Hamigerols A and B, Unprecedented Polysulfate Sterol Dimers from the Mediterranean Sponge *Hamigera hamigera*

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Two novel polysulfate sterol dimers, hamigerols A (**1**) and B (**2**), have been isolated from the Mediterranean sponge *Hamigera hamigera*. Their structures and stereochemistry have been assigned from the analysis of spectroscopic data.

A number of polyhydroxysterols and their sulfates have been described from marine sources, especially from marine Porifera and Echinodermata.[1−3] These sterol polysulfates have received considerable attention due to their anti-HIV activity.[2] Although various modifications on the steroid skeleton or its side chain have been found in these compounds, they are exclusively derivatives of monosterols and contain no more than three sulfate groups. During an effort to isolate and identify the anti-HlV active principles from the Mediterranean sponge *Hamigera hamigera*,[4] we have isolated two novel polysulfate sterol dimers, hamigerols A (**1**) and B (**2**), which contain four and five sulfate groups, respectively. In this paper we describe the structure assignment of these two compounds.

The sponge was extracted with a mixture of methanol and acetone (1:1) to give the crude extract, which showed anti-HIV activity. After solvent partitioning of the crude extract between hexane and 80% aqueous MeOH, the aqueous MeOH layer was extracted with chloroform and 1-butanol. The remaining water-soluble material was found to be the most active against HIV-l in the preliminary assay (100% inhibition at 4 μg per well). Gradient elution (H$_2$O to MeOH) of the water-soluble material on a porous ODS column followed by gel filtration (CHCl$_3$/MeOH to MeOH) of the active mixture on a Sephadex LH-20 column gave a fraction that was further purified on a reversed-phase HPLC column to yield hamigerols A (**1**) and B (**2**).[5]

Hamigerol B (**2**), colorless crystals, showed strong IR absorptions at 1225 cm^{-1}, indicating the presence of a sulfate group or groups. The positive FAB mass spectrum showed pseudomolecular ions at *m/z* 1439 [(M + K)$^+$] and 1423 [(M + Na)$^+$] and five groups of peaks at around *m/z* 1321, 1201, 1081, 961, and 841. FABMS/CID/MS indicated that these fragment peaks are due to the loss of sodium hydrogen sulfate group(s) from the parent ion, complicated by sodium/potassium exchange. Negative FABMS of **2** showed pseudomolecular ions at *m/z* 1393 [(M$^+$ + K − 2Na)$^-$] and 1377 [(M − Na)$^-$] and some desulfated fragment ions. The electrospray ionization mass spectrum (ESIMS) showed pseudomolecular ions at *m/z* 1423 [(M + Na)$^+$] and 1401 [(M + H)$^+$] as well as five sodium/proton exchanged peaks at *m/z* 1379, 1357, 1335, 1313, and 1291, suggesting a molecular mass of 1400 Da with five sodium sulfate groups for hamigerol B (**2**). This was confirmed by hydrolysis of compound **2** with HCl, which removed sodium sulfate groups to furnish a main product showing a "molecular" ion at *m/z* 890 [HRFABMS found *m/z* 913.6526, calcd for C$_{56}$H$_{90}$NaO$_8$ (M + Na)$^+$, Δ −0.7 mmu]. FDMS of **2** gave only desulfated peaks at *m/z* 819, 801 [(M − 5NaHSO$_4$ + H)$^+$] and 783. The molecular

formula C$_{56}$H$_{85}$Na$_5$O$_{23}$S$_5$, requiring 12 sites of unsaturation, was established by HRFABMS [C$_{56}$H$_{85}$Na$_5$KO$_{23}$S$_5$, (M + K)$^+$, Δ +1.0 mmu; C$_{56}$H$_{85}$Na$_6$O$_{23}$S$_5$, (M + Na)$^+$, Δ +1.6 mmu].

The ^{1}H NMR spectrum of hamigerol B (**2**) is characteristic of a steroid or terpenoid, showing 11 methyls (seven singlets and four doublets), eight oxygenated methine protons (δ 3.16−4.97), and complicated saturated aliphatic protons in the high-field region. Most of the proton signals, especially those of methyls, appeared in pairs, suggesting that hamigerol B is a bis-steroid or terpenoid that contains two similar but not identical moieties. ^{13}CNMR, DEPT, and HMQC experiments confirmed the presence of 56 carbons, including four tetrasubstituted olefinic carbons (δ 137.63, 137.34, 126.24, and 126.52), 10 oxygenated carbons (δ 71.70−88.15), four high-field sp^3 quaternary carbons (δ 39.10, 39.44, 42.51, and 42.99), 11 high-field sp^3 methines, 16 methylenes, and 11 methyls (Table 1).

Interpretation of the ^{1}H−^{1}H COSY and TOCSY spectra of hamigerol B (**2**) gave rise to two eight-carbon units, −CH$_2$−CH-(O−)−CH(O−)−CH(CH$_3$)−CH−CH(O−)−CH$_2$− (C-1−C-7 and C-28), which formed two AB ring systems of a 4-methyl-Δ^8 steroid (partial structures I and I′, Figure l), as evidenced by the following long-range ^{1}H−^{13}C couplings observed in the HMBC spectrum: H$_3$-19/C-1, C-5, C-9, C-10; H-5/C-3, C-4, C-6, C-7, C-10, C-19; H$_3$-28/C-3, C-4, C-5. One of the highest field singlet methyls, at δ 0.66, could be assigned to one of the 11-methyls coupled with a quaternary carbon at δ 42.51 (C-13), a methylene carbon at δ 38.46 (C-12), and two methine carbons at δ 53.80 (C-14) and 52.91 (C-17), respectively. COSY and TOCSY correlations indicated H-14 and H-17 are part of a D ring system unsubstituted at C-15 and C-16. Carbon-17 was also correlated with a doublet methyl (21-Me, δ 0.98), which in turn showed correlations to a methine carbon at δ 43.80 (C-20) and an oxygenated methine carbon (C-22) at δ 71.70. Since the latter oxygenated methine proton correlated with both carbons C-21 and C-17, it must be placed at C-22 (partial structure II, Figure 1). The ^{13}C chemical shifts of C and D ring carbons (C-11−C-17) were comparable to those reported for the C and D ring carbons of Δ^8-14-demethyllanosterol.[6] Similar HMBC correlations (H$_3$-19′/C-l′, C-5′, C-9′, C-10′; H-5′/C-3′, C-4′, C-6′, C-7′, C-10′, C-19′; H$_3$-28′/C-3′, C-4′, C-5′; H-18′/C-12′, C-13′, C-14′, C-17′; H$_3$-21′/C-17′, C-20′, C-22′) were observed for partial structure II′ (Figure l), except that C-22′ is a methylene (δ 36.71) instead of an oxygenated methane in partial structure I. Due to the serious overlap of proton signals in the C and C′ ring units, the connection between rings A−B and C−D was mainly based on the mass spectrometric fragmentation information (*vide infra*, also see Figure 2).

Five of the six oxygenated methines in the two A−B ring systems (C-2, 6, and 2′, 3′, 6′) appeared relatively downfield (δ 4.57−4.97;
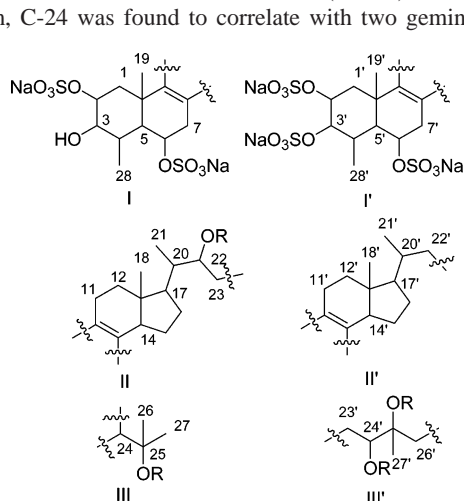
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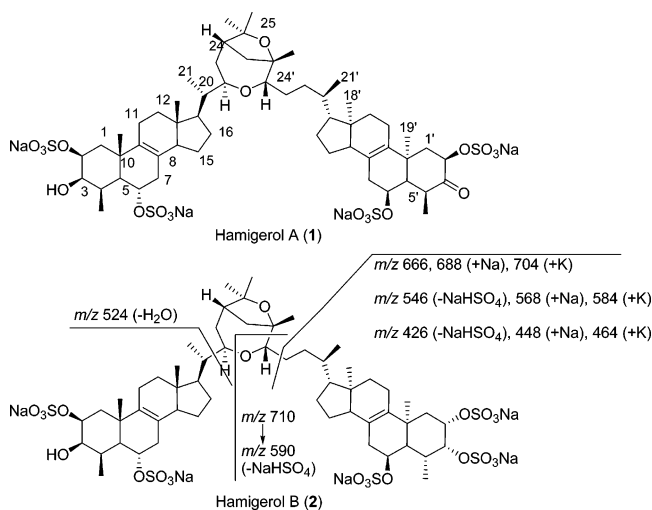
Table 1. ^{1}H and ^{13}C NMR Data for Hamigerol B (**2**, δ ppm, *J* Hz)

position	δ_H, m, *J*	δ_C, m, *J*	HMBC	position	δ_H, m, *J*	δ_C, m, *J*	HMBC
1	1.50, m	41.52, t	H-19, H-2	1′	1.79, dd, 2.5, 14.7	40.40, t	H-19
					2.51, dd, 2.9, 14.7		
2	4.81, m	79.42, d	H-1, H-3, H-4	2′	4.97, m	78.40, d	H-1′, H-3′, H-4′
3	4.00, dd, 4.0, 6.0	73.17, d	H-1, H-2, H-4, H-5, H-28	3′	4.57, dd, 4.0, 7.0	80.18, d	H-1′, H-2′, H-5′, H-28′
4	2.64, d	34.44, d	H-2, H-3, H-5, H-28	4′	2.81, m	30.30, d	H-28′, H-5′, H-3′
5	1.74, dd, 3.9, 11.7	49.95, d	H-19, H-28, H-1	5′	1.82, dd, 4.5, 12.0	49.85, d	H-19′, H-28′, H-1′
6	4.79, m	74.63, d	H-5	6′	4.82, m	74.51, d	H-5′
7	2.03, m	37.31, t	H-5	7′	2.10, m	37.24, t	H-5′
	2.89, m				2.90, m		
8		126.52, s		8′		126.24, s	
9		137.63, s	H-19	9′		137.34, s	H-19′
10		39.44, s	H-19, H-5, H-1	10′		39.10, a	H-19′, H-5′, H-4′, H-11′
11	2.11, m	22.90, t		11′	2.12, m	22.73, t	
12	1.32, m	38.46, t	H-18	12′	1.42, m	38.31, t	H-18′
	2.04, m				1.98, m		
13		42.51, s	H-18, H-12	13′		42.99, s	H-18′, H-12′
14	2.21, m	53.80, d	H-18	14′	2.21, m	42.99, s	H-18′, H-12′
15	1.40, m	25.10, t		15′	1.40, m	24.61, t	
	1.70, m				1.62, m		
16	1.30, m	30.92, t		16′	1.39, m	29.61, t	
	2.10, m				1.89, m		
17	1.50, m	52.91, d	H-18, H-21, H-22	17′	1.29, m	54.06, d	H-18′, H-21′
18	0.66, s	11.61, q		18′	0.62, s	11.11, q	
19	1.28, s	23.32, q	H-5	19′	1.31, s	23.37, q	H-5′
20	1.30, m	43.80, d	H-21, H-22	20′	1.61, m	36.25, d	H-21′
21	0.98, d, 7.5	13.11, q	H-22	21′	0.97, d, 7.5	19.47, q	
22	3.66, dd, 4.5, 11.0	71.70, d	H-21, H-24′, H-24, H-26′	22′	1.42, m	36.71, t	H-21′
23	1.23,	33.96, t	H-22, H-26′	23′	1.30, m	25.636, t	
	2.02, m				1.52, m		
24	2.13, m	45.73, d	H-26′, H-26, H-27	24′	3.17, brd, 5.5	86.62, d	H-26′, H-27′, H-22
25		85.61, s	H-26, H-27, H-26′	25′		88.15, s	H-27′, H-26′, H-24′, H-24
26	1.33, s	25.63, q	H-27	26′	2.01, m	36.48, t	H-27′
					2.20, m		
27	1.21, s	32.64, q	H-26, H-24	27′	1.13, s	27.10, q	H-26′
28	1.26, d, 5.6	10.35, q	H-3, H-5	28′	1.31, d, 5.6	10.20, q	H-3′, H-5′

δ 74.51−80.68) and could be assigned to those with sodium sulfate groups,[7] while the remaining methine at δ 4.00 and 73.17 (C-3) must be a free carbinol group.[8] After assignment of five sodium sulfate groups and one free hydroxyl group to two steroid cores, only two oxygen atoms remained to account for four oxygenated carbons, suggesting the presence in the side chain of two ether units. One of them formed, from two oxygenated methines (C-22 and C-24′, see Figure 1), could be easily recognized since there were two mutual cross-peaks (H-22/C-24′and H-24′/C-22) among their protons and carbons in the HMBC spectrum.[9] The two remaining tertiary oxygenated carbons (δ 85.61, C-25, and 88.15, C-25′) must therefore be linked through an oxygen atom. In the COSY spectrum, H-22 (δ 3.66) showed couplings to methylene protons at δ 1.23 and 2.02 (C-23, δ 33.96), while in the TOCSY spectrum the spin network was extended to the 24-methine (δ 2.13). In the HMBC spectrum, C-24 was found to correlate with two geminal singlet

methyls at δ 1.33 and 1.21, which both coupled with C-25 (δ 85.61) and with each other as well (partial structure III, Figure 1).

Partial structure III′ (C-24′ to C-27′) was assigned on the basis of the following spectroscopic data. The singlet methyl at δ 1.13 (C-27′) showed couplings to two oxygenated carbons at δ 86.62 (d, C-24′) and 88.15 (s, C-25′) and one methylene carbon at δ 36.48 (C-26′) in the HMBC spectrum. The 24′-methine proton appeared as a doublet of doublets in the ^{1}H NMR spectrum, indicating its connection to a methylene group (C-23′). On the other hand, the C-26′ methylene protons (δ 2.02 and 2.20) showed cross-peaks in the HMBC spectrum to both C-25 and C-24, thus linking it to C-24 in the partial structure III. Since connections between partial structures II and III via the C-23/C-24 bond and the oxygen bridge connections between C-25 and C-25′ and between C-22 and C-24′
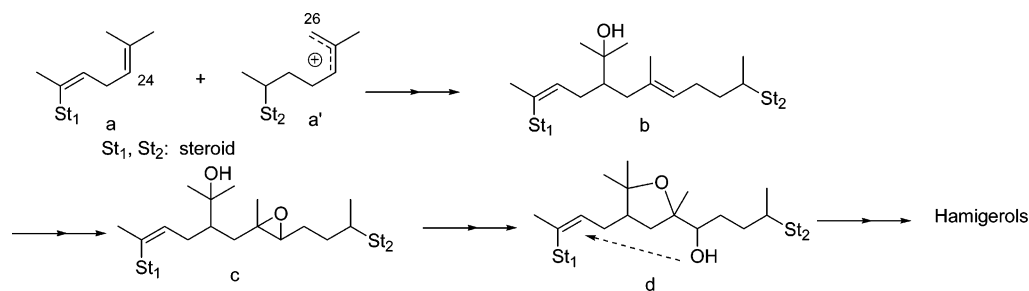

Figure 1. Partial structures of hamigerol B (**2**).

Figure 2. Complete structures of hamigerols A (**1**) and B (**2**).

Figure 3. Proposed biogenetic pathway of hamigerols.

Table 2. ^1H and ^{13}C NMR Data for Hamigerol A (**1**, δ ppm, J Hz)

position	δ_H, m, J	δ_C, m, J	HMBC	position	δ_H, m, J	δ_C, m, J	HMBC
1	1.52, dd 2.48, dd, 2.6, 14.8	41.47, t	H-19, H-2	1′	1.94, dd, 4.6, 13.2 2.77, dd, 13.2, 13.2	45.66, t	H-19′
2	4.78, brd, 3.2	80.35, d	H-1, H-3, H-4	2′	5.38, dd, 4.4, 13.2	76.02, d	H-1′, H-4′
3	3.92, dd, 3.6, 6.0	73.34, d	H-1, H-2, H-4, H-5, H-28	3′		216.05, s	H-1′, H-2′, N-28′, H-5′
4	2.58, dq, 1.2, 6.8	34.49, d	H-28, H-5, H-2, H-3	4′	2.70, brdq, 6.8	46.20, d	H-28′
5	1.84, dd, 4.2, 11.8	49.55	H-19, H-28, H-1	5′	2.23, m	50.96, d	H-19′, H-28′, H-1′
6	4.81, dt, 5.6, 11.0	74.74, d	H-5	6′	4.51, dt, 6.6, 10.3	78.13, d	H-5′
7	2.15, m 2.92, bdd, 5.6, 15.6	37.36, t	H-5	7′	2.20, m 2.67, m	36.66, t	H-5′
8		126.40, s		8′		127.95, s	
9		137.72, s	H-19	9′		134.31, s	H-19′
10		39.37, s	H-19, H-5, H-1	10′		40.53, s	H-19′, H-5′, H-4′, H-11′
11	2.15, m	23.09, t		11′	2.15m,	22.77, s	
12	1.45, m 2.05, m	38.42, t	H-18	12′	1.45, m 2.05, m	38.42, t	H-18′
13		42.80, s	H-18, H-12	13′		42.93, s	H-18′, H-12′
14	2.18, m	53.43, d	H-18	14′	2.25, m	52.79, d	H-18′
15	1.38, m 1.65, m	25.35, t		15′	1.38, m 1.65, m	25.11, t	
16	1.30, m 2.10, m	30.87, t		16′	1.40, m 1.95, m	29.69, t	
17	1.55, m	52.79, d	H-18, H-21, H-22	17′	1.30, m	54.83, d	H-18′, H-21′
18	0.66, s	11.64, q		18′	0.65, s	11.04, q	
19	1.31, s	23.20, q	H-5	19′	0.94, s	20.96, q	H-5′
20	1.35, m	43.92, d	H-21, H-22	20′	1.59, m	36.32, d	H-21′
21	0.99, d, 6.8	13.35, q	H-22	21′	0.97, d, 6.8	19.35, q	
22	3.69, dd, 3.6, 11.6	71.54, d	H-21, H-24′, H-24	22′	1.45, m 1.55, m	36.66, t	H-21′
23	1.30, m 2.04, m	34.06, t	H-22, H-26′	23′	1.30 m 1.60, m	24.65, t	
24	2.13, m	45.67, d	H-26′, H-26, H-27	24′	3.17, brd, 5.5	85.72, d	H-26′, H-27′
25		85.53, s	H-26, H-27, H-26′	25′		88.09, s	H-27′, H-26′, H-24′
26	1.32, s	25.63, q	H-27	26′	2.05, m 2.20, m	36.44, t	H-27′
27	1.22, s	32.51, q	H-26, H-24	27′	1.15, s	27.28, q	H-26′
28	1.26, d, 7.6	10.04, q	H-3	28′	1.47, d, 7.2	20.85, q	

have already been established (*vide supra*), the linkage between C-22′ and C-23′ could be made, to give a 3,8-dioxabicyclo[4.2.1]-nonane skeleton,[10] leading to structure **2** (Figure 2).

FABMS of hamigerol B (**2**) gave several characteristic peaks in the range m/z 400 to 700. A fragment ion at m/z 524 corresponds to cleavage of the C-20 and C-22 bond with loss of a water molecule, supporting the assignment of an A–B ring linked to a C–D ring. The fragment ions at m/z 710 and 590 can be explained by the cleavage of C-24′–C-25′ and C-22/O bonds. Furthermore, the three fragment ions at m/z 666, 546, and 426 correspond to fragmentation between C-23′ and C-24′, in agreement with the assigned structure (see Figure 2).

The assignment of a structure to hamigerol A (**1**) is relatively straightforward. Both positive FABMS and ESIMS showed the same pseudomolecular ion at m/z 1319 [(M + Na)$^+$]. The molecular formula $C_{56}H_{84}Na_4O_{20}S_4$, established by HRFABMS [$C_{56}H_{84}Na_4-KO_{20}S_4$, (M + K)$^+$, Δ −1.1 mmu], indicated the presence of four sulfate groups, plus one more site of unsaturation than in hamigerol B (**2**). Eleven methyls in hamigerol A (**1**) were clearly resolved, with one singlet (H$_3$-19′) being shifted upfield (A: δ 0.94; B: δ 1.31) and one doublet (H$_3$-28′) downfield (A: δ 1.47; B: δ 1.31)

relative to hamigerol B (**2**). Interpretation of 1D (^1H, ^{13}C NMR and APT) and 2D NMR spectra (COSY, TOCSY, HMQC, HMBC) revealed that one of the two steroid moieties (ABCD) is coincident with that in hamigerol B (**2**), while the other one (A′B′C′D′) contains a carbonyl group instead of a sodium sulfate unit at C-3′. The ^1H and ^{13}C data for the side chains are almost identical with those of hamigerol B (**2**).

The relative stereochemistry was assigned mainly on the basis of proton–proton coupling constants and NOE experiments. The narrow multiplicities of H-2 (δ 4.81) in hamigerol B (**2**) indicated the lack of large vicinal coupling constants, and this proton must be equatorial. The H-3 proton exhibited an NOE in the ROESY spectrum to H-5, which showed a large coupling constant with H-6 ($J_{5,6} = 11.7$ Hz). Thus, protons at C-3, C-5, and C-6 were all axial. Similarly, a small coupling constant between H-4 and H-5 ($J_{4,5} = 3.9$ Hz) suggested an equatorial orientation for H-4. This is also consistent with NOEs observed for H-6 to H$_3$-19 and H$_3$-28 in the ROESY spectrum. Similar NOEs and proton–proton coupling constants were observed for the other steroid skeleton (A′B′C′D′). The 21- and 21′-methyls were assigned as α and β on the basis of

their chemical shifts (δ 0.98 and 0.97),[11] whereas C-18 and C-18′ methyls were arbitrarily assigned the β and α configurations.

H-22 was found spatially proximate to H-26′, H$_3$-21, and H-17 since NOEs were observed for H-22 to H-26′, H$_3$-21, and H-17 in the ROSEY spectrum. The NOE for H-24′ and H$_3$-27′ justified placing H-24′ in the β position. The relatively upfield resonance of H-24′ (δ 3.17) supports the assignment of an *endo* orientation.

The stereochemistry for the ABCD steroid unit in hamigerol A (**1**) was found to be the same as that of hamigerol B (**2**). The proton H-2′ in hamigerol A (**1**), however, showed a large coupling constant with H-1′ ($J_{1',2'}$ = 13.2 and 4.4 Hz), indicating an axial orientation. Also strong NOEs were observed for H-2′ to H-4′ and H$_3$-19′. These results suggest a chair conformation of the A′ ring with H-2′, H-4′, and H$_3$-19′ axial. The sodium sulfate group at C-6′ was assigned a β (equatorial) configuration by an NOE between H-6′ and H$_3$-19′ and a large coupling constant between H-5′ and H-6′ ($J_{5',6'}$ = 10.3 Hz). The stereochemistry for the side chain ring system in hamigerol A (**1**) was identical to that in hamigerol B (**2**) on the basis of ^{1}H and ^{13}C NMR chemical shifts, ^{1}H–^{1}H coupling constants, and NOE results. Although almost all 2,3,6-trihydroxy-lsterol sulfates reported so far have 2β,3α,6α configurations,[1–3] hamigerols seem to have a different array for the C-3 sodium sulfate group. The absolute configurations for hamigerols were undetermined.

There have been only a few reports of isolation of natural bis-steroids from marine and terriestrial sources. These include cepha-lostatins, highly cytotoxic pyrazine derivatives of two steroids from a marine worm, *Cephatodisens gilchristi*,[12a] and a Diels–Alder-type bis-steroid, bistheonellasterone, from a marine sponge, *Theonella swinhoei*.[12b] An ergostane-type steroid dimer that was linked via a C-6/C-6′ bond and dimeric triterpenoid quinone ethers have been described from a fungus[13a] and a plant,[13b] respectively. Fusetani and co-workers isolated several cephalostatin-related steroid dimers from an Okinawan tunicate, *Ritterella tokioka*.[14] Unlike these above-mentioned steroid dimers, hamigerols seem to be biogenetically quite unique compounds that could be derived from two lanosterol-type steroids through an enzymatically mediated carbocation–olefin coupling. Thus, the coupling of the olefin side chain of steroid **a** with its cation derivative (**a′**) would give a linear bis-steroid intermediate **b**, which undergoes epoxidation (**c**), ring cyclization (**d**), and oxidation to give the hamigerol skeleton (Figure 3). The carbocation–olefin reaction is a common process in the formation of triterpenes from their linear precursors, but a chemically mediated carbocation–olefin reaction is not so easily achievable.[15]

Experimental Section

General Experimental Procedures. Melting points were measured on a Reichert melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP 370 polarimeter with a Na lamp using a 5 × 0.35 cm cell. Infrared spectra were taken on an IBM IR/32 FTIR spectrophotometer using KBr pellets. ^{1}H and ^{13}C NMR, APT, DEPT, ^{1}H–^{1}H COSY, TOCSY, and ROESY spectra were recorded on a GN500 (500 MHz for ^{1}H) or a Varian UNITY 400 (400 MHz for ^{1}H) spectrometer using CD$_3$OD as solvent. The 3.30 ppm resonance of residual CD$_2$HOD and 49.0 ppm signal of CD$_3$-OD were used as internal references for ^{1}H and ^{13}CNMR, respectively. Fast atom bombardment mass spectra including positive and negative FABMS were recorded on a ZAB-SE spectrometer using magic bullet[16] as matrix, and high-resolution FABMS (HRFABMS) and FABMS/CID/MS spectra on a VG 70SE-4F spectrometer. FDMS spectra were recorded on a Varian MAT 731 spectrometer. Electrospray ionization mass spectra (ESIMS) were obtained on a VG Quattro mass spectrometer using CH$_3$CN/H$_2$O (1:l) as carrier solvents. The sample was dissolved in MeOH containing 0.l% formic acid. The instrument was calibrated with CsI and scanned from 300 to 2000 Da within 10 s.

Biological Material. The sponge *Hamigera hamigera* was collected at latitude 39°53.5′ N and longitude 0°41.5′ E at Colombretes Islands, Spain.

Extraction and Isolation. The sponge was extracted with mixed solvents of methanol/acetone (1:1) to give the crude extract (35.1 g). The crude extract was partitioned between hexane (3.9 g) and 80% aqueous MeOH, and the resulting aqueous MeOH layer was subsequently extracted with CHCl$_3$ (1.03 g) and 1-BuOH (10.5 g). The remaining H$_2$O-soluble material (19.0 g) was found to be active against HIV-1 in preliminary assays (100% inhibition at ca. 4 μg per well). The H$_2$O layer was chromatographed on a porous ODS column using a H$_2$O to MeOH gradient to give five fractions. Fractions 1 to 3 (1.8 g) eluted with 10–50% MeOH in H$_2$O gave the highest activity against HIV-1 and were combined. Fractionation by gel filtration on a Sephadex LH-20 column [CHCl$_3$/MeOH (1:1 to 1:4) to 100% MeOH] afforded two major fractions. Fraction 1 was further purified on a HPLC column (Econosil C$_{l8}$, 35% MeOH in H$_2$O) to yield hamigerols A (**1**, 24.9 mg) and B (**2**, 13.5 mg).

Hamigerol A (1): mp 149−151 °C; [α]$_D$ +77 (c 0.60, MeOH); IR (KBr) ν_{max} 3400, 2925, 2850, 1710, 1225, 1050, 950 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; FABMS, ESIMS, FDMS, see Supporting Information.

Hamigerol B (2): mp 150−152 °C; [α]$_D$ +51 (c 0.37, MeOH); IR (KBr) ν_{max} 3400, 2925, 2850, 1225, 1050, 950 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; FABMS, ESIMS, FDMS, see Supporting Information.

Supporting Information Available: Copies of FABMS, ESIMS, FDMS, and HRFABMS lists and ^{1}H NMR, ^{13}C NMR, COSY, TOSCY, HMQC, and HMBC spectra of hamigerols A and B. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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(4) The sponge was identified by Dr. M. Vribe. "Hamigera" has also been used as the genus name of a fungus (e.g., *Hamigera avellanea*).

(5) Pure hamigerols proved to be inactive against HIV- 1. The active fractions containing similar steroid sulfates will be reported elsewhere.

(6) Tsuda, M.; Schroepfer, G. J., Jr. *J. Org. Chem.* **1979**, *44*, 1290−1293.

(7) O-Sulfonation causes usually a ca. 8 ppm chemical shift change for monosulfated steroids; see for example: D'Auria, M. V.; Riccio, R.; Minale, L.; BaBarne, S.; Pusset, J. I. *J. Org. Chem.* **1987**, *52*, 3947−3952. For glycol-type disulfates the shift values vary with stereochemistry and are 4−6 ppm. Calculated from reported values, the corresponding diols or similar compounds were used as standards.

(8) The presence of a free C-3 hydroxyl group in hamigerol A (**1**) was confirmed by acetylation (acetic anhydride, pyridine, rt, 1 week), which afforded a monoacetylated product: FABMS *m/z* 1361 [(M + Na)$^+$], 1259 [(M + Na − NaSO$_3$ + H)$^+$], 1241 [(M + Na − NaSO$_4$ + H)$^+$], 1139, 1019, and 959. Most proton signals in the ^{1}H NMR spectrum were unchanged, except the proton at C-3 (δ 3.92, 73.34), which was shifted downfield to δ 5.2. The CH$_3$CO methyl appeared at δ 2.10 as a singlet.

(9) A CH(O−)−CH$_n$−CH(O−) (where n = 1 or 2) sequence that could show the same mutual cross-peaks between two methines can be excluded since no coupling among protons in such a sequence was observed in either the COSY or the TOCSY spectrum. For n = 0, two oxygenated methine protons would appear as a doublet and a singlet, respectively. This is apparently not the case. Also there is no high-field sp^3 quaternary carbon left for the side chain. For details, see text.

(10) There is no report on this ring system in the literature.
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(15) See for example: Corey, E. J.; Lee, J.; Liu, D. R. *Tetrahedron Lett.* **1994**, *49*, 9149−9152.
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