Iso-, *Nor*-, and *Dinor*-Spiculoic Acids A, Polyketides from the Marine Sponge *Plakortis zyggompha*

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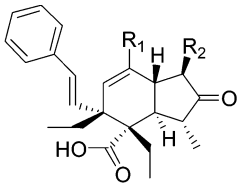
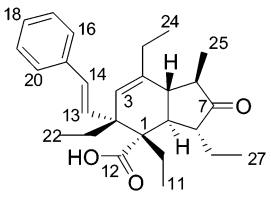
The new iso-, *nor*-, and *dinor*-spiculoic acids A (**1**, **2**, and **3**, respectively) with a rare spiculane skeleton were isolated from the marine sponge *Plakortis zyggompha*, collected in the waters south of Martinique. The structural determination of the compounds was based on 1D and 2D NMR studies and mass spectral determinations. Compounds **1** and **2** showed weak cytotoxicity against the two tumor cell lines A549 and HT29.

Various types of polyketide secondary metabolites such as cyclic peroxides,[1−3] lactones,[4−6] and bicyclic carboxylic acids[7,8] have been isolated from sponges of the genus *Plakortis*. Recently, a new family of polyketides with an uncommon spiculane skeleton was described by Andersen et al.[9] Spiculoic acids A and B were isolated from the Caribbean sponge *Plakortis angulospiculatus*, spiculoic acid A (**4**) showing moderate cytotoxicity against human breast cancer MCF-7 cells.

As part of our ongoing search of new biologically active substances from marine organisms,[10,11] we undertook the study of the sponge *Plakortis zyggompha* (order Homosclerophorida, family Plakinidae). After a biological primary screening, this organism was selected among more than 250 samples collected around Martinique Island in 2002. Fractionation of the *P. zyggompha* extract led to the isolation of three new **4** derivatives, iso-, *nor*-, and *dinor*-spiculoic acids A (**1**, **2**, and **3**). The structural elucidation of the new metabolites, as well as their cytotoxicity against two tumor cell lines, is presented below. An interpretation of their relative stereochemistry on the basis of their biogenetic pathway is given.



Isospiculoic acid A (**1**) R_1= Et, R_2= Et
Nor-spiculoic acid A (**2**) R_1= Et, R_2= Me
Dinor-spiculoic acid A (**3**) R_1= Me, R_2= Me



Spiculoic acid A (**4**)

Results and Discussion

The CH_2Cl_2−MeOH (1:1) extract of the marine sponge *P. zyggompha*[12] was subjected to a bioassay-guided fractionation using cytotoxicity against three cancer cell lines: A549, HT29, and MDA-MB-231. After an H_2O−CH_2Cl_2 partition, the bioactive CH_2Cl_2 layer was successively purified by silica gel chromatography (hexane to MeOH) and C_{18} reversed-phase HPLC (CH_3CN−H_2O) to afford three new derivatives, **1**, **2**, and **3**.

Isospiculoic acid A (**1**) was isolated as a colorless oil, and its molecular formula $C_{27}H_{36}O_3$ was deduced from HREIMS and supported by ^{13}C NMR data. The formula required 10 degrees of unsaturation including, after interpretation of the ^1H−^1H COSY, HSQC, and HMBC experiments, two carbonyl resonances (δ_C 177.4, C-12; 219.2, C-7), one monosubstituted phenyl group (δ_C 137.7, C-15; 126.5, C-16/20; 128.7, C-17/19; 127.3, C-18), one disubstituted *trans* double bond [δ_C 136.4, C-13; 131.9, C-14; δ_H 6.00 (1H, d, J = 15.9 Hz, H-13), 6.19 (1H, d, J = 15.9 Hz, H-14)], and another trisubstituted one [δ_C 123.0, C-3; 140.6, C-4; δ_H 5.23 (1H, bs, H-3)]. These observations and the comparison of the ^{13}C NMR data of **1** with the litterature values revealed a high level of similarity between compound **1** and spiculoic acid A (**4**) (Table 1). They allowed us to assign all the ^{13}C signals of **1** except those around the cyclopentane ring. The COSY and HSQC experiments indicated the presence of one methyl and four ethyl groups as in compound **4**. Thus, the observed differences were due to another distribution of these substituents around the cyclopentane ring. Signals at δ_C 40.8 (C-5) and δ_H 2.31 (H-9) were unambiguously assigned by the successive key correlations: C-5/H-3 HMBC, H-5/H-9 COSY, and C-10/H-9 HMBC (Figure 1). COSY correlations H-5/H-6, H-6/H-25, and H-25/H-25′ allowed the assignments of the signals at δ_H 2.00 (H-6), 2.11 (H-25a), 1.74−1.68 (H-25b), and 0.94 (H-25′). Together with the HMBC correlations C-7/H-26 and C-7/H-25b, this confirmed the positions of an ethyl substituent (C-25, C-25′) at C-6 and of the remaining methyl group (C-26) at C-8. Because of the overlapping of the H-8 and H-9 signals, the relative stereochemical assignments of compound **1** were not possible to determine by interpretation of the ^1H NMR scalar coupling constants, but were assessed in the 2D NOESY experiment (Figure 2). H-5/H-10, H-5/H-25′, and H-10/H-21 NOE correlations positioned each of these protons on the same side of the molecule. Furthermore, despite H-8 and H-9 having the

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Table 1. ^{13}C NMR Spectral Data of Compounds **1**−**3**[a] and **4**[9]

C#	**1**		**2**		**3**		**4**
1	54.0	qC	54.1	qC	54.2	qC	54.1
2	50.7	qC	50.7	qC	51.0	qC	51.1
3	123.0	CH	123.0	CH	125.1	CH	123.2
4	140.6	qC	140.4	qC	134.7	qC	140.9
5	40.8	CH	46.5	CH	47.7	CH	46.0
6	52.0	CH	46.8	CH	46.7	CH	47.8
7	219.2	qC	220.3	qC	220.0	qC	220.3
8	49.7	CH[b]	48.3	CH	48.3	CH	52.3
9	46.7	CH[b]	47.1	CH	47.0	CH	42.2
10	22.5	CH_2	22.6	CH_2	22.7	CH_2	22.8
11	12.3	CH_3	12.2	CH_3	12.2	CH_3	12.3
12	177.4	qC	179.0	qC	179.0	qC	179.9
13	136.4	CH	136.3	CH	136.2	CH	136.4
14	131.9	CH	132.0	CH	132.1	CH	132.2
15	137.7	qC	137.7	qC	137.7	qC	137.8
16/20	126.5	CH	126.5	CH	126.5	CH	126.2
17/19	128.7	CH	128.7	CH	128.7	CH	128.5
18	127.3	CH	127.3	CH	127.3	CH	127.1
21	27.4	CH_2	27.4	CH_2	27.2	CH_2	27.2
22	9.3	CH_3	9.3	CH_3	9.3	CH_3	9.1
23	27.8	CH_2	28.3	CH_2	22.7	CH_3	28.2
24	13.1	CH_3	13.1	CH_3			13.0
25	22.7	CH_2	16.4	CH_3	16.2	CH_3	15.5
25′	10.4	CH_3					
26	14.3	CH_3	14.9	CH_3	15.0	CH_3	22.1
27							9.6

[a] All compounds were determined at 125 MHz in $CDCl_3$; chemical shift values δ are in ppm; all assignments were confirmed by 2D NMR (see Supporting Information). [b] Assignments interchangeable.

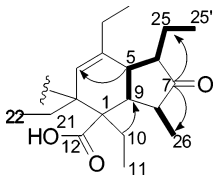

Figure 1. Key $^1H-^1H$ COSY (solid bold lines) and C−H HMBC correlations (curved arrows) of isospiculoic acid A (**1**).

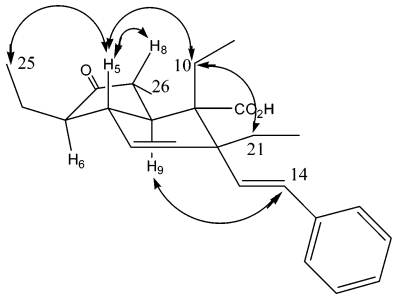

Figure 2. Key NOESY (curved arrows) correlations of **1**.

same ^1H chemical shift, the key δ_H 2.31/H-14 NOE correlation was assigned to the H-9/H-14 correlation. Indeed, molecular modeling (ChemBats3D and MM2 minimization) of **1** showed that H-8 and H-14 were spatially too far to allow any NOE. Finally, the H-8/H-5 NOE correlation allowed the relative stereochemical assignment of the C-26 methyl group.

Nor-spiculoic acid A (**2**) was isolated as a colorless oil, and its molecular formula was determined as $C_{26}H_{34}O_3$ from HREIMS. In comparison with **1**, ^{13}C NMR and mass spectra evidenced the lack of one methylene group. Furthermore, the disappearance of the triplet methyl signal at δ_H 0.94 and the presence of a new doublet methyl signal at δ_H 1.37 (J = 6.9 Hz, H-25) suggested the loss of the methylene unit from C-6. Our assumption was confirmed by examination of the $^1H-^1H$ COSY experiment, allowing the assembly of the structural fragment H-25/6/5/9/8/26. In this case, the scalar coupling constants between H-5/H-9, H-5/H-6, and H-8/H-9 (J = 11.4, 11.4, and 12.6 Hz, respectively) could be measured. Such high values required a pseudoaxial/pseudoaxial coupling for these protons and consequently corroborated the relative stereochemistry of compound **1** described herein.

Dinor-spiculoic acid A (**3**) was isolated as a colorless oil, and its molecular formula was determined as $C_{25}H_{32}O_3$ from HREIMS. In comparison with **2**, ^{13}C NMR and mass spectra evidenced the lack of one methylene group. The disappearances of both signals at δ_H 2.28 (multiplet) and 1.16 (triplet) together with the presence of a new singlet signal at δ_H 1.95 (H-23) indicated the loss of the methylene unit from C-4. The relative stereochemistry of **3**, deduced from the scalar coupling constant values and 2D NOESY data, was the same as in compounds **1** and **2**.

The structures of the three new spiculoic acid A derivatives **1**, **2**, and **3** are consistent with the polyketide origin of compound **4** proposed by Andersen et al.[9] Here also butyrate and propionate units intervene in the biogenetic pathway, and their alternate successive incorporations induce the structural differences between compounds **1**, **2**, **3**, and **4**. In the same way, a similar biosynthetic pathway is proposed for plakotenins,[7,8] secondary metabolites isolated from marine sponges of the same Plakinidae family. The intriguing closely related stereochemistry of these two families of compounds suggests an identical alternation in the configuration of the stereogenic centers created (see Supporting Information).

The final biogenetic stage is a [4+2] cycloaddition catalyzed by a Diels−Alderase recently shown to be involved in the biosynthesis of lovastatin.[13] The relative stereochemistry of the stereogenic centers in spiculoic acids implies that the [4+2] cycloaddition occurs through an *endo* transition state leading to a *trans* ring junction.[14] In the case of plakotenins the final steps would require a *Z* double bond and an *exo* [4+2] cycloaddition to be consistent with the described relative stereochemistry.[7,8]

Compounds **1** and **2** were found to be mildly cytotoxic (GI_{50} ca. 15−20 μM) against both tumor cell lines A549 (lung carcinoma) and HT29 (colon carcinoma) and inactive (GI_{50} > 25μM) against MDA-MB-231 (breast). Compound **3** was inactive against the three tumor cell lines.

Experimental Section

General Experimental Procedures. Optical rotations were measured in CH_2Cl_2 on a Jasco P-1020 polarimeter. IR spectrum were recorded on a Perkin-Elmer Paragon 1000 FT-IR spectrophotometer. NMR experiments were performed on a Bruker DRX 500, using a standard Bruker program. Chemical shifts were reported in ppm using residual $CDCl_3$ (δ 7.26 for ^1H and 77.16 for ^{13}C) as internal reference. EIMS were recorded on a VG AutoSpec spectrometer, and ESIMS were performed on a Bruker Esquire 3000 Plus spectrometer.

Animal Material. *Plakortis zyggompha* (de Laubenfels, 1934) was collected by scuba diving in July 2002 in a cave at a depth of 20 m, near the "Rocher du Diamant" (14°26′060 N, 61°02′040 W) in the south of Martinique Island. The specimen was immediately frozen. The material was identified by Dr. Iosune Uriz (Blanes, Spain), and a voucher specimen (OR-MA008545) has been deposited at the company PharmaMar S.A.

Extraction and Isolation. The frozen sample of *P. zyggompha* (170 g) was extracted with a mixture of MeOH−CH_2Cl_2 (1:1) to give after evaporation 10 g of brown gum. The extract was partitioned between H_2O and CH_2Cl_2, and the CH_2Cl_2 layer (3 g residue) was subjected to a silica gel chromatography column using a gradient of hexane to MeOH to give 15 fractions. Half of the fraction 7 (286 mg) was further

Table 2. ^{1}H NMR Spectral Data of Compounds **1**−**3**[a] and **4**[9]

C#	1	2	3	4
3	5.23 (bs)	5.26 (bs)	5.26 (bs)	5.25 (d, 2.4)
5	2.44 (m)	2.19 (dd, 11.4, 11.4)	2.09 (dd, 11.4, 11.4)	2.15 (dd, 11.8, 11.4)
6	2.00 (m)	1.99 (m)	1.98 (m)	1.88 (m)
8	2.31 (m)	2.43 (dq, 12.6, 6.7)	2.43 (dq, 12.7, 6.7)	2.45 (ddd, 11.8, 5.0, 3.4)
9	2.31 (m)	2.37 (dd, 12.6, 11.4)	2.39 (dd, 12.7, 11.4)	2.64 (dd, 11.8, 11.8)
10	2.32 (m)	2.32 (m)	2.32 (m)	2.25 (m)
	1.74−1.68 (m)	1.68 (dq, 14.4, 7.4)	1.70 (dq, 14.4, 7.4)	1.67 (m)
11	1.10 (t, 7.4)	1.11 (t, 7.4)	1.10 (t, 7.4)	1.07 (t, 7.3)
13	6.00 (d, 15.9)	6.01 (d, 15.9)	5.97 (d, 15.9)	6.03 (d, 16.0)
14	6.19 (d, 15.9)	6.21 (d, 15.9)	6.22 (d, 15.9)	6.25 (d, 16.0)
16/20	7.29 (m)	7.30 (bd, 7.6)	7.30 (bd, 7.1)	7.28 (dd, 7.3, 1.9)
17/19	7.27[b]	7.25[b]	7.26[b]	7.22 (t, 7.3)
18	7.18 (tt, 7.0, 1.5)	7.18 (bt, 7.1)	7.18 (tt, 7.2, 1.3)	7.15 (tt, 7.3, 1.9)
21	1.78 (dq, 13.3, 7.4)	1.82 (dd, 13.5, 7.4)	1.81 (dq, 13.6, 7.4)	1.83 (m)
	1.63 (dq, 13.3, 7.4)	1.65 (dd, 13.5, 7.4)	1.65 (dq, 13.6, 7.4)	1.65 (m)
22	0.86 (t, 7.4)	0.87 (t, 7.4)	0.85 (t, 7.4)	0.86 (t, 7.3)
23	2.24 (m)	2.28 (m)	1.95 (s)	2.25 (m)
24	1.16 (t, 7.4)	1.16 (t, 7.4)		1.13 (t, 7.3)
25	2.11 (dqd, 14.2, 7.4, 3.1)	1.37 (d, 6.9)	1.36 (d, 6.9)	1.33 (t, 6.5)
	1.74−1.68 (m)			
25′	0.94 (t, 7.4)			
26	1.07 (d, 6.1)	1.12 (d, 6.6)	1.12 (d, 6.6)	1.83 (m)
				1.58 (m)
27				0.63 (t, 7.3)

[a] All compounds were determined at 500 MHz in $CDCl_3$; chemical shift values δ are in ppm, and coupling constant values J in Hz. [b] Overlapped with $CDCl_3$ signal.

purified by HPLC on a C_{18} semipreparative column (SymmetryPrep C_{18} 7.8 × 300 mm, 7 μm) with 85% CH_3CN in H_2O to yield isospiculoic acid A (**1**, 2.7 mg, 1.6×10^{-3} % wet wt), *nor*-spiculoic acid A (**2**, 8.3 mg, 4.9×10^{-3} % wet wt), and *dinor*-spiculoic acid A (**3**, 4.8 mg, 2.8×10^{-3} % wet wt).

Isospiculoic acid A (1): colorless oil; $[\alpha]^{24}_D$ +134.4° (*c* 0.16, CH_2Cl_2); ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 1; ESIMS (MeOH) *m/z* 407 $[M - H]^-$; HREIMS $[M]^+$ *m/z* 408.2663 (calcd for $C_{27}H_{36}O_3$, 408.2664).

***Nor*-Spiculoic acid A (2):** colorless oil; $[\alpha]^{24}_D$ +147.8° (*c* 0.11, CH_2Cl_2); IR (film) 2954, 1734, 1688, 1461, 1379 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 1; ESIMS (MeOH) *m/z* 393 $[M - H]^-$; HREIMS $[M]^+$ *m/z* 394.2515 (calcd for $C_{26}H_{34}O_3$, 394.2508).

***Dinor*-Spiculoic acid A (3):** colorless oil; $[\alpha]^{24}_D$ +65.4° (*c* 0.09, CH_2Cl_2); ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 1; ESIMS (MeOH) *m/z* 379 $[M - H]^-$; HREIMS $[M]^+$ *m/z* 380.2342 (calcd for $C_{25}H_{32}O_3$, 380.2351).

Biological Activity. A colorimetric assay using sulforhodamine B has been adapted for a quantitative measurement of cell growth and viability following the technique described in the literature.[15] The in vitro activity of the compounds was evaluated against three tumor cell lines, including lung carcinoma A 549, colon carcinoma HT29, and breast MDA-MB-231.

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Note Added after ASAP Publication: An error in the ^{1}H NMR data for compound **4** was present in the version posted on March 29, 2005. The corrected value in Table 2 appears in the version posted on April 6, 2005.

Supporting Information Available: ^{13}C, ^{1}H, HMBC, COSY, and NOESY NMR data of iso-, *nor*-, and *dinor*-spiculoic acid A (**1**, **2**, and **3**, respectively), and proposed biogenesis of plakotenin and spiculoic acid families (Scheme S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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