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Spiculoic Acids A and B, New Polyketides Isolated from the Caribbean Marine Sponge *Plakortis angulospiculatus*

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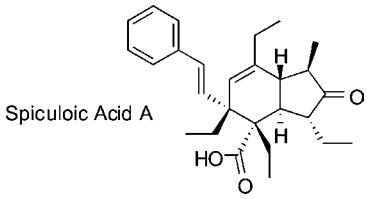
ABSTRACT



Two novel polyketides, spiculoic acids A (1) and B (2), have been isolated from extracts of the Caribbean marine sponge *Plakortis angulospiculatus*. The structures of 1 and 2 were elucidated by detailed analysis of spectroscopic data. Spiculoic acid A (1) showed in vitro cytotoxicity against human breast cancer MCF-7 cells. It has a putative polyketide biogenetic origin that involves incorporation of four butyrate units and a Diels Alderase catalyzed intramolecular [4 + 2] cycloaddition reaction.

Marine sponges in the genus *Plakortis* have proven to be a rich source of polyketide secondary metabolites that frequently exhibit interesting biological activities.[1] As part of an ongoing program to screen marine invertebrate extracts for antimitotic[2] and cytotoxic[3] metabolites, we have investigated the MeOH extract of the Caribbean sponge *Plakortis angulospiculatus* (Carter). Fractionation of the crude extract led to the isolation of the novel cytotoxic metabolite, spiculoic acid A (**1**), and the inactive analogue, spiculoic acid B (**2**). Spiculoic acid A (**1**) has the unprecedented polyketide-derived spiculane (**3**) carbon skeleton. Details of the isolation, structure elucidation, proposed biogenesis, and biological activity of **1** and **2** are presented below.

Specimens of *P. angulospiculatus*[4] were collected by hand using scuba on reefs off the coast of Dominica, frozen on site, and transported to Vancouver over dry ice. Freshly thawed sponge tissue (50 g) was exhaustively extracted with MeOH, and the gum obtained by evaporating the combined MeOH extracts in vacuo was partitioned between EtOAc and

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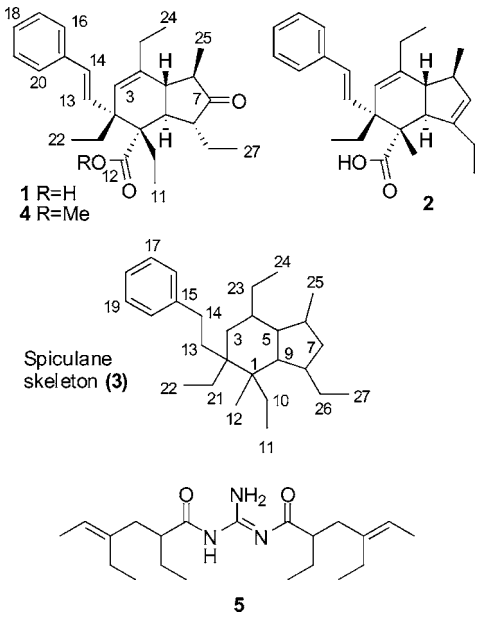


H_2O. The bioactive EtOAc-soluble portion was fractionated via flash silica gel chromatography using step gradient elution (hexanes to EtOAc) to give pure samples of spiculoic acids A (**1**) (25 mg) and B (**2**) (18 mg).

Spiculoic acid A (**1**) was isolated as an optically active oil ($[\alpha]_D = 110$; $c = 0.1$, CH_2Cl_2) that gave an $[M - H]^-$ ion at *m*/*z* 407.2594 in the ESIHRMS appropriate for a molecular formula of $C_{27}H_{36}O_3$ (calcd 407.2586 for $C_{27}H_{35}O_3$), requiring 10 sites of unsaturation. Twenty-five resonances ($6 \times$ C; $10 \times$ CH; $4 \times CH_2$; $5 \times CH_3$) were observed in the ^{13}C NMR spectrum of **1** (Table 1), indicating that there was an element of symmetry in the molecule. Three deshielded resonances in the ^{1}H NMR spectrum (δ 7.28, dd, $J = 7.3$, 1.9 Hz (H-16/20); 7.22, t, $J = 7.3$ Hz (H-17/19); 7.15, tt, $J = 7.3$, 1.9 Hz (H-18)) and four deshielded resonances in the ^{13}C NMR spectrum (δ 137.8 (C-15); 126.2 (C16/20); 128.5 (C-17/19); 127.1 (C-18)) were readily assigned to a monosubstituted phenyl residue by analysis of their ^{1}H scalar coupling patterns and the corresponding COSY, HMQC, and HMBC correlations. The monosubstituted phenyl ring accounted for the symmetry indicated by the ^{13}C NMR data and four of the 10 required sites of unsaturation. Four additional olefinic resonances (δ 123.2 (C-3); 140.9 (C-4); 136.4 (C-13); 132.2 (C-14)) and two carbonyl resonances (δ 179.9 (C-12); 220.3 (C-7)) in the ^{13}C NMR spectrum accounted for four sites of unsaturation, and the lack of ^{13}C NMR evidence for further unsaturated functionality indicated the presence of two additional rings in **1**.

A pair of deshielded doublets (δ 6.03, $J = 16$ Hz (H-13); 6.25, $J = 16$ Hz (H-14)) in the ^{1}H NMR spectrum showed COSY correlations to each other and HMBC correlations to the phenyl carbon resonance at δ 137.8 (C-15), which identified a *trans*-styryl fragment (C-13 to C-20) in **1**. Four aliphatic methyl triplets (δ 1.07 (C-11); 0.86 (C-22); 1.13 (C-24); 0.63 (C-27)) in the ^{1}H spectrum of **1** were assigned by analysis of the COSY, HMQC, and HMBC data to ethyl appendages (C-10/C11, C-21/22, C-23/24, and C-26/27). The COSY data further showed that the ethyl fragments associated with the methyl resonances at δ 1.07 (C-10/11) and 0.86 (C-21/22) were each isolated ^{1}H spin systems. A weak COSY correlation, attributed to allylic coupling, was observed between the methylene resonance at δ 2.25 (H-23) and an olefinic doublet at 5.25 ($J = 2.4$ Hz; H-3), indicating that the corresponding ethyl residue (C-23/24) was attached to an olefinic carbon.

Table 1. NMR Data for Spiculoic Acids A (**1**) and B (**2**) Recorded in $CDCl_3$

C no.	δ C (**1**)	δ H (**1**)	δ C (**2**)	δ H (**2**)
1	54.1		49.7	
2	51.1		49.6	
3	123.2	5.25, d, 2.4	121.4	5.11, d, 2.4
4	140.9		141.0	
5	46.0	2.15, dd, 11.8, 11.4	53.2	1.93, dd, 11.8, 9.5
6	47.8	1.88, m	39.7	2.36, m
7	220.3		132.4	5.28, bs
8	52.3	2.45, ddd, 3.4, 5.0, 11.8	147.2	
9	42.2	2.64, dd, 11.8, 11.8	52.0	3.06, d, 11.8
10	22.8	1.67, m; 2.25, m	14.6	1.19, s
11	12.3	1.07, t, 7.3		
12	179.9		181.7	
13	136.4	6.03, d, 16.0	136.7	5.94, d, 15.9
14	132.2	6.25, d, 16.0	131.7	6.30, d, 15.9
15	137.8		137.8	
16/20	126.2	7.28, dd, 7.3, 1.9	126.3	7.31, dd, 7.3, 1.9
17/19	128.5	7.22, t, 7.3	128.4	7.23, t, 7.3
18	127.1	7.15, tt, 7.3, 1.9	126.9	7.14, tt, 7.3, 1.9
21	27.2	1.65, m; 1.83, m	27.1	1.63, m; 1.82, m
22	9.12	0.86, t, 7.3	9.1	0.86, t, 7.3
23	28.2	2.25, m	28.0	2.25, m
24	13.0	1.13, t, 7.3	12.9	1.13, t, 7.3
25	15.5	1.33, d, 6.5	20.4	1.25, d, 6.6
26	22.1	1.58, m; 1.83, m	22.3	1.83, m; 2.05, m
27	9.6	0.63, t, 7.3	11.8	0.99, t, 7.3

The resonances (δ 1.58/1.83; H26/26′) assigned to the methylene protons of the final ethyl fragment both showed COSY correlations to a methine resonance at δ 2.45 (H-8), demonstrating that this ethyl residue was part of an extended ^{1}H spin system. COSY correlations expanded the spin system in a linear sequence from the methine resonance at δ 2.45 (H-8) to methine resonances at 2.64 (H-9), 2.15 (H-5), and 1.88 (H-6), finally terminating in the methyl doublet at 1.33 (Me-25). HMBC correlations observed between a carbon resonance at δ 220.3 (C-7) and both the methyl resonance at δ 1.33 (Me-25) and the methylene resonance at 1.58 (H-26) showed that the methine carbons (C-6 and C-8) bearing the methyl (C-25) and ethyl (C-26/27) groups had to be bridged by a saturated ketone to give a cyclopentanone ring (C-5 to C-9).

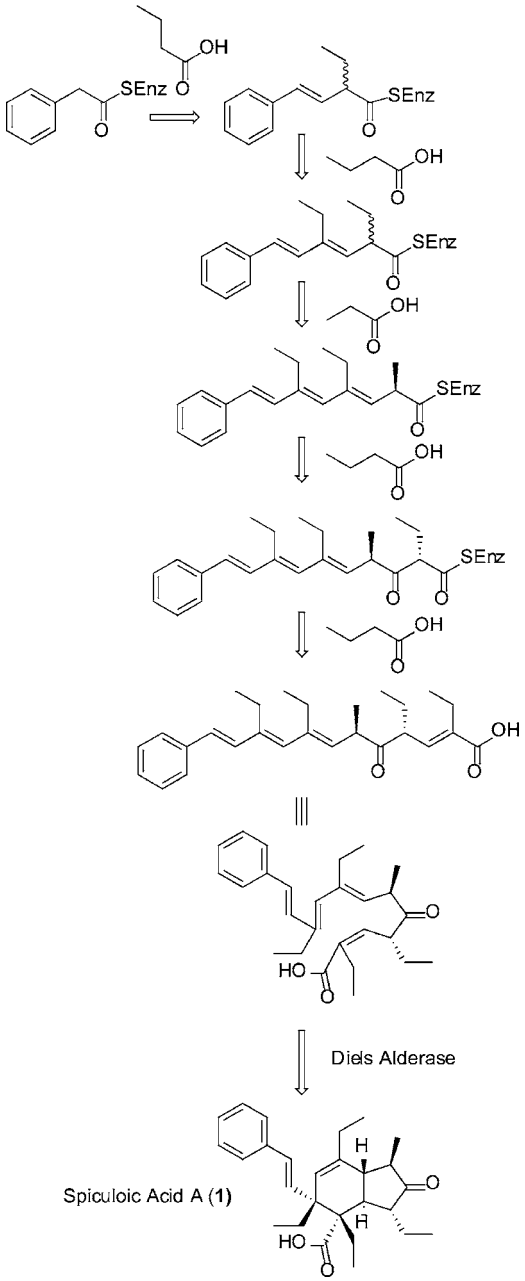
HMBC correlations between the nonprotonated olefinic carbon resonance at δ 140.9 (C-4) and all of the ^{1}H resonances at δ 2.64 (H-9), 2.15 (H-5), 1.88 (H-6), 5.25 (H-3), and 1.13 (Me-24), and between the olefinic ^{1}H resonance at δ 5.25 (H-3) and the ^{13}C methine resonance at δ 46.0 (C-5), demonstrated that the ethyl-bearing olefinic carbon had to be bonded to C-5 of the cyclopentanone ring. A series of HMBC correlations between δ 5.25 (H-3) and 51.1 (C-2), 136.4 (C-13), and 27.2 (C-21); between 6.03 (H-13) and

51.1 (C-2), 123.2 (C-3), and 27.2 (C-21); between 1.65/1.83 (H-21/21′) and 51.1 (C-2), 136.4 (C-13) and 123.2 (C-3); and between 6.25 (H-14) and 0.86 (Me-22) and 51.1 (C-2) showed that the *trans*-styryl, the C-21/22 ethyl, and the olefinic methine carbon (C-3) all had to be bonded to the same quaternary carbon (C-2). Similarly, HMBC correlations between δ 2.64 (H-9) and 54.1 (C-1), 51.1 (C-2), 22.8 (C-10), and 179.9 (C-12); between 1.67/2.25 (H-10/10′) and 54.1 (C-1), 51.1 (C-2), 42.2 (C-9), and 179.9 (C-12); and between 1.07 (Me-11) and 54.1 (C-1) indicated that C-9 had to be bonded to a quaternary carbon (C-1) that was in turn bonded to an adjacent quaternary carbon (C-2) to complete the final ring required in **1**, and also that C-1 had ethyl (C-10/C-11) and carbonyl substituents. The carbonyl substituent on C-1 had to be a carboxylic acid in order to account for the remaining atoms in the molecular formula of spiculoic acid A (**1**). This was confirmed by converting **1** to its methyl ester **4** with (trimethylsilyl)diazomethane.[5]

The relative stereochemistry of **1** was determined by analysis of NOESY and scalar coupling constant data. The vicinal coupling constant between H-9 (δ 2.64) and H-5 (δ 2.15) was 11.8 Hz, requiring that both protons are axial and, therefore, the ring junction is trans. A NOESY correlation between H-9 (δ 2.64) and H-6 (δ 1.88) showed that they are both α, and a NOESY correlation between H-5 (δ 2.15) and H-8 (δ 2.45) showed that they are both β as drawn. These relative configurations about the cyclopentanone ring are consistent with anti relationships between H-5 and H-6 and between H-9 and H-8 as required by the large H-5/H-6 (J = 11.4 Hz) and H-9/H-8 (J = 11.8 Hz) vicinal coupling constants. NOESY correlations between Me-11 (δ 1.07) and both H-5 (δ 2.15) and H-8 (δ 2.45) showed that the C-10/11 ethyl substituent is β and a NOESY correlation between H-9 (δ 2.64) and H-14 (δ 6.25) showed that the styryl substituent is α at C-2 as shown in **1**.

Spiculoic acid B (**2**) was also isolated as an optically active oil ($[\alpha]_D = -22$; c = 0.1, CH_2Cl_2) that gave an $[M - H]^-$ ion at m/z 377.2481 in the ESIHRMS appropriate for a molecular formula of $C_{26}H_{34}O_2$ (calcd 377.2481 for $C_{26}H_{33}O_2$), requiring 10 sites of unsaturation. The molecular formula of spiculoic acid B (**2**) differed from that of spiculoic acid A(**1**) by the loss of CH_2O. Comparison of the ^{1}H and ^{13}C NMR data obtained for **2** with the data obtained for **1** (Table 1) showed that the molecules were closely related. The major differences in the ^{1}H NMR of **2** compared to **1** were the replacement of one of the aliphatic methyl triplets with a methyl singlet (δ 1.19; Me-10) and the appearance of one additional olefinic methine resonance (δ 5.28; H-7). The ^{13}C NMR spectrum of **2** was missing the ketone carbonyl resonance present in the spectrum of **1**, but it contained two additional olefinic resonances at δ 132.4 (C-7) and 147.2 (C-8). Analysis of the COSY, HMQC, and HMBC data obtained for **2** showed the differences in the NMR and MS data described above were consistent with the presence of a $\Delta^{7,8}$ olefin in **2** in place of the C-7 ketone/C-8 methine in **1**, and a methyl substituent (C-10) on C-1 in **2** in place of the ethyl substituent (C10/11) at the same position in **1**. Examination NOESY and scalar coupling constant data

Scheme 1. Proposed Biogenesis of Spiculoic Acid A (**1**)



(4) A voucher specimen (ZMAPOR 17511) has been deposited at the University of Amsterdam.

(5) ^{1}H NMR data (400 MHz, $CDCl_3$) for ester **4**: δ 5.22, d, J = 2.4 Hz (H-3); 2.14, dd, J = 11.8, 11.4 Hz (H-5); 1.87, dq, J = 11.4, 6.5 (H-6); 2.38, ddd, J = 3.4, 5.0, 11.8 Hz (H-8); 2.62, dd, J = 11.8, 11.8 Hz (H-9); 1.60, m (H-10); 2.25, m (H-10′); 0.98, t, J = 7.3 Hz (H-11); 5.91, d, J = 16.0 Hz (H-13); 6.21, d, J = 16.0 Hz (H-14); 7.29, dd, J = 7.3, 1.9 Hz (H-16/20); 7.28, t, J = 7.3 Hz (H-17/19); 7.18, tt, J = 7.3, 1.9 Hz (H-18); 1.59, m (H-21); 1.70, m (H-21′); 0.82, t, J = 7.3 Hz (H-22); 2.25, m (H-23); 1.12, t, J = 7.3 Hz (H-24); 1.33, d, J = 6.5 Hz (H-25); 1.40, m (H-26); 1.74, m (H-26′); 0.65, t, J = 7.3 Hz (H-27); 3.70, s (OMe).

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showed that the relative stereochemisty of **2** at C-1, C-2, C-5, C-6, and C-9 was identical with that found in **1**. Spiculoic acid B (**2**) has an 11-norspiculane skeleton.

The structure of spiculoic acid A (**1**) is consistent with a polyketide origin as outlined in Scheme 1. A number of features of the proposed pathway leading to the new spiculane skeleton found in **1** stand out because they are only rarely encountered. The vast majority of polyketides made in nature are assembled from acetate and propionate building blocks. Among marine invertebrates, there is experimental evidence for the intact incorporation of butyrate units into triophamine (**5**) by the dorid nudibranch *Triopha* catalinae,[6] and many of the metabolites isolated from sponges in the genus *Plakortis*, with apparent polyketide origins, contain ethyl branches, suggesting that they are assembled in part from butyrate units.[1] Four butyrate units are required to construct the spiculane skeleton of spiculoic acid A (**1**).

The location of the olefin functionality formed by reduction of the β ketone and dehydration after condensation of the phenyl acetic acid starter unit with the first butyrate is unusual. Normally, the dehydration step in polyketide biosynthesis would yield an α,β unsaturated ester. In the proposed pathway to **1**, dehydration occurs in the opposite direction, leading to conjugation between the olefin and the phenyl ring. Once this pattern is set, the next two dehydrations also take place in the abnormal direction, leading to a fully conjugated polyene. This sequence is broken when the third butyrate is added and the resulting β ketone functionality is not reduced. The last condensation, reduction, and dehydration cycle, which adds the fourth butyrate unit, then leads to an olefin that is conjugated to the terminal carboxyl group in the normal fashion.

The final step in the proposed biogenesis of spiculoic acid A (**1**) is an intramolecular [4 + 2] cycloaddition reaction, presumably catalyzed by a Diels Alderase. Vederas et al. have recently characterized the first example of a Diels Alderase, which is part of the fungal PKS that makes lovastatin.[7] There is also circumstantial structural evidence, similar to the case presented for spiculoic acid A (**1**) in Scheme 1, that Diels Alderases are involved in the biosynthesis of many other polyketides.[8] The proposed Diels Alderase catalyzed reaction in the formation of spiculoic acid A (**1**) is noteworthy because it generates adjacent quaternary centers at C-1 and C-2.[9]

Spiculoic acid A (**1**) showed in vitro cytotoxicity against the human breast cancer MCF-7 cell line with an IC_{50} of 8 μg/mL, while spiculoic acid B (**2**) was inactive.

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Supporting Information Available: NMR spectra for spiculoic acids A (**1**) and B (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) A similar biogenesis, involving a different folding of a related linear polyketide intermediate and a final Diels Alderase catalyzed intramolecular [4 + 2] cycloaddition reaction, can lead to the plakotenins (see ref 1a,b).