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The marine sponge *Plakortis zyggompha*: a source of original bioactive polyketides

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Abstract—Three new spiculoic acids **1–3** and two members of a new closely related family of natural products named zyggomphic acids **4** and **5** were isolated from the very little studied marine sponge *Plakortis zyggompha*. Both families of compounds share a unique *trans*-hydrindan-2-one skeleton with six stereogenic centers. A total of 15 new metabolites were isolated from this sponge, all are of polyketide origin. The structures were elucidated using LC–MS, 1D, and 2D NMR methods. The absolute stereochemistry was determined by circular dichroism. The large number of close bioactive analogues allowed us to propose preliminary structure–activity relationships as antitumoral and antimycobaterial agents.

1. Introduction

Among the different classes of bioactive natural products, polyketides play an essential role in terms of structural diversity and biological activity.[1] This class of secondary metabolites is mostly produced by various microorganisms such as bacteria and fungi and can also be found in plants or marine invertebrates. In marine sponges, symbiotic bacteria could be the real producers of these metabolites.[2] The production of biosynthetic precursors of polyketides by different symbiotic microorganisms in the marine sponges *Plakortis* could explain the large chemical diversity found in this genus.[3] A sponge of an intense blue color identified as *Plakortis zyggompha*, whose extracts showed significant cytotoxicity on human tumoral cell lines, was collected off the coast of the Martinique in 2002. Only two reports refer to *P. zyggompha* in literature: Faulkner and Ravi described small polyketides isolated from an organism collected off the coast of Belize,[4] and Phillipson and Rinehart roughly identified a cyclic endoperoxide, named plakortic acid, from a Caribbean specimen.[5] Our first chemical studies on our specimens led to the identification of two classes of polyketides: seven new cyclic endoperoxides from the plakortide family, characterized as the major bioactive constituents of the extracts,[6] and three new spiculoic acids named dinor- (**6**), nor- (**7**), and isospiculoic acids A (**8**) isolated as minor constituents.[7] These three minor constituents of *P. zyggompha*, belonged to the recent polyketides family of spiculoic acid A (**9**) described in 2004 by Huang et al.[8] In addition to interesting antitumoral activity, these polyketides showed unusual structural features, especially the six stereogenic centers on the indane skeleton (four contiguous on the lower half, and two in the upper half of the molecules), which have raised the interest of synthetic organic chemists.[9,10]

In order to fully characterize the secondary metabolites of this species, we decided to look further into its minor constituents. The mildly bioactive fractions were explored and led to the isolation of three new spiculoic acids **1–3** and two new zyggomphic acids **4** and **5**, along with the known spiculoic acid A (**9**) and isospiculoic acid A (**8**).[8,9] The zyggomphic acids are the first examples of indane-type polyketides, which have integrated six propionate or butyrate units. Herein, we describe the isolation and structural elucidation of these compounds. Circular dichroism and the application of the octant rule allowed us to confirm the absolute configuration of this class of compounds, already described by total synthesis.[11] Antitumoral and antimycobacterial bioassays were performed on the new compounds.

Keywords: Natural products; Marine sponge; Polyketides; Spiculoic acid; Antitumoral.
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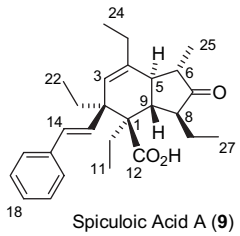
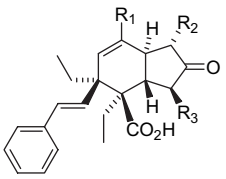
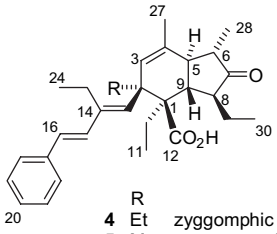




	R_1	R_2	R_3	
1	Me	Me	Et	24-norspiculoic acid A
2	Me	Et	Me	24-norisospiculoic acid A
3	Et	Et	Et	homospiculoic acid A
6	Me	Me	Me	dinorspiculoic acid A
7	Et	Me	Me	norspiculoic acid A
8	Et	Et	Me	isospiculoic acid A



	R	
4	Et	zyggomphic acid
5	Me	norzyggomphic acid

2. Results and discussion

In a previous publication, we reported that the bioguided fractionation of *P. zyggompha* (Homosclerophorida: Plakinidae) led to the isolation of three new members of the spiculoic acid family: the dinor- (**6**), nor- (**7**), and isospiculoic acid A (**8**),[8] together with seven new cyclic peroxides named plakortides Q.[7] These 10 new metabolites were isolated from the most bioactive fraction no. 7 collected after a silica gel flash chromatography of the CH_2Cl_2 sponge extract eluted in a gradient from hexane to MeOH. Because the HPLC–UV–ELSD profile of the mildly bioactive fraction no. 6 was similar to fraction no. 7 and suggested the presence of both families of spiculoic acids and plakortides Q, the chemical study of this fraction was undertaken. The endoperoxide plakortides Q were also the major constituents of this fraction and we drove our attention to the more polar compounds showing UV maxima at 254 or 290 nm. The peaks with UV maxima around 254 nm were assigned to the spiculoic acid family and we anticipated that compounds absorbing at 290 nm belonged to another structurally related family. To confirm this assumption an LC–MS experiment was carried out on the fraction no. 6 in similar HPLC conditions (Fig. 1).

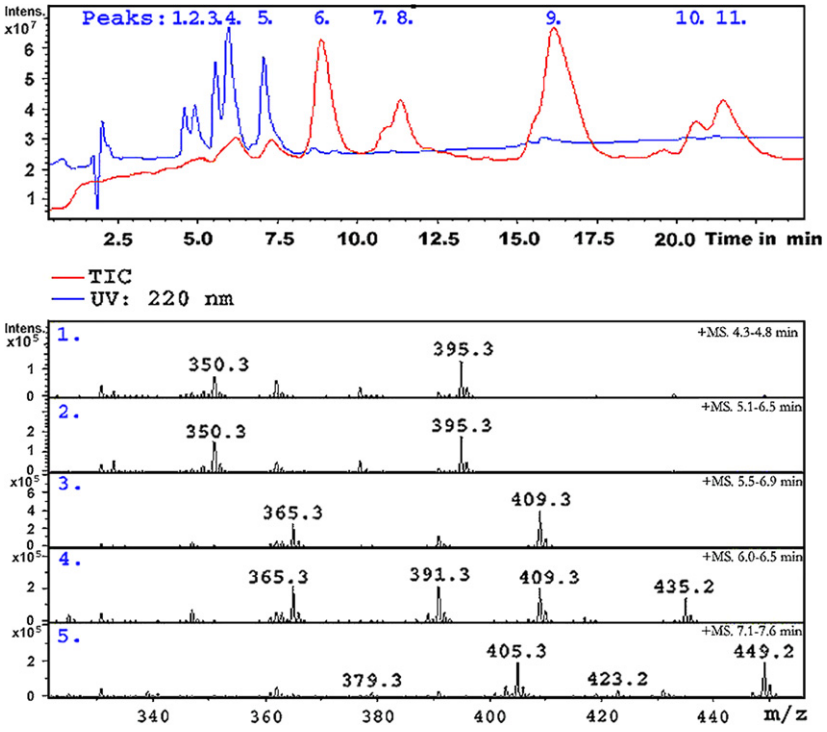


Figure 1. HPLC–(+)-APCIMS profiles of the fraction no. 6. C18 Column (Symmetry 150×4.6 mm, 3.5 μm) isocratic CH_3CN/H_2O (90:10) (0.1% formic acid).

Comparing retention times and mass patterns, the peaks 1–3 were confirmed as members of the spiculoic acid family. Peak 3 exhibited the mass profile of isospiculoic acid A [**8**, *m/z* 409 $[M+H]^+$, 365 $(-CO_2)$], whereas the mass profiles of peaks 1 and 2 were similar to the norspiculoic acid A one [**7**, *m/z* 395 $[M+H]^+$, 351 $(-CO_2)$]. The compounds corresponding to peaks 4 and 5, with strong absorbances at 290 nm, differed by one methylene unit (Δm=14 amu). For both compounds, the easy loss of CO_2 (−44 amu) suggested the presence of a carboxylic acid moiety, but their relation with spiculoic acids could not be evidenced at this stage. Interestingly, the new minor signals at *m/z* 423 $[M+H]^+$ and 379 $(-CO_2)$ in the spectrum of peak 5 suggested the presence of a homospiculoic acid in a mixture. The LC–MS analysis also allowed the quick identification of the previously described plakortides Q, related to the peaks 6–11.[7] A semi-preparative RP-HPLC separation of this fraction was finally necessary to draw the exact structure of all compounds. The purification of peaks 1–3 by RP-HPLC led to the isolation of the known spiculoic acid A (**9**), together with two new analogues **1** and **2**. The HRESIMS data analysis (*m/z* 393.2422 and 393.2449 $[M-H]^-$, respectively) of **1** and **2**, in accordance with the molecular formula $C_{26}H_{34}O_3$, showed them to be both isomers of the norspiculoic acid A (**7**). However, NMR data of compounds **1**, **2**, and **7**, showed differences, particularly in the proton and carbon resonances around the indane ring (Table 1).

Five methyls were clearly identified in the ^{1}H NMR spectra of compounds **1** and **2**, two of them, at δ_H 0.85 and 1.10 ppm (t, H_3-22 and H_3-11, respectively) being present in both compounds. A clear difference between the ^{1}H NMR spectra of **1**, **2**, and **7** was the replacement of the triplet at δ_H 1.16 ppm (H_3-24) in **7** by a singlet at δ_H 1.95 ppm (H_3-23) in **1** and **2**. The loss of a methylene unit at C-4 was confirmed by the key correlations: H_3-23/H-3 in the COSY spectrum, C-3/H_3-23 and C-5/H_3-23 in the HMBC spectrum. As isomers of **7**, the structure of both compounds **1** and **2** should finally include one methylene homologation. The upshielded triplet at δ_H 0.65 ppm (H_3-27) in **1** was reminiscent of an ethyl chain at C-8, as in spiculoic acid A (**9**),[9] and consequently placed the homologation at this position. The doublet at δ_H 1.08 ppm (H_3-26) in **2** confirmed the presence of a methyl at C-8, while a new triplet at δ_H 0.93 ppm (H_3-25′) suggested the presence of an ethyl chain at C-6, also present in isospiculoic acid A (**8**).[8] 2D NMR correlations further supported these conclusions, and compounds **1** and **2** were consequently named 24-norspiculoic acid A and 24-norisospiculoic acid A, respectively.

The ^{1}H NMR data analysis of peaks 4 and 5 indicated that each peak was a mixture of two compounds. Besides the characteristic vinylic signals (H-3) of the spiculoic acids, two other downfield shielded vinylic AX systems appeared in each spectrum at δ_H 6.48 and 6.60 or 6.56 ppm,

Table 1. ^{1}H and ^{13}C NMR data of spiculoic acids **1**, **2**, **3**, and norspiculoic acid A (**7**), in $CDCl_3$

Position	δ_H (mult. in Hz)				δ_c (mult. in Hz)			
	1	**2**	**3**	**7**[b]	**1**	**2**	**3**	**7**[b]
1					54.3 (C)	54.0 (C)	54.1 (C)	54.1 (C)
2					51.5 (C)	50.9 (C)	51.2 (C)	50.7 (C)
3	5.27 (br s)	5.25 (br s)	5.24 (br s)	5.26 (br s)	125.4 (CH)	125.1 (CH)	123.2 (CH)	123.0 (CH)
4					135.4 (C)	135.0 (C)	141.2 (C)	140.4 (C)
5	2.07 (br dd, 12.0, 12.0)	2.38 (m)	2.45 (br dd, 12.1, 12.1)	2.19 (dd 11.4 11.4)	47.3 (CH)	41.7 (CH)	40.5 (CH)	46.5 (CH)
6	1.90 (dq, 12.2, 6.7)	2.00 (m)	1.94 (ddd, 12.1, 5.7, 2.5)	1.99 (m)	47.9 (CH)	52.0 (CH)	53.1 (CH)	46.8 (CH)
7					220.4 (C)	219.6 (C)	220.1 (C)	220.3 (C)
8	2.45 (ddd, 12.2, 5.5, 3.7)	2.34 (m)	2.33 (m)	2.43 (dq, 12.6, 6.7)	52.5 (CH)	49.7 (CH)	53.7 (CH)	48.3 (CH)
9	2.65 (dd, 12.0, 12.0)	2.32 (m)	2.60 (dd, 12.1, 12.1)	2.37 (dd, 12.6, 11.4)	42.2 (CH)	46.7 (CH)	42.3 (CH)	47.1 (CH)
10	2.28 (dq, 14.5, 7.3)	2.31 (m)	2.28 (m)	2.31 (m)	23.1 (CH_2)	22.8 (CH_2)	23.2 (CH_2)	22.6 (CH_2)
	1.70 (dq, 14.5, 7.3)	1.72 (dq, 14.6, 7.1)	1.75 (m)	1.68 (dq, 14.4, 7.4)				
11	1.06 (t, 7.5)	1.10 (t, 7.1)	1.08 (t, 7.3)	1.11 (t, 7.4)	12.4 (CH_3)	12.2 (CH_3)	12.5 (CH_3)	12.2 (CH_3)
12					177.8 (C)	177.3 (C)	178.2 (C)	179.0 (C)
13	6.03 (d, 15.7)	5.97 (d, 15.8)	6.06 (d, 15.8)	6.01 (d, 15.9)	136.6 (CH)	136.3 (CH)	136.8 (CH)	136.3 (CH)
14	6.28 (d, 15.7)	6.21 (d, 15.8)	6.25 (d, 15.8)	6.21 (d, 15.9)	132.4 (CH)	132.0 (CH)	132.2 (CH)	132.0 (CH)
15					137.9 (C)	137.7 (C)	138.0 (C)	137.7 (C)
16/20	7.31 (br d, 7.2)	7.30 (br d, 7.8)	7.31 (m)	7.30 (br d, 7.6)	126.4 (CH)	126.5 (CH)	126.5 (CH)	126.5 (CH)
17/19	7.26[a]	7.27[a]	7.27[a]	7.25[a]	128.6 (CH)	128.7 (CH)	128.6 (CH)	128.7 (CH)
18	7.18 (tt, 7.2, 1.4)	7.19 (t, 7.2)	7.19 (m)	7.18 (br t, 7.1)	127.2 (CH)	127.3 (CH)	127.2 (CH)	127.3 (CH)
21	1.81 (m)	1.78 (m)	1.80 (m)	1.82 (dd, 13.5, 7.4)	27.2 (CH_2)	27.3 (CH_2)	27.4 (CH_2)	27.4 (CH_2)
	1.63 (dq, 14.4, 7.3)	1.63 (dq, 14.6, 7.3)	1.65 (m)	1.65 (dd, 13.5, 7.4)				
22	0.85 (t, 7.3)	0.84 (t, 7.3)	0.87 (t, 7.2)	0.87 (t, 7.4)	9.3 (CH_3)	9.2 (CH_3)	9.3 (CH_3)	9.3 (CH_3)
23	1.95 (s)	1.95 (s)	2.27 (m)	2.28 (m)	22.9 (CH_3)	22.4 (CH_3)	27.8 (CH_2)	28.3 (CH_2)
			2.22 (m)					
24			1.15 (t, 7.3)	1.16 (t, 7.4)			13.2 (CH_3)	13.1 (CH_3)
25	1.33 (d, 6.7)	2.10 (dqd, 14.6, 7.5, 2.9)	2.09 (m)	1.37 (d, 6.9)	15.4 (CH_3)	22.3 (CH_2)	21.7 (CH_2)	16.4 (CH_3)
		1.75 (m)	1.77 (m)					
25′		0.93 (t, 7.5)	0.93 (t, 7.4)			10.4 (CH_3)	10.4 (CH_3)	
26	1.85 (m)	1.08 (d, 5.9)	1.82 (m)	1.12 (d, 6.6)	22.3 (CH_2)	14.4 (CH_3)	21.7 (CH_2)	14.9 (CH_3)
	1.57 (m)		1.57 (m)					
27	0.65 (t, 7.3)		0.67 (t, 7.2)		9.8 (CH_3)		10.0 (CH_3)	

[a] Overlapped with $CDCl_3$.
[b] See Ref. 8.

respectively, together with a new singlet at δ_H 5.21 or 5.24 ppm. This deshielding was linked with the bathochromic shift observed (254–290 nm), and consequently, with a change in the conjugated styryl system. The separation of both mixtures realized with a phenyl bonded HPLC column led to the isolation of the new homospiculoic acid A (**3**) and zyggomphic acid (**4**) from peak 5, and of the new norzyggomphic acid (**5**) and the known isospiculoic acid A (**8**) from the peak 4.

The HRESIMS analysis of zyggomphic acid (**4**) (*m/z* 447.2918 $[M-H]^-$) was indicative of the molecular formula $C_{30}H_{39}O_3$, requiring 11 unsaturations, one more than spiculoic acids. The additional unsaturation was attributed to a third double bond as evidenced by the six vinylic resonances in the ^{13}C NMR spectrum. Furthermore, the new singlet at δ_H 5.21 ppm (H-13) was consistent with a trisubstituted double bond (Table 2).

Due to the bathochromic shift of the styryl UV absorption, an extra conjugation with the new double bond was evidenced by the H_3-13/H-15 COSY, C-13/H_2-23, and C-13/H_2-25 HMBC correlations. The remaining ^1H and ^{13}C NMR signals were very similar to those of the spiculoic acids and the key chemical shifts of the methyl and ethyl chains were used to establish the substituent distribution around the indane skeleton. The ^1H NMR signals at δ_H 1.89 (s, H-27), 1.33 (d, H-28), and 0.72 (t, H-30) revealed a methyl at C-4, a methyl at C-6, and an ethyl at C-8, respectively. The presence of an ethyl group on the new unsaturation C-13/C-14, new triplet at δ_H 1.03 (H-24), was confirmed by the HMBC correlations H-23a/H-15 and H-23b/H-13.

The structural determination of the homospiculoic acid (**3**) was more troublesome because of the presence of a minor product, as evidenced in the ^1H NMR spectrum by two uncommon doublets at δ_H 6.54 and 7.94. Fortunately, this minor product slowly disappeared from the spectrum and after 2 weeks in $CDCl_3$, its ratio decreased from around 8/10 to 2/10, which made the structural determination of homospiculoic acid (**3**) feasible. The (+)-ESI-MS at *m/z* 423 $[M+H]^+$ associated with the key chemical shifts of the methyls at δ_H 1.15 (t, H_3-24), 0.92 (t, H_3-25′), and 0.67 (t, H_3-27), confirmed the presence of three ethyl chains at C-4, C-6, and C-8, respectively, and consequently the structure of the homospiculoic acid (**7**). The initial minor product of the mixture was then examined and was observed to precipitate gradually in the NMR tube, explaining the disappearance of the unknown signals. Its characterization could not be achieved because of the low amounts of products, but the ^1H and COSY NMR data of the mixture showed strong analogy with zyggomphic acid (**4**). The unstability was attributed to a slow polymerization process occurring in slightly acidic media (e.g., $CDCl_3$), a phenomenon quite common for styryl derivatives. The propensity of zyggomphic acids to undergo polymerization was confirmed during the structural determination of norzyggomphic acid (**5**) isolated from peak 4. After separation from isospiculoic acid (**8**), compound **5** polymerized rapidly in all tested solvents, which rendered its characterization in its pure form

Table 2. NMR data of zyggomphic acids **4** and **5**, in $CDCl_3$

Atom no.	Zyggomphic acid (**4**)				Norzyggomphic acid (**5**)	
	δ_C (mult. in Hz)	δ_H (mult. in Hz)	COSY	HMBC (C-H)	δ_C	δ_H
1	56.5 (C)			3, 9, 10a,b, 11, 13	55.6	
2	52.9 (C)			3, 9, 13, 25a,b, 26	48.6	
3	126.5 (CH)	5.59 (br s)	5, 27	3, 25b, 27	130.9	5.50 (br s)
4	133.7 (C)			5, 6, 9, 27	132.7	
5	47.4 (CH)	2.09 (br dd, 12.6, 11.8)	3, 6, 9, 27	3, 6, 9, 27, 28	47.1	[a]
6	47.1 (CH)	1.96 (dq, 12.6, 6.7)	5, 28	28	46.9	[a]
7	220.4 (C)				220.4	
8	52.7 (CH)	2.43 (ddd, 11.8, 5.7, 3.2)	9, 29a,b	9, 29b, 30	52.6	[a]
9	43.5 (CH)	2.63 (dd, 11.8, 11.8)	5, 8	5, 10a	43.5	2.59 (dd, 11.5, 11.5)
10a	23.0 (CH_2)	2.21 (dq, 14.6, 7.4)	10b, 11	11	23.1	[a]
10b		1.65 (dq, 14.6, 7.4)	10a, 11			[a]
11	12.2 (CH_3)	0.99 (t, 7.4)	10a,b	10a,b	12.1	1.01 (t, 7.3)
12	176.7 (C)			10a,b	178.2	
13	137.3 (CH)	5.21 (s)	15	15, 23a,b, 25a,b	138.6	5.24 (br s)
14	142.1 (C)			13, 16, 23a,b, 24	139.9	
15	134.7 (CH)	6.61 (d, 15.8)	13, 16	13, 23a,b	134.7	6.56 (d, 16.1)
16	125.9 (CH)	6.48 (d, 15.8)	15	18/22	126.2	6.48 (d, 16.1)
17	138.0 (C)			15, 16, 19/21	137.9	
18/22	126.4 (CH)	7.38 (d, 7.7)	19/21	16, 20	126.4	7.36 (d, 7.7)
19/21	128.7 (CH)	7.29 (dd, 7.7, 7.7)	18/22, 20	19/21	128.7	[a]
20	127.1 (CH)	7.18 (t, 7.7)	19/21	18/22	127.2	
23a	19.3 (CH_2)	2.73 (dq, 14.0, 7.2)	23b, 24	13, 15, 24	19.8	2.69 (dq, 14.1, 7.3)
23b		2.38 (dq, 14.0, 7.2)	23a, 24			[a]
24	14.4 (CH_3)	1.03 (t, 7.2)	23a,b	13, 23a,b	14.2	1.06 (t, 7.3)
25a	28.8 (CH_2)	1.84 (dq, 14.2, 7.4)	25b, 26	3, 13, 26	25.2	1.30 (s)
25b		1.50 (dq, 14.2, 7.4)	25a, 26			
26	9.5 (CH_3)	0.89 (t, 7.4)	25a,b	25a,b		
27	22.8 (CH_3)	1.89 (s)	3, 5	3, 9	22.5	1.87 (s)
28	15.3 (CH_3)	1.33 (d, 6.7)	6	5, 6	15.3	1.33 (d, 6.7)
29	23.4 (CH_2)	1.91 (dqd, 14.2, 7.4, 3.2)	8, 29b, 30	8, 9	23.4	1.91 (dqd, 14.2, 7.4, 3.2)
		1.68 (m)	8, 29a, 30			1.68 (m)
30	10.1 (CH_3)	0.72 (t, 7.4)	29a,b	8, 29b	10.1	0.72 (t, 7.4)

[a] Overlapped with signals of isospiculoic acid A (**8**).

impossible. Nevertheless, the structural determination of norzyggomphic acid (**5**) was achieved using LC–(+)-APCI-MS analysis (*m/z* 435 $[M+H]^+$) of the fraction no. 6, which was consistent with a loss of a methylene unit comparing with zyggomphic acid (**4**). Strong similarities between both compounds were also confirmed by very close UV spectra. Analysis of the ^1H and ^{13}C NMR data of peak 4 before purification and after subtraction of the signals related to isospiculoic acid A (**8**) led to the identification of a new singlet at δ_H 1.30 (s, H-25) that did not fit with the usual methyl chemical shifts. Because the triplet at δ_H 0.90 (t, H-26) was absent, the conclusion was made that the ethyl at C-2 was, for the first time, replaced by a methyl substituent, and was confirmed by changes in the ^{13}C chemical shifts of the neighboring carbon atoms: C-1, C-2, C-3, and C-13.

The geometry of the *exo* disubstituted double bonds was assigned as *E* for all compounds, based on the large H,H-coupling constants. For the *exo* trisubstituted double bond of zyggomphic acids **4** and **5**, a strong H-13/H-15 NOESY correlation also required an *E* configuration. Key NOESY correlations allowed to confirm that the relative stereochemistry of the six asymmetric carbons was the same as in spiculoic acid A (**9**). To determine the absolute stereochemistry a series of CD experiments were run on isospiculoic acid A (**8**). The hydrindanone skeleton had already been studied for the n–π* electronic transition of the carbonyl moiety, which allowed easy interpretation using the octant rule.[11] A strong positive Cotton effect was observed at 304 nm for the n–π* carbonyl transition influenced by the α methyl and ethyl substituents. The interpretation of the octant rule suggested a (*S*,*S*) configuration at C-6 and C-8 (Fig. 2). The same CD pattern was observed for all compounds, which suggested them to have the same absolute stereochemistry.

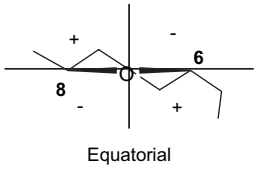


Figure 2. Octant rule applied to isospiculoic acid A (**8**).

3. Conclusion

The marine sponge *P. zyggompha*, or some associated bacteria, has been shown to produce a large family of polyketides named spiculoic and zyggomphic acids. In total, nine derivatives were isolated from our specimens. Spiculoic acids are formed through the condensation of five propionate or butyrate units whereas one more butyrate unit is necessary for zyggomphic acids. The biosynthesis finishes in a stereocontrolled Diels–Alder type cyclization to build the hydrindan-2-one skeleton. The starter unit must be derived from phenylacetic acid and it is noteworthy that the first and the last condensation steps always involve a butyrate unit. Amazingly, seven from the possible eight spiculoic acids resulting from a circular permutation of a methyl or an ethyl at C-4, C-6, and C-8, were isolated and characterized. This result can be related to a low specificity of the polyketide synthase multienzymes toward a butyrate or a propionate unit for these condensations.

All the pure compounds were tested for antitumoral and antimycobacterial activities, and the results are shown in Table 3.

In a global manner, antitumoral activity increases with the lipophilicity of the compounds, zyggomphic acid (**4**) being the most bioactive: 1.2 μM (IC50) against MDA-MB-231 breast cell lines. More precisely, a methyl at C-6 and an ethyl at C-8 seem to improve the activity, while a methyl at C-8 decreases the activity. Although moderate, the antimycobacterial activity appears to evolve in the opposite direction, the less lipophilic compound being the most active: 25–50 μg/mL (MIC99).

4. Experimental

4.1. General experimental procedures

Optical rotations were measured in $CHCl_3$ on a Jasco P-1020 polarimeter. IR spectra were recorded on a Perkin–Elmer Paragon 1000 FT-IR spectrophotometer. UV spectra were obtained with a Perkin–Elmer lambda 15 UV/Vis spectrophotometer. NMR experiments were performed on a Bruker DRX 500, using standard Bruker program. Chemical shifts were reported in parts per million using residual $CDCl_3$ (δ 7.26 for ^1H and 77.16 for ^{13}C) as internal reference. HRESIMS were recorded on an Applied Biosystems QSTAR spectrometer, and ESI and APCIMS were performed on a Bruker Esquire 3000 Plus spectrometer.

4.2. Animal material

P. 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

Table 3. Antitumoral and antimycobacterial activities of compounds **1**, **2**, **4**, and **6–9**

	INH	1	2	4	6[b]	7[b]	8[b]	9
log P[a]		6.62	6.62	7.73	6.21	6.62	7.04	7.04
Cell lines		Antitumoral activities IC50 (μM)						
MDA-MB-231		>25	>25	1.2	>25	>25	>25	2.4
A549		11.9	9.4	3.3	>25	16.5	21.5	4.6
HT29		>25	17	3.6	>25	>25	22.8	8.1
Strains		Antimycobacterial activities MIC99 (μg/mL)						
Mycobacterium smegmatis	12	50	>100	>100	25	45	50	>100
Mycobacterium tuberculosis	0.1	—	50	>100	50	50	>100	50

[a] Calculated from ChemDraw Ultra 10.
[b] See Ref. 8.

south of Martinique Island. The specimen was immediately frozen. The material was identified by Dr. Iosune Uriz (Blanes, Spain) and a voucher specimen (ORMA008545) has been deposited at the company PharmaMar SA.

4.3. 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 of 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 silica gel chromatography column using a gradient of hexane to MeOH to give 15 fractions. The fraction no. 6 (264 mg) was further purified by HPLC on a C_{18} semi-preparative column (SymmetryPrep C_{18} 300×7.8 mm, 7 μm) with 90% CH_3CN in H_2O to yield 24-norspiculoic acid A (**1**, 2.5 mg, 1.4×10^{-3}% wet wt), 24-norisospiculoic acid A (**2**, 2.3 mg, 1.3×10^{-3}% wet wt), and spiculoic acid A (**9**, 5.4 mg, 3.2×10^{-3}% wet wt) and the impure peaks 4 and 5. The peak 5 mixture was separated on a PhenylHexyl semi-preparative column (Luna, 250×10 mm, 5 μm) with 75% CH_3CN in H_2O to afford zyggomphic acid (**4**, 2.5 mg, 1.5×10^{-3}% wet wt), and another mixture where the homospiculoic acid (**3**, 0.7 mg, 0.4×10^{-3}% wet wt) has been identified after precipitation of an unknown compound. The peak 4 mixture was also separated on a PhenylHexyl semi-preparative column (Luna, 250×10 mm, 5 μm) with 75% CH_3CN in H_2O to afford isospiculoic acid (**8**, 1.4 mg, 0.8×10^{-3}% wet wt), and norzyggomphic acid (**5**), which was observed to precipitate in all used solvents.

4.3.1. 24-Norspiculoic acid A (1). Colorless oil; $[\alpha]_D^{24}$ +66.5 (*c* 0.05); UV (CH_3CN/H_2O 90:10) λ_{max} 249.8 nm; CD_{MeOH} (*c* 1.27×10^{-3} M) $[\theta]_{300}$ +5708; IR (film) ν_{max} 2954, 1734, 1688, 1461, 1379 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; ESIMS (MeOH) *m/z* 393 $[M-H]^-$; HRESIMS (*m/z*) calcd for $C_{26}H_{33}O_3$, 393.2435; found, 393.2422 $[M-H]^-$.

4.3.2. 24-Norisospiculoic acid A (2). Colorless oil; $[\alpha]_D^{24}$ +41.5 (*c* 0.05); UV (CH_3CN/H_2O 90:10) λ_{max} 249.8 nm; CD_{MeOH} (*c* 8.38×10^{-4} M) $[\theta]_{300}$ +4343; ^1H and ^{13}C NMR, see Table 1; ESIMS (MeOH) *m/z* 393 $[M-H]^-$; HRESIMS (*m/z*) calcd for $C_{26}H_{33}O_3$, 393.2435; found, 393.2449 $[M-H]^-$.

4.3.3. Homospiculoic acid A (3). UV (CH_3CN/H_2O 90:10) λ_{max} 249.8 nm; ^1H and ^{13}C NMR, see Table 1; ESIMS (MeOH) *m/z* 421 $[M-H]^-$; HRESIMS (*m/z*) calcd for $C_{28}H_{38}O_3$, 421.2743; found, 421.2757 $[M-H]^-$.

4.3.4. Zyggomphic acid (4). Colorless oil; $[\alpha]_D^{24}$ +85.9 (*c* 0.05); UV (CH_3CN/H_2O 75:25) λ_{max} 291.1 nm; ^1H and ^{13}C NMR, see Table 2; ESIMS (MeOH) *m/z* 447 $[M-H]^-$; HRESIMS (*m/z*) calcd for $C_{30}H_{39}O_3$, 447.2904; found, 447.2918 $[M-H]^-$.

4.3.5. Norzyggomphic acid (5). UV (CH_3CN/H_2O 75:25) λ_{max} 291.1 nm; ^1H and ^{13}C NMR, see Table 2; APCIMS (MeOH) *m/z* 435 $[M+H]^+$.

4.3.6. Isospiculoic acid A (8). Colorless oil; UV (CH_3CN/H_2O 75:25) λ_{max} 249.8 nm; CD_{MeOH} (*c* 2.45×10^{-3} M) $[\theta]_{304}$ +4730; ^1H and ^{13}C NMR, see Ref. 8.

4.4. 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.[12] 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. The antimycobacterial activity was screened on the fast growing saprophyte *Mycobacterium smegmatis* mc^2155 and on the virulent strain *Mycobacterium tuberculosis* H37Rv, using the new microdilution resazurin assay (MRA).[13] The minimal inhibitory concentration (MIC99) is defined as the amount of compound required for >99% inhibition of bacterial growth. MIC determinations: MICs were determined using the resazurin test. Resazurin salt powder (Sigma) was prepared at 0.01% (wt/vol) in distilled water, sterilized by filtration through a 0.4 μm membrane, and stored at 4 °C for a week. Drug stock solutions were prepared in dimethylsulfoxide (DMSO) at concentration of 50 mg/mL and frozen until used. The inoculums were prepared from growing strains of *M. tuberculosis* in Dubos medium supplemented with 10% ADC enrichment (Difco). One microliter of two-fold serial dilutions of each drug were prepared in 100 μL of Dubos medium directly in 96-well plates at concentrations from 0.9 to 500 μg/mL. Growth controls containing DMSO and isoniazid (from 1 μg/mL to 1 ng/mL) were also included. The plates were covered, sealed, and incubated during 37 °C. After 48 h for *M. smegmatis* or 6 days for *M. tuberculosis*, 30 μL of resazurin solution was added to each well and plates were allowed to incubate at 37 °C for an additional 24 h. A change from blue to pink indicates reduction of resazurin and therefore bacterial growth. The MIC was defined as the lowest drug concentration that prevented this color change. The optical density of each well was measured at 530–630 nm using a multi-well plate reader. The 99% inhibition concentrations for the most active compounds (MIC99≤50 μg/mL) were determined by curve fitting.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.12.048.

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