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Jaspines A and B: two new cytotoxic sphingosine derivatives from the marine sponge *Jaspis* sp.

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Abstract—The ethanolic extract of the sponge *Jaspis* sp. collected in Vanuatu showed cytotoxicity against KB tumour cells ($IC_{95}=10$ μg/ml). A bioassay-guided fractionation led to the isolation of two new cytotoxic sphingosine derivatives, jaspine A and jaspine B. Structures were elucidated by spectroscopic methods, and absolute configuration by chemical derivatisation. The cytotoxicity of jaspine B hydrochloride was evaluated against the A549 lung tumour cell line ($IC_{50}=3.4\times10^{-7}$ M).

The marine sponge genus *Jaspis* has been shown to yield cytotoxic compounds, such as isomalabaricanes,[1,2] jaspamides,[3] jaspisamides,[4] toyocamycin and 5-methoxycarbonyl tubercidin.[5] In recent years, it has been reported that jaspamides possess remarkable biological properties such as antiproliferative (cytotoxic and antimicrobial), anthelminthic, insecticidal, and ichtyototoxic activities.[6]

In our continuing work on bioactive compounds from marine sponges collected in Vanuatu, we investigated the cytotoxic ethanolic extract from a new species of *Jaspis* ($IC_{95}=10$ μg/ml, KB cell line). The bioguided fractionation of this extract using the brine shrimp bioassay led us to isolate two new cytotoxic compounds identified as jaspine A (**1**) and jaspine B (**2**), the first natural anhydrosphingosine derivatives. We describe here their isolation and their structure determination.

The freeze-dried sponge (50 g) was successively extracted with aqueous EtOH, EtOH and $CHCl_3$. The $CHCl_3$ extract was evaporated under reduced pressure to give extract A (900 mg). The concentrated crude EtOH extract was subjected to a partition between $CHCl_3$ and H_2O to yield $CHCl_3$ extract (B, 3 g), and aqueous extract (C, 2 g dry weight, desalted). All extracts showed marked toxicity on *Artemia salina*. The $CHCl_3$ extract A was fractionated on silicagel (C_6H_{12}/$CHCl_3$/THF/TEA 40:30:15:0.1; MeOH [0 to 40%]), to afford a bioactive fraction (5% MeOH), further purified by normal-phase HPLC (LiChrospher Si 60, 5 μm, 250×25 mm, C_6H_{12}/$CHCl_3$/THF/TEA 40:30:15:0.1, MeOH [0 to 5%]) to yield jaspine A (**1**) (0.5%, w/w). The chloroform extract B was fractionated on silicagel (C_6H_{14}:$CHCl_3$ 50:50, $CHCl_3$:MeOH 100:0 to 0:100), leading to a bioactive fraction (25% MeOH) that afforded pure jaspine B (**2**) (0.9%, w/w). The aqueous extract C (500 mg) was chromatographed by normal-phase centrifugal partition ($CHCl_3$/MeOH/H_2O 25.5:46.5:28, descending mode) to yield jaspine B hydrochloride (0.1%, w/w) as the bioactive component. Compounds **1**, **2** and the hydrochloride of **2** were toxic at concentrations as low as 0.1 μg/ml (100%) in the *A. salina* bioassay.

Jaspines A (**1**) and B (**2**) were obtained as amorphous white powders. Jaspine A (**1**) {$[\alpha]_D^{20}$ +25 (*c* 1, $CHCl_3$)} showed a quasimolecular ion peak at *m/z* 370.33202 $[M+H]^+$, [HRFABMS $C_{22}H_{44}O_3N$ (Δ+0.4 mmu)], and jaspine B (**2**) {$[\alpha]_D^{20}$ +7 (*c* 0.1, $CHCl_3$)} showed a quasimolecular ion at *m/z* 300.29015 $[M+H]^+$, [HRFABMS $C_{18}H_{38}O_2N$ (Δ+0.3 mmu)]. ESIMS analysis also showed for both products trace amounts of compounds at M+14 mu or M−14 mu, differing from the major component by one more or less methylene unit on an aliphatic chain. These trace compounds were not detected by analytical chromatography.

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Table 1. ^{13}C NMR ($CDCl_3$, 125 MHz) and ^{1}H NMR ($CDCl_3$, 500 MHz) data assignments of jaspine A (**1**) and jaspine B (**2**); HMBC correlations

	Jaspine A (**1**)			Jaspine B (**2**)		
	^{13}C	^{1}H	HMBC	^{13}C	^{1}H	HMBC
1a	73.6	3.68 (1H, m)	2, 3	72.2	3.54 (1H, dd, $J_{1a-2}=7.0$ Hz, $J_{1a-1b}=8.5$ Hz)	2
1b	73.6	3.92 (1H, d, $J_{1b-1a}=10.0$ Hz)	2, 3	72.2	3.95 (1H, dd, $J_{1b-2}=7.0$ Hz, $J_{1b-1a}=8.5$ Hz)	2
2	63.3	4.05 (1H, t, $J_{2-3}=J_{2-1a}=6.0$ Hz)	1a, 1b	54.2	3.68 (1H, dt, $J_{2-3}=5.0$ Hz, $J_{2-1a}=J_{2-1b}=7.0$ Hz)	1a, 1b
3	80.8	4.36 (1H, dd, $J_{3-4}=3.5$ Hz, $J_{3-2}=6.0$ Hz)	1, 4, 1′	71.6	3.88 (1H, dd, $J_{3-4}=3.5$ Hz, $J_{3-2}=5.0$ Hz)	1a, 1b, 4, 1′
4	83.8	3.41 (1H, dt, $J_{4-2}=3.5$ Hz, $J_{4-1'}=7.0$ Hz)	1b, 2, 3, 1′	83.1	3.75 (1H, ddd, $J_{4-3}=3.5$ Hz, $J_{4-1'}=7.0$ and 7.5 Hz)	1a, 1b, 2, 3, 1′
1″	93.6	4.52 (1H, bt, $J=5.0$ Hz)	2, 3, 2″			
2″	31.3	1.75 (2H, m)	4″			
3″	28.7	1.75 (2H, m)	1″			
4″	62.6	3.70 (2H, m)	2″			
1′	29.1	1.71 (2H, dd, $J_{1'-4}=7.0$ Hz, $J_{1'-2'}=7.5$ Hz)	4	29.3	1.71 (2H, m)	4
2′–13′	22.0–31.8	1.24–1.50 (24H, m)		22.0–31.0	1.20–1.70 (24H, m)	
CH_3	14.0	0.90 (3H, t, $J=6.5$ Hz)		14.0	0.90 (3H, t, $J=6.5$ Hz)	
OH, NH_2		2.60 (bs)			2.10 (bs)	

The NMR spectra of compounds **1** and **2** (Table 1) showed a resemblance corroborated by the slow acid hydrolysis of jaspine A (**1**) into jaspine B (**2**).

The ^{1}H NMR spectrum of jaspine B (**2**) (Table 1) displayed five deshielded signals between δ_H 3.54 and 3.95 ppm, several aliphatic protons and a broad exchangeable signal (δ_H 2.10). The HSQC experiment showed that the methine signals (δ_H 3.68, dt, $J=5.0$ and 7.0 Hz; δ_H 3.75, ddd, $J=3.5$, 7.0 and 7.5 Hz; δ_H 3.88, dd, $J=3.5$ and 5.0 Hz) and a methylene signal (δ_H 3.54 and 3.95, each dd, $J=7.0$ and 8.5 Hz) correlated respectively with δ_C 54.2, 83.1, 71.6 and 72.2, indicating that these carbons were linked to heteroatoms. This spectrum also showed signals for an alkyl long chain (clusters of methylene signals: δ_H 1.2–1.7, δ_C 22.0–31.0; methyl group: δ_H 0.90, t, $J=6.5$ Hz and δ_C 14.0). The COSY and HMBC experiments (Table 1) of jaspine B (**2**) showed a single spin system for the methine and the deshielded methylene: they were all found to correlate with each other, and the methine δ_H 3.75 furthermore correlated with a methylene group of the aliphatic chain (δ_H 1.71, m; δ_C 29.3). This led us to define a sphingosine type structure with carbons 1, 3 and 4 substituted by oxygen atoms and carbon 2 substituted by a nitrogen atom. Observation of a HMBC correlation between the hydrogens of the terminal methylene at C-1 and C-4 led us to the proposal of a tetrahydrofuran ring in an anhydrosphingosine derivative.

The observation of a medium coupling constant between H-2 an H-3 (5–6 Hz) in interconverting compounds **1** and **2** strongly suggested that the substituents of C-2 and C-3 were *cis* to each other (a 10 Hz coupling constant would have been expected for the more strained *trans* isomer). The literature[7] describes a partially synthetic related compound (2*R*,3*S*,4*S*)-4-acetamido-3-acetoxyanhydrosphingosine (**6**). In order to compare the compounds, **1** was acetylated with acetic anhydride in pyridine into the *N*,*O*-diacetylated derivative **3** (IR: ν 1741 cm^{-1}, *O*-Ac; IR: ν 1673 cm^{-1}, *N*-Ac), which showed marked differences with **6** in the ^{1}H NMR spectra (Table 2). As far as relative configurations are concerned, compounds **1**–**3** are therefore '*all-cis*' derivatives (Table 1).

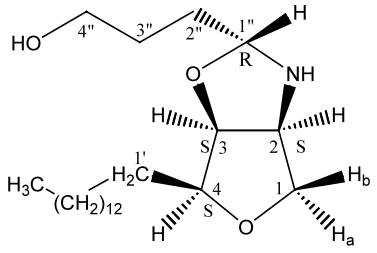
NMR data for on jaspine A (**1**) showed that this compound is closely related to jaspine B (**2**). The MS data indicated an additional C_4H_6O group, appearing on the HSQC spectrum as a deshielded methine (δ_C

Table 2. ^{1}H NMR data of the diacetyljaspine B (**3**) (500 MHz, $CDCl_3$) and of the synthetic diacetylanhydrosphingosine (**6**) (270 MHz, $CDCl_3$)

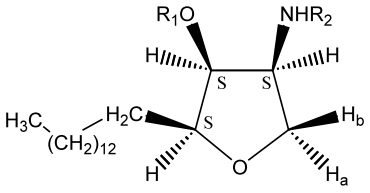
	3	**6**
1a	4.11 (1H, dd, $J_{1a-2}=J_{1a-1b}=8.2$ Hz)	4.17 (1H, dd, $J_{1a-2}=6.9$ Hz, $J_{1a-1b}=8.6$ Hz)
1b	3.62 (1H, dd, $J_{1b-2}=J_{1b-1a}=8.2$ Hz)	3.52 (1H, dd, $J_{1b-2}=6.9$ Hz, $J_{1b-1a}=8.6$ Hz)
2	4.85 (1H, qd, $J_{2-3}=5.3$ Hz, $J_{2-NH}=J_{2-1a}=J_{2-1b}=8.2$ Hz)	3.86 (1H, m)
3	5.42 (1H, dd, $J_{3-4}=3.5$ Hz, $J_{3-2}=5.3$ Hz)	4.91 (1H, dd, $J_{3-4}=2.6$ Hz, $J_{3-2}=5.9$ Hz)
4	3.93 (1H, ddd, $J_{4-3}=3.5$ Hz, $J_{4-1'}=5.3$ and 8.2 Hz)	4.65 (1H, m)
NH	5.59 (1H, d, $J_{NH-2}=8.2$ Hz)	5.66 (1H, d, $J_{NH-2}=8.6$ Hz)
OAc	2.20 (3H, s)	2.13 (3H, s)
NAc	2.05 (3H, s)	2.01 (3H, s)
CH_2CH_3	0.90 (3H, t, $J=6.8$ Hz)	0.88 (3H, t, $J=6.6$ Hz)

Table 3. ^{1}H NMR($CDCl_3$, 500 MHz) data assignments of (*S*)- and (*R*)-MTPA derivatives of *N*-acetyljaspine B (**5**)

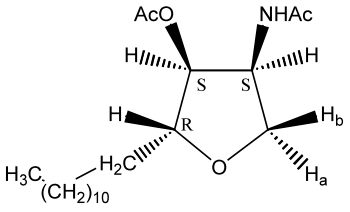
	(*S*)-MTPA (**5**)	$\Delta\delta$ (*R*)-MTPA (**5**)	
1a	4.07 (1H, dd, $J_{1a-1b}=J_{1a-2}=8.5$ Hz)	−0.05	4.12 (1H, dd, $J_{1a-1b}=J_{1a-2}=8.5$ Hz)
1b	3.67 (1H, dd, $J_{1b-1a}=J_{1b-2}=8.5$ Hz)	−0.03	3.70 (1H, dd, $J_{1a-1b}=J_{1b-2}=8.5$ Hz)
2	4.85 (1H, qd, $J_{2-3}=6.0$ Hz, $J_{2-NH}=J_{2-1a}=J_{2-1b}=8.5$Hz)	−0.04	4.89 (1H, qd, $J_{2-3}=6$ Hz, $J_{2-NH}=J_{2-1a}=J_{2-1b}=8.5$ Hz)
3	5.50 (1H, dd, $J_{3-4}=3.4$ Hz, $J_{3-2}=6.0$ Hz)	+0.07	5.43 (1H, dd, $J_{3-4}=3.4$ Hz, $J_{3-2}=6.0$ Hz)
4	3.93 (1H, ddd, $J_{4-3}=3.4$ Hz, $J_{4-1'}=5.0$ and 8.0 Hz)	+0.01	3.92 (1H, ddd, $J_{4-3}=3.4$ Hz, $J_{4-1'}=5.0$ and 8.0 Hz)



1 : jaspine A



2 $R_1 = R_2 = H$: jaspine B
3 $R_1 = R_2 =$ Ac
4 $R_1 = H$; $R_2 =$ Ac
5 $R_1 =$ MTPA ; $R_2 =$ Ac



6

93.6, δ_H 4.52, bt, $J=5.0$ Hz), two methylenes (δ_C 31.3 and δ_C 28.7; δ_H 1.75, m) and a hydroxymethylene (δ_C 62.6, δ_H 3.70, m). HOHAHA data showed two spin systems as the jaspine B moiety and the additional C-4 fragment. The additional methine was correlated on the HMBC spectrum with the tetrahydrofuran methines 2 and 3. The additional fragment of jaspine A was thus assigned to a hydroxybutanal chain branched on the 3-hydroxy and 4-amino groups of jaspine B (**2**) through an oxazolidine ring, and the structure **1** proposed for jaspine A. The signals of C-1″, C-2 and C-3 appeared to be split, indicating that jaspine A (**1**) existed as a mixture of C-1″ epimers in an approximative 1:9 ratio. The ROESY showed through-space correlations between H-1″ and H-1b, which allowed us to fix the relative configuration of the pseudo-anomeric C-1″ of jaspine A as shown in **1**. The ROEs were not detected on the minor epimer.

The absolute stereostructures of **1** and **2** were determined following (*S*)- and (*R*)-MTPA derivatisation on the *N*-monoacetylated jaspine B (**4**). Compound **3** was hydrolysed with TEA in aqueous MeOH to afford a *N*-monoacetylated derivative **4** (δ_H 2.0, IR: ν 1643 cm^{-1}, *N*-Ac). (*S*)- and (*R*)-MTPA derivatives were prepared on **4** and their ^{1}H NMR spectra analysis (Table 3) allowed us to propose the absolute configuration 2*S*,3*S*,4*S* for jaspine A (**1**) and jaspine B (**2**), i.e. the D-*lyxo* form, and 1″*R* for the additional carbon of jaspine A (**1**).

Jaspine B (**2**) has not previously been described as a natural product, though similar compounds, obtained by corn phosphatide[8] or cerebrin[9] acidic hydrolysis, or by total synthesis have been reported.[10] Jaspine B hydrochloride displayed marked cytotoxicity (IC_{50} 0.24 μM) against the A549 human lung carcinoma cell line using the ATPlite assay. The cytotoxicities of **1** and **2** were not determined because of their poor solubility in DMSO. Jaspine B hydrochloride proved the most potent compound yet isolated from the *Jaspis* genus on this cell line, i.e. pectenotoxin II (IC_{50} >10 μM),[11] bengamide Y ($IC_{50}=12.8$ μM),[12] bengamide Z ($IC_{50}=10.5$ μM).[12] Jaspine B (**2**) is an anhydrosphingosine, and it is well-known that sphingosine 1-phosphate induces a rapid and relevant release of arachidonic acid,[13] and increases phospholipase D[14] activity in A549 cells; these effects were similar to those elicited by bradykinin, a pro-inflammatory agonist.

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