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# Jasisoquinolines A and B, Architecturally New Isoquinolines, from a Marine Sponge *Jaspis* sp.

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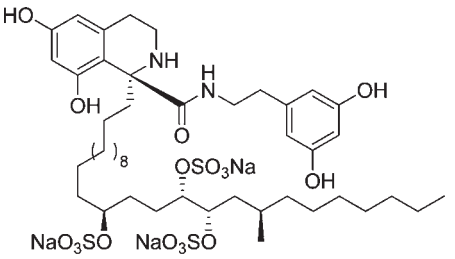
## ABSTRACT




***Jasisoquinoline A***

**Two architecturally new isoquinolines, jasisoquinolines A and B, were isolated from a marine sponge *Jaspis* sp. as cathepsin B inhibitors. Their structures were determined by a combination of spectroscopic analyses and chemical methods. Both jasisoquinolines A and B inhibit cathepsin B with an $IC_{50}$ value of 10 $\mu$g/mL.**

Cathepsin B occupies a central node of the proteolytic signal amplification network in mammals.[1] Enhanced levels of genes and proteins of this papain family of lysosomal cysteine protease in tumor cells,[2,3] together with the diminution of invasive nature of metastatic tumor cells in the gene knockout and knockdown experiments of this enzyme,[4,5] justify cathepsin B to be a target for anticancer therapy.[6] In the course of our screening of the extracts of marine invertebrates for cathepsin B inhibitors, we found activity in a marine sponge *Jaspis* sp. Bioassay-guided fractionation of the extract led us to isolate new isoquinoline alkaloids, named jasisoquinolines A (**1**) and B (**2**). In this paper, we describe the isolation and structure elucidation of jasisoquinolines.

The MeOH and EtOH extracts of the marine sponge (400 g, wet weight) were combined and subjected to solvent partitioning, silica gel column chromatography, ODS flash chromatography, and RP-HPLC sequentially to afford jasisoquinoline A (**1**, 2.0 mg) and

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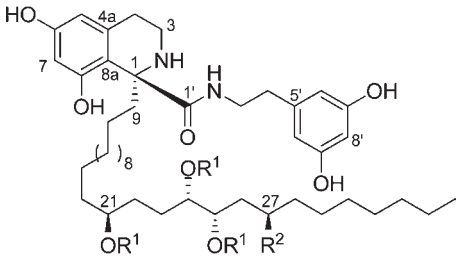
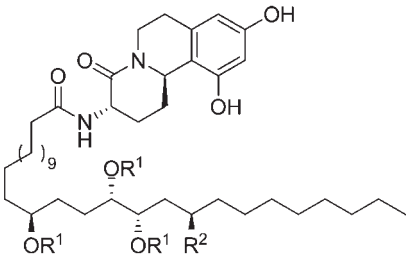
jasisoquinoline B (**2**, 0.7 mg), together with the known schulzeines A (**3**, 4.0 mg) and C (**4**, 1.5 mg).[7,8]



**1** ($R^1$ = $SO_3Na$, $R^2$ = Me)
**2** ($R^1$ = $SO_3Na$, $R^2$ = H)



**3** ($R^1$ = $SO_3Na$, $R^2$ = Me)
**4** ($R^1$ = $SO_3Na$, $R^2$ = H)

Jasisoquinoline A (**1**) had a molecular formula of $C_{45}H_{71}O_{17}N_2S_3Na_3$ as determined by HRFABMS. Two fragment ion peaks due to desulfation (*m*/*z* 951 and 849) in the negative FABMS suggested the presence of three sulfate groups, because the latter ion was presumed to contain one negatively charged sulfate group.[9] An analysis of the $^1$H NMR data in combination with the HSQC spectrum showed the presence of five aromatic protons, three oxygenated methines, two nitrogenous methylenes, one doublet methyl, one triplet methyl, and a large methylene envelope (Table S1). One aromatic resonance integrated for 2H ($\delta$ 6.10, H-6′ and H-10′) was *meta*-coupled to another aromatic signal ($\delta$ 6.12, H-8′), suggesting the presence of a 5-substituted resorcinol. In the HMBC spectrum these aromatic protons were correlated to oxygenated aromatic carbons ($\delta$ 159.4, C-7′ and C-9′) and a carbon at $\delta$ 142.1 (C-5′); the latter was connected to a 2-substituted ethyl group. The substituent was shown to be a nitrogen on the basis of the $^1$H and $^{13}$C chemical shift data ($\delta_C$ 41.9, C-3′; $\delta_H$ 3.51 and 3.43, $H_2$-3′; Figure 1a, substructure **A**). The remaining aromatic signals were *meta*-coupled to each other. Interpretation of the COSY and HMBC data demonstrated the presence of a 4,5-disubstituted resorcinol moiety. This resorcinol moiety was also linked to a 2-substituted ethyl group, the substituent being assigned as a nitrogen on the basis of the $^1$H and $^{13}$C chemical shifts of the methylene group ($\delta_C$ 40.9, $\delta_H$ 3.39 and 3.03, substructure **B**). Substructure **C** encompassed three oxymethines, one branched methyl, and six methylenes. The planar structure of this moiety, which was identical with a partial structure of schulzeine A (**3**),[7] was assigned by analysis of 2D NMR data (Figure 1a). The coincidence of the $^1$H and $^{13}$C chemical shifts suggested the identity of the relative configurations of these units.

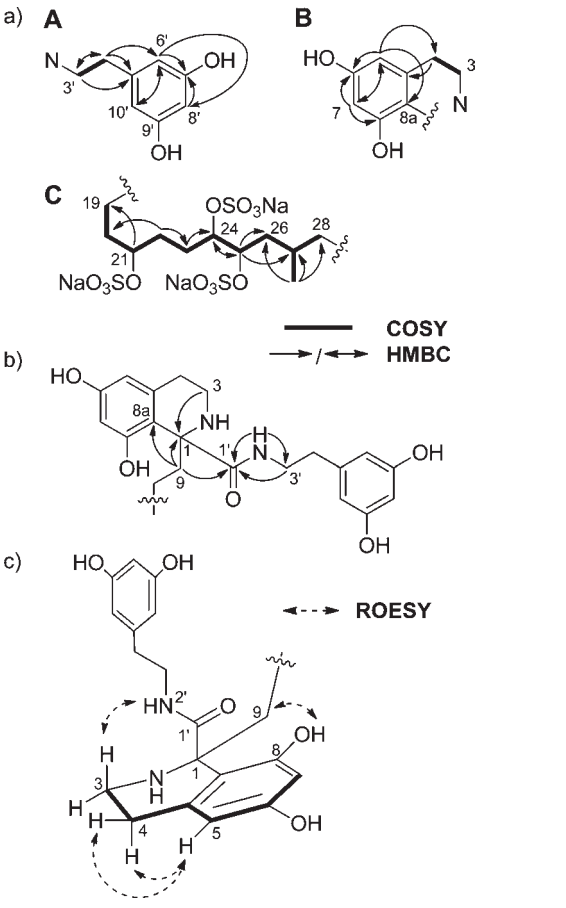
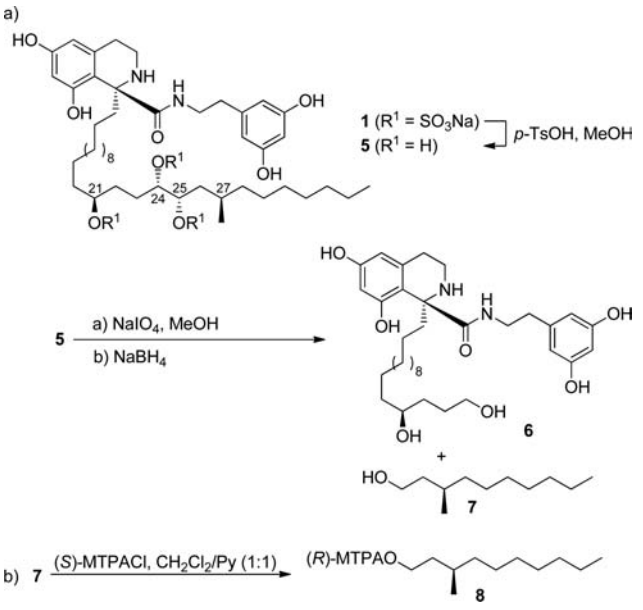



**Figure 1.** Partial structures of jasisoquinoline A (**1**).

There was a pair of methylene protons ($H_2$-9) coupled only to another pair of methylene protons ($H_2$-10). In the HMBC spectrum $H_2$-9 was correlated to a nitrogenous quaternary carbon (C-1), an aromatic carbon (C-8a), and a carbonyl carbon (C-1′). Additional HMBC correlations from $H_2$-3 to C-1, and NH-2′ and $H_2$-3′ to C-1′ demonstrated the formation of an isoquinoline ring by incorporating C-1 to which was attached C-9 and C-1′ amide carbonyl carbon (Figure 1b). ROESY cross peaks between NH-2′ and H-3ax, between 8-OH and $H_2$-9, and between $H_2$-4 and H-5 supported the structure of the isoquinoline moiety (Figure 1c).

There remained one terminal methyl and 13 methylene carbons unassigned. Therefore, substructure **C** should be placed in the middle of a long alkyl chain emanating from C-9. The location and orientation of substructure **C** in the alkyl chain was determined by taking advantage of the presence of a vicinal disulfate.[7] Sulfate esters in jasisoquinoline A (**1**) were removed with *p*-TsOH,[10] and the resulting triol was oxidized with $NaIO_4$ (Scheme 1a). The product was reduced with $NaBH_4$ to furnish diol **6** and 3-methyldecanol (**7**). The FABMS data of **6** [*m*/*z* 601 $(M + H)^+$] were consistent with the oxidation at C-21, C-24, and C-25 in **5**. This assignment was supported by the tandem FABMS data of **5** (Figure S17−S22). The absolute configuration of C-27 was determined by inspection of the $^1$H NMR data of **7** after

**Scheme 1.** Chemical Degradation of Jasisoquinoline A (**1**)




converting to the (*R*)-MTPA ester **8** (Scheme 1b).[11] The $^1$H NMR spectra of the (*S*)- and (*R*)-MTPA esters of 3-methyl-1-alkanols give charasteristic patterns for the oxygenated methylene protons by reflecting the configuration of the methyl branch.[7,12] The $^1$H NMR data of **8** were consistent with the corresponding ester of the 3*R*-alkanols, indicating the 27*R* configuration. Because the relative configuration of the C-21 to C-27 portion was suggested to be identical with that of the corresponding portion of **3**, configurations at C-21, C-24, and C-25 were deduced to be all *S*.

We initially anticipated determining the absolute configuration of the C-1 quaternary carbon by applying the exciton chirality method[13] because of the presence of two chromophoric resorcinol moieties. However, in the CD spectrum of **1** (Figure 2), no exciton split was observed near the absorption maximum of resorcinol rings (280 nm). Instead a positive $^1L_b$ transition was observed at 280 nm.[14] The conformation of the tetrahydropyridine ring in schulzeine A had been assigned on the basis of the ROESY data, and its absolute configuration had been determined by the modified Mosher analysis.[7] A positive $^1L_b$ band of schulzeine A (Figure S23) was consistent with the *P*-helicity of the tetrahydropyridine ring. On the other hand the *M*-helicity of the tetrahydropyridine ring and a negative $^1L_b$ band were observed for schulzeine B (Figure S23). It is well documented that the sign of the $^1L_b$ band of the fused benzene system is basically determined by the helicity of the cyclohexene, tetrahydropyridine, or dihydropyran ring (Figure 3), but the sign could be reversed by factors such as (1) polar substituent(s) of the benzene ring and (2) an axial substituent at the benzylic position.[14] Judging from the signs of $^1L_b$ bands in schulzeines, it was demonstrated that substitution of the benzene ring by two OH groups did not reverse the relationship between the helicity and the sign of the $^1L_b$ band in isoquinolines: *P*- or *M*-helicity corresponds to a positive or negative $^1L_b$ band, respectively.[15] We were able to assign the conformation of the tetrahydropyridine ring of jasisoquinoline A (**1**) on the basis of the ROESY data (Figure 1c) and observed a positive $^1L_b$ band. However, the sign of the $^1L_b$ band is variable due to the presence of an axial substituent at the benzylic position. Because of the lack of suitable examples, it is not possible to assess the effect of quaternalization of the benzylic position toward the sign of the $^1L_b$ band. Therefore, we tentatively draw the structure of **1** by taking into account the positive $^1L_b$ band and by presuming that its sign was not reversed by substituents at C-1 (Figure 3).

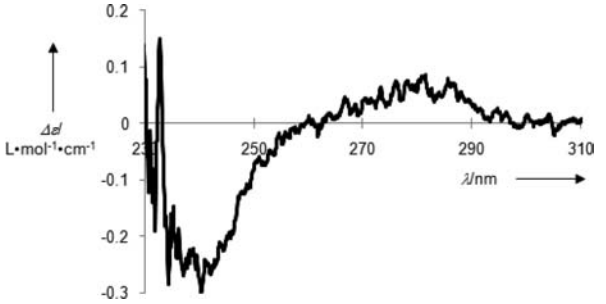



**Figure 2.** CD spectrum of jasisoquinoline A (**1**).

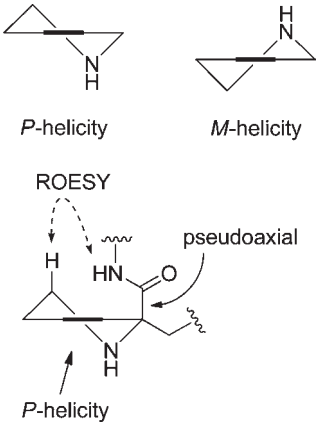



**Figure 3.** Helicity of the tetrahydropyridine ring of jasisoquinoline A (**1**). The bold lines represent the benzene rings.

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Jasisoquinoline B (**2**) was smaller than **1** by a $CH_2$ unit. The $^1H$ NMR spectrum of **2** was almost identical with that of **1** except for the absence of the secondary methyl signal. Interpretation of the COSY, HSQC, and HMBC data of **2** suggested that **2** was the 27-desmethyl analog of **1**. This was confirmed by the ESIMS of the $NaIO_4$ oxidation product of the desulfated **2**.

Both jasisoquinolines A (**1**) and B (**2**) inhibit cathepsin B with an $IC_{50}$ value of 10 $\mu$g/mL. Schulzeines A (**3**) and C (**4**) also showed inhibitory activity against cathepsin B with $IC_{50}$ values of 5.0 and 12 $\mu$g/mL, respectively. Upon desulfation the cathepsin B inhibitory activity of **1** was no longer detected, indicating the involvement of sulfate esters in the activity.

Jasisoquinolines are new members of isoquinolines from marine invertebrates. They are traced to a polyketide pathway, considering the substitution pattern in the benzene ring: biosynthesis of isoquinolines via a polyketide pathway is well-documented.[16] Diversification of the structures of schulzeines to jasisoquinolines, by α-oxidation of the fatty acid to be used as a substrate for a Pictet−Spengler reaction, is intriguing. It is worth noting that jasisoquinolines are, to the best of our knowledge, the first examples of natural products containing the amide form of 5-(2-aminoethyl)resorcinol.

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**Supporting Information Available.** Experimental details; NMR data tables for **1** and **2**; 1D and 2D NMR spectra for **1** and **2**; $^1H$ NMR spectra for **3**, **4**, and **8**; tandem FABMS spectrum for **1**; and CD spectra for schulzeines. This material is available free of charge via in Internet at http://pubs.acs.org.

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