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Wondonins A and B, new bis(dihydroxystyryl)imidazoles from a two-sponge association

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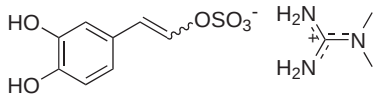
Abstract—Wondonins A and B, two alkaloids of an unprecedented structural class, along with the previously described dihydroxystyryl sulfates, have been isolated from an association of the sponges *Poecillastra wondoensis* and *Jaspis* sp. collected from Keomun Island, Korea. The structures of these compounds have been determined as imidazole alkaloids containing a bis(dihydroxystyrene) moiety by combined spectroscopic methods. The new compounds exhibited antiangiogenic activity against human umbilical vein endothelial cells.

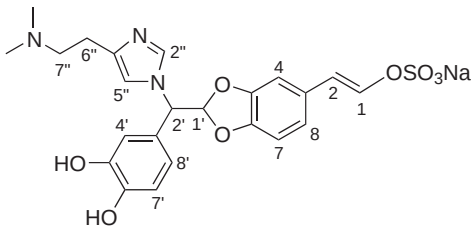
During the course of our search for bioactive substances from marine organisms, we encountered a close association of the sponges *Poecillastra wondoensis* and *Jaspis* sp. from Keomun Island, Korea.[1] The crude organic extract of the specimen exhibited remarkable cytotoxicity (LC_{50} 63 μg/mL) against the human leukemia cell line K562. Subsequently, bioassay-guided fractionation employing solvent/solvent partitioning and various chromatographic methods resulted in the isolation of several aromatic compounds. Herein we describe the structure elucidation and bioactivity of four styrene-derived metabolites including two alkaloids of an unusual structural class.

The two-sponge association was collected at Bacdo (depth 15–30 m), Keomun Island. The specimens were lyophilized (dry wt. 2.3 kg), macerated, and sequentially extracted with MeOH, CH_2Cl_2, and acetone. The combined crude extract (68.5 g) was partitioned between CH_2Cl_2 and H_2O, then the latter layer re-partitioned between *n*-BuOH and H_2O. An aliquot (5.2 g) of the BuOH layer (16.3 g) was subjected to C_{18} reversed-phase vacuum flash chromatography using stepped gradient mixtures of MeOH and H_2O as eluents. The fraction eluted with 50% aqueous MeOH was separated by HP20 adsorption chromatography sequentially eluting with H_2O, 50% aqueous MeOH, 50% aqueous



1 (*E*)-narain

2 (*Z*)-narain



3 and **4** (1'- or 2'-epimer)

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acetone, and MeOH. The aqueous MeOH fraction was further separated by reversed-phase HPLC (70% aqueous MeOH) to yield (*E*)-narain (**1**) and (*Z*)-narain (**2**) as the major metabolites; yield: 652.0 and 62.6 mg of **1** and **2**, respectively. Compounds **1** and **2**, the *N*,*N*-dimethylguanidium styryl sulfates and their structural analogs were previously isolated from the Japanese sponge *Jaspis* sp.[2–5] The spectral data for these compounds were in good agreement with those reported previously.[6]

The aqueous acetone fraction from HP20 adsorption chromatography was separated by reversed-phase HPLC (60% aqueous MeOH, then 70% aqueous MeCN) to afford wondonins A (**3**) and B (**4**) as a yellow gum; yield: 11.3 and 4.2 mg of **3** and **4**, respectively. The molecular formula of **3** was deduced as $C_{23}H_{24}N_3O_8S$ on the basis of HRFABMS (obsd *m*/*z* 502.1291, Δ −0.7 mmu) and ^{13}C NMR spectrometry.[7] Preliminary examination of the ^1H and ^{13}C NMR data showed several signals in the downfield region. Eighteen carbon resonances in the δ 150–100 region in the ^{13}C NMR spectra, coupled with the corresponding proton resonances in the δ 7.6–6.1 region in the ^1H NMR spectra, revealed the presence of two or more aromatic rings in the molecule. Characteristic absorption bands at 3400 (broad) and 1245 cm^{-1} in the IR data were indicative of the hydroxyl and sulfate groups, respectively.

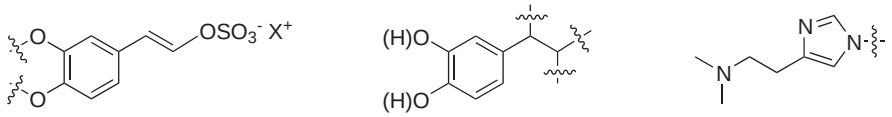
With the aid of this information, the structure determination of **3** was accomplished by extensive NMR analysis. The ^1H COSY and gradient HSQC data revealed the presence of a 1,2,4-trisubstituted benzene moiety. The gradient HMBC data aided the assignment of the NMR signals at the benzene ring as well as the attachment of oxygens at the adjacent positions; ^{13}C δ 149.0 and 147.5 (Table 1). The combined 2-D NMR data also showed the presence of a disubstituted double bond consisting of proton signals at δ 7.14 and 6.11 and its attachment at C-3. The significantly differentiated chemical shifts of the olefinic carbons at δ 140.0 and 115.4, as observed for **1** and **2**, coupled with the large vicinal coupling constant (J=12.5 Hz), revealed the attachment of a sulfate group at this (*E*)-double bond (J=12.7 and 7.3 Hz for **1** and **2**, respectively). This interpretation was further supported by absorption maxima at 264 and 210 nm in the UV spectra. Thus, a partial structure (**a**) of **3** was defined as (*E*)-styryl sulfate attached by oxygens at C-5 and C-6.

A combined ^1H COSY and HSQC analysis showed the presence of another 1,2,4-trisubstituted benzene ring. The placement of two oxygens at the adjacent positions (C-5′ and C-6′) was aided by HMBC correlations between the quaternary carbons at δ 147.3 and 146.9 and aromatic protons. The 2-D NMR data also showed the presence of another AB proton spin system consisting of signals at δ 6.81 and 5.58, as well as the attachment of the methine carbons bearing these protons at C-3′ of the benzene ring, identical to **a**. However, the chemical shift of the methine carbons at δ 110.9 and 64.3 in the ^{13}C NMR spectra suggested that the (*E*)-double bond of **a** was transformed to a different functionality in **b**.

High-resolution mass analysis of **3** showed the presence of three nitrogen atoms in the molecule. The chemical shifts of three carbons at δ 139.4 (CH), 138.1 (C), and 118.4 (CH), combined with the large C-H coupling

Table 1. ^1H and ^{13}C NMR assignments for wondonins A (**3**) and B (**4**) in CD_3OD

No.	**3**			**4**	
	$^{\delta}H$	$^{\delta}C$	HMBC	$^{\delta}H$	$^{\delta}C$
1	7.14 (d, 12.5)	140.0	C-2, C-3	7.13 (d, 12.7)	140.0
2	6.11 (d, 12.5)	115.4	C-1, C-4, C-8	6.09 (d, 12.7)	115.3
3		130.3			130.6
4	6.79 (d, 1.5)	105.9	C-2, C-6, C-8	6.74 (br s)	105.9
5		149.0			148.9
6		147.5			147.6
7	6.60 (d, 8.1)	109.6	C-3, C-5	6.66 (s)	109.2
8	6.65 (dd, 8.1, 1.5)	121.3	C-2, C-4, C-6	6.66 (d, 2.0)	121.0
1′	6.81 (d, 2.0)	110.9	C-5, C-6	6.79 (d, 2.4)	110.9
2′	5.58 (d, 2.0)	64.3	C-1′, C-3′, C-4′, C-8′, C-2″, C-5″	5.58 (d, 2.4)	64.3
3′		126.5			126.4
4′	7.00 (d, 2.2)	116.9	C-5′, C-6′	6.99 (d, 2.2)	116.9
5′		146.9			146.8
6′		147.3			147.3
7′	6.81 (d, 8.3)	116.6	C-3′, C-5′, C-6′	6.80 (d, 8.3)	116.5
8′	6.90 (dd, 8.3, 2.2)	121.5	C-4′, C-6′	6.89 (dd, 8.3, 2.2)	121.4
2″	7.61 (s)	139.4	C-4″, C-5″	7.56 (d, 1.0)	139.0
4″		138.1			138.2
5″	6.92 (s)	118.4	C-2′, C-2″, C-4″	6.98 (br s)	118.7
6″	2.68 (m); 2.72 (m)	24.9	C-4″, C-7″	2.68 (m); 2.71 (m)	25.0
7″	2.82 (2H, t, 7.1)	59.3	C-4″, C-6″, N-Me	2.76 (2H, m)	59.4
N-Me	2.62 (6H, s)	44.2	C-7″, N-Me	2.55 (6H, s)	44.3



a b c

constants of these with the attached protons ($^1J_{CH}$ = 216.7 and 192.9 Hz for the carbons at δ 139.4 and 118.4, respectively), were interpreted as an imidazole moiety. The remaining nitrogen was assigned to form a *N*,*N*-dimethylamino group on the basis of a carbon signal at δ 44.2 and corresponding proton signal at δ 2.62 (6H, s) in the NMR data. The connection of the *N*,*N*-dimethylamino group at C-4″ of the imidazole ring via an ethylene group in this partial structure (**c**) was also determined by combined 2-D NMR analysis.

The connectivity of the partial structures **a**–**c** was established by gradient HMBC experiments. The long-range correlations of H-1′ at δ 6.81 with C-5 and C-6 at 149.0 and 147.5, respectively, showed the connection between **a** and **b** through a five-membered cyclic acetal linkage. Similarly, the connection between the C-2′ and N-1″ of the imidazole ring was determined on the basis of the correlations of H-2′ at δ 5.58 with C-2″ and C-5″ at δ 139.4 and 118.4, respectively. The counter ion was determined as sodium on the basis of combined atomic absorption spectroscopy and ^{23}Na NMR analysis. Thus, the planar structure of wondonin A (**3**) was determined as an imidazole alkaloid possessing two dihydroxystyrene moieties.

The molecular formula of wondonin B (**4**) was deduced as $C_{23}H_{24}N_3O_8S$, identical to that of **3**, by HRFABMS analysis.[8] The spectral data of this compound were very similar to those of **3**. Despite the slight differences in the chemical shifts of a few signals in the ^{1}H and ^{13}C NMR spectra, combined 2-D NMR methods revealed that wondonin B had the same planar structure as wondonin A (Table 1). The counter ion was also determined as sodium, identical to **3**, by atomic absorption spectroscopy and ^{23}Na NMR analysis. Thus, **4** was defined as an epimer of **3** at either C-1′ or C-2′ that was supported by the specific rotation ($[\alpha]_D^{25}$ −4.8 and +3.6° for **3** and **4**, respectively). To assign the stereochemistry of these, the wondonins were extensively analyzed by NOESY and 1-D ROESY experiments in which strong correlations were observed between the protons (H-1′ and H-2′) attached at the asymmetric and aromatic centers.[9] Due to the large spatial distance among the aromatic moieties and severe overlapping of signals in the ^{1}H NMR spectra, however, correlations between the protons at different ring systems were not unambiguously analyzed. A three-dimensional model study also showed that spatial crowding forced benzene, imidazole, and ketal moieties to be tilted toward the molecular plane, preventing the occurrence of significant NOE among the aromatic rings. Similar results were also obtained for **4** and the stereochemistry of the wondonins remained to be determined.

Sponges have produced a wide variety of secondary metabolites.[10] However, compounds based on a dihydroxystyryl sulfate moiety have only been isolated from a few specimens of the genus *Jaspis*.[2–5] Furthermore, the connection of 4-(2-*N*,*N*-dimethylaminoethyl)-imidazole with dihydroxystyryl dimer, as found in wondonins, is unprecedented among marine natural products to the best of our knowledge.

Sponge-derived dihydroxystyryl sulfates showed ecological roles such as inhibition of fertilization of sea urchin gametes and inducement of metamorphosis of ascidian larvae.[2–5] In our assessment of the pharmacological activity, compounds **1** and **2** exhibited cytotoxicity against the human leukemia cell line K562 with LC_{50} 0.9 and 0.4 μg/mL, respectively, while **3** and **4** were inactive (LC_{50} >100 μg/mL). Thus, cytotoxicity of the crude extract was largely attributed to **1** and **2**. Instead **3** and **4** were found to possess an antiangiogenic effect. In assays using HUVEC (human umbilical vein endothelial cells), these compounds inhibited tube-forming induced by bFGF (basic fibroblast growth factor) at a concentration of 10 μg/mL without showing significant cytotoxicity toward the cell (after 48 h, 97 and 94% viability for cells treated with **3** and **4**, respectively). Details of the antiangiogenic activity of wondonins and their mode of action are currently under investigation and will be reported in due course.

Acknowledgements

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1. Two sponges appeared to be one animal since they were attached tightly to each other. Based upon the morphological features, the thin and bright yellow outer layer was identified as *P. wondoensis* while the golden yellow inner layer was identified as *Jaspis* sp. The inner specimen was similar to *J. wondoensis* in its spicule type, but different in spicule size and color (Sim, C. J.; Kim, Y. A. *Korean J. Syst. Zool.* **1995**, *11*, 147–158). A voucher specimen of this two-sponge association (Registry No. Por. 32) is deposited at the Natural History Museum, Hannam University, Korea under the curatorship of C.J.S.
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5. Ohta, S.; Kobayashi, H.; Ikegami, S. *Tetrahedron Lett.* **1994**, *35*, 4579–4580.
6. The presence of *N*,*N*-dimethylguanidium as the counter ion in **1** and **2** was determined by combined HRFABMS and NMR analysis.
7. **3**: $[\alpha]_D^{25}$ −4.8° (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.16), 264 (3.94) nm; IR (KBr) ν_{max} 3400 (br, OH), 1650, 1495, 1245 (SO_2), 1035 cm^{-1}; for ^1H and ^{13}C NMR data, see Table 1; HRFABMS *m*/*z* 502.1291 $[M]^-$ (calcd for $C_{23}H_{24}N_3O_8S$: 502.1284, Δ −0.7 mmu).
8. **4**: $[\alpha]_D^{25}$ +3.6° (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.23), 264 (3.25) nm; IR (KBr) ν_{max} 3400 (br), 1645, 1495, 1245, 1035 cm^{-1}; for ^1H and ^{13}C NMR data, see Table 1; HRFABMS *m*/*z* 502.1297 $[M]^-$ (calcd for $C_{23}H_{24}N_3O_8S$: 502.1284, Δ −1.3 mmu).
9. NOE was observed for H-1′/H-2′, H-1′/H-8′, H-2′/H-4′, H-2′/H-8′, H-2′/H-2″, and H-2′/H-5″.
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