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Ianthesines A–D, Four Novel Dibromotyrosine-Derived Metabolites from a Marine Sponge, *Ianthella* sp.

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Abstract—Novel dibromotyrosine-derived metabolites, ianthesines A, B, C, and D, were isolated from an Australian marine sponge of the genus *Ianthella* sp. Their structures were elucidated using chemical and spectroscopic techniques. Ianthesines A, B, and D were derived from two dibromotyrosines. Ianthesine C is a tetramer possessing eight bromine atoms and its molecular weight is 1606. Ianthesines B–D showed Na,K-ATPase inhibitory activity in the range of 50–440 μM, whereas ianthesine A is inactive.

Introduction

Marine sponges of the Verongida order, which include the genera *Aplysina*, *Ianthella*, *Verongia*, *Psammaplysilla*, etc. have proved to be a rich source of secondary metabolites derived from dibromotyrosine,[1] such as aeroplysinin-1,[2] aerothionin,[3] fistularins,[4] bastadins,[5,6] araplysillins,[7] purealin,[8] aerophobin-1,[9] lipopurealins,[10] purealidins,[11,12] and others.[13,14] Some of them show biological activities such as antibacterial, cytotoxic, and ATPase modulating activities. During the course of our search for biologically active compounds in marine invertebrates, our attention became focused on the Na,K-ATPase inhibitory constituents of a sponge of the genus *Ianthella*, because the organic extract of the sponge (class Demospongiae, order Verongida, family Ianthellidae) collected in Australia showed a potent Na,K-ATPase activity. We have recently reported a novel dimeric polybrominated benzofuran, iantheran A, as an active principal component from this sponge.[15] This paper describes the isolation of four novel dibromotyrosine-derived metabolites, named ianthesines A (**1**), B (**2**), C (**3**), and D (**4**), from the same sponge and their structural determination by spectral analysis and chemical derivatizations (Scheme 1).

Results and Discussion

The MeOH extract of the sponge (1.8 kg wet weight), collected at the Great Barrier Reef in Australia, was partitioned between EtOAc and water. The EtOAc portion, which inhibited the dog kidney Na,K-ATPase with an IC_{50} of 10 μg/ml, was repeatedly subjected to bioassay-guided fractionation by silica gel chromatography ($CHCl_3$/MeOH/H_2O system) and reversed-phase HPLC to give ianthesines A (**1**) (0.069% of wet sponge), B (**2**) (0.019%), C (**3**) (0.019%), and D (**4**) (0.011%).

Ianthesine A (**1**), mp 154–156°C, showed pseudomolecular ions at *m/z* 786, 788, 790, 792, and 794 $(M+H)^+$ in the ratio of ca. 1:4:6:4:1 in the positive FABMS spectrum, indicating the presence of four bromine atoms in the molecule. The molecular formula of **1** was determined to be $C_{24}H_{27}Br_4N_3O_7$ based on the high-resolution positive FABMS and elemental analysis, implying 11 of unsaturation index. The IR bands at 1660 and 1540 cm^{-1} showed the presence of a secondary amide group. The proton–carbon connectivities were determined as shown in Tables 1 and 2 by the HMQC experiment. The ^1H–^1H COSY spectrum of **1** revealed the presence of a 1,3-disubstituted propane moiety (cross-peaks of H-10/H-11 and H-11/H-12) and a 1,1,2-trisubstituted ethane moiety (cross-peaks of H-19a/H-19b, H-19a/H-20, and H-19b/H-20). The proton spin–spin coupling of the AB quartet at δ 3.09 and 3.79 could be assigned to an isolated methylene group that is adjacent to a π-electron system because of the large geminal *J* value of 18.2 Hz.[16] Since there were numerous quaternary carbons, extensive HMBC experiments were required for determining the carbon–carbon connectivities in **1**. The H–C correlations obtained by the HMBC spectrum are summarized in Fig. 1, revealing the presence of a 1,2,3,5-tetrasubstituted benzene and a 1,2,3,5,5,6-hexasubstituted 1,3-cyclohexadiene moiety. The position of the oxygen substituents was determined to be C-1, C-3, C-6, C-12, C-13, and 3-OMe based on the relatively low-field shifts of these carbons. The relatively high-field shifts at the sp^2

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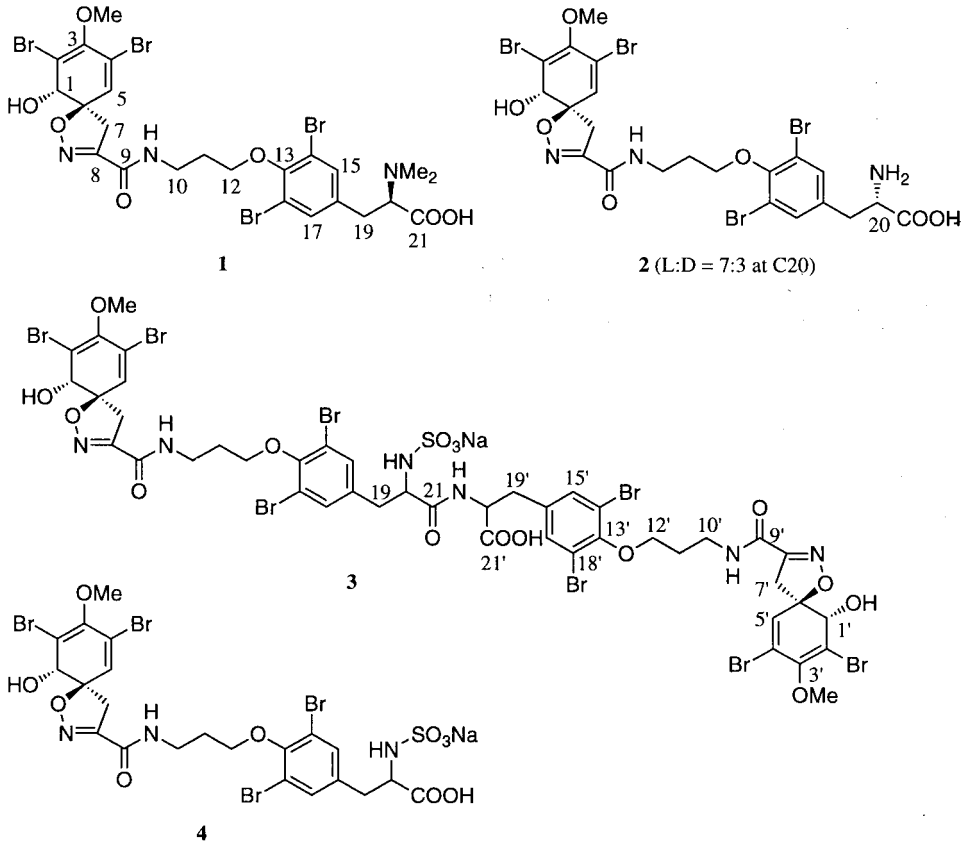

Scheme 1. Structures **1**–**4**.

carbons of C-2, C-4, C-14, and C-18 indicated the location of four bromine atoms at these carbons. The HMBC correlations of OMe/C-3 and H-12/C-13 indicated the connectivity between C-3 and OMe and between C-12 and C-13 via ether bonds. The HMBC correlations of H-10/C-9 and H-20/NMe_2 revealed the connectivities between C-9 and C-10 and between C-20 and NMe_2 via a nitrogen atom. The presence of a spiroisoxazoline structure was deduced by comparing the ^1H and ^{13}C NMR of **1** with those previously reported for the Verongida sponge constituents.[2–13] The *trans* stereochemistry of the vicinal oxygen atoms at C-1 and C-6 was established by the chemical shifts of H-1 and

Table 1. ^1H NMR data for **1**–**4**. Spectra were recorded at 400 MHz in methanole-d_4 for **1** and **2** or at 600 MHz in DMSO-d_6 for **3** (at 55°C) and **4** (at 40°C)

Position	**1**	**2**	**3**[a]	**4**
1	4.10 s	4.07 s	3.96 brs	3.94 brs
5	6.40 s	6.41 s	6.54 s	6.56 s
7a	3.09 d (18.2)	3.09 d (18.2)	3.64 d (17.5)	3.63 d (18.2)
7b	3.79 d (18.2)	3.77 d (18.2)	3.17 d (17.5)	3.20 d (18.2)
10	3.57 t (7.0)	3.58 t (7.0)	3.40 q (6.6)	3.40 dt (5.8, 6.5)
11	2.09 m	2.11 m	2.00 quint (6.6)	1.99 quint (6.5)
12	4.02 t (6.0)	4.07 t (6.0)	3.96 m	3.95 m
15,17	7.57 s	7.55 s	7.52 s, 7.44 s	7.53 s
19a	3.09 dd (8.8, 13.9)	2.98 dd (8.1, 14.7)	2.94 m, 2.79 m	2.98 dd (4.0, 13.0)
19b	3.17 dd (5.2, 13.9)	3.20 dd (4.7, 14.7)	2.79 m, 3.01 dd (4.7, 13.6)	2.86 dd (4.0, 13.0)
20	3.74 dd (5.2, 8.8)	3.75 dd (4.7, 8.1)	3.87 m, 3.89 m	3.82 brs
1-OH			6.24 brs	6.32 brs
3-OMe	3.71 s	3.72 s	3.66 s	3.65 s
9-NH			8.35 m	8.46 t (5.8)
20-NH			4.91 brs, 4.52 brs	- (not detected)
20-Nme_2 (right half of **3**)	2.89 s			
15′,17′			7.38 s, 7.47 s	
19′a			2.95 m, 2.64 dd (7.5, 14.0)	
19′b			2.86 dd (6.2, 13.4), 2.81 m	
20′			4.26 m, 4.24 m	
20′-NH			7.90 brd (6.4), 8.06 brs	

[a] **3** is a 3:2 diastereomeric mixture (the former is the major isomer).

Table 2. ^{13}C NMR data for **1**–**4**. Spectra were recorded at 100 MHz in methanol-d_4 for **1** and **2** or at 150 MHz in DMSO-d_6 for **3** (at 55°C) and **4** (at 40°C)

Position	1	2	3[a]	4
1	75.4 d	75.5 d	73.7 d	73.7 d
2	114.2 s	114.2 s	113.0 s	113.1 s
3	149.2 s	149.3 s	147.1 s	147.2 s
4	122.7 s	122.8 s	120.6 s	120.7 s
5	132.3 d	132.3 d	131.3 d	131.4 d
6	92.4 s	92.4 s	90.3 s	90.3 s
7	40.2 t	40.2 t	39.3 t	39.4 t
8	155.3 s	155.3 s	154.3 t	154.5 t
9	161.4 s	161.6 s	158.9 s	159.0 s
10	38.0 t	38.0 t	36.2 t	36.3 t
11	30.6 t	30.6 t	29.4 t	29.5 t
12	72.2 t	72.2 t	71.2 t	71.2 t
13	153.3 s	153.7 s	150.6, 150.7, 150.8 s[b]	150.4 s
14,18	119.1 s	119.4 s	116.7, 116.8, 116.9 s[b]	116.5 s
15,17	134.9 d	135.0 d	133.5 d	134.0 d
16	136.9 s	136.6 s	138.0, 138.2 s[b]	138.8 s
19	33.7 t	36.8 t	36.7, 37.0 t	36.3 t
20	72.6 d	57.1 d	57.9, 57.3 d	57.2 d
21	171.4 s	173.0 s	171.8, 172.5 s	174.3 s
OMe	60.4 q	60.4 q	59.5 q	59.6 q
20-NMe_2 (right half of **3**)	42.3 q			
19′			36.7, 36.2 t	
20′			54.5, 54.5 d	
21′			172.0, 172.3 s	

[a] **3** is a 3:2 diastereomeric mixture (the former is the major isomer).
[b] Assignments for major/minor isomer and right/left half are unclear.

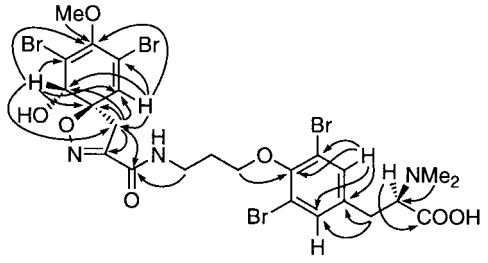

Figure 1. HMBC correlations of **1**.

H-8[17] and no NOE observation between H-1 and H-7. The absolute stereochemistry of the spirocyclohexadienylisoxazoline moiety was deduced to be 1*S*,6*R* based on a large minus optical rotation, $[\alpha]_D^{22}=-118$, and the negative Cotton effects at 248 and 285 nm in the CD spectrum, which agreed with those of the known compound aerothionin,[3] whose absolute configuration was established by X-ray analysis and CD spectroscopy.[18] The configuration of the chiral center at C-20 has been determined as follows. Ianthesine A (**1**) was hydrolyzed with hydrobromic acid to give 3,5-dibromo-*N*,*N*-dimethyltyrosine, which was subjected to catalytic hydrogenation to afford *N*,*N*-dimethyltyrosine.[19] Chiral HPLC analysis using synthetic samples revealed that the obtained tyrosine had a D configuration. The structure of ianthesine A was thus elucidated as shown by formula **1**.

Ianthesine B (**2**), mp 154–157°C, showed pseudomolecular ions at *m/z* 758, 760, 762, 764, and 766 $(M+H)^+$ in the ratio of ca. 1:4:6:4:1 in the positive FABMS spectrum, indicating the presence of four bromine atoms like ianthesine A (**1**). The molecular formula of **2** was determined to be $C_{22}H_{23}Br_4N_3O_7$ based on the high-resolution positive FABMS and elemental analysis. The ^1H and ^{13}C NMR data (Tables 1 and 2) for **2** were similar to those for **1** except for the absence of the *N*,*N*-dimethyl resonance and for the observed higher field shift of C-20 (δ_C 57.1) than that (δ_C 72.6) of **1**. The HMBC and ^1H–^1H COSY spectra of **2** suggest that **2** was the *N*,*N*-didesmethyl derivative of **1**. The absolute stereochemistry of the spirocyclohexadienylisoxazoline moiety of **2** was deduced to be the same as that of **1** based on a minus optical rotation and the negative Cotton effects in the CD spectrum of **2**. The configuration at C-20 has been determined as follows. Ianthesine B (**2**) was subjected to the same reaction sequence as that for **1** to afford tyrosine, which was found to be a mixture of the L- and D-forms in the ratio of 7:3. These findings indicate that **2** is a 7:3 mixture of two diastereomers (1*S*,6*R*,20*S* and 1*S*,6*R*,20*R*). The chiral HPLC analysis of **2** itself also showed two peaks in the ratio of 7:3 and no epimerization during the hydrolysis.

Ianthesine C (**5**), mp 200°C (dec.), showed a symmetrical isotope peak pattern around *m/z* 1583 $(M-Na)^-$ and 1503 $(M-SO_3Na)^-$ in the negative FABMS spectrum, indicating the presence of several bromine atoms and a sulfate group in the molecule. The presence of SO_3^- was supported by the intense bands at 1257 and 1047 cm^{-1} in the IR spectrum. It was difficult to determine the molecular formula of **3** by high-resolution MS due to the low intensity of the lowest-weight isotope peak of the pseudomolecular ions $(M-Na)^-$ at *m/z* 1575 containing eight ^{79}Br atoms. This compound **3** appears to be an unsymmetrical dimer of ianthesine B (**2**)

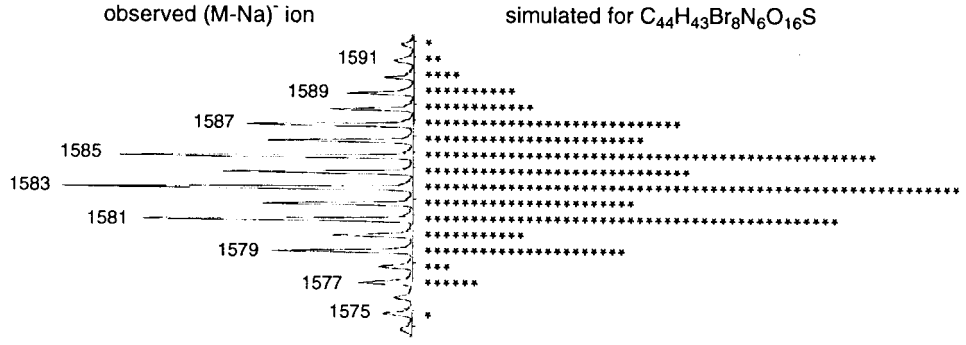

Figure 2. The observed (left) and simulated (right) FABMS patterns for the $(M-Na)^-$ ion of **3**.

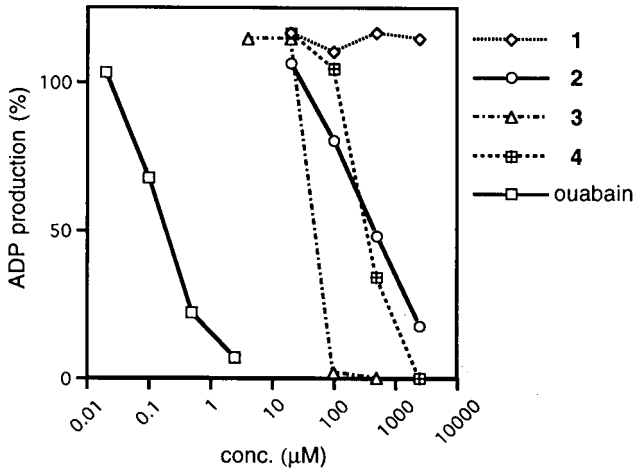

Figure 3. Na,K-ATPase inhibition by **1**–**4**. IC_{50} values for **1**–**4** and ouabain are >2,500, 440, 50, 280, and 0.2 μM, respectively.

based on the NMR data (Tables 1 and 2), and the ion peak at *m/z* 1583 $(M-Na)^-$ of **3** coincides with '2×761 (molecular weight of **2**)−18 (H_2O)+80 (SO_3)−H'. These data suggest that **3** is a dipeptide comprised of two molecules of **2** and one sulfate group, and consequently, the molecular formula of **3** is $C_{44}H_{43}Br_8NaN_6O_{16}S$. This was proved from the elemental analysis and the good agreement between the observed FABMS and a simulated isotope pattern (Fig. 2). The observation of one NH proton at δ 4.91/4.52 (3:2 ratio) indicates that the sulfate group is located on the amino group at C-20. The NMR chemical shifts of the left half of the molecule (acid part as a dipeptide) were quite similar to or partially the same as the corresponding chemical shifts of the right half (amino part). However, we could assign most of the signals as shown in Tables 1 and 2 based on the observation of an NOE between the inter-residual αH (H20) and 20′-NH, which is well-known in the NMR of peptides. Ianthesine C (**3**) seems to be a diastereomeric mixture in the ratio of ca. 3:2, because several NMR signals around the central part of the molecule (positions 13–21) make a pair in the ratio of 3:2. The configurations of C-20 and C-20′ of these diastereomers are unclear. The stereochemistry of the two spirocyclohexadienylisoxazoline moieties of **3** were deduced to be the same as those of **1** and **2** because of the approximately 2-fold intensity of the Cotton effects as those of **1**.

Ianthesine D (**4**), mp 190°C (dec.), showed NMR spectra quite similar to those of ianthesine B (**2**) (Tables 1 and 2). The ion peaks at *m/z* 836, 838, 840, 842, and 844 $(M-Na)^-$ in the ratio of ca. 1:4:6:4:1 in the negative FABMS spectrum indicated the presence of four bromine atoms like **2**. The molecular formula of **4**, $C_{22}H_{22}Br_4NaN_3O_{10}S$, which was determined by the high-resolution FABMS and elemental analysis, is more than that of **2** by 'SO_3Na–H'. The presence of a sulfate group was supported by the fragment peaks of $(M-SO_3Na)^-$ at *m/z* 756, 758, 760, 762, and 76 and the intense bands at 1217 and 1046 cm^{-1} in the IR spectrum. Although the proton signal due to 20-NH could not be observed, the position of the sulfate group was assumed to be on the amino group at C-20 based on the presence of the signals due to both 1-OH and 9-NH in the ^1H NMR. The configuration at C-20 is unclear. The stereochemistry of the spirocyclohexadienylisoxazoline moiety was deduced to be the same as those of **1**–**3** from the comparison of the NMR data and CD spectra.

A considerable number of dibromotyrosine-derived metabolites with a spirocyclohexadienylisoxazoline ring have been isolated from the marine Verongida sponges. Most of these known compounds are the terminal amine-type, whereas, to the best of our knowledge, ianthesines A–D (**1**–**4**) are the first examples of the amino acid-type among this class of metabolites. Some of these types of metabolites are known to show Na,K-ATPase inhibitory activity.[7,8,10] The dose response curves for **1**–**4** against the dog kidney Na,K-ATPase is shown in Fig. 3. The IC_{50} values of **2**, **3** and **4** are 440, 50 and 280 μM, respectively, while **1** was inactive (>2.5 mM), whereas a cardiac steroid glycoside, ouabain, showed an IC_{50} of 0.2 μM under the same conditions. The amino group of the terminal α amino acid (in **1**, **2** and **4**) or the dipeptide moiety (in **3**) seems to be important for the inhibitory activity, because the activity decrease in the order, $-NHSO_3^-$ (**3**), $-NHSO_3^-$ (**4**), $-NH_2$ (**2**), $-NMe_2$ (**1**).

Experimental

General methods

Melting points were uncorrected. HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph. IR spectra were recorded on a JASCO FT/IR-7000S. UV spectra were recorded on a JASCO Ubest-50 UV/VIS spectrophotometer. Optical rotations were measured on a JASCO DIP-370 digital polarimeter using a 10 cm cell. CD spectra were recorded on a JASCO J-720WI spectrometer. NMR spectra were recorded on a Brucker-ARX400 (400 MHz) or Brucker-AMX600 (600 MHz) spectrometer. Chemical shifts were referenced to the solvent peak at δ_H 3.30 (residual CHD_2OD), 2.49 (residual CD_3SOCD_3), δ_C 49.0 (CD_3OD), or 39.7 (CD_3SOCD_3). FAB Mass spectra were recorded on a JMS DX-705 or a MStation JMS-700 (high-resolution) mass spectrometer using glycerol or thioglycerol as the matrix.

Na,K-ATPase inhibitory assay

To 70 μl of 30 mM Tris–HCl buffer (pH 7.4) supplemented with 5 mM $MgCl_2$, 100 mM NaCl, 10 mM KCl, and 3 mM EDTA was added a sample solution (10 μl) in DMSO (or 10 μl of DMSO as a control). This mixture was kept at 37°C for 10 min. Na,K-ATPase (0.002 units, from dog kidney, Sigma) in 10 μl of Tris–mannitol buffer (250 mM mannitol, 20 mM Tris–HCl, 1 mM EDTA, pH 7.4) was added to the mixture, and after pre-incubation for 8 min at 37°C, 10 mM ATP (Oriental Yeast) in water (10 μl) was added. The resulting mixture was incubated for 1.5 h at 37°C. The reaction mixture was quenched with 20% trichloroacetic acid (2 μl) and cooled on ice, then analyzed by reversed-phase HPLC (Develosil ODS-UG-5 (4.6 i.d.×250 mm, Nomura Chemical) with 50 mM sodium phosphate buffer (pH 7.0) at a flow rate of 1.0 ml/min, detected at 260 nm) to quantify the content of ADP (t_R=5.9 min) and ATP (t_R=5.0 min). The inhibition of the ADP production from

ATP was given by the following equation: $(1-[ADP]_{sample}/[ADP]_{control})\times 100\%$, [ADP] indicates the peak area of ADP.

Collection, extraction, and isolation

The sponge *Ianthella* sp. (1.8 kg wet weight), collected at a depth of 12 m in the Great Barrier Reef, Australia, was homogenized in MeOH (4 l), and the resulting mixture was then filtered. The residue was washed three times with MeOH (2 l). The filtrates were combined and concentrated to 1 l, and the resulting aqueous residue was extracted three times with EtOAc (1.5 l). The combined EtOAc layers were evaporated to give a pale brown powder (14.4 g), which inhibited Na,K-ATPase with an IC_{50} of 10 μg/ml. A portion (4.0 g) of the EtOAc extract was chromatographed on silica gel (200 g) with $CHCl_3$–MeOH–H_2O (7:3:1 lower phase, 65:35:10 lower phase, and then 6:4:1) to give seven fractions. The third fraction was pure **1** [182 mg, R_f=0.43 on silica gel TLC developed with $CHCl_3$–MeOH–H_2O (65:35:10 lower phase)]. The second fraction (687 mg) was chromatographed on silica gel (137 g) with $CHCl_3$–MeOH–H_2O (10:3:1 lower phase). The fractions containing **1**, **2** (R_f=0.33 under the same conditions), and **3** (R_f=0.28 under the same conditions) were combined and then chromatographed on silica gel (63 g) to give **1** (163 mg), **2** (63 mg), and **3** (94 mg) as powders. An other portion (3.0 g) of the above EtOAc extract was chromatographed on silica gel in the same manner to give seven fractions. The third fraction (503 mg) contained **1**, **2**, and **3**. The fourth fraction (990 mg) contained iantheran A[15] (R_f=0.37 under the same conditions) as the major component. The fifth fraction (322 mg) containing **4** (R_f=0.13 under the same conditions) was chromatographed on silica gel (97 g) with $CHCl_3$–MeOH–H_2O (7:3:1 lower phase and then 65:35:10 lower phase) to give four fractions. A portion (41.4 mg) of the third fraction (total 86.4 mg) was purified by reversed-phase HPLC [Develosil ODS-HG-5 (10 i.d.×250 mm, Nomura Chemical) with MeOH–H_2O (3:2) at a flow rate of 2.5 ml/min, detected at 254 nm] to give **4** (20 mg, t_R=6.8 min) as a powder.

Ianthesine A (1). Colorless fine crystals; mp 154–156°C (MeOH–H_2O); $[\alpha]_D^{22}=-118$ (*c* 1.02, MeOH); IR (KBr) ν_{max} 3700–2300 (br), 1660, 1630, 1540, 1260, and 1045 cm^{-1}; UV (MeOH) λ_{max} 210 (ε 29500) and 283 nm (7390); CD (MeOH) λ_{ext} 248 nm (Δε −10.2), 285 (−9.94); ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS (matrix: thioglycerol) *m/z* 786, 788, 790, 792, and 794 (rel. int. 1:4:6:4:1) $(M+H)^+$; HRMS (FAB) calcd for $C_{24}H_{28}{}^{79}Br_4N_3O_7$ (M+H) *m/z* 785.8661, found 785.8649. Anal. Calcd for $C_{24}H_{27}Br_4N_3O_7$: C, 36.53; H, 3.45; N, 5.33%. Found: C, 36.53; H, 3.18; N, 5.23%.

Ianthesine B (2). Colorless fine crystals; mp 154–157°C (MeOH–H_2O); $[\alpha]_D^{22}=-97$ (*c* 0.58, MeOH); IR (KBr) ν_{max} 3700–2300 (br), 1660, 1635, 1540, 1260, and 1045 cm^{-1}; UV (MeOH) λ_{max} 207 (ε 45400) and 283 nm (7300); CD (MeOH) λ_{ext} 248 nm (Δε −11.6), 284 (−11.2); ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS (matrix: thioglycerol) *m/z* 758, 760, 762, 764, and 766 (rel. int. 1:4:6:4:1) $(M+H)^+$; HRMS (FAB) calcd for $C_{22}H_{24}{}^{79}Br_4N_3O_7$ (M+H) *m/z* 757.8348, found 757.8359. Anal. Calcd for $C_{22}H_{23}Br_4N_3O_7$: C, 34.72; H, 3.05; N, 5.52%. Found: C, 34.73; H, 2.80; N, 5.23%.

Ianthesine C (3). Yellow powder; mp 200°C (dec.) (precipitated from H_2O–DMSO); $[\alpha]_D^{26}$ −93 (*c* 0.86, DMSO); IR (KBr) ν_{max} 3700–2500 (br), 1660, 1584, 1541, 1257, 1218, 1183, 1047, and 990 cm^{-1}; UV (MeOH–0.5% DMSO) λ_{max} 231 (ε 26900) and 283 (11900) nm; CD (MeOH) λ_{ext} 252 nm (Δε −16.0), 289 (−16.9); ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS (matrix: glycerol), see Fig. 2. Anal. Calcd for $C_{44}H_{43}Br_8N_6O_{16}SNa$: C, 32.90; H, 2.70; N, 5.23%. Found: C, 32.48; H, 2.81; N, 4.84%.

Ianthesine D (4). Colorless powder; mp 190°C (dec.); $[\alpha]_D^{25}=-69$ (*c* 0.19, MeOH); IR (KBr) ν_{max} 3700–2500 (br), 1662, 1596, 1541, 1258, 1217, and 1046 cm^{-1}; UV (MeOH) λ_{max} 206 (ε 53200), 220 (sh, 25000), and 282 nm (6760); CD (MeOH) λ_{ext} 254 nm (Δε 9.14), 289 (−8.74); ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS (matrix: glycerol) *m/z* 836, 838, 840, 842, and 844 (rel. int. 1:4:6:4:1) $(M-Na)^-$. Anal. Calcd for $C_{22}H_{22}Br_4N_3O_7SNa$: C, 30.61; H, 2.57; N, 4.87%. Found: C, 30.60; H, 2.53; N, 4.87%.

Degradation of ianthesine A (1)

A solution of **1** (10.7 mg) in 48% hydrobromic acid (1 ml) was heated under reflux for 24 h. The resulting solution was evaporated, and the residue was purified by reversed-phase HPLC [Develosil ODS-HG-5 (10 i.d.×250 mm), 33% aq. MeOH, flow rate 2.5 ml/min, detected at 210 nm] to afford 3,5-dibromo-*N*,*N*-dimethyltyrosine (3.9 mg, t_R=10 min) as a white powder: ^1H NMR (400 MHz, CD_3OD) δ 2.66 (s, 6H), 2.93 (m, 1H), 3.05 (m, 1H), 3.49 (m, 1H), and 7.41 (s, 2H). A solution of 3,5-dibromo-*N*,*N*-dimethyltyrosine (3.9 mg) in MeOH–H_2O (1:2) (1.0 ml) was stirred with 10% Pd–C (1.7 mg) under a hydrogen atmosphere at room temperature for 1.5 h. After filtration, the filtrate was evaporated to give a crude product, which was purified by reversed-phase HPLC [Develosil ODS-HG-5 (10 i.d.×250 mm), 33% aq. MeOH, flow rate 2.5 ml/min, detected at 210 nm] to afford *N*,*N*-dimethyltyrosine (0.9 mg, t_R=5.7 min) as a white powder: $[\alpha]_D^{23}=-73$ (*c* 0.075, water); IR (KBr) ν_{max} 3700–2500 (br), 1615, 1595, 1515, and 1250 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 2.79 (s, 6H), 3.09 (dd, *J*=6.9, 14.7 Hz, 1H), 3.22 (dd, *J*=6.7, 14.7 Hz, 1H), 3.74 (dd, *J*=6.7, 6.9 Hz, 1H), 6.73 (d, *J*=8.5 Hz, 2H), and 7.16 (d, *J*=8.5 Hz, 2H); HRMS (FAB) calcd for $C_{11}H_{16}NO_3$ (M+H) *m/z* 210.1130, found 210.1146.

The absolute stereochemistry was determined to be D by chiral HPLC analysis [CHIRALPAK WH (4.6 i.d.×250 mm, Daicel Chemical), column temp. 50°C, 0.25 mM $CuSO_4$, flow rate 1.0 ml/min, detected at 230 nm], in which the retention times of the authentic samples of *N*,*N*-dimethyl-D and L-tyrosines[19] were 13.3 and 25.4 min, respectively.

Degradation of ianthesine B (2)

A solution of **2** (10.3 mg) in 48% hydrobromic acid (1 ml) was heated under reflux for 24 h. The resulting solution was

evaporated, and the residue was purified by reversed-phase HPLC under the same conditions as those for **1** to afford 3,5-dibromotyrosine (2.8 mg, t_R=12.0 min) together with 3-bromotyrosine (1.1 mg, t_R=8.0 min) as white powders. 3,5-Dibromotyrosine: ^{1}H NMR (400 MHz, CD_3OD) δ 2.90 (dd, J=8.3, 14.6 Hz, 1H), 3.16 (dd, J=4.4, 14.6 Hz, 1H), 3.70 (dd, J=4.4, 8.3 Hz, 1H), and 7.43 (s, 2H); 3-bromotyrosine: ^{1}H NMR (400 MHz, CD_3OD) δ 2.89 (dd, J=8.4, 14.7 Hz, 1H), 3.17 (dd, J=4.4, 14.7 Hz, 1H), 3.69 (dd, J=4.4, 8.4 Hz, 1H), 6.86 (d, J=8.3 Hz, 1H), 7.10 (dd, J=2.1, 8.3 Hz, 1H), and 7.42 (d, J=2.1 Hz, 1H).

The two products were combined and dissolved in MeOH–H_2O (1:2) (1.0 ml). The solution was stirred with 10% Pd–C (0.4 mg) under a hydrogen atmosphere at room temperature for 1.5 h. After filtration, the filtrate was evaporated to give tyrosine (4.4 mg) as a white powder: $[\alpha]_D^{23}=-9.0$ (c 0.057, 1N HCl); ^{1}H NMR (400 MHz, CD_3OD) δ 3.05 (dd, J=7.8, 14.7 Hz, 1H), 3.22 (dd, J=5.2, 14.7 Hz, 1H), 4.17 (dd, J=5.2, 7.8 Hz, 1H), 6.78 (d, J=8.5 Hz, 2H), and 7.11 (d, J=8.5 Hz, 2H); FABMS m/z 182 $(M+H)^+$.

The chiral HPLC analysis of the ianthesine B-derived tyrosine was performed using the same conditions as those for *N*,*N*-dimethyltyrosine, showing two peaks at the retention times of 15.5 and 38.9 min in the ratio of ca. 3:7 corresponding to the D and L forms, respectively.

The chiral HPLC analysis of **2** itself [CHIRALPAK WH (4.6 i.d.×250 mm), column temp. 50°C, CH_3CN–0.25 mM $CuSO_4$ (1:9), flow rate 1.5 ml/min, detected at 220 nm] was also carried out, indicating two peaks at the retention times of 16.8 and 32.8 min in the ratio of 3:7.

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