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Iantherans A and B, Unique Dimeric Polybrominated Benzofurans as Na,K-ATPase Inhibitors from a Marine Sponge, *Ianthella* sp.

Yoshihiro Okamoto,[a,†] Makoto Ojika,[a,*] Shingo Suzuki,[b] Masahiro Murakami[b] and Youji Sakagami[a]

[a]*Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa-ku, Nagoya 464-8601, Japan*
[b]*Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan*

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Abstract—Two novel tetrabrominated benzofuran derivatives, named iantherans A and B, were isolated from an Australian marine sponge of the genus *Ianthella*. The unique structures comprised of 2,3-bis(sulfooxy)-1,3-butadiene and two brominated benzofuran moieties were determined by spectroscopic and chemical methods. Iantheran A has a (*Z,Z*)-1,3-butadiene moiety, whereas iantheran B is the geometric isomer possessing a (*Z,E*)-1,3-butadiene moiety. The inhibitory activities of the iantherans and their derivatives against Na,K-ATPase as well as the efficacy of iantheran A against other several enzymes were evaluated.

Introduction

The sodium–potassium-activated adenosine triphosphatase (Na,K-ATPase) is a ubiquitous sodium pump that is expressed in the membrane of most eukaryotic cells.[1] The pump is the only known receptor for the cardiac glycosides, e.g. *Digitalis* toxins such as digoxin, used to treat congestive heart failure and cardiac arrhythmias. Even though the cardiac glycosides are widely used in the treatment of such heart diseases, the therapeutic index is low. From the need for relatively safe agents for the successful treatment of the failing heart, researchers have made their efforts to find novel inhibitors of the sodium pump.[1,2] In our search program for Na,K-ATPase inhibitors of marine origin, we recently isolated a quite unique tetrabrominated benzofuran metabolite, iantheran A (**1**),[3] and four novel dibromotyrosine derivatives, ianthesines,[4] from an Australian marine sponge of the genus *Ianthella* as active components. The sponges of the genus *Ianthella* (order Verongida, family Ianthellidae) and the others belong to the Verongida order and are well known to produce several bromotyrosine-derived metabolites, such as the ianthesines,[4] purealin,[5] and others,[6] whereas **1** is a quite novel type of polybrominated metabolite. A further investigation of an active fraction of the sponge extract resulted in the isolation of a geometric isomer of **1**, iantheran B (**2**), which also showed a similar Na,K-ATPase inhibitory activity. This paper describes the structure elucidation of **2** as well as the full details of the isolation and biological evaluation of **1** and **2** (Fig. 1).

Results and Discussion

The EtOAc soluble fraction[4] obtained from the sponge was subjected to bioassay-guided fractionation using column chromatography on silica gel ($CHCl_3$–MeOH–H_2O system) to give iantheran A (**1**) (0.12% of wet sponge) and iantheran B (**2**) (0.01%).

The major component, iantheran A (**1**), showed the characteristic isotope peaks in the negative FABMS spectrum at *m/z* 995, 997, 999, 1001, and 1003 $(M-Na)^-$ in the ratio of ca. 1:4:6:4:1 due to four bromine atoms in the molecule. The molecular formula of **1** was determined to be $C_{32}H_{16}Br_4Na_2O_{12}S_2$ based on the high-resolution FABMS (Fig. 2) and elemental analysis. The structural elucidation was performed by standard spectroscopic analysis and chemical methods as described in our previous paper.[3]

*Corresponding author. Tel.: +81-52-789-4117; fax: +81-52-789-4118; e-mail: ojika@agr.nagoya-u.ac.jp
†Present address: Agroscience Research Laboratories, Sankyo Co., Ltd., Yasu-cho, Yasu-gun, Shiga 520-2342, Japan.

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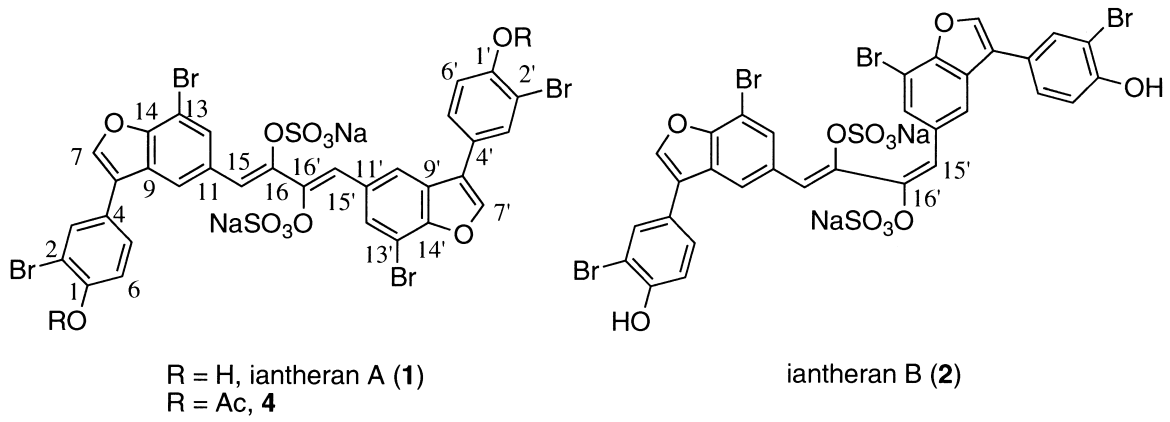

Figure 1. Structures of iantherans A (**1**), B (**2**), and iantheran A diacetate (**4**).

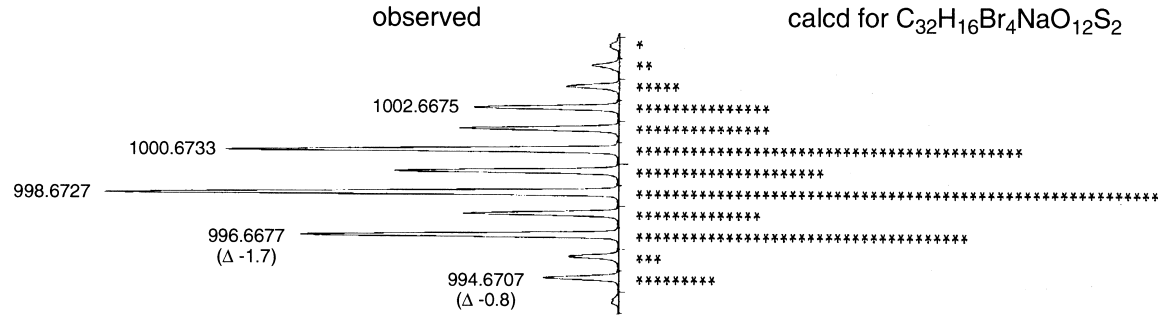

Figure 2. A high-resolution FABMS spectrum of **1** around the $(M-Na)^-$ ion peak (left) and a simulated graph (right).

The minor component, iantheran B (**2**), also showed the characteristic FABMS ion peaks at *m/z* 995, 997, 999, 1001, and 1003 $(M-Na)^-$ in the ratio of ca. 1:4:6:4:1. The fragment peaks at *m/z* 893, 895, 897, 899, and 901 $(M-Na-SO_3Na+H)^-$, and 813, 815, 817, 819, and 821 $(M-2SO_3Na+H)^-$ suggest the presence of two sulfate groups. The molecular formula of **2** was determined to be $C_{32}H_{16}Br_4Na_2O_{12}S_2$ based on the high-resolution FABMS [*m/z* 994.6692 $(M-Na)^-$, Δ −2.2 mmu]. These MS data and the molecular formula precisely agree with those for iantheran A (**1**). The ^1H and ^{13}C NMR data (Table 1) for **2** are similar to those for **1** and most of the signals appear as doublets, suggesting that **2** is a stereoisomer of **1** but, unlike **1**, not symmetrical. Thus, the stereochemistry of the 1,3-butadiene moiety of **2** should be 15*Z*,15′*E*. The NOESY correlations (Fig. 3) between H-15 and H-10′, 12′ support the 15′*E* geometry and enable us to assign all the NMR signals to the left (position 1-16) and right (position 1′-16′) parts. Although the assignment of the chemical shifts for C-11/11′ and C-16/16′ in Table 1 is tentative, we believe this assignment because of the higher field shifts of most of the right half signals due to the anisotropic effect. The 15*Z*,15′*E* geometry was further confirmed on the basis of the C–H long-range coupling constants ($^{2,3}J_{CH}$). In gated decoupling experiments, the signal due to C-16 and 16′ (δ 145.6) in **1** is observed as a triplet with a small J_{CH} (3.6 Hz), indicating $^2J_{CH}=^3J_{CH}=3.6$ Hz (Fig. 4(a)). Since the *cis*-vicinal $^3J_{CH}$ values (3–9 Hz) of the substituted olefins are usually smaller than the corresponding *trans*-vicinal $^3J_{CH}$ (8–16 Hz),[7] the geometry of the butadiene moiety of **1** was determined to be *Z,Z*. On the other hand, the signals due to C-16 (δ 146.4) and C-16′ (δ 143.1) in **2** are both observed as multiplets of dd with $J_{CH}=3.5$, 8.0 Hz and $J_{CH}=4.1$, 8.5 Hz, respectively. These coupling constants were assigned as shown in Figure 4(b) based on the splitting width of the following correlation peaks in the HMBC spectra of **2**: the cross peaks of H-15′/C-16 and H-15′/C-16′ are both split with the J_{C-H} value of ca. 8 Hz, whereas the cross peaks of H-15/C-16 and H-15/C-16′ are not split (small *J* values). Thus, the large $^3J_{C16-H15'}$ value of 8.0 Hz and the small $^3J_{C16'-H15}$ value of 4.1 Hz imply the *trans*-vicinal and *cis*-vicinal C–H orientations, respectively, namely, the 15*Z*,15′*E* geometry. The unusually large geminal $^2J_{C16'-H15'}$ value of 8.5 Hz in **2** could be explained by the *cis* relationship between H-15′ and the oxygen substituent at C-16′.[8,9] The fact that the mild hydrolysis of **1** and **2** in wet dioxane[10] provided the common α-hydroxy enone **3** (Fig. 5) supports the structural relationship between **1** and **2**, that is, geometric isomers of each other about the butadiene moiety. The structure of **3** was mainly elucidated by the NMR spectroscopic analysis. When the NMR spectra of **3** were measured in methanol-d_4,[3] we observed that the signals of H-15′ and C-15′ gradually disappeared due to the deuterium exchange. In the present study, the NMR spectra measured in acetone-d_6 clearly demonstrated these signals (Table 1) and the HMBC correlations (Experimental).

The metabolites containing the 2,3-bis(sulfooxy)-1,3-butadiene moieties such as the iantherans are quite rare, and a marine sponge metabolite, aplysillin A,[11] is the only example besides the iantherans. The dose–response

Table 1. NMR data for **2** and **3** (100 MHz for ^{13}C and 400 MHz for ^{1}H)[a]

Position	**2** (Methanol-d_4)		**3** (Acetone-d_6)	
	^{13}C	^{1}H	^{13}C	^{1}H
1	154.9 s		154.8 s	
2	111.2 s		110.9 s	
3	132.6 d	7.66 d (2.1)	132.7 d	7.84 d (2.1)
4	125.3 s		124.9 s	
5	129.1 d	7.52 dd (8.4, 2.1)	128.7 d	7.56 dd (8.3, 2.1)
6	117.7 d	6.95 d (8.4)	117.7 d	7.17 d (8.3)
7	143.3 d	7.93 s	143.9 d	8.20 s
8	123.1 s		122.5 s	
9	128.6 s		128.5 s	
10	122.1 d	8.10 d (1.1)	122.4 d	8.40 d (1.1)
11	133.2 s[b]		132.9 s	
12	130.3 d	7.98 d (1.1)	130.2 d	8.29 d (1.1)
13	104.9 s		104.7 s	
14	153.5 s		152.9 s	
15	123.5 d	6.57 s	113.6 d	7.15 s
16	146.4 s[c]		147.9 s	
1′	154.8 s		154.7 s	
2′	111.2 s		110.9 s	
3′	132.5 d	7.62 d (2.1)	132.5 d	7.82 d (2.1)
4′	125.2 s		124.8 s	
5′	128.9 d	7.38 dd (8.4, 2.1)	128.7 d	7.55 dd (8.3, 2.1)
6′	117.6 d	6.70 d (8.4)	117.7 d	7.13 d (8.3)
7′	143.2 d	7.89 s	143.7 d	8.18 s
8′	123.1 s		122.2 s	
9′	128.5 s		128.5 s	
10′	121.4 d	8.13 d (1.1)	121.6 d	7.89 s
11′	132.5 s[b]		132.7 s	
12′	130.3 d	7.79 d (1.1)	129.9 d	7.59 s
13′	104.5 s		104.4 s	
14′	153.2 s		152.5 s	
15′	121.0 d	6.87 s	41.8 t	4.47 s
16′	143.1 s[c]		195.5 s	

[a]Data for **1** are reported in ref 3. Coupling constants (Hz) are in parentheses.
[b,c]Tentative assignments within the same superscripts.

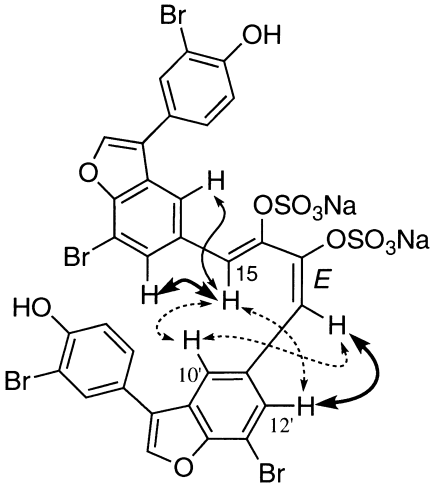

Figure 3. Partial NOESY correlations for **2**. The bold, solid, and dashed lines represent strong, medium, and weak NOEs, respectively.

curves for **1**–**3** against a Na,K-ATPase are shown in Figure 6. The IC_{50} values for **1**, **2** and **3** are 4, 7, and 7 μM, respectively. Iantheran A diacetate (**4**) shows an IC_{50} of 10 μM (dose–response curve not shown). A cardiac steroid glycoside, ouabain, inhibited the enzyme at an IC_{50} of 0.2 μM under the same conditions. Unex-

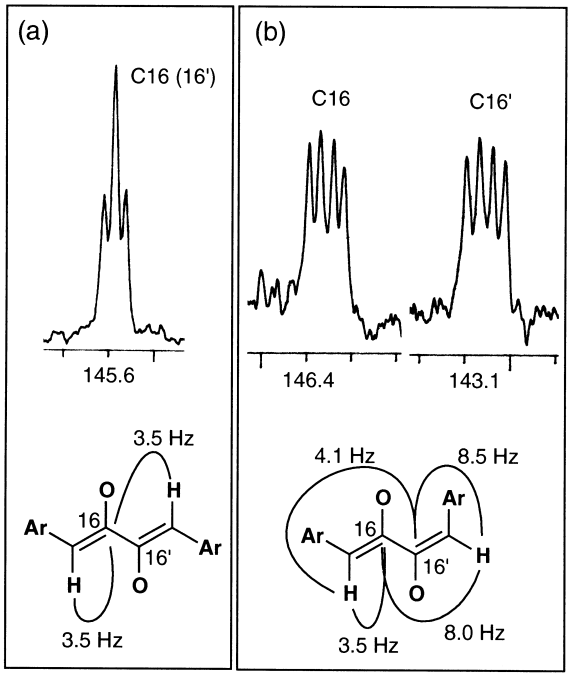

Figure 4. Partial gated decoupling spectra and J_{C-H} values of **1** (a) and **2** (b).

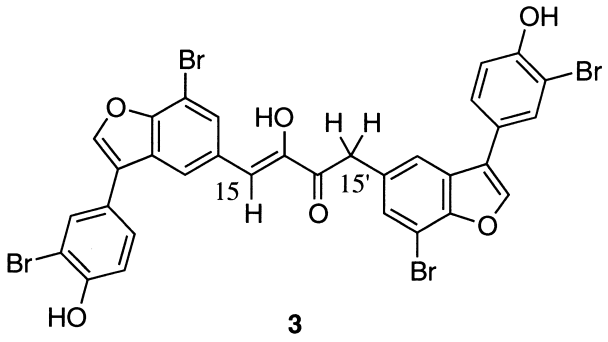

Figure 5. Structure of hydrolyzed product **3** derived from **1** and **2**.

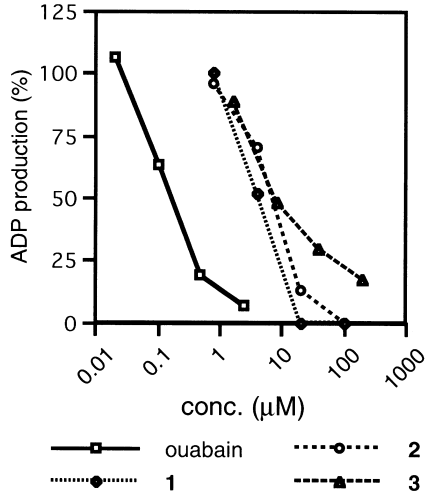

Figure 6. Na,K-ATPase inhibition by **1**–**3**. IC_{50}s for **1**, **2**, **3**, and ouabain are 4, 7, 7, and 0.2 μM, respectively.

Table 2. Inhibition of enzymes by iantherans A (**1**) and B (**2**), and a derivative **3**

Enzyme	IC_{50} (μM)		
	1	**2**	**3**
Na,K-ATPase	4	7	7
Farnesyltransferase	10	10	>100
DNA topoisomerase I[12]	3	3	20
Plasmin	0.45	nd[a]	nd
Elastase	17.5	nd	nd
Acetylcholinesterase	0.42	nd	nd
α-Glucosidase	0.37	nd	nd
Others[b]	>100	nd	nd

[a]nd: not determined.
[b]Papain, trypsin, chymotrypsin, thrombin, carboxypeptidase A, and tyrosinase were tested.

pectedly, the hydrolyzed product **3** and diacetate **4** retain the inhibitory activity, suggesting that the sulfate and phenol groups are not very important for the inhibitory activity. The enzyme inhibitory activity appears to be non-specific, because **1** (**2** and **3**) also inhibits six of the 12 other enzymes tested as shown in Table 2 (experimental details not shown). The structural units responsible for the enzyme inhibitions and the common feature of the enzymes that the iantherans inhibit are unclear.

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. NMR spectra were recorded on a Brucker-ARX400 (400 MHz). NMR chemical shifts were referenced to the solvent peak of δ_H 3.30 (residual CHD_2OD), 2.04 (residual CHD_2COCD_3), δ_C 49 for CD_3OD or 29.8 ppm for CD_3COCD_3. Mass spectra were recorded on a JMS DX-705L (FABMS) or an Mstation JMS-700 (for high-resolution FABMS) mass spectrometer using thioglycerol as the matrix. A mixture of polyethylene glycols 600 and 1000 was used as the standard for the high-resolution MS. The method for the Na,K-ATPase inhibition assay is described in our previous paper.[4]

Isolation

The collection and extraction of the sponge *Ianthella* sp. (1.8 kg wet weight) are described in our previous paper.[4] A portion (4.0 g) of the EtOAc extract (total 14.4 g) was chromatographed on silica gel (200 g) eluted with $CHCl_3$:MeOH:H_2O (7:3:1 lower phase, 65:35:10 lower phase, and then 6:4:1) to give 7 fractions. The fifth fraction (616 mg), obtained as a pale yellow powder, showed the single spot of **1** at an R_f value of 0.37 on a silica gel TLC developed with $CHCl_3$:MeOH:H_2O (65:35:10 lower phase). An analytical grade sample of **1** was obtained by recrystallization from aqueous MeOH and showed a single peak at a retention time of 20 min in 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]. The sixth fraction (121 mg), which contained **2** (R_f=0.22 under the same conditions), was chromatographed on silica gel (13 g) with $CHCl_3$:MeOH:H_2O (65:35:10 lower phase) to give **2** (50 mg) as a pale yellow powder.

Iantheran A (1). Pale yellow fine crystals; mp 172–173 °C (dec.) (MeOH–H_2O); IR (KBr) ν_{max} 3425 (br), 1635, 1495, 1450, 1415, 1240, 1095, 1060, and 1020 cm^{-1}; UV (MeOH) λ_{max} 220 (ε 43100), 244 (32500), 287 (27400), 325 (36700), 337 (39700), and 352 nm (sh) (27000); ^1H and ^{13}C NMR, ref 3; FABMS *m/z* 995, 997, 999, 1001, and 1003 $(M-Na)^-$ (1:4:6:4:1), 893, 895, 897, 899, and 901 $(M–SO_3Na–Na+H)^-$ (1:4:6:4:1), and 813, 815, 817, 819, and 821 $(M–2SO_3Na+H)^-$ (1:4:6:4:1); HRMS (FAB), Figure 2. Anal. calcd for $C_{32}H_{16}Br_4Na_2O_{12}S_2$: C, 37.60; H, 1.58; N, 0.00%. Found: C, 37.45; H, 1.87; N, 0.13%.

Iantheran B (2). Pale yellow fine crystals; mp 155–156 °C (dec.) (MeOH–H_2O); IR (KBr) ν_{max} 3440 (br), 1665, 1495, 1455, 1405, 1245, 1095, 1050, and 1015 cm^{-1}; UV (MeOH) λ_{max} 215 (ε 62500), 242 (44900), 279 (sh, 28900), 321 (25300), 331 (sh, 25000), and 355 nm (sh, 12900); ^1H and ^{13}C NMR, Table 1; HMBC correlations: H-3/C-1, 2, 8, 11; H-5/C-1, 3, 11; H-6/C-1, 2, 4; H-7/C-4, 8, 9, 14; H-10/C-8, 12, 13, 14, 15; H-12/C-10, 13, 14, 15; H-15/C-10, 12, 16, 16′; H-3′/C-1′, 2′, 8′, 11′; H-5′/C-1′, 3′, 11′; H-6′/C-1′, 2′, 4′; H-7′/C-4′, 8′, 9′, 14′; H-10′/C-8′, 12′, 13′, 14′, 15′; H-12′/C-10′, 13′, 14′, 15′; H-15′/C-16, 10′, 12′, 16′; FABMS *m/z* 995, 997, 999, 1001, and 1003 $(M-Na)^-$ (1:4:6:4:1), 893, 895, 897, 899, and 901 $(M–SO_3Na–Na+H)^-$ (1:4:6:4:1), and 813, 815, 817, 819, and 821 $(M–2SO_3Na+H)^-$ (1:4:6:4:1); HRMS (FAB) calcd for $C_{32}H_{16}{}^{79}Br_4NaO_{12}S_2$ $(M-Na)$ *m/z* 994.6714, found 994.6692.

α-Hydroxy enone 3. A solution of **1** (52 mg) in dioxane (not dried, 10 mL) was stirred at room temperature for 3 h. The mixture was diluted with EtOAc (40 mL) and filtered. The filtrate was concentrated then redissolved in water (10 mL), and the resulting aqueous solution was extracted three times with ether (10 mL). The combined extracts were concentrated and subjected to reversed-phase HPLC [Develosil ODS-HG-5 (10 i.d.×250 mm), MeOH:H_2O (95:5), flow rate 2.5 mL/min, detected at 254 nm] to afford **3** [15.3 mg, t_R=12.5 min; R_f=0.51 on TLC developed with hexane:EtOAc (1:1)] as a pale yellow solid: IR (KBr) ν_{max} 3410 (br), 1700, 1665, 1630, 1585, 1495, 1290, 1275, 1240, 1180, 1095, and 1040 cm^{-1}; UV (MeOH) λ_{max} 215 (ε 69800), 244 (sh, 43200), 280 (sh, 27300), 291 (sh, 25200), and 319 nm (24400); ^1H and ^{13}C NMR, Table 1; HMBC correlations (J=8 Hz): H-3/C-1, 2, 5, 8; H-5/C-1, 3, 8; H-6/C-1, 2, 4; H-7/C-8, 9, 14; H-10/C-8, 12, 14, 15; H-12/C-10, 13, 14, 15; H-15/C-10, 12, 16, 16′; H-3′/C-1′, 2′, 5′, 8′; H-5′/C-1′, 3′, 8′; H-6′/C-1′, 2′, 4′; H-7′/C-8′, 9′, 14′; H-10′/C-8′, 12′, 14′, 15′; H-12′/C-10′, 13′, 14′, 15′; H-15′/C-10′, 12′, 16, 16′; FABMS *m/z* 813, 815, 817, 819, and 821 (1:4:6:4:1) $(M-H)^-$; HRMS (FAB) calcd for

$C_{32}H_{17}{}^{79}Br_4O_6$ (M−H) m/z 812.7759, found 812.7745.

Diacetate 4. The treatment of **1** (25 mg) with acetic anhydride (2 mL) and pyridine (2 mL) followed by reversed-phase HPLC [Develosil ODS-HG-5 (10 i.d. ×250 mm), 64% MeOH, flow rate 2.5 mL/min] afforded the diacetate **4** (11.6 mg) as pale yellow fine crystals: mp 152–153 °C (dec.) (MeOH–H_2O); IR (KBr) ν_{max} 3430 (br), 1760, 1625, 1490, 1245, 1210, 1095, 1055, and 1020 cm^{-1}; UV (MeOH) λ_{max} 210 (ϵ 60900), 259 (31200), 274 (27200), 326 (35500), 338 (37900), and 354 nm (24900); ^1H NMR (400 MHz, methanol-d_4) δ 8.48 (s, 2 H, H-10), 8.12 (s, 2 H, H-7), 8.01 (d, J = 2.0 Hz, 2 H, H-3), 8.01 (s, 2 H, H-12), 7.95 (dd, J = 8.3, 2.0 Hz, 2 H, H-5), 7.34 (d, J = 8.3 Hz, 2 H, H-6), 7.08 (s, 2 H, H-15), 2.35 (s, 6 H, Ac); ^{13}C NMR, ref 3; FABMS m/z 1079, 1081, 1083, 1085, and 1087 (rel. int. 1:4:6:4:1) $(M–Na)^-$; HRMS (FAB) calcd for $C_{36}H_{20}{}^{79}Br_4NaO_{14}S_2$ (M–Na) m/z 1078.6926, found 1078.6913.

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