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Iantheran A, a Dimeric Polybrominated Benzofuran as a Na,K-ATPase Inhibitor from a Marine Sponge, *Ianthella* sp.

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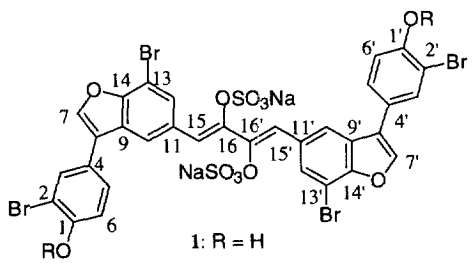
Abstract

A novel dimeric polybrominated benzofuran, iantheran A (**1**), was isolated from an Australian marine sponge, *Ianthella* sp., as a Na,K-ATPase inhibitor. The unique structure of **1** was determined on the basis of chemical and spectroscopic evidence.

Keywords: benzofuran; enzyme inhibitors; marine metabolites

Na,K-ATPase is a ubiquitous sodium pump in the membrane of most eukaryotic cells. Since the pump is the only known receptor for the toxic cardiac glycosides used to treat some heart diseases, new types of natural regulators of this pump might be useful for clinical purposes [1]. In our search for bioactive marine natural products [2-4], we have found that the organic extract of an Australian sponge of the genus *Ianthella* showed potent inhibitory activity against Na,K-ATPase. This paper communicates the isolation of a novel dimeric polybrominated benzofuran, named iantheran A (**1**), from the sponge and its structure elucidation by spectroscopic analysis including chemical derivatizations.



1: R = H

2: R = Ac

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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 soluble portion, which inhibited a dog kidney Na,K-ATPase with an IC_{50} of 10 μg/ml, was subjected to bioassay-guided fractionation using a silica gel column ($CHCl_3$/MeOH/H_2O system, step gradient) twice, followed by reversed-phase HPLC (MeOH/H_2O 3:2) to give iantheran A (**1**) (0.23% yield from wet sponge) as pale yellow fine crystals.

Iantheran A (**1**), mp 172–173 °C (dec.), showed the characteristic ion peaks in the ratio of about 1:4:6:4:1 at *m/z* 995, 997, 999, 1001, and 1003 $(M–Na)^-$, 893, 895, 897, 899, and 901 $(M\text{-}SO_3Na–Na+H)^-$, and 813, 815, 817, 819, and 821 $(M–2SO_3Na+H)^-$, in the negative FABMS spectrum, indicating the presence of four bromine atoms and two sulfate groups in the molecule. The molecular formula of **1** was determined to be $C_{32}H_{16}Br_4Na_2O_{12}S_2$ by high-resolution negative FABMS [*m/z* 994.6707 $(M–Na)^-$, Δ –0.8 mmu] and elemental analysis [Calcd. %: C, 37.60; H, 1.58; N, 0.00. Found : C, 37.45; H, 1.87; N, 0.13.], implying 26 degrees of unsaturation. The presence of the sulfate groups was supported by the intense IR bands at 1240 and 1060 cm^{-1} (KBr). All the ^1H and ^{13}C NMR signals appeared in the olefinic and aromatic region, and the signals of the protonated carbons were assigned as shown in Table 1 by a ^1H-^{13}C COSY experiment. Since only 16 carbon and seven proton signals were observed, **1** must be a symmetrical molecule. The proton spin-spin coupling data for **1** revealed only the presence of a 1,2,4-trisubstituted benzene moiety (ortho-coupling between H5 and H6, and meta-coupling between H3 and H5) and a 1,2,3,5-tetrasubstituted benzene moiety (meta-coupling between H10 and H12). The remaining proton signals were two singlets. Since there were numerous quaternary carbons, extensive

Table 1
NMR Data for **1** and its derivatives **2** and **3**[a]

Position	1		2	3
	^{13}C	^1H[b]	^{13}C	^{13}C
1, 1'	155.1 s		149.2 s	155.2 s
2, 2'	111.4 s		117.7 s	111.4 s
3, 3'	132.8 d	7.79 d (2.1)	132.8 d	132.9 d
4, 4'	125.5 s		132.5 s	125.3 s, 125.2 s
5, 5'	129.3 d	7.72 dd (8.3, 2.1)	129.3 d	128.9 d
6, 6'	117.8 d	7.06 d (8.3)	125.7 d	117.7 d
7, 7'	143.3 d	7.98 s	144.5 d	143.8 d, 143.7 d
8, 8'	123.4 s		122.8 s	123.1 s, 122.8 s
9, 9'	128.6 s		128.2 s	128.8 s
10, 10'	122.4 d	8.39 br d (1.0)	122.2 d	122.6 d, 121.7 d
11, 11'	133.5 s		133.9 s	133.2 s, 133.0 s
12, 12'	131.0 d	8.03 br d (1.0)	131.4 d	130.7 d, 130.3 d
13, 13'	104.6 s		104.6 s	105.1 s, 105.0 s
14, 14'	153.3 s		153.4 s	153.5 s, 153.2 s
15, 15'	119.3 d	7.05 s	119.3 d	114.6 d, 42.5 t
16, 16'	145.6 s		145.9 s	148.7 s, 196.6 s
1,1'-OCOMe			170.1 s / 20.6 q	

[a] Spectra were recorded at 400 MHz for ^1H and at 100 MHz for ^{13}C in methanol-d_4. Chemical shifts are in δ values.
[b] Coupling constants (Hz) are in parentheses.

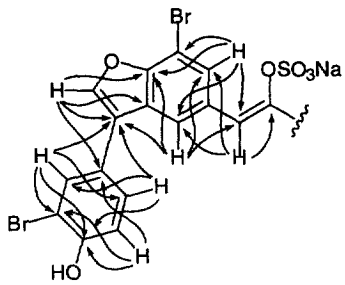


Figure 1
HMBC correlations of **1**

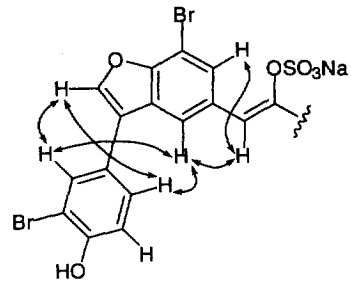
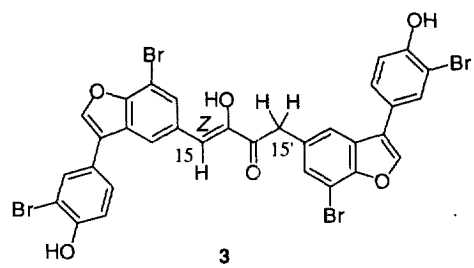


Figure 2
ROESY correlations of **1**

HMBC experiments were required for the determination of the carbon connectivities. The H-C correlations obtained by the HMBC spectra are summarized in Figure 1.

The position of the oxygen substituents was determined to be at C1, C7, C14, and C16 by the relatively low-field shifts of these carbons. The hydroxyl group was inferred to be substituted at C1 in comparison with the chemical shifts calculated using the substituent increments of polysubstituted benzenes [5]. This was confirmed by comparison of the chemical shifts between **1** and diacetate **2**, which was obtained by the treatment with acetic anhydride-pyridine; an upfield shift at C1 and downfield shifts at C2, C4, and C6 carbons were observed (Table 1). The relatively high-field shifts at C2 and C13 indicate the location of two bromine atoms on these carbons. The HMBC correlation of H7/C14 shows that two oxygen-bearing carbons, C7 and C14, are connected via ether bonds, confirming the presence of the benzofuran moiety. The remaining oxygen functionality, the sulfate group, should consequently be located at C-16. The position of the sulfate group was confirmed as follows. Thus, hydrolysis of **1** in dioxane containing trace water at room temperature [6] provided α-hydroxy enone **3**, $C_{32}H_{18}Br_4O_6$ (high-resolution FABMS: *m/z* 812.7794 (M-H), Δ +3.5 mmu), in which the NMR signals corresponding to the enol sulfate group (C15, C16, H15) disappeared and a ketone (C16', δ_C 196.6) and a methylene (C15', δ_C 42.5 and δ_H 4.27) signals were observed (Table 1). The geometry of the double bond at C15 of **3** was shown to be *Z* from an NOE correlation between H15 and H15'. The ROESY correlations summarized in Figure 2, though providing no information on the geometry of the butadiene moiety, were consistent with the gross structure of **1** obtained from the above findings.



3

Since the structure of iantheran A (**1**) is symmetrically dimeric, the stereochemistry of the 1,3-butadiene moiety must be either *Z,Z* or *E,E*. In a gated decoupling experiment of **1**, the signal assigned to C16 showed a triplet (J_{CH} = 3.6 Hz), indicating that the long-range ^{13}C-^{1}H coupling constants for C16/H15 ($^{2}J_{CH}$) and C16/H15' ($^{3}J_{CH}$) were both 3.6 Hz. Since it was reported that *cis*-vicinal $^{3}J_{CH}$ values (3-9 Hz) of substituted olefins were smaller than *trans*-vicinal $^{3}J_{CH}$ values (8-16 Hz) [7], the geometry of the butadiene moiety must be *Z,Z*. A definite fine structure at 300 to 360 nm [three maxima at 325 (ε 36700), 337 (39700), and 352 (27000) nm] in the UV spectrum of **1** supports the (*Z,Z*)-1,4-diarylbutadiene structure [8]. Thus, the structure of iantheran A was elucidated as shown in the formula **1**.

Remarkable structural features of **1** are the benzofuran and 2,3-dihydroxy-1,3-butadiene disulfate moieties. To our knowledge, benzofuran-type metabolites such as **1** are quite rare in marine natural products, e.g., furoventalene [9], and the unique butadiene moiety is only known in the marine sponge metabolite, aplysillin A [10].

Iantheran A (**1**) and its derivatives **2** and **3** showed moderate inhibitory activities against Na,K-ATPase with IC_{50}s of 2.5, 5.0, and 10 μM, respectively,[1] whereas a typical cardiac glycoside, ouabain, inhibited the enzyme at an IC_{50} of 0.1 μM under the same conditions.

Acknowledgements

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[1] In a typical assay, the reaction mixture consisted of 23 mM Tris-HCl (pH 7.4), 25 mM mannitol, 70 mM NaCl, 7 mM KCl, 3.5 mM $MgCl_2$, sample solution dissolved in 10% DMSO, Na,K-ATPase (from dog kidney, Sigma), and 1 mM ATP. The mixture was incubated at 37 °C for 90 min, and analyzed by reversed-phase HPLC (50 mM sodium phosphate buffer, pH 7.0) to quantify ADP and ATP.