Aplidioxins A and B, Two New Dibenzo-*p*-dioxins from the Ascidian *Aplidiopsis ocellata*

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Two new substituted dibenzo-*p*-dioxins, aplidioxins A (**1**) and B (**2**), were isolated from the ascidian *Aplidiopsis ocellata*. The structures of these compounds were elucidated by a combination of 1D and 2D NMR spectroscopy and an X-ray analysis of aplidioxin A.

Ascidians have yielded a wide variety of compounds including aromatic alkaloids, depsipeptides, and novel quinones.[1,2] Cytotoxicity and other types of bioactivity have been reported for many of these compounds. In the course of our continuing search for anticancer drugs from marine sources we have analyzed extracts of the ascidian *Aplidiopsis ocellata* Monniot and Monniot 1996 (Ascidiaceae), collected in the Philippines in 1996. Previous work on another species of this genus has yielded aplidiamine, a zwitterionic benzyl adenine.[3] We wish to report here the isolation of two new dizenzo-*p*-dioxins, aplidioxins A (**1**) and B (**2**), from *A. ocellata*. Dibenzo-*p*-dioxin is an uncommon skeleton in nature. It is present in the phlorotanins such as eckol isolated from the brown alga *Ecklonia kurome* Okumura.[4] Eckol has antiplasmin activity.[4] Two polybromodibenzo-*p*-dioxins have been isolated from cyanobacteria-containing sponges.[5]

The dibenzo-*p*-dioxin skeleton is probably best known due to the notorious environmental toxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin,[6] a byproduct in the synthesis of 2,4,5-trichlorophenoxyacetic acid. On the other hand, dibenzo[1,4]dioxin-carboxamides have been reported as DNA-intercalating agents and potential antitumor drugs.[7]

Dibenzo-*p*-dioxins **1** and **2** were isolated by conventional extraction and chromatographic procedures from *A. ocellata* collected in the Philippines in 1996. The ^{1}H NMR ($CDCl_3$) of compound **1** ($C_{15}H_{10}O_6$, HRFABMS) exhibited signals attributable to one exchangeable proton [δ 11.22 (s)], two aldehyde protons [δ 10.39 (s), 10.27 (s)], two separate sets of aromatic ortho-positioned protons, and one methoxy group [δ 3.86 (s)]. The presence of the former functionalities was supported by IR data [3400 (OH), 1683 (free CHO), 1662 cm^{-1} (hydrogen-bonded CHO)] and ^{13}C NMR CHO signals at δ 192.0 and 187.5 (HMQC data). The presence of 12 aromatic signals in the ^{13}C NMR spectrum considered in conjunction with 11 degrees of unsaturation prompted formulation of two benzene rings connected by two ether bridges to give a dibenzo-*p*-dioxin. The sequence of substitution in each ring was easily determined by HMQC and HMBC experiments, but the data could not distinguish between the possible structures **1** and **1′**. No NOE was observed between the aldehyde proton signals in a NOESY experiment, but this negative information was insufficient to resolve the regiochemical question. The natural product was reduced with $NaBH_4$ to give diol **3**, but no helpful NOE interactions were detected in the product. Because these results failed to establish the stereochemistry definitively, an X-ray diffraction analysis was carried out, and this confirmed **1** as the structure of aplidioxin A (Figure 1).

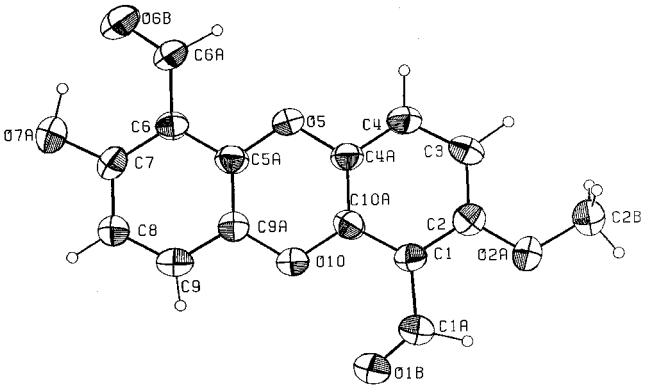
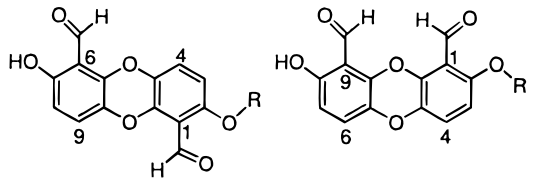
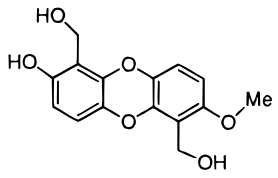


Figure 1. ORTEP drawing of **1**.



1 R = Me (Aplidioxin A) **1'**

2 R = H (Aplidioxin B)



3 Aplidioxin A diol

The calculated ^{1}H NMR data[11] for **1** were close to the observed: $\Delta\delta$ 0.23, 0.14 ppm for 4-H and 9-H, $\Delta\delta$ 0.01, 0.03 ppm for 3-H and 8-H, respectively. The calculated shifts for H-4 and H-6 of isomer **1′** were also close to those observed for H-4, H-9 of **1**, but the calculated shifts for H-3 and H-7 of **1′** differed significantly from the observed signals for H-3, H-8 of **1** ($\Delta\delta$ ca. 0.5 ppm).

Aplidioxin B (**2**) eluted from silica gel over a wide range of solvent polarity (10−100% MeOH in $CHCl_3$) possibly due to competition between intramolecular hydrogen bonding and hydrogen bonding to the adsorbent. Final purification of **2** was accomplished by reversed-phase HPLC (28% H_2O−

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MeOH). Pure **2** was sparingly soluble in a variety of NMR solvents ($CDCl_3$, MeOH-d_4, pyridine-d_5, TFA-*d*), whereas mixtures of **1** and **2** were readily soluble in $CDCl_3$.

The EIMS of **2** exhibited its highest mass ion at *m*/*z* 272, but the NMR spectrum showed signals for only four protons (OH, δ 11.28; aldehyde, δ 10.3; *o*-aromatic protons 6.53, 7.09) and seven carbons (one aldehyde and six aromatic carbons). Because the chemical shifts of **2** were nearly identical to those in the hydroxyaldehyde half of **1**, a symmetrical structure for **2** was indicated. This accounts for the molecular weight and the NMR data. Attempts to correlate **1** with **2** by cleavage of the methyl ether **1** with LiCl in DMF[12] or by monomethylation of **2** using NaH−MeI (in Et_2O or DMSO) failed. However, the regiochemistry shown in **2** appears highly likely by analogy to **1**. Also, because the proton NMR shifts of **2** closely parallel those of the hydroxy half of **1** and these match the calculated shift values for **1** as well, the regiochemistry of **2** appears supported by NMR shift data.

The structure of aplidioxin A was established by X-ray crystallography. This established the nearly symmetrically 1,2,6,7-substituted dibenzo-*p*-dioxin structure. The tricyclic ring system is planar, as found in the unsubstituted dibenzo-*p*-dioxin (DPDO) and several halogenated DPDO structures.[8,9] The rms deviation from the least-squares plane through the 14-ring atoms is 0.013 Å. The substituents lie very close to this plane, with the methoxy carbon showing the maximum deviation (0.1 Å). Some of the highly substituted DPDOs are nonplanar, with a dihedral angle between the two benzene rings as high as 30°.[10] The molecules form an intramolecular O7A−H···O6B hydrogen bond (2.660 Å).

Experimental Section

General Experimental Procedures. All solvents were redistilled. Chromatography was performed with Merck Si gel 60 (230−240 mesh) for vacuum flash chromatography and Si gel-coated rotors on a Chromatotron (model 7924, Harrison Research, Palo Alto, CA). IR spectra were obtained on a Bio-Rad 3240-SPC FT instrument. MS were measured with either Hewlett−Packard 5985B or VG ZAB E mass spectrometers. NMR experiments were conducted with a Varian VXR-500 instrument equipped with a 3-mm $^1H/^{13}C$ switchable gradient microprobe (MDG-500-3) and a pulsed field gradient driver; signals are reported in parts per million (ppm).

Animal Material. *Aplidiopsis ocellata* Monniot and Monniot 1996 was collected in 1996, near Davao in the Philippines, at −7 to −27 m, and frozen shortly after collection. A voucher specimen is maintained at the University of Oklahoma (6-PH-96).

Extraction and Isolation. Freshly thawed specimens of the ascidian (770 g wet wt, 19 g dry wt after extraction) were cut into small pieces and soaked in MeOH (2 × 750 mL) and then MeOH−CH_2Cl_2 (1:1, 2 × 750 mL). All extracts were combined, the solvents removed in vacuo, and the residue subjected to solvent partitioning as described previously[13] to afford hexane- (0.63 g), CH_2Cl_2- (0.58 g), and *n*-BuOH- (0.86 g) soluble fractions. The CH_2Cl_2 solubles were fractionated by vacuum flash chromatography over Si gel using increasing amounts of MeOH in $CHCl_3$ as eluent (16% MeOH to 100% MeOH in 10% increments) to yield four fractions. The first fraction contained a 1:1 mixture of aplidioxin A (**1**) and aplidioxin B (**2**), while the following three fractions consisted mainly of aplidioxin B (**2**). Rechromatography of the first fraction on Si gel using a Chromatotron ($CHCl_3$ as eluent) afforded pure aplidioxin A (**1**, 7.7 mg). Flash vacuum chromatography of the hexane extract on Si gel using increasing amounts of EtOAc in hexane afforded six fractions containing mixtures of aplidioxins A (**1**) and B (**2**). Pure aplidioxin B (**2**, 1−2 mg) was obtained by reversed-phase HPLC (28% H_2O−MeOH) of mixtures of **1** and **2**.

Aplidioxin A (1): yellow crystals from C_6H_6; mp 198 °C (subl.); IR (film) ν_{max} 3400 (br), 1683 (s), 1662 (s), 1265 (s), 828 (s), 812 (s), 895 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.22 (s, 7-OH), 10.39 (s, 1-CHO), 10.27 (s, 6-CHO), 7.12 (d, J = 8.8 Hz, 9-H), 7.05 (d, J = 8.8 Hz, 4-H), 6.49 (d, J = 8.8 Hz, 3-H), 6.48 (d, J = 8.8 Hz, 8-H), 3.86 (s, 2-O−CH_3); ^1H NMR (C_6D_6) δ 11.61, 10.52, 9.87, 6.56 (d, J = 8.5 Hz, H-9), 6.32 (d, J = 8.5 Hz, H-4) 6.16 (d, J = 8.5 Hz, H-8), 5.71 (d, J = 8.5 Hz, H-3); ^{13}C NMR ($CDCl_3$) δ 192.0 (d, 6-CHO), 187.5 (d, 1-CHO), 158.5 (s, C-7), 158.0 (s, C-2), 143.7 (s, C-10a), 143.5 (s, C-5a), 134.2 (s, C-4a), 132.4 (s, C-9a), 125.8 (d, C-9), 121.6 (d, C-4), 113.6 (s, C-1), 111.4 (d, C-8), 109.2 (s, C-6), 105.2 (d, C-3), 56.3 (q, 2-OMe); EIMS *m*/*z* (%) 286 (100) $[M]^+$, 272 (93), 140 (15); HRFABMS *m*/*z* 287.0559 $[M + H]^+$ (calcd for $C_{15}H_{11}O_6$ 287.0556)

Aplidioxin A Diol (3). Reaction of 3 mg of aplidioxin A (**1**) with $NaBH_4$ in 5 mL MeOH at 0 °C yielded the corresponding diol **3** after the usual workup: ^1H NMR (DMSO-d_6) δ 6.84 (d, J = 8.8 Hz), 6.58 (d, J = 8.8 Hz), 6.52 (d, J = 8.8 Hz), 6.05 (d, J = 8.8 Hz), 4.57 (s, 2H), 4.44 (s, 2H), 3.72 (s, 3H).

Aplidioxin B (2): yellow powder; ^1H NMR ($CDCl_3$) δ 11.28 (s, 2,7-OH), 10.31 (s, 1,6-CHO), 7.09 (d, J = 8.8 Hz, 4,9-H), 6.53 (d, J = 8.8 Hz, 3,8-H); ^{13}C ($CDCl_3$) δ 192.1 (d, −CHO), 158.8 (s, C-2,7), 143.8 (s, C-5a,10a), 132.2 (s, C-4a,9a), 125.3 (d, C-4,9), 111.6 (d, C-3,8), 109.4 (s, C-1,6); EIMS *m*/*z* (%) 272 (100); HREIMS *m*/*z* 272.0328 (calcd for $C_{14}H_8O_6$ 272.0321).

X-ray Crystallography. Aplidioxin A (**1**) was crystallized from a benzene solution by slow solvent evaporation. A prismatic crystal, 0.45 × 0.24 × 0.15 mm, was selected for all crystallographic measurements. Cell dimensions were obtained by least-squares fit to $\pm 2\theta$ values of 38 reflections measured at room temperature using Mo $K_{\alpha 1}$ radiation. All X-ray measurements were carried out on a Enraf−Nonius CAD-4 diffractometer.

Crystal Data for 1: $C_{15}H_{10}O_6$, MW = 286.4, triclinic, *P*1, a = 7.479(2), b = 11.629(2), c =7.036(1) Å, α = 88.76(2), β = 110.20(4), γ = 90.04(2)°, V = 611.0 (6) $Å^3$, Z = 2, D_x = 1.194 g cm^{-3}, F(000) = 296, λ (Mo Kα) = 0.71073 Å, μ (Mo Kα) = 0.8 cm^{-1}. The intensity data of all the unique reflections within 2θ range 0−46° were collected at 273 K using Mo Kα radiation and employing $\theta-2\theta$ scan technique with a variable scan width of (0.90 + 0.20 tan θ)° and horizontal aperture of (3.5 + 0.86 tan θ) mm. Three standard reflections were monitored every 2 h of X-ray exposure, and they showed maximum variation of 1.0%. The crystal orientation was checked regularly by three control reflections. A total of 1706 unique reflections was recorded, of which 924 reflections were considered observed on the basis $I \geq 2\sigma(I)$. The intensities were corrected for Lorentz and polarization factors, but no absorption correction was made. The structure was solved by direct methods with the use of the program MITHRILL[14] and refined by a full-matrix least-squares routine, SHELX76,[15] in which the quantity $\sum w(F_o - F_c)^2$ is minimized, where $w = 1/\sigma 2(F_o)$. All the hydrogen atoms were located from difference Fourier maps, and hydrogen parameters were refined. In the final stages of refinement, nonhydrogen atoms were given anisotropic thermal parameters. The refinement converged to a final R = 0.050, R_w = 0.047 for 923 observations and 230 parameters, S = 2.1, Δ/σ = 0.05; electron density in the final difference map was ± 0.2 e/$Å^3$.[16]

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(16) Crystallographic data for the structure(s) reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: +44-(0)1223−336033 or E-mail: deposit@ccdc.cam.ac.uk].

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