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Floresolides, new metacyclophane hydroquinone lactones from an ascidian, *Aplidium* sp.

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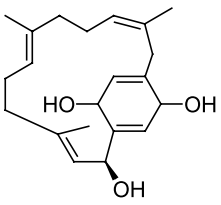
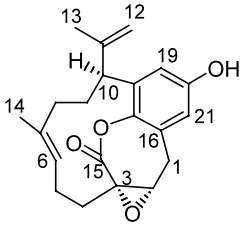
Abstract—Three new cyclofarnesylated hydroquinones, floresolides A–C (**2**–**4**), have been isolated from an ascidian, *Aplidium* sp. and the structures determined by spectroscopic and X-ray diffraction analysis. Floresolides possessing an ε-lactone bridging the aromatic ring and a [10]metacyclophane band are unique members of the class of compounds known as longithorones and longithorols from *Aplidium longithorax*.

Since the first report of longithorone A, a dimeric prenylated quinone, by Schmitz and co-workers in 1994,[1] 16 related monomeric and dimeric compounds have been described from the ascidian *Aplidium longithorax* collected in Palau and Great Barrier Reef.[2] These compounds known as longithorones and longithorols, e.g. **1**, are characterized by the presence of a paracyclophane and/or metacyclophane system built in a farnesyl quinone or hydroquinone. In our collaborative research in quest of bioactive compounds from Indonesian marine invertebrates, an extract of an ascidian of the genus *Aplidium* exhibited moderate cytotoxicity. Examination of the extract gave rise to three new active metabolites, floresolides A–C (**2**–**4**), which were found to be unique relatives of the longithorols. In this paper we report the isolation and structure elucidation of these compounds.



Longithorol C (**1**)



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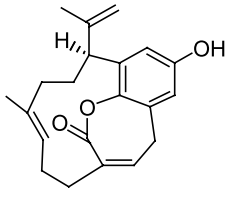

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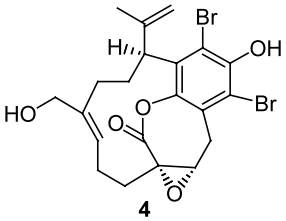

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A sample (70 g wet weight) of *Aplidium* sp.,[3] collected in Pungu Besar, Flores Island in 2001, was extracted with methanol. After concentration the residue was partitioned between EtOAc and water to give 1.31 g of organic matter which was chromatographed on silica gel (hexane/CH_2Cl_2/EtOAc/MeOH) followed by purification by HPLC using RP-18 (MeOH/H_2O) to furnish floresolides A–C (**2**–**4**) in yields of 35.6, 14.4, and 3.4 mg, respectively.

Floresolide A (**2**) was obtained as colorless crystals, mp 170°C, $[\alpha]_D^{30}$ +164° (*c* 0.2, $CHCl_3$). HREIMS (*m*/*z* 340.1663, Δ −1.0 mmu) established the molecular formula of **2** as $C_{21}H_{24}O_4$. The IR absorption bands (3365, 1722 cm^{-1}) indicated the presence of a hydroxyl and a carbonyl group. Analysis of the NMR data (Table 1) revealed that **2** contained a 1,2,3,5 tetra-substituted benzene ring [δ 6.59 (d, *J*=3.0 Hz), 6.69 (d, *J*=3.0 Hz)], an epoxy group (δ 3.45 dd; δ 63.7 d, 59.1 s), an ester (lactone) carbonyl (δ 169.1 s), a tri-substituted double bond (δ 4.82 m; δ 123.4 d, 137.4 s), and a terminal methylene (δ 4.72 brs, 4.84 brs; δ 149.3 s, 109.4 t). These structural elements and the unsaturation requirement suggested that **2** must be tetracyclic. COSY data gave connectivity for C-1/C-2, C-4/C-6, and C-8/C-10, while HMBC analysis allowed connection of

Keywords: marine natural product; cyclofarnesylated hydroquinone; metacyclophane; cytotoxin; X-ray diffraction.
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Table 1. NMR data for floresolides recorded at 125 MHz (^{13}C) and 500 MHz (^{1}H) in $CDCl_3$

#	A (**2**)		B (**3**)		C (**4**)	
	^{13}C	^{1}H (J = Hz)	^{13}C	^{1}H (J = Hz)	^{13}C	^{1}H (J = Hz)
1	33.9 t	α 2.72 dd (7.3, 14.0), β 3.16 dd (6.1, 14.0)	30.1 t	α 2.84 dd (8.5, 14.0), β 3.55 m	34.0 t	α 2.63 dd (7.3, 14.0), β 3.93 dd (6.1, 14.0)
2	63.7 d	3.45 dd (6.1, 7.3)	137.8 d	6.45 ddd (1.5, 5.8, 8.5)	62.5 d	3.48 dd (6.1, 7.3)
3	59.1 s		136.1 s		58.3 s	
4	33.4 t	2.66 ddd (4.0, 10.0, 14.0), 1.34 ddd (4.0, 7.3, 14.0)	31.4 t	2.10 m, 2.82 m	32.6 t	α 2.62 m, β 1.47 ddd (5.5, 8.0, 14.0)
5	24.0 t	1.88 m, 2.17 m	25.0 t	1.90 m, 2.40 m	23.2 t	2.04 m, 2.42 m
6	123.4 d	4.82 m	124.6 d	4.52 br s	126.7 d	4.88 t (6.7)
7	137.4 s		135.8 s		141.2 s	
8	39.3 t	2.08 m	37.4 t	1.75 m, 2.11 m	35.4 t	2.14 m, 2.31 m
9	29.6 t	2.04 m	29.3 t	1.87 m, 2.10 m	25.0 t	2.17 m, 2.80 m
10	43.2 d	3.51 br t (6.4)	41.5 d	3.50 m	44.4 d	3.97 m
11	149.3 s		149.2 s		143.9 s	
12	109.4 t	4.72 br s, 4.84 br s	108.9 t	4.78 d (1.2), 4.88 s	111.8 t	4.77 br s, 4.94 br
13	21.9 q	1.60 s	22.4 q	1.67 s	22.5 q	1.58 s
14	14.9 q	1.16 s*	13.6 q	1.32 s	58.3 t	3.77 d (12.2), 3.82 d (12.2)
15	169.1 s		166.6 s		167.8 s	
16	137.3 s		135.7 s		128.8 s	
17	142.7 s		144.2 s		142.9 s	
18	128.9 s		132.7 s		133.7 s	
19	114.0 d	6.59 d (3.0)	112.6 d	6.50 d (2.7)	112.2 s	
20	153.0 s		152.6 s		150.1 s	
21	114.2 d	6.69 d (3.0)	112.1 d	6.54 d (2.7)	109.5 s	
OH		5.86 br s		5.42 br s		

* This unusually highfield signal for methyl protons on a trisubstituted olefin is due to the shielding effect contributed mainly by the carbonyl group as indicated by CS Chem3D molecular model.

these parts with the remaining parts of the molecule to establish a planar structure. Main HMBCs observed were H-2/C-1, C-3, C-4; H-4/C-3, C-15; H-14/C-6, C-7, C-8; H-13/C-10, C-11; H-10/C-18, C-19; H-1/C-16, C-17, C-21; H-19/C-17, C-20; H-21/C-17, C-19, C-20. The ^{13}C chemical shift (δ 14.9) of C-14 indicated the Z configuration of the C-6/C-7 double bond. These informations enabled us to depict a planar structure for **2** as a unique merosesquiterpene containing a [10]metacyclophane, an endocyclic ε-lactone with an α,β-epoxy function at the bridgehead.

Floresolide B (**3**), mp 160–163°C, $[\alpha]_D^{30}$ +26° (*c* 0.6, $CHCl_3$), had a molecular formula $C_{21}H_{24}O_3$ as determined by HREIMS (m/z 324.1704, Δ −2.0 mmu). Similar structural features of **2** were suggested by the IR and ^{1}H and ^{13}C NMR spectra. An obvious difference of the NMR data (Table 1) was the presence of signals (δ 6.45; δ 136.1 s, 137.8 d) for an additional double bond and absence of those corresponding to the epoxy moiety observed in **2**, suggesting that **3** was a de-epoxy derivative of **2**. The planar structure of **3** was concluded by 2D NMR analysis.

Floresolide C (**4**) was also obtained as colorless crystals, mp 178–180°C, $[\alpha]_D^{30}$ +20° (*c* 0.4, $CHCl_3$). The molecular formula $C_{21}H_{22}Br_2O_5$ as determined by HREIMS (m/z 511.9832, Δ +1.5 mmu) indicated the replacement of two hydrogen atoms with two bromine atoms and presence of an additional oxygen atom in the formula of **2**. It also showed similar features of the IR and NMR spectra to those of **2**. Analysis of the NMR data (Table 1) revealed that **4** contained a fully substituted benzene ring, an epoxy function, a lactone, a terminal methylene, a tri-substituted double bond, and a primary alcohol (δ 3.77 d, 3.82 d; δ 58.3 t). The bromine atoms were placed in the benzene ring and the hydroxyl group of the primary alcohol on C-14 on the structure of **2** by 2D NMR analysis, establishing the planar structure of floresolide C (**4**). The structure **4** was confirmed by single crystal X-ray diffraction analysis (Fig. 1) which also revealed its absolute stereostructure (2*S*,3*S*,10*S*).[4]

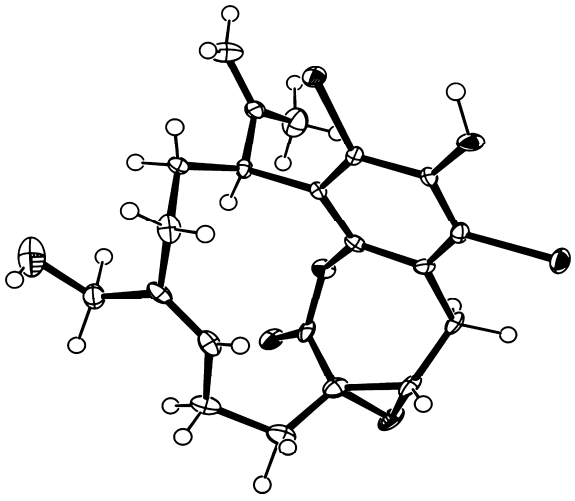

Figure 1. Computer-generated ORTEP drawing of floresolide C (**4**).

Based on the X-ray result and spectral correlation we propose the same stereochemistry at the corresponding chiral centers in **2** and **3**. The floresolides are the first examples of the longithorone/longithorol class terpenoids having an endocyclic lactone. All of the floresolides showed moderate cytotoxicity (IC_{50} 1–10 μg/mL) against KB cells.

Acknowledgements

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3. The ascidian has tentatively been identified as *Aplidium* sp. by Dr. F. Monniot, Museum national d'histoire Naturalle, Paris. A voucher specimen (No. 01F67) is deposited in our laboratory at University of the Ryukyus.
4. Crystals suitable for the X-ray analysis were obtained by recrystallization from aqueous methanol. The crystal (0.2×0.6×0. 4 mm) belongs to the orthorhombic system, space group $P2_12_12_1$, with $a=9.9669(2)$, $b=12.0275(3)$, $c=16.3930(5)$ Å, $V=1965.12(7)$ Å^3, $Z=4$, $D_{calcd}=1.738$ g cm^{-3}, λ (Mo Kα)$=0.71069$ Å. Intensity data were measured on a Rigaku RAXIS-RAPID diffractometer up to 2θ of 55°. A total of 2571 reflections were collected. The structure was solved by direct method (SIR-92) and refined by a full matrix least squares procedure. The non-hydrogen atoms were given anisotropic thermal parameters. The refinement converged to a final $R=0.034$, $R_w=0.023$ for 2248 observed reflections [$I>3.00\sigma(I)$] and 257 variable parameters. Flack parameter was calculated to be 0.0515 (0.00030). Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 193560. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].