Longithorones J and K, Two New Cyclofarnesylated Quinone Derived Metabolites from the Australian Ascidian *Aplidium longithorax*

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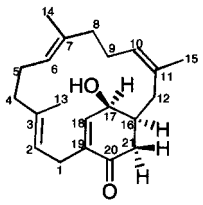
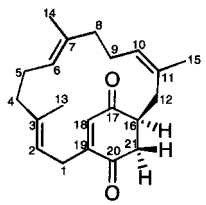
Received June 24, 1998

Chemical investigation of a Great Barrier Reef ascidian, *Aplidium longithorax*, has resulted in the isolation of two new cyclofarnesylated quinone-derived compounds, longithorones J (**1**) and K (**2**). Longithorone J (**1**) is the first example of a γ-hydroxy-cyclohexenone in this structure class. Longithorone K (**2**) is a dihydro analogue of longithorone B.

Ascidians are renowned for their overwhelming bias toward the production of nitrogenous secondary metabolites.[1] However, with the continued chemical interest in these marine invertebrates, an increasing number of non-nitrogen-containing metabolites are being isolated. Examples include the didemnenones,[2] lissoclinolide,[3] the didemnaketals,[4] and the rubrolides.[5] More recently, nine cyclofarnesylated quinones have been isolated from a Paluan ascidian *Aplidium longithorax*.[6,7] In our continuing chemical investigations of Great Barrier Reef ascidians we report the isolation of two new cyclofarnesylated quinone-derived metabolites, longithorones J (**1**) and K (**2**).



(1)



(2)

A freeze-dried sample of *A. longithorax* Monniot, 1987 (family Polyclinidae) was exhaustively extracted with CH_2Cl_2 then chromatographed on diol bonded silica using hexane and increasing amounts of *i*-PrOH. This yielded longithorone J (**1**, 1.28 mg, 0.064% dry wt) and a less polar fraction. The early-eluting fraction was subjected to C18 semipreparative HPLC using a MeOH and H_2O gradient to yield longithorone K (**2**, 0.69 mg, 0.035% dry wt).

Longithorone J (**1**) was obtained as an unstable white solid. A molecular ion in the HREIMS at *m/z* 314.2257 was consistent for the molecular formula $C_{21}H_{30}O_2$. Twenty-one signals were observed in the ^{13}C NMR spectrum (see Table 1 for NMR data). Analysis of the ^1H and HMQC spectra indicated that six of the 21 ^{13}C NMR signals were quaternary, five were methine, seven were methylene, and three were methyl. Three olefinic protons [δ 5.08 (t, J = 8.4 Hz, 1H), 4.99 (t, J = 7.2 Hz, 1H), and 5.34 (t, J = 6.9 Hz, 1H)] and three olefinic methyls [δ 1.63 (s, 3H), 1.59 (s, 3H), and 1.75 (s, 3H)] were present in the ^1H NMR spectrum. Gradient HMBC and COSY correlations for the three olefinic proton and methyl signals established the presence of three trisubstituted double-bond units. Two of these units contained shielded olefinic methyl resonances (14.9 and 16.9 ppm) and hence were assigned *E*-geometry, the remaining unit contained a downfield olefinic methyl resonance (22.8 ppm) and was assigned *Z*-geometry. Furthermore, 2D NMR data linked together the three trisubstituted double-bond units to form a farnesyl chain.

With the farnesyl chain elucidated, only six resonances in both the ^1H and ^{13}C NMR spectra were unassigned. These included two quaternary carbons at 138.4 and 196.8 ppm, a methine proton at δ 2.74 (38.0 ppm), an oxymethine proton at 4.84 (71.5 ppm), an exchangeable proton at 2.62, two diastereotopic protons at 2.38 and 2.45 (42.0 ppm), and an olefinic proton at 6.58 (147.1 ppm). The two quaternary carbons at 196.8 and 138.4 ppm and the olefinic proton at δ 6.58 (147.1 ppm) indicated the presence of an α-substituted α,β-unsaturated ketone moiety.

The IR spectrum that contained a band at 1654 cm^{-1} and the UV spectrum that had an absorption band at λ_{max} 233 nm confirmed the presence of the α,β-unsaturated ketone moiety. The methylene protons at δ 2.54 and 3.24, which were attached to C-1 of the farnesyl chain, showed HMBC correlations to all three carbons of the α,β-unsaturated ketone moiety. C-1 was, therefore, attached to the α carbon of the α,β-unsaturated ketone.

The methylene protons at δ 2.38 and 2.45 both showed $^2J_{CH}$ correlations to the ketone carbon (196.8 ppm) and $^3J_{CH}$ correlations to the oxymethine carbon (71.5 ppm) and the methylene carbon (29.0 ppm), which was C-12 of the farnesyl chain. The proton resonance at δ 2.45 had an additional $^3J_{CH}$ correlation to the quaternary carbon at 138.4 ppm to which the C-1 end of the farnesyl chain was attached, and δ 2.38 had another $^2J_{CH}$ correlation to the methine carbon at 38.0 ppm. Hence, the two diastereotopic methylene protons were positioned between the ketone carbon (C-20) and the methine carbon (C-16), with the latter carbon being linked to C-12 of the farnesyl chain.

A strong COSY correlation between the oxymethine proton at δ 4.84 and the exchangeable proton at δ 2.62 indicated that the molecule contained a secondary alcohol. HMBC correlations from δ 4.84 (H-17) to two of the α,β-unsaturated ketone carbons [147.1 and 138.4 ppm] as well as COSY correlations to δ 6.58 (H-18) and 2.74 (H-16) confirmed that δ 4.84 (H-17) was attached to a carbon γ to the ketone. Hence, a *para* bridging cyclofarnesyl-γ-hydroxy-cyclohexenone (**1)** was assigned.

The relative stereochemistry about the six-membered ring of **1** was determined by rotating frame NOE correlations (ROE) and coupling constants. ROE correlations from the proton at δ 4.84 (H-17) to the protons at δ 2.74 (H-16) and δ 2.38 (H-21α) confirmed that these three protons were on the same face of the six-membered ring. The proton at

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10.1021/np980275g CCC: $18.00
Published on Web 11/11/1998

Table 1. NMR Data for Longithorone J (**1**)[a]

position[b]	$^{13}C(\delta)$	^{1}H (δ, mult., J in Hz)	COSY	HMBC	ROESY[c]
1a	28.2	2.54 (dd, 15.0, 8.4)	1b, 18, 2, 17	20, 18, 19, 3, 2	1b, 2, 18, 13
1b		3.24 (dd, 15.0, 8.4)	1b, 18, 2, 17	20, 18, 19, 3, 2	1a, 13
2	122.3	5.08 (t, 8.4)	1a, 1b, 2	1, 4, 13	1a, 4a, 4b
3	135.3				
4a	39.5	2.17 (m)	4b	3, 6, 5, 13	4b, 13
4b		2.05 (m)	4a	2, 6, 13	4a, 2
5a	23.9	2.08 (m)	5b, 6	7, 6, 4	5b, 14
5b		2.20 (m)	5a, 6	7, 6, 4	5a, 14
6	123.9	4.99 (t, 7.2)	5a, 5b, 14	8, 5, 14	5a, 8b
7	134.9				
8a	39.8	1.81 (m)	8b	6, 14, 7	8b
8b		1.89 (m)	8b	6, 7, 9, 10, 14	8a
9a	29.0	1.89 (m)	9b	7, 8	9b
9b		2.00 (m)	9a	8	9a, 12a
10	128.2	5.34 (t, 6.9)	9a, 9b, 15	15, 9, 12	9a, 15
11	133.8				
12a	29.0	2.48 (dd, 13.8,8.4)	16, 12b	11, 10, 21, 16, 15	12b, 16, 15, 9b
12b		2.05 (m)	12a, 16	10, 11, 15, 16, 17, 21	12a, 16, 15
13	14.9	1.63 (s)	2	2, 3, 4	1b, 4a, 1a
14	16.1	1.59 (s)	6	6, 7, 8	5a
15	22.8	1.75 (s)	10	10, 11, 12	16, 12a, 12b, 10
16	38.0	2.74 (m)	17, 12a, 12b, 21α, 21β	17, 18, 20, 21	12a, 12b, 15, 17, 21α, 21β
17	71.5	4.84 (m)	1a, 1b, 16, 17-OH	18, 19	16, 21α, 18
17-OH		2.62 (d, 9.0)	17		
18	147.1	6.58 (s)	1a, 1b, 16, 17	1, 16, 20	17, 1a
19	138.4				
20	196.8				
21α	42.0	2.38 (dd, 17.4, 4.8)	16, 21β	12, 16, 17, 20	16, 17, 21β
21β		2.45 (dd, 17.4, 2.4)	16, 21α	12, 17, 19, 20	12b, 16, 21α

[a] Spectra were recorded in $CDCl_3$ at 30 °C. [b] Numbering for structures of longithorones J and K is identical to that used for longithorones A−I.[6,7] [c] Data obtained from ROESY experiment with mixing time of 600 ms.

Table 2. NMR Data for Longithorone K (**2**)[a]

position[b]	$^{13}C(\delta)$	^{1}H (δ mult., J in Hz)	COSY	HMBC	ROESY[c]
1a	28.3	2.70 (dd, 15.0, 7.8)	1b, 2, 13, 18	2, 3, 18, 19, 20	1b, 2, 13, 18
1b		3.36 (dd, 15.0, 7.8)	1a, 2, 13, 18	2, 3, 18, 19, 20	1a, 2, 13
2	120.5	5.07 (t, 7.8)	1a, 1b, 13	1, 4, 13	1a, 1b
3	137.6				
4a	39.4	2.17 (m)	4b	2, 3, 5, 6, 13	4b, 13
4b		2.10 (m)	4a	2, 3, 6, 13	13, 4a
5a	23.9	2.13 (m)	5b, 6, 14	3, 6, 7	14, 5a
5b		2.17 (m)	5a, 6, 14	3, 4, 6, 7	5b, 14
6	123.0	4.90 (t, 6.3)	5a, 5b, 14	8, 14	5a, 5b
7	135.3				
8a	39.6	1.74 (m)	8b	6, 7, 9, 14	8b, 14
8b		1.88 (m)	8a	6, 7	8a, 14
9a	28.6	1.67 (m)	9b	11	9b
9b		1.83 (m)	9a	8	9a
10	128.8	5.32 (t, 7.5)	9a, 9b, 15	12, 15	15
11	130.5				
12a	36.9	2.23 (dd, 13.8, 9.3)	12b, 16	10, 11, 15, 16, 17, 21	12b, 15
12b		2.45 (dd, 13.8, 7.2)	12a, 16	10, 11, 15, 16, 17, 21	12a, 15, 16, 21α
13	14.8	1.64 (s)	1a, 1b, 2	2, 3, 4	1a, 1b, 4b, 4a
14	16.2	1.57 (s)	5a, 5b, 6	6, 7, 8	5a, 8a, 8b, 5b
15	22.5	1.70 (s)	10	10, 11, 12	10, 12a, 12b, 16
16	46.5	2.99 (dddd, 9.3, 7.2, 5.4, 1.2)	12a, 12b, 18, 21α	12, 17, 20	12a, 12b, 15, 21β, 21α
17	200.7				
18	136.4	6.47 (d, 1.2)	1a, 1b, 16	1, 16, 20	1a
19	151.7				
20	197.1				
21α	42.0	2.96 (dd, 16.8, 5.4)	16, 21β	12, 16, 20	21β, 12b, 16
21β		2.74 (br d, 16.8)	21α	12, 16, 17, 20	21α, 16

[a] Spectra were recorded in $CDCl_3$ at 30 °C. [b] Numbering for structures of longithorones J and K is identical to that used for longithorones A−I.[6,7] [c] Data obtained from ROESY experiment with mixing time of 600 ms.

δ 2.74 (H-16) had small couplings of 4.8 and 2.4 Hz with the protons at δ 2.38 (H-21α) and δ 2.45 (H-21β), respectively, which, together with the ROE correlations between all three protons, proved that H-16 and H-21β were equatorial and, therefore, H-17 and H-21α were axial. This established the relative stereochemistry of longithorone J (**1**).

Longithorone K (**2**) was obtained as an unstable yellow gum. A molecular ion in the HREIMS at m/z 312.2079 was consistent for the molecular formula $C_{21}H_{30}O_2$. Comparison of the ^{13}C NMR data (see Table 2) of **2** with **1** showed 16 similar chemical shifts (deviation <3 ppm) of which 14 were assigned to the farnesyl chain and the other two carbons were assigned to the ketone at C-20 and the

methylene at C-21. The remaining five ^{13}C chemical shifts included two quaternary carbons (151.7 and 200.7 ppm), two methine carbons (136.4 and 46.5 ppm), and one methylene carbon (36.9 ppm). The presence of two ketone resonances at 200.7 and 196.8 ppm and the absence of the hydroxymethine carbon resonance at 71.5 ppm indicated that longithorone K (**2**) was the 17-keto analogue of longithorone J (**1**). The UV spectrum of **2** compared to **1** contained a bathochromically shifted band at λ_{max} 237 nm consistent with an extension of conjugation, which the 17-keto auxochrome provided. The IR spectrum of **2** showed absorbance at 1680 cm^{-1}, which also agrees with the 2-cyclohexen-1,4-dione. Hence, structure **2** was assigned to longithorone K.

Due to the minute quantities isolated and the instability of longithorones J (**1**) and K (**2**) in $CDCl_3$, no further stereochemical analysis of these compounds was possible. None of the previously isolated longithorones A−I [6,7] were detected or isolated in the animal that we investigated.

Experimental Section

General Experimental Procedures. NMR spectra were recorded at 30 °C on a Varian 600 MHz Unity INOVA at 599.926 MHz for ^1H and 149.98 MHz for ^{13}C. The ^1H and ^{13}C chemical shifts were referenced to the solvent peak ($CHCl_3$−$CDCl_3$) at δ 7.26 and 77.00 ppm, respectively. HREIMS was recorded on a Kratos mass spectrometer. FTIR and UV spectra were recorded on a Perkin−Elmer 1725X spectrophotometer and a GBC UV/vis 916 spectrophotometer, respectively. CD spectra and $[\alpha]_D$ values were measured on a JASCO J-715 spectropolarimeter and a JASCO P-1020 polarimeter, respectively. A Waters 600 pump equipped with a Waters 996 PDA detector and Waters 717 autosampler were used for MPLC and HPLC. Alltech Davisil 30−40 μm 60 Å diol was used for packing the Waters AP-2 prep column (20-mm D × 180-mm L) and a Hewlett−Packard Zorbax 5 μm 80 Å C18 (9.4-mm D × 250-mm L) was used for HPLC semipreparative work. All solvents used for HPLC, UV, CD, $[\alpha]_D$, and MS were Merck Omnisolv grade, and the H_2O used was Millipore Milli-Q PF filtered. Standard parameters were used for the 2D NMR spectra obtained, which included gradient COSY, HMQC, and HMBC and the phase-sensitive ROESY.

Animal Material. A specimen of *A. longithorax* was collected by scuba diving (−24 m) off Gannet Cay, at the Swains Reefs, and kept frozen prior to freeze-drying and extraction. Voucher specimen QMG305411 has been deposited at the Queensland Museum, South Brisbane, Queensland, Australia.

Extraction and Isolation. The freeze-dried ascidian (2.0 g dry wt) was extracted with CH_2Cl_2 (3 × 40 mL), then concentrated under vacuum to yield a dark red gum (46 mg). This gum was chromatographed using MPLC (254 nm) on a diol-packed Waters AP-2 prep column (20-mm D × 180-mm L) initially using isocratic conditions of 1% *i*-PrOH−99% hexane for 2 min followed by a linear gradient to 8% *i*-PrOH−92% hexane in 10 min at a flowrate of 16 mL/min. This afforded 5 fractions, of which fraction 5 contained pure longithorone J (**1**, 1.28 mg, 0.064% dry wt). The less polar fractions 1 and 2 were combined and chromatographed on a Waters HPLC (254 nm) using a Zorbax 5 μm 80 Å C18 column (9.4-mm D × 250-mm L) at a flowrate of 4 mL/min with a gradient from 70% MeOH−30% H_2O−100% MeOH in 10 min followed by isocratic condition of 100% MeOH for 3 min. Longithorone K (**2**, 0.69 mg, 0.035% dry wt) eluted from the column in 100% MeOH.

Longithorone J (1): isolated as an unstable white solid; $[\alpha]_D$ +170° (*c* 0.085, CH_2Cl_2); UV (MeOH) λ_{max} (ϵ) 233 nm (6800); CD (MeOH) 209 ($\Delta\epsilon$ −12.6), 245 ($\Delta\epsilon$ 19.5), 339 nm ($\Delta\epsilon$ −1.8); IR ν_{max} (KBr disk) 3388, 2927, 1654 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HREIMS *m*/*z* 314.2257 (calcd for $C_{21}H_{30}O_2$, 314.2245).

Longithorone K (2): isolated as an unstable pale yellow gum; $[\alpha]_D$ +61° (*c* 0.046, CH_2Cl_2); UV (MeOH) λ_{max} (ϵ) 237 nm (6600); CD (MeOH) 217 ($\Delta\epsilon$ 1.9), 251 ($\Delta\epsilon$ 1.7), 376 nm ($\Delta\epsilon$ −0.4); IR ν_{max} (KBr disk) 2919, 2851, 1680, 1440 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HREIMS *m*/*z* 312.2079 (calcd for $C_{21}H_{28}O_2$, 312.2089).

Acknowledgment. We wish to thank John Kennedy of the Sessile Marine Invertebrate group at the Queensland Museum for the collection of the ascidian specimen. Dr. P. Mather (Kott) is acknowledged for the identification of the *Aplidium longithorax*. Thanks are also extended to Noel Davies of the University of Tasmania for the HREIMS analysis. One of us (R. A. D.) acknowledges the support of the Australian Research Council in the form of an Australian Postgraduate Award.

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NP980275G