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Longithorone A: Unprecedented Dimeric Prenylated Quinone from the Tunicate *Aplydium longithorax*

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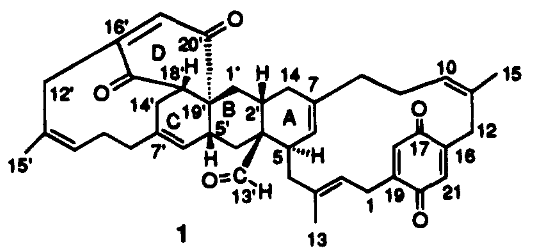
Tunicates have attracted considerable attention as potential sources of anticancer drug leads due to the variety of novel and cytotoxic nitrogenous compounds which they have yielded.[1,2] To date, the most notable metabolites in this regard are didemnin B[3] and the ecteinascidins.[4,5] Only a small number of non-nitrogenous metabolites have been reported from tunicates, and among these are approximately 10 structurally simple prenylated quinones and hydroquinones.[2] In the course of our continuing search for antitumor agents from marine sources, we have isolated an unprecedented type of prenylated benzoquinone, designated longithorone A, in which a farnesyl unit forms a macrocycle by bridging the 2,5-positions of benzoquinone, and two such units are combined to form a new carbocyclic skeleton containing 6-, 10-, and 16-membered rings. Longithorone A is weakly cytotoxic to P388 murine leukemia cells, $ED_{50} \sim 10$ μg/mL.

Longithorone A, **1**, mp 195−6 °C, α_D −87.5° (*c* 2.73, $CHCl_3$), $C_{42}H_{46}O_5$ by HRFABMS,[6] was isolated from the combined MeOH and MeOH/dichloromethane (DCM) extracts of freshly thawed specimens of *Aplydium longithorax* (Monniot) collected in Palau. The concentrated combined extracts were partitioned between hexane and $MeOH/H_2O$ (9:1). The hexane solubles were chromatographed over silica gel (hexane → hexane/EtOAc). A selected fraction was passed over a silica gel Sep-Pak (DCM) to remove most sterols, and the major fraction therefrom was resolved by HPLC, first with silica gel and 5% acetone in DCM and finally with silica gel and acetone−DCM (1:80) to give 54 mg of **1** (from ~250 g dry weight). Longithorone A was crystallized from MeOH/DCM: IR (neat) 1712, 1710, 1655 (vs) cm^{-1}; UV (EtOH) λ_{max} 250 (ϵ 14 700), 300 (3500).



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^1H and ^{13}C NMR data (see Table 1), revealed that **1** contained eight carbon−carbon double bonds and five carbonyl groups, including one aldehyde [δ 9.60 (s); 206.6 (d)], thus requiring the presence of seven carbocycles to satisfy the unsaturation number revealed by the mass spectral data. The presence of one quinone ring was also indicated by UV absorption at 250 nm, ^{13}C NMR signals at δ 187.2 and 187.6, and strong IR absorption at 1655 cm^{-1}.

Table 1. NMR Data for Longithorone A

position	$^{13}C^{a,b}$	$^1H^c$	HMBC (H no.)
1	27.6 (t)	3.75 (t, *J* = 11), 2.61 (dd, *J* = 11, 5)	18
2	124.7 (d)	4.91 (dd, *J* = 11, 5)	1, 4, 13
3	136.4 (s)		1, 4, 13
4	40.1 (t)	2.14 (m), 1.66 (m)	6, 13
5	36.1 (d)	2.94 (m)	4, 6
6	119.1 (d)	4.96 (brs)	4, 14
7	134.8 (s)		5, 14
8	37.0 (t)	1.74 (m)	14
9	26.2 (t)	2.00 (m), 1.72 (m)	
10	126.4 (d)	5.04 (br m)	12
11	132.1 (s)		12
12	35.3 (t)	3.18 (dd, *J* = 16.7, 1.8), 2.92 (br d, *J* = 16.7)	15, 21
13	15.1 (q)	1.83 (s)	4
14	32.8 (t)	2.30 (m), 1.56 (d, *J* = 18.3)	6
15	27.5 (q)	1.81 (s)	
16	149.4 (s)		12, 18
17	187.2 (s)[d]		12, 18, 21
18	131.5 (d)	6.54 (br s)	1
19	148.3 (s)		1,21
20	187.6 (s)[d]		1, 18, 21
21	133.4 (d)	6.63 (br s)	
1′	37.7 (t)	2.05 (m), 1.37 (dd, *J* = 14.2, 2.6)	
2′	30.6 (d)	2.20 (m)	14
3′	49.4 (s)		1′, 6, 13′,14
4′	27.4 (t)	2.32 (dd, *J* = 13.9, 4.6); 1.67 (m)	
5′	38.1 (d)	2.12 (m)	
6′	125.7 (d)	5.29 (br m)	8′, 14′
7′	143.9 (s)		14′, 18′
8′	29.7 (t)[e]	2.20 (m), 1.98 (m)	
9′	34.1 (t)[e]	2.16 (m), 2.02 (m)	
10′	130.5 (d)	5.35 (br dd, *J* = 12.1, 5.0)	12′
11′	131.5 (s)		12′, 15′
12′	34.6 (t)	3.02 (dd, *J* = 14.2, 1.8); 2.96 (br d, *J* = 14.2)	15′, 21′
13′	206.6 (d)	9.60 (s)	4′
14′	30.9 (t)	2.45 (m), 2.16 (m)	
15′	27.1 (q)	1.79 (s)	12′
16′	155.8 (s)		12′, 18′
17′	201.5 (s)[f]		12′, 14′, 18′
18′	55.9 (d)	2.84 (br d, *J* = 10)	1′
19′	52.9 (s)		4′, 14′, 21′
20′	202.1 (s)[f]		1′, 18′, 21′
21′	139.1 (d)	6.29 (d, *J* = 1.8)	12′

[a] ^{13}C spectrum was recorded at 75 MHz in $CDCl_3$, referenced to $CDCl_3$ (77.0 ppm). [b] ^{13}C multiplicities were determined by a DEPT experiment and assignments confirmed by HETCOR, HMQC, and HMBC experiments. [c] ^1H spectrum recorded at 500 MHz in $CDCl_3$, referenced to residual $CHCl_3$ (7.26 ppm); *J* in hertz; assignments were determined from COSY, RELAY, and NOESY experiments and H/C correlations. [d,e,f] Signals with identical letters may be interchanged.

The molecular structure of longithorone A was determined by single-crystal X-ray diffraction.[7] A stereo ORTEP plot of a single molecule of longithorone A is shown in Figure 1. The absolute configuration shown in Figure 1 was determined by the Bijvoet method using anomalous dispersion of Cu radiation by the Cl atoms of the methylene chloride solvent in the crystal.[8]

The X-ray structure revealed an unprecedented polycyclic molecule consisting of seven different rings: cyclohexane (B), two cyclohexenes (A and C), dihydrobenzoquinone (D), benzoquinone, and 10- and 16-membered alkyl macrocycles. All the central six-membered rings (A, B, C, and D) are under severe strain due to complicated ring fusions and deviate from

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(6) HRFABMS: $[M + 2H]^+$ *m/z* 632.3415, Δ 7.4 mmu; $[M + 3H]^+$, *m/z* 633.3480, Δ 8.7 mmu. For MS of quinones, cf.: Das, B. C.; Lounasma, M.; Tendille, C.; Lederer, E. *Biochem. Biophys. Res. Commun.* **1965**, *21*, 318.

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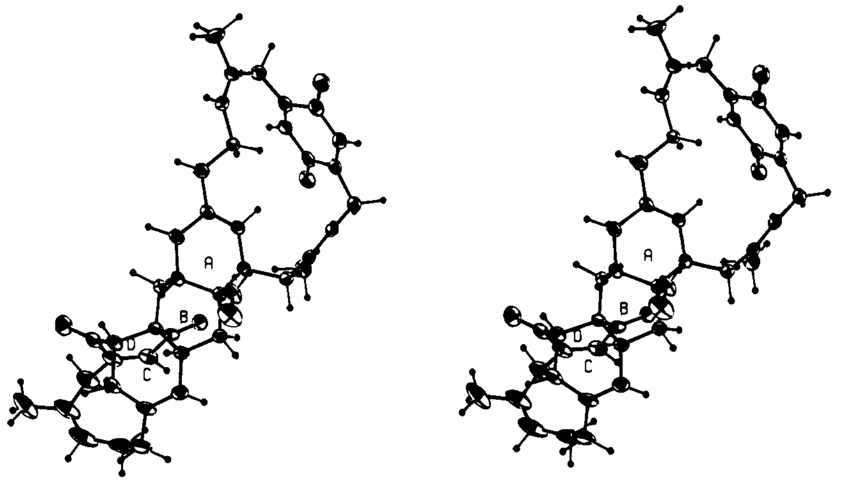
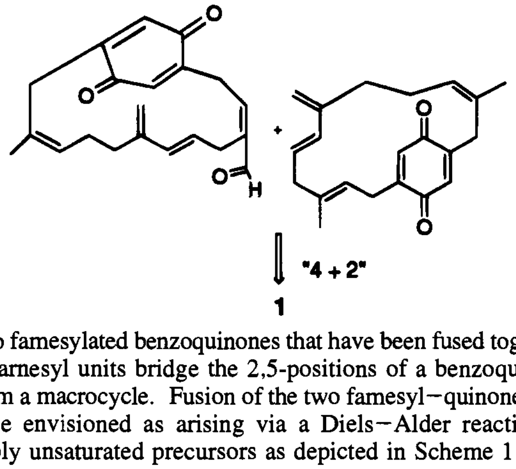


Figure 1. A stereo ORTEP plot of longithorone A. Thermal ellipsoids shown at the 30% probability level.

symmetric conformations. The saturated ring B is *cis*-fused with ring A [H−C2′−C3′−C13′ = −52(2)°], *trans*-fused with ring C [H−C5′−C19′−C20′ = −168(2)°], and attached with ring D at C19′. In addition, ring C is *cis*-fused [H−C18′−C19′−C1′ = −27(2)°] with ring D. Obviously, strain is greatest between rings A and B and least between B and C. Rings B and C assume distorted boat conformations while rings A and D are in distorted half-chair conformations. The 16-membered macrocycle includes an *E* [C2−C3 = 1.344(7) Å] and a *Z* [C10−C11 = 1.315(7) Å] double bond and also embodies the benzoquinone ring. The atoms of the macrocycle lie on three planar sections, the plane of the benzoquinone, the plane of the C2−C3 double bond, and the approximate plane through the atoms C5 to C11. The dihedral angle between the plane of the benzoquinone ring and the plane of the C2−C3 double bond is 84°. The 10-membered ring which contains a *cis* double bond [C10′−C11′ = 1.331(12) Å] assumes a highly folded conformation. Bond distances and bond angles in the longithorone A molecule are close to expected values. Bonds involving quaternary carbons (C3′ and C19′) are elongated and range between 1.548 and 1.578 Å. The benzoquinone ring deviates from perfect planarity (root mean square deviation of 0.05 Å), and the ring dimensions vary somewhat from those of known benzoquinone structures.[9] In the dihydrobenzoquinone ring, the two oxygen atoms deviate significantly (0.61 and 0.45 Å for O1′ and O2′, respectively) from the plane through the atoms C17′−C16′−C21′−C20′.

Structure **1** represents an unprecedented natural product carbocyclic arrangement. Noncoplanarity of the 1,4-enedione ring in **1** is evident from the X-ray analysis and is manifested by the downfield shift of the ^{13}C NMR signals for the carbonyl groups in this ring. Longithorone A is composed fundamentally of two farnesylated benzoquinones that have been fused together. The farnesyl units bridge the 2,5-positions of a benzoquinone to form a macrocycle. Fusion of the two farnesyl−quinone units can be envisioned as arising via a Diels−Alder reaction of suitably unsaturated precursors as depicted in Scheme 1. The stereochemistry of **1** is consistent with such a fusion. A similar intermolecular Diels−Alder reaction has been suggested to explain the origin of the tetraterpenoid methyl isosartortuate.[10] Rings B and C could arise by an intramolecular Diels−Alder reaction. Intramolecular "4 + 2" cycloadditions represent a key step in the proposed biosyntheses of the mangamines[11] and other complex polycyclic alkaloids exemplified by recent examples such as xestocyclamine,[12] the ingenamines,[13] and mandangamine.[14]

Scheme 1



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Supplementary Material Available: Tables of atomic parameters for the non-hydrogen atoms, bond distances and angles, torsion angles, anisotropic thermal parameters, and hydrogen atom parameters and ^{1}H and ^{13}C NMR spectra for longithorone A (12 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(7) One of the thin large platy crystals of the compound obtained from MeOH/CH_2Cl_2 was cut into a suitable size of 0.54 × 0.36 × 0.03mm for all X-ray measurements. Crystallographic data: $C_{42}H_{46}O_5 + CH_2Cl_2 + H_2O$, FW = 733.7, orthorhombic, $P2_12_12_1$, a = 10.597(2) Å, b = 38.097(10) Å, c = 9.396(2) Å, V = 3793.3 Å^3, Z = 4, D_{calcd} = 1.284 gm/cm^3, μ(Cu Kα) = 18.24 cm^{-1}, final $R(R_w)$ = 0.071(0.079) for 3486 observed reflections [$I \geq 2\sigma(I)$] and 605 variable parameters. The intensity data for 4410 unique reflections ($2\theta \leq 150°$) were collected on an Enraf-Nonius CAD-4 diffractometer with Cu Kα radiation at 163 ± 2 K. The structure was determined by direct methods and refined by least squares. The aldehyde group was found to be disordered (40−60%). All the hydrogen atoms belonging to the longithorone A molecule were located from difference Fourier maps and were refined with bond-length constraints and with fixed isotropic thermal parameters. The hydrogen atoms of the solvent molecules were not located.

(8) Twenty Friedel pairs with the highest Bijvoet differences were selected, and their intensities were measured repeatedly at 163 K. The observed and calculated Bijvoet differences of all 20 pairs were in agreement with the absolute configuration reported.

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