Bolinaquinone: A Novel Cytotoxic Sesquiterpene Hydroxyquinone from a Philippine *Dysidea* Sponge

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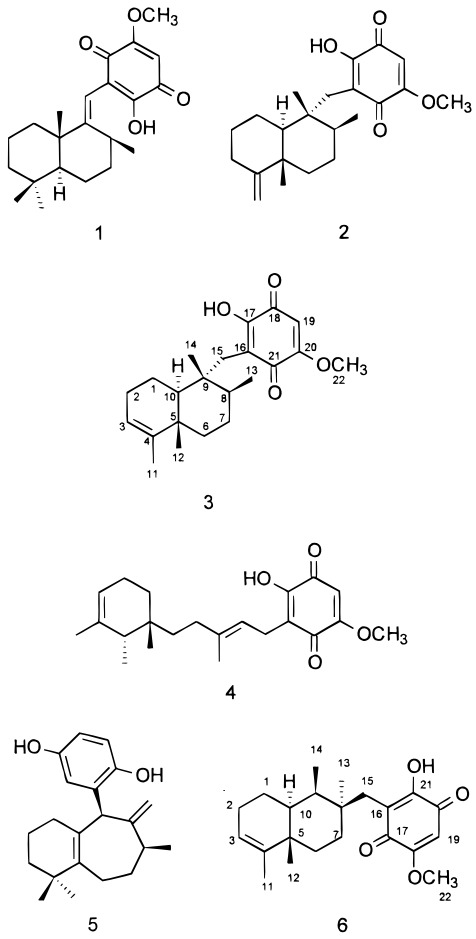
Sesquiterpene hydroxyquinones and hydroquinones have been isolated from various sponges.[3−16] The sesquiterpene moiety of these metabolites usually have the normal drimane skeleton, as exemplified by spongiaquinone (**1**),[3] a rearranged drimane skeleton, as in ilimaquinone (**2**)[4] and isospongiaquinone (**3**),[3,5] or a monocyclic sesquiterpenoid skeleton, as in metachromin C (**4**)[6] (Chart 1). Recently, Patil and co-workers isolated related sesquiterpene derivatives, frondosins A−E, which feature a 6,7-bicyclic skeleton, as exemplified by frondosin A (**5**).[7] We report here the isolation and structure determination of a cytotoxic sesquiterpene hydroxyquinone, bolinaquinone (**6**), which showed a rearranged drimane skeleton but with a different position for the hydroxyquinone moiety.

Two sponge samples, identified as *Dysidea* sp.,[17] were collected from the northern and southern parts of the Philippines. Both sponge samples yielded bolinaquinone (**6**) as the major metabolite, after column chromatography in Sephadex LH-20 of the MeOH−$CHCl_3$ extract or the Kupchan $CHCl_3$ partition layer of the MeOH extract.

Chart 1



HREIMS determined the molecular formula of bolinaquinone to be $C_{22}H_{30}O_4$ (Δ −0.6 mmu). The presence of a hydroxyquinone moiety in **6** was suggested by the observation of a mass spectral fragment ion at *m/e* 168 ($C_8H_8O_4$) as well as characteristic UV absorptions at 288 and 424 nm (log ϵ 3.70, 2.16), which shifted to 290 and 518 nm upon addition of base.[3,12] The presence of the hydroxyquinone moiety was also confirmed by the observation of ^{1}H NMR resonances at δ 7.38 (1H, br s), 3.82 (3H, s), and 5.82 (1H, s) and ^{13}C resonances at δ 102.0, 117.7, 152.8, 161.3, 182.2, 182.5, and 56.7. All these data compared satisfactorily with the NMR data observed for 3-substituted 2-hydroxy-5-methoxy-2,5-cyclohexadien-1,4-dione moieties of related compounds.[3,4] Further proof of the presence of this hydroxyquinone moiety was also shown by HMBC, which suggested the presence of partial structure A (Chart 2).

HMBC correlations shown by the methyl groups at δ 0.97, 0.98, 1.02, and 1.55 indicate partial structures B−E. Partial structure A can be connected to D through the carbon at δ 34.2, while C can be further attached to D through the carbons at δ 39.7 and 45.3. Structure C is also attached to B through the carbon at δ 41.1, and B is further attached to E through the carbons at δ 143.5 and

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(3) Kazlauskas, R.; Murphy, P. T.; Warren, R. G.; Wells, R. J.; Blount, J. F. *Aust. J. Chem*. **1978**, *31*, 2685−2697.
(4) Luibrand, R. T.; Erdman, T. R.; Vollmer, J. J.; Scheuer, P. J. *Tetrahedron* **1979**, *35*, 609−612.
(5) Capon, R. J. *J. Nat. Prod*. **1990**, *53*, 753−756.
(6) Kobayashi, J.; Murayama, T.; Ohizumi, Y.; Ohta, T.; Nozoe, S.; Sasaki, T. *J. Nat. Prod*. **1989**, *52*, 1173−1176.
(7) Patil, A. D.; Freyer, A. J.; Killmer, L.; Offen, P.; Carte, B.; Jurewicz, A. J.; Johnson, R. K. *Tetrahedron* **1997**, *53*, 5047−5060.
(8) McConnell, O. J.; Longley, R.; Gunasekera, M. *Experientia* **1992**, *48*, 891−892.
(9) Wright, A. E.; Rueth, S. A.; Cross, S. S. *J. Nat. Prod*. **1991**, *54*, 1108−1111.
(10) Hirsch, S.; Kashman, R. Y.; Loya, Y. *J. Nat. Prod*. **1991**, *54*, 92−97.
(11) Urban, S.; Capon, R. J. *J. Nat. Prod*. **1992**, *55*, 1638−1642.
(12) Swersey, J. C.; Barrows, L. R.; Ireland, C. M. *Tetrahedron Lett*. **1991**, *32*, 6687−6690.
(13) Minale, L.; Riccio, R.; Sodano, G. *Tetrahedron Lett*. **1974**, 3401−3404.
(14) Rodriguez, J.; Quinoa, E.; Riguera, R.; Peters, B. M.; Abrell, L. M.; Crews, P. *Tetrahedron* **1992**, *48*, 6667−6680.
(15) Kondracki, M.-L.; Guyot, M. *Tetrahedron* **1989**, *45*, 1995−2004.
(16) Carte, B.; Rose, C. B.; Faulkner, D. J. *J. Org. Chem*. **1985**, *50*, 2785−2787.

(17) Identification was made by Mary Kay Harper of Scripps Institution of Oceanography, La Jolla, CA. A sample of the sponge is deposited at the Scripps Institution of Oceanography repository.

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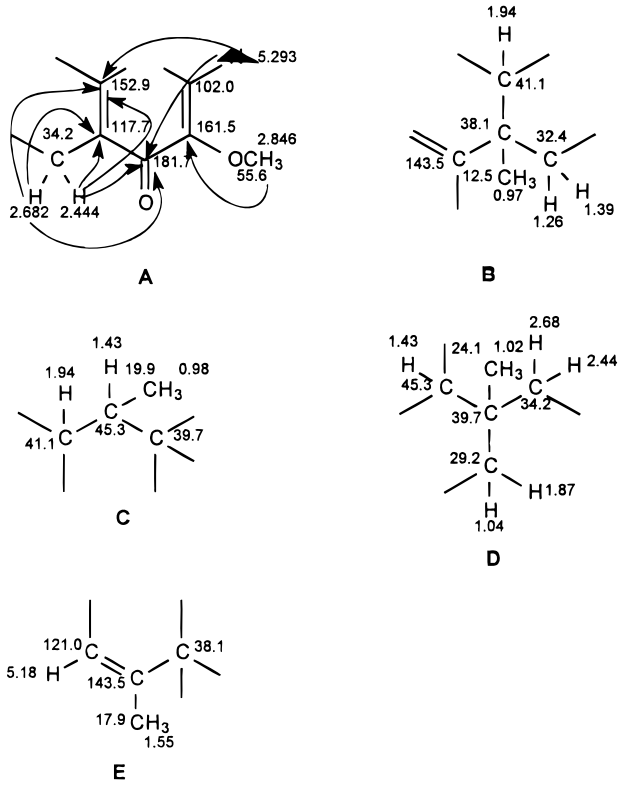
Table 1. NMR Data for Bolinaquinone in C_6D_6 and $CDCl_3$

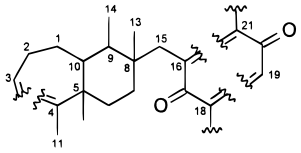
C no.	δ(C)[a]	δ(H)[a]	mult, J (Hz)	COSY, H no.	HMBC, C no.	ROESY, H no.	δ(C)[b]	δ(H)[b]
1	24.9	1.72 ax	qd, 12.5, 7	1eq, 2, 10	2, 5, 10	1eq, 2, 12	24.5	1.73
		1.12 eq	dd, 12.5, 6	1ax, 2	3	1ax, 2, 9		1.17
2	27.1	2.01	m	2, 3	1, 3, 4, 10	1ax, 1eq, 3	26.6	2.01
3	121.0	5.18	s	2, 11	1, 5, 11	2, 11	120.4	5.11
4	143.5						143.7	
5	38.1						37.9	
6	32.4	1.39 ax	dt, 13.5, 2	6eq, 7ax	5, 7, 8, 10, 12	6eq, 7eq, 11	31.9	1.40
		1.26 eq	td, 13.5, 3	6ax, 7ax	4, 5, 7, 12	6ax, 7ax, 11		1.22
7	29.2	1.87 ax	td, 14.5, 3.5	6eq, 7eq,	6, 8, 13, 15	6eq, 7eq, 12	28.7	1.68
		1.04 eq	m	7ax	5, 12	6ax, 7ax		0.94
8	39.7						39.6	
9	45.3	1.43 eq	m	14	5, 7, 8, 10, 13, 14	1eq, 10, 13	44.8	1.45
10	41.1	1.94 ax	dd, 12, 2.5	1ax, 9	1, 2, 4, 5, 9, 12, 14	9	40.6	1.85
11	17.9	1.55	s	2	3, 4, 5	3, 6ax, 6eq, 12	17.6	1.50
12	19.9	0.97	s		4, 5, 6, 10	2, 7ax, 11	19.7	0.93
13	24.1	1.02	s		8, 9, 14, 15	9eq, 15a	23.8	0.88
14	12.5	0.98	d, 10.5	9	8, 9, 10	15b	12.2	0.96
15	34.2	2.44 a	d, 12.5	15b	7, 8, 9, 13, 16, 17, 21	13, 15b	33.8	2.31
		2.68 b	d, 12.5	15a	7, 8, 9, 13, 16, 17, 21	14, 15a		2.54
16	117.7						117.7	
17	152.9	7.46 (OH)	br s				152.8	7.38 (OH)
18	182.7						182.5	
19	102.1	5.29	s		16, 17, 20, 21	22	102.0	5.82
20	161.5						161.3	
21	181.7						182.2	
22	53.6	2.85	s		20	19	56.7	3.82

[a] In C_6D_6. [b] In $CDCl_3$.

Chart 2



38.1. The methine proton at δ 1.94 was also coupled to the methylene hydrogens at δ 1.72 and 1.12, which, in turn, are coupled to the methylene protons at δ 2.01. These hydrogens at δ 2.01 also showed HMBC correlations with the carbons at δ 121.0 and 143.5, thus allowing closure of the cyclohexenyl ring. The proton at δ 1.04 was long-range coupled to the carbon at δ 38.1, while the proton at δ 1.87 correlated to the carbon at δ 32.4. These observations allowed closure of the cyclohexyl ring giving the gross structure for bolinaquinone.



The rearranged drimane skeleton of bolinaquinone did not appear to follow the usual biosynthetic rearrangement giving rise to such sesquiterpene hydroxyquinones such as isospongiaquinone and ilimaquinone. Thus, further proof was necessary to support the unique placement of the hydroxyquinone moiety in bolinaquinone. This was afforded by an INADEQUATE experiment, optimized for $J = 45$ Hz.

On the basis of INADEQUATE data, the cyclohexyl ring formed by connection of C-5 to C-10 (ring B of a reaaranged drimane skeleton) was established. From the INADEQUATE data, C-5 and C-10 were found to be the ring junction carbons. Carbon-9, which was directly bonded to C-10, was a methine carbon with Me-14 bonded to it. The methylene C-15 to which the hydroxyquinone is bonded was connected to C-8, which in turn is directly attached to the methine C-9. These connectivities support the placement of the hydroxyquinone side chain on C-8 of a rearranged drimane skeleton instead of the usual substitution at C-9.

The *trans*-fusion of the bicyclic system was indicated by the chemical shift of the Me-12 group (δ 19.7).[11−14] ROESY correlations were found between H-10 and Me-13, H-9 and Me-13, and between CH_2-15 and Me-14. These data suggest the relative stereochemistry for bolinaquinone to be that given in **6**.

Bolinaquinone yielded IC_{50} values of 1.9 μg/mL against human colon tumor cell line HCT116 and mild inhibition of *Bacillus subtilis* at 80 μg/disk. Enhanced cytotoxicity toward the DNA repair-deficient CHO cell-line xrs-6,[18]

(18) Jeggo, P. A.; Caldecott, K.; Pidsley, S.; Banks. G. R. *Cancer Res.* **1989**, *49*, 7057−7063.

as well as a Rec differential[19] of 1 mm (at 80 μg/disk), suggests that bolinaquinone exhibits its cytotoxicity by interfering with or damaging DNA.[20]

Experimental Section

Organism. The first sponge specimen was collected by scuba from shallow reef waters off Cape Bolinao, Pangasinan, Philippines, on Oct 1990. The second sponge sample was collected by scuba at a depth of 25−50 ft on 11 Nov 1996 near Pujada Island, Davao, Mindanao, Philippines, and frozen shortly after collection. Voucher samples of this sponge are kept at the Marine Science Institute, University of the Philippines, Diliman, Quezon City, at the Department of Medicinal Chemistry, University of Utah, Salt Lake City, UT, and at the Scripps Institution of Oceanography, La Jolla, CA.

Extraction and Isolation of Bolinaquinone. The sponge sample from Cape Bolinao was extracted repeatedly with MeOH−$CHCl_3$ solvent mixtures, and the concentrated extract was partitioned by Sephadex LH-20 column chromatography using MeOH−$CHCl_3$−TFA (1:1:0.01). Bolinaquinone (11.0 mg, 0.1%) eluted as an orange band after approximately one column volume.

The sponge (360 g) from Davao was homogenized in a blender and repeatedly extracted with MeOH. The dark violet MeOH extract was partitioned against hexane and then against $CHCl_3$. The $CHCl_3$ partition layer (369.8 mg) was chromatographed on a column of Sephadex LH-20, yielding the pure compound, bolinaquinone (111 mg) as the major metabolite.

Bolinaquinone: yellow glass; $[\alpha]_D$ −106° (*c* 0.4, $CHCl_3$); HREIMS *m*/*z* 358.2138, $C_{22}H_{30}O_4$ requires 358.2144; IR (neat) 3343, 2964, 2934, 2858, 1645, 1608, 1380, 1351, 1241, 1225, 1208, 1039, 840 799 cm^{-1}; UV (MeOH) λ 288 nm (log ϵ 3.70), 424 nm (log ϵ 2.16); UV (MeOH) upon addition of base λ 290 nm (log ϵ 3.67), 518 nm (log ϵ 2.70); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1.

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Supporting Information Available: ^{1}H, ^{13}C, HMQC, HMBC, and ROESY NMR spectra and INADEQUATE data for bolinaquinone (**6**) (11 pages). This material is contained in libraries on microfiche, immediately follows the 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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(19) Barrows, L. R.; Borchers, A. H.; Paxton, M. B. *Carcinogenesis* **1987**, *8*, 1853−1859.

(20) Copp, B. R.; Ireland, C. M.; Barrows, L. R. *J. Org. Chem*. **1991**, *56*, 4596−4597.