Hydroquinone Antioxidants from the Indian Ocean Tunicate *Aplidium savignyi*

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Three hydroquinone compounds have been isolated from the Indian Ocean tunicate *Aplidium savignyi*. Two are already known: geranylhydroquinone and the 2-(3-hydroxy-3,7-dimethyloct-6-enyl)-1,4-benzenediol as a new stereoisomer 2-(2*Z*)-(3-hydroxy-3,7-dimethyloct-2,6-dienyl)-1,4-benzenediol. The structures were determined using spectroscopic data (^{1}H NMR, ^{13}C NMR, MS, UV, and IR). These compounds are potential natural antioxidants.

Keywords: *Tunicates; Aplidium savignyi; hydroquinone compounds; geranylhydroquinone; natural antioxidants*

INTRODUCTION

If world fisheries production from capture remains stable at around 100 million tons over the next few decades, world aquaculture predictions indicate that future production will grow from 20 million tons in the year 2000 to around 60 million tons in the year 2025. In 1992 the Food and Agriculture Organization (FAO) published the first collection of statistics on world aquaculture production (FAO, 1992). If well-known fish species, crustaceans, and shrimps are to gain importance in the future, various kinds of bivalves such as mussel (*Mytilus edulis*) and oyster (*Crassostrea gigas*, *Ostrea edulis*) will represent a large industry in Southern Europe and will continue to grow. Algal industry coming, in particular, from brown and red seaweeds has preceded the development of marine invertebrates chemistry, either for food development or for biologically active research compounds mainly from sponges and more recently from tunicates which can be cultured.

In the course of our continuing study on biologically active metabolites from marine organisms (Combres et al., 1986; Aknin et al., 1992), we investigated the lipophilic extract of a tunicate from the Indian Ocean, *Aplidium savignyi*. Here we report the isolation and characterization of three antioxidant isoprenoid hydroquinones which can found on rocks at 15 m depth in the lagoon of Mayotte, Comoro Islands.

MATERIALS AND METHODS

Materials. The colonial tunicate *Aplidium savignyi* (Michaelsen, 1919) (Aplousobranchia order, Polyclinidae) was collected in the Indian Ocean at a depth of 15 m at prevoyante reef in the lagoon of Mayotte, Comoro Islands, northwest of Madagascar. Voucher samples are deposited at the Museum National d′Histoire Naturelle, Paris, France.

Compound Purifications. A sample of freeze-dried tunicate (180 g) was homogenized and extracted at room temperature with methanol−chloroform 1:2, v/v (500 mL × 4) to give a brown gum (1.3 g) after solvent evaporation. Part of the crude extract (300 mg) was partitioned between aqueous MeOH and *n*-hexane, and CCl_4 and $CHCl_3$. The CCl_4-soluble fraction (80 mg) was chromatographed in petroleum ether/ MeOH/$CHCl_3$ (2:1:1, v/v/v) over Sephadex LH-20 to give 15 mg of compound **2** (R_f = 0.9 in petroleum ether/ethyl acetate 2:3, v/v) and 1 mg of **3** (R_f = 0.85 in petroleum ether/ethyl acetate 2:3, v/v). The $CHCl_3$-soluble fraction (33 mg) was chromatographed over on Sephadex LH-20 with the petroleum ether/MeOH/$CHCl_3$ (1:1:1 v/v/v) to give 6 mg of geranylhydroquinone **1** (R_f = 0.9 in pure ethyl acetate).

General Experimental Procedures. IR spectra were recorded on a Nicolet 205 FT-IR spectrophotometer. UV spectra were recorded on a Perkin-Elmer spectrophotometer. LRMS and HRMS were recorded on a Fisons, Autospec Q instrument. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AMX-360 and ARX-500 spectrometers. All chemical shifts are reported with respect to TMS (δ_H = 0) and $CDCl_3$ (δ_C = 77). Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter using a 1-cm microcell.

RESULTS AND DISCUSSION

The colonial tunicate *A. savignyi* was extracted with chloroform−methanol yielding a brown gum which was solvent partitioned and then chromatographed over Sephadex LH-20 to give three compounds.

The major compound **1** (R_f = 0.9 in pure ethyl acetate) showed a molecular ion at *m*/*z* 246 in the EI mass spectra which indicated a formula of $C_{16}H_{22}O_2$ with six degrees of unsaturation. The IR spectrum revealed the presence of a phenolic function (3400, 1680, and 1610 cm^{-1}). This function was confirmed by the ions at *m*/*z* 123 (100%), 161, and 163 in the mass spectrum which are characteristic fragments of terpenoid derivatives of phenolic compounds. The ion at *m*/*z* 161 is particularly significant since it results from an intramolecular condensation of a phenolic OH group ortho to the first isoprene unit of the terpenoid chain. The ^{1}H NMR spectrum in $CDCl_3$ (Table 1) showed the following substructures: Ar−CH_2−CH=C(Me)− and −C−CH_2−

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Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Data Including H−H and H−C Correlations of Geranylhydroquinone 1[a]

C	δ ^{13}C (ppm)	H	δ ^{1}H (ppm)	J (Hz)	HMBC (H to C)	COSY (^{1}H−^{1}H)
1′	147.7				1	
2′	148.6				3′	
3′	116.2	3′	6.62 d	8.5	4′	3′→ 4′
4′	114.3	4′	6.52 dd	8.4, 2.85	3′	4′→3′,6′
5′	148.2				6′,4′	
6′	116.6	6′	6.55 d	2.5	1	6′→ 4′
1	30	1(×2)	3.24 d	8	2	1→ 2,4
2	120.8	2	5.24 t	7.1	1,10	2→ 1,(10)
3	128.1				1,2	
4	40	4(×2)	2.03 t	5.8	5,6,2	4→5
5	26.7	5(×2)	2.06 t	6.1	4	5→ 4,6
6	123.6	6	5 t	6.5	8,9,4	6→ 4/5,(8,9)
7	122				5,6,8,9	
8	26.6	8(×3)	1.63 s		6	8→(6)
9	18.1	9(×3)	1.54 s		6	9→(6)
10	15.5	10(×3)	1.7 s		2,4	10→(2)

[a] $CDCl_3$ Bruker ARX 500 instrument, chemical shifts refer to TMS ($\delta_H = 0$) for ^{1}H NMR and to $CDCl_3$ ($\delta_C = 77.6$) for ^{13}C NMR.

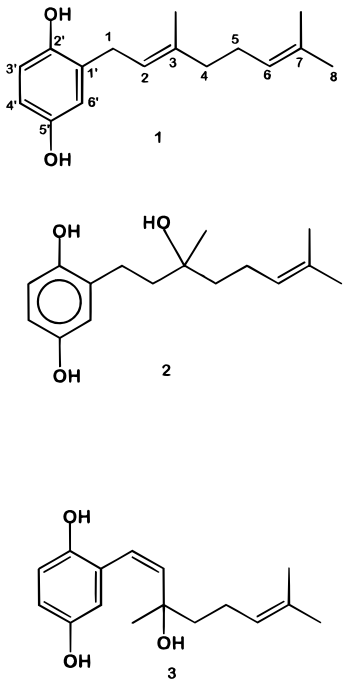

Figure 1. Structure and carbon numbering of isolated compounds from *A. savignyi*.

CH_2−CH=$C(Me)_2$. In addition there were peaks corresponding to three aromatic hydrogens, two with an ortho coupling (J = 8.5 Hz) centered at δ 6.62 (d) and 6.52 (dd) and one with the meta coupling (J = 2.5 Hz) centered at δ 6.55 (d). The ^{13}C NMR spectrum (Table 1) of this compound **1** showed peaks corresponding to 16 carbon atoms, in agreement with the MS data. All of these spectroscopic data are in agreement with a monoterpene structures with a C-1′, C-2′, and C-5′ trisubstituted aromatic ring bearing two hydroxyl groups and a monoterpenoid side chain. Compound **1** was therefore identified as 2-(2*E*)-(3,7-dimethyl-2,6-octadienyl)-1,4-benzenediol or geranylhydroquinone (Figure 1). The correlations observed in the ^{1}H−^{13}C long-range coupling by inverse detection (HMBC) for **1** are summarized in Table 1. These data allowed the unambiguous assignment of all carbon signals as well as the substitution pattern of the aromatic ring. If this compound was previously found in another tunicate, *Aplidium* sp. collected near Puerto Vallarta, Mexico (Fenical, 1974), our work completes the missing spectral from the work of Fenical (1974) and Reynolds and Rodriguez (1979) for this compound.

Table 2. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Data Including H−H Correlations of 2-(3-Hydroxy-3,7-dimethyloct-2,6-enyl)-1,4-benzene Diol 2[a]

C	δ ^{13}C (ppm)		H	δH (ppm)		J (Hz)	COSY (^{1}H−^{1}H)
1′	123.8	s					
2′	147.9	s					
3′	116.9	s	3′	6.7	d	8.3	3′→4′
4′	113.8	d	4′	6.56	dd	8.45, 2.9	4′→3′,6′
5′	148.9	s					
6′	116.6	d	6′	6.60	d		6′→4′
1	41.4	t	1a,1b	1.75			1a,1b→2a,2b
2	24.5	t	2a,2b	2.64			2a,2b→1a,1b
3	73.8	s					
4	41.7	t	4a,4b	1.56			4a,4b→5a,5b
5	22.9	t	5a,5b	2.07			5a,5b→4a,4b,6
6	124	d	6	5.1	t	6.7	6→5a,5b
7	132.4	s					
8	25.7	q	8(×3)	1.7	s		8→(6)
9	17.7	q	9(×3)	1.62	s		9→(6)
10	26.6	q	10(×3)	1.23	s		10→(2)

[a] $CDCl_3$ Bruker ARX 500 instrument, chemical shifts refer to TMS ($\delta_H = 0$) for ^{1}H NMR and to $CDCl_3$ ($\delta_C = 77.6$) for ^{13}C NMR.

Table 3. ^{1}H NMR (500 MHz) Data Including H−H Correlations of (2*E*)-(3-Hydroxy-3,7-dimethyloct-2,6-dienyl)-1,4-benzenediol[a]

H	δ ^{1}H (ppm)		J (Hz)	COSY (^{1}H−^{1}H)
3′	6.64	d	8.49	4′
4′	6.57	dd	8.56, 2.93	3′,6′
6′	6.47	d	2.81	4′
1	6.28	d	9.81	2
2	5.60	d	9.80	1
4a,4b	1.70	t	6.78	5a,5b
5a,5b	2.10 (2.04−2.15)	m		4a,4b
6	5.09	t	6.89	5a,5b, (8,9)
8	1.66	s		(6)
9	1.60	s		(6)
10	1.36	s		

[a] $CDCl_3$ Bruker ARX 500 instrument, chemical shifts refer to TMS ($\delta_H = 0$).

The structure of compound **2** ($C_{16}H_{24}O_3$, *m*/*z* 264 by EIMS), an optically inactive compound [UV absorption (EtOH, λ_{max} 290 nm, ϵ = 3000) and IR absorption (ν_{max} = 3400 cm^{-1} (OH), 1600 cm^{-1} (aromatic ring))], was established by comparison of its spectra properties with those of geranylhydroquinone **1** (Table 2). The ^{13}C NMR spectrum of **2** in $CDCl_3$ differed from that of **1** by replacement of two sp^2 carbon by one methylene carbon at δ 24.5 and one quaternary carbon bearing an hydroxyl group at δ 73.8. The COSY NMR experiment (Table 2) allowed for placement of this methylene group at C-2 and the quaternary alcohol carbon at C-3. This compound was identified as 2-(3-hydroxy-3,7-dimethyloct-6-enyl)-1,4-benzenediol (Figure 1) by comparison with the same compound isolated from the tunicate *Amaroucium multiplicatum* (Sato et al., 1989).

Compound **3** ($C_{16}H_{22}O_3$, *m*/*z* 262 by EIMS) was also an optically inactive compound with UV and IR spectra similar with that of compound **2**. Comparison of ^{1}H NMR spectrum (Table 3) of this compound show some similarities with that of 2-(2*E*)-(3-hydroxy-3,7-dimethyloct-2,6-dienyl)-1,4-benzenediol isolated from *A. multiplicatum* except for chemical shifts and coupling constants of the two ethylenic protons H-1 and H-2. They appeared as two cis olefinic proton signals (δ 5.60 ppm,

d, (H-2) and δ 6.28, d, (H-1), $J = 9.8$ Hz) in place of the trans olefinic signals ($J = 17.0$ Hz) [δ 6.07 ppm, d, (H-2) and δ 6.84, d, (H-1)]. This natural cis structure (Figure 1) is new to the best of our knowledge and should be a 2*H*-1-benzopyran (chromen) precursor of other antioxidant metabolites found in particular in *A. multiplicatum* tunicate (Sato et al., 1989).

Tunicates of this genus have been previously reported to synthesize geranylhydroquinone (Davidson et al., 1993). A broad range of marine organisms including algae (Praud et al., 1995; Mesguiche et al., 1997), tunicates (Fenical, 1974; Howard et al., 1979; Sato et al., 1989), soft corals (Bowden and Coll, 1981), and sponges (Djara et al., 1980) have been shown to contain terpene hydroquinones.

Some terrestrial phyla such as *Phacelia crenulata* contain geranylhydroquinone, in which this compound is the major contact allergen in trichome exudate (Reynolds and Rodriguez, 1979). From the heartwood of *Cordia elaegnoides*, five hydroquinone terpenoids including geranylhydroquinone were isolated (Manners, 1983).

Formation of an excess of lipid peroxide and its accumulation may contribute to cardiovascular diseases as arteriosclerosis, hypertension, and cardiac insufficiency (Sato et al., 1989). It has been reported that antioxidant compounds are shown inhibitors of lipid peroxide formation in rat liver microsomes and to soybean 15-lipoxygenase, and Rous sarcoma and mammary carcinoma could not be induced in test animals (Fenical, 1974). The occurrence of terpene hydroquinones in *A. savignyi* tunicate appears to be an interesting source of these compounds, known to be more potent antioxidants than standards such as α-tocopherol acetate or 2,6-di-*tert*-butyl-*p*-cresol (Sato et al., 1989).

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