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Desmethylubiquinone Q_2 from the Far-Eastern ascidian *Aplidium glabrum*: structure and synthesis

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Abstract—Two new diprenylquinones, glabruquinone A (desmethylubiquinone Q_2) having cancer preventive properties and its minor isomer Glabruquinone B were isolated from the ascidian *Aplidium glabrum*. Their structures have been elucidated by NMR and mass spectra and confirmed by synthesis.
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Polyprenylated 1,4-benzoquinones and hydroquinones such as ubiquinones, plastoquinones, and tocopherols are widespread in plants and animals, in which they play important roles in electron transport, photosynthesis, and as antioxidants.[1,2] Naturally occurring marine prenyl benzoquinones and hydroquinones having a terpenoid portion ranging from one to nine isoprene units and differing structurally from the above-mentioned groups have been described from marine organisms, and especially from brown algae of the order Fucales, sponges, and ascidians. Many algae contain tetraprenyl, triprenyl-, and diprenylhydroquinones.[3,4] Sponges[5–8] contain linear unsubstituted polyprenylated hydroquinones and benzoquinones with longer side chains and moderate antimicrobial activity[9] as well as ATPase inhibiting sulfated prenylhydroquinones.[10–12] Ascidians of the genus *Aplidium* have previously yielded about a dozen prenylated quinones[13–16] including the most simple of them, monoprenylbenzoquinone.[14]

New diprenylquinones, glabruquinone A **1** [yellow oil, HREIMS *m/z* 304.1655 $[M]^+$, calcd for $C_{18}H_{24}O_4$ 304.1675] and a minor isomer, glabruquinone B **2** [yellow oil, HREIMS *m/z* 304.1649 $[M]^+$, calcd for $C_{18}H_{24}O_4$ 304.1675] from the ethanolic extracts of *Aplidium glabrum* were isolated and purified by repeated HPLC on a silica gel column using a hexane–ethyl acetate system (6:1; a total yield of 0.09% on wet weight). The ratio of **1** to **2** was 95:5. The EIMS showed the typical peak for quinones (M^++2) at *m/z* 306 along with the molecular ion peak at *m/z* 304. The UV and IR spectra of **1** and **2** showed a maximum absorption at 264 nm (ε = 15,000) and IR absorption bands at 1675, 1657, 1603 cm^{-1} corresponding to the *p*-benzoquinone moiety. The ^1H NMR spectrum of **1** was similar to that of verapliquinone A from the *Aplidium* sp.[13] and differed in having an additional singlet signal at 4.02 ppm, which is typical for a MeO group. Quartets at 61.2 and 61.3 ppm in the ^{13}C NMR spectrum indicated the presence of two methoxyls in **1**. The attachment of a terpenoid fragment to C-5 of the benzoquinone moiety and methoxyls at the 2,3-positions were established by a detailed inspection of the NMR spectra, including ^1H–^1H-COSY, NOESY, and HMBC (see Scheme 1). Especially important were the multiplicity of H-6 (a narrow triplet at 6.34 ppm) and the cross peaks corresponding to H-6/H-1′ allylic coupling in ^1H–^1H-COSY and H-6/H-1′ interaction in NOESY. The signals of two trisubstituted double bonds, three methyl groups attached to these bonds and three methylene groups in the ^{13}C NMR spectrum clearly showed the presence of a diprenyl side chain. A comparison of the NMR spectra of **1** and **2** showed that glabruquinones A and B contain geranyl

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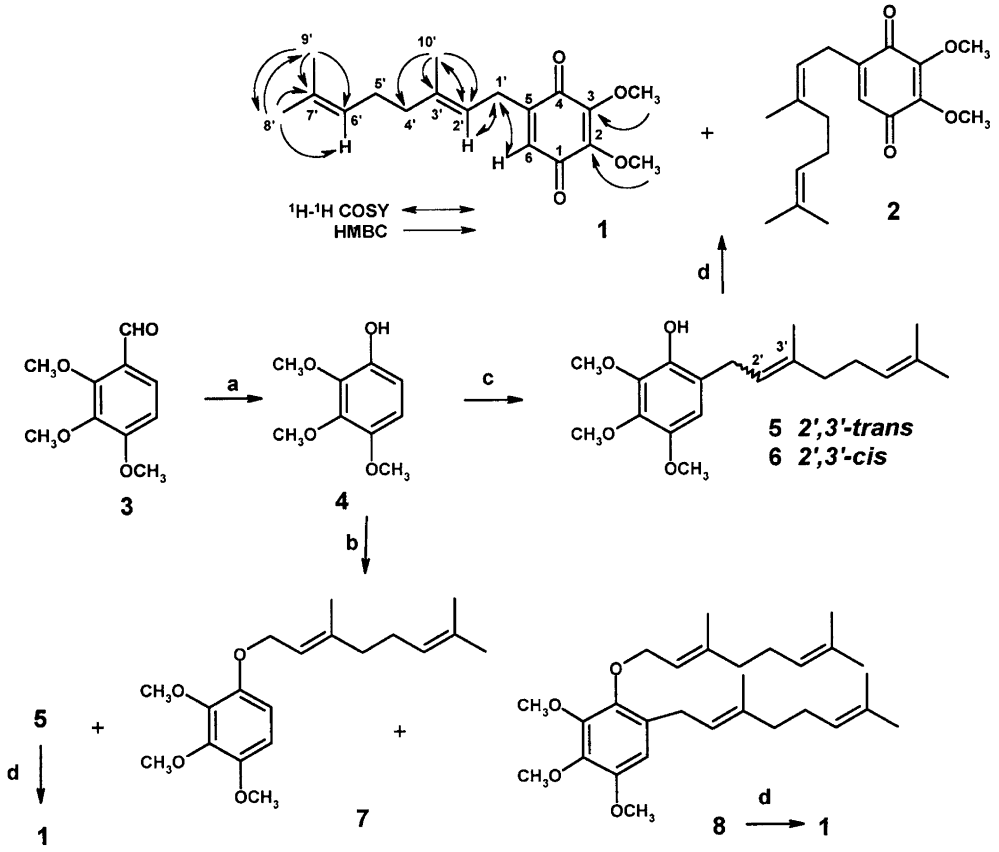

Scheme 1. Structures and synthesis of **1** and **2**. Reagents and conditions: (a) H_2O_2, H_2SO_4, MeOH, 2 h; (b) geranyl bromide, Na, ether (or benzene), 25 h, reflux; (c) geraniol, $BF_3{\cdot}Et_2O$, ether, 18 h; (d) CAN, CH_3CN, H_2O, 1 h.

and neryl types of side chains, respectively (Table 1). In fact, these compounds differ from each other by the configurations of the C2′, C3′-double bond as was established on the basis of the chemical shifts of C-10′. In the sterically more congested *E*-isomers, this signal was observed at a higher field when compared with the *Z*-isomers.[17] The C-10′ chemical shifts of **1** and **2** differ significantly, 16.2 and 22.8 ppm in spectra of **1** and **2**, respectively. Note that neryl derivatives like **2** are very rare in nature whilst the majority of natural linear benzoquinones from marine organisms are of the geranyl type.

Table 1. ^{13}C- and ^{1}H NMR data for **1** and **2** in $CDCl_3$ at 75.5 and 300 MHz, respectively

Atom	**1**		**2**	
	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)
1	184.38* s		184.38* s	
2	144.91** s		144.91** s	
3	145.16** s		145.16** s	
4	184.54* s		184.54* s	
5	146.92 s		146.92 s	
6	130.45 d	6.34 t, J = 1.7, 1H	130.45 d	6.34 t, J = 1.7, 1H
1′	27.17 t	3.10 dd, J = 7.3, 1.7, 2H	27.27 t	3.11 br d, J = 7.1, 1.2, 2H
2′	117.78 d	5.13 t sext, J = 7.3, 1.2, 1H	117.78 d	5.13 m, 1H
3′	140.17 s		140.17 s	
4′	39.72 t	2.08 m, 2H	32.01 t	2.04 m, 2H
5′	26.52 t	2.09 m, 2H	26.52 t	2.04 m, 2H
6′	123.98 d	5.08 t sept, J = 6.8, 1.2, 1H	123.98 d	5.07 m, 1H
7′	1131.95 s		131.95 s	
8′	25.77 q	1.70 d, J = 1.2, 3H	25.77 q	1.66 br d, J = 1.2, 3H
9′	17.79 q	1.60 br s, 3H	17.79 q	1.59 d, J = 1.2, 3H
10′	16.2 q	1.62 d, J = 1.2, 3H	22.76 q	1.75 q, J = 1.2, 3H
OCH_3	61.3 q; 61.2 q	4.00 s; 4.02 s	61.3 q; 61.2 q	4.00 s; 4.02 s

*,**—Values can be interchanged.

The structures **1** and **2** were confirmed by synthesis from commercially available 2,3,4–trimethoxybenzaldehyde **3** using the route shown in Scheme 1. The corresponding trimethoxyphenol **4** was obtained by the previously described method.[18] The base-catalyzed alkylation of phenol **4** with geranyl bromide led to geranylphenol **5**[19] (18% total yield) purified by HPLC. The yield was less than in other reported similar transformations.[9,20,21] Modification of the reaction conditions did not increase the yield, but led to the formation of *O*-alkylated side products (**7**, **8**).[22]

A higher yield (55%) was achieved using the conditions c, when a partial isomerization of **5** into **6** takes place (85:15). Oxidative demethylation of geranyl phenol **5** by CAN yielded glabruquinone **1** (33%), which was identical to the natural product (comparison of NMR spectra and biological activities). A similar oxidative demethylation of the mixture of **5** and **6** resulted in a mixture of quinones **1** and **2**. Synthetic **2** was separated by HPLC from this mixture and identified with natural glabruquinone B by comparison of their NMR spectra.

Recently, **1** was synthesized from 2,3-dimethoxybenzaldehyde in five steps. However, the total yield of **1** was not given.[23,24]

In our opinion, of special interest is the observation that glabruquinone A is structurally more closely related to the ubiquinones than other linear polyprenyl quinones from sponges and ascidians. Because glabruquinone A does not contain a methyl group in the quinoid moiety in contrast with ubiquinones, **1** can be named desmethylubiquinone Q_2. Glabruquinone A showed cancer preventive activity in the anchorage-independent transformation assay against mouse JB6 P^+ Cl 41 cells transformed with epidermal growth factor, inhibiting the number of colonies with an IC_{50} ($INCC_{50}$) of 7.3 μM. Its $INCC_{50}$ were of 12.7, 17.5, and 50.5 μM against HCT-116, MEL-28, and HT-460 human tumor cells, respectively. At 10 μM concentration, **1** increased the UVB-induced p53-dependent transcriptional activity of JB6 P^+ Cl 41 cells 2.5 times as much. Results of the studies on the biological activities of **1** will be published in detail elsewhere.

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19. Compound **5**: pale yellow oil, IR (CCl_4): 3541, 2935, 1498, 1464 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ: 6.44 (s, 1H), 5.45 (s, 1H), 5.31 (m, 1H), 5.11 (m, 1H), 3.95 (s, 3H), 3.86 (s, 6H), 3.79 (s, 3H), 3.31 (br d, J = 7.1, 2H), 2.07 (m, 4H), 1.72 (d, J = 1.2, 3H), 1.67 (d, J = 1.2, 3H), 1.60 (d, J = 0.7, 3H); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ: 16.12 (q, C-10′), 17.66 (q, C-9′), 25.66 (q, C-8′), 26.73 (t, C-5′), 27.90 (t, C-1′), 39.75 (t, C-4′), 56.62 (q, OMe), 60.89 (q, OMe), 61.16 (q, OMe), 108.30 (d, C-6), 121.61 (s, C-5), 121.98 (d, C-2′ or C-6′), 124.20 (d, C-6′ or C-2′), 128.89 (s, C-4), 131.41 (s, C-7′), 136.59 (s, C-1), 140.04 (s, C-3′), 140.81 (s, C-2 or C-3), 146.14 (s, C-3 or C-2).
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22. Compound **7**: yellow oil, IR ($CDCl_3$) 2937, 1491, 1435 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ: 6.61 (d, J = 9.0), 6.55 (d, J = 9.0, 1H), 5.50 (m, 1H), 5.09 (m, 1H), 4.53 (d, J = 6.6, 2H), 3.91 (s, 3H), 3.90 (s, 3H), 3.82 (s, 3H), 2.08 (m, 4H), 1.60 (d, J = 1.0, 3H), 1.68 (d, J = 1.2, 3H), 1.71 (d, J = 1.2, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ: 16.62 (q, C-10′), 17.71 (q, C-9′), 25.69 (q, C-8′), 26.33 (t, C-5′), 39.54 (t, C-4′), 56.41 (q, OMe), 61.14 (q, OMe), 61.22 (q, OMe), 66.66 (t, C-1′), 106.45 (d, C-5 or C-6), 109.05 (d, C-6 or C-5), 120.11 (d, C-2′ or C-6′), 123.88 (d, C-6′ or C-2′), 131.74 (s, C-7′), 140.59 (s, C-3′), 143.20 (s, C-1 or C-2, or C-3, or C-4), 144.21 (s, C-2, or C-1, or C-3, or C-4), 146.94 (s, C-3, or C-1, or C-2 or C-4), 147.94 (s, C-4, or C-1, or C-2, or C-3). Compound **8**: yellow oil, IR ($CDCl_3$) 2935, 1489, 1459, 1434, 1415 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ: 6.45 (s, 1H), 5.55 (m, 1H), 5.28 (m, 1H), 5.10 (m, 2H), 4.46 (d, J = 7.1, 2H), 3.93 (s, 3H), 3.87 (s, 3H), 3.81 (s, 3H), 3.33 (d, J = 7.3, 2H), 2.08 (m, 8H), 1.72 (d, J = 1.2, 3H), 1.70 (d, J = 1.2, 3H), 1.69 (d, J = 1.0, 3H), 1.67 (d, J = 1.0, 3H), 1.61 (d, J = 0.7, 3H), 1.60 (d, J = 0.7, 3H).

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