New Prenylhydroquinone Glycosides from *Phagnalon rupestre*

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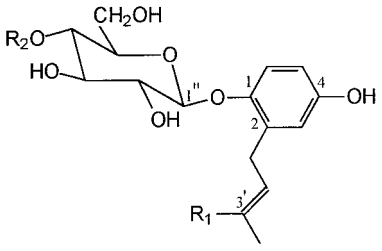
Three new hydroquinone glycosides were isolated from the MeOH extract of the aerial parts of *Phagnalon rupestre*. Their structures were elucidated as 1-*O*-*β*-glucopyranosyl-1,4-dihydroxy-2-(3′,3′-dimethylallyl)-benzene (**1**), 1-*O*-*β*-glucopyranosyl-1,4-dihydroxy-2-(3′-hydroxymethyl-3′-methylallyl)benzene (**2**), and 1-*O*-(4″-*O*-caffeoyl)-*β*-glucopyranosyl-1,4-dihydroxy-2-(3′,3′-dimethylallyl)benzene (**3**) by spectroscopic methods.

The genus *Phagnalon* (Asteraceae) is represented by 30 Eurasiatic species, six of which are typical of the European Mediterranean region.[1,2] The phytochemistry of *Phagnalon rupestre* is not well known, although some studies have revealed the presence of flavonoids such as apigenin, apigenin 7-glucoside and luteonin,[3] quinones,[4] fatty substances such as *n*-octadecane, trimethyldodecane, and other *n*-paraffin components, and essential oil constituents such as α-terpineol, thymol, and hexahydrofarnesyl acetone.[5,6]

The bark of *Phagnalon rupestre* is widely used to induce deliberate burns for the healing of various ailments,[7] and some extracts of this plant have been shown to possess antimicrobial properties.[8] On the other hand, the naturally occurring quinones present in *Phagnalon* sp. can act as contact allergens.[4]



	R_1	R_2
(**1**)	CH_3	H
(**2**)	CH_2OH	H
(**3**)	CH_3	caffeoyl

Air-dried and powdered aerial parts of *P. rupestre* were macerated with MeOH, and the resulting extract was liquid−liquid partitioned with solvents of increasing polarity. After gel filtration followed by vacuum liquid chromatography (VLC) and low-pressure liquid chromatography three compounds (**1**−**3**) were isolated from the ethyl acetate fraction. The least polar of them, compound **1**, was analyzed for $C_{17}H_{24}O_7$ (FABMS) and showed ions at *m*/*z* 340 $[M]^+$ and *m*/*z* 363 corresponding to $[M + Na]^+$. The ^1H NMR spectrum displayed the signal pattern typical of an alkylhydroquinone.[9] Three aromatic protons (H-3, H-5, and H-6) exhibited an ABX system (*δ* 6.55, 6.52, and 6.98, respectively), one oleofinic proton at *δ* 5.30, two aliphatic protons at *δ* 3.43, and two methyl groups at *δ* 1.70 and 1.73 arising from a prenyl residue. An anomeric proton at *δ* 4.71 and two double doublets at 3.66 and 3.85 indicated the presence of a *β*-glucopyranose residue (see Table 1). This was confirmed by analysis of the ^{13}C NMR spectrum, which exhibited 17 carbon signals, 11 corresponding to the aglycone and six to the sugar moiety. A NOE experiment showed correlations between H-1′ and H-1″ and between H-6 and H-1″, thus making it possible to confirm the locations of the prenyl and sugar residues. The structure of **3** was elucidated to be 1-*O*-*β*-glucopyranosyl-1,4-dihydroxy-2-(3′,3′-dimethylallyl)benzene. Full assignments of ^1H and ^{13}C NMR signals were accomplished using NOE experiments (Tables 1 and 2).

Compound **2** ($C_{17}H_{24}O_8$) possessed one more oxygen than compound **1**. The ^{13}C NMR spectrum of **2** was similar to that of **1**, with the only difference arising from the appearance of a $-CH_2OH$ carbon signal at *δ* 68.8 in **2** together with the absence of one of the two signals corresponding to a methyl group in **1**. This oxymethylene group was placed at C-4′, as shown by the upfield shift of C-5′ (*δ* 13.9) and the presence of a carbinolic proton signal at *δ* 3.85 (Tables 1 and 2). Thus **2** was identified as 1-*O*-*β*-glucopyranosyl-1,4-dihydroxy-2-(3′-hydroxymethyl-3′-methylallyl)-benzene.

Compound **3** gave an ion in the FABMS at *m*/*z* 525 $[M + Na]^+$, suggesting the molecular formula $C_{26}H_{30}O_{10}$. An intensive fragment ion at *m*/*z* 325 indicated the loss of a caffeoyl moiety. The ^1H spectrum showed an ABX pattern proton signal at *δ* 7.05, 6.76, and 6.93 and two olefinic protons at *δ* 6.27 and 7.57. The ^{13}C NMR spectrum showed one carbonilic signal at *δ* 168.5, two olefinic carbons at *δ* 114.6 and 147.6, and six aromatic carbons, two of them hydroxylated (*δ* 149.6 and 146.7). These signals indicated one caffeoyl moiety. Comparison of the sugar signals in the ^{13}C NMR spectrum of **3** with data on compounds **1** and **2** allowed identification of a 4″-*O* substitution on the glucose residue. The C-4 signal of the sugar at *δ* 72.2 was 0.8 ppm downfield from that of compound **1**, and C-3 and C-5 were shifted upfield (Tables 1 and 2). The structure of **3** was identified as 1-*O*-(4″-*O*-caffeoyl)-*β*-glucopyranosyl-4-hydroxy-2-(3′,3′-dimethylallyl)benzene.

To our knowledge, this is the first report of the occurrence of the simplest dimethylallyl-hydroquinone in glycosidic form. The sugar moiety appears to stabilize the molecule, indirectly preventing dehydrogenation to give the analogue prenyl-benzoquinone, which had previously been identified in the plant. Closely related to *Phagnalon* phenolics are some biologically active hydroquinone deriva-

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Table 1. ^{1}H NMR Spectral Data (δ) of Compounds **1**, **2**, and **3** (CD_3OD; 400 MHz)[a]

H	1	2	3
3	6.55 (1H, d, J = 2.8)	6.47 (1H, d, J = 2.8)	6.56 (1H, d, J = 2.7)
5	6.52 (1H, dd, J = 8.6, 2.8)	6.43 (1H, dd, J = 8.8, 2.8)	6.52 (1H, dd, J = *b*, 3)
6	6.98 (1H, d, J = 8.4)	6.88 (1H, d, J = 8.4)	7.01 (1H, d, J = 8.4)
1′	3.43 (2H, d, J = 7.6)	3.32 (2H, d, J = 7.6)	3.57 (2H, d, J = 7.5)
2′	5.30 (1H, t, J = 7.6)	5.47 (1H, t, J = 7.2)	5.30 (1H, t, J = 7.5)
4′	1.70 (3H, s)	1.64 (3H, s)	1.70 (3H, s)
5′	1.73 (3H, s)	3.85 (2H, s)	1.72 (3H, s)
1″	4.71 (1H, d, J = 7.6)	4.64 (1H, d, J = 7.2)	4.79 (1H, d, J = 7.5)
2″, 3″	3.32−3.37 (2H, m)	3.24−3.36 (4H, m)	3.31−3.37 (2H, m)
4″	3.29 (1H, m)	3.24−3.36 (4H, m)	3.30 (1H, m)
5″	3.40 (1H, m)	3.24−3.36 (4H, m)	3.53 (1H, m)
6″	3.66 (1H$_a$, dd, J = 12, 4.8)	3.57 (1H$_a$, dd, J = 11.6, 5.2)	3.62−3.75 (2H, m)
	3.85 (1H$_b$, dd, J = 11.4, 2)	3.75 (1H$_b$, dd, J = 11.6, 1.6)	
2‴			6.27 (1H, d, J = 15.9)
3‴			7.57 (1H, d, J = 15.9)
5‴			7.05 (1H, d, J = 1.9)
8‴			6.76 (1H, d, J = 7.8)
9‴			6.93 (1H, broad doublet)

[a] Coupling constants (Hz) are in parentheses. [b] Overlapped.

Table 2. ^{13}C NMR Spectral Data (δ) of Compounds **1**, **2**, and **3** (CD_3OD; 100 MHz)

C	1	2	3
1	150.0	150.1	150.0
2	133.9	133.4	134.0
3	113.8	114.0	113.8
4	153.5	153.6	153.6
5	116.8	118.4	116.8
6	118.3	117.0	118.5
1′	29.1	29.0	29.1
2′	123.9	125.2	123.9
3′	133.2	136.6	133.2
4′	17.9	68.8	17.9
5′	25.9	13.9	25.9
1″	103.9	103.9	103.9
2″	75.0	75.0	75.2
3″	78.1	78.1	75.9
4″	71.4	71.3	72.2
5″	77.9	77.9	76.0
6″	62.5	62.5	62.2
1‴			168.5
2‴			114.6
3‴			147.6
4‴			127.6
5‴			115.1
6‴			149.6
7‴			146.7
8‴			116.4
9‴			123.0

tives bearing polyisoprene, often hydroxylated, chains of variable length that have been reported from marine sponges, e.g., *Ircinia* sp.[10] Avarol from *Disidea avara*[11] and siphonodictyal from *Siphonodyction coralliphagum*[12] possess a hydroquinone nucleus bound to a bicyclic terpenoid moiety. Other related compounds such as the heptadecenylhydroquinones from *Tapirira guaianensis* (Anacardiaceae)[9] and glaziovianol from *Auxemma glazioviana* (Boraginaceae)[13] have been identified in higher plants.

Experimental Section

General Experimental Procedures. NMR spectra were run on a 400 MHz (δ, ppm) Brucker AMX instrument in CD_3OD. FABMS were carried out in a VG Auto Spec (Fisons). Optical rotations were measured with a Perkin-Elmer 241 polarimeter. UV spectra were obtained on a Shimadzu UV-2101 PC spectrophotometer. IR spectra were obtained using KBr disks on a Mattson Satellite FTIR spectrophotometer. Analytical TLC was carried out on Merck Si gel F_{254} and RP-18 aluminum sheets visualized with 1% sulfuric acid-anisaldehyde.

Plant Material. The flowering aerial parts of *Phagnalon rupestre* (L.) DC. were collected in Sierra de Corbera (Valencia, Spain). A voucher specimen (DF7) of the plant is kept in the Department of Pharmacology, University of Valencia.

Extraction and Isolation. The air-dried, powdered aerial part of *P. rupestre* (660 g) was extracted by stirring with MeOH (4 × 2 L) for 24 h at room temperature. The solvent was removed under reduced pressure. The methanolic extract (100.0 g) was suspended in H_2O and fractionated with EtOAc to obtain the extract (17.8 g). This EtOAc extract was filtered over Sephadex LH-20 with MeOH to yield 12 fractions. The fourth fraction (3.8 g) was subjected to VLC on a Si gel 60 (Merck) column and eluted with CH_2Cl_2−MeOH mixtures and MeOH. The fraction eluted with CH_2Cl_2−MeOH (9:1) (fraction IV-6) was rechromatographed on a Lobar B column of RP-18 (Merck) with MeOH−H_2O (6:4), and **1** (1.2 g) was obtained from the fifth fraction. Fraction IV-7 was chromatographed using Si gel 60 with CH_2Cl_2−MeOH (9:1) to yield **2** (8 mg). Fractionation of the sixth fraction (0.9 g) over Si gel with CH_2Cl_2−MeOH mixtures followed by purification on a Lobar B column of Si gel (Merck) with CH_2Cl_2−MeOH (95:5) gave **3** (50 mg).

1-*O*-*β*-Glucopyranosyl-1,4-dihydroxy-2-(3′,3′-dimethylallyl)benzene (1): amorphous powder, $[\alpha]_D$ −58° (MeOH; *c* 0.1); UV λ_{max} (MeOH) 289, 232 nm, (+NaOH) 304, 243 nm; IR ν_{max} cm^{-1}: 3400, 2973, 2928, 2907, 1644, 1606; ^{1}H and ^{13}C NMR, Tables 1 and 2; FABMS *m/z* [M + Na]$^+$ 363, [M]$^+$ 340, [glucopyranoside]$^+$ 180.

1-*O*-*β*-Glucopyranosyl-1,4-dihydroxy-2-(3′-hydroxymethyl-3′-methylallyl)benzene (2): amorphous powder, $[\alpha]_D$ −34° (MeOH; *c* 0.1); UV λ_{max} (MeOH) 293, 231 nm, (+NaOH) 306, 226; IR ν_{max} cm^{-1}: 3400, 2926, 2856, 1731, 1637; ^{1}H and ^{13}C NMR, Tables 1 and 2; FABMS *m/z* [M + Na]$^+$ 379, [M]$^+$ 356.

1-*O*-(4″-*O*-Caffeoyl)-*β*-glucopyranosyl-1,4-dihydroxy-2-(3′,3′-dimethylallyl)benzene (3): amorphous powder, $[\alpha]_D$ −26° (MeOH; *c* 0.1); UV λ_{max} (MeOH) 331, 242 nm, (+NaOH) 380, 311, 238 nm; IR ν_{max} cm^{-1}: 3400, 2925, 2856, 1694; ^{1}H and ^{13}C NMR, Tables 1 and 2; FABMS *m/z* [M + Na]$^+$ 525, [M]$^+$ 502, [M − caffeoyl + 2H]$^+$ 325.

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