PERGAMON

Phytochemistry 59 (2002) 857–860

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Phenolic glycosides from *Phagnalon rupestre*

Luis Góngora[a], Salvador Máñez[a], Rosa M. Giner[a], M. Carmen Recio[a], Alexander I. Gray[b], José-Luis Ríos[a,*]

[a]*Departament de Farmacologia, Facultat de Farmàcia, Universitat de València, Avinguda Vicent Andrés Estellés s/n, 46100 Burjassot, València, Spain*
[b]*Department of Pharmaceutical Sciences, University of Strathclyde, SIBS, 27 Taylor Street, Glasgow G4 ONR, Scotland, UK*

Received 13 September 2001; received in revised form 18 December 2001

Abstract

Analysis of the butanol-soluble fraction from the methanolic extract of the aerial parts of *Phagnalon rupestre* (Asteraceae) has led to the isolation of seven phenolic compounds. Three have been identified on the basis of their NMR spectra as new natural compounds: the lignan 7,7′-bis-(4-hydroxy-3,5-dimethoxyphenyl)-8,8′-dihydroxymethyl-tetrahydrofuran-4-*O*-β-glucopyranoside (**1**), the prenylhydroquinone glycoside 1-*O*-β-glucopyranosyl-1,4-dihydroxy-2-(3′-hydroxy-3′-methylbutyl) benzene (**2**) and the acetophenone glycoside 12-*O*-β-glucopyranosyl-9β,12-dihydroxytremetone (**3**). The known flavonoids apigenin-7-*O*-β-glucoside, luteolin-7-*O*-β-glucoside, luteolin-7-*O*-β-glucuronide and the acetophenone picein were also isolated.

Keywords: *Phagnalon rupestre*; Asteraceae; Lignan glycoside; Prenylhydroquinone glycoside; Acetophenone glycoside

1. Introduction

Phagnalon is one of the Euro-Mediterranean genera of the worldwide distributed subtribe Gnaphaliinae (tribe Inuleae, Asteraceae). *Phagnalon rupestre* (L.) DC. was recently characterized by the presence of new prenylhydroquinone glycosides (Góngora et al., 2001). Continuing phytochemical investigation on this species led to the isolation and identification of seven phenolic derivatives, three of them being here reported as new natural compounds.

2. Results and discussion

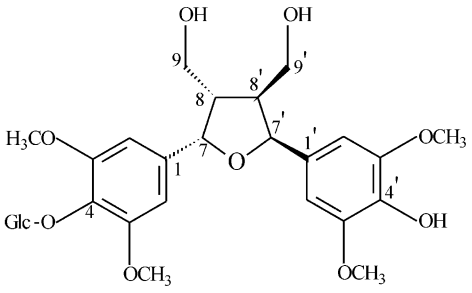
The phenolic compounds were obtained directly by precipitation or by gel filtration from the butanolic fraction of the methanolic extract of the aerial parts of *P. rupestre* followed by purification on silica gel and reverse phase liquid chromatography.

The ^{1}H and ^{13}C NMR spectra of **1** (Table 1) along with 2-D experiments (COSY, HMBC, HMQC) revealed the presence of a β-glucopyranoside with an aglycone portion consisting of 22 carbons. The latter portion gave proton and carbon shifts compatible with a bis-(dimethoxyphenylpropanoid) skeleton and was closely similar to those of the lignan icariol-A_2 previously encountered in *Epimedium sagittatum* (Berberidaceae) (Matsushita et al., 1991), except that most of the signals for the lignan skeleton of the latter were duplicated. The most striking differences were observed in the chemical shifts of the carbons of the two aromatic rings of **1**. For example, the significant differences between C-1 (139.9 ppm) and C-1′ (133.4 ppm) and between C-3/C-5 (154.8 ppm) and C-3′/C-5′ (149.7 ppm) indicated attachment of the glucose residue at C-4 (Della Greca et al., 1998). The protons at C-7 (C-7′) and C-8 (C-8′) appear to be *cis* orientated because the coupling constant between H-7 (H-7′) and H-8 (H-8′) is 4 Hz, which correspond to an angle of 25° according to the Insight II software applied. This configuration is opposite to that of the same protons in icariol A_2 for which *J* values (*trans*) are 8 Hz (Matsushita et al., 1991). This structure was confirmed by HREIMS, which gave a base peak at *m*/*z* 418 attributable to $C_{22}H_{26}O_8$ [M-glucose]$^{+}$. So, this compound was identified as 7,7′-bis-(4-hydroxy-3,5-dimethoxyphenyl)-8,8′-dihydroxymethyl-tetrahydrofuran-4-*O*-β-glucopyranoside.

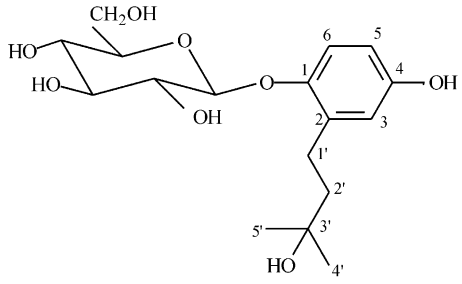
Compound **2**, analyzed for $C_{17}H_{26}O_8$ by means of FABMS, showed a molecular ion at *m*/*z* 359 and 381 corresponding to $[M+Na]^{+}$. The ^{1}H NMR spectrum displayed the signal pattern similar to that of a prenylhydroquinone previously identified in the EtOAc fraction

* Corresponding author. Tel./Fax: +34-96-3864973.
E-mail address: riosj1@uv.es (J.-L. Ríos).

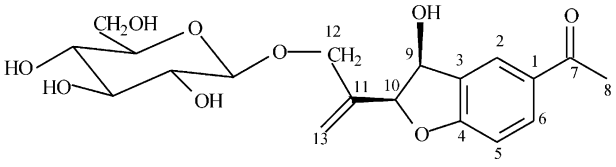
0031-9422/02/$ - see front matter
PII: S0031-9422(02)00011-0



1



2



3

of the methanol extract of this plant (Góngora et al., 2001). It showed three protons (H-3, H-5 and H-6) with an ABX system (6.56, *d*, $J=2.8$ Hz; 6.52, *dd*, $J=8.8$, 3.2 Hz; and 7.00, *d*, $J=8.8$ Hz). Four aliphatic protons at δ 2.68 (2H, *m*) and 1.69 (2H, *m*), and two methyl groups at δ 1.24 and 1.25, derived from a prenyl chain. The presence of an hexose residue was deduced from the anomeric proton signal at δ 4.68 (*d*, $J=8$ Hz) and two double doublets at δ 3.86 ($1H_b$, $J=12.0$, 2.0 Hz) and 3.67 ($1H_a$, $J=12.0$, 4.8 Hz). The ^{13}C NMR spectrum confirmed this structure, which showed six aromatic signals, two of them hydroxylated (δ 153.6 and 150.3), five aliphatic carbons, one of these quaternary and hydroxylated at δ 71.5, and six which corresponded to the sugar and matched with those of a β-glucopyranosyl moiety. Full assignments of ^1H and ^{13}C NMR signals were accomplished using NOE experiments (Table 2). The structure of **2** was elucidated to be 1-*O*-β-glucopyranosyl-1,4-dihydroxy-2-(3′-hydroxy-3′-methylbutyl) benzene.

Compound **3**, analyzed for $C_{19}H_{24}O_9$ by means of FABMS, showed a molecular ion at *m*/*z* 397 and 419

Table 1
^1H and ^{13}C NMR spectral data of compound **1** (CD_3OD; 400 and 100 MHz)

	^1H	^{13}C
1	–	139.9
2	6.70 (1H, *s*)	105.2
3	–	154.8
4	–	135.9
5	–	154.8
6	6.70 (1H, *s*)	105.2
7	4.75 (1H, *d*, $J=4.0$ Hz)	87.6
8	3.12 (1H, *m*)	55.9[a]/55.7[a]
9	3.86–3.92 ($1H_a$, *m*)	
	4.24–4.30 ($1H_b$, *m*)	73.2[a]/73.3[a]
OCH_3	3.83 (3H, *s*)	57.2[a]/57.4[a]
OCH_3	3.83 (3H, *s*)	57.2[a]/57.4[a]
1′	–	133.4
2′	6.65 (1H, *s*)	104.8
3′	–	149.7
4′	–	136.6
5′	–	149.7
6′	6.65 (1H, *s*)	104.8
7′	4.70 (1H, *d*, $J=4.0$ Hz)	88.0
8′	3.12 (1H, *m*)	55.7[a]/55.9[a]
9′	3.86–3.92 ($1H_a$, *m*)	
	4.24–4.30 ($1H_b$, *m*)	73.2[a]/73.3[a]
OCH_3	3.84 (3H, *s*)	57.2[a]/57.4[a]
OCH_3	3.84 (3H, *s*)	57.2[a]/57.4[a]
1″	4.76 (1H, *d*, $J=7.2$ Hz)	105.7
2″	3.47 (1H, *m*)	76.1
3″	3.40 (1H, *t*, $J=7.2$ Hz)	78.2
4″	3.41 (1H, *t*, $J=7.2$ Hz)	71.7
5″	3.19 (1H, *m*)	78.7
6″	3.66 ($1H_a$, *dd*, $J=12.0$, 4.8 Hz)	
	3.77 ($1H_b$, *dd*, $J=12.0$, 2.4 Hz)	62.9

[a] Interchangeable.

Table 2
^1H and ^{13}C NMR spectral data of compound **2** (CD_3OD; 400 and 100 MHz)

	^1H	^{13}C
1	–	153.6
2	–	135.2
3	6.56 (1H, *d*, $J=2.8$ Hz)	113.9
4	–	150.3
5	6.52 (1H, *dd*, $J=8.8$, 3.2 Hz)	117.2
6	7.00 (1H, *d*, $J=8.8$ Hz)	118.5
1′	1.69 (2H, *m*)	26.1
2′	2.68 (2H, *m*)	45.2
3′	–	71.5
4′	1.24 (3H, *s*)	28.9
5′	1.25 (3H, *s*)	29.4
1″	4.68 (1H, *d*, $J=8.0$ Hz)	104.2
2″	3.34–3.37 (2H, *m*)	75.1
3″	3.34–3.37 (2H, *m*)	78.1
4″	3.29 (1H, *m*)	71.6
5″	3.40 (1H, *m*)	78.0
6″	3.67 ($1H_a$, *dd*, $J=12.0$, 4.8 Hz)	
	3.86 ($1H_b$, *dd*, $J=12.0$, 2.0 Hz)	62.6

corresponding to $[M+Na]^+$. The ^1H NMR spectrum (Table 3) which displayed a signal pattern of a substituted acetophenone, presented three aromatic protons (H-2, H-5 and H-6) with a typical ABX system (δ 7.99, *d*, $J=2.0$ Hz; 6.99, *d*, $J=8.5$ Hz; and 7.87, *dd*, $J=8.4$, 2.0 Hz). Two heterocyclic protons (H-9 and H-10), were seen at δ 5.21 (*d*, $J=6.0$ Hz) and 5.03 (*d*, $J=6.0$ Hz), respectively, and one methylene group as an ABq system δ 4.23 and 4.40 ($J=12.0$ Hz). Finally, two geminal olefinic protons showed at δ 5.40 and δ 5.48, and one methyl group at δ 2.53. Moreover, this spectrum showed a signal for an anomeric proton at δ 4.32 and two double doublets at δ 3.78 and 3.68, which indicated the presence of a glucosyl residue. The ^{13}C NMR spectrum (Table 3) showed 19 carbons, 13 corresponding to the aglycone and six to the sugar moiety. It showed two olefinic carbons, C-11 and C-13, at δ 140.7 and 113.4, respectively, and carbonyl signal at δ 196.4. An hydroxylated carbon (C-9) of the heterocycle appeared at δ 70.2 while the signal at δ 88.4 corresponded to C-10 of the same ring. The value of the coupling constant between H-9 and H-10 ($J=6.0$ Hz) is indicative of eclipsed protons, and corresponds to the cisoid configuration 9β-hydroxy-10β-isopropenyl, since its is twice greater than that described by Hänsel et al. (1980) for 13-acetoxy-toxol, a 9α-hydroxy-10α-isopropenyl derivative isolated from *Helichrysum italicum* (Compositae). The structure of **3** was then elucidated to be 12-*O*-β-glucopyranosyl-9β,12-dihydroxytremetone.

Table 3
^1H and ^{13}C NMR spectral data of compound **3** ($CDCl_3$; 400 and 100 MHz)

	^1H	^{13}C
1	–	131.1
2	7.99 (1H, *d*, $J=2$ Hz)	131.7
3	–	130.9
4	–	163.3
5	6.99 (1H, *d*, $J=8.5$ Hz)	110.1
6	7.87 (1H, *dd*, $J=8.4$, 2.0 Hz)	127.3
7	–	196.4
8	2.53 (3H, *s*)	26.8
9	5.21 (1H, *d*, $J=6.0$ Hz)	70.2
10	5.03 (1H, *d*, $J=6.0$ Hz)	88.4
11	–	140.7
12	4.23 ($1H_a$, *d*, $J=12.0$ Hz)	
	4.40 ($1H_b$, *d*, $J=12.0$ Hz)	69.7
13	5.40 ($1H_a$, *s*)	
	5.48 ($1H_b$, *s*)	113.4
1′	4.32 (1H, *d*, $J=7.6$ Hz)	103.1
2′	3.24–3.59 (4H, *m*)	73.8
3′	3.24–3.59 (4H, *m*)	76.8
4′	3.24–3.59 (4H, *m*)	70.5
5′	3.24–3.59 (4H, *m*)	77.2
6′	3.78 ($1H_a$, *dd*, $J=12.0$, 2.8 Hz)	
	3.68 ($1H_b$, *dd*, $J=12.0$, 4.8 Hz)	61.2

The remaining compounds were identified as apigenin-7-*O*-β-glucoside (**4**), luteolin-7-*O*-β-glucoside (**5**), luteolin-7-*O*-β-glucuronide (**6**) and 4 - *O* - β - glucopyranosyl - 4 - hydroxyacetophenone (picein, **7**) by comparison of their UV and NMR data with those of the literature (Agrawal and Bansal, 1989; Dommisse et al., 1986).

Although the Asteraceae are not a family characterized by a high occurrence of lignans, different members of substituted tetrahydrofurans and, more frequently, 2,6-diaryl-3,7-dioxabicyclo[3.3.0]octanes, such as the pinoresinol derivatives from *Mikania saltensis* (Cuenca and Catalán, 1991) and the sesamin-type lignans from *Artemisia arborescens* (Marco et al., 1997) and *Podolepis* species (Jaensch et al., 1989) have been described. Among the tetrahydrofurans, a 3,4-dibenzyltetrahydrofuran from *Artemisia chamaemelifolia* (Marco et al., 1996) and the 2-aryl-4-benzyltetrahydrofurans 2-hydroxyolivil from *Carduus assoi* (Fernández et al., 1991) and acuminatin from *Helichrysum acuminatum* (Jakupovic et al., 1987) were identified. However, this is the first time, to our knowledge, that a 2,5-diphenyltetrahydrofuran derivative is found in this family. Concerning the appearance of furan-acetophenones, which are in fact one of the most characteristic chemical features of the Asteraceae, they can be found in many species of the family, not random, but mainly located in the tribes Astereae, Eupatorieae, Heliantheae, Inuleae and Senecioneae (Proksch and Rodríguez, 1983). So, the present report of the occurrence of compounds **3** and, to a lesser extent, **7** strengthens the links between *Phagnalon* and *Helichrysum*, possibly one of the best studied genus within the Inuleae. Other phenolic glycosides, such as the flavone apigenin and luteolin derivatives are ubiquitous in Asteraceae.

3. Experimental

3.1. General experimental procedures

NMR spectra were run on a 400 MHz (δ, ppm) Bruker AMX instrument in CD_3OD. FABMS and HREIMS 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 recorded as KBr disks on a Mattson Satellite FTIR spectrophotometer. Analytical TLC was carried out on Merck silica gel F_{254} and RP-18 aluminum sheets visualized with 1% sulfuric acid-anisaldehyde.

3.2. Plant material

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

3.3. Extraction and isolation procedures

The BuOH subextract (19 g) of the methanolic extract of the dry aerial parts of *P. rupestre* (660 g), obtained after successive liquid partition with hexane, CH_2Cl_2, EtOAc and BuOH, was filtered over Sephadex LH-20 with MeOH to yield 16 fractions. The eighth fraction (fr. VIII, 3.15 g) was further fractionated over silica gel 60 (Merck) column and eluted with CH_2Cl_2–MeOH (9:1) to obtain 12 subfractions. The fifth fraction (fr. VIII-5) gave compound **1** (6.1 mg). The seventh fraction (fr. VIII-7, 227 mg) was re-chromatographed on a Lobar B column of RP-18 (Merck) with MeOH–H_2O (25:75) to obtain 8 fractions and the last one yielded compound **2** (13.2 mg). The eighth fraction (fr. VIII-8, 141 mg) gave compound **4** (18 mg) which precipitated after adding methanol. The methanol-soluble portion of fraction VIII-8 was re-chromatographed on a Lobar B column of RP-18 (Merck) with MeOH–H_2O (2:8) to give compound **3** (6.2 mg). Compounds **5** (902.8 mg) and **6** (98.6 mg) were directly obtained by precipitating from fractions ninth (fr. IX) and 11th (fr. XI), respectively. The ninth fraction (fr. VIII-9, 234 mg) was purified over Lobar B column of RP-18 (Merck) with MeOH–H_2O (15:85) to yield compound **7** (5.1 mg).

3.3.1. 7,7′-Bis-(4-hydroxy-3,5-dimethoxyphenyl)-8,8′-dihydroxymethyl-tetrahydrofuran-4-O-β-glucopyranoside (**1**)

Amorphous powder; $[\alpha]_D$ −13° (MeOH; *c* 0.1); UV λ_{max} (MeOH) 212, 273 nm; IR ν_{max} (KBr) 3394, 2925, 2854, 1596, 1519, 1468, 1224, 1122 cm^{-1}; HREIMS *m/z* 418.16 (100), 388.15 (17), 181.04 (55).

3.3.2. 1-O-β-Glucopyranosyl-4-hydroxy-2-(3′-hydroxy-3′-methylbutyl) benzene (**2**)

Amorphous powder; $[\alpha]_D$ −39° (MeOH; *c* 0.1); UV λ_{max} (MeOH) 217, 283 nm (+NaOH) 238, 298 nm; IR ν_{max} (KBr) 3409, 2967, 2927, 1627, 1500, 1451, 1384, 1297, 1215, 1075 cm^{-1} FABMS *m/z* $[M+Na]^+$ 381, $[M]^+$ 359.

3.3.3. 12-O-β-Glucopyranosyl-9,12-dihydroxytremetone (**3**)

Amorphous powder; $[\alpha]_D$ −5° (MeOH; *c* 0.1); UV λ_{max} (MeOH) 222, 274 nm (+NaOH) 211, 275 nm; IR ν_{max} (KBr) 3417, 2926, 1667, 1608, 1493, 1444, 1360, 1265 cm^{-1}; FABMS *m/z* $[M+Na]^+$ 419, $[M]^+$ 397.

Acknowledgements

The authors thank Dr. J.B. Peris, Department of Plant Biology, University of Valencia, for his help in botanical aspects. This work was supported by the Secretaría de Estado de Universidades e Investigación of the Spanish Government (grant PM98–0206).

References

Agrawal, P.K., Bansal, M.C., 1989. Flavonoid glycosides. In: Agrawal, P.K. (Ed.), Carbon-13 NMR of Flavonoids. Elsevier, Amsterdam, pp. 283–364.

Cuenca, M.R., Catalán, C.A.N., 1991. Monoterpenes and lignans from *Mikania saltensis*. J. Nat. Prod. 54, 1162–1164.

Della Greca, M., Ferrara, M., Fiorentino, A., Monaco, P., Previtera, L., 1998. Antialgal compounds form *Zantedeschia aethiopica*. Phytochemistry 49, 1299–1304.

Dommisse, R.A., Van Hoof, L., Vlietinck, A.J., 1986. Structural analysis of phenolic glucosides from Salicaceae by NMR spectroscopy. Phytochemistry 25, 1201–1204.

Fernández, I., García, B., Pedro, J.R., Varea, A., 1991. Lignans and flavonoids from *Carduus assoi*. Phytochemistry 30, 1030–1032.

Góngora, L., Giner, R.M., Máñez, S., Recio, M.C., Ríos, J.L., 2001. New prenylhydroquinone glycosides from *Phagnalon rupestre*. J. Nat. Prod. 64, 1111–1113.

Hänsel, R., Cybulski, E.M., Çubukçu, B., Meriçli, A., Bohlmann, F., Zdero, C., 1980. Neue Pyron-Derivative aus *Helichrysum*-Arten. Phytochemistry 19, 639–644.

Jaensch, M., Jakupovic, J., King, R.M., Robinson, H., 1989. Pyrones and other constituents from *Podolepis* species. Phytochemistry 28, 3497–3501.

Jakupovic, J., Pathak, V.P., Bohlmann, F., King, R.M., Robinson, H., 1987. Obliquin derivatives and other constituents from Australian *Helichrysum* species. Phytochemistry 26, 803–807.

Marco, J.A., Sanz-Cervera, J.F., Morante, M.D., García-Lliso, V., Vallés-Xirau, J., Jakupovic, J., 1996. Tricyclic sesquiterpenes from *Artemisia chamaemelifolia*. Phytochemistry 41, 837–844.

Marco, J.A., Sanz-Cervera, J.F., García-Lliso, V., Vallés-Xirau, J., 1997. Sesquiterpene lactones and lignans from *Artemisia arborescens*. Phytochemistry 44, 1133–1137.

Matsushita, H., Miyase, T., Ueno, A., 1991. Lignan and terpene glycosides from *Epimedium sagittatum*. Phytochemistry 30, 2025–2027.

Proksch, P., Rodríguez, E., 1983. Chromones and benzofurans of the Asteraceae, their chemistry and biological significance. Phytochemistry 22, 2235–2248.