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# A SAPONIN FROM THE ROOTS OF *GYPSOPHILA BERMEJOI*

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**Key Word Index**—*Gypsophila bermejoi*; Caryophyllaceae; root; saponins; triterpenoids; carbohydrates; gypsogenin.

**Abstract**—A new saponin was isolated from the methanol extract of the roots of *Gypsophila bermejoi* and identified by a combination of chemical degradation and spectral methods, which included negative FABMS and extensive 1D and 2D-NMR analysis (DQCOSY, TOCSY, ROESY, HMQC and HMBC), as gypsogenin 28-O-$\beta$-D-glucopyranosyl(1 → 2)-[$\beta$-D-glucopyranosyl (1 → 6)]-$\beta$-D-glucopyranoside. 

## INTRODUCTION

The genus *Gypsophila* is well known to contain saponins of industrial interest with various applications. For example, the saponins from the roots of *G. paniculata* and *G. arrostii* have been used as detergent and expectorant [1]. *G. struthium* is known as a source of saponins since the antiquity and is also used in gastronomy in Arabic countries [2]. The structures of saponins isolated from *G. paniculata*, *G. arrostii, G. struthium*, *G. pacifica* and *G. capillaris* have been investigated [2–6].

Plants of the genus *Gypsophila* mainly grow in the Mediterranean zone. *G. bermejoi* is a Spanish endemism, found in Cuenca, Segovia and Madrid. It grows in saline soils, between 700 and 800 m of elevation height. We now report on the isolation and structure elucidation of a new saponin isolated from *G. bermejoi*.

## RESULTS AND DISCUSSION

The methanol extract of roots of *G. bermejoi* was fractionated by a silica gel column to give several fractions, from which further purification by preparative TLC, yielded a new saponin (**1**).

The FAB mass spectrum was registered in the negative-ion mode and gave a $[M–H]^-$ ion at $m/z$ 955, corresponding to a molecular formula $C_{48}H_{76}O_{19}$. On alkaline hydrolysis, saponin **1** produced gypsogenin and an oligosaccharide, while on acidic hydrolysis, **1** furnished gypsogenin and glucose as the sole carbohydrate.

The high resolution $^1$H NMR spectrum of **1** contained an aldehyde singlet ($\delta$ 9.47, s), an ethylenic triplet ($\delta$ 5.45, $J$ = 3.5 Hz), a double doublet ($\delta$ 3.04, $J$ = 4.2; $J$ = 13.6 Hz), characteristic of an axial (ring E) H-18 proton, and six methyl singlets. The pattern of the carbohydrate zone of protons showed three doublets in the anomeric region, with coupling constants characteristic of $\beta$-anomers ($J_{1,2}$, *ca.* 7.8 Hz). The three anomeric protons were labelled **G1**–**G3** from low to high field. The rest of the glycosidic protons were resolved and allowed the measurement of most of the coupling constants. Assignment of the different resonances of the three residues was achieved through COSY and TOCSY (mixing time = 85 ms) experiments. The large coupling constants found for protons H-2, H-3 and H-4 of the spin systems of each sugar unit were consistent with those of glucose moieties ($J_{2,3}$; $J_{3,4}$; $J_{4,5} > 9$ Hz; see Table 2).

The $^{13}$C NMR spectrum gave peaks for 48 carbons and supported the deductions derived from the $^1$H NMR spectrum. Compound **1** showed two carbonyl peaks ($\delta$ 207.1, aldehyde, and $\delta$ 178.5, carboxylic), The rest of carbon chemical shifts were assigned by heteronuclear multiple quantum corre-

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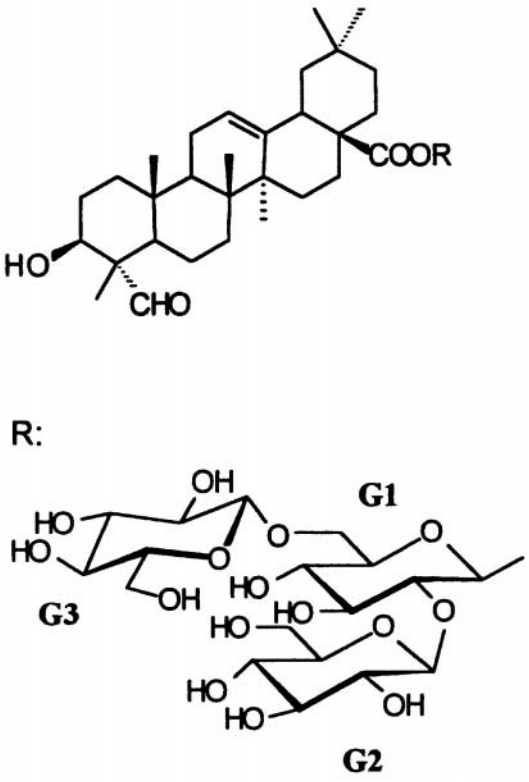


Table 1. $^{1}H$ NMR and $^{13}C$ NMR chemical shifts ($\delta$) and proton–proton coupling constants ($J$, Hz) for the aglycone moiety of compound **1** in methanol-$d_4$

| Atom | $^{1}H$* | $^{13}C$ |
|---|---|---|
| 1 | 1.89–1.28 | 39.7 |
| 2 | 1.87–n.d. | 27.3 |
| 3 | 3.95 (*dd*, $J$ = 4.6, 11.9) | 73.1 |
| 4 | – | 57.1 |
| 5 | 1.51 | 48.3 |
| 6 | 1.72–1.10 | 22.1 |
| 7 | 1.73–1.44 | 33.7 |
| 8 | – | 41.4 |
| 9 | 1.90 | 49.0 |
| 10 | – | 37.3 |
| 11 | 2.12 | 24.8 |
| 12 | 5.45 (*t*, $J$ = 3.5) | 123.7 |
| 13 | – | 145.4 |
| 14 | – | 43.2 |
| 15 | 1.94–1.20 | 30.1 |
| 16 | 2.20–2.04 | 23.8 |
| 17 | – | n.d. |
| 18 | 3.04 (*dd*, $J$ = 4.2, 13.6) | 42.8 |
| 19 | 1.91–1.33 | 47.5 |
| 20 | – | 31.9 |
| 21 | 1.60–1.41 | 35.2 |
| 22 | 1.89–1.83 | 33.4 |
| 23 | 9.47 (*s*) | 208.7 |
| 24 | 1.21 (*s*) | 9.7 |
| 25 | 1.19 (*s*) | 16.5 |
| 26 | 0.99 (*s*) | 18.1 |
| 27 | 1.38 (*s*) | 26.8 |
| 28 | – | 178.4 |
| 29 | 1.10 (*s*) | 33.8 |
| 30 | 1.18 (*s*) | 24.5 |

*Protons are multiplets, unless otherwise stated. When two values are given, the first is the equatorial one.

Table 2. $^{1}H$ NMR and $^{13}C$ NMR chemical shifts ($\delta$) and proton–proton coupling constants ($J$, Hz) for the sugar moiety of compound **1** in $CD_3OD$

| | **1** | |
|---|---|---|
| | $^{1}H$ | $^{13}C$ |
| Glucose 1 (**G1**) | | |
| 1 | 5.61 (*d*, $J_{1,2}$ = 8.2) | 94.3 |
| 2 | 4.03 (*dd*, $J_{2,3}$ = 9.2) | **78.4** |
| 3 | 3.84 (*dd*, $J_{3,4}$ = 9.7) | 79.0 |
| 4 | 3.67 (*dd*, $J_{4,5}$ = 9.2) | 71.1 |
| 5 | 3.71 (*m*, $J_{5,6a}$ = 2, $J_{5,6b}$ = 4.9) | 78.1 |
| 6a | 4.29 (*dd*, $J_{6a,6b}$ = − 11.6) | **69.9** |
| 6b | 4.09 (*dd*) | |
| Glucose 2 (**G2**) | | |
| 1 | 4.98 (*d*, $J_{1,2}$ = 7.7) | 104.0 |
| 2 | 3.37 (*dd*, $J_{2,3}$ = 9.4) | 76.1 |
| 3 | 3.53 (*dd*, $J_{3,4}$ = 8.8) | 78.3 |
| 4 | 3.30 (*dd*, $J_{4,5}$ = 10.8) | 72.8 |
| 5 | 3.44 (*m*, $J_{5,6a}$ = 1.6, $J_{5,6b}$ = 7.0) | 78.3* |
| 6a | 4.08 (*dd*, $J_{6a,6b}$ = − 11.6) | 64.0 |
| 6b | 3.80 (*dd*) | |
| Glucose 3 (**G3**) | | |
| 1 | 4.52 (*d*, $J_{1,2}$ = 7.6) | 105.0 |
| 2 | 3.39 (*dd*, $J_{2,3}$ = 9.1) | 75.5 |
| 3 | 3.54 (*dd*, $J_{3,4}$ = 9.2) | 78.4 |
| 4 | 3.47 (*dd*, $J_{4,5}$ = 9.4) | 71.9 |
| 5 | *ca.* 3.49 | 78.5* |
| 6a | 4.04 | 63.1 |
| 6b | *ca.* 3.85 | |

The bold underlined values correspond to glycosylation points.
*These values may be interchanged.

lation (HMQC) (see Tables 1 and 2). The values for the aglycone moiety agree with those published for similar saponins, and quaternary carbons were assigned accordingly. The chemical shift of the anomeric carbon for **G1** (*ca.* $\delta$ 94.0) indicated that it was linked as an ester to the C-28 carboxyl group of the aglycon [7–10]. In addition, C-2 and C-6 of the same unit gave values shifted downfield to those expected for unsubstituted glucopyranoses [11] which indicated that **G1** was *O*-glycosylated at those positions by the other two (**G2** and **G3**). Unequivocal demonstration of the glycosidic linkages was deduced from a heteronuclear multiple bond correlation experiment (HMBC) which, in addition to expected intraresidue peaks for all different moieties, gave crosspeaks H-1(**G2**)/C-2(**G1**), H-1(**G3**)/C-6(**G1**), and O–C=O/H-1(**G1**). The crosspeaks confirmed also the chemical shifts of quaternary carbons. All these facts allowed us to propose for saponin **1** the structure shown.

## EXPERIMENTAL

### *General*

NMR spectra were recorded in methanol-$d_4$, on a Varian Unity 500 instrument (proton: 500 MHz, carbon: 125 MHz) at 25°C. Chemical shifts refer to the methanol-$d_4$ multiplet (proton: 3.30 ppm, carbon: 49.0 ppm). FABMS was carried out in a VG AutoSpec (Fisons). Analytical TLC was carried out

on Merck Si gel $F_{254}$ aluminium sheets, eluted with *n*-BuOH–HOAc–$H_2O$ (4:1:5), visualized with 1% vanillin in MeOH–$H_2SO_4$ (50:50). Carbohydrates were identified by chromatographic comparison with an authentic sample of glucose.

*Plant material*

*G. bermejoi* roots were collected in September 1993 from Cuenca (Spain) and it was identified by Dra. C. Bartolomé, Department of Vegetal Biology, Faculty of Sciences, University of Alcalá de Henares, Madrid, Spain. A voucher specimen was deposited at the Herbarium of the University of Alcalá.

*Extraction and isolation*

Air-dried and powdered roots of *G. bermejoi* (900 g) were treated for 24 h at room temperature with 80% MeOH (9 l). The MeOH was removed *in vacuo* and the resulting aq. solution was extracted first with $CH_2Cl_2$ and then with *n*-BuOH. The alcohol was removed under vacuum giving a solid (25.1 g), 4.4 g of which were chromatographed over a silica gel column, eluting with $CHCl_3$–MeOH–$H_2O$ (55:37:6), monitoring the fractions on TLC. The appropriate fractions were rechromatographed on prep. TLC (silica gel-60) with *n*-BuOH–HOAc–$H_2O$ 55–37–6), from which a fraction was collected as a single spot, giving 33.6 mg of saponin **1**.

*Acid hydrolysis of* **1**

Compound **1** (2 mg) was refluxed with 10% HCl (4 ml) for 4 h. After extraction of the solution ($Et_2O$), the aq. layer was neutralized (10% *N*,*N*-dioctylmethylamine in $CHCl_3$) and concentrated under reduced pressure. The residue was compared with standard sugars by TLC with $CH_2Cl_2$/MeOH/$H_2O$ (50:25:5), showing that glucose was the only one present. The organic layer was TLC chromatographed with $CHCl_3$–$CH_3COCH_3$ (50:6). Comparison with standard aglycones showed gypsogenin was the prosapogenin of **1**.

*Alkaline hydrolysis*

Compound **1** (2 mg) was hydrolyzed with 0.2% KOH (4 ml) at 100°C for 1.5 h and after neutralization (HCl), extraction ($Et_2O$) and removal of the organic solvent *in vacuo*, sapogenin gypsogenin was obtained, as compared with an authentic sample by TLC, as above.

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