Constituents of *Chenopodium pallidicaule* (Cañihua) Seeds: Isolation and Characterization of New Triterpene Saponins

Luca Rastrelli,[†] Francesco De Simone,[‡] Oreste Schettino,[§] and Antonio Dini*[,†]

Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli "Federico II", via D. Montesano 49, 80131 Napoli, Italy, Facoltà di Farmacia, Università di Salerno, Penta di Fisciano, 84084 Salerno, Italy, and Dipartimento di Chimica Farmaceutica e Tossicologica, Università di Napoli "Federico II", via D. Montesano 49, 80131 Napoli Italy

Seven triterpenoid saponins were isolated from the seeds of *Chenopodium pallidicaule* (Chenopodiaceae). Of these compounds, four were identified as known saponins of oleanolic acid and phytolaccagenic acid. The other three compounds, named **1**−**3**, are new saponins. The structures of these saponins were determined to be: (**1**) hederagenin 3-*O*-[α-L-arabinopyranosyl-(1→3)-*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranoside; (**2**) phytolaccagenic acid 3-*O*-[*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl]-28-*O*-*β*-D-glucopyranoside; and (**3**) phytolaccagenic acid 3-*O*-[*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl]-28-*O*-*β*-D-glucopyranoside. All structures were elucidated by chemical and spectroscopic methods.

Keywords: *Cañihua; Chenopodium pallidicaule; chenopodiaceae; South American crop; triterpene glycosides; ^{1}H NMR, ^{13}C NMR, 2D NMR*

INTRODUCTION

Cañihua (*Chenopodium pallidicaule*) is an annual herbaceous plant that grows in Peru and Bolivia at elevations up to 4400 m in dry desert or semi-desert climates. Cañihua was tamed as an alimentary plant by Andean people prior to the Spanish conquest. Once considered a variety of *Chenopodium quinoa*, it was recognized as a new plant in 1929 (Gade, 1970). It requires minimal care during its growth and produces acceptable yields on poor or depleted soils where other crops yield essentially nothing. Therefore, cañihua can be used to take advantage of marginal lands. The seeds are used in making soups or are ground into flour to prepare breads, cakes, and fermented drinks. Cañihua grain has been identified as a very promising food crop because of its exceptional nutritive value as judged by its protein and lipid content, as well as by its essential amino acid composition with a high lysine content (Gross et al., 1989). However, other than among the Andean Indians, cañihua is pratically unknown and is not utilized as a food grain. This plant is also used in indigenous medicine; the leaves are used for the treatment of dysentery and the seeds are used for the care of blennorrhea and urinary ailments (Girault, 1987).

In a previous paper (Rastrelli et al., 1995), we reported the isolation and the structure determination of 10 flavonol glycosides from the seeds of *C. pallidicaule*. As a continuation of our chemical studies on the constituents of alimentary plants from South America (Rastrelli et al., 1995), we have now isolated three new triterpenoid saponins, together with known saponins, from the same source, and this paper deals with the elucidation of their structures. Cañihua saponins can be divided into three different saponin groups; namely, groups containing either oleanolic acid, hederagenin, or phytolaccagenic acid as the aglycon. Saponins are potential antinutrients, and a general toxic effect of saponins is due to their tendency to alter the cell wall permeability (Basu et al., 1967). Bisdesmosidic glycosides of the same aglycons have been isolated from *C. quinoa* seeds (Mizui et al., 1990). These compounds were shown to possess bitter taste, to be membranolitic against cells of the small intestine, and to cause an increase in mucosal permeability (Gee et al., 1993).

EXPERIMENTAL PROCEDURES

Material. The plant material, supplied by Central Peruviane de Servicios, was collected in Ayakawa, Peru in 1990. A voucher specimen is deposited in the Herbario de Museo de Historia Natural "J. Prado" Un. H. S. Lima (Peru).

Apparatus. Fast-atom bombardment mass spectra (FABMS), in negative ion mode, were obtained by dissolving the samples in a glycerol−thyoglycerol matrix and placing them on a copper probe tip prior to bombardment with Ar atoms of energy 2−6 kV in a Kratos MS 902 spectrometer equipped with a Kratos FAB source.

The NMR spectra were obtained in CD_3OD with Bruker WM-250 and Bruker AMX-500 spectrometers. The DEPT experiments (Bendall et al., 1983) were performed with a pulse of 135° to obtain positive signals for CH and CH_3 and negative ones for CH_2; an average CH coupling constant of 135 Hz was assumed. COSY-90 experiments were conducted with the conventional pulse sequence. The COSY spectrum was obtained with a data set (t1 × t2) of 1024 × 1024 points for a spectral width of 1165 Hz (relaxation delay 1 s). The data matrix was processed by an unshifted sine bell window function, followed by transformation to give a magnitude spectrum with symmetrization (digital resolution in both F2 and F1 dimension 1.13 Hz for point). The 2D NOESY experiment was performed in the phase-sensitive mode. The spectral width (t2) was 1002 Hz; 512 experiments of 80 scans each (relaxation delay 1.5 s, mixing time 300 m) were acquired in 2K data points.

The gas chromatography-mass spectroscopy (GC-MS) analyses were made with a Hewlett-Packard 5890 gas chromatograph equipped with an HP-5 column (25 m × 0.2 mm, i.d.; 0.33-*μ*m film) coupled to an HP 5970B mass selective detector, with a HP 59970 MS Chemstation computer attached.

* Author to whom correspondence should be addressed.

[†] Dipartimento di Chimica delle Sostanze Naturali.

[‡] Facolta di Farmacia.

[§] Dipartimento di Chimica Farmaceutica e Tossicologica.

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HPLC separations were performed on a Hewlett-Packard HP 1050 series pumping system equipped with a Whatman Partisil 10 ODS-2 column and a Varian RI-4 refractive index detector.

Extraction and Isolation. The whole flour from the seeds (1.0 kg) was extracted successively with petroleum ether, $CHCl_3$, and MeOH (39 g). Part of the MeOH extract (13 g) was partitioned between *n*-BuOH and H_2O to afford an *n*-BuOH-soluble portion (6.0 g) that was chromatographed on a Sephadex LH-20 column (100 × 5 cm), with MeOH as eluent. Fractions (9 mL) were collected and checked by TLC [Si-gel plates in *n*-BuOH−HOAc−H_2O (60:15:25)]. Fractions 31−41 (550 mg) containing the crude glycosidic mixture were chromatographed on silica gel with $CHCl_3$−MeOH−H_2O (30:10:1, 10:5:1, 6:4:1, and 4:6:1) to give five fractions. These fractions were further separated by reversed-phase HPLC with MeOH−H_2O (60:40) at a flow rate of 2.5 mL/min as eluent to yield pure compounds **2** (16 mg; R_t,, 23.2 min), **3** (18 mg; R_t, 19.5 min), and **4** (20 mg; R_t, 25.6 min) from fraction 3, and **1** (12 mg; R_t, 11.5 min), **5** (11 mg; R_t, 14.5 min), **6** (14 mg; R_t, 17.5 min), and **7** (18 mg; R_t, 18.5 min) from fraction 5.

Acid Hydrolysis of 1−7, Carbohydrate Constituents. A solution of each compound (5 mg) in 10% H_2SO_4−EtOH (1:1, 3.5 mL) was refluxed for 4 h. The reaction mixture was diluted with H_2O and then extracted with Et_2O. The Et_2O layer was dried with anhydrous Na_2SO_4 and evaporated to dryness. The H_2O layer was neutralized with Amberlite MB-3 ion-exchange resin and evaporated to dryness. The resulting monosaccharides were reacted with TRISIL-Z (Pierce) and analyzed by GC−MS. Retention times were identical to those of the authentic trisil sugars.

Alkaline Hydrolysis. Glycosides **1**−**3** (5 mg) were heated separately in 0.5 aqueous KOH (1 mL) at 110 °C in a stoppered reaction vial for 2 h. The reaction mixture was adjusted to pH 7 with HCl and then extracted with *n*-BuOH. The organic phase was evaporated to dryness, dissolved in CD_3OD, and analyzed by ^1H and ^{13}C NMR.

Compound 1. ^1H NMR (CD_3OD) δ 0.73 (3H, s, Me-24), 0.84 (3H, s, Me-26), 0.96 (3H, s, Me-30), 0.98 (3H, s, Me-29), 1.10 (3H, s, Me-25), 1.21 (3H, s, Me-27), 2.89 (1H, dd, J = 13.7 and 5 Hz, H-18), 5.29 (1H, m, H-12). Sugar signals are shown in Table 4.

Compound 2. ^1H NMR (CD_3OD) δ 0.75 (3H, s, Me-24), 0.84 (3H, s, Me-26), 1.03 (3H, s, Me-25), 1.18 (3H, s, Me-29), 1.22 (3H, s, Me-27), 2.74 (1H, dd, J = 13.7 and 5 Hz, H-18), 3.74 (3H, s, OCH_3), 5.36 (1H, m, H-12), 4.46 (1H, d, J = 7.5 Hz, H-1 glucose), 4.48 (1H, d, J = 7.5 Hz, H-1 glucose) 5.39 (1H, d, J = 7.5 Hz, H-1 glucose linked at C-28).

Compound 3. ^1H NMR (CD_3OD) δ 0.76 (3H, s, Me-24), 0.84 (3H, s, Me-26), 1.02 (3H, s, Me-25), 1.18 (3H, s, Me-29), 1.22 (3H, s, Me-27), 2.74 (1H, dd, J = 13.7 and 5 Hz, H-18), 3.74 (3H, s, OCH_3), 5.36 (1H, m, H-12), 4.46 (1H, d, J = 7.5 Hz, H-1 glucose), 4.58 (1H, d, J = 7.5 Hz, H-1 glucose), 4.78 (1H, d, J = 7.5 Hz, H-1 glucose), 5.39 (1H, d, J = 7.5 Hz, H-1 glucose linked at C-28).

Compound 4. ^1H NMR (CD_3OD) δ 0.75 (3H, s, Me-24), 0.84 (3H, s, Me-26), 1.03 (3H, s, Me-25), 1.18 (3H, s, Me-29), 1.22 (3H, s, Me-27), 2.74 (1H, dd, J = 13.7 and 5 Hz, H-18), 3.74 (3H, s, OCH_3), 5.36 (1H, m, H-12), 4.44 (1H, d, J = 7.5 Hz, H-1 glucose linked at C-3), 5.39 (1H, d, J = 7.5 Hz, H-1 glucose linked at C-28).

Compound 5. ^1H NMR (CD_3OD) δ 0.84 (3H, s, Me-26), 0.88 (3H, s, Me-24), 0.95 (3H, s, Me-30), 0.97 (3H, s, Me-29), 0.99 (3H, s, Me-23), 1.11 (3H, s, Me-25), 1.19 (3H, s, Me-27), 2.90 (1H, dd, J = 13.7 and 5 Hz, H-18), 5.29 (1H, m, H-12), 4.46 (1H, d, J = 7.5 Hz, H-1 glucose) 4.52 (1H, d, J = 7.5 Hz, H-1 glucose), 4.76 (1H, d, J = 7.5 Hz, H-1 glucose), 5.42 (1H, d, J = 7.5 Hz, H-1glucose linked at C-28).

Compound 6. ^1H NMR (CD_3OD) δ 0.84 (3H, s, Me-26), 0.89 (3H, s, Me-24), 0.96 (3H, s, Me-30), 0.98 (3H, s, Me-29), 0.99 (3H, s, Me-23), 1.10 (3H, s, Me-25), 1.21 (3H, s, Me-27), 2.90 (1H, dd, J = 13.7 and 5 Hz, H-18), 5.28 (1H, m, H-12), 4.40 (1H, d, J = 7.5 Hz, H-1 glucuronic acid), 4.58 (1H, d, J = 7 Hz, H-1 arabinose), 5.40 (1H, d, J = 7.5 Hz, H-1 glucose linked at C-28).

Compound 7. ^1H NMR (CD_3OD) δ 0.84 (3H, s, Me-26), 0.88 (3H, s, Me-24), 0.95 (3H, s, Me-30), 0.97 (3H, s, Me-29), 0.99 (3H, s, Me-23), 1.11 (3H, s, Me-25), 1.19 (3H, s, Me-27), 2.89 (1H, dd, J = 13.7 and 5 Hz, H-18), 5.29 (1H, m, H-12), 4.46 (1H, d, J = 7.5 Hz, H-1 glucose linked at C-3), 5.42 (1H, d, J = 7.5 Hz, H-1 glucose linked at C-28).

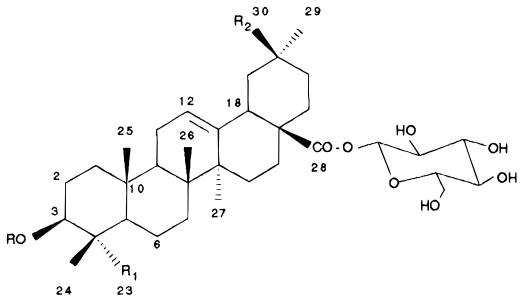
RESULTS AND DISCUSSION

C. pallidicaule grains were successively extracted with petroleum ether, $CHCl_3$, and MeOH. The MeOH extract was then partitioned into a mixture of *n*-BuOH and H_2O to afford the *n*-BuOH-soluble portion, which was subjected to Sephadex LH-20. The fractions containing the glycosidic mixture, checked by TLC, were separated by chromatography on silica gel columns, followed by repeated fractionation by HPLC, affording seven saponins (**1**−**7**; Figure 1). The structures and molecular formulae were determined by negative ion FABMS spectra (Table 1) and ^1H, ^{13}C, and ^{13}C DEPT NMR data (Tables 2 and 3).

Compound **1** showed an $[M - H]^-$ ion at *m/z* 941 and prominent fragments at *m/z* 779 $[(M - H) - 162]^-$ and *m/z* 763 $[(M - H) - 178]^-$, which were interpreted as the cleavage of a glucose moiety without and with the glycosidic oxygen, respectively; and at *m/z* 647 $[(M - H) - (162 + 132)]^-$, due to subsequent loss of a pentose unit. The ^{13}C and ^{13}C DEPT NMR spectra showed 47 signals, of which 17 were assigned to the saccharide portion and 30 to a triterpenic moiety. Inspection of the ^1H and ^{13}C NMR spectra indicated that saponin **1** was the 3,28-*O*-bisdesmoside of hederagenin, with three monosaccharide units. On acidic hydrolysis, **1** afforded hederagenin [which was identified by ^1H, ^{13}C, and ^{13}C DEPT NMR spectra (Table 2) and by comparison with literature data (Tori et al., 1974)] and glucuronic acid, arabinose, and glucose in a 1:1:1 ratio.

The ^1H and ^{13}C NMR spectra (Table 3) confirmed the presence of glucuronic acid, glucose, and arabinose. The presence of a β-glucose unit linked at the carboxyl group (C-28) of the aglycon was consistent with the anomeric signals at δ 5.42 (1H, d, J = 7.5 Hz) in the ^1H NMR spectrum and at 96.5 ppm in the ^{13}C NMR spectrum and in full agreement with the results of alkaline hydrolysis of **1**. The ether glycosidation site was shown to be at C-3 on the basis of the downfield shift exhibited in the ^{13}C NMR spectrum by C-3 (82.1 ppm) and the upfield shifts experienced by C-2 (26.4 ppm) and C-4 (42.8 ppm) when compared with corresponding signals in hederagenin, and was subsequently confirmed by 2D NMR experiments.

The interglycosidic linkage and the sequential arrangement of the disaccharide moiety linked at C-3 of the aglycon was determined by a combination of a two-dimensional ^1H correlation (COSY) and two-dimensional nuclear Overhauser effect (NOESY) spectroscopy. The COSY experiment allowed the sequential assignment of most resonances for each sugar ring starting from the anomeric signals (Table 4). On the basis of these assignments, NOESY experiments were carried out to determine the position of glycosyl bond. Diagnostic NOE were observed from H-3 of the aglycon (δ 3.42) to H-1_{glucA} (δ 4.52) and from H-3_{glucA} (δ 3.58) to H-1_{glucA} (δ 4.52) and H-1_{ara} (δ 4.58), indicating a glycosidic linkage at C-3 of glucuronic acid of arabinose. Chemical shifts, multiplicity of the signals, absolute values of the coupling constants, and their magnitudes in the ^1H NMR spectrum (Table 4) as well as ^{13}C NMR data (Table 3) indicated the β configuration at the anomeric



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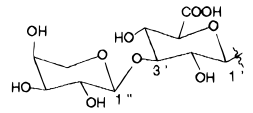
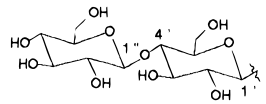
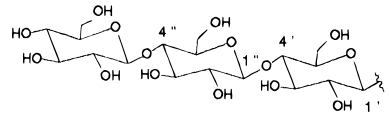
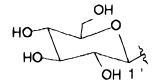
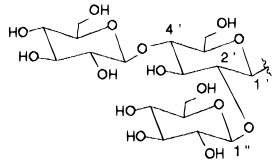
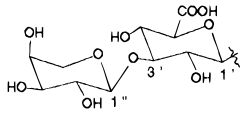
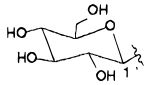
1		CH_2OH	CH_3
2		CH_2OH	$COOCH_3$
3		CH_2OH	$COOCH_3$
4		CH_2OH	$COOCH_3$
5		CH_3	CH_3
6		CH_3	CH_3
7		CH_3	CH_3

Figure 1. Structure of triterpene saponins isolated from *C. pallidicaule* seeds: (**1**) hederagenin 3-*O*-[α-L-arabinopyranosyl-(1→3)-*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranoside; (**2**) phytolaccagenic acid 3-*O*-[*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl]-28-*O*-*β*-D-glucopyranoside; (**3**) phytolaccagenic acid 3-*O*-[*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl]-28-*O*-*β*-D-glucopyranoside; (**4**) phytolaccagenic acid-3-*O*-*β*-D-glucopyranosyl-28-*O*-*β*-D-glucopyranoside; (**5**) oleanolic acid 3-*O*-[2′-4′-di-*O*-(*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl]-28-*O*-*β*-D-glucopyranoside; (**6**) oleanolic acid 3-*O*-[α-L-arabinopyranosyl-(1→3)-*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranoside; (**7**) oleanolic acid 3-*O*-*β*-D-glucopyranosyl-28-*O*-*β*-D-glucopyranoside.

positions for glucuronopyranosyl ($J_{H1-H2} = 7.5$ Hz) and glucopyranosyl ($J_{H1-H2} = 7.5$ Hz) units. Moreover nOe observed between H-1_{ara} and H-3_{ara} (δ 3.62) and H-5_{ara} (δ 4.00) were consistent with an α configuration for the arabinopyranosyl group (Waltho et al., 1986).

From all these data, the structure of **1** was concluded to be hederagenin 3-*O*-[α-L-arabinopyranosyl-(1→3)-*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranoside.

Compound **2** showed an $[M - H]^-$ ion at *m/z* 1001 and prominent fragments at *m/z* 839 $[(M - H) - 162]^-$ and *m/z* 823 $[(M - H) - 178]^-$, which were interpreted as the cleavage of a glucose moiety without and with the glycosidic oxygen, respectively, and at *m/z* 677 $[(M - H) - (162+162)]^-$. Another fragment at *m/z* 515 $[(M - H) - (162 \times 3)]^-$, resulting from the cleavage of a third glucose unit, was ascribable to aglycon. The ^{13}C and ^{13}C DEPT NMR spectra showed 49 signals, of which 18 were assigned to the saccharide portion and 31 to a triterpenic moiety. On acidic hydrolysis, **2** afforded phytolaccagenic acid [which was identified by ^1H, ^{13}C, and ^{13}C DEPT NMR (Table 2) spectra and by comparison with literature data (Bandara et al., 1989)] and glucose. The ^1H NMR spectrum of **2** exhibited five methyl signals at $\delta = 0.75-1.22$, together with one *O*-methyl singlet at $\delta = 3.74$, an olefinic proton at $\delta = 5.36$, and three anomeric protons signals at $\delta = 4.46$

Table 1. FABMS Spectral Data for Compounds 1−7[a]

compd	formula	spectral data
1	$C_{47}H_{74}O_{19}$	941 $[M-H]^-$, 779 $[(M-H)-162]^-$, 763 $[(M-H)-178]^-$, 647 $[(M-H)-(162+132)]^-$
2	$C_{49}H_{78}O_{21}$	1001 $[M-H]^-$, 839 $[(M-H)-162]^-$, 823 $[(M-H)-178]^-$, 677 $[(M-H)-(162+162)]^-$, 515 $[(M-H)-(162\times3)]^-$
3	$C_{55}H_{88}O_{26}$	1163 $[M-H]^-$, 1001 $[(M-H)-162]^-$, 985 $[(M-H)-178]^-$, 839 $[(M-H)-(162+162)]^-$, 677 $[(M-H)-(162\times3)]^-$, 515 $[(M-H)-(162\times4)]^-$
4	$C_{43}H_{68}O_{16}$	839 $[M-H]^-$, 677 $[(M-H)-162]^-$, 661 $[(M-H)-178]^-$, 515 $[(M-H)-(162+162)]^-$
5	$C_{54}H_{88}O_{23}$	1103 $[M-H]^-$, 941 $[(M-H)-162]^-$, 925 $[(M-H)-178]^-$, 779 $[(M-H)-(162+162)]^-$, 617 $[(M-H)-(162\times3)]^-$, 455 $[(M-H)-(162\times4)]^-$
6	$C_{47}H_{74}O_{18}$	925 $[M-H]^-$, 763 $[(M-H)-162]^-$, 747 $[(M-H)-178]^-$, 631 $[(M-H)-(162+132)]^-$
7	$C_{42}H_{68}O_{13}$	779 $[M-H]^-$, 617 $[(M-H)-162]^-$, 601 $[(M-H)-178]^-$

[a] The mass unit lost corresponded to fragments indicated in the text.

Table 2. ^{13}C NMR Chemical Shift Assignments (δ in CD_3OD) of Aglycon Moieties of Compounds 1−7 and Related Compounds

carbon	DEPT	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	CH_2	39.4	39.5	39.5	39.5	39.7	39.7	39.6	39.5
2	CH_2	26.4	26.3	26.2	26.2	26.9	26.8	26.8	26.2
3	CH	82.1	83.4	84.1	83.4	91.3	90.7	90.8	84.1
4	C	42.8	42.9	42.9	42.9	40.4	40.2	40.6	42.9
5	CH	48.3	48.1	48.3	47.2	56.7	57.0	56.7	48.3
6	CH_2	18.8	18.8	18.8	18.8	19.2	19.3	19.3	18.8
7	CH_2	30.7	31.3	31.3	31.3	33.1	30.5	33.1	31.3
8	C	40.5	40.6	40.6	40.0	40.0	40.7	40.0	40.6
9	CH	48.0	48.6	48.5	48.1	48.0	48.0	48.0	48.5
10	C	37.6	37.6	37.6	37.7	37.8	37.8	37.7	37.6
11	CH_2	24.6	24.1	24.1	24.1	24.4	24.5	24.4	24.1
12	CH	123.6	124.3	124.3	124.3	123.6	123.8	123.6	124.3
13	C	144.7	144.5	144.5	144.7	144.8	144.7	144.8	144.5
14	C	43.7	42.8	43.3	42.9	42.6	42.9	42.6	43.3
15	CH_2	28.9	28.9	28.9	28.9	28.3	28.9	28.2	28.9
16	CH_2	24.6	24.5	24.5	24.5	26.1	24.5	26.2	24.5
17	C	47.4	47.4	47.3	47.4	48.0	47.1	47.8	47.3
18	CH	42.7	43.8	44.1	43.8	42.4	42.6	42.3	44.1
19	CH_2	47.2	43.2	43.6	43.2	47.2	47.2	47.2	43.6
20	C	31.4	40.6	40.6	40.5	31.5	31.5	31.6	40.6
21	CH_2	35.0	31.3	31.2	31.2	34.7	34.9	34.7	31.2
22	CH_2	33.2	33.4	33.3	33.3	33.2	33.2	33.2	33.3
23	CH_2, CH	64.8	64.8	64.6	64.7	28.2	28.4	28.2	64.6
24	CH_3	13.4	13.4	13.3	13.4	16.0	16.0	16.0	13.3
25	CH_3	16.3	16.5	16.5	16.5	16.7	16.9	16.7	16.5
26	CH_3	17.6	17.7	17.7	17.7	17.5	17.7	17.6	17.7
27	CH_3	26.4	26.2	26.5	26.5	26.1	26.2	26.1	26.5
28	C	177.8	178.7	178.7	178.7	178.0	178.1	178.0	182.7
29	CH_3	33.3	28.6	28.6	28.6	33.3	33.4	33.4	28.6
30	CH_3, C	23.8	177.5	177.5	177.5	23.8	23.9	23.8	180.5
OCH_3			52.3	52.3	52.3				

(1H, d, $J = 7.5$ Hz), 4.48 (1H, d, $J = 7.5$ Hz), and 5.39 (1H, d, $J = 7.5$ Hz). These results suggested that **2** was a bisdesmoside that contains three molecules of glucose. The coupling constants of three anomeric protons indicated a β configuration for the C-1 atoms of glucose. The ^{13}C NMR spectrum of **2** showed significant glycosidation shifts for the C-3 (+ 9.7) of the aglycon and for the C-4′ (+ 10.6) of a glucopyranosyl moiety. Consequently, **2** was suggested to posses a glucopyranosyl residue attached to the 4′-OH function in another glucopyranosyl moiety. A carbon signal at $\delta = 95.7$ ppm also indicated that a glucose moiety was linked in a ester linkage with the 28-COOH function. It remained, therefore, to establish the relative sugar positions. The alkaline hydrolysis of **2** yielded a compound whose ^{1}H and ^{13}C NMR spectra showed no signals ascribable to a β-D-glucose in an ester linkage but exhibited the signals ascribable to a *O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl virtually unshifted with respect to **2**. From these results, the structure of **2** was established as phytolaccagenic acid 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl]-28-*O*-β-D-glucopyranoside.

Compound **3** showed an $[M-H]^-$ ion at *m*/*z* 1163, which was 162 mass units higher than that of **2**, and prominent fragments at *m*/*z* 1001 $[(M-H)-162]^-$, 839 $[(M-H)-(162+162)]^-$, 677 $[(M-H)-(162\times3)]^-$, and 515 $[(M-H)-(162\times4)]^-$, which were interpreted as the cleavage of four glucose moieties. On acidic hydrolysis, **3** afforded phytolaccagenic acid and glucose. The ^{1}H and ^{13}C NMR resonances of **3**, in comparison with those of **2**, showed the presence of another anomeric proton and carbon signals ($\delta_H = 4.78$, 1 H, d, $J = 7.5$ Hz; $\delta_C = 104.3$ ppm), whereas the other signals remained almost unshifted. The attachment of the fourth glucose unit at the C-4 position of another glucopyranosyl moiety was deduced by comparison of the carbon chemical shifts observed in **3** with those of the corresponding methyl pyranosides and taking into account the known effects of glycosidation (Breitmaier et al., 1987). Thus, the observed carbon resonances of C-4′ at δ 80.4 ppm and C-4″ at δ 80.6 ppm were consistent with the presence of two C-4 glycosylated glucopyranosyl units.

These spectral data left two possible sequences for the glycoside chain of **3**; namely, phytolaccagenic acid 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl]-28-*O*-β-D-glucopyranoside and phytolaccagenic acid 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-

Table 3. ^{13}C NMR Chemical Shift Assignments (δ in CD_3OD) of Sugar Moieties of Compounds 1−7

carbon	DEPT	1	2	3	4	5	6	7
C-3 sugar								
GlucA −1	CH	104.7					105.5	
−2	CH	74.6					72.9	
−3	CH	86.5					86.5	
−4	CH	71.0					71.0	
−5	CH	77.4					77.5	
−6	C	176.1					177.0	
Ara −1	CH	105.4					106.4	
−2	CH	74.9					74.9	
−3	CH	73.8					73.8	
−4	CH	70.8					71.0	
−5	CH_2	67.3					67.1	
Glu inner −1	CH		104.6	104.6	105.7	104.5		105.8
−2	CH		74.9	73.9	73.8	82.8		73.8
−3	CH		76.6	77.9	78.2	76.4		78.5
−4	CH		80.6	80.4	71.0	80.3		71.6
−5	CH		76.3	77.0	77.7	77.1		78.1
−6	CH_2		62.3	62.3	62.3	62.0		62.4
Glu other −1	CH		105.5	104.5	104.3	104.7	105.2	
−2	CH		74.0	72.0	76.1	73.9	73.8	
−3	CH		78.1	77.8	78.4	78.3	78.1	
−4	CH		71.0	80.6	71.3	71.2	71.1	
−5	CH		77.9	76.3	78.1	77.9	77.7	
−6	CH_2		62.0	62.0	62.4	62.6	62.3	
C-28 sugar								
Glu −1	CH	95.6	95.7	95.7	95.7	95.7	95.7	95.6
−2	CH	73.6	75.6	75.0	75.2	75.1	75.0	74.8
−3	CH	78.2	78.7	78.7	78.7	78.1	78.7	78.8
−4	CH	71.7	71.5	71.0	71.3	71.0	71.1	71.4
−5	CH	78.2	78.3	78.3	78.2	78.2	78.3	78.3
−6	CH_2	62.3	62.6	63.0	62.4	62.8	62.4	62.6

Table 4. ^1H NMR[a] Data for the Oligosaccharide Moiety of 1 in CD_3OD

proton	glucuronic acid	arabinose	glucose
H-1	4.52, d, $J = 7.5$	4.58, d, $J = 7.0$	5.42, d, $J = 7.5$
H-2	3.40, dd, $J = 7.5, 9.5$	3.44, t, $J = 7.5, 9.0$	3.32, dd, $J = 7.5, 9.5$
H-3	3.58, t, $J = 9.5, 9.5$	3.62, dd, $J = 9.0, 3.5$	3.20, t, $J = 9.5, 9.5$
H-4	3.64, t, $J = 9.5, 9.5$	3.92, m	3.35, t, $J = 9.5, 9.5$
Ha-5	3.70, d, $J = 4$	3.28, dd, $J = 11, 1.5$	3.40, m
Hb-5	4.00, dd, $J = 11, 3.5$		
Ha-6			3.78, dd, 12.0, 3.5
Hb-6			3.68, dd, $J = 12.0, 5.0$

[a] From 2D COSY; ^1H-^1H coupling constants in the sugar spin system were measured from COSY spectrum and are reported in Hz.

glucopyranosyl]-28-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranoside. On alkaline hydrolysis, **3** gave a prosapogenin **8** that was identified by ^1H, ^{13}C, and ^{13}C DEPT NMR (Table 2) spectra as phytolaccagenic acid 3-*O*-[*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl]. This hydrolysis allowed us to differentiate between the two proposed structures and led to the formulation of **3** as phytolaccagenic acid 3-*O*-[*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl]-28-*O*-*β*-D-glucopyranoside.

The saponins **4**−**7** showed spectroscopic and hydrolytic data identical with those of phytolaccagenic acid-3-*O*-*β*-D-glucopyranosyl-28-*O*-*β*-D-glucopyranoside (**4**), isolated previously only from the stem of *Diploclisia glaucescens* (Bandara et al., 1989); oleanolic acid 3-*O*-[2′-4′-di-*O*-(*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl]-28-*O*-*β*-D-glucopyranoside (**5**), isolated before from the berries of *Phytolacca dodecandra* (Domon et al., 1984); oleanolic acid 3-*O*-[*β*-D-glucuronopyranosyl-(1→3)-α-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside (**6**), isolated previously from the root of *Momordica cochinchinensis* (Iwamoto et al., 1985); and oleanolic acid 3-*O*-*β*-D-glucopyranosyl-28-*O*-*β*-D-glucopyranoside (**7**). These structures were confirmed by FABMS (Table 1), ^1H NMR (see Experimental Procedures) and ^{13}C and ^{13}C DEPT NMR (Tables 2 and 3) spectral analyses.

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