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JENISSEENSOSIDES C AND D, BIOLOGICALLY ACTIVE ACYLATED TRITERPENE SAPONINS FROM *SILENE JENISSEENSIS*

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Key Word Index—*Silene jenisseensis*; Caryophyllaceae; triterpene-saponin; quillaic acid glycosides; *cis-p*-methoxycinnamoyl; *trans-p*-methoxycinnamoyl; immunological activity.

Abstract—We previously reported the isolation and structure elucidation of a new *trans-p*-methoxycinnamoyl triterpene-saponin along with its *cis-p*-methoxycinnamoyl isomer as an inseparable mixture from the roots of *Silene jenisseensis*. In a continuing study on this plant, two additional new acylated triterpene-saponins were obtained as an inseparable mixture. Their structures have been established by chemical means and spectroscopic methods including 1D and 2D homonuclear and heteronuclear correlation NMR spectroscopy as 3-*O*-[β-D-galactopyranosyl-(1 → 2)-β-D-glucuronopyranosyl]-28-*O*-[{α-L-rhamnopyranosyl-(1 → 2)}-{4-*O*-*trans-p*-methoxycinnamoyl}-β-D-fucopyranosyl] quillaic acid and its *cis*-isomer, respectively. They showed a significant enhancement of the granulocyte phagocytosis *in vitro*.

INTRODUCTION

The root of *Silene jenisseensis* Willd [1] is well-known in the traditional Chinese medicine as a substitute for the Chinese drug Yin-Chai-Hu (root of *Stellaria dichotoma* var. *lanceolata* Bge) and is used to treat fever due to Yin-deficiency and fever in infant malnutrition [2, 3].

In the course of our investigation on new biologically active saponins from Caryophyllaceae [4, 5], we previously reported the isolation and structure elucidation of a new *trans-p*-methoxycinnamoylglycoside of quillaic acid (**1**) along with its *cis*-isomer (**2**), respectively, as an inseparable mixture and as major components of the root of *S. jenisseensis* [5]. Continuation of this work led to the isolation of two additional minor acylated triterpenoidal saponins as an inseparable mixture (**1a, 2a**). This paper deals with the isolation, structure elucidation and immunological properties of these new compounds named jenisseensosides B and C whereas we propose to name jenisseensosides A and B compounds **1** and **2**.

RESULTS AND DISCUSSION

The ethanol extract of the dried roots of the plant was separately subjected to three partitions between water and petrol ether, then between water and ethyl acetate and finally between water and *n*-butanol. The butanol-soluble portion was subjected to normal silica gel column chromatography to give the saponin fraction that was further separated by repeated MPLC over reversed phase and normal silica gel yielding compounds **1a** and **2a** as an inseparable mixture. They gave only one spot by HPTLC, but two peaks by HPLC.

Acid hydrolysis of **1a** and **2a** with TFA 2 N provided D-glucuronic acid, D-galactose, D-fucose, L-rhamnose (TLC) and an aglycone identified as quillaic acid (TLC, ^{1}H and ^{13}C NMR) [5]. The UV spectra after HPLC/UV chromatography displayed absorption maxima at 311, 291 sh, 230 nm for **1a** and at 303, 230 sh nm for **2a** characteristic of cinnamoyl chromophores.

The negative FAB-mass spectrum of **1a** and **2a** gave a molecular ion peak at *m/z* 1275 $[M-H]^-$, compatible with the molecular formula $C_{64}H_{92}O_{26}$, and fragment ions peaks at *m/z* 1115 $[M-H-160]^-$, 823 $[M-H-160-146-146]^-$, 661 $[M-H-160-2\times 146-162]^-$ and 485 $[M-H-160-2\times 146-162-176]$, corresponding to the successive loss of a *p*-methoxycinnamoyl group, two desoxyhexosyl moieties, one hexosyl moiety and one hexosyluronic moiety, respectively. The IR spectrum showed absorptions at

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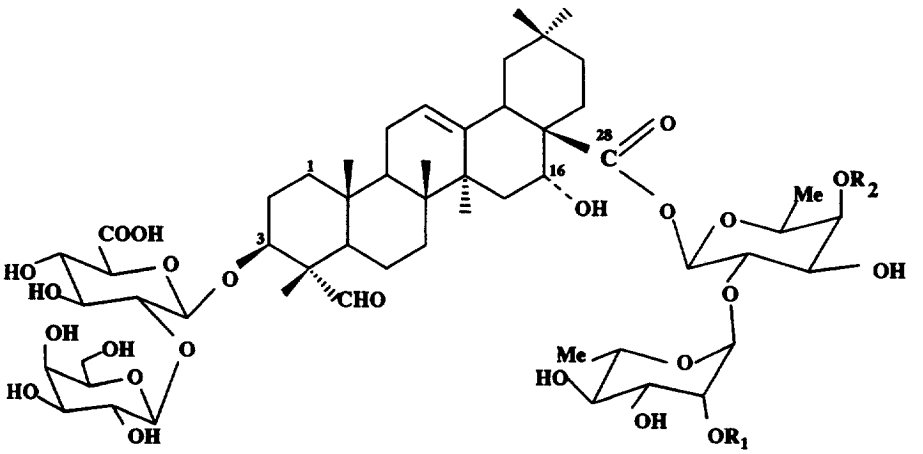
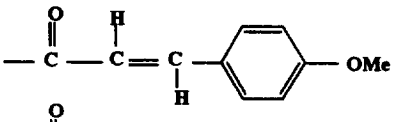
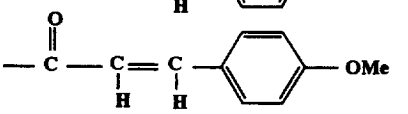
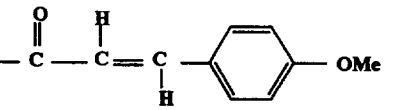
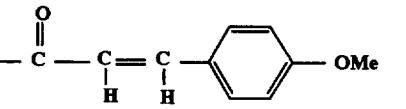


1 (Jenisseensoside A) R_1=Glc R_2= 

2 (Jenisseensoside B) R_1=Glc R_2= 

1a(Jenisseensoside C) R_1=H R_2= 

2a(Jenisseensoside D) R_1=H R_2= 

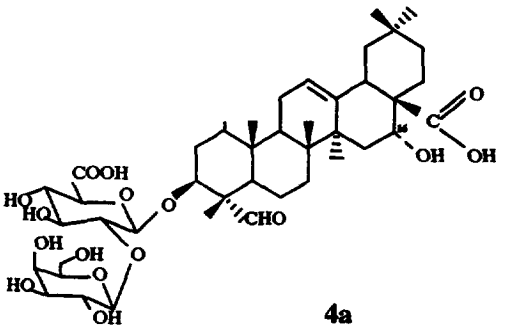
3a R_1=H R_2= H

3410 (OH), 1730 (ester) and 1715 cm^{-1} (carboxyl) while the ^1H NMR spectrum displayed signals for six tertiary methyl groups at δ 0.68, 0.82, 0.89, 0.91, 1.05, 1.32 and two secondary methyl groups (δ 0.95, 1.11), one olefinic proton (δ 5.25, *m*) one aldehydic proton (δ 9.5, *s*), five anomeric protons in the δ 5.50–4.15 range and two singlets at δ 3.79 and 3.83 due to two carbomethoxy groups. The ^1H-^1H COSY experiment permitted us to identify the *trans*-olefinic protons of the methoxycinnamoyl moiety which appeared as two doublets at δ 6.59 and 7.61 (1H each, J = 16 Hz), the *cis*-olefinic protons which appeared as two doublets at δ 5.96 and 6.92 (1H each, J = 12.4 Hz) and the *para*-substituted benzene ring protons (δ 6.95, 7.80, 2H each, *d*, J = 8 Hz; δ 7.00, 7.71, 2 H each, *d*, J = 8 Hz).

These findings indicated that **1a** and **2a** are a mixture of *trans*- and *cis*-*p*-methoxycinnamoyl triterpeneglycosides (11:7, respectively, from relative NMR and HPLC intensities). This mixture was homogeneous by TLC but was separated into *cis*- and *trans*-isomers by HPLC. All attempts to separate **1a** and **2a** by preparative HPLC were unsuccessful. Such a phenomenon could be explained by the geometrical structures of the *p*-methoxycinnamoyl group in **1a** and **2a** which showed tautomer-like behaviour under light in aqueous methanolic solution. Such isomerization of *cis*- and *trans*-methoxycinnamoyl derivatives has already been observed in *E*- and *Z*-mixtures of senegasaponins [6].

A mild alkaline hydrolysis of **1a** and **2a** with 1% KOH (30 min at room temperature) yielded *trans*- and *cis*-*p*-methoxycinnamic acid (^1H NMR, HPLC/UV) and a deacylated saponin **3a** which was homogeneous according to TLC and HPLC. The FAB mass spectrum of **3a** exhibited a quasi molecular ion $[M-H]^-$ at *m/z* 1115, compatible with the molecular formula $C_{54}H_{84}O_{24}$, and other significant peaks at *m/z* 823 $[M-H-146-146]^-$ corresponding to the loss of two desoxyhexosyl moieties, and at *m/z* 485, corresponding to the sapogenin quillaic acid. The alkaline hydrolysis of **3a** with KOH 5% (1 hr at 100°) gave the prosapogenin **4a**.

HPLC/UV analysis, the FAB-mass spectrum (thio-



4a

glycerol matrix, negative ion mode) and ^{1}H NMR and ^{13}C NMR spectral data of **4a** were in good agreement with those of the prosapogenin **4** of **1–2** which has been previously identified as 3-*O*-[*β*-D-galactopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyl] quillaic acid [5].

The above data indicated that **1a** and **2a** must be acylated bidesmosidic saponins in which two sugars are bound by a glycosidic linkage to the aglycone at C-3 whilst the two remaining sugars must be bound to the genin by a glycosidic ester linkage at C-28.

We confirmed that saponins **1a** and **2a** contained four sugar residues as shown by HMQC experiments (correlation via one-bond coupling) which revealed four correlations between carbons from anomeric C signals in *δ* 105-93 range and anomeric H signal resonating between *δ* 4.0 and 5.5.

The anomeric C signals at *δ* 104.7, 101.4, 100.5 and 93.2, which give correlations with anomeric protons at *δ* 4.25 (*d*, *J* = 7.8 Hz), 4.13 (*d*, *J* = 8.0 Hz) 5.1 (*s*) and 5.35 (*d*, *J* = 7.6 Hz), were assigned to anomeric signals of *β*-D-galactopyranose, *β*-D-glucuronopyranose, *α*-L-rhamnopyranose and *β*-D-fucopyranose, respectively. After subtraction of the anomeric signals of the sugars linked at the C-3 position from the total spectrum, the signals of two sugars linked to the aglycone by an ester linkage remained (fucose and rhamnose). The correlation in the HMQC spectrum situated apart from other δ_C/δ_H 93.2/5.35 (*d*, *J* = 7.6 Hz) showed that the fucose residue was attached to the acidic function of the aglycone by an ester linkage. This conclusion was confirmed by the heteronuclear multiple bond connectivity (HMBC) experiments which showed a correlation between the fucose H-1 (*δ* 5.35) and C-28 (*δ* 175.1) of the aglycone, confirming the attachment of fucose at C-28. Another correlation in the HMQC spectrum between the carbon at *δ* 73.7 and the deshielded H at *δ* 5.05 (*m*) corresponded to the geminal proton of one secondary alcoholic function esterified by the *p*-methoxycinnamic acid. From the cross peak in the COSY spectrum of **1a–2a** it was easy to recognize from the anomeric proton of fucose (*δ* 5.35) the H-2, H-3, deshielded H-4 (*δ* 5.05, *m*) H-5 and H-6. Furthermore, the HMBC experiment showed long range couplings between the deshielded Fuc-H-4 (*δ* 5.05) and fuc-C-2 (^3J) (*δ* 73.9), fuc-C-3 (*δ* 72.0), fuc-C-5 (*δ* 69.1), between the fuc-H-Me-6 (*δ* 0.95) and fuc-C-5 (^2J) (*δ* 69.1), fuc-C-4 (*δ* 73.7) (^3J), confirming that fuc-C-4 was esterified by the *p*-methoxycinnamic acid. Other correlations between rha-H-1 (*δ* 5.11) and fuc-C-2 (*δ* 73.9) indicated that the rhamnose was linked to the fucose by a 1 → 2 linkage. The attachment point of *p*-methoxycinnamoyl to C-4 of fucose was confirmed by comparison between the ^{13}C NMR spectral data of **1a–2a** and **3a** obtained after mild alkaline hydrolysis of **1a–2a**. All signals due to the sapogenin and sugar moieties appeared at almost the same positions. With regard to the fucose carbon region, on going from **3a** to **1a–2a** the signal for C-4 was displayed downfield by +2.6 ppm while both signals due to C-3 and C-5 were shielded by −2.4 and −1.5 ppm, respectively. Such change in the chemical shifts can only be explained if the hydroxyl group at the C-4 position of the fucose moiety is acylated [7].

Based on the above results, and the assumption that the fucose and galactose are members of the commonly found D-series and rhamnose of the L-series, the structures of **1a** and **2a** are represented at 3-*O*-[*β*-D-galactopyranosyl-(1→2)-*β*-D-glucuronopyranosyl]-28-*O*-[{*α*-L-rhamnopyranosyl-(1→2)}-{4-*O*-*trans*-*p*-methoxycinnamoyl}-*β*-D-fucopyranosyl] quillaic acid and its *cis*-isomer which have been named jenisseensosides C and D. According to previous reports on triterpene-saponins having a 4-*p*-methoxycinnamoylfucopyranosyl residue [5, 6, 8], **1a** and **2a** are new natural compounds.

Since some triterpene-saponins have been reported to exert immunostimulating activities [9], saponins **1a** and **2a** were tested in the *in vitro* granulocyte phagocytosis assay according to the method in refs [10–12] and in the *in vitro* T-cells activation assay according to the method in ref. [13]. In the concentration range of 10–100 $\mu g\ ml^{-1}$, the saponins **1a–2a** showed a significant enhancement of the granulocyte phagocytosis (70–37%). In the concentration range of 100 $ng\ ml^{-1}$–1 $pg\ ml^{-1}$, the same substances exerted an immunosuppressive effect (60–20%) in the T-cell activation assay.

EXPERIMENTAL

General methods. The NMR spectra were obtained with a Brucker DRX 500 spectrometer (500 MHz for ^{1}H and 2D ^{1}H-^{1}H COSY spectra and 125 MHz for ^{13}C spectra). The carbon type (Me, CH_2, methine) was determined by DEPT experiments. The ^{1}H-detected one-bond and multiple bond ^{13}C multiple-quantum coherence spectra (HMQC and HMBC), respectively, were measured at 500 MHz with a DRX 500 spectrometer which was equipped to allow inverse detection. The magnitude of the delay for optimising one-bond correlations in the HMQC spectrum and suppressing them in the HMBC spectrum was 3.45 msec, and the evolution delay for long range couplings in the latter was set to 60 msec. All 1D and 2D spectra were recorded using standard software and data

manipulation of the 2D spectra was performed on a Silicon Graphics Indigo computer. All chemical shifts (δ) are given in ppm and the samples were solubilized in DMSO-d_6. IR spectra (KBr disc) were recorded on a Perkin Elmer 281 spectrophotometer. Fast atom bombardment (FAB-MS): negative ion mode, thioglycerol matrix. Jeol DX 300 with JMA-3500 system. The target was bombarded with 6 keV Xe atoms. TLC and HPTLC employed precoated silica gel plates $60F_{254}$ (Merck). The following TLC solvent systems were used: for saponins, (a) $CHCl_3$–MeOH–AcOH–H_2O (15:8:3:2); for sapogenins; (b) toluene–Me_2CO (4:1); for monosaccharides, (c) $CHCl_3$–MeOH–H_2O (8:5:1); for *p*-methoxycinnamic acid, (d) toluol–Et_2O (1:1, satd with HOAc 10%). Spray reagents for the saponins were: Komarowsky reagent, a mixt. (5:1) of *p*-hydroxybenzaldehyde (2% in MeOH) and H_2SO_4 50%; for the sugars: diphenylaminephosphoric acid reagent. Analytical HPLC of saponins: Waters 600 E liquid chromatograph with Waters 990 photodiode array detection system. Lichrospher (10 g) CH-18 (5 μm) column (125 × 4 mm ID; Merck); eluent: linear gradient from 30 to 60% MeCN–H_2O with 1% 0.1 N phosphoric acid during 30 min, flow rate 1 ml min^{-1}; detection wavelength 210 nm. Isolations were carried out using a medium pressure liquid chromatography (MPLC) system [pump Gilson M 303, head pump 25SC, manometric module M 802, Injector Rheodyne 7125, Büchi column (460 × 25 mm), Büchi precolumn (110 × 15 mm), stationary phase: Lichroprep C-18 (25–40 μm), silica gel 60 (15–40 μm). For column chromatography, silica gel 60 (70–230 mesh, Merck) was used.

Plant material. The root of *Silene jenisseensis* was collected in July 1990 at the Xintai (Heipei, China). A voucher specimen no. 5001 is deposited in the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Beijing Medical University, Beijing 100083, P.R. China. Plants were identified by Dr Z. H. Cui.

Extraction and isolation of saponins. The ethanolic (95%) extract (300 g) obtained by maceration of 6 kg of dried roots was suspended in H_2O (5 l) and submitted to successive extractions by petrol, EtOAc and *n*-BuOH. After evapn under red. pres. of the solvent, 37 g of a petrol extract, 42 g of the EtOAc extract and 50 g of the BuOH extract were obtained. The BuOH extract was submitted to CC on silica gel 60 eluted successively by $CHCl_3$–MeOH (1:1 → 0–100%) and by $CHCl_3$–MeOH–H_2O (6:4:1) yielding 2.8 g of a crude saponin fr. containing **1a** and **2a**. This fr. was further purified by MPLC on reverse phase material RP-18 (solvent: MeOH–H_2O, 1:1 → 3:1) and on silica gel (15–40 μm) ($CHCl_3$–MeOH–H_2O, 8:5:1) yielding **1a** and **2a** as an inseparable mixt. (35 mg). All attempts to separate them by prep. HPLC were unsuccessful.

Compounds **1a** *and* **2a**. 3-*O*-β-D-Galactopyranosyl-(1→2)-β-D-glucuronopyranosyl-28-*O*-[{α-L-rhamnopyranosyl-(1→2)}-{4-*O*-*trans*-*p*-methoxycinnamoyl}-β-D-fucopyranosyl] quillaic acid and its *cis*-isomer. Amorphous powder. TLC R_f 0.49 (system a). Grey violet spots by spraying with Komarowsky reagent. IR ν_{max} cm^{-1}: 3500–3300 (OH), 2930 (CH), 1730 (CO ester), 1715 (CO carboxylic acid). HPLC/UV **1**: R_t 13.75; λ_{max} nm: 210, 230, (299 sh), 311; **2**: R_t 14.52; λ_{max} nm: (230 sh), 303; **1a**: R_t 14.86; λ_{max} nm: 215, 235, (300 sh), 315; **2a**: R_t 16.01; λ_{max} nm: (235 sh), 305. Negative FAB-MS *m/z*: 1275 $[M-H]^-$, 1115 $[M-H-160]^-$, 823 $[M-H-160-2\times146]^-$, 661 $[M-H-160-2\times146-162]^-$, 485 $[M-H-160-2\times146-162-176]^-$. ^1H NMR (DMSO-d_6, 500 MHz): δ 0.68, 0.82, 0.89, 0.91, 1.05, 1.32 (each 3H, *s*, Me of C-26, C-29, C-25, C-30, C-24 and C-27 of the aglycone; 0.95 (3H, *d*, J = 6.34 Hz), Me of fuc); 1.11 (3 H, J = 6.06 Hz, Me of rha); 5.25 (1H, *m*, H-12 of the aglycone; 9.50 (1H, *s*, aldehydic proton of the aglycone); protons of the *cis*-*p*-methoxycinnamoyl group: δ 7.80 (H-2″, H-6″, *d*, J = 8 Hz); 6.95 (H-3″-5″, *d*, J = 8 Hz), 5.96 (Hα, *d*, J = 12.4 Hz), 6.92 (Hβ, *d*, J = 12.4 Hz); protons of the *trans*-*p*-methoxycinnamoyl group: δ 7.71 (H-2″–H-6″, *d*, J = 8 Hz), 7.00 (H-3″–H-5″, *d*, J = 8 Hz), 6.59 (Hα, *d*, J = 16 Hz), 7.61 (Hβ, *d*, J = 16 Hz); anomeric protons: δ 4.13 (GlcA-H-1, *d*, J = 8.0 Hz), 4.25 (Gal-H-1, *d*, J = 7.8 Hz), 5.35 (Fuc-H-1, *d*, J = 7.6 Hz), 5.1 (Rha-H-1, *s*). ^{13}C NMR (DMSO-d_6, 125 MHz): see Tables 1 and 2. Long range correlations in the HMBC spectrum used for defining the aglycone of **1a**–**2a**: δ 1.09 (Me-24) → C-3 (82.1), C-5 (46), C-4 (54); δ 0.68 (Me-26) → C-7 (31.8), C-8 (38.9), C-9 (47.4) δ 1.32 (Me-27) → C-8 (38.9), C-13 (144.3), C-14 (40.6), C-15 (34.8); δ 0.82 (Me-29) → C-19 (46.5), C-20 (30.1), C-21 (34.9), C-30 (24.3); δ 0.91 (Me-30) → C-20 (30.1), C-21 (34.9), C-29 (32.8).

Acid hydrolysis of **1a** *and* **2a**. A soln of **1a**–**2a** (8 mg) in H_2O (10 ml) and 2N aq. TFA (20 ml) was refluxed on a water bath for 3 hr. After this period, the reaction mixt. was diluted with H_2O (15 ml) and extracted with CH_2Cl_2 (3 × 5 ml). The combined CH_2Cl_2 extracts were washed with H_2O and dried with Na_2SO_4; evapn of the solvent gave quillaic acid (4 mg) which was identical TLC, MS, IR, ^1H and ^{13}C NMR with lit. data and with an authentic sample. After repeated evap. of the solvent of the aq. layer by adding MeOH to remove the acid, the sugars were analysed by TLC (solvent system c).

Mild alkaline hydrolysis of **1a** *and* **2a**. Compounds **1a** and **2a** (12 mg) were hydrolysed with 1% KOH at room temp. After 30 min the mixt. was neutralized with dilute HCl and extracted with Et_2O. The Et_2O layer gave *cis*- and *trans*-*p*-methoxycinnamic acid identified by TLC (R_f 0.40 solvent system d), HPLC/UV and ^1H NMR. The aq. layer was extracted with *n*-BuOH yielding the deacylated saponin **3a** (8 mg).

Alkaline hydrolysis of **3a**. Compound **3a** (8 mg) was refluxed with 5% aq. KOH (10 ml) for 1 hr. The reaction mixt. was adjusted to pH 6 with dilute HCl, and then extracted with H_2O–satd *n*-BuOH (3 × 10 ml). The combined BuOH extracts were washed

(H_2O). Evapn of the BuOH gave the prosapogenin **4a** (5 mg).

Compound **3a**. Amorphous powder. TLC R_f 0.39 (system a), grey spot with Komarowsky reagent. Negative FAB-MS *m/z* 1115 $[M-H]^-$, 823 $[M-H-146-146]^-$, 485 $[M-H-146-146-162-176]^-$. ^1H NMR (DMSO-d_6, 500 MHz): 4.17 (1H, *d*, $J = 7.8$ Hz, anomeric H of β-D-glucuronic acid), 4.25 (1H, *d*, $J = 8$ Hz, anomeric H of β-galactopyranose), 5.25 (1H, *d*, $J = 7.5$ Hz, anomeric proton of β-D-fucopyranose, 5.35 (1H, *s*, anomeric H of α-L-rhamnopyranose), 0.65, 0.86, 0.91, 0.92, 1.10, 1.29 (each 3H, *s* of the Me of the quillaic acid), 1.10 (3H, *d*, $J = 6.5$ Hz, Me of fucose), 1.19 (3H, *d*, $J = 6$ Hz, Me of rhamnose), 5.15 (1H, *br s*, olefinic H); ^{13}C NMR (DMSO-d_6, 125 MHz): see Table 1 and 2. Acid hydrolysis of **3a** under the same conditions as described for **1a**–**2a** furnished the aglycone identified as quillaic acid and the sugars identified as galactose, rhamnose, fucose, glucuronic acid (TLC).

Compound **4a**. The spectral data are almost superimposable with those described for the prosapogenin **4** of compounds **1**–**2** in our previous report [5].

Table 1. ^{13}C NMR chemical shifts of aglycones of saponins **1a**–**4a** (δ ppm, DMSO-d_6 125 MHz)*

C	DEPT	1a	2a	3a	4a
1	CH_2	37.7	37.7	37.6	37.5
2	CH_2	24.1	24.1	24.2	24.1
3	CH	82.1	82.1	82.2	82.2
4	C	54.0	54.0	54.0	53.9
5	CH	46.0	46.0	46.0	46.0
6	CH_2	19.8	19.8	19.7	19.7
7	CH_2	31.8	31.8	31.7	31.6
8	C	38.9	38.9	40.5	40.0
9	CH	47.4	47.4	47.3	47.4
10	C	35.5	35.5	35.5	35.2
11	CH_2	22.9	22.9	22.8	22.8
12	CH	121.2	121.2	121.3	120.7
13	C	143.3	143.3	143.3	144.3
14	C	40.6	40.6	41.0	41.1
15	CH_2	34.8	34.8	34.8	34.6
16	CH	72.5	72.5	72.5	72.5
17	C	48.0	48.0	47.9	47.9
18	CH	41.1	41.1	41.0	41.1
19	CH_2	46.5	46.5	46.4	46.5
20	C	30.1	30.1	30.1	30.3
21	CH_2	34.9	34.9	35.1	34.6
22	CH_2	30.8	30.8	31.3	32.0
23	CHO	209.0	209.0	209.5	207.9
24	Me	10.3	10.3	10.3	10.3
25	Me	15.5	15.5	15.5	15.4
26	Me	16.8	16.8	16.6	17.0
27	Me	26.3	26.3	26.3	26.5
28	C	175.1	175.1	175.0	177.1
29	Me	32.8	32.8	32.8	32.9
30	Me	24.3	24.3	24.3	24.4

*Carbon-13 chemical shifts are referenced to dimethylsulfoxide at δ 39.5 ppm.

Multiplicities were assigned from DEPT spectra.

Table 2. ^{13}C NMR chemical shifts of sugar moieties of compounds **1a**–**4a** (δ ppm, DMSO-d_6, 125 MHz)*

		1a	2a	3a	4a
3-*O*-					
Glc-A	1	101.4	101.4	101.4	101.4
	2	81.5	81.5	81.5	81.6
	3	75.2	75.2	75.2	75.2
	4	71.8	71.8	72.3	71.8
	5	76.4	76.4	76.4	76.4
	6	173.2	173.2	172.2	173.1
Gal	1	104.7	104.7	104.9	104.7
	2	72.4	72.4	72.3	72.3
	3	72.9	72.9	72.9	72.9
	4	67.7	67.7	67.7	67.5
	5	73.9	73.9	74.0	73.8
	6	59.7	59.7	59.8	59.6
28-*O*-					
Fuc	1	93.2	93.2	93.0	
	2	73.9	73.9	74.2	
	3	72.0	72.0	74.4	
	4	73.7	73.7	71.1	
	5	69.1	69.1	70.6	
	6	16.1	16.1	15.9	
Rha	1	100.5	100.5	99.4	
	2	70.2	70.2	70.0	
	3	70.4	70.4	70.1	
	4	72.4	72.4	72.3	
	5	68.9	68.9	68.3	
	6	18.2	18.2	18.1	
p-Methoxy cinnamoyl					
	1″	126.7	126.9		
	2″–6″	130.3	132.4		
	3″–5″	114.4	113.5		
	4″	161.2	160.2		
	α	115.4	116.5		
	β	144.7	142.9		
	CO	166.6	165.8		
	oMe	55.3	55.4		

*The assignments were based on the HMBC, HMQC, COSY and DEPT experiments.

Glc-A, β-D-glucuronopyranosyl; Gal, β-D-galactopyranosyl; Rha, α-L-rhamnopyranosyl; Fuc, β-D-fucopyranosyl.

Bioassays. The granulocyte phagocytosis assay was performed according to refs [10, 11]. The T-cell activation assay was performed according to refs [12, 13].

Corrigenda. We give the structures of **1** and **2** which were incorrectly printed in ref. [5]. The ester glycosyl chain at C-28 was wrongly positioned at C-22.

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REFERENCES

1. Tu, Y., *Zhong Yao Zhi*, Vol. 2. The People's Medical Publishing House, Beijing, 1982, p. 520.

2. Jiansu New Medicinal College, *Great Chinese Medicinal Plant Dictionary*. Shangai, China, 1977, p. 2170.
3. *Chinese Pharmacopoeia*, 1990 edn.
4. Lacaille-Dubois, M. A., Hanquet, B., Rustaiyan, A. and Wagner, H., *Phytochemistry*, 1993, **34**, 489.
5. Lacaille-Dubois, M. A., Hanquet, B., Cui, Z.-H., Lou, Z.-C. and Wagner, H., *Phytochemistry*, 1995, **40**, 509.
6. Yoshikawa, M., Murakami, T., Ueno, T., Kadoya, M., Matsuda, H., Yamahara, J. and Murakami, N., *Chemical and Pharmaceutical Bulletin*, 1995, **43**, 2115.
7. Terui, Y., Tori, K. and Tsuji, N., *Tetrahedron Letters*, 1976, **8**, 621.
8. Hostettmann, K. and Marston, A., *Saponins, Chemistry and Pharmacology of Natural Products Series*, ed. J. D. Phillipson, D. C. Ayres and H. Baxter. Cambridge University Press, Cambridge, 1995.
9. Lacaille-Dubois, M. A. and Wagner, H., *Phytomedicine*, 1996, **2**, 363.
10. *Phagotest*. Becton Dickinson, Heidelberg, 1990.
11. Ogle, J. D., Nowl, J. G., Strankovski, R. M., Ogel, C. K. and Alexander, J. W., *Journal of Immunological Methods*, 1988, **115**, 17.
12. Jurcic, K. and Wagner, H., *Second International Congress on Phytomedicine*, Munich, Germany, 1996, SL17.
13. Maino, V. C., Suni, M. A. and Ruitenberg, J. J., *Cytometry*, 1995, **20**, 127.