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ACYLATED TRITERPENE SAPONINS FROM *SILENE JENISSEENSIS*

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Key Word Index—*Silene jenisseensis*; Caryophyllaceae; triterpene saponin.

Abstract—From the roots of *Silene jenisseensis* a new *trans-p*-methoxycinnamoyl triterpene saponin has been isolated along with its *cis-p*-methoxycinnamoyl isomer as an inseparable mixture. Their structures were 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*-[*β*-D-glucopyranosyl-(1 → 2)-*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-4-*O*-*trans-p*-methoxycinnamoyl-fucopyranosyl] quillaic acid and its *cis*-isomer, respectively. They did not show any activity in the *in vitro* chemoluminescence granulocytes assay, but exhibited only a weak inhibitory effect in the cyclooxygenase inhibition assay.

INTRODUCTION

The root of *Silene jenisseensis* Willd [1] is well known in traditional chinese medicine as a substitute for the Chinese drug Yin-Chai-Hu (root of *Stellaria dichotoma* var. *lanceolata* Bge) and it is utilized to treat fever due to Yin-deficiency and fever in infant malnutrition [2, 3]. The presence of quillaic acid has been mentioned [4] but there are no reports of either phytochemical or pharmacological work on this plant.

In the course of our investigation on new biologically active saponins from Caryophyllaceae [5], we report herein the isolation and structure elucidation of two new triterpene saponins which have been obtained as an inseparable mixture from the root of *S. jenisseensis*.

RESULTS AND DISCUSSION

The ethanol extract of the dried roots of the plant was separately subjected to three partitions between water and petrol, then between water and ethyl acetate and finally between water and butanol, followed by repeated column chromatography and preparative HPLC over reverse phase silica to obtain compounds **1** and **2** as an inseparable mixture. They gave only one spot by HPTLC but two peaks by HPLC.

Acid hydrolysis of **1** and **2** with 2N TFA provided D-glucuronic acid, D-galactose, D-fucose, L-rhamnose, D-glucose (TLC, GLC of the alditol acetates) and an aglycone identified as quillaic acid (TLC, ^{1}H and ^{13}C NMR) [6–9]. The UV spectra after HPLC/UV chromatography displayed absorption maxima at 311, 291sh, 230 nm for **1** and at 303, 230sh nm for **2**, characteristic of cinnamoyl chromophores.

The negative FAB-mass spectra of **1** and **2** gave an $[M - H]^-$ ion peak at *m/z* 1437, compatible with the molecular formula $C_{70}H_{102}O_{31}$, and fragment ions peaks at *m/z* 823 and 485, corresponding to hexosyl–hexosyluronic acid–quillaic acid and quillaic acid, respectively. The IR spectrum showed absorptions at 3410 (OH), 1735 (ester) and 1710 cm^{-1} (carbonyl) while the ^{1}H NMR spectrum displayed signals for six tertiary methyl groups at *δ*0.62, 0.85, 0.89, 0.90, 1.05, 1.30 and two secondary methyl groups (*δ*0.98, 1.15), one olefinic proton (*δ*5.25, *m*), one aldehydic proton (*δ*9.9, *s*), five anomeric protons in the *δ*5.50–4.15 range and two singlets at *δ*3.81 and 3.84 due to two carbomethoxy groups. The ^{1}H–^{1}H COSY experiment permitted us to identify the *trans*-olefinic protons of a cinnamoyl moiety which appeared as two doublets at *δ*6.61 and 7.65 (1H each, $J = 16$ *Hz*), *the cis*-olefinic protons which appeared as two doublets at *δ*6.00 and 6.90 (1H each, $J = 12$ Hz) and the *para*-substituted benzene ring protons (*δ*6.98, 7.82, 2 H each, *d*, $J = 8$ Hz; *δ*7.02, 7.75, 2 H each, *d*, $J = 8$ Hz).

These findings indicated that **1** and **2** comprise a mixture of *trans-* and *cis-p*-methoxycinnamoyl triterpene-glycosides (11:9, respectively, from relative NMR and HPLC intensities).

This mixture was homogeneous by TLC but was separated into *cis-* and *trans*-isomers by HPLC. Further evidence was obtained from the mild alkaline hydrolysis

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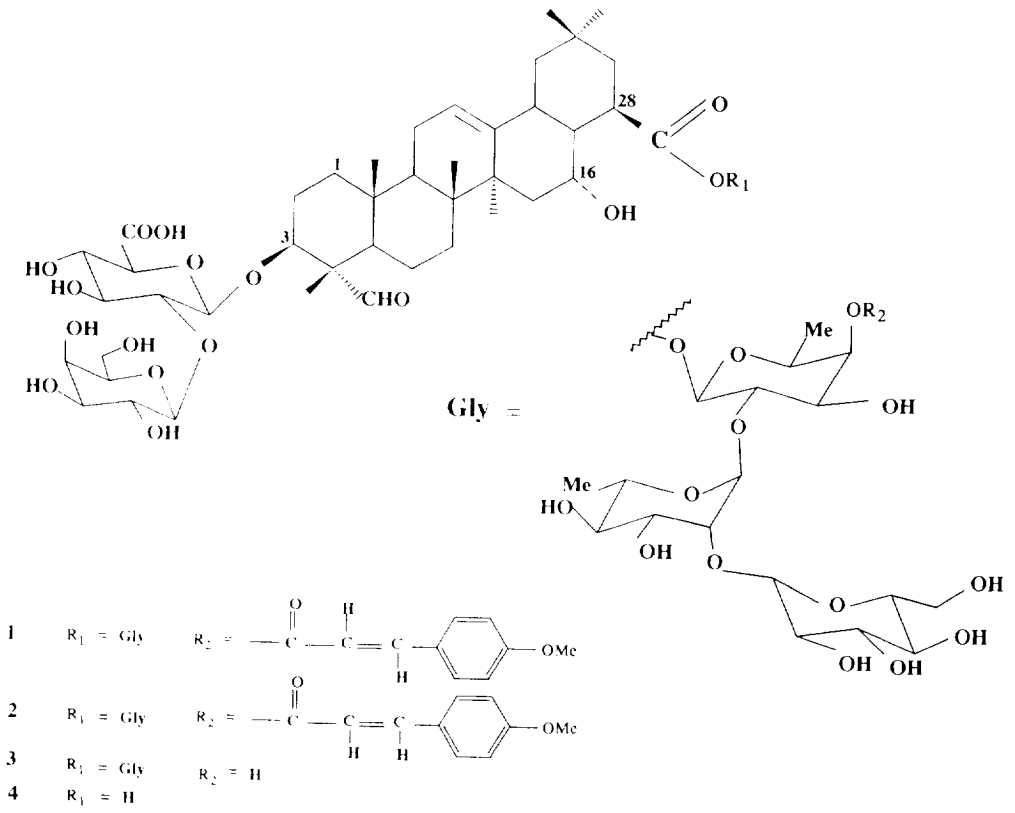

of **1** and **2** with KOH 1% (1 hour at room temperature) which yielded *trans*- and *cis*- *p*-methoxycinnamic acid (^{1}H NMR, HPLC/UV) and a deacylated saponin **3**, which was homogeneous according to TLC and HPLC. The FAB-mass spectrum of **3** exhibited a quasi-molecular ion $[M - H]^-$ at *m/z* 1277, compatible with the molecular formula $C_{60}H_{94}O_{29}$, and other significant peaks at *m/z* 1115 $[M - H - 162]^-$, 969 $[M - H - 162 - 146]^-$ and 823 $[M - H - 162 - 146 - 146]^-$, corresponding to the successive loss of one hexosyl, one desoxyhexosyl and other desoxyhexosyl moieties, and at *m/z* 485, corresponding to the sapogenin quillaic acid. The alkaline hydrolysis of **3** with KOH 5% (1 hour at 100°) gave the prosapogenin **4**.

The FAB-mass spectrum (thioglycerol matrix, negative ion mode) of **4** exhibited a quasi-molecular ion $[M - H]^-$ at *m/z* 823 with other significant peaks at *m/z* 661 and 485, corresponding to the successive loss of one hexosyl and one hexosyluronic acid, respectively. Subsequent acid hydrolysis of **4** with 2M TFA gave an aglycone which was identified as quillaic acid by comparison of its ^{13}C NMR spectrum with reported data [6–9] and galactose (co-TLC with authentic samples and GLC of the alditol acetates) and glucuronic acid (co-TLC with an authentic sample).

The above data indicated that **1** and **2** must be acylated bidesmosidic saponins in which two sugars should be bound by a glycosidic linkage to the aglycone at C-3, whilst the three remaining sugars must be bound to the genin by a glycosidic ester linkage at C-28. The ^{1}H NMR of **4** showed two anomeric protons signals at δ4.16 (*d*, J = 7.5 Hz) and 4.25 (*d*, J = 7.8 Hz) indicating β-glycosidic linkages of a β-glucuronopyranose and β-galactopyranose, which were supported by the chemical shifts of the anomeric carbon (Table 1).

In the ^{13}C NMR spectrum of **4** we could recognize the signals of the aglycone and of the two sugars. The downfield chemical shift at δ82.2 was assigned to C-3, which indicated that the sugar linkage is at this position. The (1 → 2) linkage of Gal with Glc-UA is supported by comparing the ^{13}C NMR data given in the literature for such linked sugar moieties [5, 9]. Thus the downfield chemical shifts at δ81.6 confirmed the attachment of Gal at C-2 of GlcUA. The ^{13}C NMR assignments were further confirmed by an HMQC experiment for **1** and **2**. The signal at δ81.6 assigned to C-2 of glucuronic acid showed connectivity with H-2 at δ3.12, and this in turn showed connectivities with the signal at δ4.1 due to H-1 of glucuronic acid in the ^{1}H–^{1}H COSY spectrum. The above data supported the structure for **4** of 3-*O*-β-D-galactopyranosyl-(1 → 2)-β-D-glucuronopyranosyl quillaic acid.

The next step in the structure elucidation of **1** and **2** was the determination of the interglycosidic linkages of the three remaining sugars, fucose, rhamnose, glucose and the position of the *p*-methoxycinnamoyl group, which were established by analysis of the 1D NMR spectra (^{1}H, ^{13}C NMR, DEPT) and 2D NMR spectra [^{1}H–^{1}H COSY, HMQC (correlation via one bond coupling) [10] and HMBC (correlation via two or three bond

couplings) [11] experiments]. We can confirm that saponins **1** and **2** contain five sugar residues as shown by one-bond chemical shift experiments which revealed five correlations between carbons from anomeric C signals in the δ106–94 range and anomeric H signals resonating between δ4.0 and 5.5 ppm. Even at high field the 1D and 2D COSY 45 ^{1}H NMR spectra of **1** and **2** are complex and a full assignment could not be achieved.

The anomeric C signals at δ105.8, 104.7, 101.5, 99.5 and 92.7, which give correlations with anomeric protons at δ4.26 (*d*, J = 8 Hz), 4.25 (*d*, J = 8Hz), 4.18 (*d*, J = 7.73 Hz), 5.48 (*s*) and 5.38 (*d*, J = 7.8 Hz) were assigned to anomeric protons of β-D-glucopyranose, β-D-galactopyranose, β-D-glucoronopyranose, 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 three sugars linked to the aglycone by an ester linkage remain (fucose, rhamnose and glucose). The correlation in the HMQC spectrum situated apart from other δ_C/δ_H 92.7/5.38 (*d*, J = 8 Hz) showed that the fucose residue is 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 δ5.38) and C-28 (δ175.0) of the aglycone, confirming the attachment of fucose at C-28. Another correlation in the HMQC spectrum between the carbon at δ73.4 and the deshielded H at δ5.0 (*m*) corresponds to the geminal proton of one secondary alcoholic function esterified by the *p*-methoxycinnamic acid. From the cross-peak in the COSY spectrum of **1** and **2** it was easy to recognize from the anomeric proton of fucose (δ5.38) the H-2, H-3, deshielded H-4 (δ5.0, *m*), H-5 and H-6. Furthermore, the HMBC experiment showed long-range couplings between the deshielded Fuc-H-4 (δ5.0) and fuc-C-2 (^3J) (δ73.9), between the fuc-H-Me-6 and fuc-C-5 (^2J), fuc-C-4 (δ73.4) (^3J), confirming that fuc-C-4 was esterified by the *p*-methoxycinnamic acid. Other correlations between rha-H-1 (δ5.4) and fuc-C-2 (δ73.9) and between Glc-H-1 (δ4.25) and rha-C-2 (δ81.3) indicate that the rhamnose is linked to the fucose by a 1 → 2 linkage and the glucose to the rhamnose by a 1 → 2 linkage. Furthermore, in the ^{1}H–^{1}H COSY spectrum, the anomeric proton of rhamnose (δ5.4) is directly correlated to the H-2 of rhamnose at δ3.8 which is correlated to C-2 of rhamnose (δ81.3) in the HMQC spectrum, confirming that the glucose is linked to the rhamnose by a 1 → 2 linkage. The attachment point of *p*-methoxycinnamoyl to C-4 of fucose was confirmed by comparison between ^{13}C NMR spectral data of **1** and **2** and **3** obtained after mild alkaline hydrolysis of **1** and **2**. 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 **3** to **1** and **2** the signal for C-4 was displayed downfield by + 2.1 ppm, while both signals due to C-3 and C-5 were shielded by − 1.9 and − 1.4 ppm, respectively. Such change in the chemical shifts can only be explained if the hydroxyl group at the 4-position of the fucose moiety is acylated [12].

Based on the above results and the assumption that the fucose, glucose and galactose are members of the commonly found D-series and rhamnose of the L-series, the structures of **1** and **2** are represented as 3-*O*-[β-D-galactopyranosyl-(1 → 2)-β-D-glucuronopyranosyl]-28-*O*-[β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranosyl]-(1 → 2)-β-D-4-*O*-*trans*-*p*-methoxycinnamoyl-β-D-fucopyranosyl] quillaic acid and its *cis*-isomer. According to one report on a triterpene saponin having a 4-*p*-methoxycinnamoylfucopyranosyl residue [13], and several reviews on the distribution of quillaic acid glycosides in plants [14, 15], **1** and **2** are new natural compounds.

Since some triterpene saponins have been reported to exert immunostimulating activities [16, 17] saponins **1** and **2** were tested in the *in vitro* assay for chemoluminescence of granulocytes according to the method of Wagner and Jurcic [18]. They showed no significant burst enhancement. The potential anti-inflammatory response of **1** and **2** was tested by subjecting the mixture to the *in vitro* cyclo-oxygenase inhibition test [19, 20]. It showed only a very weak inhibition effect in this test system (9.5% inhibition at a concentration of 50 μM).

EXPERIMENTAL

Instruments and general methods. The NMR spectra were obtained with a Brucker AM 400 spectrometer (400 MHz for ^{1}H and 2D ^{1}H–^{1}H COSY spectra and 100 MHz for ^{13}C spectra). The carbon type (methyl, methylene, methine) was determined by DEPT experiments. The ^{1}H detected one-bond [8] and multiple bond ^{13}C multiple-quantum coherence spectra [9] (HMQC and HMBC), respectively, were measured at 500 MHz with an AMX 500 spectrometer, which was modified to allow inverse detection. The magnitude of the delay for optimizing 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 80 msec. All 1D and 2D spectra were recorded using standard software and data manipulation of the 2D spectra was performed on a Brucker Aspect X32 Data station. All chemical shifts 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. GLC analysis: Perkin–Elmer 900 B, glass column (200 × 0.3 cm) packed with 0 V 225, carrier gas: Ar, 30 ml min^{-1}. TLC and HPTLC employed pre-coated silica gel plates 60F_{254} (Merck). The following TLC solvent systems were used: for saponins, (*a*) $CHCl_3$–MeOH–HOAc–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–ether (1:1, satd with HOAc 10%). Spray reagents for the saponins were: Komarowsky reagent, a mixture (5:1) of *p*-hydroxy benz-

aldehyde (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) i.d. 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 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)]. For column chromatography, silica gel 60 (70–230 mesh, Merck) was used.

Plant material. The root of *Silene jenissensis* was collected in July 1990 at the Xintai (Heipei, China). A voucher specimen n° 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 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 **1** and **2**. This fr. was further purified by MPLC on reverse-phase material RP-18 (solvent: MeOH–H_2O, 1:1 → 3:1) yielding **1** and **2** as an inseparable mixt. (100 mg). All attempts to separate them by prep. HPLC were unsuccessful.

Compounds **1** and **2**. 3-*O*-[β-D-galactopyranosyl-(1 → 2)-β-D-glucuronopyranosyl]-28-*O*-[β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranosyl]-(1 → 2)-β-D-4-*O*-*trans*-*p*-methoxycinnamoyl-fucopyranosyl] quillaic acid and its *cis*-isomer. Amorphous powder. TLC R_f 0.44 (system *a*). Grey spots by spraying with Komarowsky reagent. IR ν_{max} cm^{-1}: 3500–3300 (OH), 2930 (CH), 1725 (CO ester), 1710 (CO carboxylic acid). HPLC/UV **1**: t_R 13.62; λ_{max}^{MeOH} nm: 210, 230, (299sh), 311; **2**: t_R 14.37; λ_{max}^{MeOH} nm: (230sh), 303. Negative FAB-MS *m/z*: 1437 $[M-H]^-$, 1275 $[M-H-162]^-$, 1115 $[M-H-162-160]^-$, 823 $[M-H-162-160-2\times146]^-$, 661 $[M-H-162-160-2\times146-162]^-$, 485 $[M-H-162-160-2\times146-162-176]^-$. ^{1}H NMR (DMSO-$d_6$, 400 MHz): δ0.62, 0.85, 0.89, 0.9, 1.05, 1.3 (each, 3H, *s*, Me of C-26, C-29, C-25, C-30, C-24 and C-27 of the aglycone), 0.98 (3H, *d*, J = 6.34 Hz, Me of fuc), 1.15 (3H, *d*, J = 6.06 Hz, Me of rha), 5.24 (1H, *m*, H-12 of the aglycone), 9.90 (1H, *s*, aldehydic proton of the aglycone); protons of the *cis*-*p*-methoxycinnamoyl group: δ7.80 (H-2″, H-6″, *d*, J = 8 Hz), 6.96 (H-3″-5″, *d*, J = 8 Hz), 6.0 (Hα, *d*, J = 12.6 Hz), 7.0 (Hβ, *d*, J = 12.6 Hz); protons of the *trans*-*p*-methoxycinnamoyl group: δ7.75 (H-2″–H-6″, *d*, J = 8 Hz), 7.02 (H-3″–5″, *d*, J = 8 Hz), 6.62 (Hα, *d*, H = 16 Hz), 7.65 (Hβ, *d*, J = 16 Hz); anomeric protons: δ4.18 (GluUA-H-1, *d*, J = 7.73 Hz), 4.25 (Glc-H-1, *d*, J = 8 Hz), 4.26 (Gal-H-1, *d*, J = 8 Hz), 5.38 (Fuc-H-1, *d*, J = 7.8 Hz), 5.48 (Rha-H-1, *s*). ^{13}C NMR (DMSO-d_6, 100 MHz): see Tables 1 and 2.

Acid hydrolysis of **1** *and* **2**. A soln of **1–2** (15 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 (8 mg) which exhibited identical TLC, MS, IR, ^{1}H and ^{13}C NMR with literature data and with an authentic sample. After repeated evapns of the solvent of the aq. layer by adding MeOH to remove the acid, the sugars were analysed by TLC (solvent system c). The

Table 1. ^{13}C NMR chemical shifts of sugar moieties of compounds **1–4**

	C	1	2	3	4
3-*O*-					
Glc-A	1	101.5	101.5	101.4	101.4
	2	81.6	81.6	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	72.3	72.3	72.3	72.3
	3	72.9	72.9	72.9	72.9
	4	67.7	67.7	67.7	67.5
	5	73.8	73.8	74.0	73.8
	6	59.8	59.8	59.8	59.6
28-*O*-					
Fuc	1	92.7	92.7	92.9	
	2	73.9	73.9	74.4	
	3	72.5	72.5	74.6	
	4	73.4	73.4	71.3	
	5	69.0	69.0	70.6	
	6	16.0	16.0	15.9	
Rha	1	99.5	99.5	99.4	
	2	81.3	81.3	81.5	
	3	70.1	70.1	70.1	
	4	72.0	72.0	72.4	
	5	68.7	68.7	68.5	
	6	18.1	18.1	18.0	
Glc	1	105.8	105.8	105.9	
	2	73.4	73.4	73.1	
	3	76.3	76.3	76.5	
	4	70.0	70.0	70.0	
	5	76.6	76.6	76.8	
	6	61.2	61.2	61.2	
p-Methoxy cinnamoyl					
	1″	126.7	126.9		
	2″–6″	130.2	132.3		
	2″–5″	114.3	113.5		
	4″	161.1	160.1		
	α	115.4	116.6		
	β	144.6	143.2		
	CO	166.6	165.8		

Table 2. ^{13}C NMR chemical shifts of aglycones of saponins **1–4** (δ ppm, DMSO-d_6 as solvent and interference δ39.5, multiplicities assigned by DEPT spectra)

C	DEPT	1	2	3	4
1	CH_2	37.5	37.5	37.6	37.5
2	CH_2	24.2	24.2	24.2	24.1
3	CH	82.2	82.2	82.2	82.2
4	C	53.8	53.8	54.0	53.9
5	CH	45.8	45.8	46.0	46.0
6	CH_2	19.7	19.7	19.7	19.7
7	CH_2	31.7	31.7	31.7	31.6
8	C	38.9	38.9	40.5	40.0
9	CH	47.3	47.3	47.3	47.4
10	C	35.4	35.4	35.5	35.2
11	CH_2	22.7	22.7	22.8	22.8
12	CH	121.2	121.2	121.3	120.7
13	C	143.1	143.1	143.3	144.3
14	C	41.0	41.0	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	47.8	47.8	47.9	47.9
18	CH	41.0	41.0	41.0	41.1
19	CH_2	46.3	46.3	46.4	46.5
20	C	30.0	30.0	30.1	30.3
21	CH_2	34.8	34.8	35.1	34.6
22	CH_2	31.6	31.6	31.9	32.0
23	CHO	209.2	209.2	209.5	207.9
24	Me	10.1	10.1	10.3	10.3
25	Me	15.3	15.3	15.5	15.4
26	Me	16.6	16.7	16.6	17.0
27	Me	26.2	26.2	26.3	26.5
28	C	175.0	175.0	175.0	177.1
29	Me	32.7	32.7	32.8	32.9
30	Me	24.3	24.3	24.3	24.4

saponins **1** and **2** (5 mg) was hydrolysed with 2 N TFA in a sealed serum vial at 100° for 3 hr. Sugars in the hydrolysates were converted to the alditol acetates and then subjected to GLC analysis according to the method previously described [5].

Mild alkaline hydrolysis of **1** *and* **2**. Compounds **1** and **2** (25 mg) were hydrolysed with KOH 1% 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 **3** (12 mg).

Alkaline hydrolysis of **3**. Compound **3** (10 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 **4** (5 mg).

Compound **3**. Amorphous powder. TLC R_f 0.18 (system *a*), grey spot with Komarowsky reagent. Negative FAB-MS *m/z*: 1277 $[M - H]^-$, 1115 $[M - H - 162]^-$, 969 $[M - H - 162 - 146]^-$ and 823 $[M - H - 162 - 146 - 146]^-$, 485 $[M - H - 162 - 146 - 146 - 162 - 176]^-$. ^1H NMR (DMSO-d_6, 400 MHz): δ4.15 (1H, *d*, J = 7 Hz, anomeric H of β-D-glucuronic acid), 4.22 (1H, *d*, J = 7.8 Hz, anomeric proton of β-D-glucopyranose), 4.23 (1H, *d*, J = 7.8 Hz, anomeric H of β-galactopyranose), 5.20 (1H, *d*, J = 8 Hz, anomeric proton of β-D-fucopyranose), 5.45 (1H, *s*, anomeric H or α-L-rhamnopyranose), 0.65, 0.86, 0.90, 0.91, 1.08, 1.25 (each 3H, *s* of the Me of the quillaic acid), 1.11 (3H, *d*, J = 6.5 Hz, Me of fucose), 1.29 (3H, *d*, J = 6.1 Hz, Me of rhamnose), 5.15 (1H, *br s*, olefinic H); ^{13}C NMR (DMSO-d_6, 100 MHz): see Tables 1 and 2. TLC R_f 0.35 (solvent system *a*), violet spot with Komarowsky reagent. Acid hydrolysis of **3** under the same conditions as described for **1** and **2** provided the aglycone identified as quillaic acid and the sugars identified as glucose, galactose, rhamnose, fucose, glucuronic acid (TLC, GLC of the alditol acetates).

Compound **4**. Amorphous powder. Negative FAB-MS *m/z*: 823 $[M - H]^-$, 661 $[M - H - 162]^-$, 485 $[M - H - 162 - 176]^-$. ^1H NMR (DMSO-d_6, 400 MHz): δ4.16 (1H, *d*, J = 7.5 Hz, anomeric H of β-D-glucuronic acid), 4.25 (1H, *d*, J = 7.8 Hz, anomeric H of β-D-galactopyranose), 0.7, 0.87, 0.94, 0.95, 1.09, 1.31 (each 3H, *s* of the Me of the quillaic acid), 9.50 (1H, *s*, aldehydic H of the aglycone). ^{13}C NMR (DMSO-d_6, 100 MHz): see Tables 1 and 2. TLC R_f 0.75 (solvent system *a*), violet spot with Komarowsky reagent. Acid hydrolysis of **4** under the same conditions as described for **1** and **2** produced quillaic acid, glucuronic acid and galactose.

Bioassays. The *in vitro* chemoluminescence test was performed according to Wagner and Jurcic [18]. The *in vitro* cyclooxygenase inhibition test was performed according to ref. [19, 20].

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