Sulfated Lupane Triterpene Derivatives and a Flavone *C*-Glycoside from *Gypsophila repens*

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A new sulfated lupane triterpene, Gypsophilin (1), and its glucosyl ester, Gypsophilinoside (2) were isolated from the roots of *Gypsophila repens* whereas a new flavone *C*-glycoside (3) was obtained from the aerial parts. Their structures were established as (3β)-3-*O*-(sulfo)lup-20(29)-en-23,28-dioic acid (1), (3β)-3-*O*-(sulfo)lup-20(29)-en-23,28-dioic acid -28-*O*-β-D-glucopyranosyl ester (2) and luteolin-7-*O*-α-L-arabinopyranosyl-6-*C*-β-glucopyranoside (3) by spectroscopic methods such as 1D and 2D NMR, HR-ESI-MS and FAB-MS.

Key words *Gypsophila repens*; Caryophyllaceae; sulfated lupane triterpene; saponin; flavone *C*-glycoside

The genus *Gypsophila* (Caryophyllaceae), has more than 100 species and shows a wide distribution in Europa and Asia and is well known to contain saponins. Some species are used in folk medicine as remedies for cough, colds, ailments of the upper respiratory tract and also as a source of various products in industry such as detergents.[1)] The commercial Merck saponin which has been widely utilized as a standard for hemolytic test was obtained from the roots of several *Gypsophila* species. As part of our continuing investigation on the saponins of Caryophyllaceae family,[2—5)] we have examined the roots and aerial parts of *Gypsophila repens* which is a perennial plant widely distributed in Europe[6)] (France, Swisserland, Belgium) and in the South West of Asia. This plant was reported to contain a gypsogenin glycoside,[7)] but no detailed phytochemical study was described. This paper deals with the isolation and structure elucidation of a new sulfated lupane triterpene (**1**) and its glucosyl ester (**2**) from the 90% methanolic extract of the roots, in addition to one new flavone-*C*-glycoside (**3**) from the 90% methanolic extract of the aerial parts of *G. repens*. Their structures were determined mainly on the basis of spectroscopic methods such as 1D, and 2D NMR, HR-ESI-MS and FAB-MS.

Results and Discussion

The 90% MeOH extract from the roots of *Gypsophila repens* was partitioned with $CHCl_3$, EtOAc and H_2O-saturated *n*-BuOH. The *n*-BuOH fraction was suspended in MeOH and purified by precipitation with Et_2O yielding a crude saponin mixture which was subjected to multiple chromatographic steps over Si RP-18, Sephadex LH-20 and Si gel to afford **1** and **2**. Furthermore, a concentrated *n*-BuOH-soluble fraction of the 90% MeOH extract of the aerial parts of *G. repens* was purified by precipitation with diethyl ether and successively chromatographed over Si-RP18 and Sephadex LH-20 to yield compound **3**.

Gypsophilin (**1**) was obtained as a white amorphous powder and its molecular formula was assigned as $C_{30}H_{46}O_8S$ indicated by the $[M+Na]^+$ ion peak at *m/z* 589.2840 (Calcd 589.2811) in HR-ESI-MS. Its FAB-MS (negative-ion mode) showed a quasi-molecular ion peak at *m/z* 565 $[M-H]^-$, indicating a molecular weight of 566. The fragment ion at *m/z* 485 $[M-H-80]^-$ showed the presence of a sulfate group $[SO_3]^-$ which was corroborated by the presence of a peak at *m/z* 97 $[OSO_3H]^-$. The IR spectrum indicated the presence of hydroxyl groups (3400 cm^{-1}), a carboxylic group (1712 cm^{-1}) and a double bond (1636 cm^{-1}). The presence of SO group was indicated by the absorption band at 1220 cm^{-1}.

The ^{1}H-NMR spectrum of **1** displayed four methyl signals at δ 0.65, 0.85, 0.91 and 1.38 (3H each, s), five proton signals of an isoprenyl moiety at δ 1.70 (3H, s), 4.66 and 4.75 (1H each, br s). In the ^{13}C-NMR and DEPT spectra, 30 carbon signals were observed, including five methyls, 10 methylenes, 6 methines, 5 quaternary carbons as well as two carboxylic carbons and two olefinic carbons (one $=CH_2$ and one quaternary carbon) (Table 1). An extensive 2D NMR analysis of **1** is in favour of a triterpenic lup-20(29)-ene structure which is in good agreement with betulinic acid[8)] except some signals of ring A. The downfield shift observed in the HSQC spectrum at δ 5.41 (dd, J=7.9, 2.7 Hz)/δ 83.6 suggested that the aglycone residue was esterified by the SO_3H group at the position C-3, that was in good agreement for similar sulfated triterpenoids.[9)] The configuration of the OSO_3H group was established to be β by the NOESY spectrum which showed a significant cross peak between δ 5.41 (dd, J=7.9, 2.7 Hz, H-3) and δ 1.82 (m, H-5). Furthermore, the HMBC experiment showed cross-peaks between the ^{1}H-NMR signal at δ 1.38 (3H, s, H-24) and the carboxylic carbon signal at δ 184.9, between δ 1.38 (3H, s, H-24), and δ 83.6 (C-3), δ 53.5 (C-4), and δ 51.5 (C-5), indicating the presence of a C-23 carboxylic function. This was confirmed by the NOESY experiment which showed spatial correlations between H_3-24 and H_3-25 indicating that the C-24 methyl group was β-axial, which in turn indicated the C-23 carboxyl group to be connected as α-equatorial at C-4 (see Fig. 2). On basis of these results, compound **1** was elucidated as (3β)-3-*O*-(sulfo)lup-20(29)-en-23,28-dioic acid, a new natural compound named gypsophilin.

Gypsophilinoside (**2**) was obtained as a white amorphous powder. The molecular formula was determined to be $C_{36}H_{56}O_{13}S$ from the molecular ion peak at *m/z* 751.3315 $[M+Na]^+$ (Calcd 751.3339) in the HR-ESI-MS. Its FAB-MS (negative-ion mode) showed a quasi-molecular ion peak at *m/z* 727 $[M-H]^-$, indicating a molecular weight of 728.

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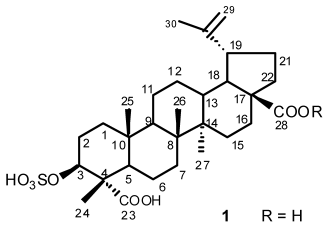
Table 1. ^{1}H- (600 MHz) and ^{13}C-NMR (150 MHz) Spectral Data of **1** and **2** in Pyridine-d_5 [a,b,c]

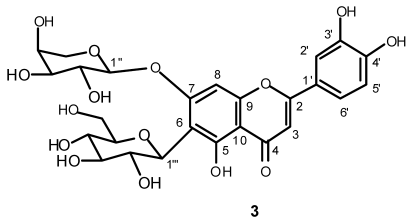
Position	Mult.	**1**		**2**	
		δ_H	δ_C	δ_H	δ_C
1	CH_2	0.90	38.5	1.44	38.5
		1.48		1.90	
2	CH_2	1.84	24.5	1.83	24.4
		2.58		2.58	
3	CH	5.41 dd (7.9, 2.7)	83.6	5.40 dd (9.1, 3.1)	83.7
4	C		53.5		53.6
5	CH	1.82	51.5	1.81	51.6
6	CH_2	1.46	20.9	1.40	20.8
		nd		nd	
7	CH_2	1.08 br d (10.7)	34.0	1.05 br d (10.5)	33.8
		1.42		1.39	
8	C		40.9		41.0
9	CH	1.28	50.3	1.26	50.3
10	C		36.0		36.2
11	CH_2	1.12	20.7	1.12	20.6
		nd		nd	
12	CH_2	1.02	25.6	0.98	25.4
		1.74		1.72	
13	CH	2.52 t-like	38.1	2.47 t-like	38.0
14	C		42.1		42.3
15	CH_2	1.01	29.7	1.01	29.6
		1.63		1.63	
16	CH_2	1.40	32.4	1.40	31.8
		2.46 d (12.4)		2.53 d (11.9)	
17	C		56.2		56.6
18	CH	1.61	47.3	1.63	49.4
19	CH	3.33 m	49.3	3.24 m	47.0
20	C		151.0		150.5
21	CH_2	1.38	30.7	1.32	30.4
		2.08 m		2.02 m	
22	CH_2	1.43	37.1	1.42	36.4
		2.10 t-like		2.10 t-like	
23	COOH		184.9		184.9
24	CH_3	1.38 s	12.4	1.40 s	12.6
25	CH_3	0.65 s	16.2	0.64 s	16.2
26	CH_3	0.85 s	15.8	0.91 s	15.7
27	CH_3	0.91 s	14.6	0.87 s	14.6
28	COOH		179.2		175.0
29	CH_2	4.68 br s	109.5	4.66 br s	109.7
		4.78 br s		4.75 br s	
30	CH_3	1.70 s	19.1	1.66 s	19.0
Glc					
1	CH			6.18 d (8.1)	94.8
2	CH			4.03 t (8.5)	73.4
3	CH			4.21	77.8
4	CH			4.15 t (9.1)	70.4
5	CH			3.92 m	78.6
6	CH_2			4.19	61.5
				4.30 d (12.1)	

a) δ ppm; values, coupling constants (J in parentheses) are given in Hz; assignments were confirmed by 1D and 2D NMR experiments (COSY ^{1}H–^{1}H, TOCSY, NOESY, HSQC and HMBC). *b*) Multiplicities were assigned from DEPT spectra. *c*) Overlapped ^{1}H-NMR signals are reported without designated multiplicity. nd: not detezmined

Other significant fragment peaks appeared at *m/z* 565 $[(M-H)-162]^-$, corresponding to the loss of one hexosyl unit, and at *m/z* 485 $[(M-H)-162-80]^-$, corresponding to the quasi-molecular ion of the aglycone.

The ^{1}H-NMR spectrum of **2** (C_5D_5N) exhibited one anomeric ^{1}H-NMR signal at δ 6.18 (1H, d, J=8.1 Hz) which correlated in the HSQC spectrum with the ^{13}C-NMR signal at δ 94.8. The complete assignment of the glycosidic NMR signals was achieved by analysis of COSY, TOCSY, HSQC and HMBC experiments (Table 1). Evaluation of spin–spin couplings and chemical shifts allowed the identification of one β-glucopyranosyl (Glc) unit. The D-configuration was determined by GC.[10] Most of the ^{1}H- and ^{13}C-NMR signals corresponding to the aglycone part of **2** were superimposable with those formerly described for **1**. Moreover, comparison of 2D-NMR data of **1** and **2** revealed that the only difference between these two compounds is the presence in **2** of an additional β-glucopyranosyl unit. The linkage of this β-glucopyranosyl unit at C-28 of the aglycone was deduced by the HMBC correlation observed between the anomeric ^{1}H-NMR signal at δ 6.18 (1H, d, J=8.1 Hz, Glc H-1) and the carboxylic ^{13}C-NMR signal at δ 175.0 (C-28) showing a upfield shift due to the esterification at this position. Therefore, the structure of Gypsophilinoside (**2**) was established as (3β)-3-





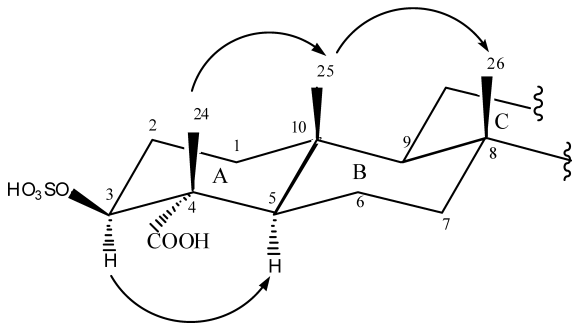


Fig. 1. Selected NOESY Correlations within Rings A and B for Compound **1**

Table 2. ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) and Spectral Data of **3** (CD_3OD)[a,b,c]. δ ppm, (J) in Hz

Position	Mult.	δ_H	δ_C
Agly			
2	C		166.1
3	CH	6.52 s	103.9
4	C		183.9
5	C		160.0
6	C		109.2
7	C		165.3
8	CH	6.44 s	95.0
9	C		158.8
10	C		105.1
1′	C		123.5
2′	CH	7.34 s-like	114.1
3′	C		147.0
4′	C		151.0
5′	CH	6.88 d (8.8)	116.9
6′	CH	7.36 br d (8.7)	120.3
Ara			
1″	CH	4.36 d (5.9)	106.6
2″	CH	3.51 dd (8.6, 6.6)	73.4
3″	CH	3.43 dd (8.6, 3.4)	74.2
4″	CH	3.58 m	69.1
5″	CH_2	3.07 dd (12.4, 1.8)	66.4
		3.26 dd (12.5, 3.3)	
Glc			
1‴	CH	4.94 d (9.8)	73.2
2‴	CH	3.43 dd (8.6, 8.4)	73.2
3‴	CH	3.65 t (9.0)	80.5
4‴	CH	3.49 dd (9.0, 8.8)	71.6
5‴	CH	3.40 m	82.5
6‴	CH_2	3.72 dd (12.0, 5.5)	62.9
		3.86 dd (12.0, 2.0)	

a) The assignments are based on the COSY, TOCSY, NOESY, HSQC and HMBC experiments. *b*) Multiplicities were assigned from DEPT spectra. *c*) Overlapped ^{1}H-NMR signals are reported without designated multiplicity.

O-(sulfo)lup-20(29)-en-23,28-dioic acid-28-*O*-β-D-glucopyranosyl ester, a new natural compound.

Although two 3-*O*-β-sulfated hederagenin and gypsogenin glycosides were already reported from the roots of *Gypsophila bermejoi*,[9] and one 3-*O*-β-sulfated gypsogenic acid glycoside from *Arenaria juncea*,[5] this is the first report of a sulfated triterpene and its glucosyl ester with lupane-type skeleton from the Caryophyllaceae, but also from the plant kingdom.

A 90% methanolic extract of the aerial parts of *G. repens* was separated into $CHCl_3$, EtOAc, and *n*-BuOH fractions by solvent partition. The *n*-BuOH extract after precipitation in Et_2O was further fractionated on a C18 reversed phase column, and Sephadex LH-20 affording **3** as a main constituent. Compound **3**, a yellow powder, had the molecular formula $C_{26}H_{28}O_{15}$ as determined from the HR-ESI-MS (molecular ion peak $[M+Na]^+$ at *m/z* 603.1534 (Calcd 603.1506)), the ^{13}C-NMR and DEPT spectral data. Its FAB-MS (negative-ion mode) showed a quasi-molecular ion peak at *m/z* 579 $[M-H]^-$, indicating a molecular weight of 580. Other significant fragment peaks appeared at *m/z* 447 $[(M-H)-132]^-$, corresponding to the loss of one pentosyl unit. The UV spectrum of **3** showed absorption maxima at 270 and 350 nm suggesting that **3** is a flavone derivative. The non permanent bathochromic shift on addition of $AlCl_3$, $AlCl_3$/HCl, NaOAc, and NaOAc/H_3BO_3 suggested the presence of an ortho dihydroxy group on the B ring which was confirmed by three aromatic ^{1}H-NMR signals at δ 7.34 (s-like), 6.88 (d, J=8.8 Hz) and 7.36 (br d, J=8.7 Hz) in the ^{1}H-NMR spectrum assignable to H-2′, H-5′ and H-6′. A detailed analysis of the 2D NMR spectra (Table 2) displayed the presence of luteolin[11] as aglycone together with two sugar residues, which were identified as one β-glucopyranosyl (Glc) and one α-arabinopyranosyl (Ara) units. The two anomeric ^{1}H-NMR signals at δ 4.94 (1H, d, J=9.8 Hz) and 4.36 (1H, d, J=6.0 Hz) which correlated in the HSQC spectrum with two anomeric ^{13}C-NMR signals at δ 73.2 and 106.6, respectively, corresponded to a *C*-linked glucopyranosyl and a *O*-linked arabinopyranosyl moiety, respectively. The analysis of the ^{13}C-NMR and DEPT spectra allowed the assignment of the quaternary carbon signal at δ 109.2 to be the C-6 position of the aglycone substituted by a sugar residue. This was confirmed by the HMBC correlation between δ 4.94 (d, J=8 Hz) (Glc-H-1) and δ 109.2 and 160.0 correponding to C-6 and C-5 of the aglycone. An extensive analysis of the 2D NMR spectra revealed all the proton and carbon signals corresponding to the flavone-*C*-glycosidic part of the molecule were in good agreement with those of isoorientin (6-*C*-β-glucopyranosyl-luteolin).[12] The binding site of Ara to the aglycone at OH (C-7) was deduced by the HMBC correlation between δ 4.36 (d, J=6 Hz, Ara H-1) and δ 165.3 (C-7 of the aglycone). This was supported by the chemical shift value of C-4 of the aglycone at δ 183.9. If this glycosylation had occurred at the

5-hydroxyl group, the hydrogen bonding between the 5-hydroxyl group and the carbonyl group would be broken leading to a significant upfield shift of C-4 and downfield shift of C-3.[13,14] The linkage position of the Ara at the OH (C-7) was also confirmed by the bathochromic shift of the band II in the UV spectrum ($\Delta\lambda$ II$=+3$ nm) in the presence of NaOAc, indicating a substituted 7-OH group. The L-configuration of Ara was determined after acid hydrolysis of **3** and GC analysis of the derivatized sugar.[10] On the basis of the above observations, the structure of **3** was determined as luteolin-7-*O*-α-L-arabinopyranosyl-6-*C*-β-glucopyranoside, a new natural compound.

Experimental

General Optical rotations were measured with a Perkin-Elmer 241 polarimeter. IR spectra (KBr disc) were recorded on a Perkin-Elmer 281 spectrophotometer. UV spectra were recorded on a Kontron UVIKON 860 spectrophotometer. High resolution ESI mass spectrometry (HR-ESI-MS) (positive-ion mode) was carried out on a Q-TOF 1-micromass spectrometer. FAB-MS (negative-ion mode, glycerol matrix) was measured on a JEOL SX 102 mass spectrometer. The 1D and 2D NMR spectra (^{1}H–^{1}H COSY, TOCSY, NOESY, HSQC and HMBC) were performed using a UNITY-600 spectrometer at the operating frequency of 600 MHz on a Varian INOVA 600 instrument equipped with a SUN 4 L-X computer system (600 MHz for ^{1}H- and 150 MHz for ^{13}C-NMR spectra). Conventional pulse sequences were used for COSY, HSQC and HMBC. TOCSY spectra were acquired using the standard MLEV17 spin-locking sequence and 90 ms mixing time. All chemical shifts (δ) are given in ppm and the samples were solubilized in C_5D_5N (**1**, **2**), and CD_3OD (**3**). GC analysis was carried out on Thermoquest gas chromatograph using a DB-1701 capillary column (30 m×0.25 mm, i.d.) (J&W Scientific); detection, FID; detector temperature, 250 °C; injection temperature, 230 °C; initial temperature was maintained at 80 °C for 5 min and then raised to 270 °C at the rate of 15 °C/min; carrier gas, He. Isolations were carried out using a MPLC system [Gilson pump M 305, head pump 25 SC, manometric module M 805, Büchi column (460×25 mm and 460×15 mm), Büchi precolumn (110×15 mm)]. TLC and HPTLC employed precoated silica gel plates 60 F_{254} (Merck). For saponins, the TLC solvent system $CHCl_3$/MeOH/H_2O (65 : 35 : 10, lower phase) was used. The detection of saponins was made with the Komarowsky reagent (2% 4OH-benzaldehyde in MeOH/50% H_2SO_4, 4 : 1). Elution of flavonoid was monitored by TLC on silica gel eluted with EtOAc/HCOOH/CH_3COOH/H_2O (100 : 11 : 11 : 26). The detection was made by observation of the plate under UV light at 254 and 365 nm, and spraying with the Neu's reagent (2% methanolic solution of diphenylboric aminoethyl ester).

Plant Material Roots of *Gypsophila repens* L. (Caryophyllaceae) were collected in May 1996 at Col des Prés, Massif du Mercantour, Alpes Maritimes, France and identified by G. Ducerf. A voucher specimen (N° 5544) is deposited in the herbarium of the Laboratory of Pharmacognosy, University of Burgundy, Dijon, France.

Extraction and Isolation Air dried powdered roots (260 g) were extracted with 90% MeOH under reflux (3×3 l). After the removal of solvent in vacuum, the residue was dissolved in H_2O (300 ml) and then partitioned successively with $CHCl_3$, EtOAc and H_2O-saturated *n*-BuOH, (3×300 ml, for each solvent). The *n*-BuOH soluble extract was dissolved in MeOH (20 ml) and purified by precipitation with Et_2O (4×250 ml) yielding a crude saponin mixture (2.3 g). Of this extract (2.2 g) was submitted to a VLC on RP-C_{18} silica gel (100% H_2O and 100% MeOH), to eliminate the free sugars. The MeOH fraction was further subjected to gel filtration chromatography on Sephadex LH-20 eluted with MeOH to give 15 fractions. Fraction 6 (45 mg) and 5 (92 mg) were purified by repeated MPLC (silica gel 60, 15—40 μm; $CHCl_3$/MeOH/H_2O 65 : 35 : 10, lower phase) to afford (**1**) (11 mg) and (**2**) (9 mg), respectively.

The dried aerial parts (400 g) were repeatedly refluxed in 90% MeOH (3×2 l). After evaporation to dryness in vacuum, the residue was dissolved in H_2O (300 ml) and partitioned successively with $CHCl_3$, EtOAc and H_2O-saturated *n*-BuOH, (3×300 ml, for each solvent). After evaporation of the solvent in vacuum, the *n*-BuOH fraction was suspended in MeOH (20 ml) and purified by precipitation with Et_2O (3×300 ml) to remove very non polar compounds yielding (3.1 g) of a yellowish crude material. An aliquote of this crude extract (3.0 g) was subjected to a reversed-phase VLC on RP C_{18} silica gel, eluted by H_2O 100% and MeOH 100% and then the MeOH fraction was chromatographed on Sephadex LH-20 (MeOH) to afford 13 fractions (frs. 1—13). Fraction 8 (60 mg) was submitted to a column chromatography on Sephadex LH-20 (MeOH) to give compound **3** (20 mg) in pure form.

Gypsophilin (**1**): White amorphous powder. $[\alpha]_D^{24}$ −25.0° (c=0.06, MeOH); IR (KBr) ν_{max} cm^{-1}: 3400 (OH), 2944 (CH), 1712 (CO carboxylic acid), 1636 (C=C), 1602, 1515, 1220 (SO); positive HR-ESI-MS *m/z*: 589.2840 $[M+Na]^+$ (Calcd for $C_{30}H_{46}O_8NaS$, 589.2811); negative FAB-MS *m/z*: 565 $[M-H]^-$; ^{1}H- and ^{13}C-NMR data, (600 and 150 MHz, respectively, C_5D_5N), see Table 1.

Gypsophilinoside (**2**): White amorphous powder. $[\alpha]_D^{24}$ −41.0° (c=0.12, MeOH); IR (KBr) ν_{max} cm^{-1}: 3400 (OH), 2944 (CH), 1740 (CO ester groups), 1710 (CO carboxylic acid), 1640 (C=C), 1600, 1515, 1220, 920; positive HR-ESI-MS *m/z*: 751.3315 $[M+Na]^+$ (Calcd for $C_{36}H_{56}O_{13}NaS$, 751.3339); negative FAB-MS *m/z*: 727 $[M-H]^-$; ^{1}H- and ^{13}C-NMR data, (600 and 150 MHz, respectively, C_5D_5N), see Table 1.

Compound **3**: Yellow amorphous powder. $[\alpha]_D^{24}$ −13.0° (c=0.115, MeOH); UV λ_{max} nm (MeOH): 270, 350; (NaOH) 240, 266, 280, 344, 408; ($AlCl_3$) 276, 303, 334, 428; ($AlCl_3$/HCl) 265, 280, 297, 362, 384; (NaOAc) 273, 326, 372; (NaOAc/H_3BO_3) 264, 374, 430; positive HR-ESI-MS *m/z*: 603.1534 $[M+Na]^+$ (Calcd for $C_{26}H_{28}O_{15}Na$, 603.1506); negative FAB-MS *m/z*: 579 $[M-H]^-$; ^{1}H- and ^{13}C-NMR data, (600 and 150 MHz, respectively, CD_3OD), see Table 2.

Acid Hydrolysis of 2 and 3 Compound **2** (2 mg) was hydrolyzed with 2 N aqueous CF_3COOH (5 ml) at 110 °C for 3 h. After this period, the reaction mixture was diluted with H_2O (10 ml) and extracted with CH_2Cl_2 (3×5 ml) (sapogenin). After repeated evaporations to dryness of the aqueous layer with MeOH until neutral, the residue of sugars was dissolved in anhydrous pyridine (100 ml) and L-cysteine methyl ester hydrochloride (0.06 mol/l) was added.[10] The mixture was stirred at 60 °C for 1 h, then 150 μl of HMDS-TMCS (hexamethyldisilazane–trimethylchlorosilane, 3 : 1) was added, and the mixture was stirred at 60 °C for another 30 min. The precipitate was centrifuged off, and the supernatant was concentrated under a N_2 stream. The residue was partitioned between *n*-hexane and H_2O (0.1 ml each), and the hexane layer (1 μl) was analyzed by GC. D-glucose (18.64 min) was detected by co-injection with a standard sugar derivative.

Compound **3** was refluxed with 10% HCl in 75% EtOH (3 ml) for 3 h. After this period, the reaction mixture was diluted with H_2O (10 ml) and extracted with H_2O saturated *n*-BuOH (3×10 ml). The combined BuOH extracts were washed with H_2O and then evaporated to dryness in vacuum yielding two *C*-glucosylflavones, isoorientin and orientin (Wessely-Moser isomerization). After neutralisation of the hydrolysate, the residue of sugars was treated by the same manner as for **1**. L-arabinose (11.88 min) was detected.

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