New Triterpenoidal Saponins from *Gypsophila repens*

by **Mohamed Elbandy**[a]), **Tomofumi Miyamoto**[b]), and **Marie-Aleth Lacaille-Dubois***[a])

[a]) Laboratoire de Pharmacognosie, Unité de Molécules d'Intérêt Biologique (UMIB, UPRES-EA 3660), Faculté de Pharmacie, Université de Bourgogne, 7 Bd Jeanne d'Arc, BP 87900, F-21079 Dijon Cedex (phone: +33-3-80-39-32-29; fax: +33-3-80-39-33-00; e-mail: m-a.lacaille-dubois@u-bourgogne.fr)

[b]) Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812-8582, Japan

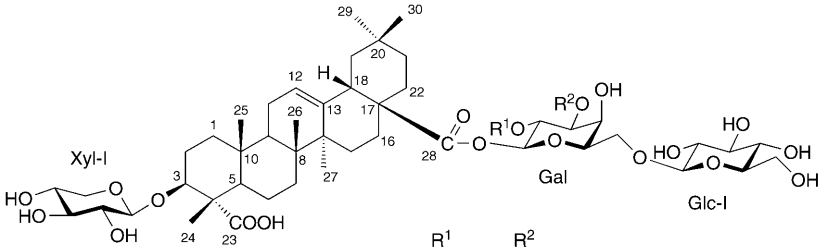
Six new triterpene glycosides, repensosides A–F (**1**–**6**, resp.), were isolated from the roots of *Gypsophila repens* L. Their structures, established by extensive 1D- and 2D-NMR spectroscopic experiments as well as MS analyses, were found to be based on gypsogenic acid (or gypsogenin) as aglycone, with three to nine branched or unbranched sugar moieties.

Introduction. – *Gypsophila* species (Caryophyllaceae) are among the plants that have been investigated first for saponins. Some species are known in folk medicine, where they are used against cough, cold, and as ailment of the upper respiratory tract. *Gypsophila* saponins are also used in industry, for example as detergents [1]. The commercial *Merck* saponin, used as a standard in hemolytic tests, is obtained from the roots of *G. paniculata* and several other species.

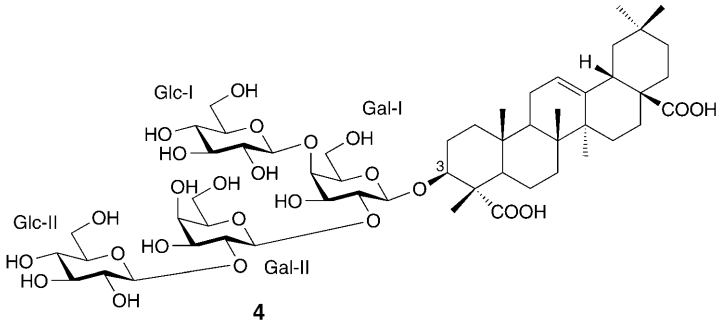
In continuation of our search of biologically active saponins from plants of the Caryophyllaceae family [2–9], we have examined the saponin fraction of the roots of *G. repens* L. One saponin had previously been isolated from this species, with a gypsogenin aglycone and galactose, xylose, arabinose, rhamnose, fructose, and glucose as sugars [10]. Herein, we report the isolation and structure elucidation of six new triterpene glycosides, repensosides A–F (**1**–**6**, resp.).

Results and Discussion. – The roots of *G. repens* were extracted with 90% aqueous MeOH. After solvent removal, the extract was successively partitioned between H_2O and $CHCl_3$, AcOEt, and BuOH, respectively. After evaporation of the solvent, the BuOH extract was suspended in MeOH, and precipitated with Et_2O. The resulting crude saponin mixture was further fractionated by column chromatography (*Sephadex LH-20*) and repeated medium-pressure liquid chromatography (MPLC; silica gel), followed by semi-preparative reverse-phase HPLC, yielding compounds **1**–**6**. Their structures were elucidated by extensive NMR spectroscopy, including a series of 2D-NMR experiments (^{1}H,^{1}H-COSY, TOCSY, NOESY, HSQC, HMBC), in combination with HR-ESI and FAB-MS.

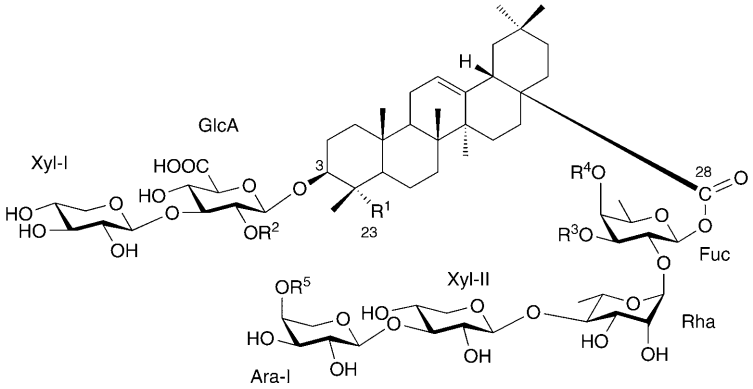
Compounds **1**–**6** were isolated as amorphous powders. Acid hydrolyses of **1**–**5** afforded the same aglycone, gypsogenic acid (=(3β)-3-hydroxyolean-12-ene-23,28-dioic acid), as identified by NMR. Acid hydrolysis of **6** led to gypsogenin (=(3β)-3-hydroxy-23-oxoolean-12-ene-28-oic acid) as aglycone. Most of the observed NMR signals



	R^1	R^2
1	H	H
2	Xyl-II	H
3	H	Glc-II



4



	R^1	R^2	R^3	R^4	R^5
5	COOH	Glc	H	Qui	Ara-II
6	CHO	Gal	Ac	Ac	H

of these aglycones were superimposable with those reported in the literature [2][11]. The sugars obtained by aqueous acid hydrolyses, identified by TLC comparison with authentic samples, were identified as xylose (Xyl), glucose (Glc), and galactose (Gal)

in the case of **1**–**3**; as Glc and Gal in the case of **4**; as rhamnose (=6-deoxymannose; Rha), fucose (=6-deoxygalactose; Fuc), Xyl, arabinose (Ara), Glc, quinovose (=6-deoxyglucose; Qui), and glucopyranosiduronic acid (GlcA) in the case of **5**; and as Rha, Fuc, Xyl, Ara, Gal, and GlcA in the case of **6**. Alkaline hydrolysis of **1**–**3**, **5**, and **6** afforded prosapogenins, whereas compound **4** remained intact under these conditions. These results suggested that **1**–**3**, **5**, and **6** are bisdesmosidic saponins, whereas **4** is a monodesmosidic glycoside.

Repensoside A (**1**). High-resolution electrospray-ionization mass spectrometry (HR-ESI-MS) of **1** exhibited the $[M+Na]^+$ peak at *m/z* 965.4678 (calc. 965.4722), consistent with the molecular formula $C_{47}H_{74}O_{19}$. Negative-ion FAB-MS displayed the $[M-H]^-$ signal at *m/z* 941, indicating a molecular weight of 942 D. Other significant ion peaks were observed at *m/z* 779 ($[M-H-162]^-$) and 617 ($[(M-H-162-162]^-$), indicating the loss of two hexose units, respectively. The signal at *m/z* 485 ($[M-H-162-162-132]^-$) pointed to the loss of an additional pentose unit and, at the same time, corresponded to the pseudomolecular ion of the aglycone (gypsogenic acid) [11]. On the basis of detailed 1D- and 2D-NMR analyses (*Tables 1* and *2*), the structure of repensoside A (**1**) was, thus, elucidated as (3*β*)-3-*O*-(*β*-D-xylopyranosyl)-gypsogenic acid 28-{*β*-D-glucopyranosyl-(1 → 6)-*β*-D-galactopyranosyl} ester.

The ^{1}H-NMR spectrum of **1** exhibited signals for three anomeric H-atoms at *δ*(H) 6.01 (*d*, *J*=8.3 Hz), 4.92 (*d*, *J*=7.4 Hz), and 4.82 (*d*, *J*=7.8 Hz), which showed HSQC correlations with *δ*(C) 95.0, 105.2, and 103.9, resp., indicating the presence of three sugar units in the *β*-anomeric form. The ring H-atoms of the monosaccharide residues were assigned starting from the anomeric H-atoms by means of COSY, TOCSY, HSQC, and HMBC plots (*Table 2*), and the sequence of the oligosaccharide chains was obtained from HMBC and NOESY experiments. Evaluation of spin-spin couplings and chemical shifts allowed the identification of the sugars in their pyranosyl form as one 6-substituted *β*-Gal, one terminal *β*-Xyl (Xyl-I), and one terminal *β*-Glc (Glc-I) unit, resp. The D-configuration for Gal, Xyl, and Glc was assumed, since most often encountered among plant glycosides. From 1D- and 2D-NMR experiments and the results of alkaline hydrolysis, it was concluded that **1** was a 3,28-bisdesmosidic saponin, with one terminal Xyl at C(3) (*δ*(C) 85.0) of the aglycone, the two other monosaccharides being linked at C(28) (*δ*(C)176.6) through an ester bond. The linkage of Xyl-I at C(3) of the aglycone was defined from the observed HMBC correlation between *δ*(H) 4.92 (*d*, *J*=7.4 Hz, H–C(1) of Xyl-I) and *δ*(C) 85.0 (C(3) of aglycone). This was further confirmed by the NOESY correlation between the anomeric H-atom of Xyl-I at *δ*(H) 4.92 (*d*, *J*=7.4 Hz) and H–C(3) of the aglycone at *δ*(H) 3.98. The sugar chain attached at C(28) of the aglycone was established from HMBC correlations between *δ*(H) 6.01 (*d*, *J*=8.3 Hz, H–C(1) of Gal) and the ^{13}C-NMR signal at *δ*(C) 176.6 (C(28) of aglycone), between *δ*(H) 4.16, 4.54 (CH_2(6) of Gal) and *δ*(C) 103.9 (C(1) of Glc-I), and between *δ*(H) 4.82 (*d*, *J*=7.9 Hz, H–C(1) of Glc-I) and *δ*(C) 68.4 (C(6) of Gal). This indicated that Gal was linked at C(28), and that Glc-I was linked to Gal by a 1 → 6 linkage. Further confirmation was obtained by NOESY cross-peaks between *δ*(H) 4.16, 4.54 (CH_2(6) of Gal) and *δ*(H) 4.82 (*d*, *J*=7.9 Hz, H–C(1) of Glc-I).

Repensoside B (**2**). In the positive-ion HR-ESI mass spectrum of **2**, the $[M+Na]^+$ signal was observed at *m/z* 1097.5189 (calc. 1097.5145), consistent with the molecular formula $C_{52}H_{82}O_{23}$. Negative-ion FAB-MS showed the $[M-H]^-$ peak at *m/z* 1073, indicating a molecular weight of 1074 D, 132 mass units higher than that of **1**. This indicated the presence of one additional pentose unit. Significant fragment peaks appeared at *m/z* 941 ($[M-H-132]^-$), 779 ($[M-H-132-162]^-$), 617 ($[M-H-132-162-162]^-$), and 485 ($[M-H-132-162-162-132]^-$), corresponding to the loss of one pentose, two hexoses, and another pentose unit, respectively. On the basis of extensive 2D-

Table 1. *^{13}C-NMR Data of the Aglycones of* **1**–**6**. At 150 MHz in (D_5)pyridine; δ in ppm.

Atom	**1**	**2**	**3**	**4**	**5**	**6**
CH_2(1)	38.2	38.2	38.2	38.2	38.2	37.6
CH_2(2)	26.8	26.7	26.8	26.5	26.3	24.2
H–C(3)	85.0	85.0	85.1	84.0	84.3	84.3
C(4)	53.3	53.4	53.3	54.0	54.0	55.0
H–C(5)	51.3	52.3	52.0	51.3	51.3	47.8
CH_2(6)	23.0	23.1	23.0	23.2	23.1	19.5
CH_2(7)	32.3	32.1	32.1	32.6	32.6	32.1
C(8)	39.6	39.6	39.5	40.1	39.6	39.6
H–C(9)	47.8	47.7	47.6	47.6	48.3	47.2
C(10)	36.1	36.1	36.0	36.2	36.1	35.6
CH_2(11)	23.4	23.3	23.2	23.6	23.7	23.8
H–C(12)	122.0	121.9	122.0	122.1	122.0	122.1
C(13)	143.8	143.8	143.6	144.0	143.8	143.5
C(14)	41.6	41.5	41.5	42.1	42.0	41.8
CH_2(15)	27.6	28.0	27.4	28.0	28.0	27.0
CH_2(16)	21.5	22.4	22.6	21.8	21.7	23.7
C(17)	46.6	46.6	46.6	46.6	46.6	46.6
H–C(18)	41.1	41.0	41.0	41.1	41.2	41.8
CH_2(19)	45.5	45.7	45.7	46.1	46.3	45.2
C(20)	30.2	30.1	30.0	30.9	30.1	30.1
CH_2(21)	33.4	33.4	33.2	33.2	33.5	33.2
CH_2(22)	31.8	31.7	31.7	31.8	31.8	31.6
C(23)	181.0	181.3	180.8	180.6	180.7	210.0
Me(24)	12.8	12.7	12.6	12.8	12.9	10.3
Me(25)	15.5	15.5	15.3	15.7	15.2	15.2
Me(26)	16.8	16.8	16.7	17.5	16.8	16.6
Me(27)	25.6	25.6	25.5	27.2	26.5	25.4
C(28)	176.6	176.7	176.6	181.6	176.7	175.5
Me(29)	32.6	32.6	32.5	33.3	32.5	32.6
Me(30)	23.2	23.1	23.0	23.1	23.9	23.1

NMR spectroscopic analyses (*Tables 1* and *2*), the structure of **2** was, thus, determined as (3β)-3-*O*-(β-D-xylopyranosyl)gypsogenic acid 28-{β-D-xylopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 6)]-β-D-galactopyranosyl} ester.

The assignments of the ^{1}H- and ^{13}C-NMR data of **2** (*Tables 1* and *2*), determined on the basis of extensive 2D-NMR experiments, were in good agreement with those of **1**, except for the appearance of an additional pentose unit. Evaluation of spin-spin couplings and chemical shifts allowed the identification of a second β-Xyl unit (Xyl-II). The site of linkage of this monosaccharide was determined by HMBC experiments, which showed a long-range correlation between δ(H) 5.15 (*d*, *J* = 7.6 Hz, H–C(1) of Xyl-II) and δ(C) 78.6 (C(2) of Gal)), and a reverse correlation between δ(H) 4.08 (H–C(2) of Gal) and δ(C) 104.4 (C(1) of Xyl-II). These results corroborated the attachment of Xyl-II to Gal by a (1 → 2) linkage, as further confirmed by a NOESY cross-peak between δ(H) 5.15 (*d*, *J* = 7.6 Hz, H–C(1) of Xyl-II) and δ(H) 4.08 (H–C(2) of Gal).

Repensoside C (**3**). The positive-ion HR-ESI mass spectrum of **3** exhibited the $[M+Na]^+$ peak at *m*/*z* 1127.5210 (calc. 1127.5250), consistent with the molecular for-

Table 2. *^1H- and ^{13}C-NMR Data of the Sugar Moieties of* **1**–**3**. At 600/150 MHz, resp., in (D_5)pyridine; δ in ppm, J in Hz. Overlapped ^{1}H-NMR signals are reported without multiplicities.

Atom	**1**		**2**		**3**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ (H)
Xyl-I:						
H–C(1)	105.2	4.92 (d, J=7.4)	105.1	4.85 (d, J=7.3)	104.9	4.84 (d, J=7.5)
H–C(2)	74.3	3.80	74.2	3.78	74.1	3.74
H–C(3)	77.0	4.07	76.8	3.98	76.5	3.93
H–C(4)	70.2	4.06	70.1	4.06	69.9	4.01
CH_2(5)	66.1	3.57, 4.21	66.0	3.54, 4.20	65.8	3.54, 4.16
Gal:						
H–C(1)	95.0	6.01 (d, J=8.3)	92.9	5.87 (d, J=7.8)	94.2	5.97 (d, J=7.8)
H–C(2)	72.9	4.02	78.6	4.08	72.1	4.05
H–C(3)	77.2	4.14	77.4	4.16	86.0	4.18
H–C(4)	69.8	4.17	70.7	3.88	68.0	4.06
H–C(5)	76.8	3.98	77.0	3.86	76.2	3.93
CH_2(6)	68.4	4.16, 4.54	68.1	4.14, 4.46	68.0	4.08, 4.41
Glc-I:						
H–C(1)	103.9	4.82 (d, J=7.9)	104.0	4.80 (d, J=7.8)	103.7	4.74 (d, J=7.8)
H–C(2)	74.2	3.83	74.1	3.84	73.9	3.76
H–C(3)	77.4	4.06	77.0	4.04	76.7	4.04
H–C(4)	70.7	3.94	70.1	3.93	70.5	3.86
H–C(5)	77.4	3.73	77.2	3.73	77.0	3.70
CH_2(6)	61.7	4.09, 4.28	61.7	4.08, 4.27	61.4	3.98, 4.21
Xyl-II:						
H–C(1)			104.4	5.15 (d, J=7.6)		
H–C(2)			74.9	3.82		
H–C(3)			77.1	4.05		
H–C(4)			69.2	4.14		
CH_2(5)			66.2	3.56, 4.22		
Glc-II:						
H–C(1)					103.9	5.15 (d, J=7.4)
H–C(2)					74.3	3.85
H–C(3)					76.7	4.00
H–C(4)					70.4	3.82
H–C(5)					77.2	3.79
CH_2(6)					61.2	4.04, 4.30

mula $C_{53}H_{84}O_{24}$. Negative-ion FAB-MS showed the $[M-H]^-$ peak at m/z 1103, indicating a molecular mass of 1104, 162 mass units higher than for **1**. Three significant fragments appeared at m/z 941 ($[M-H-162]^-$), 617 ($[M-H-132-162-162]^-$), and 485 ($[M-H-162-162-162-132]^-$), corresponding to the loss of three hexoses and one pentose, respectively. Extensive study of the 2D-NMR spectra of **3** led to the establishment of its structure as (3β)-3-*O*-(β-D-xylopyranosyl)gypsogenic acid 28-{β-D-glucopyranosyl(1 → 3)-[β-D-glucopyranosyl-(1 → 6)]-β-D-galactopyranosyl} ester.

The ^{1}H- and ^{13}C-NMR data of **3** (*Tables 1* and *2*) were similar to those of **1**, except for the appearance of an additional hexose moiety, which was identified as a terminal β-Glc unit (Glc-II) from spin–spin cou-

plings and chemical shifts. The linkage of Glc-II at C(3) of Gal was deduced from the HMBC correlation between δ(H) 5.15 (*d*, *J* = 7.4 Hz, H–C(1) of Glc-II) and δ(C) 86.0 (C(3) of Gal), and by a reverse HMBC correlation between δ(H) 4.18 (H–C(3) of Gal) and δ(C) 103.9 (C(1) of Glc-II). Further confirmation was obtained by a NOESY cross-peak between δ(H) 5.15 (*d*, *J* = 7.4 Hz, H–C(1) of Glc-II) and δ(H) 4.18 (H–C(3) of Gal).

Repensoside D (**4**). The positive-ion HR-ESI mass spectrum of **4** exhibited the $[M+Na]^+$ signal at *m*/*z* 1157.5321 (calc. 1157.5356), consistent with the molecular formula $C_{54}H_{86}O_{25}$. Negative-ion FAB-MS showed the $[M-H]^-$ signal at *m*/*z* 1133, indicating a molecular weight of 1134 D. Significant fragments appeared at *m*/*z* 971 ($[M-H-162]^-$), 809 ($[M-H-162-162]^-$), and 485 ($[M-H-162-162-162-162]^-$), corresponding to the loss of four hexose units. On the basis of 2D-NMR spectroscopic data and hydrolysis, the structure of **4** was, thus, established as (3β)-3-*O*-{β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 4)]-β-D-galactopyranosyl}gypsogenic acid.

The ^{13}C-NMR chemical shifts of C(3) (δ(C) 84.0) and C(28) (δ(C) 181.6) of the aglycone, in combination with the result of alkaline hydrolysis, indicated that **4** was a monodesmosidic glycoside. The ^{1}H- and ^{13}C-NMR signals of **4** (*Table 3*) allowed the identification of four monosaccharide units. The anomeric signals resonated at δ(H) 5.23 (*d*, *J* = 7.4 Hz), 5.16 (*d*, *J* = 7.8 Hz), 4.91 (*d*, *J* = 8.1 Hz), and 4.74 (*d*, *J* = 7.8 Hz), and showed HSQC correlations with δ(C) 102.3, 105.0, 103.9, and 102.8, resp. After extensive 2D-NMR spectroscopic analysis, two β-Glc units (Glc-I and Glc-II) and two β-Gal units (Gal-I and Gal-II) were identified, assuming common D-configurations. Their sequence was determined by HMBC and NOESY experiments. The HMBC correlation between δ(H) 4.74 (*d*, *J* = 7.9 Hz, H–C(1) of Gal-I) and δ(C) 84.0 (C(3) of aglycone) showed that Gal-I was linked to the O-atom at C(3) of gypsogenic acid, as confirmed by a NOESY cross-peak between δ(H) 4.74 (*d*, *J* = 7.9 Hz, H–C(1) of Gal-I) and δ(H) 3.98 (H–C(3) of aglycone). The HMBC correlation between δ(H) 5.23 (*d*, *J* = 7.4 Hz, H–C(1) of Gal-II) and δ(C) 79.8 (C(2) of Gal-I) indicated that Gal-II was linked to Gal-I at C(2). This was further supported by a NOESY cross-peak between δ(H) 5.23 (*d*, *J* = 7.4 Hz, H–C(1) of Gal-II) and δ(H) 4.00 (*d*, *J* = 7.4 Hz, H–C(2) of Gal-I). The HMBC correlations between δ(H) 4.91 (*d*, *J* = 8.1 Hz, H–C(1) of Glc-I) and δ(C) 78.8 (C(4) of Gal-I), and the reverse correlation between δ(H) 4.05 (H–C(4) of Gal-I) and δ(C) 103.9 (C(1) of Glc-I) proved that Glc-I was linked to Gal-I at C(4). This sugar linkage was confirmed by a NOESY cross-peak between δ(H) 4.91 (*d*, *J* = 8.1 Hz, H–C(1) of Glc-I) and δ(H) 4.05 (H–C(4) of Gal-I). An HMBC correlation between δ(H) 5.16 (*d*, *J* = 7.9 Hz, H–C(1) of Glc-II) and δ(C) 81.8 (C(2) of Gal-II), and the reverse correlation between δ(H) 4.30 (H–C(2) of Gal-II) and δ(C) 105.0 (C(1) of Glc-II) indicated that Glc-II was attached to Gal-II at C(2), as confirmed by a NOESY cross-peak between H–C(1) of Glc-II and H–C(2) of Gal-II.

Repensoside E (**5**). Positive-ion HR-ESI-MS of **5** showed the $[M+Na]^+$ ion at *m*/*z* 1813.0178 (calc. 1813.0125), consistent with the molecular formula $C_{80}H_{158}O_{42}$. Negative-ion FAB-MS showed the $[M-H]^-$ peak at *m*/*z* 1789, indicating a molecular weight of 1790 D. Full ^{1}H- and ^{13}C-NMR assignment (*Table 4*) was possible with the aid of 2D-NMR experiments. Thus, the structure of **5** was established as (3β)-3-*O*-{β-D-glucopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranuronosyl}gypsogenic acid 28-{α-L-arabinopyranosyl-(1 → 4)-α-L-arabinopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-[β-D-quinovopyranosyl-(1 → 4)]-β-D-fucopyranosyl} ester.

The ^{1}H-NMR spectrum of **5** (*Tables 1* and *4*) displayed nine anomeric signals at δ(H) 6.04 (br. *s*), 5.76 (*d*, *J* = 7.6 Hz), 5.27 (*d*, *J* = 7.6 Hz), 5.13 (*d*, *J* = 7.6 Hz), 4.97 (*d*, *J* = 7.1 Hz), 4.94 (*d*, *J* = 7.1 Hz), 4.86 (*d*,

Table 3. *^1H- and ^{13}C-NMR Data of the Sugar Moieties of* **4**. At 600/150 MHz, resp., in (D_5)pyridine; δ in ppm, J in Hz. Overlapped ^{1}H-NMR signals are reported without multiplicities.

Atom	δ(C)	δ(H)
Gal-I:		
H–C(1)	102.8	4.74 (d, J=7.9)
H–C(2)	79.8	4.00
H–C(3)	76.1	4.36
H–C(4)	78.8	4.05
H–C(5)	76.2	3.82
CH_2(6)	61.2	4.25, 4.28
Gal-II:		
H–C(1)	102.3	5.23 (d, J=7.4)
H–C(2)	81.8	4.30
H–C(3)	77.1	4.02
H–C(4)	69.7	4.25
H–C(5)	77.7	3.81
CH_2(6)	62.0	4.08, 4.30
Glc-I:		
H–C(1)	103.9	4.91 (d, J=8.1)
H–C(2)	74.5	3.80
H–C(3)	77.2	4.03
H–C(4)	70.9	3.84
H–C(5)	78.1	3.79
CH_2(6)	61.9	4.07, 4.22
Glc-II:		
H–C(1)	105.0	5.16 (d, J=7.9)
H–C(2)	75.6	3.87
H–C(3)	77.2	4.04
H–C(4)	70.7	3.88
H–C(5)	78.2	3.80
CH_2(6)	61.9	3.99, 4.13

J=8.0 Hz), 4.80 (d, J=7.0 Hz), and 4.67 (d, J=7.3 Hz), which correlated in the HSQC spectrum with δ(C) 100.1, 93.8, 102.8, 103.6, 104.2, 105.4, 105.0, 105.6, and 102.6, resp. The identity of the monosaccharides and the determination of their sequence were carried out by a combination of COSY, TOCSY, NOESY, HSQC, and HMBC plots. Evaluation of spin–spin couplings and chemical shifts allowed the identification of one α-Rha, one β-Fuc, one β-Glc, one β-GlcA, two α-Ara (Ara-I and Ara-II), one β-Qui, and two β-Xyl (Xyl-I and Xyl-II) units, assuming D-configurations for Fuc, GlcA, Qui, Gal, Xyl, and Glc, but L-configurations for Rha and Ara, as commonly encountered in saponins. Detailed NMR analysis, assigning the chemical shifts of C(3) (δ(C) 84.3) and C(28) (δ(C) 176.7), in combination with the results of alkaline hydrolysis, indicated that **5** was a bisdesmosidic glycoside. The HMBC correlations between δ(H) 5.27 (d, J=7.6 Hz, H–C(1) of Glc) and δ(C) 77.1 (C(2) of GlcA), between δ(H) 5.13 (d, J=7.6 Hz, H–C(1) of Xyl-I) and δ(C) 85.0 (C(3) of GlcA), and between δ(H) 4.67 (d, J=7.1 Hz, H–C(1) of GlcA) and δ(C) 84.3 (C(3) of aglycone) showed that the trisaccharide moiety Glc-(1 → 2)-[Xyl-(1 → 3)]-GlcA was linked to C(3) of the aglycone. Further confirmation was obtained by NOESY cross-peaks between δ(H) 5.27 (d, J=7.6 Hz, H–C(1) of Glc) and δ(H) 4.18 (H–C(2) of GlcA), between δ(H) 5.13 (d, J=7.6 Hz, H–C(1) of Xyl-I) and δ(H) 4.17 (H–C(3) of GlcA), and also between δ(H) 4.69 (d, J=7.1 Hz, H–C(1) of GlcA) and δ(H) 3.94 (H–C(3) of aglycone).

The sequence of the sugars attached to the O-atom of the COOH group at C(28) of the aglycone was determined by HMBC and NOESY correlations. The HMBC correlations between δ(H) 5.76 (d, J=7.6 Hz, H–C(1) of Fuc) and δ(C) 176.7 (C(28) of aglycone) confirmed that Fuc was linked to C(28) of the

aglycone. The HMBC correlation between δ(H) 4.38 (H–C(2) of Fuc) and δ(C) 100.1 (C(1) of Rha) indicated that Rha was attached to Fuc by a 1 → 2 linkage. This was confirmed by a NOESY cross-peak between δ(H) 6.04 (br. *s*, H–C(1) of Rha) and δ(H) 4.38 (H–C(2) of Fuc). HMBC correlations between δ(H) 4.94 (*d*, *J* = 7.1 Hz, H–C(1) of Xyl-II) and δ(C) 83.4 (C(4) of Rha) proved that Xyl-II was linked to C(4) of Rha. This was also confirmed by a NOESY cross-peak between δ(H) 4.94 (*d*, *J* = 7.1 Hz, H–C(1) of Xyl-II) and δ(H) 4.14 (H–C(4) of Rha). The HMBC correlation between δ(H) 4.97 (*d*, *J* = 7.1 Hz, H–C(1) of Ara-I) and δ(C) 85.4 (C(3) of Xyl-II) indicated that Ara-I was linked to C(3) of Xyl-II. Further confirmation was obtained by a NOESY cross-peak between δ(H) 4.97 (*d*, *J* = 7.1 Hz, H–C(1) of Ara-I) and δ(H) 3.92 (H–C(3) of Xyl-II). The HMBC correlation between δ(H) 4.80 (*d*, *J* = 7.0 Hz, H–C(1) of Ara-II) and δ(C) 78.1 (H–C(4) of Ara-I) established that Ara-II was attached to Ara-I at C(4). This was supported by a NOESY cross peak between δ(H) 4.80 (*d*, *J* = 7.0 Hz, H–C(1) of Ara-II) and δ(H) 4.21 (H–C(4) of Ara-I). Another HMBC correlation between δ(H) 4.86 (*d*, *J* = 8.0 Hz, H–C(1) of Qui) and δ(C) 82.8 (C(4) of Fuc) confirmed the attachment of Qui to Fuc at C(4).

Repensoside F (**6**). The positive-ion HR-ESI mass spectrum of **6** exhibited the $[M+Na]^+$ signal at *m/z* 1603.6802 (calc. 1603.6780), consistent with the molecular formula $C_{73}H_{112}O_{37}$. Negative-ion FAB-MS showed the $[M-H]^-$ signal at *m/z* 1579, indicating a molecular weight of 1580 D. Significant fragment peaks appeared at *m/z* 1447 ($[M-H-132]^-$), 1315 ($[M-H-132-132]^-$), 939 ($[M-H-132-132-146-146-42-42]^-$), and 469 ($[M-H-132-132-146-146-42-42-162-132-176]^-$), corresponding to the loss of two pentose, two deoxyhexose, two acetyl (Ac), one hexose, one pentose, and a glucopyranuronosyl (GlcA) unit(s), resp. The ion peak at *m/z* 469 was assigned to the pseudomolecular ion of gypsogenin [5]. Based on extensive 1D- and 2D-NMR analyses, the structure of **6** was established as (3β)-3-*O*-{β-D-galactopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranuronosyl}gypsogenin 28-{α-L-arabinopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-(3-*O*,4-*O*-diacetyl-β-D-fucopyranosyl)} ester.

The ^{1}H- and ^{13}C-NMR data of **6**, assigned by extensive 2D-NMR analyses, allowed the identification of gypsogenin as the aglycone (*Table 1*). The presence of seven monosaccharide units was suggested by the seven anomeric resonances at δ(H) 4.69 (*d*, *J* = 7.5 Hz), 5.30 (*d*, *J* = 7.4 Hz), 5.14 (*d*, *J* = 7.4 Hz), 5.93 (*d*, *J* = 8.1 Hz), 5.56 (br. *s*), 4.91 (*d*, *J* = 7.4 Hz), and 5.00 (*d*, *J* = 7.1 Hz), which were HSQC-correlated with δ(C) 103.1, 103.1, 103.7, 93.6, 101.0, 105.6, and 104.5, resp. (*Table 4*). Comparison of the 2D-NMR spectra of **6** with those of a related compound isolated from *Acanthophyllum glandulosum* [7] revealed that the terminal sugar of the glycosyl ester was Ara in the case of **6**.

Experimental Part

General. Column Chromatography (CC): *Sephadex LH-20* (*Pharmacia*). Vacuum liquid chromatography (VLC): *RP-18* gel (25–40 μm; *Merck*). Medium-pressure liquid chromatography (MPLC): silica gel *60* (15–40 μm; *Merck*), *Gilson M-305* pump, *Büchi* precolumn (110 × 15 mm), and *Büchi* glass column (460 × 25 or 460 × 15 mm). Semi-prep. and anal. HPLC: *Gilson M-305* and *-306* pumps, *Gilson-234* autoinjector, *Gilson-151* UV/VIS detector, *Merck-Hitachi D-7500* integrator, *Dionex Vydac RP-18* (5 μm, 300 Å; 10 × 250 mm) column, eluent: 26% MeCN in H_2O containing 0.06% TFA, flow 4 ml/min; detection at 210 nm. TLC and HPTLC: silica gel *60* F_{254} (*Merck*); solvent system for saponins (*A*): $CHCl_3$/MeOH/AcOH/H_2O 15 : 8 : 3 : 2; for sapogenins (*B*): $CHCl_3$/MeOH 9 : 1; for monosaccharides (*C*): $CHCl_3$/MeOH/H_2O 8 : 5 : 1; spray reagent for saponins: *Komarowsky* reagent (5 : 1 mixture of 2% 4-hydroxybenzaldehyde in MeOH and 50% aq. H_2SO_4); for sugars: diphenylamine/H_3PO_4. IR Spectra (KBr): *Perkin-*

Table 4. *^1H- and ^{13}C-NMR Data of the Sugar Moieties of* **5** *and* **6**. At 600/150 MHz, resp., in (D_5)pyridine; δ in ppm, J in Hz. Overlapped ^{1}H-NMR signals are reported without multiplicities.

Atom	**5**		**6**	
	δ(C)	δ(H)	δ(C)	δ(H)
GlcA:				
H–C(1)	102.6	4.67 (*d*, *J* = 7.3)	103.1	4.69 (*d*, *J* = 7.5)
H–C(2)	77.1	4.18	77.3	4.18
H–C(3)	85.0	4.17	85.2	4.13
H–C(4)	68.5	4.14	68.0	4.16
H–C(5)	76.8	3.99	77.1	4.00
C(6)	170.0	not obs.	170.0	not obs.
Gal:				
H–C(1)			103.1	5.30 (*d*, *J* = 7.4)
H–C(2)			72.5	4.20
H–C(3)			74.3	3.98
H–C(4)			70.0	4.24
H–C(5)			76.0	3.80
CH_2(6)			61.4	4.14, 4.28
Glc:				
H–C(1)	102.8	5.27 (*d*, *J* = 7.6)		
H–C(2)	75.6	4.14		
H–C(3)	78.3	4.00		
H–C(4)	69.7	4.21		
H–C(5)	76.8	3.98		
CH_2(6)	61.4	4.10, 4.26		
Xyl-I:				
H–C(1)	103.6	5.13 (*d*, *J* = 7.6)	103.7	5.14 (*d*, *J* = 7.4)
H–C(2)	74.2	3.77	74.3	3.81
H–C(3)	76.8	4.10	78.1	4.22
H–C(4)	69.8	3.98	70.4	4.01
CH_2(5)	66.0	3.59, 4.17	66.2	3.58, 4.09
Fuc:				
H–C(1)	93.8	5.76 (*d*, *J* = 7.6)	93.6	5.93 (*d*, *J* = 8.1)
H–C(2)	74.4	4.38	71.9	4.43
H–C(3)	75.6	4.14	74.3	5.40
H–C(4)	82.8	4.01	71.0	5.47
H–C(5)	71.0	3.94	69.6	4.11
Me(6)	17.5	1.41 (*d*, *J* = 6.0)	16.9	1.14 (*d*, *J* = 5.5)
3-*O*-Ac			20.1, 171.0	2.03 (*s*, 3 H)
4-*O*-Ac			20.3, 170.0	2.10 (*s*, 3 H)
Rha:				
H–C(1)	100.1	6.04 (br. *s*)	101.0	5.56 (br. *s*)
H–C(2)	70.6	4.58	70.8	4.39
H–C(3)	71.4	4.39	72.1	4.35
H–C(4)	83.4	4.14	83.5	4.14
H–C(5)	67.8	4.24	68.5	3.86
Me(6)	17.8	1.53 (*d*, *J* = 6.1)	17.9	1.58 (*d*, *J* = 6.1)
Xyl-II:				
H–C(1)	105.4	4.94 (*d*, *J* = 7.1)	105.6	4.91 (*d*, *J* = 7.4)
H–C(2)	74.4	3.81	74.3	3.82

Table 4 (cont.)

Atom	**5**		**6**	
	δ(C)	δ(H)	δ(C)	δ(H)
H–C(3)	85.4	3.92	85.8	3.93
H–C(4)	68.0	3.88	68.5	4.16
CH_2(5)	65.7	3.38, 4.02	65.9	3.42, 4.06
Ara-I:				
H–C(1)	104.2	4.97 (*d*, *J* = 7.1)	104.5	5.00 (*d*, *J* = 7.1)
H–C(2)	72.0	4.28	71.9	4.36
H–C(3)	73.0	4.11	73.5	4.04
H–C(4)	78.1	4.21	67.8	4.38
CH_2(5)	65.7	3.70, 4.36	66.5	3.70, 4.18
Ara-II:				
H–C(1)	105.6	4.80 (*d*, *J* = 7.0)		
H–C(2)	71.8	4.24		
H–C(3)	73.0	4.00		
H–C(4)	68.5	4.14		
CH_2(5)	66.0	3.63, 4.08		
Qui:				
H–C(1)	105.0	4.86 (*d*, *J* = 8.0)		
H–C(2)	74.6	3.86		
H–C(3)	74.2	3.94		
H–C(4)	75.8	3.80		
H–C(5)	69.8	3.52		
Me(6)	17.3	1.14 (*d*, *J* = 5.5)		

Elmer 281 spectrophotometer; in cm^{-1}. 1D- and 2D-NMR Spectra: *Varian Unity-600* and *Inova-600* instruments equipped with a *Sun-4-L-X* computer system, at 600 (^{1}H) and 150 MHz (^{13}C); for details, see [2]. FAB-MS (neg.): *Jeol SX-102*; in *m*/*z*. HR-ESI-MS (pos.): *Micromass Q-TOF-1* apparatus; in *m*/*z*.

Plant Material. The roots of *Gypsophila repens* L. (Caryophyllaceae) were collected in May 1996 in Col des Prés, Massif du Mercantour, Alpes Maritimes, France, and identified by Mr. *G. Ducerf*. A voucher specimen (No. 55-44) was deposited at the Herbarium of the Laboratory of Pharmacognosy, Faculty of Pharmacy, University of Burgundy, Dijon, France.

Extraction and Isolation. The air-dried, finely powdered roots (260 g) were first macerated for 4 h with MeOH/H_2O 9 : 1 (3 × 4 l) and then heated at reflux. After cooling, the soln. was filtered and concentrated *in vacuo*. The resulting extract was dissolved in H_2O (300 ml) and successively extracted with 3 × 300 ml of $CHCl_3$, AcOEt, and BuOH (sat. with H_2O), resp. After evaporation of the solvent, the BuOH extract was solubilized in MeOH (10 ml) and purified by precipitation with Et_2O (3 × 250 ml) to afford a crude saponin mixture (2.3 g). An aliquot (2.2 g) was submitted to VLC (*RP-18*; H_2O/MeOH gradient) and then further fractionated by CC (*Sephadex LH-20*; MeOH) and successive MPLC (SiO_2; $CHCl_3$/MeOH/H_2O 13 : 7 : 2, lower phase), followed by semi-prep. HPLC (see *General*) to afford **1** (6 mg), **2** (7 mg), **3** (10 mg), **4** (8.5 mg), **5** (8 mg), and **6** (7.5 mg).

Repensoside A (= (3β)-3-O-(*β*-D-*Xylopyranosyl*)*gypsogenic Acid 28-*{*β*-D-*Glucopyranosyl*-(1 → 6)-*β*-D-*galactopyranosyl*} *Ester*; **1**). Colorless, amorphous powder. TLC (*A*): R_f 0.50. IR (KBr): 3405, 2926, 1750, 1740, 1710, 1634, 1610, 1560. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. FAB-MS (neg.): 941 ([*M* – H]$^-$), 779 ([*M* – H – 162]$^-$), 617 ([*M* – H – 162 – 162]$^-$), 485 ([*M* – H – 162 – 162 – 132]$^-$). HR-ESI-MS (pos.): 965.4678 ([*M* + Na]$^+$; calc. 965.4722).

Repensoside B (= (3β)-3-O-(*β*-D-*Xylopyranosyl*)*gypsogenic Acid 28-*{*β*-D-*Xylopyranosyl*-(1 → 2)-[*β*-D-*glucopyranosyl*-(1 → 6)]-*β*-D-*galactopyranosyl*} *Ester*; **2**). Colorless, amorphous powder. TLC (*A*): R_f

0.34. IR (KBr): 3406, 2927, 1740, 1723, 1710, 1636, 1580, 1420. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. FAB-MS (neg.): 1073 ($[M-H]^-$), 941 ($[M-H-132]^-$), 779 ($[M-H-132-162]^-$), 617 ($[M-H-132-162-162]^-$), 485 ($[M-H-132-162-162-132]^-$). HR-ESI-MS (pos.): 1097.5189 ($[M+Na]^+$; calc.1097.5145).

Repensoside C (=(3*β*)-*3*-O-*(β*-D-*Xylopyranosyl)gypsogenic Acid 28-{β*-D-*Glucopyranosyl(1 → 3)-[β*-D-*glucopyranosyl-(1 → 6)]-β*-D-*galactopyranosyl} Ester*; **3**). Colorless, amorphous powder. TLC (*A*): R_f 0.35. IR (KBr): 3404, 2927, 1740, 1723, 1710, 1636, 1580, 1500, 1420. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. FAB-MS (neg.): 1103 ($[M-H]^-$), 941 ($[M-H-162]^-$), 617 ($[M-H-132-162-162]^-$), 485 ($[M-H-162-162-162-132]^-$). HR-ESI-MS (pos.): 1127.5210 ($[M+Na]^+$; calc.1127.5250).

Repensoside D (=*(3β)-3*-O-*{β*-D-*Glucopyranosyl-(1 → 2)-β*-D-*galactopyranosyl-(1 → 2)-[β*-D-*glucopyranosyl-(1 → 4)]-β*-D-*galactopyranosyl}gypsogenic Acid*; **4**). Colorless, amorphous powder. TLC (*A*): R_f 0.34. IR (KBr): 3404, 2927, 1723 1740, 1710, 1636, 1580, 1500, 1420. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *3*. FAB-MS (neg.): 1133 ($[M-H]^-$), 971 ($[M-H-162]^-$), 809 ($[M-H-162-162]^-$), 485 ($[M-H-162-162-162-162]^-$). HR-ESI-MS (pos.): 1157.5321 ($[M+Na]^+$; calc. 1157.5356).

Repensoside E (=(3*β*)-*3*-O-*{β*-D-*Glucopyranosyl-(1 → 2)-[β*-D-*xylopyranosyl-(1 → 3)]-β*-D-*glucopyranuronosyl}gypsogenic Acid 28-{α*-L-*Arabinopyranosyl-(1 → 4)-α*-L-*arabinopyranosyl-(1→3)-β*-D-*xylopyranosyl-(1→4)-α*-L-*rhamnopyranosyl-(1→2)-[β*-D-*quinovopyranosyl-(1→4)]-β*-D-*fucopyranosyl} Ester*; **5**). Colorless, amorphous powder. TLC (*A*): R_f 0.10. IR (KBr): 3404, 2927, 1740, 1723, 1710, 1636, 1580, 1500, 1420. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *4*. FAB-MS (neg.): 1789 ($[M-H]^-$). HR-ESI-MS (pos.): 1813.0178 ($[M+Na]^+$; calc. 1813.0125).

Repensoside F (=(3*β*)-*3*-O-*{β*-D-*Galactopyranosyl-(1→2)-[β*-D-*xylopyranosyl-(1→3)]-β*-D-*glucopyranuronosyl}gypsogenin 28-{α*-L-*Arabinopyranosyl-(1→3)-β*-D-*xylopyranosyl-(1→4)-α*-L-*rhamnopyranosyl-(1→2)-(3*-O,*4*-O-*diacetyl-β*-D-*fucopyranosyl)} Ester*; **6**). Colorless, amorphous powder. TLC (*A*): R_f 0.33. IR (KBr): 3404, 2927, 1740, 1723, 1710, 1636, 1580, 1500, 1420. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *4*. FAB-MS (neg.): 1579 ($[M-H]^-$), 1447 ($[M-H-132]^-$), 1315 ($[M-H-132-132]^-$), 939 ($[M-H-132-132-146-146-42-42]^-$), 469 ($[M-H-132-132-146-146-42-42-162-132-176]^-$). HR-ESI-MS (pos.): 1603.6802 ($[M+Na]^+$; calc. 1603.6780).

Acid Hydrolysis. A soln. of the isolate (2 mg) in H_2O (2 ml) was treated with 2N aq. CF_3COOH (5 ml) and heated at reflux for 3 h. The mixture was extracted with $CHCl_3$ (3 × 5 ml) (sapogenin, TLC, solvent *B*), the aq. layer was separated, treated with MeOH, and repeatedly evaporated until neutral, and then analyzed by TLC (solvent *C*) by comparison with standard sugars.

Alkaline Hydrolysis. The appropriate saponin (2 mg) in 5% aq. KOH soln. (10 ml) was heated at reflux for 2 h. The mixture was adjusted to pH 6 by adding dil. aq. HCl, and then extracted with H_2O-sat. BuOH (3 × 10 ml). The combined BuOH extracts were washed with H_2O and evaporated to afford the corresponding prosapogenin.

REFERENCES

[1] Z. Jia, K. Koike, N. Sahu, T. Nikaido, in 'Studies in Natural Products Chemistry', Ed. Atta-Ur-Rahman, Elsevier, Amsterdam, 2002, Vol. 26, p. 3.
[2] G. Gaidi, T. Miyamoto, A. Rustaiyan, V. Laurens, M.-A. Lacaille-Dubois, *J. Nat. Prod.* **2000**, *63*, 1497.
[3] G. Gaidi, T. Miyamoto, A. Rustaiyan, M.-A. Lacaille-Dubois, *J. Nat. Prod.* **2001**, *64*, 920.
[4] G. Gaidi, T. Miyamoto, M.-A. Lacaille-Dubois, *J. Nat. Prod.* **2001**, *64*, 1533.
[5] G. Gaidi, T. Miyamoto, V. Laurens, M.-A. Lacaille-Dubois, *J. Nat. Prod.* **2002**, *65*, 1568.
[6] M. Haddad, T. Miyamoto, M. Ramezani, M.-A. Lacaille-Dubois, *Helv. Chim. Acta* **2004**, *87*, 73.
[7] G. Gaidi, T. Miyamoto, M. Ramezani, M.-A. Lacaille-Dubois, *J. Nat. Prod.* **2004**, *67*, 1114.
[8] G. Gaidi, T. Miyamoto, M.-A. Lacaille-Dubois, *Pharmazie* **2005**, *60*, 635.
[9] M. F. Melzig, P. Hebestreit, G. Gaidi, M.-A. Lacaille-Dubois, *Planta Med.* **2005**, *71*, 1088.
[10] B. Pasich, K. Terminska, J. Pasich, *Pharmazie* **1983**, *38*, 489.
[11] Z. Jia, K. Koike, T. Nikaido, *J. Nat. Prod.* **1999,** *62*, 449.

Received November 10, 2006