Triterpenoid Saponins from the Fruits of *Caryocar glabrum*

Abdulmagid Alabdul Magid,[†] Laurence Voutquenne,*[,†] Christian Moretti,[‡] Christophe Long,[§] and Catherine Lavaud[†]

Laboratoire de Pharmacognosie, FRE CNRS 2715, IFR 53 Biomolécules, Bâtiment 18, BP 1039, 51687 Reims Cedex 2, France, IRD, Unité No. S84 Biodival, Technoparc, 5 Rue du Carbone, 45072 Orléans Cedex 2, France, and Institut de Recherche Pierre Fabre, ISTMT, UMS CNRS 2579, 3 Rue des Satellites, BP 94244, 31432 Toulouse Cedex, France

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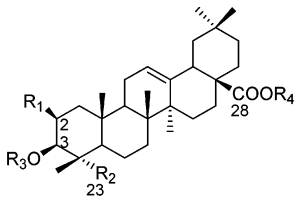
Twenty-one new triterpenoid saponins, named caryocarosides (**1**−**21**), glycosides of 2β-hydroxyoleanolic acid, hederagenin, bayogenin, and gypsogenic acid, have been isolated from the fruits of *Caryocar glabrum* along with nine known triterpenoid saponins (**22**−**30**) that are described for the first time from a plant in the Caryocaraceae. Their structures were established by 1D and 2D NMR techniques (^{13}C, COSY, TOCSY, HSQC, HMBC, and ROESY experiments), ESIMS, and acid hydrolysis. The isolated compounds could be classified into two series: glucosides (**1**−**8**, **22**, **27**, and **30**) derived from the 3-*O*-monoglucoside and glucuronides (**9**−**21**, **23**−**26**, **28**, and **29**) derived from the 3-*O*-monoglucuronide. In 22 of the saponins (**1**−**8**, **12**−**22**, and **24**−**26**), a galactose moiety was linked to C-3 of a glucuronic acid or a glucose moiety. The galactose was substituted in position 3 by a second galactose unit (**6**, **7**, **20**, and **21**) or by a xylose unit (**8**). Seven saponins (**4**, **5**, **16**−**19**, and **26)** were found to be bidesmosides with one glucose unit linked to C-28 of the aglycon. The hemolytic activity of the major saponins (**2**, **3**, **5**, **12**−**15**, **17**, **24**, and **28**) was measured on sheep erythrocytes in order to establish structure−activity relationships based on the type of sugar attached to the aglycon and on the structure of this aglycon.

The Caryocaraceae is a small family of magnificent trees comprising only two genera, *Anthodiscus* (9 species) and *Caryocar* (16 species), distributed in neotropical and tropical America.[1] The *Caryocar* genus, which is called "pequi"[2,3] by the local Indian tribes, is exploited for the oil content of the pulp and the seeds. The resultant edible fats are of potential commercial value for cooking as a substitute for butter and used in the cosmetic industry and for homemade soaps.[4,5] Apart from scattered reports about lipid composition,[2,3,5] the phytochemistry of the Caryocaraceae has not been studied exhaustively. A short report about *C. microcarpum* mentioned the presence of a large quantity of gallotanin in addition to ellagic acid, gallic acid, and methyl gallate, as well as the occurrence of glycosides of oleanolic acid and its hydroxylated derivatives, 2β-hydroxyoleanolic acid, hederagenin, and bayogenin, in the leaves.[6−7]

Caryocar glabrum (Aubl.) Pers. sp. *glabrum* (local name: "sawa" and "peke'a là") is a large tree (up to 30 m high) that grows in the primary humid forests of northern South America and occurring wild in the northern Amazonia and in Guyana.[1] The fruit is irregularly globular, approximately 5−8 cm in diameter. The peel is yellowish-gray colored and easy to remove. The fleshy pulp (mesocarp) generally surrounds one or two kidney-shaped seeds. The fruit and the seed are reported to be edible, fresh or cooked. The fruit and the stem bark have been used traditionally by the Indian tribes of French Guyana, Colombia, Venezuela, and Brazil as a remedy for skin problems and as a fish poison.[7]

We herein report the isolation and structural elucidation of 30 saponins. The compounds **1**−**8**, **22**, **27**, and **30** are glucoside triterpenoids and compounds **9**−**21**, **23**−**26**, **28**, and **29** are glucuronide triterpenoids. The structures of these saponins were established by spectroscopic methods (1D and 2D NMR, ESIMS), by acid hydrolysis, and by comparison with literature data for known compounds. To the best of our knowledge, compounds **22**−**30** are known, whereas the remaining 21 isolates were found to be new saponins, tentatively named caryocarosides. The Roman numerals I to V indicate the aglycon type—oleanolic acid (I), hederagenin (II), bayogenin (III), 2β-hydroxyoleanolic acid (IV), and gypsogenic acid (V)—and the Arabic number is related to the structure of the glycoside chain. In a program based on the evaluation of the hemolytic activity of saponins and their structure−activity relationship,[8,9] we have measured the hemolytic activity of selected saponins in order to evaluate the influence of a glucuronic acid as compared to a glucose in the sugar chain. Thus, the hemolytic activity of the 10 major saponins (**2, 3**, **5**, **12**−**15**, **17**, **24**, and **28**) was measured on sheep erythrocytes and some structure−activity relationships were established.



	R_1	R_2	R_3	R_4
1	H	COOH	Gal-(1→3)-Glc-	H
2	H	CH_2OH	Gal-(1→3)-Glc-	H
3	OH	CH_2OH	Gal-(1→3)-Glc-	H
4	H	CH_2OH	Gal-(1→3)-Glc-	Glc
5	OH	CH_2OH	Gal-(1→3)-Glc-	Glc
6	H	CH_2OH	Gal-(1→3)-Gal-(1→3)-Glc-	H
7	OH	CH_2OH	Gal-(1→3)-Gal-(1→3)-Glc-	H
8	OH	CH_2OH	Xyl-(1→3)-Gal-(1→3)-Glc-	H
9	OH	CH_3	GlcA-	H
10	OH	CH_2OH	GlcA-	H
11	OH	CH_3	6-*O*-methyl-GlcA-	H
12	OH	CH_3	Gal-(1→3)-GlcA-	H
13	H	CH_2OH	Gal-(1→3)-GlcA-	H
14	OH	CH_2OH	Gal-(1→3)-GlcA-	H
15	OH	CH_3	Gal-(1→3)-6-*O*-methyl-GlcA-	H
16	OH	CH_3	Gal-(1→3)-GlcA-	Glc
17	H	CH_2OH	Gal-(1→3)-GlcA-	Glc
18	OH	CH_2OH	Gal-(1→3)-GlcA-	Glc
19	H	CH_2OH	Gal-(1→3) -6-*O*-methyl-GlcA-	Glc
20	OH	CH_3	Gal-(1→3)-Gal-(1→3)-GlcA-	H
21	H	CH_2OH	Gal-(1→3)-Gal-(1→3)-GlcA-	H
24	H	CH_3	Gal-(1→3)-GlcA-	H
28	H	CH_2OH	GlcA-	H

* To whom correspondence should be addressed. Tel: +33 (0)-326918208. Fax: +33 (0)326913596. E-mail: laurence.voutquenne@univ-reims.fr.

[†] Université de Reims.

[‡] IRD.

[§] UMS CNRS 2579.

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Results and Discussion

The fruits of *Caryocar glabrum* were collected in French Guyana at Cayena Island. The dried peel and pulp of fruits were extracted separately with methanol, and the methanol extract was evaporated to dryness. Analytical HPLC and TLC analysis of the two methanol extracts revealed that the chromatographic profiles of the peel and the pulp of fruit were qualitatively identical but quantitatively different with a higher amount of bidesmosides in the pulp. The pulp extract was then purified by a combination of silica gel column or RP-18 column chromatography and finally by semipreparative HPLC or by preparative TLC to afford 30 saponins (**1**−**30**).

Acid hydrolysis of the crude extract yielded five aglycons. Among them, three were identified as oleanolic acid, hederagenin, and bayogenin by TLC with authentic samples. Structural confirmation was achieved by analysis of 1D and 2D NMR spectra of each purified saponin. ^{13}C NMR spectra were in accordance with data reported in the literature.[10,11] The two remaining aglycons were identified by analysis of their NMR spectral data in each intact saponin as 2β-hydroxyoleanolic acid[12] and gypsogenic acid.[10,13] The monosaccharides obtained from the acid hydrolysis were identified as D-xylose, D-glucose, D-galactose, and D-glucuronic acid by TLC with authentic samples and measurement of their optical rotation after purification. The ^{1}H and ^{13}C NMR data of intact saponins indicated a β configuration for the anomeric position of each sugar (Tables 1−3).

All of the saponins with oleanolic acid as an aglycon were known compounds. These saponins were identified as 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-glucopyranosyloleanolic acid (arvensoside B) (**22**),[14−16] 3-*O*-β-D-glucuronopyranosyloleanolic acid (glycoside F) (**23**),[14,15,17−25] 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-glucuronopyranosyloleanolic acid (glycoside D) (**24**),[14,15,17] 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-methylglucopyranosiduronate oleanolic acid (**25**),[17,26] and 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-glucuronopyranosyloleanolic acid 28-*O*-β-D-glucopyranosyl ester (glycoside C) (**26**).[14,15] The other known saponins were identified as 3-*O*-β-D-glucopyranosylhederagenin (colchiside 4) (**27**),[18,27−29] 3-*O*-β-D-glucuronopyranosylhederagenin (**28**),[18−20] 3-*O*-β-D-methyl glucopyranosiduronate hederagenin (**29**),[17,18] and 3-*O*-β-D-glucopyranosylbayogenin (**30**).[28]

The aglycon was identified as 2β-hydroxyoleanolic acid in compounds **9**, **11**, **12**, **15**, **16**, and **20**. The negative ESIMS^2 of these compounds showed the same ion fragment at *m*/*z* 471 [M − H − glycosidic chain]$^-$. The ^{1}H NMR spectra exhibited seven signals due to tertiary methyl groups, resonating as singlets at δ 0.85, 0.93, 0.97, 1.10 (6H), 1.18, and 1.27 (δ ±0.02 ppm). The ethylene H-12 signal was observed at δ 5.27 ± 0.01 ppm (t, J = 3.6 ± 0.2 Hz). The two proton signals of a vicinal diol (H-2 and H-3) were observed at δ 4.22 (q, J = 3.3 ± 0.4 Hz) and 3.20 (d, J = 3.3 ± 0.4 Hz), respectively (Tables 2 and 3). The small coupling constant between H-2 and H-3 ($J_{2,3}$ = 3.3 ± 0.4 Hz) indicated that the two hydroxyl groups adopted a vicinal *cis*-β configuration, like in bayogenin. The assignments of other proton and carbon signals of the aglycon were accomplished by analysis of the usual 2D COSY, ROESY, HSQC, and HMBC experiments. The ^{1}H and ^{13}C NMR values were in full agreement with those reported in the literature for 2β-hydroxyoleanolic acid.[12]

Caryocaroside V-1 (**1**) was the only saponin containing gypsogenic acid as aglycon. The ^{1}H NMR spectrum of **1** exhibited six signals due to tertiary methyl groups, resonating as singlets at δ 0.83, 0.93, 0.96, 1.00, 1.17, and 1.19, one hydroxymethine signal (H-3) at δ 4.11 (dd, J = 11.8−4.5 Hz), and one ethylene proton signal at δ 5.27 (t, J = 3.6 Hz). The ^{13}C NMR spectrum of this compound indicated the presence of two carbonyl groups at δ 181.9 and 182.0 that were assigned to C-23 and C-28 by analysis of the HMBC experiment (Table 1). The ^{13}C NMR values of the aglycon were in good agreement with those reported for gypsogenic acid.[10,13] The positive ESIMS of **1** exhibited a molecular ion peak at *m*/*z* 811 [M + H]$^+$ and a positive fragment at *m*/*z* 487 [aglycon + H]$^+$ attributed to the loss of a disaccharide moiety consisting of two hexoses. Further analysis of the ^{1}H and ^{13}C NMR spectra of **1** revealed the presence of two anomeric protons at δ 4.37 and 4.52 correlated in the HSQC spectrum with two anomeric carbons at δ 104.9 and 105.7, respectively (Table 1). Complete assignment of each glycoside proton system was achieved by analysis of COSY and TOCSY experiments. A β-D-glucose unit was identified starting from the anomeric proton at δ 4.37 (d, J = 7.7 Hz). The second monosaccharide whose anomeric proton resonates at δ 4.52 (d, J = 7.6 Hz) was identified as a β-D-galactose, characterized by its equatorial proton H-4″ at δ 3.82 (d, $J_{3,4}$ = 3.5 Hz) (Table 1). The deshielding of C-3′ (δ 87.3) of glucose suggested the linkage site with the galactose (Table 1). In the HMBC experiment, the cross-peaks observed between H-1″ of galactose and C-3′ of glucose and between H-1′ of glucose and C-3 of the gypsogenic acid (δ 85.5) led to the assignment of caryocaroside V-1 (**1**) as 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-glucopyranosylgypsogenic acid.

Caryocarosides II-1 (**2**) and III-1 (**3**) exhibited molecular ion peaks [M − H]$^-$ at *m*/*z* 795 and 811 in the negative ESIMS. The MS^2 experiments of the [M − H]$^-$ ion of both **2** and **3** gave the same negative fragments [M − H − 162]$^-$ and [M − H − 2 × 162]$^-$ attributed to the successive loss of two hexoses. The ^{1}H and ^{13}C NMR spectra of **2** and **3** indicated two anomeric carbons at δ 105.1 and 105.5 for **2** and at δ 105.2 and 105.8 for **3**, which were correlated in the HSQC experiment with anomeric protons at δ 4.48 (d, J = 7.9 Hz) and 4.52 (d, J = 7.7 Hz) for **2** and 4.52 (d, J = 7.8 Hz) and 4.55 (d, J = 7.7 Hz) for **3**. Comparison of the glycosidic ^{1}H and ^{13}C NMR values for **2** and **3** with those of **1** showed that all these compounds contained the same glycoside chain (β-D-galactopyranosyl-(1→3)-β-D-glucopyranoside). Thus, caryocarosides II-1 (**2**) and III-1 (**3**) were concluded to be 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-glucopyranosylhederagenin and 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-glucopyranosylbayogenin, respectively.

Caryocarosides II-2 (**4**) and III-2 (**5**) gave a molecular ion peak [M − H]$^-$ at *m*/*z* 957 and 973 in the negative ESIMS, and the MS^2 experiments of these ions gave the same fragment [M − H − 162]$^-$ at *m*/*z* 795 and 811, respectively, suggesting a supplementary hexose unit compared to **2** and **3**. The ^{13}C NMR spectrum of **4** exhibited three anomeric carbons at δ 95.7, 105.3, and 105.7, correlated in the HSQC spectrum with three anomeric protons at δ 5.40, 4.48, and 4.52, respectively (Table 1). Further analysis of the 2D NMR experiments revealed that the sugar chain linked at C-3 of the hederagenin was β-D-galactopyranosyl-(1→3)-β-D-glucopyranoside as in compounds **1**, **2**, and **3**. The values δ_H 5.40 and δ_C 95.7 belonged to the anomeric signals of a glycoside ester unit, and the ^{13}C NMR signal due to the C-28 of the aglycon moiety (δ 178.1) indicated a glycosylation of the carboxyl group. Consequently, compound **4** was a bidesmoside saponin. The COSY and TOCSY experiments led to the assignment of the glycoside ester unit as a β-D-glucose unit. A cross-peak was observed in the HMBC experiment between H-1‴ of this glucose and C-28 of hederagenin. The above evidence led to the assignment of caryocaroside II-2 (**4**) as 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-glucopyranosylhederagenin 28-*O*-β-D-glucopyranosyl ester. Comparison of the ^{1}H and ^{13}C NMR values of the saccharide part in compounds **4** and **5** showed that they contain the same glycoside chains linked at the C-3 and C-28 positions. Thus, the structure of caryocaroside III-2 (**5**) was concluded to be 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-glucopyranosylbayogenin 28-*O*-β-D-glucopyranosyl ester.

Caryocarosides II-3 (**6**) and III-3 (**7**) displayed a molecular ion peak [M − H]$^-$ at *m*/*z* 957 and 973, respectively, in the negative ESIMS. The MS^2 experiment gave negative fragments at *m*/*z* 795, 633, and 471 for **6** and at *m*/*z* 811, 649, and 487 for **7** attributed to the successive losses of three hexoses, [M − H − 162]$^-$, [M − H − 2 × 162]$^-$, and [M − H − 3 × 162]$^-$. The ^{1}H NMR spectrum

Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1**−**8** (CD_3OD)

	1		**2**		**3**		**4**		**5**		**6**		**7**		**8**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
aglycon																
2	1.71 (m)	26.4	1.77 (m)	26.2	4.34 (q, 3.1)	71.2	1.78 (m)	26.3	4.32 (q, 2.9)	70.7	1.77 (td, 14, 4)	26.4	4.34 (q, 3.5)	71.1	4.34 (q, 3.5)	71.2
2	1.99 (m)		1.94 (dq, 13.5, 4.4)				1.96 (dq, 14.1, 3.8)				1.95 (m)					
3	4.11 (dd, 11.8, 4.5)	85.8	3.67 (d, 8.5, 4.7)	83.7	3.64 (d, 3.8)	84.0	3.67 (dd, 12.2, 4)	83.5	3.52 (d, 3.6)	84.1	3.65 (m)	83.6	3.64 (d, 3.5)	84.0	3.64 (d, 3.5)	84.0
12	5.27 (t, 3.6)	123.4	5.26 (t, 3.6)	123.4	5.25 (t, 3.6)	122.5	5.27 (t, 3.7)	123.8	5.30 (t, 3.4)	123.5	5.27 (t, 3.3)	122.3	5.25 (t, 3.5)	122.4	5.25 (t, 3.6)	122.4
18	2.87 (dd, 14.2, 4)	42.7	2.86 (dd, 13.7, 4.2)	42.6	2.93 (dd, 13.7, 4.5)	43.5	2.88 (dd, 13.1, 3.4)	42.6	2.87 (dd, 13.7, 4.2)	42.3	2.93 (dd, 13.6, 3.2)	43.5	2.93 (dd, 13.1, 3.5)	43.5	2.94 (dm, 13)	43.5
23		181.9	3.31 (d, 11.4)	65.1	3.26 (d, 11.3)	65.7	3.27 (d, 11.8)	65.0	3.25 (d, 11.3)	65.8	3.31 (d, 11.1)	65.0	3.26 (d, 12.2)	65.7	3.26 (d, 11.5)	65.8
23			3.65 (d, 11.4)		3.64 (d, 11.5)		3.66 (d, 11.8)		3.61 (d, 11.4)		3.63 (d, 11.1)		3.64 (d, 12)		3.64 (d. 11.5)	
24	1.17 (s)	12.0	0.73 (s)	13.4	0.96 (s)	14.7	0.73 (s)	13.4	0.95 (s)	14.5	0.73 (s)	13.4	0.96 (s)	14.7	0.96 (s)	14.7
25	1.00 (s)	16.2	1.00 (s)	16.4	1.30 (s)	17.5	1.01 (s)	16.5	1.28 (s)	17.4	1.00 (s)	16.5	1.30 (s)	17.5	1.29 (s)	17.5
26	0.83 (s)	17.6	0.83 (s)	17.7	O.91 (s)	18.3	0.82 (s)	17.8	0.81 (s)	17.6	0.92 (s)	18.3	0.92 (s)	18.7	0.92 (s)	18.3
27	1.19 (s)	26.4	1.19 (s)	26.5	1.17 (s)	26.6	1.19 (s)	26.3	1.16 (s)	26.4	1.17 (s)	26.6	1.17 (s)	26.6	1.17 (s)	26.6
28		182.0		182.2		185.9		178.1		178.0		186.0		186.3		180.2
29	0.93 (s)	33.6	0.92 (s)	33.6	0.90 (s)	34.0	0.96 (s)	33.5	0.91 (s)	33.5	0.90 (s)	34.0	0.90 (s)	34.0	0.90 (s)	34.0
30	0.96 (s)	24.0	0.96 (s)	24.0	0.98 (s)	24.4	0.96 (s)	24.0	0.94 (s)	23.9	0.98 (s)	24.5	0.98 (s)	24.6	0.98 (s)	24.4
β-D-glucose (at C-3)																
1′	4.37 (d, 7.7)	104.9	4.48 (d, 7.9)	105.1	4. 52 (d, 7.8)	105.2	4.48 (d, 7.9)	105.3	4.49 (d, 7.9)	104.6	4.48 (d, 7.9)	105.3	4.51 (d, 7.9)	105,2	4.51 (d, 7.5)	105.2
2′	3.33 (t, 8.4)	74.3	3.40 (dd, 8.9, 7.9)	74.7	3.50 (dd, 9, 7.8)	74.7	3.39 (dd, 8.9, 7.9)	74.8	3.50 (t, 8.4)	74.2	3.40 (dd, 8.9, 7.9)	74.7	3.51 (dd, 8.9, 7.9)	74,6	3.50 (dd, 9, 7.5)	74.8
3′	3.35 (t, 9)	87.3	3.57 (t, 8.9)	88.0	3.60 (t, 8.9)	87.8	3.57 (t, 9)	88.1	3.58 (t, 8.3)	87.8	3.59 (t, 8.9)	87.9	3.62 (t, 8.9)	87,8	3.61 (t, 9)	87.7
4′	3.42 (t, 8.8)	70.0	3.45 (dd, 9.6, 8.9)	69.8	3.51 (dd, 9.6, 8.9)	69.5	3.45 (dd, 9.6, 9)	69.9	3.53 (t, 8.7)	69.2	3.45 (dd, 9.6, 8.9)	69.8	3.52 (t, 9)	69.5	3.52 (t, 8.9)	69.5
5′	3.28 (ddd, 10, 5.1, 2)	77.4	3.34 (ddd, 9.6, 5.1, 2.4)	77.1	3.35 (ddd, 9.6, 4.6, 2.3)	77.3	3.33 (m)	77.3	3.33 (m)	76.9	3.33 (m)	77.3	3.35 (m)	77.3	3.35 (m)	77.3
6′a	3.70 (dd, 11.6, 4.7)	62.6	3.72 (dd, 12, 5.1)	62.5	3.74 (dd, 12, 4.6)	62.2	3.72 (dd, 11.5, 4.3)	62.6	3.74 (dd, 12, 4.5)	61.9	3.72 (m)	62.6	3.75 (m)	62,2	3.74 (dd, 12, 4.5)	62.2
6′b	3.87 (dd, 12, 2.3)		3.87 (dd, 12, 2.4)		3.83 (dd, 12, 2.3)		3.87 (dd, 11.5, 2)		3.83 (dd, 12, 3.6)		3.87 (dd, 12, 2)		3.84 (m)		3.83 (dd, 12, 2)	
β-D-galactose (at C-3′)																
1″	4.52 (d, 7.6)	105.7	4.52 (d, 7.7)	105.5	4.55 (d, 7.7)	105.8	4.52 (d, 7.7)	105.7	4.50 (d, 7.8)	105.4	4.62 (d, 7.8)	105.3	4.63 (d, 7.8)	105,3	4.62 (d, 7.5)	106.3
2″	3.60 (dd, 9.8, 7.7)	73.1	3.63 (dd, 9.7, 7.7)	72.9	3.64 (dd, 9.6, 7.8)	73.0	3.63 (dd, 9.6, 7.7)	73.0	3.64 (dd, 9.6, 7.8)	72.7	3.80 (dd, 9.4, 7.6)	72.2	3.81 (dd, 9, 8)	72,1	3.80 (t, 8)	72.2
3″	3.51 (dd, 9.8, 3.6)	74.7	3.54 (dd, 9.7, 3.3)	74.6	3.54 (dd, 9.6, 3.4)	74.7	3.53 (dd, 9.6, 3.3)	74.7	3.54 (dd, 9.6, 3.2)	74.4	3.68 (dd, 9.4, 2.9)	84.5	3.69 (dd, 9.3, 3)	84,6	3.67 (dd, 7, 3)	84.1
4″	3.82 (d, 3.5)	70.3	3.83 (dd, 3.3, 0.1)	70.2	3.83 (d, 3.4)	70.3	3.82 (d, 3.3)	70.3	3.83 (d, 3.2)	69.9	4.13 (d, 3)	70.2	4.15 (d, 3.3)	69,8	4.04 (dd, 3, 0.5)	69.8
5″	3.56 (m)	77.1	3.60 (ddd, 9.6, 5.8, 1)	77.0	3.60 (dd, 7.7, 4.4)	77.2	3.59 (ddd, 7.7, 3.3, 1)	77.2	3.58 (m)	76.8	3.62 (m)	76.7	3.63 (m)	76,7	3.61 (m)	76.7
6″a	3.70 (dd, 11.6, 4.6)	62.6	3.72 (dd, 11.6, 4.5)	62.5	3.71 (dd, 11.5, 4.4)	62.6	3.71 (dd, 11.4, 3.3)	62.5	3.71 (dd, 11.5, 4)	62.3	3.71 (dd, 11.5, 2.7)	62.6	3.74 (dd, 11.5, 2.7)	62,6	3.70 (dd, 11, 4)	62.5
6″b	3.79 (dd, 11.6, 7.6)		3.81 (dd, 11.6, 7.8)	-	3.80 (dd, 11.5, 7.7)		3.80 (dd, 11.4, 7.7)		3.81 (dd, 11.5, 7.8)		3.81 (dd, 11.5, 7.4)		3.80 (dd, 11.5, 5)		3.80 (dd, 11, 8)	
sugar (at C-28 or C-3″)							β-D-glucose (at C-28)		β-D-glucose (at C-28)		β-D-galactose (at C-3″)		β-D-galactose (at C-3″)		β-D-xylose (at C-3″)	
1‴							5.40 (d, 8.1)	95.7	5.40 (d, 8.1)	95.4	4.52 (d, 7.6)	106.3	4.53 (d, 7.8)	106.9	4.53 (d, 7)	105.2
2‴							3.34 (dd, 9.3, 8.1)	73.9	3.35 (t, 8.5)	73.5	3.64 (m)	73.0	3.64 (m)	72.9	3.32 (t, 9)	75.2
3‴							3.43 (t, 9.3)	78.3	3.45 (dd, 9.1, 8.5)	77.9	3.52 (dd, 9.7, 3.3)	74.6	3.53 (dd, 9.7, 3.6)	74.8	3.36 (t, 9)	77.6
4‴							3.38 (t, 9.3)	71.1	3.40 (t, 9.3)	70.8	3.82 (d, 3.4)	69.8	3.85 (d, 3)	70.2	3.52 (m)	71.2
5‴/5‴a							3.37 (m)	78.7	3.37 (m)	78.2	3.56 (m)	76.8	3.57 (m)	76.8	3.24 (t, 11.5)	66.9
5‴b															3.89 (dd, 11.5, 5)	
6‴a							3.70 (dd, 11.4, 3)	62.4	3.71 (dd, 11.6, 3.4)	62.2	3.75 (m)	62.5	3.74 (m)	62.6		
6‴b							3.84 (dd, 11.4, 2)		3.83 (dd, 11.6, 2.5)		3.75 (m)		3.74 (m)			

Table 2. ^{1}H and ^{13}C NMR Data of Compounds **9**−**11**, **14** and **15** (CD_3OD), and **12** and **13** (DMSO-*d*6)

	9		**10**		**11**		**12**		**13**		**14**		**15**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
aglycon														
2	4.22 (q, 3.3)	71.2	4.32 (q, 3.4)	71.1	4.17 (q, 2.9)	71.2	4.08 (q, 3.7)	69.6	1.59 (m)	26.4	4.28 (q, 3.3)	71.5	4.08 (q, 3.3)	69.9
2									1.78 (m)					
3	3.20 (d, 3.7)	91.1	3.63 (d, 3.5)	83.8	3.20 (d, 3.7)	91.0	3.04 (d, 2.2)	90.0	3.58 (dd, 13.3, 4)	79.6	3.66 (d, 3.3)	83.9	3.02 (d, 3.4)	89.6
12	5.28 (t, 3.6)	123.9	5.28 (t, 3.7)	123.7	5.27 (t, 3.6)	123.6	5.12 (t, 3.7)	123.0	5.16 (t, 3.5)	123.0	5.27 (t, 3.4)	123.7	5.12 (t, 3.7)	122.2
18	2.87 (dd, 14, 3)	42.7	2.87 (dd, 13.4, 4.4)	42.8	2.88 (dd, 13.5, 4)	42.9	2.87 (dd, 13.4, 3)	42.6	2.76 (dd, 12.1, 3)	42.4	2.87 (dd, 14, 4.1)	42.7	2.87 (dd, 13.9, 4)	41.3
23	1.10 (s)	29.9	3.24 (d, 11.3)	65.3	1.10 (s)	29.9	0.99 (s)	30.6	3.04 (d, 10.5)	63.9	3.25 (d, 11.3)	65.3	1.10 (s)	28.4
23			3.64 (d, 11.4)						3.48 (d, 11.1)	-	3.64 (d, 11.3)			
24	1.10 (s)	18.5	0.97 (s)	14.6	1.10 (s)	18.5	0.97 (s)	19.5	0.97 (s)	14.3	0.97 (s)	14.6	1.10 (s)	17.1
25	1.27 (s)	16.8	1.30 (s)	17.4	1.27 (s)	16.8	1.16 (s)	17.2	0.89 (s)	17.0	1.30 (s)	17.4	1.27 (s)	15.4
26	0.85 (s)	17.8	0.85 (s)	17.8	0.86 (s)	17.8	0.74 (s)	18.7	0.73 (s)	18.4	0.85 (s)	17.8	0.85 (s)	16.4
27	1.18 (s)	26.4	1.17 (s)	26.5	1.18 (s)	26.4	1.06 (s)	27.0	1.10 (s)	27.0	1.20 (s)	26.5	1.18 (s)	25.1
28	-	181.9	-	182.0	-	181.8	-	179.7	-	182.0	-	182.0	-	181.0
29	0.93 (s)	33.4	0.93 (s)	33.6	0.93 (s)	33.6	0.84 (s)	34.4	0.87 (s)	34.2	0.93 (s)	33.6	0.93 (s)	32.1
30	0.97 (s)	24.0	0.97 (s)	24.0	0.97 (s)	24.0	0.80 (s)	24.9	0.87 (s)	24.7	0.97 (s)	24.0	0.96 (s)	22.5
β-D-glucuronic acid (at C-3)														
1′	4.46 (d, 7.5)	106.5	4.51 (d, 7.7)	105.3	4.47 (d. 7.5)	106.7	4.40 (d, 8.1)	104.6	4.38 (d, 7.9)	104.2	4.60 (d, 7.8)	105.5	4.53 (d, 7.8)	104.2
2′	3.36 (dd, 9.2, 7.7)	75.0	3.33 (dd, 9.1, 7.7)	75.1	3.36 (t. 7.6)	75.0	3.27 (t, 8.4)	74.2	3.18 (t, 8.5)	73.5	3.56 (dd, 9.5, 8.4)	74.4	3.59 (t, 7.8)	73.0
3′	3.41 (t, 9.2)	77.6	3.42 (t, 9.1)	77.8	3.40 (dd, 9.5, 7.6)	77.4	3.50 (m)	85.7	3.44 (t, 9.5)	86.3	3.65 (m)	86.5	3.66 (m)	84.8
4′	3.53 (t, 9.5)	73.2	3.49 (t, 9.1)	73.3	3.54 (t, 9.5)	73.2	3.30 (t, 9.2)	71.8	3.32 (t, 10.1)	72.0	3.64 (t, 9)	73.0	3.66 (m)	71.6
5′	3.83 (d, 9.7)	76.0	3.77 (d, 10.2)	76.1	3.90 (d, 9.6)	76.5	3.38 (d, 9.9)	75.0	3.38 (d, 9.5)	76.1	3.90 (d, 9)	76.0	3.95 (d, 9.6)	74.6
6′		173.0		171.0		171.5		174.2		175.0	-	174.2		169.7
$-OCH_3$					3.78 (s)	52.9							3.80 (s)	51.5
β-D-galactose (at C-3′)														
1″							4.33 (d, 7.7)	104.9	4.36 (d, 7.9)	105.1	4.55 (d, 7.7)	105.6	4.57 (d, 7.7)	105.0
2″							3.46 (dd, 9.5, 2.7)	71.8	3.47 (t, 8.4)	71.7	3.63 (dd, 9.6, 7.7)	71.9	3.64 (dd, 9.6, 3.4)	70.4
3″							3.33 (dd, 8.5, 3.3)	74.2	3.30 (dd, 9.7, 3)	74.2	3.55 (dd, 9.7, 3.2)	74.7	3.53 (dd, 8.5, 3.3)	73.2
4″							3.62 (d, 3.3)	69.6	3.63 (d, 3)	69.5	3.83 (d, 3.2)	70.4	3.80 (d, 3.3)	68.9
5″							3.42 (m)	77.2	3.44 (m)	77.3	3.59 (ddd, 7.7, 4.3, 3.2)	77.2	3.65 (m)	75.7
6″a							3.51 (m)	61.9	3.51 (m)	61.8	3.71 (dd, 11.5, 4.3)	62.7	3.70 (dd, 11.8, 4.3)	61.2
6″b							3.51 (m)		3.51 (m)		3.81 (dd, 11.5, 7.7)		3.79 (dd, 11.8, 5.5)	

Table 3. ^{1}H and ^{13}C NMR Data of Compounds **16**−**21** (CD_3OD)

	16		**17**		**18**		**19**		**20**		**21**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
aglycon												
2	4.21 (q, 3.7)	71.3	1.77 (m)	26.2	4.28 (q, 3.1)	71.5	1.77 (m)	26.4	4.22 (m)	70.9	1.77 (m)	26.4
2			1.97 (m)				1.95 (m)				1.89 (ddd, 13.4, 2.9, 1.3)	
3	3.22 (d, 3.7)	91.2	3.70 (dd, 11, 2.7)	82.2	3.65 (d, 3.4)	83.9	3.63 (dd, 11, 2.7)	83.5	3.22 (d, 3.3)	91.2	3.68 (dd, 9.7, 3.3)	83.2
12	5.28 (t, 3.8)	123.9	5.27 (t, 3.6)	123.8	5.28 (t, 3.5)	123.8	5.27 (t, 3.6)	123.8	5.28 (t, 3.4)	124.0	5.26 (t, 3.5)	123.8
18	2.88 (dd, 14, 4.9)	42.6	2.87 (dd, 13.4, 4.3)	42.6	2.87 (dd, 13.5, 4.2)	42.6	2.87 (dd, 13.4, 4.3)	42.6	2.87 (dd, 14, 3)	42.7	2.87 (dd, 13.6, 4.2)	42.8
23	1.09 (s)	29.8	3.27 (d, 11.4)	64.9	3.25 (d, 11.3)	65.3	3.27 (d, 11.4)	64.7	1.10 (s)	29.9	3.30 (d, 11.3)	64.8
23			3.66 (d, 11.4)		3.63 (d, 11.3)		3.66 (d, 11.4)				3.64 (d, 11.3)	
24	1.09 (s)	18.5	0.72 (s)	13.4	0.96 (s)	14.7	0.72 (s)	13.3	1.10 (s)	18.6	0.72 (s)	13.4
25	1.27 (s)	16.9	1.00 (s)	16.5	1.30 (s)	17.5	1.00 (s)	16.5	1.27 (s)	16.8	1.00 (s)	16.4
26	0.83 (s)	17.8	0.82 (s)	17.8	0.83 (s)	17.8	0.82 (s)	17.8	0.85 (s)	17.8	0.84 (s)	17.8
27	1.18 (s)	26.3	1.19 (s)	26.3	1.19 (s)	26.4	1.19 (s)	26.4	1.18 (s)	26.4	1.20 (s)	26.2
28		178.0		178.1		178.1		178.1		181.9		182.0
29	0.93 (s)	33.5	0.93 (s)	33.5	0.93 (s)	33.5	0.93 (s)	33.5	0.93 (s)	33.6	0.93 (s)	33.6
30	0.96 (s)	24.0	0.96 (s)	24.0	0.95 (s)	24.0	0.96 (s)	24.0	0.97 (s)	24.0	0.96 (s)	24.0
β-D-glucuronic acid (at C-3)												
1′	4.53 (d, 7.8)	106.3	4.51 (d, 7.9)	104.6	4.60 (d, 7.9)	105.5	4.54 (d, 7.9)	105.6	4.53 (d, 7.7)	106.3	4.54 (d, 7.9)	105.4
2′	3.57 (dd, 9.5, 7.8)	74.5	3.47 (dd, 9.1, 8)	74.6	3.56 (t, 8.2)	74.4	3.43 (dd, 9.1, 8)	74.6	3.59 (dd, 10.1, 7.5)	74.6	3.47 (dd, 9.7, 7.5)	74.6
3′	3.64 (t, 9.2)	86.4	3.67 (t, 9)	86.7	3.65 (m)	86.5	3.64 (t, 9)	86.8	3.67 (m)	85.9	3.64 (m)	87.3
4′	3.65 (t, 9.2)	71.9	3.57 (dd, 9.9, 8.9)	72.1	3.65 (m)	71.8	3.63 (t, 8.9)	71.8	3.67 (m)	71.2	3.63 (m)	71.2
5′	3.90 (d, 9.4)	76.0	3.66 (d, 9.9)	76.8	3.91 (d, 8.7)	75.4	3.91 (d, 9.7)	76.3	3.80 (m)	nd	3.83 (d, 9)	76.6
6′		172.2		176.7		172.0		171.0		174.2		174.2
-OCH_3							3.80 (s)	52.9				
β-D-galactose (at C-3′)												
1″	4.57 (d, 7.7)	105.6	4.60 (d, 7.8)	105.5	4.56 (d, 7.7)	105.6	4.53 (d, 7.7)	105.6	4.66 (d, 7.9)	105.1	4.64 (d, 7.8)	105.2
2″	3.63 (dd, 9.7, 7.7)	73.1	3.65 (dd, 9.8, 7.8)	72.8	3.63 (dd, 10.1, 7.7)	73.0	3.63 (dd, 9.6, 7.7)	73.0	3.81 (dd, 9.6, 7.9)	72.1	3.80 (dd, 9.8, 8)	72.1
3″	3.53 (dd, 9.7, 3.3)	74.7	3.53 (dd, 9.8, 3.4)	74.7	3.53 (dd, 10.1, 3.4)	74.7	3.57 (dd, 9.6, 3.3)	74.7	3.69 (dd, 9.6, 3.2)	84.5	3.68 (dd, 9.5, 3.1)	84.5
4″	3.82 (dd, 3.3, 0.8)	70.4	3.82 (dd, 3.4, 1.2)	70.5	3.82 (dm, 3.4)	70.4	3.81 (d, 3.4)	70.3	4.14 (d, 2.8)	69.9	4.13 (d, 3.1)	69.9
5″	3.58 (ddd, 7.8, 4.1, 0.8)	77.2	3.61 (ddd, 7.8, 4.4, 1.2)	77.2	3.59 (dd, 7.8, 4.5)	77.2	3.59 (ddd, 7.8, 4.4, 1.2)	77.2	3.63 (dd, 7.1, 5)	76.8	3.63 (m)	76.8
6″a	3.70 (dd, 11.5, 4.1)	62.7	3.70 (dd, 11.9, 4.5)	62.6	3.70 (dd, 11.8, 4.5)	62.7	3.70 (dd, 11.9, 4.5)	62.2	3.71 (dd, 10.8, 5)	62.6	3.71 (dd, 11.9, 4.3)	62.6
6″b	3.80 (dd, 11.5, 7.8)		3.82 (m)		3.81 (dd, 11.8, 7.8)		3.82 (brd, 11.8)		3.82 (dd, 10.8, 7.1)		3.77 (m)	
sugar at C-28 or C-3″	β-D-glucose (at C-28)		β-D-glucose (at C-28)		β-D-glucose (at C-28)		β-D-glucose (at C-28)		β-D-galactose (at C-3″)		β-D-galactose (at C-3″)	
1‴	5.40 (d, 8.1)	95.7	5.40 (d, 8.1)	95.7	5.40 (d, 8.2)	95.7	5.40 (d, 8.1)	95.7	4.53 (d, 7.6)	106.3	4.53 (d, 7.6)	106.3
2‴	3.38 (dd, 9, 8.1)	73.9	3.34 (dd, 9.1, 8.1)	73.9	3.34 (t, 8.6)	73.9	3.35 (dd, 9.1, 8.1)	73.9	3.64 (dd, 9.7, 7.7)	73.0	3.64 (dd, 9.5, 7.7)	73.3
3‴	3.43 (t, 9)	78.3	3.43 (t, 9.1)	78.3	3.43 (t, 8.6)	78.3	3.43 (t, 9.1)	78.3	3.52 (dd, 9.7, 3.3)	74.6	3.52 (dd, 9.5, 3.4)	74.6
4‴	3.37 (t, 9.5)	71.1	3.38 (t, 9.5)	71.1	3.38 (t, 9.5)	71.1	3.38 (t, 9.5)	71.1	3.84 (d, 3.3)	70.2	3.84 (dd, 3.4, 0.5)	70.3
5‴	3.37 (m)	78.7	3.37 (m)	78.7	3.37 (m)	78.7	3.37 (m)	78.7	3.56 (ddd, 7, 5, 1)	76.8	3.56 (ddd, 7.3, 5, 0.5)	76.8
6‴a	3.70 (dd, 11.5, 4.1)	62.4	3.70 (dd, 11.5, 4.4)	62.4	3.70 (dd, 11.8, 4.2)	62.4	3.70 (dd, 11.5, 4.4)	61.9	3.72 (dd, 11.3, 5)	62.6	3.72 (dd, 11.5, 5)	62.6
6‴b	3.83 (dd, 11.5, 1.8)		3.84 (m)		3.84 (brd, 11.8)		3.84 (brd, 11.8)		3.77 (dd, 11.3−7.2)		3.77 (m)	

of **6** exhibited three anomeric protons at δ 4.48, 4.52, and 4.62, correlated in the HSQC experiment with their respective anomeric carbons at δ 105.3, 106.3, and 105.3 (Table 1). Complete assignment of each glycoside proton system was achieved by analysis of COSY, TOCSY, and ROESY experiments. The sugar units with anomeric protons at δ 4.62 (d, $J = 7.8$ Hz) and 4.52 (d, $J = 7.6$ Hz) corresponded to two β-D-galactose units. The third glycosidic unit with the anomeric proton at δ 4.48 (d, $J = 7.9$ Hz) was identified as a β-D-glucose unit (Table 1). The downfield shifts of C-3″ (δ 84.5) of the inner galactose moiety and C-3′ (δ 87.9) of the glucose moiety and their correlations in the HMBC spectrum observed between H-1‴ (δ 4.52) of the terminal galactose and C-3″ of the inner galactose, between H-1″ (δ 4.62) of this galactose and C-3′ of the glucose, and between H-1′ (δ 4.48) of the glucose and C-3 (δ 83.6) of hederagenin showed that caryocaroside II-3 (**6**) is 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→3)-β-D-glucopyranosylhederagenin. Comparison of the ^{1}H and ^{13}C NMR values of the trisaccharide chain of compounds **6** and **7** showed that **7** contained the same trisaccharide. Thus, caryocaroside III-3 (**7**) was concluded to be 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→3)-β-D-glucopyranosylbayogenin.

The MS^2 experiment of the $[M - H]^-$ molecular ion peak of caryocaroside III-4 (**8**) observed at *m*/*z* 943 in the negative ESIMS gave a negative fragment at *m*/*z* 811 $[M - H - 132]^-$, suggesting a supplementary terminal pentose unit compared to **7**. Analysis of 2D NMR experiments of **8** revealed that the nature of the supplementary pentose was a terminal β-D-xylose with anomeric signals at δ_H 4.53 (d, $J = 7$ Hz) and δ_C 105.2 (Table 1). The ROE interactions observed in the ROESY spectrum between H-1‴, H-3‴, and H-5‴ of the xylose unit confirmed the α-axial orientation of these three protons and the β-anomeric configuration. The sequence of the trisaccharide chain was deduced from the cross-peaks observed in the HMBC spectrum between H-1‴ of the terminal xylose and C-3″ of the galactose, H-1″ of the galactose and C-3′ of the glucose, and H-1′ of the glucose and C-3 of bayogenin. This sequence was confirmed by the observation of the ROE interactions between the protons involved in the interglycosidic linkages: H-1‴ of xylose/H-3″ of galactose, H-1″ of galactose/H-3′ of glucose, and H-1′ of glucose/H-3 of bayogenin. On the basis of the foregoing evidence, the structure of caryocaroside III-4 (**8**) was established as 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-galactopyranosyl-(1→3)-β-D-glucopyranosylbayogenin.

The ^{1}H NMR spectrum of caryocarosides IV-5 (**9**) and III-5 (**10**) exhibited anomeric proton doublets at δ 4.46 ($J = 7.5$ Hz) and 4.51 ($J = 7.7$ Hz), respectively, which gave correlations with anomeric carbons at δ 106.5 and 105.3 in the HSQC experiment (Table 2). Analysis of COSY and HSQC experiments identified a β-D-glucuronic acid with the carbonyl C-6′ at δ 173.0 (**9**) and 171.0 (**10**). In addition, the $[M - H - 176]^-$ negative ion observed at *m*/*z* 471 (**9**) and 487 (**10**) after MS^2 fragmentation of the molecular ion $[M - H]^-$, in the negative ESIMS, confirmed the presence of the glucuronic acid. The cross-peaks observed in the HMBC experiments between C-3 of the aglycon and H-1′ of glucuronic acid and the ROE interactions observed in the ROESY experiments between H-1′ of glucuronic acid and H-3 of the aglycon indicated that the glucuronic acid was attached to C-3 of 2β-hydroxyoleanolic acid in **9** and to C-3 of bayogenin in **10**. From these data, the structures of caryocarosides IV-5 (**9**) and III-5 (**10**) were established as 3-*O*-β-D-glucuronopyranosyl-2β-hydroxyoleanolic acid and 3-*O*-β-D-glucuronopyranosylbayogenin, respectively.

Caryocaroside IV-6 (**11**) showed a molecular ion peak $[M - H]^-$ at *m*/*z* 661 that gave ion fragments at *m*/*z* 647 $[M - H - 15]^-$ and 471 $[M - H - (175 + 15)]^-$ in the negative ESIMS^2, attributed to the losses of a methyl group and a methyl hexosuronate. These data suggested an additional methyl group compared to **9**. This hypothesis was confirmed by the presence of the NMR signal of a methoxy group at δ_H 3.78 (s, 3H) and δ_C 52.9 (Table 2). Nearly all of the ^{13}C NMR signals were superposable with those of **9** except for C-6′ of glucuronic acid, which exhibited an upfield shift at δ 171.5. Moreover, in the HMBC experiment, a cross-peak was observed between the protons of the methoxy group and the carbonyl C-6′ of glucuronic acid. Thus, caryocaroside IV-6 (**11**) is 3-*O*-β-D-methyl glucopyranosiduronate-2β-hydroxyoleanolic acid.

The negative ESIMS experiments of caryocarosides IV-7 (**12**) and II-7 (**13**) gave both molecular ion peaks $[M - H]^-$ at *m*/*z* 809, whereas the molecular ion peak was observed at *m*/*z* 825 for caryocaroside III-7 (**14**). The MS^2 experiments of the $[M - H]^-$ ion of these saponins gave the same ion fragment $[M - H - 162 - 176]^-$ attributed to the loss of a disaccharide consisting of a hexose and a hexosuronic acid. The ^{1}H and ^{13}C NMR spectra of **12** confirmed the presence of two sugar residues with their anomeric carbons at δ 104.6 and 104.9 correlating in the HSQC experiment with the anomeric protons at δ 4.40 (d, $J = 8.1$ Hz) and 4.33 (d, $J = 7.7$ Hz) (Table 2). Analysis of 2D NMR experiments revealed the presence of a terminal β-D-galactose (δ_H 4.33) and a β-D-glucuronic acid (δ_H 4.40) monosubstitued at position C-3′ (δ_C 85.7). The HMBC spectrum showed cross-peaks between H-1″ of galactose and C-3′ of glucuronic acid and between H-1′ of glucuronic acid and C-3 of 2β-hydroxyoleanolic acid. This evidence led to the assignment of caryocaroside IV-7 (**12**) as 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-glucuronopyranosyl-2β-hydroxyoleanolic acid. Comparison of the ^{1}H and ^{13}C NMR spectral data of the disaccharide moieties of **13** and **14** with those of **12** showed that hederagenin (**13**) and bayogenin (**14**) were both substituted at C-3 by the same disaccharide chain (Table 2). Thus, the structure of caryocaroside II-7 (**13**) was elucidated as 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-glucuronopyranosylhederagenin and caryocaroside III-7 (**14**) as 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-glucuronopyranosyl-bayogenin.

As observed for caryocaroside IV-6 (**11**), the ^{1}H and ^{13}C NMR spectra of caryocaroside IV-8 (**15**) showed an additional methoxy group compared to **12** (Table 2). The MS^2 experiment of its $[M - H]^-$ molecular ion at *m*/*z* 823 gave fragments at *m*/*z* 791 $[M - H - CH_3OH]^-$ and 471 $[M - H - 162 - (175 + 15)]^-$ due to the elimination of the methoxy group and to the loss of a disaccharide consisting of a hexose and a methyl hexosuronate. The methoxy group observed in the ^{1}H NMR at δ_H 3.80 (s, 3H) was correlated in the HMBC spectrum with the carbonyl C-6′ of glucuronic acid. Thus, caryocaroside IV-8 (**15**) is 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-methyl glucopyranosiduronate-2β-hydroxyoleanolic acid.

In the negative ESIMS, the molecular ion peaks $[M - H]^-$ of caryocarosides IV-9 (**16**) and II-9 (**17**) were both observed at *m*/*z* 971, whereas in caryocaroside III-9 (**18**) the molecular ion peak was observed at *m*/*z* 987. The MS^2 experiment of these ions gave fragments suggesting an additional hexose compared to **12**, **13**, and **14**, respectively. Analysis of the ^{1}H and ^{13}C NMR spectra of compounds **16**, **17**, and **18** revealed that the C-3 of the aglycon was substituted by the disaccharide [β-D-galactopyranosyl-(1→3)-β-D-glucuronopyranoside] as in compounds **12**, **13**, and **14**. The additional hexose unit was identified as a terminal β-D-glucopyranose with anomeric signals at δ_H 5.40 and δ_C 95.7 (Table 3), linked to C-28 of the aglycon as deduced from the cross-peaks observed in the HMBC spectrum between its H-1‴ and the C-28 (δ 178.1) of the aglycon. On the basis of the foregoing evidence, the structures of caryocarosides IV-9, II-9, and III-9 were concluded to be 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-glucuronopyranosyl-2β-hydroxyoleanolic acid 28-*O*-β-D-glucopyranosyl ester (**16**), 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-glucuronopyranosylhederagenin 28-*O*-β-D-glucopyranosyl ester (**17**), and 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-glucuronopyranosylbayogenin 28-*O*-β-D-glucopyranosyl ester (**18**), respectively.

Comparison of the ^{1}H and ^{13}C NMR and ESIMS of caryocaroside II-10 (**19**) with those of II-9 (**16**) showed that **19** also possessed an additional methoxy group. Furthermore, in the HMBC experiment,

Table 4. Hemolytic Activity of the 10 Major Saponins and the Methanol Extract of the Pulp (MEP1) and the Peel (MEP2)

	HD_{50}		HD_{100}	
compound	μM	μg/mL	μM	μg/mL
2	56	45	94	75
3	18	15	24	25
5			>513	>500 [10%][a]
28	93	60	116	75
24	19	15	31	25
12	32	26	49	40
13	45	37	93	75
14	66	55	121	100
15	157	130	303	250
17	309	300	514	500
MEP1		25		100
MEP2		25		100
Sigma D		7.5		25

[a] Hemolytic percentage observed at this concentration.

a cross-peak was observed between the protons of this methyl (δ_H 3.80) and the carbonyl C-6′ (δ_C 171.0) of glucuronic acid. Thus, caryocaroside II-10 **(19)** was elucidated as 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-methylglucopyranosiduronate hederagenin 28-*O*-β-D-glucopyranosyl ester.

The negative ESIMS of caryocarosides IV-11 (**20**) and II-11 **(21)** showed that they were isomers to **16** and **17**. The ^{13}C NMR spectrum of **20** and **21** showed three anomeric carbon signals at δ 105.1, 106.3 (2C) for **20** and 105.2, 105.4, and 106.3 for **21**. Analysis of the 2D NMR experiments allowed full identification of a terminal β-D-galactose (δ_H 4.53 and δ_C 106.3), a β-D-galactose (δ_H 4.66 or 4.64 and δ_C 105.1 or 105.2) monosubstituted at position C3″ (δ_C 84.5), and a β-D-glucuronic acid monosubstituted at position C-3′ (δ_C 85.9 or 87.3) (Table 3). The sequence of the trisaccharide chain was deduced from the cross-peaks observed in the HMBC experiments between H-1‴ of the terminal galactose and C-3″ of the inner galactose, between H-1″ of the inner galactose and C-3′ of the glucuronic acid, and between H-1′ of the glucuronic acid and C-3 of the aglycon. This evidence led to the assignment of caryocaroside IV-11 (**20**) as 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→3)-β-D-glucuronopyranosyl-2β-hydroxyoleanolic acid and caryocaroside II-11 (**21**) as 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→3)-β-D-glucuronopyranosylhederagenin.

The ability of the methanol extracts of the pulp and the peel of *C. glabrum* fruits and of the 10 major isolated saponins (**2, 3**, **5**, **12**−**15**, **17**, **24**, and **28**) to lyse sheep erythrocytes (10% suspension phosphate buffer) were evaluated in vitro using the method previously described.[8] The tested saponins were generally less active than the dialyzed saponin mixture from Sigma (Sigma D) used as a reference (Table 4). The hemolytic activity of the monodesmosidic saponins **3** and **13** was much higher than the corresponding bidesmosidic saponins **5** and **17**. Comparison of the hemolytic activity of saponins **28** and **13** showed that the disaccharide saponin **13** was more active than the corresponding monosaccharide **28**. These results confirmed the fact that bidesmoside saponins are generally less hemolytic than monodesmoside saponins and that hemolytic activity increases with the number of sugar units linked at position 3 of the aglycon.[8] In our previous study, we established that hemolytic activity increased with the polarity of ring A (hydroxyl group at C-23 and/or C-2). This observation was confirmed with saponins containing a β-D-glucose linked at position C-3 of the aglycon. Caryocaroside III-1 (**3**) was 3-fold more active than caryocaroside II-1 (**2**). To evaluate the influence of a glucose and a glucuronic acid on the hemolytic activity, we compared the activity of saponins **2** and **3** with **13** and **14**, respectively. With hederagenin as aglycon (**2** and **13**), the presence of a glucuronic acid slightly enhanced hemolytic activity, but with bayogenin (**3** and **14**), we observed the reverse effect; glucuronic acid decreased the hemolytic activity. It is known that the effect of the sugar residue is not transferable from one aglycon to one another.[30] With saponins including a β-D-glucuronic acid linked at position 3 of the aglycon (**24**, **12**, **13**, and **14**), the hemolytic activity decreased when the polarity of ring A increased. We suggest that the addition of a hydroxyl group of ring A (at C-23 and/or C-2) might interfere with the free carboxylic acid group of the glucuronic acid in the hemolytic mechanism. Caryocaroside IV-7 (**12**) was 5 times more hemolytic than caryocaroside IV-8 (**15**), which contains a methoxy group attached to C-6′ of the glucuronic acid. The esterified glucuronic acid decreased the polarity of the saponin and led to a decrease in the hemolytic activity, confirming the important role of polarity of ring A on hemolytic activity.

Experimental Section

General Experimental Procedures. Optical rotations were measured in MeOH or H_2O with a Perkin-Elmer 241 polarimeter. ^1H and ^{13}C NMR spectra were recorded in CD_3OD or DMSO-d_6 on a Bruker Avance DRX-500 spectrometer (^1H at 500 MHz and ^{13}C at 125 MHz), and 2D-NMR experiments were performed using standard Bruker microprograms (XWIN NMR version 2.6 software). ESIMS and MS-MS experiments were performed using a Bruker Esquire-LC ion trap mass spectrometer. The samples were introduced by infusion in a solution of MeOH. TLC was carried out on precoated silica gel 60 F_{254} (Merck), with $CHCl_3$−MeOH−H_2O (14:6:1), and spots were visualized by spraying with 50% H_2SO_4. Column chromatography (CC) was carried out on Kieselgel 60 (63−200 μm, Merck) or LiChroprep RP-18 (40−63 μm, Merck). HPLC was performed on a Dionex apparatus equipped with an ASI-100 automated sample injector, a STH 585 column oven, a P580 pump, a UVD 340S diode array detector, and the Chromeleon software. A prepacked C_{18} reversed-phase column (201SPTM, 4.6 × 250 mm, 5 μm, 90 Å, Dionex, vydac, France) was used for analytical HPLC with a binary gradient elution (solvent A: H_2O−TFA, 0.025%, solvent B: MeCN) and a flow rate of 1 mL min^{-1}, and the chromatogram was monitored at 205 and 210 nm. A prepacked C_{18} reversed-phase column (201SP510, 10 × 250 mm, 5 μm, 90 Å, Dionex, vydac, France) was used for semipreparative HPLC with a binary gradient elution (solvent A: H_2O−TFA 0.0025%, solvent B: MeCN) and a flow rate of 3 mL min^{-1}, and the chromatogram was monitored at 205 and 210 nm.

Plant Material. The fruits of *C. glabrum* were collected in two localities of French Guyana, the Amirande forest near Matoury, and the Ecerex forest station near Sinnamary, in October 2001. The species was identified by M. F. Prevost of the botany laboratory of the IRD Centre of Cayena (French Guyana), and a herbarium specimen (Prevost MFP 4864) was deposited in the Herbier of Guyana.[31]

Hemolytic Assays. This assay was performed as described previously.[8] The 10% sheep erythrocyte suspension (10%) was obtained by dilution of a commercial 50% suspension from Biomerieux, Lyon, with phosphate-buffered saline (PBS). Saponins **2**, **3**, **5**, **12**−**15**, **17**, **24**, and **28** were prepared in triplicate with concentrations ranging from 1 to 500 μg/mL in PBS. Erythrocyte suspension (25 μL) was added to 1 mL of the sample and rapidly stirred. Absorbance of the supernatant was measured at 540 nm after 60 min of incubation and centrifuged for 5 min at 3000 rpm. HD_{50} and HD_{100} values were the concentrations of sample that cause 50% and 100% hemolysis. The hemolytic activity was measured with regarded to a dialyzed saponin from Sigma used as reference standard, which caused 100% hemolysis at 25 μg/mL.

Extraction and Isolation. The air-dried powdered pulp (mesocarp) (35 g) and peel (pericarp) (84 g) of fruit were boiled under reflux in methanol (1 and 2 L, respectively) for 3 h. After cooling and filtration, the methanol extract was evaporated to provide the saponin mixture as a brown residue (20 g, 57% yield for the pulp; 26 g, 31% yield for the peel). The saponin mixture was analyzed by TLC on silica gel using the solvent mixture $CHCl_3$−MeOH−H_2O (14:6:1) and by analytical HPLC over C_{18} with the gradient elution program 30 to 50% B for 75 min.

A part of the methanol extract of the pulp (6 g) was subjected to column chromatography on silica gel (240 g, 2.5 × 38 cm) using a gradient of $CHCl_3$−MeOH−H_2O (95:5:0 to 60:40:7) to give 191 fractions of 250 mL. Fractions 56−58 eluted with $CHCl_3$−MeOH (8:2) were purified by preparative TLC in $CHCl_3$−MeOH (75:25) to give **27** (19 mg) and **30** (11 mg). Fraction 63, eluted with $CHCl_3$−MeOH

(8:2), contains compound **2** (103 mg). Fractions 64−69 eluted with $CHCl_3$−MeOH (8:2) were purified by preparative TLC in $CHCl_3$−MeOH−H_2O (70:30:3) to give **3** (28 mg) and **22** (6 mg). Fractions 83−86, eluted with $CHCl_3$−MeOH (7:3), were purified on a silica gel column using the gradient $CHCl_3$−MeOH (85:15 to 7:3), and fractions eluted with $CHCl_3$−MeOH (85:15) were purified further by reversed-phase C_{18} column chromatography using a gradient of MeOH−H_2O (5:5 to 7:3) to give **4** (11 mg) and **7** (5 mg). Fractions 87−90, eluted with $CHCl_3$−MeOH (7:3), were purified by RP-18 column chromatography, eluting with MeOH−H_2O (5:5 to 7:3), to give **5** (29 mg). Fractions 91−100 eluted with $CHCl_3$−MeOH−H_2O (70:30:1) were passed through an ion exchange IRN 77 (H^+) Amberlite resin column before purification by RP-18 column chromatography, eluting with MeOH−H_2O (5:5 to 8:2), to give **6** (5 mg), **8** (13 mg), and **29** (11 mg). Fractions 101−105, eluted with $CHCl_3$−MeOH−H_2O (70:30:1), were purified by RP-18 column chromatography, using a gradient of MeOH−H_2O (5:5 to 8:2), to give **23** (12 mg) and **28** (30 mg). Fractions eluted with MeOH−H_2O (5:5) were further purified by semipreparative HPLC with a linear gradient (40 to 55% B) for 15 min to afford 2 mg of **1** (t_R = 12.4 min), and fractions eluted with MeOH−H_2O (8:2) were purified by semipreparative HPLC with a linear gradient (30 to 43% B) for 30 min to afford 7 mg of **9** (t_R = 12.4 min). Fractions 119−124, eluted with $CHCl_3$−MeOH−H_2O (70:30:1), were purified by RP-18 column chromatography eluting with MeOH−H_2O (45:55 to 8:2), to give **12** (17 mg), **13** (58 mg), and **24** (21 mg), while fractions eluted with MeOH−H_2O (45:55) were purified by semipreparative HPLC with a linear gradient (35 to 41% B) for 25 min to give 4 mg of **10** (t_R = 10.5 min). Fractions 125−137, eluted with $CHCl_3$−MeOH−H_2O (70:30:2), were purified by RP-18 column chromatography using a gradient of MeOH−H_2O (35:65 to 8:2) to give **25** (11 mg). Fractions eluted with MeOH−H_2O (7:3) were further purified by semipreparative HPLC, with a linear gradient (30 to 43% B) for 30 min, to give 8 mg of **14** (t_R = 23.6 min) and 2 mg of **19** (t_R = 19.9 min), and fractions eluted with MeOH−H_2O (8:2) were further purified by silica gel column chromatography to give **11** (2 mg), **15** (18 mg), and **24** (6 mg). Fractions 152−155, eluted with $CHCl_3$−MeOH−H_2O (70:30:5), were purified by RP-18 column chromatography, eluting with MeOH−H_2O (3:7 to 8:2), to give **16** (3 mg) and **17** (18 mg). Fractions eluted with MeOH−H_2O (6:4) were then purified by semipreparative HPLC with a linear gradient of 30 to 38% B for 30 min to give 3 mg of **26** (t_R = 25.9 min). Fractions 156−170, eluted with $CHCl_3$−MeOH−H_2O (70:30:5), were purified by RP-18 column chromatography, using a gradient of MeOH−H_2O (3:7 to 8:2). The fraction eluted with MeOH−H_2O (3:7) was further purified by semipreparative HPLC with a linear gradient (30 to 35% B) for 20 min to give 4 mg of **18** (t_R = 12.9 min) and 7 mg of **21** (t_R = 13.9 min). Fractions eluted with MeOH−H_2O (4:6) were then purified by semipreparative HPLC with a linear gradient (40 to 50% B) for 20 min to give 4 mg of **20** (t_R = 15.5 min).

Acid Hydrolysis of Saponin Mixture. An aliquot of the crude saponin mixture (1 g) was refluxed with 60 mL of 2 N HCl for 4.5 h. The sapogenin mixture was extracted with EtOAc (3 × 30 mL), washed with H_2O, and evaporated to dryness. Oleanolic acid, hederagenin, and bayogenin were identified from the sapogenin residue with authentic samples by TLC in $CHCl_3$−MeOH (98:2). The acid aqueous layer was neutralized with 0.5 M NaOH and freeze-dried. Four sugars were identified and compared with authentic samples by TLC using MeCOEt−i-PrOH−Me_2CO−H_2O (20:10:7:6) as xylose, glucose, galactose, and glucuronic acid. After preparative TLC of the sugar mixture (100 mg) in this solvent, the optical rotation of each purified sugar was measured.

Caryocaroside V-1 (1): white powder; $[\alpha]^{20}_D$ +16.7 (*c* 0.17, MeOH); ^{1}H NMR of the aglycon (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR of the aglycon (CD_3OD, 125 MHz) δ 12.0 (C-24), 16.2 (C-25), 17.6 (C-26), 21.9 (C-6), 24.0 (C-30), 24.0 (C-16), 24.5 (C-11), 26.4 (C-27), 26.4 (C-2), 28.9 (C-15), 31.6 (C-20), 33.6 (C-29), 33.8 (C-7), 34.0 (C-22), 35.0 (C-21), 37.4 (C-10), 39.7 (C-1), 40.8 (C-8), 42.7 (C-18), 42.9 (C-14), 47.2 (C-19), 47.6 (C-17), 49.0 (C-9), 52.9 (C-5), 54.2 (C-4), 85.8 (C-3), 123.4 (C-12), 145.2 (C-13), 181.9 (C-23), 182.0 (C-28); ^{1}H and ^{13}C NMR of the glycosidic part, see Table 1; ESIMS (positive-ion mode) *m/z* 833 $[M + Na]^+$, 811 $[M + H]^+$, 487 $[M + Na - Glc - Gal]^+$.

Caryocaroside II-1 (2): white powder; $[\alpha]^{20}_D$ +31.9 (*c* 0.83, MeOH); ^{1}H NMR of the aglycon (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR of the aglycon (CD_3OD, 125 MHz) δ 13.4 (C-24), 16.4 (C-25), 17.7 (C-26), 18.8 (C-6), 24.0 (C-16), 24.0 (C-30), 24.5 (C-11), 26.2 (C-2), 26.5 (C-27), 28.7 (C-15), 31.5 (C-20), 33.3 (C-7), 33.7 (C-22), 33.9 (C-29), 34.9 (C-21), 37.6 (C-10), 39.3 (C-1), 40.4 (C-8), 42.6 (C-18), 42.9 (C-14), 43.7 (C-4), 47.2 (C-19), 47.6 (C-17), 48.2 (C-5), 48.8 (C-9), 65.1 (C-23), 83.7 (C-3), 123.4 (C-12), 145.2 (C-13), 182.2 (C-28); ^{1}H and ^{13}C NMR of the glycosidic part, see Table 1; ESIMS (negative-ion mode) *m/z* 795 $[M - H]^-$; ESIMS-MS MS^2 (795) *m/z* 633 $[M - H - Gal]^-$, 471 $[M - H - Gal - Glc]^-$; ESIMS (positive-ion mode) *m/z* 835 $[M + K]^+$, 819 $[M + Na]^+$; ESIMS-MS MS^2 (819) *m/z* 775 $[M + Na - CO_2]^+$, 657 $[M + Na - Gal]^+$, 613 $[M + Na - CO_2 - Gal]^+$.

Caryocaroside III-1 (3): white powder; $[\alpha]^{20}_D$ +24.9 (*c* 1, MeOH); ^{1}H NMR of the aglycon (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR of the aglycon (CD_3OD, 125 MHz) δ 14.7 (C-24), 17.5 (C-25), 18.3 (C-26), 18.7 (C-6), 24.4 (C-30), 24.5 (C-16), 24.7 (C-11), 26.6 (C-27), 29.2 (C-15), 31.8 (C-20), 33.6 (C-7), 34.0 (C-29), 34.4 (C-22), 35.5 (C-21), 37.6 (C-10), 40.6 (C-8), 43.0 (C-4), 43.3 (C-14), 43.5 (C-18), 44.4 (C-1), 47.5 (C-17), 48.1 (C-19), 48.2 (C-5), 49.4 (C-9), 65.7 (C-23), 71.2 (C-2), 84.0 (C-3), 122.5 (C-12), 146.8 (C-13), 185.9 (C-28); ^{1}H and ^{13}C NMR of the glycosidic part, see Table 1; ESIMS (negative-ion mode) *m/z* 811 $[M - H]^-$; ESIMS-MS MS^2 (811) *m/z* 649 $[M - H - Gal]^-$, 487 $[M - H - Gal - Glc]^-$; ESIMS (positive-ion mode) *m/z* 835 $[M + Na]^+$; ESIMS-MS MS^2 (835) *m/z* 791 $[M + Na - CO_2]^+$, 673 $[M + Na - Gal]^+$, 629 $[M + Na - CO_2 - Gal]^+$.

Caryocaroside II-2 (4): white powder; $[\alpha]^{20}_D$ +20.7 (*c* 0.75, MeOH); ^{1}H NMR of the aglycon (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR chemical shift values of the aglycon (CD_3OD, 125 MHz) were identical to those described for **2** (±0.6 ppm) except for C-28 at δ 178.0; ^{1}H and ^{13}C NMR of the glycosidic part, see Table 1; ESIMS (negative-ion mode) *m/z* 957 $[M - H]^-$; ESIMS-MS MS^2 (957) *m/z* 795 $[M - H - Gal]^- = [M - H - Glc]^-$, MS^3 (795) *m/z* 634 $[M - H - Glc - Gal]^-$, 471 $[M - H - 2\ Glc - Gal]^-$; ESIMS (positive-ion mode) *m/z* 981 $[M + Na]^+$, 997 $[M + K]^+$; ESIMS-MS MS^2 (981) *m/z* 819 $[M + Na - (Glc\ or\ Gal)]^+$, MS^3 (819) *m/z* 775 $[M + Na - Glc - CO_2]^+$, 613 $[M + Na - Glc - CO_2 - Gal]^+$.

Caryocaroside III-2 (5): white powder; $[\alpha]^{20}_D$ +28.8 (*c* 1, MeOH); ^{1}H NMR of the aglycon (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR chemical shift values of the aglycon (CD_3OD, 125 MHz) were identical to those described for **3** (±0.6 ppm) except for C-28 at δ 178.0; ^{1}H and ^{13}C NMR of the glycosidic part, see Table 2; ESIMS (negative-ion mode) *m/z* 973 $[M - H]^-$; ESIMS-MS MS^2 (973) *m/z* 811 $[M - H - Gal]^- = [M - H - Glc]^-$; ESIMS (positive-ion mode) *m/z* 1013 $[M + K]^+$, 997 $[M + Na]^+$; ESIMS-MS MS^2 (997) *m/z* 835 $[M + Na - (Gal\ or\ Glc)]^+$, MS^3 (835) *m/z* 791 $[M + Na - CO_2 - (Gal\ or\ Glc)]^+$, 673 $[M + Na - Gal - Glc)]^+$, 629 $[M + Na - Gal - CO_2 - Glc]^+$.

Caryocaroside II-3 (6): white powder; $[\alpha]^{20}_D$ +15.6 (*c* 0.42, MeOH); ^{1}H NMR of the aglycon (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR chemical shift values of the aglycon (CD_3OD, 125 MHz) were identical to those described for **2** (±0.6 ppm); ^{1}H and ^{13}C NMR of the glycosidic part, see Table 1; ESIMS (negative-ion mode) *m/z* 957 $[M - H]^-$; ESIMS-MS MS^2 (957) *m/z* 795 $[M - H - Gal]^+$, 633 $[M - H - Gal - Gal]^-$, 471 $[M - H - 2\ Gal - Glc]^-$.

Caryocaroside III-3 (7): white powder; $[\alpha]^{20}_D$ +15 (*c* 0.42, MeOH); ^{1}H NMR of the aglycon (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR chemical shift values of the aglycon (CD_3OD, 125 MHz) were identical to those described for **3** (±0.4 ppm); ^{1}H and ^{13}C NMR of the glycosidic part, see Table 1; ESIMS (negative-ion mode) *m/z* 973 $[M - H]^-$; ESIMS-MS MS^2 (973) *m/z* 811 $[M - H - Gal]^-$, 649 $[M - H - 2\ Gal]^-$, 487 $[M - H - 2\ Gal - Glc]^-$.

Caryocaroside III-4 (8): white powder; $[\alpha]^{20}_D$ +17.5 (*c* 0.42, MeOH); ^{1}H NMR of the aglycon (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR chemical shift values of the aglycon (CD_3OD, 125 MHz) were identical to those described for **3** (±0.4 ppm); ^{1}H and ^{13}C NMR of the glycosidic part, see Table 1; ESIMS (negative-ion mode) *m/z* 943 $[M - H]^-$; ESIMS-MS MS^2 (943) *m/z* 811 $[M - H - Xyl]^-$, 649 $[M - H - Xyl - Gal]^-$, 487 $[M - H - Xyl - Gal - Glc]^-$; ESIMS (positive-ion mode) *m/z* 967 $[M + Na]^+$; ESIMS-MS MS^2 (967) *m/z* 923 $[M + Na - CO_2]^+$, 835 $[M + Na - Xyl]^+$, 673 $[M + Na - Xyl - Gal]^+$, MS^3 (923) *m/z* 791 $[M + Na - CO_2 - Xyl]^+$, 629 $[M + Na - CO_2 - Xyl - Gal]^+$.

Caryocaroside IV-5 (9): white powder; $[\alpha]^{20}_D$ +26.2 (*c* 0.58, MeOH); ^{1}H NMR of the aglycon (CD_3OD, 500 MHz), see Table 2; ^{13}C NMR of the aglycon (CD_3OD, 125 MHz) δ 18.5 (C-24), 16.8 (C-25), 17.8 (C-26), 19.1 (C-6), 24.0 (C-30), 24.0 (C-16), 24.5 (C-11), 26.4 (C-27), 28.7 (C-15), 29.9 (C-23), 31.6 (C-20), 33.4 (C-29), 33.8

(C-22), 34.0 (C-7), 34.9 (C-21), 37.7 (C-10), 39.4 (C-4), 40.6 (C-8), 42.7 (C-18), 43.0 (C-14), 44.5 (C-1), 47.2 (C-19), 47.6 (C-17), 49.3 (C-9), 57.0 (C-5), 71.2 (C-2), 91.1 (C-3), 123.9 (C-12), 145.2 (C-13), 181.9 (C-28); ^{1}H and ^{13}C NMR of the glycosidic part, see Table 2; ESIMS (negative-ion mode) *m/z* 647 $[M - H]^-$; ESIMS-MS MS^2 (647) *m/z* 471 $[M - H - GlcA]^-$; ESIMS (positive-ion mode) *m/z* 671 $[M + Na]^+$, 687 $[M + K]^+$; ESIMS-MS MS^2 (671) *m/z* 495 $[M + Na - GlcA]^+$, 451 $[M + Na - GlcA - CO_2]^+$.

Caryocaroside III-5 (10): white powder; $[\alpha]^{20}_D$ +16.8 (*c* 0.25, MeOH); ^{1}H NMR of the aglycon (CD_3OD, 500 MHz), see Table 2; ^{13}C NMR chemical shift values of the aglycon (CD_3OD, 125 MHz) were identical to those described for **5** (±0.2 ppm); ^{1}H and ^{13}C NMR of the glycosidic part, see Table 2; ESIMS (negative-ion mode) *m/z* 663 $[M - H]^-$; ESIMS-MS MS^2 (663) *m/z* 487 $[M - H - GlcA]^-$; ESIMS (positive-ion mode) *m/z* 709 $[M - H + 2 Na]^+$, 687 $[M + Na]^+$; ESIMS-MS MS^2 (709) *m/z* 691 $[M - H + 2 Na - H_2O]^+$, 533 $[M - H + 2 Na - GlcA]^+$.

Caryocaroside IV-6 (11): white powder; $[\alpha]^{20}_D$ +12 (*c* 0.17, MeOH); ^{1}H NMR of the aglycon (CD_3OD, 500 MHz), see Table 2; ^{13}C NMR chemical shift values of the aglycon (CD_3OD, 125 MHz) were identical to those described for **9** (±0.4 ppm); ^{1}H and ^{13}C NMR of the glycosidic part, see Table 2; ESIMS (negative-ion mode) *m/z* 661 $[M - H]^-$; ESIMS-MS MS^2 (661) *m/z* 647 $[M - H - CH_3]^-$, 471 $[M - H - (CH_3GlcA)]^-$; ESIMS (positive-ion mode) *m/z* 701 $[M + K]^+$, 685 $[M + Na]^+$; ESIMS-MS MS^2 (685) *m/z* 495 $[M + Na - Me - GlcA]^+$.

Caryocaroside IV-7 (12): white powder; $[\alpha]^{20}_D$ +8.4 (*c* 0.17, MeOH); ^{1}H NMR of the aglycon (DMSO-d_6, 500 MHz), see Table 2; ^{13}C NMR of the aglycon (DMSO-d_6, 125 MHz) δ 17.2 (C-25), 18.7 (C-26), 19.5 (C-24), 19.2 (C-6), 24.9 (C-30), 23.6 (C-16), 24.5 (C-11), 27.0 (C-27), 28.8 (C-15), 30.6 (C-23), 31.9 (C-20), 34.4 (C-29), 34.0 (C-22), 34.0 (C-7), 35.2 (C-21), 37.7 (C-10), 39.3 (C-4), 40.3 (C-8), 42.6 (C-18), 43.0 (C-14), 44.8 (C-1), 47.7 (C-19), 47.7 (C-17), 49.0 (C-9), 52.0 (C-5), 69.6 (C-2), 90.0 (C-3), 123.0 (C-12), 146.0 (C-13), 179.7 (C-28)**;** ^{1}H and ^{13}C NMR of the glycosidic part, see Table 2; ESIMS (negative-ion mode) *m/z* 809 $[M - H]^-$; ESIMS-MS MS^2 (809) *m/z* 647 $[M - H - Gal]^-$, 471 $[M - H - Gal - GlcA]^-$; ESIMS (positive-ion mode) *m/z* 855 $[M - H + 2Na]^+$; ESIMS-MS MS^2 (855) *m/z* 693 $[M - H + 2 Na - Gal]^+$, 517 $[M - H + 2Na - Gal - GlcA]^+$, MS^3 (693) *m/z* 675 $[M - H + 2 Na - Gal - H_2O]^+$, 517 $[M - H + 2 Na - Gal - GlcA]^+$.

Caryocaroside II-7 (13): white powder; $[\alpha]^{20}_D$ +11.4 (*c* 0.44, MeOH); ^{1}H NMR of the aglycon (DMSO-d_6, 500 MHz), see Table 2; ^{13}C NMR of the aglycon (DMSO-d_6, 125 MHz) δ 14.3 (C-24), 17.0 (C-25), 18.4 (C-26), 18.7 (C-6), 24.1 (C-16), 24.7 (C-30), 24.4 (C-11), 26.4 (C-2), 27.0 (C-27), 28.7 (C-15), 31.8 (C-20), 33.6 (C-7), 33.5 (C-22), 34.2 (C-29), 34.8 (C-21), 37.5 (C-10), 39.5 (C-1), 40.4 (C-8), 42.4 (C-18), 42.8 (C-14), 43.8 (C-4), 47.0 (C-17), 47.2 (C-19), 47.5 (C-5), 48.6 (C-9), 63.9 (C-23), 79.6 (C-3), 123.0 (C-12), 145.4 (C-13), 182.0 (C-28);^{1}H and ^{13}C NMR of the glycosidic part, see Table 2; ESIMS (negative-ion mode) *m/z* 809 $[M - H]^-$; ESIMS-MS MS^2 (809) *m/z* 471 $[M - H - Gal - GlcA]^-$; ESIMS (positive-ion mode) *m/z* 847 $[M + K]^+$.

Caryocaroside III-7 (14): white powder; $[\alpha]^{20}_D$ +26.2 (*c* 0.67, MeOH); ^{1}H NMR of the aglycon (CD_3OD, 500 MHz), see Table 2; ^{13}C NMR chemical shift values of the aglycon (CD_3OD, 125 MHz) were identical to those described for **3** (±0.6 ppm);^{1}H and ^{13}C NMR of the glycosidic part, see Table 2; ESIMS (negative-ion mode) *m/z* 825 $[M - H]^-$; ESIMS-MS MS^2 (825) *m/z* 487 $[M - H - Gal - GlcA]^-$; ESIMS (positive-ion mode) *m/z* 871 $[M - H + 2 Na]^+$, 849 $[M + Na]^+$; ESIMS-MS MS^2 (871) *m/z* 709 $[M - H + 2 Na - Gal]^+$, 533 $[M - H + 2 Na - Gal - GlcA]^+$, MS^3 (709) *m/z* 691 $[M - H + 2 Na - Gal - H_2O]^+$, 533 $[M - H + 2 Na - Gal - GlcA]^+$.

Caryocaroside IV-8 (15): white powder; $[\alpha]^{20}_D$ +30 (*c* 1, MeOH); ^{1}H NMR of the aglycon (CD_3OD, 500 MHz), see Table 2; ^{13}C NMR chemical shift values of the aglycon (CD_3OD, 125 MHz) were identical to those described for **9** (±0.2 ppm). ^{1}H and ^{13}C NMR of the glycosidic part, see Table 2; ESIMS (negative-ion mode) *m/z* 823 $[M - H]^-$; ESIMS-MS MS^2 (8423) *m/z* 791 $[M - H - CH_3OH]^-$, 601 $[M - H - Gal - (COOCH_3)]^-$, 471 $[M - H - Gal - CH_3GlcA]^-$; ESIMS (positive-ion mode) *m/z* 863 $[M + K]^+$, 847 $[M + Na]^+$; ESIMS-MS MS^2 (847) *m/z* 685 $[M + Na - Gal]^+$, 641 $[M + Na - Gal - CO_2]^+$.

Caryocaroside IV-9 (16): white powder; $[\alpha]^{20}_D$ +12 (*c* 0.5, MeOH); ^{1}H NMR of the aglycon (CD_3OD, 500 MHz), see Table 3; ^{13}C NMR chemical shift values of the aglycon (CD_3OD, 125 MHz) were identical to those described for **9** (±0.6 ppm) except for C-28 at δ 178.0; ^{1}H and ^{13}C NMR of the glycosidic part, see Table 3; ESIMS (negative-ion mode) *m/z* 971 $[M - H]^-$; ESIMS-MS MS^2 (971) *m/z* 809 $[M - H - Gal]^- = [M - H - Glc]^-$, MS^3 (809) *m/z* 647 $[M - H - Gal - Glc]^-$, 471 $[M - H - Gal - Glc - GlcA]^-$; ESIMS (positive-ion mode) *m/z* 1017 $[M - H + 2Na]^+$; ESIMS-MS MS^2 (1017) *m/z* 855 $[M - H + 2 Na - (Glc or Gal)]^+$, MS^3 (855) *m/z* 693 $[M - H + 2 Na - Glc - Gal]^+$, 517 [aglycon $- H + 2 Na]^+$.

Caryocaroside II-9 (17): white powder; $[\alpha]^{20}_D$ +11.9 (*c* 1, MeOH); ^{1}H NMR of the aglycon (CD_3OD, 500 MHz), see Table 3; ^{13}C NMR chemical shift values of the aglycon (CD_3OD, 125 MHz) were identical to those described for **4** (±0.6 ppm); ^{1}H and ^{13}C NMR of the glycosidic part, see Table 3; ESIMS (negative-ion mode) *m/z* 971 $[M - H]^-$; ESIMS-MS MS^2 (971) *m/z* 809 $[M - H - Gal]^- = [M - H - Glc]^-$, MS^3 (809) *m/z* 647 $[M - H - Gal - Glc]^-$, 471 $[M - H - Gal - Glc - GlcA]^-$; ESIMS (positive-ion mode) *m/z* 1009 $[M - H + K]^+$; ESIMS-MS MS^2 (1009) *m/z* 847 $[M - H + K - (Glc or Gal)]^+$, 685 $[M - H + K - Glc - Gal]^+$, MS^3 (847) *m/z* 803 $[M - H + K - (Glc or Gal) - CO_2]^+$, 685 $[M - H + K - Glc - Gal]^+$, 641 $[M - H + K - Glc - Gal - CO_2]^+$.

Caryocaroside III-9 (18): white powder; $[\alpha]^{20}_D$ +15.6 (*c* 1, MeOH); ^{1}H NMR of the aglycon (CD_3OD, 500 MHz), see Table 3; ^{13}C NMR chemical shift values of the aglycon (CD_3OD, 125 MHz) were identical to those described for **5** (±0.4 ppm); ^{1}H and ^{13}C NMR of the glycosidic part, see Table 3; ESIMS (negative-ion mode) *m/z* 987 $[M - H]^-$; ESIMS-MS MS^2 (987) *m/z* 825 $[M - H - Gal]^- = [M - H - Glc]^-$, MS^3 (825) *m/z* 663 $[M - H - Gal - Glc]^-$, 487 $[M - H - Gal - Gal - GlcA]^-$; ESIMS (positive-ion mode) *m/z* 1027 $[M + K]^+$, 1011 $[M + Na]^+$; ESIMS-MS MS^2 (1027) *m/z* 863 $[M + K - (Glc or Gal)]^+$, MS^3 (863) *m/z* 819 $[M + K - (Glc or Gal) - CO_2]^+$, 701 $[M + K - Glc - Gal]^+$, 657 $[M + K - Glc - Gal - CO_2]^+$.

Caryocaroside II-10 (19): white powder; $[\alpha]^{20}_D$ +10.8 (*c* 0.17, MeOH); ^{1}H NMR of the aglycon (CD_3OD, 500 MHz), see Table 3; ^{13}C NMR chemical shift values of the aglycon (CD_3OD, 125 MHz) were identical to those described for **5** (±0.4 ppm); ^{1}H and ^{13}C NMR of the glycosidic part, see Table 3; ESIMS (positive-ion mode) *m/z* 1009 $[M + Na]^+$, 847 $[M + Na - (Glc or Gal)]^+$; ESIMS-MS MS^2 (1009) *m/z* 979 $[M + Na - CH_3OH]^+$, 847 $[M + Na - (Glc or Gal)]^+$, 685 $[M + Na - Glc - Gal]^+$, MS^3 (847) *m/z* 685 $[M + Na - Glc - Gal]^+$, 641 $[M + Na - Glc - Gal - CO_2]^+$.

Caryocaroside IV-11 (20): white powder; $[\alpha]^{20}_D$ +12 (*c* 0.29, MeOH); ^{1}H NMR of the aglycon (CD_3OD, 500 MHz), see Table 3; ^{13}C NMR chemical shift values of the aglycon (CD_3OD, 125 MHz) were identical to those described for **9** (±0.4 ppm); ^{1}H and ^{13}C NMR of the glycosidic part, see Table 3; ESIMS (negative-ion mode) *m/z* 971 $[M - H]^-$; ESIMS-MS MS^2 (971) *m/z* 809 $[M - H - Gal]^-$; 647 $[M - H - Gal - Gal]^-$, 471 $[M - H - 2 Gal - GlcA]^-$.

Caryocaroside II-11 (21): white powder; $[\alpha]^{20}_D$ +11.5 (*c* 0.42, MeOH); ^{1}H NMR of the aglycon (CD_3OD, 500 MHz), see Table 3; ^{13}C NMR chemical shift values of the aglycon (CD_3OD, 125 MHz) were identical to those described for **2** (±0.4 ppm); ^{1}H and ^{13}C NMR of the glycosidic part, see Table 3; ESIMS (negative-ion mode) *m/z* 971 $[M - H]^-$; ESIMS-MS MS^2 (971) *m/z* 809 $[M - H - Gal]^-$, 647 $[M - H - 2 Gal]^-$, 471 $[M - H - 2 Gal - GlcA]^-$.

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