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Triterpenoid Saponins from the Fruits of *Caryocar villosum*

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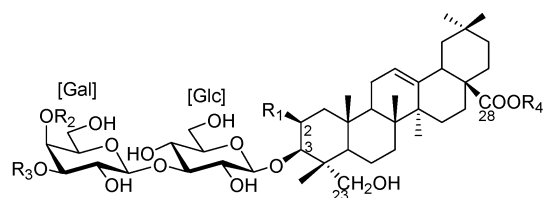
Fourteen new triterpenoid saponins (**1–14**) were isolated from the methanol extract of the fruits of *Caryocar villosum* along with 10 known saponins. Their structures were established on the basis of extensive NMR (¹H, ¹³C, COSY, TOCSY, ROESY, HSQC, and HMBC) and ESIMS studies. The toxicity of the methanolic extracts of the peel and the pulp of fruits and the crude saponin fraction of the peel was assessed using the *Artemia salina* test. The antimicrobial activities of caryocarosides IV-21 (**14**), II-1 (**16**), III-1 (**17**), and IV-9 (**20**) and of saponin **23** were also studied in vitro on *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Mycobacterium smegmatis*, and *Enterococcus faecalis* bacteria.

Caryocaraceae is a small family, distributed in neotropical and tropical America.¹ The fruits of some plants of the *Caryocar* genus furnish an edible oil that is used for cooking as a substitute for butter, for homemade soaps, and in the cosmetic industry.^{2–4} Our laboratory has recently been interested in the phytochemical investigation of some *Caryocar* species, and we have reported in a previous study the isolation and structural determination of 21 triterpenoid saponins, named caryocarosides, from the fruits of *Caryocar glabrum*.⁵ In a continuation of this study, we have examined the chemical composition of the fruits of *Caryocar villosum* (Aubl.) Pers., a very large tree (up to 40–50 m high) that grows in the forests of the Amazon and mainly in the region near the Amazon estuary.^{2,3}

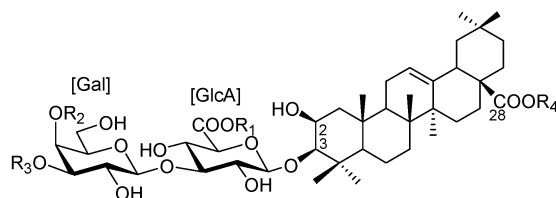
The fruits of *C. villosum* are irregularly globular, approximately 7–9 cm in diameter. The seeds are edible, fresh or cooked, and the pulp is edible after cooking. The local industry produces an edible oil from the fruit that is used as a salad oil or for cooking purposes.^{2,3} Traditionally, the pulp and the peel were used by the local inhabitants of French Guyana, as a fish poison, whereas, in the rural household of Northeast Brazil, the oil of piquià fruit is used for the treatment of dermatophytoses such as ringworm (*Tinea capitis*) and athletes foot (fungal skin diseases).^{3,4} The pulp has a relatively low water content, approximately 50%, and high raw fat content, approximately 65%, in dry weight. The major components of the oil are palmitic and oleic acid, each amounting to almost 50% of the total fatty acid.² In the fruit, the sugar and organic acid amounts were found to be very low, while the selenium content was noticeably high.²

In this report we describe the isolation and structure elucidation of 24 saponins, among which, compounds **1–14** were found to be new and are named here caryocarosides in a continuation of our previous study.⁵ The structures of these saponins were determined by using 1D and 2D NMR analyses, ESIMS, and acid hydrolysis. The toxicity of the methanolic extracts from the peel and from the pulp of the fruits and of the crude saponin fraction of the peel was evaluated using the *Artemia salina* test. The specific antimicrobial activity of caryocarosides IV-21 (**14**), II-1 (**16**), III-1 (**17**), and IV-9

(**20**) and **23** was also studied using the agar diffusion method, against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Mycobacterium smegmatis*, and *Enterococcus faecalis* bacteria.



	R ₁	R ₂	R ₃	R ₄
1	H	H	Xyl-	Glc'-
2	OH	H	Xyl-	Glc'-
3	H	H	Gal'-	Glc'-
4	OH	H	Gal'-	Glc'-
5	OH	H	Xyl-(1→3)-Gal'-	Glc'-
6	H	Xyl-(1→3)-Gal''-(1→3)-Gal'-	H	Glc'-
7	OH	Xyl-(1→3)-Gal''-(1→3)-Gal'-	H	Glc'-
8	OH	Xyl-(1→3)-Gal''-(1→3)-Gal'-	H	H
16	H	H	H	H
17	OH	H	H	H



	R ₁	R ₂	R ₃	R ₄
9	-CH ₃	H	H	Glc-
10	H	H	Xyl-	Glc-
11	-CH ₃	H	Xyl-	Glc-
12	H	Xyl-(1→3)-Gal'-	H	H
13	H	Xyl-(1→3)-Gal''-(1→3)-Gal'-	H	H
14	H	Xyl-(1→3)-Gal''-(1→3)-Gal'-	H	Glc-
20	H	H	H	Glc-

Results and Discussion

The dried peel and pulp of fruits were extracted separately with MeOH, and the MeOH extract was evaporated to dryness. Analytical HPLC of the MeOH extracts revealed that the chromatographic profiles of the peel and the pulp were qualitatively identical but quantitatively different, with higher amounts of caryocarosides IV-

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Table 1. ^1H and ^{13}C NMR Data of the Sugar Moieties of Compounds **1–4** (CD_3OD)

	1		2		3		4	
	δ_{H} (m, J Hz)	δ_{C}	δ_{H} (m, J Hz)	δ_{C}	δ_{H} (m, J Hz)	δ_{C}	δ_{H} (m, J Hz)	δ_{C}
<i>β-D-glucose (at C-3)</i>								
1	4.48 (d, 7.9)	105.3	4.51 (d, 7.9)	105.2	4.48 (d, 7.9)	105.3	4.51 (d, 7.9)	105.2
2	3.40 (dd, 9.1, 8.1)	74.9	3.50 (dd, 9, 7.9)	74.7	3.40 (dd, 9.1, 7.8)	74.9	3.49 (t, 9.1)	74.8
3	3.58 (t, 9.1)	87.9	3.61 (t, 8.9)	87.7	3.58 (t, 9.1)	87.9	3.61 (t, 8.7)	87.7
4	3.44 (t, 9.6)	69.8	3.52 (t, 8.8)	69.5	3.45 (t, 9.5)	69.8	3.52 (t, 8.4)	69.5
5	3.33 (m)	77.3	3.34 (m)	77.3	3.33 (m)	77.3	3.34 (m)	77.3
6a	3.71 (dd, 11.8, 4.8)	62.6	3.74 (dd, 12, 4.6)	62.2	3.72 (dd, 11.4, 4.5)	62.6	3.74 (dd, 11.4, 5.1)	62.4
6b	3.83 (dd, 11.8, 2.2)		3.83 (dd, 12, 2.7)		3.86 (dd, 11.4, 2.5)		3.83 (brd, 11.4)	
<i>β-D-galactose (at C-3 Glc)</i>								
1	4.60 (d, 7.8)	105.2	4.62 (d, 7.8)	105.2	4.60 (d, 7.8)	105.2	4.63 (d, 7.8)	105.2
2	3.80 (dd, 9.6, 8)	72.2	3.81 (dd, 9.7, 7.6)	72.2	3.80 (dd, 10.1, 7.8)	72.2	3.81 (dd, 9.7, 7.7)	72.2
3	3.66 (dd, 9.6, 3.3)	84.0	3.67 (dd, 9.6, 3.4)	84.1	3.68 (dd, 9.8, 3.3)	84.5	3.69 (dd, 9.7, 3.6)	84.5
4	4.03 (d, 3.3)	69.8	4.04 (d, 3.4)	69.8	4.13 (d, 3.3)	69.8	4.14 (d, 3)	69.8
5	3.61 (dd, 7.8, 5.3)	76.7	3.61 (ddm, 7.6, 5.1)	76.7	3.63 (m)	76.7	3.63 (m)	76.7
6a	3.70 (dd, 11.3, 5.3)	62.5	3.70 (dd, 11.9, 4.7)	62.5	3.75 (dd, 11.6, 7)	62.6	3.74 (dd, 11.5, 5)	62.6
6b	3.80 (dd, 11.5, 7.5)		3.83 (dd, 11.9, 7.6)		3.81 (dd, 11.4, 7.7)		3.81 (dd, 11.5, 7.8)	
<i>β-D-xylose (at C-3 Gal)</i>								
1	4.52 (d, 7.6)	106.3	4.54 (d, 7.1)	106.3				
2	3.32 (m)	75.2	3.32 (m)	75.2				
3	3.36 (t, 8.3)	77.5	3.36 (t, 8.4)	77.5				
4	3.53 (ddd, 10.2, 8.3, 5.4)	71.0	3.52 (ddd, 10.3, 8.4, 5.4)	71.0				
5a	3.24 (dd, 11.4, 10.3)	66.9	3.24 (dd, 11.4, 10.3)	66.9				
5b	3.88 (dd, 11.4, 5.4)		3.89 (dd, 11.4, 5.4)					
<i>β-D-galactose' (at C-3 Gal)</i>								
1					4.52 (d, 7.6)	106.3	4.53 (d, 7.5)	106.3
2					3.64 (dd, 9.6, 7.7)	72.9	3.64 (m)	73.0
3					3.52 (dd, 9.6, 3.4)	74.6	3.52 (dd, 9.5, 3.5)	74.6
4					3.85 (d, 3.4)	70.2	3.84 (d, 3.7)	70.2
5					3.56 (dd, 7.1, 5.1)	76.8	3.56 (dd, 7.8, 5)	76.8
6a					3.71 (m)	62.6	3.73 (dd, 11.3, 5)	62.6
6b					3.79 (m)		3.81 (dd, 11.3, 7.8)	
<i>β-D-glucose' (at C-28)</i>								
1	5.40 (d, 8.1)	95.7	5.41 (d, 8.1)	95.7	5.40 (d, 8.1)	95.7	5.40 (d, 8.1)	95.7
2	3.34 (t, 8.4)	73.9	3.34 (m)	73.9	3.34 (t, 8.3)	73.9	3.34 (m)	73.9
3	3.43 (t, 9)	78.3	3.43 (t, 9)	78.3	3.43 (t, 9.2)	78.3	3.43 (t, 9.1)	78.3
4	3.38 (t, 9.3)	71.1	3.38 (t, 9)	71.1	3.38 (t, 9.4)	71.1	3.37 (t, 9.6)	71.1
5	3.37 (m)	78.7	3.37 (m)	78.7	3.37 (m)	78.7	3.36 (m)	78.7
6a	3.70 (dd, 12, 4.7)	62.6	3.70 (dd, 11.9, 4.5)	62.4	3.70 (dd, 12, 3.3)	62.4	3.70 (dd, 11.8, 5)	62.6
6b	3.83 (m)		3.84 (dd, 12.2, 1.9)		3.85 (dd, 11.8, 4.7)		3.84 (brd, 11.8)	

10 (**9**), IV-17 (**10**), II-2 (**18**), and IV-9 (**20**) and compound **23** in the pulp. The methanolic extract of the peel of fruits of *Caryocar villosum* was subjected to VLC over reversed-phase C_{18} to afford a crude saponin-rich fraction. This fraction was then purified by a series of column chromatography over silica gel and reversed-phase C_{18} and finally by semipreparative HPLC over C_{18} or by preparative TLC to give 24 saponins.

The sugar components were determined, by TLC after acid hydrolysis of the crude saponin mixture, to be D-xylose, D-glucose, D-galactose, and D-glucuronic acid, after verification of their optical rotations, and the structures of the aglycons were identified by TLC by comparison with authentic samples. Structural confirmation of the aglycons and of the sugar moieties in the intact saponins was achieved by analysis of 1D and 2D NMR experiments.

The 10 known saponins were identified by detailed NMR analysis and comparison of the data with reference samples as 3-*O*- β -D-glucopyranosyl hederagenin (**15**),^{5–7} 3-*O*- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-glucopyranosyl hederagenin (caryocaroside II-1) (**16**),⁵ 3-*O*- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-glucopyranosyl bayogenin (caryocaroside III-1) (**17**),⁵ 3-*O*- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-glucopyranosyl hederagenin-28-*O*- β -D-glucopyranosyl ester (caryocaroside II-2) (**18**),⁵ 3-*O*- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-glucopyranosyl bayogenin-28-*O*- β -D-glucopyranosyl ester (caryocaroside III-2) (**19**),⁵ 3-*O*- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-glucuronopyranosyl-2 β -hydroxyoleanolic acid-28-*O*- β -D-glucopyranosyl ester (caryocaroside IV-9) (**20**),⁵ 3-*O*- β -D-glucopyranosyl hederagenin-28-*O*- β -D-glucopyranosyl ester (**21**),⁸ 3-*O*- β -D-glucopyranosyl bayogenin-28-*O*- β -D-glucopyranosyl ester (**22**),⁹ 3-*O*- β -D-glucopyranosyl-(1 \rightarrow 2)- β -D-galactopyranosyl hederagenin-28-*O*- β -D-glucopyranosyl

ester (**23**),¹⁰ and 3-*O*- β -D-glucopyranosyl-(1 \rightarrow 2)- β -D-galactopyranosyl oleanolic acid-28-*O*- β -D-glucopyranosyl ester (**24**).¹¹

In the 14 new caryocarosides, the aglycons were identified as hederagenin (II) [$\delta_{\text{H}-23}$ 3.30, 3.66 ± 0.01 , $\delta_{\text{C}-23}$ 65.0 ± 0.3] in compounds **1**, **3**, and **6**, as bayogenin (III) [$\delta_{\text{H}-2}$ 4.35, $\delta_{\text{C}-2}$ 71.1 ± 0.3 and $\delta_{\text{H}-23}$ 3.24, 3.64 ± 0.01 , $\delta_{\text{C}-23}$ 65.7 ± 0.3] in compounds **2**, **4**, **5**, **7**, and **8**, and as 2 β -hydroxyoleanolic acid (IV) [$\delta_{\text{H}-2}$ 4.22 ± 0.05 , $\delta_{\text{C}-2}$ 71.3 ± 0.3] in compounds **9–14**. These data and other spectroscopic data were in agreement with those reported in the literature.^{12–15}

Caryocarosides II-12 (**1**) and III-12 (**2**) respectively displayed molecular ion peaks [$\text{M} + \text{Na}$]⁺ at m/z 1113.5466 and 1129.5419 in the positive HRESIMS, in accordance with empirical molecular formulas of $\text{C}_{53}\text{H}_{86}\text{O}_{23}\text{Na}$ and $\text{C}_{53}\text{H}_{86}\text{O}_{24}\text{Na}$, respectively. The ESIMS² experiment of the [$\text{M} + \text{Na}$]⁺ ion peaks gave positive fragments at m/z 951, 819, 657, and 495 for **1** and at m/z 967, 835, 673, and 511 for **2**, attributed to successive losses of a hexose, a pentose, and two hexose moieties. The ^1H NMR spectrum of **1** displayed four anomeric proton signals at δ_{H} 4.48, 4.52, 4.60, and 5.40 correlated in the HSQC spectrum with four anomeric carbons at δ_{C} 105.3, 106.3, 105.2, and 95.7 in the ^{13}C NMR spectrum, respectively (Table 1). Complete assignment of each glycosidic proton system was achieved by analysis of COSY, TOCSY, ROESY, and HSQC experiments. Two β -D-glucosyl units were assigned starting from the anomeric protons at δ_{H} 4.48 (d, $J = 7.9$) and 5.40 (d, $J = 8.1$); the first (Glc) is substituted at position 3 ($\delta_{\text{C}-3}$ 87.9), and the second is in a terminal position (Glc'). A β -D-galactose unit, characterized by its equatorial proton H-4 (d, $J_{3,4} = 3.3$ Hz), was identified starting from its anomeric proton

signal at δ_{H} 4.60 (d, $J = 7.8$). The last unit possessing an anomeric proton signal at δ_{H} 4.52 (d, $J = 7.6$) was identified as a terminal β -D-xylose (Table 1). The chemical shifts of the signals due to C-3 ($\delta_{\text{C}-3}$ 83.5) and C-28 ($\delta_{\text{C}-28}$ 178.1) of the aglycon, in the ^{13}C NMR spectra, indicated that **1** was a bidessmoside of hederagenin.⁶ In the HMBC spectrum, long-range couplings ($^3J_{\text{H,C}}$) were observed between the proton signals at δ_{H} 4.48 (Glc-H-1) and 5.40 (Glc'-H-1) and C-3 and C-28 of hederagenin, respectively, whereas the proton signals at δ_{H} 4.52 (Xyl-H-1) and 4.60 (Gal-H-1) were correlated with carbon signals at δ_{C} 84.0 (Gal-C-3) and 87.9 (Glc-C-3), respectively. Thus, caryocaroside II-12 (**1**) was concluded to be 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 3)- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-glucopyranosyl hederagenin-28-*O*- β -D-glucopyranosyl ester. Comparison of the ^1H and ^{13}C NMR values of the saccharidic portion of **1** and **2** and the analysis of the HMBC correlations showed that **2** contained the same sugar moieties linked to C-3 of bayogenin instead of hederagenin (Table 1), thus confirming the structure of caryocaroside III-12 (**2**) to be 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 3)- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-glucopyranosyl bayogenin-28-*O*- β -D-glucopyranosyl ester.

Caryocarosides II-13 (**3**) and III-13 (**4**) displayed molecular ion peaks $[\text{M} + \text{Na}]^+$ at m/z 1143.5553 and 1159.5505 in the positive HRESIMS mode, in agreement with empirical molecular formulas of $\text{C}_{54}\text{H}_{88}\text{O}_{24}\text{Na}$ (**3**) and $\text{C}_{54}\text{H}_{88}\text{O}_{25}\text{Na}$ (**4**). The ESIMS² experiment of the $[\text{M} + \text{Na}]^+$ ion peaks observed at m/z 1143 (**3**) and 1159 (**4**) gave positive fragments $[\text{M} + \text{Na} - 2 \times 162]^+$ at m/z 819 and 835, respectively, attributed to the successive losses of two hexoses. These ion fragments suggested that the terminal xylose in **1** and **2** was replaced by a hexose in **3** and **4**. Complete ^1H and ^{13}C NMR assignments of the spin systems of two β -D-glucosyl units ($\delta_{\text{H}-1}$ 4.48, d, $J = 7.9$ and 5.40, d, $J = 8.1$ Hz) and two β -D-galactosyl units ($\delta_{\text{H}-1}$ 4.60, d, $J = 7.8$ Hz and 4.52, d, $J = 7.6$ Hz) were based on analysis of a combination of 2D-NMR experiments (Table 1). The spectroscopic features indicated that **3** was closely related to **1** and that the two glucose units were directly linked to C-3 and C-28 of hederagenin. The long-range couplings observed in the HMBC experiment of **3** showed that the inner galactose unit ($\delta_{\text{H}-1}$ 4.60) was linked to C-3 ($\delta_{\text{C}-3}$ 87.9) of the glucose attached to C-3 of the hederagenin, and the terminal galactose ($\delta_{\text{H}-1}$ 4.52) was attached to C-3 ($\delta_{\text{C}-3}$ 84.5) of the inner galactose. The structure of caryocaroside II-13 (**3**) was elucidated as 3-*O*- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-glucopyranosyl hederagenin-28-*O*- β -D-glucopyranosyl ester. Comparison of the ^1H and ^{13}C NMR data of the saccharide moieties of **4** with those of **3** showed that bayogenin in **4** was substituted at C-3 by the same trisaccharide chain and at C-28 by a β -D-glucosyl unit (Table 1). On this basis, the structure of caryocaroside III-13 (**4**) was proposed to be 3-*O*- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-glucopyranosyl bayogenin-28-*O*- β -D-glucopyranosyl ester.

Caryocaroside III-14 (**5**) displayed a molecular ion peak $[\text{M} + \text{Na}]^+$ at m/z 1291.5944 in the positive HRESIMS, in accordance with an empirical molecular formula of $\text{C}_{59}\text{H}_{96}\text{O}_{29}\text{Na}$. The positive ESIMS² suggested the presence of five sugar units, one pentose, and four hexoses, and this was confirmed by the presence of five anomeric proton and carbon signals in the ^1H and ^{13}C NMR spectra (Table 2). The detailed analysis of the 2D NMR spectra led to the identification of one terminal β -D-xylose unit, of two β -D-galactose units substituted in position 3 ($\delta_{\text{C}-3}$ 84.4 and 83.3), and of two β -D-glucose units, one of which being substituted at C-3 ($\delta_{\text{C}-3}$ 87.7) and the other one being terminal and linked to C-28 (Table 2). The sequence of the tetrasaccharide chain at C-3 was deduced from the HMBC experiment, which showed long-range correlations between Xyl-H-1 (δ_{H} 4.54)/Gal'-C-3 (δ_{C} 83.9), Gal'-H-1 (δ_{H} 4.61)/Gal-C-3 (δ_{C} 84.4), Gal-H-1 (δ_{H} 4.63)/Glc-C-3 (δ_{C} 87.7), and Glc-H-1 (δ_{H} 4.52)/C-3 of bayogenin (δ_{C} 84.0). The ester chain was constituted by a single β -D-glucose unit, as indicated by the cross-peak between Glc'-H-1 (δ_{H} 5.41) and C-28 of the bayogenin (δ_{C}

178.2). These findings led to the assignment of caryocaroside III-14 (**5**) as 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 3)- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-glucopyranosyl bayogenin-28-*O*- β -D-glucopyranosyl ester.

The positive HRESIMS of caryocarosides II-16 (**6**) and III-16 (**7**) showed molecular ion peaks $[\text{M} + \text{Na}]^+$ at m/z 1437.6528 and 1453.6476, corresponding to $\text{C}_{65}\text{H}_{106}\text{O}_{33}\text{Na}$ and $\text{C}_{65}\text{H}_{106}\text{O}_{34}\text{Na}$ molecular formulas, respectively. The ESIMS² experiment of the $[\text{M} + \text{Na}]^+$ ion of **7** gave a positive fragment $[\text{M} + \text{Na} - 162]^+$ at m/z 1291, suggesting an additional hexose compared to **5**. The six sugar units of **6** were identified by analysis of COSY, TOCSY, ROESY, and HSQC experiments as three β -D-galactose units (Gal, Gal', and Gal''), two β -D-glucose units (Glc and Glc'), and one β -D-xylose unit (Xyl) (Table 2). The first β -D-galactose unit was substituted at the C-4 position (Gal $\delta_{\text{C}-4}$ 79.9), whereas the two others were substituted at the C-3 position (Gal' $\delta_{\text{C}-3}$ 85.0 and Gal'' $\delta_{\text{C}-3}$ 83.9) as in compound **5**. The sequences of the saccharide chains at C-3 and C-28 were indicated by the following significant long-range couplings in the HMBC spectrum: between Xyl-H-1 (δ_{H} 4.54)/Gal''-C-3 (δ_{C} 83.9), Gal''-H-1 (δ_{H} 4.59)/Gal'-C-3 (δ_{C} 85.0), Gal'-H-1 (δ_{H} 4.56)/Gal-C-4 (δ_{C} 79.9), Gal-H-1 (δ_{H} 4.54)/Glc-C-3 (δ_{C} 88.3), and Glc-H-1/C-3 (δ_{C} 83.1) of hederagenin and between Glc'-H-1/C-28 (δ_{C} 178.1) of the aglycon. On the basis of the aforementioned evidence, the structure of caryocaroside II-16 (**6**) was elucidated as 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 3)- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-glucopyranosyl hederagenin-28-*O*- β -D-glucopyranosyl ester. Comparison of the ^1H and ^{13}C NMR values of the glycosidic portions of **6** and **7** and analysis of the HMBC correlations showed that **6** and **7** contained the same sugar moieties, linked to C-3 and C-28 of bayogenin instead of hederagenin (Table 2). Thus, caryocaroside III-16 (**7**) was concluded to be 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 3)- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-glucopyranosyl bayogenin-28-*O*- β -D-glucopyranosyl ester.

Caryocaroside III-15 (**8**) displayed a molecular ion peak $[\text{M} + \text{Na}]^+$ at m/z 1291.5924 in the positive HRESIMS, in accordance with a molecular formula of $\text{C}_{59}\text{H}_{96}\text{O}_{29}\text{Na}$, thus, an isomer of **5**. The five sugar units of **8** were characterized by their anomeric carbon signals at δ_{C} 105.2, 105.6, 105.9, 106.3, and 106.5 in the ^{13}C NMR spectrum. The chemical shifts of C-3 (δ_{C} 83.9) and C-28 (δ_{C} 182.0) of the aglycon showed that **8** was a monodesmoside of bayogenin with a pentasaccharide chain linked to C-3 as in **7**. Comparison of the ^1H and ^{13}C NMR values of the pentasaccharidic portion of **8** and **7** and the analysis of the HMBC correlations showed that **8** contained the same sugar moieties linked to C-3 of bayogenin (Table 2). Thus, the structure of caryocaroside III-15 (**8**) was elucidated to be 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 3)- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-galactopyranosyl bayogenin.

Caryocaroside IV-10 (**9**) showed a $[\text{M} + \text{Na}]^+$ ion peak at m/z 1009.5009, in the positive HRESIMS, in accordance with an empirical molecular formula of $\text{C}_{49}\text{H}_{78}\text{O}_{20}\text{Na}$. The positive ion $[\text{M} + \text{Na}]^+$ gave in the ESIMS² ion fragments at m/z 847 $[\text{M} + \text{Na} - 162]^+$, 685 $[\text{M} + \text{Na} - 2 \times 162]^+$, and 495 $[\text{M} - \text{H} - 2 \times 162 - (175 + 15)]^-$, assigned to the successive losses of two hexoses and a methyl hexosuronate. In the ^1H NMR spectrum, three anomeric proton signals were observed at δ_{H} 4.53 (d, $J = 7.8$ Hz), 4.57 (d, $J = 7.7$ Hz), and 5.40 (d, $J = 8.2$ Hz) (Table 3). The deshielded anomeric proton signal at δ_{H} 5.40 suggested the presence of an ester sugar linked to C-28, which was identified as a terminal β -D-glucose on the basis of the ^1H and ^{13}C NMR data. The sugar with an anomeric proton signal at δ_{H} 4.53 was identified as a β -D-glucuronic acid, characterized by a five-spin system, a doublet for an axial H-5 proton ($J_{\text{H4-H5}} = 9.5$ Hz), and a carbonyl C-6 at δ_{C} 172.2. This β -D-glucuronic acid was esterified by a methyl group, as shown by the cross-peak correlation between the carbonyl C-6

Table 2. ^1H and ^{13}C NMR Data of the Sugar Moieties of Compounds **5–8** (CD_3OD)

	5		6		7		8	
	δ_{H} (m, J Hz)	δ_{C}	δ_{H} (m, J Hz)	δ_{C}	δ_{H} (m, J Hz)	δ_{C}	δ_{H} (m, J Hz)	δ_{C}
β -D-glucose (at C-3)								
1	4.52 (d, 7.9)	105.2	4.47 (d, 7.8)	105.3	4.51 (d, 7.7)	105.1	4.51 (d, 7.8)	105.2
2	3.50 (dd, 9.1, 7.2)	74.8	3.39 (dd, 9.2, 7.9)	74.8	3.50 (m)	74.7	3.49 (dd, 8.8, 7.8)	74.7
3	3.62 (t, 9)	87.7	3.55 (t, 9)	88.3	3.57 (t, 8.9)	88.0	3.57 (t, 8.9)	88.0
4	3.52 (t, 9.3)	69.5	3.43 (t, 9)	69.8	3.50 (m)	69.5	3.49 (t, 8.4)	69.4
5	3.34 (m)	77.3	3.32 (m)	77.3	3.33 (m)	77.3	3.33 (m)	77.3
6a	3.75 (dd, 12.1, 5.1)	62.4	3.71 (dd, 11, 5)	62.6	3.74 (dd, 11.8, 2.9)	62.4	3.73 (m)	62.1
6b	3.84 (brd, 12)		3.84 (brd, 12)		3.83 (dd, 11.8, 2.1)		3.82 (brd, 11.6)	
β -D-galactose (at C-3 Glc)								
1	4.63 (d, 7.8)	105.2	4.54 (d, 7.6)	106.3	4.56 (d, 7.7)	105.6	4.55 (d, 7.8)	105.6
2	3.82 (dd, 9.7, 8.0)	72.1	3.63 (m)	73.8	3.64 (m)	73.8	3.63 (m)	73.8
3	3.71 (dd, 9.6, 3.6)	84.4	3.64 (m)	75.1	3.63 (m)	75.2	3.63 (m)	75.2
4	4.13 (brs, $w_{1/2} = 3$)	69.9	4.07 (brs, $w_{1/2} = 3$)	79.9	4.08 (brs, $w_{1/2} = 3$)	79.9	4.07 (brs, $w_{1/2} = 3$)	80.0
5	3.63 (m)	76.7	3.64 (m)	76.2	3.64 (m)	76.2	3.64 (m)	76.2
6a	3.72 (dd, 11.4, 4.6)	62.6	3.75 (dd, 11.6, 6.6)	61.8	3.75 (dd, 11.4, 6.8)	61.8	3.73 (m)	61.8
6b	3.83 (dd, 11.4, 3.3)		3.88 (dd, 11.6, 6.2)		3.89 (dd, 11.4, 6.9)		3.88 (dd, 11.4, 5.7)	
β -D-galactose' (at C-3 Gal)								
1	4.61 (d, 7.7)	105.9						
2	3.83 (dd, 9.5, 7.5)	72.1						
3	3.67 (dd, 9.6, 3.2)	83.9						
4	3.05 (d, 3.2)	69.7						
5	3.59 (m)	76.4						
6a	3.75 (dd, 11.4, 4.5)	62.4						
6b	3.83 (m)							
β -D-galactose' (at C-4 Gal)								
1			4.55 (d, 7.8)	106.5	4.56 (d, 7.7)	106.5	4.55 (d, 7.8)	106.5
2			3.80 (dd, 9.1, 7.8)	72.3	3.81 (dd, 9.5, 7.7)	72.3	3.80 (dd, 9.5, 7.8)	72.3
3			3.68 (dd, 9, 3)	85.0	3.68 (dd, 9.5, 3)	85.1	3.67 (dd, 9.5, 3)	85.0
4			3.11 (d, 3.3)	69.9	4.12 (d, 12.2, 2.9)	69.9	4.10 (brd, 2.7)	69.9
5			3.59 (m)	76.4	3.60 (m)	76.4	3.59 (m)	76.6
6a			3.75 (m)	62.6	3.74 (m)	62.5	3.75 (dd, 12.1, 3.6)	62.4
6b			3.80 (m)		3.80 (dd, 12.5, 5.6)		3.80 (dd, 11.4, 4.6)	
β -D-galactose'' (at C-3 Gal')								
1			4.59 (d, 7.7)	105.9	4.59 (d, 7.7)	105.9	4.58 (d, 7.7)	105.9
2			3.82 (dd, 9.4, 7.8)	72.1	3.82 (dd, 9.4, 7.7)	72.1	3.82 (dd, 9.2, 7.7)	72.1
3			3.62 (dd, 9.7, 3)	83.9	3.66 (dd, 9.8, 3.3)	83.9	3.66 (dd, 9.2, 3.4)	83.9
4			4.05 (d, 3.2)	69.7	4.06 (d, 3)	69.7	4.05 (d, 2.6)	69.7
5			3.59 (ddm, 8, 5)	76.6	3.59 (m)	76.6	3.58 (m)	76.6
6a			3.71 (dd, 11.5, 5.1)	62.4	3.71 (dd, 11.8, 6.6)	62.4	3.71 (m)	62.4
6b			3.83 (m)		3.82 (dd, 11.8, 5.9)		3.82 (m)	
β -D-xylose (at C-3 Gal')								
1	4.54 (d, 7.2)	106.3	β -D-xylose (at C-3 Gal'')		β -D-xylose (at C-3 Gal'')		β -D-xylose (at C-3 Gal'')	
2	3.33 (m)	75.2	4.54 (d, 7.2)	105.6	4.54 (d, 7.1)	106.3	4.53 (d, 7.3)	106.3
3	3.36 (t, 7.9)	77.5	3.32 (m)	75.1	3.33 (m)	75.2	3.33 (m)	75.2
4	3.52 (m)	71.1	3.36 (t, 8.2)	77.5	3.36 (t, 8.5)	77.5	3.36 (m)	77.5
5a	3.25 (t, 11.5)	66.9	3.52 (ddd, 10.4, 8.3, 5.4)	71.0	3.52 (m)	71.0	3.53 (m)	71.0
5b	3.89 (dd, 11.5, 5.3)		3.24 (dd, 11.4, 10.4)	66.9	3.24 (dd, 11.3, 10.1)	66.9	3.24 (dd, 11.4, 10.3)	66.9
			3.89 (dd, 11.4, 5.3)		3.89 (dd, 11.4, 5.2)		3.89 (dd, 11.4, 5.5)	
β -D-glucose' (at C-28)								
1	5.41 (d, 8.1)	95.7	5.40 (d, 8.1)	95.7	5.40 (d, 8.1)	95.7		
2	3.34 (m)	73.9	3.34 (dd, 9.2, 8.1)	73.9	3.34 (t, 8.2)	73.9		
3	3.43 (t, 8.9)	78.3	3.43 (t, 9.2)	78.3	3.43 (dd, 9.1, 8.7)	78.3		
4	3.38 (t, 9.4)	71.0	3.38 (t, 9.4)	71.1	3.38 (t, 9.3)	71.1		
5	3.37 (m)	78.7	3.37 (m)	78.7	3.36 (m)	78.7		
6a	3.70 (dd, 11.7, 4.6)	62.4	3.71 (dd, 11.9, 4.5)	62.4	3.71 (dd, 12.2, 3.1)	62.4		
6b	3.84 (dd, 11.7, 4)		3.83 (dd, 12.5, 1.6)		3.81 (dd, 12.5, 1.6)			

and a methoxycarbonyl signal at δ_{H} 3.80 (s, 3H), in the HMBC experiment. The third unit, with its anomeric proton at δ_{H} 4.57 (d, $J = 7.7$ Hz), was identified as a terminal β -D-galactose. The cross-peaks observed in the HMBC spectrum between GlcA-H-1/C-3 of the aglycon ($\delta_{\text{C}-3}$ 91.1), Gal-H-1/GlcA-C-3 ($\delta_{\text{C}-3}$ 86.3), and GlcA-H-1/C-28 ($\delta_{\text{C}-28}$ 178.0) led to the assignment of caryocaroside IV-10 (**9**) as 3-*O*- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-methyl glucopyranosiduronate-2 β -hydroxyoleanolic acid-28-*O*- β -D-glucopyranosyl ester. This saponin was the methyl ester of caryocaroside IV-9 (**20**) and was probably an artifact that originated during extraction with MeOH.¹⁶ Although the same extraction process was previously used for the fruits of *C. glabrum*,⁵ only caryocaroside IV-9 was detected in the saponin extract of this last species.

Caryocaroside IV-17 (**10**) gave a molecular ion peak $[\text{M} + \text{Na}]^+$ at m/z 1127.5225 in the positive HRESIMS ($\text{C}_{55}\text{H}_{84}\text{O}_{24}\text{Na}$). The positive ESIMS² of the $[\text{M} + \text{Na}]^+$ ion peak suggested the presence

of four sugar moieties and that was confirmed in the ^1H NMR spectrum by their anomeric proton signals at δ_{H} 4.52 (d, $J = 7.6$), 4.54 (d, $J = 7.1$), 4.64 (d, $J = 7.8$), and 5.40 (d, $J = 8.1$). As previously seen, the sugar units in **10** were determined to be a monosubstituted β -D-glucuronic acid ($\delta_{\text{H}-1}$ 4.52 and $\delta_{\text{C}-3}$ 86.2), a monosubstituted β -D-galactose ($\delta_{\text{H}-1}$ 4.64 and $\delta_{\text{C}-3}$ 84.0), a terminal β -D-xylose ($\delta_{\text{H}-1}$ 4.54), and a terminal β -D-glucose ($\delta_{\text{H}-1}$ 5.40) (Table 3). The sequence of the sugar chain at C-3 was determined by the following long-range couplings in the HMBC spectrum: Xyl-H-1/Gal-C-3, Gal-H-1/GlcA-C-3, and GlcA-H-1/C-3 of 2 β -hydroxyoleanolic acid. The linkage of the glucose unit at C-28 of the aglycon was confirmed by the HMBC correlations between GlcA-H-1 and C-28. Analysis of all the above evidence led to the assignment of caryocaroside IV-17 (**10**) as 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 3)- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-glucuronopyranosyl-2 β -hydroxyoleanolic acid-28-*O*- β -D-glucopyranosyl ester.

Table 3. ^1H and ^{13}C NMR Data of the Sugar Moieties of Compounds **9–14** (CD_3OD)

	9		10		11		12		13		14	
	δ_{H} (m, J Hz)	δ_{C}	δ_{H} (m, J Hz)	δ_{C}	δ_{H} (m, J Hz)	δ_{C}	δ_{H} (m, J Hz)	δ_{C}	δ_{H} (m, J Hz)	δ_{C}	δ_{H} (m, J Hz)	δ_{C}
β -D-glucuronic acid (at C-3)												
1	4.53 (d, 7.8)	106.3	4.52 (d, 7.6)	106.3	4.53 (d, 7.8)	106.5	4.52 (d, 7.5)	106.3	4.44 (d, 7.8)	106.0	4.51 (d, 7.6)	106.1
2	3.57 (dd, 9.4, 7.8)	74.5	3.58 (dd, 8.7, 8.1)	74.5	3.59 (t, 7.8)	74.3	3.57 (dd, 9.9, 7.6)	74.5	3.56 (dd, 8.6, 8)	74.7	3.57 (t, 7.6)	74.6
3	3.63 (t, 9.4)	86.3	3.65 (m)	86.2	3.66 (m)	86.0	3.62 (m)	86.6	3.63 (t, 8.3)	86.5	3.64 (m)	86.2
4	3.64 (t, 9.4)	71.9	3.65 (m)	71.8	3.66 (m)	71.8	3.64 (m)	71.8	3.58 (t, 8.5)	72.2	3.63 (m)	72.0
5	3.90 (d, 9.5)	76.0	3.89 (d, 7.3)	76.5	3.95 (d, 9.6)	76.1	3.88 (d, 9.3)	76.1	3.66 (d, 9.5)	76.8	3.85 (m)	76.2
6		172.2		173.0		172		174.2		176.0		173.0
—OCH ₃	3.80 (s)	52.8			3.80 (s)	52.9						
β -D-galactose (at C-3 Glc)												
1	4.57 (d, 7.7)	105.6	4.64 (d, 7.8)	105.1	4.63 (d, 7.8)	105.1	4.59 (d, 7.5)	105.5	4.61 (d, 7.8)	105.3	4.59 (d, 7.7)	105.7
2	3.63 (dd, 9.7, 7.7)	73.0	3.80 (dd, 9, 7.8)	72.2	3.80 (m)	72.2	3.63 (m)	73.8	3.68 (dd, 8.8, 8)	73.6	3.64 (m)	73.8
3	3.53 (d, 3.4)	74.7	3.67 (dd, 9, 3.2)	84.0	3.67 (dd, 7.6, 3)	84.0	3.63 (m)	75.1	3.63 (dd, 8.8, 3.6)	75.2	3.63 (m)	75.2
4	3.82 (brd, 3.9)	70.4	4.03 (d, 3.3)	69.8	4.03 (d, 3)	69.8	4.06 (m)	79.9	4.06 (d, 2.9)	80.0	4.07 (m)	80.0
5	3.58 (m)	77.2	3.61 (m)	76.7	3.61 (m)	76.7	3.63 (m)	76.6	3.63 (m)	76.3	3.63 (m)	76.4
6a	3.70 (dd, 11.8, 4.4)	62.7	3.79 (m)	62.6	3.79 (m)	62.5	3.76 (dd, 11.5, 7)	62.0	3.78 (dd, 11.7, 4.8)	62.0	3.76 (dd, 11.4, 2.6)	62.0
6b	3.85 (dd, 11.5, 6.1)		3.80 (m)		3.80 (m)		3.85 (dd, 11.5, 6.1)		3.88 (dd, 11.7, 6.2)		3.88 (dd, 11.4, 6)	
β -D-galactose' (at C-4 Gal)												
1							4.55 (d, 7.9)	106.5	4.56 (d, 7.9)	106.5	4.56 (d, 7.9)	106.4
2							3.79 (dd, 9.5, 7.9)	72.3	3.80 (dd, 9.4, 7.9)	72.3	3.80 (m)	72.3
3							3.64 (m)	84.7	3.68 (m)	85.0	3.67 (dd, 8.9, 3.4)	85.0
4							4.02 (d, 2.9)	69.9	4.11 (d, 3.5)	70.0	4.12 (d, 3.6)	70.0
5							3.57 (m)	76.7	3.59 (m)	76.6	3.59 (m)	76.7
6a							3.71 (dd, 11.2, 4.7)	62.4	3.73 (dd, 11.2, 4.6)	62.5	3.74 (dd, 11.2, 7)	62.4
6b							3.78 (dd, 11.2, 7.8)		3.81 (dd, 10.9, 7.4)		3.80 (m)	
β -D-galactose'' (at C-3 Gal')												
1									4.59 (d, 7.7)	105.9	4.59 (d, 7.7)	105.8
2									3.82 (dd, 9.6, 7.9)	72.1	3.82 (dd, 9.6, 7.7)	72.1
3									3.66 (m)	83.9	3.66 (dd, 9.7, 3.5)	83.9
4									4.05 (d, 4.6)	69.7	4.06 (d, 3.5)	69.7
5									3.58 (m)	76.4	3.59 (m)	76.6
6a									3.72 (dd, 11.3, 6.5)	62.4	3.73 (dd, 11.3, 7.8)	62.4
6b									3.79 (dd, 11.6, 6.9)		3.79 (dd, 11.3, 5.4)	
β -D-xylose												
			at C-3 Gal		at C-3 Gal		at C-3 Gal'		at C-3 Gal''		at C-3 Gal''	
1			4.54 (d, 7.1)	106.3	4.54 (d, 7.1)	106.3	4.51 (d, 7.0)	106.3	4.53 (d, 7.2)	106.3	4.54 (d, 7.2)	106.2
2			3.32 (m)	75.2	3.32 (m)	75.2	3.32 (t, 8.9)	75.2	3.32 (m)	75.2	3.32 (m)	75.2
3			3.35 (m)	77.5	3.35 (m)	77.5	3.35 (m)	77.5	3.36 (t, 9)	77.5	3.36 (m)	77.5
4			3.52 (ddd, 10.2, 8.5, 5.2)	71.0	3.52 (ddd, 10.2, 8.5, 5.2)	71.0	3.51 (ddd, 10.5, 8.2, 5.1)	71.0	3.52 (ddd, 9.9, 8.6, 5.5)	71.0	3.52 (ddd, 10.2, 8.5, 5.5)	71.0
5a			3.24 (t, 11.2)	66.9	3.24 (t, 11.2)	66.9	3.23 (t, 11)	66.9	3.24 (dd, 11.1, 10.7)	66.9	3.24 (dd, 11.4, 10.5)	66.9
5b			3.88 (dd, 11.3, 5.3)		3.88 (dd, 11.3, 5.3)		3.87 (dd, 11.3, 5.2)		3.88 (dd, 11.4, 5.4)		3.89 (dd, 11.4, 5.3)	
β -D-glucose (at C-28)												
1	5.40 (d, 8.2)	95.7	5.40 (d, 8.1)	95.7	5.40 (d, 8.1)	95.7					5.40 (d, 7.7)	95.7
2	3.34 (m)	73.9	3.34 (m)	73.9	3.34 (m)	73.9					3.34 (m)	73.9
3	3.43 (t, 8.9)	78.3	3.43 (t, 8.8)	78.3	3.43 (t, 8.8)	78.3					3.43 (t, 9.1)	78.3
4	3.38 (t, 9.5)	71.1	3.38 (m)	71.1	3.38 (m)	71.0					3.38 (t, 8.3)	71.1
5	3.37 (m)	78.7	3.37 (m)	78.7	3.37 (m)	78.7					3.34 (m)	78.7
6a	3.70 (dd, 11.7, 4.4)	62.4	3.70 (dd, 12, 4.4)	62.4	3.70 (dd, 12, 4.4)	62.4					3.70 (dd, 11, 3.7)	62.4
6b	3.84 (dd, 11.7, 1.5)		3.83 (brd, 12)		3.83 (brd, 12)						3.84 (dd, 12, 1)	

The ^1H and ^{13}C NMR spectra of caryocaroside IV-18 (**11**) showed an additional methoxy group compared to **10**, suggesting that **11** was the methyl ester of **10** (Table 3). The molecular ion peak $[\text{M} + \text{Na}]^+$ at m/z 1141.5402 ($\text{C}_{54}\text{H}_{86}\text{O}_{24}\text{Na}$) in the positive HRESIMS was 14 mass units higher than that in **10**. The methoxycarbonyl group observed in the ^1H NMR spectrum at δ_{H} 3.80 (s, 3H) gave a correlation in the HMBC spectrum with the carbonyl C-6 of glucuronic acid (δ_{C} 172.0). Thus, caryocaroside IV-18 (**11**) was concluded to be 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 3)- β -

D-galactopyranosyl-(1 \rightarrow 3)- β -D-methylglucopyranosiduronate-2 β -hydroxyoleanolic acid-28-*O*- β -D-glucopyranosyl ester and could be considered as an artifact formed from **10** during extraction with methanol, as observed with **20**.

Caryocaroside IV-19 (**12**) displayed a molecular ion peak $[\text{M} + \text{Na}]^+$ at m/z 1127.5240 in the positive HRESIMS ($\text{C}_{53}\text{H}_{84}\text{O}_{24}\text{Na}$). The ^{13}C NMR data showed that **12** was a monosaccharide saponin since C-28 of 2 β -hydroxyoleanolic acid was deshielded at δ_{C} 182.2. The ^1H and ^{13}C NMR data of **12** displayed signals for four anomeric

protons at δ_H 4.51 (d, $J = 7.0$ Hz), 4.52 (d, $J = 7.5$ Hz), 4.55 (d, $J = 7.9$ Hz), and 4.59 (d, $J = 7.5$ Hz) and attached carbons at δ_C 106.3 (2C), 106.5, and 105.5, respectively (Table 3). Conventional analysis of COSY, TOCSY, and HSQC experiments allowed full identification of the four spin systems. The unit with an anomeric proton at δ_H 4.51 was identified as a terminal β -D-xylose, whereas the units with anomeric protons at δ_H 4.55 and 4.59 corresponded to two β -D-galactoses monosubstituted at positions C-3 (Gal') and C-4 (Gal), respectively. The last unit possessing an anomeric proton signal at δ_H 4.52 was a C-3-monosubstituted glucuronic acid (Table 3). Sequencing of the glycoside chain in **12** was achieved by analysis of the HMBC spectrum, which showed correlations between Xyl-H-1/Gal'-C-3 (δ_C 84.7), Gal'-H-1/Gal-C-4 (δ_C 79.9), Gal-H-1/GlcA-C-3 (δ_C 86.6), and GlcA-H-1/C-3 of the aglycon. Thus, the structure of caryocaroside IV-19 (**12**) was elucidated as 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 3)- β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-glucuronopyranosyl-2 β -hydroxyoleanolic acid.

In the positive HRESIMS, caryocaroside IV-20 (**13**) displayed a molecular ion peak $[M + Na]^+$ at m/z 1289.5764, whereas caryocaroside IV-21 (**14**) displayed a molecular ion peak $[M - H + 2Na]^+$ at m/z 1473.6127, in accordance with molecular formulas of $C_{59}H_{94}O_{29}Na$ (**13**) and $C_{65}H_{103}O_{34}Na_2$ (**14**). Comparison of the ESIMS and NMR spectral data of **14** with those of **13** indicated that **14** possessed one supplementary terminal hexose unit (Table 3). This hexose was identified as a β -D-glucose ester (δ_{H-1} 5.40, δ_{C-1} 95.7), suggesting that **14** was the bidesmoside of **13**. Further analysis of the ESIMS and the 1H and ^{13}C NMR data showed that the sugar chain linked to C-3 of 2 β -hydroxyoleanolic acid was identical for **13** and **14**. The positive ESIMS² experiment of the pseudomolecular ion $[M + Na]^+$ of **13** gave a fragment at m/z 1157 $[M + Na - 132]^+$ corresponding to the loss of a terminal pentose unit. Comparison of the NMR data for the glycoside chain of **13** and **12** indicated that **13** possessed one additional C-3-monosubstituted β -D-galactose unit (Gal'') (Table 3). The cross-peaks observed in the HMBC experiment between Xyl-H-1 (δ_H 4.53)/Gal''-C-3 (δ_C 83.9), Gal''-H-1 (δ_H 4.59)/Gal'-C-3 (δ_C 85.0), Gal'-H-1 (δ_H 4.56)/Gal-C-4 (δ_C 80.0), and Gal-H-1/GlcA-C-3 (δ_C 86.5) led to the assignment of caryocaroside IV-20 (**13**) as 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 3)- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-glucuronopyranosyl-2 β -hydroxyoleanolic acid. Consequently, the structure of caryocaroside IV-21 (**14**) was 3-*O*- β -D-xylopyranosyl-(1 \rightarrow 3)- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-galactopyranosyl-(1 \rightarrow 3)- β -D-glucuronopyranosyl-2 β -hydroxyoleanolic acid-28-*O*- β -D-glucopyranosyl ester.

The toxicity of the methanol extracts of the pulp and the peel of *C. villosum* fruits and of the crude saponin fraction of the peel was evaluated in the brine shrimp assay (*Artemia salina*). The two methanol extracts and the saponin fraction showed good larvicidal activity according to Meyer, who classified crude extracts and pure substances into toxic (LC_{50} value $< 1000 \mu g/mL$) and nontoxic (LC_{50} value $> 1000 \mu g/mL$).¹⁷ The methanol extract of the pulp with a LC_{50} of $100 \mu g/mL$ was more toxic than the extract of the peel ($500 \mu g/mL$). The saponin fraction of the peel (LC_{50} $100 \mu g/mL$) showed 17% death at a concentration of $10 \mu g/mL$ and was also more active than the methanol extracts. These results suggested that the toxicity of the peel and the pulp of *C. villosum* fruits was due to the presence of saponins, despite the small percentage (0.5%) of saponin content. This toxicity reveals the fruits as potential pesticidal and antitumoral agents and explains the traditional use as a fish poison.¹⁸

The antimicrobial activity of caryocarosides IV-21 (**14**), II-1 (**16**), III-1 (**17**), and IV-9 (**20**) and **23** was studied using the agar diffusion method. These compounds did not exhibit any antimicrobial activity at a dose of $50 \mu g$ against *Escherichia coli*, *Staphylococcus aureus*,

Pseudomonas aeruginosa, *Mycobacterium smegmatis*, and *Enterococcus faecalis* bacteria.

Experimental Section

General Experimental Procedures. Optical rotations were measured in MeOH or H₂O with a Perkin-Elmer 241 polarimeter. 1H and ^{13}C NMR spectra were recorded in CD₃OD 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 ESIMS² experiments were performed using a Bruker Esquire LC-MS ion trap mass spectrometer. The samples were introduced by infusion in a solution of MeOH. HRESIMS experiments were performed using a Micromass Q-TOF micro instrument (Manchester, UK) with an electrospray source. TLC were carried out on precoated silica gel 60 F₂₅₄ (Merck), with CHCl₃-MeOH-H₂O (13:7:1), and spots were visualized by heating after spraying with 50% H₂SO₄. Column chromatography 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 C₁₈ reversed-phase column (Dionex, 201 SPTM, 4.6×250 mm, $5 \mu m$, 90 Å) was used for analytical HPLC with a binary gradient elution (solvent A: H₂O-TFA, 0.025%, solvent B: MeCN) and a flow rate of $1 mL \cdot min^{-1}$; the chromatogram was monitored at 205 and 210 nm. A prepacked C₁₈ reversed-phase column (Dionex, 201 SPTM, 10×250 mm, $5 \mu m$, 90 Å) was used for semipreparative HPLC with a flow rate of $3 mL \cdot min^{-1}$.

Plant Material. The fruits of *C. villosum* were collected at Orstom Centre, Cayena Island, in Guyana in January 2000. The species was identified by M. F. Prevost of the botany laboratory of the IRD Centre of Cayena (French Guyana), and an herbarium specimen (Prevost MFP 3777) was deposited in the Herbarium of Guyana.¹⁹

Brine Shrimp Test. Brine shrimp eggs (*Artemia salina* Leach) were hatched in seawater for 48 h.^{20,21} The eggs were placed in one of the two compartments of a tank and covered in order to keep the eggs in a dark ambient. The second compartment of the tank was illuminated in order to attract shrimps through perforations at the boundary plate. After 24 h, the phototropic shrimps, which went to the illuminated compartment, were collected by pipet and incubated under illumination for 24 h at room temperature. The MeOH extract of the peel and the pulp of the fruits and the saponin fraction were prepared in triplicate at concentrations of 1000, 100, and $10 \mu g/mL$ in H₂O. Shrimps were added to 5 mL of the sample and incubated for 24 h. After this time, survivors were counted, the percentage of deaths at each concentration was recorded, and the LC_{50} was evaluated.

Extraction and Isolation. The air-dried and powdered peel (450 g) and pulp (440 g) of fruit were boiled under reflux in MeOH (1.5 L) for 3 h. After cooling and filtration, the MeOH extract was evaporated to provide the saponin mixture as a brown residue (182 g, 40% yield for the peel; 230 g, 52% yield for the pulp). The saponin mixture was analyzed by TLC on silica gel using the solvent mixture CHCl₃-MeOH-H₂O (13:7:1) and by analytical HPLC over C₁₈ with the gradient elution program 30 to 50% B for 75 min.

A part of the MeOH extract of the peel (60 g) was subjected (6×10 g) to vacuum liquid chromatography (VLC) over C₁₈, eluted successively with 40, 60, and 80% MeOH in H₂O and MeOH. The fractions eluted with 80% MeOH in H₂O were combined to give 2.2 g of a saponin mixture (0.5% yield). This was then purified by reversed-phase C₁₈ column chromatography (CC) (60 g) using a gradient of MeOH-H₂O (5:5 to 8:2) and then MeOH. Fractions 26 and 27, eluted with 6:4 MeOH-H₂O, were purified by silica gel CC to afford **5** (11 mg) and **8** (7 mg). Fractions 28 and 29, eluted with MeOH-H₂O (6:4), were subjected to silica gel CC using a gradient of CHCl₃-MeOH-H₂O (85:15:0 to 60:40:7). Fractions eluted with CHCl₃-MeOH (75:25) were further purified by semipreparative HPLC with a linear gradient (31 to 33% B) for 15 min to give 8 mg of **2** and 8 mg of **19**, and fractions eluted with CHCl₃-MeOH (7:3) were purified by HPLC with a linear gradient (26 to 30% B) for 30 min to give 7 mg of **4**. Fraction 30, eluted with MeOH-H₂O (65:35), was purified by silica gel CC using a gradient of CHCl₃-MeOH-H₂O (9:1:0 to 14:6:1) to give 4 mg of **22**. Fractions 31 and 32, eluted with MeOH-H₂O (65:35), were passed through an ion exchange IRN 77 H⁺ Amberlite resin

column before purification by silica gel CC eluting with CHCl_3 –MeOH– H_2O (9:1:0 to 14:6:1) to give 33 mg of **23**. Fractions eluted with CHCl_3 –MeOH– H_2O (70:30:2) were further purified by HPLC with a linear gradient (29 to 30% B) for 30 min to give 9 mg of **7**. Fractions 33 and 34, eluted with MeOH– H_2O (65:35), were purified by silica gel CC eluting with CHCl_3 –MeOH– H_2O (9:1:0 to 70:30:3) to give 11 mg of **18**. Fractions eluted with CHCl_3 –MeOH– H_2O (70:30:1) were further purified by HPLC with a linear gradient (31 to 32% B) to afford 10 mg of **3** and 11 mg of **1**. Fractions 37–39, eluted with MeOH– H_2O (65:35), were purified by silica gel CC using a gradient of CHCl_3 –MeOH– H_2O (9:1:0 to 60:40:7) to give 9 mg of **21**. Fractions eluted with CHCl_3 –MeOH– H_2O (60:40:7) were further purified by HPLC with a linear gradient (31 to 33% B) to afford 7 mg of **14**. Fractions 40–42, eluted with MeOH– H_2O (65:35), were purified by HPLC with a linear gradient (32 to 34% B) for 20 min to give 6 mg of **10** and 8 mg of **20**. Fractions 47–49, eluted with MeOH– H_2O (7:3), were purified by silica gel CC eluting with CHCl_3 –MeOH– H_2O (9:1:0 to 70:30:5) to afford 36 mg of **24**. Fractions 52–54, eluted with MeOH– H_2O (7:3), were purified by silica gel CC using a gradient of CHCl_3 –MeOH– H_2O (9:1:0 to 70:30:5) to give 3 mg of **6**. Fractions eluted with CHCl_3 –MeOH (8:2) were further purified by HPLC with a linear gradient (35 to 39% B) for 20 min to give 2 mg of **9** and 2 mg of **11**. Fractions 55–66, eluted with MeOH– H_2O (8:2), were purified by silica gel CC using a gradient of CHCl_3 –MeOH– H_2O (9:1:0 to 14:6:1) to give 2 mg of **15**, 6 mg of **16**, and 14 mg of **13**. Fractions eluted with CHCl_3 –MeOH (8:2) were further purified by HPLC with a linear gradient (34 to 40% B) for 30 min to afford 2 mg of **17**, and fractions eluted with CHCl_3 –MeOH– H_2O (70:30:1) were purified by HPLC with a linear gradient (35 to 42% B) for 22 min to give 4 mg of **12**.

Acid Hydrolysis of Saponin Mixture. An aliquot of the crude saponin mixture (200 mg) was refluxed with 30 mL of 2 N HCl for 4 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–Me₂CO– H_2O (20:10:7:6) as xylose, glucose, galactose, and glucuronic acid. After a preparative TLC of the sugar mixture (100 mg) in this solvent, the optical rotation of each purified sugar was measured, and all were determined to be D sugars.

Caryocaroside II-12 (1): white powder; $[\alpha]_D^{25} +12.7$ (c 0.83, MeOH); ^1H NMR of aglycon (CD_3OD , 500 MHz) δ 0.73 (3H, s, H-24), 0.83 (3H, s, H-26), 0.93 (3H, s, H-29), 0.95 (3H, s, H-30), 1.01 (3H, s, H-25), 1.19 (3H, s, H-27), 2.87 (1H, dd, $J = 14, 4$ Hz, H-18), 3.31 (1H, d, $J = 11.5$ Hz, H-23a), 3.65 (1H, d, $J = 11.5$ Hz, H-23b), 3.66 (1H, dd, $J = 9.1, 3$ Hz, H-3), 5.27 (1H, t, $J = 3.6$ Hz, H-12); ^{13}C NMR of the aglycon (CD_3OD , 125 MHz) δ 13.4 (C-24), 16.5 (C-25), 17.8 (C-26), 18.9 (C-6), 24.0 (C-16), 24.0 (C-30), 24.6 (C-11), 26.2 (C-2), 26.3 (C-27), 28.9 (C-15), 31.5 (C-20), 33.4 (C-7), 33.1 (C-22), 33.5 (C-29), 34.9 (C-21), 37.7 (C-10), 39.5 (C-1), 40.7 (C-8), 42.6 (C-18), 43.0 (C-14), 43.9 (C-4), 47.2 (C-19), 48.0 (C-17), 48.2 (C-5), 49.0 (C-9), 65.0 (C-23), 83.5 (C-3), 123.7 (C-12), 144.9 (C-13), 178.1 (C-28); ^1H and ^{13}C NMR of the glycosidic part (CD_3OD), see Table 1; ESIMS (positive-ion mode) m/z 1113 $[\text{M} + \text{Na}]^+$; ESIMS² (1113) m/z 951 $[\text{M} + \text{Na} - \text{Glc}]^+$, 819 $[\text{M} + \text{Na} - \text{Glc} - \text{Xyl}]^+$, 657 $[\text{M} + \text{Na} - \text{Glc} - \text{Xyl} - \text{Gal}]^+$, 495 $[\text{M} + \text{Na} - \text{Glc} - \text{Xyl} - 2 \times \text{Gal}]^+$; ESIMS (negative-ion mode) m/z 1089 $[\text{M} - \text{H}]^-$; ESIMS² (1089) m/z 927 $[\text{M} - \text{H} - \text{Glc}]^-$, 795 $[\text{M} - \text{H} - \text{Glc} - \text{Xyl}]^-$, 633 $[\text{M} - \text{H} - \text{Glc} - \text{Xyl} - \text{Gal}]^-$; HRESIMS (positive-ion mode) m/z 1113.5466 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{53}\text{H}_{86}\text{O}_{23}\text{Na}$, 1113.5458).

Caryocaroside III-12 (2): white powder; $[\alpha]_D^{25} +23.7$ (c 0.59, MeOH); ^1H NMR of the aglycon (CD_3OD 500 MHz), δ 0.83 (3H, s, H-26), 0.93 (3H, s, H-29), 0.95 (3H, s, H-30), 0.96 (3H, s, H-24), 1.19 (3H, s, H-27), 1.31 (3H, s, H-25), 2.88 (1H, dd, $J = 14, 4$ Hz, H-18), 3.25 (1H, d, $J = 11.5$ Hz, H-23a), 3.64 (1H, d, $J = 11.5$ Hz, H-23b), 3.64 (1H, d, $J = 3.5$ Hz, H-3), 4.35 (1H, q, $J = 3.5$ Hz, H-2), 5.29 (1H, t, $J = 3.6$ Hz, H-12); ^{13}C NMR of the aglycon (CD_3OD , 125 MHz) δ 14.7 (C-24), 17.5 (C-25), 17.8 (C-26), 18.6 (C-6), 24.0 (C-30), 24.0 (C-16), 24.7 (C-11), 26.4 (C-27), 28.8 (C-15), 31.5 (C-20), 33.1 (C-22), 33.4 (C-7), 33.5 (C-29), 34.9 (C-21), 37.5 (C-10), 40.8 (C-8), 43.1 (C-4), 43.2 (C-14), 42.6 (C-18), 44.4 (C-1), 47.2 (C-19), 48.0 (C-17), 48.1 (C-5), 49.3 (C-9), 65.7 (C-23), 71.1 (C-2), 83.9 (C-3), 123.8 (C-12), 145.0 (C-13), 178.1 (C-28); ^1H and ^{13}C NMR of the

glycosidic part (CD_3OD), see Table 1; ESIMS (positive-ion mode) m/z 1129 $[\text{M} + \text{Na}]^+$; ESIMS² (1129) m/z 967 $[\text{M} + \text{Na} - \text{Glc}]^+$, 835 $[\text{M} + \text{Na} - \text{Glc} - \text{Xyl}]^+$, 673 $[\text{M} + \text{Na} - \text{Glc} - \text{Xyl} - \text{Gal}]^+$, 511 $[\text{M} + \text{Na} - \text{Glc} - \text{Xyl} - \text{Gal} - \text{Glc}]^+$; ESIMS (negative-ion mode) m/z 1105 $[\text{M} - \text{H}]^-$; ESIMS² (1105) m/z 943 $[\text{M} - \text{H} - \text{Glc}]^-$, 811 $[\text{M} - \text{H} - \text{Glc} - \text{Xyl}]^-$, 649 $[\text{M} - \text{H} - \text{Glc} - \text{Xyl} - \text{Gal}]^-$, 487 $[\text{M} - \text{H} - \text{Glc} - \text{Xyl} - \text{Gal} - \text{Glc}]^-$; HRESIMS (positive-ion mode) m/z 1129.5419 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{53}\text{H}_{86}\text{O}_{24}\text{Na}$, 1129.5407).

Caryocaroside II-13 (3): white powder; $[\alpha]_D^{25} +16.3$ (c 0.83, MeOH); ^1H and ^{13}C NMR chemical shift values of the aglycon (CD_3OD) were identical to those described for **1** within ± 0.05 and ± 0.3 ppm, respectively; ^1H and ^{13}C NMR of the glycosidic part (CD_3OD), see Table 1; ESIMS (positive-ion mode) m/z 1143 $[\text{M} + \text{Na}]^+$; ESIMS² (1143) m/z 981 $[\text{M} + \text{Na} - \text{Glc}]^+$, 819 $[\text{M} + \text{Na} - \text{Glc} - \text{Gal}]^+$, 657 $[\text{M} + \text{Na} - \text{Glc} - 2 \times \text{Gal}]^+$; ESIMS (negative-ion mode) m/z 1119 $[\text{M} - \text{H}]^-$; HRESIMS (positive-ion mode) m/z 1143.5553 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{54}\text{H}_{88}\text{O}_{24}\text{Na}$, 1143.5563).

Caryocaroside III-13 (4): white powder; $[\alpha]_D^{25} +23$ (c 0.5, MeOH); ^1H and ^{13}C NMR chemical shift values of the aglycon (CD_3OD) were identical to those described for **2** within ± 0.05 and ± 0.2 ppm, respectively; ^1H and ^{13}C NMR (CD_3OD) of the glycosidic part, see Table 1; ESIMS (positive-ion mode) m/z 1159 $[\text{M} + \text{Na}]^+$; ESIMS² (1159) m/z 997 $[\text{M} + \text{Na} - \text{Glc}]^+$, 835 $[\text{M} + \text{Na} - \text{Glc} - \text{Gal}]^+$, 673 $[\text{M} + \text{Na} - \text{Glc} - 2 \times \text{Gal}]^+$; ESIMS (negative-ion mode) m/z 1135 $[\text{M} - \text{H}]^-$; ESIMS² (1135) m/z 973 $[\text{M} - \text{H} - (\text{Glc or Gal})]^-$, 811 $[\text{M} - \text{H} - \text{Glc} - \text{Gal}]^-$, 649 $[\text{M} - \text{H} - \text{Glc} - 2 \times \text{Gal}]^-$; HRESIMS (positive-ion mode) m/z 1159.5505 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{54}\text{H}_{88}\text{O}_{25}\text{Na}$, 1159.5512).

Caryocaroside III-14 (5): white powder; $[\alpha]_D^{25} +19.9$ (c 0.83, MeOH); ^1H and ^{13}C NMR chemical shift values of the aglycon (CD_3OD) were identical to those described for **2** within ± 0.05 and ± 0.2 ppm, respectively; ^1H and ^{13}C NMR (CD_3OD) of the glycosidic part, see Table 2; ESIMS (positive-ion mode) m/z 1291 $[\text{M} + \text{Na}]^+$; ESIMS² (1291) m/z 1129 $[\text{M} + \text{Na} - \text{Glc}]^+$, 997 $[\text{M} + \text{Na} - \text{Glc} - \text{Xyl}]^+$, 835 $[\text{M} + \text{Na} - \text{Glc} - \text{Xyl} - \text{Gal}]^+$, 673 $[\text{M} + \text{Na} - \text{Glc} - \text{Xyl} - 2 \times \text{Gal}]^+$; ESIMS (negative-ion mode) m/z 1267 $[\text{M} - \text{H}]^-$; ESIMS² (1267) m/z 1105 $[\text{M} - \text{H} - \text{Glc}]^-$, 973 $[\text{M} - \text{H} - \text{Glc} - \text{Xyl}]^-$; HRESIMS (positive-ion mode) m/z 1291.5944 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{59}\text{H}_{96}\text{O}_{29}\text{Na}$, 1291.5935).

Caryocaroside II-16 (6): white powder; $[\alpha]_D^{25} +18$ (c 0.75, MeOH); ^1H and ^{13}C NMR chemical shift values of the aglycon (CD_3OD) were identical to those described for **1** within ± 0.05 and ± 0.3 ppm, respectively; ^1H and ^{13}C NMR of the glycosidic part (CD_3OD), see Table 2; ESIMS (positive-ion mode) m/z 1437 $[\text{M} + \text{Na}]^+$; ESIMS² (1437) m/z 1275 $[\text{M} + \text{Na} - \text{Glc}]^+$, 1143 $[\text{M} + \text{Na} - \text{Glc} - \text{Xyl}]^+$, 981 $[\text{M} + \text{Na} - \text{Glc} - \text{Xyl} - \text{Gal}]^+$, 819 $[\text{M} + \text{Na} - \text{Glc} - \text{Xyl} - 2 \times \text{Gal}]^+$; ESIMS (negative-ion mode) m/z 1413 $[\text{M} - \text{H}]^-$; ESIMS² (1413) m/z 1251 $[\text{M} - \text{H} - \text{Glc}]^-$, 1119 $[\text{M} - \text{H} - \text{Glc} - \text{Xyl}]^-$, 957 $[\text{M} - \text{H} - \text{Glc} - \text{Xyl} - \text{Gal}]^-$, 795 $[\text{M} - \text{H} - \text{Glc} - \text{Xyl} - 2 \times \text{Gal}]^-$, 633 $[\text{M} - \text{H} - \text{Glc} - \text{Xyl} - 3 \times \text{Gal}]^-$, 471 $[\text{M} - \text{H} - \text{Glc} - \text{Xyl} - 3 \text{ Gal} - \text{Glc}]^-$; HRESIMS (positive-ion mode) m/z 1437.6528 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{65}\text{H}_{106}\text{O}_{33}\text{Na}$, 1437.6514).

Caryocaroside III-16 (7): white powder; $[\alpha]_D^{25} +17.3$ (c 0.37, MeOH); ^1H and ^{13}C NMR chemical shift values of the aglycon (CD_3OD) were identical to those described for **2** within ± 0.05 and ± 0.3 ppm, respectively; ^1H and ^{13}C NMR of the glycosidic part (CD_3OD), see Table 2; ESIMS (positive-ion mode) m/z 1453 $[\text{M} + \text{Na}]^+$; ESIMS² (1453) m/z 1291 $[\text{M} + \text{Na} - \text{Glc}]^+$, 1159 $[\text{M} + \text{Na} - \text{Glc} - \text{Xyl}]^+$, 997 $[\text{M} + \text{Na} - \text{Glc} - \text{Xyl} - \text{Gal}]^+$, 835 $[\text{M} + \text{Na} - \text{Glc} - \text{Xyl} - 2 \times \text{Gal}]^+$; ESIMS (negative-ion mode) m/z 1429 $[\text{M} - \text{H}]^-$; ESIMS² (1429) m/z 1267 $[\text{M} - \text{H} - \text{Glc}]^-$; HRESIMS (positive-ion mode) m/z 1453.6476 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{65}\text{H}_{106}\text{O}_{34}\text{Na}$, 1453.6463).

Caryocaroside III-15 (8): white powder; $[\alpha]_D^{25} +30$ (c 0.25, MeOH); ^1H and ^{13}C NMR chemical shift values of the aglycon (CD_3OD) were identical to those described for **2** within ± 0.05 and ± 0.2 ppm, respectively, except for C-28, which was observed at δ 182.0 ppm; ^1H and ^{13}C NMR of the glycosidic part (CD_3OD), see Table 2; ESIMS (positive-ion mode) m/z 1291 $[\text{M} + \text{Na}]^+$; ESIMS² (1291) m/z 1159 $[\text{M} + \text{Na} - \text{Xyl}]^+$, 997 $[\text{M} + \text{Na} - \text{Glc} - \text{Xyl}]^+$, 835 $[\text{M} + \text{Na} - \text{Glc} - \text{Xyl} - \text{Gal}]^+$, 673 $[\text{M} + \text{Na} - \text{Glc} - \text{Xyl} - 2 \times \text{Gal}]^+$; ESIMS (negative-ion mode) m/z 1267 $[\text{M} - \text{H}]^-$; ESIMS² (1267) m/z 1135 $[\text{M} - \text{H} - \text{Xyl}]^-$, 973 $[\text{M} - \text{H} - \text{Glc} - \text{Xyl}]^-$, 811 $[\text{M} - \text{H} - \text{Xyl} - 2 \times \text{Gal}]^-$, 649 $[\text{M} - \text{H} - \text{Xyl} - 3 \times \text{Gal}]^-$, 487 $[\text{M} - \text{H} - \text{Xyl} - 3 \times \text{Gal} - \text{Glc}]^-$; HRESIMS (positive-ion mode) m/z 1291.5924 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{59}\text{H}_{96}\text{O}_{29}\text{Na}$, 1291.5935).

Caryocaroside IV-10 (9): white powder; $[\alpha]^{22}_D +12$ (c 0.5, MeOH); ^1H of the aglycon (CD_3OD , 500 MHz) δ 0.83 (3H, s, H-26), 0.93 (3H, s, H-29), 0.95 (3H, s, H-30), 1.09 (3H, s, H-24), 1.17 (3H, s, H-27), 1.26 (3H, s, H-25), 2.87 (1H, dd, $J = 14, 4$ Hz, H-18), 3.21 (1H, d, $J = 3.5$ Hz, H-3), 4.21 (1H, q, $J = 3.2$ Hz, H-2), 5.28 (1H, t, $J = 3.6$ Hz, H-12); ^{13}C NMR of the aglycon (CD_3OD , 125 MHz) δ 16.9 (C-25), 17.8 (C-26), 18.5 (C-24), 19.1 (C-6), 24.0 (C-30), 24.0 (C-16), 24.6 (C-11), 26.3 (C-27), 28.9 (C-15), 29.8 (C-23), 31.5 (C-20), 33.2 (C-22), 33.5 (C-29), 34.0 (C-7), 34.9 (C-21), 37.7 (C-10), 39.5 (C-4), 40.8 (C-8), 42.6 (C-18), 43.1 (C-14), 44.6 (C-1), 47.2 (C-19), 48.0 (C-17), 49.2 (C-9), 57.0 (C-5), 71.3 (C-2), 91.1 (C-3), 123.9 (C-12), 144.9 (C-13), 178.0 (C-28); ^1H and ^{13}C NMR of the glycosidic part (CD_3OD), see Table 3; ESIMS (positive-ion mode) m/z 1009 $[\text{M} + \text{Na}]^+$; ESIMS² (1009) m/z 847 $[\text{M} + \text{Na} - (\text{Glc or Gal})]^+$, 685 $[\text{M} + \text{Na} - \text{Glc} - \text{Gal}]^+$, 495 $[\text{M} + \text{Na} - \text{Glc} - \text{Gal} - \text{CH}_3 - \text{GlcA}]^+$; ESIMS (negative-ion mode) m/z 985 $[\text{M} - \text{H}]^-$; HRESIMS (positive-ion mode) m/z 1009.5009 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{49}\text{H}_{78}\text{O}_{20}\text{Na}$, 1009.4984).

Caryocaroside IV-17 (10): white powder; $[\alpha]^{22}_D +17$ (c 0.5, MeOH); ^1H and ^{13}C NMR chemical shift values of the aglycon (CD_3OD) were identical to those described for **9** within ± 0.07 and ± 0.2 ppm, respectively; ^1H and ^{13}C NMR of the glycosidic part (CD_3OD), see Table 3; ESIMS (positive-ion mode) m/z 1143 $[\text{M} + \text{K}]^+$; ESIMS² (1143) m/z 981 $[\text{M} + \text{K} - \text{Glc}]^+$, 849 $[\text{M} + \text{K} - \text{Glc} - \text{Xyl}]^+$, 687 $[\text{M} + \text{K} - \text{Glc} - \text{Xyl} - \text{Gal}]^+$; ESIMS (negative-ion mode) m/z 1103 $[\text{M} - \text{H}]^-$; HRESIMS (positive-ion mode) m/z 1127.5225 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{53}\text{H}_{84}\text{O}_{24}\text{Na}$, 1127.5250).

Caryocaroside IV-18 (11): white powder; $[\alpha]^{22}_D +17$ (c 0.5, MeOH); ^1H and ^{13}C NMR chemical shift values of the aglycon (CD_3OD) were identical to those described for **9** within ± 0.07 and ± 0.2 ppm, respectively; ^1H and ^{13}C NMR of the glycosidic part (CD_3OD), see Table 3; ESIMS (positive-ion mode) m/z 1141 $[\text{M} + \text{Na}]^+$; ESIMS² (1141) m/z 979 $[\text{M} + \text{Na} - \text{Glc}]^+$, 847 $[\text{M} + \text{Na} - \text{Glc} - \text{Xyl}]^+$, 685 $[\text{M} + \text{Na} - \text{Glc} - \text{Xyl} - \text{Gal}]^+$; ESIMS (negative-ion mode) m/z 1117 $[\text{M} - \text{H}]^-$; HRESIMS (positive-ion mode) m/z 1141.5402 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{54}\text{H}_{86}\text{O}_{24}\text{Na}$, 1141.5407).

Caryocaroside IV-19 (12): white powder; $[\alpha]^{22}_D +5.2$ (c 0.29, MeOH); ^1H and ^{13}C NMR chemical shift values of the aglycon (CD_3OD) were identical to those described for **9** within ± 0.07 and ± 0.2 ppm, respectively, except for C-28, which was observed at δ 182.2 ppm; ^1H and ^{13}C NMR of the glycosidic part (CD_3OD), see Table 3; ESIMS (positive-ion mode) m/z 1143 $[\text{M} + \text{K}]^+$; ESIMS² (1143) m/z 1011 $[\text{M} + \text{K} - \text{Xyl}]^+$, 849 $[\text{M} + \text{K} - \text{Xyl} - \text{Gal}]^+$, 687 $[\text{M} + \text{K} - \text{Xyl} - 2 \times \text{Gal}]^+$; ESIMS (negative-ion mode) m/z 1103 $[\text{M} - \text{H}]^-$; HRESIMS (positive-ion mode) m/z 1127.5240 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{53}\text{H}_{84}\text{O}_{24}\text{Na}$, 1127.5250).

Caryocaroside IV-20 (13): white powder; $[\alpha]^{22}_D +13$ (c 1, MeOH); ^1H and ^{13}C NMR chemical shift values of the aglycon (CD_3OD) were identical to those described for **9** within ± 0.07 and ± 0.2 ppm, respectively, except for C-28, which was observed at δ 182.1 ppm; ^1H and ^{13}C NMR of the glycosidic part (CD_3OD), see Table 3; ESIMS (positive-ion mode) m/z 1311 $[\text{M} - \text{H} + 2\text{Na}]^+$; ESIMS² (1311) m/z 1179 $[\text{M} - \text{H} + 2\text{Na} - \text{Xyl}]^+$, 1017 $[\text{M} - \text{H} + 2\text{Na} - \text{Xyl} - \text{Gal}]^+$, 855 $[\text{M} - \text{H} + 2\text{Na} - \text{Xyl} - 2 \times \text{Gal}]^+$, 693 $[\text{M} - \text{H} + 2\text{Na} - \text{Xyl} - 3 \times \text{Gal}]^+$; ESIMS (negative-ion mode) m/z 1265 $[\text{M} - \text{H}]^-$; ESIMS² (1265) m/z 1071 $[\text{M} - \text{H} - \text{H}_2\text{O} - \text{CO}_2 - \text{Xyl}]^-$, 909 $[\text{M} - \text{H} - \text{H}_2\text{O} - \text{CO}_2 - \text{Xyl} - \text{Gal}]^-$; HRESIMS (positive-ion mode) m/z 1289.5764 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{59}\text{H}_{94}\text{O}_{29}\text{Na}$, 1289.5778).

Caryocaroside IV-21 (14): white powder; $[\alpha]^{22}_D +10.8$ (c 0.25, MeOH); ^1H and ^{13}C NMR chemical shift values of the aglycon (CD_3OD) were identical to those described for **9** within ± 0.07 and ± 0.2 ppm, respectively; ^1H and ^{13}C NMR of the glycosidic part (CD_3OD), see Table 3; ESIMS (positive-ion mode) m/z 1473 $[\text{M} - \text{H} + 2\text{Na}]^+$; ESIMS² (1473) m/z 1311 $[\text{M} - \text{H} + 2\text{Na} - \text{Glc}]^+$, 1017 $[\text{M} - \text{H} + 2\text{Na} - \text{Glc} - \text{Xyl}]^+$, 855 $[\text{M} - \text{H} + 2\text{Na} - \text{Glc} - \text{Xyl} - \text{Gal}]^+$, 693 $[\text{M} - \text{H} + 2\text{Na} - \text{Glc} - \text{Xyl} - 2 \times \text{Gal}]^+$; ESIMS (negative-ion mode) m/z 1427 $[\text{M} - \text{H}]^-$; ESIMS² (1427) m/z 1265 $[\text{M} - \text{H} - \text{Glc}]^-$, 1071 $[\text{M} - \text{H} - \text{Glc} - \text{H}_2\text{O} - \text{CO}_2 - \text{Xyl}]^-$, 908 $[\text{M} - \text{H} - \text{Glc} - \text{H}_2\text{O} - \text{CO}_2 - \text{Xyl} - \text{Gal}]^-$; HRESIMS (positive-ion mode) m/z 1473.6127 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{65}\text{H}_{103}\text{O}_{34}\text{Na}_2$, 1473.6126).

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