Arboreasides A−E, Triterpene Saponins from the Bark of *Cussonia arborea*

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Five new triterpene saponins, arboreasides A−E (**1**−**5**), and two known saponins, ciwujianoside C_3 and 23-hydroxyursolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, were isolated from the bark of *Cussonia arborea*. The structures were established using extensive 1D and 2D NMR spectroscopic analyses and mass spectrometry.

Cussonia arborea Hochst (Araliaceae) is a tree widely distributed in the savanna from western into the central and eastern areas of Africa. Different parts of this plant are used for the treatment of eye injuries, paralysis, epilepsy, convulsions, spasms, veneral diseases, diarrhea, dizziness, and women's infertility, and for social purposes.[1,2] Previous phytochemical investigations on the genus *Cussonia* revealed the presence of triterpene saponins,[3−5] diterpene glycosides,[6] and flavonoids.[7,8] A literature survey showed that no significant chemical and biological work has been done on *C. arborea*.

In continuation of our study on the glycosides from Araliaceae plants,[9,10] we describe herein the isolation and structure elucidation of five new triterpene glycosides, arboreasides A−E (**1**−**5**), as well as two known saponins from *C. arborea* bark.

Results and Discussion

The methanol extract of the dried bark of *C. arborea* was subjected to multiple chromatographic steps (VLC and MPLC; see Experimental Section), affording five new bidesmosidic triterpene saponins, arboreasides A−E (**1**−**5**), as well as two known saponins, ciwujianoside C_3[11] and the 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside of 23-hydroxyursolic acid.[12] Their structures were elucidated using chemical and spectroscopic methods including 1D and 2D NMR experiments, HRESIMS, and FABMS.

Compounds **1**−**5** were isolated as white, amorphous powders. The sugars obtained by aqueous acid hydrolysis of each compound were identified by comparison on TLC with authentic samples as glucose, galactose, and rhamnose (in the case of **1** and **2**), glucose, xylose, arabinose, and rhamnose (in the case of **3**), glucose, galactose, arabinose, and rhamnose (in the case of **4**), and glucose, galactose, and rhamnose (in the case of **5**). The absolute configurations of the sugars were determined to be D for galactose, glucose, and xylose and L for arabinose and rhamnose by GC analysis of chiral derivatives of the sugars in the hydrolysate of each compound (see Experimental Section). In the ^{1}H NMR spectra of the compounds, the relatively large $^3J_{H\text{-}1,H\text{-}2}$ values of the Gal, Glc, Xyl, and Ara (between 7.0 and 8.3 Hz) moieties indicated a β anomeric proton for Gal, Glc, and Xyl and an α anomeric proton for Ara,[13] whereas the small $^3J_{H\text{-}1,H\text{-}2}$ value (J = 4.2 Hz) of the Glc indicated an α anomeric proton for Glc. The broad singlet of the anomeric proton indicated an α-orientation for Rha. In the ^{13}C NMR spectrum of **1**−**5**, the downfield chemical shift at δ_C 82.1−88.9 (Agly-3) and the upfield chemical shift at δ_C 176.1−176.5 (Agly-28) (Table 1) indicated that **1**−**5** are bidesmosidic saponins.

Arboreaside A (**1**) had the molecular formula $C_{54}H_{88}O_{23}$, as determined by HRESIMS (positive-ion mode), showing a pseudomolecular ion peak at *m/z* 1127.5620 $[M + Na]^+$ (calcd for 1127.5614). Its FABMS (negative-ion mode) displayed a quasimolecular ion peak at *m/z* 1103 $[M - H]^-$, indicating a molecular weight of 1104. Further fragment ion peaks were observed at *m/z* 957 $[(M - H) - 146]^-$, 633 $[(M - H) - 146 - 162 - 162)]^-$, and 471 $[(M - H) - 146 - 162 - 162 - 162]^-$, corresponding to the successive losses of one deoxyhexosyl and three hexosyl moieties, respectively. The fragment ion peak at *m/z* 471 is related to the pseudomolecular ion of the aglycone.

The aglycone of **1** was identified as hederagenin by comparison of ^{1}H and ^{13}C NMR spectroscopic data obtained from the 2D NMR spectra of **1** with reported data.[14] The presence of four sugar residues was confirmed from the observation of four anomeric protons at δ_H 4.84 (d, J = 7.8 Hz), 5.04 (d, J = 8.3 Hz), 5.60 (br s), and 6.00 (d, J = 8.0 Hz), giving HSQC correlations with four anomeric carbons at δ_C 104.0, 105.1, 102.0, and 95.0, respectively. Complete assignments of the resonances of each sugar unit were achieved by extensive 1D and 2D NMR analyses. Evaluation of chemical shifts and spin−spin couplings allowed the identification of two β-glucopyranosyl (Glc I and Glc II), one β-galactopyranosyl (Gal), and one α-rhamnopyranosyl (Rha) unit in **1**. The absolute configuration of Glc and Gal was determined as D and that of Rha as L (see above).

The sequence of the oligosaccharidic chains of **1** was determined by HMBC and NOESY experiments. The HMBC correlations between the anomeric proton at δ_H 5.60 (br s, Rha H-1) and the carbon at δ_C 78.1 (Glc I C-4) and between the proton at δ_H 5.04 (d, J = 8.3 Hz, Glc I H-1) and the carbon at δ_C 82.1 (Agly C-3) proved the sequence of the sugar chain at C-3 to be α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl. This sequence was confirmed by the observation of NOESY cross-peaks between the protons at δ_H 5.60 (Rha H-1) and 4.14 (Glc I H-4) and between the protons at δ_H 5.04 (Glc I H-1) and 4.11 (Agly H-3). Other HMBC correlations between the proton at δ_H 4.84 (d, J = 7.8 Hz, Gal H-1) and the carbon at δ_C 68.6 (Glc II C-6) and between the proton at δ_H 6.00 (d, J = 6.0 Hz, Glc II H-1) and the carbon at δ_C 176.5 (Agly C-28) and a NOESY cross-peak between the proton at δ_H 4.84 (Gal H-1) and the proton at δ_H 4.54 (Glc II H-6a) allowed the sequencing of sugars at C-28 as β-D-galactopyranosyl-(1→6)-β-D-glucopyranosyl. Thus, the structure of arboreaside A (**1**) was

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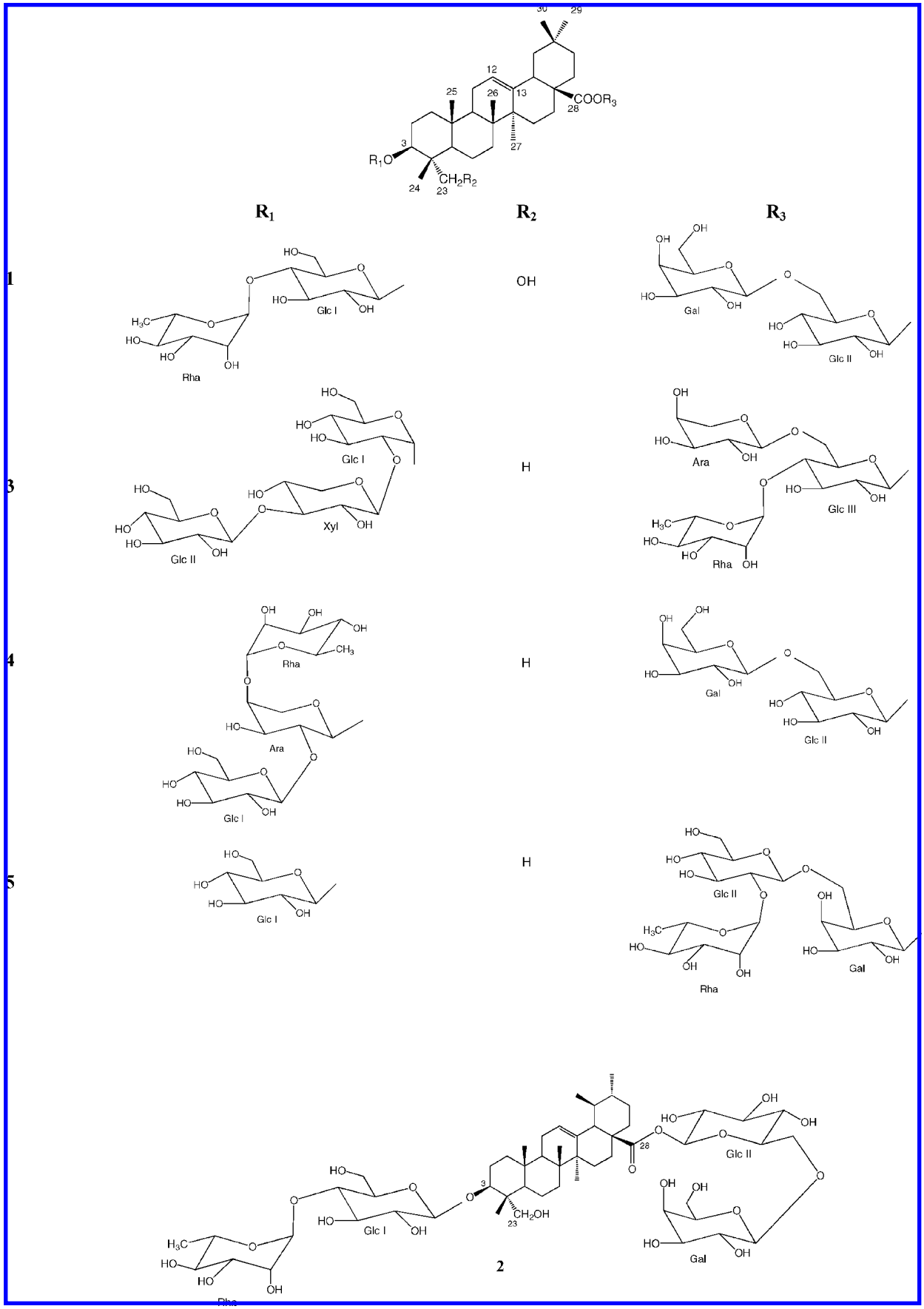
Chart 1



concluded to be 3-*O*-α-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-hederagenin-28-*O*-*β*-D-galactopyranosyl-(1→6)-*β*-D-glucopyranosyl ester.

The molecular formula of arboreaside B (**2**) was determined as $C_{54}H_{88}O_{23}$ by HR-ESIMS, showing a pseudomolecular ion peak at *m/z* 1127.5618 $[M + Na]^+$ (calcd for 1127.5614). Its FABMS

Table 1. ^{13}C and ^{1}H NMR Data for the Aglycone Moieties of **1**−**5**[a]

	1		**2**		**3**		**4**		**5**	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	38.3	0.84, 1.38	38.4	0.84, 1.38	38.4	0.76, 1.38	38.4	0.82, 1.42	38.6	0.78, 1.45
2	25.6	1.82, 2.22	25.6	1.82, 2.22	25.9	1.74, 1.89	26.5	1.78, 1.98	26.9	[b], 1.82
3	82.1	4.13 m	82.1	4.13 m	88.9	3.11	88.8	3.14 m	88.8	3.31
4	41.2		42.2		39.1		39.5		39.5	
5	46.9	1.50	47.7	1.54	55.4	0.63 m	55.5	0.64 d (11.9)	55.5	0.68
6	17.7	1.20, 1.55	17.9	1.22, 1.52	18.2	1.15, 1.33	18.2	1.16, 1.34	18.2	1.26, 1.34
7	32.1	1.66, 1.76	32.1	1.66, 1.76	32.8	1.24, 1.36	32.8	1.26, 1.34	33.6	1.20, [b]
8	39.9		39.5		39.5		39.5		39.5	
9	47.6	1.62	47.7	1.62	47.6	1.49 m	47.7	1.52 m	48.1	1.62 m
10	36.4		36.5		36.6		36.9		36.4	
11	23.3	0.80, 1.82	23.4	0.80, 1.82	23.3	1.09, 1.80	23.5	1.10, 1.82	23.4	1.82, 1.84
12	122.6	5.35 br s	125.7	5.34 br s	123.3	5.34 br s	123.1	5.35 br s	122.5	5.35 br s
13	143.8		138.1		143.8		143.8		143.5	
14	42.1		42.2		41.8		42.0		42.1	
15	32.4	1.22, 1.58	32.4	1.22, 1.58	27.8	1.15, 2.19	27.9	1.14, 2.19	27.9	1.20, 2.19
16	24.1	1.84, 1.94	24.2	1.84, 1.94	23.0	1.94, 2.00	23.0	[b], 1.82	23.0	[b]
17	48.0		48.1		46.7		46.7		46.7	
18	41.7	3.05 br d (13.0)	52.9	2.38 d (10.7)	41.3	3.09	41.8	3.09	41.3	3.08 br d (13.1)
19	45.8	1.12, 1.62	39.0	1.29 m	45.9	1.15, 1.66	45.9	1.16, 1.64	45.9	1.14, 1.62
20	30.2		38.7	0.79 m	30.3		30.4		30.3	
21	36.2	1.63, 1.80	36.4	1.66, 1.80	33.6	1.01, 1.24	33.8	1.24,[b]	32.4	1.26, 1.50
22	32.6	1.12, 1.56	33.6	[b]	32.1	1.63, 1.80	32.1	1.66, 1.79	32.1	1.66, 1.79
23	63.5	3.56 d (11.2), 4.16	64.0	3.58 d (11.0), 4.18	27.8	1.09 s	27.9	1.14 s	27.9	1.22 s
24	13.2	0.84 s	13.2	0.88 s	16.4	0.92 s	16.4	0.96 s	16.7	0.92 s
25	15.7	0.83 s	15.8	0.84 s	15.2	0.76 s	15.2	0.78 s	15.7	0.88 s
26	17.1	0.99 s	17.2	1.01 s	17.1	0.98 s	17.1	1.00 s	17.2	1.02 s
27	25.6	1.12 s	23.3	1.04 s	25.7	1.15 s	25.7	1.16 s	25.7	1.16 s
28	176.5		176.5		176.5		176.5		176.1	
29	32.6	0.82 s	17.0	0.84	32.8	0.82 s	32.8	0.81 s	32.7	0.82 s
30	23.2	0.79 s	20.9	0.81 d (5.7)	23.3	0.81 s	23.3	0.82 s	23.3	0.82 s

[a] 600 MHz (^{1}H), 125 MHz (^{13}C), pyridine-d_5, δ (ppm), coupling constants (*J*) in Hz are given in parentheses, overlapped signals are reported without designated multiplicity. [b] Not determined.

(negative-ion mode) displayed a quasimolecular ion peak at *m/z* 1103 $[M - H]^-$, indicating a molecular weight of 1104. Further fragment ion peaks were observed at *m/z* 957 $[(M - H) - 146]^-$, 633 $[(M - H) - 146 - 162 - 162)]^-$, and 471 $[(M - H) - 146 - 162 - 162 - 162]^-$, corresponding to the successive loss of one desoxyhexosyl and three hexosyl moieties, respectively.

Comparison of the ^{1}H and ^{13}C NMR data of **2** with **1** (Tables 1 and 2) showed that they differ only in the aglycone part. Thus, a set of olefinic carbon signals at δ_C 125.6 (C-12) and 138.0 (C-13) and a tertiary carbon signal at δ_C 52.8 (C-18) was observed in **2** instead of δ_C 122.6 (C-12), 143.8 (C-13), and 41.7 (C-18) in **1**. Furthermore, two tertiary carbons at δ_C 39.0 (C-19) and 38.7 (C-20), showing HSQC correlations with protons at δ_H 1.29 (m, H-19) and 0.79 (m, H-20), respectively, and a COSY correlation between these two resonances, are in good agreement with an ursane-type skeleton. Aditionally, the proton at δ_H 2.38 (d, $J = 10.7$ Hz, H-18) showing a long-range correlation in the HMBC spectrum with the carbons at δ_C 125.6 (C-12), 138.0 (C-13), 48.0 (C-17), and 39.0 (C-19) confirmed the proposed skeleton. Thus, the structure of the aglycone of **2** was elucidated as 23-hydroxyurs-12-en-28-oic acid, which was confirmed by extensive 2D NMR experiments.[4,15] According to the above data, arboreoside B (**2**) was determined to be 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl 23-hydroxyurs-12-en-oic acid 28-*O*-β-D-galactopyranosyl-(1→6)-β-D-glucopyranosyl ester.

Arboreaside C (**3**) had the molecular formula $C_{64}H_{104}O_{30}$ by HRESIMS ($[M + Na]^+$ at *m/z* 1375.6519, calcd for 1375.6510). The FABMS (negative-ion mode) displayed a quasimolecular ion peak at *m/z* 1351 $[M - H]^-$, indicating a molecular weight of 1352. Further fragment ion peaks were observed at *m/z* 779 $[(M - H) - 162 - 132 - 132 - 146)]^-$ and 455 $[(M - H) - 162 - 132 - 132 - 146 - 162 - 162]^-$, corresponding to the loss of three hexosyl, one deoxyhexosyl, and two pentosyl moieties. The fragment ion peak at *m/z* 455 is related to the pseudomolecular ion of aglycone.

The aglycone of **3** was identified as oleanolic acid by comparison of ^{1}H and ^{13}C NMR spectroscopic data obtained from the 2D NMR spectra of **3** with reported data.[16]

The presence of six sugar residues was confirmed from the observation of six anomeric protons at δ_H 4.88 (d, $J = 6.5$ Hz), 4.96 (d, $J = 4.2$ Hz), 5.12 (d, $J = 7.1$ Hz), 5.13 (d, $J = 7.1$ Hz), 5.65 (br s), and 6.09 (d, $J = 8.0$ Hz), giving correlations in the HSQC spectrum with six anomeric carbons at δ_C 104.0, 103.8, 103.0, 106.2, 102.2, and 95.2, respectively. Complete assignments of each sugar by extensive 1D and 2D NMR spectroscopic analysis allowed the identification of one α-glucopyranosyl (Glc I), two β-glucopyranosyl (Glc II and Glc III), one β-xylopyranosyl (Xyl), one α-rhamnopyranosyl (Rha), and one α-arabinopyranosyl (Ara) moiety. The absolute configuration of Xyl and Glc was determined as D and that of Rha and Ara as L (see above).

The sequence of the oligosaccharidic chains of **3** was determined by HMBC and NOESY experiments. HMBC correlations between the proton at δ_H 5.12 (d, $J = 7.1$ Hz, Glc II H-1) and the carbon at δ_C 84.0 (Xyl C-3), between the proton at δ_H 5.13 (d, $J = 7.1$, Xyl H-1) and the carbon at δ_C 79.2 (Glc I C-2), and between the proton at δ_H 4.96 (d, $J = 4.2$, Glc I H-1) and the carbon at δ_C 88.9 (Agly C-3) proved the sugar chain sequence at C-3 to be β-D-glucopyranosyl-(1→3)-β-D-xylopyranosyl-(1→2)-α-D-glucopyranosyl. This was confirmed by NOESY cross-peaks between the proton at δ_H 5.12 (Glc II H-1) and the proton at δ_H 3.99 (Xyl H-3), between the proton at δ_H 4.46 (Glc I, H-2) and the proton at δ_H 5.13 (Xyl H-1), and between the proton at δ_H 4.96 (Glc I H-1) and the proton at δ_H 3.11 (Agly H-3). Other HMBC correlations between the proton at δ_H 5.65 (Rha H-1) and the carbon at δ_C 78.1 (Glc III C-4), between the proton at δ_H 4.88 (Ara H-1) and the carbon at δ_C 68.5 (Glc III C-6), and between the proton at δ_H 6.09 (Glc III H-1) and the carbon at δ_C 176.5 (Agly C-28) suggested the sequence at C-28 to be [α-L-arabinopyranosyl-(1→6)]-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl. This was confirmed by the HMBC reverse correlation between the proton at δ_H 4.23 (Glc III H-6b)

Table 2. ^{13}C and ^{1}H NMR Data for Sugar Moieties of **1**−**5**[a]

	1		**2**		**3**		**4**		**5**	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
3-O	Glc I		Glc I		Glc I		Ara		Glc I	
1	105.1	5.04 d (8.3)	105.1	5.02 d (8.3)	103.8	4.96 d (4.2)	104.4	4.86 d (6.4)	106.1	4.82 d (8.0)
2	75.7	3.97	75.7	3.97	79.2	4.46	79.7	4.52 t (6.6)	75.6	3.96 m
3	77.6	4.20	77.4	4.20	77.1	4.13 m	73.0	4.29	77.6	4.21
4	78.1	4.16	78.1	4.16	70.0	4.24	78.1	4.21	70.2	4.21
5	77.4	3.85	77.4	3.85	77.8	3.85	64.6	3.75 l d (9.7)	77.5	3.99
								4.22		
6	60.7	3.94	60.7	3.94	62.0	4.27			60.8	3.95
		4.07		4.07		4.42				4.06
					Xyl		Glc I			
1					106.2	5.13 d (7.1)	105.0	5.10 d (7.6)		
2					75.9	4.00	75.6	3.98 m		
3					84.0	3.99	77.5	4.11		
4					71.2	4.09	71.0	4.12		
5					66.8	3.65 t (10.9)	77.7	3.73		
						4.36				
6							60.9	3.98, 4.10		
					Glc II					
1					103.0	5.12 d (7.1)				
2					75.3	3.97 m				
3					76.2	4.00				
4					70.7	4.04				
5					77.4	3.68 m				
6					60.7	3.97, 4.07				
	Rha		Rha				Rha			
1	102.0	5.60 br s	102.0	5.60 br s			102.2	5.66 br s		
2	71.7	4.54 m	71.7	4.54			71.9	4.56 m		
3	71.8	4.42 m	71.8	4.42 m			72.0	4.44 m		
4	73.1	4.21	73.1	4.21			73.8	4.24		
5	69.8	4.72	69.4	4.72			70.0	4.76 m		
6	17.9	1.56 d (5.9)	17.9	1.56 d (5.9)			17.9	1.60 d (6.1)		
28-O	Glc II		Glc II		Glc III		Glc II		Gal	
1	95.0	6.00 d (8.0)	95.0	6.05 d (8.0)	95.2	6.09 d (8.0)	95.2	6.10 d (8.0)	95.1	6.09 d (8.0)
2	73.1	4.03	73.1	4.03	73.2	4.05	73.3	4.06 t (8.0)	72.9	4.06 m
3	77.6	4.16	77.6	4.16	77.8	4.23	77.5	4.19	75.9	4.00
4	70.9	4.07	70.9	4.07	78.1	4.17 m	70.5	4.24	70.0	4.74 m
5	77.4	3.98	77.4	3.98	77.4	3.98	77.5	4.00	76.6	3.42
6	68.6	4.21, 4.54	68.4	4.21, 4.54	68.5	4.23, 4.56	68.6	4.24, 4.56	68.5	4.22, 4.55
	Gal		Gal		Ara		Gal		Glc II	
1	104.0	4.84 d (7.8)	104.1	4.84 d (7.8)	104.0	4.88 d (7.6)	104.0	4.90 d (7.8)	104.2	4.88 d (7.8)
2	74.4	3.83	74.4	3.83	74.6	3.83	74.6	3.85 t (8.5)	78.1	4.18
3	75.7	4.00	75.7	4.00	75.9	4.02	75.9	4.02	77.6	4.20
4	70.0	4.18	69.8	4.18 m	70.2	4.20	67.8	4.32	72.0	4.42
5	76.5	3.50 m	76.5	3.50 m	64.0	3.82 l d (8.3)	76.8	3.53	76.8	3.52
						4.23				
6	62.0	4.21, 4.35	62.0	4.21, 4.35			62.0	4.25, 4.34	62.5	4.25 m, 4.46 m
		l d (11.5)		l d (11.5)						
					Rha					
1					102.2	5.65 br s				
2					71.8	4.56				
3					72.0	4.42				
4					73.2	4.23				
5					69.9	4.76 m				
6					17.9	1.58 d (6.1)				
									Rha	
1									102.1	5.64 br s
2									72.0	4.55 m
3									72.9	4.06 m
4									73.2	4.04 m
5									69.6	4.74 m
6									18.0	1.58 d (5.9)

[a] 600 MHz (^{1}H), 125 MHz (^{13}C), pyridine-d_5, δ (ppm), coupling constants (J) in Hz are given in parentheses, overlapped signals are reported without designated multiplicity.

and the carbon at δ_C 104.0 (Ara C-1) and by NOESY cross-peaks between the proton at δ_H 4.88 (Ara H-1) and the proton at δ_H 4.56 (Glc III H-6a) and between the proton at δ_H 5.65 (Rha H-1) and the proton at δ_H 4.17 (Glc III, H-4). Thus, the structure of arboreaside C (**3**) was elucidated as 3-*O*-β-D-glucopyranosyl-(1→3)-β-D-xylopyranosyl-(1→2)-α-D-glucopyranosyloleanolic acid 28-*O*-[α-L-arabinopyranosyl-(1→6)]-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl ester.

The molecular formula of arboreaside D (**4**) was determined as $C_{59}H_{96}O_{26}$ by HRESIMS, showing the $[M + Na]^+$ ion peak at *m*/*z* 1243.613 (calcd for 1243.6088). Its FABMS (negative-ion mode) displayed a quasimolecular ion peak at *m*/*z* 1219 $[M - H]^-$, indicating a molecular weight of 1220. Further fragment ion peaks were observed at *m*/*z* 749 $[(M - H) - 162 - 162 - 146]^-$ and 455 $[(M - H) - 162 - 162 - 146 - 132 - 162]^-$, corresponding to the successive loss of two hexosyl, one deoxyhexosyl, one

pentosyl, and one hexosyl moiety, respectively. The fragment ion peak at *m/z* 455 is related to the pseudomolecular ion of aglycone.

Extensive studies of 1D and 2D NMR spectra data led to the identification of the aglycone of **4** as oleanolic acid.[16]

The ^{1}H NMR spectrum of **4**, showing five anomeric protons at δ_H 4.86 (d, J = 6.4 Hz), 4.90 (d, J = 7.8 Hz), 5.10 (d, J = 7.6 Hz), 5.66 (br s), and 6.10 (d, J = 8.0 Hz), giving correlations in the HSQC spectrum with five anomeric carbons at δ_C 104.4, 104.0, 105.0, 102.2, and 95.2 respectively, indicated the presence of five sugar moieties. Units of one α-rhamnopyranosyl (Rha), one α-arabinopyranosyl (Ara), two β-glucopyranosyl (Glc I and Glc II), and one β-galactopyranosyl (Gal) were identified. The absolute configuration of the sugar residues was D for Glc and Gal and L for Rha and Ara (see above).

HMBC correlations between the proton at δ_H 5.66 (br s, Rha H-1) and the carbon at δ_C 78.1 (Ara C-4), between the proton at δ_H 5.10 (d, J = 7.6 Hz, Glc I H-1) and the carbon at δ_C 79.7 (Ara C-2), and between the proton at δ_H 4.86 (d, J = 6.4 Hz, Ara H-1) and the carbon at δ_C 88.8 (Agly C-3) suggested the sequence at C-3 as [α-L-rhamnopyranosyl-(1→4)]-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl. This was confirmed by NOESY correlations between the proton at δ_H 5.66 (Rha H-1) and the proton at δ_H 4.21 (Ara H-4), between the proton at δ_H 5.10 (Glc I H-1) and the proton at δ_H 4.52 (Ara H-2), and between the proton at δ_H 4.86 (Ara H-1) and the proton at δ_H 3.14 (Agly H-3). Other HMBC correlations between the proton at δ_H 4.90 (Gal H-1) and the carbon at δ_C 68.6 (Glc II C-6) and between the proton at δ_H 6.10 (Glc II H-1) and the carbon at δ_C 176.5 (Agly C-28) and NOESY cross-peaks between the proton at δ_H 4.90 (d, J = 7.8 Hz, Gal H-1) and the proton at δ_H 4.24 (Glc II H-6a) allowed the sugar sequence at C-28 as β-D-galactopyranosyl-(1→6)-β-D-glucopyranosyl. Thus, arboreaside D (**4**) was elucidated as 3-*O*-[α-L-rhamnopyranosyl-(1→4)]-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyloleanolic acid 28-*O*-β-D-galactopyranosyl-(1→6)-β-D-glucopyranosyl ester.

The molecular formula of arboreoside E (**5**) was determined as $C_{54}H_{88}O_{22}$ by HRESIMS, showing the pseudomolecular ion peak $[M + Na]^+$ at *m/z* 1111.5671 (calcd for 1111.5665). The FABMS (negative-ion mode) displayed a quasimolecular ion peak at *m/z* 1087 $[M - H]^-$, indicating a molecular weight of 1088. Further fragment ion peaks were observed at *m/z* 925 $[(M - H) - 162]^-$, 617 $[(M - H) - 162 - 146 - 162]^-$, and 455 $[(M - H) - 162 - 146 - 162 - 162]^-$, corresponding to the sucessive loss of one hexosyl, one deoxyhexosyl, and two hexosyl moieties, respectively. The fragment ion peak at *m/z* 455 is related to the pseudomolecular ion of aglycone.

The 1D and 2D NMR spectroscopic data led to the conclusion that **5** is a bidesmosidic saponin having an oleanolic acid as aglycone (Tables 1 and 2).[16] The ^{1}H NMR spectrum of **5** showed four anomeric protons at δ_H 4.82 (d, J = 8.0 Hz), 4.88 (d, J = 7.8 Hz), 5.64 (br s), and 6.09 (d, J = 8.0 Hz), which gave correlations in the HSQC spectrum with four anomeric carbons at δ_C 106.1, 104.2, 98.4, and 95.1, respectively. 2D NMR data allowed the identification of one α-rhamnopyranosyl (Rha), two β-glucopyranosyl (Glc I and Glc II), and one β-galactopyranosyl (Gal) unit. The absolute configuration was D for Glc and Gal and L for Rha (see above).

The substitution of Agly C-3 by the terminal Glc I compared to oleanolic acid was suggested by HMBC correlations between the proton at δ_H 4.82 (d, J = 8.0 Hz, Glc I H-1) and the carbon at δ_C 88.8 (Agly C-3). This was confirmed by a NOESY cross-peak between the proton at δ_H 4.82 (Glc I H-1) and the proton at δ_H 3.31 (Agly H-3).

Additional HMBC correlations between the proton at δ_H 5.64 (br s, Rha H-1) and the carbon at δ_C 78.1 (Glc II C-2), between the proton at δ_H 4.88 (d, J = 7.8 Hz, Glc II H-1) and the carbon at δ_C 68.5 (Gal C-6), and between the proton at δ_H 6.09 (d, J = 8.0 Hz, Gal H-1) and the carbon at δ_C 176.1 (Agly C-28) established the sugar chain at C-28 as α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)-β-D-galactopyranosyl. The structure of arboreaside E (**5**) was thus elucidated as 3-*O*-β-D-glucopyranosyloleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)-β-D-galactopyranosyl ester.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on an AA-OR automatic polarimeter. IR spectra (KBr disk) were recorded on a Perkin-Elmer 281 spectrometer. 1D and 2D NMR spectra were recorded in pyridine-d_5 using a Varian INOVA-600 (600 MHz) NMR spectrometer. Solvent signals were used as internal standard (pyridine-d_5: δ_H 7.21, δ_C 123.5 ppm), and the coupling constants (J) are in Hz. HRESIMS (positive-ion mode) was carried out on a Q-TOF 1-micromass spectrometer and FABMS (negative-ion mode, glycerol matrix) on a Jeol-SX-102 mass spectrometer. GC analysis was carried out on a Thermoquest gas chromatograph. TLC and HPTLC were carried out on precoated silica gel plates $60F_{254}$ (Merck) ($CHCl_3$−MeOH−H_2O−HOAc, 65:32:6.5:0.5 and 70:30:5:0.5). Saponins were detected with the Komarowsky reagent. Isolations were carried out using column chromatography (CC) on silica gel 60 (Merck, 70−200 μm), CC on Sephadex LH-20, vacuum-liquid chromatography (VLC) on reversed-phase RP-18 (25−40 μm), medium-pressure liquid chromatography (MPLC) on silica gel 60 (Merck, 15−40 μm) (Gilson apparatus),[13] and flash chromatography (Combiflash, Serlabo; silica gel Redisep flash column, 15−40 μm, 3.5 × 14 cm, 40 g).

Plant Material. The bark of *C. arborea* was collected in the village Bangoua near Bangangté located in the Ndé Division of the Western Highlands of Cameroon in April 2007 and identified by Dr. P. Nana, botanist of the National Herbarium of Cameroon (NHC), Yaoundé, where a voucher specimen (N1545) was deposited.

Extraction and Isolation. Dried and finely powdered bark of *C. arborea* (1.0 kg) was macerated with MeOH (3 L) for 48 h. After evaporation of the solvent in vacuo, a dark residue of 140 g was obtained. A 13 g aliquot of this MeOH extract was submitted to VLC on RP-18 silica gel using H_2O (3 × 100 mL), MeOH−H_2O (5:5, 3 × 100 mL), and MeOH (200 mL). The MeOH-soluble portion was evaporated to dryness to afford a yellowish powder (7.6 g). An aliquot of 2.5 g was then fractionated by flash chromatography on silica gel using $CHCl_3$−MeOH−H_2O (62:32:6.5) as eluent, affording 10 fractions, F-1 (66.3 mg), F-2 (114.4 mg), F-3 (22.1 mg), F-4 (223.5 mg), F-5 (24.7 mg), F-6 (110.2 mg), F-7 (120 mg), F-8 (90.7 mg), F-9 (80.4 mg), and F-10 (10 mg).

F-8 (90.7 mg) was purified by successive MPLC on RP-18 silica gel eluted with MeOH−H_2O (40% → 100%), yielding **1** (29.1 mg), **2** (6.8 mg), and **4** (3.5 mg). F-9 (80.4 mg) was purified under the same conditions to yield **3** (10 mg). F-4 (223.5 mg) was purified by repeated MPLC on silica gel using a gradient system of solvent, $CHCl_3$−MeOH−H_2O (70:30:5 → 80:20:2), followed by MPLC on RP-18 silica gel with a MeOH−H_2O gradient system to yield **5** and ciwujianoside C_3.[11]

F-3 (22.1 mg) was obtained as a pure compound identified as 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside of 23-hydroxyursolic acid, identified by comparison of the reported spectroscopic data.[12]

Acid Hydrolysis and GC Analysis. Each compound (3 mg) was hydrolyzed with 2 N aqueous TFA (5 mL) for 3 h at 95 °C. After extraction with CH_2Cl_2 (3 × 5 mL), the aqueous layer was repeatedly evaporated to dryness with MeOH until neutral and then analyzed by TLC over silica gel ($CHCl_3$−MeOH−H_2O, 8:5:1) by comparison with authentic samples. The trimethylsilyl thiazolidine derivatives of the sugar residue of each compound were prepared and analyzed by GC.[17] The absolute configurations were determined by comparing the retention times with thiazolidine derivatives prepared in a similar way from standard sugars (Sigma-Aldrich). The following sugars were detected: D-glucose, D-galactose, L-rhamnose for **1** and **2**; D-glucose, D-xylose, L-arabinose, and L-rhamnose for **3**; D-glucose, D-galactose, L-arabinose, and L-rhamnose for **4**, and D-glucose, D-galactose, and L-rhamnose for **5**.

Arboreaside A (1): white, amorphous powder; $[\alpha]^{21}_D$ −33.5 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3440, 1726, 1655, 1260, 1074 cm^{-1}; ^{1}H NMR and ^{13}C NMR (pyridine-d_5), see Tables 1 and 2; FABMS (negative-ion mode) *m/z* 1103 $[M - H]^-$, 957 $[(M - H) - 146]^-$, 633 $[(M - H) - 146 - 162 - 162)]^-$, 471 $[(M - H) - 146 - 162 - 162 - 162]$;

HRESIMS (positive-ion mode) *m/z* 1127.5620 $[M + Na]^+$ (calcd for $C_{54}H_{88}O_{23}Na$, 1127.5614).

Arboreaside B (2): white, amorphous powder; $[\alpha]^{21}_D$ +52.2 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3450, 1736, 1649, 1250, 1072 cm^{-1}; ^1H NMR and ^{13}C NMR (pyridine-d_5), see Tables 1 and 2; FABMS (negative-ion mode) *m/z* 1103 $[M - H]^-$, 957 $[(M - H) - 146]^-$, 633 $[(M - H) - 146 - 162 - 162]^-$, 471 $[(M - H) - 146 - 162 - 162 - 162]^-$; HRESIMS (positive-ion mode) *m/z* 1127.5620 $[M + Na]^+$ (calcd for $C_{54}H_{88}O_{23}Na$, 1127.5614).

Arboreaside C (3): white, amorphous powder; $[\alpha]^{21}_D$ +20.9 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3445, 1730, 1640, 1250, 1076 cm^{-1}; ^1H NMR and ^{13}C NMR (pyridine-d_5), see Tables 1 and 2; FABMS (negative-ion mode) *m/z* 1351 $[M - H]^-$, 779 $[(M - H) - 162 - 132 - 132 - 146]^-$, 455 $[(M - H) - 162 - 132 - 132 - 146 - 162 - 162]^-$; HRESIMS (positive-ion mode) *m/z* 1375.6519 $[M + Na]^+$ (calcd for $C_{64}H_{104}O_{30}Na$, 1375.6510).

Arboreaside D (4): white, amorphous powder, $[\alpha]^{21}_D$ +25 (*c* 0.16, MeOH); IR (KBr) ν_{max} 3435, 1728, 1655, 1255, 1080 cm^{-1}; ^1H NMR and ^{13}C NMR (pyridine-d_5), see Tables 1 and 2; FABMS (negative-ion mode) *m/z* 1219 $[M - H]^-$, 749 $[(M - H) - 162 - 162 - 162]^-$; HRESIMS (positive-ion mode) *m/z* 1243.613 $[M + Na]^+$ (calcd for $C_{59}H_{96}O_{26}Na$, 1243.6088).

Arboreaside E (5): white, amorphous powder; $[\alpha]^{21}_D$ +30 (*c* 0.22, MeOH); MeOH); IR (KBr) ν_{max} 3435, 1735, 1660, 1260, 1078 cm^{-1}; ^1H NMR and ^{13}C NMR (pyridine-d_5), see Tables 1 and 2; FABMS (negative-ion mode) *m/z* 108 $[M - H]^-$, 925 $[(M - H) - 162]^-$, 617 $[(M - H) - 162 - 146 - 162]^-$, 455 $[(M - H) - 162 - 146 - 162 - 162]^-$; HRESIMS (positive-ion mode) *m/z* 1111.5671 $[M + Na]^+$ (calcd for $C_{54}H_{88}O_{22}Na$, 1111.5665).

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