TRITERPENE GLYCOSIDES FROM *Cussonia paniculata*. I. ISOLATION AND STRUCTURE DETERMINATION OF GLYCOSIDES A, B_1, B_2, C, D, G_2, H_1, AND H_2 FROM LEAVES OF *Cussonia paniculata*

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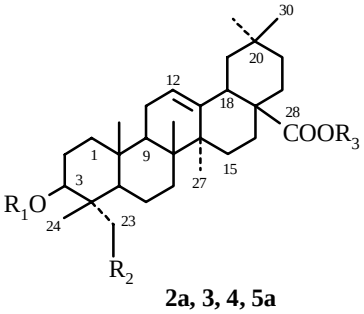
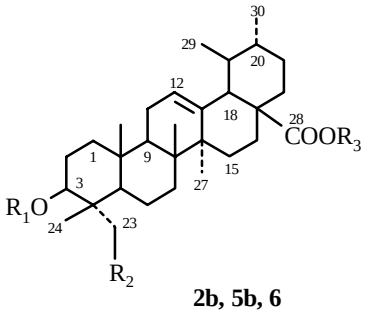
*The 3-O-β-D-glucopyranoside of β-sitosterol (**1**) and the known triterpene glycosides 3-O-α-L-arabinopyranosides of oleanolic (**2a**) and ursolic (**2b**) acids and hederagenin (**3**), 3-O-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside of oleanolic acid (**4**), 3-O-α-L-arabinopyranosyl-28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosides of oleanolic (**5a**) and ursolic (**5b**) acids and the new glycoside 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranoside of 23-hydroxyursolic acid (**6**) were isolated from leaves of* Cussonia paniculata *(Araliaceae). Their structures were established using chemical methods and NMR spectroscopy.*

Key words: triterpene glycosides, *Cussonia paniculata*, Araliaceae.

Cussonia paniculata Eckl. et Zeih. (Araliaceae Juss.) grows on Madagascar and in southeastern Africa. The *Cussonia* genus includes about 40 species, of which the phytochemistry was previously studied for *C. spicata* [1], *C. barteri* [2], *C. racemosa* [3, 4], *C. bancoensis* [5], *C. bojeri* [6], and *C. vantsilana* [7]. Triterpene glycosides were found in *C. spicata*, *C. barteri*, and *C. bancoensis*; diterpene glycosides of the *ent*-caurane type; in *C. bojeri* and *C. vantsilana*. Both triterpene [3] and diterpene glycosides of the *ent*-caurane, clerodane, and labdane types [4] were isolated from *C. racemosa*. Plants of the *Cussonia* genus are used in folk medicine to treat malaria, rheumatism, diarrhea, and psychic illnesses [3, 4]. Glycosides of *C. spicata* are known as molluscocides and spermicides [1]; of *C. barteri*, as sedatives [2]; of *C. bancoensis*, as inhibitors of nitrogen oxide (NO) formation in biotests [5].



2a, 3, 4, 5a



2b, 5b, 6

	R_1	R_2	R_3
2a:	α-L-Arap→	H	H
2b:	α-L-Arap→	H	H
3:	α-L-Arap→	OH	H
4:	β-D-Glcp-(1→2)-α-L-Arap→	H	H

	R_1	R_2	R_3
5a:	α-L-Arap→	H	←β-D-Glcp-(6←1)-β-D-Glcp-(4←1)-α-L-Rhap
5b:	α-L-Arap→	H	←β-D-Glcp-(6←1)-β-D-Glcp-(4←1)-α-L-Rhap
6:	H	OH	←β-D-Glcp-(6←1)-β-D-Glcp-(4←1)-α-L-Rhap

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TABLE 1. Chemical Shifts for ^{13}C of Algycons of Glycosides B_1 (**2a**), B_2 (**2b**), C (**3**), D (**4**), H_1 (**5a**), H_2 (**5b**), G_2 (**6**), and Protons of the Aglycon of Glycoside G_2 (**6**) (δ, ppm, 0 = TMS, C_5D_5N)

C atom	Compound						
	2a, **4**	**2b**	**3**	**5a**	**5b**	**6**	**6** (^1H)
1	38.8	39.0	38.9	38.9	39.1	39.1	1.57, 1.01
2	26.5	26.6	26.0	26.6	26.7	27.6	1.94, 1.89
3	88.7	88.7	82.0	88.8	88.8	73.7	4.15
4	39.5	39.6	43.5	39.6	39.4	42.7	-
5	56.0	56.0	47.8	56.1	56.0	48.6	1.43
6	18.5	18.5	18.3	18.6	18.6	18.7	1.59, 1.38
7	33.3	33.5	33.0	33.2	33.6	33.3	1.64, 1.34
8	39.8	40.0	39.8	40.0	40.2	40.3	-
9	48.0	48.0	48.2	48.1	48.1	48.2	1.66
10	37.0	36.9	36.9	37.0	37.1	37.2	-
11	23.8	23.7	23.8	23.8	23.8	23.9	1.96, 1.94
12	122.5	125.6	122.6	122.9	126.1	126.2	5.44
13	144.8	139.3	144.8	144.1	140.5	138.6	-
14	42.2	42.6	42.1	42.2	42.5	42.9	-
15	28.3	28.7	28.3	28.3	28.8	28.9	2.36, 1.13
16	23.8	25.0	23.8	23.5	24.7	24.7	2.04, 1.94
17	46.7	48.1	46.7	47.1	48.5	48.7	-
18	42.0	53.6	42.0	41.8	53.3	53.4	2.48
19	46.6	39.5	46.5	46.3	39.1	39.5	1.39
20	30.9	39.4	30.9	30.8	39.5	39.2	0.89
21	34.3	31.2	34.3	34.1	30.3	31.0	1.37, 1.24
22	33.3	37.5	33.2	32.6	36.8	36.9	1.90, 1.75
23	28.2	28.2	64.4	28.3	28.4	68.0	4.11, 3.66
24	17.0	17.0	13.7	17.0	17.0	13.2	1.02
25	15.5	15.6	16.1	15.7	15.8	16.4	1.00
26	17.4	17.4	17.4	17.6	17.7	17.9	1.14
27	26.3	23.9	26.2	26.1	23.9	23.9	1.13
28	180.1	179.8	180.2	176.6	176.3	176.6	-
29	33.3	17.5	33.2	33.2	17.5	17.5	0.93
30	23.8	21.4	23.8	23.7	21.4	21.4	0.89

Here and in Table 2 in columns containing data for several compounds, average values of chemical shifts are given that differ from the individual compounds on the average by less than ±0.15 ppm.

We investigated the glycoside composition of the previously unstudied species *Cussonia paniculata* Eckl. et Zeih. TLC of the alcohol extract of leaves from this plant detected 12 groups of glycosides designated from A through L in the order of increasing polarity. Two-dimensional (2D) chromatography [8] established the presence in the extract of the usual mono- and bisdesmoside glycosides, a significant quantity of glycosides containing native acyl groups, and a small amount of acidic glycosides with glucuronic acid units.

Chromatographic separation of the purified total glycosides over silica gel produced pure glycosides designated A (**1**), B (**2**), C (**3**), D (**4**), and H (**5**), respectively. Fraction G was rechromatographed over Silpearl microsphere silica gel to produce glycosides G_1, G_2 (**6**), and G_3. The structures of the glycosides were determined by chemical methods and NMR spectroscopy.

TABLE 2. ^{13}C Chemical Shifts of Carbohydrates of Glycosides B_1 (**2a**), B_2 (**2b**), C (**3**), D (**4**), H_1 (**5a**), H_2 (**5b**), and G_2 (**6**) (δ, ppm, 0 = TMS, C_5D_5N)

C atom	Carbohydrate part on aglycon C-3			C atom	Carbohydrate part on aglycon C-28		
	2a, **2b**, **5a**, **5b**	**3**	**4**		**5a**	**5b**	**6**
Ara-1	107.1	106.7	104.7	Glc-1	95.6	95.6	95.7
2	72.8	73.2	80.7	2	73.8	73.8	73.7
3	74.5	74.8	73.4	3	78.6	78.6	78.6
4	69.3	69.7	68.2	4	70.8	71.0	70.9
5	66.4	66.9	64.7	5	78.0	77.9	77.9
				6	69.2	69.2	69.4
Glc-1			105.7	Glc-1	104.8	104.9	104.8
2			76.2	2	75.3	75.3	75.2
3			78.2	3	76.5	76.5	76.5
4			71.5	4	78.5	78.5	78.6
5			78.1	5	77.1	77.1	77.1
6			62.5	6	61.3	61.3	61.4
				Rha-1	102.7	102.7	102.7
				2	72.5	72.5	72.5
				3	72.7	72.7	72.7
				4	73.9	73.9	73.9
				5	70.4	70.4	70.4
				6	18.5	18.5	18.5

TLC analysis of **1**, **3**, and **4** showed that they were identical to known samples of the 3-O-β-D-glucopyranoside of β-sitosterol, the 3-O-α-L-arabinopyranoside of hederagenin, and the 3-O-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside of oleanolic acid. The structures of **3** and **4** were additionally confirmed by total acid hydrolysis with subsequent TLC analysis of the resulting sugars and aglycons and by PMR and ^{13}C NMR spectra (Tables 1 and 2), which were identical to those in the literature [9].

Total acid hydrolysis of **2** produced arabinose and the aglycon, which coincided in chromatographic mobility with oleanolic acid. Signals in the ^{13}C NMR spectrum of the α-L-arabinopyranosyl fragment were assigned according to the literature [10]. However, analysis of the aglycon part of the spectrum, as previously described [11], showed the presence of two aglycons, oleanolic and ursolic acids in a 3:2 mole ratio. This followed from the relative signal strengths.

Thus, **2** is a mixture of two chromatographically inseparable glycosides designated B_1 (**2a**) and B_2 (**2b**) that are the known 3-O-α-L-arabinopyranosides of oleanolic and ursolic acids.

Total acid hydrolysis of **5** produced arabinose, glucose, rhamnose, and the aglycon, which had the same chromatographic mobility as oleanolic acid. The progenin of this glycoside obtained from alkaline hydrolysis was identical by TLC with **2**. Signals in the ^{13}C NMR spectrum of **5** for α-L-arabinopyranosyl and α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl fragments were assigned based on the literature [10]. Like for **2**, analysis of the aglycon part of the ^{13}C NMR spectrum showed the presence of two aglycons, oleanolic and ursolic acids in a 3:2 mole ratio. Thus, glycoside **5** is a mixture of two previously known isomeric glycosides H_1 (**5a**) and H_2 (**5b**), 3-O-α-L-arabinopyranosyl-28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosides of oleanolic and ursolic acids. Tables 1 and 2 list the ^{13}C NMR spectra of **5a** and **5b**.

Total acid hydrolysis of **6** produced glucose, rhamnose, and the aglycon, which had the same chromatographic mobility as hederagenin but differed from it in the color of the chromatographic band upon development by phosphotungstic acid. A compound that formed upon alkaline hydrolysis had the same chromatographic mobility as the aglycon, which indicated the lack of a carbohydrate chain on the C-3 hydroxyl of the aglycon. Signals of the α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl fragment were easily assigned in the ^{13}C NMR spectrum of **6** by comparison with much literature data, e.g., [10]. The structure of the aglycon was established as follows. It was assumed based on the

chromatographic mobility being identical to that of hederagenin that the aglycon was isomeric with hederagenin. This was confirmed by the similarity of the chemical shifts of the C atoms of rings A and B of the aglycon to those of C atoms in rings A and B of hederagenin. It was also noted that the signals for C atoms in rings C, D, and E of the aglycon were similar to those of C atoms in rings C, D, and E of 28-O-glycosylated ursolic acid [10, 11]. Therefore, the structure 23-hydroxyursolic acid was proposed for the aglycon. Then this was confirmed by comparing the subspectrum of the aglycon part of **5** with literature data for 23-hydroxyursolic acid and its glycosides, which were isolated from *Patrinia scabiosaefolia* (Valerianaceae) [12]. Signals in the aglycon part of **5** were also unambiguously assigned by combined analysis of 2D HSQC, COSY, and TOCSY spectra beginning with several unambigous signals for C atoms (C-2, C-3, C-7, C-12, C-18, C-21, etc.). Assignments of pairs of closely lying signals for C-10 and C-22 and C-26 and C-29 were corrected by comparison with the literature [12]. Thus, glycoside G_2 (**6**) is the 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranoside of 23-hydroxyursolic acid and is a new triterpene glycoside. Tables 1 and 2 contain the PMR and ^{13}C NMR data for **6**.

EXPERIMENTAL

NMR spectra were obtained on a Bruker-500DRX (500 MHz for ^{1}H and 125 MHz for ^{13}C) instrument in deuteropyridine. Standard Bruker programs were used for 2D HSQC, COSY, and TOCSY experiments. TLC monitoring was carried out on Silufol plates using solvent systems $CHCl_3$:CH_3OH:H_2O (100:40:7 and 100:30:5) and $CHCl_3$:CH_3OH:NH_4OH (25%) (100:50:15, 100:40:10, 100:30:6, and 100:20:3). Glycosides and aglycons were detected with alcoholic phosphotungstic acid (10%); sugars, anilinium acid phthalate with subsequent heating of the chromatograms. Preparative separation was performed on silica gel L (40-100 μm) and Silpearl microsphere silica gel.

Isolation of Glycosides. Dried leaves (5.6 g) of *Cussonia paniculata* were obtained from the Botanical Garden of the Botanical Institute of the Russian Academy of Sciences, ground, and defatted with benzene (3×100 mL). Glycosides were extracted with isopropanol (80%, 4×150 mL). Evaporation of the alcohol extract in vacuum gave a dry solid (1.6 g) that was dissolved in water-saturated butanol (250 mL) and washed with water (3×100 mL). Evaporation of the butanol layer gave purified total triterpene glycosides (0.85 g).

Separation of Glycosides. The purified total triterpene glycosides were separated by chromatography over silica gel L with gradient elution by water-saturated $CHCl_3$:isopropanol (6:1→1:1) to afford 12 fractions of triterpene glycosides A (20 mg), B (17 mg), C (45 mg), D (26 mg), E (90 mg), F (57 mg), G (324 mg), H (45 mg), I (181 mg), J (27 mg), K (27 mg), and L (30 mg). Fraction G was separated over Silpearl microsphere silica gel with elution by water-saturated $CHCl_3$:isopropanol (3:1) to produce glycosides G_1 (32 mg), G_2 (149 mg), and G_3 (143 mg).

Total Acid Hydrolysis. Aglycons were determined by dissolving glycoside (1 mg) in a mixture (1:1, 0.2 mL) of aqueous H_2SO_4 (2 N) and CH_3OH with heating at 100°C for 2 h. Aglycons were extracted with $CHCl_3$. The organic layer was washed with water until neutral and analyzed by TLC using $CHCl_3$:CH_3OH:NH_4OH (25%) (100:20:3) and comparison with known samples.

Sugars were determined by heating glycoside (1 mg) with CF_3CO_2H (1 N) in dioxane (0.2 mL) at 100°C for 2 h. The hydrolysate was analyzed without working it up by TLC using $CHCl_3$:CH_3OH:NH_4OH (25%) (100:40:10) and known carbohydrate samples.

Alkaline Hydrolysis. Glycoside (2 mg) was dissolved in KOH (0.5 mL, 10%) in CH_3OH:H_2O (1:1), heated at 100°C for 2 h, and neutralized with H_2SO_4 (1 N) until weakly acidic. Progenins were extracted with butanol. The butanol layer was washed with water until neutral and analyzed by TLC by comparison with known samples of monodesmoside glycosides.

REFERENCES

1. J. Gunzinger, J. D. Msonthi, and K. Hostettmann, *Phytochemistry*, **25**, 2501 (1986).
2. M. A. Duboiss, M. Ilyas, and H. Wagner, *Planta Med.*, 80 (1986).
3. L. Harinantenaina, R. Kasai, and K. Yamasaki, *Chem. Pharm. Bull.*, **50**, 1290 (2002).
4. L. Harinantenaina, R. Kasai, and K. Yamasaki, *Chem. Pharm. Bull.*, **50**, 268 (2002); L. Harinantenaina, R. Kasai, and K. Yamasaki, *Phytochemistry*, **60**, 339 (2002).

5. L. A. Taponjou, D. Lontsi, B. L. Sondengam, F. Shaheen, M. I. Choudhary, Atta-ur-Rahman, F. R. van Heerden, H. J. Park, and K. T. Lee, *J. Nat. Prod.*, **66**, 1266 (2003).
6. L. Harinantenaina, R. Kasai, and K. Yamasaki, *Chem. Pharm. Bull.*, **50**, 1122 (2002).
7. L. Harinantenaina, R. Kasai, and K. Yamasaki, *Phytochemistry*, **61**, 367 (2002).
8. V. I. Grishkovets, *Khim. Prir. Soedin.*, 53 (2001).
9. V. I. Grishkovets, E. A. Sobolev, A. S. Shashkov, and V. Ya. Chirva, *Khim. Prir. Soedin.*, 395 (2000).
10. A. S. Stolyarenko, V. I. Grishkovets, N. N. Arnautov, S. V. Iksanova, and V. Ya. Chirva, *Khim. Prir. Soedin.*, 136 (2000).
11. V. I. Grishkovets, *Khim. Prir. Soedin.*, 627 (1999).
12. A. Inada, M. Yamada, H. Murata, M. Kobayashi, H. Toya, Y. Kato, and T. Nakanishi, *Chem. Pharm. Bull.*, **36**, 4269 (1988).