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TRITERPENE GLYCOSIDES FROM *Cussonia paniculata*. III. STRUCTURE OF GLYCOSIDES I_1, I_2, J_{1a}, J_{1b}, J_2, K, L_1, AND L_2 FROM *C. paniculata* LEAVES

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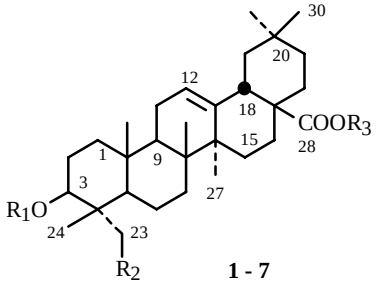
*Structures of eight triterpene glycosides, of which the 28-*O*-(2-*O*-acetyl- and 3-*O*-acetyl-α-L-rhamnopyranosyl)-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl esters of hederagenin 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranoside (J_{1a} and J_{1b}) were new, from* Cussonia paniculata *(Araliaceae) leaves were established using chemical and NMR spectroscopic methods.*

Key words: triterpene glycosides, oleanolic acid and hederagenin glycosides, *Cussonia paniculata*, Araliaceae.

We reported previously [1, 2] on the principal triterpene glycosides from leaves of *Cussonia paniculata* Eckl. et Zeih. Herein we report the establishment of the structures of minor glycosides from leaves of this plant.

Glycosides I_1 (**1**) and I_2 (**2**) were prepared by separating fraction I [2]. According to TLC and comparison with authentic samples, **1** was oleanolic acid 3-*O*-β-D-glucuronopyranoside; **2**, the 28-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl ester of hederagenin 3-*O*-α-L-arabinopyranoside. Chemical shifts in the ^{13}C NMR spectra of **1** and **2** agreed with the literature data [3, 4] and confirmed the structures of these glycosides.



1 - 7

	R_1	R_2	R_3
1:	β-D-GlcUA*p*→	H	H
2:	α-L-Ara*p*→	OH	←β-D-Glc*p*-(6←1)-β-D-Glc*p*-(4←1)-α-L-Rha*p*
3a:	β-D-Glc*p*-(1→2)-α-L-Ara*p*→	OH	←β-D-Glc*p*-(6←)-β-D-Glc*p*-(4←1)-α-L-Rhap^2 ←OAc
3b:	β-D-Glc*p*-(1→2)-α-L-Ara*p*→	OH	←β-D-Glc*p*-(6←)-β-D-Glc*p*-(4←1)-α-L-Rhap^3 ←OAc
4:	β-D-GlcUA*p*→	OH	←β-D-Glc*p*
5:	β-D-Glc*p*-(1→2)-α-L-Ara*p*→	H	←β-D-Glc*p*-(6←1)-β-D-Glc*p*-(4←1)-α-L-Rha*p*
6:	β-D-Glc*p*-(1→2)-α-L-Ara*p*→	OH	←β-D-Glc*p*-(6←1)-β-D-Glc*p*-(4←1)-α-L-Rha*p*
7:	β-D-Glc*p*-(1→2)-β-D-GlcUA*p*	H	←β-D-Glc*p*

Fraction J was prepared by separating the purified total triterpene glycosides [1]. According to two-dimensional (2D) TLC [5], fraction J contained both neutral and acidic triterpene glycosides. Furthermore, the neutral glycosides (also using 2D TLC with intermediate storage in ammonia vapor) also contained acyl groups. Therefore, fraction J was separated over basic $MgCO_3$ [6] to afford glycosides J_1 and J_2.

Glycoside J_1 (**3**) was a chromatographically pure compound. Total acid hydrolysis of **3** produced glucose, rhamnose, arabinose, and hederagenin. Alkaline hydrolysis of **3** produced hederagenin 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranoside according to TLC [7]. Deacylation of **3** gave the 28-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl ester of hederagenin 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranoside according to TLC and comparison with an authentic sample [7]. The structures of the carbohydrate chains and the nature and location of the acyl

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TABLE 1. ^{13}C Chemical Shifts of Aglycons of Glycosides **1**, **2**, **3a**, **3b**, **4**, **5**, **6**, and **7** (δ, ppm, 0 = TMS, C_5D_5N)

C atom	**1**	**2, 3a, 3b, 4, 6**	**5, 7**	C atom	**1**	**2, 3a, 3b, 4, 6**	**5, 7**
1	38.8	38.9	38.7	16	23.8	23.7	23.4
2	26.5	26.0	26.6	17	46.7	47.0	47.0
3	88.7	82.0	89.1	18	42.0	41.9	41.8
4	39.5	43.5	39.6	19	46.6	46.3	46.2
5	56.0	47.8	55.8	20	30.9	30.9	30.8
6	18.5	18.3	18.5	21	34.3	34.1	34.0
7	33.3	33.0	33.1	22	33.3	32.6	32.6
8	39.8	39.8	39.9	23	28.2	64.6	28.2
9	48.0	48.2	48.0	24	17.0	13.7	16.7
10	37.0	36.9	36.9	25	15.5	16.2	15.5
11	23.8	23.8	23.8	26	17.4	17.6	17.5
12	122.5	122.6	122.9	27	26.3	26.2	26.1
13	144.8	144.2	144.1	28	180.1	176.6	176.5
14	42.2	42.1	42.2	29	33.3	33.2	33.1
15	28.3	28.3	28.2	30	23.8	23.8	23.7

group in **3** were confirmed using PMR and ^{13}C NMR spectra and 2D COSY, TOCSY, HSQC, HMBC, and ROESY methods, as previously described [2]. This showed that **3** was not a pure compound but a chromatographically inseparable mixture of two isomeric glycosides J_{1a} (**3a**) and J_{1b} (**3b**) that were the 28-*O*-(2-*O*-acetyl- and 3-*O*-acetyl-α-L-rhamnopyranosyl)-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl esters of hederagenin 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranoside, respectively, These are new triterpene glycosides and were present in the fraction in a 1:1 mole ratio according to the ratio of integrated intensities of the signals of identical C atoms in **3a** and **3b**, respectively.

The acid hydrolysate of J_2 (**4**) contained glucose, glucuronic acid, and hederagenin. Alkaline hydrolysis of **4** produced hederagenin 3-*O*-β-D-glucuronopyranoside. The ^{13}C NMR spectrum of **4** contained signals of β-D-glucuronopyranosyl, β-D-glucopyranosyl, and 3,28-disubstituted hederagenin moieties according to the literature [8, 9]. Thus, **4** was the known 28-*O*-β-D-glucopyranosyl ester of hederagenin 3-*O*-β-D-glucuronopyranoside.

Fraction K [1] was chromatographically pure glucoside K (**5**). Total acid hydrolysis of **5** gave arabinose, rhamnose, and glucose in addition to oleanolic acid. The progenin of **5** was identified using TLC and an authentic sample of oleanolic acid 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranoside [7]. Glycoside **5** itself was identified using TLC and ^{13}C NMR as the 28-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl ester of oleanolic acid 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranoside [7].

Fraction L [1] was also separated over basic $MgCO_3$ into neutral L_1 (**6**) and acidic L_2 (**7**) glycosides.

Acid hydrolysis of **6** produced glucose, rhamnose, arabinose, and hederagenin. The progenin of **6** was identified using TLC as hederagenin 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranoside [7]. Glycoside **6** itself was according to TLC and ^{13}C NMR spectroscopy the known 28-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl ester of hederagenin 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranoside [7].

The acid hydrolysate of **7** contained glucose, glucuronic acid, and the aglycon oleanolic acid. Glucose and glucuronic acid were found in the progenin from the alkaline hydrolysate of **7**. The ^{13}C NMR spectrum of **7** (Tables 1 and 2) was compared with the literature [9] in order to assign signals of the β-D-glucopyranosyl fragment, which was bound to the aglycon carboxylic group, and 3,28-disubstituted oleanolic acid. A signal in the low-field part of the spectrum at 172.5 ppm was assigned to the carboxylic group of glucuronic acid. The structure of the carbohydrate bound to the aglycon C-3 hydroxyl was established as follows. After signals of anomeric protons in the ^{13}C NMR spectrum of the 28-*O*-glucopyranosyl fragment were assigned, there remained two unassigned signals at 105.3 and 105.9 ppm. The HSQC spectrum was used to find the anomeric protons (at 5.02 and 5.42 ppm) corresponding to these. Both of these signals in the one-dimensional PMR spectrum were doublets with SSCC of 8 Hz. Because only two carbohydrates were found in the acid hydrolysate of the progenin, these signals belonged to β-D-glucose and β-D-glucuronic acid. Signals in the PMR spectrum (Table 3) were fully assigned using a combination of COSY and TOCSY spectra. Signals in the ^{13}C NMR spectrum were assigned using proton signals and the HSQC spectrum.

TABLE 2. ^{13}C Chemical Shifts of Carbohydrates of Glycosides **1**, **2**, **3a**, **3b**, **4**, **5**, **6**, and **7** (δ, ppm, 0 = TMS, C_5D_5N)

Aglycon C-3						Aglycon C-28				
C atom	**2**	**3a, 3b, 5, 6**	**1**	**4**	**7**	C atom	**4, 7**	**2, 5, 6**	**3a**	**3b**
	Ara-	-Ara-	GlcUA-	GlcUA-	-GlcUA-		Glc-	-Glc-	-Glc-	-Glc-
1	106.7	104.7	107.1	106.1	105.3	1	95.6	95.7	95.7	95.7
2	73.2	80.7	75.4	75.5	82.7	2	74.1	73.8	73.7	73.7
3	74.8	73.4	78.0	78.0	77.4	3	78.8	78.6	78.4	78.4
4	69.7	68.2	73.3	73.3	73.1	4	71.1	70.8	70.6	70.7
5	66.9	64.7	76.9	77.7	77.7	5	79.2	78.0	77.9	77.8
6			172.9	172.9	172.5	6	62.2	69.4	69.0	69.1
		Glc-			Glc-			-Glc-	-Glc-	-Glc-
1		105.7			105.9	1		104.9	104.7	104.9
2		76.2			77.0	2		75.3	75.4	75.3
3		78.2			77.9	3		76.5	76.2	76.2
4		71.5			71.7	4		78.4	77.2	78.1
5		78.1			78.3	5		77.1	77.1	76.9
6		62.5			62.8	6		61.3	61.0	61.2
								Rha-	Rha-	Rha-
						1		102.8	98.9	102.4
						2		72.5	74.3	70.0
						3		72.7	70.3	76.1
						4		73.9	74.2	70.7
						5		70.4	70.0*	70.4*
						6		18.6	18.2	18.3
						-OAc			20.9**	21.1**
						-OAc			171.0	171.0

Signals marked (*) and (**) are assigned arbitrarily.

Columns in Tables 1 and 2 that list several compounds give averaged chemical shifts; deviations from the mean for individual compounds are less than ±0.1 ppm.

TABLE 3. ^{1}H Chemical Shifts of Carbohydrates in **7** (δ, ppm, 0 = TMS, C_5D_5N)

Carbohydrate fragment			
aglycon C-3		aglycon C-28	
H atom	chemical shift	H atom	chemical shift
	-GlcUA-		Glc-
1	5.02	1	6.32
2	4.37	2	4.22
3	4.61	3	4.29
4	4.57	4	4.35
5	4.41	5	4.05
		6A	4.47
		6B	4.40
	Glc-		
1	5.42		
2	4.14		
3	4.26		
4	4.31		
5	3.95		
6A	4.51		
6B	4.47		

Glycosylation effects in the ^{13}C NMR spectrum revealed the presence of a 1→2 bond between the terminal glucopyranose and glucuronic acid when compared with an unsubstituted 3-*O*-*β*-D-glucuronopyranosyl moiety. This was also confirmed by the 2D ROESY and HMBC spectra. Thus, **7** was the known 28-*O*-*β*-D-glucopyranosyl ester of oleanolic acid 3-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-*β*-D-glucuronopyranoside.

The glycoside composition of *C. paniculata* leaves is the same relative to the content of many glycosides as that of *Fatsia japonica* leaves [7], despite the fact that the systematic genera *Fatsia* and *Cussonia* in the Araliaceae family are not close [10].

EXPERIMENTAL

General comments have been published [1].

NMR spectra were obtained on a Bruker-500 DRX instrument (500 MHz for ^1H; 125 MHz, ^{13}C) in C_5D_5N. 2D HSQC, COSY, and TOCSY experiments used standard Bruker programs.

The following solvent systems were used for 2D TLC to determine acidic glycosides: $CHCl_3$:CH_3OH:H_2O (100:30:5) in the first direction and the same system with 3-5% added formic acid in the second (perpendicular) direction. Glycosides containing acyl groups were determined using $CHCl_3$:CH_3OH:H_2O (100:40:7) or $CHCl_3$:CH_3OH:NH_4OH (25%) (100:40:10) in both directions by storing the plates in a closed chamber with ammonia vapor for 2 h before chromatography in the second direction. Spots in 2D chromatograms were developed using the usual developers (for example, phosphotungstic acid) after careful removal of traces of formic acid or ammonia by blowing the plates with hot air.

Separation of Triterpene Glycosides. Fractions J and L [1] were separated over basic $MgCO_3$ with elution by water-saturated $CHCl_3$: isopropanol (2:1) and (1:1), respectively, to afford J_1 (**3**, 15 mg), J_2 (**4**, 12 mg), L_1 (**6**, 12 mg), and L_2 (**7**, 18 mg).

Identification and Establishment of the Glycoside Structures. Glucosides **1** and **2** and progenins from **3**-**6** that were obtained by alkaline hydrolysis were identified preliminarily using TLC and authentic samples [3, 4, 7, 8]; monosaccharides and aglycons of **3**-**6**, using TLC with authentic samples after acid hydrolysis.

Tables 1 and 2 give the ^{13}C NMR spectra of **1**-**7**. Table 3 lists the PMR spectrum of **7**.

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