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TRITERPENE GLYCOSIDES OF *Scheffleropsis angkae.* II. STRUCTURE OF GLYCOSIDES L-E_1, L-E_2, L-K_1, AND L-K_2

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The structures of four triterpene glycosides from leaves of Scheffleropsis angkae *(Araliaceae) are established using chemical and NMR methods. The structures 3-O-β-D-glucopyranosyl-(1→3)-O-α-L-arabinopyranosides of oleanic and ursolic acids and their 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-gentiobiosyl ethers are proposed for L-E_1, L-E_2, L-K_1, and L-K_2, respectively. L-K_1 and L-K_2 are new triterpene glycosides.*

Key words: *Scheffleropsis angkae*, triterpene glycosides, 3-O-β-D-glucopyranosyl-(1→3)-O-α-L-arabinopyranosyl and -28-O-α-L-rhamnopyranosyl-(1→4)-O-β-gentiobiosyl ethers of oleanic and ursolic acids.

We previously described the isolation of the total glycosides from leaves of *Scheffleropsis angkae* (Craib.) Grushv. et N. Skvorts. (Araliaceae), the separation of it into fractions L-A through L-K, and the determination of the structures of glycosides L-B_1, L-B_2, L-H_1, and L-H_2 [1].

In the present article, results are presented from a study of fractions L-E and L-K, which, according to TLC using various solvent systems, contains pure saponins. The colors of the spots of these compounds in the chromatograms were pink, like for glycosides of oleanic acid, upon detection by a solution of phosphotungstic acid and brownish-green upon detection by acidic reagents with an excess of *p*-hyhdroxybenzaldehyde, like for ursolic acid and its glycosides. Analogous observations were made for glycosides L-B and L-H, which are mixtures of isomeric saponins of oleanic and ursolic acids. We propose the same genins for L-E and L-K.

Total acid hydrolysis of L-E (**1**) produced arabinose and glucose and a mixture of oleanic and ursolic acids, which were identified by TLC. Glycoside L-E is methylated by CH_2N_2 and is not hydrolyzed by alkali. This suggests that this saponin is a monodesmoside and that the saccharide chain is located on C-3 of the aglycones. Partial acid hydrolysis yields the starting glycoside and the aglycones in addition to a chromatographically inseparable mixture of the 3-O-α-L-arabinopyranosides of oleanic and ursolic acids (glycosides L-B_1 and L-B_2) [1]. This defines the sequence of sugars in the chain.

The nature of the glycosidic bond between the glucose and arabinose residues was studied by comparing **1** with authentic samples of 3-O-β-D-glycopyranosyl-O-α-L-arabinopyranosides of oleanic acid with 1→2 and 1→4 bonds. Neither of these had an R_f value identical to that of L-E. Then, the proposed 1→3 bond was proved using periodate oxidation, which destroyed only the glucose and formed the saponin L-B. Chromatographic and chemical methods demonstrated that glycoside L-E is probably a mixture of the 3-O-glucosyl-(1→3)-O-α-L-arabinopyranosides of oleanic and ursolic acids (**1a** and **1b**, respectively).

The structure of L-E was further established using NMR spectroscopy. The low-field part of the PMR spectrum of **1** contains a pseudotriplet at 5.45 ppm that is assigned to vinylic H-12 of the aglycone, a doublet for the anomeric proton at 5.15 ppm with spin—spin coupling constant (SSCC) 8 Hz, and two closely lying doublets near 4.7 ppm that have identical SSCCs of 6 Hz with a 1H total integrated intensity divided into a 2:1 ratio. Obviously the doublet at 5.15 ppm belongs to H-1″ of the glucose; the two signals with δ 4.68 and 4.67, to H-1′ of the arabinose bonded to the isomeric aglycones.

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TABLE 1. Chemical Shifts of ^{13}C Atoms of Glycosides E_1 (**1a**), E_2 (**1b**), K_1(**2a**), and K_2 (**2b**) (δ, ppm, 0 = TMS, C_5D_5N)

C atom	Compound				C atom	Compound			
	1a	**1b**	**2a**	**2b**		**1a**	**1b**	**2a**	**2b**
					Ara′				
1	38.9	39.0	38.9	39.1	1	107.0	107.0	107.0	107.0
2	26.6	26.6	26.6	26.6	2	71.6	71.6	71.5	71.5
3	88.7	88.7	88.7	88.7	3	83.9	83.9	83.9	83.9
4	39.6	39.6	39.5	39.5	4	69.1	69.1	69.0	69.0
5	56.0	56.0	55.9	55.9	5	66.6	66.6	66.6	66.6
6	18.5	18.5	18.5	18.5	Glc″				
7	33.2	33.5	33.1	33.5	1	105.8	105.8	105.8	105.8
8	39.8	40.0	39.9	40.1	2	75.5	75.5	75.5	75.5
9	48.0	48.0	48.1	48.1	3	78.2	78.2	78.1	78.1
10	37.1	36.9	37.0	36.9	4	71.7	71.7	71.7	71.7
11	23.7	23.6	23.7	23.7	5	78.4	78.4	78.4	78.4
12	122.5	125.6	122.6	126.1	6	62.7	62.7	62.6	62.6
13	144.8	139.2	144.1	138.4	Glc‴				
14	42.2	42.5	42.1	42.5	1			95.5	95.5
15	28.2	28.7	28.2	28.7	2			73.7	73.7
16	23.7	24.9	23.4	24.6	3			78.5	78.5
17	46.7	48.1	47.0	48.4	4			70.8	71.0
18	42.1	53.6	41.7	53.2	5			77.9	77.7
19	46.5	39.5	46.3	39.1	6			69.1	69.4
20	30.9	39.4	30.7	39.3	Glc⁗				
21	34.3	31.1	34.0	30.8	1			104.6	104.7
22	33.2	37.4	32.5	36.8	2			75.1	75.1
23	28.2	28.2	28.2	28.2	3			76.4	76.4
24	17.0	17.0	16.9	16.9	4			78.5	78.5
25	15.6	15.6	15.6	15.7	5			77.0	77.0
26	17.4	17.4	17.5	17.6	6			61.4	61.4
27	26.1	23.9	26.0	23.7	Rha′′′′′				
28	180.1	179.8	176.5	176.3	1			102.6	102.6
29	33.2	17.5	33.1	17.3	2			72.4	72.4
30	23.8	21.4	23.7	21.2	3			72.6	72.6
					4			73.8	73.8
					5			70.2	70.2
					6			18.3	18.3

The signals for the remaining framework protons of the monosaccharides were assigned based on two-dimensional COSY spectra and are listed in the Experimental section. The nature of the splitting of these protons and the SSCCs are consistent with β-gluco- and α-arabinopyranoses. Signals for carbohydrate C atoms in the ^{13}C NMR spectrum of **1** were unambiguously assigned using two-dimensional heteronuclear correlation spectroscopy (HETCOSY). The data are listed in Table 1. Compared with the chemical shift of an unsubstituted arabinose [1], C-3′ of arabinose in L-E undergoes a significant positive α-effect (9.4 ppm). Neighboring C atoms experience negative β-effects (1.2 and 0.2 ppm). This is consistent with a 1→3 glycosidic bond between the monosaccharides.

The 1→3 bonding was also confirmed by two-dimensional spectroscopy using the nuclear Overhauser effect in a rotating coordinate system ROESY. Structurally informative cross-peaks between glucose H-1″ and arabinose H-3′ and between arabinose H-1′ and aglycone H-3 were easily identified. Furthermore, the absolute values of the α- and β-effects of glycosylation of the aglycone and the arabinose, which were analyzed according to the literature [2, 3], confirmed that L-arabinose and D-glucose were present. Therefore, the saccharide portion of **1** is a 3-O-β-D-glucopyranosyl-(1→3)-O-α-L-arabinopyranosyl residue.

Two signals for carboxyl C atoms were present (1:2 ratio) in the low-field ^{13}C NMR spectrum of **1** in addition to distinctly different signals for olefinic C atoms of the oleanic and ursolic acids. Signals of remaining C atoms of the aglycones in **1a** and **1b** were assigned based on ^{13}C NMR spectra with ART editing, as described by us previously [1] and given in Table 1.

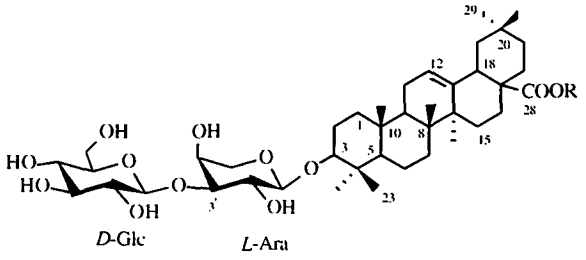
Thus, glycoside L-E is a chromatographically inseparable mixture of 3-O-β-D-glucopyranosyl-(1→3)-O-α-L-arabinopyranosides of oleanic and ursolic acids. Both of these glycosides were recently isolated from leaves of *Ilex paraguariensis* (Aquifoliaceae) as an inseparable mixture [4].

Arabinose, glucose, and rhamnose were identified in the total acid hydrolysate of L-K (**2**). Furthermore, the aglycones were a mixture of oleanic and ursolic acids. Alkaline hydrolysis of **2** gives the progenin, which is identical to **1** by TLC and NMR (^1H and ^{13}C).

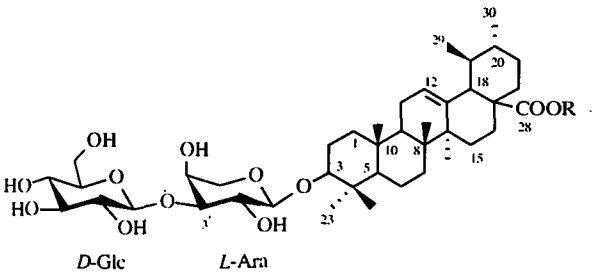
The PMR and ^{13}C NMR spectra of L-K showed that it contains five monosaccharides, three of which form a chain at C-28 of the aglycone with an acylglycoside bond. Usually this trisaccharide in the aralia family is α-L-rhamnopyranosyl-(1→4)-O-β-gentiobiose. Additional signals for C atoms in the spectrum of **2** unambiguously correspond to the aforementioned fragment according to chemical shifts [1].

Assignment of ^{13}C signals in the spectrum of the aglycone of **2** was carried out analogously as for L-H and indicates that the genins of L-K are oleanic and ursolic acids in approximately a 1:2 ratio, like for glycoside L-E.

Thus, the structures 3-O-β-D-glucopyranosyl-(1→3)-O-α-L-arabinopyranosyl-28-O-α-L-rhamnopyranosyl-(1→4)-O-β-gentiobiosyl ethers of oleanic and ursolic acids are proposed for L-K_1 and L-K_2, respectively. These compounds are new triterpene glycosides.



1a, 2a



1b, 2b

1a, 1b: R=H

2a, 2b: R=←β-*D*-Glc*p*←6β-*D*-Glc*p*←4α-*L*-Rha*p*

EXPERIMENTAL

General remarks and isolatation of fractions L-E and L-K have been reported [1].

NMR spectra were obtained on a Bruker DRX-500 (500 MHz for ^1H and 125 MHz for ^{13}C) in C_5D_5N.

Partial acid hydrolysis was performed in 1 N H_2SO_4 in a water—methanol (1:1) mixture at 100° C for 30 min with subsequent extraction of products by butanol.

Periodate decomposition was carried out by treating glycoside (2 mg) with $NaIO_4$ solution (1 ml, 2%) at room temperature for 48 h. The mixture was treated with ethyleneglycol (0.1 ml) and $NaBH_4$ (100 mg) and left for 18 h at 4° C, after which the product was extracted with *n*-butanol. The organic phase was treated with H_2SO_4 (0.5 N, 1:5 vol. ratio) and held for 3 h at 50° C. The mixture was diluted with water. The decomposition product was extracted with $CHCl_3$ and analyzed by TLC.

Glycosides L-E were purified by chromatography on SiO_2 with elution by $CHCl_3$—isopropanol (4:1) saturated with aqueous ammonia (25%). This yielded 50 mg of pure glycoside **1** from 150 mg of fraction L-E. Purification of fraction L-K (800 mg) gave **2** (600 mg) upon elution by $CHCl_3$—isopropanol (17:8) saturated with aqueous ammonia (25%). Furthermore, a more polar fraction designated L-L (80 mg) was separated during purification of L-K.

Glycoside L-E (1) is a chromatographically inseparable mixture of **1a** and **1b**. The total acid hydrolysate of **1** contained arabinose, glucose and oleanic and ursolic acids. L-E is methylated by CH_2N_2 in ether (TLC monitoring). Periodate

decomposition of L-E gave L-B.

PMR of **1** (500 MHz, C_5D_5N, ppm, *J*, Hz): 5.45 (pt, $W_{1/2}$ = 7.5, H-12), 5.15 (d, $J_{1,2}$ = 8, H-1″), 4.68 (d, $J_{1,2}$ = 6, H-1′ in **1b**), 4.67 (d, $J_{1,2}$ = 6, H-1′ in **1a**), 4.41 (dd, $J_{2,3}$ = 9, H-2′), 4.38 (m, H-4′), 4.36 (dd, $J_{5,6a}$ = 3, $J_{6a,6b}$ = 11.5, H-6a″), 4.18 (dd, $J_{5,6b}$ = 5.5, H-6b″), 4.16 (dd, $J_{5a,4}$ = 2, H-5a′), 4.12 (dd, $J_{3,4}$ = 3.5, H-3′), 4.07 (t, *J* = 9, H-3″), 4.00 (t, *J* = 9, H-4″), 3.91 (t, *J* = 8.5, H-2″), 3.84 (m, H-5″), 3.69 (d, $J_{5a,5a}$ = 11.5, H-5e′), 3.34 (dd, $J_{3,2e}$ = 4, $J_{3,2a}$ = 11.5, H-3), 3.24 (dd, $J_{18,19e}$ = 4, $J_{18,19a}$ = 13.5, H-18 in **1a**).

Compound **1** is 0.11% of the dry mass.

Glycoside L-K (2) is a chromatographically inseparable mixture of **2a** and **2b**. The total acid hydrolysate of **2** contains arabinose, glucose, rhamnose, and oleanic and ursolic acids. Alkaline hydrolysis of **2** gives **1**. Periodate decoposition of L-K gives L-B.

PMR of **2** (500 MHz, C_5D_5N, ppm, *J*, Hz): 6.38 (d, $J_{1,2}$ = 8, H-1‴ in **2a**), 6.30 (d, $J_{1,2}$ = 8, H-1‴ in **2b**), 5.69 (s, H-1⁗), 5.30 (pt, $W_{1/2}$ = 7.5, H-12), 5.22 (d, $J_{1,2}$ = 7.5, H-1″), 4.81 (d, $J_{1,2}$ = 8, H-1⁗), 4.62 (d, $J_{1,2}$ = 6, H-1′), 1.54 (d, $J_{5,6}$ = 5.5, H-6⁗).

The content of **2** is 1.4% of the dry mass.

^{13}C NMR spectra of **1a**, **1b**, **2a**, and **2b** are listed in Table 1.

REFERENCES

1. A. S. Stolyarenko, V. I. Grishkovets, N. N. Arnautov, S. V. Iksanova, and V. Ya. Chirva, *Khim. Prir. Soedin.*, 136 (2000).
2. Y. Tsuda, M. Kaneda, N. Yasufuku, and Y. Shimizu, *Chem. Pharm. Bull.*, **29**, No. 8, 2123 (1981).
3. A. S. Shashkov, G. M. Lipkind, Y. A. Knirel, and N. K. Kochetkov, *Magn. Reson. Chem.*, **26**, 735 (1988).
4. E. P. Schenkel, J. A. Montanha, and G. Gosmann, *Saponin Used in Food and Agriculture*, Plenum Press, New York (1996), p. 47.