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TRITERPENE GLYCOSIDES OF *Schefflеropsis angkae*. IV. STRUCTURE OF GLYCOSIDES L-C_2 AND L-I_2

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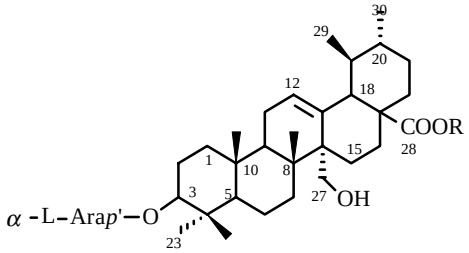
Two new triterpene glycosides of the α-amyrin series, L-C_2 and L-I_2, 27-hydroxyursolic acid 3-O-α-L-arabinopyranoside and its 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl ester, are isolated from leaves of Scheffleropsis angkae *(Araliaceae). The structures of the glycosides are established using chemical methods and NMR techniques (^1H, ^{13}C, ^{13}C-APT, COSY, TOCSY, HSQC, HMBC, and ROESY).*

Key words: *Schefflеropsis angkae*, Araliaceae, triterpene glycosides, 27-hydroxyursolic acid glucosides, NMR.

We described the method for isolating glycoside L-I_2 (**1**) from the L-I fraction of glycosides from leaves of *Schefflеropsis angkae* (Craib.) Grushv. et N. Skvorts. (Araliaceae) [1]. According to acid hydrolysis, **1** contains arabinose, rhamnose, glucose, and an unidentified aglycone of triterpene nature, the chromatographic mobility of which in various solvent systems is similar to that of hederagenin and echinocystic acid, i.e., triterpenes containing one carboxylic and two hydroxyls groups.

Alkaline hydrolysis of **1** produces its progenin **2**. Acid hydrolysis indicates that **2** contains only arabinose and the same aglycone as in **1**. Cleaving of **1** with LiI in a mixture of 2,6-lutidine and methanol afforded **2** and the anomeric mixture of methyl-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-D-glucopyranosides, which were identified by TLC with an authentic sample obtained previously [1]. This suggested that the trisaccharide α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranose was bonded to the carboxyl in **1** and that α-L-arabinopyranose was possibly bonded to the C-3 hydroxyl of the aglycone.

The structure of **1** was established by NMR techniques. Signals of four anomeric C atoms in the range 95-107 ppm were readily identified in the ^{13}C NMR spectrum. The corresponding signals of the anomeric protons were determined in the HSQC spectrum. Then signals of the remaining skeletal protons were identified using TOCSY and COSY. The nature of the splitting and the magnitude of the spin—spin coupling constants confirm that α-L-arabinopyranose, α-L-rhamnopyranose, and β-D-glucopyranose are present. The structures of the carbohydrate fragments and their sites of attachment were confirmed by analyzing the chemical shifts (CS) of C atoms compared with extensive literature data for α-L-arabinopyranose and the trisaccharide α-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→6)-β-D-Glc*p*, e.g. [2], and the HMBC and ROESY spectra as previously reported [1]. The NMR CS of ^1H and ^{13}C atoms in the carbohydrates are listed in Table 1.



1: R = ←β-D-Glc*p*"-(6←1)-β-D-Glc*p*"'-(4←1)-α-L-Rha*p*""
2: R = H

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TABLE 1. Chemical Shifts of ^1H and ^{13}C in L-I_2 (**1**) and L-C_2 (**2**) (δ, ppm, 0 = TMS, C_5D_5N)

Atom	Compound			Atom	Compound		
	1		**2**		**1**		**2**
	^{13}C	^1H	^{13}C		^{13}C	^1H	^{13}C
1	39.0	1.56, 1.00	39.1	Ara′			
2	26.6	2.13, 1.86	26.6	1	107.1	4.71	107.1
3	88.8	3.27	88.8	2	72.2	4.38	72.8
4	39.5	-	39.6	3	74.4	4.21	74.5
5	55.8	0.93	55.9	4	69.3	4.32	69.3
6	18.9	1.47, 1.30	18.7	5	66.4	4.28, 3.73	66.4
7	34.4	2.12, 1.56	34.3	Glc′′			
8	41.0	-	41.0	1	95.6	6.17	
9	48.4	2.10	48.3	2	73.8	4.31	
10	37.1	-	37.0	3	78.5	4.29	
11	24.0	2.00, 2.00	23.9	4	70.8	4.28	
12	130.0	5.73	129.6	5	77.8	4.08	
13	135.4	-	136.0	6	96.3	4.64, 4.29	
14	48.2	-	48.1	Glc′′′			
15	23.1	2.36, 1.88	23.1	1	104.7	4.92	
16	25.1	2.21, 2.06	25.5	2	75.1	3.90	
17	48.4	-	48.0	3	76.4	4.09	
18	53.3	2.62	53.7	4	78.5	4.30	
19	39.0	1.48	39.3	5	76.9	3.63	
20	39.1	0.87	39.3	6	61.3	4.20, 4.14	
21	30.5	1.35, 1.20	30.8	Rha′′′′			
22	36.8	1.92, 1.78	37.5	1	102.6	5.75	
23	28.1	1.18	28.3	2	72.4	4.64	
24	16.9	0.96	16.9	3	72.6	4.53	
25	16.1	0.94	16.0	4	73.7	4.30	
26	18.9	1.16	18.8	5	70.3	4.83	
27	64.1	4.04, 4.04	64.3	6	18.5	1.68	
28	176.7	-	180.3				
29	17.9	1.13	18.1				
30	21.2	0.82	21.3				

We were able to identify in the ^{13}C NMR spectrum of the aglycone of **1** a signal for the carbonyl C in the COOR (d 176.7 ppm), two signals for olefinic C at 135.4 and 130.0 ppm, preliminarily assigned to the $D^{12,13}$ double bond of the triterpene *a*-amyrin series according to CS [2], and signals for two more deshielded C, one of which (d 88.8 ppm) was unambiguously assigned to C-3 with a glycosylated OH; the other (d 64.1 ppm), to another aglycone C with an OH. According to the ^{13}C NMR spectrum taken using the APT method and CS, this C belongs to CH_2OH, which is possible if one of the seven methyls of the *a*-amyrin is hydroxylated.

A comparison of the ^{13}C NMR spectrum of the aglycone of **1** with that of an ursolic acid bisdesmoside glycoside (L-H_2 from leaves of *S. angkae* [2]) showed good agreement for signals of rings A, B, and E, and significant differences for atoms of rings C and D. Signals in the PMR spectrum of the aglycone part of **1** were completely assigned as follows. Signals of protons in the isolated spin systems H-1—H-3, H-9, H-11, H-12, H-18—H-22, H-29, and H-30 were identified starting with the unambiguously interpreted signals of H-3, H-12, and H-18 in the TOCSY and COSY spectra. The remaining signals were assigned to spin systems H-5—H-7 and H-15 and H-16. The CS of the corresponding C atoms were found using the HSQC spectra. Signals of quaternary methyls H-23 and H-24 were assigned using the HMBC spectrum and the C-3—C-5 cross-peaks. The singlet with CS 1.18 ppm is assigned to H-23 according to correlations in the ROESY with axial H-3 and H-5. Therefore, the singlet with CS 0.96 ppm belongs to H-24. The signal for C-26 in the HMBC spectrum was assigned analogously using

correlation with H-7 and H-25. Assignments of doublets for H-29 and H-30 were confirmed by correlation with C-5. The lack of a signal for H-27 at high-field indicates that the OH is bonded to C-27, the signal for which has CS 64.1 ppm. Although informative cross-peaks for H-27 or C-27 are not observed in the HMBC spectrum, cross-peaks between H-27 and H-7a, H-9, H-15e, and H-19 are unambiguously interpreted in the ROESY spectrum. This proves the position of the OH in the aglycone. Table 1 contains the complete assignment of the aglycone signals of **1**.

Thus, the aglycone in **1** is 27-hydroxyursolic acid; glycoside L-I_2 (**1**), 27-hydroxyursolic acid 3-O-α-L-arabinopyranosyl-28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranoside, is a new triterpene glycoside.

Glycosides L-C_2 (**2**) and L-C_1 were isolated from fraction L-C [2] by chromatography on microspherical silica gel. The total acid hydrolysate of **2** contains arabinose and 27-hydroxyursolic acid. Alkaline hydrolysis does not affect **2**. It is methylated by CH_2N_2. The chromatographic mobility of **2** and the color of its spot after developing are the same as those for the progenin of **1**.

These data indicated to us that **2** is 27-hydroxyursolic acid 3-O-α-L-arabinopyranoside. The ^{13}C NMR spectrum of **2** was analyzed by comparing it with that of **1**. Signals of α-L-arabinopyranose were assigned unambiguously; those of the aglycone, taking into account the well-known effects of glycosylating C-28 [2, 3], which appeared on C atoms of rings D and E.

Therefore, L-C_2 is 27-hydroxyursolic acid 3-O-α-L-arabinopyranoside, also a new triterpene glycoside.

The only known 27-hydroxyursolic acid glycoside was isolated previously from the aerial part of *Fagonia arabica* (Zygophyllaceae) [4]. A comparison of the aglycone part of the ^{13}C NMR spectra of **1** and that of 27-hydroxyursolic acid 3-O-β-D-xylopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-α-L-arabinopyranosyl-28-O-β-D-glucopyranoside from *F. arabica* shows that most signals agree with the exception of small differences (up to 0.3 ppm) for C-2, C-4, C-23, and C-24, which are due to a different carbohydrate chain on C-3. However, the previous signal assignments [4] for C-9, C-14, C-17; C-4, C-19, C-20; C-11, C-15, C-16; C-6 and C-26; and C-24 and C-25 do not agree with ours. The previous study used only very rudimentary literature data for the ^{13}C NMR of free ursolic acid in $CDCl_3$ [5] and took into account effects from adding an OH. Despite the use of COSY, HSQC, HMBC, and NOESY to completely assign the carbohydate parts, they were not used to assign signals in the aglycone part. Therefore, erroneous assignments were made for the closely spaced signals mentioned above.

EXPERIMENTAL

Data for the isolation of L-I_2 and fraction L-C and general comments have been published [1, 2].

Isolation of L-C_2. Fraction L-C (1.14 g) was chromatographed over L (40-100 μ) silica gel with elution by $CHCl_3$—$(CH_3)_2CHOH$ (4:1) saturated with aqueous ammonia (25%) to give a mixture of L-C_1 and L-C_2 (100 mg) that was purified of most accompanying compounds. This mixture was separated over Silpearl microspherical silica gel using water-saturated $CHCl_3$—$(CH_2)_2CHOH$ (5:1) to give L-C_1 (25 mg) and L-C_2 (18 mg).

Glycoside L-C_2 (2). Yield, 0.04% of dry mass. The total acid hydrolysate of **2** contains arabinose and 27-hydroxyursolic acid. Table 1 lists the ^{13}C NMR data.

Glycoside L-I_2 (1). Yield, 0.11% of dry mass. The acid hydrolysate of **1** contains arabinose, rhamnose, glucose and 27-hydroxyursolic acid.

Cleavage of 1 by LiI in a mixture of 2,6-lutidine and CH_3OH was performed as before [1] and gave **2** and an anomeric mixture of methyl-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-D-glucopyranosides. Table 1 lists the CS of signals in the PMR and ^{13}C NMR.

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