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TRITERPENOID GLYCOSIDES OF *Schefflеropsis angkae*. III. STRUCTURE OF GLYCOSIDE L-I_1

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A new triterpenoid glycoside L-I_1, the 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl ester of 3β,23,27-trihydroxylup-20(29)-en-28-oic acid, is isolated from the leaves of Scheffleropsis angkae *(Araliaceae). Its structure is established by chemical methods and various NMR techniques (^{1}H, ^{13}C, APT, COSY, TOCSY, ROESY, HSQC, HMBC). 3β,23,27-Trihydroxylup-20(29)-en-28-oic acid is a new triterpenoid aglycone.*

Key words: *Schefflеropsis angkae*, new triterpenoid glycoside, 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl ester of 3β,23,27-trihydroxylup-20(29)-en-28-oic acid, NMR (^{1}H, ^{13}C, APT, COSY, TOCSY, ROESY, HSQC, HMBC).

We have previously described the isolation of the total glycosides from leaves of *Schefflеropsis angkae* (Craib.) Grushv. et N. Skvorts. (Araliaceae) and the separation of them into fractions [1]. The structures of the predominant glycosides and their progenins were also reported [1, 2].

In the present article, the isolation of glycoside L-I_1 from fraction L-I and the elucidation of its structure using chemical and physicochemical methods are reported.

Fraction L-I was separated by chromatography into the pure components L-I_1 and L-I_2 as the total acetates with subsequent deacetylation and additional chromatographic purification. According to acid hydrolysis, L-I_1 contains glucose and rhamnose in addition to an unknown aglycone which is yellow, like the glycoside itself, upon development of the TLC with phosphotungstic acid and *p*-hydroxybenzaldehyde. This behavior is similar to betulinic acid, which prompted us to propose that the aglycone belongs to the lupane series. Alkaline hydrolysis or decomposition by LiI in a mixture of 2,6-lutidine—methanol yields the same aglycone and, in the second instance, also an anomeric mixture of methyl-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosides from the carbohydrate fragment, which is usually bonded to C-28 of the aglycone in bisdesmoside glycosides of Araliaceae. The production of the same aglycone upon acid and alkaline hydrolysis indicates that the saccharide chain is bonded only to the carboxylic acid whereas the hydroxyl on C-3 of the aglycone is free.

The ^{13}C NMR of L-I_1 exhibits a low-field signal for the substituted carboxylic acid at 175.4 ppm and signals at 151.0 and 110.0 ppm that are assigned to vinylidene atoms according to the chemical shifts and APT-analysis of the spectrum.

Signals for three anomeric C atoms of the carbohydrate that is bonded to the carboxylic acid are observed at 95-105 ppm. The signals of the trisaccharide part of the glycoside in the PMR and ^{13}C NMR were completely assigned by analyzing COSY and TOCSY two-dimensional homonuclear spectra and HSQC heteronuclear spectra (Table 1).

The nature of the splitting of the PMR signals confirms that two monosaccharides are β-glucose and one is α-rhamnose. The chemical shifts of the carbohydrate C atoms indicate that one of the glucose residues is substituted at C-6; the other, at C-4. The rhamnose is terminal. The bond types and sequence of monosaccharides were confirmed by the presence of the corresponding correlation peaks in the ROESY two-dimensional spectrum between the spatially proximal anomeric protons and protons bonded to glycosylated C atoms of neighboring monosaccharides. Furthermore, the HMBC two-dimensional heteronuclear spectrum exhibits cross-peaks across multiple bonds for the anomeric C atoms and protons on glycosylated C

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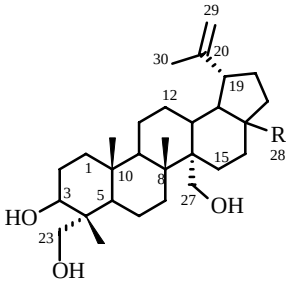
TABLE 1. ^{1}H and ^{13}C Chemical Shifts for Glycoside L-I_1 (δ, ppm, 0 = TMS,C_5D_5N)

Aglycone			Carbohydrate		
	^{13}C	^{1}H	atom	^{13}C	^{1}H
1	39.2	1.68, 1.01	Glc′		
2	27.6	1.92, 1.92	1	95.2	6.34
3	73.4	4.17	2	73.9	4.11
4	42.9	-	3	78.4	4.25
5	49.0	**1.54**	4	70.7	4.30
6	18.6	1.64, 1.43	5	77.9	4.10
7	35.6	2.32, 1.61	6	69.3	4.66, 4.18
8	42.0	-	Glc′′		
9	52.3	1.68	1	104.8	4.93
10	37.5	-	2	75.1	3.92
11	21.4	1.44, 1.19	3	76.4	4.11
12	25.6	1.87, 1.07	4	78.4	4.31
13	39.1	2.82	5	76.9	3.63
14	46.7	-	6	61.3	4.18, 4.07
15	24.0	2.40, 1.93	Rha′′′		
16	33.4	2.83, 1.94	1	102.6	5.77
17	56.9	-	2	72.4	4.64
18	50.2	2.09	3	72.6	4.53
19	47.5	3.41	4	73.8	4.32
20	151.0	-	5	70.3	4.87
21	30.9	2.17, 1.42	6	18.4	1.67
22	36.7	2.26, 1.51			
23	67.8	4.12, 3.62			
24	12.8	1.04			
25	17.2	0.92			
26	17.0	1.22			
27	60.0	4.58, 4.15			
28	175.4	-			
29	110.1	4.88, 4.73			
30	19.5	1.68			

atoms and, on the other hand, between glycosylated C atoms and anomeric protons. The chemical shifts of the carbohydrate C atoms of L-I_1 correspond completely with the literature data for such a trisaccharide fragment [3].

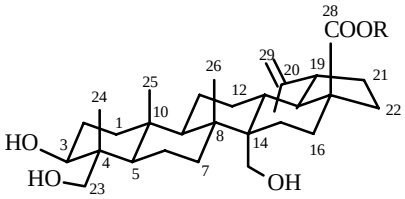
The structure of the aglycone was elucidated as follows. Three signals of C atoms belonging to the aglycone and presumably containing OH groups are found at 60-75 ppm in the ^{13}C NMR spectrum. The HSQC two-dimensional heteronuclear spectrum shows ^{1}H–^{13}C correlations through one bond. Thus, the signals with chemical shifts 67.8 and 60.0 ppm correspond to C atoms of primary alcohols because cross peaks with pairs of nonequivalent protons are observed. However, the signal at 73.4 ppm corresponds to C-3 of a nonglycosylated secondary alcohol because a cross peak with the single H-3 proton is observed for it.



R= COO-β-*D*-Glc*p*-(6←1)-β-*D*-Glc*p*-(4←1)-α-*L*-Rha*p*

Analysis of the COSY correlation spectrum and the TOCSY total correlation spectrum identified signals of H-1a,b and H-2a,b (multiplets) that form together with H-3 (doublet of doublets) a closed spin system. The large (17.5 Hz) SSCC of the signal for H-3 is consistent with an axial orientation of this proton in ring *A* and the β-orientation of the OH group on C-3.

Signals of C-1 and C-2 are located in the HSQC spectrum by using the chemical shifts of H-1 and H-2. The position of the signal for C-4 and the methyl (12.8 ppm) and primary alcohol (67.8 ppm) C atoms bonded to it are located in the HMBC spectrum by using H-3 cross peaks. Cross peaks for the framework protons of these groups were used to find the signal for C-5. Then, the ROESY spectrum revealed an axial orientation for H-5 in ring *A* because a strong cross peak between it and axial H-3 is observed. Judging from the nature of the splitting with neighboring H-6a and H-6b (doublet of doublets with one large, 14 Hz, and one small SSCC), H-3 is axial in ring *B*. This unambiguously determines the *trans*-fusion of rings *A* and *B*.



The location of the OH on C-23 or C-24, i.e., the configuration of C-4, was determined using HMBC two-dimensional spectroscopy. The spectrum of L-I_1 exhibits a strong correlation peak between H-3 and the methyl C atom and a much weaker peak bewteen H-3 and the C atom of the CH_2OH group. This is consistent with the large ^{1}H-3/$^{13}CH_3$ SSCC compared with ^{1}H-3/$^{13}CH_2OH$. Therefore, H-3 and CH_3 are trans-oriented. This means that the methyl is axial (β) and the CH_2OH is equatorial (α).

The chemical shifts found for ^{13}C atoms of the aglycone of L-I_1 were compared with the literature values for hederagenin and 4-epihederagenin taking into account the effects of glycosylation. Good agreement was found for the chemical shifts of C atoms in rings *A* and *B* and those in unsubstituted hederagenin (OH on C-23) [3]. There was a significant discrepancy for 4-epihederagenin (OH on C-24) [4], especially for C-3, C-5, C-6, C-23, and C-24. This also confirms the proposed configuration for C-4.

Signals for protons and C atoms of ring *B* were found using the signal of H-5, like for ring *A*. Assignments were made analogously for rings *C*, *D*, and *E*. The ROESY spectrum exhibits cross peaks of H-7b, H-16a, and H-18 with framework protons of the primary alcohol with δ 4.85 and 4.15 ppm, which form a closed AX-system. Therefore, the C atom of this group should be assigned as C-27.

The nature of the splitting and the SSCC of signals for H-9, H-13, and H-18 in the COSY spectrum indicate *trans*-fusion of rings *B* and *C*, *C* and *D*, and *D* and *E*. This is confirmed by cross peaks in the ROESY spectrum between H-5 and H-9, H-13 and H-26, and H-18 and H-27b. The nature of the splitting of the H-19 signal in ring *E* defines the axial (β) orientation for this proton. Therefore, the isopropenyl group has the α-orientation. Signals for its atoms were found in COSY, TOCSY, HMBC, and HSQC spectra. The presence in the HMBC spectrum of cross peaks of the carbonyl C and H-22a, H-18, H-16b, and H-22b indicate that it is C-28.

Thus, the aglycone belongs to the lupane series and is 3β,23,27-trihydroxylup-20(29)-en-28-oic or 23,27-dihydroxybetulinic acid. The isolated glycoside, 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl ester of 3β,23,27-trihydroxylup-20(29)-en-28-oic acid, is a new triterpenoid glucoside of a new aglycone. As far as is known, this aglycone has not been found as a free triterpene.

EXPERIMENTAL

General remarks and the isolation of fraction L-I have been reported [1].

NMR spectra were obtained on a Bruker DRX-500 instrument (500 MHz for ^{1}H and 125 MHz for ^{13}C) in C_5D_5N at 30°C. The scrambling time (spin-locking) in the ROESY experiment was 100 ms.

Total acetylation was achieved by treating fraction L-I (350 mg) with a mixture of C_5H_5N (5 ml) and Ac_2O (4.8 ml) at room temperature for 12 h. The reaction mixture was evaporated to dryness to give the total acetates (415 mg). These were separated by chromatography on SiO_2 with elution by CCl_4—acetone (10:1) to give L-I_1 acetate (224 mg) and L-I_2 acetate (61 mg).

Deacetylation was achieved in absolute MeOH in the presence of catalytic amounts of NaOMe for 2 days at room temperature with subsequent removal of Na^+ by cation-exchanger KU-2-8 in the H^+-form. Pure glycosides were purified by chromatography with elution by water-saturated $CHCl_3$—isopropanol (5:2) to give pure L-I_1 (180 mg) and L-I_2 (50 mg).

Cleavage of acylglycoside bonds was performed by a modified literature method [5]. L-I_1 (25 mg) was dissolved in 2,6-lutidine (0.6 ml), treated with dry MeOH (1 ml) and anhydrous LiI (25 mg), and stored in a sealed ampul at 100°C for 24 h. The salt was removed by ion-exchanger KU-2-8 in the H^+-form and AV-17-8 in the OH^--form. The reaction mixture was evaporated to dryness. The solid was separated on SiO_2 with elution by water-satured $CHCl_3$-isopropanol (5:2) to yield aglycone (6 mg) and an anomeric mixture of methyl-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-D-glucopyranosides (7 mg), which were identified by TLC using various solvent systems and authentic samples obtained by this method from cleaved H_2 tauroside from *Hedera taurica* [6].

Glycoside L-I_1. TLC of the total acid hydrolysate detected sugar, glycose and rhamnose, in addition to aglycone, which was also formed by alkaline hydrolysis.

The content of L-I_1 was 0.41% of the dry mass.

PMR and ^{13}C NMR data are listed in Table 1.

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