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TRITERPENE GLYCOSIDES FROM *Tupidanthus calyptratus.* I. STRUCTURE OF GLYCOSIDES B_1, B_2, F_1, AND F_2 FROM LEAVES OF HOODED TUPIDANTHUS

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Chromatographically inseparable mixtures of oleanolic and ursolic 3-O-α-L-rhamnopyranosyl-(1→2)-O-α-L-arabinopyranosides (glycosides B_1 and B_2) and their 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl esters (glycosides F_1 and F_2) are isolated from the leaves of Tupidanthus calyptratus *Hook. f. (Araliaceae). The structures of the isolated glycosides are established from chemical methods and ^1H and ^{13}C NMR spectra. Glycoside F_2 is a new triterpene glycoside.*

The monotypical genus Tupidanthus (Tupidanthus Hook f. et Thoms., Araliaceae) has one species, hooded tupidanthus (*Tupidanthus calyptratus* Hook. f.), that is distributed among mountainous forests of India (eastern Himalayas), China (Yun-Nan province), Thailand, Burma, and Vietnam [1]. Tupidanthus is one of the most primitive genera of aralia [2]. The glycoside composition of this plant is exceedingly interesting from the viewpoint of phylogenetics.

In the present article, we describe the isolation of triterpene glycosides from the leaves of hooded tupidanthus and the structure determination of the predominant glycosides B_1, B_2, F_1, and F_2. The triterpene glycosides were isolated by extraction of ground and defatted ($CHCl_3$) dry leaves with 80% isopropanol in water and separation of the extracted compounds on silica gel using gradient elution with $CHCl_3$-ethanol mixtures (10:1 → 1:1) saturated with water. Glycoside fractions A-H were obtained, of which B and F were predominant. The fractions were additionally purified of a significant amount of phenolic glycosides by rechromatographing on silica gel with elution by $CHCl_3$-ethanol mixtures saturated with 10% aqueous ammonia.

TLC analysis on Silufol, Polygram, and Merck $60F_{254}$ high-efficiency plates in various solvent systems demonstrated that fractions B and F are pure glycosides that have chromatographic mobilities identical to authentic samples of oleanolic 3-O-α-L-rhamnopyranosyl-(1→2)-O-α-L-arabinopyranoside (tauroside C [3]) and its 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl ester (tauroside G_2 [4]), respectively. However, the color of the spots for the isolated glycosides and the authentic samples differed somewhat after detection with phosphotungstic acid. This became especially noticeable if other detecting reagents were used (vanillin-sulfuric acid, vanillin-perchloric acid, *p*-hydroxybenzaldehyde-sulfuric acid). This phenomenon prompted us to carry out a detailed structure determination of the isolated glycosides. Thus, the progenin of glycoside F, which was obtained by alkaline hydrolysis, was identical to glycoside B. Total acid hydrolysis of glycoside B showed the presence of arabinose and rhamnose; in the contents of glycoside F-arabinose, rhamnose, and glucose. The aglycone of both glycosides had the same chromatographic mobility as oleanolic acid but differed from an authentic sample in the color of the spot using the detecting reagents mentioned above.

The structures of B (**1**) and F (**2**) were established using ^1H and ^{13}C NMR spectroscopy. Thus, a comparison of the PMR spectra of **2** (see Experimental) and tauroside G_2 [4] are identical in the low-field region (3.0-6.5 ppm), which contains signals for the framework protons of the carbohydrates in the glycosides, with one exception. The doublet for the anomeric proton H-1′″ of the glucose, which is bound through an acylglycoside bond to the aglycone and is unambiguously recognized in the spectrum owing to its very low-field position (6.20 ppm), has approximately half of the integrated intensity compared with signals of the remaining anomeric protons. An analogous doublet of half intensity with a spin—spin coupling constant (SSCC) $J_{1,2} = 8.0$ Hz is located next to it (at 6.15 ppm). In the high-field region of the PMR for **2**, there are two easily recognized doublets for the H-6 protons of the two rhamnose units. However, instead of the expected seven singlets for the methyl groups on the quaternary

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TABLE 1. Chemical Shifts of ^{13}CAtoms in Aglycones of Glycosides B_1(1a), B_2(1b),F_1 (2a), and F_2 (2b) (δ, ppm, 0 = TMS, C_5D_5N)

C-atom	Compound				C-atom	Compound			
	1a	1b	2a	2b		1a	1b	2a	2b
1	39.0	39.0	39.0	39.0	16	23.7	24.9	23.3	24.6
2	26.4	26.4	26.3	26.3	17	46.7	47.9	47.0	48.4
3	88.8	88.8	88.9	88.9	18	41.9	53.5	41.6	53.2
4	39.4	39.4	39.3	39.3	19	46.5	39.3	46.2	39.0
5	55.9	55.9	55.9	55.9	20	30.9	39.3	30.6	39.3
6	18.5	18.5	18.5	18.5	21	34.3	31.1	34.0	30.8
7	33.2	33.5	33.0	33.4	22	33.3	37.4	32.5	36.7
8	39.8	40.1	39.9	40.0	23	28.2	28.2	28.1	28.1
9	48.0	48.0	48.0	48.0	24	16.8	16.8	16.8	16.8
10	37.1	37.0	37.0	36.9	25	15.6	15.6	15.5	15.5
11	23.7	23.6	23.6	23.6	26	17.3	17.3	17.4	17.6
12	122.5	125.6	122.5	126.0	27	26.2	23.8	25.9	23.6
13	144.7	139.1	144.0	138.3	28	180.0	179.8	176.5	176.2
14	42.2	42.5	42.1	42.4	29	33.3	17.3	33.0	17.2
15	28.2	28.6	28.1	28.6	30	23.7	21.2	23.6	21.1

TABLE 2. Chemical Shifts ^{13}CAtoms in Carbohydrates of Glycosides B_1(1a), B_2(1b),F_1 (2a), and F_2 (2b) (δ, ppm, 0 = TMS, C_5D_5N)

C-atom	Compound		C-atom	Compound	
	1a, 1b	2a, 2b		2a	2b
Ara ′			Glc′′′		
1	104.1	104.2	1	95.5	95.5
2	76.1	76.0	2	73.7	73.7
3	72.8	72.8	3	78.6	78.6
4	67.8	67.9	4	70.8	70.9
5	63.6	63.7	5	77.7	77.6
			6	69.2	69.4
Rha ′′			Glc ′′′′		
1	101.5	101.5	1	104.4	104.5
2	72.0	72.0	2	75.0	75.0
3	72.3	72.0	3	76.3	76.3
4	73.7	73.8	4	78.4	78.4
5	69.7	69.8	5	76.8	76.8
6	18.2	18.2	6	61.3	61.3
			Rha ′′′′′		
			1	102.5	102.5
			2	72.2	72.2
			3	72.5	72.5
			4	73.7	73.7
			5	70.2	70.2
			6	18.3	18.3

C-atoms of the oleanolic acid, eight more or less resolved singlets (the others overlap) with approximately half of the required intensity and two doublets at 1.05 and 0.86 ppm with SSCCs of 6.0 Hz are observed.

These data suggest that the chromatographically pure F glycoside is a nearly equimolar mixture of two glycosides, which are denoted F_1 (**2a**) and F_2 (**2b**), with identical carbohydrate chains but different aglycones. Information about the structure

of the aglycones of **2a** and **2b** and additional confirmation of the structure of the carbohydrate part were obtained by analyzing the ^{13}C NMR spectrum of **2** (Tables 1 and 2). Thus, the region of carboxylic C atoms (C-28 aglycones) contains two signals at 176.5 and 176.2 ppm. The region of olefinic C atoms has the expected signals for C-12 and C-13 of oleanolic acid at 122.5 and 144.0 ppm and two more signals at 126.0 and 138.3 ppm with chemical shifts that correspond to those for C-12 and C-13 of ursane (α-amyrin) triterpenoids [5].

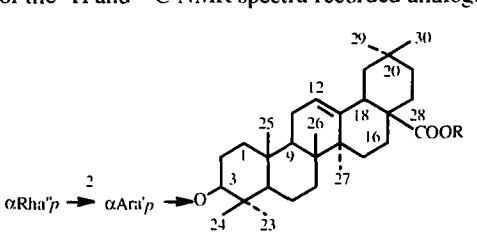
Most of the signals for the aglycone C atoms in the range 20-60 ppm were unambiguously assigned to oleanolic acid in **2a** by comparison with the literature [4, 6]. The remaining unassigned signals in this region apparently belong to the aglycone of the accompanying glycoside **2b**. Considering the identical chromatographic mobility of **2a** and **2b**, the aglycone of **2b** is proposed to be ursolic acid (the most widely distributed isomer of oleanolic acid, which differs only in the position of the methyl groups in ring *E*). Comparing the chemical shifts of the remaining unassigned signals for the aglycone with literature data for ursolic acid and its glycosides [7-9] enabled them to be unambiguously assigned.

We note that the most important differences in the chemical shifts for the isomeric glycosides **2a** and **2b** are observed only for the C atoms of ring *E* and its closest neighbors. The chemical shifts of the remaining C atoms are either identical, which is evident in the spectrum as signals of double intensity, or insignificantly different by only 0.1-0.2 ppm. In these instances, assignments for **2a** and **2b** were made using literature data [9], where isomeric oleanolic and ursolic glycosides were obtained pure.

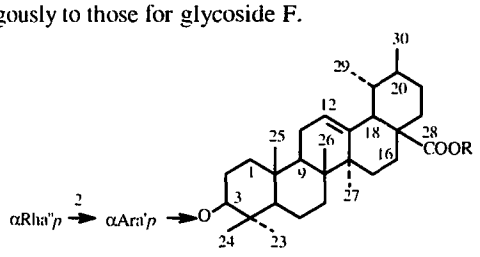
Signals for the C atoms of the carbohydrates of **2a** and **2b** were unambiguously assigned by comparison with the literature data [4]. It is interesting that the ^{13}C NMR signals for C-4 through C-6 of the glucose directly bonded to the aglycone and even for C-1 of the next glucose unit are doubled by 0.1-0.2 ppm. This is additional confirmation that two isomeric glycosides are present. It becomes understandable why the PMR spectrum contains two close doublets for the anomeric proton of the glucose bonded to the aglycone and two doublets in the high-field region for the methyl groups on C-19 and C-20 of the ursolic acid that are split into doublets by spin—spin coupling with the protons on C-19 and C-20.

Thus, the isolated glycoside F is a chromatographically inseparable mixture of F_1 and F_2, which are 3-O-α-L-rhamnopyranosyl-(1→2)-O-α-L-arabinopyranosyl-28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl esters of oleanolic and ursolic acids, respectively.

Because the isolated glycoside B was identical to progenin F in chromatographic mobility and spot color using various detecting reagents, we hypothesized that it also is a nearly equimolar mixture of two isomeric glycosides B_1 (**1a**) and B_2 (**1b**), the 3-O-α-L-rhamnopyranosyl-(1→2)-O-α-L-arabinopyranosides of oleanolic and ursolic acids, respectively. This was confirmed by an analysis of the ^1H and ^{13}C NMR spectra recorded analogously to those for glycoside F.



1a, 2a



1b, 2b

1a	R=H
2b	R=H
1a	R=H→βGlc*p*''→(6→1)-βGlc*p*''''→(4→1)-αRha*p*'''''
2b	R=H→βGlc*p*''→(6→1)-βGlc*p*''''→(4→1)-αRha*p*'''''

According to the literature it is difficult to separate mixtures of isomeric ursane and oleanane glycosides [10]. In particular, isomeric pairs of patrinia glycosides (*Patrinia scabiosaefolia*) were separated using repeated and carefully performed HPLC [9, 11]. Isomeric holly glycosides (*Ilex paraguariensis*) could not be separated even after total conversion to the acetates [8].

Oleanolic 3-O-α-L-rhamnopyranosyl-(1→2)-O-α-L-arabinopyranoside and its 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl ester have been observed more than once in plants of the aralia family. Ursolic 3-O-α-L-rhamnopyranosyl-(1→2)-O-α-L-arabinopyranoside rather recently was found in *Patrinia scabiosaefolia* [9] and leaves

of *Ilex paraguariensis* [8]. The F_2 glycoside described by us is a new triterpene glycoside.

Ursolic glycosides are rarely encountered in natural sources. They have been found in plants of the aralia family only in the roots of *Aralia decaisneana* [12]. Glycosides of α-amyrin have been found in Araliaceae plants only in two species of *Schefflera* (*S. impressa* and *S. octophylla*). These are glycosides of 23-hydroxyursolic, asiatic, and 3-α-hydroxyurs-12-en-23,28-dioic acids [13, 14].

The presence of significant quantities of ursolic glycosides can be hypothesized to be a chemical marker of philogenetic primitiveness (antiquity) of a taxon (in this instance, a genus) because ursolic glycosides have not been found in many other well studied and rather advanced genera of Araliaceae (*Acanthopanax*, *Eluetherococcus*, *Fatsia*, *Hedera*, *Kalopanax*, *Oplopanax*, *Panax*, *Polyscias*, *Tetrapanax*, and most studied species of *Aralia*). The observation of ursolic glycosides in *Aralia decaisneana* [12] and the predominance of oleanolic glycosides can be considered to be a vestigial chemical marker because the genus *Aralia* is considered to be one of the most advanced in the family [2].

EXPERIMENTAL

NMR spectra were obtained on a Varian VXR-300 instrument. Solutions of glycosides in pyridine-D_5 were used.

Total acid hydrolysis for sugar determination was performed by dissolving glycosides in a dioxane-CF_3COOH (4 N, aqeous) mixture (1:1) and heating at 100°C for 2 h. The sugar in the hydrolysate was identified without preliminary preparation by TLC on Silufol plates using $CHCl_3$-CH_3OH-water (100:40:7) or $CHCl_3$-CH_3OH-aqueous ammonia (25%) (100:40:10) mixtures and authentic samples. Aglycones were identified using total acid hydrolysis by dissolving the glycosides in a CH_3OH-H_2SO_4 (2 N, aqueous) mixture and heating at 100°C for 2 h with subsequent dilution of the hydrolysate with a three-fold excess of water, extraction of the aglycones with $CHCl_3$, TLC analysis, and comparison with authentic samples using $CHCl_3$-CH_3OH-ammonia (25%) (100:20:3) or benzene-acetone (4:1).

Alkaline hydrolysis was carried out by dissolving the glycosides in 10% KOH in a water-CH_3OH mixture (1:1), heating at 100°C for 2 h with subsequent dilution with water, neutralization with aqueous H_2SO_4 until the solution was weakly acidic, extraction of the progenins by butanol, and TLC analysis using $CHCl_3$-CH_3OH-water (100:30:5) or $CHCl_3$-CH_3OH-ammonia (100:30:6).

Spots of glycosides, progenins, and aglycones on the chromatograms were detected using the usual phosphotungstic acid and vanillin-H_2SO_4, vanillin-perchloric acid [15], and *p*-hydroxybenzaldehyde-H_2SO_4 [16]. Spots of sugars were detected by acid phthalate anyline.

Isolation and Separation of Glycosides. Dried leaves (30 g) of hooded tupidanthus that were obtained from the Botanical Garden of the Botanical Institute, Russian Academy of Sciences (St. Petersburg), were carefully ground and defatted by treatment with three portions (200 ml) of $CHCl_3$. The glycosides were extracted by 80% aqueous isopropanol (3×200 ml). Evaporation of the combined alcohol extracts produced a total of 10 g of triterpenes, phenolic glycosides, and other extracted substances. The glycosides were purified of free sugars, salts, and other highly polar compounds by dissolving them in butanol (300 ml) saturated with water and washing with water (3×150 ml). Evaporation of the butanol solution gave 6 g of purified triterpene glycosides.

The triterpene glycosides were separated by chromatography on silica gel L (40-100 μm) using gradient elution by $CHCl_3$-ethanol (10:1 → 1:1) saturated with water. Glycosides A (0.25 g), B (1.5 g), C and D (0.2 g of a mixture), E (0.3 g), F (1.7 g), and G and H (0.3 g of a mixture) were obtained.

Glycoside F (**2**) was additionally purified by rechromatography on silica gel using $CHCl_3$-ethanol (1:1) saturated with 10% aqueous ammonia. This yielded 0.8 g of **2** free of phenolic compounds. TLC using $CHCl_3$-CH_3OH-water (100:40:7) and $CHCl_3$-CH_3OH-ammonia (25%) (100:40:10) showed that **2** has the same R_f value as tauroside G_2 [4].

The total acid hydrolysate of **2** contained arabinose, rhamnose, glucose, and a chromatographically inseparable mixture of oleanolic and ursolic acids.

^{1}H NMR spectrum of **2** (**2a** and **2b** mixture), (δ, ppm, 0 = TMS, C_5D_5N): 6.20 (d, $J_{1,2}$ = 8.0 Hz, H-1′″ in **2a**), 6.15 (d, $J_{1,2}$ = 8.0 Hz, H-1′″ in **2b**), 6.08 (d, $J_{1,2}$ = 1.5 Hz, H-1″), 5.80 (d, $J_{1,2}$ = 1.5 Hz, H-1′″″), 5.40 (pent, H-12), 4.95 (d, $J_{1,2}$ = 7.5 Hz, H-1″″), 4.93 (m, H-5′″″), 4.88 (d, $J_{1,2}$ = 5.0 Hz, H-1′), 4.73 (dd, $J_{2,3}$ = 3.5 Hz, H-2″), 4.65 (dd, $J_{2,3}$ = 3.5 Hz, H-2′″″), 4.60 (dd, $J_{3,4}$ = 9.5 Hz, H-3″), 4.59 (m, H-5″), 4.52 (m, H-2′, H-3′″″), 4.35 (t, $J_{4,5}$ = 9.0 Hz, H-4″″), 4.30 (t, $J_{4,5}$ = 9.5 Hz, H-4′″″), 4.2-4.3 (m H-3′, H-4′, H-5e′, H-4″), 4.0-4.2 (m, H-2′″, H-3′″, H-3″″), 3.92 (t, $J_{2,3}$ = 8.0 Hz, H-2″″), 3.82 (m, $J_{5a,5e}$

= 11.0 Hz, H-5a'), 3.59 (m, H-5‴), 3.21 (m, H-3), 1.65 (d, $J_{5,6}$ = 6.0 Hz, H-6′‴), 1.60 (d, $J_{5,6}$ = 6.0 Hz, H-6‴), 1.23, 1.18, 1.14, 1.11, 1.04, 0.92, 0.90, 0.86 (all s, CH_3 groups on quaternary C atoms in **2a** and **2b**, signals of the remaining quaternary CH_3 groups overlap in the range 0.8-0.9 ppm), 1.05 and 0.86 (both d, $J_{29,19} = J_{30,20}$ = 6.0 Hz, H-29 and H-30).

Glycoside B (**1**) was additionally purified by chromatography on silica gel using $CHCl_3$-ethanol (3:1) saturated with 10% aqueous ammonia. This yielded **1** (0.9 g) free of phenolic compounds. TLC using $CHCl_3$-CH_3OH-water (100:30:5) and $CHCl_3$-CH_3OH-ammonia (25%) (100:30:6) showed that **1** has an R_f value identical to that of tauroside C [3]. The total acid hydrolysate of **1** contained arabinose, rhamnose, and a chromatographically inseparable mixture of oleanolic and ursolic acids.

The ^{13}C NMR spectra of **1** and **2** are listed in Tables 1 and 2.

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