Chemistry of Natural Compounds, Vol. 38, No. 3, 2002

TRITERPENE GLYCOSIDES AND THEIR GENINS FROM *Tragacantha stipulosa*. STRUCTURE OF CYCLOSTIPULOSIDE C

T. N. Kaipnazarov,[1] K. K. Uteniyazov,[1] V. V. Kachala,[2] Z. Saatov,[1] and A. S. Shashkov[2]

UDC 547.918:547.926

*A new cycloartane glycoside of the cyclostipuloside C series, **3**-O-β-D-xylopyranoside-6,16-di-O-β-D-glucopyranoside-20S-24R-epoxycycloartan-3β,6α,16β,25-tetraol is isolated from aerial organs of* Tragacantha stipulosa *Boriss.*

Key words: cycloartanes, cyclostipuloside C.

In continuation of the study of cycloartane triterpenoids of *Tragacantha stipulosa* Boriss (Leguminosae) [1], we isolated from the butanol extract of the aerial organs a new cycloartane glycoside, cyclostipuloside C (**1**). The roots of this plant yielded four compounds: cyclostipuloside A [2], askendoside G, askendoside D, and cycloglobiseposide B (**2**) [1].

The PMR spectra (Table 1) of **1** in the strong-field region contain signals for protons of seven methyls and 1H doublets of an AB-splitting system at 0.18 and 0.52 ppm with J = 3.5 Hz that are characteristic of methylene protons of a cyclopropane ring. The presence of a three-membered ring was also confirmed by an absorption band at 3050 cm^{-1} in the IR spectrum of **1**.

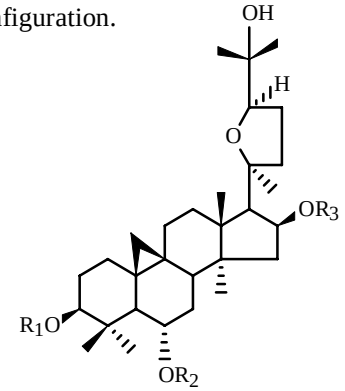
Acid hydrolysis of **1** showed that xylose and glucose occur in the carbohydrate components.

The ^1H and ^{13}C NMR spectra of **1** exhibit signals of three anomeric protons at 4.78, 4.85, and 4.88 ppm and three anomeric C atoms of monosaccharide units that resonate at 106.46, 107.68, and 105.60 ppm.

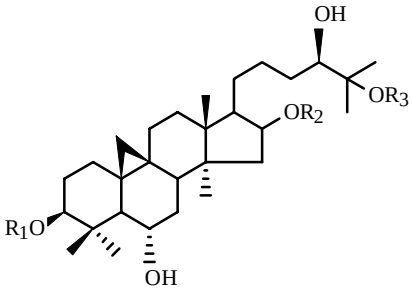
These data indicate that the glycoside of **1** is a trioside.

^{13}C NMR spectra of **1** indicate that the signals of the aglycone part agree mainly with spectral properties of cyclosiversigenin (**3**) [3]. However, differences in the positions of the signals for C-21—C-24 are observed.

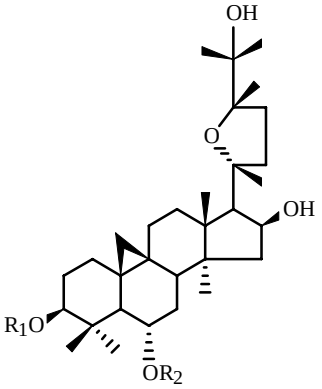
Signals for C-22 and C-24 in the ^{13}C NMR spectrum of **1** are shifted to weak field by +4.04 and +2.90 ppm whereas the resonance for CH_3-21 shifts to strong field by -2.37 ppm. Therefore, the asymmetric center C-24 in **1** has the R-configuration.



1, 5



2



3, 4

1: R_1 = β-D-Xyl *p*; $R_2 = R_3$ = β-D-Glc*p*
2: R_1 = β-D-Xyl *p*; $R_2 = R_3$ = β-D-Glc*p*
5: $R_1 = R_2 = R_3$ = H

3: R_1 = β-D-Xyl *p*; R_2 = β-D-Glc*p*
4: $R_1 = R_2$ = H

1) S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (99871) 120 64 75; 2) N. D. Zelinskii Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, Leninskii pr., 47. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 228-230, May-June, 2002. Original article submitted June 17, 2002.

 0009-3130/02/3803-0272$27.00

TABLE 1. ^{1}H and ^{13}C Chemical Shifts in NMR Spectra of Cyclostipuloside C (**1**), Cycloglobiseposide B (**2**), Cyclosiversioside F (**3**), and Cyclosiversigenin (**4**) (C_5D_5N, 0 = TMS, δ, ppm)

No.	Compound					
	1		2[1]		3[7]	4
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	32.29	1.54; 1.22	32.55	1.63; 1.25	34.60	33.01
2	30.22	2.35; 1.96	30.30	2.38; 2.00	29.03	31.70
3	88.66	3.52	88.80	3.63	88.55	78.53
4	42.65	-	42.70	-	42.67	42.69
5	52.60	1.87	53.90	1.76	52.56	52.20
6	79.96	3.73	66.90	3.67	79.20	68.57
7	35.05	2.37; 1.78	38.25	1.75; 1.61	34.92	39.08
8	45.99	1.92	46.90	1.73	46.24	47.50
9	21.17	-	21.25	-	21.13	21.19
10	29.26	-	29.25	-	28.89	30.11
11	26.46	1.83; 1.23	26.25	1.91; 1.17	26.49	26.51
12	33.07	1.90; 1.85	32.85	1.61; 1.61	33.41	33.65
13	46.79	-	45.65	-	45.08	45.28
14	47.30	-	46.85	-	46.24	46.41
15	46.79	2.49; 2.15	47.30	2.21; 1.99	45.75	46.99
16	83.50	4.53	82.85	4.39	73.41	73.69
17	59.83	2.46	57.45	1.84	58.23	58.64
18	21.16	1.60	19.05	1.23	21.13	21.37
19	29.48	0.52; 0.18	30.30	0.45; 0.25	30.23	31.21
20	87.04	-	31.70	2.19	87.20	87.48
21	26.26	1.72	18.20	1.00	28.60	28.81
22	38.71	2.26; 2.18	34.50	2.42; 1.11	32.24	35.16
23	25.81	2.17; 2.03	29.90	2.19; 1.64	26.20	26.69
24	84.34	4.06	78.30	3.87	81.69	81.93
25	71.31	-	80.85	-	71.29	71.48
26	26.46	1.47	22.10	1.55	28.21	27.38
27	27.50	1.41	23.55	1.52	28.60	28.44
28	20.21	0.92	20.20	0.96	27.10	20.44
29	28.78	2.04	28.85	1.99	16.65	29.67
30	16.16	1.36	16.70	1.34	19.87	16.38
	β-D-Xyl*p* (1→3Agl)		*β*-D-Xyl*p* (1→3Agl)		*β*-D-Xyl*p* (1→3Agl)	
1	107.68	4.85	107.45	4.89	107.56	
2	75.61	4.08	75.40	4.06	75.61	
3	78.53	4.13	78.20	4.17	78.15	
4	71.31	4.22	71.10	4.22	71.28	
5	67.06	4.36; 3.70	67.90	4.36; 3.72	67.07	
	β-D-Glc*p* (1→6Agl)		*β*-*D*-Glc*p* (1→16Agl)		*β*-D-Glc*p* (1→6Agl)	
1	105.60	4.88	106.10	4.77	105.46	
2	75.61	4.03	75.80	3.92	75.61	
3	79.29	4.20	78.60	4.20	79.31	
4	71.94	4.17	71.40	4.20	71.29	
5	77.96	3.94	77.90	3.88	78.55	
6	63.30	4.61; 4.35	62.75	4.41; 4.29	63.10	
	β-D-Glc*p* (1→16Agl)		*β*-D-Glc*p* (1→25Agl)			
1	106.46	4.87	98.60	5.14		
2	75.60	3.98	75.15	4.00		
3	78.86	4.18	78.40	4.25		
4	72.09	4.14	71.70	4.21		
5	78.36	3.96	78.00	3.94		
6	62.94	4.59; 4.38	62.70	4.49; 4.26		

Chemical shifts of C-24 in ^{13}C NMR spectra of dammarane triterpene glycosides differ for the 24R- and 24S-series by 2.7-3.3 ppm, with C-24 of the R-configuration resonating at weaker field. This confirms that the asymmetric center C-24 in **1** has the R-configuration [4]. Such a configuration for C-24 is observed in the cycloartane cyclogalegigenin [3, 5, 6] (Table 1).

A comparison of the ^{13}C NMR spectra of this compound and **1** confirms that its aglycone is cyclogalegigenin.

Further spectral analysis showed that carbinol atoms C-3, C-6, and C-16 in **1** are glycosylated and resonate at 88.66, 79.96, and 83.50 ppm, respectively. Thus, it is assumed that the sugars are bonded to the genin hydroxyls on C-3, C-6, and C-16.

According to the magnitudes of the H′, H″, and H‴ spin—spin coupling constants (SSCC) (7.5 Hz), the monosaccharide units have the β-configuration, the C-1 conformation, and a pyranose ring.

HMBC and ROESY methods defined the location of the carbohydrate residues on the genin. The results showed that **1** is 3-O-β-D-xylopyranoside-6,16-di-O-β-D-glucopyranoside-20S,24R-epoxycycloartan-3β,6α,16β,25-tetraol.

EXPERIMENTAL

We used silica gel containing 10% gypsum and Silufol plates for TLC; silica gel (KSK) of particle size 0.1-0.08 and 0.16-0.1 mm, for column chromatography. Cycloartanes and their derivatives were detected using methanolic phosphotungstic acid (20%) with heating at 120°C for 5-10 min. IR spectra were recorded on a Perkin—Elmer System 2000 FT IR Fourier spectrometer in KBr pellets.

^1H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 instrument at working frequencies 500.13 and 125.27 MHz, respectively, in C_5D_5N at 30°C with TMS standard. Two-dimensional spectra were recorded using standard Bruker methods. The relaxation time between recording TOCSY and ROESY spectra was 0.2 sec. The accuracy of the measured chemical shifts of ^1H and ^{13}C was ±0.01 ppm; $^1H/^1H$ SSCC, 0.2 Hz.

Paper chromatography (PC) was performed on FN-11 paper. Sugars were developed by anilinium phthalate.

We used systems 1) $CHCl_3$—CH_3OH—H_2O (70:23:3) and 2) butanol—pyridine—water (6:4:3) for chromatography.

Isolation of Cycloartanes. Air-dried ground aerial organs (2.5 kg) of *T. stipulosa* Boriss (Leguminosae) that were collected in October 1995 in the Baisunsk region of Surkhandar′insk region were extracted five times with methanol (11 L). The extract was condensed. The remainder was diluted with water. The solid was separated. The rest of the methanol was distilled. The aqueous solution was extracted with ethylacetate and butanol. Evaporation of solvents in vacuo gave ethylacetate (25.15 g) and butanol (76.23 g) fractions.

Separation of the Butanol Fraction. The butanol fraction was chromatographed over a column with elution by system 1 to give a mixture of three compounds (14 g). The fraction was rechromatographed using the same system to isolate **1**, 916 mg, 0.036% (here and hereafter the yield is calculated based on air-dried material).

Cyclostipuloside C (1). $C_{47}H_{77}O_{18}$, mp 232-235°C (methanol), $[\alpha]_D^{22}$ +38.4±2° (*c* 0.5, $CHCl_3$—CH_3OH, 4:1). IR spectrum (KBr, ν, cm^{-1}): 3393 (OH), 3050 (cyclopropane ring).

^1H and ^{13}C NMR spectra are listed in Table 1.

Acid Hydrolysis. Compound **1** (5 mg) was hydrolyzed in methanolic H_2SO_4 (5 mL, 0.5%) at 70°C for 3 h. Neutralization by $BaCO_3$, evaporation, and PC using system 2 detected D-xylose and D-glucose in the hydrolysate by comparison with authentic samples.

REFERENCES

1. R. Zh. Karimov, V. V. Kachala, N. Sh. Ramazanov, Z. Saatov, and A. S. Shashkov, *Khim. Prir. Soedin.*, 448 (2001).
2. R. Zh. Karimov, R. U. Umarova, Z. Saatov, M. G. Levkovich, and N. D. Abdullaev, *Khim. Prir. Soedin.*, 670 (1998).
3. V. Kh. Kravtsov, V. N. Biyushkin, O. N. Rebrova, M. I. Isaev, M. B. Gorovits, M. D. Alaniya, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 541 (1988).

4. O. Tanaka and S. Yahara, *Phytochemistry*, **17**, 1353 (1978).
5. M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 156 (1989).
6. M. D. Alaniya, M. I. Isaev, M. B. Gorovits, N. D. Abdullaev, E. P. Kemertelidze, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 332 (1983).
7. K. K. Uteniyazov, Z. Saatov, N. D. Abdullaev, and M. G. Levkovich, *Khim. Prir. Soedin.*, 509 (1998).