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

T. N. Kaipnazarov, K. K. Uteniyazov, and Z. Saatov

UDC 547.918:547.926

*The known compound trojanoside A (**1**) and a new cycloartane glycoside cyclostipuloside E (**2**) were isolated from the aerial parts of* Tragacantha stipulosa *Boriss. The structure of cyclostipuloside E was proposed as 24R-cycloartan-3β,6α,16β,24,25-pentaol 6,16,25-tri-O-β-D-glucopyranoside 3-O-β-D-xylopyranoside based on physicochemical data and chemical transformations.*

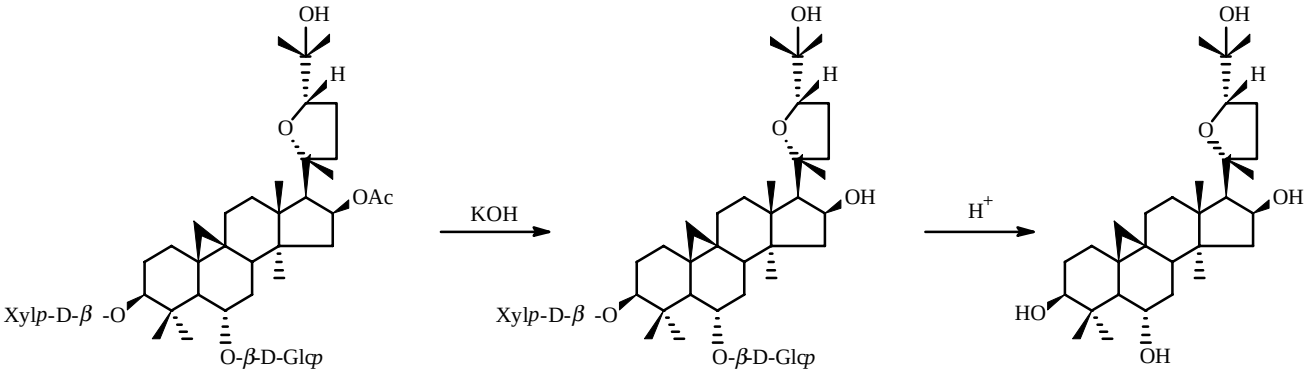
Key words: cycloartanes, trojanoside A, cyclosiversioside F, cyclosiversigenin, cyclostipuloside E, cycloasgenin C, cycloasgenin C monoside.

In continuation of research on cycloartane triterpenoids from *Tragacantha stipulosa* Boriss (Leguminosae) [1], we isolated the known cycloartane glycoside trojanoside A (**1**) and a new compound, cyclostipuloside E (**2**), from the butanol fraction of the methanol extract of the aerial parts. Six compounds were previously isolated from this plant: cycloglobiseposide B [2], cyclostipuloside A, askendoside G, askendoside D [3], cyclostipuloside D [4], and cyclostipuloside C [1].

The PMR spectrum of **1** [5] contains signals for seven methyls at 0.89, 0.94, 0.98, 1.02, 1.19, 1.24, and 1.24 ppm in addition to signals for two 1H doublets of an AB system at 0.17 and 0.47 ppm, which are unambiguously assigned to methylene H atoms of the cyclopropane ring. Therefore, **1** is a cycloartane.

The PMR spectrum of this compound contains a 3H singlet at 1.95 ppm that indicates the molecule has a single acetyl. This is also evident in the ^{13}C NMR spectrum, in which resonances of C atoms of a single acetate are clearly visible at 21.24 and 171.25 ppm. This is confirmed by the presence in the IR spectrum of an absorption band for an ester at 1717 cm^{-1}.

Saponification of **1** by base produced the deacetylated derivative **3**, which coincided on TLC with cyclosiversioside F [6]. Acid hydrolysis showed that the genin is cyclosiversigenin (**4**) whereas the carbohydrate consists of xylose and glucose.



1 **3** **4**

Comparison of the ^{13}C NMR spectra of **1** and **3** revealed that all signals in general coincide. The only difference is that the signal for C-16 in the spectrum of **1** is shifted to weak field by +1.54 ppm.

It can be concluded from the above that the acetyl in **1** is located on C-16 of the aglycon.

S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, 700170, Tashkent, fax (99871) 120 64 75, e-mail: root@icps.org.uz. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 35-38, January-February, 2004. Original article submitted November 3, 2003.

 0009-3130/04/4001-0040

TABLE 1. ^{13}C NMR Chemical Shifts (ppm) of Cyclostipuloside E (**2**), Cycloasgenin C (**5**), and its Monoside (**6**) in C_5D_5N

C atom	Compound		
	2	**5**	**6**
1	32.57	32.81	32.61
2	30.05	31.48	30.10
3	88.77	78.84	88.75
4	42.72	42.43	42.71
5	54.04	53.88	54.10
6	79.32	68.25	67.89
7	38.39	38.56	38.40
8	46.86	47.02	46.92
9	21.28	21.07	21.15
10	29.60	29.55	29.62
11	26.26	26.26	26.25
12	32.83	34.37	32.80
13	45.63	45.55	45.61
14	46.86	46.90	46.92
15	47.30	47.82	47.39
16	82.84	71.77	71.75
17	57.51	57.19	57.15
18	18.21	18.08	18.52
19	30.37	30.30	30.25
20	31.74	31.96	31.95
21	19.04	19.18	19.14
22	30.22	30.63	30.49
23	34.55	34.37	34.57
24	78.86	79.96	78.82
25	80.79	72.70	72.65
26	22.32	25.42	24.83
27	23.50	26.39	25.18
28	20.23	20.25	20.24
29	28.92	29.44	29.04
30	16.74	16.23	16.65
	3-O-*β*-D-Xyl*p*		
1′	107.60		107.59
2′	75.62		75.63
3′	78.04		78.44
4′	71.85		71.33
5′	67.05		67.05
	6-O-*β*-D-Glc*p*		
1″	105.68		
2″	75.85		
3″	77.62		
4″	72.27		
5″	78.79		
6″	62.27		

TABLE 1. (Continued)

C atom	Compound		
	2	**5**	**6**
	16-O-β-D-Glc*p*		
1‴	106.28		
2‴	75.94		
3‴	78.49		
4‴	71.27		
5‴	78.14		
6‴	62.94		
	25-O-β-D-Glc*p*		
1⁗	98.75		
2⁗	75.35		
3⁗	78.72		
4⁗	71.85		
5⁗	78.32		
6⁗	62.87		

The structure of **1** was confirmed using PMR, ^{13}C NMR, and two-dimensional spectra (COSY, TOCSY). Comparison of the ^{13}C NMR spectrum of the genin of compound **1** with those in the literature established that it is identical to that of cyclosiversigenin (**4**) [6, 7].

Thus, the structure of trojanoside A (**1**) is 16-O-acetyl-20*R*,24*S*-epoxycycloartan-3β,6α,16β,25-tetraol 3-O-β-D-xylopyranoside 6-O-β-D-glucopyranoside.

The PMR spectrum of the new compound, cyclostipuloside E (**2**), has at strong field signals for protons of seven methyls in addition to 1H doublets for an AB system at 0.25 and 0.45 ppm with ^{2}J = 3.9 Hz, which are characteristic of methylene protons of a cyclopropane ring. The presence of a three-membered ring was confirmed by an absorption band at 2972 cm^{-1} in the IR spectrum of **2**.

Acid hydrolysis of **2** produced the genin, which was identified using spectral and literature data as cycloasgenin C (**5**) [2]. Paper chromatography (PC) of the hydrolysates detected D-xylose and D-glucose.

The PMR and ^{13}C NMR spectra of **2** exhibit signals for four anomeric protons at 4.78, 4.84, 4.92, and 5.20 ppm and four anomeric C atoms that resonate at 107.60, 105.68, 106.28, and 98.75 ppm, respectively (Table 1). These data indicate that **2** is a tetraoside.

Comparison of the ^{13}C NMR spectra of **5** [2] and **2** showed that four C atoms of the genin (C-3, C-6, C-16, and C-25) are affected by glycosylation and resonate at 88.77, 79.32, 82.84, and 80.79 ppm, respectively.

Thus, it can be assumed that the sugars are bonded to the genin through hydroxyls on C-3, C-6, C-16, and C-25.

Enzymatic hydrolysis of **2** by gastric juice of the snail *Helix plectotropis* produced progenin **6** [2].

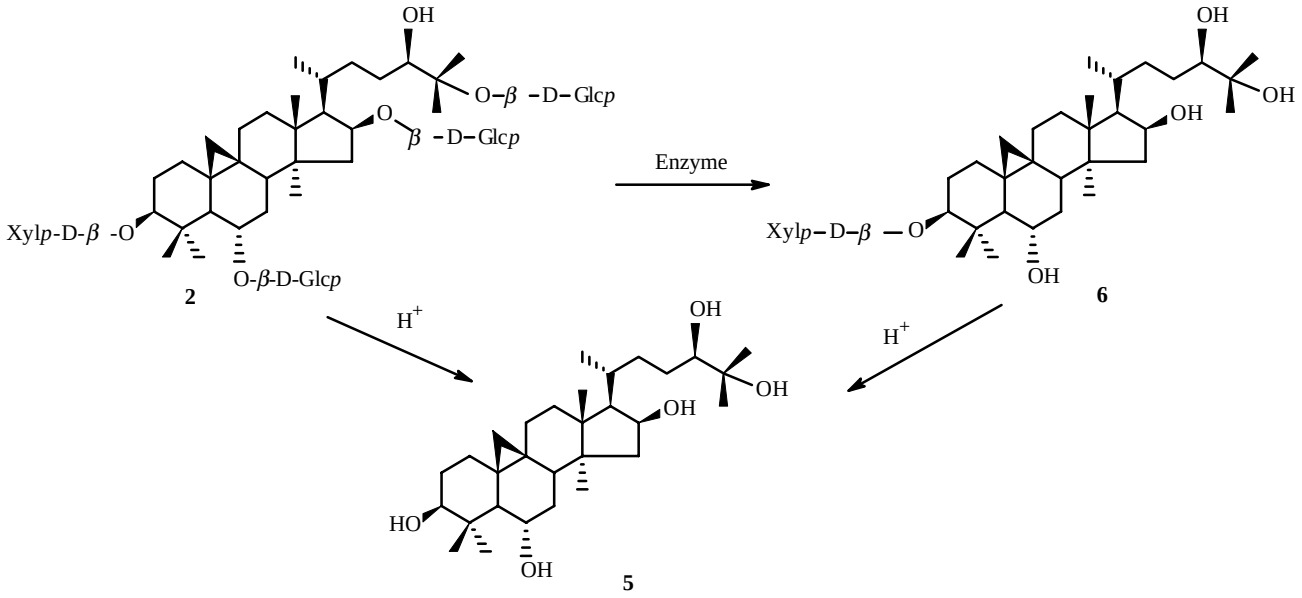
Acid hydrolysis of **6** gave cycloasgenin C (**5**) [2]. PC of the hydrolysate detected D-xylose.

The PMR spectrum of **6** [2] exhibits a signal for an anomeric proton as a doublet at 4.86 ppm with SSCC ^{2}J = 7.7 Hz. Comparison of the ^{13}C NMR spectra of **6** [2] and **5** [2] found that the signal for C-3 undergoes a paramagnetic shift by 9.93 ppm compared with the genin (Table 1).

Therefore, **6** [2] contains D-xylose bonded to the genin through the C-3 hydroxyl and is cycloasgenin C 3-O-β-D-xylopyranoside [2].

These data indicate that the three glucoses in **2** are bonded to hydroxyls on C-6, C-16, and C-25. This conclusion was confirmed using PMR and ^{13}C NMR spectra of **2**. The SSCC of the anomeric protons are consistent with the $^4C_1$-conformation of the pyranose rings and the β-configuration of the glycoside centers.

Thus, **2** has the structure 24*R*-cycloartan-3β,6α,16β,24,25-pentaol 6,16,25-tri-O-β-D-glucopyranoside 3-O-β-D-xylopyranoside.



EXPERIMENTAL

For general comments, see [1].

Separation of the Butanol Fraction. The butanol fraction was chromatographed over a column using $CHCl_3$:CH_3OH:H_2O (70:23:3) to afford trojanoside A (**1**, 1.5 g, 0.06%) [5] and **2** (90 mg, 0.0036%) (here and hereinafter yields are calculated per air-dried raw material).

Trojanoside A (1). $C_{43}H_{70}O_{15}$, mp 292-285°C (CH_3OH), $[\alpha]_D^{25}$ +20.1° (*c* 0.1, CH_3OH). IR spectrum (KBr, ν_{max}, cm^{-1}): 3416 (OH), 3030 (cyclopropane), 1717 (C=O), 1272 and 1043 (C–O–C).

PMR spectrum (DMSO, δ, ppm, 0 = TMS): 1.44, 1.16 (H-1), 1.82, 1.49 (H-2), 3.05 (H-3), 1.46 (H-5), 3.38 (H-6), 1.73, 1.45 (H-7), 1.77 (H-8), 1.76, 1.31 (H-11), 1.65, 1.60 (H-12), 2.11, 1.25 (H-15), 5.23 (H-16), 2.38 (H-17), 0.94 (H-18), 0.47, 0.17 (H-19), 1.24 (H-21), 2.13, 1.51 (H-22), 1.80, 1.69 (H-23), 3.61 (H-24), 3.81 (OH-25), 1.02 (H-26), 0.98 (H-27), 1.24 (H-28), 1.19 (H-29), 0.89 (H-30), 4.13 (H-1′-Xyl*p*), 2.96 (H-2′), 3.07 (H-3′), 3.24 (H-4′), 3.65, 3.00 (H-5′), 4.15 (H-1″-Glc*p*), 2.94 (H-2″), 3.11 (H-3″), 3.03 (H-4″), 3.05 (H-5″), 3.62, 3.44 (H-6″), 1.95 (CH_3COO).

^{13}C NMR spectrum (DMSO, δ, ppm, TMS): 31.32 (C-1), 29.08 (C-2), 87.16 (C-3), 41.47 (C-4), 51.19 (C-5), 77.27 (C-6), 32.57 (C-7), 44.03 (C-8), 20.38 (C-9), 27.95 (C-10), 25.51 (C-11), 31.88 (C-12), 45.31 (C-13), 46.66 (C-14), 44.33 (C-15), 74.95 (C-16), 56.69 (C-17), 19.44 (C-18), 27.33 (C-19), 84.78 (C-20), 26.86 (C-21), 36.32 (C-22), 25.69 (C-23), 82.08 (C-24), 70.11 (C-25), 26.79 (C-26), 24.87 (C-27), 19.72 (C-28), 27.22 (C-29), 15.78 (C-30), 105.98 (C-1′), 73.77 (C-2′), 76.59 (C-3′), 69.60 (C-4′), 65.50 (C-5′), 103.13 (C-1″), 74.02 (C-2″), 77.27 (C-3″), 70.22 (C-4″), 76.61 (C-5″), 61.29 (C-6″), 21.24, 171.25 (CH_3COO).

Alkaline Hydrolysis. Compound **1** (100 mg) was saponified by KOH solution (25 mL, 0.5%). The reaction mixture was left at room temperature for 1 d, diluted with water (25 mL), and neutralized with acetic acid. The methanol was evaporated. The solution was extracted with butanol. The solid after distilling off the butanol was chromatographed over a silica-gel column with elution by $CHCl_3$:CH_3OH:H_2O (70:23:3) to afford **3** (70 mg) [6], $C_{41}H_{68}O_{14}$, mp 260-261°C (CH_3OH). IR spectrum (KBr, ν, cm^{-1}): 3382 (OH), 2941 (cyclopropane).

^{13}C NMR spectrum (C_5D_5N, δ, ppm, TMS): 34.66 (C-1), 29.03 (C-2), 88.27 (C-3), 42.67 (C-4), 52.56 (C-5), 79.20 (C-6), 34.92 (C-7), 46.24 (C-8), 21.13 (C-9), 28.93 (C-10), 26.49 (C-11), 33.41 (C-12), 45.08 (C-13), 46.24 (C-14), 45.77 (C-15), 73.41 (C-16), 58.23 (C-17), 21.13 (C-18), 30.23 (C-19), 87.27 (C-20), 28.60 (C-21), 32.24 (C-22), 26.20 (C-23), 81.69 (C-24), 71.29 (C-25), 28.24 (C-26), 28.60 (C-27), 27.10 (C-28), 16.65 (C-29), 19.87 (C-30), 107.56 (C-1′), 75.61 (C-2′), 78.15 (C-3′), 71.18 (C-4′), 67.07 (C-5′), 105.46 (C-1″), 75.61 (C-2″), 79.31 (C-3″), 71.29 (C-4″), 78.55 (C-5″), 63.19 (C-6″).

Acid Hydrolysis. Compound **3** (50 mg) was hydrolyzed for 3 h in methanolic H_2SO_4 (20 mL, 0.5%), cooled, and treated with water (20 mL). The methanol was distilled off. The solution was heated for another 2 h, cooled, and extracted with

$CHCl_3$. The $CHCl_3$ extracts were washed with water and evaporated to dryness in a rotary evaporator. The solid was chromatographed over a silica-gel column with elution by $CHCl_3$:CH_3OH (9:1) to afford **4** (30 mg) [6, 7]. Paper chromatography using butan-1-ol:pyridine:water (6:4:3) of the aqueous part of the hydrolysate detected D-xylose and D-glucose by comparison with authentic specimens.

Literature data for **4** [6, 7]: mp 229-231°C (ethylacetate), $[\alpha]_D^{20}$ +67.1° (*c* 0.192, CH_3OH).

Cyclostipuloside E (2). $C_{53}H_{94}O_{24}$, mp 272-274°C (CH_3OH).

IR spectrum (KBr, ν, cm^{-1}): 3390 (OH), 2972 (cyclopropane).

PMR spectrum (500 MHz, C_5D_5N, δ, ppm, J/Hz, 0 = TMS): 0.25 and 0.45 (1H each, d, J = 3.9, 2H-19), 0.87, 0.97, 1.23, 1.35, 1.52, 1.68, 2.02 (s, 3H each, tertiary methyls), 3.64 (1H, dd, J = 11.6 and 4.6, H-3), 3.75 (1H, m, H-6), 4.78 (1H, d, J = 7.6, xylose H-1′), 4.84, 4.92, and 5.20 (3H, d, J = 7.4, 7.6, and 7.8, glucose H-1″, H-1‴, and H-1⁗).

For the ^{13}C NMR spectrum, see Table 1.

Acid Hydrolysis. Glycoside **2** (40 mg) was hydrolyzed as described above to afford **5** (10 mg), $C_{30}H_{52}O_5$, mp 244-246°C (acetone), $[\alpha]_D^{23}$ +33.7 ± 2° (*c* 1.18, CH_3OH). These constants in addition to a comparison with an authentic specimen identified the aglycon as cycloasgenin C (**5**) [2]. PC of the hydrolysate using butan-1-ol:pyridine:water (6:4:3) detected D-xylose and D-glucose by comparison with authentic specimens.

Enzymatic Hydrolysis. An aqueous solution of **2** (30 mg) was treated with *H. plectotropis* enzyme, held for 2 months at 38°C, diluted with water (15 mL), and extracted with butanol (5 × 5 mL). The solvent was evaporated. The solid was chromatographed over a silica-gel column with elution by $CHCl_3$:CH_3OH:H_2O (40:7.5:1) to afford progenin **6** (12 mg) [2], $C_{35}H_{58}O_9$, mp 253-255°C (CH_3OH).

PMR spectrum (C_5D_5N, δ, ppm, J/Hz, 0 = TMS): 0.34 and 0.56 (1H each, d, ^2J = 4.0, 2H-19), 0.99, 1.02 (d, J = 6.5), 1.25, 1.40, 1.47, 1.53, 1.94 (s, 3H each, tertiary methyls), 3.70 (1H, dd, ^3J = 11.6 and 4.7, H-3), 3.74 (2H, m, H-6 and H-24), 4.86 (1H, d, ^3J = 7.7, xylose H-1′).

For the ^{13}C NMR, see Table 1.

Further elution of the column with the same solvent system isolated starting substance **2** (13 mg).

Acid Hydrolysis of Progenin 6. Compound **6** (12 mg) was hydrolyzed as described above to afford cycloasgenin C (**5**, 4 mg). PC of the hydrolysate detected D-xylose by comparison with an authentic specimen.

ACKNOWLEDGMENT

We thank the leader of the NMR spectroscopy group Dr. A. S. Shashkov and his coworkers for recording the PMR and ^{13}C NMR spectra.

REFERENCES

1. T. N. Kaipnazarov, K. K. Uteniyazov, Z. Saatov, V. V. Kachala, and A. S. Shashkov, *Khim. Prir. Soedin.*, 228 (2002).
2. K. K. Uteniyazov, Z. Saatov, M. G. Levkovich, and N. D. Abdullaev, *Khim. Prir. Soedin.*, 216 (1999).
3. R. Zh. Karimov, V. V. Kachala, N. Sh. Ramazanov, Z. Saatov, and A. S. Shashkov, *Khim. Prir. Soedin.*, 448 (2001).
4. T. N. Kaipnazarov, K. K. Uteniyazov, Z. Saatov, V. V. Kachala, and A. S. Shashkov, in: 5th International Symposium on the Chemistry of Natural Compounds, Tashkent, 21-23 May, 2003.
5. E. Bedir, I. Calis, R. Aquino, S. Piacente, and C. Pizza, *J. Nat. Prod.*, **62**, 563 (1999).
6. K. K. Uteniyazov, Z. Saatov, N. D. Abdullaev, and M. G. Levkovich, *Khim. Prir. Soedin.*, 509 (1998).
7. A. N. Svechnikova, R. U. Umarova, M. B. Gorovits, K. L. Seitanidi, Ya. V. Rashkes, M. R. Yagudaev, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 67 (1981).