LITERATURE CITED

1. B. A. Imomnazarov and M. I. Isaev, Khim. Prir. Soedin., 227 (1992).
2. M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 156 (1989).
3. K. Nakanishi, Infrared Absorption Spectroscopy. Practical, Holden-Day, San Francisco (1962).
4. M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 431 (1985).
5. M. I. Agzamova, M. I. Isaev, M. B. Gorovits, N. K. Abubakirov, Khim. Prir. Soedin., 719 (1986).

TRITERPENE GLYCOSIDES OF ASTRAGALUS AND THEIR GENINS

XLII. CYCLOARTANES OF ASTRAGALUS TRAGACANTHA

M. I. Isaev, B. A. Imomnazarov, Yu. M. Fadeev, and P. A. Kintya

UDC 547.918:547.926

Another six components of Astragalus tragacantha Habl. have been identified on the basis of spectral characteristics and chemical transformations. We have previously described cyclocanthagenin and its 3-O-β-D-xylopyranoside – cyclocanthoside A – as products of the acid hydrolysis of cyclocanthoside D. Cyclocanthosides B, C, E, and G are here described for the first time and are (24S)-cycloartane-3β,6α,16β,24,25-pentol 3-O-(4-O-acetyl-β-D-xylopyranoside) 6-O-β-D-glucopyranoside, (24S)-cycloartane-3β,6α,16β,24,25-pentol 6-O-(6-O-acetyl-β-D-glucopyranoside) 3-O-β-D-xylopyranoside, (24S)-cycloartane-3β,6α, 16β,24,25-pentol 6-O-β-D-glucopyranoside, and (24S)-cycloartane-3β, 6α, 16β, 24, 25-pentol 6-O-β-D-glucopyranoside 3-O-[O-β-D-glucopyranosyl-(1→2)-β-D-xylopyranoside], respectively.

Continuing a study of the cycloartane triterpenoids and their glycosides of Astragalus tragacantha Habl. (Leguminosae) [1, 2] we have isolated another two products which have been designated as substances (3) and (12A). The present paper is devoted to a proof of the structures of four new compounds and the identification of two known compounds isolated from Astragalus tragacantha [1, 2]. The substances under consideration were assigned to the cyclartane triterpenoid series on the basis of their ^1H and ^{13}C NMR spectra [3, 4].

Substance (3) was identified as cyclocanthogenin (I), which we had obtained previously from cyclocanthoside D [1]. Substances (6), (9), (10), (12A), and (14) were glycosides, and we have called them cyclocanthosides A, B, C, E, and G, respectively. Cyclocanthoside A is cyclocanthogenin 3-O-β-D-xylopyranoside, identical with the progenin obtained from cyclocanthoside B [2] (see top of following page).

On acid hydrolysis, cyclocanthoside E (III) formed cyclocanthogenin (I). GLC [5] showed that glycoside (III) contained D-glucose and D-xylose residues in a ratio of 1:1. This conclusion also followed from its ^1H and ^{13}C NMR spectra (Tables 1 and 2).

It became clear from a comparison of the ^{13}C NMR spectra of compounds (I) and (III), that the C-3 and C-6 atoms of the genin moiety of glycoside (III) experienced the glycosylation effect, resonating at 88.59 and 79.13 ppm. Consequently, cyclocanthoside E was a bisdesmoside. The SSCCs of the anomeric protons and the chemical shifts of the carbon atoms of the monosaccharide residue showed the β-configuration, the C1-conformation, and the pyranose form of the D-xylose and D-glucoside residues.

Institute of Chemistry of Plant Substances, Uzbekistan Academy of Sciences, Tashkent. Institute of Ecological Genetics, Moldavian Academy of Sciences, Kishenev. Translated from Khimiya Prirodnykh Soedinenii, Nos. 3,4, pp. 360-367, May-August, 1992. Original article submitted September 6, 1991.

0009-3130/92/0304-0315$12.50

TABLE 1. Chemical Shifts (δ, ppm), Multiplicities, and SSCCs (J, Hz) of the Protons of Cyclocanthosides E (III), B (IV), and C (V), (C_5D_5N, 0 – TMS)*

Position of the protons	Compound		
	III	IV	V
H-3	3,49 dd (12; 5)	3,48 dd (12; 4)	3,47 dd (12; 4)
H-6	3,77 td (9; 3)	3,78 td (9; 4)	3,78 m
H-16	4,68 q (7; 7; 7;)	4,69 q (7; 7; 7)	4,74 q (7; 7; 7)
2H-19	0,18; 0,57 d (4)	0,18; 0,56 d (4)	0,16; 0,60 d (4)
H-24	3,91 dd (11; 3)	3,91 dd (12; 3)	3,91 dd (11; 3)
CH_3 groups	0,97 s	0,96 s	1,08 d (6,4) CH_3=21
	1,06 d (6,3) CH_3-21	1,06 d (6,4) CH_3-21	1,10 s
	1,32 s	1,31 s	1,28 s
	1,37 s	1,38 s	1,39 s
	1,43 s	1,43 s	1,43 s
	1,45 s	1,45 s	1,45 s
	1,96 s	1,98 s	1,91 s
CH_3COO	—	1,94 s	2,08 s
	β-D-Xylp residue		
1	4,79 d (7,3)	4,80 d (7,5)	4,78 d (7,4)
2	4,00 dd (9; 7,3)	4,01 dd (9; 7,5)	4,01 dd (9; 7,4)
3	4,08 t (9)	4,20 t (9)	4,11 t (9)
4	4,18 td (9; 5)	5,35 td (9; 5,6)	4,19 td (9; 5)
5a	3,65 dd (10; 9)	3,51 dd (10; 9)	3,65 dd (10; 9)
5e	4,31 dd (10; 5)	4 29 dd (10; 5,6)	4,32 dd (10; 5)
	β-D-Glcp residue		
1	4,86 d (7,6)	4,87 d (7,7)	4285 d (7,8)
2	3,98 dd (9; 7,6)	3.99 dd (9; 7,7)	3 98 dd (9; 7,8)
3	4,15 t (9)[a]	4,18 t (9)	4,18 t (9)
4	4,14 t (9)[a]	4.14 t (9)	4.15 t (9)
5	3,85ddd (9; 6; 3)	3 85 ddd (9; 6; 3)	3,94 m
6	4,25dd (12; 6)	4,27 dd (12; 6)	4,57 dd (11; 6)
6′	4,42dd (12; 3)	4,43 dd (12; 3)	4,97 dd (11; 2)

*The assignment of the signals of the protons of the carbohydrate residues is based on the double homonuclear resonance experiments.

[a]Signals superposed upon one another.

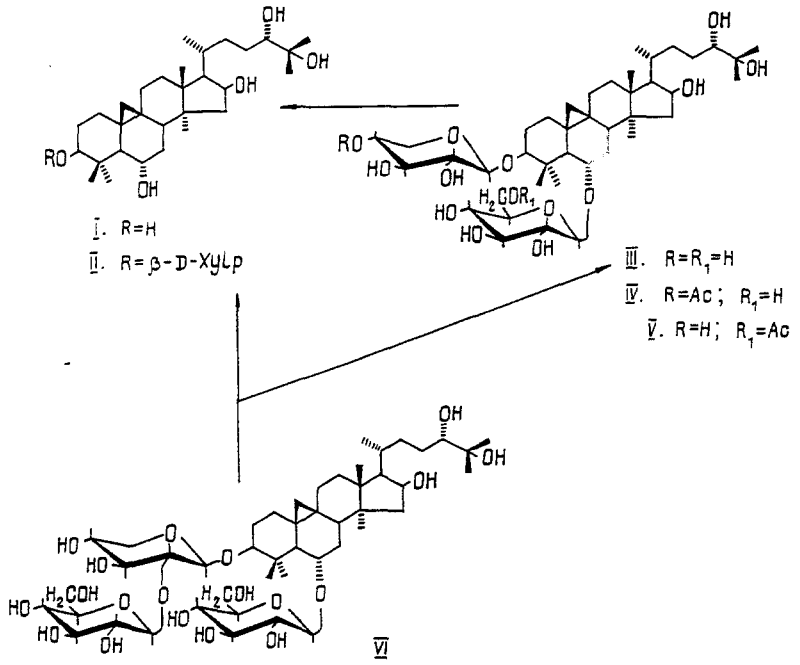

TABLE 2. Chemical Shifts of the Carbon Atoms of Compound (I-VI) (δ, ppm, 0 – TMS, C_5D_5N)

C atom	Compound					
	I	II	III	IV	V	VI
1	32,78	32,52	32,27	32,19	32,09	32,10
2	31,34	28,65	28,77	28,74	28,52	28,56
3	78,34	88,74	88,59	88,61	88,44	88,39
4	42,33	42,71	42,68	42,64	42,60	42,63
5	53,96	54,11	52,52	52,48	52,19	52,28
6	68,29	67,93	79,13[a]	79,15[a]	78,71[a]	78,87
7	38,45	38,42	34,32	34,35	33,59	33,95
8	47,17	48,36	45,61	45,65	44,93	45,09
9	21,35	21,36	21,45	21,41	21,46	21,43
10	30,37	30,35	30,20	30,09	30,12	30,06
11	26,43	26,28	26,30	26,26	26,31	26,29
12	33,33	33,19	33,21	33,18	33,18	33,19
13	45,76	45,73	45,82	45,79	45,83	45,82
14	46,85	46,81	46,94	46,91	46,82	46,89
15	48,41	46,99	47,88	47,88	47,53	47,69
16	72,02	72,01	72,02	71,93	72,05	71,98
17	57,39	57,35	57,20	57,19	57,16	57,11
18	18,24	18,28	18,37	18,48	18,08	18,23
19	29,70	29,53	28,21	28,26	27,85	27,88
20	28,78	28,86	28,57	28,52	28,70	28,23
21	18,97	18,91	18,48	18,32	18,32	18,34
22	33,07	33,03	33,00	32,97	33,04	32,98
23	27,99	27,91	27,90	27,87	27,14	27,70
24	77,22	77,19	77,14	77,11	77,18	77,12
25	72,49	72,54	72,58	72,52	72,55	72,55
26	25,43*	25,71*	25,77*	25,74*	25,63*	25,75*
27	26,46*	26,47*	26,50*	26,46*	26,49*	26,43*
28	20,18	20,14	19,84	19,82	19,77	19,72
29	29,17	29,25	28,65	28,61	28,30	28,64
30	15,98	16,15	16,71	16,62	16,69	16,56
	β-D-Xylp residue					
1		107,59	107,60	107,38	107,54	105.36(−2,24)
2		75,61	75,61	75,74	75,55	83.86(+8,25)
3		78,49	78,48	74,91(−3,57)	78,48	76,93(−1,55)
4		71,26	71,25	73.23(+1,98)	71,24	70,90
5		67,04	67,02	63,17 (−3,85)	67,01	66,58
	6-O-β-D-Glcp residue					
1			105.18	105,15	105,07	105,11
2			75,56	75,60	75,49	75,66[a]
3			79,13[a]	79,15[a]	78,71[a]	78,85
4			71,95	71,98	71,51	71,95
5			78,06	78,07	74.98(−3,09)	77,97
6			63,20	63,17	65,06(+1,89)	63,20
	β-D-Glcp residue located at C-2 of β-D-Xylp					
1						106,25
2						75,66[a]
3						78,15
4						71,91
5						77,79
6						63,02
COO				170,57	170,97	
CH_3				20,86	21,03	

Signals marked with asterisks have been assigned ambiguously, and those with the same letters are superposed upon one another.

The difference PMR spectrum, from the measurement of the NOEs in the one-dimensional variant on the irradiation of the H-3 atom of the genin moiety, revealed a negative Overhauser effect on the signal of the anomeric proton in the β-D-xylopyranoside residue. This means that the D-xylopyranose residue was located at C-3 and the D-glucopyranose residue at C-6 of cyclocanthogenin [6]. In actual fact, from the products of the acid hydrolysis of cyclocanthoside E, together with cyclocanthogenin, we isolated a progenin (II) which was identical with cyclocanthoside A.

Thus, cyclocanthoside E is (24S)-cycloartane-3β,6α,16β,24,25-pentol 3-O-β-D-xylopyranoside.

Cyclocanthosides B (IV) and C (V) likewise formed cyclocanthogenin (I) and the same set of monosaccharides and this in the same ratios as cyclocanthoside E. The IR spectra of these glycosides showed the absorption bands of ester groups. The PMR spectra of glycosides (IV) and (V), containing three-proton singlets at 1.94 and 2.08 ppm, respectively, showed the presence in the molecules of each of the glycosides under investigation of one acetic acid residue. This was also shown by the ^{13}C NMR spectra of glycosides (IV) and (V), in which signals were observed at 170.57, 20.86 and 170.97, 21.03 ppm, respectively.

The alkaline hydrolysis of cyclocanthosides B and C gave one and the same glycoside (III), identical with cyclocanthoside E.

The positions of the acetyl groups were revealed by a study of the ^{1}H and ^{13}C NMR spectra.

In the PMR spectrum of glycoside (IV), the proton geminal to the acetoxy group resonated at 5.35 ppm in the form of a triplet of doublets (Table 1). This signal was unambiguously assigned to H-4 of the β-D-xylopyranoside residue on the basis of double homonuclear resonance experiments. Consequently, the acetyl group was located at C-4 of the pentose residue. An analysis of the ^{13}C NMR spectrum led to an identical conclusion.

As can be seen from Table 2, the α-effect of acetylation was experienced by C-4 of the β-D-xylopyranose residue, and its signal underwent a downfield shift by +1.98 ppm as compared with that in the spectrum of cyclocanthoside E. As was to be expected, the C-3 and C-5 atoms of the same monosaccharide residue experienced β-effects of acetylation, and their signals were shifted upfield by −3.57 and −3.85 ppm in comparison with the corresponding signals of the spectrum of glycoside (III). This meant that the acetyl group in glycoside (IV) was located at C-4 of the β-D-xylopyranoside residue, and cyclocanthoside B has the structure of (24S)-cycloartane-3β,6α,16β,24,25-pentol 3-O-(4-O-acetyl-β-D-xylopyranoside).

In the PMR spectrum of cyclocanthoside C, the signals of the H-6 and H-6' protons of the β-D-glucopyranoside residues, forming the AB part of an ABX system, were shifted downfield and appeared at 4.57 and 4.97 ppm. This fact permitted the assumption that the acetyl group in the molecule of glycoside (V) was located at C-6 of the glucopyranoside residue. The ^{13}C NMR spectrum of cyclocanthoside C could serve as a confirmation of this, showing a downfield shift of the C-6 signals of the hexose residue by +1.89 ppm and an upfield shift of the C-5 signal by −3.09 ppm.

Thus, we are justified in concluding that cyclocanthoside C has the structure of (24S)-cycloartane-3β,6α,16β-24,25-pentol 6-O-(6-O-acetyl-β-D-glucopyranoside) 3-O-β-D-xylopyranoside.

Cyclocanthoside G (VI) was the most polar glycoside among the components isolated from the plant Astragalus tragacantha. Acid hydrolysis of cyclocanthoside G gave cyclocanthogenin (I). GLC [5] showed that glycoside (VI) contained D-glucose and D-xylose residues in a ratio of 2:1. The ^{1}H and ^{13}C NMR spectrum agreed with the GLC results.

The enzymatic hydrolysis of cyclocanthoside G performed with the gastric juice of the grape snail (Helix pomatia) led to cyclocanthoside A. Consequently, the D-xylose residue was attached at C-3 of cyclocanthogenin and had the β- configuration, the C1 conformation and also, of course, the pyranose form.

A comparative analysis of the ^{13}C NMR spectra of genin (I) and glycoside (VI) showed that in the glycoside (VI) molecule the C-3 and C-6 atoms of the genin moiety experienced glycosylation effects and, consequently, cyclocanthoside G was a bisdesmoside.

The partial hydrolysis of glycoside of (VI) yielded cyclocanthoside E (III), in addition to cyclocanthogenin and cyclocanthoside A. Consequently, one of the D-glucose residues was located at C-6 and had the β- configuration, the C1 conformation, and the pyranose form. The cyclocanthoside E molecule was based on that of cyclocanthoside G, to which an additional D-glucose residue was attached. The position of the latter was revealed by a comparative study of the ^{13}C NMR spectra of cyclocanthosides E and G.

The anomeric carbon atom of the D-xylose residue in the ^{13}C NMR spectrum of cyclocanthoside E resonated at 107.60 ppm. In the spectrum of cyclocanthoside G, the signal of the carbon atom under consideration had undergone an upfield shift of −2.24 ppm and was observed

at 105.36 ppm. This was possible if the second D-glucose residue were present at C-2 of D-xylose [7, 8]. In actual fact, the C-2 atom of the latter experienced a glycosylation effect and resonated at 83.86 ppm while the C-3 atom, like the C-1 atom experienced the β-effect of glycosylation (−1.55 ppm). These facts unambiguously determined the position of the second D-glucose residue at C-2 of the D-xylose residue. The SSCC of the anomeric proton and the chemical shifts of the carbon atoms of the D-glucose residue under consideration showed the β-configuration, the C1 conformation, and the pyranose form of the latter.

Thus, cyclocanthoside G has the structure of (24S)-cycloartane-3β,6α,16β-24,25-pentol 6-O-β-D-glucopyranoside 3-O-[O-β-D-glucopyranosyl-(1→2)-β-D-xylopyranoside).

EXPERIMENTAL

For general remarks, see [1]. The following solvent systems were used: 1) chloroform–methanol (15:1); 2) chloroform–methanol–water (70:12:1); 3) chloroform–methanol–water (70:23:4); and 4) n-butanol–pyridine–water (6:4:3).

PC was conducted on FN-11 paper. For the GLC conditions, see [5].

The ^1H and ^{13}C NMR spectra were recorded on Bruker WM-250 and Bruker AM-400 instruments (δ, ppm, 0 – TMS). The ^{13}C NMR spectra were also recorded under the conditions of J-modulation.

The isolation and separation of the isoprenoids of Astragalus tragacantha are described in [1]. On continuing the separation of the fractions containing substance (3) and the intermediate fractions collected after the elution of substance (12) and partially containing the latter, substance (3) and a substance designated as (12A) were isolated.

Cyclocanthogenin (I) – substance (3), $C_{30}H_{52}O_5$, mp 194-195°C (from methanol); $[\alpha]_D^{24}$ +57 ± 2° (c 0.9; methanol) [1].

Cyclocanthoside A (II) – substance (6), $C_{35}H_{60}O_9$, mp 154-155°C (from ethyl acetate); $[\alpha]_D^{24}$ +27 ± 2° (c 0.8; methanol) [2].

Cyclocanthoside B (IV) – substance (9), $C_{43}H_{72}O_{15}$, mp 235-237°C (from methanol); $[\alpha]_D^{23}$ +12.6 ± 2° (c 0.95; methanol). ν_{max}^{KBr}, cm^{-1}: 3510-3320 (OH); 1730, 1255 (ester group). It was found by the GLC method [5] that glycoside (IV) contained D-glucose and D-xylose residues in a ratio of 1.00:0.80. For the ^1H and ^{13}C spectra, see Tables 1 and 2.

Cyclocanthoside C (V) – substance (10), $C_{43}H_{72}O_{15}$, mp 240-242°C (from chloroform–methanol (1:1)); $[\alpha]_D^{20}$ +30.6 ± 2° (c 1.11; methanol). ν_{max}^{KBr}, cm^{-1}: 3570-3350 (OH); 1740, 1270 (ester group). GLC [5] showed the presence in glycoside (V) of D-glucose and D-xylose residues in a ratio of 1.00:0.95. For its ^1H and ^{13}C NMR spectra, see Tables 1 and 2.

Cyclocanthanoside E (III) – substance (12A), $C_{41}H_{70}O_{14}$, mp 282-284°C (from ethanol); $[\alpha]_D^{28}$ +23.5 ± 2° (c 0.5; pyridine). ν_{max}^{KBr}, cm^{-1}: 3550-3230 (OH), 3060 (CH_2 of a cyclopropane ring). It was found by the GLC method [5] that cyclocanthoside E contained D-glucose and D-xylose residues in a ratio of 1.00:0.87. For its ^1H and ^{13}C NMR spectra, see Tables 1 and 2.

Cyclocanthoside G (VI) – substance (14), $C_{47}H_{80}O_{19}$, mp 190-195°C (from system 3); $[\alpha]_D^{28}$ ±3° (c 1.18; methanol), ν_{max}^{KBr}, cm^{-1}: 3600-3230 (OH). GLC [5] showed that glycoside (VI) contained D-glucose and D-xylose residues in a ratio of 1.00:0.42. PMR spectrum (C_5D_5N): 0.15 and 0.56 (2H-19, d, ^2J = 4 Hz), 1.06 (CH_3-21, d, ^3J = 6.4 Hz), 0.99; 1.37; 1.39; 1.43; 1.46, 1.86 (6 × CH_3, s), 3.40 (H-3, dd, $^3J_1$ = 12 Hz, $^3J_2$ = 5 Hz), 4.66 (H-16, q, $^3J_1 = {}^3J_2 = {}^3J_3$ = 7 Hz), 4.80 (H-1 of D-xylose, d, ^3J = 6.2 Hz), 4.83 (H-1 of D-glucose at C-6, d, ^3J = 7.7 Hz), 5.30 (H-1 of a D-glucose residue at C-2 of D-xylose, d, ^3J = 7.6 Hz). For the ^{13}C NMR spectrum, see Table 2.

Cyclocanthogenin (I) from (III). Cyclocanthoside E (100 mg) was hydrolyzed with 20 ml of 0.1% methanolic sulfuric acid at 60°C for 8 h. The reaction mixture was diluted with water and extracted with chloroform. The chloroform extract was washed with water and evaporated. The residue was chromatographed on a column with elution by system 1. This led to the isolation of 47 mg of cyclocanthogenin (I), mp 194-195°C (from methanol), $[\alpha]_D^{23}$ +57 ± 2° (c 1.0; methanol), also identified by its PMR spectrum.

Partial Hydrolysis of Cyclocanthoside E (III). Glycoside (III) (100 mg) was hydrolyzed with 20 ml of 0.05% methanolic sulfuric acid at 60°C for 6 h. After the usual working up and chromatography on a column in system 1, the reaction product yielded 19 mg of cyclocanthogenin (I), mp 194-195°C (from methanol), $[\alpha]_D^{23}$ +56.5 ± 2° (c 0.9; methanol), and 25 mg of cyclocanthoside A (II), mp 154-155°C (from ethyl acetate), $[\alpha]_D^{23}$ +27 ± 2° (c 1.0; methanol) [2]. Products (I) and (II) were identified by direct comparison with authentic samples.

Cyclocanthogenin (I) from (IV) and (V). Cyclocanthosides B (IV) and C (V) (50 mg in each case) were hydrolyzed under the conditions given above. Cyclocanthogenin (I) was isolated from the products of the acid hydrolysis of both glycosides and was identified by the usual expedients.

D-Glucose and D-xylose were detected in the carbohydrate fractions of the hydrolysates by PC in system 4.

Cyclocanthoside E (III), from (IV) and (V). Cyclocanthosides B (IV) and C (V) (25 mg in each case) were hydrolyzed with 2 ml of 0.1% of methanolic sodium hydroxide at room temperature for 1 h. After working up and chromatography on a column in system 3, cyclocanthoside E was isolated from the products of alkaline hydrolysis in both cases and was identified.

Cyclocanthogenin (I) from (VI). Glycoside (VI) (100 mg) was hydrolyzed with 25 ml of 0.5% methanolic sulfuric at 60°C for 30 min. After the usual working up and chromatography on a column in system 1, the genin fraction of the hydrolysate yielded 27 mg of genin (I), mp 194-195°C (from methanol), $[\alpha]_D^{23}$ +57 ± 2° (c 0.8; methanol), and this was identified as cyclocanthogenin [1].

Enzymatic Hydrolysis of Cyclocanthoside G (VI). Glycoside (VI) (182 mg) in 20 mg of water was treated with 0.5 ml of the gastric juice of the grape snail and a few drops of toluene, and the mixture was left at room temperature for 30 days. After this it was treated with n-butanol. The residue after the evaporation of the butanolic extract was chromatographed on a column with elution by system 2. This led to the isolation of 35 mg of the monoside (II), mp 154-155°C (from ethyl acetate), $[\alpha]_D^{20}$ +26 ± 2° (c 0.8; methanol). The monoside (II) was also identified as cyclocanthoside A from the characteristics of its PMR and IR spectra.

Partial Hydrolysis of Cyclocanthoside G (VI). Glycoside (VI) (200 mg) was hydrolyzed with 30 ml of 0.05% methanolic sulfuric acid at 60°C for 8 h. After working up, the reaction products were chromatographed on a column with elution by systems 1 and 2, successively. This led to the isolation of 35 mg of cyclocanthogenin (I), 13 mg of cyclocanthoside A (II), and 50 mg of the bioside (III) which was identified as cyclocanthoside E.

LITERATURE CITED

1. Yu. M. Fadeev, M. I. Isaev, Yu. A. Akimov, P. K. Kintya, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 817 (1987).
2. Yu. M. Fadeev, M. I. Isaev, Yu. A. Akimov, P. K. Kintya, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 73 (1988).
3. M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 431 (1985).
4. M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 156 (1989).
5. M. A. Agzamova, M. I. Isaev, B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 719 (1986).
6. H. K. Wang, K. He, L. Ji, Y. Tezuka, T. Kikuchi, and I. Kitagawa, Chem. Pharm. Bull., 37, 2041 (1989).
7. A. N. Svechnikova, R. U. Umarova, N. D. Abdullaev, M. B. Gorovits, T. T. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 460 (1983).
8. I. Kitagawa, N. K. Wang, M. Saito, and M. Yoshikawa, Chem. Pharm. Bull., 31, 709 (1983).