TRITERPENE GLYCOSIDES AND THEIR GENINS FROM Thalictrum foetidum.

III. THE STRUCTURE OF CYCLOFOETOSIDE A

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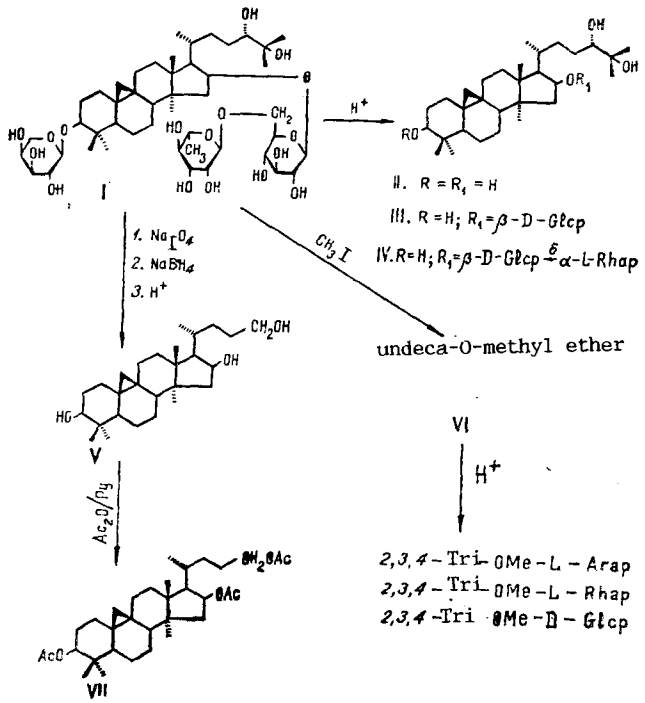
A new glycoside of the cycloartane series – cyclofoetoside A – has been isolated from the epigeal part of Thalictrum foetidum L., and its structure has been established on the basis of chemical transformations and spectral characteristics as 24S-cycloartane-3β,16β,24,25-tetraol 3-O-α-L-arabinopyranoside 16-O-[O-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside].

In the present paper we consider the determination of the structure of glycoside A which we isolated from Thalictrum foetidum L. (Ranunculaceae) [1] and have called cyclofoetoside A (I).

The acid hydrolysis of cyclofoetoside A (I) led to a triterpenoid of the cycloartane series – cyclofoetigenin A (II) [2]. In the hydrolysate, and also in the products of the methanolysis of glycoside (I), D-glucose, L-rhamnose, and L-arabinose were found by TLC and GLC [3] in a ratio of 1:1:1.



The stepwise hydrolysis of trioside (I) yielded two progenins – a monoside (III) and a bioside (IV). The carbohydrate moiety of progenin (III) consisted of D-glucose, while bioside (IV) contained D-glucose and L-rhamnose residues (1:1).

The Hakomori methylation [4] of cyclofoetoside A (I) led to the undeca-O-methyl ether (VI). In the products of the methanolysis of the permethylate (VI), 2,3,4-tri-O-methyl-L-

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TABLE 1. Chemical Shifts of the Carbon Atoms of Cyclofoetoside A (I) and Its Derivatives (δ, ppm, O – TMS; C_5D_5N)

C atom	Multiplicity	Compound			
		I	II	III	IV
1	t	32,5	32,5	32,5	32,5
2	t	29,9	31,3	31,3	31,3
3	d	88,7	78,0	78,1	78,1
4	s	41,3	41,1	41,1	41,1
5	d	47,8	47,6	47,6	47,7
6	t	21,2	21,5	21,4	21,5
7	t	26,4	26,5	26,3	26,4
8	d	48,2	48,4	48,2	48,4
9	s	20,1	20,0	20,0	20,0
10	s	26,4	26,8	26,7	26,7
11	t	26,4	26,5	26,3	26,4
12	t	33,6	33,3	33,5	33,6
13	s	45,8	45,8	45,9	45,9
14	s	47,1	47,1	47,1	47,1
15	t	48,2	48,8	48,2	48,4
16	d	82,7	72,0	82,7	82,8
17	d	57,8	57,5	57,8	57,9
18	q	17,5	18,3	17,6	17,6
19	t	30,2	30,3	30,3	30,4
20	d	29,7	28,7	29,6	29,6
21	q	19,5	19,4	19,6	19,6
22	t	33,1	33,1	33,1	33,1
23	t	28,9	27,9	28,9	29,0
24	d	78,1	77,2	78,1	78,1
25	s	72,8	72,5	72,8	72,8
26	q	26,3	26,2	26,3	26,3
27	q	25,8	25,6	25,7	25,7
28	q	20,5	20,3	20,5	20,6
29	q	25,6	26,5	26,3	26,3
30	q	15,4	14,8	14,8	14,8
D-Glucose					
1	d	106,5		106,7	106,7
2	d	75,5		75,7	75,6
3	d	78,4		78,6	78,5
4	d	71,9*		72,0	71,9*
5	d	76,6		78,1	76,7
6	t	68,4		63,1	68,5
L-Rhamnose					
1	d	102,2			102,3
2	d	72,7*			72,8*
3	d	72,2*			72,3
4	d	74,0			74,0
5	d	69,5			69,6
6	q	18,6			18,6
L-Arabinose					
1	d	106,9			
2	d	72,7*			
3	d	74,4			
4	d	69,1			
5	t	66,2			

*Assignment ambiguous within a column.

arabinopyranose, 2,3,4-tri-O-methyl-L-rhamnopyranose, and 2,3,4-tri-O-methyl-D-glucopyranose were identified by GLC [5]. The results of methylation showed that cyclofoetoside A (I) was a bisdesmosidic glycoside.

The trioside (I) was subjected to Smith degradation [6], after which compound (V) with M^+ 418 was isolated. A comparison of the PMR spectra of substance (V), its triacetate (VII), and the genin (II) showed that (V) contained two secondary hydroxy groups (at C-3 and C-16) and one primary hydroxy group. The production of the triol (V) with a molecular weight 58 units lower than that of cyclofoetigenin A (II, M^+ 476) indicated the cleavage of a C-24–C-25 diol system. From this it is obvious that the fourth hydroxy group in the nor compound (V) was present at C-24.

The formation of the 3,16,24-triol (V) showed that in cyclofoetoside A (I) the hydroxy groups at C-24 and C-25 are not substituted by sugar residues. On the basis of the bisdesmosidic structure of glycoside (I) it must be considered that the sugar residues are located at C-3 and C-16.

It is known that the Smith degradation of glycosides of such a structure, having free hydroxy groups at C-16, C-24, and C-25, takes place with the cyclization of the side chain, leading to 16,24-epoxy-25-norcycloartanes [7, 8]. The absence of a corresponding 16,24-epoxy derivative in this case also presupposes the presence of a carbohydrate chain at C-16.

A comparison of the ^{13}C NMR spectra of compounds (I-IV) confirmed this hypothesis. The chemical shifts of the carbinol atoms (C-3, C-16, C-24, and C-25) of glycoside (I) and genin (II) showed that the C-3 and C-16 atoms had undergone a glycosylation effect (Table 1).

It follows from the ^{13}C NMR spectra of the genin (II) and the monoside (III) that the D-glucose molecule was attached to the hydroxy group at C-16 ($\Delta\delta$ = +10.7 ppm). In the spectra of the bioside (IV) and of the genin (II), the C-3 carbinol atoms had practically identical values of their chemical shifts. On passing from the monoside (III) to the bioside (IV) only the signal of the C-6 atom of the D-glucose molecule underwent a paramagnetic shift ($\Delta\delta$ = +5.4 ppm), which indicates the glycosylation of this atom by the L-rhamnose. It is obvious that the L-arabinose was attached to the hydroxy group at C-3 of genin (II). In actual fact, in a comparison of the spectra of progenin (IV) and glycoside (I) it can be seen that the chemical shifts of the signals of all the carbon atoms apart from C-3 are close. In the spectrum of glycoside (I), the signal of the C-3 carbinol atom has shifted downfield by 10.7 ppm, appearing at 88.7 ppm.

In the PMR spectrum of the permethylate (VI), the anomeric protons of the D-glucose and L-arabinose residues resonated in the form of doublets with SSCCs 8.1 and 7.0 Hz, respectively, and the anomeric proton of the L-rhamnose residue in the form of a broadened singlet. These facts, and also the chemical shifts of the carbon atoms of the carbohydrate components in the ^{13}C NMR spectrum of cyclofoetoside A show the β configuration of the glycosidic bond of D-glucose residue and the α stereochemistry of the anomeric centers of the L-arabinose and L-rhamnose residues [9].

Thus, cyclofoetoside A (I) has the structure of 24S-cycloartane-3β,16 β,24,25-tetraol 3-O-β-L-arabinopyranoside 16-O-[O-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside].

EXPERIMENTAL

For general observations, see [1]. The following solvent systems were used: 1) chloroform–methanol (15:1); 2) chloroform–methanol (20:1); 3) chloroform–methanol–water (70:12:1); 4) butanol–ethanol–water (5:3:2); 5) benzene–acetone (7:1).

PMR spectra were taken on XL-200 (Varian) and JNM-4H-100/100 MHz instruments in deuterochloroform or in deuteropyridine (δ, ppm, O – TMS and HMDS), and ^{13}C NMR spectra on a Varian CFT-20 instrument (δ, ppm, O – TMS).

For the isolation of the triterpene glycosides of Thalictrum foetidum, see [1].

Cyclofoetoside A (I). Substance A [2], $C_{47}H_{80}O_{17}$, mp 265-266°C (from methanol), $[\alpha]D^{24}$ +22 ± 2° (c 1.1; pyridine); ν_{max}^{KBr}, cm^{-1}: 3500-3300 (OH), 3040 (CH_2 of a cyclopropane ring). The methanolysis of glycoside (I) in anhydrous methanol containing 5% of HCl for 12 h followed by the analysis of the carbohydrates with the aid of GLC [3] showed the presence of D-glucose, L-arabinose, and L-rhamnose in a ratio of 1.00:0.95:0.91.

Cyclofoetigenin A (II) from (I). A mixture of 300 mg of cyclofoetoside A and 50 ml of 0.5% sulfuric acid in methanol was heated in the boiling water bath for 6.5 h. Then it was diluted with a twofold volume of water, and the methanol was evaporated off. The aqueous solution was extracted with chloroform. The chloroform extract was washed with water and was dried and evaporated to dryness, and the residue was chromatographed on a column. Elution with system 1 yielded 30 mg of a compound with mp 182-184°C (from methanol), $[\alpha]D^{21}$ +68.2 ± 2° (c 1.32; methanol), which was identified as cyclofoetigenin (II) [2]. The aqueous fraction after acidification with sulfuric acid to 5% was boiled for 5 h. The reaction mixture was neutralized with ARA-8p anion-exchange resin and evaporated. D-Glucose, L-arabinose, and L-rhamnose were detected by TLC (system 4).

Partial Hydrolysis of Cyclofoetoside A (I). A mixture of 500 mg of cyclofoetoside A and 50 ml of 0.25% methanolic sulfuric acid was boiled in the water bath for 5 h. Then it was diluted with water, the methanol was evaporated off, and the products were extracted with butanol. The butanolic extract was washed with water and evaporated. The residue was chromatographed on a column of silica gel with elution by system 2, which yielded 31 mg of cyclofoetigenin (II) with mp 182-184°C (from methanol), $[\alpha]D^{21}$ +68.2 ± 2° (c 1.32; methanol). Fur-

ther elution of the column with system 3 led to the progenins (III) (70 mg) and (IV) (57 mg).

Cyclofoetigenin A 16-O-β-D-Glucopyranoside (III). $C_{36}H_{62}O_9$, mp 248-250°C (from methanol), $[\alpha]D^{24}$ +70.5 ± 2° (c 0.82; methanol). νmax^{KBr}, cm^{-1}: 3550-3250 (OH), 3050 (CH_2 of a cyclopropane ring). According to GLC [3], glycoside (III) contained one D-glucose residue. PMR (C_5D_5N, δ, ppm, O – TMS): 0.29 and 0.47 (1 H each, d, 2 H-19, ^2J = 4.0 Hz); 1.05 (3 H, d, CH_3-21, ^3J = 7.7 Hz); 0.91, 1.10, 1.23, 1.30 (3 H each, s, 4 CH_3); 1.53 (6 H, s, 2 CH_3); 3.5-4.6 (m, H-3, H-15, H-24, and the protons of the sugar residues); 4.76 (1 H, d, anomeric proton of the D-glucopyranose residue, ^3J = 7.7 Hz).

Cyclofoetigenin A 16-O-[O-α-L-Rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside] (IV). $C_{42}H_{72}O_{13}$, mp 168-170°C (from methanol), $[\alpha]D^{26}$ +42.3 ± 2° (c 0.85; methanol). νmax^{KBr}, cm^{-1}: 3350-3250 (OH), 3045 (CH_2 of a cyclopropane ring). According to GLC [3], glycoside (IV) contained D-glucose and L-rhamnose residues in a ratio of 1.00:1.18. PMR (C_5D_5N, δ, ppm, O – TMS): 0.29 and 0.55 (1 H each, d, 2 H-19, ^2J = 4.0 Hz); 1.00, 1.09, 1.19, 1.29 (3 H each, s, 4 CH_3); 1.53 (6 H, s, 2 CH_3); 1.03 (3 H, d, CH_3-21, ^3J = 7.8 Hz); 1.67 (3 H, d, CH_3 of a L-rhamnopyranose residue, ^3J = 6.1 Hz); 3.5-4.7 (m, H-3, H-16, H-24, and the protons of the sugar residues); 4.71 (1 H, d, anomeric proton of the D-glucopyranose residue, ^3J = 7.8 Hz); 5.50 (1 H, br.s, anomeric proton of the L-rhamnopyranose residue).

Undeca-O-methyl Ether of Cyclofoetoside A (VI) from (I). In small portions, 400 mg of sodium hydride was added to 400 mg of cyclofoetoside A (I) in 60 ml of dry dimethyl sulfoxide, and the mixture was stirred at room temperature for an hour. Then 6 ml of methyl iodide was added to it dropwise and stirring was continued for another 6 h. The reaction products were poured into 40 ml of 2% sodium hyposulfite solution, and this was diluted with water and extracted with butanol, after which the butanolic extract was washed with water and was evaporated to dryness. The dry residue was methylated again in a similar manner to that described above. The reaction mixture was extracted with chloroform, the extract was evaporated, and the dry residue was chromatographed on a column of silica gel. Elution with system 5 gave 81 mg of the amorphous methyl ether (VI), $C_{58}H_{102}O_{17}$, $[\alpha]D^{27}$ +23.1 ± 2° (c 0.64; methanol). The IR spectrum of compound (VI) lacked the absorption of hydroxy groups. M^+ 1070. PMR ($CDCl_3$, δ, ppm, O – TMS): 0.33 and 0.54 (1 H each, d, 2 H-19, ^2J = 4.1 Hz); 0.85 (s, 2 CH_3); 0.93 (d, ^3J = 6 Hz, CH_3-21); 0.99 (s, CH_3); 1.14 (s, 2 CH_3); 1.17 (s, CH_3); 1.28 (d, ^3J = 6.3 Hz, CH_3 of L-rhamnopyranose residue); 3.24, 3.44, 3.49 (s, 3 OCH_3), 3.51 (s, 4 OCH_3), 3.54 (s, 2 OCH_3), 3.59, 3.61 (s, 2 OCH_3); 4.13 (1 H, d, ^3J = 8.1 Hz, anomeric proton of D-glucopyranose residue); 4.25 (1 H, d, ^3J = 7.0 Hz, anomeric proton of L-arabinopyranose residue); 4.83 (1 H, br.s, anomeric proton of L-rhamnopyranose residue).

Identification of the Methylated Sugars. The methanolysis of 10 mg of the methyl ether (VI) was carried out by boiling it in 10 ml of dry methanol containing 7% of HCl for 4 h. 2,3,4-Tri-O-methyl-L-arabinopyranose, 2,3,4-tri-O-methyl-L-rhamnopyranose, and 2,3,4-tri-O-methyl-D-glucopyranose were identified in the reaction products by GLC [5].

25-Norcycloartane-3β,16β,24-triol (V) from (I). A solution of 250 mg of sodium periodate in 5 ml of water was added to 100 mg of the trioside (I) in 10 ml of methanol, and the mixture was stirred at room temperature for 6.5 h. Then it was diluted with 10 ml of water, and 2.5 ml of glycerol was added. The reaction products were extracted with chloroform. The chloroform extract was washed with water and evaporated. The residue was dissolved in 25 ml of methanol, and this solution was treated with 250 mg of sodium tetrahydroborate and heated in the water bath for 1 h. After this, the reaction mixture was acidified with dilute sulfuric acid to pH 1 and was left at room temperature for 24 h. Then 20 ml of water was added, the methanol was evaporated off, and the reaction products were extracted with chloroform. The chloroform extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue was chromatographed on a column of silica gel. Elution of system 2 yielded 33 mg of substance (V), $C_{27}H_{46}O_3$, mp 194-195°C (from methanol); $[\alpha]D^{26}$ +80.8 ± 2° (c 0.7; methanol). νmax^{KBr}, cm^{-1}: 3570-3225 (OH); 3060 (CH_2 of a cyclopropane ring. Mass spectrum, m/z (%): M^+ 418 (17.4), 403 (28.3), 400 (69.6), 385 (91.3), 382 (13.0), 367 (21.7), 357 (39.1), 341 (8.7), 331 (17.4), 313 (60.9), 299 (15.2), 295 (13.0), 278 (100), 263 (43.5), 245 (30.4), 241 (15.2), 229 (26.9), 219 (21.7), 203 (65.2). PMR C_5D_5N, δ, ppm, O – HMDS): 0.44 (1 H at C-19, d, ^2J = 4 Hz); 0.83 (3 H, s, CH_3); 0.93 (3 H, d, ^3J = 6 Hz, CH_3-21); 0.95 (3 H, s, CH_3); 1.10 (3 H, s, CH_3); 1.31 (3 H, s, CH_3); 3.37 (H-3, m); 3.78 (2 H-24, m); 4.53 (H-16, m).

25-Norcycloartane-3β,16β,24-triol 3,16,24-Triacetate (VII) from (VI). Substance (VI) (17 mg) was acetylated with 0.1 ml of acetic anhydride in 0.2 ml of pyridine at room tempera-

ture for 12 h. The residue after the solvents had been evaporated off was recrystallized from acetone, giving 14 mg of the acetate (VII), $C_{33}H_{52}O_6$, mp 179-181°C, $[\alpha]D^{27}$ +63 ± 2° (c 0.2; methanol). ν_{max}^{KBr}, cm^{-1}: 3060 (CH_2 of a cyclopropane ring); 1730, 1250 (ester groups). Mass spectrum, m/z (%): M^+ 544 (0.9), 484 (50.0), 469 (27.5), 441 (40.0), 424 (20.0), 409 (100), 381 (10.0), 373 (12.5), 362 (24.0), 355 (55.0), 341 (15.0), 313 (7.5), 302 (15.0), 295 (32.5), 287 (20.0), 281 (17.5). PMR ($CDCl_3$, δ, ppm, O − TMS): 0.36 and 0.59 (1 H each, d, ^2J = 4 Hz, 2 H-19); 0.84 (3 H, CH_3, s); 0.88 (3 H, CH_3, s); 0.92 (3 H, CH_3, s); 0.94 (3 H, CH_3-21, d, ^3J = 6 Hz); 1.14 (3 H, s); 2.02 (3 H, CH_3—COO, s); 2.06 (6 H, 2 CH_3—COO, s); 4.0 (2 H-24, m); 4.56 (H-3, m); 5.24 (H-16, m).

SUMMARY

A new glycoside of the cycloartane series − cyclofoetoside A − has been isolated from the epigeal part of Thalictrum foetidum L. (Ranunculaceae); it has the structure of 24S-cycloartane 3β,16β,24,25-tetraol 3-O-α-L-arabinopyranoside 16-O-[O-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside].

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