TRITERPENE GLYCOSIDES AND THEIR GENINS FROM Thalictrum foetidum.

II. THE STRUCTURE OF CYCLOFOETIGENIN A

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UDC 547.918:547.926

A glycoside isolated from the epigeal parts of Thalictrum foetidum (Ranunculaceae) has yielded a new genin – cyclofoetigenin A, the structure of which has been established on the basis of chemical transformations and spectral characteristics as 24S-cycloartane-3β,16β,25,25-tetraol.

We have previously reported the structure of an oleanolic acid pentaoside – foetoside C – isolated from the epigeal part of the plant Thalictrum foetidum L. (Ranunculaceae) [1]. The present paper is devoted to a proof of the structure of the genin obtained by the hydrolysis of glycoside A [1], which we have called cyclofoetigenin A (I, scheme 1).

The molecular formula of genin (I) is $C_{30}H_{52}O_4$. The presence in the PMR spectrum of two one-proton doublets at 0.20 and 0.45 ppm (Table 1) linked with one another in the manner of an AB system and also that of the signals of seven methyl groups permitted cyclofoetigenin A to be assigned to the triterpenoids of the cycloartane series [2]. This was also shown by an absorption band at 3055 cm^{-1} in the IR spectrum of cyclofoetigenin A, which is characteristic of a cyclopropane ring [3], and a mass-spectrometric fragmentation of the new genin characteristic for cycloartanes (scheme 2) [4, 5]. Compounds of this class have been isolated previously from Thalictrum minus L. [6].

The acetylation of cyclofoetigenin A (I) with acetic anhydride in pyridine gave a triacetate (IV) and a tetraacetate (III). The PMR spectrum of cyclogenin (I) showed the signals of three protons geminal to secondary hydroxy functions, at 3.43, 3.83, and 4.63 ppm. This was confirmed by the paramagnetic shifts of the signals of the protons under consideration in the PMR spectrum of the triacetate (IV) and the tetraacetate (III). The formtion of the tetraacetate (III) showed that the fourth oxygen atom was present in a tertiary hydroxy group. It followed from the facts given and the molecular formula, $C_{30}H_{52}O_4$, that the side chain of cyclofoetigenin A has an acyclic structure.

The mass spectrum of cyclofoetigenin A (M+ 476) showed the peaks of ions with m/z 336 and 313. The ion with m/z 313 ($C_{22}H_{33}O$) was formed as the result of the cleavage of the C-17–C-20 bond and the splitting out of one molecule of water. This fragment indicated that there were two hydroxy groups in the polycyclic part of the molecule and two in the side chain. The appearance of a fragment with m/z 336 ($C_{21}H_{36}O_3$) was due to the cleavage of ring A and showed the presence of one of the hydroxy groups in this ring. It is obvious that the hydroxy group concerned was present at C-3, since the ^{13}C NMR spectrum of genin (I) (Table 2) had the signals of a quaternary atom at 41.1 ppm and of a secondary carbinol carbon at 78.0 ppm, belonging, respectively, to C-4 and C-3 [7].

The proton geminal to this hydroxy group resonated at 3.43 ppm in the form of a quartet with the spin–spin coupling constants (SSCCs) $^3J_1$ = 11.4 and $^3J_2$ = 4.8 Hz and, to judge from these SSCC values, occupied the α position. These facts determined the β orientation of the hydroxyl at C-3.

The periodate oxidation of the tetraol (I) led to compound (II) with a molecular weight of 416, the structure of which is discussed below. The loss of 60 units on passing from the genin (I) (M+ 476) to substance (II) (M+ 416) indicated that the cyclofoetigenin A had an α-diol group located in the side chain at C-24–C-25.

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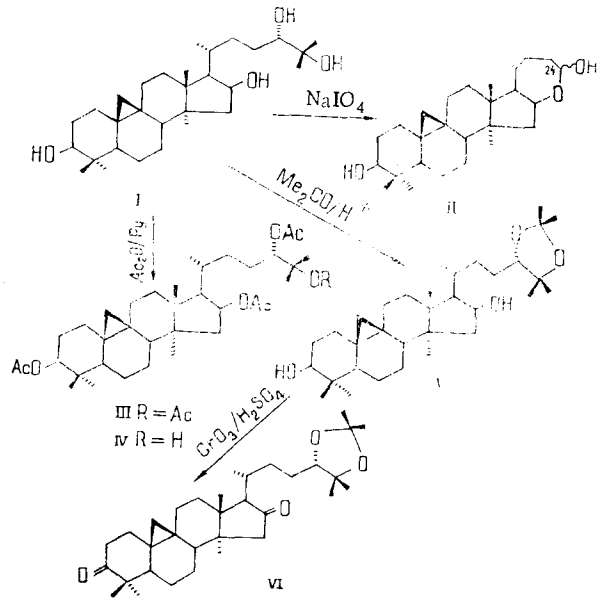
Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 370-375, May-June, 1985. Original article submitted July 5, 1984.

0009-3130/85/2103-0345$09.50

TABLE 1. Chemical Shifts of the Protons of Cyclofoetigenin A (I) and Its Derivatives (δ, ppm, 0 – HMDS)

Compound	Positions of the protons					
	H-3	H-16	2H-19	H-24	CH_3 groups	OAc
I	[3,43 q ^3J=11,4 and 4 8 Hz]	[4,63 sx ^3J=7,9; 7,5 and 8,6 Hz]	[0,20; 0,45 d, ^2J=4,4 Hz]	[3,83 q ^3J=10,9 and 2,6 Hz]	[0 83; 0,98; 0 99 d; 1,11; 1,33; 1,35; 1,37]	—
II	3,20 q	4,14 m	0,55 d $^2J\approx4$ Hz	4,58 q	0,76; 0,83*; ~0,85* d; 0,91*; 1,08	—
III	4,49 q ^3J=4,5 and 10,8 Hz	5,14 m	0,30; 0,53 d ^2J=4,4 Hz	4,95 q ^3J=3,0 and 10,0 Hz	0,78; 0,82; 0,85; 0,86 d; 1,07; 1,37; 1,40	1,89; 1,93; 1,99; 2,01
IV	4,3—4,6 m*	5,10 m	0,27; 0,52 d, $^2J\approx4$ Hz	4,3--4,6 m*	0,77; 0,81; 0,84; ~0,86 d; 1,07; 1,11 (2×CH_3)	1,90; 1,97; 2,01
	[4,65 m]	[5,30 m]	[0,40]	[5,04 m]	[0,70; 0,79; 0,86; ~0,88 d; 1,06; 1,31 (2×CH_3)]	[1,94; 1,98; 2,02]
V	3,21 q	4,41 m	0,30 d ^2J=4 Hz	3 80 q	0,77; 0,83; ~0,9 d; 0,93; 1,07; 1,12; 1,21; 1,28; 1,36	—
VI	—	—		3,67 m	1,00—1,13 (6×CH_3); 1,24; 1,29; 1,38	—

Note. The spectra were taken in $CDCl_3$ or C_5D_5N. The indices given in square brackets were obtained with the use of C_5D_5N. The signals in the horizontal rows marked with an asterisk are superposed upon one another. The signals of the methyl groups have a singlet nature, with the exception of that of the CH_3 at C-20 which is a doublet; d – doublet; q – quartet; sx – sextet; m – multiplet.



Scheme 1

In solution in dry acetone, the genin (I) formed a 24,25-monoacetonide (V). The Jones oxidation [8] of the acetonide (V) gave a diketone (VI), the IR spectrum of which showed absorption bands at 1732 and 1705 cm^{-1}, which are characteristic for five- and six-membered cyclic ketones, respectively. Consequently, one of the keto functions and the secondary hydroxy group corresponding to it was present in ring D.

In the ^{13}C NMR spectra of cycloartanes with a cyclic side chain but without hydroxy groups in ring D the C-16 atoms resonate at 26.5 ppm and the C-17 atoms at 52.2 ppm [7]. In the spectrum of cyclofoetigenin A (I) signals at 72.0 and 57.5 ppm corresponded to the C-16 and C-17 atoms (Table 2). Such considerable changes in the values of the chemical shifts are due to the presence of a hydroxy group at C-16. The signals of the proton geminal to the C-16 hydroxy group in the PMR spectrum of the genin (I) was observed at 4.63 ppm in the form of a sextet with the SSCCs $^3J_1$ = 7.9, $^3J_2$ = 7.5, $^3J_3$ = 4.6 Hz. These parameters show the α orientation f H-16 [9]. Thus, the hydroxy group present in ring D is located at C-16 and has the β orientation.

The configuration of the C-24 asymmetric center was determined by comparing the ^{13}C NMR spectra of the compound under study (I) and of cycloasgenins B and C, each of which has a side chain of similar structure [9, 10]. In the ^{13}C NMR spectra of cycloasgenis B and C the C-24 carbon atom resonates at 80.5 ppm [10]. We may note that in these compounds the C-24 atom has the R configuration. The signal of the carbon atom under consideration in the spectrum of cyclofoetigenin A was located at 77.2 ppm. The difference in the value of the C-24 chemical shifts of cycloasgenis B and C and of cyclofoetigenin A, which is 3.3 ppm, is probably due to a difference in the C-24 configurations in the compounds being compared. This gave grounds for considering that the C-24 chiral center of cyclogenin A has the S configuration.

Thus, the experimental facts given permit the conclusion that cyclogenin A has the structure of 24S-cycloartane-3β,16β,24,25-tetraol. The proposed structure agrees with the ^{13}C NMR spectrum of the compound and its derivatives (Table 2).

As mentioned above, the periodate oxidation of cyclofoetigenin A (I) gave the nor compound (II). The IR spectrum of this compound lacked carbonyl absorption. The signal of the anomeric proton was easily detected in the PMR spectrum of substance (II) at 4.58 ppm. These facts permitted the assumption that product (II) was the 24,16-demiacetal. This was also shown by the 0.27 ppm diamagnetic shift of the H-16 signal in the spectrum of substance (II) as compared with the corresponding signal in the spectrum of the acetonide (V). Thus, substance (II) had the structure of 16β,24ξ-epoxy-25-norcycloartane-3β,24-diol. Compounds of similar structure have been obtained from cycloasgenins B and C [9, 10].

TABLE 2. Chemical Shifts of the Carbon Atoms of Cyclofoetigenin A (I) (C_5D_5N), Its Tetraacetate (III) ($CDCl_3$), and Its Acetonide (V) ($CDCl_3$)

Atom C	δ, ppm			Atom C	δ, ppm			Atom C	δ, ppm		
	I	III	V		I	III	V		I	III	V
1	32,5	31,6	32,0	12	33,6[c]	32,5[c]	32,9[c]	23	27,9	25,9	24,9
2	31,3	30,2	30,5[a]	13	45,8	45,8	45,5	24	77,2	77,8	81,4
3	78,0	80,6	78,9	14	47,1	46,2	46,7	25	72,5	82,7	80,3
4	41,1	39,5	40,6	15	48,8	47,1	47,3	26	26,2	22,3	26,2
5	47,6[b]	47,2[b]	47,3[b]	16	72,0	74,7	72,6	27	26,5	22,2	23,3
6	21,5	20,8	21,1	17	57,5	54,2	57,0	28	20,3	20,0	20,1
7	26,5	26,2	26,3	18	18,3	18,0	17,7	29	25,6	25,4	25,5
8	48,4[b]	48,0[b]	48,1[b]	19	30,3	30,2	30,2[a]	30	14,8	15,1	14,1
9	20,0	19,3	20,0	20	28,7	26,2	28,7	CH_3 CH_3			26,9 27,7
10	26,8	26,8	26,3	21	19,4	18,5	18,9	O—C—O			106,8
11	26,5	26,3	26,3	22	33,1[c]	32,3[c]	32,5[c]	C=O CH_3		170,9 170,6 170,3 170,0 22,0 21,3 21,2 20,9	

Note. Ambiguous assignments are shown by identical letters within the columns.

EXPERIMENTAL

For general observations, see [1]. The following solvent systems were used: 1) chloroform–methanol (20:1); 2) benzene–ethyl acetate (5:1); 3) hexane–ethyl acetate (8:1); 4) hexane–ethyl acetate (9:1).

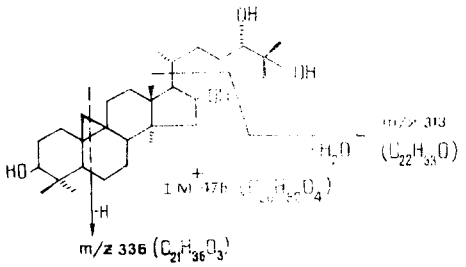
PMR spectra were taken on XL-200 (Varian) and JNM-4H-100/100 MHz instruments in deuterochloroform or in deuteropyridine (δ, ppm, 0 – HMDS), and ^{13}C NMR spectra on a CFT-20 instrument (Varian) (δ, ppm, 0 – TMS).

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

Cyclofoetigenin A (I). Glycoside A [1] (650 mg) was hydrolyzed in 200 ml of a 0.25% methanolic solution of sulfuric acid at the boiling point for 5 h. Then the reaction mixture was diluted with two volumes of water, and part of the methanol was evaporated off. The products were extracted with butanol. The butanolic extract was washed with water and evaporated. The residue was chromatographed on a column with elution by system 1. This gave 55 mg of cyclofoetigenin A (I), $C_{30}H_{52}O_4$, mp 182-184°C (from ethanol), $[\alpha]_D^{21}$ +68.2 ± 2° (c 1.32; methanol). ν_{max}^{KBr}, cm^{-1}: 3520-3310 (OH); 3055 (CH_2 of a cyclopropane ring). Mass spectrum, m/z(%): M^+ 476 (30.0), 458 (45.0), 443 (55.0), 440 (30.0), 425 (60.0), 415 (25.0), 407 (25.0), 399 (37.5), 381 (35.0), 371 (17.5), 353 (20.5), 336 (47.5), 313 (67.5), 295 (22.5), 203 (47.5), 173 (65.0), 159 (62.5), 43 (100).

The 3,16,24-Triacetate (IV) and the 3,16,24,25-Tetraacetate (III) of Cyclofoetigenin A from (I). Cyclofoetigenin A (80 mg) was acetylated with 1 ml of acetic anhydride in 2 ml of pyridine at room temperature for 48 h. The residue after the solvents had been evaporated off was chromatographed on a column, with elution by system 2. This gave 6 mg of the 3,16,24,25-tetraacetate (III), $C_{38}H_{60}O_8$, mp 145-147°C (from methanol), $[\alpha]_D^{22}$ +78 ± 2° (c 0.6; methanol). ν_{max}^{KBr}, cm^{-1}: 3040 (CH_2 of a cyclopropane ring); 1740, 1250 (ester group). Mass spectrum, m/z (%): M^+ 644 (3.0), 584 (100), 569 (22.7), 541 (22.7), 524 (54.5), 509 (63.6), 471 (36.4), 464 (31.8), 455 (27.7), 449 (31.8), 411 (36.4), 402 (45.5), 355 (63.6), 295 (54.5), 203 (81.8).

Continuing the elution of the column with the same solvent system led to the isolation of 60 mg of the 3,16,24-triacetate (IV), $C_{36}H_{58}O_7$, mp 89-91°C (from methanol), $[\alpha]_D^{22}$ +78.4 ± 2° (c 1.02; methanol). ν_{max}^{KBr}, cm^{-1}: 3550-3440 (OH); 3040 (CH_2 of a cyclopropane ring); 1740, 1250 (ester group). Mass spectrum, m/z (%): M^+ 602 (5.2), 584 (4.3), 542 (100),



Scheme 2

527 (24.1), 524 (12.0), 509 (6.0), 499 (19.8), 482 (82.8), 467 (75.9), 439 (19.0), 420 (22.4), 407 (49.1), 355 (77.6), 295 (56.8), 203 (51.7).

Cyclofoetigenin A 24,25-Acetonide (V) from (I). A mixture of 70 mg of cyclofoetigenin A (I) and 2.5 ml of acetone containing 2% of sulfuric acid was shaken at room temperature for 3 h, after which it 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 3. This gave 66 mg of cyclofoetigenin A 24,25-acetonide (V), $C_{33}H_{56}O_4$, mp 198-200°C (from methanol), $[\alpha]_D^{21}$ +70 ± 2° (c 1.2; methanol). ν_{max}^{KBr}, cm^{-1}: 3540-3330 (OH),; 3060 (CH_2 of a cyclopropane ring). Mass spectrum, m/z (%): M+ 516 (4.1), 501 (24.3), 498 (10.8), 483 (7.4), 455 (6.8), 440 (16.2), 425 (37.8), 407 (18.2), 385 (12.8), 376 (9.5), 353 (12.8), 313 (32.4), 303 (8.8), 295 (8.1), 285 (10.1), 273 (8.1), 203 (14.9), 173 (24.3), 149 (100).

24,25-Dihydroxy-24S-cycloartane-3,16-dione 24,25-Acetonide (VI) from (V). The acetonide (V) (35 mg) in 5 ml of acetone was oxidized with 0.5 ml of the Jones reagent [8] at −5°C for 10 min. The excess of oxidizing agent was decomposed with 1 ml of methanol. The reaction mixture was diluted with water and treated with chloroform. The chloroform extract was washed with water and evaporated. The residue was chromatographed on a column with elution by system 4. This gave 27 mg of the diketone (VI), $C_{33}H_{52}O_4$, mp 149-151°C (from methanol), $[\alpha]_D^{22}$ −56.6 ± 2° (c 0.61; methanol). ν_{max}^{KBr}, cm^{-1}: 3055 (CH_2 of a cyclopropane ring); 1732 (C=O at C-16); 1705 (C=O at C-3). Mass spectrum, m/z (%): M^+ 512 (2.6), 497 (76.9), 454 (43.6) 439 (92.3), 398 (12.8), 383 (100), 355 (11.5), 327 (5.1), 313 (17.9), 271 (46.2), 258 (12.8).

16β,24ξ-Epoxy-25-norcycloartane-3β,24-diol (II) from (I). A solution of 100 ml of sodium periodate in 0.5 ml of water was added to 31 mg of cyclofoetigenin A (I) in 3 ml of methanol, and the mixture was left at room temperature for 2.5 h. The excess of oxidizing agent was decomposed by the addition of a few drops of ethylene glycol. the reaction mixture was dilute with water and treted with chloroform. The chloroform extract was washed with water, and the chloroform was evaporated off. The residue was recrystallized from a mixture of chloroform and methanol (1:1), giving 20 mg of compound (II), $C_{27}H_{44}O_3$, mp 151-153°C, $[\alpha]_D^{21}$ +22.2 ± 2° (c 0.63; methanol). ν_{max}^{KBr}, cm^{-1}: 3510-3350 (OH); 3050 (CH_2 of a cyclopropane ring). Mass spectrum, m/z (%): M+ 416 (14.6), 401 (31.3), 398 (43.8), 383 (68.8), 365 (16.7), 355 (43.8), 329 (16.7), 311 (18.8), 279 (35.4), 149 (100).

SUMMARY

The hydrolysis of a glycoside isolated from the epigeal part of Thalictrum foetidum L. has yielded a new genin of the cycloartane series – cyclofoetigenin A. It has the structure of 24S-cycloartane-3β,16β,24,25-tetraol.

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DETECTION AND SEPARATION OF TWO FORMS OF COTTON-PLANT PYROPHOSPHATASE

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UDC 633.511.157.153

Two pyrophosphatase isoenzymes with identical molecular weights but differing in the pH optima of their action and also in their senstivity to some bivalent metal ions have been isolated from extracts of the cotyledons of three-day cotton-plant shoots by gel filtration and ion-exchange chromatography.

Inorganic pyrophosphatase (EC 3.6.1.1.) is a polyfunctional enzyme. Its participation in the phosphorus metabolism and also in the energy metabolism of the cell is well known [1]. Recently, another function of the enzyme has been revealed which is connected with the utilization of condensed phosphates representing an effective form of phosphorus fertilizers for plants and, in particular, for the cotton plant [2]. The utilization of condensed phosphate takes place with the participation of enzymes liberated into the external medium by the roots of the plant and hydrolyzing pyrophosphatase bonds [3]. We have previously reported on the presence and isolation of a pyrophosphatase enzyme from cotton-plant tissues [4]. In the present paper we give information obtained in the purification of the enzyme. The object of the investigations were three-day shoots of cotton plants of variety 108-F.

Gel filtration of the enzyme extract gave two fractions possessing pyrophosphatase activity (Fig. 1). They were combined and concentrated and used for the subsequent chromatographic stages. It must be mentioned here that although the pyrophosphatase activity in fraction 1 was 1.5 times greater than in fraction 2, the specific acitivity of the former was far smaller than that of the latter. The use of this procedure permits a large amount of ballast substances to be eliminated, since in fraction 1, together with pyrophosphate activity, a very large amount of ATPase activity, exceeding the pyrophosphatase activity by a factor of 3-4, and also tripolyphosphatase and phosphatase activities were detected. Consequently, we subsequently investigated mainly the second fraction. When it was chromatographed on DEAE-cellulose, the proteins were separated in two peaks (Fig. 2). Pyrophosphatase activity was detected in fractions 6-10 and 17-18. At the same time, the specific activity of the enzyme increased several fold. Together with this, in fractions 6-10, with a fairly high specific pyrophosphatase activity, ATPase activity (10-14% of the pyrophosphatase activity) was also detected. Conversely, fractions 8-10 possessed a high pyrophosphatase activity unaccompanied by other activities. When they were rechromatographed (Fig. 3), a single protein peak possessing only pyrophosphatase activity and eluting at a low ionic strength of NaCl was obtained. The enzyme exhibited its maximum activity at pH 8.6, and the addition of activator metals ($MgCl_2$) to it led to a six- to seven-fold increase in the specific activity of the enzyme. The enzyme eluted in fractions 17-18, in contrast to that in fractions 17–18, was active in the acid pH range and did not require the addition of activator metals for the manifestation of its activity. Furthermore, this pyrophosphatase was capable of hydrolyzing p-nitrophenyl phosphate well:

V. I. Lenin Tashkent State University. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 375-378, May-June, 1985. Original article submitted June 25, 1984.

 0009-3130/85/2103-0350$09.50