TRITERPENE GLYCOSIDES OF Caltha silvestris

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Caltha silvestris Worosch.* is a perennial plant of the family Ranunculaceae which is endemic in the southern part of the Soviet Far East [1, 2]. The chemical composition of the plant has not been studied previously. From the results of thin-layer chromatography on silica gel, a methanolic extract of the roots of C. silvestris contains ten triterpene glycosides which we have named in order of increasing polarity calthosides A-J. As a result of repeated partition chromatography [3] of a methanolic extract, calthoside D was isolated in the pure state.† The full methyl ether of calthoside D, synthesized by Kuhn's method [4], on acid hydrolysis, gave 23-O-methylhederagenin methyl ester [5], 2,3,4,6-tetra-O-methyl-D-glucose [6], and 3,4-di-O-methyl-L-arabinose [7]. The latter was identified with particular care because of the absence of an authentic sample. In contrast to information in the literature [8], methyl 3,4-di-O-methyl-L-arabinose obtained in the usual way appears on a gas-liquid chromatogram in the form of two peaks corresponding to the α and β anomers. The mixture was separated by adsorption chromatography on silica gel. The mass spectra of the compounds isolated were identical and corresponded to the usual direction of fragmentation of pentapyranoses [9]. For additional confirmation, the mass spectrum of the trimethylsilyl ether of 3,4-di-O-methyl-L-arabinose [10] was also recorded, the mass numbers and relative intensities of the characteristic fragments being given below:

Type of ions	m/e	Relative intensity, %	Type of ions	m/e	Relative intensity, %
	73	100		131	11
	147	6	B_2	189	1,3
	88	6		247	0,1
H_1	146	17	B_{T_2}	175	0,6
	204	0,4		233	0,6
	75	13	A_2	143	1,8
J_1	133	39		201	0,2
	191	0,2		115	4
	101	4	C_2	173	1,8
F_1	159	48		231	0,7
	217	0,6	T_2	217	0,6
K_1	58	9		275	0,9
	116	5	T_3	243	0,2
T_1	307	0,1	$M-Si(CH_3)_3OH$	232	1,5
	176	0,2	M	322	0,02
B_{T_1}	219	0,2			
	277	0,2			

Thus, a 1 → 2 bond between the terminal glucose and the arabinose was established. On the basis of the NMR spectrum of calthoside D acetate, this linkage has the β configuration, as is shown by the doublet signal of the proton at C-1 (δ 4.56 ppm, J = 7.3 Hz) and by the magnitude of the chemical shift of the proton at C-3 (δ < 5.4 ppm) [11] of the glucose residue. In addition, calculation by Klyne's method [12] shows that

*The samples of C. silvestris were collected and determined by P. G. Gorov (Institute of Biologically Active Substances of the Far-Eastern Scientific Center).

†From the results of I. V. Dardymov and É. I. Khasina (Laboratory of Pharmacology and Experimental Therapy, Institute of Biologically Active Substances, Far-Eastern Scientific Center), in a model experiment with hexokinase, calthoside D in a concentration of $1 \cdot 10^{-6}$ g/ml increases the activity of the enzyme by a factor of two.

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the optimum variant has the α configuration of the bond of the L-arabinosyl residue with the hederagenin. Consequently, calthoside D is hederagenin 3-[O-β-D-glucopyranosyl-(1 → 2)-α-L-arabopyranoside], and is identical with the saponin B isolated previously from Caulophyllum robustum Maxim. [13].

EXPERIMENTAL

The analyses of all the compounds corresponded to the calculated figures. The mass spectra were taken on an MKh-1303 instrument with the direct introduction of the sample into the ion source at an evaporator temperature of 50°C for the sugar derivatives and 125°C for the aglycones. The GLC analysis was performed on a Tswett-2 instrument (Experimental Design Bureau for Automation, Dzerzhinsk). The columns were filled with 10% of poly(tetramethylene succinate) on Chromosorb W (45-60 mesh) AWDMCS. The rate of feed of nitrogen and hydrogen was 60 ml/min. Analysis was performed with temperature programming: 100 → 225°C (10°C/min). The temperature of the evaporator was 280°C. Chromatography was performed with silica gel of type KSK (200-270 mesh) and the following solvent systems: 1) toluene–butan-1-ol/H_2O; 2) toluene–ethanol (9 : 1); 3) benzene–ethyl acetate; and 4) benzene–ethanol (100 : 0 → 100 : 5). The glycosides and aglycones were revealed with conc. H_2SO_4 and the sugars with a saturated solution of aniline phthalate in butan-1-ol.

Isolation of Calthoside D. The evaporated methanolic extract (100 g) was chromatographed on a column of silica gel in system 1 (4 : 1 → 0 : 1). This gave calthoside D with mp 248-253°C (butan-1-ol), $[\alpha]_D^{20}$ + 39.2 ± 0.5° (c 0.96; dimethyl sulfoxide). The chromatographically homogeneous full acetate of calthoside D did not crystallize.

Methylation of Calthoside D. Calthoside D (1 g) was methylated by Kuhn's method [4]. The course of methylation was monitored by thin-layer chromatography in silica gel in system 2. The product was chromatographed on a column (50 × 3 cm) of silica gel in system 3 (1 : 0 → 0 : 1). This gave 0.74 g of the full methyl ether, $[\alpha]_D^{20}$ + 28.4 ± 0.8° (c 0.633; benzene). IR spectrum ($CHCl_3$): OH absent. The methyl ether of calthoside D (0.2828 g) and 10 ml of a mixture of 42% $HClO_4$ and methanol (1 : 5) was heated. Chromatography of the aglycone fraction on silica gel in system 3 (100 : 0 → 100 : 2) gave 0.096 g of 23-O-methylhederagenin methyl ester with mp 191-193°C (ethanol), $[\alpha]_D^{20}$ + 76 ± 3° (c 0.38; benzene), M^+ 500 m/e 395, 262, 208* (mass spectrometry). In addition, 0.1160 g of a mixture of methylated sugars was obtained from which, after chromatography on silica gel in system 4, 0.0509 g of 2,3,4,6-tetra-O-methyl-D-glucose, mp 79-84°C (hexane, sublimation), $[\alpha]_D^{20}$ + 115 → + 84.7° (c 0.133; benzene) and 0.0484 g of 3,4-di-O-methyl-L-arabinose, $[\alpha]_D^{20}$ + 128.3° ± 1.3° (c 0.306; water) were isolated.

Methyl α- and β-3,4-Di-O-methyl-L-arabinosides. A solution of 10 mg of 3,4-di-O-methyl-L-arabinose in 0.5 ml of absolute methanol was heated in the presence of Amberlite IR-120 (H^+) for 6 h and was then chromatographed on a column of silica gel (12 × 2 cm) in system 4. Fraction 1: $V_{R\ rel}$ 1.13; fraction 2, $V_{R\ rel}$ 1.43.

Trimethylsilyl Ether of 3,4-Di-O-methyl-L-arabinose. To 7 mg of 3,4-di-O-methyl-L-arabinose were added 0.5 ml of hexamethyldisilazane and five drops of chlorotrimethylsilane. The solid matter was centrifuged off. The supernatant liquid was evaporated to dryness. The dry residue was purified on a column of silica gel (12 × 2 cm) in system 4. The fraction containing the α and β trimethylsilyl ethers of 3,4-di-O-methyl-L-arabinose, after drying, was subjected to mass spectrometry.

The NMR spectrometry and the GLC analysis were performed by V. V. Isakov and I. N. Krasikova.

SUMMARY

It has been established that calthoside D is hederagenin 3-[O-β-D-glucopyranosyl-(1 → 2)-α-L-arabopyranoside] and is identical with caulosaponin B.

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