ASCASIDE – A NEW FLAVONOID GLYCOSIDE OF *Astragalus caucasicus*

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The isolation of flavonoid substances from some species of the genus Astragalus L. of the Georgian flora has been reported previously [1]. Continuing investigations of the flavonoids of plants of this genus, from Astragalus caucasicus Pall., we have obtained a substance which we have called ascaside.

Ascaside gives the reactions specific for flavonoids [2]. Its flavonoid nature was confirmed by characteristic absorption bands in the IR spectrum (3400, 3030, 2900, 1690, 1515, 1550, and 1580 cm^{-1}) and UV spectrum ($\lambda_{max}^{C_2H_5OH}$ 352, 255 nm).

The NMR spectrum of the trimethylsilyl ether of the glycoside showed the following signals: doublet at 6.8 ppm with J = 8.5 Hz due to the protons in positions 3' and 5' of the flavonoid nucleus, and doublets at δ 6.11 ppm (J = 2.5 Hz) and 6.25 ppm (J = 2.5 Hz) which can be assigned to the protons in positions 6 and 8. The presence of signals corresponding to three anomeric protons in the δ 5.49, 4.73, and 4.25 ppm regions shows that there are three sugar residues in ascaside [3].

The substance under investigation was readily hydrolyzed by 2% sulfuric acid, forming D-galactose, L-rhamnose, and an aglycone which was identified from its elementary composition, physicochemical properties, and IR and UV spectra as kaempferol.

The NMR spectrum of the glycoside showed doublets at 1.15 and 0.8 ppm belonging to the two methyl groups of L-rhamnose residues. From the results obtained, the carbohydrate moiety of the glycoside can be represented by two molecules of L-rhamnose and one of D-galactose, which was confirmed by the results of qualitative hydrolysis.

The position of attachment of the sugars was shown by UV spectroscopy with diagnostic reagents (Table 1). A comparison of the bathochromic shift of the aglycone and of the glycoside under investigation on the addition of aluminum chloride showed that in the glycoside the 3-hydroxy group of the aglycone molecule is substituted.

The action of the enzymes of the grape snail [4] and of rhamnodiastase [5, 6] on ascaside did not change its molecule. This gives grounds for assuming that it does not contain D-galactose as a terminal sugar and that a galacto <6→1>-L-rhamnose bond can be excluded.

The D-galactose found in the reaction mixture after periodate oxidation and hydrolysis of the reaction product shows the substitution of the C_3 hydroxy group by one of the L-rhamnose residues [7] and the formation of an acetonide shows the presence of free hydroxy groups of L-rhamnose residues at C_2 and C_3 [8].

When the glycoside was methylated [9] and then hydrolyzed, 3-hydroxy-4',5,7-trimethoxyflavone was obtained, while when the carbohydrate fraction was chromatographed on paper in the water-saturated methyl ethyl ketone system and other systems [9, 12], two methylated sugars were found which from their R_g values corresponded to 2,6-di-O-methyl-D-galactose and 2,3,4-tri-O-methyl-L-rhamnose.

EXPERIMENTAL METHOD

The UV spectra were obtained on a Hitachi spectrometer in ethanol, the IR spectra on a UR-10 instrument in the form of tablets with KBr, and the NMR spectra on a Hitachi–Perkin–Elmer R 20 A instru-

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TABLE 1. Results of a UV-spectral Analysis of Ascaside and Its Aglycone

Substance	Absorption band	0.001% soln. in ethanol	+ sodium acetate		+ aluminum chloride		+ boric acid + sodium acetate		+ sodium ethoxide	
		λ, nm	λ, nm	Δλ, nm	λ, nm	Δλ, nm	λ, nm	Δλ, nm	λ, nm	Δλ, nm
Aglycone	I	360	375	15	415	55	368	8	410	50
	II	270	275	5	285	15	280	10	282	12
Ascaside	I	352	368	16	402	50	350	−2	410	58
	II	255	274	19	272	17	275	20	272	17

ment (at 60 MHz) with TMS as standard.

Isolation. The flavonoids were extracted from 1.3 kg of the air-dry comminuted leaves of A. caucasicus with 13 liters of 80% ethanol. The extract was evaporated under vacuum until the ethanol had been completely eliminated. The aqueous residue was filtered and treated with chlorofom. The aqueous liquid was mixed with 7 liters of acetone. The resinous substances that precipitated were separated off. The acetone was distilled off from the aqueous acetone filtrate, and the aqueous residue (2.7 liters) was extracted with 2.3 liters of ethyl acetate and three liters of a mixture of ethyl acetate and ethanol (4 : 1). The extracts were concentrated separately and subjected to qualitative analysis by PC in various systems. It was found that the two residues had identical flavonoid compositions and each contained three substances.

The total material (10 g) was dissolved in 5 ml of ethanol, the solution was mixed with 8 g of polyamide, and the resulting product was dried at room temperature. The dry powder was deposited on a column (3.5 × 40 cm) of polyamide sorbent. The column was washed first with pure chloroform and then with mixtures of chloroform and ethanol in various ratios. The fractions containing ascaside were combined, the solvent was distilled off, and the residue was then separated by preparative chromatography on paper in the butan-1-ol–acetic acid–water (4 : 1 : 2) system. The ascaside zone (R_f 0.47) was cut out and eluted with aqueous ethanol, the ethanol was distilled off, and the aqueous liquid was rechromatographed on a column of Sephadex LH-20 (2 × 20 cm).

The fractions containing ascaside were combined, the solvent was distilled off, and the flavonoid was crystallized from ethanol. This gave 0.17 g of yellow acicular crystals of ascaside, $C_{33}H_{40}O_{19}$, mp 180-182° C, $[\alpha]_D^{20}$ −84° (c 0.1; absolute ethanol).

In the cyanidin reaction the glycoside was colored bright red, and Bryant's test showed its glycosidic nature [2].

Acid Hydrolysis of Ascaside. An accurately weighed 50-mg sample of the glycoside was hydrolyzed with 5 ml of 2% H_2SO_4. The aglycone that deposited was separated off by filtration, washed with water, and dried. The weight of aglycone was 19 mg, or 38%, which corresponds to a triglycoside.

The aglycone crystallized from 60% ethanol in the form of yellow acicular crystals with mp 273-274° C. It was identified as kaempferol [1].

The acid hydrolyzate after the separation of the aglycone was neutralized with AV-17 anion-exchange resin (OH^-). The eluates were distilled to give a syrupy residue (30 mg), and this was chromatographed on paper in the butan-1-ol–acetic acid–water (4 : 1 : 2) and the pyridine–benzene–butanol–water (3 : 1 : 5 : 3) systems. The chromatograms showed the presence of two carbohydrates at the levels of authentic samples of D-galactose and L-rhamnose.

Enzymatic Hydrolysis of Ascaside. A solution of 20 mg of the substance in 3 ml of water was treated with 10 mg of an enzyme preparation of Helix plectotropis [4] in 2 ml of water or with rhamnodiastase [5, 6], and the mixture was kept at 37° C for 48 h. In both cases the starting material was recovered.

Periodate Oxidation of Ascaside. The compound under investigation (10 mg) was added to 30 mg of $NaIO_4$ in 25 ml of water and the mixture was left for three days in a dark place [7]. Then 0.1 ml of ethylene glycol was added and, after 1 h, the mixture was deionized with KU-2 cation-exchange resin. The liquid was filtered and the solvent was driven off under vacuum. Then the reaction mixture was worked up as described in the literature [11].

Ascaside Acetonide. A solution of 10 mg of the glycoside in 5 ml of absolute acetone was treated with 100 mg of anhydrous copper sulfate and the mixture was boiled with a calcium chloride tube for 4 h. On a paper chromatogram, the acetonide formed appeared as a dark red spot, and its mobility (R_f 0.39) differed sharply from that of the initial substance (R_f 0.79).

Kuhn Methylation of Ascaside [9]. The flavonoid (100 mg) was dissolved in 3 ml of dimethyl formamide which had been freshly distilled over phosphorus pentoxide and had been previously heated to 40° C, 3 ml of methyl iodide was added, and then, with stirring, 1 g of silver oxide was introduced in small portions over 30 min. The reaction mixture was then worked up as described by Patkhulaeva et al. [10]. The methylated glycoside was hydrolyzed with 2% H_2SO_4. The hydrolyzate deposited crystals of the methyl derivative of the aglycone with mp 159-164° C, which we identified as 4',5,7-tri-O-methylkaempferol [13]. The carbohydrate fraction of the hydrolyzed methylated product was chromatographed on paper in the systems given by Aspinall and Wood [12] in parallel with 2,3,4,6-tetra-O-methyl-D-glucose to determine the values of R_g (g = 2,3,4,6-tetra-O-methyl-D-glucose). Two methylated sugars were found corresponding to 2,6-di-O-methyl-D-galactose and 2,3,4-tri-O-methyl-L-rhamnose.

SUMMARY

A new flavonoid glycoside has been isolated from Astragalus caucasicus Pall. and has been called ascaside; its most probable structure has been established as kaempferol 3-O-[3,4-di-O-α-L-rhamnosyl-galactopyranoside].

LITERATURE CITED

1. M. D. Alaniya, N. F. Komissarenko, and E. P. Kemertelidze, Soobshch. AN GruzSSR, 6, 82, 357 (1972).
2. B. T. Bryant, J. Amer. Chem. Soc., 39, 481 (1950).
3. T. J. Mabry et al., The Systematic Identification of Flavonoids, Springer, New York (1970), p. 265.
4. P. I. Gvozdyak and V. I. Litvinenko, Med. Prom. SSSR, 18, 5 (1964).
5. F. W. Parrisch and A. S. Perllin, Nature (London), 187, 1110 (1960).
6. L. Norhammer and R. Hansel, Arch. Pharm., 286, 425 (1953).
7. P. K. Kintya, N. E. Isaeva, et al., Khim. Prirodn. Soedin., 308 (1972).
8. N. K. Kochetkov et al., Carbohydrate Chemistry [in Russian], Moscow (1970).
9. R. Kuhn and I. Löw, Ber., 77, 202 (1944).
10. M. Patkhulaeva, L. G. Mzhel'skaya, and N. K. Abubakirov, Khim. Prirodn. Soedin., 38 (1973).
11. V. G. Bukharov, V. V. Karlin, and V. A. Talan, Khim. Prirodn. Soedin., 25 (1969).
12. G. O. Aspinall and T. M. Wood, J. Chem. Soc., 3, 1683 (1963).
13. Y. Takino, H. Imagarva, and H. Yoshida, J. Agr. Chem. Soc. Japan, 28, 186 (1954).