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# CHEMICAL STUDY OF PLANTS OF THE MONGOLIAN FLORA. COUMARINS OF *Stellera chamaejasme*: THE STRUCTURE OF CHAMAEJASMOSIDE — A NEW BICOUMARIN GLYCOSIDE

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*The coumarin composition of the epigeal part of* Stellera chamaejasme *has been studied. Together with the known coumarins daphnoretin, daphnetin, daphnin, and daphnorin, we have isolated the new bicoumarin glycoside chamaejasmoside and have established its structure by chemical transformations and a study of its spectral properties. Of the known coumarins, this is the first time that daphnorin and daphnin have been isolated from this plant.*

We have studied the epigeal part of *Stellera chamaejasme* gathered in the flowering phase in the environs of Ulan-Bator (Mongolia). By ethanolic extraction and separation of chloroform and ethyl acetate fractions on a silica gel column we isolated daphnoretin [1], daphnetin [1], daphnin, and daphnorin, identified from their physicochemial properties and spectral characteristics, and also compound (**1**), which we have called chamaejasmoside. This is the first time that daphnin and daphnorin hve been isolated from this plant.

Chamaejasmoside, with the composition $C_{25}H_{22}O_{12}$, mp 204-205°C (methanol), had absorption maxima in the UV spectrum at $\lambda_{max}^{CH_3OH}$, 228, 284, 324, 336 nm, which are characteristic for bicoumarins. In the IR spectrum there were absorption bands at $\nu_{max}$ 1720, 1610, 1500 $cm^{-1}$. The PMR spectrum taken in DMSO-$d_6$ showed signals in the form of one-proton singlets in the 7.82. 7.25, and 7.22 ppm regions, which are characteristic for the H-4, H-5, and H-8 protons, respectively, and a three-proton singlet from a methoxy group at 3.82 ppm. Two one-proton doublets at 8.03 and 6.37 ppm with SSCCs of 9.5 Hz corresponded to the H-4′ and H-3′ protons, and the signal of one proton at 7.73 ppm (J = 9.5 Hz) to H-5′. The H-6′ proton appeared in the form of a doublet of doublets at 7.14 and 7.15 ppm with SSCC of 8 and 2.5 Hz, while a one-proton doublet at 7.17 ppm ( J = 2.5 Hz) belonged to the proton in position 8. The PMR spectrum of chamaejasmoside resembled that of daphnoretin, isolated previously from the plant under study.

Its mobility in TLC and the presence in its PMR spectrum of the signals of the protons of a carbohydrate moiety in the 4.0—5.3 ppm region, including the signal of an anomeric proton at 5.12 ppm with a SSCC of 8 Hz, permitted the assumption of the glycosidic nature of chamaejasmoside and — more specifically — of the β- configuration of its glycosidic center. The acid hydrolysis of chamaejasmoside led to the formation of daphnoretin and the monosaccharide D-galactose. The sugar component was identified by paper chromatography with an authentic specimen.

The mass fragmentation of the compound, and, namely, fragments with m/z (%) 352 (100), 337 (6), 324 (7), 309 (14), 295 (5), 179 (31), 164 (12), 145 (9), 89 (30), showed that chamaejasmoside was a dicoumarin.

*Deceased.

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TABLE 1. Chemical Shifts of the Carbon Nuclei of Chamaejasmoside (**1**), Daphnoretin (**2**), and Quercetin 3-O-Galactoside (**3**)

| Atom C | 1 | 2 | 3 |
|---|---|---|---|
| 2 | 159.55 | 161.10 | 156.2 |
| 3 | 137.10 | 136.85 | 133.8 |
| 4 | 129.17 | 132.02 | 177.5 |
| 4a | 113.75 | 115.52 | 104.0 |
| 5 | 103.17 | 103.94 | 161.2 |
| 6 | 154.76 | 151.54 | 98.6 |
| 7 | 148.80 | 154.60 | 164.0 |
| 8 | 113.46 | 110.56 | 93.4 |
| 8a | 143.64 | 145.08 | 156.3 |
| 2′ | 158.08 | 160.81 | |
| 3′ | 112.15 | 111.31 | |
| 4′ | 146.35 | 146.84 | |
| 4′a | 114.41 | 114.98 | |
| 5′ | 129.65 | 130.98 | |
| 6′ | 104.29 | 105.09 | |
| 7 | 156.40 | 156.13 | |
| 8′ | 109.98 | 114.55 | |
| 8′a | 146.47 | 148.58 | |
| 1″ | 99.68 | | 102.3 |
| 2″ | 72.89 | | 71.3 |
| 3″ | 73.7 | | 73.4 |
| 4″ | 69.57 | | 68.0 |
| 5″ | 76.3 | | 75.8 |
| 6″ | 62.74 | | 60.8 |
| $-OCH_3$ | 56.12 | | |

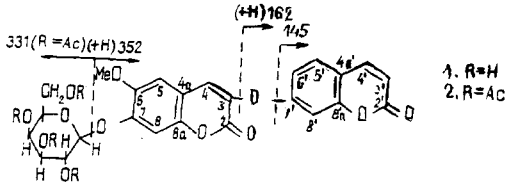
Additional information on the structure, including the site of attachment of the carbohydrate moiety in chamaejasmoside, was obtained (Table 1) as a result of an analysis of the characteristics of the $^{13}C$ NMR spectra of chamaejasmoside (**1**), daphnoretin (**2**), and quercetin 3-O-galactoside (**3**) [3].

The spectrum of chamaejasmoside showed the signals of the carbon atoms of a galactopyranoside ring. The anomeric carbon atom C1″ resonated at 99.68 ppm.

It is known from the literature that that the glycosylation of phenolic hydroxy groups of coumarins and flavonoids leads to paramagnetic shifts of the signals of the $\alpha$-carbon atoms by 1-3 ppm, while the $\beta$-carbon atoms undergo a weak shift of of the order of 1-2.5 ppm [4]. Such a pattern is observed for the C-7 position in chamaejasmoside.

Thus, the structure of daphnoretin 7-O-$\beta$-D-galactopyranoside applies to chamaejasmoside. This proposed structure was confirmed by a mass-spectrometric study.

We obtained an acetate derivative of chamaejasmoside and took its mass spectrum. In the general mass spectrum of chamaejasmoside peracetate the peak of the molecular ion was absent, but there was the peak of a $(M - AcOH)^+$ ion, with m/z 622. The presence of a peracetylated galactose residue was confirmed by the peak of an ion with m/z 331 ($C_{14}H_{19}O_9$) and peaks of its dcomposition products with m/z 271, 229, 211, 169, 109; and the presence of a dicoumarin residue by an intense peak of an ion with m/z 352 ($C_{19}H_{12}O_7$). The subsequent decomposition of this ion took place by the elimination of ($CH_3$ + CO) from the C-6 position and the splitting out of CO from the $\alpha$-pyrone rings. Cleavage of the bonds of the ester bridge led to an ion with m/z 162 ($C_9H_6O_3$) and a pair of ions with m/z 145 and 207 (scheme).




The peak of the last-mentioned ion was weak. This ion broke down with the elimination of CO and the formation of an ion with m/z 179 ($C_9H_7O_4$). The majority of the transitions mentioned were confirmed by metastable defocusing (MD)

spectra. In addition, this method showed the junction of the peracetylgalactose and the dicoumarin residues: in the MD spectrum of the ion with m/z 352 a transition from an ion with m/z 394, formed by a route characteristic for such structures [5], was recorded.

## EXPERIMENTAL

Thin-layer chromatography was conducted on Silufol UV-254 plates in the solvent system ethyl acetate–methanol–water (12:1:1), and paper chromatography on Whatman No. 3 (Czechoslovakia). For column chromatography we used Silica Geli Sigma brand silica gel. The UV spectrum was taken on a Shimadzu UV-160 spectrophotometer in methanol, the IR spectrum on a UR-20 instrument in paraffin oil, and the PMR and $^{13}C$ NMR spectra on a Varian VXR-500S spectrometer in DMSO-$d_6$ with TMS as internal standard. Mass spectra were recorded on a MKh-1310 instrument with a system for direct injection into the ion source, the temperature of the evaporator bulb being 160°C, and that of the ionization chamber 150°C. The ionizing potential was 80 V, and the collector current 60 $\mu$A. The elementary compositions of the ions were measured at R = 10,000, the reference material being perfluorokerosine. For the conditions of recording the MD spectra, see [6].

**Isolation of the Coumarins.** The air-dry comminuted epigeal part of *Stellera chamaejasme* (15 kg) gathered in the flowering period in the environs of Ulan-Bator (Mongolia) was extracted four times with ethanol at room temperature. The concentrated extract obained by distilling off the ethanol was diluted with water in a ratio of 1:1 and was extracted successively with hexane, chloroform, ethyl acetate, and butanol. The chloroform and ethyl acetate fractions were chromatographed on columns of silica gel. When the column with the chloroform fraction was eluted with the solvent systems chloroform and chloroform—methanol (99:1), daphnoretin and daphnetin were obtained. When the column with the ethyl acetate fraction was eluted with mixtures of chloroform and methanol, the coumarins daphnorin, daphnin, and chamaejasmoside were isolated.

**Chamaejasmoside**, $C_{25}H_{22}O_{12}$, mp 204-205°C (from methanol), $\lambda_{max}^{CH_3OH}$: 228, 284, 324, 336; $\nu_{max}$ ($cm^{-1}$); 1720, 1610, 1500. PMR spectrum (DMSO-$d_6$, $\delta$-scale): 3.82 (s, $OCH_3$); 4.0-5.3 (partial sucrose proton); 5.12 (d, 8 Hz, H-1″), 7.82 (s, H-4), 7.25 (s, H-5), 7.22 (s, H-8), 8.03 (d, 9.5 Hz, H-4′), 6.37 (d, 9.5 Hz, H-3′), 7.73 (d, 9.5 Hz, H-5′), 7.14 (dd, 8 and 2.5 Hz, H-6′), 7.17 (d, 2.5 Hz, H-8′). Mass spectrum m/z (%): $M^{+\cdot}$ 352 (100), 337(6), 324(7), 309(14), 295(5), 202(2), 179(31), 164(12), 145(9), 89(30).

**Chamaejasmoside Peracetate.** A mixture of 0.03 g of chamaejasmoside in 1.2 ml of acetic anhydride and 0.4 ml of pyridine was kept at room temperature for 48 h. The excess of acetic anhydride was evaporated off, and the residue was dissolved in water, whereupon the acetate precipitated. Mass spectrum of the acetate, m/z (%): 109(70), 145(21), 162(66), 169(100), 179(13), 207(5), 211(17), 271(6), 309(5), 331(46), 352(49), 394(2), 622($M^{+\cdot}$, 0.5).

**Acid Hydrolysis of Chamaejasmoside**. A solution of 10 mg of the glycoside chamaejasmoside in 3 ml of a 3% solution of sulfuric acid was heated in the water bath for 4 h. The reaction product was extracted with ethyl acetate. The extract was washed with water and evaporated to dryness, to give a compound with mp 245-247°C, identical with an authentic sample of daphnoretin.

The aqueous solution was neutralized with anion-exchange resin, filtered, and evaporated. D-Galactose was identified in the residue by paper chromatography in the presence of authentic samples.

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