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FLAVONOIDS OF *Stachys spectabilis*

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From the epigeal part of *Stachys spectabilis* we have isolated three flavonoids: the known stachyflaside and isostachyflaside, and the new spectabiflaside (I). On the basis of chemical transformations and an analysis of spectral characteristics, for (I) we propose the structure of 4',5,8-trihydroxy-7-[O-β-D-mannopyranosyl-(1 → 2)-β-D-glucopyranosyloxy]-3'-methoxyflavone (I).

Continuing an investigation of plants of the genus *Stachys* L., we have studied the flavonoid composition of *Stachys spectabilis* Choisy et DC, which is widely distributed in the subalpine zone of Transcaucasia. For the investigation we used raw material collected in the flowering period in the region of Aragats, Armenian SSR.

The flavonoids were isolated by a method described previously [1]. As a result, three substances of flavonoid nature were obtained. Two of them were identified as stachyflaside [2] and isostachyflaside [3], while the third substance was a new compound, which we have called spectabiflaside.

On a paper chromatogram, spectabiflaside was detected in the form of a light brown spot with R_f 0.31 in system 3.

To establish the structure of spectabiflaside we obtained the PMR spectra of the glycoside and of its aglycone in DMSO-D_6 and also of their acetyl and trimethylsilyl derivatives (in $CDCl_3$ and in CCl_4, respectively). The UV spectra with ionizing additives were also obtained [4]. Hydrolysis was carried out with 5% sulfuric acid, as a result of which we iso-

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lated an aglycone $C_{16}H_{12}O_7$ with mp 310-312°C. Chromatography on paper in system 1 showed the presence of two shoulders corresponding in R_f values on parallel chromatography to glucose and mannose.

The spectrum of the aglycone contained the signals of four hydroxy groups at δ (ppm) 12.37 (5-OH), 10.47 (7-OH), 9.42 (4'-OH), and 8.71 (8-OH), and also the signal of a methoxy group at δ 3.89 ppm. This was confirmed by the presence of the signals of four acetyl groups in the PMR spectrum of the acetate of the aglycone.

A comparison of the chemical shifts of the aromatic protons of ring B with those calculated theoretically for analogous models containing an unsubstituted phenyl radical by an additive scheme using the substituent constants for solution in DMSO [5] showed that the lateral residue was substituted by —OCH_3 and —OH groups in positions 3' and 4', respectively

To confirm these results we subjected the aglycone to alkaline degradation. Paper chromatography (system 2) showed the presence of vanillic acid among the degradation products. The PMR spectra of the aglycone also contained the signals of the H-3 proton at δ 6.71 ppm and of the H-6 proton at δ 6.30 ppm. These signals were identified by comparing the PMR spectra of the complete trimethylsilyl ethers and of the 5-OH trimethylsilyl ethers of the glycoside and of the aglycone [6].

Thus, the structure of the aglycone can be represented as 4',5,7,8-tetrahydroxy-3'-methoxyflavone.

The presence of three aromatic acetyl groups (δ 2.49, 2.42, and 2.34 ppm) in the spectrum of spectabiflaside acetate shows the bioside nature of this glycoside.

The position of attachment of the sugar component to the aglycone was shown by a method described in the literature [4, 6]. The sugars obtained by hydrolysis were readily fermented by yeast [7] which confirms that they belonged to the D-series of carbohydrates.

The stability of the glycoside to hydrolysis by the enzymes of the grape snail, by rhamnodiastase, and by a solution of alkali [8, 9] shows a 1 → 2 bond between the sugar residues. The spectra of the trimethylsilyl either of spectabiflaside showed the signals of the anomeric protons of two glycosidic residues: glucose (d, J = 7 Hz, δ 4.91 ppm) and mannose (d, J = 3.0 Hz, δ 5.67 ppm). From the size of the splitting constants of the anomeric protons and the values of their chemical shifts it is possible to deduce the β-configuration of the carbohydrate components [6]. The facts given show that the structure of the sugar component is the same as in other flavonoid biosides isolated from plants of the genus *Stachys* [2, 3].

Thus, the structure of spectabiflaside can be represented as 4',5,8'trihydroxy-7-[O-β-D-mannopyranosyl-(1 → 2)-β-D-glucopyranosyloxy]-3'-methoxyflavone.

EXPERIMENTAL

The UV spectra were taken on a EPS-3 instrument and the PMR spectra on a R-20A (60 MHz) instrument, with tetramethylsilane as internal standard. Optical activities were determined on a Al-EPL polarimeter. For column chromatography we used a polyamide sorbent. For paper chromatography we used the following systems: 1) liquid phenol; 2) 5% acetic acid; 3) butan-1-ol—acetic acid—water (4:1:2). Melting points were determined on a Kofler block.

Isolation of the Flavonoids. The flavanoids were extracted with 80% ethanol from 3.8 kg of air-dry epigeal part of the plant. The extract obtained was evaporated and was purified by a method described previously. This gave 126 g of purified total flavonoids. The combined substances were separated by chromatography on polyamide sorbent, using as eluent chloroform containing ethanol (3 and 6% by volume). We isolated stachyflaside and isostachyflaside, identical with substances obtained previously [2, 3], and spectabiflaside — a new flavonoid glycoside. The analyses of the compounds corresponded to the calculated figures.

Spectabiflaside, $C_{28}H_{32}O_{17}$, a yellow crystalline powder with mp 270-272°C, $[\alpha]_D^{20}$ −80° (c 0.1, methanol—dimethylformamide (9:1)).

Acetylation of Spectabiflaside. A solution of 0.3 g of the substance in 8 ml of pyridine was treated with 10 ml of acetic anhydride and the mixture was left at 20°C for 15 h.

The subsequent working up was carried out as described previously [1]. This gave 0.18 g of spectabiflaside acetate which was purified and recrystallized from methanol.

Spectabiflaside acetate formed a white crystalline powder with mp 133-135°C, $[\alpha]_D^{20}$ −40° (c 0.1, methanol).

Acid hydrolysos. A mixture of 0.5 g of spectabiflaside, 15 ml of methanol, and 15 ml of 10% sulfuric acid was heated on the boiling water bath for 12 h. The completeness of hydrolysis was determined by paper chromatography in system 2. The aglycone that deposited after concentration was filtered off and recrystallized from methanol. Its yield was 0.19 g. The filtrate after the separation of the aglycone was purified on alumina, neutralized with AV-17 anion-exchange resin, and evaporated. By paper chromatography in system 1 two sugars were detected, with R_f 0.4 and 0.43, which were identified as glucose and mannose, respectively.

Enzymatic Hydrolysis. The hydrolysis was carried out by a known method [8] with the enzyme of the grape snail and with rhamnodiastase at 36°C for 48 h. After fermentation, the initial substance was recovered.

Alkaline Hydrolysis. A mixture of 10 mg of spectabiflaside and 2 ml of 0.5% caustic potash solution was heated on the boiling water bath for 4 h. The mixture was neutralized and was chromatographed in system 3. No sugars were detected in the hydrolysis products.

The aglycone, $C_{16}H_{12}O_7$, formed yellow acicular crystals with mp 316-318°C (decomp.).

The aglycone was acetylated in the same way as the glycoside. From 60 mg of aglycone was obtained 46 mg of its tetraacetate recrystallized from methanol. The tetraacetate of the aglycone ($C_{24}H_{20}O_{11}$) formed a white crystalline powder with mp 241-243°C.

Alkaline Degradation of the Aglycone. A solution of 50 mg of the aglycone in 10 ml of 20% caustic potash solution was heated on the boiling water bath for 1 h [9]. Vanillic acid was detected in the hydrolysis products in system 2.

SUMMARY

Three substances of flavonoid nature have been isolated from the epigeal part of *Stachys spectabilis*. Two of the flavonoids have been identified as stachyflaside and isostachyflaside, and the third, which we have called spectabiflaside, is a new compound with the structure of 4',5,8-trihydroxy-7-[O-β-D-mannopyranosyl-(1 → 2)-β-D-glucopyranosyloxy]-3'-methoxyflavone.

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