ACETYLSPECTABIFLASIDE FROM *Stachys atherocalyx*

O. I. Kostyuchenko, N. F. Komissarenko,
I. P. Kovalev, A. I. Derkach,
and V. G. Gordienko

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From the herbage of *Stachys atherocalyx* C. Koch, a new flavonoid glycoside has been isolated — acetylspectabiflaside, with mp 237-242°C. On the basis of acetylation, the formation of a hydroxamic acid, alkaline, and enzymatic hydrolysis, and oxidative degradation, and also the results of UV, IR, and PMR spectroscopy, the structure of 4',5,7,8-tetrahydroxy-3'-methoxyflavone 7-O-{acetyl-[O-β-D-glucopyranosyl-(1→2)-β-D-mannopyranoside]} has been proposed for acetylspectrabiflaside.

Continuing an investigation of the flavonoid composition of *Stachys atherocalyx* C. Koch, using column chromatography on polyamide sorbent, from the combined flavonoids we have isolated four substances, three of which have been identified as diacetylspectabiflaside, spectabiflaside [2], and stachyflaside [1], while the fourth, which we have called acetylspectabiflaside, has not previously been isolated.

On paper chromatography, acetylspectabiflaside appears in UV light in the form of a dark spot with R_f 0.44 in system 1 and 0.37 in system 2. The UV spectrum of acetylspectabiflaside has maxima at 337, 275, and 255 nm, which is characteristic for flavonoids having the 3',4'-type of substition [3]. The IR spectrum of the substance has an absorption band at 1750 cm^{-1}, which shows the presence of an ester grouping in the acetylspectabiflaside molecule. We also obtained the PMR spectra of acetylspectabiflaside and of its aglycone in DMSO-d_6, of their acetyl derivatives in $CDCl_3$, and of the trimethylsilyl ether of the glycoside in CCl [3]. The spectrum of the glycoside contains the signals of three hydroxy groups at δ 12.35 (5-OH), 9.42 (4-OH), and 8.71 ppm (8-OH) and three-proton singlets at δ 3.90 ($-OCH_3$), and 1.87 ppm. The latter indicates the presence of an acetyl group in a carbohydrate residue.

The protons of ring B of acetylspectabiflaside give signals characteristic for the 3',4'-type of substitution. A comparison of the chemical shifts of the aromatic protons of ring B with those calculated theoretically for analogous models containing a unsubstituted phenyl radical by the additive scheme with the use of the substituent constants for solutions in DMSO [4] has shown that the side chain is substituted by $-OCH_3$ and $-OH$ groups in positions 3' and 4', respectively.

Acetylspectabiflaside was hydrolyzed by 5% sulfuric acid with the formation of the aglycone $C_{16}H_{12}O_7$, mp 311-313°C (decomp.) and two sugars which were identified as D-glucose and D-mannose.

From the results of alkaline degradation and on the basis of PMR spectroscopy, the aglycone was identified as 4',5,7,8-tetrahydroxy-3'-methoxyflavone, which has been isolated previously from *Stachys spectabilis* [2].

The spectrum of spectabiflaside acetate showed the signals of three aromatic acetyl groups (δ 2.49, 2.42, and 2.34 ppm) which indicates the biosidic nature of the compound. To confirm this, the glycoside was subjected to oxidative degradation, as a result of which a biose was formed [5].

To determine the nature of the acyl substituent we obtained the hydroxamic acid derivative of the acyl residue of acetylspectabiflaside. A negative hydroxyaminolysis reaction for the aglycone and a positive one for the glycoside confirmed the presence of an acetyl substituent in the carbohydrate moiety of the glycoside. Acetylspectabiflaside was hydrolyzed by the esterases of the grape snail with the formation of a more polar intermediate substance with R_f 0.31 (system 1) and acetic acid. The de-acyl derivative was identified by the re-

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sults of UV, IR, and PMR spectroscopy and by comparison with an authentic sample of spectabiflaside, which had been isolated previously from *Stachys spectabilis* [2].

The position of attachment of the carbohydrate component to the aglycone of the glycoside was determined by UV spectroscopy from the absence of a bathochromic shift with sodium acetate [3], and the fact that the sugars belong to the D series was confirmed by their ready digestibility with yeast enzymes [7].

Acetylspectabiflaside was not hydrolyzed by a 0.5% solution of caustic soda, which shows the 1→2 order of the bonds between the sugar residues [8]. In the spectrum of the trimethylsilyl ether of the glycoside the signals of the anomeric protons of two carbohydrate residues were detected: glucose (δ 4.88 ppm, J = 7.5 Hz), and mannose (δ 5.58 ppm, J = 3.0 Hz). From the magnitudes of the coupling constants of the anomeric protons and the values of their chemical shifts the β configuration of the carbohydrate components can be deduced [3]. The structure of the carbohydrate component of acetylspectabiflaside is analogous to that of other flavonoid biosides isolated from the genus *Stachys* [9, 10].

Thus, the structure of acetylspectabiflaside can be represented as 4',5,7,8-tetrahydroxy-3'-methoxyflavone 7-O-[O-β-D-mannopyranosyl-(1→2)-β-D-glucopyranoside] acylated with acetic acid.

EXPERIMENTAL

UV spectra were taken on a SF-4A instrument, IR spectra on a Hitachi instrument, and PMR spectra on a R20 A (60 MHz) instrument with tetramethylsilane as internal standard. Optical activities were determined on a A 1-EPL polarimeter. Melting points were determined on Kofler block. For paper chromatography we used the solvent systems: 1) butan-1-ol—acetic acid—water (4:1:2), 2) 15% acetic acid; 3) liquid phenol; and 4) butan-1-ol saturated with ammonia. For column chromatography we used a polyamide sorbent precipitated with acetic acid.

Isolation of the Flavonoids. The air-dry threshed berbage (2 kg) was extracted three times with chloroform—ethanol (3:1). The resulting extract was evaporated in vacuum to 500 ml, and the polyphenols were precipitated with a sevenfold amount of ether. This gave 98 g of crystalline flavonoids.

Separation of the Combined Flavonoids. A solution of 10 g of the combined flavonoids in ethanol was mixed with 15 g of Kapron [nylon-6] powder, dried, and deposited on a prepared column containing polyamide sorbent (60 × 4 cm). Elution was performed with chloroform containing from 1 to 10% of ethanol by volume. This gave diacetylspectabiflaside (6 g), acetylspectabiflaside (1.12 g), spectabiflaside (2.34 g), and stachyflaside (0.87 g).

Acetylspectabiflaside formed yellow acicular crystals with mp 237-242°C having the empirical formula $C_{30}H_{34}O_{18}$.

Acetylation of Acetylspectabiflaside. A solution of 0.1 g of the substance in 2.5 ml of pyridine and 2 ml of acetic anhydride was kept at room temperature for 18 h. The subsequent procedure was as described previously [11]. This gave 60 mg of colorless crystals of the decaacetate of the glycoside with the empirical formula $C_{48}H_{52}O_{27}$, mp 133-135°C (methanol), $[\alpha]_D^{20}$ −40° (c 0.1; methanol).

Acid Hydrolysis. A solution of 0.3 g of acetylspectabiflaside in 50 ml of ethanol containing 5% of sulfuric acid was hydrolyzed on the boiling water bath for 3 h. The completeness of hydrolysis was checked by paper chromatography in system 2. The yield of aglycone was 137 mg (44.6%). In the hydrolysate, purified on polyamide and neutralized with AV-17 anion-exchange resin, two sugars were detected, with R_f 0.41 and 0.44 in system 3, corresponding to D-glucose and D-mannose. According to PMR spectroscopy and the products of its alkaline degradation, the aglycones was identified as 4',5,7,8-tetrahydroxy-3'-methoxyflavone [2].

Enzymatic Hydrolysis. Portions of 10 mg in each case of acetylspectabiflaside were hydrolyzed with the enzymes of the grape snail, with rhamnodiastase, and with emulsin [5] at 36°C for 48 h. In the last two cases, the initial substance was recovered. Under the influence of the esterases of the grape snail enzymes, an intermediate compound was obtained with R_f 0.37 (in system 1) and mp 270-272°C which was identified as spectabiflaside. The hydrolysate was treated with ether, the resulting extracts were concentrated at 37°C, and the residue was chromatographed in system 4. An acid was detected the R_f value of which corresponded to that of an authentic sample of acetic acid (R_f 0.12).

Hydroxylaminolysis. A mixture of 10 mg of acetylspectabiflaside and three drops of the hydroxylamine reagent was heated to the boil [6]. The hydroxamic acid so obtained was chromatographed in systems 1 and 2. After the chromatogram had been treated with a 1% solution of ferric chloride containing 1% of hydrochloric acid, acetohydroxamic acid was revealed from the nature of the lilac coloration of the spots with R_f 0.57 and 0.92, respectively.

Alkaline Hydrolysis. Acetylspectabiflaside (10 mg) was hydrolyzed as described by Litvinenko and Makarov [8]. The hydrolysis products contained no sugars according to the results of chromatography in system 3.

Oxidative Degradation. A solution of 0.05 g of acetylspectabiflaside in 25 ml of acetone was treated with three drops of 0.1 N ammonia solution and 12.5 ml of 0.5 N potassium permanganate solution. The subsequent procedure was as described by Chandler and Harper [5]. The degradation products were found to contain a biose R_f 0.57 (in system 1).

CONCLUSION

The presence of flavonoid glycosides in the epigeal part of *Stachys atherocalyx* has been established, and from them four individual compounds have been isolated, three of which have been identified as diacetylspectabiflaside, stachiflaside, and spectabiflaside, while for the fourth, which has been acetylspectabiflaside, the structure of 4',5,7,8-tetrahydroxy-3'-methoxyflavone 7-O-{acetyl-[O-β-D-mannopyranosyl-(1→2)-glucopyranoside]} has been proposed.

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