FLAVONOID GLYCOSIDES OF Stachys annuae

I. P. Sheremet and N. F. Komissarenko

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In a previous study of the aglycone composition of the flavonoid compounds of the epigeal part of Stachys annuae (L.) (hedgenettle betony), we isolated 4'-methoxyscutellarein [1].

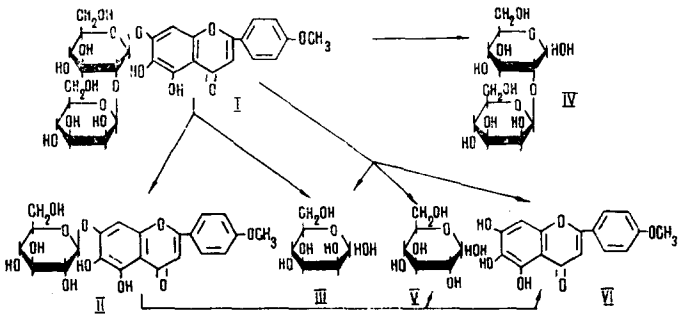
In a further investigation of this group of substances by paper chromatography, no less than five flavonoid glycosides have been detected; these have been named provisionally substances A-E and have been separated on a polyamide sorbent. Four of them (A-D) were obtained in the individual state (Table 1).

We have established the structures of substances A and B, which have been called stachannin and stachannoside, respectively, while for C and D we have determined the empirical formulas and some physicochemical properties.

From its physicochemical properties, molecular weight, and elementary composition, stachannin A (II) is a monoside. UV spectroscopy with ionizing and complex-forming reagents showed the presence of a free OH group in position 5 of A. From the characteristic shape of the spectral curve, the presence of another hydroxyl in position 6 of this substance is possible. On acid hydrolysis, the monoside was split into the aglycone 4'-methoxyscutellarein (VI) and D-glucose (V), while the UV spectra and negative Bargellini reaction [2] of stachannin A show that the sugar residue in it is present in position 7. On the basis of a comparison of molecular rotations and IR spectra [3], the sugar residue of the glycoside has a β-glycosidic linkage and a pyranose oxide ring.

The investigations performed have shown that the structure of stachannin can be given as 7-O-β-D-glucopyranosyl-5,6-dihydroxy-4'-methoxyflavone (II).

Scheme of the transformations of stachannoside B



Stachannoside B is a bioside whose acid hydrolysis formed 4'-methoxyscutellarein (VI), D-glucose (V), and D-mannose (III). After the oxidative splitting off of the carbohydrate moiety [4], the biose (IV) was found in the reaction products. As in stachannin, the sugar component had replaced the OH group at C-7 of the aglycone.

The sequence of addition of the sugars in the glycoside was found by stepwise hydrolysis with formic acid in cyclohexanol [5]. This gave a monoside identical with stachannin (II) and D-mannose (III). Both sugars of stachannoside B were found [3] to have β-glycosidic linkages and pyranose oxide rings. However,

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TABLE 1. Physicochemical Properties of the Flavonoids of Stachys annuae

Substance	Formula	mp, °C	$[\alpha]_D$, deg	Coloration with ferric chloride
Stachannin A	$C_{22}H_{22}O_{11}$	238–240	—70	Brown-green, changing to brown
Stachannoside B	$C_{28}H_{32}O_{16}$	286–289	—102	
C	$C_{37}H_{38}O_{18}$	260–263	—90	Green, changing to brown
D	$C_{27}H_{30}O_{16}$	220–224	—60	Green

glycoside B did not undergo hydrolysis with rhamnodiastase, as has been observed for the rhamnosyl-(1→6) glucoside (rutinoside), the rhamnosyl-(1→6)-galactoside (robinobioside) [6], the arabinosyl-(1→6)-glucoside [7], or the arabinosyl-(1→6)-galactoside [8], nor with emulsin [9] or the enzymes of Aspergillus oryzae [10, 11], which shows that the biose does not include (1→6) and (1→4) bonds.

The destruction of the D-glucose, as well as the D-mannose, in substance (I) on periodate oxidation excludes a (1→3) bond between them.

The steric difficulties of enzymatic hydrolysis [13, 14] for the stachannoside and also the results of periodate oxidation give grounds for assuming that the D-mannose is attached to the D-glucose by a (1→2) bond, as for the sugar linkage in neohesperidose [15]. To confirm this, we made use of the property of phenol glycosides of being hydrolyzed by alkalis [16]. It is known that in an alkaline medium the ionization of the 2-hydroxy group of the sugar takes place, which then interacts with the glycosidic center and causes hydrolysis. Where the 2-hydroxy group is substituted, no hydrolysis takes place.

We found no biose in the products of the alkaline cleavage of (I) which is characteristic for phenol biosides with a (1→2) linkage.

Thus, the structure of stachannoside B can be given as 4'-methoxyscutellarein 7-[O-β-D-mannopyranosyl-(1→2)-β-D-glucopyranoside].

EXPERIMENTAL

For analysis, the substances were dried over P_2O_5 in vacuum at 110-115°C for 6 h. The specific rotations were determined on an SPU-M spectropolarimeter, and the melting points on a Kofler block. The UV spectra were recorded on a Hitachi spectrophotometer and the IR spectra on a UR-10 instrument.

For all the compounds, the analyses corresponded to the calculated figures.

Isolation of the Flavonoids. The total flavonoid compounds were obtained as described in the literature [1]. To isolate the individual substances, 5 g of the combined flavonoids was chromatographed on a column of polyamide sorbent (d 4 cm, h 50 cm). Elution was performed with mixtures of ethanol and chloroform in various ratios. The process of separation was checked by paper chromatography in the benzene–ethyl acetate–acetic acid (23.5 : 74.5 : 2) system. The fractions of the individual substances were evaporated under vacuum. From the eluates containing 2% of methanol 0.7 g of the crystalline substance C deposited; from the 7% eluates, 0.2 g of B; from the 10% eluates, 0.1 g of A; and from the 20% eluates, 0.35 g of substance D.

Stachannin A (II). Yellow crystals with the composition $C_{22}H_{22}O_{11}$, soluble in methanol, ethanol, formamide, and dimethylformamide. On the addition of sodium ethoxide to a solution of the substance in absolute ethanol, no green coloration was observed (the Bargellini reaction) (see Table 1). IR spectrum, cm^{-1}: 3420 (carbohydrate OH); 2930, 2860 (hydrocarbon CH or methoxy group); 1670 (γ-pyrone C=O); 1620, 1512 (C=C of an aromatic system); 840 cm^{-1} (absorption due to a para substitution in the lateral phenyl radical).

In the IR spectrum of the glycoside (II), obtained without the aglycone moiety, the three bands appeared in the 1100-1010 cm^{-1} region that are characteristic for the pyranose form of the sugar component, while an absorption band at 890 cm^{-1} showed the β configuration of the glycosidic linkage.

UV spectra, nm: $\lambda_{max}^{+CH_3OH}$ 370,* 325,* 307, 280, 222; λ_{max}^{+AcONa} 367,* 313, 269; $\lambda_{max}^{+CH_5ONa}$ 330, 285; $\lambda_{max}^{+AlCl_3}$ 420,*, 350, 325, 287, 223; $\lambda_{max}^{+H_3BO_3+AcNa}$ 365, 325,† 305, 278.

*Here and below – shoulder.

†Weak maximum.

Acid Hydrolysis of (II). The glycoside (500 mg) was hydrolyzed in aqueous alcohol containing 5% of sulfuric acid at 100°C for 5 h. The hydrolysis was monitored by paper chromatography in the benzene–ethyl acetate–acetic acid (23.5 : 74.5 : 2) system. The genin 4'-methoxyscutellarein (VI), $C_{16}H_{12}O_6$, mp 250-253°C [1], was isolated, while paper chromatography in the liquid phenol system showed the presence of D-glucose (V).

Stachannoside B (I). Yellow crystals with the composition $C_{28}H_{32}O_{16}$, sparingly soluble in methanol and ethanol, more soluble in a mixture of methanol and chloroform, soluble in formamide and dimethylformamide. As for (II), the Bargellini reaction was negative (see Table 1). IR spectrum, cm^{-1}: 3430 (carbohydrate OH); 2940, 2860 (carbohydrate CH or methoxy group); 1670 (γ-pyrone C=O); 1620, 1512 (C=C of an aromatic system); 840 (absorption due to para substitution in the lateral phenyl radical). The spectrum of (I) taken without substance (II) also exhibited three strong bands in the 1100-1010 cm^{-1} and the 890 cm^{-1} regions, which shows the pyranose form of D-mannose and the β configuration of its linkage.

UV spectra, nm: $\lambda_{max}^{+CH_3OH}$ 370,* 325,* 307, 280, 222; λ_{max}^{+AcONa} 380,* 337,*, 310, 280; $\lambda_{max}^{+CH_3ONa}$ 335,† 290; $\lambda_{max}^{+AlCl_3}$ 425,* 350, 324, 285, 223; $\lambda_{max}^{+H_3BO_3+AcNa}$ 360,* 325,† 305, 278.

Acid Hydrolysis of (I). This was performed as for the stachannin (II). The aglycone 4'-methoxyscutellarein (VI) and D-glucose (V) and D-mannose (III) were obtained.

Oxidative Splitting out of the Biose (IV). With heating, 200 mg of the glycoside B was dissolved in 100 ml of acetone, and 0.5 ml of a 0.1 N solution of ammonia and 50 ml of a 0.5 N solution of potassium permanganate was added. The subsequent treatment was as described by Chandler and Harper [4]. On paper chromatography in the liquid phenol system, the biose (IV) was detected.

Stepwise Hydrolysis of (I). In a flask with a reflux condenser a solution of 250 mg of the substance in 36 ml of boiling cyclohexanol was treated with 12 ml of 85% formic acid, added through the condenser. Hydrolysis was performed at 120-130°C for 10 h. The hydrolysis products were separated by the method of Fox et al. [5]. The monoside II, identical with stachannin A, and D-mannose (III) were isolated.

Periodate Oxidation of B. To a solution of 60 mg of the glycoside under investigation in 10 ml of acetate buffer with pH 4.5 was added 36 mg of periodic acid, and the reaction mixture was left in a dark place for three days. Then the excess of periodic acid was decomposed by the addition of a few drops of ethylene glycol, the solvent was distilled off in vacuum, and the oxidation products were extracted with ethanol. After the solvent had been driven off, the residue was hydrolyzed with 5% sulfuric acid at 100°C for 4 h. The reaction mixture was neutralized with AV-17 anion-exchange resin. After evaporation of the hydrolysate and its chromatography, no sugars were found.

Alkaline Hydrolysis of (I). The stachannoside (40 mg) was dissolved in 10 ml of 0.5% aqueous KOH and hydrolyzed by a published method [16]. No biose was found in the products of alkaline cleavage.

SUMMARY

Four flavonoid compounds have been isolated from the herb Stachys annuae (L.). stachannin A, stachannoside B, and substances C and D.

It has been established that stachannin A is 7-O-β-D-glucopyranosyl-5,6-dihydroxy-4'-methoxyflavone, and stachannoside B is 4'-methoxyscutellarein 7-[O-β-D-mannopyranosyl-(1→2)-glucopyranoside].

Both glycosides are new compounds.

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