Daurinol Methyl Ether. An ethereal solution of diazomethane was added to a suspension of 40 mg of (II) in 5 ml of anhydrous chloroform. After 6 h, the solvent was distilled off. The reaction product was separated from the initial substance by chromatography on a column of alumina. mp 231-233°C (methanol), R_f 0.68. A mixture with justicidin B gave no depression of the melting point, and their IR spectra were identical.

SUMMARY

Justicidin B and a new arylnaphthalene lignan daurinol have been isolated from *Haplophyllum dauricum*. On the basis of chemical and spectral characteristics using double resonance it has been ascribed the structure (IIa or b).

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MICRANTHOSIDE — A NEW GLYCOSIDE FROM *Eupatorium micranthum*

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From the epigeal parts of the plant *Eupatorium micranthum* Less., introduced into the Sukhumi Botanical Garden, a new glycoside has been isolated which has been called micranthoside and which has the structure of 4',5-di-O-β-glucosyl-7-O-methyldihydrokaempferol or 4',5-di-O-β-D-glucosyl-7-O-methylaromadendrin.

The epigeal part of *Eupatorium micranthum* Less. syn. *E. ligustrinum* DC., family Asteraceae (Compositae), introduced into the Sukhumi Botanical Garden (Georgian SSR) [1], has proved to be rich in flavonoid compounds [2] (Scheme, following page, top).

From an aqueous alcoholic extract of the plant we have quantitatively isolated the main substance, a flavonoid glycoside which has been named micranthoside (I).

Micranthoside (I), $C_{28}H_{34}O_{16}$, gives the reactions specific for flavonoids. In the UV spectrum $\lambda_{max}^{C_2H_5OH}$ 280 nm (log ε 4.4). The IR spectrum shows the absorption bands characteristic for a hydroxy group (3420 cm^{-1}), for the carbonyl of a γ-pyrone ring (1680 cm^{-1}), for aromatic rings (1615, 1580, 1520 cm^{-1}), and for a methoxy group (2920 cm^{-1}).

The assignment of the PMR spectrum of compound (I) taken in deuteropyridine (Fig. 1a) permitted it to be ascribed to the group of flavonol diglycosides.

A complex multiplet in the 3.8-4.4 ppm region has an integral intensity corresponding to the protons of the two glucose residues. At 5.30 ppm there is the signal of one anomeric proton in the form of a doublet with a constant of 6.5 Hz (the use of double resonance showed that on suppression of the resonance at 4.20 ppm the doublet splitting was eliminated). Such

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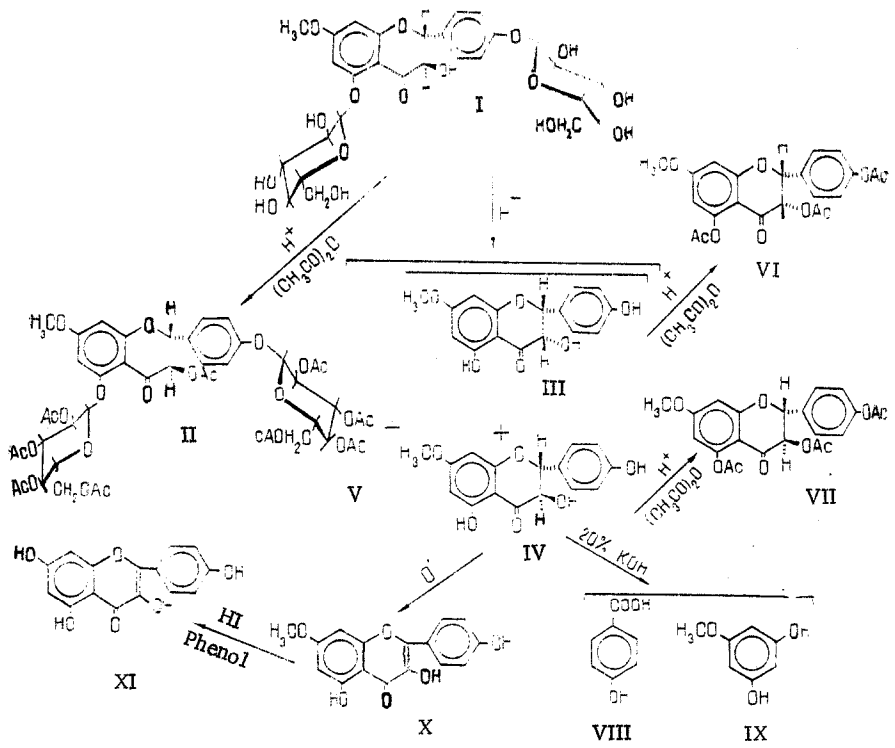

Chemical transformation of micranthoside

a signal is characteristic for a sugar residue in the C1 conformation attached to the aglycone through oxygen by a β-glycosidic bond.

In the weak-field part of the spectrum of compound (I) there are the signals of protons 2', 6', 3', and 5' in the form of a distorted spectrum of the AB type (a AA'BB' system) and doublet signals (spin-spin coupling constant 2.75 Hz) of the H-8 and H-6 protons (6.85 and

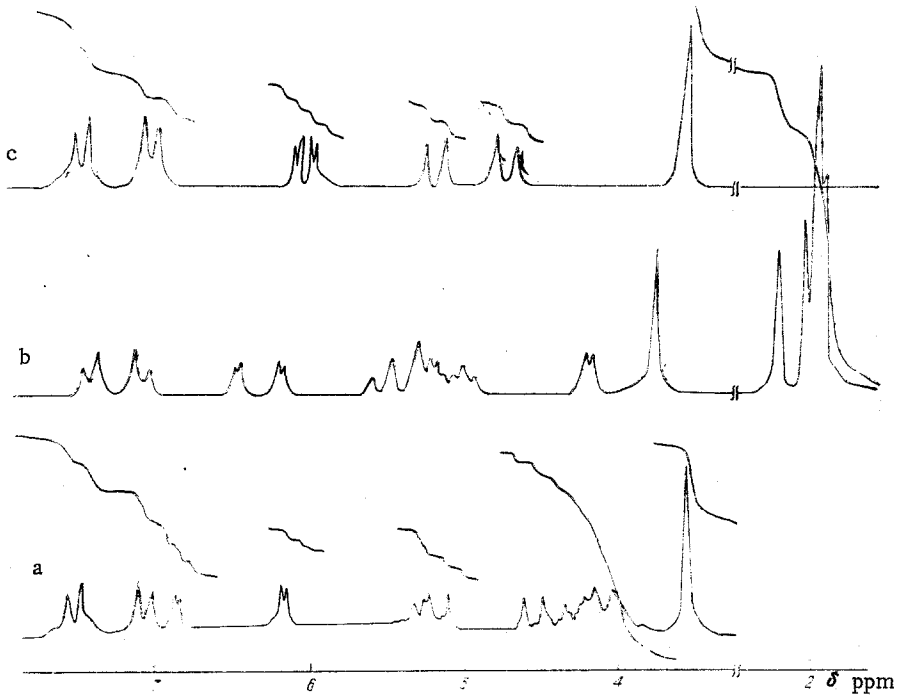

Fig. 1. PMR spectrum of micranthoside (a) and its acetate (b) and the aglycone-2 (c).

6.18 ppm). Resonance of the H-2 and H-3 protons is observed in the form of signals characteristic for an AB system, which was confirmed by double resonance. The spin-spin coupling constant between these vicinal protons amounts to 11.8 Hz, which shows a conformation of the $H_{(2)}$—C—C—$H_{(3)}$ fragment close to the trans conformation [6, 7].

The signal of a methoxy group appears at 3.58 ppm.

The acetylation of micranthoside in acetic anhydride in pyridine gave the acetate (II) with mp 215-217°C, in the IR spectrum of which the absorption band at 3420 cm^{-1} had disappeared while bands corresponding to acetyl groups had appeared at 1760 and 1240 cm^{-1}.

The PMR spectrum of the acetate of the glycoside was recorded in the form of a solution in $CDCl_3$ (Fig. 1b). Integration of the signals showed that eight sugar acetyl groups and one acetyl group in a γ-pyrone ring were resonating. The signals of the sugar protons were shifted downfield, with the exception of the signal of the second anomeric proton (4.19 ppm) with doublet splitting of 6.5 Hz, which permits us to conclude that the two sugar residues are attached in the same way.

Micranthoside readily underwent acid and enzymatic hydrolysis with the formation of D-glucose and the aglycone (yield 40%), which shows the biosidic or diglycosidic nature of the glycoside.

Since the molecule of a flavanonol contains two asymmetric carbon atoms [8], it is possible to obtain stereoisomeric forms. In actual fact, the acid hydrolysis of micranthoside gave two genins — aglycone-1 (II) with a yield of 2% and aglycone-2 (IV) with a yield of 3.8%. In the aqueous fraction of the hydrolysate after its neutralization with silver carbonate, only D-glucose was detected.

The enzymatic hydrolysis of micranthoside gave an optically inactive aglycone.

The stereochemistry of the aglycones obtained by acid and enzymatic hydrolysis was determined on the basis of their physicochemical properties. In order to establish the structure of the aglycone of micranthoside we studied the predominating substance — aglycone-2 (IV) — in detail.

Aglycone-1, with the composition $C_{16}H_{14}O_6$, mp 175-178°C, $[\alpha]_D^{20}$ −50° (c 0.5; ethanol), formed yellow acicular crystals soluble in ethanol and chloroform. The acetyl derivative of aglycone-1 with the composition $C_{28}H_{34}O_{16}$ melted at 110-112°C. From the physicochemical properties of the aglycone itself and of its acetyl derivative, aglycone-1 was identified as fol-orogenin (III), which, according to the literature, is the cis isomer of 7-methoxyaromadendrin. In all probability, aglycone-1 is a product of the isomerization of aglycone-2 [9].

Aglycone-2, with the composition $C_{16}H_{14}O_6$, mp 184-184°C, $[\alpha]_D^{20}$ +17.3° (c 0.95; ethanol) formed white acicular crystals soluble in ethanol, chloroform, and ethyl acetate. UV spectrum: $\lambda_{max}^{C_2H_5OH}$ 290 nm (log ε 4.5).

The PMR spectrum of the aglycone-2 (IV) (Fig. 1c) taken in $CDCl_3$, in contrast to that of the glycoside (I), lacked the characteristic signals of anomeric and sugar protons. The spin-spin coupling constant of the H-2 and H-3 protons was J = 11.8 Hz. The formation of compound (X) by the oxidation of aglycone-2 (IV) [10] and its conversion by demethylation into kaempferol [11, 12] shows the trans-diaxial arrangement of these protons [6, 7]. In the spectrum of the aglycone-2 taken in dimethyl sulfoxide, the free hydroxyl at C-5 can be seen in the 11.65 ppm region, and that at C-3 in the 9.6 ppm region.

The fusion of aglycone-2 with potassium hydroxide led to the formation of p-hydroxybenzoic acid (VIII) and methylphloroglucinol, which is one of the products of the degradation of rhamnetin [13].

On the basis of the results obtained, it may be assumed that aglycone-2 (IV) is 7-O-methyldihydrokaempferol or 7-O-methylaromadendrin, which has been isolated previously from *Eucalyptus maculata, Eupatorium capillifolium, E. macrocephalus, E. perfoliatum,* and *Prunus avium* [14-17].

The aglycone obtained as the result of the enzymatic hydrolysis of micranthoside with mp 182-186°C is probably, according to its physicochemical properties, a racemate of the above-mentioned aglycones [16].

A comparison of the UV-spectral characteristics obtained with the aid of diagnostic reagents for the aglycone and the glycoside showed the presence of free hydroxyls in the agly-

cone in the C-3, C-5, and C-4' positions, and substitution at C-7. The absence of bathochromy in the UV spectrum of the glycoside when sodium ethanolate was added and its appearance in the spectrum of the aglycone shows that one molecule of the sugar component is present in the C-4' position. No bathochromy was observed in the UV spectrum of the glycoside when zirconyl chloride was added, while in the case of the aglycone this led to a slight shift (30 nm) which disappeared in the presence of citric acid. This is characteristic for derivatives of a flavanonol having substitution in position 5 [8] and, consequently, a free hydroxyl in position 3, as was confirmed by the conversion of aglycone-2 (IV) into the flavonol (X), the demethylation of which led to the formation of kaempferol [11]. A proof of this is a broadened signal at 9.6 ppm unambiguously assigned to the hydroxyl at C-3.

Substitution of the hydroxy group at C-5 was confirmed by the absence from the PMR spectrum of the glycoside of a signal in the 11-12 ppm region. It must be assumed that the second molecule of glucose is attached at this position.

The presence in the IR spectrum of micranthoside of four absorption bands at 1100, 1080, 1040, and 890 cm^{-1} and the ease of enzymatic hydrolysis indicates the pyranose form of the oxide ring of the glucose and the β configuration of the glycosidic bond [18-20].

On the basis of the facts given above, the most probable structure of micranthoside has been established as 4',5-di-O-β-glucosyl-7-O-methyldihydrokaempferol or 4'-5-di-O-β-glucosyl-7-O-methylaromadendrin.

EXPERIMENTAL

IR spectra were taken on a UR-20 instrument in KBr and paraffin oil, UV spectra on a SF-4 instrument, and PMR spectra on a Perkin-Elmer R-32 spectrometer (in C_5D_5N, $CDCl_3$, and DMSO) with HMDS as internal standard (δ scale). Melting points were determined on a Kofler block. For the paper chromatographic analysis of the flavonoids and carbohydrates we used the following solvent systems: 1) butanol–acetic acid–water (4:1:2); 2) 15% acetic acid; 3) benzene–ethyl acetate–acetic acid (23.5:74.5:2); and 4) pyridine–benzene–butanol–water (3:1:5:3). The flavonoids were revealed with a 10% ethanolic solution of NaOH, and the carbohydrates with the aniline phthalate reagent. Molecular weights were determined by the spectrophotometric method.

Isolation of Micranthoside (I). The air-dry comminuted epigeal parts of *Eupatorium micranthum* (0.5 kg) were extracted with 80% methanol. The alcohol was distilled off from the extract and the aqueous liquid was purified with chloroform. The substance that deposited in the aqueous phase in this process was separated off and recrystallized from aqueous ethanol. This gave 9.2 g of micranthoside (I) with mp 238-241°C, $C_{28}H_{34}O_{16}$ (mol. wt. 626.36) $[\alpha]_D^{20}$ −49° (c 1.0; dimethylformamide); soluble in 45% ethanol, dimethyl sulfoxide, dimethylformamide, and pyridine; insoluble in water, chloroform, and ether.

UV spectrum, nm: $\lambda_{max}^{C_2H_5OH}$ 280 (log ε 4.4); (+NaOAc) 280; [+$ZrO(NO_3)_2$] 280; (+NaOMe) 280.

IR spectrum, ν_{max}^{KBr}, cm^{-1}: 3420 (OH^-), 2920 (−OCH_3), 1680 (C=O of a γ-pyrone), 1615, 1580, 1520 (C=C), etc.

PMR spectrum in deuteropyridine (ppm) (see Fig. 1a): 7.55 (d, J = 8 Hz, H-2', 6'), 7.08 (d, J = 8 Hz, H-3', 5'), 6.85 (d, J = 2.75 Hz, H-8), 6.18 (d, J = 2.75 Hz, H-6), 5.18 (d, J = 11.8 Hz, H-2), 4.56 (d, J = 11.8 Hz, H-3), 3.58 (s, −OCH_3), 5.30 (d, J = 6.5 Hz, H'), 3.8-4.4 (m, sugar protons and H").

Acetylation of Micranthoside (I). A solution of 150 mg of the substance in pyridine was treated with 5 ml of acetic anhydride and the mixture was heated in a water bath for 0.5 h. After cooling, it was poured into a double volume of ice water and was extracted with ethyl ether. The concentrated extract was recrystallized from ethanol. This gave 240 mg of white acicular crystals of (II) with mp 215-217°C.

IR spectrum (in paraffin oil), cm^{-1}: 1760, 1240 (−OAc groups).

PMR spectrum in $CDCl_3$ (ppm) (see Fig. 1b): 7.40 (d, J = 8 Hz, H-2', 6'), 7.08 (d, J = 8 Hz, H-3', 5'), 6.46 (d, J = 2.7 Hz, H-8), 6.18 (d, J = 2.7 Hz, H-6), 5.54 (d, J = 11.8 Hz, H-2), 4.49 (d, J = 11.8 Hz, H-3), 3.34 (s, OCH_3), 5.03-5.35 (m, sugar protons and H'), 4.19 (d, J = 6 Hz, H"), 2.22 (s, acetate at C-3), and 1.92-2.04 (m, acetyl groups in sugar residues).

Quantitative Acid Hydrolysis of the Glycoside (I). A solution of 516 mg of the glycoside (I) in 40 ml of 2N HCl was heated in the water bath. The course of the reaction was monitored by PC in systems 1 and 2. Hydrolysis was complete after 40 min. When the reaction mixture cooled, fine crystals of two different colors deposited which were readily separated from one another mechanically and were recrystallized from ethanol. This gave 10 mg of yellow crystals of the aglycone-1 (III) and 195 mg of white crystals of the aglycone-2 (IV).

Aglycone-1 (III): $C_{16}H_{14}O_6$, mp 175-178°C $[\alpha]_D^{20}$ −50° (c 0.5 ethanol). R_f 0.90 in systems 1 and 3.

Aglycone-2 (IV): $C_{16}H_{14}O_6$, mp 184-186°C, $[\alpha]_D^{20}$ +17.3° (c 0.95; ethanol), R_f 0.90 in systems 1 and 3. UV spectrum, nm: $\lambda_{max}^{C_2H_5OH}$ 290; (log ε 4.51); (+ NaOAc) 290; [+$ZrO(NO_3)_2$] 320; [+$ZrO(NO_3)_2$ + $C_6H_8O_7$] 290 (+NaOMe) 350.

IR spectrum (in paraffin oil), cm^{-1}: 3470, 3375, 3210, 1650, 1580, 1530.

PMR spectrum in $CDCl_3$ (ppm) (see Fig. 1c): 7.45 (d, J = 8 Hz, H-2', 6'), 6.98 (d, J = 8 Hz, H-3', 5'), 6.06 (d, J = 2.5 Hz, H-8), 5.94 (d, J = 2.5 Hz, H-6), 5.16 (d, J = 11.8 Hz, H-2), 4.70 (d, J = 11.8 Hz, H-3), 3.52 (s, OCH_3).

In the carbohydrate part of the hydrolysate, after neutralization with silver carbonate [21] and evaporation to 0.5 ml, D-glucose (V) was detected by PC analysis in systems 1 and 4.

Acylation of Aglycone-1 (III). A solution of 7 mg of the substance in acetic anhydride was treated with one drop of concentrated H_2SO_4. After 5 minutes, the mixture was diluted with water and the acetyl derivative was isolated as described for the glycoside (I). This gave white acicular crystals of (VI) with mp 110-112°C.

Acetylation of Aglycone-2 (IV). The substance (30 mg) was acetylated by the method described above. This gave the acetate (VII) with mp 144-145°C.

Alkaline Fusion of Aglycone-2 (IV). A solution of 40 mg of the substance in 10 ml of 20% KOH was boiled at 130°C for 1.5 h. The cooled solution was treated with 10% H_2SO_4 to pH 4-5. The degradation products were extracted with ethyl ether. The extracts were evaporated and the residue was dissolved in 1 ml of ethanol and chromatographed on paper in systems 2 and 3. p-Hydroxybenzoic acid (VIII) and methylfluoroglucinol (IX) were detected.

Oxidation of Aglycone-2 (IV). A solution of 100 mg of the substance in 16 ml of 1 N sulfuric acid was heated in the boiling water bath and a slow current of air was passed over the surface of the solution for 4 h [5, 10]. The yellow precipitate that deposited was filtered off, washed with cold water, and recrystallized from 70% ethanol. This gave yellowish acicular crystals of (X) with mp 219-221°C, $\lambda_{max}^{C_2H_5OH}$ 270, 365 nm. R_f 0.52 and 0.92 in systems 2 and 3, respectively.

Demethylation of the Oxidized Aglycone-2 (X). A mixture of 50 mg of the substance, 0.5 ml of acetic anhydride, 0.3 ml of liquid phenol, and 0.5 ml of hydriodic acid [11, 12] was heated at 130°C for 30 min, and after cooling it was diluted with water and extracted with ethyl acetate. The concentrated extract was recrystallized from ethanol. The demethylation product — yellow acicular crystals with mp 272-274°C; $\lambda_{max}^{C_2H_5OH}$ 270, 360 nm — appeared on PC at the level of authentic kaempferol.

Enzymatic Hydrolysis of Micranthoside (I). With heating, 50 mg of the substance was dissolved in 5 ml of 20% ethanol, and the solution was treated with 20 mg of the enzyme of the grape snail in 5 ml of water. Hydrolytic decomposition (monitored with the aid of PC in systems 1 and 2) was complete in 10 h. The enzyme was precipitated with 96% ethanol, the ethanol was evaporated off from the liquid, and the aglycone was extracted with ethyl ether. This was an optically inactive aglycone with mp 182-186°C. In the aqueous residue evaporated to 1 ml, D-glucose was detected by PC analysis in systems 1 and 4.

SUMMARY

A new flavonoid diglycoside which has been called micranthoside has been isolated from the epigeal part of *Eupatorium micranthum* Less. On the basis of a study of its chemical and spectral properties, its most probable structure has been established as 4',5-di-O-β-glucosyl-7-O-methyldihydrokaempferol or 4',5-di-O-β-glucosyl-7-O-methylaromadendrin.

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