A NEW FLAVONOL GLYCOSIDE FROM Azara microphylla

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The leaves of Azara microphylla Hook., introduced into the Sukhami Botanical Garden of the Academy of Sciences of the Georgian SSR, have yielded a new glycoside, which has been called azamicroside and its structure has been established as myricetin 3-O-L-dirhamnoside.

A new flavonoid glycoside, which has been called azamicroside (I) has been isolated from an aqueous alcoholic extract of the leaves of Azara microphylla Hood. (family Flacourtiaceae) introduced into the Sukhumi Botanical Garden of the Academy of Sciences of the Georgian SSR [1].

Azamicroside (I), $C_{27}H_{30}O_{16}$, gives a positive Bryant cyanidin reaction [2], which shows its glycosidic nature. IR spectra, $\lambda_{max}^{C_2H_5OH}$, nm: 358, 300 (sh.), 260 (log ε 4.18, 4.14, 4.19). The IR spectrum shows absorption bands characteristic for hydroxy groups (3270 cm^{-1}), the carbonyl group of a γ-pyrone ring (1660 cm^{-1}), of aromatic rings (1580, 1560, 1510 cm^{-1}), and others.

The PMR spectrum of (I) taken in deuteromethanol (Fig. 1a) shows: a two-proton singlet corresponding to the H-2' and H-6' protons (δ 6.91 ppm), doublet signals with SSCCs of 1 and 8 Hz of the H-8 and H-6 protons (6.30 and 6.13 ppm, respectively), a complex multiplet in the 3.3-3.9 region, corresponding to eight protons of sugar residues, and also the signals of two anomeric protons at 5.27 and 4.20 ppm [3].

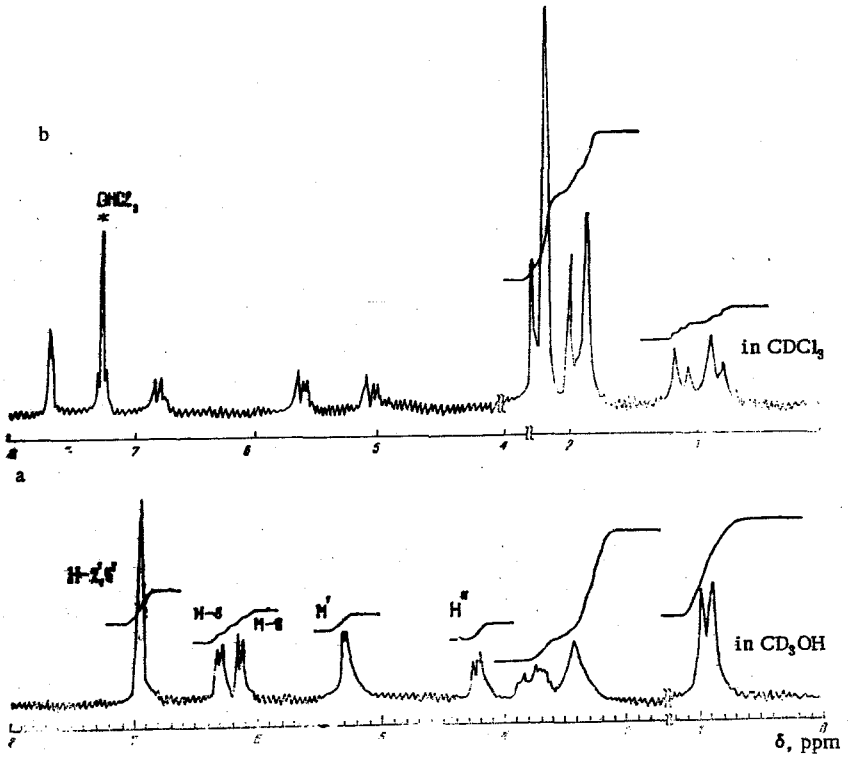

Fig. 1. PMR spectra of azamicroside (a) and its acetate (b).

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The acetylation of (I) with acetic anhydride in pyridine yielded the acetate (II), in the IR spectrum of which the absorption band at 3270 cm^{-1} had disappeared and bands had appeared at 1760 and 1200 cm^{-1}, corresponding to acetyl groups.

The PMR spectrum of (II) was recorded in the form of a solution in $CDCl_3$ (Fig. 1b). Integration of the signals showed that in the strong-field part of the spectrum ten acetyl groups resonated in the 1.8-2.4 ppm region, while two signals with an SSCC of 6 Hz at 0.85 and 1.15 ppm corresponded to the protons of two methyl groups of L-rhamnose residues. The PMR spectrum of the glycoside (Fig. 1a) also had a signal at 0.95 ppm corresponding in its integral intensity to the six protons of two methyls of L-rhamnose residues. In addition, the IR spectrum had an absorption band at 970 cm^{-1}, which is characteristic for the methyl group of a L-rhamnose residue.

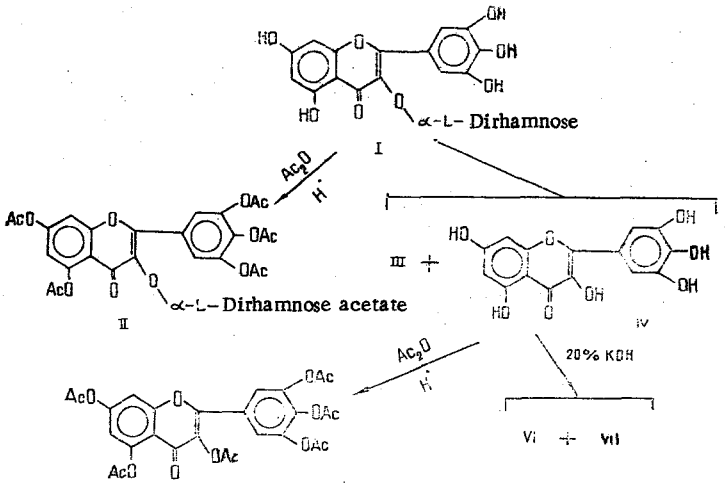
Azamicroside readily underwent acid hydrolysis with the formation of L-rhamnose (III) and an aglycone (IV) with a yield of 45%, which shows the biosidic or diglycosidic nature of the glycoside. This was confirmed by a comparison of the PMR spectra of the glycoside and its aglycone. The PMR spectrum of the aglycone taken in CD_3OD, unlike the spectrum of the glycoside (I) (Fig. 1a) lacked the characteristic signals of anomeric and sugar protons.

Fusion of the aglycone (IV) with potassium hydroxide led to the formation of gallic acid (VI) and phloroglucinol (VII). Acetylation of the aglycone yielded (V), identical in its physicochemical properties and spectral characteristics with myricetin acetate (3,3',4',5,5',7-hexaacetylflavone).

A comparison of the UV spectral characteristics obtained with the addition of diagnostic reagents of the aglycone and the glycoside, and also the qualitative Hörhammer–Hänsel [4], Bargellini [5], and Wilson [6] reactions and that with diazotized sulfanilic acid [7] showed the presence of free hydroxyls in the aglycone at C-3,3',4',5,5',7, while in the glycoside the hydroxyls at C-3 and either at C-5' or C-3' had disappeared. Thus, one molecule of L-rhamnose was attached to position 3 of the aglycone. The position of the second rhamnose residue in the side chain was proposed as C-5' on the basis of qualitative reactions and spectral characteristics, but this was unjustified since the hydroxyls at C-5' and C-3' are arranged symmetrically, and 3-O-glycosides of myricetin give a negative Bargellini reaction [8]. The presence of the signals of the anomeric protons of L-rhamnose residues at 5.27 and 4.20 ppm with a difference of 1.07 ppm is evidence in favor of a biosidic structure of the glycoside. This gave grounds for considering that both rhamnose residues were present in position 3.

The stability of azamicroside (I) to the hydrolytic action of the enzymes of the grape snail showed the α configuration of the glycosidic bond. Absorption bands in the IR spectra at 1080, 1070, 1050, and 1030 cm^{-1} indicated the pyranose form of the L-rhamnose residues [9].

On the basis of the facts given above, the most probable structure of azamicroside has been established as myricetin 3-O-α-L-dirhamnopyranoside.



Scheme of chemical conversion of azamicroside.

EXPERIMENTAL

IR spectra were taken on a UR-20 instrument in paraffin oil, UV spectra on a Specord UV-VIS, and PMR spectra on a Varian-56/60 A spectrometer (in CD_3OD and $CDCl_3$) 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) ethyl acetate–benzene–acetic acid (74.5:23.5:2); and 4) pyridine–benzene–butanol–water (3:1:5:3). The flavonoids were revealed with a 1% solution of aluminum chloride, and the carbohydrates with the aniline phthalate reagent.

Molecular weights were determined by the spectrophotometric method [10].

Isolation of Azamicroside (I). The air-dry comminuted leaves of Azara microphylla (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 precipitated in the aqueous phase under this treatment was separated off and recrystallized from ethanol. This gave 5.5 g of azamicroside (I) in the form of white acicular crystals. The substance was soluble in ethanol, dimethyl sulfoxide, and dimethylformamide, and practically insoluble in chloroform, diethyl ether, and petroleum ether. mp 190-193°C, $C_{27}H_{30}O_{16}$, MM 610.26, $[\alpha]_D^{19}$ −145.5 ± 2° (c 0.5; ethanol–dimethylformamide (99.5:0.5)); R_f 0.64 and 0.42 in systems 1 and 2, respectively.

UV spectrum (nm): $\lambda_{max}^{C_2H_5OH}$ 358; 300; 260 (log ε 4.18; 4.14; 4.19); (+NaOAc) 358, 300, 262; (+NaOAc + H_3BO_3) 370, 305, 263; (init.+ $AlCl_3$) 370; 300, 260; (+ $AlCl_3$ + HCl) 364, 300, 260, (+ NaOMe) 400, 278.

IR spectrum (cm^{-1}): ν_{max} 3270 (−OH); 1660 (C = O of a γ-pyrone); 1580, 1560, 1510 (C=C); and others.

PMR spectrum in CD_3OD (ppm) (Figure 1a): 6.91 (s, H-2', 6'); 6.30 (d, J = 1.8 Hz, H-8); 6.13 (d, J = 1.8 Hz, H-6); 5.27 (d, J = 1 Hz, anomeric H''); 4.20 (m, anomeric H'''); 3.3-3.9 (m, 8 H, sugar protons); 0.95 (6 H, d, J = 6 Hz, 2 CH_3 of rhamnose residues).

Acetylation of Azamicroside (I). A solution of 140 mg of the substance in pyridine was treated with 5 ml of acetic anhydride and the mixture was heated on the water bath for 20 min. After cooling, it was poured into a double volume of iced water and was extracted with ethyl acetate. The concentrated extract was crystallized from ethanol to give 236 mg of white acicular crystals of (II), composition $C_{47}H_{50}O_{26}$, mp 124-127°C. IR spectrum (cm^{-1}): ν_{max} 1770, 1200 (−OAc groups).

PMR spectrum in $CDCl_3$ (Figure 1b): 7.65 (s, H-2', 6'); 7.20 (d, J = 1.8Hz, H-8); 6.78 (d, J = 1.8 Hz, H-6); 5.63 (m, anomeric H''); 5.07 (m, anomeric H'''); 1.8-2.4 (m, 10 acetyl groups); 0.85 (d, J = 6 Hz, $-CH_3$ of a rhamnose residue); and 1.15 (d, J = 6 Hz, $-CH_3$ of a rhamnose residue).

Quantitative Acid Hydrolysis of the Glycoside (I). With heating on the water bath under reflux, 450 mg of azamicroside was hydrolyzed in 30 ml of 2% sulfuric acid solution: The course of the reaction was monitored by PC in systems 1 and 2. Hydrolysis was complete in 4 min. After cooling, the reaction mixture was exhaustively extracted with ethyl acetate. The ethyl acetate extract was washed with water to neutrality, dried, and concentrated. The residue was recrystallized from ethanol. This gave 202 mg of the aglycone or 45% on the weight of the initial substance (I).

The aglycone (IV), with the composition $C_{15}H_{10}O_8$, mp 350-353°C, had R_f 0.47 and 0.25 in systems 1 and 3, respectively. UV spectrum (nm): $\nu_{max}^{C_2H_5OH}$ 375, 305, 257; (+ NaOAc) 365, 300, 260; (+ NaOAc + H_3BO_3) 392, 260; (+ $AlCl_3$) 440, 268; (+ $AlCl_3$ + HCl) 365, 265. IR spectrum (cm^{-1}): ν_{max} 3100–3500 (OH); 1660 (C = O), 1610, 1520 (=C=C=).

PMR spectrum in CD_3OD (ppm): 7.30 (s, H-2',6'); 6.36 (d, J = 1.8 Hz, H-8); 6.14 (d, J = 1.8 Hz, H-6).

The acid hydrolysate after extraction of the aglycone was neutralized with AV-17 anion-exchange resin and filtered, and the liquid was evaporated to a syrupy mass. The residue was dissolved in 1 ml of distilled water and chromatographed in systems 1 and 2 in the presence of authentic monosaccharides. The presence of L-rhamnose (III) was shown.

Acetylation of the Aglycone (IV). Three drops of concentrated H_2SO_4 was added to a solution of 4 mg of the aglycone in 2 ml of acetic anhydride, and the mixture was left for 5 min, after which it was poured into 30 ml of ice water and the acetyl derivative was isolated as described for glycoside (I). This gave 70 mg of white acicular crystals (V) with mp 208-210°C.

Alkaline Fusion of the Aglycone (IV). A solution of 45 mg of the substance in 10 ml of 20% potassium

hydroxide solution was boiled at 130°C for 1.5 h and was then neutralized with 10% H_2SO_4 solution to pH 4-5. The degradation products were extracted with ethyl ether. The ethereal extract was evaporated and the residue was dissolved in 1 ml of ethanol and chromatographed in systems 1 and 2. Gallic acid (VI) and chloroglucinol (VII) were detected.

SUMMARY

The leaves of Azara microphylla Hook., have yielded a new flavonoid glycoside which has been called azamicroside. From its physicochemical and spectral characteristics its most probable structure has been established as myricetin 3-O-α-L-dirhamnoside.

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TERPENOIDS OF THE ESSENTIAL OIL OF *Ledum palustre*

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The chemical composition of the essential oil of crystal tea ledum of the ordinary variety growing in Western Siberia has been studied. Eighteen terpenoids have been isolated and identified from their spectral characteristics. Linalyl acetate, α-terpenyl acetate, carvone, geraniol, β-farnesene, calamenene, p-mentha-1(7)-8-dien-2α-ol, and p-mentha-1(7)-8-dien-2β-ol have been isolated from the essential oil of crystal tea ledum for the first time. Compounds of the p-menthane and aromadendrane series were found as the main components.

The officinal medicinal plant Ledum palustre L. (crystal tea ledum) (family Ericaceae) is widely distributed in the territory of the Soviet Union [1]. The main active principle, possessing an antitussive action, is the sesquiterpene ledol [2]. For medical purposes, the preparation of the raw material (leafy shoots) is carried out mainly in Belorussia and in the north of the European part of the USSR. In order to expand the raw materials basis, we have studied the composition of the mono- and sesquiterpenoids of the essential oil of Ledum palustre L. var. vulgare Ledeb. (crystal tea ledum, common variety), which grows widely in the territory of Western Siberia [3].

The composition of the monoterpene hydrocarbons (19.6%) was determined by gas–liquid chromatography (GLC); the main component was limonene (17.6%). From the fraction of sesquiterpene hydrocarbons (4.0%) we isolated and identified from their spectral characteristics α-humulene, alloaromadendrene, calamenene, β-farnesene, and δ-cadinene, and we also isolated an unidentified hydrocarbon with a molecular weight of 204.

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