PHENYLPROPANOID GLYCOSIDES OF Teucrium polium

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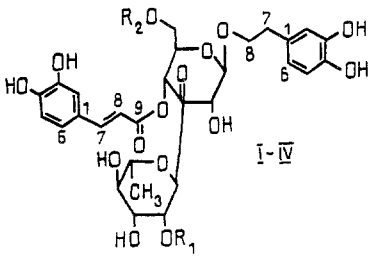
Together with poliumoside we have isolated for the first time from golden germander verbascoside and a new phenylpropanoid glycoside (teupolioside) for which the structure of 2-(3,4-dihydroxyphenyl)ethyl O-β-D-galactopyranosyl-(1→2)-O-α-L-rhamnopyranosyl-(1→3)-4-O-caffeoyl-β-D-glucopyranoside is proposed. A mixture of poliumoside and teupolioside revealed a moderate antibacterial activity.

The isolation of 3',4',5'-trihydroxy-6,7-dimethoxyflavone and of 4',5'-dihydroxy-6,7-dimethoxyflavone from the golden germander Teucrium polium L. has been reported previously [1]. Continuing an investigation of the chemical composition of this plant, which is widely used in Armenian folk medicine [2-4], we have studied its hydrophilic components.

By column chromatography (CC) on polyamide and silica gel of the polar part of a methanolic extract of the plant, three phenylpropanoid glycosides (I, II, and III) were isolated. On the basis of their IR, UV, and ^1H and ^{13}C NMR spectra, the mass spectra of their peracetates, a study of the products of acid hydrolysis and, in the case of glycoside (I), a comparison with an authentic sample, glycosides (I) and (II) were identified as the known phenylpropanoid glycosides verbascoside (I) [5] and poliumoside (II) [6]. Poliumoside (II) is characteristic for germanders of the Polium section [7], but this is the first time that verbascoside (I) has been found in golden germander.

On mild hydrolysis, glycoside (III), the most polar of the three isolated, formed galactose (according to PC and TLC), and on severe acid hydrolysis it yielded galactose, rhamnose, glucose, dihydroxyphenylethanol, and caffeic acid. The UV and IR spectra also indicated the presence of a caffeoyl fragment in glycoside (III).

Analysis of the ^1H NMR spectrum of glycoside (III) using the methods of double resonance and NOE enabled the correct assignment of the signals of the protons to be made (Table 1) and the sequence of linkage of the sugar residues to be determined. Glycoside (III) is, thus, a new phenylpropanoid glycoside, which we have provisionally named teupolioside, and is 2-(3,4-dihydroxyphenyl)ethyl O-β-D-galactopyranosyl-(1→2)-O-α-L-rhamnopyranosyl-(1(1→3)-4-O-caffeoyl-β-D-glucopyranoside (III).



I-IV

I. $R_1=R_2=H$
II. $R_1=H$, $R_2=$ α-L-rhamnopyranosyl
III. $R_1=$ β-D-galactopyranosyl, $R_2=H$

A study of the ^{13}C NMR spectrum of teupolioside (see the Experimental part) in comparison with those of the known phenylpropanoid glycosides purpureaside C (IV, R_1 = H; R_2= β-D-galactopyranosyl) [8], teucrioside (IV, R_1 = α-L-lyxopyranosyl, R_2 = H) [9], and lavandulifolioside (IV, R_1 = α-L-arabinopyranosyl, R_2 = H) [10] also led to structure (III) for teupolioside.

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TABLE 1. Chemical Shifts and Spin–Spin Coupling Constants of the Protons in the PMR Spectrum of Teupolioside (III) (Py-d_5–D_2O (19:1); 0 – TMS)*

Part of the molecule	H	δ, ppm	J, Hz
Caffeic acid fragment	2	7,25 d	$J_{2,6}$=2,0
	5	7,31 d	
	6	6,71 dd	$J_{5,6}$=8,4
	7	8,00 d	
	8	6,65 d	$J_{7,8}$=15,7
Aglycon moiety	2	7,69	$J_{2,6}$=2,0
	5	7,26 d	$J_{5,6}$=8,5
	6	7 16 dd	
	7	2,96 br.m	
	8	3,93 m; 4,27 m	J≈8
Glucosyl moiety	1	4,89 d	$J_{1,2}$=8,1
	2	3,95 dd	$J_{2,3}$=9,0
	3	3,99 m	$J_{3,4}$=9,0
	4	5,57 m	$J_{4,5}$=9,0
	5	3,95 m	
	6	— †	
Rhamnosyl moiety	1	5,37 d	$J_{1,2}$=1,5
	2	4,84 dd	$J_{2,3}$=3,5
	3	4,52 dd	$J_{3,4}$=9,5
	4	4,14 m	$J_{4,5}$=9,5
	5	4,32 q	
	6	1,53 d	$J_{5,6}$=6,2
Galactosyl moiety	1	5 17 d	$J_{1,2}$=8,0
	2	4,51 dd	$J_{2,3}$=9,9
	3	4,09 dd	$J_{3,4}$=3,5
	4	4,42 br.d	
	5	4,03 m	$J_{4,5}$<2
	6	—†	

*d) Doublet; m) multiplet; q) quartet; dd) doublet of doublets; br.d) broadened doublet.
†Signal undifferentiated.

We studied the antibacterial action of an evaporated methanolic extract of golden germander, the polar fraction of the methanolic extract, and also a mixture of poliumoside (II) and teupolioside (III) in in vitro experiments by the method of twofold serial dilutions in nutrient agar [11]. As the test culture we used Staphylococcus aureus (strains 209p, Smith and 25923), Flexner's bacillus (Shigella paradysenteriae) (6858 and 121), Friedländer's bacillus (Klebsiella pneumoniae), enterobacteria, and a proteus. All the samples tested showed the same activity: They retarded the growth of the dysentery bacillus in concentrations of 0.3-0.6 mg/ml, depending on the strain, and at higher doses (1.2-2.5 mg/ml) they suppressed the growth of the staphylococci 25925 and 209p, Friedländer's bacillus, and the enterobacteria, and in doses greater than 2.5 mg/ml they also retarded the growth of Pr. vulgaris.

EXPERIMENTAL

UV spectra were taken on a Specord UV-Vis instrument, IR spectra on a UR-20 instrument, ^1H NMR spectra on a Bruker WM-250 instrument, ^{13}C NMR spectra on a Bruker AM-300 instrument, and mass spectra on a MK-1320 spectrometer. Optical activities were determined on a Polarmat A instrument. Chromatography was conducted on type S paper (PC) in the following systems: 1) butanol–acetic acid–water (4:1:5); and 2) butanol–benzene–pyridine–water (5:1:3:3) and on Silufol UV-254 plates (TLC) in ethyl acetate–methanol–water (16:2:1) system. Sugars were detected on the chromatograms with benzidine reagent (0.5 g of benzidine, 20 ml of acetic acid, 80 ml of ethanol), and phenylpropanoid glycosides, caffeic acid, and 3,4-dihydroxyphenylethanol with a 1% aqueous solution of ferric chloride and with UV light. The peracetyl derivatives of the glycosides were obtained by the usual method – acetylation with a mixture of pyridine and acetic anhydride (1:2) at room temperature.

Isolation of Glycosides (I-III). The comminuted air-dried plant (2.0 kg) was exhaustively extracted with methanol. The methanolic extract was concentrated to a volume of 0.5 liter, diluted with 0.5 liter of water, filtered, and washed in turn with benzene (4 × 0.3 liter), ethyl acetate (5 × 0.2 liter), and chloroform–ethanol (3:1) (6 × 0.3 liter). The washed aqueous solution was chromatographed on a column containing 300 g of polyamide powder (Serva). The column was washed with 0.4 liter of water and the eluate was evaporated under reduced pressure to dryness, giving 4.0 g of a hydrophilic fraction which was transferred to a column containing 120 g of silica gel (KSK, 63-100 μm). Elution with ethyl acetate–methanol–water (90:7:3) led to the isolation of 0.19 g of verbascoside (I), R_f 0.40 (TLC). Ethyl acetate–methanol–water (16:2:1) eluted successively from the column 0.109 g of poliumoside (II), R_f 0.32 (TLC), and 0.278 g of teupolioside (III).

Teupolioside (III) – a yellowish amorphous substance with $[\alpha]_D^{22}$ –44.0° (c 1.18; ethanol).

UV spectrum (λ_{max}^{MeOH}, nm): 222 sh, 250 sh, 291, 333. IR spectrum (ν_{max}^{KBr}, cm^{-1}): 3400, 1740, 1665, 1635, 1550, 1475. 1400.

^{13}C NMR spectrum (in CD_3OD solution; 0 – TMS, δ, ppm): aglycon moiety – 131.62 (C-1), 117.16 (C-2), 146.1 (C-3), 144.7 (C-4), 116.54 (C-5), 121.29 (C-6), 72.14 (C-7), 36.48 (C-8); acid moiety – 127.67 (C-1), 114.70 (C-2), 147.4 (C-3), 149.85 (C-4), 115.27 (C-5), 123.18 (C-6), 147.96 (C-7), 116.32 (C-8), 167.24 (C-9); glucose fragment – 104.10 (C-1), 75.94 (C-2), 82.88 (C-3), 70.45 (C-4), 76.91 (C-5), 62.33 (C-6); rhamnosyl fragment – 102.35 (C-1), 82.88 (C-2), 74.84 (C-3), 74.14 (C-4), 70.45 (C-5), 18.39 (C-6); galactosyl fragment – 107.46 (C-1), 71.84 (C-2), 74.88 (C-3), 70.32 (C-4), 76.11 (C-5), 62.85 (C-6). For the ^1H NMR spectrum, see Table 1. When the anomeric proton of the galactosyl fragment (5.17 ppm) was preirradiated, the signals of the H-2, H-3, and H-5 protons of the galactosyl moiety and H-2 of the rhamnosyl moiety gave a response. When H-1 of the rhamnosyl fragment (6.37 ppm) was preirradiated, the signals of the H-2 proton of the rhamnosyl and the H-3 proton of the glucosyl fragments responded. When H-1 of the glucosyl fragment was preirradiated the signals of the H-2, H-3, and H-5 protons of the glucosyl and the H-1 proton of the aglycon moieties responded.

Peracetate, R_f 0.52 [TLC in the chloroform–methanol (19:1) system; UV light]; $[\alpha]_D^{25}$ –62.70° (c 2.7; chloroform).

Hydrolysis of Teupolioside (III). A solution of 85 mg of teupolioside (III) in 1.7 ml of a 0.1 N solution of hydrochloric acid was heated at 70°C for 1 h, cooled, and extracted with butanol (3 × 5 ml). The acid aqueous solution was neutralized with $BaCO_3$ and evaporated. PC with markers revealed a single spot with R_f 0.22 (system 1) and 0.28 (system 2) corresponding to galactose.

The butanolic extract was evaporated to dryness under reduced pressure. This gave 53 mg of a substance showing in TLC two spots with R_f 0.26 (teupolioside) and 0.40 (verbascoside). The substance was dissolved in 2 ml of 2 N hydrochloric acid. The solution was heated in the boiling water bath for 4 h and was treated as described above. In the acidic aqueous fraction of the hydrolysate galactose, glucose, and rhamnose were detected with the aid of PC (R_f values in system 1: 0.22, 0.24, and 0.38; and in system 2: 0.28, 0.33, 0.58, respectively), and in the butanolic fraction by the TLC method, substances with R_f values of 0.82 and 0.93 corresponding to caffeic acid and to dihydroxyphenylethanol.

LITERATURE CITED

1. G. B. Oganesyan and V. A. Mnatsakanyan, Arm. Khim. Zh., No. 1, 57 (1985).
2. A. Kh. Rollov, Wild-Growing Plants of the Caucasus, Their Distribution, Properties, and Use [in Russian], Tiflis (1908).
3. Medicinal Plants of Armenia and Medicinal Preparations Based on Them [in Russian], Izd. Akad. Nauk ArmSSR, Erevan (1949), p. 73.
4. S. Ya. Zolotnitskaya, Medicinal Resources of the Armenian Flora [in Russian], Izd. Akad. Nauk ArmSSR, Erevan, Vol. 2 (1965), p. 238.
5. É. Yu. Agababyan, L. S. Arutyunyan, V. A. Mnatsakanyan, É. Gach-Baitts, and L. Radich, Arm. Khim. Zh., No. 11, 709 (1985).
6. C. Andary, R. Wylde, A. Heitz, J. P. Rascol, J. L. Roussel, and C. Lafitte, Phytochemistry, 24, No. 2, 362 (1985).

7. C. Andary, J. P. Rascol, S. Peuch, J. L. Roussel, and G. Privat, Can. J. Bot., 66, No. 12, 1007 (1988).
8. H. Sasaki, H. Nishimura, M. M. Chin (Zhengxiong Chen), and H. Mitsunashi, Phytochemistry, 28, No. 23, 875 (1989).
9. G. A. Cross, M. F. Lahloub, C. Anklin, H. R. Schulten, and O. Sticher, Phytochemistry, 27, No. 5, 1459 (1988).
10. A. A. Basaran, J. Calis, C. Ankin, S. Nishibe, and O. Sticher, Helv. Chim. Acta, 71, No. 6, 1483 (1988).
11. V. A. Bandyukova, V. S. Cherevatnyi, T. N. Vashchenko, and N. V. Postnikova, Rast. Res., No. 4, 607 (1987).

ISOLATION OF OXYGEN-CONTAINING MONOTERPENOIDS OF ESSENTIAL OILS BY PREPARATIVE ADSORPTION CHROMATOGRAPHY WITH GRADIENT ELUTION

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A simple and effective procedure for the comparative chromatography of 1-2 ml of essential oils on a 40 × 250 mm column of silica gel with gradient elution by hexane–diethyl ether has been developed. A simple system of preparing the mobile phase permits the creation of a continuous gradient during chromatography. The efficacy of the procedure has been shown taking as an example the isolation of fenchone from fennel essential oil.

Oxygen-containing monoterpenoids possess a wide spectrum of action and a higher biological activity and lower toxicity than monoterpene hydrocarbons and sesquiterpenoids [1]. The isolation of these substances from essential oils (EOs) in fairly large amounts (more than 0.1 g) is frequently necessary for their identification (by the methods of UV, IR, NMR, and mass spectrometry), the study of their biological activity, and their use as comparative substances for the identification and the quantitative determination of the components of EOs by chromatographic methods.

Our aim was the development of an effective procedure for the preparative chromatographic separation of oxygen-containing monoterpenoids of EOs that would permit the isolation of a substance with a purity of not less than 95% in amounts (more than 0.1 g) sufficient for use as comparison substances for GLC analysis.

The simplest and most effective method of fractionating terpenoids is their adsorption chromatography on silica gel [2]. In the separation on a silica gel column of a mixture of substances with close polarities, such as monoterpene hydrocarbons [3], isocratic elution with pentane is used. The collection of a number of small fractions leads to the separation or enrichment of certain substances in different fractions. To separate mixtures of substances differing appreciably in polarity, the method of gradient elution is used [4], this consisting in the fact that during chromatography the eluting power of the mobile phase is gradually increased. This is achieved by the successive use of a number of solvents ("eluotropic series") or mixtures of two solvents with gradual increase in the proportion of the more polar component. A continuous gradient changes the values of the partition coefficients of substances between the mobile and stationary phases. The increasing eluting power of the mobile phase compresses the band of the sample, as the result of which the peaks become narrower and the "tail" of a peak decreases even with large loads on the column.

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