COUMARIN GLYCOSIDES OF *Haplophyllum perforatum*

M. P. Yuldashev, E. Kh. Batirov, and V. M. Malikov

UDC 577.15/17:582.89

From the epigeal part of *Haplophyllum perforatum* we have isolated the coumarins scopoletin (I), scopoletin 7-O-β-D-glucopyranoside (II) and the new coumarin glycoside haploperoside A (III), mp 212-213°C, $[\alpha]_D^{22}$ −37° (c 0.24, CH_3OH). The acid hydrolysis of (III) formed (I) and the monosaccharides D-glucose and L-rhamnose. Partial hydrolysis of (III) with 10% acetic acid led to (II) and L-rhamnose. On the basis of the results of a study of UV, IR, and PMR spectra, and also periodate oxidation and polarimetric analysis the structure of 6-methoxy-7-[O-α-L-rhamnopyranosyl-(2→1)-β-D-glucopyranosyloxy]coumarin has been established for (III). Details of the IR, UV, PMR, and mass spectra are given.

Many species of plants of the family *Rutaceae* contain coumarins [1]. A number of alkaloids has previously been isolated from *Haplophyllum perforatum*, which belongs to this family [2]. By qualitative reactions, we also detected the presence of coumarins and flavonoids in it. On studying the chemical composition of the epigeal part of *H. perforatum* collected in the flowering period (environs of Mount Alim-Tau, Chimkent province, KazSSR) we isolated four compounds of coumarin nature. In the present paper we give the results of an investigation of these substances.

From the results of IR and UV spectroscopy, substance (I) was assigned to the group of 6,7-disubstituted coumarins. On the basis of the results of a study of the mass spectrum (M^+ 192), and also by direct comparison with an authentic sample, it was identified as scopoletin [3].

According to the results of IR and PMR spectroscopy, substance (II) with the composition $C_{16}H_{18}O_9$ was a glycoside. It was found by acid hydrolysis and enzymatic hydrolysis with emulsin that compound (II) contained an aglycone identical with (I) and glucose in equimolar proportions. A study of spectral characteristics and the results of a polarimetric analysis showed that (II) was scopoletin 7-O-β-glucopyranoside (scopolin) [3, 4]. Glycoside (III) proved to be new, and we have called it haploperoside A. The UV spectrum of (III) (λ_{max}, nm, 230, 252 sh, 281, 344) is characteristic for 6,7-disubstituted coumarin derivatives [5].

The IR spectrum of haploperoside A showed the absorption bands of hydroxy groups (3200-3600 cm^{-1}), of an α-pyrone carbonyl (1710 cm^{-1}), of a methoxy group (2930 cm^{-1}), and of the C–O vibrations of glycosides (1020-1120 cm^{-1}). On acid hydrolysis, (III) formed scopoletin and the sugars glucose and rhamnose.

By acetylating (III) with acetic anhydride in the presence of pyridine we obtained a hexacetate with mp 84-85°C. Consequently, haploperoside A is a bioside. This was confirmed by the results of a study of the PMR spectrum of (III) in deuteropyridine, which showed the signals of the anomeric protons of glucose (5.48 ppm, J = 6.5 Hz) and of rhamnose (5.26 ppm, J = 2 Hz), of the CH_3 group of rhamnose (1.45 ppm, J = 5 Hz), of 10 protons of the carbohydrate moiety in the 3.70-4.65 ppm interval, of a methoxy group (3.57 ppm), and of the protons of a coumarin nucleus (Fig. 1).

The partial hydrolysis of (III) with 10% acetic acid led to the formation of rhamnose and of a glycoside identical with (II). Consequently, rhamnose is the terminal sugar and the glucose is attached directly to the aglycone. To determine the position of attachment of the rhamnose to the glucose, we used the periodate oxidation of haploperoside A followed

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenni, No. 2, pp. 168-172, March-April, 1980. Original article submitted November 16, 1979.

0009-3130/80/1602-0125$07.50 © 1980 Plenum Publishing Corporation

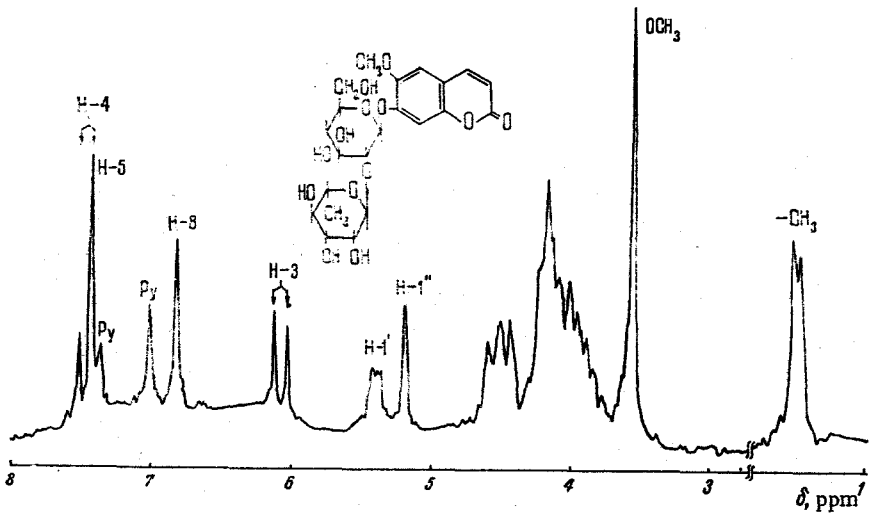

Fig. 1. PMR spectrum of haploperoside A in deuteropyridine.

by further oxidation with nitric acid [6]. The destruction of both sugars on oxidation with periodic acid showed the absence of a 1→3 bond [7], and the absence of tartaric acid from the products of complete oxidation excluded a 1→4 bond between the sugars. The resistance of the glycoside to alkaline hydrolysis gave grounds for assuming that the rhamnose was attached to the glucose by a 1 → 2 bond [8]. The sizes of the oxide rings at the configurations of the glycosidic bonds were determined by comparing molecular rotations and by PMR spectroscopy [9-11]. It was established that the rhamnose is linked to the glucose by an α-glycosidic bond and both sugars have the pyranose form of the oxide rings. The latter agrees well with the rate of acid hydrolysis [12].

Thus, the structure of haploperoside A can be represented as 6-methoxy-7-[-O-αL-rhamnopyranosyl-(1 →2)-β-D-glucopyranosyloxy]coumarin.

As can be seen, haploperoside A is a bioside the carbohydrate moiety of which consists of neohesperidose. Neohesperidosides of some flavonoids have been found previously in plants of the genus *Citrus*, which also belongs to the family Rutaceae [13, 14].

EXPERIMENTAL

The spectral characteristics were obtained on the following instruments: a UR-20 (tablets with KBr, IR), a Hitachi EPS-3T spectrophotometer (UV, in ethanol), a JNM-4H-100/100 MHz, internal standard, HMDS (PMR), and a MKh-1303 at 40 V (mass spectrum). Melting points were determined in an instrument with an electric heater, angles of rotation were determined on a J-20 spectropolarimeter.

Chromatographic monitoring was performed by TLC (Silufol UV-254) in the following systems: 1) chloroform–methanol (8:2); 2) chloroform–petroleum ether (1:1); and 3) toluene–ethanol–ethyl acetate (1:1:1) and by PC in the butan-1-ol–pyridine–water (6:4:3) system. To separate the coumarins on a column, we used KSK and Woelm (GFR) silica gels.

Isolation. The air-dry comminuted raw material (3 kg) was extracted five times with ethanol, and the extract was concentrated in vacuum. The concentrated extract was diluted with water (1:1), the resulting precipitate of chlorophyll was filtered off, and the filtrate was extracted successively with chloroform, ether, ethyl acetate, and butanol.

The ethyl acetate extract (20.0 g) was subjected to chromatographic separation on a column of silica gel in the petroleum ether–chloroform system. At a composition of the mixture of 1:1, scopoletin was eluted from the column, and at a composition of 25:75 substance (II) was eluted.

Part of the butanolic extract (35.0 g) was separated by the method described above in the chloroform–propanol system. At a composition of the mixture of 8:2 (fractions 42-52) substance (II) was isolated, and then (fractions 62-70) substance (III).

Scopoletin 7-O-β-glucopyranoside (II), mp 207-209°C (melting point of the tetraacetate 157-159°C), $[\alpha]_D^{22}$ −6.5° (c 1.13; dimethylformamide), R_f 0.69 (system 1); IR spectrum (cm^{-1}): 3250-3600 (OH group), 2930 (OCH_3), 1735 (α-pyrone C=O), 1610, 1596, 1518 (C=C bonds in rings), 1082, 1054, 1012 (C–O vibrations of glycopyranosides).

UV spectrum, nm, $\lambda_{max}^{ethanol}$: 229, 280, 339 (log ε 3.87, 3.28, 2.78).

PMR spectrum in [D]pyridine (ppm): 7.57 (d, 10 Hz, H-4), 7.35 (s, H-5), 6.91 (s, H-8) 6.21 (d, 10 Hz, H-3), 4.91 (br.s, H-1'), 3.95-4.45 (m, 6 H of glucose), 3.63 (s, OCH_3).

6-Methoxy-7-[O-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyloxy]coumarin (III), white crystalline substance soluble in ethanol and water, mp 212-213°C, $[\alpha]_D^{22}$ −37° (c 0.24, CH_3OH), R_f 0.49 (I). IR spectrum (cm^{-1}): 3200-3600 (OH group), 2930 (OCH_3), 1710 (C=O of an α-pyrone), 1617, 1570, 1518 (C=C bonds in rings), 1459, 1429, 1390, 1281, 1254, 1145, 1120-1015 (C–O vibrations of glycosides), 940, 918, 870, 835, 816, 766, 755.

UV spectrum, $\lambda_{max}^{ethanol}$, nm: 230, 252 sh., 260 sh., 281, 344 (log ε 4.16, 3.56, 3.64, 3.80, respectively).

PMR spectrum in [D] pyridine (ppm): 7.58 (d, 10 Hz, H-4), 7.54 (s, H-5), 6.93 (s, H-8), 6.21 (d, 10 Hz, H-3), 5.48 (m, 6.5 Hz, H-1'), 5.26 (br.s, H-1"), 3.70-4.65 (m, 10 H of the sugar moiety), 3.57 (s, $-OCH_3$), 1.45 (d, 5 Hz, CH_3 group of rhamnose).

Acid Hydrolysis of (III). A solution of 24 mg of (III) in 3 ml of 5% sulfuric acid was treated in the boiling water bath for 2 h. The cooled mixture was diluted with water and extracted with chloroform. The chloroform extracts were washed with water, dried with anhydrous sodium sulfate, filtered, and distilled. The residue was recrystallized from ethanol. This gave 7 mg of an aglycone which was identified as scopeletin by IR and mass spectroscopy (M^+ 192) and comparative chromatography. When the aqueous residue was neutralized with AN-1 anion-exchange resin and evaporated, D-glucose and L-rhamnose were detected by PC with markers.

Partial Hydrolysis of (III). A mixture of 30 mg of (III) and 6 ml of 10% acetic acid was heated on the water bath. The course of the reaction was followed by TLC (system 1).

After 6 h, the reaction mixture was neutralized with 10% $NaHCO_3$ solution and was evaporated in vacuum, and L-rhamnose was detected by the PC method with markers. The dried residue was chromatographed on a column of silica gel in the chloroform–ethanol (4:1) system. This gave 9 mg of monoglucoside, which was identified as substance (II) by direct comparison.

Acetylation of (III). A mixture of 44 mg of compound (III), 0.5 ml of pyridine, and 3 ml of acetic anhydride was kept at room temperature for 48 h. Then it was poured into ice water, and the resulting precipitate was filtered off and washed with water. After recrystallization from ethanol, 40 mg of the hexacetate with mp 84-85°C was obtained.

Periodate Oxidation of Haploperoside A. A solution of 50 mg of (III) in 10 ml of 50% ethanol was treated with 0.06 g of periodic acid and was left in a dark place at room temperature for 48 h. Then the mixture was treated with a small amount of ethylene glycol and was neutralized on AV-10G anion-exchange resin (OH form) and it was evaporated to 3 ml. Of this concentrated solution, 1 ml was hydrolyzed with 5% hydrochloric acid, neutralized on anion-exchange resin, and chromatographed on paper with glucose.

Another part of the solution was mixed with 1 ml of 20% nitric acid and the mixture was evaporated with the addition of small amounts of water until the oxides of nitrogen had been completely eliminated. The residue was chromatographed on paper with tartaric acid. No glucose or tartaric acid were detected.

Action of Alkali on (III). A solution of 15 mg of (III) in 5 ml of 0.5% KOH solution was heated in the boiling water bath for 3 h. Then the mixture was neutralized with 2% hydrochloric acid and was evaporated in vacuum. On chromatography (TLC, system 1) it was found that the initial substance had not changed.

SUMMARY

The presence of coumarin glycosides in *Haplophyllum perforatum* has been established. From the epigeal part of this plant we have isolated scopoletin, scopolin, and a new coumarin glycoside — haploperoside A — for which the structure of 6-methoxy-7-[O-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyloxy]coumarin is suggested.

LITERATURE CITED

1. M. G. Pimenov, List of Plants that are Sources of Coumarin Compounds [in Russian], Leningrad (1971), p. 43.
2. S. Yu. Yunusov, Alkaloids [in Russian], Tashkent (1974), p. 181.
3. G. A. Kuznetsova, Natural Coumarins and Furocoumarins [in Russian], Leningrad (1967), p. 74.
4. L. I. Kosheleva and G. K. Nikonov, Farmatsiya, No. 4, 78 (1969).
5. M. E. Perel'son, Yu. N. Sheinker, and A. A. Savina, The Spectra and Structures of Coumarins, Chromones, and Xanthones [in Russian], Moscow (1975), p. 9.
6. T. A. Sergienko, L. S. Kazarnovskii, and V. I. Litvinenko, Khim. Prir. Soedin., 166 (1966).
7. B. N. Stepanenko, The Chemistry and Biochemistry of Carbohydrates (Polysaccharides) [in Russian], Moscow (1978), p. 17.
8. V. I. Litvinenko and V. A. Makarov, Khim. Prir. Soedin., 366 (1969).
9. T. A. Sergienko, L. S. Kazarnovskii, and V. I. Litvinenko, Farmatsiya, 34 (1967).
10. B. N. Stepanenko, The Chemistry and Biochemistry of Carbohydrates (Monosaccharides) [in Russian], Moscow (1977), p. 90.
11. T. J. Mabry, K. R. Markham, and M. B. Thomas, The Systematic Identification of Flavonoids, Springer, New York (1970), p. 268.
12. V. N. Spiridonov, I. P. Kovalev, and A. P. Prokopenko, Khim. Prir. Soedin., 5 (1969).
13. R. M. Horowitz and B. Gentili, Tetrahedron, 19, 773 (1963).
14. J. B. Harborne and T. J. Mabry, The Flavonoids, Chapman and Hall, London (1975), p. 398.

FLAVONOIDS OF *Stachys spectabilis*

A. I. Derkach, N. F. Komissarenko,
V. G. Gordienko, I. P. Sheremet,
I. P. Kovalev, and D. A. Pakaln

UDC 547.918

From the epigeal part of *Stachys spectabilis* we have isolated three flavonoids: the known stachyflaside and isostachyflaside, and the new spectabiflaside (I). On the basis of chemical transformations and an analysis of spectral characteristics, for (I) we propose the structure of 4',5,8-trihydroxy-7-[O-β-D-mannopyranosyl-(1 → 2)-β-D-glucopyranosyloxy]-3'-methoxyflavone (I).

Continuing an investigation of plants of the genus *Stachys* L., we have studied the flavonoid composition of *Stachys spectabilis* Choisy et DC, which is widely distributed in the subalpine zone of Transcaucasia. For the investigation we used raw material collected in the flowering period in the region of Aragats, Armenian SSR.

The flavonoids were isolated by a method described previously [1]. As a result, three substances of flavonoid nature were obtained. Two of them were identified as stachyflaside [2] and isostachyflaside [3], while the third substance was a new compound, which we have called spectabiflaside.

On a paper chromatogram, spectabiflaside was detected in the form of a light brown spot with R_f 0.31 in system 3.

To establish the structure of spectabiflaside we obtained the PMR spectra of the glycoside and of its aglycone in DMSO-D_6 and also of their acetyl and trimethylsilyl derivatives (in $CDCl_3$ and in CCl_4, respectively). The UV spectra with ionizing additives were also obtained [4]. Hydrolysis was carried out with 5% sulfuric acid, as a result of which we iso-

Kharkov Scientific-Research Institute of Pharmaceutical Chemistry. Ukrainian Zonal Experimental Station for Medical Plants of VILR, Lubny. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 172-174, March-April, 1980. Original article submitted September 14, 1979.

 0009-3130/80/1602-0128$07.50 © 1980 Plenum Publishing Corporation