4. M. E. Stansby, World Rev. Nutr. Diet, 11, 46 (1969).
5. M. E. Stansby, J. Am. Diet Assoc., 63, 625 (1973).
6. K. Vuorela, I. K. Kaitaranta, and R. R. Linko, J. Sci. Food Agric., 30, 921 (1979).
7. I. K. Kaitaranta, J. Sci. Food. Agric., 31, 1303 (1980).
8. V. I. Lizenko, U. S. Sidarov, and O. I. Potapova, J. Icthyol., 13, 253 (1973).
9. E. L. Bligh and W. I. Dyer, Can. J. Biochem. Physiol., 37, No. 8, 911 (1958).
10. S. A. Aristarkhova, E. B. Buralkova, and N. G. Khrapova, Biofizika, 4, 688 (1974).
11. F. M. Rzhavskaya, T. A. Dubrovskaya, and L. V. Pravdina, Trudy VNIRO, 95, 111 (1974).
12. F. M. Rzhavskaya, The Fats of Fish and Marine Mammals [in Russian], Pishchevaya Prom-st' (1976), p. 467.
13. M. Kates, Techniques of Lipidology, North-Holland, Amsterdam/American Elsevier, New York (1972).
14. A. J. Gordon and R. A. Ford, The Chemist's Companion, Wiley-Interscience, New York (1975).
15. P. R. Genter and A. Haasemann, Fette, Seifen, Anstrichmittel, 81, No. 9, 357 (1979).
16. M. Alter and T. Guteinger, Riv. Ital. Sostanze Grasse, 59, No. 1, 14 (1982).

COUMARIN GLYCOSIDES OF *Haplophyllum perforatum*. STRUCTURES OF HAPLOPEROSIDES C, D, AND E

M. P. Yuldashev, É. Kh. Batirov,
A. D. Bdovin, V. M. Malikov, and M. R. Yagudaev

UDC 547.15/17:582.89

New coumarin glycosides — haploperosides C, D, and E — have been isolated from the epigeal part of the *Haplophyllum perforatum* (MB) Kar et Kir. On the basis of chemical transformations and spectral characteristics, haploperoside D has the structure of 6-methoxy-7-[O-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyloxy]-2H-benzopyran-2-one, and haploperoside C that of 6-methoxy-7-[O-α-L-rhamnopyranosyl-(1 → 6)-(2-O-acetyl-β-D-glucopyranosyloxy)]-2H-benzopyran-2-one. The structure of haploperoside E has been established as 7-[O-α-L-rhamnopyranosyl-(1 → 2)-O-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyloxy]-2H-benzopyran-2-one. The structures of haploperosides A and B have been refined. An assignment has been made of the carbon signals in the ^{13}C NMR spectra of haploperosides A, D, C, and E.

We have previously reported the isolation of two coumarin glycosides from *Haplophyllum perforatum* [1-3]. Continuing this investigation we have isolated another three glycosides, which we have called haploperosides C, D, and E. In the present paper we give proofs of their structures.

The UV spectrum of haploperoside D (I) is characteristic for 6,7-di-O-substituted coumarins and is similar to the spectra of scopolin and of haploperoside A [1]. It was established by the GLC method that the molecule of (I) contained D-glucose and L-rhamnose residues in a ratio of 1:1. The acid hydrolysis of (I) gave, in addition to the monosaccharides mentioned above, an aglycone identified as scopoletin. The acetylation of haploperoside D led to a hexaacetate with the composition $C_{34}H_{40}O_{19}$, M^+ 752. Consequently, haploperoside D is a scopoletin bioside.

The mass spectrum of the acetate of (I) contained strong peaks of ions with m/z 273, 213, and 153, showing that in the haplopenoside D molecule the L-rhamnose is the terminal sugar residue [4]. This was confirmed by the production, on partial hydrolysis, of a monoglucoside which was identified as scopoletin 7-O-β-D-glucopyranoside (scopolin) [1]. To determine the structure of the carbohydrate chain we performed the Hakomori methylation of glycoside (I) [5]. In a hydrolysate of the methylation product we identified by GLC

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 27-35, January-February, 1985. Original article submitted March 27, 1984.

0009-3130/85/2101-0025$09.50

2,3,4-tri-O-methyl-L-rhamnose and 3,4,6-tri-O-methyl-D-glucose [6]. Thus, the terminal L-rhamnose sugar residue was attached to the D-glucose residue by a 1 → 2 bond.

In the PMR spectrum of haploperoside D, the anomeric protons of the glucose and the rhamnose resonated at 5.54 and 4.59 ppm in the form of doublets with spin—spin coupling constants ^3J = 8 and 2 Hz, respectively. This shows that C1 conformation of the glucopyranose ring and the 1C conformation of the rhamnopyranose ring and, consequently, the β-configuration of the glycosidic center of the D-glucose residue and the α-configuration of the L-rhamnose residue [7, 8].

Thus, haploperoside D has the structure of 6-methoxy-7-[O-α-L-rhamnopyranosyl-(1 → 2)-β-B-glucopyranosyloxy]-2H-benzopyran-2-one (I).

We had previously proposed a similar structure for haploperoside A [1], and this made it necessary to reconsider the structure of the latter. Haploperoside A and D possibly differ by the position of attachment of the rhamnose residue to the glucose residue. For an unambiguous answer to this question, haploperoside A (II) was also subjected to Hakomori methylation. From a hydrolysate of the methylation products we isolated and identified by the TLC and GLC methods 2,3,4-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-L-rhamnose [6]. Consequently, in the (II) molecule the L-rhamnose residue by a 1 → 6 bond and haploperoside A has the structure of 6-methoxy-7-[O-α-L-rhamnopyranosyl)-(1 → 6)-β-D-glucopyranosyloxy]-2H-benzopyran-2-one (II).

We have previously effected the passage from haploperoside B to (II) [2] and therefore haploperoside B has the structure of 7-[O-(4"-O-acetyl-α-L-rhamnopyranosyl)-(1 → 6)-β-D-glucopyranosyloxy]-6-methoxy-2H-benzopyran-2-one (III). The position of the acetyl group at C-4" of the L-rhamnose residue had been established previously by the INDOR method [3].

The structures of glycosides (I) and (II) were also confirmed by a study of the ^{13}C NMR spectra obtained under the conditions of complete and incomplete (off-resonance) decoupling from protons. In the spectra of haploperosides A and D in the 102.8-160.5 ppm region nine signals appeared in each case from sp^2-hybridized carbon atoms of coumarin nucleus and 12 signals in each case from sp^3-hybridized carbon atoms of the sugar moiety (δ 17.7-100.2 ppm). In the assignment of the signals of the carbon atoms in the ^{13}C NMR spectra of (I) and (II) we made use of literature information on the CSs of the carbon atoms for 7-β-D-glucopyranosyloxycoumarin [9] taking into account the influence of methoxylation in the C-6 position [10] and the contribution of glycosylation to the values of the chemical shifts [11, 12], and also making a comparison with the characteristics of the spectra of rhamnoglucosides [13-15]. The results of the assignment of the ^{13}C spectra are given in Table 1. In the spectrum of (II), the C-6' signal appears at δ 65.8 ppm, which confirms the 1 → 6 order of the bond between the monosaccharide residues [14]. The upfield shift of the signal of the anomeric carbon atom of the D-glucose residue of (I) as compared with that of haploperoside A is evidence in favor of the 1 → 2 order of the bond between the rhamnose and glucose residues in the molecule of haploperoside D [11, 12].

On the basis of an analysis of its UV and PMR spectra, haploperoside C (IV) was also assigned to the coumarin glycosides. In fact, on acid hydrolysis (IV) was split with the formation of scopoletin, D-glucose, and L-rhamnose. The presence of the absorption band of an ester carbonyl at 1749 cm^{-1} in the IR spectrum and of a three-proton singlet at 1.98 ppm in the PMR spectrum indicated that haploperoside C was an acylated coumarin glycoside and contained one acetate group.

The alkaline hydrolysis of (IV) with a 0.5% solution of caustic soda led to the formation of a glycoside which was identified as haploperoside A. The acetylation of (IV) gave an acetyl derivative which similarly proved to be identical with the hexacetate of haploperoside A. Consequently, (IV) was a natural monoacetyl derivative of haploperoside A.

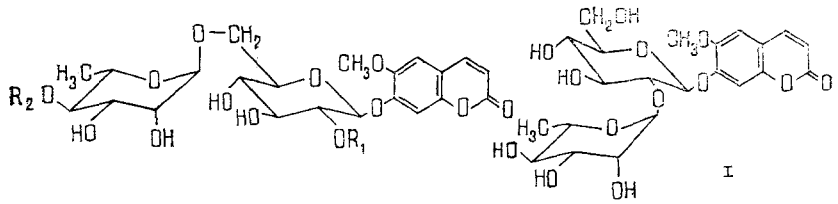
In the PMR spectrum of glycoside (IV), the proton geminal to the ester function resonated at 5.62 ppm in the form of a triplet with $^3J_1 = {}^3J_2$ = 9 Hz. In the same spectrum, the signals of the anomeric protons of the D-glucose and L-rhamnose residue appeared at 5.31 ppm (d, ^3J = 9 Hz) and 5.18 ppm (br.s), respectively. The position of the acetyl group cannot be determined from the multiplicity and spin—spin coupling constants of the geminal proton, although it is possible to exclude the C-2" and C-3" positions of the L-rhamnose residue. On the superposition of an additional radiofrequency field with ν = 531 Hz (H-1'), the triplet at 5.62 ppm was converted into a doublet with ^3J = 9 Hz. Conversely, irradiation with a frequency ν = 562 Hz (H-2') led to the conversion of the doublet signal of the anomeric proton of the glucose residue

TABLE 1. Details of the ^{13}C NMR Spectra of the Coumarin Glycosides in DMSO-d_6 (δ, 0 — TMS)

Carbon atom	Haploperoside A	Haploperoside C	Haploperoside D	Haploperoside E	Multiplicity
2	160,5	159,3	158,9	160,1	*s*
3	113,0	112,9	112,4	112,6	*d*
4	144,0	143,0	142,7	143,6	*d*
5	109,6	109,9	109,1	108,8	*d*
6	145,8	145,3	145,4	145,5	*s*
7	148,5	147,4	147,6	147,8	*s*
8	103,1	104,1	102,8	102,8	*d*
9	149,6	148,3	148,5	148,6	*s*
10	112,2	112,5	111,6	112,0	*s*
1′	100,2	98,1	97,7	97,5	*d*
2′	72,9	73,2	77,1	77,4	*d*
3′	76,8	72,7	75,5	75,2	*d*
4′	69,5	69,4	69,8	69,8	*d*
5′	75,4	75,1	76,5	76,5	*d*
6′	65,8	65,4	60,5	65,9	*t*
1″	99,8	99,7	99,1	99,5	*d*
2″	70,2	70,0	70,2	70,2	*d*
3″	70,5	70,2	70,2	70,2	*d*
4″	71,7	71,3	71,7	71,7	*d*
5″	68,1	67,8	67,8	68,1	*d*
6″	17,7	17,8	17,8	18,0	*q*
1‴				99,9	*d*
2‴				70,2	—
3‴				70,2	—
4‴				71,2	—
5‴				69,8	—
6‴				18,3	*q*
$CH_3C=O$	—	167,7	—	—	*s*
CH_3CO	—	20,6	—	—	*q*
CH_3O—	56,0	56,2	55,9	55,9	*q*

into a singlet. Thus, the triplet at 5.62 ppm was due to the H-2' proton and, consequently, the acetyl group occupies the C-2' position of the D-glucose residue. A comparative analysis of the chemical shifts of the carbon atoms of the glucopyranose moieties in the ^{13}C spectra of haploperosides A and C, and also of dauroside B [16] confirmed the conclusion concerning the position of the acetyl group. The C-2' signal underwent a slight additional downfield shift by 0.3 ppm on passing from haploperoside A to haploperoside C, while the signals of the anomeric carbon of the glucose residue (C-1') and of C-3' underwent diamagnetic shifts by −2.1 and −3.8 ppm, respectively, at the same time. This is possible when the O-acetyl group is present at the C-2' carbon atom [14, 17, 18].

Thus, the facts given above determine haploperoside C as 6-methoxy-7-[0-α-L-rhamnopyranosyl-(1 → 6)-(2-O-acetyl-β-D-glucopyranosyloxy)]-2H-benzopyran-2-one (IV).

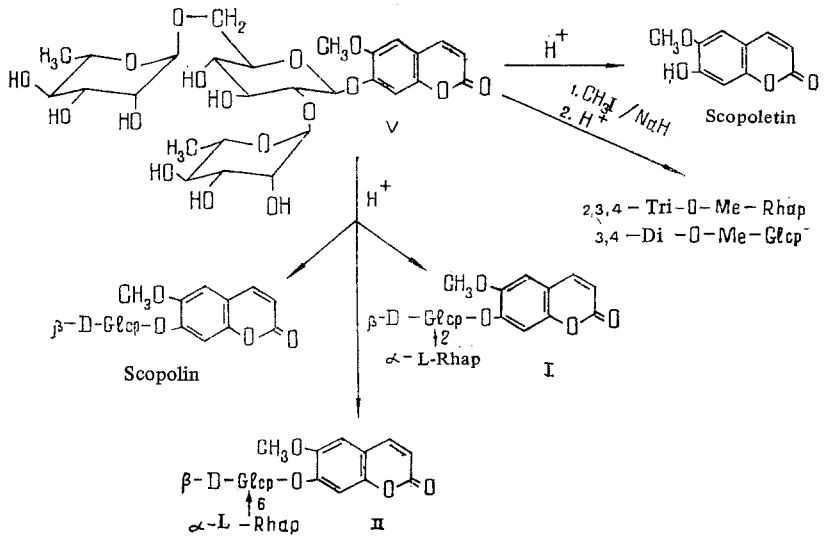


I

II. $R_1=R_2=H$
III. $R_1=H$, $R_2=COCH_3$
IV. $R_1=COCH_3$, $R_2=H$

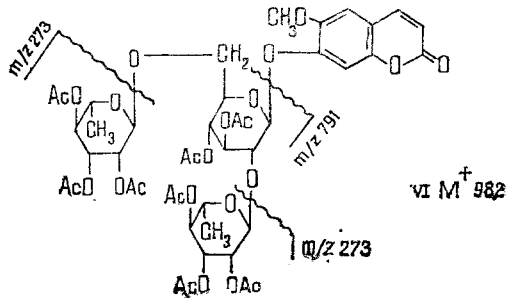
Haploperoside E (V) is the most polar glycoside from the plant studied. Its UV spectrum is almost identical with those of (I) and (II). The hydrolysis of glycoside (V) in 5% sulfuric acid yielded scopoletin, D-glucose, and L-rhamnose. It was established by the GLC method that the ratio of the D-glucose and L-rhamnose residues in the molecule of (V) was 1:2. This was confirmed in a study of the mass spectrum of the peracetate of (V) — (VI) — which showed the peak of the molecular ion with m/z 982, and also the peaks of ions with m/z 791, 561, 519, and others [19]. The presence in the PMR spectrum of the signals of the protons from two methyl groups of rhamnose at 1.48 ppm (3 H, m) and 1.67 (3 H, d, 6 Hz) also showed the presence of two L-rhamnose residues in (V).

Haploperoside E was methylated by Hakomori's method, and the resulting product was subjected to acid hydrolysis. Analysis of the carbohydrate fractions of the hydrolysate with the aid of GLC and TLC showed the presence in them of 2,3,4-tri-O-methyl-L-rhamnose and a dimethyl ether of D-glucose. The formation of 2,3,4-tri-O-methyl-L-rhamnose proved that the two L-rhamnose residues were terminal and the D-glucose residue was a center of branching. Confirmation of this was given by the results of the partial hydrolysis of glycoside (V). This gave three progenins, which were identified by direct comparison on scopolin and haploperosides A and D.



Consequently, haploperoside E has structure (V) and is 6-methoxy-7-{[O-α-L-rhamnopyranosyl-(1 → 2)][O-α-L-rhamnopyranosyl-(1 →6)]-β-D-glucopyranosyloxy -2H-benzopyran-2-one.

In the ^{13}C NMR spectrum of haploperoside E, as a result of the multiple superposition of signals, 11 signals of carbon atoms of the sugar moiety are observed in the 18.0-99.9 ppm region, which complicates their assignment. The same circumstances prevented the determination of the multiplicities of the signals of the C-2′, C-3′, C-4′, C-5′, C-2″, C-3″, C-4″, C-5″, C-2‴, C-3‴, C-4‴ , and C-5′′′′ carbon atoms in the off-resonance spectra. Consequently, the assignment of the signals to the above-mentioned carbon atoms were made from the ^{13}C NMR spectra with complete decoupling (see Table 1).



Haploperoside E is the first coumarin glycoside containing three monosaccharide residues. The flavonoids manghaslin and clitorin, having sugar moieties analogous to that of haploperoside E, have been isolated from the leaves of *Cerbera mangas* L. by Japanese workers [19].

EXPERIMENTAL

For general remarks, see [1]. The following solvent systems were used: 1) chloroform–methanol (8:2); 2) butanol–pyridine–water (6:4:3); 3) chloroform–methanol (50:1); and 4) chloroform–acetone (15:2). The sugars and their derivatives were detected on PC and TLC by spraying with o-toluidine salicylate followed by heating at 90-100°C for 3-5 min.

Gas–liquid chromatography was performed on Chrom-5 and Tsvet-101 chromatograms. The monosaccharides were analyzed in the form of trimethylsilyl derivatives of methyl glycosides and aldonitrile acetates. A column (3.7 m × 3 mm) containing Chromaton N-AW impregnated with 5% of the silicone phase SE-30 was used. The thermostat temperature as 190°C, and the carrier gas was helium at the rate of 45 ml/min.

The methyl glycosides, which were obtained by boiling the methyl esters of the monosaccharides in 6% methanolic HCl solution (4 h) were chromatographed on a column (1.2 m × 3 mm) containing 20% of poly(butane-1,4-diyl succinate) on Celite. The thermostat temperature was 160°C and the rate of flow of helium 30 ml/min. The retention times (T_{rel}) for the methylated methyl glycosides were calculated in relation to the retention times of 2,3,4,6-tetra-O-methyl-β-D-glucopyranose.

^{13}C NMR spectra were taken in DMSO-d_6 on a Varian XL-100, 25.2 MHz, instrument (δ, ppm; 0 – TMS).

Isolation of the Glycosides. The air-dry comminuted epigeal part of *Haplophyllum perforatum* (M. B.) Kar. et Kir. (7.3 kg) collected on May 5, 1980, on the foothills of the Alim-Tau (Chimkent province) in the budding stage were extracted seven times with ethanol at room temperature. The extract was evaporated in vacuum to 2 liters and was diluted with water (1:1), and the resulting precipitate was filtered off. The filtrate was extracted successively with chloroform, with ethyl acetate, and with butanol. The butanolic solution, after the solvent had been distilled off, yielded 110 g of total extractive substances. Since the aqueous solution after extraction continued to exhibit intense blue fluoresence in UV light, it was evaporated in vacuum, to give 250 g of an oily residue. The compositions of the coumarins from the butanolic extract and the residue from the aqueous solution were identical according to the results of TLC, and they were therefore combined and chromatographed on a column of silica gel using the chloroform–methanol (97:3–80:20) system. Individual fractions were rechromatographed on silica gel and recrystallized from ethanol to give, in addition to haploperosides A and B, haploperosides C (1.0 g), D (0.38 g), and E (10.0 g).

Haploperoside D. $C_{22}H_{28}O_{13}$ (I), mp 249–251°, $[\alpha]_D^{20}$ –37,8° (c 1,0; DMFA), ν_{max}^{KBr} (cm^{-1}): 3200 – 3600 (OH), 2920 (OCH_3), 1695 (C=O of an α-pyrone), 1617, 1568, 1519 (aromatic C=C bonds), 1105-1000 (C–O vibrations of glycosides); $\lambda_{max}^{CH_3OH}$, nm: 230, 253 sh., 261 sh, 292, 345 (log ε 4.03, 3.54, 3.48, 3.60, 3.76). PMR spectrum (C_5D_5N): 1.63 (3 H, d, 6 Hz, $-CH_3$); 3.72 (3 H, s, $-OCH_3$); 3.75-4.80 (10 H of the sugar moiety); 4.59 (1 H, d, 2 Hz, H-1"); 5.54 (1 H, d, 8 Hz, H-1'); 6.30 (1 H, d, 9.5 Hz, H-3); 6.92 (1 H, s, H-8); 7.36 (1 H, s, H-5); 7.51 (1 H, d, 9.5 Hz, H-4).

Acid Hydrolysis of (I). Glycoside (I) (25 mg) was hydrolyzed with 5% sulfuric acid in the water bath for 3 h. The reaction mixture was diluted with water and was exhaustively extracted with chloroform. The chloroform extract was washed with water, dried with anhydrous sodium sulfate, and distilled. After recrystallization from ethanol, 8 mg of aglycone was obtained which was identified by means of TLC and IR and mass spectroscopy (M^+ 192) as scopoletin. In the hydrolysate that had been neutralized on AN-1-anion-exchange resin and evaporated, D-glucose and L-rhamnose were detected by PC (system 2) with markers. The ratio of the sugars according to GLC was 1:1.07.

Partial Hydrolysis of (I). Glycoside (I) (80 mg) was hydrolyzed with a 0.25% solution of sulfuric acid on the water bath for 3 h. The reaction mixture was diluted with water and was extracted exhaustively with ethyl acetate. The ethyl acetate extract was washed with water and evaporated. The residue was chromatographed on a column of silica gel in the chloroform–methanol (97:3) system. This yielded 18 mg of scopoletin and 24 mg of scopoletin 7-O-β-D-glucopyranoside with mp 212-214°C, $[\alpha]_D^{25}$ –65.6° (c 1.13 ; DMFA), which was identified by a comparison of its R_f value on TLC (system 1) and its IR spectrum with those of an authentic sample.

Acetylation of (I). A mixture of 45 mg of glycoside (I), 1 ml of pyridine, and 3 ml of acetic anhydride was heated in the water bath for 30 min. Then it was distilled with water and extracted with chloroform. The chloroform extract was washed three times with 5% sulfuric acid and then with water to neutrality and was dried with anhydrous sodium sulfate and evaporated. The residue was recrystallized from ethanol, to give 53 mg of a hexaacetate with mp 212-214°C.

PMR spectrum ($CDCl_3$): 1.28 (3 H, d, 6 Hz, CH_3); 1.84 (3 H, s), 1.88 (6 H, s), 1.92 (3 H, s), 1.97 (3 H, s), 2.07 (3 H, s, the signals of six $COCH_3$ groups); 3.70 (3 H, s, $-OCH_3$); 3.08-4.90 (6 H); 5.06-5.87 (6 H); 6.18 (1 H, d, 10 Hz, H-3); 6.87 (1 H, s, H-8); 7.16 (1 H, s, H-5); 7.46 (1 H, d, 10 Hz, H-4).

Mass spectrum, *m/z* (%): M^+ 752(0.2), 693(0.13), 619(0.2), 561(65), 509(0.6), 501(0.8), 459(0.6), 415(0.6), 399(0.8), 381(0.85), 355(1.51), 339(1), 331(4), 317(6), 273(100), 229(9), 213(18.5), 193(28), 192(20), 171(24), 169(26), 153(31), 139(13), 129(15), 127(20), 111(30), 109(24).

Hakomori Methylation of (I). A solution of 60 mg of glycoside (I) in 12 ml of dry dimethyl sulfoxide was treated with 60 mg of sodium hydride in small portions. The resulting mixture was stirred at room temperature for 1 h. Then 5 ml of methyl iodide was added dropwise to the reaction mixture and it was stirred for another 3 h, after which it was poured into 100 ml of a 2% solution of sodium hyposulfite and extracted with chloroform. The chloroform extract was washed with water and was dried with anhydrous sodium sulfate. After the solvent had been distilled off and the residue had been dried, 82 mg of methylation product was obtained in the IR spectrum of which there was no band of the absorption of hydroxy groups.

Acid Hydrolysis of the Product of the Methylation of (I). The product of the methylation of (I) (10 mg) was boiled on the water bath in 4 ml of a 6% methanolic solution of hydrogen chloride for 4 h. The reaction mixture was neutralized with silver carbonate, the resulting precipitate was filtered off, and the filtrate was evaporated to dryness. 2,3,4-Tri-O-methyl-L-rhamnose and 3,4,6-tri-O-methyl-D-glucose were identified in the residue by GLC.

Haploperoside C, $C_{24}H_{30}O_{14}$ (IV). mp 155 – 157°, $[\alpha]_D^{20}$ –27.43° (*c* 1.02; CH_3OH), ν_{max}^{KBr} (cm^{-1}): 3200 – 3600 (OH), 2930 (OCH_3), 1749 (C=O of an ester); 1716 (C=O of an α-pyrone); 1617, 1574, 1531 (aromatic C=C bonds); 1105-1000 (C–O vibrations of glycosides). $\lambda_{max}^{CH_3OH}$, nm: 229, 252 sh, 260 sh, 291, 344 (log ε 4.41; 3.58; 3.77; 3.99; 4.13).

PMR spectrum (C_5H_5N): 1.44 (3 H, d, 5 Hz, $-CH_3$); 1.98 (3 H, s, $COCH_3$); 3.57 (3 H, s, $-OCH_3$), 3.70-4.62 (10 H of the sugar moiety); 5.18 (1 H, br.s, H-1''); 5.31 (1 H, d, 9 Hz, H-1'); 5.62 (1 H, t, $J_1 = J_2 = 9$ Hz, H-2'); 6.15 (1 H, d, 10 Hz, H-3); 6.84 (1 H, s, H-8); 7.48 (1 H, s, H-5); 7.52 (1 H, d, 10 Hz, H-4).

Acid Hydrolysis of (IV). Glycoside (IV) (30 mg) was hydrolyzed with 5% sulfuric acid in the water bath for 3 h. After the removal of the scopoletin and neutralization, D-glucose and L-rhamnose were detected in the hydrolysates by PC in system 2. The ratio of the sugars according to GLC was 1:1.02.

Acetylation of (IV). Glycoside (IV) (50 mg) was acetylated with acetic anhydride in pyridine at room temperature for 48 h. After the usual working up, an acetate with mp 125-126°C was obtained which, according to TLC in system 3 and IR spectroscopy, was identical with the hexaacetate of haploperoside A.

PMR spectrum ($CDCl_3$): 1.12 (3 H, d, 6 Hz, $-CH_3$); 1.91 (3 H, s), 1.97 (9 H, s), 2.00 (6 H, s, the signals of 6 $COCH_3$ groups); 3.77 (3 H, s, $-OCH_3$), 3.40-3.90 (5 H); 4.59 (1 H, br.s, H-1''); 4.77-5.25 (6 H); 6.17 (1 H, d, 9.5 Hz, H-3); 6.78 (1 H, s, H-8); 6.95 (1 H, s, H-5); 7.48 (1 H, d, 9.5 Hz, H-4).

Mass spectrum, *m/z* (%): M^+ 752(6), 561(10), 509(9), 311(11), 305(11), 273(8.5), 259(15), 229(9), 215(13), 213(16), 211(10), 193(24), 192(13), 187(19), 171(31), 169(60), 157(21), 155(24), 153(100), 145(49), 139(28), 129(30), 127(45), 115(58), 111(76), 109(57), 103(51), 101(54).

Haploperoside A from (IV). A solution of 30 mg of haploperoside C in 10 ml of 0.5% caustic soda solution was kept at room temperature for 30 min. Then it was neutralized with 5% hydrochloric acid and was extracted exhaustively with n-butanol. The butanolic solution was washed with water and was evaporated in vacuum. The residue was chromatographed on a column of silica gel in the chloroform–methanol (9:1) system. This gave 16 mg of haploperoside A with mp 212-213°C, $[\alpha]_D$ –37° (*c* 0.24; methanol), which was identified both from its R_f value on TLC (system 1) and by its IR spectrum.

Determination of the Structure of the Sugar Moiety of Haploperoside A. Haploperoside A (100 mg) was methylated by Hakomori's method. After the appropriate working up, 112 mg of methylation product was obtained. This was hydrolyzed with a 3% methanolic solution of sulfuric acid in the water bath for 4 h. After the usual working up, 2,3,4-tri-O-methyl-

L-rhamnose and 2,3,4-tri-O-methyl-D-methyl-D-glucose were identified in the carbohydrate fraction of the hydrolysate by TLC in system 4 in the presence of markers and with the aid of GLC.

Haploperoside E, $C_{28}H_{38}O_{17}$ (V). mp 175 – 177°, $[\alpha]_D^{20}$ – 56.41° (*c* 0.53; CH_3OH), ν_{max}^{KBr} (cm^{-1}): 3600 – 3250 (OH), 2920 (OCH_3), 1709 (C=O of an α-pyrone), 1615, 1567, 1516 (aromatic C=C bonds), 1122-1000 (C–O vibrations of glycosides); $\lambda_{max}^{C_2H_5OH}$, nm: 231, 252 sh., 261 sh., 289, 345 (log ε 4.04; 3.51; 3.47; 3.54; 3.61).

PMR spectrum (C_5D_5N): 1.48 (3 H, br.s, $-CH_3$); 1.67 (3 H, d, 6 Hz, $-CH_3$); 3.52-5.01 (15 H); 3.74 (3 H, s, $-OCH_3$); 5.19 (1 H, br.s, H-1'''); 5.38 (1 H, d, 9 Hz, H-1'); 6.39 (1 H, d, 10 Hz, H-3); 6.78 (1 H, s, H-8); 7.45 (1 H, s, H-5); 7.55 (1 H, d, 10 Hz, H-4).

PMR spectrum (DMSO-d_6): 1.01 (3 H, d, 6 Hz, $-CH_3$); 1.11 (3 H, m, $-CH_3$); 3.72 (3 H, s, $-OCH_3$); 2.85-3.84 and 4.10-5.37 (protons of the sugar moiety); 6.23 (1 H, d, 9.5 Hz, H-3); 7.07 (1 H, s, H-8); 7.18 (1 H, s, H-5); 7.85 (1 H, d, 9.5 Hz, H-4).

Acid Hydrolysis of (V). The trioside (V) (30 mg) was hydrolyzed with 5% sulfuric acid in the water bath for 4 h. After the separation of the scopoletin and neutralization, D-glucose and L-rhamnose were identified in the hydrolysate by the PC method in system 2.

Acetylation of (V). Glycoside (V) (100 mg) was acetylated with acetic anhydride in pyridine at room temperature for 24 h. After the usual working up and purification by preparative TLC in system 3, 60 mg of an acetate $C_{44}H_{54}O_{25}$ (VI) with mp 145-146°C was obtained. Mass spectrum, *m/z* (%): M^+ 982(1), 791(4), 561(2), 547(3), 273(100), 213(16), 192(11), 171(17.5), 153(40.5), 111(30).

Partial Hydrolysis of the Trioside (V). A solution of 400 mg of the glycoside (V) in 50 ml of 0.25% aqueous sulfuric acid was heated in the water bath for 4 h. Then it was diluted with water and exhaustively extracted with butanol. The butanolic extract was washed with water and evaporated. Chromatography on a column of silica gel in a chloroform–methanol gradient system, and also preparative TLC, led to the isolation of scopoletin, scopoletin 7-O-β-D-glucopyranoside with mp 212-214°C, haploperoside A with mp 212-213°C, and haploperoside D with mp 249-251°C. All the substances were identified from their R_f values on TLC in system 1 and by their IR spectra in comparison with authentic samples.

Determination of the Structure of the Sugar Moiety of Trioside (V). Trioside (V) (70 mg) was methylated by Hakomori's method. After the appropriate working up, 82 mg of methylation product was obtained which showed no absorption bands of hydroxy groups in the IR spectra. Hydrolysis of the methylation product was carried out with a 5% methanolic solution of sulfuric acid at the boiling point of the reaction mixture for 4 h. After the usual working up, 2,3,4-tri-O-methyl-L-rhamnose was identified in the carbohydrate fraction of the hydrolysate by TLC in system 4 and by GLC. In addition to methylated rhamnose, TLC showed the presence of a more polar component – 3,4-di-O-methyl-D-glucose.

SUMMARY

Three new coumarin glycosides – haploperosides C, D, and E – have been isolated from the epigeal part of *Haplophyllum perforatum* (M. B.) Kar. et Kir. (family Rutaceae). Their structures have been determined on the basis of chemical and spectral characteristics, and the structures of haploperosides A and B have been refined.

LITERATURE CITED

1. M. P. Yuldashev, É. Kh. Batirov, and V. M. Malikov, Khim. Prir. Soedin., 168 (1980).
2. M. P. Yuldashev, É. Kh. Batirov, and V. M. Malikov, Khim. Prir. Soedin., 412 (1980).
3. M. P. Yuldashev, É. Kh. Batirov, V. M. Malikov, and M. E. Perel'son, Khim. Prir. Soedin., 718 (1981).
4. H. Budziekiwicz, C. Djerassi, and D. H. Williams, Structure Elucidation of Natural Products by Mass Spectroscopy, Holden-Day, San Francisco, Vol. 2 (1964), p. 204.
5. S. Hakomori, J. Biochem. (Tokyo), 55, 205 (1964).
6. G. O. Aspinall, J. Chem. Soc., 1676 (1963).
7. J. M. Van der Veen, J. Org. Chem., 28, 564 (1963).
8. G. G. Zapesochnaya, Khim. Prir. Soedin., 695 (1982).
9. N. J. Cussans and T. N. Huckerby, Tetrahedron, 31, 2719 (1975).
10. H. Duddeck and M. Kaiser, Org. Magn. Reson., 20, No. 2, 55 (1982).

11. K. Yoshimoto, Y. Itatani, K. Shibata, and Y. Tsuda, Chem. Pharm. Bull., 28, 208 (1980).
12. L. I. Strigina and V. V. Isakov, Khim. Prir. Soedin., 847 (1980).
13. K. R. Markham and B. Ternai, Tetrahedron, 32, 2607 (1976).
14. K. R. Markham, B. Ternai, R. Stanley, H. Geiger, and T. J. Mabry, Tetrahedron, 34, 1389 (1978).
15. H. Ripperger, Phytochemistry, 20, No. 7, 1757 (1981).
16. D. Batsuran, E. Kh. Batirov, V. M. Malikov, and M. R. Yagudaev, Khim. Prir. Soedin., 142 (1983).
17. K. Yamasaki, R. Kasai, Y. Masaki, M. Okihara, O. Tanaka, H. Oshio, Sh. Takagi, M. Yamaki, K. Masuda, G. Nokana, M. Tsuboi, and I. Nishioka, Tetrahedron Lett., 1231 (1977).
18. K. Yoshimoto, Y. Iatani, and Y. Tsuda, Chem. Pharm. Bull., 28, No. 7, 2065 (1980).
19. A. Sakushimo, H. Hisada, Y. Ogihara, and S. Nishibe, Chem. Pharm. Bull., 28, No. 4, 1219 (1980).

STRUCTURES OF TWO NEW FLAVANONES FROM *Vexibia alopecuroides*

É. Kh. Batirov, S. S. Yusupova,
Sh. V. Abdullaev, A. D. Vdovin,
V. M. Malikov, and M. R. Yagudaev

UDC 547.972

Two new flavanones — vexibinol and vexibidin — have been isolated from the chloroform fraction of an ethanolic extract of the roots of *Vexibia alopecuroides* by column chromatography on silica gel. Their structures have been established on the basis of chemical and spectral characteristics. Their IR, UV, ^1H, and ^{13}C NMR, and mass spectra are given.

We have reported the isolation of four flavonoids from the roots of *Vexibia alopecuroides* (L) Yakovl. (*Sophora alopecuroides*) [1]. Continuing this investigation, from the chloroform fraction of an alcoholic extract we have isolated two new flavonoids which have been called vexibinol and vexibidin. Vexibinol, $C_{25}H_{28}O_6$ (I), M^+ 424, gives positive reactions with $FeCl_3$ solution and with magnesium in hydrochloric acid. Its IR spectrum has the absorption bands of hydroxy groups (3366 cm^{-1}), of a carbonyl group (1632 cm^{-1}), and of aromatic C=C bonds (1604, 1519 cm^{-1}). The UV spectrum of vexibinol ($\lambda_{max}^{C_2H_5OH}$ (nm) 293, 340 sh; log ε 4.23, 3.69) is characteristic for flavanones [2]. The results of a study of spectra taken in the presence of CH_3COONa, $AlCl_3$, and CH_3ONa showed the presence of phenolic hydroxy groups at C-7, C-5, and C-4'.

The acetylation of (I) with acetic anhydride in pyridine gave an acetyl derivative (II) the PMR spectrum of which showed the signals of the protons of the methyls of four Ar—$OCOCH_3$ groups (Table 1). Consequently, vexibinol contains four phenolic hydroxy groups, and the signals of their protons appeared at 9.37, 9.63, 10.67, and 12.13 ppm (C_5—OH) in the PMR spectrum of (I) taken in DMSO-d_6.

The presence of the characteristic signals of the H-2 and H-3 protons in the spectrum confirmed that (I) was flavanone [2, 3]. Furthermore, signals of four aromatic protons and the protons of the side chain were detected in the spectra (Table 1). According to the PMR and ^{13}C NMR spectra (Table 2), the side chain of (I) had the structure of 2-isopropenyl-5-methylhex-4-enyl. Flavanones and chalcones having such a side chain are rare but have been isolated from some species of plants of the genera *Sophora* and *Ammothamnus* [4-6]. In the mass spectrum of vexibinol there are the peaks of ions with m/z 163 and 136, which shows the presence of two phenolic hydroxy groups in ring B [3, 7].

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. I. P. Pavlov Samarkand State Medical Institute. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 35-41, January-February, 1985. Original article submitted February 28, 1984.

 0009-3130/85/2101-0032 $09.50