NEW COUMARINS OF *Haplophyllum obtusifolium*

É. Kh. Batirov, A. D. Matkarimov,
V. M. Malikov, M. R. Yagudaev,
and E. Seitmuratov

UDC 577.15/17:582.89

Two coumarins have been isolated from an ethanolic extract of the epigeal part of *Haplophyllum obtusifolium* Lebed.: capensin (I) and the new coumarin obtusicin, $C_5H_{16}O_6$, (II), mp 81-91°C (CH_3OH). The acid hydrolysis of (I) and (II) leads to fraxetin. On the basis of the results of a study of acetylation products and the spectral characteristics (IR, UV, PMR, and mass spectra) it has been established that obtusicin has the structure of 8-hydroxy-7-(4'-hydroxy-3'-methyl-but-2'-enyl-oxy)-6-methoxycoumarin.

Continuing a chemical study of coumarins of *Haplophyllum obtusifolium* Ledeb. [1, 2], from the chloroform-soluble fraction of an ethanolic extract we have isolated two coumarins: $C_{15}H_{16}O_5$ (I) M^+ 276, and $C_{15}H_{16}O_6$ (II), M^+ 292.

The UV spectra of (I) [$\lambda_{max}^{C_2H_5OH}$ 230, 259, 315, nm (log ε 4.10, 3.66, 3.94)] and (II) [$\lambda_{max}^{C_2H_5OH}$ 229, 258, 315 nm (log ε 4.15, 3.71, 3.95)] are characteristic for 7-alkoxycoumarins [3].

The positive reaction with $FeCl_3$ and the bathochromic shift of the maxima in an alkaline medium showed that each of these coumarins contained a phenolic hydroxy group.

The IR spectra of (I) and (II) each show absorption bands of hydroxy groups [(I) — 3140-3410 cm^{-1}; (II) — 3250, 3405 cm^{-1}], of a carbonyl in an α-pyrone ring [(I) — 1698 cm^{-1}; (II) - 1696 cm^{-1}], and an aromatic nucleus [(I) — 1617, 1572, 1500 cm^{-1}; (II) — 1614, 1577, 1510 cm-

The acid hydrolysis of each of the coumarins gave a substance with mp 228-230°C, M^+ 208 (III). We have isolated compound (III) from the same plant and on the basis of the closeness of the melting point and of the PMR spectra we previously considered it to be isofraxetin (5,(dihydroxy-7-methoxycoumarin) [1]. Further investigations showed that some properties of (III) do not coincide with the properties of isofraxetin [4].

In the PMR spectrum of the diacetyl derivative of (III) (IV) the position of the signal of the H-4 proton had scarcely changed, and the signal of the sole aromatic proton had undergone a paramagnetic shift by 0.38 ppm.

If there were a hydroxy group at C-5, the signal of the H-4 proton in the spectrum of (IV) should have shifted upfield by 0.5-0.6 ppm [5, 6].

The interaction of (III) with methylene iodide in the presence of potassium carbonate gave a methylenedioxy derivative with mp 217-218°C, M^+ 220 (V). Consequently, the hydroxy groups of (III) have the ortho position. Furthermore, a nuclear Overhauser effect was observed between the protons of the CH_3O group and the aromatic proton, i.e., on irradiation with an additional radiofrequency field have a frequency of ν_2 = 362 Hz corresponding to the resonance transitions of the protons of the methoxy group. The integral intensity of the signal at 6.52 ppm increased by 20%.

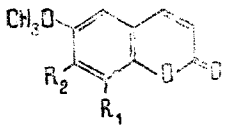
On the basis of the facts presented, compound (III) must have the structure of 7,8-dihydroxy-6-methoxycoumarin and be identical with fraxetin [7]. This was confirmed by the preparation of 6,7,8-trimethoxycoumarin (VI) [8] when (III) was methylated with methyl iodide in the presence of K_2CO_3. Consequently, (I) and (II) are fraxetin derivatives.

Complex Institute of Natural Sciences of the Karakalpak Branch of the Academy of Sciences of the Uzbek SSR, Nukus, and Institute of the Chemistry of Plant Substances of the Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Pridodnykh Soedinenii, No. 6, pp. 785-789, November-December, 1980. Original article submitted July 8, 1980.

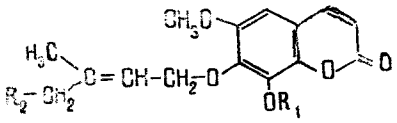
 0009-3130/80/1606-0558$07.50

In the PMR spectrum of (I), together with the signals of the H-3, H-4, and H-5 protons and the methoxy group, the signals of the protons of two vinylmethyl groups (1.56 ppm, 6 H, singlet), a methylene group bound to oxygen (4.53 ppm, 2 H, doublet, J = 7 Hz), and an olefinic proton (5.32 ppm, 1 H, multiplet) are observed. This means that in (I) there is a side chain consisting of an isopentenyloxy ($OCH_2-CH=C<^{CH_3}_{CH_3}$) group. The position of the side chain was established from a study of the PMR spectrum of the acetyl derivative of (I) (VII), which differed from the spectrum of (I) by a shift of the H-5 signal downfield by 0.46 ppm and by the signal of the CH_3CO group at 2.18 ppm, as in the case of the coumarin brosiparin and its acetate [3, 9]. Consequently, the phenolic hydroxy group occupies the C_8 position, and the isopentyloxy group the C_7 position.

Thus, (I) has the structure of 8-hydroxy-7-(3,3'-dimethylallyloxy)-6-methoxycoumarin and is identical with capensin [10]



III. $R_1=R_2=OH$
IV. $R_1=R_2=OCOCH_3$
V. $R_1=R_2=CH_2O_2$
VI. $R_1=R_2=OCH_3$



I. $R_1=R_2=H$
II. $R_1=H$, $R_2=OH$
VII. $R_1=COCH_3$, $R_2=H$
VIII. $R_1=COCH_3$, $R_2=OCOCH_3$

Coumarin (II) (obtusicin) contains one oxygen atom more than (I). On acetylation with acetic anhydride in pyridine, (II) gave a diacetyl derivative (VIII). The PMR spectrum of (II) (Fig. 1) was close to that of (I), differing only by the signals of the protons of the side chain. The presence of the signals of protons at 1.77 ppm (3 H, broadened singlet, $=C-CH_3$), 4.29 ppm (2 H, singlet, $-CH_2-OH$), 4.86 ppm (2 H, doublet, J = 7 Hz, $=CH-CH_2-O$) and 5.68 ppm (1 H, multiplet, $=CH-CH_2$) shows that the side chain of (II) has the structure $-O-CH_2-CH=C(CH_2-OH)-CH_3$. The positions of the phenolic hydroxy groups of (I) and (II) must be identical, since their IR spectra are similar in both neutral and alkaline media. The paramagnetic shift of the H-5 signal ($\Delta\delta$ 0.35 ppm) on acetylation confirms this hypothesis.

The spectrum of (VIII) also shows the signals of the protons of two acetyl groups (1.86 and 2.25 ppm, 3 H each), and the signal of the methylene group is shifted downfield and appears at 4.56 ppm ($\Delta\delta$ 0.27 ppm).

It follows from this that obtusicin is a new coumarin and has the structure of 8-hydroxy-7-(4'-hydroxy-3'-methylbut-2'-enyloxy)-6-methoxycoumarin (II).

The mass spectra of (I), (II), and (VIII) also confirms the structures suggested for them. The maximum peak of the ion with m/e 208 in the mass spectrum of (I), which is formed from the molecular ion (M^+ 276) as the result of the ejection of a molecule of isoprene, confirms the presence of an isopentenyloxy group. The further breakdown of the ion with m/e 208 leads to the formation of ions with m/e 193, 180, 165, 137, 109, which are characteristic for the fragmentation of the molecular ion of fraxetin. Analogous ion peaks were also found in the spectrum of (II). The ion with m/e 127 in the mass spectrum of (VIII) formed as the result of β-cleavage confirms the structure of the obtusicin side chain.

It must be mentioned that a coumarin of similar structure, haptusinol, has been isolated from the same plant by other authors [11].

EXPERIMENTAL

UV spectra were recorded on an EPS-3T spectrophotometer in ethanolic solutions, IR spectra on a UR-20 instrument (tablets with KBr), and PMR spectra on a JNM-C-60HL spectrometer (JEOL) with a working frequency of 60 MHz in [D]pyridine solution (0 — HMDS). The NOE was measured on a Varian XL-100-15 spectrometer and the mass spectra were recorded on MKh-1303 and MKh-1310 instruments.

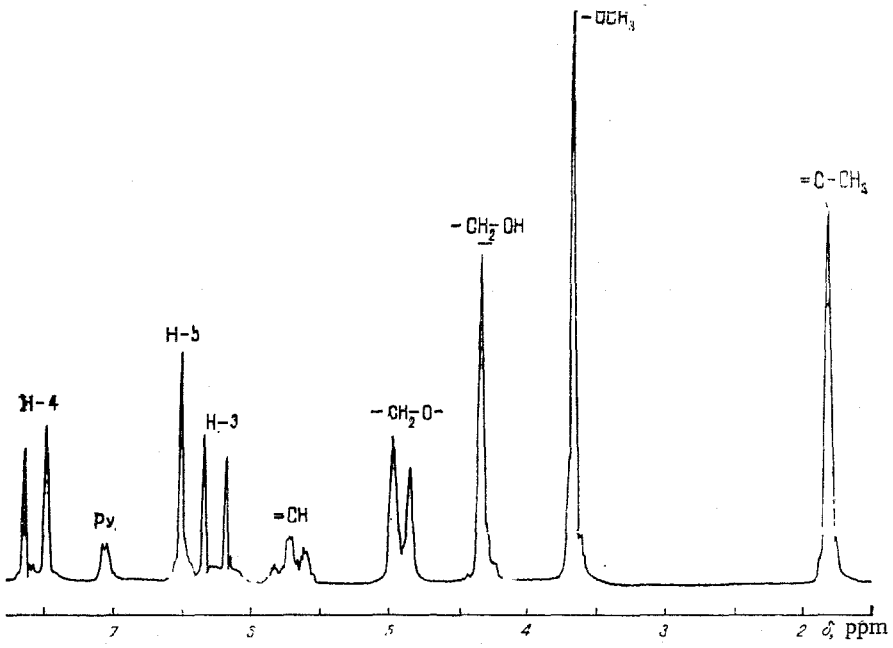


Fig. 1. PMR spectrum of obtusicin in deuteropyridine.

The homogeneity of the substances was checked by thin-layer chromatography on Silufol in systems 1) ethyl acetate—ethanol—water (13:5:2), and 2) chloroform—petroleum ether—methanol (8:4:1).

Isolation of the Coumarins. An ethanolic extract from the epigeal part of the plant [1] was concentrated in vacuum to 2 liters and was diluted with water (1:1). The resulting precipitate of chlorophyll was separated off and the filtrate was extracted successively with chloroform and ethyl acetate. When the solvents had been distilled off, 190 g of chloroform fraction and 58.0 g of ethyl acetate fraction were obtained. Part of the chloroform fraction (75.0 g) was chromatographed on a column of silica gel (130 × 4.5 cm; 1100 g) and was eluted with a mixture of petroleum ether and chloroform with increasing concentrations of the latter, 250-ml fractions being collected. At a mixture composition of (4:1), 0.92 g of 6-methoxy-7-(3',3'-dimethylallyloxy)coumarin [2] (from fractions 20-32) and 3.98 g of capensin (from fractions 33-44) were isolated. The mixture with the (19:1) ratio eluted 0.11 g of obtusinol [2]. Then chloroform and chloroform—ethanol (19:1 and 9:1) eluted 8.44 g of obtusinin [1], 0.16 g of fraxetin, and 0.97 g of obtusicin.

Capensin, mp 137-139°C (methanol), R_f 0.81 (system 2). PMR spectrum (ppm): 1.56 (6 H, s, $=C(CH_3)_2$), 3.63 (3 H, s, $-OCH_3$), 4.53 (2 H, d, 7 Hz, $=CH-CH_2O$); 5.32 (m, $=CH-CH_2$), 6.08 (d, 9.5 Hz, H-3), 6.35 (s, H-5) 7.41 (d, 9.5 Hz, H-4).

Mass spectrum, m/e (%): 276 (M^+, 12), 261 (3), 209 (12), 208 (100), 193 (13), 180 (6), 165 (5), 153 (6), 137 (5), 81 (6), 69 (30), 67 (8), 53 (9).

Obtusicin, mp 89-91°C (methanol), R_f 0.41 (system 2), PMR spectrum (ppm): 1.77 (3 H, s, $=C-CH_3$), 3.62 (s, $-OCH_3$), 4.29 (2 H, s, $-CH_2OH$), 4.86 (2 H, d, 7 Hz, $-CH-CH_2-O-$), 5.68 (m, $=CH-CH_2$), 6.22 (d, 9.5 Hz, H-3), 6.46 (s, H-5), 7.52 (d, 9.5 Hz, H-4).

Mass spectrum: 292 (M^+, 3), 209 (7), 218 (47), 193 (12), 180 (6), 165 (6), 137 (9), 109 (12), 95 (7), 85 (9), 84 (100), 83 (22), 81 (14), 69 (17).

Acid Hydrolysis of (I) and (II). A solution of 0.1 g of the substance in 2.5 ml of glacial acetic acid was treated with two drops of concentrated sulfuric acid and the mixture was heated in the water bath for 30 min. After the usual working up, compound (III) was obtained: $C_{10}H_8O_5$, M^+ 208, mp 228-230°C (methanol), R_f 0.74 (system 1).

PMR spectrum of (III) (ppm): 3.62 (s, $-OCH_3$), 6.13 (d, 9 Hz, H-3), 6.51 (s, H-5), 7.51 (d, 9 Hz, H-4).

PMR spectrum of (IV) (ppm): 2.15 and 2.18 (s, 2 $COCH_3$), 3.55 (s, $-OCH_3$), 6.24 (d, 9.5 Hz, H-3), 6.89 (s, H-5), 7.49 (d, 9.5 Hz, H-4).

Aceylation of (I) to (VII). A solution of 0.05 g of (I) in 1 ml of pyridine was treated with 1.5 ml of acetic anhydride. After a day the acetyl derivative (VII) was isolated in the usual way: $C_{17}H_{18}O_6$, mp 97-99°C.

PMR spectrum of (VII) (ppm): 1.52 [6 H, s, $=C(CH_3)_2$], 2.18 (s, $-COCH_3$), 3.61 (s, $-OCH_3$), 4.58 (d, 7.5 Hz, $=CH-CH_2$), 5.64 (m, $=CH-CH_2$), 6.16 (d, 9.5 Hz, H-3), 6.81 (s, H-5), 7.48 (d, 9.5 Hz, H-4).

Acetylation of (II) to (VIII). Compound (II) (0.04 g) was acetylated by the method described above. This gave compound (VIII) with the composition $C_{19}H_{20}O_8$, M^+ 376, mp 72-74°C.

PMR spectrum of (VIII) (ppm): 1.66 (3 H, br.s. $=C-CH_3$), 1.86 (s, $-CH_2-OCOCH_3$), 2.25 (s, $Ar-O-COCH_3$), 3.62 (s, $-OCH_3$), 4.56 (s, $-CH_2-Ac$), 4.68 (d, 7.5 Hz, $=CH-CH_2$), 5.64 (m, $=CH-CH_2$), 6.17 (d, 9.5 Hz, H-3), 6.81 (s, H-5), 7.49 (d, 9.5 Hz, H-4).

The Methylenedioxy Derivative of (III) (V). A solution of 0.1 g of (III) in 15 ml of acetone was treated with 0.5 g of anhydrous potassium carbonate and 1 ml of methylene iodide. The mixture was boiled in the water bath for 14 h, and then the acetone was distilled off and the residue was diluted with water and was extracted with chloroform. The residue from the chloroform extract was chromatographed on silica gel. This gave 17 mg of substance with mp 217-218°C, M^+ 220.

Methylation of (III) to (VI). Compound (III) (0.1 g) was methylated with methyl iodide in the presence of potassium carbonate as described by Gashimov and Kuznetsova [12]. This gave a substance with mp 103-104°C (petroleum ether).

SUMMARY

The epigeal part of *Haplophyllum obtusifolium* has yielded capensin and the new coumarin obtusicin. On the basis of the results of chemical transformations and spectral characteristics the structure of obtusicin has been established as 8-hydroxy-7-(4'-hydroxy-3'-methylbut-2'-enyloxy)-6-methoxycoumarin.

LITERATURE CITED

1. A. D. Matkarimov, É. Kh. Batirov, V. M. Malikov, and E. Seitmuratov, Khim. Prir. Soedin., 328 (1980).
2. A. D. Matkarimov, É. Kh. Batirov, V. M. Malikov, and E. Seitmuratov, Khim. Prir. Soedin., 565 (1980).
3. M. E. Perel'son, Yu. N. Sheinker, and A. A. Savina, Spectra and Structure of Coumarins, Chromones, and Xanthones [in Russian], Moscow (1975), p. 34, 109.
4. M. V. Artem'eva, G. K. Nikonov, and M. O. Karryev, Khim. Prir. Soedin., 493 (1973).
5. E. V. Lassak and J. T. Pinhey, Aust. J. Chem., 22, 2175 (1969).
6. A. Arnone, G. Cirdillo, L. Merlini, and R. Mondelli, Tetrahedron Lett., 4201 (1967).
7. F. E. King, I. R. Housley, and T. J. King, J. Chem. Soc., 1392 (1954).
8. G. A. Kuznetsova, Natural Coumarins and Furocoumarins [in Russian], Leningrad (1967), p. 81.
9. R. Braz Filho, A. F. Magalhaes, and O. R. Gottlieb, Phytochemistry, 11, 3307 (1972).
10. W. E. Campbell and G. M. L. Crangg, Phytochemistry, 18, 688 (1979).
11. A. Z. Abyshev and N. F. Gashimov, Khim. Prir. Soedin., 845 (1979).
12. N. F. Gashimov and G. A. Kuznetsova, Khim. Prir. Soedin., 303 (1974).