OBTUSININ — A NEW COUMARIN FROM *Haplophyllum obtusifolium*

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

UDC 577.15/17:582.89

The epigeal part of *Haplophyllum obtusifolium* collected in Ustyurt has yielded β-sitosterol, isofraxetin, and a new coumarin — obtusinin — with the composition $C_{15}H_{18}O_6$, mp 135-137°C (methanol), $[\alpha]_D^{20}$ +140.9° (c 0.44; chloroform). The structure of obtusinin has been established on the basis of the results of a study of UV, IR, PMR, and mass spectra and also of some chemical transformations (hydrolysis, acetylation).

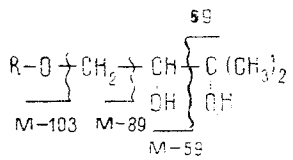
A number of coumarins have been isolated previously from the plant *Haplophyllum obtusifolium* (family Rutaceae) [1]. In order to study the coumarins and flavonoids, the epigeal part of this plant collected in Ustyurt (Karakalpak ASSR) at the end of the flowering period was repeatedly extracted with ethanol, and then with aqueous ethanol. The concentrated aqueous ethanolic extract was chromatographed on a column of silica gel, and elution with organic solvents yielded two coumarins. The first coumarin, with the composition $C_{15}H_{18}O_6$ (I), M^+ 294, which we have called obtusinin, gave a UV spectrum typical for 6,7-disubstituted coumarins: $\lambda_{max}^{C_2H_5OH}$, nm, 230, 253 sh, 297, 347 (log ε 4.14, 3.75, 3.73, and 4.00). The UV spectrum of (I) did not change in an alkaline medium, which shows the absence of a phenolic hydroxy group in it.

The IR spectrum of (I) showed, together with other bands, absorption bands at (cm^{-1}) 3500, 3314-3385 (OH groups), 1728 (α-pyrone C=O), 1620, 1567, and 1520 (C=C bonds of aromatic system).

Obtusinin forms a monoacetyl derivative (II) and is readily hydrolyzed by a mixture of sulfuric and glacial acetic acids. Scopoletin was isolated from the products of alkaline hydrolysis. Consequently, compound (I) contains a substituent with the composition $C_5H_{11}O_2$ in position 7. The structure of this substituent was established from its PMR and mass spectra. The PMR spectrum of (I) taken in deuterochloroform showed, in addition to the signals of a 6,7-disubstituted coumarin nucleus at (ppm) 6.19 (1 H, d, 10 Hz, H-3), 6.74 (2H, s, H-5 and H-8), and 7.52 (1 H, d, 10 Hz, H-4), the signals of protons in the following groups: $-O-C(CH_3)_2$ (1.28 and 1.32 ppm, singlets), $O-CH_2$ (3.98-4.35 ppm, 2 H, multiplet), and O—CH (3.63-3.87 ppm).

In the spectrum of (II), the signal of the methine proton undergoes a paramagnetic shift ($\Delta\delta$ = 1.41 ppm) and appears at 5.16 ppm in the form of a one-proton quartet. Consequently, the side chain of (I) has the structure $-O-CH_2-\underset{OH}{\underset{|}{CH}}-\underset{OH}{\underset{|}{C}}(CH_3)_2$. It follows from this that obtusinin and its acetate have structures (I) and (II) (Figure 1).

The structure of (I) is also confirmed by its mass spectrum, which contains the peaks of ions with m/e 294 (M^+), 279 $(M-15)^+$, 276 $(M-18)^+$, 261 $(M-18-15)^+$, 235 $(M-59)^+$, 217 (235 −18), 205 $(M-89)^+$, 192, 191 (M − 103) and others.



Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Combined Institute of Natural Sciences of the Karakalpak Branch, Academy of Sciences of the Uzbek SSR, Nukus. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 328-330, May-June, 1980. Original article submitted Februarv 22, 1980.

 0009-3130/80/1603-0240$07.50

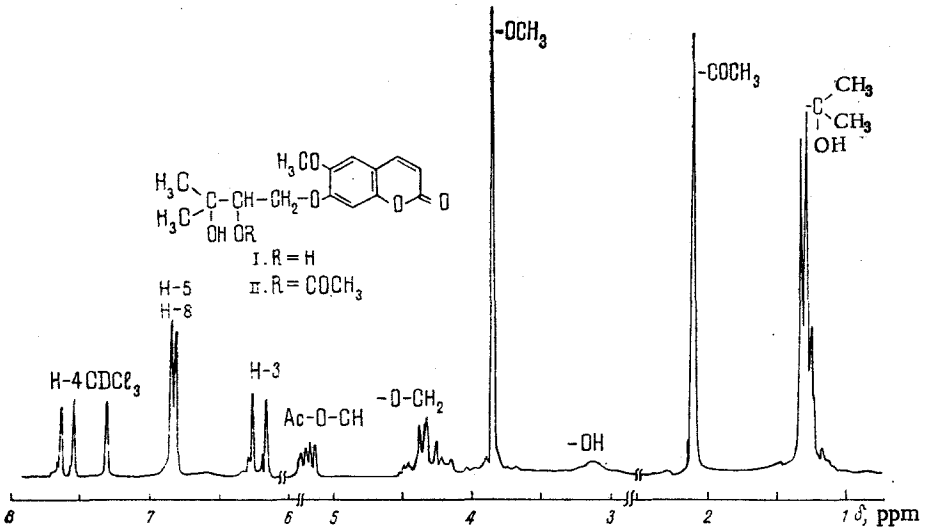


Fig. 1. PMR spectrum of obtusinin acetate in $CDCl_3$.

Similar ion peaks are observed in the spectra of mexoticin [2], evoxine, and halfordine [3, 4], which have the same side chain.

The second coumarin, with the composition $C_{10}H_8O_5$ (III), M^+ 208 does not fluoresce in UV light and, according to its UV spectrum (λ_{max}, nm, 230, 262 sh, 345: log ε 4.17, 3.62, 4.07) it belongs to the 5,6,7-trihydroxycoumarin derivatives.

The IR spectrum of the (III) had the characteristic absorption bands of hydroxy groups (3250-3440 cm^{-1}), of a carbonyl in an α-pyrone ring (1693 cm^{-1}), and of an aromatic nucleus (1612, 1583, 1514 cm^{-1}).

The PMR spectrum of (III) in deuteropyridine is close to the spectrum of isofraxetin [5]. The acetylation of (III) formed a diacetyl derivative (IV) the PMR spectrum of which (in $CDCl_3$) showed the signals of the protons of two acetyl groups (2.29 and 2.35 ppm), of a methoxy group (3.79 ppm) and of a coumarin nucleus: H-3 (6.24 ppm, d, 10 Hz), H-8 (6.73 ppm, s), and H-4 (7.52 ppm, 10 Hz). Thus, the second coumarin is 5,6-dihydroxy-7-methoxycoumarin and is identical with isofraxetin [5].

When the concentrated ethanolic extract was diluted with water, a precipitate deposited with mp 136-138°C (methanol), M^+ 414. On the basis of the results of a study of its IR and mass spectra and of a direct comparison with an authentic sample, it was identified as β-sitosterol [6, 7].

EXPERIMENTAL

The UV spectra were taken on a Hitachi spectrophotometer, the IR spectra on a UR-20 instrument (KBr), the mass spectra on a MKh-1310 instrument, and the PMR spectra on a JNM-4H-100 instrument at 100 MHz, the chemical shifts being given in the δ scale from the signal of HMDS taken as zero.

The substances were chromatographed in a thin layer of silica gel L 5/40 μ in the ethyl acetate–ethanol–water (13:5:2) system.

Isolation of the Coumarins. The comminuted epigeal part of the plant under investigation (10 kg) was extracted five times with ethanol and then another five times with 50% ethanol. The aqueous ethanolic extract was freed from flavonoids by passage through a column of polyamide and was concentrated in vacuum. This gave 700 g of residue, 150 g of which was chromatographed on a column containing silica gel (1:18). Elution was performed with chloroform and with chloroform–ethanol.

The chloroform eluates yielded 0.43 g of obtusinin, and elution with chloroform–ethanol (19:1) gave 0.39 g of isofraxetin with mp 228-230°C (methanol), R_f 0.74.

Obtusinin has mp 135-137°C (methanol), $[\alpha]_D^{20}$ +140.9° (c 0.44; chloroform), R_f 0.73.

Acid Hydrolysis of (I). One drop of concentrated sulfuric acid was added to a solution of 50 mg of (I) in 1.2 ml of glacial acetic acid and the mixture was heated in the water bath

at 80°C for 20 min. After the usual working up and chromatographic purification, 17 mg of a substance with mp 202-204°C, M^+ 192, identical with scopoletin, was obtained.

Acetylation of (I). A solution of 45 mg of (I) in 1 ml of pyridine was treated with 2 ml of acetic anhydride. After a day the reaction mixture was diluted with water and extracted with chloroform. After drying and the removal of the solvent by distillation, an acetyl derivative with mp 98-99°C was obtained.

Acetylation of Isofraxetin. Compound (III) (40 mg) was acetylated with 2 ml of acetic anhydride in the presence of pyridine (1 ml) at room temperature. After the usual working up, a diacetate was obtained with mp 180-182°C (ethanol).

SUMMARY

β-Sitosterol, isofraxetin, and a new coumarin $C_{15}H_{18}O_6$ with mp 135-137°C, which has been called obtusinin, have been isolated from the epigeal part of *Haplophyllum obtusifolium*. The structure of obtusinin has been established on the basis of chemical transformations and spectral characteristics.

LITERATURE CITED

1. A. Z. Abyshev and N. F. Gashimov, Khim. Prir. Soedin., 401, 403, 845 (1979).
2. D. P. Chakraborty, B. K. Chowdhyry, and B. C. Das, Tetrahedron Lett., No. 36, 3471 (1967).
3. V. I. Akhmedzhanova, I. A. Bessonova, and S. Yu. Yunusov, Khim. Prir. Soedin., 272 (1975).
4. W. D. Crow, J. H. Hodgkin, and J. S. Shannon, Austral. J. Chem., 18, 1433 (1965).
5. M. V. Art'emova, G. K. Nikonov, and M. O. Karryev, Khim. Prir. Soedin., 493 (1973).
6. A. Mallabaev, I. M. Saitbaeva, and G. P. Sidyakin, Khim. Prir. Soedin., 320 (1969).
7. T. V. Zinchenko and A. Ya. Kobzar, Farm. Zh., No. 5, 76 (1979).

HAPLOSIDE A — A NEW ACYLATED FLAVONOL GLYCOSIDE FROM *Haplophyllum perforatum*

É. Kh. Batirov and V. M. Malikov

UDC 547.972

The epigeal part of *Haplophyllum perforatum* has yielded a new acylated flavonol glycoside, haploside A, for which the structure of 3,4',5,7,8-pentahydroxy-3'-methoxyflavone 7-O-(6"-O-acetyl-β-D-glucopyranoside) has been established. The aglycone of the glycoside isolated — 3,4',5,7,8-pentahydroxy-3'-methoxyflavone — has also proved to be new and it has been called haplogenin. The structures of the compounds mentioned have been established on the basis of UV, IR, PMR, and mass spectra and the products of acetylation and of acid and alkaline hydrolysis.

According to the literature, the flavonoids of the plants of the genus *Haplophyllum* (family Rutaceae) have not been studied [1]. We have begun the study of the flavonoids of *H. perforatum* growing in the foothills of the Alim-Tau mountains (Southern Kazakhstan). From an ethanolic extract of the epigeal part of the plant collected in the flowering period (June, 1978) a flavonoid with the composition $C_{24}H_{24}O_{14}$ (I) has been isolated by adsorption chromatography on a column of silica gel. It has proved to be new, and we have called it haploside A. According to qualitative reactions [2] and UV spectroscopy [3] (λ_{max}, nm, 261, 280 sh, 343, 389), the flavonoid isolated belongs to the flavonol group.

Compound (I) is a glycoside, as was shown by Bryant's qualitative cyanidin reaction, spectral characteristics, and the existence of optical rotation. In actual fact, the acid hydrolysis of (I) gave D-glucose and an aglycone with mp 218-221°C, M^+ 332.

A study of the PMR spectrum of haploside A permitted it to be assigned to the monoglycosides. In the spectrum taken in deuteropyridine (Fig. 1), in addition to the signals of

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 330-334, May-June, 1980. Original article submitted December 14, 1979.

 0009-3130/80/1603-0242$07.50