A NEW COUMARIN – OBTUSIFOL FROM Haplophyllum obtusifolium

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We have previously established that plants of the genus Haplophyllum (family Rutaceae) contain mainly derivatives of 6,7-dihydroxycoumarin (esculetin). From Haplophyllum pedicellatum, scopoletin, 6-methoxymarmin, 7-geranyloxy-6-methoxycoumarin, and pedicellone have been isolated [1-3]. The present paper gives the results of a study of the structure of a new coumarin from the epigeal mass of Halophyllum obtusifolium Ledeb., which we have called obtusifol (I). Obtusifol with mp 149.5-150°C $[\alpha]_D^{19}$ +60.39° (c 1.45; ethanol), has the molecular formula $C_{15}H_{16}O_6$, mol. wt. 292 (mass spectrometry). Substance (I) possesses the properties of coumarins. On a paper chromatogram it gives a spot with R_f 0.77 (blue in UV light, system 1).

The nature of the absorption of (I) in the UV and IR region shows its coumarin structure. Obtusifol (I) gives a UV spectrum characteristic for 5,6,7- or 6,7,8-trisubstituted coumarins with maxima at λ_{max} 210, 230 and 330 nm (log ε 4.50, 4.18, and 3.92). As compared with the 6,7-disubstituted hydroxy- and methoxy coumarins, it shows a hypsochromic shift of the long-wave maximum and the disappearance of the maximum in the 280-300-nm region.

The IR spectrum of (I) (Fig. 1) shows absorption bands at 3420 cm^{-1} (OH group), 1702 cm^{-1} (C=O of a δ lactone), and 1618, 1575, and 1510 cm^{-1} (aromatic system). In the IR spectrum of obtusifol acetate, the band of the hydroxy group has disappeared and the broader band of the carbonyl groups of a δ lactone and of an acetyl group can be seen at 1710-1722 cm^{-1}. Obtusifol gives no reaction with ferric chloride, which excludes the presence of phenolic hydroxyls in it. The hydroxy group is obviously tertiary, since (I) is not oxidized by chromium trioxide. However, when it was subjected to prolonged heating m pyridine with acetic anhydride, obtusifol monoacetate (II) with mp 187-188°C was obtained.

When (I) was treated with hydrobromic acid, a substance (III) with the composition $C_{10}H_8O_5$ mp 195-196°C was isolated from the reaction products. This substance gives UV and IR spectra that are characteristic for trisubstituted coumarins. UV spectrum of (III): λ_{max} 210, 338 nm (log ε 4.47, 4.20). In the IR spectrum of (III) the C=O absorption band of a δ lactone is in the region of lower frequencies (1690 cm^{-1}), which is characteristic of hydroxy- and methoxycoumarins. A broad band of hydroxy groups (3300-3400

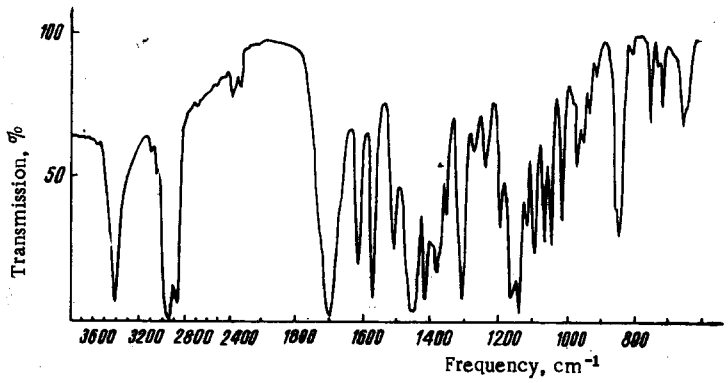

Fig. 1. IR spectrum of obtusifol.

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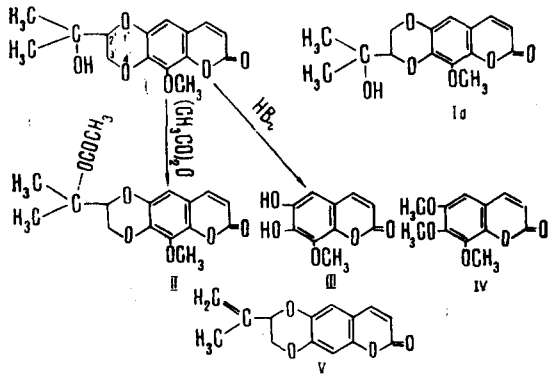
cm^{-1}) shows the presence of free and bound OH groups. On reaction with ferric chloride, substance (III) gives a green color which is characteristic of o-dihydroxybenzenes. All that has been said above permits the conclusion that (III) is 6,7-dihydroxy-5-hydroxymethoxycoumarin or the corresponding 8-methoxy compound. The structure of 7,8-dihydroxy-6-methoxycoumarin is excluded, since the melting point of this compound (fraxetin) is 227-228°C [4].

This structure is confirmed by the production on the methylation of (III) with methyl iodide of a trimethoxycoumarin (IV), $C_{12}H_{12}O_5$ with mp 104-104.5°C. The IR spectrum of (IV) lacks the absorption band of a OH group and has the absorption bands of a δ-lactone C=O (1725 cm^{-1}) and of an aromatic ring (1600, 1570, and 1500 cm^{-1}). From its melting point (IV) corresponds to 6,7,8-trimethoxy coumarin (literature data: mp 104°C) [4]. It follows from this that the treatment of obtusifol with hydrobromic acid splits off a $C_5H_9(OH)$ chain and 6,7-dihydroxy-8-methoxycoumarin is formed. Consequently, obtusifol is a derivative of 6,7,8-trihydroxymarmin.

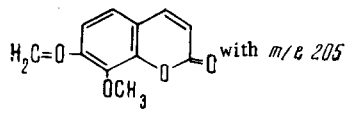
The negative reaction of (I) with ferric chloride shows that all the hydroxyls in the nucleus are etherified. The methoxy group is present at position 8 of (I), and the hydroxyls in positions 6 and 7 form a cyclic dioxane system with the $C_5H_9(OH)$ chain. On the basis of the results of chemical and spectral investigations, one of the two structures (I) or (Ia), which are close to the structure of the natural coumarin obliquin (V) [5, 6] is proposed for obtusifol.



The results of a study of the NMR spectra of obtusifol and the products of its cleavage confirm the proposed structure (I or Ia). The NMR spectrum of (I) (Fig. 2a) has a series of peaks denoted in Fig. 1 by a, b, c, d, e, g, h. The doublets *a* (7.56 ppm, J = 10 Hz) and c (6.24 ppm, J = 10 Hz) relate to the protons in positions 4 and 3 of the coumarin nucleus. The singlet b (6.48 ppm) relates to a proton in position 5 or 8. Consequently, the structure of obtusifol can be represented as a 5,6,7- or a 6,7,8-trisubstituted coumarin. Peak e (3.88 ppm, 3H) is due to the protons of a methoxy group. The singlet signals h (1.36 and 1.42 ppm, intensities of 3 proton units each) correspond to a gem-dimethyl group. The singlet signal g (2.95, 1H) is caused by the proton of a hydroxy group. The multiplet d in the range from 3.92 to 4.70 ppm with an intensity of 3 proton units may be assigned to the methine and methylene protons in the $-O-CH-CH_2-O-$ grouping. This agrees well with literature information for a compound with a structure close to that of obliquin [6].

The NMR spectrum of obtusifol acetate shows the peak of the acetate methyl group (2.03 ppm) and the signal of a proton of the OH group has disappeared (Fig. 2b). In the NMR spectrum of the trimethoxycoumarin (IV) there are doublets at 7.60 ppm, J = 10 Hz, and 6.33 ppm, J = 10 Hz, corresponding to protons in positions 4 and 3 of the coumarin nucleus and a singlet signal at 6.66 ppm for the proton in position 5. Furthermore, three singlet signals are observed at 4.04, 3.98 and 3.89 ppm (each with an intensity of three proton units) which are due to three methoxy groups in positions 6, 7, and 8 (Fig. 2c).

Thus, the results of a chemical investigation and of studies of the UV, IR, and NMR spectra has permitted the structure of 2'-(1-hydroxy-1-methylethyl)-8-methoxy[1,4]dioxano[g]coumarin (I) or (Ia) to be proposed for obtusifol. The choice between (I) and (Ia) can be made after the synthesis of obtusifol has been performed. By analogy with obliquin, for which the definitive structure (V) has been proposed on the basis of the results of synthesis [6], it may be assumed that structure (I) is more probable. The results of a study of the mass spectrum of (I) and a comparison of it with literature data for obliquin is also in favor of structure (I). The mass spectrum of obtusifol has the strong peak of the molecular ion with m/e 292 (97%) and ions with m/e 277 (11%), 234 (100%), 59 (32%), and 205 (45%). The latter is formed by the cleavage of the bond of the dioxane ring and the splitting out of $(CH_3)_2-C(OH)-C=O$ from the coumarin nucleus with the formation of the fragment



The mass spectrum of obtusifol acetate has a strong peak of the molecular ion with m/e 334.

Obtusifol—a coumarin with a dioxane ring—we have found for the first time in plants of the family Rutaceae. Obliquin, with a structure close to that of obtusifol, we isolated from Ptaeroxylon obliquum [5, 6]. The inclusion of this plant in a particular family has not yet been definitively established. It is assumed that this genus belongs to the family Sapindaceae or Meliaceae. Dean and Parton [5] have not excluded a connection of this genus with the family Rutaceae, since the presence of esculetin derivatives is characteristic for plants of the family Rutaceae. However, no chromones found together with obliquin in Ptaeroxylon obliquum have been detected in plants of the family Rutaceae. Consequently, these authors [5] considered

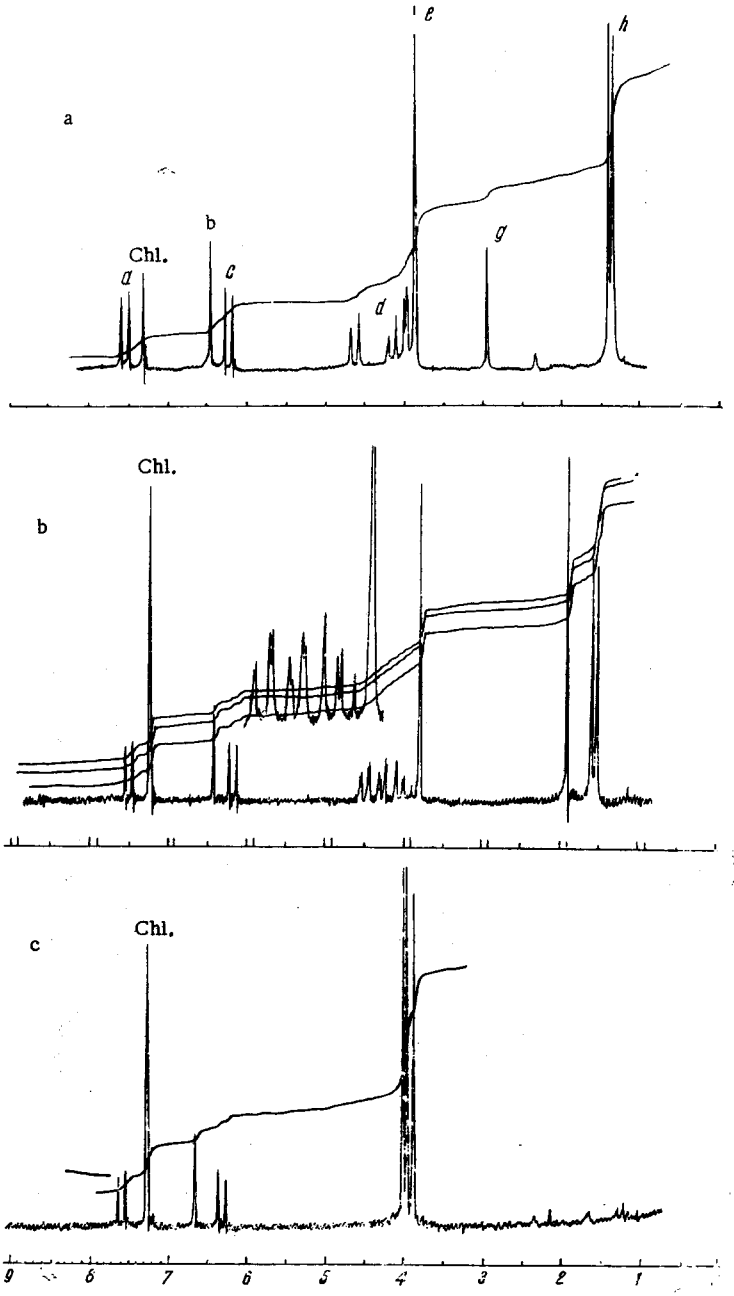


Fig. 2. NMR spectra of obtusifol (a), obtusifol acetate (b), and 6,7,8-trimethoxymarmin (c).

that this is insufficient for assigning the genus Ptaeroxylon to the family Rutaceae. The series of esculetin derivatives that we have found in plants of the genus Haplophyllum [1-3] and, in particular, obtusifol, with a structure similar to that of obliquin, form evidence in favor of the assignment of the genus Ptaeroxylon to the family Rutaceae and show the closeness of the genus Ptaeroxylon to the genus Halophyllum.

EXPERIMENTAL

Chromatography was performed on neutral alumina (activity grade III) and on type "B" ["fast"] paper of the Leningrad mill with ethylene glycol as the stationary phase and petroleum ether as the mobile phase (system 1), and also with formamide as the stationary phase and benzene as the mobile phase (system 2). The UV spectra were taken on a SF-16 spectrophotometer, the IR spectra were obtained by T. V. Bukreeva on a UR-10 instrument and the NMR spectra by V. A. Gindin on a Varian HA, 100 MHz, instrument in deuterochloroform. The chemical shifts in the δ scale were calculated relative to HMDS. The mass spectra were taken by B. V. Rozynov on a MKh-1303 mass spectrometer at 70-100°C (direct introduction at an energy of the ionizing electrons of 70 eV).

The elementary analyses were performed by E. A. Sokolova. The plant material of Haplophyllum obtusifolium was gathered in the foothills of the Kopet-Dagh range in the Ashkhabad region in the flowering period.

Isolation of Obtusifol (I). A concentrated acetone extract (33.5 g of resin) from the epigeal mass (3kg) of H. obtusifolium was separated on alumina (1100 g). Fractions 1-15 were eluted with petroleum ether, fractions 16-28 with petroleum ether–chloroform (2 : 1), fractions 29-38 with petroleum ether–chloroform (1 : 1), and finally fractions 39-51 with chloroform. The volume of each fraction was 200 ml. Fractions 39-51 yielded obtusifol with mp 149.5-150°C (after 4 recrystallizations from benzene and 2 from chloroform and petroleum ether).

The reaction of (I) with ferric chloride was negative: R_f 0.77 (system 2): deep-blue fluorescence in UV light, $[\alpha]_D^{19}$ +60.39° (c 1.45; ethanol).

Found %: C 61.44; 61.50; H 5.38; 5.42, mol. wt. 292 (mass spectrometry). $C_{15}H_{16}O_6$. Calculated %: C 61.64; H 5.47; mol. wt. 292.

Obtusifol Acetate (II). A mixture of 0.3 g of obtusifol, 2 ml of acetic anhydride and 2 ml of pyridine was heated for 5 h. Then it was cooled, diluted with water (50 ml), and extracted with chloroform (5 × 30 ml). The chloroform extracts were washed with water and dried. Then the chloroform was distilled off and the residue was chromatographed on alumina. Elution was performed with chloroform, and the solvent was distilled off. After recrystallization from chloroform and petroleum ether, obtusifol acetate (II) was obtained with mp 187-188°C, R_f 0.91 (system 2) and 0.15 (system 1) with a blue fluorescence in UV light. The elementary analysis and the molecular weight of 334 (mass spectrometry) corresponded to a molecular formula for obtusifol acetate of $C_{17}H_{18}O_7$.

Action of HBr on Obtusifol. A solution of obtusifol (0.6 g) in 5 ml of concentrated HBr was heated at the boiling point for 10 min and was then poured into water containing ice and was extracted with chloroform. The chloroform extract was dried and the solvent was distilled off. Recrystallization of the residue from chloroform and petroleum ether yielded substance (III) with mp 195-196°C, giving no green coloration with ferric chloride and having, according to elementary analysis, the molecular formula $C_{10}H_8O_5$, i.e., 6,7-dihydroxy-8-methoxycoumarin.

6,7,8-Trimethoxycoumarin (IV). To 0.06 g of (III) in 5 ml of ethanol were added 0.6 g of potassium acetate and 0.2 ml of methyl iodide and the mixture was heated for 12 h. After the end of the reaction, the mixture was diluted with water and extracted with chloroform. The chloroform extract was dried, the solvent was distilled off, and the residue was chromatographed on silica gel (30 g). Elution with chloroform yielded substance (IV) with mp 104-104.5°C (after recrystallization from chloroform and petroleum ether). According to elementary analysis, (IV) has the molecular formula $C_{12}H_{12}O_5$ and its melting point corresponds to that of 6,7,8-trimethoxycoumarin (literature data: mp 104°C [4]). The melting point of 5,6,7-trimethoxycoumarin is 76-77°C [7].

CONCLUSIONS

A new coumarin obtusifol, $C_{15}H_{16}O_6$, with mp 149.5-150°C has been isolated from Haplophyllum obtusifolium for the first time.

The structure of 2'- or 3'-(1-hydroxy-1-methylethyl)-8-methoxy[1,4]dioxano[g]coumarin has been proposed for obtusifol.

It has been shown that the genus Haplophyllum is close in respect of its coumarin composition to the genus Ptaeroxylon.

LITERATURE CITED

1. G. A. Kuznetsova and N. F. Gashimov, Abstracts of Lectures at an International Symposium on "The Chemistry of the Natural Compounds of the Balkan Flora," Bulgaria (1971), p. A22.
2. G. A. Kuznetsova and N. F. Gashimov, Khim. Prirodn. Soedin., 666 (1972).
3. G. A. Kuznetsova and N. F. Gashimov, Khim. Prirodn. Soedin., 113 (1973).
4. G. A. Kuznetsova in: Natural Coumarins and Furocoumarins [in Russian], Leningrad (1967), p. 81.
5. F. M. Dean, and D. A. H. Taylor, J. Chem. Soc., (C), 114 (1966).
6. F. M. Dean and B. Parton, J. Chem. Soc. (C), 526 (1969).
7. E. Spath, and Z. Jermanowska, Ber. 70, 698 (1937).