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PHENOLIC COMPONENTS OF *Empetrum nigrum* EXTRACT AND THE CRYSTAL STRUCTURE OF ONE OF THEM

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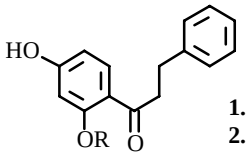
Four phenolic components are isolated from the $CHCl_3$ extract of Empetrum nigrum *L. Three of them are known from this plant. The fourth (6,8-dimethylpinocembrine) is isolated from crowberry for the first time. The previously proposed structure for 2′-methoxy-4′-hydroxy-α,β-dihydrochalcone is confirmed by x-ray structure analysis.*

Key words: *Empetrum nigrum* L., 6,8-dimethylpinocembrine, 2′,4′-dihydroxychalcone, 2′,4′-dihydroxy-α,β-dihydrochalcone, 2′-methoxy-4′-hydroxy-α,β-dihydrochalcone, x-ray structure analysis.

Black crowberry (*Empetrum nigrum* L., Empetraceae) is a small bush that is widely distributed in the northern hemisphere [1] and in the Russian Federation [2]. Its aerial part (shoots) is used in folk medicine to cure liver and kidney disease. Its extracts exhibit diuretic and spasmolytic properties [3]. Certain chemical components of crowberry possess antibacterial and fungicidal activity [4]. The chemical composition of this plant is rather complicated. It includes alkanes [5], flavonoids [6], cycloalkanes [7], aliphatic ethers [8], bisbenzyl and 9,10-dihydrophenanthrene derivatives [4, 9], chalcones and dihydrochalcones [9], and dihydrostilbenes [10]. In continuation of studies on the chemical composition of the aerial part of this plant, we isolated from the acetone-soluble part of the $CHCl_3$ extract the previously described 2′,4′-dihydroxychalcone, 2′,4′-dihydroxy-α,β-dihydrochalcone* (**1**), and two other phenolic compounds. One of them was 6,8-dimethyl-5,7-dihydroxyflavanone (demethoxymatteucinol, 6,8-dimethylpinocembrine), which was previously unknown from crowberry. Its structure was elucidated by spectra data [12] and an x-ray structure analysis [13]. It was previously found in leaves of *Ceratiola ericoides* [13].



1. R=H
2. R=Me

The second compound corresponds in melting point and spectral data with a component of crowberry that was proposed as 2′-methoxy-4′-hydroxy-α,β-dihydrochalcone (**2**) [9] on the basis of mass, UV, and PMR spectra. The shape and multiplicity of the signals for H-3′ and H-5′ remained unknown owing to the low working frequency of the NMR spectrometer that was used (200 MHz). In order to prove unambiguously the correctness of the proposed structure, we performed an x-ray structure analysis of this compound. The structure found corresponds to that previously proposed [9] and is shown in Fig. 1.

*The conventional atomic numbering for chalcone is used [11].

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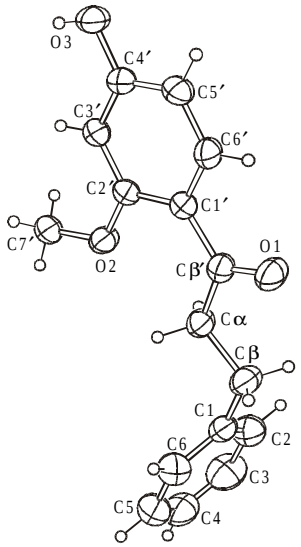

Fig. 1. Crystal structure of compound **2**.

The molecule of **2**, except for the unsubstituted phenyl ring, is practically planar (±0.059 Å). The dihedral angle between the planes of the two phenyl rings is 80.3(1)°. The bond lengths correspond to the expected values [14] and are very close to the analogous ones in 2′,4′-dihydroxy-4,6′-dimethoxy-α,β-dihydrochalcone [15]. Molecules of compound **2** in the crystal are connected by H-bonds.

The contents of dihydrochalcones **1**, **2**, and 2′,4′-dihydroxychalcone in the acetone-soluble part of the $CHCl_3$ extract of black-crowberry shoots are 5.2, 2.9, and 5.4%, respectively, according to HPLC using markers of these compounds. The retention coefficients (k') are 4.78, 3.56, and 5.69.

EXPERIMENTAL

General Comments. NMR spectra were recorded on a Bruker DRX-500 spectrometer (working frequency 500.13 MHz for ^1H and 125.76 MHz for ^{13}C, $CDCl_3$ solvent, δ-scale). Mass spectra were obtained on Finnigan MAT 8200 (for compound **2**) and LKB-2091 instruments.

HPLC was carried out on a Millichrom (Ob'-4) microcolumn liquid chromatograph equipped with a 64×2 mm column packed with 5-μm Lichrosorb RP-18 (Merck) stationary phase. The mobile phase was a mixture of MeOH and 0.05 M aqueous H_3PO_4 (85:15, respectively, by volume). The detector operated at 200 nm.

The aerial part of black crowberry was collected in July, 1995, in Kosh-Agachskii region of the Altai Republic and was ground and dried in air.

Isolation of Dihydrochalcone 2 and Accompanying Substances. Ground and air-dried shoots of crowberry (2 kg) were extracted with $CHCl_3$ (3×6 l) with heating (~60°C). The combined extracts were filtered and evaporated to dryness. The solid (200 g) was washed with acetone (2 l). The insoluble part (60 g) was filtered off. The acetone was evaporated to give a dark brown syrupy mass (140 g), part of which (120 g) was chromatographed on SiO_2 (1.5 kg). The eluent was a hexane—chloroform mixture with a constantly increasing content of the latter (from 40 to 100%). Fractions were successively isolated, from which crystallization from $CHCl_3$ gave 6,8-dimethylpinocembrine (0.08 g, 0.07%), substance **2** (0.32 g, 0.27%), substance **1** {0.40 g, 0.33%, mp 62-63°C (hexane), lit. 80-81°C (aqueous ethanol) [9]}, and 2′,4′-dihydroxychalcone {0.43 g, 0.36%, mp 140-142°C, lit. 145-146°C (benzene) [9]}

6,8-Dimethylpinocembrine (6,8-dimethyl-5,7-dihydroxyflavanone). Light-yellow crystals, mp 200-201°C (lit. 202-204°C [16]). UV spectrum (C_2H_5OH, λ_{max}, nm): 210, 300, 350 (log ε 4.5, 4.3, 3.6). Mass spectrum (EI, 70 eV) *m*/*z* (I_{rel}, %): 284 (M^+, 100), 180 (92), 152 (77). The PMR spectrum corresponds to that published [12, 13].

2′-Methoxy-4′-hydroxy-α,β-dihydrochalcone (2). Colorless crystals, mp 135-136°C (lit. 130-131°C [9]). UV spectrum (C_2H_5OH, λ_{max}, nm): 230, 269, 304 (log ε 4.7, 4.7, 4.6). Mass spectrum *m*/*z* (%): 256 (M^+, 25), 151 (100), 91 (6).

TABLE 1. Coordinates ($\times 10^4$) and Equivalent Isotropic Parameters ($Å^2 \times 10^3$) of Nonhydrogen Atoms in **2**

Atom	*x*	*y*	*z*	U_{eq}
C1	5359(1)	3241(8)	3960(4)	69(1)
Cβ	5875(1)	4267(9)	4198(4)	78(1)
Cα	6241(1)	2779(8)	3345(4)	65(1)
Cβ′	6768(1)	3662(8)	3547(3)	62(1)
C2	5151(2)	1310(10)	4739(5)	92(1)
C3	4671(2)	329(13)	4472(7)	119(2)
C4	4424(2)	1325(18)	3455(8)	123(2)
C5	4629(2)	3187(18)	2699(6)	130(2)
C6	5096(2)	4168(12)	2939(5)	103(2)
C1′	7178(1)	2350(8)	2838(3)	59(1)
C2′	7142(1)	299(7)	1883(3)	57(1)
C3′	7555(1)	-734(7)	1296(3)	61(1)
C4′	8019(1)	235(8)	1636(4)	61(1)
C5′	8067(1)	2306(9)	2547(3)	68(1)
C6′	7651(1)	3292(8)	3128(4)	67(1)
C7′	6627(1)	-2530(9)	566(4)	73(1)
O1	6858(1)	5492(6)	4320(3)	85(1)
O2	6680(1)	-627(6)	1576(3)	77(1)
O3	8432(1)	-753(7)	1085(3)	77(1)

High resolution mass spectrometry gave *m*/*z* 256.11035; calc. for $C_{16}H_{16}O_3$, 256.10994.

PMR (δ, ppm, J, Hz): 6.48 (1H, dd, H-5′, J = 8.7 and 2.4), 6.55 (1H, d, H-3′, $J_{3',5'}$ = 2.4), 7.64 (1H, d, H-6′, $J_{5',6'}$ = 8.7).

^{13}C NMR: 30.62 t, 45.10 t, 55.38 q, 98.94 d, 107.87 d, 120.50 s, 125.76 d, 128.27 d, 128.33 d, 132.88 d, 141.71 s, 161.19 s, 161.30 s, 200.14 s.

X-ray structure determination of compound 2 was performed on a Syntex $P2_1$ diffractometer (Cu Kα-radiation, graphite monochromator, 2θ/θ-scanning in the range $2\theta < 140^\circ$). A crystal of dimensions 0.2×0.3×1.0 mm was used. The crystals are orthorhombic, *a* = 27.183(5), b = 4.755(1), c = 10.607(2) Å, V = 1371.0(5) $Å^3$, space group $Pca2_1$, Z = 4, $C_{16}H_{16}O_3$, d_{calc} = 1.242 g/cm^3, μ = 0.689 mm^{-1}. Intensities of 1375 independent reflections were measured. Corrections for absorption were made taking into account the actual dimensions of the crystal (transmission 0.76-0.91). The structure was solved by direct methods using the SHELXS-86 programs [17]. Positions of H atoms were calculated geometrically. Final refinement of the structure factors for all F^2 using full-matrix anisotropic (isotropic for H) least-squares methods and the SHELXL-93 programs [18] gave wR_2 = 0.1215 (R = 0.0445 for 1074 $F > 4\sigma$). Atomic coordinates and equivalent thermal factors for nonhydrogen atoms are listed in Table 1.

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