

NAPHTHAZARINS FROM *ONOSMA HETEROPHYLLA*

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ABSTRACT.—Four naphthazarins were isolated from the roots of *Onosma heterophylla*. Three of these have been encountered before. The fourth pigment was characterized by chemical and spectroscopic methods as the 5,8-dihydroxy-2-(1'- β,β -dimethylacryloyloxy-4'-acetoxy-4'-methyl)pentyl-1,4-naphthalenedione [**1**].

Onosma heterophylla Griseb. (Boraginaceae) is a shrub that grows wild in northern Greece. The Boraginaceae family is known to yield alkannin and its esters (1). These esters have been reported to display a remarkable ability in regenerating necrotic tissue and are regarded as a new class of drugs (2,3). The biological importance of these compounds prompted us to examine various species of the Boraginaceae. In this paper we report on the isolation and structure elucidation of a new isohexylnaphthazarin, 5,8-dihydroxy-2-(1'- β,β -dimethylacryloyloxy-4'-acetoxy-4'-methyl)pentyl-1,4-naphthalenedione [**1**], in addition to three known naphthazarins.

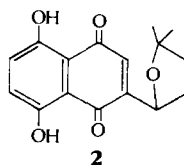
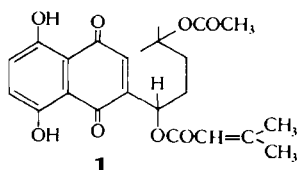
RESULTS AND DISCUSSION

The residue from a hexane extract of the dried roots of *O. heterophylla* was extracted with MeOH, and the pigments in this extract were separated from the interfering fluorescent compounds by precipitation as insoluble Cu-chelates. The Cu-complexes were decomposed with dilute HCl to give the free pigments. The mixture of the pigments was fractionated on Si gel columns.

Repeated cc afforded three known naphthazarins: deoxyalkannin mp 94-95° [lit. mp 95° (4)], alkannin β,β -dimethylacrylate mp 115-116° [lit. mp 116-117° (5)], and alkannin isovalerate mp 93-94° [lit. mp 94-95° (6)], which were identified by comparing ir, nmr, and mass spectral data (4,6,7).

Further fractions from the column gave a deep red pigment. Elemental analysis for C, H, and N indicated absence of nitrogen, and the analytical data obtained conform with the formula C₂₃H₂₆O₈. Absorption bands in the ir spectrum indicated the presence of free and bonded -OH and carbonyls belonging to ester, α,β -unsaturated ester, and chelated quinone. Additional information obtained from the ¹H-nmr spectrum suggested that the most likely structure of the new pigment is **1**. In fact, the ¹H-nmr (CDCl₃) spectrum was characterized by two singlets at δ 12.20 and 12.30, D₂O exchangeable, for two chelated phenolic hydroxyl protons and a one proton doublet at δ 7.00 (*J*, 1.1 Hz) for the quinone proton. A two proton singlet at δ 7.16 was assignable to two equivalent aromatic protons.

In addition to the above mentioned signals, the ¹H-nmr spectrum showed a singlet (6H) at δ 1.50 (>CMe₂), two singlets (3H each) at δ 1.60 and 1.70 (=CMe₂), a singlet (3H) at δ 2.00 (-OCOMe), two multiplets (2H each) at δ 2.25 and 2.40 for methylene protons of the side chain, a singlet (1H) at δ 5.95 (vinylic proton of the acrylic acid moiety), and a triplet (1H) at δ 6.04 (methine proton α to the quinone ring).



The mass spectrum was in accordance with the suggested structure. The loss of CH_3COOH from the molecular ion M^+ 430 results in the formation of alkannin β,β -dimethylacrylate. Subsequent fragmentation follows the pattern exhibited by alkannin and its β,β -dimethylacrylic ester (7).

Further confirmation for the proposed structure was obtained by alkaline hydrolysis of **1** affording hydroxyalkannin that then under treatment with HCl in dry C_6H_6 yielded the tetrahydrofuran **2** (8).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were taken on a Kofler hot stage and are uncorrected. Ir spectra were recorded with a Perkin Elmer 567 spectrophotometer. ^1H -nmr spectra were obtained with a Brüker-Physic HF80s spectrometer with TMS as an internal standard. Mass spectra were run on a Varian MAT 311A spectrometer at an ionizing voltage of 70 eV. Pigments were chromatographed on Kieselgel 60, 70-230 mesh (Merck); tlc was performed on Kieselgel 60F₂₅₄ plates (Merck) with C_6H_6 - CHCl_3 - Me_2CO (50:50:1) as the developing solvent.

PLANT MATERIAL.—The plant material was collected in the summer of 1978 from the south coast of the lake M. Prespa, Northern Greece, and a specimen has been deposited at GZU, Herbarium des Institutes für Botanik der Universität Graz, Austria. Its identity was established morphologically, anatomically, and by chromosomal examination and was shown to be *O. heterophylla* (chromosomal number $2n=26$, $n=13$).

EXTRACTION AND ISOLATION.—Air-dried roots (800 g) were extracted thoroughly with hexane at room temperature. The solvent was removed at reduced pressure at a temperature below 50° . The dark viscous residue was extracted with cold MeOH to separate the soluble pigments and fluorescing compounds from the insoluble waxes. The methanolic solution was then treated with cupric acetate, whereupon the pigments precipitated as insoluble Cu-chelates. These were washed exhaustively first with hexane and then with MeOH to ensure removal of fluorescing impurities. The mixture of free pigments, obtained by decomposition of the purified Cu-complexes with 10% HCl, was chromatographed on Si gel. The column was eluted successively with hexane and hexane/ C_6H_6 mixtures of increasing polarity and finally with C_6H_6 . The hexane fraction was a mixture of three compounds, deoxyalkannin, alkannin β,β -dimethylacrylate, and alkannin isovalerate, which were isolated by repeated cc. Elution with hexane- C_6H_6 (1:4) afforded the new pigment **1**.

5,8-DIHYDROXY-2-(1'- β,β -DIMETHYLACRYLOYLOXY-4'-ACETOXY-4'-METHYL)PENTYL-1,4-NAPHTHALENEDIONE [1].—It was isolated as a deep red viscous solid that could not be induced to crystallize. Rf 0.18. Found: C, 63.70; H, 6.18% ($\text{C}_{23}\text{H}_{26}\text{O}_8$ required; C, 64.18; H, 6.09%). ^1H nmr (80 MHz, CDCl_3): see text; ir ν max (Nujol) cm^{-1} 3450 and 3280 (free and bonded -OH), 1740 (CO, ester), 1700 and 1140 (α,β -unsatur. ester), 1610 (CO, quinone); eims m/z (%) 430 (M^+ , 5), 370 (5), 270 (100), 255 (99), 229 (33), 227 (28), 220 (92), 219 (71), 191 (24), 190 (20).

5,8-DIHYDROXY-2-(5'- β,β -DIMETHYL-2'-TETRAHYDROFURYL)-1,4-NAPHTHALENEDIONE [2].—It was obtained from **1** according to the literature procedure (8) and crystallized from hexane as light red needles mp $89-90^\circ$ (lit. 91°); Rf 0.38. It was identified by comparing ir, nmr, and mass spectral data (8).

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