

5,8-Dihydroxy-2-(4-methylpent-3-enyl)-1,4-naphthoquinone and its 2-[4-Methyl-1-(2-methylcrotonoyloxy)pent-3-enyl] Analogue (Shikonin Angelate) from *Alkanna hirsutissima*

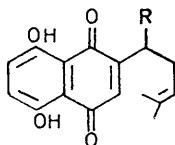
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The title compounds have been isolated and their structures established.

Alkanna hirsutissima (Boraginaceae) grows abundantly in Northern Iraq and its presence has also been reported all along the Mediterranean coast and in the Syrian deserts. The Boraginaceae family is known to yield shikonin (III) and its esters.¹⁻⁵ We have now isolated and characterised two metabolites of this plant which have not been reported previously.

The crude pigment extract was fractionated on silica gel columns. Early fractions furnished a mixture of two compounds which could not be separated by t.l.c. They were eventually separated by repeated column chromatography.

The first pigment, C₁₆H₁₆O₄, was optically inactive and showed a u.v.-visible absorption spectrum similar to that of naphthazarin.⁶ Its i.r. spectrum showed bands at 1660 and 1615 cm⁻¹, expected for quinonoid C=C and hydrogen bonded C=O groups. Its n.m.r. spectrum showed two one-proton singlets τ -2.45 and -2.35, removed by D₂O and assigned to chelated phenolic hydroxy-functions. Aromatic (2H) and quinone proton (1H) signals appeared at τ 2.7 and 3.1, respectively. A broad triplet (1H) at τ 4.8 could be attributed to an olefinic proton of the isopentenyl side chain and a multiplet (4H) at τ 7.5 indicated methylene protons coupled and deshielded by olefinic groups. Two singlets (each 3H) at τ 8.3 and 8.4 confirmed the presence of the isopentenyl side chain. The compound, therefore, has structure (I). This was supported by the mass spectrum, which showed the expected fragmentations involving the isopentenyl side chain. Compound (I) was also obtained by removing the hydroxy-group from the side chain of shikonin (III) with Raney nickel.



(I) R = H

(II) R = MeCH: CMe·CO·O

(III) R = OH

The second pigment showed u.v.-visible and i.r. absorptions similar to the first. Its n.m.r. spectrum

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¹ R. Mamima and S. Kuroda, *Acta Phytochim.*, 1922, **1**, 43.

² A. S. Romanova and A. I. Ban'Kovskii, *Khim. prirod. Soedinenii*, 1965, 266.

³ A. S. Romanova, N. V. Tareeva, and A. I. Ban'Kovski, *Khim. prirod. Soedinenii*, 1967, 71.

⁴ S. Miura, *Syoyakugaku Zasshi*, 1963, **17**, 45.

showed τ -2.4 and -2.6 (each 1H, s, exchangeable, phenolic OH), 2.9 (2H, s, aromatic), 3.1 (1H, d, J 2 Hz, quinone H), 3.96 [2H, distorted q, benzylic and olefinic (ester) protons], 4.8 (1H, t, Me₂C:CH), 7.4-7.9 (8H, complex, C:C-CH₂ and MeCH:CMe), and 8.02 (6H, 2s, Me₂C), in agreement with structure (II) (shikonin angelate). This assignment was confirmed by alkaline hydrolysis to give shikonin (III) and angelic acid, identical with authentic samples. Alkanin angelate (the enantiomer of shikonin angelate) has been isolated by Brand *et al.*⁷

EXPERIMENTAL

N.m.r. spectra were determined for solutions in CDCl₃ with a Varian A60 instrument. Mass spectra were measured on an A.E.I. MS9 spectrometer at 70 eV with source temperature 250 °C. U.v. spectra refer to ethanolic solutions and i.r. spectra to solutions in chloroform. R_F Values refer to t.l.c. on Kieselgel G (Merck) in benzene-methanol-acetic acid (20 : 2 : 1 v/v). Light petroleum had b.p. 40-60 °C.

Extraction and Purification of the Pigments.—Finely milled roots of *Alkanna hirsutissima* (1 kg) were extracted (Soxhlet) until no more pigment was removed. The extracts were evaporated to give a red viscous oil (5.2 g), which was subjected to gradient elution on an acid-washed silica gel column. Elution with light petroleum gave a red gum which was further purified on a series of columns (very slow elution). The resulting bright red gum crystallised from n-hexane as fine red needles (57 mg) of 5,8-dihydroxy-2-(4-methylpent-3-enyl)-1,4-naphthoquinone (I), m.p. 92-93°; R_F 0.77 (red spot) (Found: C, 70.5; H, 5.7%; M^+ , 272. C₁₆H₁₆O₄ requires C, 70.6; H, 5.8%). Resonance stabilisation of an allyl cation leads to β -fission⁸ and this resulted in a base peak at m/e 41 in the mass spectrum. A major peak at m/e 68 was a result of hydrogen transfer leading to the formation of methylbutadiene cation radical and 5,8-hydroxy-3-methylnaphthoquinone.

Later fractions from the original column contained impure shikonin angelate (II). This was further purified by column chromatography on acid-washed silica gel to furnish 1-(5,8-dihydroxynaphthoquinon-2-yl)-4-methylpent-3-enyl 2-methylcrotonate (II) as a viscous oil (1.3 g), R_F 0.77 (red spot) (Found: C, 68.0; H, 6.1%; M^+ 370. C₂₁H₂₂O₆ requires C, 68.1; H, 5.9%).

Alkaline Hydrolysis of Shikonin Angelate (II).—The pigment (II) (500 mg) was shaken with aqueous 2N-sodium

⁵ I. Morimoto, T. Kishi, S. Ikegami, and Y. Hurata, *Tetrahedron Letters*, 1965, 4737.

⁶ I. Singh, R. T. Ogata, R. E. Moore, C. W. J. Chang, and P. J. Scheuer, *Tetrahedron*, 1968, **24**, 6053.

⁷ K. Brand and A. Lohmann, *Ber.*, 1935, **68**, 1487.

⁸ K. Biemann, 'Mass Spectrometry; Organic Chemical Applications,' McGraw-Hill, New York, 1962, p. 83.

hydroxide (300 ml) at 20 °C for 25 h. The blue solution was filtered, cooled in ice, and acidified with dilute sulphuric acid. The red suspension was extracted with chloroform (3 × 150 ml), which was then extracted with saturated aqueous sodium hydrogen carbonate. Acidification of the alkaline extract and extraction with ether gave an oil which crystallised from n-pentane as prisms of angelic acid, m.p. 46—47° (lit.,⁹ 46°), identical (i.r. spectrum) with an authentic sample.

The phenolic hydrolysis product was obtained as a red solid by evaporation of the chloroform solution remaining after alkaline extraction. Repeated crystallisation from ether-petroleum yielded shikonin as red prisms (150 mg), R_F 0.44 (red spot), m.p. 147—148° (lit.,⁴ 147°) (Found: C, 67.0; H, 5.5%; M^+ , 288. Calc. for $C_{16}H_{16}O_5$; C, 66.7; H, 5.6%), identical with an independently isolated product from *Jatropha glandulifera*.¹⁰

Partial Reduction of Shikonin.—Shikonin (200 mg) in dry

ethanol (150 ml) was heated under reflux in the presence of Raney nickel (W-2; 350 mg) for 4 h. The product was worked up in the usual way and chromatographed on an acid-washed silica gel column. Elution with light petroleum yielded a red gum which crystallised from n-hexane as fine needles (35 mg), m.p. and mixed m.p. 92—93°, identical (i.r. spectrum) with the natural product (I), in all respects.

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⁹ W. G. Young, R. T. Dillon, and H. J. Lucas, *J. Amer. Chem. Soc.*, 1929, **51**, 2528.

¹⁰ J. A. Ballantine, *Phytochemistry*, 1969, **8**, 1587.