The Biosynthesis of the Plant Phenalenone Haemocorin

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Summary The plant phenalenone, haemocorin, is shown to be derived from phenylalanine and tyrosine, in contrast to the known fungal phenalenones, which are biosynthesised *via* the acetate–polymalonate pathway.

HAEMOCORIN, a constituent of the rhizome of *Haemodorum corymbosum*, was the first phenalenone (perinaphthenone) derivative to be characterised from plant sources.[1] Two alternative schemes for the biosynthesis of the aglycone, (Ia)⇌(Ib), have been suggested; in one (Path a, Scheme), the carbon skeleton is derived from two C_6–C_3 units and one molecule of acetate,[2] whereas in the alternative mechanism (Path b, Scheme), the phenalenone nucleus is considered to arise primarily from acetate or malonate units,[3] *via* a pathway which parallels that previously established for the fungal phenalenone norherqueinone.[2]

Evidence supporting the derivation of haemocorin aglycone from C_6–C_3 units has now been obtained following labelled precursor feeding studies. Aqueous suspensions of freshly sliced rhizomes of *H. corymbosum* were incubated individually with solutions of [2-^{14}C]tyrosine, [1-^{14}C]- and [2-^{14}C]-phenylalanine, sodium [1-^{14}C]- and [2-^{14}C]-acetate, and sodium [^{14}C]formate. After 20 h at room temperature, the rhizome residues were extracted with methanol and the concentrates fractionated by preparative chromatography on Whatman 3MM paper (75% propanol in water). In addition to the orange haemocorin band (R_F 0·65), two other major orange components were detected (R_F 0·4 and 0·8) which gave an orange-green fluorescence in u.v. light, in contrast to the intense red-orange fluorescence of haemocorin. Radioautographs of both the [^{14}C]tyrosine- and the [^{14}C]phenylalanine-derived extracts, clearly showed activity coincident with the haemocorin band, although the incorporations into the two unknown orange components were significantly higher.

Haemocorin aglycone was also found to be present in the rhizome extract, appearing as a purple zone near the front of the chromatogram; this fraction was eluted with acetone, diluted with inactive aglycone, and then twice recrystallised from aqueous acetone. On assaying for ^{14}C-activity, the following efficiencies of incorporation of precursors into the undiluted aglycone fractions were calculated: [2-^{14}C]tyrosine, 0·50%; [1-^{14}C]- and [2-^{14}C]-phenylalanine, 0·30 and 0·29%, respectively; [1-^{14}C]- and [2-^{14}C]-acetate 0·09 and 0·12%, respectively; [^{14}C]formate 0·05%.

The C_6–C_3 pathway (Path a) requires that the aglycone, derived from [2-^{14}C]tyrosine, should be specifically labelled at C-5, the activity of which could be determined by utilising the established degradative sequence of Cooke and his collaborators.[1]

Two tautomeric structures for haemocorin aglycone, (Ia) and (Ib), are possible, and methylation has been shown to yield a mixture of the corresponding dimethyl ethers, (IIa) and (IIb).[1] The mixture obtained on methylation of the diluted [2-^{14}C]tyrosine-derived aglycone was found to resolve satisfactorily following t.l.c. on silica gel (10% phenol in benzene).

The identities of the radioactive methyl ethers (IIa) and (IIb) were confirmed by comparison with samples of the authentic compounds (supplied by Dr. R. G. Cooke), and by examination of their mass spectra, which were shown to differ in a highly characteristic manner. It was observed that the most abundant ion in the mass spectrum of the aglycone (determined by Dr. J. S. Shannon) corresponds to an $(M - 1)^+$ ion, possibly (III).[4] This could arise from a

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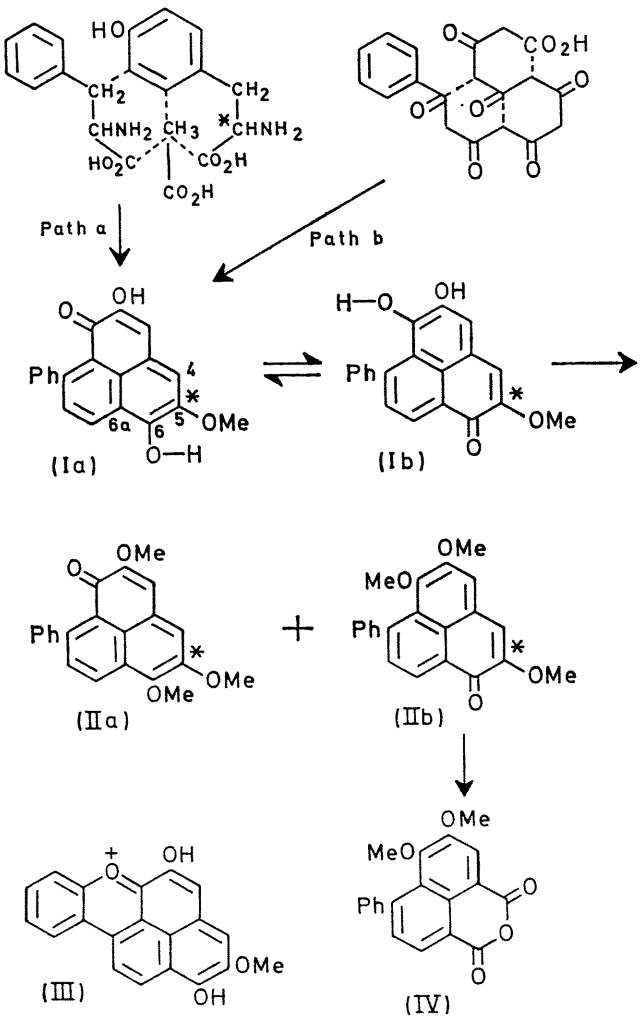

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hydrogen atom elimination initiated by the phenalenone carbonyl group, which is *peri* to the phenyl substituent in (Ia) but not (Ib), and accordingly it was observed that the mass spectra of (IIa) and (IIb) (determined by Dr. E. S. Waight) exhibited base peaks corresponding to $(M - 1)^+$ and M^+ ions, respectively. 1-Arylanthraquinones have similarly been shown to produce a characteristic $(M - 1)^+$ ion, apparently due to the loss of a hydrogen atom from the *ortho* position of the phenyl group,[5] while a compound closely resembling (III) is a probable intermediate in the formation of a photochemical oxidation product of the phenalenone lachnanthocarpone.[6]

Labelled methyl ether (IIa), after dilution with unlabelled (IIa) and repeated crystallisation to constant specific activity (429 d.p.m./mg), was oxidised to yield the naphthalic anhydride (IV)[1] (3 d.p.m./mg), thus specifically locating the [2-^{14}C]tyrosine-derived activity at C-5, as required by the C_6–C_3 pathway. Suitable small-scale degradations for examining the positions of labelling of the [1- and 2-^{14}C]phenylalanine-derived aglycones were not available, but the observed efficiencies of incorporation, which approached that of [2-^{14}C]tyrosine, were consistent with the predicted pathway. The incorporation of [2-^{14}C]-acetate was also in accord with Path a; however, the low activity precluded attempts to determine the predicted preferential labelling at C-6a, and since the apparent incorporation of [1-^{14}C]acetate was only slightly less under these feeding conditions, the present data do not allow an unequivocal assignment of the role of this precursor.

These results, in conjunction with the established polyketide origin of norherqueinone, support the view that the known plant and fungal phenalenones are biosynthetically unrelated.

I thank Dr. J. M. Edwards for informing me of his results, prior to publication, which confirm the operation of the C_6–C_3 pathway in the biosynthesis of the structurally related plant phenalenones of the genus *Lachnanthes* (Haemodoraceae).[7]

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