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THE BIOSYNTHESIS OF 7-OXYGENATED COUMARINS IN HYDRANGEA AND LAVENDER

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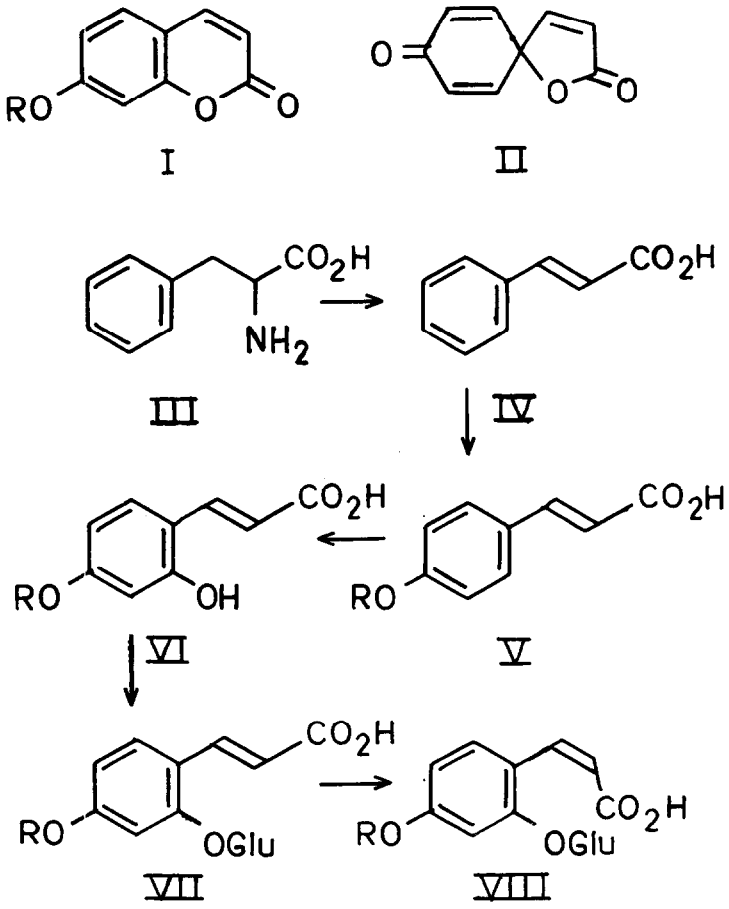
Recently there was reported an *in vitro* synthesis of 7-oxygenated coumarins by rearrangement of spirolactones such as (II), which was obtained from *cis*-p-hydroxycinnamic acid (1). These results were used to fortify a theory (2), developed from Haworth's earlier proposal (3), to explain the biosynthesis of 7-oxygenated coumarins from p-hydroxycinnamic acids. Isotopic tracer studies of the mould coumarin novobiocin have also given rise to the suggestion of an intermediate oxidative step either through a similar spirolactone or by a direct carboxyl attack *meta* to the established phenolic grouping (4). (See also (5)). All of these oxidative cyclisation theories demand the participation of a *cis* rather than a *trans*-cinnamic acid precursor.

We now present evidence (Table I) which negates this premise of the oxidative cyclisation theories, but accords rather with a generalisation of Brown's scheme (III→VIII) for the biosynthesis of herniarin (I; R=CH_3). This involves

ortho-hydroxylation of trans-p-methoxycinnamic acid followed by glucosylation and isomerisation of the double bond to give 2-glucosyl-4-methoxy-cis-cinnamic acid (VIII; R=CH_3) (6). This closely resembles the previously established path to coumarin (7).



The fact that p-hydroxycinnamic acid is a reportedly 'good' precursor for umbelliferone (I; R=H) in Hydrangea (8), combined with our finding of a high yield of umbelliferone from Hydrangea Macrophylla (var. 'Blue Wave') made this plant a particularly suitable choice for the present study. One-year old plants were wick fed with 1% aqueous solutions of the sodium salts of cis- (2-C^{14})--p-hydroxycinnamic acid, trans-(2-C^{14})-p-hydroxycinnamic acid, and trans-(2-C^{14})-cinnamic acid. The corresponding 2-C^{14}-labelled spirolactone (II) was wick fed as a 1% aqueous solution. Extraction of the fed stems and leaves followed by purification involving emulsin hydrolysis and thin-layer chromatography produced umbelliferone (identified with authentic material) which was counted as an infinitely thin layer.

TABLE I

Wick Feedings to Hydrangea Macrophylla (var. 'Blue Wave')

Compounds fed (activity - cpm/mmole)	Cpm fed	% Incorporation into umbelliferone 1 day	3 days	5 days
trans-p-hydroxy-cinnamic acid (1.60×10^{6})	1.97×10^{4}	4.0, 2.6	4.2, 2.7	4.9, 5.1
cis-p-hydroxy-cinnamic acid (1.60×10^{6})	1.95×10^{4}	0.49 -	0.33, 0.26	0.65, 1.16
Spirolactone (II) (1.19×10^{6})	1.40×10^{4}	0.062	0.094	0.146
trans-cinnamic acid (1.82×10^{6})	2.35×10^{4}	0.24	-	0.33

These results demonstrate in a gratifyingly clear-cut manner that trans-p-hydroxycinnamic acid proved to be an excellent precursor for umbelliferone whereas cis-p-hydroxycinnamic acid was only about one-seventh as efficient. This latter incorporation may in fact be the result of isomerisation (light-induced or otherwise) to the trans-acid, probably in the leaves. The spirolactone (II), a theoretical intermediate in the Grisebach and Ollis theory (2), was still less efficient, with an incorporation about one-fortieth of the trans-acid. It is possible that the incorporation of the spirolactone may be due to prior hydrogenolysis of this compound to cis-p-hydroxycinnamic acid since examination of the p-hydroxycinnamic acid isolated from these feedings showed appreciable radioactivity (TABLE 2).

TABLE 2

% Incorporation of Radioactivity into p-Hydroxycinnamic Acid

Compounds fed	1 day	3 days	5 days
trans-p-hydroxy-cinnamic acid	8.1, 18.9	-, 6.9	12.4 12.5
cis-p-hydroxy-cinnamic acid	8.9, 12.9	15.8, 13.2	19.2, 22.4
Spirolactone (II)	6.7	3.6	5.9

The observed rapid binding of administered hydroxycinnamic acids as glucose esters and glucosides in plants (9) provides an explanation of the slow increase in incorporation of the trans acid into umbelliferone after an initial fast conversion. The acid, once bound, may be converted only slowly into a form suitable for enzymic ortho-hydroxylation.

The incorporation of cinnamic acid itself into umbelliferone is in accord with the fact that phenylalanine, rather than tyrosine, is the precursor for the p-hydroxycinnamic system in plants other than grasses (10).

A similar pattern of results was obtained by analysis of the herniarin (I; R=CH_3) isolated from root-fed Lavandula (Munstead) plants which showed relative incorporations (average of three feedings) of 5:2.5:1 for trans--p-hydroxycinnamic acid, cis-p-hydroxycinnamic acid, and the spirolactone (II) respectively. These results were not taken as definitive because of the low specific incorporation of the trans-p-hydroxycinnamic acid (ca. 0.02%).

Analysis of the 'free' and 'bound' umbelliferone from a one-day feeding of trans-p-hydroxycinnamic acid to Hydrangea showed that over 85% of the umbelliferone was in a bound form. The activity of the 'free' compound was slightly lower than that of the 'bound'.

Paper chromatograms of Hydrangea extracts have not revealed the presence of 7-β-D-glucosyloxycoumarin (I; R=Glu) and the bound form may possibly be 2,4-di-β-D-glucosyloxy-cis--cinnamic acid (VIII; R=Glu).

We feel that the O-glucosylation of the product of enzymic orthohydroxylation of the trans-cinnamic system plays a key part in the ensuing stereomutation to the cis-o-glucoside. Inspection of models of the cis and trans-o-glucosyloxy-cinnamic acid shows that only in the cis acid can there exist a strongly intramolecularly hydrogen bonded system, between the carboxyl group and the 2-hydroxyl group of the glucose. Experimental investigation of this stabilising factor as indicated by the cis:trans ratio under equilibrating conditions (induced by light or by an isomerase) is now in progress on this and other systems. The great preponderance of the cis-structure in equilibrated systems of this type is strongly suggested by already reported results (11, 12, 13).

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