Chrysophanol (1,8-Dihydroxy-3-methylanthraquinone) Biosynthesis in Higher Plants

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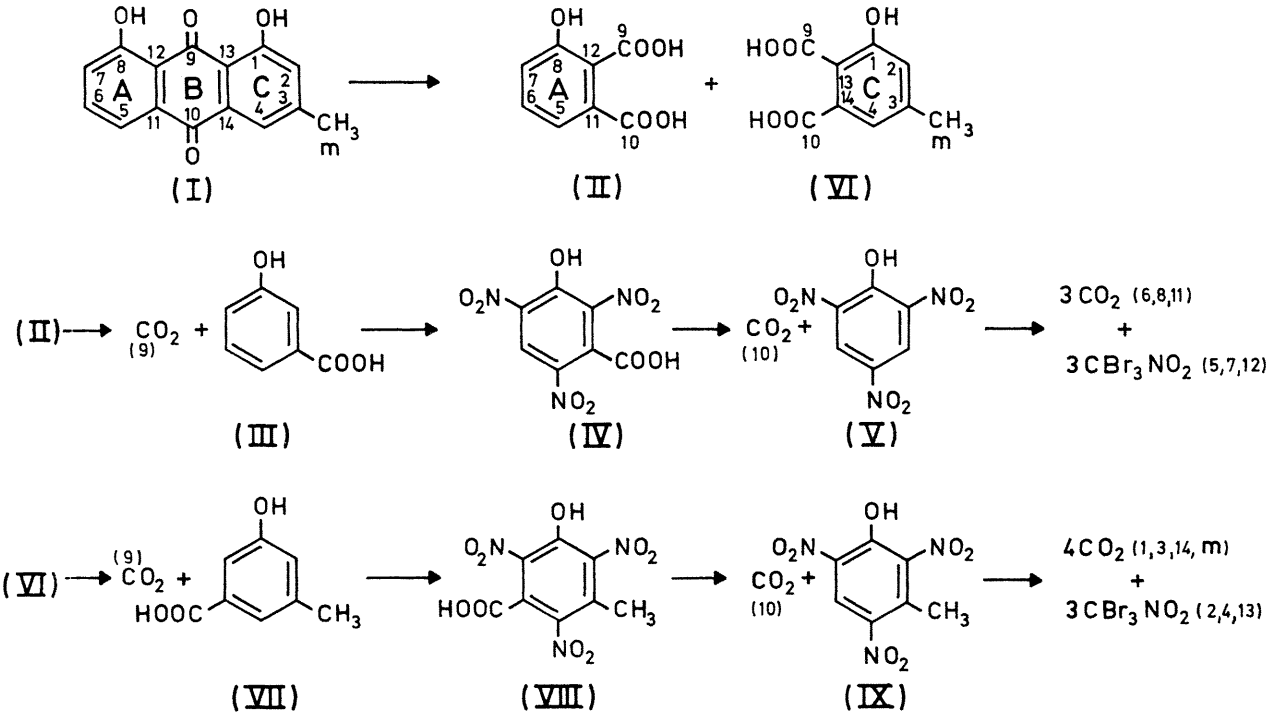
IN fungi, anthraquinones of the emodin type (ring-A hydroxylated) are synthesized *via* the acetate-plus-malonate pathway.[1] In higher plants, however, an independent pathway for the formation of ring-A unsubstituted anthraquinones has recently been demonstrated, involving shikimate[2] and melavonate.[3,4] It has been suggested that this latter pathway operates also for the biosynthesis of emodin-type anthraquinones in higher plants;[5] most recently this has been supported by the finding[6] that labelled shikimate is incorporated into chrysophanol and emodin in *Rheum palmatum* (*Polygonaceae*). [^{14}C]Acetate incorporation into chrysophanol was reduced markedly by the addition of unlabelled mevalonate, indicating mevalonate to be an intermediate in the biosynthesis of chrysophanol by the shikimate pathway.

We present experimental evidence that this assumption is not tenable, and showing that in higher plants the biosynthesis of emodin-type anthraquinones proceeds *via* the polyacetate–malonate pathway.

Young growing leaves of *Rumex alpinus* (*Polygonaceae*) were fed through the cut ends with potential ^{14}C-labelled precursors in complete darkness. After 24 hr., chrysophanol (I) was isolated from the leaves and purified to constant specific activity. The quinone was diluted (*ca.* 1 : 100) with carrier material and degraded by alkaline hydrogen peroxide to give acids (II) and (VI). Acid (II) was degraded by standard procedures (II → V), and for acid (VI) a similar route (VI → IX) was followed. The following results were obtained:

(1) No incorporation of (±)-[1,2-^{14}C]shikimic acid and (±)-[2-^{14}C]mevalonate into the emodin-type anthraquinones occurred, although both acids were extensively metabolized by the leaf tissue. [1-^{14}C]- and [2-^{14}C]-Acetate, however, were incorporated into chrysophanol (0·4 and 0·6%, respectively; specific activity chrysophanol $2{\cdot}3 \times 10^5$ d.p.m./μmole and $1{\cdot}6 \times 10^5$ d.p.m./μmole).

(2) Extensive chemical degradation of the [^{14}C]chrysophanol molecule from the labelled acetate feeding



experiments shows the location of radioactivity to be in complete agreement with the polyacetate–malonate pathway (see Table).

Similar results have been obtained with *Rhamnus frangula*. It can hence be considered as established that higher plants have developed two completely separate

Distribution of radioactivity in degradation products of chrysophanol after application of [1-^{14}C]- *and* [2-^{14}C]-*acetate*

Compound	$CH_3{\cdot}^{14}CO_2H$ theory %	$CH_3{\cdot}^{14}CO_2H$ found %	$^{14}CH_3{\cdot}CO_2H$ theory %	$^{14}CH_3{\cdot}CO_2H$ found %
Chrysophanol (I)	100	100	100	100
3-Hydroxyphthalic acid (II)	57·2	55·7	50	48·9
CO_2 *ex* (II) (=C-9)	14·3	13·2	0	2·9
CO_2 *ex* (IV) (=C-10)	0	1·6	12·5	10·8
Picric acid (V)	42·8	45·3	37·5	34·9
CO_2 *ex* (V) (=C-6,-8,-11)	42·8	48·3	0	3·3
CBr_3NO_2 *ex* (V) (=C-5,-7,-12)	0	2·3	37·5	30·7
3-Hydroxy-5-methylphthalic acid	57·2	58·3	62·5	64·2
CO_2 *ex* (VIII) (=C-10)	0	0	12·5	10·9
Trinitro-*m*-cresol (IX)	42·8	49·3	50	48·3
CO_2 *ex* (IX) (=C-1,-3,-14,-m)	42·8	47·2	12·5	12·5
CBr_3NO_2 *ex* (IX) (=C-2,-4,-13)	0	2·2	37·5	41·1

(3) Kuhn–Roth oxidation of the labelled chrysophanol molecule (from [2-^{14}C]acetate) yielding C-3 and C-m as acetate [1/8 of the activity of (I) was found in C-3 + C-m; C-m containing 95%] is in complete agreement with the acetate-malonate pathway. The ratio of ^{14}C of ring A (V) to ring C (IX) in the [1-^{14}C]acetate feeding experiment was 1 : 1.

pathways for the synthesis of the anthraquinone carbon skeleton, *i.e.*, the shikimate and the polyacetate–malonate pathways.

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