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BIOSYNTHESIS OF PLUMBAGIN (5-HYDROXY-2-METHYL-1,4-NAPHTHOQUINONE) VIA THE ACETATE PATHWAY IN HIGHER PLANTS.

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Naphthoquinones are formed in higher plants either by direct incorporation of shikimate [1,2] or via the homogentisic acid pathway involving the condensation of mevalonic acid and most probably toluhydroquinone [1,3]. 2-Methyl-juglone (plumbagin) by its striking structural similarity with juglone and menadion was expected to be formed via the shikimate pathway, by C-methylation of juglone in the 2 position[4]. Both the genus Drosera[5] and the genus Plumbago[6] are known to contain plumbagin. One species of each genus was used as experimental material to study the course of biosynthesis of this naphthoquinone. Feeding experiments with young shoots of Plumbago europaea L. showed that neither shikimate -7-^{14}C,L-$^{14}CH_3$-methionine, DL-tyrosine-ß-^{14}C, DL-phenylalanine (ring-1-^{14}C), nor DL-mevalonic acid-5-^{14}C were incorporated into plumbaginto a significant extent (see Table 1). In contrast, acetate-1-^{14}C, 2-^{14}C and malonate-2-^{14}C labelled this naphthoquinone heavily. This fact suggested that none of the above mentioned routes leads to the formation of plumbagin but rather that this molecule is formed by the well known polyacetate-malonate pathway. To examine this possibility plumbagin labelled either from acetate-1-^{14}C or-2-^{14}C was subjected to extensive chemical degradation. Plumbagin was oxidized (H_2O_2/OH^-) to 3-hydroxyphthalic acid which was further degraded according to known procedures[7]. Furthermore, radioactivity of C-2 and C-11 of plumbagin was determined after Kuhn - Roth oxidation.
The results are given in Table 2 and confirm the suggestion that the polyacetate-malonate pathway is the route used in the biosynthesis of plumbagin.
Similar results have been obtained by acetate feeding to different Droseraceae

species and degradation of labelled plumbagin. The co-occurring 7-methyl-juglone[5] in Drosera plants is also formed via the polyacetate-malonate route.

Plumbagin and 7-methyl-juglone are the first napthoquinones in higher plants shown to be formed according to the acetate pathway which has long been known for the formation of naphthoquinones in fungi[8]. A hexaacetyl chain leads to the formation of these naphthalenes. It can hence be considered as established that higher plants have developed at least three separate pathways for the synthesis of the naphthoquinone carbon skeleton, i.e. the shikimate, the homogentisate and the polyacetate-malonate pathways.

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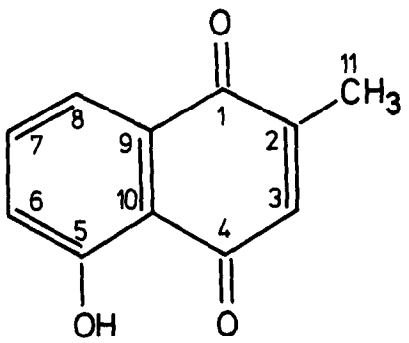
precursor applied			Plumbagin		
	µMol	total act. dpm	incorp. %	spec.act. dpm/µMol	dilution
D-Shikimic acid-7-^{14}C	0.5	8.4×10^6	0.05	6.23×10^2	26976
L-Methionine-$^{14}CH_3$	0.4	5.5×10^6	0.08	3.94×10^2	35009
DL-Tyrosine-ß-^{14}C	0.4	2.2×10^6	0.03	3.99×10^2	17600
DL-Phenylalanine (ring-1-^{14}C)	3.5	1.7×10^7	0.02	6.65×10	71975
DL-Mevalonic acid-5-^{14}C	0.4	1.1×10^7	0.05	1.8×10^2	144142
Acetate-1-^{14}C	1.3	1.1×10^8	0.65	1.37×10^5	640
Acetate-2-^{14}C	0.9	1.1×10^8	1.99	2.18×10^5	55
Malonate-2-^{14}C	0.4	1.1×10^7	3.49	5.26×10^4	506

Table 1. Incorporation of potential ^{14}C-labelled precursors into plumbagin in young shoots of Plumbago europaea L. after 24 hours.



Plumbagin

Degradation product	CH_3-$^{14}CO_2H$ as precursor		$^{14}CH_3$-CO_2H as precursor	
	theory %	found %	theory %	found %
Plumbagin (=C-1 - C-11)	100.0	100.0	100.0	100.0
C-2 (Kuhn - Roth)	20.0	18.2	0.0	0.1
C-3 (by difference)	0.0	0.2	16.7	17.0
C-11 (Kuhn - Roth)	0.0	1.1	16.7	14.8
3-Hydroxyphthalic acid (=C-1,4,5,6,7,8,9,10)	80.0	80.5	66.7	68.1
CO_2 ex 3-Hydroxyphthalic acid (=C-4)	20.0	14.7	0.0	0.7
m-Hydroxybenzoic acid (=C-1,5,6,7,8,9,10)	60.0	65.7	66.7	62.3
CO_2 ex m-Hydroxybenzoic Acid (=C-1)	0.0	0.0	16.7	17.3
Picric acid (=C-5,6,7,8,9,10)	60.0	60.0	50.0	46.6
CO_2 ex Picric acid (=C-5,7,9)	0.0	0.0	50.0	41.5
$CBr_3NO_2.C_6H_{12}N_4$ (=C-6,8,10)	60.0	51.3	0.0	4.1

Table 2. Distribution of radioactivity in chemical degradation products of plumbagin synthesized from acetate 1-^{14}C and 2-^{14}C in Plumbago europaea L.