Tetrahedron Letters No. 44, pp 4151 - 4155, 1971. Pergamon Press. Printed in Great Britain.

p-HYDROXYBENZOIC ACID AND MEVALONIC ACID AS PRECURSORS OF THE PLANT NAPHTHOQUINONE ALKANNIN.

H.V.Schmid and M.H.Zenk
Lehrstuhl für Pflanzenphysiologie
Ruhr-Universität, 463 Bochum, Germany

(Received UK 20 September 1971; accepted for publication 1 October 1971)

Alkannin [5.8-dihydroxy-2-((S)-1'-hydroxy-4'-methylpent-3'-enyl)-1,4-naphthoquinone] is a highly colored dyestuff found in a number of Boraginaceae species[1,2] and also recently reported in one species of the family Euphorbiaceae[3]. This 1,4-naphthoquinone is rather unique in its six carbon atom isoprenoid sidechain. Three different pathways for the biosynthesis of naphthoquinones in higher plants have been described. First, the incorporation of shikimic acid into the benzenoid naphthoquinone ring[4,5] with retention of the carboxyl group; second, the incorporation of the ring- and ß-C-atoms of tyrosine into the quinoid portion of a naphthoquinone[4,6]; and thirdly the synthesis of the entire quinone molecule from acetate units[7].

A study of the biosynthesis of alkannin was undertaken because of the interesting structural features of this molecule.

Potential ^{14}C-labelled precursors of alkannin or the (R) enantiomer shikonin, when fed to root systems of Alkanna tinctoria Tausch. and Lithospermum erythrorhizon Sieb. et Zucc., gave very low rates of incorporation which were unsuitable for degradation. The pigment, however, is also present on the leaf surface of Plagiobothrys arizonicus (A.Gray) Greene, and since better incorporation values were obtained, this plant was used as the experimental material.

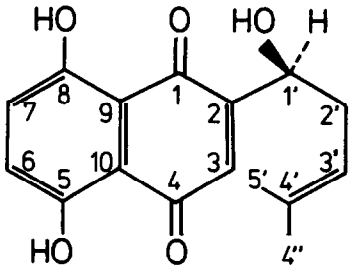
Table 1 shows the incorporation of supposed precursors into alkannin. Surprisingly, neither shikimic acid-7-^{14}C nor tyrosine-U-^{14}C was significantly incorporated into the pigment. However, phenylalanine- and cinnamic acid-ring-1-^{14}C labelled the naphthoquinone, suggesting a new biosynthetic route for the

formation of this compound. For the degradation of the molecule, use was made of the Diels-Alder addition of 2,3-dimethyl-1,3-butadiene to the tautomeric form of alkannin. The resulting anthraquinone[8] was subjected to permanganate oxidation which afforded 4,5-dimethylphthalic acid containing the C-5 and C-8 of alkannin as carboxyl groups. The phthalic acid thus derived from alkannin which was labelled during a cinnamic acid-ring-1-^{14}C feeding was shown to have 95.8 % of the specific activity of the alkannin. The entire radioactivity (49.2 %) was confined to one carboxyl group. Analogous results were obtained with alkannin labelled from p-hydroxybenzoic acid-ring-1,2,6-^{14}C. Thus it can be concluded that the hydroxylated aromatic ring of alkannin (C-atoms 5-10) is derived from the ring-C-atoms of p-hydroxybenzoic acid. Hydroquinone-2,3,5,6-^{14}C, the decarboxylation product of p-hydroxybenzoic acid, is not, however, incorporated to any significant extent.

Mevalonic acid-2-^{14}C was well incorporated into alkannin (table 1). Degradation[9] of the labelled naphthoquinone yielded acetone from the sidechain containing 51.38 % of the specific activity of the quinone. The label was confined entirely (50.24 %) to the methyl groups of acetone corresponding to 4" and 5' of alkannin. This result, as well as that from the double-label experiment with mevalonic acid-5-^{14}C and -^{3}H (table 1), suggested that two molecules of mevalonate take part in the biosynthesis of alkannin. Alkannin labelled by mevalonate-2-^{14}C was degraded by ozone-oxidation[9]. Unlabelled 3,6-dihydroxyphthalic acid was obtained, thus demonstrating that neither keto group (C-1 or C-4) of the naphthoquinone is formed from carbon atom 2 of mevalonate. Ozonolysis of the same alkannin in acetic acid followed by treatment with hydrogen peroxide[10] gave L-(-)malic acid (carbon atoms 2,1',2',3' of alkannin) which contained 50.08 % of the radioactivity of the naphthoquinone molecule. Further degradation of this acid showed that the radioactivity was entirely (50.27 %) confined to the original carbon atom 1' and thus established the position of the second prenyl unit.

We therefore postulate a fourth biosynthetic sequence for the formation of the naphthoquinone carbon skeleton in higher plants. The pathway for the biosynthesis of alkannin must involve the prenylation of p-hydroxybenzoic acid with two molecules of prenylpyrophosphate in succession or with geranylpyrophosphate (or a hydroxylated derivative thereof), subsequent decarboxylation, and finally ring closure of the diprenylhydroquinone, which is reminiscent of chimaphilin[6] or alizarin[11,12] biosynthesis.

We are grateful to Dr.E.Bloss, Tuscon, Arizona, for providing us with seeds of P.arizonicus, to Miss G.Busch for excellent technical assistance and to the "Bundesminister für Bildung und Wissenschaft", Bonn, for financial support.



Alkannin

precursor applied			Alkannin		
	µMol	total act. dpm	incorp. %	spec.act. dpm/µMol	dilution
D-Shikimic acid-7-^{14}C	1.52	7.35×10^6	0.001	6.00×10^1	80590
L-Tyrosine-U-^{14}C	1.37	1.06×10^7	0.002	8.26×10^1	93670
DL-Phenylalanine-ring-1-^{14}C	2.91	2.20×10^7	0.660	3.51×10^4	215
trans-Cinnamic acid-ring-1-^{14}C	1.45	1.10×10^7	0.484	6.29×10^4	121
p-Hydroxybenzoic acid-ring-1,2,6-^{14}C	1.44	1.10×10^7	1.363	2.60×10^4	294
Hydroquinone-2,3,5,6-^{14}C	1.67	2.20×10^7	0.005	4.43×10^2	29740
DL-Mevalonic acid-2-^{14}C	1.72	2.22×10^7	0.514	1.33×10^4	970
DL-Mevalonic acid-5-^{3}H	0.40	$3.34 \times 10^{8*}$	0.044	$1.65 \times 10^{5*}$	1416
and					
DL-Mevalonic acid-5-^{14}C	1.03	$2.68 \times 10^{7*}$	0.089	$2.65 \times 10^{4*}$	707

Table 1. Incorporation of potential ^{14}C- and ^{3}H-labelled precursors into alkannin in young plants of Plagiobothrys arizonicus after 48 hours feeding (* T/C ratio: precursor 12,48:1; product: 6,23:1).

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