Tetrahedron Letters, Vol.22, No.22, pp 2097 - 2098, 1981
Printed in Great Britain

0040-4039/81/222097-02$02.00/0

BIOSYNTHESIS OF A RETROCHALCONE, ECHINATIN: A FEEDING STUDY WITH ADVANCED PRECURSORS[1]

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Abstract: Feeding experiments with ^{14}C-labeled compounds established that a dibenzoylmethane, licodione(3), is an obligate intermediate in the biosynthesis of a retrochalcone, echinatin(1).

Echinatin(1)[2a,c], a component of the tissue culture of Glycyrrhiza echinata, has been proven by tracer experiments[3] to be a retrochalcone, in which origins of two aromatic rings are reversed to normal flavonoids, i.e., the A ring of 1 originates from cinnamate while the B ring is formed via acetate-malonate pathway. Incorporation of [^{3}H]-isoliquiritigenin(2) into 1[3] has suggested that the biosynthetic course involves the inversion of α,β-unsaturated ketone unit. Isolation of a new dibenzoylmethane, licodione(3)[2b,c], has led us to assume that 3 may be an intermediate of this process. This view was strongly supported by the detection of an O-methyltransferase(LMT)[4] which catalyzes the position specific 2'-O-methylation of 3 in the cells, but a direct proof of biosynthetic linkage between 1 and 3 has been lacking. Here we report a feeding study with 3 and other compounds labeled with ^{14}C resulting in the establishment of this pathway.

^{14}C-Labeled compounds were synthesized from [carbonyl-^{14}C]-resacetophenone(4) which was prepared from resorcinol and $CH_3{}^{14}COOH$ in the presence of $ZnCl_2$. Acylation of [carbonyl-^{14}C]-4-O-benzyl-4 by p-benzyloxybenzoyl chloride followed by a rearrangement mediated by alkali and hydrogenolytic deprotection gave [1-^{14}C]-3 (19% yield from 4), and treatment of this compound with HCl afforded [carbonyl-^{14}C]-7,4'-dihydroxyflavone(5)(52% yield). Condensation of [carbonyl-^{14}C]-4-O-methoxymethyl-4 and p-methoxymethoxybenzaldehyde followed by acid treatment to remove the protecting group gave [carbonyl-^{14}C]-2 (57% yield from 4).

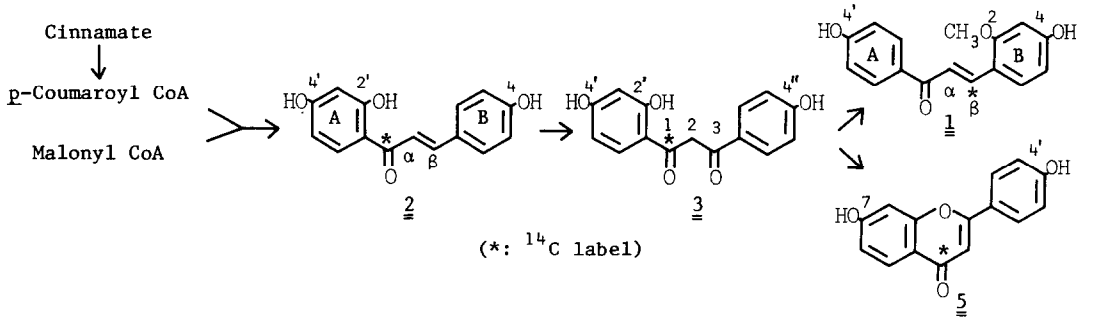
Labeled compounds were fed separately to suspension culture of 3 weeks old G.echinata callus and were incubated for 2 days. Echinatin(1) was isolated and recrystallized with cold carrier to a constant specific activity. Table 1 shows clearly that 3 was effectively incorporated into 1 while 2 and 5 were incorporated but to less extents. Echinatin from the cells fed with 3 was further degraded with alkali to yield p-hydroxyacetophenone(6) and 2-methoxy-4-hydroxybenzaldehyde(7). Radioactivity was shown to exist only in 7 within an experimental error (2.7% in 6 and 104% in 7 of original activity of 1). This indicates that the ^{14}C label at carbonyl in 3 was intactly converted into the label at ß-position of 1. Thus, the last steps of 1 biosynthesis would consist of the following reactions; 2'-O-methylation of 3 (catalyzed by LMT), reduction of 1-keto group to a benzyl alcohol and dehydration to yield the retrochalcone.

A relatively low incorporation of 5 into 1 compared with that of 2 implies that 2 is the preferred precursor of 3, and hydration of 5, if present, is a minor pathway to form licodione (3)[5]. In contrast, 5 isolated from the cells fed with 3 is considerably radioactive (see Table

1), suggesting that dehydration of 3 is involved in 5 biosynthesis. Metabolic role of dibenzoylmethanes as precursors of flavones has been repeatedly postulated[7], and the result presented here is the first in vivo demonstration of the conversion of a dibenzoylmethane into a flavone.

Table 1. Incorporation of ^{14}C-labeled compounds into echinatin and 7,4'-dihydroxyflavone in G.echinata cell culture

	Precursors (Specific activity; 1.6 x 10^9 dpm/mM)			
	[1-^{14}C]-Licodione(3)		[Carbonyl-^{14}C]-Isoliquiritigenin(2)	[Carbonyl-^{14}C]-7,4'-Dihydroxyflavone(5)
Compound isolated	Echinatin(1)	7,4'-Dihydroxyflavone(5)	Echinatin(1)	Echinatin(1)
Specific activity (dpm/mM)	8.2 x 10^6	1.6 x 10^6	6.8 x 10^5	2.2 x 10^5
Specific incorporation ratio (%)	0.51	0.097	0.043	0.014
Total incorporation ratio (%)	0.25	0.017	0.11	0.006



References and Notes

1) Part 35 in the series "Studies on Plant Tissue Cultures". For Part 34, see ref.4.
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4) S.Ayabe, T.Yoshikawa, M.Kobayashi and T.Furuya, Phytochemistry, 19, 2331(1980).
5) We have pointed out a possibility[1c] of a biosynthetic course (liquiritigenin→5→3→1) which does not involve a chalcone intermediate, regarding the absence of 2 in the cell culture and Hahlbrock's previous observation[6a] that the first obligate intermediate in flavonoid biosynthesis is a flavanone but not a chalcone. Recent correction of "flavanone synthase" to "chalcone synthase"[6b] and our result described herein made our earlier hypothesis unlikely.
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(Received in Japan 18 February 1981)