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BIOSYNTHESIS OF ECHINATIN

A NEW BIOSYNTHETICAL SCHEME OF RETROCHALCONE

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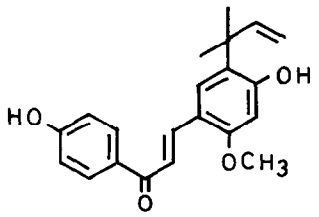
As mentioned in the preceding report[1], licochalcone A (1) and licochalcone B (2)[1] isolated from Shinkiang Licorice, and echinatin (3)[2] produced by the tissue culture of Glycyrrhiza echinata L are noted by their unusual dispositions of O-functional groupings in contrast with the normal chalcones so far known Referring an additional example of a peculiar isoflavone, licoricone[3], of North-Eastern Chinese Licorice, the name of retroflavonoid has been proposed for these compounds in assuming a new biosynthetical scheme[1].



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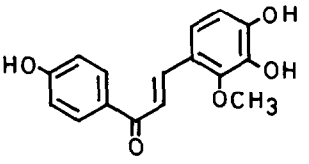

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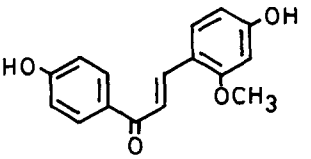

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For the purpose of proving the existence of such a scheme in living cells, a series of experiments on the biosynthesis of echinatin which is assumed to be retrochalcone has been performed using a suspension culture of calli derived from the seedling of G. echinata .

[3-^{14}C] Cinnamic acid and [1-^{14}C] cinnamic acid were fed to the culture to investigate the position of the labelled carbon incorporated into echinatin After shaking the culture in the White medium for 6 days in the dark at 26°, echinatin was isolated from the calli to measure the incorporation of ^{14}C The radioactive echinatin thus obtained was degraded with alkali into p-hydroxyacetophenone and 2-methoxy-4-hydroxybenzaldehyde to determine the distribution of radioactivity (Table I).

Table I Incorporation of ^{14}C-labelled cinnamic acid into echinatin and distribution of radioactivity (^{14}C) in echinatin

	Precursors	
	[3-^{14}C] Cinnamic acid (0.05 mCi)	[1-^{14}C] Cinnamic acid (0.05 mCi)
Echinatin		
Specific activity (dpm/mM)	1.46 x 10^6	5.22 x 10^6
Total incorporation ratio (%)	0.08	0.52
Specific incorporation ratio (%)	0.0013	0.056
Echinatin		
Specific activity (dpm/mM)	1.46 x 10^6	5.22 x 10^6
Distribution (%)	100	100
p-Hydroxyacetophenone		
Specific activity (dpm/mM)	1.82 x 10^6	nil
Distribution (%)	125	0
2-Methoxy-4-hydroxybenzaldehyde		
Specific activity (dpm/mM)	0.09 x 10^6	5.53 x 10^6
Distribution (%)	6	106

The radioactivity of echinatin incorporated from [3-^{14}C] cinnamic acid was localized to the carbonyl and that incorporated from [1-^{14}C] cinnamic acid to the carbon atom in the β-position These results indicate that the A-ring of echinatin is derived from p-coumaroyl CoA.

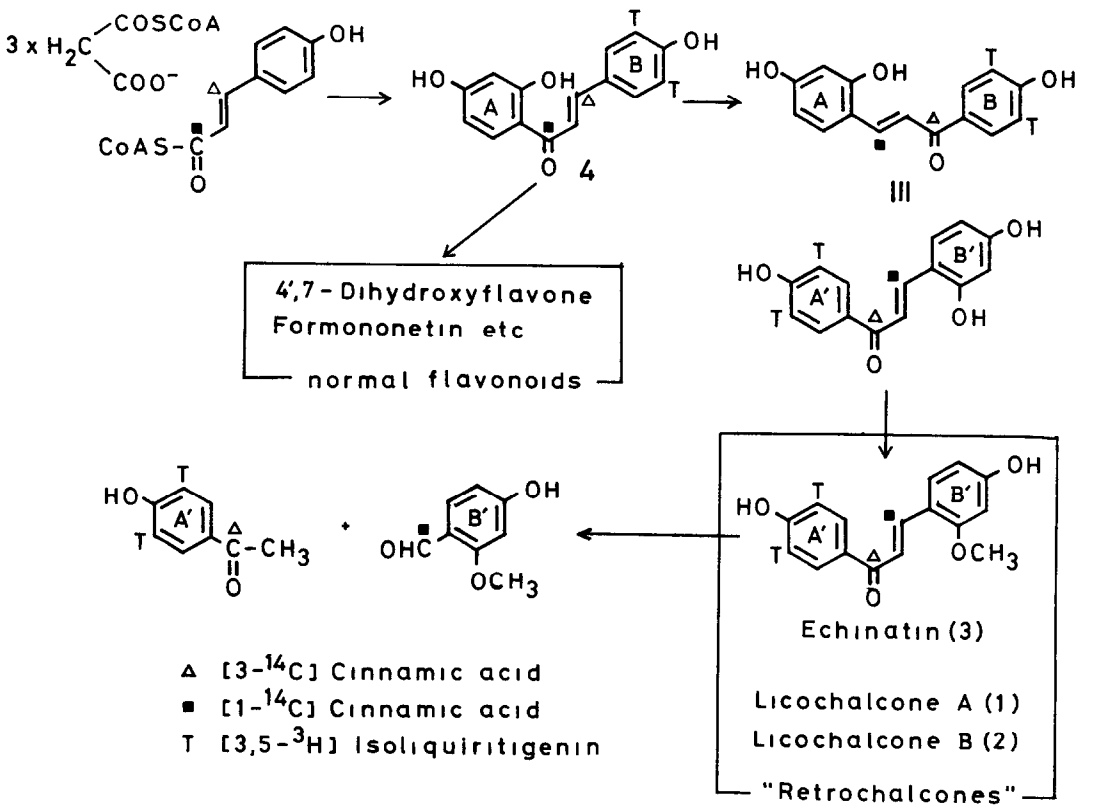
In considering the co-occurence of normal flavonoid compounds with the unusual one in licorice roots as well as in the tissue culture of G. echinata*, normal chalcone has been presumed as an intermediate precursor of retrochalcone to design following experiment [3,5-T] Isoliquiritigenin (4) synthesized by the condensation of [3,5-T] p-hydroxybenzaldehyde and 2,4-dihydroxy-resacetophenone was fed to the culture of calli of G echinata suspended in the White medium under shaking for 2 days in the dark at 26° The results reveal that [3,5-T] isoliquiritigenin is highly incorporated into echinatin, in which the radioactivity is distributed into the A-ring presumably at 3' and 5'-positions (Table II)

* Along with echinatin, formononetin (7-hydroxy-4'-methoxyisoflavone)[2], 7,4'-dihydroxyflavone and licoflavone A (7,4'-dihydroxy-6-γ,γ-dimethylallylflavone)[4] have been isolated from the callus of G. echinata[5].

Table II Incorporation of [3,5-T] isoliquiritigenin into echinatin and distribution of radioactivity (T) in echinatin

Isoliquiritigenin	Total activity	8 56 mCi
Echinatin	Specific activity (dpm/mM)	2.63×10^8
	Total incorporation ratio (%)	0.11
	Specific incorporation ratio (%)	1.38
Echinatin*	Specific activity (dpm/mM)	4.55×10^5
	Distribution (%)	100
p-Hydroxyacetophenone		
	Specific activity (dpm/mM)	3.89×10^5
	Distribution (%)	85.5
2-Methoxy-4-hydroxybenzaldehyde		
	Specific activity (dpm/mM)	nil
	Distribution (%)	0

* Diluted with carrier.



Consequently, it has been established by the present experiments that isoliquiritigenin, a normal chalcone, is an efficient precursor of echinatin whose A-ring is derived from the B-ring of isoliquiritigenin by the conversion of carbonyl.

Further studies on the mechanism of the conversion forming retrochalcone are in progress.

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