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CONVERSION OF FLAVANONOL TO ISOFLAVONE

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It has been demonstrated that isoflavones are synthesized from chalcones of the corresponding hydroxylation pattern and that cinnamic acid serves as a precursor of the chalcones in leguminous plants (1,2, 3). Thus, 2',4',4-trihydroxychalcone-4'-glucoside is converted to formononetin, but not to biochanin A, which has an additional hydroxyl group at the 5 position. Although some mechanistic speculations on phenyl group rearrangement in the isoflavone biosynthesis have been discussed (4,5), the details of the pathway from chalcone to isoflavone are still unknown. Based on the acid catalyzed rearrangement of α,β-epoxyketones (6), Grisebach et al. suggested the migration of the aryl group at the chalcone epoxide stage (7). An alternative mechanism, which includes a flavanonol as an intermediate as a stage of the rearrangement was postulated from an analogy of a conversion of tetramethoxycatechin to tetramethoxyisoflavene (5).

Recently, Grisebach et al. reported that labeled 4',5,7-trihydroxyflavanone was converted into biochanin A, but not into formononetin (8).

The enzymatic conversion of chalcone to flavanone has been reported in several plants (9); however, the aryl group migration at the flavanone stage without an accompanying oxidation process is mechanistically unlikely. Rather, an equilibration between the flavanone and the chalcone, which serves as a precursor on the main pathway of the isoflavone biosynthesis, is the most likely process involving the flavanone.

Establishment of the presence of 4',7-dihydroxyflavanon-3-ol (garbanzol) along with isoflavones and flavonols of the same hydroxylation pattern in chana seedlings (Cicer arietinum) called attention to a hypothesis that isoflavone would be formed from chalcone via flavanonol, at which the rearrangement of the 2 phenyl group to the 3 position occurs (10).

Our present result shows that garbanzol-4-^{14}C serves as a precursor of formononetin, but not of biochanin A.

Garbanzol-4-^{14}C was synthesized by the aqueous acetone and sodium carbonate treatment of 2'-hydroxy-4,4'-dibenzyloxychalcone-α-^{14}C-dibromide, which was derived from acetic-1-^{14}C anhydride and resorcinol (overall yield 19%). The specific radioactivity was 0.87 mc/mmole and the purity was checked by thin-layer chromatography.

Chana seeds, purchased from the local market, were allowed to germinate in Vermiculite moistened with a nutrient solution at 25° in the dark. After 3 days, the seedlings were transferred to hydroponic culture. In the case of experiments with etiolated seedlings, the culture was continued for 3 days in the dark; and when green seedlings were used, the plants were maintained under continuous fluorescent light for 8 days. Garbanzol-4-^{14}C was fed either through the cut end of the stems or through the roots. Feeding periods were 2 or 5 days. In the feeding through the cut end, all the radioactive solution was absorbed within 6 hours and water was supplied thereafter. About 75% of the radioactivity was taken up by the seedlings when feeding was through the roots. Extraction of phenolic substances was carried out by a method similar to that used previously (10), except that acid hydrolysis of the extract with 1 N HCl for 1 hour on a steam bath was added before ether extraction. Separation and partial purification of the isoflavones were carried out by repetition of thin-layer chromatography (Silica Gel H, $CHCl_3$-MeOH, 95:5 $^{v}/v$), paper chromatography (Whatman 3MM, 70% aq. MeOH) and then thin-layer chromatography (Silica Gel H, $CHCl_3$-MeOH, 95:5 $^{v}/v$ or benzene-EtOH, 90:10 $^{v}/v$). To the formononetin or biochanin A fraction was added 100 mg of the respective non-radioactive isoflavone as carrier and the isoflavone was purified by repeated recrystallization from 70% EtOH or 60% acetone. Radioactivity was measured by a Nuclear Chicago liquid scintillation spectrometer, with dioxane-PPO-POPOP-naphthalene system as a phosphor solution.

TABLE I

Incorporation of Garbanzol-4-^{14}C into Formononetin in Chana Seedlings

Dose (μc)		28	28	34
Seedlings		etiolated	green	green
Conditions of feeding		cut end dark 2 days	cut end light 2 days	root light 5 days
Radioactivity of				
Formononetin dpm/mmole	X3*	5174	6455	2100
	X4	5062	6080	1909
	X5	5074	6317	2060
Biochanin A dpm/mmole	X4	50		
	X5	64	229	164

* Xn: After n times recrystallization

As shown in the table, formononetin was labeled from garbanzol-4-^{14}C, while labeling of biochanin A was very small under different conditions. Although degradation of the labeled formononetin and the incorporation of the garbanzol into other flavonoid constituents are under way, the selective incorporation of the radioactive garbanzol into formononetin suggests the direct conversion of the flavanonol to the corresponding isoflavone, therefore, the phenyl group rearrangement after the pyrone ring formation is plausible. It should be noted, however, that under certain conditions the transformation 3-hydroxyflavanone $\rightleftarrows$ chalcone epoxide is possible.

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