The Role of Dihydroflavonols in Flavonoid Biosynthesis

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Dihydroflavonols have been postulated as intermediates in the biosynthesis of flavonols, anthocyanins and isoflavones.[1] We have now shown experimentally that a dihydroflavonol is an efficient precursor in flavonol and anthocyanin biosynthesis but not in isoflavone biosynthesis.

3,5,7,4'-Tetrahydroxyflavanone (dihydrokaempferol) (I) was labelled with tritium by a modified Wilzbach technique[2] and purified to radio-purity. By oxidation[3] to kaempferol-[T] (II) it was shown that 41·5% of the total activity of (I) was located at carbon atoms 2 and 3. In parallel experiments (I) or (II) together with [1-^{14}C]-phenylalanine as "internal standard" was fed to buckwheat seedlings and the ^{14}C and tritium activity were determined in the isolated[4] quercetin and cyanidin (Table 1).

[1] H. Grisebach, "Chemistry and Biochemistry of Plant Pigments" (T. W. Goodwin, ed.), Academic Press, London, 1965, p. 279.
[2] H. Wollenberg and M. Wenzel, *Z. Naturforsch.* 1963, **18**b, 8.
[3] H. Pachéco, *Compt. rend.* 1960, **251**, 1077.
[4] L. Patschke, W. Barz, and H. Grisebach, *Z. Naturforsch.* 1964, **19**b, 110.

The results prove that (I) but not (II) is a very efficient precursor for quercetin and cyanidin. comparison with other precursors were given by these workers.

TABLE 1

Incorporation of dihydrokaempferol-[T], kaempferol[-T], and [1-^{14}C]-phenylanine into quercetin and cyanidin

Precursor or compound	Dilution* ^{14}C	Dilution* T	Incorporation (%) ^{14}C	Incorporation (%) T	T/^{14}C
Dihydrokaempferol-[T] } [1-^{14}C]-Phenylalanine					9·5
Quercetin	2330	315	0·42	0·58	12·9
Cyanidin	227	48	0·43	0·38	8·3
Kaempferol-[T] } [1-^{14}C]-Phenylalanine					7·0
Quercetin	4890	4690	0·22	0·075	1·8
Cyanidin	219	539	0·44	0·045	0·7

Incorporations and dilutions are corrected for loss of tritium at C-2 and C-3 of (I)

* $\frac{\text{specific activity of precuror}}{\text{specific activity of product}}$

TABLE 2

Incorporation of dihydrokaempferol-[T], kaempferol-[T,] and [1-^{14}C]-phenylalanine into the isoflavones of chana

Precursor or compound	Dilution ^{14}C	Dilution T	Incorporation (%) ^{14}C	Incorporation (%) T	T/^{14}C
Dihydrokaempferol-[T]					
Biochanin-A	—	$1·48 \times 10^5$	—	$1·57 \times 10^{-3}$	
Formononetin	—	$2·98 \times 10^4$	—	$3·04 \times 10^{-3}$	
Dihydrokaempferol-[T] } [1-^{14}C]-Phenylalanine					2·6
Biochanin A	2957	$3·47 \times 10^5$	0·25	$1·45 \times 10^{-3}$	$1·5 \times 10^{-2}$
Formononetin	230	$1·09 \times 10^5$	1·3	$3·86 \times 10^{-3}$	$7·4 \times 10^{-3}$
Kaempferol-[T] } [1-^{14}C]-Phenylalanine					2·87
Biochanin-A	3135	$1·5 \times 10^5$	0·21	$2·8 \times 10^{-3}$	$3·6 \times 10^{-2}$
Formononetin	219	$2·0 \times 10^5$	1·1	$7·8 \times 10^{-4}$	$1·9 \times 10^{-5}$

Incorporations and dilutions are corrected for loss of tritium at C-2 and C-3 of (I)

In similar experiments (I) or (II) with or without the simultaneous addition of [1-^{14}C]-phenylalanine was fed to chana seedlings (*Cicer arietinum*) and the incorporation into the isoflavones was determined[4] (Table 2).

The values demonstrate that the incorporation of (I) and (II) into the isoflavones is insignificant and unspecific.[4] These results are in contradiction to the publication by Imaseki *et al.*[5] in which the selective incorporation of [4-^{14}C]-3,7,4′-trihydroxyflavanone into formononetin in chana was reported. However, no incorporation rates, dilution values or comparison with other precursors were given by these workers.

From the above results and from our earlier investigations[1,6] the following biogenetic relations between flavonoids can be postulated:

flavanol
↑
chalcone ⇌ flavanone → dihydroflavonol
↓ (flavanone) isoflavone
↓ (dihydroflavonol) anthocyanin

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[5] H. Imaskei, R. E. Wheeler, and T. A. Geissman, *Tetrahedron Letters* 1965, 1785.
[6] H. Grisebach and S. Kellner, *Z. Naturforsch.* 1965, **20b**, 446, and previous publications.