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SHORT COMMUNICATION

BIOSYNTHESIS OF FLAVONOIDS—XVI.

DIHYDROKAEMPFEROL AND DIHYDROQUERCETIN AS PRECURSORS OF KAEMPFEROL AND QUERCETIN IN *PISUM SATIVUM**

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Abstract—The incorporation of dihydrokaempferol-[*T*] (I) and dihydroquercetin-[*T*] (II) into kaempfero and quercetin in the pea plant was investigated. (I) is a precursor for both flavonols whereas (II) is a specific precursor for quercetin. These results give further support to the postulated pathway: flavanone ⟶ dihydroflavonol ⟶ flavonol, in which the introduction of the 3′-hydroxyl group occurs at the dihydroflavonol stage.

INTRODUCTION

WE REPORTED earlier that dihydrokaempferol-[*T*] (3,5,7,4′-tetrahydroxyflavanone-[*T*]) but not kaempferol-[*T*] is a very efficient precursor for quercetin and cyanidin in buckwheat seedlings.[1] From this result, we concluded that the introduction of the 3′-hydroxyl group takes place with dihydrokaempferol but not with kaempferol as the substrate. It was therefore of interest to extend these investigations to another plant and to compare the incorporation of dihydrokaempferol and dihydroquercetin into kaempferol and quercetin. Furuya and Galston[2] have characterized the major flavonoids of the pea plant as kaempferol 3-triglucoside, quercetin 3-triglucoside and their respective *p*-coumaric acid esters. We have therefore used pea plants for our further investigations.

RESULTS

Incorporation of Dihydrokaempferol-[T] *into Kaempferol and Quercetin*

Three parallel experiments were carried out with 7-, 11- and 15-day-old pea plants. In each case 3·12 μmole dihydrokaempferol-[*T*] (11·7 μc/μmole)[3] in aqueous solution was administered to eleven excised plants during 36 hr under continuous illumination. During this time 96 per cent of the radioactivity was taken up by the plants. The aglycones were isolated as described in the experimental part and kaempferol and quercetin were separated on paper in 60 per cent acetic acid. After dilution with non-radioactive material, both flavonoids were purified to constant specific activity by recrystallization as their acetyl derivatives.

* Part XV, H. GRISEBACH and H. J. GRAMBOW, *Phytochem.*, in press.

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[1] L. PATSCHKE, W. BARZ and H. GRISEBACH, *Naturforsch.* **21b**, 45 (1966).

[2] M. FURUYA and A. W. GALSTON, *Phytochem.* **4**, 285 (1965).

[3] W. BARZ and H. GRISEBACH, *Naturforsch.* **21b**, 47 (1966).

Table 1 shows the incorporation rates into quercetin and kaempferol and the dilution values. The figures are corrected for loss of the hydrogen atoms at C-2 and C-3 of dihydrokaempferol upon transformation into the flavonols (these H-atoms contained 41·5 per cent of the radioactivity).[3]

TABLE 1. INCORPORATION OF DIHYDROKAEMPFEROL-[*T*] INTO KAEMPFEROL AND QUERCETIN IN THE PEA

Compound (μmole)	Age of plants (days)	Spec. activity (dpm/mmole)*	Dilution	Incorporation rate (%)
Kaempferol (0·25)	7	$7{\cdot}87 \times 10^5$	193	0·04
Kaempferol (1·3)	11	$1{\cdot}3 \times 10^5$	117	0·37
Quercetin (0·51)	11	$1{\cdot}14 \times 10^4$	1,340	0·01
Kaempferol (4·45)	15	$1{\cdot}04 \times 10^4$	1,455	0·10
Quercetin (3·1)	15	$1{\cdot}08 \times 10^3$	14,000	0·007

* Figures are corrected for loss of hydrogen atoms at C-2 and C-3 of dihydrokaempferol.

Incorporation of Dihydroquercetin into Kaempferol and Quercetin

The incorporation of dihydrokaempferol into quercetin should occur via dihydroquercetin.[1] Dihydroquercetin should therefore be a specific precursor for quercetin but not for kaempferol. Dihydroquercetin-[*T*] was prepared by a modified Wilzbach procedure[4] and purified to constant specific activity. In three parallel experiments, eleven 9-, 13- and 17-day-old plants were each fed 2·76 μmole dihydroquercetin (12·95 μc/μmole) in aqueous solution through the cut stems during 36 hr under continuous illumination. During this time, 96 per cent of the radioactivity was taken up by the plants. In each case the isolated kaempferol had no radioactivity. The incorporation rates into quercetin and the dilution values are recorded in Table 2.

TABLE 2. INCORPORATION OF DIHYDROQUERCETIN INTO QUERCETIN IN THE PEA

Quercetin (μmole)	Age of plants (days)	Spec. activity (dpm/mmole)*	Dilution	Incorporation rate (%)
0·31	9	$3{\cdot}13 \times 10^4$	624	0·02
0·28	13	$1{\cdot}66 \times 10^4$	1,175	0·01
0·28	17	$3{\cdot}45 \times 10^4$	565	0·02

* Figures are corrected for loss of hydrogen atoms at C-2 and C-3 of dihydroquercetin. 32 per cent of the radioactivity is located in these H-atoms.

DISCUSSION

The results with dihydrokaempferol show that in pea plants, as in buckwheat seedlings, this compound can function as a precursor for kaempferol and quercetin. The lower incorporation rate into quercetin is probably due partly to the lower rate of synthesis of this flavonol. The experiment with dihydroquercetin proves that this compound is a specific precursor for quercetin. No incorporation into kaempferol was observed. This fact also proves that dihydroquercetin is not degraded to smaller fragments which can be used for the synthesis of kaempferol.

[4] H. WOLLENBERG and M. WENZEL, *Naturforsch.* **18b**, 8 (1963).

The results reported in this paper give further support to the postulated pathway: flavanone ⟶ dihydroflavonol ⟶ flavonol in which the oxygenation pattern in ring B is determined at the dihydroflavonol stage.[1, 5, 6] In this connexion it is important to note that Maier and Metzler have recently isolated dihydrokaempferol from grapefruit where it co-occurs with the corresponding flavanone (naringenin) and kaempferol.[7]

EXPERIMENTAL

Plants

The peas ("Palerbse") were a commercial variety. They were germinated for 4 days in running water and were then grown in Vermiculite nourished with a hydroponic solution.

Isolation of Kaempferol and Quercetin

The plants were ground in a mortar with quartz sand and extracted with hot methanol. After the methanol had been removed *in vacuo*, water was added to the dry residue and the solution extracted with chloroform. HCl was then added to the aqueous phase to a concentration of 2 per cent and the solution boiled under reflux for 2 hr. The solvent was removed *in vacuo* and the residue taken up with methanol. The methanolic solution was chromatographed on paper (Schleicher and Schuell 2043b) with 60 per cent acetic acid (kaempferol R_f 0·65; quercetin R_f 0·5). After elution from the paper, the concentration of the flavonols was determined spectrophotometrically.

Dihydroquercetin-[T]

150 mg dihydroquercetin[8] were absorbed on to quartz sand and incubated with 3C tritium gas at 20° for 4 weeks.[4] The compound was eluted from the sand with methanol and the methanol removed *in vacuo*. To remove labile tritium, dihydroquercetin was dissolved in 100 ml methanol and the solvent evaporated *in vacuo*. This procedure was repeated three times. The residue was recrystallized from water. 20 mg of the substance remaining were purified six times by paper chromatography (Whatman 3 MM) with the following aqueous solvent systems: 20 per cent methanol, 10 per cent acetic acid, 15 per cent acetic acid, 25 per cent methanol, 50 per cent methanol and 70 per cent methanol. After the last purification step the dihydroquercetin-[T] had a constant specific activity of $2{\cdot}88 \times 10^{10}$ dpm/mmole.

Determination of Radioactivity

The samples were counted in a Tri-carb liquid scintillation spectrometer with a toluene or toluene/dioxane scintillator.

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[5] H. GRISEBACH and H. J. GRAMBOW, *loc. cit.*
[6] L. PATSCHKE and H. GRISEBACH, *Naturforsch.* **20b**, 1039 (1965).
[7] V. P. MAIER and D. M. METZLER, *Phytochem.* **6**, 763 (1967).
[8] J. C. PEW, *J. Am. Chem. Soc.* **74**, 3001 (1952); T. A. GEISSMAN and H. LISCHNER, *J. Am. Chem. Soc.* **74**, 3001 (1952).