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SHORT COMMUNICATION

GRAPEFRUIT PHENOLICS—I.

IDENTIFICATION OF DIHYDROKAEMPFEROL AND ITS CO-OCCURRENCE WITH NARINGENIN AND KAEMPFEROL

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Abstract—Dihydrokaempferol has been isolated from the fruit of *Citrus paradisi* Macf. where it has been shown to co-occur with naringenin and kaempferol. These findings extend the limited knowledge of the occurrence of dihydroflavonols to the Rutaceae and lend support to the postulated biosynthetic pathway for flavonols: flavanone→dihydroflavonol→flavonol.

INTRODUCTION

GLYCOSIDES of naringenin and kaempferol have been reported to occur in grapefruit (*Citrus paradisi* Macf.).[1, 2] Biosynthetically, flavonols are currently envisaged as arising from flavanones with dihydroflavonols as their immediate precursors.[3] On the basis of this postulated pathway dihydrokaempferol might be expected to occur in grapefruit.

We report here the isolation of dihydrokaempferol from grapefruit, its identification by comparison with authentic dihydrokaempferol and by oxidation to kaempferol.

RESULTS

Grapefruit dihydrokaempferol was obtained chromatographically pure by preparative TLC. It gave u.v. spectra[4] (λ_{max}^{EtOH} 293, ~330; $\lambda_{max}^{EtOH+NaOAc}$ 328; $\lambda_{max}^{EtOH+AlCl_3}$ 314 nm), R_f values (BzAW, 0·21; 10% HOAc, 0·41) and an electrophoretic migration distance (16·5 cm) identical with those of authentic dihydrokaempferol. In addition, TLC differentiated dihydrokaempferol from dihydroquercetin, naringenin, eriodictyol, and phloretin. On TLC, the grapefruit compound and dihydrokaempferol gave identical colors under u.v. light

* A laboratory of the Western Utilization and Development Division, Agricultural Research Service, U.S. Department of Agriculture. Reference to a company or product does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

[1] For reviews see:

(*a*) R. M. HOROWITZ, In *The Orange, Its Biochemistry* and *Physiology* (Edited by W. B. SINCLAIR), p. 334. University of California Press, Berkeley (1961).

(*b*) J. W. KESTERSON and R. HENDRICKSON, *University of Florida Agr. Exp. Station Bull.* No. 511A (1957).

[2] W. J. DUNLAP and S. H. WENDER, *Anal. Biochem.* 4, 110 (1962).

[3] H. GRISEBACH, In *Chemistry and Biochemistry of Plant Pigments* (Edited by T. W. GOODWIN), p. 279. Academic Press, New York (1965).

[4] L. JURD, In *The Chemistry of Flavonoid Compounds* (Edited by T. A. GEISSMAN), p. 107. Pergamon Press, Oxford (1962).

(absorbs), with *bis*-diazotized benzidene (brownish-maroon), and with borohydride-HCl[5] (light brown). The identity of the grapefruit compound was confirmed by oxidation to kaempferol with bismuth trioxide in glacial acetic acid.[6] The product was shown to be identical to authentic kaempferol by spectra (λ_{max}^{EtOH} 268, 367, $\lambda_{max}^{EtOH+AlCl_3}$ 427 nm), R_f values (BzAW, 0·23; 10% HOAc, 0·0; 50% HOAc, 0·30), its appearance in u.v. light, and by treatment with borohydride-HCl. Authentic dihydrokaempferol also yielded kaempferol when treated with bismuth trioxide.

Dihydrokaempferol was detected in enzyme hydrolyzed extracts of peel and endocarp of mature Marsh grapefruit, whole immature fruit of various sizes (1·5, 4·6 and 6·6 cm equatorial dia.), growing leaves (8–9 cm length), and young twigs (current flush of growth, maximum dia. 2 mm, green color). Naringenin and kaempferol* were also identified in these tissues and in older twigs (maximum dia. 4 mm, grey–brown color). Only a trace of dihydrokaempferol was detected in the older twigs.

DISCUSSION

Isolation of dihydrokaempferol from grapefruit (*C. paradisi* Macf.) extends the limited knowledge of the occurrence of dihydroflavonols[8, 9] to the Rutaceae. The presence of very small amounts of dihydrokaempferol in such metabolically active grapefruit tissues as leaves, fruits and young twigs and its presence in only trace amounts in more mature woody twigs suggests that it is a metabolic intermediate in grapefruit rather than an end-product. Dihydrokaempferol has been previously found in eleven plant families[7–12] primarily in the heartwood and/or bark tissues as the free phenol, although several glycosides have been reported. The only prior reports of dihydrokaempferol in leaves were those of Yasue and Hasegawa[12] and Arthur and Tam.[11]

A condition important to proof of a postulated biosynthetic pathway in higher plants is demonstration of the existence of the proposed intermediates in the tissue.[13] On this basis the co-occurrence of naringenin, dihydrokaempferol, and kaempferol in grapefruit tissues lends further support to the postulated biosynthetic pathway for flavonols: flavanone→dihydroflavonol→flavonol. (Of course their co-occurrence does not rule out other pathways.) This is the first instance in which these three flavonoids have been found to co-occur in actively metabolizing plant tissues. The only other material in which they have been reported to co-occur is wood powder.[10] The presence of dihydrokaempferol in grapefruit

* Dihydrokaempferol 7-glucoside is reported to undergo oxidation to kaempferol 7-glucoside in boiling water.[7] In view of the ease of oxidation of dihydrokaempferol grapefuit tissues were extracted and hydrolyzed under mild conditions and extracts were analyzed immediately after extraction or were held at 3° under nitrogen.

[5] R. M. HOROWITZ, *J. Org. Chem.* **23**, 1733 (1957).
[6] W. RIGBY, *J. Chem. Soc.* 793 (1951).
[7] J. B. HARBORNE and H. S. A. SHERRATT, *Biochem. J.* **78**, 298 (1961).
[8] J. E. GOWAN, E. M. PHILBIN and T. S. WHEELER, In *The Chemistry of Vegetable Tannins, A Symposium*, p. 133. Society of Leather Trades' Chemists, Croydon (1956).
[9] M. SHIMOKORIYAMA, In *The Chemistry of Flavonoid Compounds* (Edited by T. A. GEISSMAN), p. 286. Pergamon Press, Oxford (1962).
[10] T. TAKAHASHI, M. YASUE, H. IMAMURA, M. MIKAZSHI and O. HONDA, *Nippon Mokuzai Gakkaishi* **9**, 199 (1963); *Chem. Abstr.* **61**, 3297 (1964).
[11] H. R. ARTHUR and S. W. TAM, *J. Chem. Soc.* 3197 (1960).
[12] M. YASUE and M. HASEGAWA, *Nippon Ringaku Kaishi* **44**, 170 (1962); *Chem. Abstr.* **58**, 1722 (1963).
[13] T. SWAIN, In *Biosynthetic Pathways in Higher Plants* (Edited by J. B. PRIDHAM and T. SWAIN), p. 9. Academic Press, New York (1965).

tissues is of additional interest since Barz, Patschke, and Grisebach[14] have shown that dihydrokaempferol but not kaempferol is an efficient precursor of quercetin in buckwheat seedlings. In grapefruit we have found quercetin to be present in the same tissues as dihydrokaempferol.[15]

EXPERIMENTAL

Low temperature concentrated, frozen grapefruit juice (400 g, 55% solids) was extracted with acetone until free of flavonoids. After removal of acetone the aqueous residue was treated with 3 g anthocyanase to hydrolyze glycosides and esters, and the free phenols were extracted with ethyl acetate containing 10% (v/v) methanol. (Since dihydrokaempferol could not be detected in unhydrolyzed extracts it appears to occur naturally as a glycoside or ester.) Dihydrokaempferol was isolated by preparative thin-layer chromatography (TLC) using plates coated with a 1 mm layer of powdered cellulose. Chromatographic solvents in the order used were: benzene–acetic acid–water (125:72:3) (BzAW), 10% acetic acid, and water. Dihydrokaempferol was oxidized to kaempferol by refluxing in glacial acetic acid with excess bismuth trioxide for 90 min and kaempferol was isolated by preparative TLC.

Analytical TLC was carried out on plates coated with a 0·25 mm layer of micro-crystalline cellulose (Brinkmann Instruments, Inc.). For two-dimensional TLC 20 × 20 cm plates were used and the plates were developed in the first direction with BzAW and in the second direction with 10% HOAc. High voltage paper electrophoresis was carried out on Whatman No. 1 filter paper with 0·05 M borate buffer (pH 9), and 110 V/cm for 75 min.

The various grapefruit tissues were ground and exhaustively extracted with methanol. After enzyme hydrolysis the extracts were analyzed for naringenin, dihydrokaempferol, and kaempferol by two-dimensional TLC.

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[14] W. Barz, L. Patschke and H. Grisebach, *Chem. Commun.* 400 (1965).
[15] V. P. Maier and D. M. Metzler, Unpublished results.