Dihydrochalcones and Chalcones of Some Flavanone Glycosides: Qualitative and Quantitative Determinations

HARALD A. B. LINKE and DOUGLAS E. EVELEIGH*

Department of Microbiology, New York University Dental Center, New York

(Z. Naturforsch. **30b**, 940-942 [1975]; received June 19, 1975)

Dihydrochalcones, Flavanone Glycosides, Sweeteners, Thin-layer Chromatography,
Quantitative Analysis

The chalcones and dihydrochalcones of the flavanone glycosides naringin, neohesperidin and hesperidin were separated and identified by thin-layer chromatography; a similar procedure was utilized to perform preparative TLC for purification of these compounds. A new spectrophotometric method for the quantitative determination of dihydrochalcones in the concentration range from 0.005 to 0.100% in aqueous or alcoholic solution is described as well as a detection method of these compounds in agar media for microbiological studies.

Results and Discussion

Since cyclamate was banned in 1969 from the U.S. market of artificial sweeteners, and saccharin was removed from the "generally-recognized-as-safe" list of food additives by the Food and Drug Administration in 1972, the dihydrochalcone sweeteners derived from flavanone glycosides of citrus fruits have gained increased attention.

- 1: R = β -neohesperidosyl, R₁ = OH, R₂ = H,
 2: R = β -neohesperidosyl, R₁ = OCH₃, R₂ = OH,
 3: R = β -rutosyl, R₁ = OCH₃, R₂ = OH,
 4: chalcone of 1,
 5: chalcone of 2,
 6: chalcone of 3,
 7: dihydrochalcone of 1,
 8: dihydrochalcone of 2,
 9: dihydrochalcone of 3.

Requests for reprints should be sent to Dr. H. A. B. LINKE, New York University Dental Center, Department of Microbiology, 421 First Avenue, New York, N. Y. 10010, USA.

The dihydrochalcones can be easily obtained chemically from the corresponding flavanone glycosides naringin (1), neohesperidin (2) and hesperidin (3) by alkaline hydrolysis and catalytic hydrogenation *via* the chalcones¹⁻³. Naringin dihydrochalcone (7) and neohesperidin dihydrochalcone (8) are intensely sweet tasting compounds, however, hesperidin dihydrochalcone (9)** has no sweet taste⁴. In an attempt to produce these sweetening agents by microbial conversion of the flavanone glycosides⁵, some methods were explored for the quantitative determination of these compounds. The qualitative determination of some flavanone glycosides and their dihydrochalcones has already been reported⁶ as well as a quantitative method for the former compounds^{7,8}; we now describe a method for the quantitative determination of the latter compounds. The UV absorption peak in the range 407 to 409 nm of a complex with Neu's reagent (β -aminoethyl-diphenylborate)⁹ was used for their determination, since their natural UV maximum at 282 nm is interfered with by the UV absorption of the flavanone glycosides in this range. The complexes formed with the dihydrochalcones are stable for hours, whereas the complexes formed with the

* Department of Biochemistry and Microbiology, Cook College, Rutgers University, New Brunswick, N. J. 08903, USA.

** We thank Dr. R. M. HOROWITZ for a sample of hesperidin dihydrochalcone.

flavanone glycosides and their chalcones are not; with time, the absorption at the maximum increases in case of the former and rapidly decreases in case of the latter. The UV maxima of the reagent complexes with 7, 8 and 9 are at 407 nm, 408 nm and 409 nm, respectively; the absorption of the parent compounds in this range is negligible. The concentration of the dihydrochalcones can be determined by this method in aqueous or alcoholic solutions ranging from 0.005 to 0.100% (Fig. 1). An excess amount of Neu's reagent caused no interference in this method.

Fig. 1. Quantitative spectrophotometric determination of naringin dihydrochalcone (7), neohesperidin dihydrochalcone (8) and hesperidin dihydrochalcone (9) in methanol (0.2% Neu's reagent present).

Under strong alkaline conditions, *e.g.*, the synthesis of naringin chalcone (4), neohesperidin chalcone (5) and hesperidin chalcone (6)³ several

hydrolysis by-products of the flavanone glycosides are produced, which can only be removed from the desired product by column chromatography or preparative TLC. To monitor the purity of the synthesized products, thin-layer chromatography was utilized. A previously reported solvent system¹⁰ A (Table I) produced satisfactory results with silica gel thin-layer plates, although the presence of acetic acid in this system interfered with preparative TLC. For this reason the solvent system B (Table I) was developed, which produced excellent results in both TLC systems. The compounds 1 to 9 can be observed either under UV light (375 nm) or after developing with Neu's reagent (Table I). The R_f values of both systems increase in the orders flavanone glycoside < chalcone < dihydrochalcone, $6 < 5 < 4$ and $9 < 8 < 7$ (Table I). Therefore, TLC in combination with Neu's reagent can also be utilized to distinguish between the chalcones and/or dihydrochalcones. The color reaction with Neu's reagent is also useful for the qualitative determination of flavanone glycosides, chalcones and dihydrochalcones in agar media for microbiological studies; the respective colors are beige-yellow, yellow-orange and bright-yellow⁵.

Experimental

Quantitative determination of the dihydrochalcones

Standard solutions in final concentrations containing 0.005 to 0.100% of 7, 8 and 9 and 0.200% of Neu's reagent in methanol were prepared. The absorption of each colored complex that formed was measured in aliquots of these solutions in 1.0 cm quartz cuvettes using a Cary 14 Recording

Table I. Chromatographic characteristics of some flavanone glycosides, their chalcones and dihydrochalcones.

Compound	R_f value		Color Neu's reagent
	Solvent system A*	Solvent system B**	
Naringin (1)	0.59	0.57	Beige-yellow
Neohesperidin (2)	0.56	0.55	Beige-yellow
Hesperidin (3)	0.52	0.54	Beige-yellow
Naringin chalcone (4)	0.64	0.65	Yellow-orange
Neohesperidin chalcone (5)	0.57	0.59	Yellow-orange
Hesperidin chalcone (6)	0.53	0.56	Yellow-orange
Naringin dihydrochalcone (7)	0.65	0.67	Bright-yellow
Neohesperidin dihydrochalcone (8)	0.58	0.63	Bright-yellow
Hesperidin dihydrochalcone (9)	0.54	0.59	Bright-yellow

* n -BuOH:HAc:H₂O = 4:1:5 (upper phase).

** n -BuOH saturated with H₂O.

Spectrophotometer and read at the following wavelengths: 407 nm for 7, 408 nm for 8 and 409 nm for 9. Standard curves are shown in Fig. 1.

Thin-layer chromatography

Silica gel plates (Merck, 60F-254, 0.25 mm) were used for the separation of compounds 1 to 9, utilizing the solvent systems A (*n*-butanol: glacial acetic acid: H₂O = 4:1:5, upper phase) and B (H₂O-saturated *n*-butanol). The produced spots were usually detected under UV light (375 nm) or after developing by spraying the plates with 1% Neu's reagent in methanol. The *R_f* values (6 h running time at 23 °C) and colors are summarized in Table I. For preparative TLC silica gel plates (Merck, 60F-254, 2 mm) were utilized in combination with solvent system B.

Detection of flavanone glycosides, chalcones and dihydrochalcones in agar media for microbiological studies⁵

The surface of the agar plates was flooded with 1 ml of Neu's reagent (1% in methanol). After 5 minutes the colors of the complexes that formed, were evaluated in day light; flavanone glycosides, when present in the agar medium, produced a beige-yellow, chalcones a yellow-orange and dihydrochalcones a bright-yellow color.

This work was supported in part by National Science Foundation (grant number GI 34287 C). Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University - The State University of New Jersey, New Brunswick, N.J. 08903.

¹ R. M. HOROWITZ and B. GENTILI, U. S. Patent 3,087,821 (April 30, 1963).

² L. KRBECEK, G. INGLET, M. HOLIK, B. DOWLING, R. WAGNER, and R. RITER, J. Agr. Food Chem. **16**, 108 [1968].

³ H. A. B. LINKE and D. E. EVELEIGH, Z. Naturforsch. **30b**, 606 [1975].

⁴ R. M. HOROWITZ and B. GENTILI, J. Agr. Food Chem. **17**, 696 [1969].

⁵ H. A. B. LINKE, S. NATARAJAN, and D. E. EVELEIGH, Abstr. Ann. Meet. Amer. Soc. Microbiol. **1973**, 171, P 184.

⁶ B. GENTILI and R. M. HOROWITZ, J. Chromatogr. **63**, 467 [1971].

⁷ R. GOREN and S. P. MONSELISE, J. of the A. O. A. C. **47**, 677 [1964].

⁸ A. CIEGLER, L. A. LINDENFELSER, and G. E. N. NELSON, Appl. Microbiol. **22**, 974 [1971].

⁹ R. NEU, Naturwissenschaften **44**, 181 [1957].

¹⁰ T. B. GAGE, C. D. DOUGLASS, and S. H. WENDER, Analyt. Chem. **23**, 1582 [1951].