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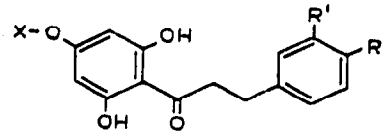
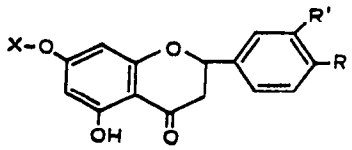
Chromatography of dihydrochalcone sweeteners and related compounds

A reagent for detecting dihydrochalcones

Continuing interest in the dihydrochalcone sweeteners[1] derived from citrus bitter principles prompts us to record some chromatographic procedures that are useful in the analysis of these and related compounds. Two of the sweeteners, naringin dihydrochalcone (I) and neohesperidin dihydrochalcone (II), are obtained by reduction of the bitter flavanone glycosides, naringin (III) and neohesperidin (IV), respectively. A third sweetener, hesperetin dihydrochalcone 4′-β-D-glucoside (V), is obtained by reduction of the tasteless glycoside hesperidin (VI) to hesperidin dihydrochalcone (VII) followed by partial acid hydrolysis. This yields a mixture of V and hesperetin dihydrochalcone (VIII).

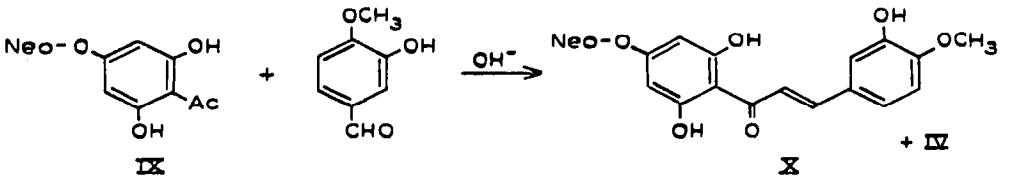




(I) R=OH; R′=H; X=β-neohesperidosyl
(II) R=OCH_3; R′=OH; X=β-neohesperidosyl
(V) R=OCH_3; R′=OH; X=β-D-glucosyl
(VII) R=OCH_3; R′=OH; X=β-rutinosyl
(VIII) R=OCH_3; R′=OH; X=H.

(III) R=OH; R′=H; X=β-neohesperidosyl
(IV) R=OCH_3; R′=OH; X=β-neohesperidosyl
(VI) R=OCH_3; R′=OH; X=β-rutinosyl.

When naringin dihydrochalcone and neohesperidin dihydrochalcone are made by the procedures mentioned, the reactions occur smoothly and the products are isolated in high yield. On a large scale, however, it is usually necessary to prepare neohesperidin dihydrochalcone by the conversion of naringin, due to the scarcity of neohesperidin. This is done by treating naringin with alkali to form an intermediate, phloracetophenone 4′-β-neohesperidoside (IX), which is then allowed to condense with isovanillin to form neohesperidin chalcone (X) and neohesperidin (IV). The reactions are as follows:



Depending on reaction conditions and the purification steps used, the final product may contain, in addition to neohesperidin dihydrochalcone, small amounts of neohesperidin, phloracetophenone 4′-β-neohesperidoside, naringin and naringin dihydrochalcone. These five closely related compounds can be cleanly separated by either the thin-layer chromatographic or paper electrophoretic procedures described below. Compounds VI, VII and VIII, which may be present as impurities in prepara-

tions of hesperetin dihydrochalcone glucoside, can also be separated by these procedures or, better, by paper chromatography.

In earlier work we showed that it is possible to detect naturally occurring flavanones by the purple or mauve colors they produce when reduced with sodium borohydride and treated with hydrogen chloride[2]. This reaction is specific for flavanones and is not given by any other class of flavonoid compound, including dihydrochalcones. We now find that the test conditions can be modified so as to give similar colors with the kinds of dihydrochalcones discussed here. The modification consists of adding a small amount of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to the acidic reagent used to develop the color. Under these conditions dihydrochalcones give immediate, brilliant colors; flavanones respond weakly; and other types of flavonoids do not respond at all. Since the reagent can also be used to detect dihydrochalcones in solution, the test should be particularly valuable in examining plant extracts for the presence of dihydrochalcones. The reactions involved in the color formation are being studied.

TABLE I

CHROMATOGRAPHIC DATA FOR DIHYDROCHALCONES AND RELATED COMPOUNDS

Compound	R_F *value*		*Electrophoretic Migration*[a]	*Color*	
	Polyamide sheets	*Paper*		*Benzidine*	*$NaBH_4$–D*
Neohesperidin (IV)	0.69	0.73	0.62	yellow	
Naringin (III)	0.57	0.74	0.73	red-brown	
Hesperidin (VI)	0.72	0.72	0.39	yellow	
Neohesperidin dihydrochalcone (II)	0.42	0.60	0.87	red-brown	blue-purpl
Naringin dihydrochalcone (I)	0.28	0.61	0.92	red-brown	blue-red
Hesperidin dihydrochalcone (VII)	0.43	0.59	0.63	red-brown	blue-purpl
Hesperetin dihydrochalcone 4′-β-D-glucoside (V)	0.49	0.44	0.60	red-brown	blue-purpl
Hesperetin dihydrochalcone (IV)	0.45	0.18	decomp.	red-brown	
Phloracetophenone 4′-β-neohesperidoside (IX)	0.48	0.81	1.14	red-brown	
Rutin			1.00	red-brown	

[a] Relative to rutin = 1.00.
[b] Flavanones give a brownish pink color that develops slowly (5–10 min).

Experimental

R_F values, electrophoretic migrations and colors of spots are shown in Table I.

Thin-layer chromatography. Eastman Chromagram polyamide sheets (type K 541 V) gave excellent separations and, at least in this application, were superior to polyamide-coated glass plates prepared in the laboratory*. A concentrated solution of the sample in pyridine or dimethyl sulphoxide was applied in a *single* spotting or streaking and was dried immediately in a current of warm air. The sheet was irrigated (ascending) with nitromethane–methanol (3:2) and, after drying it in a fume hood,

* Mention of specific products does not constitute endorsement by the U.S. Department of Agriculture.

was sprayed with diazotized benzidine[3] solution. This brings out all the phenolic compounds as yellow or red-brown spots. The borohydride–DDQ reagent can be used selectively to show the dihydrochalcones, but the polyamide sheets must be sprayed very lightly with this reagent; otherwise they become transparent and it is difficult to see the spots.

Paper chromatography. Whatman No. 1 paper was irrigated with 10% aqueous acetic acid. Either the benzidine or borohydride–DDQ spray reagent is suitable.

Paper electrophoresis. Whatman No. 1 paper was used with 0.1 *M* sodium borate solution as the electrolyte. The sample was streaked on the paper, which was then dipped in the electrolyte and placed in a water-cooled EC electrophoresis apparatus. Electrophoresis was carried out for 6 h at about 920 V. The dried paper was sprayed with diazotized benzidine solution. The borohydride–DDQ reagent was ineffective on the electrophoretic papers, probably due to the buffering action of the sodium borate. Table I lists migrations relative to rutin = 1.00.

Dihydrochalcone spray reagent (borohydride–DDQ). The chromatogram is sprayed initially with a methanolic solution of sodium borohydride (about 0.5% w/v is satisfactory). It is allowed to dry and is then sprayed with a solution made up as follows: *p*-Toluenesulfonic acid monohydrate (2 g) is dissolved in warm glacial acetic acid (5 ml). 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (2–5 mg) is added to the cooled solution. The reagent is stable for many days.

Dihydrochalcones of the type discussed here show up immediately in various shades of purple. Flavanones react more slowly but eventually appear as brownish-pink spots. Various other types of naturally occurring flavonoids that were tested failed to give colors.

Testing for dihydrochalcones in solution. The dihydrochalcone (< 1 mg) in a minimum of methanol (< 0.5 ml) is treated with solid sodium borohydride (~ 2–3 mg). After several minutes a drop of acetic acid is added to the solution to destroy excess borohydride. Solid DDQ (< 0.5 mg) is then added followed by concentrated hydrochloric acid (1–2 ml). Dihydrochalcones of the type discussed here yield purple colors, which are often stable for several days.

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