Flavonoids of Citrus. I. Isolation of Diosmin from Lemons (*Citrus limon*)

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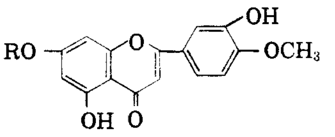
The flavonoids of *Citrus* have received considerable study in recent years, particularly in connection with their reported biological actions.[1] Although various citrus fruits have yielded a number of interesting flavonoids, the only compound of this type which has been unequivocally identified in the lemon is hesperidin (3′, 5, 7-trihydroxy-4′-methoxyflavanone 7-rhamnoglucoside).[2] There have also been reports which indicate the presence of a glycoside of eriodictyol (3′, 4′, 5, 7-tetrahydroxyflavanone) but the identity of this substance has not been rigidly established.[3]

Lemon peel is a particularly rich source of flavonoids. When a paper chromatogram of a methanolic extract of the peel is sprayed with an aluminum chloride solution and observed in ultraviolet light a complicated mixture of phenolic compounds is revealed. Of the four major spots two are flavanone glycosides (hesperidin and presumably an eriodictyol glycoside) and two are flavone glycosides. Several other flavonoid spots occur regularly but in smaller concentration. In addition, there are two spots which give a red color with vanillin-hydrochloric acid (probably catechin types) and a number of spots which fluoresce with a blue color (hydroxylated cinnamic acids and coumarins).

One of the two major flavone glycosides has been isolated and identified as diosmin (3′, 5, 7-trihydroxy-4′-methoxyflavone 7-rhamnoglucoside) (I), the flavone analog of hesperidin.[4] This compound was present in a mixture of hesperidin and other flavonoids which precipitated from a concentrated methanolic extract of the dried peel. Upon boiling this mixture for some time in alcoholic hydrochloric acid, all of the glycosides except one were hydrolyzed and dissolved. The undissolved, extremely insoluble residue proved to be diosmin. This compound could also be obtained by extracting the peel exhaustively with several portions of methanol; the final extract contained diosmin, the least soluble of the glycosides, in an essentially pure condition.



I R = Rhamnoglucosido
II R = H

The identity of the flavone glycoside as diosmin was established by comparing its melting point, absorption spectrum, and R_f values with those of an authentic sample prepared by the dehydrogenation of hesperidin.[5] Hydrolysis in concentrated hydrochloric acid yielded diosmetin (II), rhamnose, and glucose, all of which were characterized. Demethylation of the diosmetin with hydriodic acid gave the expected luteolin (3′,4′,5,7-tetrahydroxyflavone), thus confirming the pattern of hydroxylic substitution.

An extract of Valencia orange peel did not contain any diosmin other than possibly a trace amount. This is somewhat surprising since the orange is one of the citrus fruits which produces hesperidin. The presence of both diosmin and hesperidin in the lemon constitutes an additional example of a pair of flavonoids which differ only in the oxidation level of the heterocyclic ring. The occurrence of such pairs, which are fairly numerous, may plausibly be interpreted as indicating a common precursor in the biosynthesis of these compounds and suggests that the nature of the heterocyclic ring is determined in one of the late stages of the synthesis.[6]

EXPERIMENTAL

Ripened, unwaxed lemons of a commercial variety (mainly Eurekas) were obtained from a local packing house. The fruit was reamed and the chopped peel was dried in a draft oven at 37° for 36 hours.

Extraction of the peel. The dried, finely powdered peel (3.5 kg.) was extracted in a metal Soxhlet extractor with the following solvents for the specified periods: (A) petroleum ether (48 hours); (B) ether (48 hours); (C) acetone (64 hours); (D) methanol (64 hours); (E) methanol (1 week); (F) methanol (2 weeks). The largest amount of extracted constituents was contained in extract D, which, on evaporation, yielded about 1 kg. of amber colored syrup.

Isolation of diosmin. (1). Extract E contained a solid which was washed free of gum with warm water and ethanol. The resulting grey-white crystals (30 g.) were soluble in alkali and gave a purple color when treated with magnesium in alcoholic hydrochloric acid. Paper chromatography showed the presence of both flavanones and flavones. One gram of this solid was treated with methanol (250 cc.) and concentrated hydrochloric acid (30 cc.) and the mixture was boiled under reflux for 16 hours. The white residue (0.2 g.) from this treatment was taken up in warm 1% sodium hydroxide and was crystallized by saturating the solution with carbon dioxide. Three recrystallizations afforded small yellow crystals of a flavone glycoside which had an ultraviolet absorption spectrum and R_f values indistin-

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(1) Scarborough and Bacharach in Harris and Thimann, *Vitamins and Hormones*, Academic Press, Inc., New York, 1949, vol. 7, p. 1.

(2) Swingle in Webber and Batchelor, *The Citrus Industry*, University of California Press, Berkeley and Los Angeles, 1943, vol. 1, p. 392.

(3) (a) Mager, *Zeit. physiol. Chem.*, **274**, 109 (1942); (b) Higby, *J. Am. Pharm. Assn., Scientific Ed.*, **32**, 74 (1943).

(4) Diosmin was first described by Oesterle and Wander, *Helv. Chim. Acta*, **8**, 519 (1925), who isolated it from hyssop and other plants.

(5) Lorette, Gage, and Wender, *J. Org. Chem.*, **16**, 930 (1951).

(6) Geissman and Hinreiner, *Botan. Revs.*, **18**, 77 (1952).

TABLE I

ABSORPTION SPECTRA OF DIOSMIN AND DIOSMETIN

	$\lambda_{max.}$ in mμ (log E)			
Diosmin				
in Ethanol:	345 (4.30);	268 (4.25);	255 (4.28)	
in Ethanol-0.002 *N* NaOH:	390 (4.03);	328 (4.12);	268 (4.34)	242 (4.24)
Diosmetin				
in Ethanol:	345 (4.32);	268 (4.25);	253 (4.28)	
in Ethanol-0.002 *N* NaOH:	376 (4.17);	319 (4.10);	278 (4.44)	237 (4.39)

guishable from those of the diosmin prepared from hesperidin.

(2). Extract F yielded a tan solid (3.3 g.) on standing. Paper chromatography showed this to be almost pure diosmin contaminated with a trace of a flavanone. (The other flavonoids of the peel, being more soluble than diosmin, were removed almost completely in extracts C, D, and E.) The flavanone contaminant was effectively removed by heating the crude diosmin (0.5 g.) for several minutes in 6 *N* sodium hydroxide (5 cc.) (thus converting the flavanone to its chalcone) followed by acidification with excess concentrated hydrochloric acid (3 cc.) in the cold. The diosmin which came out of the acid solution on brief standing was recrystallized from 1% sodium hydroxide to which carbon dioxide was added. It was obtained as small yellow crystals having a sharp but somewhat variable melting point. Several determinations in a capillary tube gave values ranging between 258° and 275° (uncorrected). A sample of diosmin prepared from hesperidin melted within 1–2° of the naturally occurring diosmin when the determinations were made simultaneously. There was no depression of the mixture m.p. Reported values of the m.p. of diosmin range from 270°[7] to 280°.[4]

Anal. Calc'd for $C_{28}H_{32}O_{15}$: C, 55.3; H, 5.30; CH_3O, 5.10. Found: C, 54.9; H, 5.50; CH_3O, 4.96.

The identity of the natural and synthetic diosmin was established further by comparing their utraviolet absorption spectra (Table I) and their R_f values. The solvent mixtures used in two-way chromatograms were acetic acid-water (1:9) followed by *n*-butanol-acetic acid-water (20:6:15). Both the natural and synthetic diosmin had average R_f values of 0.20 and 0.60 in these solvent systems, respectively. The spots appeared yellow in ultraviolet light after spraying the chromatograms with aluminum chloride solution.

Diosmin is estimated to comprise roughly 0.3–0.5% of the weight of the dried peel. It is a white or grey-white solid as obtained initially from the plant. On contact with alkaline reagents it becomes yellow and remains so through repeated recrystallizations. When dissolved in alcoholic alkali and treated with magnesium ribbon and excess hydrochloric acid it gives a red-orange color in contrast to the blue-violet produced by hesperidin.

Diosmin was synthesized by dehydrogenating hesperidin with N-bromsuccinimide, following a procedure recorded in the literature.[5] Although this is a convenient method, the yield of glycoside is low and it contains a small quantity of a bromo derivative.

Diosmetin. Diosmin (150 mg.) was dissolved in hot concentrated hydrochloric acid (2 cc.). After 5 minutes the solution was diluted with water and the gelatinous precipitate was collected, dried, and sublimed at 1 mm. (bath temperature 240°). The sublimate was crystallized twice from anhydrous methanol to give small yellow needles containing methanol of crystallization; m.p. 258–259°; reported m.p. 253–254°.[8] The mixture m.p. with authentic diosmetin (obtained by hydrolyzing synthetic diosmin) was not depressed.

Anal. Calc'd for $C_{16}H_{12}O_6 \cdot {}^1/_2CH_3OH$: C, 62.7; H, 4.46; CH_3O, 14.8. Found: C, 62.6; H, 4.52; CH_3O, 13.8.

Diosmetin triacetate, prepared in acetic anhydride-sodium acetate, crystallized from methanol as colorless needles; m.p. and mixture m.p. 195°; reported m.p. 195–196°.[8]

Anal. Calc'd for $C_{22}H_{18}O_9$: C, 62.0; H, 4.26. Found: C, 62.0; H, 4.35.

Rhamnose and *glucose* were identified in the hydrolysate by co-chromatography with authentic samples. The hydrochloric acid solution was freed of diosmetin by extraction with ethyl acetate and was evaporated to dryness. The residue was taken up in water and spotted on paper strips. In *n*-butanol-methanol-water (5:2:4) authentic rhamnose and glucose as well as the sugars in the hydrolysate had R_f's of 0.67 and 0.54, respectively; in ethyl acetate-pyridine-water (8:2:1) the R_f's were 0.31 and 0.05, respectively. The spots were made visible by spraying with aniline phthalate dissolved in butanol.

Demethylation of diosmetin. Diosmetin from 150 mg. of diosmin was dissolved in boiling acetic anhydride (2 cc.) to which hydriodic acid (2 cc.) was added dropwise. After boiling the solution for 1 hour it was cooled and diluted with aqueous sodium bisulfite. This was extracted with ethyl acetate, the ethyl acetate was evaporated, and the residue was crystallized from methanol. Recrystallization from methanol gave a buff-colored solid; m.p. and mixture m.p. with authentic luteolin 323–325°; reported m.p. 328–330°. Both the luteolin obtained from diosmetin and authentic luteolin moved with identical R_f's in acetic acid-water (1:1) and in ethanol-acetic acid-water (40:13:30), the R_f values being 0.50 and 0.88 in these solvents, respectively.

Extraction of orange peel. Dried, powdered Valencia orange peel (100 g.) was extracted successively with petroleum ether, acetone, and methanol. A paper chromatogram of the methanol extract showed the presence of hesperidin and other flavonoids but there was only a very faint luminescence at the position expected for diosmin.

Absorption spectra. Spectral data for diosmin and diosmetin are shown in Table I. The spectra of the synthetic and naturally derived compounds were identical.

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(7) Narasimhachari and Seshadri, *Indian Acad. Sci. Proc.*, **30A**, 151 (1949).

(8) Lovecy, Robinson, and Sugasawa, *J. Chem. Soc.*, 817 (1930).