Degradation of Hesperetin and Naringenin to Phloroglucinol

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A preparative, alkaline fusion procedure has been developed for the production of phloroglucinol from hesperetin, the aglycone of hesperidin from orange, or naringenin, the aglycone of naringin from grapefruit. Separation of phloroglucinol from the accompanying aromatic acid derivative was accomplished best in each case by column chromatography using Dowex-1 resin.

Alkaline cleavage of hesperetin (I, Figure 1) or naringenin (II) has been reported to give phloroglucinol (III), isoferulic acid (IV), and *p*-hydroxybenzoic acid (V), respectively (Horowitz, 1961). These hydrolyses have been studied to elucidate the structures of the aglycones and were not intended as preparative procedures.

Phloroglucinol or *s*-trihydroxybenzene is an expensive chemical used in many chemical processes and formulations (Atkinson, 1952; Nagel, 1951; Vennootschap, 1947). It is prepared from *s*-triaminobenzene (Kalle, 1965; Kastens and Kaplan, 1950) which is available as a reduction product of the corresponding trinitro compound, which in turn is prepared from TNT. Because of the cost and importance of phloroglucinol, its preparation from hesperidin and naringin was investigated.

The isolation of hesperidin and naringin from citrus peel (Asahina and Inubose, 1929; Hendrickson and Kesterson, 1965) and their acid hydrolysis (Pulley and von Loesecke, 1939; Wilson, 1955) have been studied extensively. The published hydrolysis procedures give almost quantitative removal of the two sugars, rhamnose and glucose, and generation of aglycones I and II.

EXPERIMENTAL

The best fusion conditions to obtain optimum yields of phloroglucinol (III) were determined from a study of the fusion of naringenin (II) with potassium hydroxide. These conditions were equally applicable to the degradation of hesperetin (I) to phloroglucinol. From these studies, the following procedure was developed, which gives reproducible yields of III of 60 to 65%.

Fusion Process. Two grams of naringenin, 10 grams of potassium hydroxide pellets, and 4 ml. of water were mixed and stirred in a nickel crucible until a deep red, homogeneous solution was obtained. The crucible was immersed in a salt bath [potassium nitrate–sodium nitrite (10 to 7, w./w.)] preheated to 160° C. A uniform rate of heating was maintained with an electric heater so that the fusion melt reached a temperature of 300° C. in 30 minutes. The melt was stirred continuously with a stainless steel rod. The melt became colorless at 200° C. and started to foam. From 280° to 300° C. all foaming ceased, and the melt became fluid and "quiet." The crucible was removed from the salt bath and cooled in ice during the addition of 50 ml. of water. Sufficient concentrated hydrochloric acid was added to the resulting solution to make it strongly acidic (pH ≈ 2), and it was concentrated to dryness on a film evaporator at 55° C. (water bath temperature). The crystalline residue was triturated with methanol and filtered, and the filtrate concentrated to dryness. This procedure gave 2.48 grams of crude, crystalline fusion product, which also contained some potassium chloride.

Detection of Degradation Products. Paper chromatography was used to detect the presence of phloroglucinol (III) and the accompanying *p*-hydroxybenzoic acid (V). Solid melt samples either withdrawn on glass rods during the fusion process or obtained after fusion were dissolved in water; the solution was acidified with hydrochloric acid and spotted on Whatman No. 1 filter paper. After drying, the papers were developed (ascending) using a butanol–acetic acid–water solution (4:1:5). The dried papers were then sprayed with a solution comprising equal volumes of 5% aqueous sodium nitrite and 1% sulfanilic acid in 6*N* hydrochloric acid. A final spraying with 10% aqueous sodium carbonate solution produced a dark brown, lower spot ($R_f \approx 0.65$) for phloroglucinol (III) and a yellow, upper spot ($R_f \approx 0.86$) for *p*-hydroxybenzoic acid. This detection technique was used both to determine the best fusion procedure for optimum yields of III and also in the development of procedures for the separation of III from V.

Separation of Fusion Products. Two methods for separating phloroglucinol (III) from the accompanying aromatic acid fusion products were developed. Both procedures were equally applicable to the fusion products from hesperetin or naringenin. Typical procedures using the fusion product from naringenin are given.

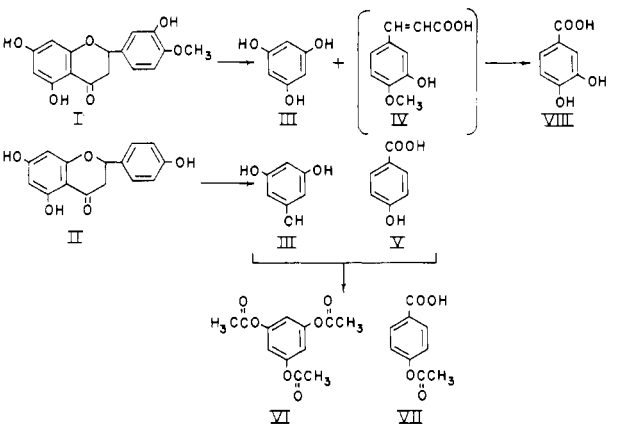

Figure 1. Products from the degradation of hesperetin and naringenin

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Column Chromatography. The crude fusion product (2.48 grams) was dissolved in about 250 ml. of water. This solution was then passed through a 13 × 120 mm. column of Dowex 1 × 8 (100- to 200-mesh) ion exchange resin in the acetate form. The fusion products were retained on the column. Phloroglucinol was removed from the column by elution with 2 liters of water acidified with acetic acid to pH 4. Approximately $3 \times 10^{-4}M$ acetic acid was sufficient to remove all phloroglucinol from the column. The eluate was collected using a fraction collector. Fractions were monitored by adding diazotized sulfanilic acid reagent, described previously, which gives a deep red color in the presence of phloroglucinol. The combined fractions, after concentration to dryness on a film evaporator at 50° C. (water bath temperature), gave 0.738 gram (79%) of tan colored crystals of crude phloroglucinol (m.p. 213–15° C.). Recrystallization from water afforded 0.603 gram (65%) of pure, anhydrous phloroglucinol (m.p. 217–19° C.). The infrared absorption of this product was identical with that of an authentic sample.

The *p*-hydroxybenzoic acid fusion product (V) remaining on the column was eluted with 150 to 200 ml. of 3*N* formic acid. Concentration of the eluate to dryness and recrystallization of the acid from water gave pure V in 80% yield, identified by melting point and infrared absorption.

Paper chromatography of the phloroglucinol and *p*-hydroxybenzoic acid obtained using this procedure showed that the two compounds were completely separated from each other.

Acetylation. The crude product (2.48 grams) from the fusion of naringenin was refluxed for 30 minutes in 10 ml. of acetic anhydride containing 1 gram of anhydrous sodium acetate. The mixture was cooled and diluted with water, and the resulting solution extracted five times with ethyl ether. The combined ether extracts were washed with 5% aqueous sodium bicarbonate solution until no more acidic material was removed. Concentration of the ether phase to dryness gave 1.21 grams (65% yield) of phloroglucinol triacetate (VI) as colorless needles (m.p. 100–102° C., reported m.p. 104° C.). The infrared absorption curve of this product was identical with that of an authentic sample of VI prepared by acetylation of phloroglucinol (98% yield). Ether extraction of the combined, acidified bicarbonate extracts gave 0.84 gram (64%) of *p*-acetoxybenzoic acid (plates, m.p. 186–9° C., reported m.p. 187–8° C.).

DISCUSSION

The course of the fusion process was followed by withdrawing samples of the melt at various times and temperatures. After weighing, these samples were made up at different concentrations in dilute hydrochloric acid and chromatographed on paper as previously described. In this way, the approximate concentration of phloroglucinol (III) in the melt could be determined at any given time. These studies showed: Phloroglucinol is not formed in appreciable amounts in the melt until the temperature is above 280° C. All phloroglucinol is rapidly destroyed by heating the melt to 360° C. When the melt is held at 300° C., there is a slow degradation of phloroglucinol and destruction is complete in about 1 hour. Direct fusion of naringin or hesperidin is not practical. The sugars char and interfere with the degradative process. All of these results indicate that the time and temperature of fusion are critical for optimum yields.

The fusion of hesperetin did not proceed as smoothly as that of naringenin, although comparable yields (60 to 65%) of phloroglucinol (III) were obtained. This was evident by more foaming and a thickening of the melt at 300° C. Also, isoferulic acid (IV) was not isolated by column chromatography of the fusion products from 2 grams of hesperetin (I). Instead, 0.85 gram of needles [m.p. 200° C. (dec.)] was isolated when the column was finally washed with 3*N* formic acid. This product did not depress the melting point (200° C. dec.) of an authentic sample of protocatechuic acid (3,4-dihydroxybenzoic acid, VIII, Figure 1) and its infrared absorption curve was identical to that of this dihydroxy acid. This represents an 85% yield of protocatechuic acid from hesperetin. The cleavage of the 4′-methoxyl group in ring B of hesperetin to hydroxyl is not surprising in view of the rigorous conditions employed in the fusion process. This is analogous to the published procedure for preparing protocatechuic acid by alkaline fusion of vanillin (Pearl, 1946).

Of the two procedures outlined for the separation of phloroglucinol (III) from the accompanying acid degradation products, column chromatography offers distinct advantages. Phloroglucinol is recovered *per se* and the extra steps of acetylation and hydrolysis of the triacetate to phloroglucinol are avoided. Also, column separation is more amenable to continuous isolation on a large scale.

The fusion of hesperetin and naringenin to give phloroglucinol, protocatechuic acid, and *p*-hydroxybenzoic acid offers a profitable outlet for two citrus by-products for which there is at present limited demand.

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