Table I.—Constants of the Volatile Oil of *Cyperus rotundus* L.

Item	Oil from Puerto Rico	Maximum and Minimum Values Reported by Other Investigators	
		Maximum	Minimum
Per cent yield (dry tuber basis)	0.40	1.0	0.45
Density 20° C.	0.9428	1.0944	0.9548
Refractive index 20° C.	1.5070	1.5175	1.4988
Optical rotation	−12.9	+35.5	−19.9
Acid value	1.7	11.3	1.0
Saponification value	22.1	31.4	6.6
Saponification value after acetylation	62.9	105.0	63.3

The first scientific article, devoted to the chemistry of this oil, was written by Joseph and Whitfield (3) in 1922. They determined, the principal constants of an oil obtained in the Sudan. In 1925 Rao, Panicker and Sudborough (4) examined several Indian oils, determining their constants and isolating mixtures of dicyclic and tricyclic sesquiterpenes. Hedge and Rao (5) continued this investigation and, in 1935, isolated for the first time the sesquiterpene-ketone α-cyperone. They also demonstrated the presence in this oil of l-pinene, cineole and of mixtures of secondary and tertiary alcohols. The structure of α-cyperone has been worked out at Simonsen Laboratory (6, 7). They have also synthesized this compound (8). In Japan Kimura and Ohtani (9) have investigated several samples of the oil distilled in that country.

EXPERIMENTAL

Eight thousand seven hundred and fifty grams of air-dried tubers, obtained in Puerto Rico, yielded 200 Gm. of petroleum ether extractive. This extractive was subjected to steam distillation and 32 cc. of volatile oil were obtained. On cohobating the aromatic water, 3 cc. of oil were recovered, making a total yield of 35 cc., that is, 17.5% of the petroleum ether extractive.

This oil has a faintly camphoraceous terebinthinate odor and a yellow color. The principal constants are reported in Table I.

SUMMARY

1. The literature pertaining to the volatile oil of *Cyperus rotundus* L. is reviewed.

2. The yield and constants of the oil, obtained from Puerto Ricon tubers, have been determined.

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The Chemical Nature of Hesperidin and Its Experimental Medical Use as a Source of Vitamin P— A Review

*By Ralph H. Higby**

The successful clinical treatment of hemorrhagic purpura, and other disorders arising from abnormal capillary fragility, by the administration of hesperidin, has recently been reported by a number of research workers. Present evidence indicates that this glucoside when administered orally fulfils the function of vitamin P, and can probably be regarded as a precursor of this vitamin. Since hesperidin is not generally well known, either to chemists or to the medical profession, it seemed worth while to review the literature dealing with the occurrence, preparation, properties and medicinal uses of this substance.

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OCCURRENCE

Hesperidin is a flavanone glucoside occurring in most varieties of citrus fruit. It was discovered by Lebreton (27) in 1828 and has been made the subject of numerous investigations since that time.

Pfeffer (36) (1874) found that hesperidin was precipitated as sphero-crystals in the cells of the sweet orange by soaking the tissue in glycerine or alcohol, or merely by desiccation. Using isolated pieces of tissue he found hesperidin in pith and bark of young twigs, in leaves and flower parts, in the parenchymatous cells of the fruit flesh and the cells of the pulp. Hesperidin was present in the lime, but not in the grapefruit or the Seville sour orange. He hypothesized that hesperidin occurred in solution in the cell sap from which it was precipitated by dehydration. Tunmann (50) (1913) confirmed this observation. Hall (14) (1925) speculated on the condition necessary for the solution of hesperidin in the cell sap, hypothesizing a glucose-hesperidin complex.

Hesperidin has been reported as occurring in many plants other than citrus. Zenetti (54) (1895) reported hesperidin in *Barosma*, Schulze (43) (1902) in *Skimmia Japonica Thunb*, Vogl (51) (1896) in *Scrophularia nodosa*, Tunmann in *Lobeliaceæ*, *Valerianceæ*, *Umbelliferæ*, *Labatæ*, *Compositæ*, *Papilionaceæ* and *Rutaceæ*. Alverson (1) (1919) found hesperidin in 250 varieties of *Labatæ*, Klein (22) (1921) in *Rubiaceæ*, but only in genus *Galium*, and Nilsson (32) (1921) reported hesperidin in a number of varieties of *Umbelliferæ*.

It is probable, however, that in many of the above instances hesperidin was not adequately identified, and that any substance depositing in the plant cell as sphero-crystals upon desiccation has been reported as hesperidin. Oesterle and Wander (33) (1925) following an extensive investigation found that many of the substances reported in the literature as hesperidin were in reality diosmin.

Hesperidin, according to Tunmann, occurs in greatest concentration in the green fruit. Harvey and Rygg (15) (1936) found a decrease from 3.07% to 2.03% in the rind of Washington Navel oranges in the period from January 5th to April 12th. These analyses are based on the dry weight of peel. Higher percentages were found in the blossom end than in the stem end. It was also shown that hesperidin increased in the fruit during storage.

Iwasaki (19) (1936) determined the amount of hesperidin in Mandarin oranges as follows: dry peel 5.68%, imbedded fiber 5.24%, dry endocarp 1.10%. The juice had only a trace. Kwang-Fong Tseng and Ren Dzin Yu (25) (1936) obtained 8.2% of pure hesperidin by the methyl alcohol extraction of dried orange peel. A related substance, neohesperidin, m. p. 244°, was obtained from bitter oranges by Kolle and Gloppe (23) (1936).

PREPARATION

Most of the early preparations of hesperidin were undoubtedly impure. Lebreton, for example, gave a melting point of 109° for his original preparation. Nearly all of the extraction methods have been similar, depending on the solubility of hesperidin in dilute alkali, and using alcohol to repress the solution of pectin. Typical methods of preparation are given below.

Pfeffer (36) (1874) soaked the crushed fruit in alkaline 50% alcohol for several hours, pressed off the liquor and neutralized with HCl, obtaining sphero-crystals. These were purified by solution in alkali and reprecipitation with acid.

Paterno and Briosi (34) (1876) followed Pfeffer's method of extraction but crystallized from boiling acetic acid, obtaining the first semblance of a pure product, m. p. 243–245°.

Hilger (16) (1876) used a modification of Pfeffer's method on dried green oranges, purifying his product by crystallization from strong alkaline alcohol through saturation with HCl gas, and a final crystallization from acetic acid.

Tiemann and Will (48) (1881) pointed out that many substances reported as hesperidin were mixtures, and that some if not all *Aurantiaceæ* contain another more soluble glucoside. In their preparation of hesperidin the peel was first extracted with large quantities of water to remove lead acetate-pre-

cipitable substances, then exhausted with 50% alcohol containing 1–2% KOH. The hesperidin was precipitated by neutralization, and the crude product cooked out with 90% alcohol to remove colored impurities. The colorless residue was dissolved in very dilute alcoholic NaOH and hesperidin precipitated by bubbling in CO_2. The melting point of the needle-shaped crystals obtained in this way was 251°. Crystallization from alcohol or from boiling glacial acetic acid gave no purer product.

Kwong-Fong Tseng and Ren Dzin Yu (25) (1936) by extracting dried orange peel with a methanol-ethanol mixture to remove impurities, then extracting the residue with large quantities of methanol, obtained hesperidin having the highest melting point yet reported, 261–262° corrected.

CHEMICAL PROPERTIES

Pure hesperidin occurs in long hair-like colorless needles, easily soluble in dilute alkali, and in pyridine, slightly soluble in methyl alcohol and in hot glacial acetic acid, almost insoluble in acetone, benzol and chloroform. In water it dissolves to the extent of one part in 50,000. Hesperidin, in common with other flavanone glucosides, has the property of forming complex crystals with other similar glucosides, which greatly affect its solubility and other physical properties, making it difficult to obtain in a pure state. For this reason much of the earlier work on structure has been misleading. Hoffman (17) (1876) reported that by acid hydrolysis hesperidin was resolved into hesperetin (m. p. 225°) and glucose. Alkaline hydrolysis was said to produce hesperetinic acid. Tiemann and Will (48) (1881) prepared hesperetin by hydrolyzing hesperidin for 3 hours in 2% H_2SO_4 in 50% alcohol, heating in a pressure vessel to 115–120° C. Dilution of the hydrolysis liquor with water precipitates the hesperetin, which is taken up in alcohol, clarified with lead acetate, and recrystallized by dilution with warm water. The crystals melted at 224–226° with decomposition. Analysis showed the formula as $C_{16}H_{14}O_6$. Alkaline hydrolysis of hesperetin yielded phloroglucinol and hesperetinic acid, m. p. 226°. "Hesperetinic acid" was shown to be identical with isoferulic acid. Later Will (52) (1887) determined that both rhamnose and glucose were obtained by the acid hydrolysis of hesperidin, the sugars were separated by the insolubility of phenyl glucosazone in acetone. Tanret (47) (1888) confirmed this finding, and suggested the probable hydrolysis reaction as follows:

(Tanret)

$$\underset{\text{Hesperidin}}{C_{50}H_{60}O_{27}} + 3H_2O \rightarrow \underset{\text{Rhamnose}}{C_6H_{14}O_6} + 2\underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Hesperetin}}{C_{32}H_{28}O_{12}}$$

This view was widely accepted and the $C_{50}H_{60}O_{27}$ formula for hesperidin is still given in many handbooks. Perkin (35) (1898) confirmed the formula for hesperetin as $C_{16}H_{14}O_6$, but assigned the substances an ester structure.

Power and Tutin (37) (1907) pointed out the close relationship between hesperetin and homoeriodictyol. Asahina and Inubose (4) (1929) studied the hydrolysis of hesperidin. They found that the reaction proceeded as follows:

(Asahina)

$$\underset{\text{(Hesperidin)}}{C_{28}H_{34}O_{15}} + 2H_2O \xrightarrow[\text{Acid}]{} \underset{\text{(Hesperetin)}}{C_{16}H_{14}O_6} + \text{glucose} + \text{rhamnose}$$

When heated with $Ba(OH)_2$ hesperidin yielded isoferulic acid plus a rhamnoglucoside. Acid hydrolysis of the latter gave glucose, rhamnose and phloroglucinol.

King and Robertson (21) (1931) confirmed these results and located the position of the sugar residue at carbon atom number seven. The structural formula for hesperidin is that of a typical flavanone as shown below (Fig. 1).

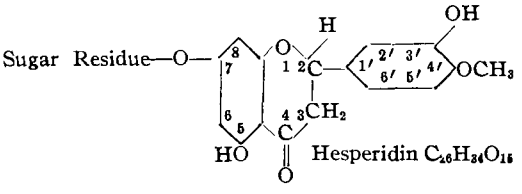

Fig. 1.

Naringin, the bitter glucoside of grapefruit, differs from hesperidin only in the absence of the methoxyl group in position 4′ and the shift of the hydroxyl group to this position (Fig. 2).

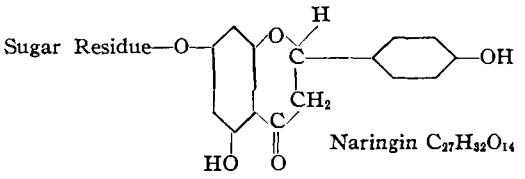

Fig. 2.

The vitamin-like activity of certain lemon peel preparations was believed by Szent-Györyi to be due to eriodictyol glucoside, a substance which had never been isolated, but which could theoretically be formed by the demethylation of hesperidin. The structural formula for this substance is that given by Gortner, shown in Fig. 3.

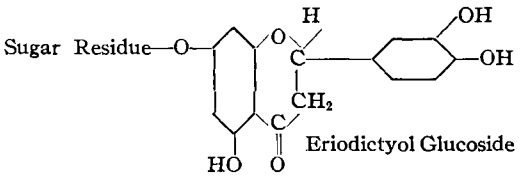

Fig. 3.

MEDICAL USES OF HESPERIDIN

In 1936 Szent-Györyi (3) and a group of his collaborators reported the beneficial effects of lemon juice, and of extracts of Hungarian red pepper, in a number of cases of *purpura hemorrhagica* which had failed to respond to large dosages of pure ascorbic acid. Because of the vitamin-like action of the curative substances in these extracts it was called vitamin P, to indicate its effect on permeability of the capillaries.

Seeking the active principle, a crystalline flavone substance was isolated from lemon juice and was named "citrin." This was shown to be physiologically effective.

Chemical studies of citrin (9, 39) showed it to be a mixed crystalline substance composed largely of hesperidin, but containing smaller amounts of eriodictyol glucoside, called by Szent-Györyi, "eriodictin." This latter was not obtained in pure crystalline form.

Szent-Györyi obtained patents (13, 46) covering the preparation of "citrin" and of "eriodictin" solution, the latter being preferred by him for administration by injection. Chromatographic absorption studies by Robeznieks (38) indicated that citrin contained a third quercitrin-like flavone in addition to hesperidin and eriodictin. This possibly explains the observation of Armentano (2) that citrin brings about a definite lowering of blood pressure which he attributed to a quercitrin-like flavone.

The favorable results reported by various workers in the administration of the several flavone preparations in a large number of clinical cases would seem to establish beyond doubt that these substances exert a valuable therapeutic effect upon capillary fragility and permeability.

In addition to the original observations of Szent-Györyi and his associates, Jersild (20) has reported the curative effect of 50 mg. of citrin daily in treatment of Shönlein-Henoch purpura; Lajos (26) found citrin effective in treatment of kidney hemorrhages; Morii (31) found the administration of 30 mg. of hesperidin daily effective in decreasing capillary permeability and increasing capillary resistance in various clinical cases suffering from pleurisy, tuberculosis, Graves' disease and beriberi; Decker (10) showed remarkable improvement in hemorrhagic diathesis, using dosages of 50–150 mg. daily of citrin, and found that recovery was accelerated by the administration of 500 mg. daily of ascorbic acid. Margitay-Becht (29) has reported a curative effect in three out of four cases of acute glomerulonephritis resulting from dosages of 55–110 mg. of citrin daily. Unfortunately in most of the foregoing work the flavone substances used are not sufficiently described, and are of unknown purity. This is especially true since the term "citrin" has been used loosely to describe either crystalline "citrin" or the "eriodictin" mother liquor.

Perhaps the most significant clinical work on vitamin P has been that of Scarborough and his associates, using hesperidin from oranges as the source of the vitamin. In 1938 Scarborough and Stewart (40) showed the value of hesperidin in the treatment of purpura resulting from the use of arsenicals in the treatment of syphilis. Scarborough (41) (1939) used pure hesperidin, crude hesperidin, soluble "citrin" prepared by Szent-Györyi's method from orange peel, and

orange and lemon juice in the treatment of a number of cases of lowered capillary resistance resulting from generalized vitamin deficiency, with uniformly successful results. Hesperidin was effective when given in doses of 1 Gm. per day orally, the soluble "citrin" was apparently equally effective when 0.2 Gm. daily was injected. Capillary resistance was not increased by administration of ascorbic acid. In a later paper (1940), Horne and Scarborough (18) have shown that erythema and dermatitis resulting from arsenical therapy are caused by lowered capillary resistance. The curative effect of hesperidin was demonstrated in one such case of toxic purpura and in one of toxic erythema.

Kugelmass (24) (1940) has also demonstrated the effectiveness of "vitamin P"[1] from oranges in curing several cases of vascular purpura.

HESPERIDIN AS VITAMIN P

Szent-Györgyi's claim for the vitamin-like nature of the flavone substances, based upon clinical evidence, appeared to be supported by the animal experiments of Bentsath (7) and his co-workers, which showed an increased survival time for those guinea pigs on a scorbutic diet that received supplements of vitamin P concentrates. Zilva (55) was unable to confirm these results, when pure hesperidin, pure eriodictyol, mixtures of these two substances, and citrin prepared according to Szent-Györgyi's directions were administered. Moll (30) likewise reported negative results, and later Bentsath (5, 6) himself was unable to check his earlier experiments. Similarly Detrick (11) failed to show any beneficial effects of citrin in scorbutic guinea pigs.

The negative results obtained by these workers were based upon the failure of the vitamin P preparations to alter the familiar symptoms of experimental scurvy. No attempt was made to measure changes in capillary resistance.

Zacho (53), however, in 1938, using a vacuum cup method, was able to show that diminution of capillary resistance in guinea-pig scurvy was not alleviated by administration of ascorbic acid, but was cured by administration of citrin.

More recently St. Rusnyak and Benko (44), using a similar technique, were able to produce diminished capillary resistance in rats maintained on a scorbutogenic diet, and were able to relieve this condition by the administration of 3 to 4 mg. of citrin per day. Thienes and Leser (49) have also reported a test method using mice as experimental animals.

These results are in accord with those of Scarborough (42) (1940) who has traced the syndrome of vitamin P deficiency in man as contrasted with that of scurvy. He was able to demonstrate a vitamin P deficiency which did not respond to treatment with vitamins A, B, D or ascorbic acid but was relieved by the oral administration of hesperidin and other citrus flavanones containing hesperidin.

These clinical and pharmacological results seem to prove the existence of a vitamin substance of flavone nature which serves to control the condition of the capillaries. Whether hesperidin itself is this vitamin or whether it is merely a precursor which is broken down in the digestive system to form the vitamin is not yet known.

Some investigators have questioned the vitamin nature of hesperidin on the grounds of the high dosage required. This is slightly less than 1 Gm. per day for an adult. It should be remembered, however, that nothing is known of the minimum protective dose. Comparing this curative dose for hesperidin with that of vitamin C (100–1000 mg. per day), it becomes evident that the two are of the same order of magnitude and therefore this argument alone is not valid.

SUMMARY

Hesperidin is not generally well known, hence a review has been made of the literature dealing with the occurrence, the chemical nature, and the medical uses of this substance. The review discloses that this citrus glucoside, has been successfully used in the treatment of disorders caused by

[1] Nature and source of the vitamin material not stated but presumably hesperidin.

abnormal capillary fragility. Clinical and pharmacological evidence indicates this substance as an source of vitamin P

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Drug Extraction. XXIV. The Effect of the Length of Drug Column on the Efficiency of Percolation of Cinchona*

By William J. Husa† and Clifford T. Pacenta‡

Recent studies of drug extraction have shown that the method of forced percolation through a long column of drug has given more efficient extraction of several drugs than could be obtained by ordinary percolation (1, 2, 3). However, little information has been available regarding the effect of variations in the length of the drug column on the efficiency of extraction. Hence some research has been carried out on this point, using cinchona as representing an important drug which offers difficulties in extraction.

EXPERIMENTAL

Materials Used.—Yellow cinchona, U. S. P., in moderately coarse (No. 40) powder, assaying 7.31% alkaloids and 8.87% moisture was used. The menstrua employed were those prescribed in the U. S. P. X for fluidextract of cinchona.

Analytical Methods.—Alkaloidal content of the percolates was determined by the U. S .P. XI method for Compound Tincture of Cinchona, with a modification in the amount of sample taken. Asbestos fiber was used as the adsorbent. Moisture was determined by the U. S. P. XI method for drugs containing no constituents volatile at 100° C. To determine total extractive, 10 cc. of the liquid were evaporated to dryness on a water bath and then heated in an oven at 105° C. until the difference between two successive weighings did not exceed 10 mg.

Apparatus and Procedure.—The apparatus described by Husa and Huyck (1) was employed, the length of the drug column being increased or decreased by varying the number of tubes and elbows. The tubes were of Pyrex glass, each tube having a length of 91 cm. and an internal diameter of 2.5 cm. The drug was moistened with 25 cc. of Menstruum I per 100 Gm. of drug and packed, in sections, with moderately firm pressure, using 20 to 25 Gm. of drug for each packing portion. After being packed with drug, the tubes and elbows were joined together by means of rubber jackets and metal joint flanges. One end of the drug column was connected to a storage tank containing Menstruum II. This menstruum was forced through the drug column by means of air pressure obtained from the compressed air line, using a reducing valve and gage to secure the desired pressure. The percolate was collected in four equal fractions, the volume of each fraction amounting to 0.5 cc. for each Gm. of drug used. Each experiment was repeated and the data in the tables represent the averages of the duplicate experiments. No breaks in the drug column were observed in any of the experiments. The experimental conditions are given in Table I and the results are summarized in Table II.

DISCUSSION OF RESULTS

Table III shows the per cent of total alkaloids extracted in the first fraction, second fraction, and total of the first two fractions of percolate. Table IV shows the amount of total extractive obtained in 100 cc. of percolate in the first and second fractions, and also the total amount obtained in these two fractions.

The results in Tables III and IV indicate that the efficiency of extraction of alkaloids and total extractive is about the same for one and two tubes. The efficiency of extraction is greater when the drug column is lengthened by using three and five tubes, respectively.

Table I.—Experimental Data

	Experiments			
	A	B	C	D
Number of tubes used	1	2	3	5
Weight of drug in Gm.	300	600	950	1650
Approximate volume of packed drug in cc.	630	1295	1955	3325
Air pressure used	Up to 3 lb.	Up to 4 lb.	Up to 4 lb.	Up to 8 lb.
Average temperature	24.5° C.	24.5° C.	24.5° C.	23.5° C.
Time required for experiment (in hours)	29.5	98.1	210.5	449.2

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