Neohesperidin dihydrochalcone: an updated review on a naturally derived sweetener and flavor potentiator

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The demand for and acceptance of citrus fruit in the daily diet of human beings is based largely on nutritional value, flavor, aroma, and other aesthetic characteristics such as color and texture. Citrus fruit is a primary source of our daily requirement of vitamin C, which man cannot synthesize, and additionally, provides, supplementary nutritional value of citrus bioflavonoids as well as amino acids, inorganic salts, and carbohydrates. Despite the worldwide use of grapefruit and other citrus derivatives in foods, drugs, beverages, and confections, surprisingly little has been written about neohesperidin dihydrochalcone (hereinafter referred to as NEO-DHC) and its many successful applications as a sweetening agent.

The principal flavonoid ingredient in grapefruit, the all-important flavonoid extractive, naringin, is the key to the successful synthesis and commercialization of NEO-DHC. It is the purpose of this paper to present the similarities and interrelationships between the naturally-occurring dihydrochalcones and neohesperidin dihydrochalcone.

Physical characteristics

NEO-DHC is a white, crystalline, nonhygroscopic solid, possessing sweetening power 1,500-2,000 times that of an equivalent amount of sucrose on a taste threshold basis. NEO-DHC is soluble in water, ethanol, acetone, and dilute alkalis while being insoluble in ether, ethyl acetate, hexane and dilute mineral acids. NEO-DHC is nonvolatile in the dry state, not being oxidized by atmospheric oxygen nor being degraded in hot water in the presence of oxygen. Its overall characteristics, extended stability, and nonhazardous nature are indicative of long shelf life and ease of transport with no expensive handling required. It has been reported that dihydrochalcones are resistant to hydrolysis by acids at pHs greater than 2 at room temperature.

Extraction of naringin

The flavor and general acceptability of grapefruit juice is adversely affected by the bitter flavonoid, naringin. Since the peel and the pulp contain significantly greater amounts of this flavonoid than the juice, great care must be taken during the juicing process to minimize the extraction of the flavanone, naringin, from the peel and pulp into the juice. The peel residue left from expressing the juice consists of two elements, the albedo or white pulpy inner portion, and the flavedo or outer yellow colored skin.

The Brown extractor, in conjunction with the Brown shaver, separates the whole reamed half peels into the shaved albedo and shaved flavedo, making the recovery of by-products and specialty products easier by segregating the citrus refuse into more distinct components.[1] The shaved albedo is subjected to a hot water or solvent leach. The hot extraction is performed at low pH values (8.9-9.0) with the addition of lime or alkali which serves two purposes: to coagulate the pectin-like materials also present which inhibit the naringin crystallization; and to solubilize naringin by virtue of alkali salt formation. The entire mixture is then squeezed in a screw press to separate the coarser particles from the extraction solvent. The solution is then refiltered and transferred to a glass-lined tank and adjusted to pH 6, during which time

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the chalcone form of naringin is converted to the flavanone and slowly crystallizes.

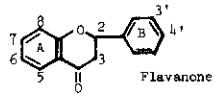
Great difficulty in extracting and obtaining acceptable yields of naringin have been documented in the literature, and at best the process remains an art as well as a science. Yields generally run from six to ten pounds per ton of wet peel, and unless great care is exercised in the process, they can be very disappointing.[2]

Naringin is one of the bitterest flavonoids known to man. Before NEO-DHC's discovery, naringin's principal use was to impart the characteristic bitter flavor to tonic waters, candy (chocolate), and marmalade. Today, because of these widespread uses, the demand for naringin exceeds the available supply. The annual grapefruit harvest from Florida alone could yield some five million pounds of naringin, but at present much of the peel is simply dried and fed to cattle, since there is no large-scale commercial extraction of naringin in Florida.

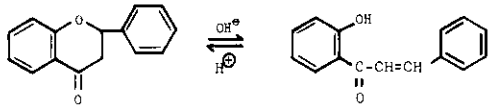
Relationship of flavonoids to NEO-DHC

Citrus fruits contain numerous flavonoid glycosides, four of which are bitter, four of which are tasteless, and all of which can be reduced to dihydrochalcones. The dihydrochalcone NEO-DHC is a typical example of the large group of plant products designated as flavonoids. Neohesperidin dihydrochalcone is the open chain (reduced) analog of the parent substances neohesperidin, a flavonone which occurs as the major constituent of the Seville orange.

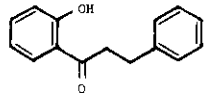
Flavanones possess the following structure:



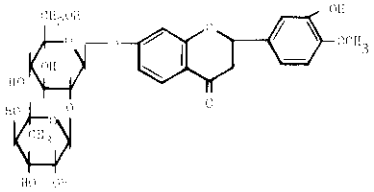
Upon addition of alkali, the hetero ring is opened to form a chalcone.



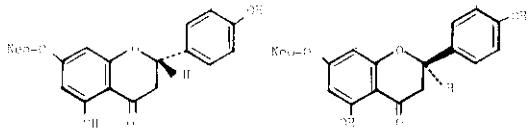
The chalcone is a highly conjugated planar compound, as attested by its orange color. If the double bond of the chalcone is hydrogenated, the colorless dihydrochalcone is formed.



When a sugar molecule is attached to a flavanone, as in naringin and neohesperidin, the resulting compounds are called flavanone glycosides, and are colorless, nonplanar compounds.

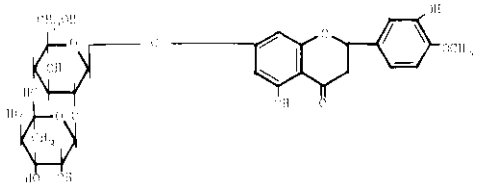


Carbon 2 of the flavanone moiety is a chiral center that can occur in either the S or R configuration. It has been found that most citrus flavanone glycosides exist in the 2S configuration.



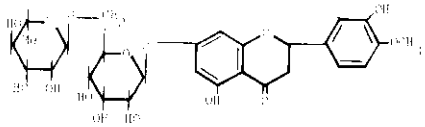
(-)-2 (S)-naringin (-)-2 (R)-naringin

Neohesperidin, which is bitter, occurs naturally in the Seville orange and has the following structure:



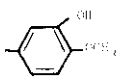
Left: Carbohydrate "portion" is the disaccharide, B-Neohesperidose (2-O-a-L-rhamnosyl-B-D-glucose). Right: Flavanone "portion" called an "aglycone" has no carbohydrate attached.

Hesperidin occurs abundantly in sweet oranges, Seville oranges, lemons and citrons. It is highly crystalline and is one of the easiest flavonoids to isolate.[5]

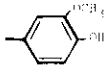


The carbohydrate "portion" is the disaccharide: B-Rutinose (6-)-a-L-Rhamnosyl-B-D-Glucose)

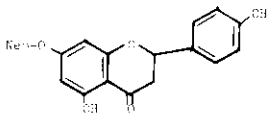
The "B" ring has the isovanillyl structure:



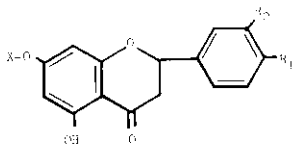
For reference, the "vanillyl" structure is:



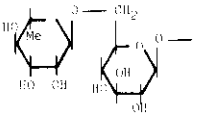
Naringin, the intensely bitter principal flavonoid in grapefruit, occurs as a colorless crystalline compound and is isolated as outlined. As little as 20 mgm per liter of water can be easily detected by taste. The carbohydrate portion is the disaccharide Neohesperidose (2-O-L-Rhamnosyl-B-D-Glucose).



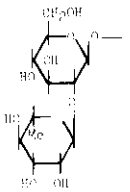
Flavanone glycosides in citrus fruits have the following structures:



X = Rutinosyl
(6-O-a-L-Ramnosyl-B-D-Glucose)

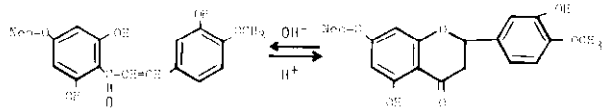


X = Neohesperidosyl
(2-O-a-L-Rhamnosyl-B-D-Glucose)

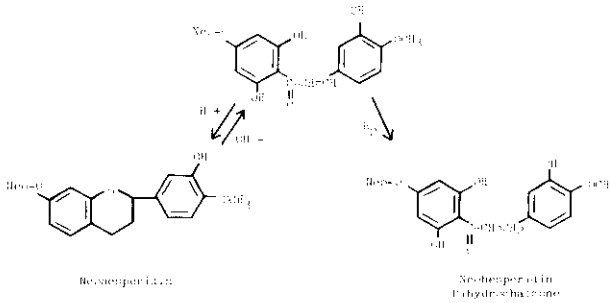


R1	R2		Occurrence		Occurrence
OCH_3	OH	Hesperidin	Sweet orange/ lemon	Neohesperidin	Seville orange
OH	H	Naringenin	Peel of navel & valencia	Naringin	Grapefruit
OCH_3	H	Isosakuranetin	Navel & valencia orange	Poncirin	Grapefruit
OH	OH	Eriocitrin	Lemons	Neoeriocitrin	Bergamots

Their occurrence in various fruits is shown in the above table. The chalcone form of neohesperidin is:



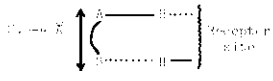
If the double bond is hydrogenated, the resulting neohesperidin dihydrochalcone is 1500 to 2000 times sweeter than cane sugar.



As a whole, the flavonoids are notoriously innocuous. They contain neither nitrogen nor sulfur, elements associated with toxicity. Most importantly, they occur in all higher plants and, therefore, always have been a common constituent of man's diet. A large number of dihydrochalcones have been found in nature.[6-15]

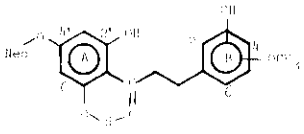
Introduction to sweetness

Shallenberger has postulated that virtually all sweet compounds have a molecular fragment designated A-H, B, in which A-H and B are proton donor and acceptor, respectively, in H-bonding partnership with the receptor site.[16]



Some evidence continues to be reported in support of this hypothesis. However, not all compounds with a structure fragment of this nature are sweet. The A-H, B moiety, then, is necessary for sweetness but does not guarantee sweetness.

After the discovery of the intensely sweet naringin dihydrochalcone, Horowitz and Gentili synthesized a large number of analogs from which the following generalizations can be made.[17]



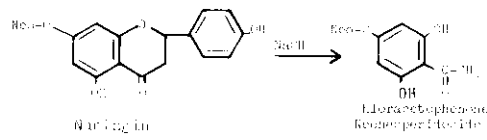
Neo=Neohesperidose
(2-O-alpha-L-rhamnosyl-beta-D-glucose)

1. A and B-ring hydroxyls are needed for sweetness, but their presence does not guarantee sweetness.
2. For hydroxy-alkoxy B-ring derivatives, the alkoxy must be the more remote from the C-2 carbon if sweetness is to be retained.
3. Tri-substituted B-ring derivatives (containing hydroxyl and alkoxyl groups) are not sweet.
4. Sweetness is possible, but diminished, with only one A-ring hydroxyl, provided the B-ring meets the requirements listed above.

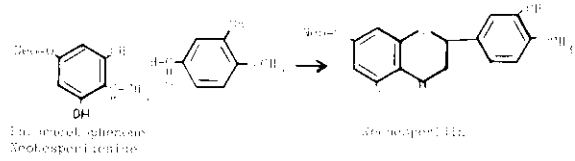
Dihydrochalcones are examples of flavonoids as mentioned previously. Flavonoids are defined by the presence of two C_6 aromatic groups, A and B, joined by a C_3 bridge (C_6 C_3 C_6). The particular type of flavonoid (anthrocyanin, aurone, flavone, flavonone, chalcone, dihydrochalcone, and so forth) is determined by the oxidation level of the C_3 bridge and by whether one of the carbon atoms of the bridge is linked through an oxygen atom to one of the aromatic units to form a third ring.

Synthesis of neohesperidin dihydrochalcone

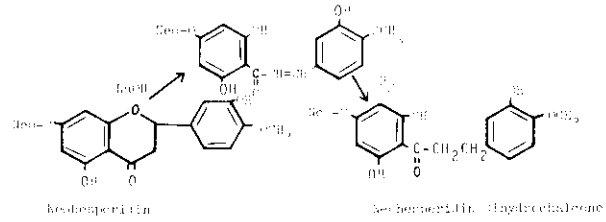
The synthesis of neohesperidin dihydrochalcone follows the general method developed by Horowitz and Gentili.[18] Naringin is degraded first to phloracetophenone 5'-neohesperidoside by alkali and high temperature.



Secondly, phloracetophenone 4'-neohesperidoside is condensed with isovanillin to produce neohesperidin.



Finally, hydrogenation of neohesperidin to neohesperidin dihydrochalcone.



Flavor formulations

Since neohesperidin dihydrochalcone is 1,500 to 2,000 times sweeter than sucrose, judicious amounts must be used in flavor formulation work to avoid the persistence associated with the overpowering effect of using high concentrations of such a powerful sweetener. Therefore, in order to successfully formulate, bulking agents are needed. Controlled amounts of NEO-DHC in conjunction with suitable bulking agents, sugar alcohols, and sugar-free carbohydrates are used with the product in question. Each application for individual products is custom-formulated, since each product requires a unique approach for successful formulation.

There are numerous other references in the literature to the use of other artificial sweeteners such as saccharin and cyclamates. The general techniques used with such sweeteners can also be successfully applied to NEO-DHC formulations.

Canned or frozen grapefruit juice is generally not widely accepted as a breakfast beverage due to its

bitter nature; the addition of NEO-DHC has been proposed to improve its organoleptic acceptance.[18] This situation is not unique to grapefruit. Other citrus fruits, notably the navel orange grown in California, could also possibly benefit from this technique, since the extracted juice also develops a bitter flavor.[19]

Toxicity studies

Extensive toxicity studies have been undertaken by the USDA testing NEO-DHC's safety for human consumption. In addition, CAFCO and Nutrilite have each submitted petitions to the FDA for approval of this new sweetener.

Beginning in 1965, the USDA conducted various in-vivo and in-vitro studies. All of these trials revealed no ill effects associated with the ingestion of dihydrochalcones.[20] Pathology reports on rats fed a diet of 2.5% and 5.0% NEO-DHC for two years revealed no occurrence of tumors or carcinogenic activity associated with the ingestion of NEO-DHC. A two year study by the USDA on young beagles produced no deleterious results attributable to NEO-DHC. The only disturbing find was that one of three dogs in the two highest dosage groups exhibited testicular atrophy. The dogs in these two groups were fed a diet of approximately 5% NEO-DHC. As of this writing, the significance of this occurrence is uncertain. No comparable testicular lesions were reported in a control group of 1,000 laboratory beagle dogs exhibiting a wide spectrum of spontaneous subclinical lesions, but there are other reports that idiopathic testicular atrophy and infertility do occur commonly and spontaneously in young dogs.[21]

In spite of the formidable evidence submitted by the USDA to the FDA, as well as the petitions of CAFCO and Nutrilite, the FDA has not approved NEO-DHC. The lack of carcinogenicity, mutagenicity, and extremely low order of toxicity of NEO-DHC as endorsed by the USDA studies are apparently not convincing enough for the FDA, probably because of the changes in protocol since the early USDA studies, as well as current FDA thinking. To remedy this problem, Congress has mandated that industry begin a program to find a suitable sweetener to replace saccharin, and the National Toxicological Program has tentatively given NEO-DHC a high priority rating for its test program in order to find a noncarcinogenic alternative sweetener.

Acknowledgement

The author appreciates the help of Dr. Robert Horowitz and Bruno Gentili, who kindly reviewed this article and whose suggestions and encouragement were invaluable. Thanks also to James Leary, who aided in the research and extensive revisions entailed in the preparation of this article. Also gratefully acknowledged is the assistance and collaboration of Dr. Samuel Klein in the extensive formulation work done on various products with neohesperidin dihy-

drochalcone as well as that of Al Passaro of the Manhattan Special Bottling Company of New York, for his contribution in using NEO-DHC in experimental soft drink formulations.

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