Advanced Organic Chemistry Laboratory
Natural products Chemistry

Isolation and Characterization of: *Experiment 1*
Hesperidin from Orange Peel[1] *Week 1*

Background Reading

Zubrick, J. W. *The Organic Chem Lab Survival Manual, 4th edition*, Wiley & Sons, Inc., New York, 1997.

Keeping a Notebook	Pg 12-24.
Extraction	Pg 147-164
NMR	Pg 339-350

Introduction to Flavonoids:

The flavonoid Compounds can be regarded as C_6-C_3-C_6 compounds, in which each C_6 moiety is a benzene ring, the variation in the state of oxidation of the connecting C3 moiety determining the properties and class of each such compound. The classes are shown below.

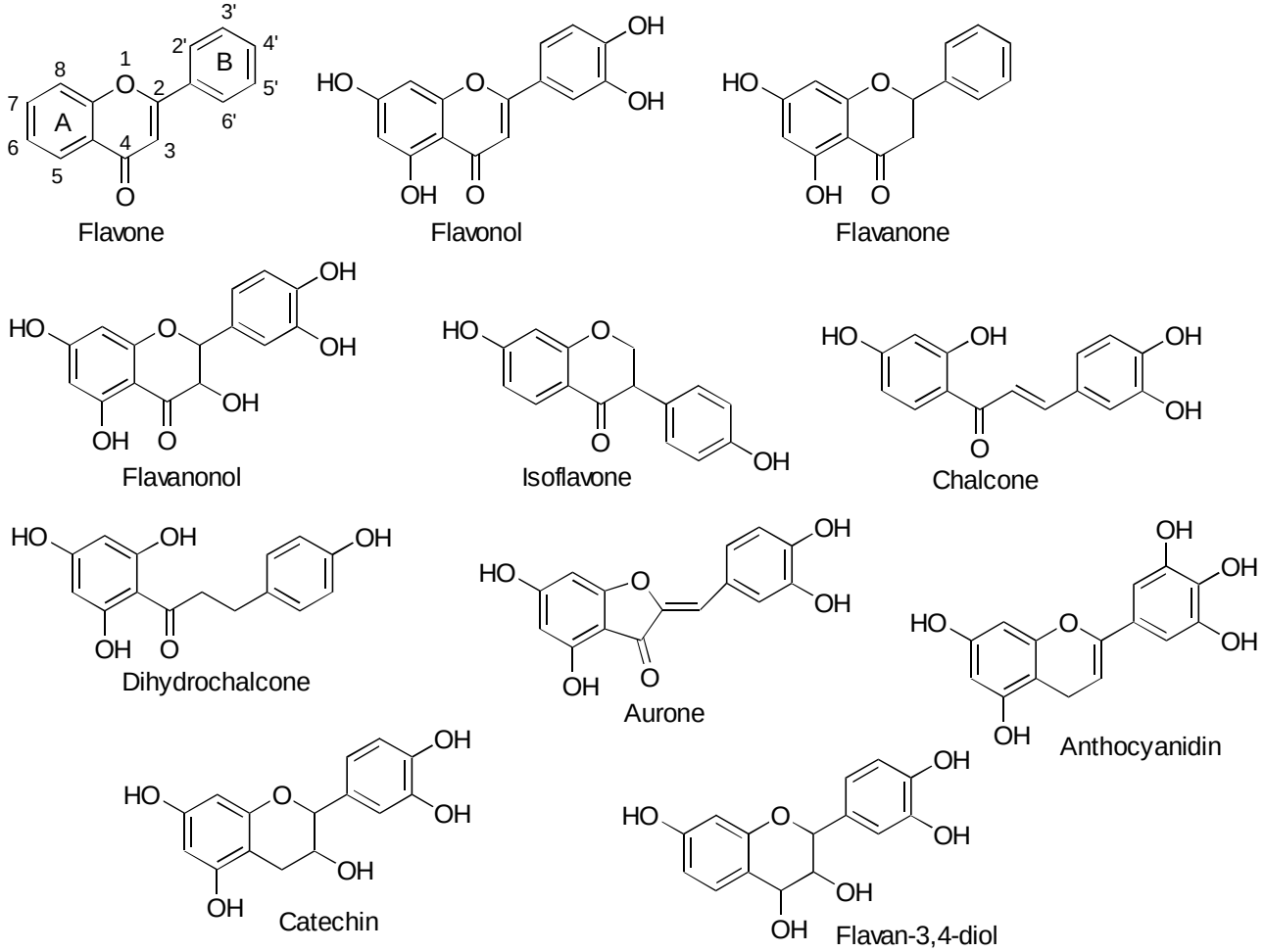


Figure 1. Classes of Flavonoids

Flavonoid compounds and the related coumarins usually occur in plants as glycosides in which one or more of the phenolic hydroxyl groups are combined with sugar residues. The hydroxyl groups are nearly always found in positions 5 and 7 in ring A, while ring B commonly carries hydroxyl or alkoxyl groups at the 4'-position or at both

[1] Experiment is a modified version of an experiment found in: Ikan, R. *Natural Products: A Laboratory Guide, 2nd Edition*, Academic Press, Inc. San Diego, 1991.

the 3'- and 4'-positions. Glycosides of flavonoid compounds may bear the sugar on any of the available hydroxyl groups.

Flavonoids occur in all parts of plants, including the fruit, pollen, roots, and heartwood. Numerous physiological activities have been attributed to them. Thus, small quantities of flavones may act as cardiac stimulants; some flavones, e.g., hesperidin, appear to strengthen weak capillary blood vessels; highly hydroxylated flavones act as diuretics and as antioxidants for fats. It is also claimed that flavones behave like auxins in stimulating the germination of wheat seeds. The flavonoids are responsible for the bright colors of insect-pollinated flowers and edible fruits. It has been postulated that the function of these colored compounds is to make these plant organs more conspicuous in order to aid seed dispersion by animals and insects.

The fundamental method in structural studies of flavonoids is alkaline (or base) hydrolysis. For example, **Figure 2** shows the alkaline degradation of chrysin to yield phoroglucinol, acetic acid, benzoic acid and a small amount of acetophenone. These types of degradations were once the most important step in the determination of the structure of natural products. Today, spectroscopy, particularly nuclear magnetic resonance (NMR) spectroscopy is the method by which structures of new natural products are determined. The main advantages of NMR over degradative methods are that much smaller amounts are required for NMR analysis and the NMR experiments can be performed quicker than planning a degradation scheme on an unknown. For flavonoids, color reactions can also play an important role in the preliminary stages of structure analysis.

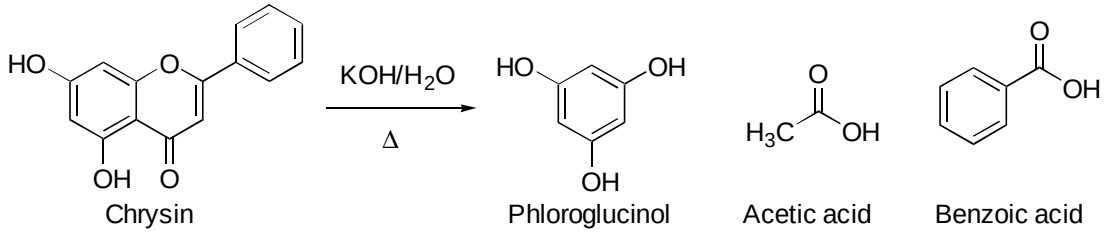


Figure 2. Alkaline Degradation of Flavones

The possibility of interconversion between the various structures shown in **Figure 1** is of considerable importance in the structural elucidation of flavonoid compounds. For example, chalcones and flavones are isomeric and can readily undergo interconversion, **Figure 3**, and flavanones may be converted into flavonols and flavones through mild oxidation-reduction steps.

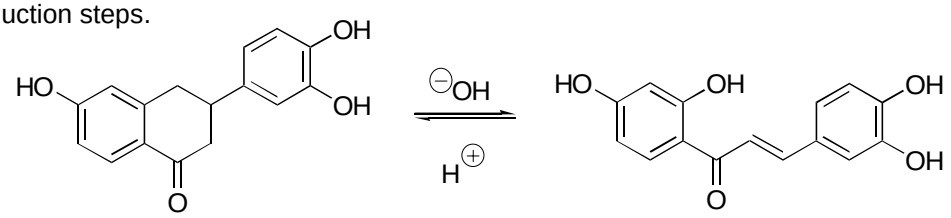


Figure 3. Interconversion of Flavanones and Chalcones

Flavones:

In flavones, ring C is basic and forms a pyrylium salt upon reaction with a strong acid such as hydrochloric acid, Figure 4. Consequently, the carbonyl group of flavone does not react normally with some carbonyl identifying reagents such as hydroxylamine. However, this same carbonyl group does react normally with Grignard reagents. The most widespread flavone is quercetin. Some flavones, such as primuletin and fisetin, have only one hydroxyl group in ring A.

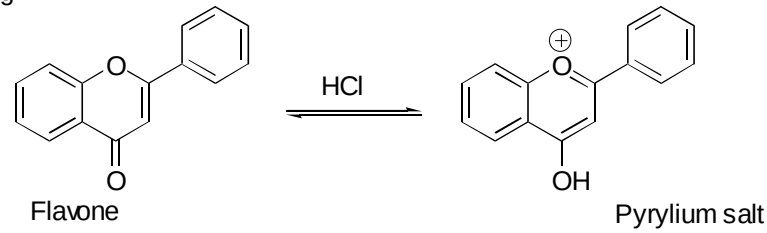
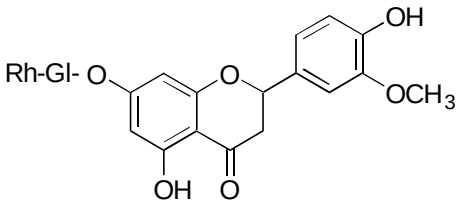


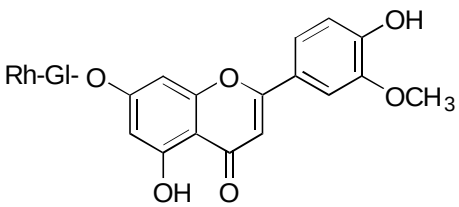
Figure 4. Protonation of Ring C in Flavones

Flavanones:

Flavanones are always hydroxylated. The non-hydroxylated flavanone skeleton has not yet been found in nature. The hydroxylated flavanones occur in nature in both the free form and as glycosides. In plants, flavanones frequently coexist with the corresponding flavones, e.g., hesperidin and diosmin in the bark of *Zanthoxylum avicennae* or rhoifolin and naringin in the peel of *Citrus aurantium*.



Hesperidin

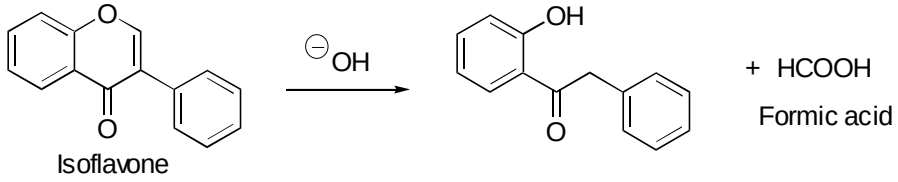


Diosmin

Unlike the unsaturated flavones, the saturated flavanones show reactivity of the 4-carbonyl group. The behavior of flavanones toward alkalis differs from that of flavones; the former decomposes into benzaldehyde, acetic acid and phenol under drastic conditions, whereas the latter yield phenol and cinnamic acid. Dehydrogenation of flavanones, e.g., conversion of hesperidin into diosmin, is of importance, as it makes possible the rapid identification of a new flavanone by reference to a known flavone.

Isoflavones:

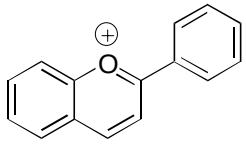
The isoflavones are 3-phenylchromones. At present about 35 isoflavones are known. Isoflavones are degraded by alkali to produce one equivalent of formic acid, which results from carbon 2 of the isoflavone. Isoflavones have shown estrogenic, insecticidal, and antifungal activity. They are also used as potent fish poisons.



Isoflavone

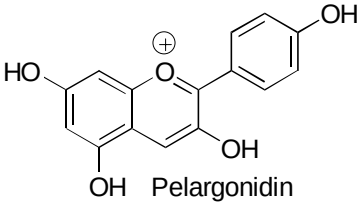
Anthocyanins:

The innumerable shades of blue, purple, and violet, and nearly all of the red colors which appear in the cell sap of flowers, fruits, leaves, and stems of plants are due to anthocyanin pigments in the dissolved state. The sugar free pigments are called anthocyanidins. The structure common to all anthocyanidins is the flavylium (2-phenylbenzopyrylium) ion.

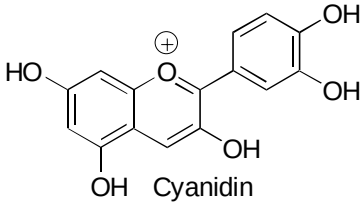


Flavylium ion

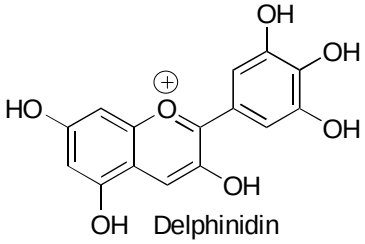
The natural anthocyanidins may be classified into three groups: pelargonidin, cyanidin, and delphinidin.



Pelargonidin



Cyanidin



Delphinidin

Various anthocyanins can be distinguished by partition between two immiscible solvents, by their absorption spectra, and by their colors in buffer solutions of graded pH. For structure determination, anthocyanidins are treated with alkali, whereupon they form phloroglucinol and a phenolic acid **Figure 4**. Several factors, such as the pH, complex forming metals, and tannins affect the actual colors of the anthocyanins.

Leucoanthocyanidins:

Leucoanthocyanidins are flavan-3,4-diols. These colorless compounds give red solutions with acids. Leucoanthocyanidins are widely distributed in the plant kingdom. Flavan-3,4-diols are sometimes obtained by reduction of flavanols and flavanonols

Biosynthesis of the Flavanoids:

Biosynthetically, the flavonoid compounds are formed by the combination of a C_6-C_3 fragment derived from shikimic acid, e.g., *p*-hydroxycinnamic acid, with a six carbon unit formed by the linear combination of three acetate units.

The general scheme outlined in **Figure 5** has been proposed for the biosynthesis of flavonoids via shikimic acid: shikimic acid → prephenic acid → *p*-hydroxyphenylpyruvic acid → *p*-hydroxyphenyllactic acid → *p*-hydroxycinnamic acid → flavones.

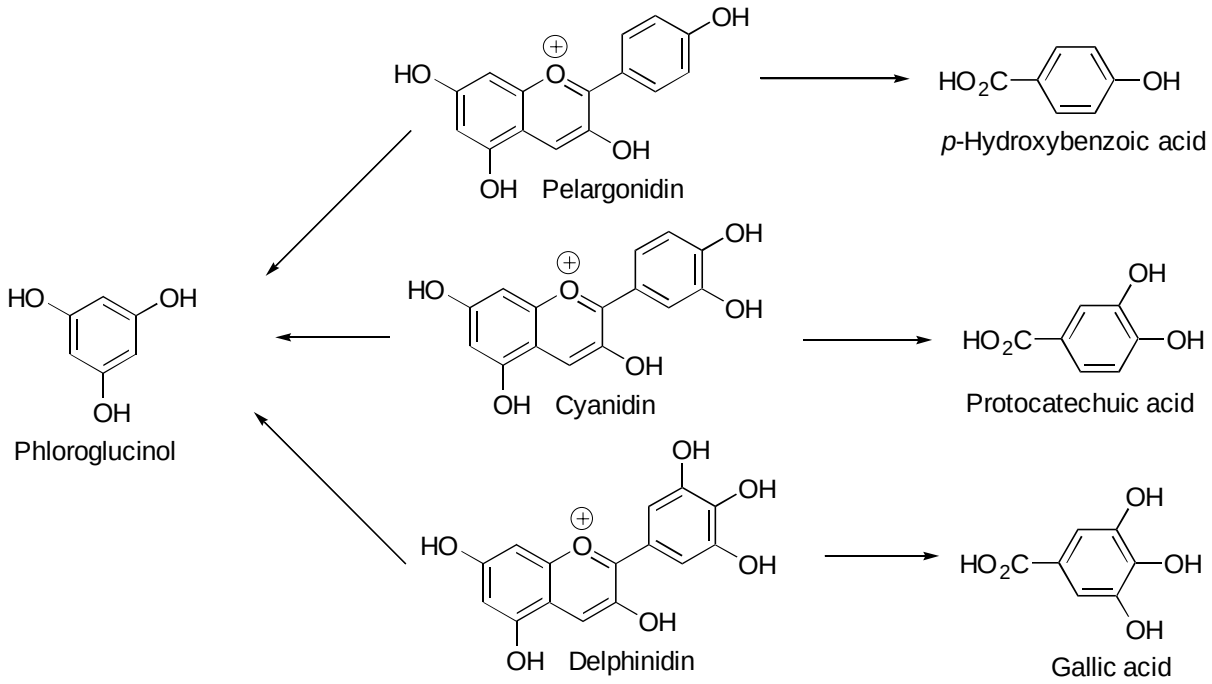
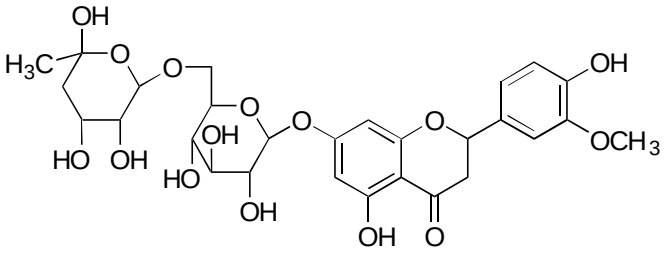


Figure 4. Alkali Degradation of Anthocyanins

Isolation of Hesperidin from Orange Peel:



Introduction:

Hesperidin was first isolated by Leberton in 1828 from the albedo (the spongy inner portion of the peel) of oranges of the family Hesperides, and was given the name hesperidin[2]. Its presence was detected in lemons by Pheffer as early as 1874[3]. Neohesperidin, an isomer of hesperidin, has been isolated together with hesperidin

[2] Leberton, P. *J. Pharm. Chim. Paris* **1828**, *14*, 377.
[3] Pheffer, W. *Bot. Ztg*. **1874**, *32*, 529.

from unripe sour oranges cultivated in Europe[4]. Horowitz and Gentili[5] isolated it from ponderosa lemon. Hesperidin was isolated from *Citrus mitis* by Sastry and Row[6]. Neohesperidin, a bitter compound, occurs in bitter orange, *Citrus aurantium*, while hesperidin, a nonbitter compound, is the predominant flavonoid in lemons and the ordinary sweet orange, *Citrus sinensis*. Biologically, hesperidin decreases the fragility of blood capillaries.

Hesperidin can be isolated by two different methods. The first method involves extracting the dried citrus peel successively with petroleum ether followed by methanol. The petroleum ether removes the essential oils in the peel and the methanol will extract the glycoside (hesperidin). The second method uses an alkaline extraction of chopped orange peel and acidification of the extract. The hesperidin can then be crystallized from the acidified extract. Because of its highly insoluble, crystalline nature, hesperidin is one of the easiest flavonoids to isolate.

Experimental Procedure:

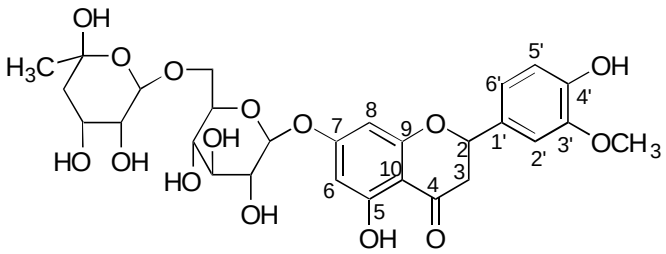
Chopped orange peel (200 g) and 750 mL of 10% calcium hydroxide solution are placed in a 2-liter Erlenmeyer flask and thoroughly mixed, then left overnight at room temperature. The mixture is filtered through a large Buchner funnel containing a thin layer of Celite on the filter paper. The yellow-orange filtrate is carefully acidified to pH 4-5 with concentrated hydrochloric acid. Hesperidin separates as an amorphous powder. If the precipitation of hesperidin on addition of HCl is slow, then the solution should be concentrated under reduced pressure (rotovap). Filter the solids from your acidic solution and wash with water. The crude hesperidin should be recrystallized from an aqueous formamide solution (20-25% v/v). Dry the hesperidin crystals and obtain a melting point and NMR spectra (^{1}H, ^{13}C, and HETCOR). Compare your results with the literature values given below.

Physical Data for Hesperidin

Hesperidin should yield white needles upon recrystallization. Melting point 252-254°C.

^{13}C NMR:

C-2	δ 78.4	C-1'	131.2
C-3	42.0	C-2'	114.3
C-4	196.7	C-3'	146.7
C-5	163.0	C-4'	148.1
C-6	96.7	C-5'	112.7
C-7	165.2	C-6'	117.8
C-8	95.8	OMe	56.0
C-9	162.5		
C-10	103.5		



Hesperidin

[4] Karrer, W. *Helv. Chim. Acta*. **1949**, *32*, 714.
[5] Horowitz, R. M.; Gentili *J. Am. Chem. Soc*. **1960**, *82*, 2803.
[6] Sastry, G. P.; Row, L. R. *J. Sci. Ind. Res. (India)* **1960**, *19B*, 500.