SOME SPECIAL FEATURES IN THE STUDY OF LEUCOANTHOCYANIDINS IN FRUITS

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LEUCOANTHOCYANIDINS form an important group of naturally occurring plant products and are known to be present in almost all parts of plants. Considerable attention has been paid in recent years to the relationship between leucoanthocyanidins and tannins and it is now recognised that under suitable conditions the monomeric flavan-3, 4-diols polymerize[1] to dimers and polymers and the latter two categories are grouped as tannins.[2] Astringency in fruits has been attributed to the presence of these leucoanthocyanidins (mostly dimers)[3] and it is believed that they affect the cross-linking of proteins and glycoproteins in the mouth and thus reduce the lubricant action and give the sensation of astringency.

In fruits, the monomeric leucoanthocyanidins are invariably accompanied by polymeric leucoanthocyanidins (tannins) in which the leucoanthocyanidin units are probably linked together in repeating units capable of depolymerisation to yield anthocyanidins with hot acids.[4] Since the only means of detecting and characterizing leucoanthocyanidins in plants is by their ability to yield anthocyanidins, the study of these compounds by this method is complicated. A systematic study thus involves the initial separation of the various polymers by the usual methods of chromatography and solvent separation followed by conversion into anthocyanidins. During the course of our investigations on leucoanthocyanidins in fruits we have made some significant observations as described below.

I. Paper Chromatography

Circular and two-dimensional chromatography has been frequently employed for the study of leucoanthocyanidins in plant materials. Among the various reagents[5-7] used as developers for these chromatograms, *p*-toluene sulphonic acid[6] has been considered to be specific for the detection of flavan-3, 4-diol and flavan-3-ol systems. When paper chromatograms of these systems are sprayed with a 5% solution of *p*-toluene sulphonic acid in absolute alcohol and then kept at 75–80° for 20 minutes, spots of various colours[8,9] ranging from dark-brown, brown-pink, pink, and scarlet are reported to be produced depending upon the molecular size and the complexity of the flavonoid system. Dihydroflavonols[9] are known to give yellow fluorescent spots visible in ultra-violet light while catechins develop spots which are dull yellow[8] or brown[9] in visible light and deep blue-mauve in ultra-violet light. This reagent has therefore been used in paper chromatography as suitable not only for detection of flavonoids but also for distinguishing between various categories.

During the course of our investigation on the leucoanthocyanidins present in fruits it has been observed that *p*-toluene sulphonic acid reagent developed grey, brown and pink spots with fruit extracts. In order to get an idea of the reaction of this reagent on components other than leucoanthocyanidins occurring in these juices, known samples of sugars, nitrogenous and non-nitrogenous acids have been examined and the results are given in Table I. Maltose, galactose, glucose, arabinose, glutamic acid, tartaric acid and citric acid do not develop any colours under the above conditions.

TABLE I

Sample	Colour (visible)	Colour (ultra-violet light)
Sucrose ..	Brown	Reddish-brown
Fructose ..	do.	do.
Sorbose ..	do.	do.
Tyrosine ..	Pale brown	Pink
Asparagine ..	Yellow (hot)	Light violet
Aspartic acid ..	No colour	Violet
Ascorbic acid ..	Light brown	Light brown

From the above data it is clear that besides flavonoids, ketoses, sucrose, ascorbic acid and even amino-acids respond to this test. The coloured spots produced by these compounds are prominent and might even superimpose the spots produced by leucoanthocyanidins. Since in edible fruits, the leucoanthocyanidins are always accompanied by sugars and many other compounds mentioned in Table I, the use of *p*-toluenesulphonic acid reagent for the detection of leucoanthocyanidins and related products could only be made with reservations.

II. Conversion of Leucoanthocyanidins into Anthocyanidins

As already mentioned, the method for the detection and identification of leucoanthocyanidins in plants is to convert them into the corresponding anthocyanidins by heating in acid solutions. During our investigations on the scope of this reaction, we have met with unexpected results. The juices of apples

(*Pyrus malus*) and wild unripe pomegranates (*Punica granatum*) seemed to contain leucoanthocyanidins. By heating with acid for a short period the deep colour of anthocyanidins can be obtained and by careful extraction the identification could be effected. But on continued refluxing with alcoholic hydrochloric acid (1 hr.) as is usually done, these juices yielded black solutions and some black precipitate also and no anthocyanidins could be isolated. Looking for the reason, these fruit juices were found to contain good amounts of fructose, glucose and/or sucrose. Consequently, the stability of anthocyanidins in the presence of these sugars was examined. It was observed that fructose, sorbose and sucrose on heating with alcoholic hydrochloric acid gave brown to black solutions. When this reaction was repeated in presence of cyanidin chloride similar coloured solutions were obtained which were devoid of anthocyanidin. Even when the solutions were kept at room temperature, without heating, the anthocyanidin chromophore was lost after two days (*see* Table II). Parallel results were obtained with sorbose and sucrose but glucose under the same conditions had no effect.

The above spectral data indicate that except luteolinidin chloride (wherein there is no 3-hydroxyl) other anthocyanidins were decomposed. What actually happens is not clear; they may be converted into other coloured products or be decolourised. It would appear that the hydroxyl group at position 3 has an important part in the above reaction and the hydroxyls at other positions have no effect. It has also been observed that cyanin, in which the 3-hydroxyl is blocked by glycosylation is stable under these conditions. Earlier studies have shown that decolourisation of anthocyanins can be brought about by means of enzymes[10] or light.[11] The decolourisation by enzymes involves[10] (*a*) enzymatic hydrolysis of the anthocyanin to sugar and aglycone, (*b*) transformation of the aglycone to colourless pseudo-base and (*c*) destruction of the pseudo-base by molecular oxygen. However, the mechanism involved in the decolourisation of anthocyanins by light is not very clear.

The above observations may throw some light on the process of browning in fruits. Ketoses are known to be responsible for browning by their decomposition. Their effect on anthocyanidins (which might arise from leucoanthocyanidins) may provide additional cause for the browning of fruits. The above observation may have interest in another connection. It has been suggested that there is loss of astringency in fruits during ripening probably due to increased polymerisation of leucoanthocyanidins and the higher polymers contributing little to astringency.[3] While that may be true, there is also the possibility of keto-sugars removing leucoanthocyanidins after they are converted into the anthocyanidin stage.

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TABLE II

Anthocyanidins (in ethanolic hydrochloric acid) + fructose + 2-3 drops of hydrochloric acid (total volume about 10 c.c.) were kept for two days at room temperature and the spectra were recorded on a Hilger spectrophotometer.

No.	Anthocyanidin	λmax in mμ	
		Before treatment	After treatment (strong)
1	Luteolinidin chloride ..	503	280, 500 (strong)
2	Cyanidin chloride ..	545	275, 450 (infl.)
3	Delphinidin chloride ..	555	280
4	5, 7-Dimethoxy-3-hydroxy flavylium chloride ..	400, 480	280, 470 (weak)
5	5, 7, 4′-Trimethoxy-3-hydroxy flavylium chloride ..	420, 510	280
6	7, 3′, 4′-Trimethoxy-3-hydroxy flavylium chloride ..	400, 510	280
7	5, 7, 3′, 4′-Tetramethoxy-3-hydroxy flavylium chloride ..	523	280, 460 (weak)
8	7, 3′, 4′, 5′-Tetramethoxy-3-hydroxy flavylium chloride ..	470	280
9	Fructose + hydrochloric acid (Blank) ..		280

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