THE USE OF METAL COMPLEXES IN IDENTIFICATION OF FLAVONOID PIGMENTS

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The flavonoids comprise a large group of yellow plant pigments which are isolated either in the form of glycosides of rhamnose, glucose, and galactose, or as aglycones. There are many difficulties encountered in the identification and characterization of these compounds. Many of the pigments do not exhibit sharp melting points but, instead, melt over a range of 10-25 degrees. The melting points of derivatives are often identical for two or more flavonoids. Quite often the problem of characterization narrows down to the location of one or more hydroxyl or methoxyl groups on the 2-phenylbenzopyrone nucleus. The classical approach to characterization generally involves the degradation of the pigment by opening of the heterocyclic ring with boiling alkali and further degradation to an aromatic acid and a phenol. Various methods of synthesis have also been developed which make possible the comparison of a known structure with the pigment under study. Link (3) has briefly reviewed methods of synthesis and degradation of flavonoids.

Another aid to the identification of flavonoid pigments is the use of ultraviolet absorption spectra. This is particularly of value in certain cases where the location of a hydroxyl or methoxyl group is involved. Aronoff (1) has reviewed the work in this field and attempted to correlate the structure of the flavonoids with their absorption spectra.

Quite often, two or more flavonoid pigments are present in the same plant source and, thus, a mixture is isolated. Many of the pigments exhibit similar solubility relationships and the problem of identification is thereby further complicated due to difficulties involved in the separation of such mixtures.

Recent investigations on flavonoid pigments at the University of Oklahoma (2, 4) have been concerned with the application of paper partition chromatography, together with ultraviolet absorption spectra data, to the problem of separation and identification of these compounds in plant extracts. The general plan has been to (1) separate the mixture by paper partition chromatography, (2) remove each individual pigment zone by leaching with some appropriate solvent, and (3) determine the ultraviolet absorption spectra of the extracted pigments. In the course of this work, it was found that aluminum chloride solutions were very effective in leaching certain flavonol glycoside pigments from the paper strips. Aluminum chloride causes a shift of the absorption spectra of most of the flavonoids toward the visible region of the spectrum. The absorption spectra of the flavonoid-aluminum chloride complexes are quite stable for at least several hours. This makes possible the use of such spectra in identification work.

EXPERIMENTAL. The ultraviolet absorption spectra of seven flavonoid pigments and their aluminum chloride complexes were obtained in the Beckman Model

DU spectrophotometer. Duplicate samples of each pigment were measured into 5 ml volumetric flasks from stock solutions of known concentration. A micro pipette was used for this purpose. One sample of each pigment was made up to volume with ethanol and transferred to 1 cm silica cells for absorption spectra measurements. The wave lengths corresponding to the principle absorption maxima of each pigment are listed in Table 1.

The second sample of each pigment was diluted to 5 ml volume with 0.5% aqueous aluminum chloride solution (pH 3.2) and the absorption spectra of the aluminum chloride complexes thus prepared were determined. The wave lengths corresponding to the principal absorption maxima of the aluminum chloride-flavonoid complexes are also listed in Table I.

TABLE I

Absorption Maxima of Flavonoid Pigments in Ethanol and in $AlCl_3$ Solutions

Flavonoid	Absorption Maxima in Ethyl Alcohol		Absorption Maxima in Aluminum Chloride Solution		
	Band I	Band II	Band I	Band II	Band III
	mμ	mμ	mμ	mμ	mμ
Rutin	258	362	272	415	
Xanthorhamnin	257	363	272	409	
Quercitrin	258	363	272	409	
Robinin	267	352	273	391	342
Isoquercitrin	258	360	270	415	
Quercetin	262	375	268	428	
Naringin	283-5	335	292-302	380	

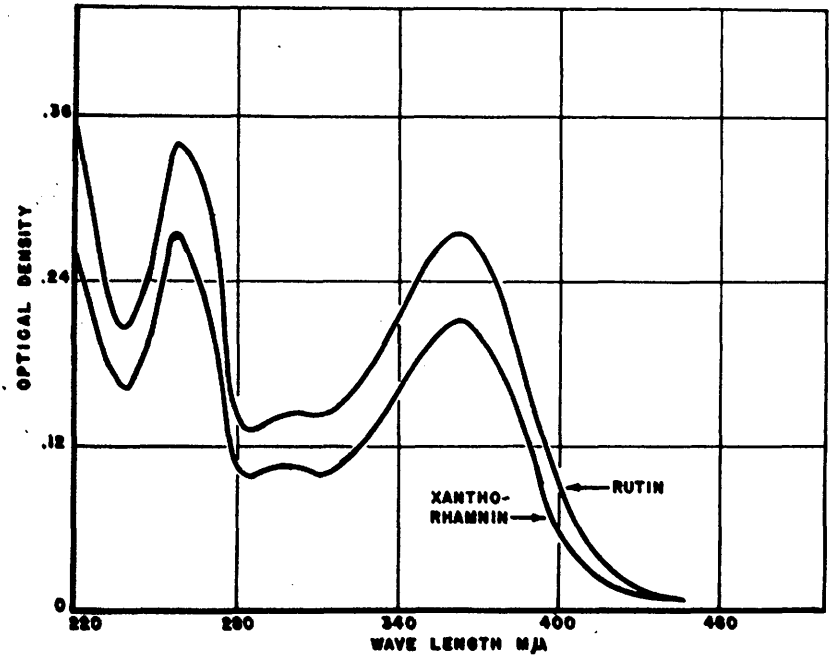

FIGURE 1. Absorption Spectra of Rutin (0.00825 g/l) and Xanthorhamnin (0.00967 g/l) in 95% Ethanol. One cm cells.

Figure 1 shows the absorption curve for two of the flavonoid pigments in ethyl alcohol solution. While rutin exhibits a somewhat stronger absorption at all wave lengths than xanthorhamnin on a weight basis, the shape of the two curves is identical. Differentiation between these two pigments on a qualitative spectral curve of unknown concentration would be impossible.

The absorption spectra curves of the aluminum chloride complexes of rutin and xanthorhamnin are presented in Fig. 2. Although the general shape of the two curves is again very similar, there is a significant difference in the location of the second absorption maxima (Band II). By forming the aluminum chloride complex, it should be possible to differentiate between these two pigments by absorption spectra studies.

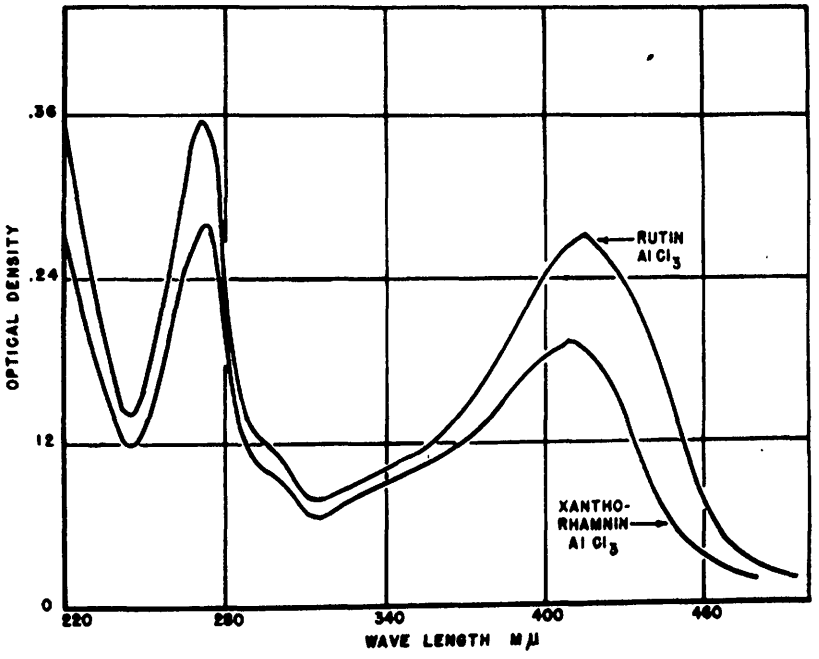

FIGURE 2. Absorption Spectra of Rutin (0.00825 g/l) and Xanthorhamnin (0.00967 g/l in 0.5% Aqueous Aluminum Chloride Solution. One cm cells.

While it would be difficult to distinguish between rutin and isoquercitrin or between quercitrin and xanthorhamnin on the basis of their spectra in alcohol or aluminum chloride solution, it should be mentioned that it is possible to differentiate between these pairs by means of paper partition chromatography (4).

The absorption spectrum of an alcoholic and an aluminum chloride solution of robinin is shown in Fig. 3. This pigment produces double maxima in the near visible region of the spectrum with aluminum chloride. The reason for this alteration in the spectrum of robinin in the presence of aluminum chloride is not known at present. The production of such a typical curve, however, should be of value in the identification of robinin.

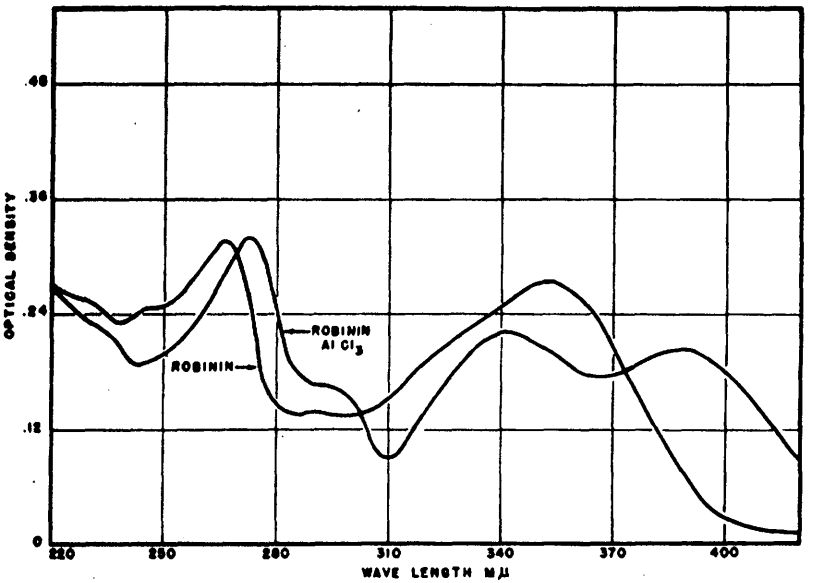

FIGURE 3. Absorption Spectrum of Robinin (0.0096 g/1) in 95% Ethanol and in 0.5% Aqueous Aluminum Chloride Solution. One cm cells.

Further studies are in progress with additional flavonoid pigments and other metal salts. The shift of the absorption maxima is only slight with the nitrates of aluminum, thorium, zirconium, and uranium. The chlorides of all of these metals, however, offer possibilities in the identification of flavonoid pigments.

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LITERATURE CITED

1. ARONOFF, S. Some structural interpretations of flavone spectra. 1940. J. Org. Chem. 5: 561-571.

2. GAGE, THOMAS B. and SIMON H. WENDER. 1949. The purification and quantitative estimation of quercetin by paper partition chromatography. Proc. Oklahoma Acad. Sci. *29*: 64-47.

3. GILMAN, HENRY. 1943. Organic Chemistry. Vol. II, 2nd Edition, 1315-1340, (Karl Paul Link) New York: John Wiley and Sons.

4. WENDER, SIMON H. and THOMAS B. GAGE. Paper chromatograhpy of flavonoid pigments. 1949. Science *109*: 287-289.