THE ULTRAVIOLET SPECTRUM OF NARINGIN BY USE OF THE BECKMAN MODEL DU SPECTROPHOTOMETER

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The ultraviolet spectrum of a flavonoid pigment should be a very useful tool in its identification and quantitative estimation, especially when used in connection with paper partition chromatography. However, for these purposes, one needs to have the correct ultraviolet absorption data on each flavonoid pigment. Preliminary studies in this laboratory have indicated that many of the spectra of these pigments which are recorded in the literature are not reproducible on the more modern instruments now in use. Hence, the determination of the ultraviolet absorption spectra of such flavonoid pigments becomes essential. Such a pigment is naringin.

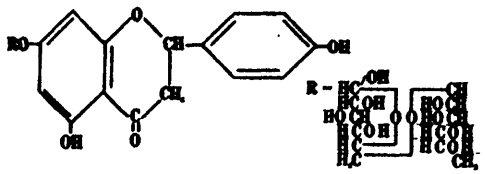

FIGURE 1. Naringin

Naringin is the rhamno-glucoside of naringenin. Its structural formula is indicated in Figure 1. It is obtained by extracting the skins of citrus fruits with alcohol (Shinoda, Uyeda 1929) or with hot water (Poore, 1934).

Shibata and Nagai (1924), in connection with some studies on the ultraviolet spectra of some chalcone derivatives determined the spectrum of naringin. The maxima obtained by these workers were at 300 and 350 millimicrons. These values are not in agreement with those which have been obtained in this laboratory using the Beckman Model DU Spectrophotometer. A possible reason for this disagreement is that the previous workers did not have available to them the highly purified naringin samples furnished us.

We have also attempted to perfect a technique whereby micro quantities of naringin may also be purified by paper partition chromatography and then used for the determination of spectra. This has been only moderately successful so far due to an impurity which is encountered during the chromatography.

EXPERIMENTAL

Highly purified naringin, which was furnished through the courtesy of the California Fruit Growers Exchange, Ontario, California, was dissolved in 95% undenatured alcohol, and this was made up to 100 cc volume to give a solution of 0.00117 M. An aliquot of this was further diluted to 0.0000585 M. This latter solution was suitable for spectrophotometric determinations.

A carefully cleaned Corex ultraviolet absorption cell was filled with the 0.0000585 M naringin solution. Another cell was filled with 95% undenatured alcohol to serve as a blank. These cells were placed in the Beckman spectrophotometer and the percentage transmission read at every 5 millimicrons.

For the attempted purification by means of paper partition chromatography, 20 microdrops of the 0.00117 M solution were used and the technique of Gage and Wender (1949) employed for the development of the chromatogram.

Two different methods were attempted for removing the naringin from the paper chromatograms. The first of these consisted of cutting out the band of naringin from the strip. It was cut to a sharp point on one end. The end which had been cut at right angles to the axis of the strip was placed between two microscope slides. The opposite ends of the slides were immersed in 95% alcohol in a Petri dish and the slides were allowed to rest on the rim of the dish. The alcohol flowed to the paper by capillary siphoning. A small beaker was provided at the pointed end of the paper as a container. An aluminum wire spring was necessary to lead the drop from the paper into the container. This method was not successful because in flowing over the wire the naringin solution became discolored. The substitution of nichrome or iron wire, even after treatment with nitric acid, yielded no better results.

The second method was to allow a blank strip to chromatograph in the same chamber with the chromatogram being developed. After development,

the strips were dried and placed together, one over the other. When the band was located the strips were cut so as to obtain the corresponding area on the blank strip. The two papers were then separated and cut into small pieces. The pieces were then placed in beakers and leached with alcohol. The leachates were then made up to 10 cc volumes. The leachate from the blank strip served as a solvent blank.

Previous treatment of the strips was necessary because of the interfering impurity. This treatment consisted of leaching once with both alcohol and ethyl acetate. Ethyl acetate was used as the dynamic phase in the development of the chromatograms after this treatment.

DISCUSSION

The spectrum of the naringin is shown in Fig. 2. Maxima occur at 285 and 330 millimicrons. This is definitely different from the previous literature maxima of 300 and 350 millimicrons.

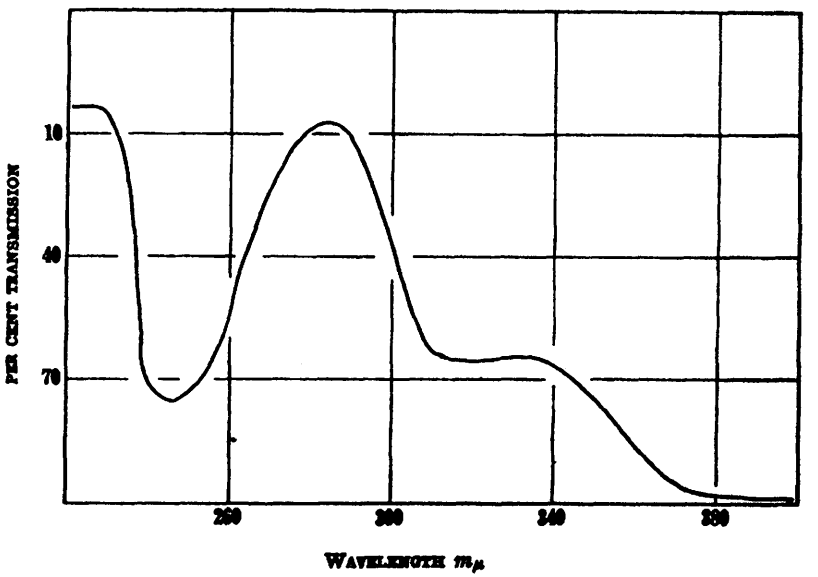

FIGURE 2. Spectrum of Naringin

The spectrum of the chromatographed naringin is shown in Fig. 3. The interfering effect of an impurity is evident in the maximum which occurs at 255 millimicrons. Gage, Douglass, and Wender (1948) have determined the ultraviolet spectrum of this impurity and found its maximum to be in the 250-280 millimicron range. Various methods were tried to eliminate this impurity. The strips were leached with alcohol *n*-butanol, ethyl acetate, and water. Other strips which had been previously leached with phenol were leached with ethyl acetate and alcohol. Several strips were extracted 24 hours in a Soxhlet apparatus with ether and with alcohol. None of the above methods was successful. As stated in the experimental portion the effect was minimized when the strips were leached with alcohol and ethyl acetate prior to the development of the chromatogram. Further work is in progress to eliminate the impurity interference.

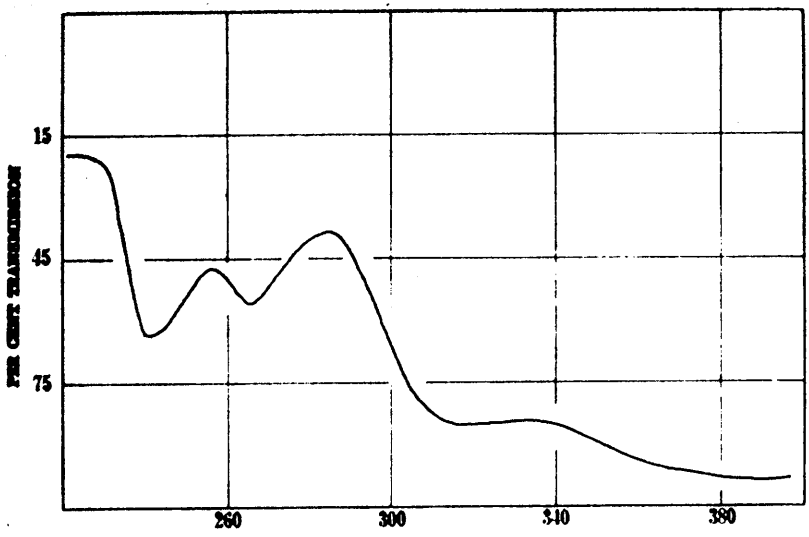

FIGURE 3. Spectrum of Chromatographed Naringin

SUMMARY

The ultraviolet spectrum of naringin has been determined by using the Beckman Model DU Spectrophotometer. The maxima obtained (285 and 330 millimicrons) were found to be in disagreement with those recorded in the literature.

An attempt was made at further purification using paper partition chromatography. An impurity was encountered which interfered with the absorption of naringin in the ultraviolet region. Methods are described in which attempts to eliminate this impurity were made.

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