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SHORT COMMUNICATION

THE OCCURRENCE OF 418 AND 444 nm CHLOROPHYLL-TYPE COMPOUNDS IN SOME GREEN PLANT TISSUES*†

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Abstract—Two green pigments, besides chlorophylls *a* and *b*, were observed in low concentrations in acetone extracts of green tissues of bell pepper (*Capsicum frutescens*), banana peel and cucumber peel. Their absorption spectra are similar to those of the chlorophylls but with absorption maxima in the "blue" region at 418 nm and 444 nm instead of 430 nm and 454 nm. They were both Molisch phase-test negative. Their relative polarity places them between chlorophylls and chlorophyllides on a two-dimensional silica gel thin-layer chromatogram when modified Bauer's solvent systems are used.

INTRODUCTION

THE disappearance of chlorophylls in green fruits or vegetables and autumn leaves is a widely observed phenomenon.[1] Noack[2] suggested several possible steps in the destruction of chlorophyll in leaves postulating that the action of chlorophyllase yielding chlorophyllides is the first step in the sequence. Although disagreement exists here, chlorophyllase is thought to act primarily in the biosynthesis of chlorophyll and it is not considered to take part in chlorophyll breakdown *in vivo*.[3–5] To date, there is still little known about the processes involved in chlorophyll biodegradation.

RESULTS AND DISCUSSION

Besides the usual green and yellow pigments, two green pigments were observed in low concentrations on thin layer chromatograms of extracts of all three plant materials used in this study. From their spot sizes and color intensities, they were estimated to represent about 5 per cent or less of total green pigments. They moved more slowly than chlorophylls under the conditions described, and were well separated between chlorophylls and chlorophyllides, when the latter were added to a pigment extract (Fig. 1). The two pigments were eluted from the silica gel more readily with MeOH than with ether which is commonly used for the chlorophylls.

Under u.v. light, both pigments displayed pink fluorescence. Under day light, the color of the higher R_f compound was bluish green. Its spectrum in diethyl ether showed maxima

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at 418 nm and 655 nm. The color of the slower-moving (low R_f) compound was green with a slightly yellowish tinge and had absorption maxima at 444 nm and 630–632 nm. For brevity they will be referred to as the 418 and 444 compounds. The visible absorption spectra are also presented in Fig. 1.

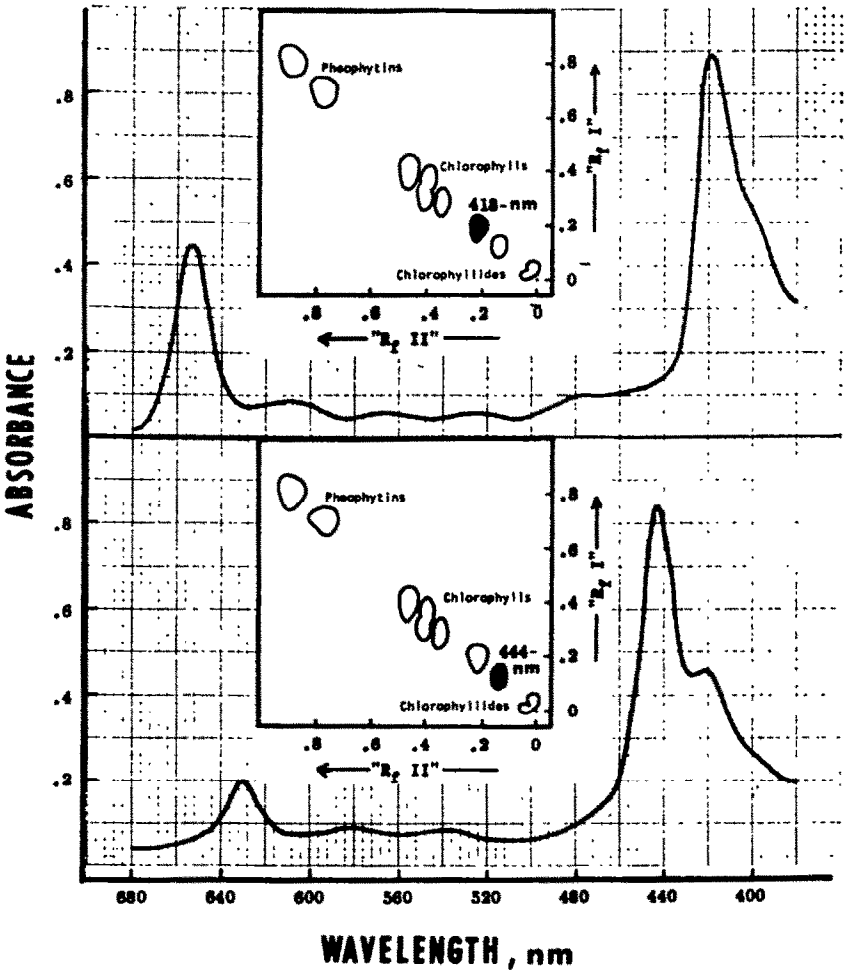

FIG. 1. QUALITATIVE ABSORPTION SPECTRA OF THE 418 AND 444 COMPOUNDS IN DIETHYL ETHER AND SCHEMATIC REPRESENTATION OF THEIR LOCATION ON A TWO-DIMENSIONAL CHROMATOGRAM.

Several steps were taken for checking the possible formation of artifacts. The plant extract was chromatographed one-dimensionally on a thin-layer plate coated with cellulose powder (Whatman CC41) following the procedure described by Bacon.[6] Chlorophylls *a* and *b* occurred as two wide but well separated zones respectively moving ahead of a weakly pink fluorescent zone which was the 444 compound. Compound 418 could not be detected. From this it can be concluded that at least the 444 compound is not an artifact formed on the surface of the silica gel plate but existed in the plant extracts. To locate the 418 compound on the

[6] M. F. BACON, *J. Chromatog.* **17**, 322 (1965).

cellulose plate a known purified 418 compound was co-chromatographed with a plant extract as well as being spotted as a single spot at the side of the extract. On this chromatogram, the plant extract separated as before into three zones (2 wider ones followed by the 444 zone). The added 418 compound did not separate from the chlorophylls. The 418 compound spotted on the side moved about as far as the chlorophyll *b* zone. However, the chlorophyll *a* zone, when eluted and rechromatographed on a silica gel plate, yielded back the added 418 compound. This variation of R_f value with the amount and even the number of compounds in the mixture was observed on cellulose by Bacon.[6] The experiments show that cellulose is not satisfactory as adsorbent using Bacon's solvent for the separation of these minor pigment constituents in plant extracts.

To determine whether 418 was formed on silica gel, the plant extract was spotted on a silica gel plate and developed as before. After the spot of 418 had been spectrally identified, all the compounds that separated other than the 418 compound were recombined and re-chromatographed on a new silica gel plate. Even after several replications no trace of the 418 compound could be found. From this it can be concluded that the amount of 418 compound found in this experiment was not an artifact under the conditions of the chromatography. The plant extracts were also chromatographed on powdered sugar columns as described by Strain,[7] but these minor constituents of the plant extract were not detected. Similar difficulties were reported by Michel-Wolwertz and Sironval.[8] Also, sugar plates were used following the method of Colman and Vishniac[9] but the separation was not satisfactory.

Both pigments gave a negative Molisch phase test, indicating that the isocyclic ring has been altered and the pigments thus are not phorbins but may be chlorins. This seems to be evident since their spectra are similar to those reported for the unstable chlorin monomethylester by Stern and Pruckner.[10]

From the R_f values, polarities, spectra and colors, it could be considered that the 418 compound is a chlorophyll *a* type, while the 444 compound is a chlorophyll *b* type. Both of them could possibly be the derivatives of chlorophylls or of chlorophyll precursors, or even be precursors of chlorophylls.

The occurrence of the 418 and 444 compounds has been observed previously in bell pepper extract.[11] However, only the spectrum of the 418 compound was reported, since the amount of the other compound that was isolated was not sufficient for analysis.

Recently, the presence of several chlorophylls in Chlorella extracts was reported by Michel-Wolwertz and Sironval[8] by using paper chromatography. The compounds in their zones a_4 and b_3 are similar to the ones found in this study with respect to the visible spectra and the negative Molisch phase test; however, the spectra of the 418 and 444 compounds of this study did not display the peaks reported by Michel-Wolwertz and Sironval for the region below 400 nm. It appears possible that these compounds which occur in lower plants, such as Chlorella, may also exist in higher plants.

If, in spite of the precautions described earlier, these two compounds are found to be artifacts, they still are of interest. Compounds formed from the chlorophylls under carefully controlled conditions might well be formed in plant tissues but this cannot be demonstrated until a block is introduced which will result in their accumulation.

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EXPERIMENTAL

Five to ten g amounts of green tissue of banana peel, cucumber peel or carpel portion of bell peppers (*Capsicum frutescens*) were extracted with acetone with the addition of approximately 0·3 g of $MgCO_3$[12] and purified glass sand in a mortar at 0° under minimum illumination. The pigments were transferred to diethyl ether as described previously.[11] Appropriate amounts of pigments were chromatographed in the dark at 15° on a thin-layer plate (8 in. × 8 in.) coated with approx. 5 g silica gel G (average thickness 250 μ).[13] The solvent used for developing the first dimension was benzene:light petroleum (b.p. 30–60°):acetone (10:2·5:2, by vol.) and that for the second dimension was benzene:light petroleum:acetone:MeOH (10:2·5:1:0·25, by vol.).[14,15] The development required only about 40 min for each dimension. The chromatograms were first examined under u.v. light (366 nm maximum output) and re-examined in daylight.

Individual pink fluorescent pigments were eluted from the silica gel with absolute methanol which was then evaporated under vacuum. The pigments were redissolved in diethyl ether and their spectra determined with a recording spectrophotometer (Bausch and Lomb Spectronic 505). A Beckman DU Spectrophotometer was used for detailed study of some spectral regions.

Appropriately cut strips of black plastic with a 10 mm horizontal slit, 1 mm high, were inserted in front of the sample and reference cuvettes. This did not affect the width of the light path but reduced the height so that a complete spectrum of the pigment solution could be obtained with only 0·1 ml in the microcuvette. (Scientific Cell Co., 10 × 3·6 mm.)

One-dimensional chromatography of plant extracts on cellulose thin-layers, approx. 0·25 mm thickness, was carried out in the dark at 15° following the procedure of Bacon.[6]

The Molisch phase test was carried out by underlayering an ether solution of pigment with an equal volume of 29–30% KOH in MeOH (w/v).

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