PIGMENTS OF THE EMBRYO OF THE YELLOW NELUMBO

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When seeds of most angiosperms are allowed to germinate in complete darkness, the seedlings contain no chlorophyll. If such plants are brought into the light, chlorophyll synthesis takes place rapidly. Smith and Young (1956) have reviewed chlorophyll formation in plants. They reported that etiolated barley or oat leaves will show the presence of chlorophyll within two hours after exposure of leaves to light. However, Lyon (1902) reported green pigments present in the embryo of *Nelumbo*. Arata, in his study of the germination of *Nelumbo* seeds (1958) mentioned that the embryos were green. Meyer and Anderson (1952: 302) stated that "In a few angiosperms such as seedlings of water lotus . . . chlorophyll can also develop in the absence of light."

Methods and Results

Collections of the developing flowers, leaves, and fruits were made from plants of *Nelumbo lutea* (Willd.) Pers. growing in Crab Orchard Lake during the summer of 1957. These tissues, if not examined immediately, were stored at —20° C. until further work could be done. Each time a collection was made, some flowers were examined for the presence of green pigments in the embryo.

Tissues were usually ground in a mortar using 85% acetone with a small amount of calcium carbonate or redistilled ether as the extracting solvent. The extract of the embryo usually had a more yellow cast to it than did leaf extracts. Identification of the chlorophylls was made by simultaneously chromatographing leaf and embryo extracts on the same paper in the same tank. Under these conditions—using a solvent of petroleum ether/acetone (90:10 v/v) the blue-green chlorophyll *a* and the green chlorophyll *b* separated from both extracts.

Four spots of chlorophyll *a* and of chlorophyll *b* were cut from sections of the paper containing the embryo extract, eluted with redistilled ether, and their absorption spectra determined in a Beckmann D.U. spectrophotometer. These, with the spectrum of the crude ether extract of the embryo pigment, are presented in Figures 1, 2, and 3.

When an 85% acetone extract of the pigments was placed on a magnesium oxide column and the various pigments separated by washing the column with 85% acetone, a band of deep yellow formed at the upper end. This pigment was held very tightly, and it remained there after the chlorophylls were carried from the column by the acetone. The column was then extruded from the tube, the yellow band cut out and its properties studied. The substance making up the yellow band was soluble in water. In the presence of a base, the yellow color became more intense; in the presence

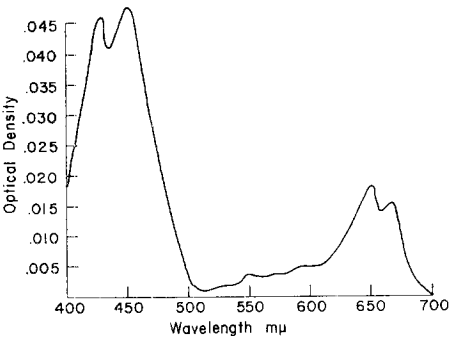

FIG. 1.—Ether extract of embryo of *N. lutea.*

of an acid the yellow color disappeared, leaving a colorless solution.

The yellow pigments were further identified. Water extracts were prepared of leaf tissue, petals, and embryonic tissues. Each extract was shaken with ethyl acetate in a separatory funnel and the water solution discarded. The ethyl acetate was removed under vacuum and the residue taken up in acetone. This acetone extract was used to spot Whatman #1 filter paper. The solvent system used was a mixture of n-butanol/acetic acid/water (6:1:2 v/v/v) and the ascending chromatogram technique was used.

Ten lambda aliquots of each extract were placed on the paper, with 12 spots in all. Paraffin was placed on the cover of the tank, heated, and the paper was fixed thereto. In this manner the paper cylinder was held above the solvent until equilibrium was established between the paper and the vapors of the solvent. After six hours the paper was freed from the paraffin by heat, placed in the solvent, and allowed to stand for 16 hours. Under the highly acidic conditions of the solvent system, the yellow color disappeared. The paper was then removed from the tank, dried at room temperature and placed in a tank in contact with ammonia fumes. In contact with ammonia the yellow color reappeared. In all cases there was evidence for two pigments having Rf values of 0.5 and 0.6.

The paper was further examined for the presence of fluorescent substances. Under ultra-violet light, fluorescent spots appeared in the flower extracts and the extract of the embryo, but there was no fluorescent material found in the leaf extract.

When acetone extracts were reduced to dryness, taken up in ethanol, and treated with metallic magnesium and concentrated HCl, a red color appeared in all cases. The formation of a red color upon reduction of magnesium with concentrated HCl is positive identification of pigments of the flavonoid type (Geissman, 1956).

DISCUSSION

The evidence presented here confirms earlier statements that the embryo of *N. lutea* contains chlorophyll pigments. From the absorption spec-

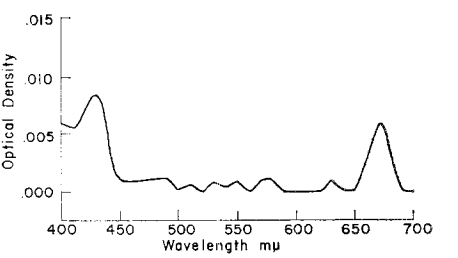

FIG. 2.—Ether extract of chlorophyll *a. N. lutea.*

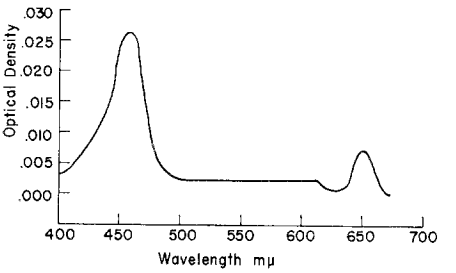

FIG. 3.—Ether extract of chlorophyll *b*, *N. lutea*.

tra presented in Figures 2 and 3, chlorophyll *a* shows two maxima at 430 mu and 665 mu; chlorophyll *b* at 455 and 650 mu. These values are in good agreement with the values published by Zscheile and Comar (1941) for ethyl-ether extracts of spinach leaves. These figures also give some indication of the quantities of the two chlorophylls present. The height of the maxima for chlorophyll *b* is about twice that of chlorophyll *a* in the embryo extract. This fact and the presence of the yellow pigments may account for the strong yellowish cast of all embryo extracts.

As collections were made, the developing ovule was examined for the presence of any green pigment in the embryo. Such pigments could be detected very early in the development of the embryo—almost as soon as the primary leaf could be distinguished. It is difficult to see how any light can come in contact with the young embryo. The pistil has a thick, solid style and the ovary is embedded in a thickened torus. There is a single ovule in the ovary, and the mature "seed" is composed of a very hard ovary wall in contact with the seed coat.

The reactions of the yellow pigments show that we are dealing with an anthoxanthone. It is interesting that the same group of pigments is found in the petal, the embryo, and the mature green leaf. The presence of fluorescent spots in the petal and embryo extract must also be indicative of a close relationship between these two tissues. Paech (1955) has discussed the evidence for the appearance of anthocyanin pigments in young leaves. He has suggested that the flavonoid pigments may be present in leaves but may be masked by other leaf pigments. Here is presented a clear-cut example of the presence of pigments of this kind. These pigments cannot be a product of cell degradation as the leaves collected were mature in a very active condition. The Rf values obtained indicate that the pigments isolated are different from any previously reported (Geissman, 1956).

SUMMARY

The embyro of *N. lutea* contains both chlorophyll *a* and chlorophyll *b*. These pigments are formed early in embryonic development and in the absence of light.

Pigments of the flavonoid type are found in the embryo, the petal of the flower, and the green leaf. The pigments isolated from all three tissues are identical. Fluorescent substances also are present in the extract of the petal and the embryo but are absent in the leaf.

ACKNOWLEDGMENT

I wish to thank Mr. W. Garrison for his help in determining the absorption spectra.

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