A CAROTENOID PIGMENT ASSOCIATED WITH ANTHERIDIAL FORMATION IN *EQUISETUM* GAMETOPHYTES[1]

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Abstract: A red pigment extracted from male gametophytes of *Equisetum arvense* and *E. hyemale* and from young vegetative stems of *E. arvense* and overwintering stems of *E. hyemale* was identified as a carotenoid similiar to but not identical with rhodoxanthin. Its specific occurrence in the gametophyte generation accompanying antheridial formation suggests a relationship between carotenoids and reproduction, a relationship also apparent in other groups of organisms but not yet elucidated.

INTRODUCTION

Antheridial gametophytes of *Equisetum* have for over a century been characterized as yellowish in color (Hofmeister, 1852), as compared to the green archegonial gametophytes. Kashyap (1914) commented on the smooth, red appearance of the antheridial portion of old hermaphroditic gametophytes, and stated further that if the gametophyte is in the sun and so becomes reddish, the antheridial portions are redder than the remainder. Chatterjee and Ram (1968) observed that in some cells the chloroplasts degenerate and a red pigment accumulates, and Duckett (1970, 1972) noted the pinkish color of antheridial gametophytes or antheridial lobes of hermaphrodites in his cultures.

During my studies of sex determination in *Equisetum*, I had noticed that the cover cells of antheridia were characterized by a pinkish coloration, even when the remainder of the gametophyte was green, thus giving the whole gametophyte when viewed macroscopically a yellow appearance. Further, it was seen that in gametophytes grown under higher light intensities or on sugar-enriched media the color development was enhanced, because cells surrounding the antheridium also became pigmented, until the whole gametophyte, seen macroscopically, looked pink. Under high magnification, I could see that the pigment was located as apparently lipoidal droplets in the plastids, which had lost their chlorophyll and frequently had accumulated starch grains.

Schimper (1885) published a picture of chromoplasts in *Equisetum arvense* fertile stems which look just like those I observed in the gametophytes. Lippmaa (1926 a, b, c) described the occurrence of a red pigment in vegetative stems of several species of *Equisetum* and called the pigment "rhodoxanthin". As far as can be determined, from his publications, he never actually extracted and identified this pigment from *Equisetum*, but rather based his determination on its similarity in appearance with the pigment of *Cryptomeria*. Prat (1924) had earlier reported rhodoxanthin from lower internodes of *E. fluviatile*. Goodwin (1965) listed *Equisetum* and *Selaginella* as containing rhodoxanthin, apparently on the basis of Prat's and Lippmaa's reports. Jagels (1971) extracted the pigment from *Selaginella* and identified it as rhodoxanthin. He described it as occurring there in lipoidal droplets within

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modified chloroplasts, associated with starch grains, and induced by high light intensity. This closely parallels the occurrence of the red pigment in *Equisetum* gametophytes.

The red pigment from gametophytes of *Equisetum hyemale* and *E. arvense*, and from overwintering stems of *E. hyemale* and young vegetative stems of *E. arvense* was extracted and tested, to determine if it is rhodoxanthin. The following procedures were carried out in the laboratory of Kenneth Simpson, of the University of Rhode Island.

METHODS

The procedure used to extract and characterize the pigment is an adaptation of the methods of Jagels (1970) and Foppen (1969).

Plant material to be extracted was homogenized in a Waring blender with acetone, and filtered through a sintered glass filter. The filtrate was diluted with petroleum ether (30°–60°C) and washed free of acetone with distilled water in a separatory funnel, then stored over anhydrous Na_2SO_4 for one hour and evaporated to dryness in a flash evaporator. The residue was taken up in 3 ml petroleum ether (30°–60°C) and chromatogrammed on a column of cellulose (Whatman cellulose CF–11). The chromatography was carried out in a dark room, using a developing solvent of petroleum ether and acetone (4:1, v:v). Acetone was used to elute the red band and the eluate was transfered to P. E., dried over Na_2SO_4 as before and was rechromatographed on a column of aluminum oxide (Woelm neutral grade a). The process above was repeated and this time the residue in 3 ml petroleum ether was used to obtain an absorption spectrum between 300 and 700 nm, with a Cary 15 recording spectrophotometer. The red pigment in petroleum ether was then stored over $NaBH_4$ for four hours, and its absorption spectrum again determined. A change in spectrum would indicate reduction of the pigment by the $NaBH_4$. Further, the partition coefficient in petroleum ether vs. 95% methanol 5% H_2O was determined.

RESULTS

The red pigment in chromoplasts of *Equisetum* gametophytes is the same as that in the overwintering stems of *E. hyemale* and the young vegetative stems of *E. arvense*. In petroleum ether it has absorption peaks at 452, 475, and 505 mm. Rhodoxanthin is reported to exhibit maxima at 456, 487, and 521 nm (Karrer and Jucker, 1950). The red pigment is not reduced by $NaBH_4$, as rhodoxanthin is reported to be, and its partition coefficient in petroleum ether: methanol is 0:100, whereas that for rhodoxanthin (in hexane: methanol) is reported as 55:45 (Quackenbush, 1965). Therefore, this red carotenoid from *Equisetum*, although apparently quite similar to rhodoxanthin in occurrence and color, is not rhodoxanthin. It remains to be identified.

DISCUSSION

The specificity of the occurrence of the red carotenoid pigmentin *Equisetum* gametophytes in association with developing antheridia is striking. Similar apparent relationships of carotenoids with male sex development are known in the fungus

Allomyces, where the male gametangia contain an orange carotenoid pigment, and in the lycopsid *Selaginella*, which often has orange microspores. Goodwin (1950) and Burnett (1965) reviewed various examples of a possible role of carotenoids in reproduction in algae, fungi, and animals. Krinksy (1971) stated (p. 704) "Although there have been many reports of the apparent relationship between the accumulation of carotenoids and the development of reproductive structure in both animals and plants, there has never been an adequate explanation for this phenomenon in terms of a functional role for carotenoids."

It is my hope that by indicating here one more example of the highly specific relationship between carotenoids and sexual development, I might interest some person knowledgeable in biochemistry of carotenoids or in the physiology of sex to discover the exact relationship between the red pigment and the development of antheridia in *Equisetum* gametophytes.

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