ANTHOCYANIN PIGMENTS OF BUCKWHEAT HYPOCOTYLS

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INTRODUCTION

Although seedlings of buckwheat (*Fagopyrum sagittatum* Gilib.) have frequently been employed in studies on anthocyanin formation, the nature of the pigment or pigments involved has not been completely elucidated. Karstens (1939) offers strong evidence that the aglycon is cyanidin, but anomalous results prevented him from reaching definite conclusions concerning the identity of the glycosidal pigment. The probable cause of this uncertainty is suggested by new studies on buckwheat hypocotyls: paper-chromatographic techniques (Bate-Smith, 1949) now indicate that actually several pigments are present in this material.

METHODS

Extracts were prepared from the red hypocotyls of Japanese buckwheat seedlings 7 to 10 days old and from stems and hypocotyls of mature field-grown plants by steeping the material for several days in 1 percent hydrochloric acid in methanol or by macerating it in the same solvent. Such solutions are designated "unhydrolyzed." Some extracts were hydrolyzed by adding equal volumes of concentrated hydrochloric acid, boiling for 10 minutes, and shaking with *n*-amyl alcohol; the amyl alcohol layers were taken as the "hydrolyzed" extracts.

Portions of the solutions to be analyzed were applied to sheets of Whatman No. 1 filter paper and the resulting chromatograms developed with various solvents—usually in two dimensions, sometimes in one. Color reactions of the spots on completed chromatograms were noted. For all of the substances mentioned in this report these were as follows: untreated, pink; with ammonia vapor, blue; after spraying with 1 percent aluminum chloride solution, violet.

RESULTS AND DISCUSSION

Results obtained with extracts of seedlings and of mature plants were identical. Representative R_f values of the substances observed are presented in table 1. In every "unhydrolyzed" extract examined three pigments (here designated "A," "B," and "C") were seen provided suitable solvent systems were employed. Substance A was the principal component; substance B occurred in lesser amounts; and substance C was present in small but significant quantities. In the organic ("acid-poor") phases of the alcoholic solvents used the color of substance C faded gradually during development of the chromatograms and eventually disappeared. In these cases the final location of this pigment could not be ascertained. In "hydrolyzed" extracts only one anthocyanidin spot was noted. Significantly, this substance was also disposed to lose color in some solvents, and its R_f values closely resembled those of substance C.

Since the only method of separation which has been effective so far is that of paper chromatography, only minute amounts of these pigments have been available and the usual chemical studies have not yet been made. However, the absorption spectra of the individual pigment zones in the wavelength region 340 to 600 mμ could be measured directly from chromatograms in a Beckman DU spectrophotometer (Bradfield and Flood, 1952; Troyer, 1955). The spectra thus obtained resemble those usually reported for anthocyanins. Locations of the principal absorption maxima are given in table 1. Spectra of substance C

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and the anthocyanidin from a "hydrolyzed" extract were almost exactly the same and different from those of substances A and B. Since substance A was present in larger quantities, some of this material was eluted from chromatograms with acid ethanol and the spectrum of the solution measured. The latter closely resembled that taken from filter paper.

It seems likely that substance C and the anthocyanidin in "hydrolyzed" extracts are identical. In order to check the possibility that the appearance of substance C may have resulted from hydrolysis during the extraction procedure, additional "unhydrolyzed" extracts were prepared at low temperatures with immediate preparation of the chromatograms. In all cases significant amounts of substance C were found.

The anthocyanin pattern in buckwheat is thus more complex than has been supposed. Under usual conditions the hypocotyl contains at least three pigments. One of these is an anthocyanidin, presumably cyanidin (Karstens, 1939). The other two are anthocyanin derivatives of the first. Obviously any study of conditions influencing anthocyanin formation in this plant should embrace a consideration of possible changes in each of the several constituents.

TABLE 1

R_f values and absorption maxima of anthocyanin pigments in extracts of buckwheat hypocotyls. Solvent composition designated by volume

SOLVENT	PIGMENTS IN UNHYDROLYZED EXTRACT			ANTHOCYANIDIN IN HYDROLYZED EXTRACT
	A	B	C	
n-Butanol–2N HCl (1–1) (upper)	.30	.27	—	.85
n-Butanol–2N HCl (1–1) (lower)	.33	.16	.06	.07
n-Amyl Alc.–2N HCl (1–1) (upper)	.08	.10	—	—
n-Amyl Alc.–2N HCl (1–1) (lower)	.30	.14	.06	.07
n-Octanol–2N HCl (1–1) (upper)	.07	.06	—	—
n-Octanol–2N HCl (1–1) (lower)	.18	.08	.04	.06
Acetic Acid–2N HCl (6–4)	.80	.70	.52	.52
Propionic Acid–2N HCl (6–4)	.81	.65	.57	.60
Absorption maximum on filter paper, wavelength in mμ	535–540	535–538	543–545	543–545

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