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SHORT COMMUNICATION

CONSTITUENTS OF THE COTTON BUD—VII.*

IDENTIFICATION OF THE ANTHOCYANIN AS CHRYSANTHEMIN

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Abstract—The only anthocyanin in cotton buds and flowers of *Gossypium hirsutum* L., var. Delta Pine Smooth Leaf, was identified as chrysanthemin, the 3-β-monoglucoside of cynidin. Buds and flowers contained 0·030 and 0·041 per cent pigment, respectively.

INTRODUCTION

As far back as 1916, Perkin[1] suggested that the red color in an alcoholic extract of red flowers of *Gossypium arboreum* L. might be attributable to an anthocyanin. Stephens[2] examined flowers of five *Gossypium* species and concluded that the anthocyanin was identical in all. Despite the efforts of several investigators, a rigorous identification has never been achieved, however.

Stephens[2, 3] described the pigment as cyanidin-3-pentose-glycoside; Parks[4] noted anthocyanin spots in alcoholic extracts of *G. hirsutum*, *G. barbadense*, and *G. arboreum* and stated that the major anthocyanidin was cyanidin. Sadykov and coworkers[5] detected an anthocyanin in the rootlets of cotton plants, and recently Ghosh and Joham[6] identified the anthocyanidin in *G. hirsutum* as cyanidin.

In the present work, we report the identification of the anthocyanin in *G. hirsutum* as chrysanthemin, the 3-β-monoglucoside of cyanidin, and give further evidence to substantiate the identification of the anthocyanidin described in the earlier reports.

RESULTS AND DISCUSSION

Isolation of the anthocyanin was accomplished by two methods. The first involved extraction of flowers or buds with methanol, precipitation with neutral lead acetate, removal

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4 C. R. PARKS, *Am. J. Botany* **52**, 309 (1965).
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6 D. GHOSH and H. E. JOHAM, *Plant Physiol.* **39**, XXI (1964).

of lead with dilute sulfuric acid, absorption on strong acid ion-exchange resin, elution with ethanol, and successive paper chromatography in four systems. This gave a product whose u.v. spectrum indicated it was free of other flavonoids. The second involved extraction with methanol, removal of nonpolar components with light petroleum ether (b.p. 30–60°) and extraction of the anthocyanin with *n*-butanol and separation by paper chromatography in three systems avoiding mineral acid.[7]

A quantitative estimation of the pigment was accomplished chromatographically[4] showing 0·030 per cent pigment from buds and 0·041 per cent from flowers (average of three determinations). R_f values are given in Table 1 and led[8] to identification of the anthocyanin as chrysanthemin.

TABLE 1. R_f VALUES OF THE COTTON ANTHOCYANIN, ANTHOCYANIDIN, AND RELATED STANDARD AUTHENTIC COMPOUNDS

	R_f values in solvent systems indicated					
	AcOH:HCl:H_2O (5:1:5)		BuOH:AcOH:H_2O (4:1:5)		BuOH:2 N HCl (upper layer)	
Compound	Observed*	Reported	Observed*	Reported	Observed*	Reported
Cotton anthocyanin	0·66	—	0·40	—	0·26	—
Chrysanthemin	—	0·61	—	0·40	—	0·27
Malvin†	0·83	0·84	0·31	0·30	0·11	0·07
Cotton anthocyanidin	0·45	—	0·46	—	0·75	—
Cyanidin†	0·45	0·34	0·48	0·68	0·74	0·69

* Chromatography on Whatman No. 1 filter paper by the ascending method at 23°.
† Obtained from K and K Laboratories, Inc., Plainview, N.Y.

The observed λ_{max} of the anthocyanin in ethanol—0·01 % HCl occurred at 282 and 537 nm; and in methanol—0·01 % HCl at 280 and 528 nm (cf. 525 nm reported for chrysanthemin in methanol—0·01 % HCl[9]). Addition of $AlCl_3$ to an anthocyanin preparation in ethanol from which mineral acid has been carefully excluded gave a bathochromic shift of Ionswave λ_{max} indicating the presence of *o*-dihydroxy groups.[9] Since the pigment had no maxima between 300 and 330 nm,[9] and the low R_f in butanolic solvents and high one in aqueous solvents[10] it is suggested that no acyl group is present. This conclusion was substantiated by the observed stability of the pigment in 2 N NaOH.[8]

The cotton anthocyanidin had λ_{max} in methanol—0·01 % HCl of 273 and 538 nm, and in ethanol—0·01 % HCl of 277 and 548 nm. A bathochromic shift of λ_{max} of Band I was produced by addition of $AlCl_3$ (cf. cyanidin[8]). The i.r. spectrum of the cotton anthocyanidin could be superimposed on the spectrum of commercial cyanidin.

Treatment of the anthocyanin with diazomethane,[11] followed by conversion to the aglycone and degradation with alkali[12] gave two spots when separated by thin-layer chromato-

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graphy (Silica Gel G, 45:8:4, benzene:methanol:acetic acid) whose R_f values and color reactions were identical to the corresponding spots produced by co-chromatography of the mixture obtained by alkaline degradation of 5,7,3',4-tetra-*o*-methylquercetin, i.e. phloroglucinol dimethyl ether and veratric acid. This provides additional evidence for assignment of glycosidation to the 3-position. Alkaline degradation of the aglycone yielded phloroglucinol and protocatechuic acid as expected.

Pigment obtained by chromatography in mineral acid free systems was degraded to the anthocyanidin and sugar and the component moieties isolated according to Lynn and Luh.[13] Found: cyanidin and glucose in ratio 1·0:1·09. Identification of the sugar as glucose was achieved by paper and thin-layer chromatographic procedures. GLC of the trimethyl silylated sugar[14] gave evidence for the beta isomer.

EXPERIMENTAL

Chromatographic Systems

Scheme 1—Whatman No. 31 Extra Thick paper in 5:1:5 acetic acid:concentrated hydrochloric acid:water, 4:1:5 1-butanol:acetic acid:water (upper phase), 1-butanol:2N hydrochloric acid (upper phase), and 5:2:3 1-butanol:acetic acid:water. Scheme 2—Whatman 3MM paper in 15:85 acetic acid:water, and 60:15:25 and 50:20:30 1-butanol:acetic acid:water.

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[14] J. Kagan and T. J. Mabry, *Anal. Chem.* **37**, 289 (1965).