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SHORT COMMUNICATION

SOME CHARACTERISTICS OF THE ADDITIONAL ANTHOCYANIDINS FORMED DURING CONVERSION OF LEUCOANTHOCYANIDINS INTO ANTHOCYANIDINS

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Abstract—During the conversion of leucoanthocyanidins into anthocyanidins with alcoholic HCl small amounts of additional anthocyanidins are formed, which appear to be simple ethers of the anthocyanidins and the alcohol used in the medium. The chromatographic characteristics of such derivatives produced from different leucoanthocyanidins have been determined.

INTRODUCTION

EARLIER workers[1–4] have remarked upon the formation of double spots during the conversion of leucoanthocyanidins into anthocyanidins. Roux and Bill[1] in the case of *iso*propanol and Kubitzki[4] in the case of *n*-butanol indicated the possibility of the formation of an ether derivative of the anthocyanidin. Some additional evidence regarding the formation of such derivatives is presented in this communication.

RESULTS AND DISCUSSION

When purified areca (*Areca catechu* L.) leucocyanidin[5] is treated with hot *n*-butanol–HCl and the products examined chromatographically, a faint pink spot can be noticed in addition to the cyanidin. This spot (referred to as additional anthocyanidin) has a higher R_f in common solvents than the anthocyanidin. Quantitative separation, elution and estimation of the additional anthocyanidin formed from areca leucocyanidin showed that its proportion of the total anthocyanidin was approximately 5 per cent. On rechromatography this additional spot was unchanged, as was cyanidin, thus ruling out the possibility that it was an artifact of chromatographic development. On purification, the additional anthocyanidin had the same colour, absorption spectra and shift with $AlCl_3$ as cyanidin. Leucofisetinidin also gave an additional spot with spectral characteristics similar to those of fisetinidin.

With different alcohols as media in the reagent, different additional anthocyanidin spots were formed, with characteristic R_fs in Forestal and formic acid solvents (Table 1). Thus, additional spots formed during the conversion of leucoanthocyanidins into anthocyanidins are simple derivatives of the anthocyanidin with the alcohol used in the medium.

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[1] D. G. ROUX and M. C. BILL, *Nature* **183**, 42 (1959).
[2] W. D. MCFARLANE and M. J. VADER, *J. Inst. Brewing* **68**, 254 (1962).
[3] V. S. GOVINDARAJAN and A. G. MATHEW, *Phytochem.* **4**, 985 (1965).
[4] K. KUBITZKI and W. VINK, *Bot. Jb.* **87**, 1 (1967).
[5] V. S. GOVINDARAJAN and A. G. MATHEW, *Phytochem.* **2**, 321 (1963).

TABLE 1. R_f VALUES OF DERIVATIVES OF CYANIDIN AND FISETINIDIN ON CONVERSION WITH DIFFERENT ALCOHOLS AND HYDROCHLORIC ACID

Additional anthocyanidin with	Cyanidin† R_f value of derivatives			Fisetinidin‡ R_f value of derivatives		
	Forestal	Formic acid	Remarks	Forestal	Formic acid	Remarks
Methanol	0·67	0·39	—	0·83	0·60	—
Ethanol	0·71	0·37	—	0·86	0·61	—
n-Propanol	0·75–0·92*	0·53–0·58*	—	0·90	0·63	—
Iso-propanol	0·88	0·54	—	0·91	0·73	—
n-Butanol	0·82	0·46	—	0·91	0·64	—
Iso-butanol	0·91	0·47	—	0·92	0·68	—
Sec-butanol	0·91	Streaking beyond 0·5	Faint	0·97	0·75	Faint
Tert-butanol	Streaking	0·4–0·6*	Very faint	0·93	0·5–0·65*	Very faint
n-Amyl alcohol	0·90	0·50	Faint	0·97	0·67	Faint
Tert-amyl alcohol	0·81	0·41	Very faint	0·95	0·5–0·7*	Very faint

2% aqueous solution of leucoanthocyanidins, converted according to the method of Swain and Hillis.[9]

* Streaking oblong spots.

† R_f in Forestal 0·50 and in formic acid 0·22.

‡ R_f in Forestal 0·75 and in formic acid 0·40.

Boiling the anthocyanidin itself with alcohol and acid did not produce any additional anthocyanidin; its formation therefore occurs before or during the actual conversion. Chromatographic analysis at different time intervals showed that the bulk of the additional spot was formed within the first 3 min. The formation of additional anthocyanidin is not reversible, as heating the eluted additional anthocyanidin with acid did not result in its hydrolysis to the original anthocyanidin.

Polymerization was not found to affect the formation of additional anthocyanidin, as polymerized areca leucocyanidin[5] behaved like the monomer. Its formation was not affected by the presence of iron in the reagent.[3]

While the additional anthocyanidin of cyanidin was readily formed those of delphinidin and pelargonidin were formed only if corresponding leucoanthocyanidins were present in substantial quantities. Thus cashew-nut testa (*Anacardium occidentale*) polyphenols[6] (the proanthocyanidins of which contain 77% leucocyanidin and 15% leucopelargonidin), when treated with *n*-butanol–HCl, gave an additional anthocyanidin spot besides that of cyanidin. This additional anthocyanidin, when purified, showed the colour and spectral characteristics of pelargonidin and was assumed to be the *n*-butyl ether of pelargonidin (R_f in Forestal 0·91 and in formic acid solvent 0·50). Similarly sapota (*Achras sapota* L.) polyphenols[7] showed the additional anthocyanidin of cyanidin and another which proved to be the *n*-butyl ether of delphinidin (R_f 0·56 in Forestal and 0·30 in formic acid). In all these cases, about 5 per cent of the total anthocyanidin was present as the alcohol ether. The derivatives of pelargonidin, cyanidin and delphinidin with *n*-butanol moved with the solvent-front when chromatographed with *n*-butanol–HCl.[8]

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[6] A. G. MATHEW, Chemical Studies of Astringency in Plant Products with Special Reference to Polyphenols, Ph.D. Thesis, University of Kerala, Trivandrum (1967).

[7] S. LAKSHMINARAYANA and A. G. MATHEW, *J. Food Sci.* **32**, 451 (1967).

[8] E. C. BATE-SMITH, *Biochem. J.* **58**, 122 (1954).

[9] T. SWAIN and W. E. HILLIS, *J. Sci. Food Agric.* **10**, 63 (1959).

EXPERIMENTAL

Conversion of leucoanthocyanidin into anthocyanidin was carried out under the conditions specified by Swain and Hillis.[9] Chromatographic procedures[3] and special tests[7] are as described in earlier communications.

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