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Note

High-pressure liquid chromatography of anthocyanidins

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The separation and identification of anthocyanidins has been achieved in the past by either paper chromatography[1], two-dimensional cellulose thin-layer chromatography[2], or column chromatography[3]. More recently, a gas chromatographic procedure has been reported[4] which separated the anthocyanidins as their corresponding silylated quinoline derivatives after reaction with trimethylchlorosilane and hexamethyldisilazane. As these methods demand hours to perform, we would like to report a simpler procedure using a modified version of the reversed-phase high-pressure liquid chromatographic system developed by Wulf and Nagel[5] for the separation of flavonoids and phenolic acids. The examination by this method of plant materials containing anthocyanins will require a prior acid hydrolysis, to set free the anthocyanidins for analysis.

EXPERIMENTAL

Chromatograms were run on a Waters liquid chromatograph (Waters Assoc., Milford, Mass., U.S.A.) using a 30 cm × 4 mm I.D. μBondapak/C_{18} column (Waters Assoc.) and a Schoeffel SF770 UV–VIS detector set at 530 nm. A flow-rate of 2 ml/min was maintained employing a mixture of water–acetic acid–methanol (71:10:19) by volume as eluent.

Authentic specimens of delphinidin, cyanidin, pelargonidin and malvidin were obtained from commercial sources. Petunidin chloride was isolated from muscadine grapes by column chromatography of a crude hydrolysate on Polyclar AT[3]. Peonidin chloride was kindly supplied by Prof. S. Sakamura of Hokkaido University, Sapporo, Japan. These compounds were used to study the chromatographic separation, by injecting 25 μl of an aqueous solution containing approximately 1 mg/ml of each component. The exact concentration of each anthocyanidin was purposely varied according to the purity of the sample, in order to obtain chromatograms showing six well-resolved peaks of equal height.

For the analysis of plant materials, a sample (about 0.5 g) was extracted at room temperature for 1 h, with 20 ml of methanol made 0.01 *N* with concentrated HCl, the extract evaporated *in vacuo* and the residue hydrolyzed for 30 min at 100°

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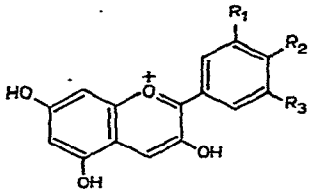
TABLE I

STRUCTURES AND RETENTION TIMES OF THE SIX MOST COMMON ANTHOCYANIDINS AS REFERRED TO IN THE TEXT



Peak No.	*Compound*	R_1	R_2	R_3	t_R *(min)*
1	Delphinidin	OH	OH	OH	5.7
2	Cyanidin	OH	OH	H	8.7
3	Petunidin	OCH_3	OH	OH	11.1
4	Pelargonidin	H	OH	H	13.6
5	Peonidin	OCH_3	OH	H	17.0
6	Malvidin	OCH_3	OH	OCH_3	20.8

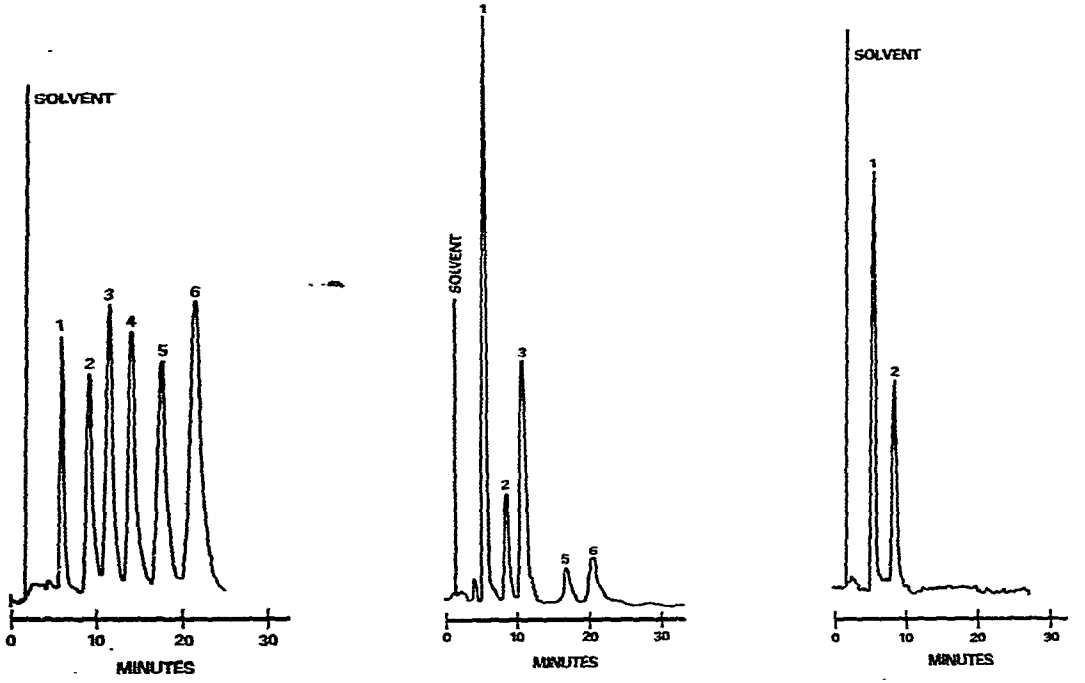


Fig. 1. Separation of the six reference anthocyanidins on μBondapak/C_{18} with water–acetic acid–methanol (71:10:19) by volume as eluent. The peak numbers are explained in Table I.

Fig. 2. Separation of *Vitis rontundifolia* anthocyanidins. The peak numbers are explained in Table I.

Fig. 3. Separation of *Hibiscus sabdariffa* anthocyanidins. The peak numbers are explained in Table I.

with 5 ml of 2 *N* HCl[6]. The hydrolysate was added to the top of a short (2 × 0.3 cm I.D.) Polyclar AT column, the column washed with two bed volumes of 0.01 *N* HCl, and the anthocyanidins eluted with 25 ml of 0.01 *N* HCl in methanol. The eluent was then concentrated to 1 ml on a rotary evaporator (< 30°) before injection on the chromatograph.

RESULTS AND DISCUSSION

Fig. 1 shows a typical chromatogram of a standard mixture of the six most common anthocyanidins. The structures and retention times of these anthocyanidins are given in Table I. The components were eluted in less than 30 min, started with the most hydrophilic one (delphinidin), and continued in order of decreasing hydrophilicity, as expected in a reversed-phase system.

Figs. 2 and 3 represent results from the injection of hydrolysates of extracts from muscadine grape skins (*Vitis rotundifolia*) and dried calyces of roselle (*Hibiscus sabdariffa*). The muscadine result corroborates previous determinations[7] which list delphinidin and petunidin as the major components and report the absence of pelargonidin. The roselle sample, in agreement with previous studies[8], showed only the presence of delphinidin and cyanidin.

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