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SHORT COMMUNICATION

THE CO-OCCURRENCE OF MONOGLUCOSIDES AND MONOGALACTOSIDES OF CYANIDIN AND PEONIDIN IN THE AMERICAN CRANBERRY, *VACCINIUM MACROCARPON**

T. FULEKI and F. J. FRANCIS

Department of Food Science and Technology, University of Massachusetts, Amherst, Massachusetts

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Abstract—Cyanidin 3-glucoside and peonidin 3-glucoside were identified as minor (<1%) pigments in cranberries. Their isolation was possible with a new developing solvent of high resolving power.

RESULTS AND DISCUSSION

CYANIDIN 3-galactoside (Cy 3-Ga), peonidin 3-galactoside (Pn 3-Ga), cyanidin 3-arabinoside (Cy 3-Ar) and peonidin 3-arabinoside (Pn 3-Ar) were identified in the American Cranberry (*Vaccinium macrocarpon* Ait.) by Sakamura and Francis[1] and Zapsalis and Francis.[2] While developing a method for the quantitative determination of each pigment[3] the presence of several minor cranberry anthocyanins was established. Two of these minor pigments have now been identified as cyanidin 3-glucoside (Cy 3-Gl) and peonidin 3-glucoside (Pn 3-Gl).

Early work by Grove and Robinson[4] indicated the presence of Pn 3-Gl in the American Cranberry, *Oxycoccus macrocarpus* Pers. (old nomenclature) and they even named the new pigment oxycoccicyanin after its source. Their identification, however, was tenuous even by the standards of those days. It was based mainly on direct comparison of the distribution numbers of purified cranberry anthocyanin (which was probably a mixture of pigments) with that of synthetic Pn 3-Gl but even these "did not agree as sharply as we anticipated"; the nature of the sugar moiety was not determined directly because of the lack of raw material. The botanical nature of the raw material may also be in question. The berries were obtained from two sources, with those from Newfoundland being "rather smaller" than those from Cape Cod. The geobotanical and morphological information indicates that they were dealing with berries of two species. The berries from Newfoundland were probably lingonberries (*V. vitis-idaea* L.) which grow wild and were exported in large quantities.[5–6]

* Part of a Ph.D. thesis by the senior author, carried out while on educational leave from the Canada Department of Agriculture, Research Station, Kentville, Nova Scotia. Present address: Food and Drug Research Laboratories, Department of National Health and Welfare, Tunney's Pasture, Ottawa, Canada.

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Harborne[7] has pointed out the difficulty there was in distinguishing the galactosides from the glucosides before the development of paper chromatographic methods. The above author cited three cases—one of them being Grove and Robinson's identification of Pn 3-Gl in cranberry—where mistakes have arisen over these monosides. It is probable that Grove and Robinson were dealing with Pn 3-Ga which is the anthocyanin present in the greatest quantity in the varieties examined (Early Black approx. 23 per cent and Howes approx. 38 per cent) while the amount of the Pn 3-Gl is less than 1 per cent of the total anthocyanin content.[8] It is misleading[9] to recommend the American Cranberry as a good source of the rather uncommon Pn 3-Gl as given in several books and review articles.[10-17]

It is interesting from a chemotaxonomical viewpoint to note that Cy 3-Gl, Cy 3-Ga, Pn 3-Gl and Pn 3-Ga were also found in the closely related lowbush blueberry, *V. angustifolium* Ait.[18] This indicates that the genus *Vaccinium* may be characterized by the presence of the monoglycosidic combination of cyanidin and peonidin with glucose, galactose and arabinose. The close chemical relationship also supports the present classification of the American Cranberry as belonging to the genus *Vaccinium*[19] and not to a separate genus (*Oxycoccus*), as was previously believed. Cyanidin arabinoside, galactoside and glucoside have also been reported in a related genus, *Rhododendron*.[20]

The co-occurrence of the monoglucoside and monogalactoside of the same aglycone is probably more frequent than the literature indicates. Due to the very similar mobility of these pigments in the solvent systems usually used for separating the anthocyanins, they remain unseparated. On hydrolysis of the unseparated glucoside and galactoside, the sugar component of the minor pigment may escape detection because of the low concentration, as happened in previous studies with cranberry anthocyanins. The separation of the closely related pigments requires repeated paper chromatographic development for extended periods and a solvent system with high resolving power such as BBFW.

EXPERIMENTAL

The anthocyanins were extracted with 1% HCl in methanol from the ripe berries of Howes variety. The pigments were separated on Whatman No. 3 paper by descending chromatography with the upper phase of 1-butanol–benzene–formic acid–water, 100:19:10:25 (BBFW).[21] The separation scheme (Fig. 1) shows that repeated chromatography with BBFW for up to 84 hr completely separated two of the minor pigments in sufficient quantity to allow their identification. These minor anthocyanins moved slightly faster than the

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pigments in the corresponding major bands (3a and 4a) and they were numbered 3b and 4b respectively. After four purifications with BBFW (series D in Fig. 1), the separated pigments were run in 1% HCl to remove free sugar and finally in BBFW to remove the arabinose paper artifact. Methanol–acetic acid–water (90:5:5) was used as eluting solvent.

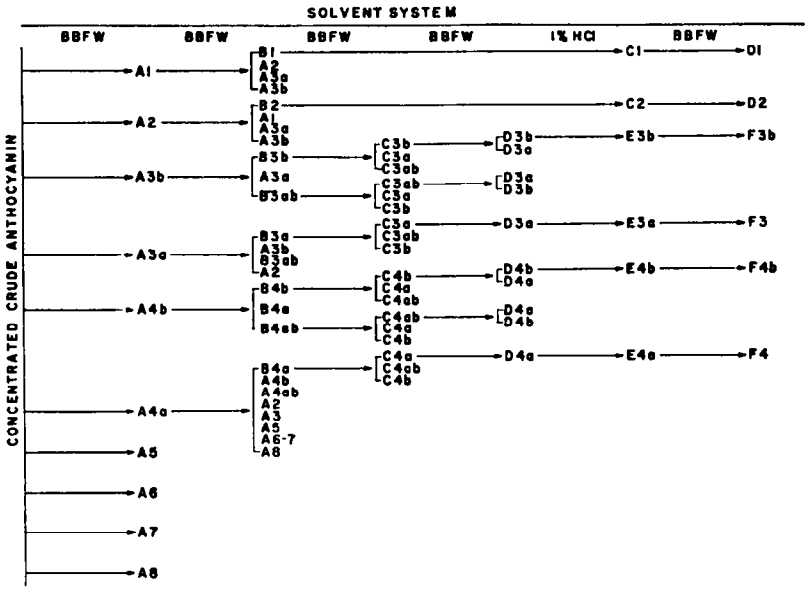

FIG. 1. SEPARATION SCHEME FOR CRANBERRY ANTHOCYANINS. THE FASTEST MOVING PIGMENT (Pn 3-Ar) WAS NUMBERED 1 AND THE CAPITAL LETTERS INDICATE THE CONSECUTIVE STAGES IN THE PURIFICATION PROCESS.

The identification of the two minor pigments followed in general the chromatographic and spectroscopic procedure described by Harborne.[22-23] The R_f values were determined using descending chromatography on Whatman No. 1 paper. Chromatographic data for the minor pigments and authentic anthocyanins (Table 1) indicated that the two unidentified pigments were monoglucosides of peonidin (Pn) and cyanidin

TABLE 1. CHROMATOGRAPHIC DATA FOR THE MINOR CRANBERRY ANTHOCYANINS

Pigment	R_f (× 100) in BAW*	BH	HAc–HCl	1% HCl
3b	48	26	32	11
4b	35	24	26	8·5
Markers				
Pn 3-galactoside	45	25	32	12
Cy 3-galactoside	35	21	28	10
Cy 3-glucoside	35	22	26	8·3
Cy 3,5-diglucoside	20	10	39	22

* Solvents: BAW = 1-butanol–HoAc–water (4:1:5), BH = 1-butanol–2 N HCl (1:1), HAc–HCl = water–HoAc–12 N HCl (82:15:3), 1% HCl = water–12 N HCl (97:3).

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(Cy). Further evidence was obtained by chromatographing the products of acid hydrolysis. The R_f values in Forestal [acetic acid–12 N HCl–water (30:3:10)] and Formic [formic acid–12 N HCl–water (5:2:3)] for the anthocyanidins produced from the minor pigments 3b and 4b corresponded with those of authentic Pn and Cy respectively. The chromatographic data with BBPW [1-butanol–benzene–pyridine–water (5:1:3:3)], phenol [phenol–water (4:1)] and BBFW for the sugar component showed that glucose was the only sugar present in both cases. The wavelength of maximum absorption in methanolic 0·01 % HCl was 528 nm for both minor anthocyanins and the E_{440}/E_{max} ratio was 27 and 23 % for pigments 3b and 4b respectively. The spectral data in the u.v. and visible range showed that the sugar was attached to the 3-hydroxyl and that neither pigment was acylated. The color changes observed under visible, short and long u.v. lights, produced with various chromogenic reagents ($AlCl_3$, $FeCl_3$, lead acetate,[24] sodium acetate) also supported the identification of 3b as Pn 3-Gl and 4b as Cy 3-Gl.

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