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ANTHOCYANINS FROM *CORNUS MAS*

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Key Word Index—*Cornus mas*; Cornaceae; anthocyanins: cyanidin-3-rhamnosylgalactoside; cyanidin-3-robinobioside.

Abstract—Cyanidin-3-rhamnosylgalactoside, a new anthocyanin, was isolated and identified from the berries of *Cornus mas* L. Cyanidin-3-galactoside and delphinidin-3-galactoside were also identified.

INTRODUCTION

OUR PRELIMINARY investigation of the anthocyanins of dogwood tree fruits, *Cornus mas* L., also known as cornelian cherries, has indicated the presence of pelargonidin-3-galactoside and pelargonidin-3-rhamnosylgalactoside.[1] The latter is a new anthocyanin representing a new class, the seventh, of 3-biosides. The sixth class of 3-biosides, arabinosylglucoside, was reported to occur in some *Viburnum* species.[2,3] This report describes the isolation and identification of other anthocyanins present in cornelian cherries.

RESULTS AND DISCUSSION

Prolonged chromatography of cornelian cherry pigment extract in butanol–formic acid–water (3 days) showed the presence of 5 pigments. The two fastest running, 1 and 2, were the orange colored, previously identified pelargonidin-3-galactoside and -3-rhamnosylgalactoside. The relative amounts of pigments present were in the following order: 1, 3, 2, 4, 5.

Pigment 3 is present in cornelian cherry in amounts second only to 1. It gave no intermediary products on partial acid hydrolysis, and yielded cyanidin and galactose as the end products. Spectral data indicated that it was a simple cyanidin-3-glycoside. It gave galactose on H_2O_2 oxidation and was identified as cyanidin-3-galactoside, by co-chromatography with an authentic sample (Table 1).

Spectral data for pigment 4 suggested that it is a 3-substituted glycoside of cyanidin without aromatic acid acylation. It yielded cyanidin, rhamnose, and galactose on acid hydrolysis. On partial hydrolysis, cyanidin-3-galactoside was formed. Hydrogen peroxide hydrolysis removed a disaccharide having mobility similar to the one isolated from pelargonidin-3-rhamnosylgalactoside. The purified disaccharide when hydrolysed gave rhamnose and galactose. Thus the anthocyanin is cyanidin-3-rhamnosylgalactoside. This pigment has close, but different, R_f values, from cyanidin-3-rutinoside. The two compounds, when co-chromatographed, could be effectively resolved by prolonged chromatography in

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butanol–formic acid–water. The isolated disaccharide reacted in a manner similar to rutinose toward a range of chromogenic reagents specific for glycosidic linkages,[4–6] and is believed to have a similar linkage. A sample of robinobiose (rhamnosyl-α,1$\rightarrow$6-galactose), obtained through the courtesy of Dr. J. B. Harborne, co-chromatographed with the disaccharide from *Cornus* in 3 solvents. This is the first time this disaccharide has been reported in an anthocyanin.

TABLE 1. R_f VALUES OF CORNELIAN CHERRY ANTHOCYANINS

Pigment	Source	R_f (×100) in HOAc-HCl	1% HCl	15% HOAc	BAW	R_{Cy3G} (×100) in BFW*
3		25	7	57	34	87
4		37	17	68	33	71
(4 hydrolysed intermediate)		26	8	—	—	86
5–1		15	3	40	—	47
5–2		77	—	—	—	42
Authentic markers:						
Cy-3-glucoside	*V. dentatum*[8]	26	6	58	35	100
Cy-3-rutinoside	*V. dentatum*[8]	37	16	69	34	88
Cy-3-galactoside	cranberry[9]	26	7	58	34	87
Dp-3-glucoside	Roselle[10]	16	3	39	—	50

* Chromatographic mobility relative to cyanidin 3-glucoside.

Two slower running minor pigments were also present in cornelian cherry. They were resolved by chromatography in 15% HOAc (5,1, 5,2). 5,1 is tentatively identified as delphinidin-3-galactoside since it has an R_f similar to delphinidin-3-glucoside and yielded delphinidin and galactose on acid hydrolysis. 5,2 has the chromatographic mobility of a trisaccharide, and, judging by its associated pigments, it may be a new pigment. No attempt was made to identify this pigment because of its very low concentration.

EXPERIMENTAL

Plant material. Ripe berries of *Cornus mas* L. were macerated with 1% HCl in MeOH, filtered, concentrated *in vacuo,* and purified on Amberlite CG-50 ion exchange resin.[11] The purified pigment extract was purified by conventional paper chromatography. Each anthocyanin band was purified in BFW, 15% HOAc, BFW, and 15% HOAc respectively.

Solvent systems. BFW (n-BuOH-HCO_2H-H_2O, 20:5:12, upper phase).[12] BAW (n-BuOH-HOAC-H_2O, 4:1:5:, upper phase), BEW (n-BuOH-EtOH-H_2O, 4:1:2·2), BBPW (n-BuOH-C_6H_6-pyridine-H_2O, 5:1:3:3) 15% HOAc, HOAc-HCl (HOAc–conc. HCl–H_2O, 15:3:82), 1% HCl, Formic (HCO_2H–conc. HCl–H_2O, 5:2:3), Forestal (HOAc conc. HCl–H_2O 30:3:10), Phenol (PhOH-H_2O, 4:1), MAW (MeOH–HOAc–H_2O, 18:1:1).

Authentic markers. Authentic pigments were obtained from previous studies. Authentic aglycones were obtained from grapes (Dp), cranberries (Cy) and strawberries (Pg).

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Hydrogen peroxide hydrolysis. The method of Chandler and Harper[13] was used with slight modification[10]. Prolonged chromatography is used for Rg measurement. For disaccharide analysis, the reaction mixture is further purified in BAW.

Pigment identification. Anthocyanin identification was carried out by standard methods.[7] Hydrolyzed aglycones were compared with authentic standards in BAW, Formic and Forestal. Sugars were run in BBPW, Phenol and BAW. Authentic pigments were spotted along with samples for all R_f measurements.

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