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Anthocyanin Pigments in the Grape Skins of Several Red *Vitis vinifera* Cultivars bred in Japan

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The anthocyanin pigments of red grapes *Vitis vinifera* cultivars, 'Kaiji', 'Sekirei ', 'Morgenshön', 'Mario' and 'Unicorn' were examined by thin-layer chromatography and reflection densitometry. Seven spots were separated, and they were identified to pigments by *Rf* values, molybdate shift and color reactions, except for one unidentified spot. In 'Kaiji', the pigments in decreasing order of concentration, were cyanidin-3-monoglucoside (92%), delphinidin-3-monoglucoside (8%), and traces of peonidin-3-monoglucoside and an unidentified pigment. 'Ruby Okuyama', 'Morgenshön' and 'Sekirei' had constitutions similar 'to 'Kaiji'. Pigments of 'Rizamat', 'Mario' and 'Unicorn', in decreasing order of concentration, were malvidin-, cyaniden-, petunidin-, delphinidin- and peonidin-3-monoglucosides, 'Mario' lacking peonidin-3-monoglucoside. In 'Kaiji', the aglycones were cyanidin (92%) and delphinidin (8%). In 'Ruby Okuyama' and 'Morgenshön', the aglycones were in about the same order, and plus petunidin in 'Sekirei'. 'Rizamat' and 'Unicorn' contained five aglycones, in decreasing order of concentration, malvidin, cyanidin, petunidin, delphinidin and peonidin, and peonidin was absent in 'Mario'.

INTRODUCTION

Investigation by Ribéreau-Gayon of *Vitis vinifera* cultivars have indicated that this species is characterized by 3-monoglucosides for five common anthocyanins (Ribérau-Gayon et al., 1955). Rankine et al. and Albach et al. reported that the pigment composition of the majority of red wine cultivars of *V. vinifera* appeared to conform to a general eight-band pattern when analyzed by paper chromatography. The 3-monoglucosides of peonidin and of cyanidin have been recently identified as major components of certain *V. vinifera* table grape cultivars (Albach et *al.*, 1963). Anthocyanins isolated from two ***V.*** *vinifera* cultivars 'Koshu' and 'Gros Colman' were identified as 3-monoglucosides only (Akuta et *al.*, 1977).

A fresh red skin color is very attractive for table grapes. In Japan, viticulture of vinifera cultivars in open fields is very difficult because of the rainy climate during the growing period. But viticulture under structures enables the production of *vinifera* cultivars such as 'Flame Tokay' or 'Rizamat' which are fresh red and of high quality. The new *V. vinifera* cultivars which were recently bred in Japan are well suitable for viticulture under structures.

The present paper is concerned with the identification and qualitative assessment of the anthocyanins of seven red table grapes grown in Japan.

MATERIALS AND METHODS

Ripe grapes of 'Kaiji', 'Sekirei', 'Morgenshön' and 'Unicorn' were supplied by N. Uehara, Director of the Uehara Grape Institute in Yamanashi. Ripe grapes of 'Ruby Okuyama', 'Rizamat' and 'Mario' were supplied by T. Simi of the Akitsu Branch Station of the Fruit Tree Research Station.

The methods of sample treatment, isolation of anthocyanins and measurement of the optical densities of the separated spots were the same as in previous report (Shiraishi et *al., 1986).*

RESULTS AND DISCUSSION

The parentages and skin colors of grape cultivars examined were shown in Table 1. The skin colors of 'Kaiji', 'Sekirei', 'Morgenshön' and 'Ruby Okuyama' were almost fresh red, but those of 'Rizamat', 'Mario' and 'Unicorn' were dull red or dark red.

Figures 1 and 2 show a tracing from a two-dimensional thinlayer chromatograms of the pigments from 'Kaiji' and 'Rizamat'. The identities of the pigments, as

Table 1. Parentages and skin color of grape cultivars examined.

Cultivar	Parent of cultivar	Skin color
Kaiji	'Flame Tokay'× 'Neo Muscat'	Red
Sekirei	A bud mutation of 'Kaiji'	Fresh purple-red
Morgenshön	'Katta Kurgan' × 'Kaiji'	Fresh red
Ruby Okuyama	A bud mutation of 'Itaria'	Fresh red
Rizamat	'Katta Krugan' × 'Parkentskij'	Purple-red
Mario	'Rizamat' **X** 'Neo Muscat'	Reddish purple-black
Unicorn	Seedling of V.*vinifera* cultivar	Dull red

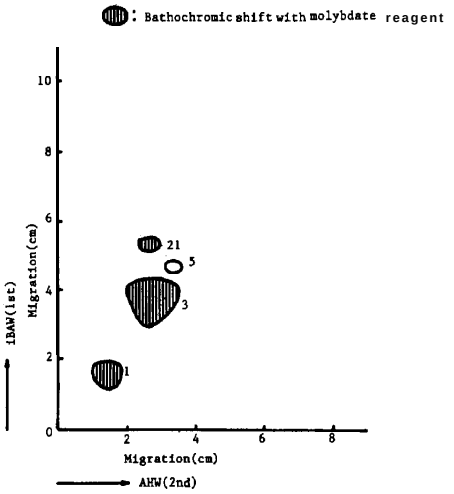

Fig. **1.** A two-dimensional thin-layer chromatogram of the anthocyanins in 'Kaiji'

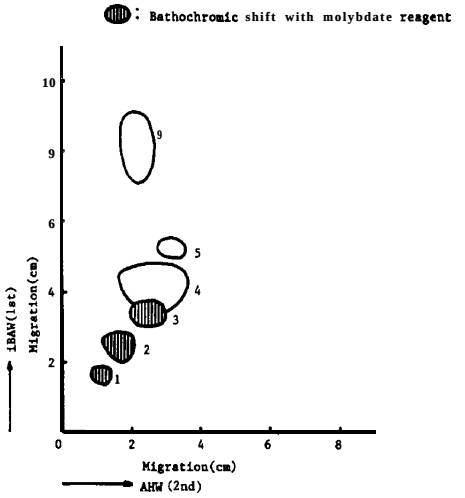

Fig. 2. A two-dimensional thin-layer chromatogram of the anthocyanins in 'Rizamat'.

indicated in Tables 2 and 3, are based on the position of the pigments on the two-dimentional chromatograms molybdate shift and color reactions, and co-chromatography with known pigment samples isolated in prior studies with *V. vinifera* pigments mixtures using column chromatography method (Ohta et al., 1978). Table 4 lists the percent distribution of the pigments of seven cultivars as determining by chromatogram scanner. Table 5 shows the aglycone constitution of seven cultivars.

In 'Kaiji', cyanidin-3-monoglucoside is by far the major anthocyanin pigment. The cyanidin-derived anthocyanin accounts for 92% of the total pigments ; the delphinidin derivative is a distant second, 8% ; and the peonidin-derived anthocyanin accounts for only a trace. Only an unknown spot at *Rf* 0.35 on *i*BAW and 0.18 in *i*AHW was also recognized and its color changed with molybdate. This spot was also recognized in a trace from 'Ruby Okuyama'. The percent distribution of pigments in 'Ruby Okuyama' and 'Morgenshön' was similar to that of 'Kaiji', the former lacking a trace amount of peonidin-3-monoglucoside, the latter lacking an unknown spot. In 'Sekirei' the major anthocyanin pigment, cyanidin-3-monoglucoside, accounted for 76 % of the total pigments ; the delphinidin derivative for 22% ; and the petunidin-derived anthocyanin accounted for only a trace. The ratio of the absorption density of crude extract in 0.1% HCl-MeOH from 'Kaiji' and 'Sekirei' was 10.4 : 28.8 at 535 nm. This result shows the improvement of red color development by a bud mutation in 'Kaiji'.

Major aglycone in 'Rizamat' is malvidin, followed by cyanidin, petunidin, peonidin and delphinidin. The major aglycone of 'Mario' is also malvidin, and in decreasing order of importance are petunidin, cyanidin and delphinidin, this cultivar lacking peonidin. Therefore, seven cultivars examined here are able to be grouped as the 'Kaiji' group, 'Kaiji', 'Sekirei', 'Ruby Okuyama' and 'Morgenshön' and the 'Rizamat' group, 'Rizamat', 'Mario' and 'Unicorn'. The 'Sekirei' group shows fresh red skin

Table 2. Comparison of *Rf* values and colors of individual anthocyanins in examined grapes

Pigment No.	*Rf* value (×*100*) at 25°C		Color in		Molybdate shift
	*i*BAW	AHW	visible light	UV light	
1	11	8	Purple	Dull purple	+
2	17	11	Reddish purple	Dull purple	+
3	21	14	Red	Dull purple	+
4	27	17	Red-purple	Dull purple	
5	32	19	Pink	Dull red-purple	
9	49	15	Red-purple	Dull purple	
21	35	18	Red	Dull purple	+

Table 3. Identification of the anthocyanins in examined grapes.

Pigment No.	Identification	Abbreviation
1	Delphinidin-3-monoglucoside	(Dl-3G)
2	Petunidin-3-monoglucoside	(Pt-3G)
3	Cyanidin-3-monoglucoside	(CY-3G)
4	Malvidin-3-monoglucoside	(Mv-3G)
5	Peonidin-3-monoglucoside	(Pn-3G)
9	Malvidin-3-monoglucoside acylated with p-coumarate	(Mv-3G-Cm)
21	Unidentified	

Table 4. Pacentages of constituent anthocyanins in skin of the examined grapes.

		Estimated percent of total anthocyanin content						
Pigment No.	Abbreviation	Kaiji	Sekirei	Ruby Okuyama	Morgenshön	Rizamat	Mario	Unicorn
1	Dl-3G	8	22	3	2	5	11	17
2	Pt-3G		2			12	14	14
3	Cy-3G	92	76	97	98	13	11	28
4	Mv-3G					36	32	26
5	Pn-3G	trace	trace			8		14
9	Mv-3G-Cm	—				26	32	
21	Unknown	trace	—	trace		—		

Table 5. Aglycone constitution of the examined grapes.

Aglycone	Estimated percent of total aglycone content						
	Morgenshön	Ruby Okuyama	Kaiji	Sekirei	Rizamat	Mario	Unicorn
Cyanidin	98	97	92	76	13	11	28
Peonidin			trace	trace	8		14
Delphinidin	2	3	8	22	5	11	17
Petunidin				2	12	14	14
Malvidin					62	64	26
Unknown		trace	trace				

color, and the 'Rizamat' group shows dull or dark red, namely, in the former cyanidin-derived anthocyanin was the major pigment, and they did not contained methylated anthocyanins, malvidin, petunidin and peonidin, or only a little. In breeding of fresh red cultivars, parents should be made use of 'Kaiji' group which contain high amounts of cyanidin and delphinidin lack methylated anthocyanins, malvidin, petunidin and peonidin.

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