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Chemical Structure of the Major Color Component from a Korean Pigmented Rice Variety

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Abstract : The major color component of a Korean pigmented rice (*Oryza sativa* var. Suwon 415) was purified with Amberlite XAD-7 column and preparative paper chromatography. The purified pigment was determined as anthocyanin by paper chromatography, UV/Vis and NMR spectroscopy. The λ_{max} of the purified anthocyanin on UV/Vis spectrum were 529 nm and 281 nm. The A_{440}/A_{529} value of the purified anthocyanin was 23% suggesting the presence of 3-glycosidic structure. The aglycone from acid hydrolysis showed bathochromic shift (18 nm) in the presence of $AlCl_3$ indicating that the anthocyanidin contained free adjacent hydroxyl groups such as cyanidin, delphinidin, petunidin or luteolinidin. The sugar moiety obtained from acid hydrolysis was determined as glucose by paper chromatography. The NMR spectra showed that the aglycone was cyanidin and the sugar was β-D-glucopyranose. Thus, the chemical structure of the purified anthocyanin was identified as cyanidin 3-*O*-β-D-glucopyranoside(Received March 29, 1996; accepted April 29, 1996).

Introduction

Anthocyanins are the most important group of water-soluble pigments in plant kingdom. They are found in flower petals, fruits, roots, stems, leaves and bracts. These pigments are responsible for pink, scarlet, red, mauve, violet and blue colors of higher plants.[1)] Anthocyanins play a definite role in the attraction of animals as pollination and seed dispersal factors. They are also important factors in the defence system against UV light and insect attack.[2)]

Pigmented rice is red or purple colored rice. Anthocyanins in pigmented rice were reported as cyanidin 3-glucoside, cyanidin 3-rhamnoside, cyanidin 3, 5-diglucoside and malvidin 3-galactoside. Especially, cyanidin 3-glucoside and malvidin 3-galactoside are the major anthocyanins in pigmented rice in Japan.[3,4)] The major color components of pigmented rice bred in Korea (*Oryza sativa* var. Suwon 415) were also tentatively identified as cyanidin 3-glucoside and malvidin 3-glucoside.[5)] In connection of previous study,[5)] we performed experiments to determine the chemical structure of the purified anthocyanin from a pigmented rice cultivar in Korea.

Materials and Methods

Materials

Pigmented rice (*Oryza sativa* var. Suwon 415) was supplied from Dr. Moon, H.-P., National Crop Experiment Station, Rural Development Administration, Suwon, Korea, and stored at room temperature. Chromatography papers were purchased from Whatman Ltd.. Amberlite XAD-7, dimethyl-d_6 sulfoxide (DMSO-d_6), trifluoroacetic acid-*d* (TFA-*d*), acetic acid and monosaccharides were bought from Sigma Chemical Co.. Other chemicals including methanol, n-butanol, amyl alcohol and ammonia solution were obtained from Hayman Ltd. and Tedia Company, Inc..

Extraction and purification

The pigmented rice (100 g) was extracted with 500 m*l* of 0.1% HCl-MeOH at room temperature for 1 hour with stirring. The filtered extract was concentrated to a small volume (10 m*l*). The concentrated extract was applied to Amberlite XAD-7 column equilibrated with H_2O. The pigment was eluted with H_2O-MeOH (4 : 1) and the elute was concentrated to a small volume (8 m*l*). The concentrate was purified by preparative paper chromatography with BAW (n-BuOH : AcOH : H_2O=4 : 1 : 5). The major bands were cut and extracted with 200 m*l* of 0.01% HCl-MeOH and the extract was dried *in vacuo*. The purified anthocyanin (35 mg) obtained was used for further studies.

Acid hydrolysis

Acid hydrolysis was carried out according to the stand-

Key words : anthocyanin, pigmented rice, cyanidin 3-*O*-β-D-glucopyranoside, ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY.
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ard procedure.[1] The hydrolysate was extracted with amyl alcohol and used for aglycone analysis. The aqueous phase after extraction was concentrated and used for sugar analysis.[6]

UV/Vis and NMR spectroscopy

The UV/Vis spectrum of the purified anthocyanin was obtained with a Varian DMS 300 spectrophotometer. The purified anthocyanin dissolved in 0.01% HCl-MeOH was scanned in the range of 200~700 nm. The ^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY spectra of the anthocyanin were measured in 90% DMSO-d_6−10% TFA-*d* with OXFORD 400 MHz FT-NMR.

Results and Discussion

Purification of anthocyanin

In the previous study,[5] the colored components extracted from Korean pigmented rice (*Oryza sativa* var. Suwon 415) were anthocyanins identified from characteristic absorption spectrum in UV-visible ranges showing maximum absorption at 280 and 530 nm. The extract of the pigmented rice with 0.1% HCl-MeOH showed a major and a minor spots on paper chromatogram developed with various solvent systems (data not shown). The major anthocyanin pigment was purified by Amberlite XAD-7 column and then preparative paper chromaography with BAW solvent system. The major band was cut and extracted with 0.01% HCl-MeOH. The purified anthocyanin was homogeneous with a single spot on paper chromatogram with various solvent systems. The R_f values of the purified anthocyanin were summarized in Table 1. These R_f values were similar with those of the cyanidin 3-glucoside from Chrysanthemum,[7] *Hibiscus syriacus*[8] and *Sambucus canadensis*.[9]

UV/Vis spectrum of the purified anthocyanin

The purified pigment showed a unique UV/Vis spectrum with absorption peaks at 281 nm and 529 nm (Fig. 1), indicating that the purified pigment is a typical anthocyanin.[7,10] A_{440}/A_{Vismax} values provide a clue to determine the position of sugar attachment to aglycone in anthocyanin pigments.[7,10-13] The A_{440}/A_{Vismax} values of cyanidin 3-glycoside and cyanidin 3, 5-diglucoside are 22~25% and 13%, respectively.[7,11] For delphinidin, the corresponding values are 17~18 and 11%, respectively.[7,11] The A_{440}/A_{529} value of the purified anthocyanin was 23% suggesting that the sugar attachment site to aglycone of anthocyanin could be a 3-glycoside. The attachment of sugar moieties at 3 and/or 5 positions causes various hypsochromic shift according to attachment patterns.[1,10] Absorption maximum of the aglycone (540 nm) was shifted hypsochromically to 529 nm (data not shown) indicating also the presence of 3-glycoside.[10-13] Anthocyanins that contain free adjacent hydroxyl groups such as cyanidin, delphinidin, petunidin or luteolinidin show bathochromic shift in the presence of $AlCl_3$[1,6,10]. The purified anthocyanin showed 39 nm of bathochromic shift (Fig. 1) in the presence of $AlCl_3$, suggesting that free adjacent hydroxyl groups exist in the anthocyanin sample molecule.[1]

Table 1. R_f values and spectral properties of the purified anthocyanin from Korean pigmented rice

R_f values (×100) in			Spectral data in 0.01% HCl-MeOH		
BAW	BuHCl	1% HCl	λ_{max} (nm)	A_{440}/A_{529} (%)	$+AlCl_3$
37	29	6	281, 529	23	+39 nm

Solvent key : BAW = n-BuOH : AcOH : H_2O (4 : 1 : 5), BuHCl = n-BuOH : 2M HCl (1 : 1), 1% HCl = conc. HCl : H_2O (3 : 97).

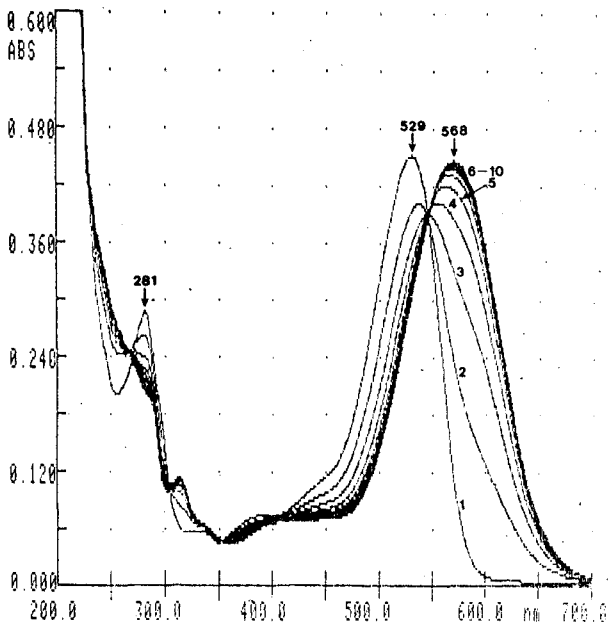

Fig. 1. UV/Vis spectra of the purified anthocyanin in 0.01% HCl-MeOH. 1 is scanned in the absence of $AlCl_3$ and 2~10 are scanned in the presence of 11 mM $AlCl_3$ with 10 min interval.

Acid hydrolysis of the purified anthocyanin

Anthocyanins can be cleaved to aglycone and sugar moieties by acid hydrolysis.[1] In order to identify the aglycone and sugar moiety, the purified anthocyanin was hydrolyzed with 2N-HCl at 100℃ for 40 min. The hydrolysate was extracted with amyl alcohol and used for aglycone analysis. The UV/Vis spectrum of the aglycone showed 18 nm of bathochromic shift in the presence of $AlCl_3$ (data not shown) suggesting that the aglycone might be cyanidin.[10] The sugar fraction liberated from acid hydrolysis of the purified anthocyanin was analyzed with paper chromatography (Fig. 2), indicating that the

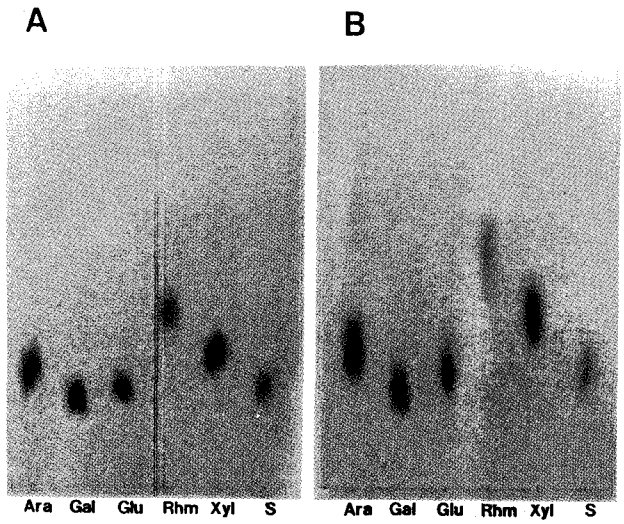

Fig. 2. Paper chromatograms of the sugar liberated from acid hydrolysis of the purified anthocyanin with BAW (A) and BTPW (n-BuOH : Toluene : Pyridine : H_2O=5 : 1 : 3 : 3) (B). S is the sugar liberated from the anthocyanin and reference sugars are arabinose (Ara), galactose (Gal), glucose (Glu), rhamnose (Rhm) and xylose (Xyl).

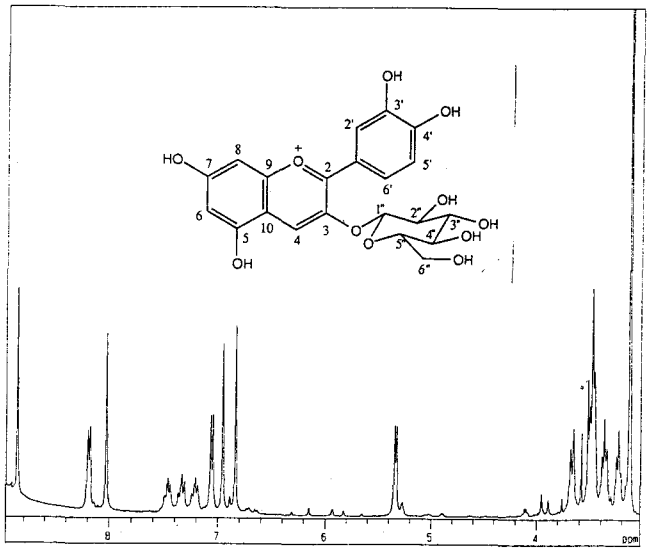

Fig. 3. ^{1}H NMR spectrum of the purified anthocyanin in 90% DMSO-d_6-10% TFA-*d*. Inset represents the chemical structure of cyanidin 3-*O*-β-D-glucopyranoside.

sugar molecule attached to anthocyanin is glucose.

NMR spectra of the purified anthocyanin

The ^{1}H NMR spectrum (Fig. 3) showed that the molar ratio of anthocyanidin to sugar is one. From the chemical shift and coupling pattern of six aglycone proton peaks in the ^{1}H NMR spectrum (Fig. 3), the aglycone of the purified anthocyanin was determined as cyanidin.[8,9,12,14,15] The proton peaks of aglycone were assigned by analysis of the 1D and 2D NMR spectra (Table 2). Analyzing the ^{1}H-^{1}H COSY spectrum (Fig. 4), H-5' appeared at δ 7.06 is coupled with H-6'(δ 8.19). The proton peaks of the sugar moiety appeared in the range of δ 3.23~5.33 (Fig. 3). The assignments of the sugar proton peaks (Table 2) were achieved on the basis of ^{1}H-^{1}H (Fig. 4) and ^{1}H-^{13}C COSY spectra (Fig. 5). The coupling constant (7.6 Hz) of the anomeric proton at δ 5.33 and the large coupling constants (8.8~10.5 Hz) of other sugar protons indicate that the sugar is the β-D-glucopyranose.[8,12,14-17] The ^{13}C peaks were assigned by comparing with reference data[8,11,12] and ^{1}H-^{13}C COSY spectrum (Fig. 5). The ^{13}C NMR spectrum showed fifteen aglycone carbon signals and six sugar carbon signals (Table 2), confirming that the isolated anthocyanin contains one glucose molecule attached to cyanidin.

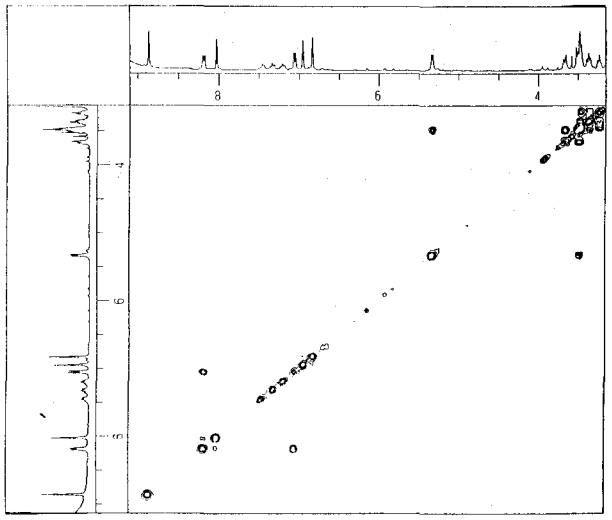

Fig. 4. ^{1}H-^{1}H COSY spectrum of the purified anthocyanin in 90% DMSO-d_6-10% TFA-*d*.

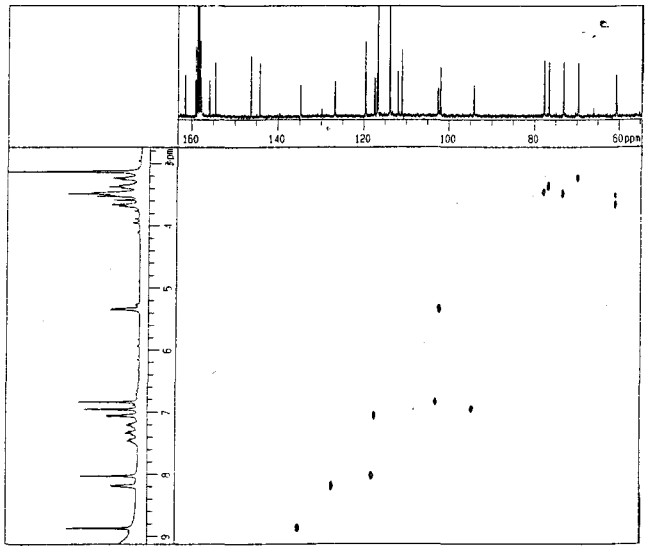

Fig. 5. ^{1}H-^{13}C COSY spectrum of the purified anthocyanin in 90% DMSO-d_6-10% TFA-*d*.

In summary, the major anthocyanin of a Korean pig-

Table 2. ^{1}H and ^{13}C NMR data of the purified anthocyanin from Korean pigmented rice (in 90% DMSO-d_6-10% TFA-*d*, chemical shifts in ppm)

	^{1}H NMR	^{13}C NMR
Cyanidin		
2		161.51
3		144.20
4	8.86 *s*	134.82
5		157.77
6	6.84 *s*	102.59
7		168.68
8	6.95 *s*	94.13
9		155.84
10		111.94
1′		119.64
2′	8.03 *s*	117.48
3′		146.29
4′		154.44
5′	7.06 *d* (8.6)	116.93
6′	8.19 *d* (8.6)	126.82
Glucose		
1″	5.33 *d* (7.6)	101.98
2″	3.48 *m*	73.14
3″	3.37 *dd* (8.8, 8.8)	76.52
4″	3.23 *dd* (8.8, 8.8)	69.64
5″	3.46 *m*	77.65
6″	3.67 *d* (10.5)	60.75
	3.51 *m*	

coupling contants (*J* in Hz) in parentheses

mented rice cultivar (Suwon 415) was purified by chromatographic techniques and identified by chemical and spectroscopical methods. Analyzing experimental results and reference data, we identified the chemical structure of the purified anthocyanin as cyanidin 3-*O*-*β*-D-glucopyranoside.

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References

1. Strack D. and V. Wray (1989) Methods in Plant Biochemistry, Vol. 1, p. 325 - 356, Academic Press, London.
2. Harborne J. B. (1993) The Flavonoids : Advances in research, p. 1-22, Chapman & Hall, London.
3. Suzushino G., A. Hatanake, Y. Suzuki and I. Nagai (1957) Anthocyanins in rice, *Nihon Daigaku Nojuigakubu Gakujutsu Kenkyu Hokoku,* **8**, 65 (CA 55, 702c).
4. Nagai I., G. Suzushino and Y. Suzuki (1960) Anthoxantins and anthocyanins in *Oryzaceae, Japan J. Breed,* **10**, 247-252.
5. Yoon H. -H, Y. -S. Paik, J. -B. Kim and T. -R. Hahn (1995) Identification of anthocyanins from Korean pigmented rice, *Agr. Chem. Biotech.* **38**, 581-583.
6. Harborne J. B. (1984a) Phytochemical Methods : A guide to modern techniques of plant analysis, p. 222-242, Chapman & Hall, London.
7. Harborne J. B. (1984b) Phytochemical Methods : A guide to modern techniques of plant analysis, p. 37-99, Chapman & Hall, London.
8. Kim J. H., G. I. Nonaka, K. Fujieda and S. Uemoto (1989) Anthocyanidin malonylglucosides in flowers of *Hibiscus syriacus, Phytochemistry,* **28**, 1503-1506.
9. Johansen O. P., O. M. Andersen, W. Nerdal and D. W. Aksnes (1991) Cyanidin 3-[6-(*p*-coumaroyl)-2-(xylosyl)-glucoside]-5-glucoside and other anthocyanins from fruits of *Sambucus canadensis, Phytochemistry,* **30**, 4137-4141.
10. Harborne J. B. (1958) Spectral Methods of characterizing anthocyanins, *Biochem. J.,* **70**, 22-28.
11. Francis F. J. (1982) Food Science Technology, p. 181-207, Academic Press, London.
12. Saito N., K. Toki, T. Honda and K. Kawase (1988) Cyanidin 3-malonylglucuronylglucoside in *Bellis* and cyanidin 3-malonylglucose in *Dendranthema, Phytochemistry,* **27**, 2963-2966.
13. Terahara N., M. Yamaguchi and K. Shizukuishi (1988) Cyanidin 3-acetylrutinoside in *Eurya japonica* berris, *Phytochemistry,* **27**, 3701-3703.
14. Saito N., M. Yokoi, M. Yamaji and J. Honda (1987) Cyanidin 3-p-coumaroylglucoside in *Camellia* species and cultivars, *Phytochemistry,* **26**, 2761-2762.
15. Toki K., N. Saito and T. Honda (1991) Cyanidin 3-glucuronylglucosides from red flowers of *Bellis perennis, Phytochemistry,* **30**, 3769-3771.
16. Toki K., N. Terahara, N. Saito, T. Honda and T. Shioji (1991) Acetylated anthocyanins in *Verbena* flowers, *Phytochemistry,* **30**, 671-673.
17. Toki K., T. Yamamoto, N. Terahara, N. Saito, T. Honda, H. Inoue and H. Mizutani (1991) Pelargonidin 3-acetylglucoside in *Verbena* flowers, *Phytochemistry,* **30**, 3828-3829.

한국산 유색미에서 분리한 안토시아닌의 화학구조

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초록 : 한국산 유색미(*Oryza sativa* var. Suwon 415)의 주요색소를 Amberlite XAD-7 및 종이 크로마토그래피를 이용하여 분리하였다. 분리된 색소를 종이 크로마토그래피, UV/Vis 및 NMR 분광분석한 결과 안토시아닌으로 밝혀졌다. 분리된 안토시아닌은 UV/Vis 스펙트럼에서 529nm와 281nm에서 최대 흡광도를 보였고 A_{440}/A_{529}는 23%였다. 따라서 이 안토시아닌에는 3-glycoside가 존재함을 알 수 있다. 산 가수분해로부터 얻은 aglycone은 $AlCl_3$ 존재하에서 18 nm의 bathochromic shift를 보였다. 이 결과는 분리된 안토시아닌에 cyanidin, delphinidin, petunidin 또는 luteolinidin과 같이 둘 이상의 hydroxyl group이 근접하여 존재함을 의미한다. 종이 크로마토그래피를 이용해 분석한 결과, 안토시아닌의 산 가수분해로부터 해리된 당은 포도당으로 확인되었다. NMR 분광분석결과 aglycone은 cyanidin이며 포도당은 β-D-configuration을 갖는 것으로 생각된다. 이상의 결과로부터 분리된 안토시아닌의 화학구조는 cyanidin 3-*O*-*β*- D-glucopyranoside로 결정되었다.

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