Note

Major Anthocyanin of Bolivian Purple Corn (*Zea mays* L.)[†]Nobuji NAKATANI,* Hitomi FUKUDA
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Recently there have been pointed out many problems on the food additives, especially on the food colorants. This study aims to develop new and safe colorants of plant origin. Our attention has been focused on the investigation of the pigments of the pericarps of Bolivian purple corn (*Zea mays* L., Maize Morado) kernels, which contain more than ten anthocyanins in our preliminary observation on paper chromatography. Several works on chemical analysis of the dark colored seed of corn have been reported so far, for example Straus¹⁾ identified two anthocyanins from 'Black Mexican Sweet Corn,' Coe²⁾ and Harborne *et al.*³⁾ discussed on the relationship between anthocyanins and color geno types. Baraud *et al.*⁴⁾ reported on the pigments of a Peruvian race and Lawanson *et al.*⁵⁾ on the pigments of maize seedlings. This paper describes the isolation and identification of the major anthocyanin present in a Bolivian purple corn.

According to the procedure shown in Scheme 1, major pigments obtained from corn kernels and cobs were purified by successive column chromatography and paper chromatography. From the spectroscopic properties and *R_f* values of paper chromatography (Table I), both of major pigments were same and also identical with the authentic cyanidin 3-glucoside. Bathochromic shifts with AlCl_3 indicated the presence of an ortho-dihydroxyl group in the B ring.⁶⁾ The ratio, $E_{440}/E_{\lambda_{\text{max}}}$, supported the hydroxy group at C-5 position free. The structure of the aglycone moiety obtained by complete acid hydrolysis was assigned to be cyanidin by the direct comparison with the authentic and synthetic sample, prepared by the condensation of phloroglucinaldehyde and 3,4, ω -triacetoxy acetophenone according to the method of Robertson.⁷⁾ The λ_{max} (339 nm) of the aglycone was identical with

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SCHEME 1. Extraction and Purification of Pigments from *Zea mays* L. (Maize Morado).

authentic cyanidin. Sugar moiety was identified as glucose by paper chromatography with different solvents and by GLC after conversion to an alditol acetate. Alternatively, enzymic hydrolysis of the pigment with β -glucosidase afforded glucose and aglycone, while with α -glucosidase the starting material was recovered. This fact revealed that the glycosidic linkage of the pigment is β .

The aglycone was subjected to alkaline hydrolysis by $\text{Ba}(\text{OH})_2$ solution. The hydrolyzate was identified as protocatechuic acid by the comparison of *R_f* values and the characteristic color, tan, with the authentic compound.

Consequently, from the basis of the above evidences, both of the structure of the major pigment of corn kernels and cobs was cyanidin 3- β -glucoside, further supported by the retention time of high pressure liquid chromatography.

EXPERIMENTAL

Materials. Purple corn (*Zea mays* L., Maize Morado) was harvested in Bolivia in 1975.

Extracton and purification of pigments. Scheme 1 shows the extraction and purification of anthocyanins from the corn kernels. Air dried pericarps (2.7 g) from kernels (100 g) were macerated in a blender with

TABLE I. PROPERTIES OF MAJOR PIGMENT OF *Zea mays* L. (Maize Morado)

	<i>R_f</i> values ($\times 100$) ^a								Spectral data		
	BAW	AWHCl	BBFW	AHW	Forest.	BuHCl	15% AcOH	1% HCl	$\lambda_{\max}$ (nm)	AlCl ₃ shift (nm)	$E_{440}/E_{\lambda_{\max}}$ ($\times 100$)
Pericarp	34	29	9	59	66	27	47	9	533	19	29
Cob	36	29	10	60	66	28	46	9	534	12	24
Authentic cyanidin 3-glucoside	36	29	9	61	66	28	46	9	534	11	26

^a On Toyo No. 51 paper. Abbreviations: BAW (*n*-BuOH-AcOH-H₂O; 4: 1: 5, v/v, upper layer); AWHCl (AcOH-H₂O-conc. HCl; 15: 82: 3, v/v); BBFW (*n*-BuOH-Benzene-HCOOH-H₂O; 100: 19: 10: 25, v/v); AHW (AcOH-conc. HCl-H₂O; 5: 1: 5, v/v); Forest. (AcOH-conc. HCl-H₂O; 30: 3: 10, v/v); BuHCl (*n*-BuOH-2*N* HCl; 1: 1, v/v, upper layer); 15% AcOH (AcOH-H₂O; 15: 85, v/v); 1% HCl (conc. HCl-H₂O; 3: 97, v/v).

0.1% HCl in MeOH solution at room temperature for 5 min and then kept in the refrigerator overnight. After the filtration of the mixture, the residue was reextracted three times in the same way. The combined extracts were concentrated to dryness *in vacuo* at 30°C and the residue (1.15 g) was partitioned between water and ether. Water layer was concentrated to a small volume, then applied to a column of Dowex 50W X4 cation exchange resin (H⁺ form).⁹⁾ The pigment mixture eluted was fractionated by a polyacrylamide (Wako, C-200) column chromatography. The separated pigments were further purified by repeated paper chromatography.

High pressure liquid chromatography. A SC-02 packed column (ODS) was used for JASCO FLC-350 High Pressure Liquid Chromatography using 85% MeOH solution as an eluent.

Spectral analysis. The UV and visible absorption spectra of pigments were recorded in 0.01% HCl in MeOH solution with HITACHI 323 Spectrophotometer. The AlCl₃ shifts were measured 1 min after adding 3 drops of 5% AlCl₃ in EtOH solution to the cuvette.

Acid hydrolysis. The purified pigment (1 mg) was heated at 100°C with 2*N* HCl solution (5 ml) for 1 hr. The resultant solution was extracted with isoamyl alcohol three times (5 ml of each), followed by concentration *in vacuo* to afford aglycone.

The aqueous portion of hydrolyzate was passed through short columns of Amberlite IR 120 (H⁺ form) and Amberlite IRA 410 (OH⁻ form) successively for deionization. The eluate was concentrated and co-chromatographed with the authentic sugar samples. The sugar spots were visualized with AgNO₃ reagent method.⁹⁾

A portion of the above concentrate was dissolved in a small volume of H₂O-MeOH (1: 1) and reduced by the addition of NaBH₄ at room temperature. After

the excess reagent was decomposed by the addition of Amberlite IR 120 until the bubble to cease, boric acid was removed by repeated evaporations with MeOH. The resulting alditol was acetylated in the usual method.

Gas liquid chromatography. The above alditol acetate was analyzed with a Shimadzu Gas Chromatograph Model GC-6AM fitted with a flameionization detector using a glass column (0.4 $\times$ 200 cm) packed with 3% ECNSS-M on Gas Chrom Q at 190°C.

Alkaline degradation. The aglycone was refluxed in saturated Ba(OH)₂ solution under N₂ for 1 hr.¹⁰⁾ After neutralizing with dil. HCl solution, the mixture was extracted with ether. The ether extract was examined on paper chromatography and sprayed with the diazotized *p*-nitroaniline reagent (to 0.25 g of *p*-nitroaniline in 50 ml of 2*N* HCl solution added 5% NaNO₂ solution until colorless), intensified the color of spot by spraying with 20% Na₂CO₃ solution.

Enzymic degradation. To the aqueous solution (2 ml) of the purified pigment (1 mg), a suspension of α - or β -glucosidase (Sigma, 1 mg) in water (0.5 ml) was added. The mixture was covered with toluene and allowed to stand for 24 hr at 37°C. The reaction mixture was extracted with isoamyl alcohol. The

aqueous and organic layer were examined by paper chromatography for sugar and aglycone moieties, respectively.

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