Note

Major Anthocyanin of the Flowers of Hibiscus (*Hibiscus rosa-sinensis* L.)

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The red pigments contained in red flowers of the *Hibiscus* species are anthocyanins, and have been widely used as coloring agents. Particularly, the red anthocyanins present in the calyces of roselle (*Hibiscus sabdariffa* L.) have been used as food colorants, and their major compounds are cyanidin-3-sambubioside and delphinidin-3-sambubioside.¹⁾ Anthocyanins contained in the red petals of hibiscus (*Hibiscus rosa-sinensis* L.) are also used as coloring agents. Concerning these, cyanidin-3-sophoroside has been suggested as the major compound.^{2,3)} However, *H. rosa-sinensis* L. consists of many strains, including interspecific hybrids, so that it is obscure whether every hibiscus strain produces cyanidin-3-sophoroside as the major floral anthocyanin. In this paper, we confirm the major anthocyanin of an old type strain of hibiscus (*H. rosa-sinensis* L.) to be cyanidin-3-sophoroside by means of chromatographic and NMR analyses.

Scarlet petals of *H. rosa-sinensis* L. cv. 'Brilliant' were collected from trees cultivated in our experimental field. The anthocyanins were extracted from lyophilized petals (150 g) with methanol containing 0.01% HCl at 4°C,

washed with *n*-hexane, and recovered as a precipitate (15 g) with five volumes of diethyl ether. By two-dimensional cellulose thin-layer chromatography (TLC), the anthocyanins in this precipitate were separated into one major and three minor spots. This major spot was estimated to involve about 90% in content of the anthocyanins by the spectrophotometric pH-differential method.⁴⁾ The precipitated anthocyanins were chromatographed on a cellulose powder column with a solvent system of isobutanol-acetic acid-water (8:2:3), and then on a Toyopearl HW-40S column with methanol containing 0.01% HCl as the solvent. The purity in each fraction was assayed by cellulose TLC, and 200 mg of the major anthocyanin (hibiscus anthocyanin) were isolated.

The hibiscus anthocyanin was completely hydrolyzed into an anthocyanidin and monosaccharide(s) by heating at 100°C in 1 N HCl for 60 min. The aglycon moiety was recovered by extracting with isoamylalcohol. The aqueous phase in the extract was neutralized with Ag₂CO₃ powder, and the AgCl precipitate was removed by centrifugation. Both the aglycon and sugar solutions were dried by evaporation. Authentic cyanidin-3-glucoside was also hydrolyzed in the same way as that already described, and cyanidin was obtained.

The *R_f* values of the hibiscus aglycon on three paper chromatograms coincided well with those of cyanidin (Table I). The monosaccharide(s) were shown to be glucose by gas-liquid chromatography after converting to the trimethylsilyl-derivative(s).

An analysis of the partially acid-hydrolyzed products of hibiscus anthocyanin by cellulose TLC indicated that cyanidin-3-glucoside was a unique intermediate during the deglycosylation reaction. This suggests that the 3-position

Table II. CHROMATOGRAPHIC MOBILITY OF DIGLUCOSIDE LIBERATED FROM HIBISCUS ANTHOCYANIN AND AUTHENTIC DIGLUCOSIDES

Thin-layer plate Solvent system	<i>R_g</i> value (× 100)	
	Cellulose BEtW	Silica gel BAEW
Hibiscus sugar	69	28
Sophorose (β-1,2)	70	28
Laminaribiose (β-1,3)	78	40
Cellobiose (β-1,4)	61	24
Gentiobiose (β-1,6)	56	16
Maltose (α-1,4)	62	32
Isomaltose (α-1,6)	57	22

Sugar spots were visualized with aniline hydrogen phthalate (110°C, 10 min). Abbreviations: BEtW, *n*-butanol-ethanol-water (4:2:2.2, v/v, upper phase); BAEW, *n*-butanol-acetic acid-diethyl ether-water (9:6:3:1, v/v).

Table I. *R_f* VALUES OF ANTHOCYANIDINS BY PAPER CHROMATOGRAPHY

Solvent system	<i>R_f</i> value (× 100)		
	HAW-3	FWH	HAW-2
Hibiscus aglycon	51	23	32
Cyanidin	49	22	34

Toyo filter paper no. 50 (40×40 cm) was used. Abbreviations: HAW-3, HCl-acetic acid-water (3:30:10); FWH, formic acid-HCl-water (5:2:3, v/v); HAW-2, HCl-acetic acid-water (1:5:5, v/v).

Fig. 1. ^1H -NMR Spectrum of Hibiscus Anthocyanin.

Ten milligrams of hibiscus anthocyanin were dissolved in 0.4 ml of CD_3OD containing 0.1% DCl. The spectrum was measured on a JEOL GX-400 FT-NMR spectrometer (400 MHz). A small signal from HDO, δ 4.93 (singlet), can be observed.

of cyanidin is the single glycosylated site by the hibiscus sugar, presumably a diglucoside. An intact hibiscus sugar attached at the 3-position was then liberated by hydrolysis with hydrogen peroxide⁵⁾ and applied to TLC. As shown in Table II, the *R_f* value of the hibiscus sugar was identical to that of sophorose.

These results suggested that hibiscus anthocyanin is cyanidin-3-sophoroside. To confirm this assumption, the ^1H -NMR spectrum of hibiscus anthocyanin was analyzed. The aromatic signals of hibiscus anthocyanin, δ 8.99 (1H, s), 8.21 (1H, dd), 8.04 (1H, d), 7.06 (1H, d), 6.90 (1H, d) and 6.66 (1H, d), showed good correspondence with those of authentic cyanidin-3-glucoside,⁶⁾ 9.01 (1H, s), 8.26 (1H, dd), 8.04 (1H, d), 7.01 (1H, d), 6.90 (1H, d) and 6.66 (1H, d). The anomeric configurations of the two glucose moieties were both indicated to be β based on the anomeric signals, 5.46 (1H, d, $J=7.5$ Hz) and 4.75 (1H, d, $J=7.5$ Hz). From this evidence, the hibiscus anthocyanin was concluded to be cyanidin-3- β -sophoroside.

Anthocyanins of the *Hibiscus* species are useful as natural coloring agents, and particularly as food additives. For large-scale production of these anthocyanin pigments, various attempts have been made to induce anthocyanin production in cultured cells of the *Hibiscus* species.

Recently, Mizukami *et al.*⁷⁾ succeeded in producing anthocyanin in callus tissues induced from seedlings of roselle. We are now examining the conditions to induce calli that express anthocyanin-synthesis pathways from *H. rosa-sinensis* L.

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