Anthocyanin of the Seedlings of a *Polygonum*. Studies on Anthocyanins, XXXII*

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The seedling of a cultivated variety of *Polygonum hydropiper* L. is remarkable in that a pronounced deep red color appears immediately after germination in the cotyledons as well as in the hypocotyl. The seedling is known as 'Benitade', i. e., red knot-grass, and is occasionally used as a garnish in Japanese dishes.

The chemical nature of this pigment has been studied as a preliminary measure for investigating the biochemical process of pigment formation in these seedlings. The pigment was finally obtained as brownish red needles which showed all the characteristics of idaein (3-monogalactoside of cyanidin). Idaein, however, is hardly distinguishable from chrysanthemin (3-monoglucoside of cyanidin) on the paper chromatogram on account of the similarity in the R_f values of both pigments with various solvent mixtures. It was found that the differentiation was achieved by means of paper electrophoresis with dilute aqueous borax solution.

Idaein, so far, has been isolated from the fruits of the following plants: *Vaccinium vitis-idaea* L.¹⁾, *Fatsia japonica* Decne. et Planch.²⁾, and *Pirus malus* L.³⁾. The present isolation is probably the first instance that the pigment has been obtained from vegetative organs.

Experimental

Fresh seedlings (900 g.) were extracted with cold 1% methanolic hydrochloric acid (2 l.) for an hour followed by filtration, and the material was again extracted with the same solvent (0.5 l.). The combined extract was carefully added with aqueous basic lead acetate, until the precipitation of the blue lead salt was complete. The precipitate was quickly filtered, washed with methanol, and dried. The conversion of the lead salt into chloride was effected as usual by treatment with 4% methanolic hydrochloric acid (200 ml.), and the product was precipitated with a large excess of ether (about 6 vols.). The amorphous red powder obtained was dissolved in hot water (60 ml.) and filtered solution was mixed with a saturated aqueous solution of picric acid. On cooling, needle-shaped crystals commenced to separate. After standing overnight, the crude picrate was collected and recrystallized from water (80 ml.) containing some picric acid. The crystals (0.48 g.) consisted of homogeneous brownish red needles, which were sparingly soluble in cold water, but very easily soluble in hot water, methanol and ethanol. Mp. 162-163° (decomp.).

Conversion into Anthocyanin Chloride.

The purified picrate was dissolved in a small amount of hot water and added with 3.5 vols. of 4% methanolic hydrochloric acid. On standing in a refrigerator overnight, straight-cut needles crystallized out. Recrystallization was repeated twice

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in a similar manner. Yield 0.082 g., viz., 0.009% of fresh plant material. The crystalline chloride is moderately soluble in cold water with brownish red color. The color of ethanolic solution is pure red. An aqueous solution of the chloride gives with aqueous ferric chloride a violet color, which turns into intense blue on dilution with ethanol. The distribution number between *iso*-amyl alcohol and 0.5% hydrochloric acid was determined as 10-13, which lies within a range found with a majority of monoglycosidic anthocyanins*. The air-dried specimen melts at 209-210° under simultaneous effervescence. It contains one molecule of water of crystallization. (Found: H₂O 3.61%. Calc. for C₂₁H₂₁O₁₁Cl·H₂O:H₂O 3.58%.) CH-Determination of the air-dried specimen gives a result that agrees with the calculated value of monohydrate of cyanidin-monohexoside. (Found: C 50.47, H 4.20%. Calc. for C₂₁H₂₁O₁₁Cl·H₂O: C 50.15, H 4.57%.)

Spectrophotometric examination: The crystalline chloride was dissolved in 60% ethanol containing 0.1% hydrochloric acid in a concentration of 5×10^{-5} mol. and the solution was subjected to spectroscopic analysis, chrysanthemin and idaein being examined in parallel. The absorptions of three anthocyanins proved to be quite identical in the entire spectral region, indicating that they were quite similar as to the glycoside bonding.

Paper chromatographic test: Developed with three different solvent mixtures by ascending method, it was found that the R_f values were not sufficient for the discrimination between chrysanthemin and idaein, as shown in the following data:

	Chrysanthemin	Idaein	<i>Polygonum</i> - anthocyanin
<i>n</i> -BuOH/AcOH/H ₂ O [4 : 1 : 5 (v/v)]	0.31	0.27	0.27
Phenol saturated with water	0.26	0.30	0.30
AcOH/conc. HCl/H ₂ O [5 : 1 : 5 (v/v)]	0.60	0.60	0.60

Paper electrophoresis: A satisfactory method of discrimination between chrysanthemin and idaein was found; that is, paper electrophoresis method in 1% borax solution (pH 11). The following electrophoresis (16 mA/cm., 650 V, Tôyô No. 52 filter paper, one-hour run) clearly demonstrated the identity of the pigment from *Polygonum*-seedling with idaein.

	Chrysanthemin	Idaein	<i>Polygonum</i> - anthocyanin
Distance of migration toward the anode	0.21 mm.	0.26 mm.	0.26 mm.

Hydrolysis of the Glycoside.

Identification of cyanidin: When the glycoside was boiled with 20% hydrochloric acid, it was easily hydrolyzed and the aglycone began to crystallize out in brownish red needles while hot; the separation was complete on cooling. The crystals were collected by filtration and air-dried. 27.77 mg. of the aglycone were obtained from 39.65 mg. anhydrous glycoside chloride. (Found: aglycone 70.0%. Calc. for C₁₆H₁₁O₆Cl·H₂O from C₂₁H₂₁O₁₁Cl: 70.3%.) The identity of this substance with cyanidin was established by careful comparison with the authentic specimen. An air-dried specimen gave analytical values corresponding to those of cyanidin chloride. (Found: C 52.89,

* Distribution number of monoglycosides: 10 (Willstätter and Mallison), 14.9 (Hayashi) for idaein, and 19.5 (Willstätter and Bolton), 18 (Hayashi) for chrysanthemin, respectively.

H 3.86%. Calc. for $C_{16}H_{11}O_6Cl \cdot H_2O$: C 52.88, H 3.58%.) Paper chromatographic data proved also the identity of this substance with cyanidin: R_f 0.31 for cyanidin, and 0.31 for *Polygonum*-anthocyanidin [Acetic acid/conc. HCl/water, 5 : 1 : 5 (v/v)].

Determination of the sugar component: The almost colorless, acidic filtrate obtained above was evaporated to dryness *in vacuo* over solid sodium hydroxide. The residue was dissolved in a minimal amount of water and analyzed by descending paper chromatography on the one hand and by paper electrophoresis on the other. By these methods, the sugar component was determined unequivocally as galactose, as seen from the following data:

Paper chromatography of sugar component (descending, Tôyô No. 52 filter paper, 15°)			
	Sugar from <i>Polygonum</i> -anthocyanin	Galactose	Glucose
Pyridin/ <i>n</i> -BuOH/ H_2O^* (4 : 6 : 3 (v/v))	172 mm.	172 mm.	192 mm.
<i>n</i> -BuOH/AcOH/ H_2O^{**} (4 : 1 : 5 (v/v))	56 mm.	56 mm.	70 mm.

* Distance of migration from the start after 40 hrs.

** Do. after 20 hrs.

Paper electrophoresis of sugar component (1.8 mA/cm., 500 V, Tôyô No. 52 filter paper, 3-hour run in 1% borax sol.)

	Sugar from <i>Polygonum</i> -anthocyanin	Galactose	Glucose
Distance of electrophoretic migration toward the anode	47 mm.	47 mm.	60 mm.

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References

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摘 要

ペニタデ (芽生え) のアントシアニン (アントシアニンの研究. 第 32 報)

菅 野 延 彦・林 孝 三

ヤナギタデ *Polygonum hydropiper* L. の栽培品で芽生えが特に赤く色づくものをペニタデと呼んで、古くから料理の「つま」に用いている。この芽生えにおける色素の生成過程をしらべるための前提として、われわれは色素の単離同定を試みた。

ペニタデの赤色はペーパークロマトグラフ的に単一のアントシアニンによるものであり、さらに結晶色素の化学分析によりイデイン (シアニジン-3-ガラクトサイド) と同定された。

一般に葉などの栄養器官に出現するアントシアンはクリサンテミン (シアニジン-3-グルコサイド) であり、今回のようにガラクトース配糖体である例は、今までのところ *Fagus sylvatica* の紅葉 [Robinson, R. and Smith, H., Nature **175**: 634 (1955)] 以外には知られていない。

なおイデインとクリサンテミンとは R_f 値が極めて接近しており、ペーパークロマトグラフィによる識別は不可能に近いが、今回われわれは硼砂水溶液中での沝紙電気泳動法によって両者の判別が可能なることを見出した。(東京教育大学理学部植物学教室)