

Grass Anthocyanins

I. On Anthocyanins in *Molinia caerulea*

GERD BENDZ and ÅSA HAGLUND

The Institute of Chemistry, University of Uppsala, Uppsala, Sweden

The inflorescences of many grasses exhibit colours varying from nearly black to brown violet or even red. On treatment with acids red solutions are formed indicating the presence of anthocyanins. Undoubtedly these pigments are responsible not only for the reddish colour but also for the black to violet tint, although these darker shades must be in part due to other factors, for instance copigmentation or metal chelation of the anthocyanins present.

The very dark blue-violet inflorescences of *M. caerulea* have been found to contain two anthocyanins. Cyanidin-3-monoglucoside was identified by comparing its spectral and chromatographical data with an authentic specimen (Table 1). On acid hydrolysis it gave glucose, identified chromatographically and cyanidin identified by co-chromatography and spectral data. The

other pigment was identified as cyanidin-3-rhamnosylglucoside by its R_F -values in four solvents (Table 1) and identification of its hydrolysis products. On controlled hydrolysis unchanged pigment, cyanidin-3-monoglucoside and cyanidin were isolated and identified by co-chromatography and spectral measurements. On acid hydrolysis it gave cyanidin, identified as before, and two sugars, glucose and rhamnose, identified chromatographically. The disaccharide component of this anthocyanin might be rutinose, but it has not been further investigated.

After completing this work we learned that Harborne¹ had made a survey of 23 different species of grasses with regard to their anthocyanin contents and found that all but one contained cyanidin-3-monoglucoside. One of these cyanidin-containing grasses was *M. caerulea* which was not, however, further investigated. Of the grasses hitherto investigated only one, *Oryza sativa*, was reported to contain cyanidin-3-rhamnosylglucoside together with malvidin-3-galactoside.²

Experimental. The absorption spectra were measured in methanol containing 0.01 % conc. HCl with a Bausch and Lomb Spectronic 505 spectrophotometer. The infrared spectra were measured in the KBr phase with a Perkin-Elmer Model 157 spectrophotometer. MN-Cellulose powder 300, Macherey, Nagel & Co., was used for TLC and Whatman No. 1 and No. 3 papers for PC. The solvents used were: BAW = butanol-acetic acid-water (4:1:5, by vol., top layer); BuHCl = butanol-2 M HCl (1:1, v/v, top layer); 1 % HCl = conc. HCl-water (3:97, v/v); HAc-HCl = acetic acid-conc. HCl-water (15:3:82, by vol.); forestal = acetic acid-conc. HCl-water (30:3:10, by vol.).

M. caerulea was collected at the parish of Transtrand in Dalecarlia and only the inflorescences were investigated. They were extracted with methanol containing 1 % conc. HCl and the pigments precipitated by addition of a tenfold volume of ether. The precipitate was dissolved in a mixture of butanol, acetic acid and water (6:1:2, by vol.) and was chromatographed on a cellulose column in the same solvent. The red fraction was evaporated and purified by repeated chromatography on Whatman No. 3 in 15 % aq. acetic acid according to the method described earlier.^{3,4} The isolated pigments were tested for homogeneity by TLC in the first four solvents. The R_F -

Table 1. R_F -values of the anthocyanins.

	BAW	BuHCl	1 % HCl	HAc-HCl
Pigment I	0.36	0.25	0.07	0.26
Cyanidin-3-glucoside ^a	0.37	0.25	0.07	0.26
Mixture of both	0.37	0.25	0.07	0.26
Pigment II	0.36	0.25	0.17	0.40
Cyanidin-3-rhamnosylglucoside ^b	0.37	0.25	0.19	0.43
Cyanidinglucoside obtained on controlled hydrolysis	0.37	0.25	0.07	0.26
Mixture with cyanidin-3-glucoside	0.37	0.25	0.07	0.26

^a Isolated from *Sedum album*.

^b Reported by Harborne.⁴

values on paper as well as those of cyanidin-3-monoglucoside (isolated from *Sedum album*) are given in Table 1.

The acid hydrolysis of the two pigments was performed as described earlier with the exception that the aglycones were extracted with butanol.³ The two anthocyanins yielded cyanidin identified by absorption and infrared spectra as well as by co-chromatography in forestal with an authentic marker. The sugars were isolated according to the method described earlier.³ They were identified by chromatography with authentic markers in ethyl acetate-pyridine-water (8:1:1, by vol.) and ethyl acetate-acetic acid-water (3:1:1, by vol). Pigment I gave glucose and pigment II glucose and rhamnose. The position of the attachment of the sugar was determined by spectral measurements.^{5,6} The controlled hydrolysis was performed by warming pigment II with 5 % HCl at 100° for 5 min. The resulting solution was then chromatographed on paper in 1 % HCl. Three spots were obtained identified by co-chromatography and spectral measurements as cyanidin, cyanidin-3-monoglucoside (Table 1) and unchanged pigment.

Acknowledgements. Our thanks are due to Professor A. Fredga for his kind interest in this work, for collecting the material used and for the facilities put at our disposal. A grant from the *Royal Swedish Academy of Science (Kemiska prisgruppens särskilda fond)* is gratefully acknowledged.

1. Clifford, H. T. and Harborne, J. B. *Proc. Linnean Soc. London* **178** 2 (1967) 125.
2. Nagai, I., Suzushino, G. and Suzuki, Y. *Japan. J. Breeding* **10** (1960) 247.
3. Bendz, G., Mårtensson, O. and Terenius, L. *Acta Chem. Scand.* **16** (1962) 1183.
4. Harborne, J. B. *J. Chromatog.* **1** (1958) 473.
5. Harborne, J. B. *Biochem. J.* **70** (1958) 22.
6. Harborne, J. B. *Phytochemistry* **2** (1963) 85.

Received September 8, 1967.

The Anthocyanins of the Berries of *Majanthemum bifolium*

GERD BENDZ and ÅSA HAGLUND

The Institute of Chemistry, University of Uppsala, Uppsala, Sweden

The *Liliaceae* is a vast and heterogeneous family and among its many genera, only a few have coloured berries instead of capsules. Earlier these genera (*Asparagus*, *Majanthemum*, *Polygonatum*, *Convallaria*, and *Paris*) formed one family, *Convallariaceae*, but owing to many fundamental differences between them they are now all grouped in the *Liliaceae* family and, according to Krause,¹ in the subfamily *Asparagordeae*.

At least one of the five genera mentioned above differs as regards its pigment content. The three species of *Polygonatum* occurring in Sweden have berries generally described as dark blue. However this impression is due to the occurrence of bloom on the berries and their real colour is dark green. As far as we have found these berries do not contain any extractable pigments. The berries of *Asparagus officinalis* and *Convallaria majalis* are, at least partly, pigmented by carotenoids. The former contain capsanthin² and physalein³ (zeaxanthin dipalmitate) whereas the latter contain α -, β -, and γ -carotene, lutein and lycopene. If they also contain anthocyanins will be investigated later on.

The berries of *Paris quadrifolia* and of *Majanthemum bifolium* owe their colour to the presence of anthocyanins. A preliminary investigation of the berries of *P. quadrifolia* has revealed the presence of at least five different anthocyanins. A fuller report of the anthocyanin content will appear later.

The unripe berries of *M. bifolium* are colourless but as they ripen they turn red, sometimes in a very short time. Since the red colour is due to the presence of anthocyanins the unripe berries ought to contain some precursor the nature of which will be further investigated.

Three anthocyanins have been isolated from the ripe berries. Cyanidin-3-monoglucoside and cyanidin-3-rhamnosylglucoside were identified from spectral data, co-chromatography with authentic specimens, and identification of their hydrolysis