Proceedings of the Iowa Academy of Science

Volume 75 | Annual Issue Article 13

1968

# Anthocyanin Pigments in the Genus Lobelia: I. Pigments in Lobelia Cardinalis L. and L. Siphilitica L.

S. Lane Wilson
*Drake University*

# Anthocyanin Pigments in the Genus Lobelia: I. Pigments in *Lobelia Cardinalis L.* and *L. Siphilitica L.*[1]

S. Lane Wilson[2]

*Abstract.* The corollas of *L. cardinalis* and *L. siphilitica* were extracted in 1 percent HCl in methanol for 24 hours. This extract (10 ml. portions) was streaked on 3 mm. Whatman filterpaper. The filterpaper was placed in a large chromatographic tank in a solvent system of n-butanol: acetic acid:water (4:1:5 v/v). After separation the bands were cut out and the pigment from each band eluted in a solution of 0.01 percent HCl in methanol. These solutions were then reduced in volume under vacuum and the absorption spectrum of each pigment determined. Aliquots of the crude extract was also spotted on Whatman No. 1 filterpaper and the Rf values of the pigments determined in four different solvent systems. From these data, the main pigment found in *L. cardinalis* is pelargonidin-3-monoglucoside, and the main pigment in *L. siphilitica* is a delphinidin-3,5-diglucoside.

Interest in pigmentation of flowers has been greatly stimulated by the development of a technique for identification using only small amounts of material. With filterpaper chromatography, it is now possible to isolate quantities of pigments suitable for complete identification of any pigment. This kind of information is of great value in helping answer questions about the relationship of plants within a genus. Brehm and Ownbey (1965) used this method successfully in studying the relationship amongst species of the *Tragopogon* complex in the Compositae.

There are some 15 species of *Lobelia* common to the eastern half of the United States. Conard (1958) lists six species native to Iowa. Of these, *L. cardinalis* L. has a scarlet corolla, all others have blue, or blue and white corollas. Because of one species having a red pigment, it seemed interesting to examine the pigments present in members of the genus *Lobelia.*

## Methods

Flowers of *L. cardinalis* and *L. siphilitica* L. were collected from sites in Polk County, Iowa, in the early fall of 1967. The corollas were removed and extracted in a 1 percent HCl in methanol. This solution was poured off and a second aliquot of extracting solution used. These were combined and stored at 5° C. until used. The blue pigment of *L. siphilitica* seemed to be somewhat unstable at room temperature in light. These methanolic solutions were used to spot chromatograms, using Whatman No. 1 filterpaper. The descending chromatographic technique was used for separating pigments using

[1]Contributions from the Biology Department, Drake University, No. 25.
[2]Biology Department, Drake University, Des Moines, Iowa 50311.

four different solvent systems. These are listed in Table 1. The n-butanol: acetic acid:water system (N-BuOH:$H_2O$ 4:1:5 v/v), hereafter referred to as B.A.W. is a very commonly used system and pigment Rf values are usually reported in this system (Harborne, 1958a) A second system is one using equal volumes of n-BuOH and 2N NCl. Both of these systems using butanol should age 24 hours before they are used (Harborne, 1958a). The Rf values obtained and the solvent systems used are summarized in Table 1.

**Table 1**

Rf Values of Pigments of *L. cardinalis* and *L. siphilitica*

| Plant | Solvent System | Spot Number 1 | 2 | 3 |
|---|---|---|---|---|
| L. cardinalis | B.A.W.[a] | 0.10 | 0.19 | 0.33 |
| | BuOH:2nHCl[a] | 0.13 | 0.26 | 0.50 |
| | 10% HAc:$H_2O$ | 0.33 | 0.42 | 0.49 |
| | 30% HAc:$H_2O$ | 0.70 | 0.90 | 0.94 |
| L. siphilitica | B.A.W.[a] | 0.03 | | |
| | BuOH:2nHCl | 0.11 | | |
| | 10% HAc:$H_2O$ | 0.58[b] | | |
| | 30% HAc:$H_2O$ | 0.86 | | |

[a]Use organic fraction after standing 24 hours.
[b]Streaking of spot.

In order to get quantities of pigment for spectroscopic examination, up to 10 ml. of crude methanolic extract was applied in a broad band on Whatman 3 mm. filterpaper, using B.A.W. and the chromatogram was allowed to run for 36 hours. The solvent eventually dripped off the end of the paper but the pigment bands were separated very cleanly. These bands were then cut out and eluted in 0.01 percent HCl in methanol. This methanolic solution proved to be the most satisfactory of a variety tried for elution of the pigment.

The pigment solutions were then reduced in volume to a small amount under vacuum at a temperature of less than 40° C. An absorption spectrum of the concentrated pigment was then obtained in a Beckmann DB Spectrophotometer and the maxima determined from the curves obtained from a recorded attached to the spectrophotometer.

**Table 2**

Spectral Maxima of Anthocyanin Pigments of *Lobelia*[a]

| Plant | Spot Number | Spectrum Maximum (M) | | | | | |
|---|---|---|---|---|---|---|---|
| L. cardinalis | 1 | 269 | | 313 | | 518 | 552[s] |
| | 2 | 269 | 285[s] | 330 | 425 | 508 | 552[s] |
| | 3 | 269 | 285 | 330 | 425 | 508 | 552[s] |
| L. siphilitica | 1 | 269 | 280[s] | 310 | | 534 | 552[s] |

[a]0.01 percent 1 HCl in methanol.
[s]shoulder.

The values obtained are presented in Table 2.

The sugar or sugars making up the anthocyanin pigment were determined according to Chandler and Harper (1961). The concentrated pigment was hydrolyzed by refluxing with 10 percent acetic acid or by treatment with potassium permanganate (0.1 N). The sugar present in the hydrolysate were identified chromatographically using the B.A.W. solvent and after separation spraying the paper with aniline hydrogen phthalate. The sugar is identified by comparison with a known sugar treated in the same manner. In all cases, the sugar identified with glucose.

## Discussion

The crude pigment of *L. cardinalis* separates into three distinct bands. The upper bands (spots 2 and 3) are the characteristic orange-red of the flower, and the main bulk of the pigment appears to be in band 2. Band 1, the lowest band in all solvent systems, has a reddish cast and it migrated much slower than the others in all the solvents used. Band 1 is present in a comparatively small amount, judging from the height of the maximum absorption peak in the absorption spectrum.

The main absorption of anthocyanin pigments in the visible is in the region of 500 - 550 m$\mu$. Bands 2 and 3 have very similar absorption maxima, with the main peak being at 508 m$\mu$. Thus, spots 2 and 3 have Pelargonidin as the aglycone. The main pigment of *L. cardinalis* is thus Pelargonidin-3-glucose. The absorption peaks agree very well with the absorption curve reported in Harborne (1958). The maximum at 508 m$\mu$ is similar to what was found in *M. cardinalis* by Pollock, Vickery, and Wilson (1967). The presence of a peak in the 420-450 region indicates that there is no glycosidic linkage at the 5-OH position (Harborne, 1958). When a few drops of $AlCl_3$ were added, there was no evident shift in the absorption maximum from 508 m$\mu$ and this is what would be expected in pigments of the pelargonidin type where there is only one hydroxyl group in the B ring in the 4′ position.

The nature of spot 3 is not yet known. As the absorption spectrum is symmetrical in the region of 400 - 600 m$\mu$ (there is no peak in the 420-450 region) the pigment is a 3,5-diglycoside and its slow movement in the B.A.W. would indicate that there may be several glycosidic moieities. This pigment may be acylated, as the high absorption peak in the 330 m$\mu$ region indicates the presence of a cinnamic acid derivitive (Jurd, in Geissman, 1962).

The solution of pigment from *L. siphilitica* is much less stable than that of *L. cardinalis*. It is more easily hydrolyzed in 10 percent acetic acid, and the streaking of the pigment spot on the filterpaper also bears this out. The behavior of spot 1 of *L. siphilitica* in the solvents, its Rf value, the absorption maximum at 534 m$\mu$, and also the lack of a peak in absorption in the 400-450 region indicates that

the pigment aglycone is Delphinidin, and the pigment itself is 3,5-diglucose-delphinidin. The presence of a high absorption peak in the region of 310 m$\mu$ (and the unstability of the pigment) is indicative of another acylated anthocyanin. According to Jurd (in Geissman, 1962) this may well indicate the presence of p-Coumaric Acid.

When $AlCl_3$ was added to the purified pigment of *L. siphilitica* the absorption maximum shifted from 534 m$\mu$ to 550 m$\mu$, and this should occur as the aglycone delphinidin has hydroxyl groups in the 3′, 4′ and 5′ positions in the B ring.

Harborne (1958b) has suggested that the pelargonidin pigment is a characteristic of the more primitive members, and it is interesting that only in *L. cardinalis* is this pigment found. There also is no indication of acylation in this pigment. Thus, *L. cardinalis* might tentatively represent the most primitve member in this genus.

## Bibliography

Conard, H. C. 1958. Plants of Iowa, Seventh Edition, Swift & Co., 90 pp.

Brehm, B. G., and M. Ownbey. 1965. Variation in chromatographic patterns in the Tragopogon dubius-pratensis-porrifolius complex (Compositae). Amer. Jour. Bot. 52:811-818.

Chandler, B. V., and K. A. Harper. 1961. Identification of saccharides in anthocyanins and other flavonoids. Aust. Jour. Chem. 14:586-595.

Harborne, J. B. 1958a. Spectral Methods for characterizing anthocyanins. Biochemical Jour. 70:22-28.

Harborne, J. B. 1958b. Chromatography of Anthocyanins. Jour. Chromat. 1: 473-488.

Jurd, Leonard. 1962. Spectral Properties of Flavonoid Compounds (pp. 107-155 in T. A. Geissman. The Chemistry of Flavonoid Compounds. Macmillan Co., New York, 666 pp.).

Pollock, H. G., R. K. Vickery, Jr., and K. G. Wilson. 1967. Flavonoid Pigments in *Mimulus cardinalis* and its related species. I Anthocyanins. Amer. Jour. Bot. 54:695-701.