Paper chromatography of anthocyanins in two species of *Aconitum* from the Tatry Mts.: *A. variegatum* (L.) Rchb., and *A. napellus* ssp. *skerisorae* (Gayer) Seitz.

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Abstract

The results of studies on phenolic compounds occurrence and variation in the two *Aconitum* species were reported in our previous paper (Szweykowski, Krzakowa, 1977a and b). A modified extraction method allowed us to get additional data on anthocyanin variation in flowers of the same species. In addition one albino plant of *A. variegatum* was also investigated in this respect.

MATERIAL AND METHODS

Flowers of both species were collected by M. K. in July and August 1972 in the Tatry Mts., from the following pure populations:

- A. variegatum* — West slope of Kobylarz Mountain by Droga Hawiarska trail (1400 m),
 - Kalatówki glade 1100 m,
 - Dolina Kościeliska Valley 1100 m (Kiry),
 - Dolina Chochołowska Valley 1150 m (Olejarnia),
 - Southern slope of Giewont Mountain 1800 m, albino form,
- A. napellus* ssp. *skerisorae*
 - Dolina Sucha Kasprowa Valley 1850 m,
 - Piekło Valley 1650 m.

400 mg of petals from each population have been used. The anthocyanins were extracted with 30 mls 2% HCl in methanol for 24 hrs at 4°C. Thereafter petals were washed with 5 mls of 2% HCl two times and extracts have been mixed. After decantation, a deep red solution

of anthocyanins was mixed with 40 ml portion of ethyl ether and left in refrigerator at 4°C for 1 hour. The mixture was centrifuged (Janetzky K-24 with refrigeration) at $10.000 \times g$ for 30 min. The precipitate was dissolved in 0.5 ml 6N HCl and hydrolyzed in 100°C by 15 min. After cooling 15 drops of n-amyl alcohol was added, shaken vigorously and left for 15 min. When two layers were clearly visible, upper layer was taken and 0.065 ml of it was applied on filter paper. Chromatograms were developed in two different ways:

1. One-dimensional descending chromatography (Whatman 3) with Formic solvent system (formic acid:conc. HCl:water = 5:2:3 v/v). Developing time about 4.5 hours,

2. Two-dimensional descending chromatography (Whatman 1). The first developing solvent was BuCl (Harborne 1967) i.e. BuOH:2N HCl = 2:2 v/v, the second solvent was Formic (see above). Developing time was about 10 hours.

The air dried chromatograms were viewed over both in visible and UV light and colour of spots recorded. They were characterised also according to their R_f values.

RESULTS

1. One-dimensional chromatography

Fifteen spots were detected in one-dimensional chromatograms (Fig. 1). Three of them (marked a, b, c) were purple-red in day light, the rest of them were visible only in UV light. Spots appearing in day light were common to both species, but, interestingly enough, they were absent in albino plant. Specific differences were observed in spots visible in UV light only: three of them (nos. 6, 8, 9) were found in flowers of *A. napellus* ssp. *skerisorae* only. Only one spot (no. 5) was observed exclusively in *A. variegatum*. The rest (spots nos. 1, 2, 3, 4, 7, 10, 11, and 12) were common to both species in question. Albino plant had typical for *A. variegatum* spot pattern excepting spot no. 11 which occurs in plants with normally coloured flowers belonging to both species. The characteristics of particular spots are to be found in Table 1.

2. Two-dimensional chromatography

Twenty-two spots were observed, the red ones visible in the day light amounted to six (nos. 1—6, Fig. 2). These six spots were common to both species. The rest 16 spots were visible in UV light only. Nine of them were common to both species, four spots occurred in *A. variegatum* only (nos. 9, 17, 19, 20) and three spots were characteristic for *A. napellus* ssp. *skerisorae* (nos. 10, 21, 22). The characteristic of particular spots are given in Table 2.

Fig. 1. One-dimensional chromatographic patterns of anthocyanins in *Aconitum napellus* ssp. *skerisorae* (ANS), *Aconitum variegatum* with normal (coloured) flowers (AV) and an albino plant of *Aconitum variegatum* (AAV). Contours in solid line — spots visible in UV light only, contours in broken lines — spots visible in day-light

DISCUSSION

Flowers of *A. variegatum* and *A. napellus* ssp. *skerisorae* differ markedly in colour. Those of *A. variegatum* are usually much paler and more bluish than very darkly violet (frequently fast black) flowers of *A. napellus* ssp. *skerisorae*. In spite of these conspicuous differences the anthocyanins that show colour in day-light are in both species the same. The differences in flower colour are caused either by mixtures of different quantities of the pigments or by a different anatomy of the perianth parts (petals). The differences in invisible (in the day-

Colours, R_f values and occurrence of spots in one-dimensional chromatograms. Colour key: b — brown, y — yellow, c — celadon, o — orange, p — purple, r — red 1 — light

[illegible]

Fig. 2. Two dimensional chromatographic patterns of anthocyanins. Characteristic spots of AV are dotted, characteristic spots for ANS are lined, the rest are spots common to both species

light) spots are distinct and consistent and will be used as markers of hybridity in mixed populations of both species (l.c.).

It is interesting to note the results of albino form investigations. The plant is morphologically typical *A. variegatum* excepting flower colour (white). The spot pattern is also typical for that species excepting six spots red in daylight and one small spot visible in UV light only — no. 11, Fig. 1. The absence of the daylight spots in the albino form and their invariable presence in the coloured flowers of the both species in question shows that these six different anthocyanin compounds are the main cause of flower colour.

Colours, R_f values, and occurrence of spots in two-dimensional chromatograms

Colour key: C — celadon, y — yellow, p — purple, v — violet, b — brown, r — red

[illegible]

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Chromatografia bibułowa antocjanów z dwóch tatrzańskich gatunków *Aconitum*: *A. variegatum* (L.) Rchb. i *A. napellus* ssp. *skerisorae* (Gayer) Seitz.

Streszczenie

Za pomocą jednokierunkowej i dwukierunkowej chromatografii bibułowej dokonano rozdziału związków antocjanowych u dwóch gatunków *Aconitum*: *A. variegatum* (L.) Rchb. i *A. napellus* ssp. *skerisorae* (Gayer) Seitz. Dla obu gatunków uzyskano plamy wspólne oraz charakterystyczne. Roślina albinotyczna charakteryzowała się brakiem czerwonych plam wizualnych, pozostałe plamy (z wyjątkiem plamy nr 11) były takie same, jak u *A. variegatum*.