The Isolation and Characterization of Tissue Extracts of *Erythronium americanum* and *Erythronium albidum*

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Abstract

Comparative thin layer silica gel chromatographic analysis of plant tissue extracts of *Erythronium americanum* Ker. and *Erythronium albidum* Nutt. revealed the presence of differentiating secondary compounds whose characterization through infrared spectrophotometry seems possible.

Introduction

Biochemical analysis of plant tissue extracts can provide a valuable basis for the confirmation or refutation of established classification schemes which attempt to represent the natural relationships among organisms (1, 12). Biochemical synthesis of organisms are determined by their DNA complement (14, 15) and the detection of chemical similarities among various taxonomic groups can be directly related to resemblances in DNA structure which may indicate a common ancestry. The plant products which are most useful for the establishment of DNA homology and organismic relatedness are the secondary compounds such as alkaloids, flavonoids, and terpenoids (3, 11, 16).

Erythronium americanum Ker. (yellow dog-tooth violet) and *Erythronium albidum* Nutt. (white dog-tooth violet) have been classified as two distinct species on the basis of flower color differences and morphological differences in the stigma (9). The purpose of this study was to gather evidence which would verify the classification of *Erythronium americanum* and *Erythronium albidum* as distinct species. Thus, plant extracts were analyzed to determine whether or not any biochemical difference exists between the two species and to attempt characterization of one or more differentiating secondary compounds.

Materials and Methods

Erythronium americanum and *Erythronium albidum* were collected while in flower from a wooded area near Muncie, Indiana. No roots were taken. The plant specimens which were collected were washed free of adhering soil and debris, dried in air, and placed between sheets of newspaper until the plant tissue became brittle from dehydration. The time required for drying was approximately 1 month. With no regard for size or age of the plant specimens, samples of *Erythronium americanum*

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and *Erythronium albidum* were placed in a desiccator charged with anhydrous calcium chloride for 48 hours.

To prepare the plant tissue extract for analysis, 0.08g of desiccated plant tissue, stem, leaves and flower parts of *Erythronium americanum* and *Erythronium albidum*, respectively, were placed into separate small vials (11). According to Fahselt and Ownby (8) no diagnostic compounds are found in roots or rhizomes and Brehm and Alston (7) have found general uniformity of compounds using different aerial plant parts taken from several different species. After pulverizing the plant tissue with a glass rod, 0.5 ml of extracting agent, methanol-concentrated hydrochloric acid (99:1 v/v) was added and the vial was sealed and placed in the dark at 20° C for 12 hours to extract secondary compounds (4, 7, 10, 11).

Silica gel plates, 9 cm x 40 cm, were prepared according to the method of Grant and Whetter (10). As a band, 50 μl of the respective plant tissue extracts of *Erythronium americanum* and *Erythronium albidum* were applied to the silica gel plates using a Hamilton microliter syringe, # 720 N. The extract spotted silica gel plates were developed by an ascending single pass using a methanol-chloroform (3:7 v/v) solvent (10, 13). The chromatographic tank was kept in a dark chamber at 20° C during the development of the chromatogram. To maintain consistency in the chromatogram development, silica gel chromatographic plates of *Erythronium americanum* and *Erythronium albidum* were developed simultaneously in the same tank. Only respective chromatogram pairs were compared for similarities and evaluation of R_f values.

After development, the chromatogram bands were visualized using a short wave length ultraviolet lamp (2, 10). R_f values were determined and Ektachrome 35 mm slides were made according to Grant and Whetter (10). Although the color slides clearly showed the differentiating bands, the low contrast attainable in black and white reproductions precludes their illustration here. A pale blue differentiating band, $R_f = 0.36$, on the chromatogram of *Erythronium americanum* was removed, extracted in ether, and isolated by flash evaporation of

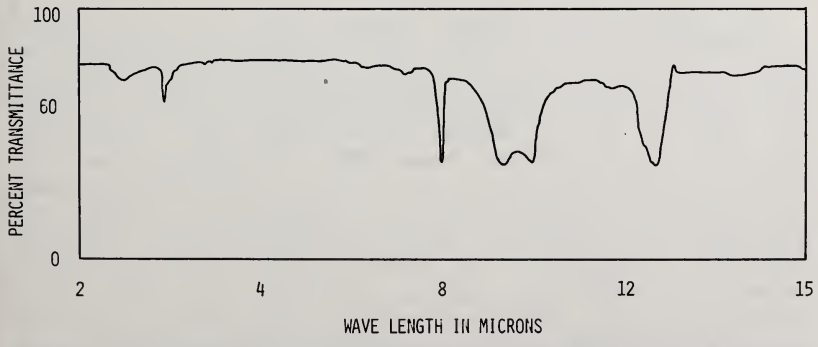

FIGURE 1. *IR-8 Spectrum of the Chromatographic band isolated from* Erythronium americanum.

the ether under a nitrogen atmosphere. The isolated chromatogram band was prepared as a KBr-pressed-disk for analysis with a Beckman model IR-8 infrared spectrophotometer (5, 6).

Results

Comparison of the IR-8 spectrum of the isolated chromatogram band with Sadtler standard spectra did not reveal the identity of the isolated material. However, the spectrum (Fig. 1) indicates the possible presence of an amide or amine group, phospho group, and a ring configuration which may be similar to dimethyl phosphoramidic acid; the spectrum may also be that of a mixture of substances characteristic of the isolated band.

As revealed by R_f value calculations and visual chromatogram comparisons, several of the ultraviolet chromatogram bands of *Erythronium americanum* and *Erythronium albidum* are similar; however, various bands appear to be distinctive for each species (Table 1).

TABLE 1. *R_f values of ultraviolet light visibilized compounds extracted from two species of* Erythronium.

Erythronium americanum	Erythronium albidum
0.89 (red)	0.89 (red)
0.83 (blue)	0.83 (blue)
0.49 (pink)	0.54 (faint pink)
0.36 (pale blue)[1]	0.47 (pink)
0.30 (pale red)	0.26 (brown-red)
0.25 (yellow)	0.18 (yellow)
0.22 (yellow)	0.14 (blue)
0.19 (orange-yellow)	0.04 (violet)
0.17 (pale violet)	
0.14 (blue)	
0.06 (pale blue)	

[1] Chromatographic band isolated for IR-8 spectrophotometric analysis

Discussion

The separation and detection procedure which was used to study the secondary compounds of *Erythronium americanum* and *Erythronium albidum* is useful as a method for the comparative study of taxonomic groups and the characterization of differentiating metabolic products. The results of this preliminary study indicate that *Erythronium americanum* and *Erythronium albidum* differ considerably in their synthesis of secondary compounds. The findings based upon the analysis of the diagnostic compounds supports the classification of *Erythronium americanum* and *Erythronium albidum* as distinct species. Continued research in the separation and detection of secondary compounds is necessary to permit their absolute identification. Continued studies should focus on the blue bands common to both species, R_f values of 0.83 and 0.14; the yellow bands characterizing *Erythronium americanum*, R_f values of 0.19, 0.22, and 0.25; and the brown-red band peculiar to

Erythronium albidum, R_f value of 0.26. The application of nuclear magnetic resonance spectroscopy and or mass spectrometry may be helpful as a method to achieve greater plant product characterization.

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