The Effects of Preservation Time on Chromatographic Patterns in *Equisetum hiemale*

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Abstract

A study was made to determine if the age of a herbarium specimen of *Equisetum hiemale* L. had any effect on the chromatographic patterns. Specimens of *E. hiemale* were analyzed employing descending paper chromatography. The amino acid and phenolic patterns were of significant value in determining if the chromatographic patterns varied as the herbarium specimen aged. The fluorescent phenolic compounds of *E. hiemale* were analyzed by placing the chromatograms under long wave ultraviolet light. The free amino acids were detected by spraying the chromatograms with ninhydrin.

The chromatographic patterns showed that the age of the oldest herbarium specimen investigated had no effect on its patterns.

The purpose of this study was to determine if variations occur in the free-amino acid and phenolic chromatographic patterns of *Equisetum hiemale* L. samples taken from herbarium specimens as compared with those obtained from fresh material.

Since the technique of paper chromatography appeared, it has contributed significantly to systematic studies (3). The works of Alston and Irwin, Heywood, Smith and Levin, and others have firmly established the study of "secondary constituents" as a valid taxonomic method (1, 4, 7).

Analyses can be carried out on fresh plants from the field, or greenhouse transplants. In the absence of fresh material, the investigator may have to resort to using herbarium specimens. Since some taxa are exceedingly rare, and some are difficult to grow under greenhouse conditions, it is significant to know whether detectable differences exist between freshly-dried specimens and ones which have been dried for long periods of time. Some workers have stated that no significant differences exist (7). Others suggest that analyses are best carried out on fresh materials (6).

Methods and Materials

Herbarium specimens of *Equisetum hiemale* were obtained from the Missouri Botanical Garden Herbarium and the Field Museum of Natural History. Those materials had been preserved for periods up to 144 years. Fresh material was collected in Richmond, Indiana.

Comparisons of free-amino acid and phenolic composition were made by means of 1-dimensional paper chromatography. Extracts were prepared by powdering dried internodes and soaking the materials in methanol:water:hydrochloric acid (7.9 : 2 :0.1) for 24 hours at room temperature. One hundred microliters of each sample were applied to 23 × 57 cm sheets of Whatman #1 paper using the spot method. The chromatograms were run using n-butanol:acetic acid: water (12 : 3 : 5) as the solvent. Following air drying at room

temperature, the chromatograms were viewed under long wave ultraviolet light in the presence of ammonia vapor. Those spots which fluoresced but did not show a ninhydrin reaction were taken to represent phenolics (1, 2, 5). Subsequently the chromatograms were sprayed with ninhydrin for the detection of free-amino acids and related substances (1, 2, 4, 5, 7).

Results

The chromatographic results of horsetails which had been preserved for periods of time ranging from four to 144 years were compared chromatographically with freshly collected materials (Table 1).

TABLE 1. Equisetum hiemale *used in chromatography and their sources.*

Date Collected	Locality	Collector	Reference Number
Sept. 1970-Feb. 1971	Richmond, Indiana	L. C. Brown	P1, A1
Jun. 1966	Cooke City, Montana	Robert G. Stolze	P2
Nov. 1958	Lafayette, Louisiana	W. D. Reese	P3
Jul. 1955	Iantha, Missouri	E. J. Palmer	A4
June 1946	Cumberland, Rhode Island	E. J. Palmer	P5, A5
Oct. 1935	Greenwich Pt. Pennsylvania	John M. Fogg, Jr.	P6
Jul. 1926	Spearfish, South Dakota	Herman E. Hayward	P7
Aug. 1911	Hull, Canada	John Macoun	P8, A8
Jun. 1902	Amesbury, Mass.	Walter Deane	P9
Oct. 1890	Flor de Maria, State of Mexico	C. G. Pringle	P10
May 1880	Preble, New York	J. H. Schueffer	P11, A11
1871	Koenigberg, Germany		P12
1862	Hock Creek, Georgia	Dr. Arthur Schott	P13
May 1858	Easton, Pa.	T. Green	P14
April 1847	Mexico	G. Gregg	P15
1826	Frankfort, Germany	G. Engelmann	P16

The ultraviolet patterns viewed in the presence of ammonia vapor were consistently characterized by the presence of nine compounds. These compounds are designated by number in Figure 1. Compound 2 was present in all except P13. Compound 1 was detected in all samples except P11, P12, and P16. Compounds 5 and 7 existed in all samples except P9 (Fig. 1).

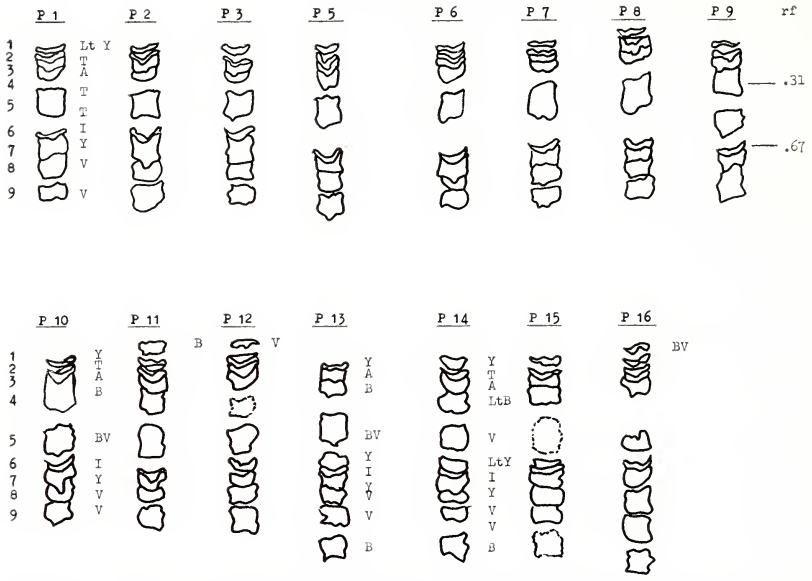

FIGURE 1. *Patterns of* Equisetum hiemale *in ultraviolet light (A=avocado, B=blue, BV=blue violet, I=ivory, LtB=light blue, LtY=light yellow, T=turquoise, V=violet, Y=yellow).*

Chromatograms sprayed with ninhydrin showed remarkable consistency in free-amino acid composition. All samples revealed nine compounds except A4 in which spot # 1 was not observed (Fig. 2).

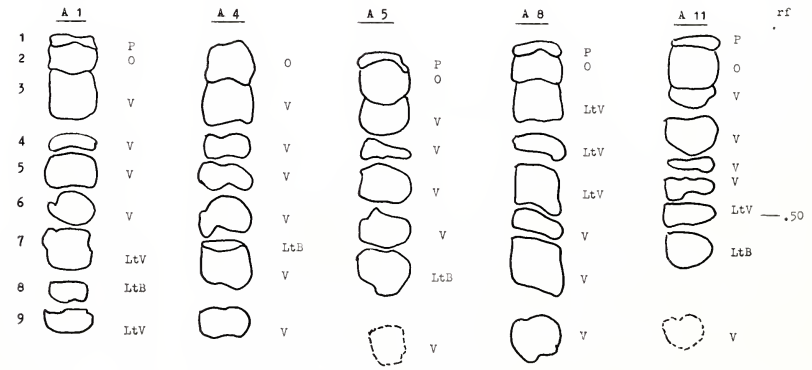

FIGURE 2. *Amino acid patterns of* Equisteum hiemale *(LtB=light blue, LtV=light violet, O=orange, P=pink).*

Discussion and Summary

The results of this study indicate that the place and aging of herbarium specimens have little effect on the free-amino acid and phenolic composition in *Equisetum hiemale.*

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