Soluble Proteins of *Dieffenbachia*

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Abstract

The soluble proteins in the stalk of *Dieffenbachia picta* 'Rudolph Roehrs' were found to be separable into 3 fractions using a DEAE cellulose column; one of which comprised the major portion of the proteins. Separation of the soluble proteins using a Sephadex G-100 column indicated the presence of 3 major protein components with the molecular weights (1) above 150,000, (2) 4,400 ± 300, and (3) between 1,000 and 4,400. The electrophoretic patterns of the soluble proteins in the stalk of 5 different species and varieties of *Dieffenbachia* show differences among themselves in any particular time of the year and also exhibit distinctive changes corresponding to the season of the year. Toxicity of the stalk was mainly due to the water insoluble matter in the stalk, especially calcium oxalate.

Introduction

Many of the numerous species and varieties of *Dieffenbachia* or dumb cane are commonly found in homes and public places in spite of the fact that ingestion of the plant can cause severe corrosive burns of the mouth, oropharynx, and stomach. The toxic components in the plant have not been fully characterized. The toxicity has been attributed to calcium oxalate and some unidentified proteinaceous matter by Occhioni and Rizzini (1958) and to a glycoside by others (Locket 1965).

This paper deals with investigations on a few species and varieties of *Dieffenbachia* of commerce, with special emphasis on the soluble proteins in the stalk, their molecular weights, and electrophoretic patterns.

Experimental

Plant Materials

The species and varieties of the plant used for this study included: *Dieffenbachia amoena*, *aurantica*, *picta*, *picta* 'Arvida', and *picta* 'Rudolph Roehrs'. These were obtained from the greenhouse of the Horticulture Department of the University of Kentucky, Lexington, Kentucky.

Column Chromatography of Proteins

Separation of soluble proteins by column chromatography of the stalk of D. picta *'Rudolph Roehrs'.*—The stalk (20 g) was ground with 10 ml 0.1 M phosphate buffer, pH 7.0, filtered, and 10 ml of the solution was chromatographed. Apart from trial separations on Corning controlled pore glass (CPG-10) columns, the separation was carried out on a column 35 cm long (ID 2.5 cm) containing DEAE cellulose. The column was eluted with 0.1 M phosphate buffer, pH 7.0, and fractions (10 ml) collected every 15 min were monitored for absorptions at 260 and 280 nm. The concentration of proteins in each fraction was calculated using the empirical formula which includes the correction for the interference of nucleotides and nucleosides showing absorption at the same wavelengths as proteins (Colowick and Kaplan 1957).

Molecular weight determination of soluble proteins in the stalk of D. picta *'Rudolph Roehrs'.*—The stalk of the plant was ground well, the juice pressed out, and centrifuged. The centrifugate was diluted twice with 0.1 M NaCl solution and 0.5 ml of the diluted juice was chromatographed on a

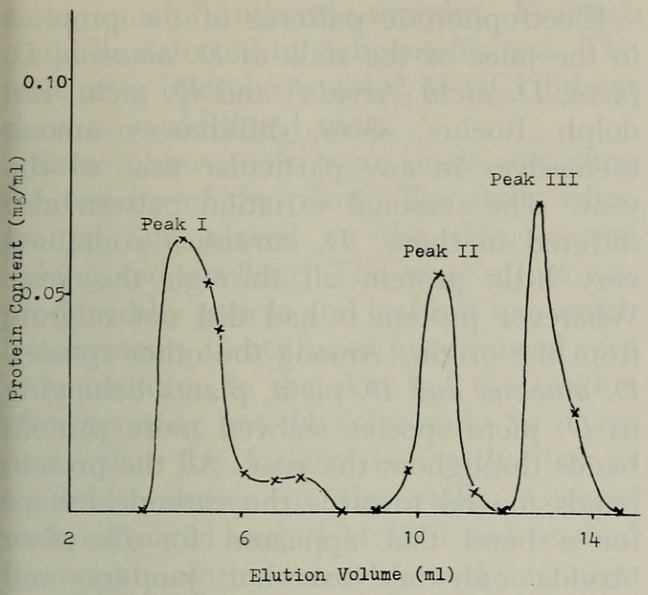

FIG. 1. Separation of soluble proteins in the stalk of *Dieffenbachia picta* 'Rudolph Roehrs' on Sephadex G-100 column.

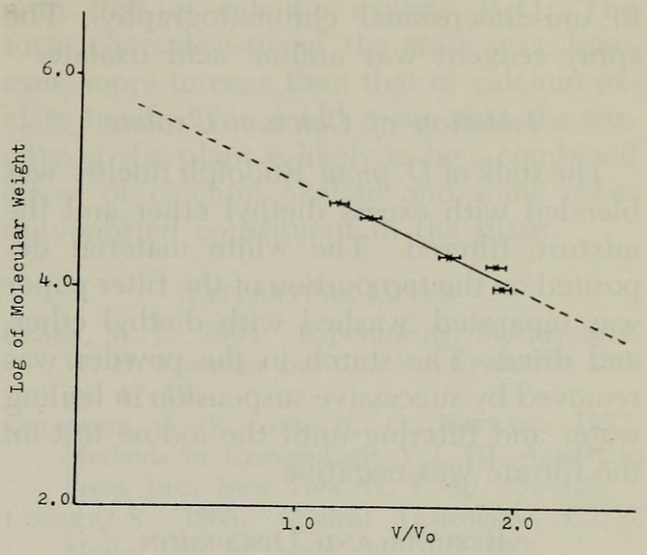

FIG. 2. Standard graph for molecular weight determination constructed using proteins of known molecular weight (see experimental part) on Sephadex G-100 column.

Sephadex column K9/30, 19 cm (G-100 and G-25). The column was eluted with 0.1 M NaCl solution at the rate of 0.2 ml/min. Fractions of 0.75 ml collected were monitored for UV absorption, and concentration of protein in them calculated. The column was calibrated with samples of known MW markers (e.g., cytochrome-c, myoglobin, chymotrypsinogen, ovalbumin, and albumin). The soluble proteins were separated into 3 main fractions, the molecular weights of which were determined by reference to a plot of the logarithm of molecular weight vs. the ratio of elution volume to the void volume of the column (V/Vo). The void volume, Vo, was determined using apoferritin which has a molecular weight higher than the upper fractionation limit of both Sephadex G-25 and G-100.

Electrophoresis of Soluble Proteins

Apart from some trials on juice pressed out from the leaves and petioles of the plant, most electrophoretic separations were carried out on the juice of the stalk of 5 species and varieties of *Dieffenbachia*. Electrophoresis was done in a Deluxe Electrophoresis Chamber (Gelman Instrument Company, Model No. 51170-1) using 2.5 × 30.5-cm cellulose polyacetate strips (Sepraphore III, Gelman Instrument Company) as support strips. The buffer used was 0.05 M Tris-Barbital sodium barbital buffer, pH 8.8 (Gelman High-Resolution Buffer). After electrophoresis the strips were stained by Ponceau S stain in 5 percent aqueous trichloracetic acid for 5–10 min and destained by successive washing in 5 percent acetic acid. The electrophoretic patterns were obtained in January, March, May, August, September, and November.

Paper Chromatography

Paper chromatography of amino acids and sugars was carried out on Whatman No. 1 and S & S 507 papers. For amino acids, the solvent used was prepared by mixing 500 ml freshly shaken mixture of equal volumes of *n*-butyl alcohol and water, with 60 ml glacial acetic acid. The upper butyl alcohol layer was used as the solvent, and 1 percent aqueous ninhydrin solution containing traces of pyridine was used as the spray reagent. For 2-dimensional chromatography of sugars, isopropyl alcohol: acetic acid:water (3:1:1) and phenol saturated with water (Clark 1964) were used as solvents. Ethyl acetate:acetic acid: water (3:1:3) (Parkinson 1954) was used

in uni-dimensional chromatography. The spray reagent was aniline acid oxalate.

Isolation of Calcium Oxalate

The stalk of *D. picta* 'Rudolph Roehrs' was blended with excess diethyl ether and the mixture filtered. The white material deposited on the top portion of the filter paper was separated, washed with diethyl ether, and dried. The starch in the powder was removed by successive suspension in boiling water and filtering until the iodine test on the filtrate was negative.

RESULTS AND DISCUSSION

Application of juice from various plant parts to the skin (forearm) of a test subject showed that the content of toxic component in the stalk of the 5 species and varieties of *Dieffenbachia* was considerably higher than that in the leaves and petioles. Separation of soluble proteins in the stalk of *D. picta* 'Rudolph Roehrs' on DEAE cellulose column showed 1 major protein fraction constituting most of the soluble proteins together with 2 minor fractions. But separation of the same on a Sephadex G-100 showed 3 major protein fractions as shown in Fig. 1. The molecular weights of the 3 fractions corresponding to peaks I, II, and III were above 150,000, 4,400 ± 300, and between 4,400 and 1,000, respectively. Peak I had an elution volume equal to the void volume of the Sephadex G-100 column used, showing that the molecular weight of this fraction was above 150,000, which is the upper fractionation limit for proteins on Sephadex G-100. Peak II corresponded to a molecular weight of 4,400 ± 300 as read from the calibration graph using the molecular weight markers. Peak III evidently had a molecular weight below 4,400. This fraction had an elution volume which corresponded to a molecular weight well inside the fractionation limit for proteins on the Sephadex G-25 tried. The fractionation limit for proteins on Sephadex G-25 columns is 5,000–1,000. Therefore, Peak III corresponded to a protein fraction with a molecular weight between 4,400 and 1,000.

Electrophoretic patterns of the proteins in the juice of the stalk of *D. amoena*, *D. picta*, *D. picta* 'Arvida', and *D. picta* 'Rudolph Roehrs' show differences among themselves in any particular time of the year. The seasonal variation pattern also differed in them. *D. aurantica* contained very little protein all through the year. Whatever protein it had did not migrate from the origin. Among the other species, *D. amoena* and *D. picta*, plants belonging to *D. picta* species showed more protein bands throughout the year. All the protein bands moved towards the cathode except for a band that appeared for *D. picta* 'Arvida' only in November, January, and March, which moved towards the anode. A maximum of 5 protein bands was observed in September and a minimum of 2 in January for *D. picta* 'Rudolph Roehrs'. The same trend was noticed for *D. picta* 'Arvida'. In September, the electrophoretic patterns of these 2 were very similar. *D. picta* showed 4 protein bands throughout the period under investigation except in September, when it had 5 clearly separated bands. *D. amoena* did not show any regular trends. It had a maximum of 5 protein bands in March, and 2 or 3 bands the rest of the year.

In addition to the electrophoretic pattern of proteins, the amino acid content of the juice of the stalk was also determined for 1 year for each of the 5 species and varieties. The amino acid content was very similar in all of them, and, moreover, the pattern did not show any significant change during this period. The maximum amino acid concentration occurred in September and was minimal in March and May. No attempts were made to identify the 6 or 7 different amino acids present.

Separation and identification of sugars in the juice pressed out from the stalk of *D. picta* 'Rudolph Roehrs' was attempted by 1- and 2-dimensional chromatography. This study showed that disaccharides were not present in detectable amounts in the juice. The juice contained glucose or galactose or both, and fructose or mannose or both, as shown by comparison with chro-

matograms of authentic samples. Fructose could be detected by Seliwanoff's test. The juice after hydrolysis with 2 M HCl did not show any additional spots.

Some observations were made regarding the toxicity of the plant. The stalk, when ground and placed on the skin, caused itching and swelling. The same effect was produced by petiole and leaf but to a much lesser extent. Centrifuged juice caused very little irritation to the skin but the residue after pressing out the juice did. So it appears that the toxicity is mostly due to insoluble matter in the stalk. Ground stalk extracted with 4 M HCl and washed thoroughly with water showed no toxic effect. Therefore, the toxic component might be calcium oxalate which is a skin irritant. The infrared spectrum of the white powder isolated from the stalk matched with that of calcium oxalate $\cdot H_2O$. The toxic characteristic of the stalk was, however, more intense than that of calcium oxalate itself. This could mean that the toxicity of the plant is likely to be a combined effect of calcium oxalate and some other unidentified constituent in the plant.

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