

## *Allergens of Timothy Pollen*

A. L. MCNEIL AND G. H. STEWART

*Department of Chemistry, Gonzaga University  
Spokane, Washington*

THE LACK of pure allergens of natural origin has impeded attempts to establish standards for allergen sensitivity. Electrophoresis, precipitation methods, dialysis, ultracentrifugation, and chromatography have been used extensively (Loveless, et al., 1951; Newell, 1943; Abramson, et al., 1942; Abramson, 1947; Robbins, et al., 1959) in attempts at isolation of the active allergenic constituents of grass pollens with consistently poor results. The dearth of reports (Robbins, et al., 1959; Bookman and Wax, 1954) on the use of ion-exchange materials in this field led the authors to investigate the use of DEAE cellulose for the fractionation of extracts of timothy pollen. Further characterization of chromatographically homogeneous fractions substantiates the suitability of this method.

### *Experimental*

#### **Preparation of Extract**

Timothy pollen (0.5 g.) was extracted with two 10 ml. aliquots of ether and air dried. The defatted pollen was slurried with 5 ml. of 0.04N phosphate-citrate buffer (pH 8.0) and shaken mechanically for a period of 16 hours at room temperature. The supernatant extract was decanted after a 30-minute centrifugation, and the residue was mixed and recentrifuged with sufficient buffer to make the final extract volume 5 ml. The pH of the extract was adjusted to pH 8 with 0.04N NaOH and diluted with distilled water to 10 volumes (final volume approximately 80 ml.). The final extract was 0.004N in Na ion.

#### **Column Fractionation**

The columns were 0.9 x 16 cm. (compact) and contained 2.2 g. of DEAE cellulose. The sample was allowed to flow on to the column at a rate of 100 ml. per hour and was followed by buffer solution of pH 8.0 and 0.004N in Na ion. Ten ml. samples were collected on an automatic fraction collector, and absorbance was read at 280  $m\mu$  on a Beckman DU. The buffer elution was continued until all visibly chromatographing bands were eluted and the fractions had an absorbance less than 0.025. The collection of chromatographable material in this manner gave fractions (I, II, and III in Figure 1), which were relatively salt-free, obviating the need for dialysis.

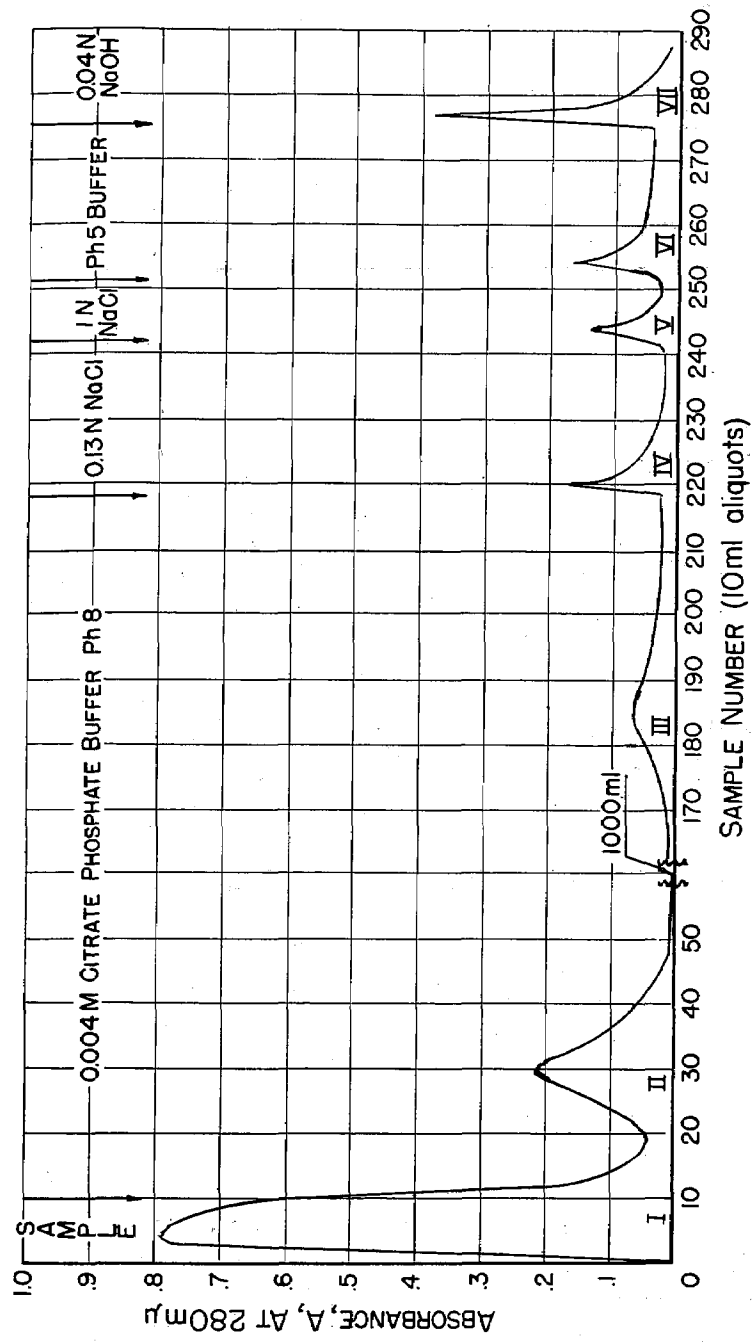


Figure 1. Absorbance at 280 mμ of samples eluted from DEAE column.

Salt gradients (NaCl in pH 8 buffer) of varying slope revealed minor components, the largest being eluted at a salt concentration of 0.13N. It was decided to combine the minor constituents into two fractions by eluting with 0.13N and 1.0 N NaCl solutions, respectively. An acid gradient (0.02M citric acid against 0.02M buffer) showed only one distinct fraction. The diffuseness of this fraction on gradient elution was avoided by eluting with pH 5 buffer. A final fraction was gained with 0.04N NaOH. The elution diagram for the final protocol is given in Figure 1.

The elution reported in Figure 1 was performed in a cold room at 4° C.

Use of a column 3.5 x 16 cm. allowed fractionation of the extract from 10 g. of pollen with no apparent loss in resolution.

#### Characterization

Fractions were tested for allergen activity by skin tests on individuals sensitive to whole extract, and by the Oudin precipitin test and gel diffusion against rabbit antisera. Fractions were observed for pigmentation, and the presence of carbohydrates was determined by the orcinol reaction. Homogeneity was tested by paper chromatography, paper electrophoresis and gel diffusion.

#### Amino Acids

The amino acids of Fraction 2 were determined by the method of Stein and Moore (1958).

#### *Results*

The characteristics of the fractions displayed in Figure 1 are summarized in Table 1 below.

The apparent homogeneity and the ease of preparation of Fractions II and III fit them as suitable allergens for studies relating structure to allergenicity. Preliminary to these studies we have characterized Fraction II in terms of its amino acid composition (Table 2). On the basis of this assay the minimum molecular weight of the compound is 6433.

#### *Discussion*

The use of DEAE cellulose chromatography has led to the isolation of two allergen fractions which appear to be homogeneous compounds. These fractions are allergenic and are readily isolated in reasonable quantities. The minimum molecular weight determined for Fraction II is of the order of magnitude expected on the basis of rate of diffusion in gel and the difficulty in establishing sharp boundaries with this material in a Tiseleus electrophoresis apparatus.

TABLE 1

CHARACTER		ALLERGENICITY				HOMOGENEITY	
<i>Fraction</i>	<i>Pigment</i>	<i>Orcinol</i>	<i>Skin Test</i>	<i>Oudin</i>	<i>Gel Diff.</i>	<i>Chromatography</i>	<i>Electrophoresis</i>
I	yellow	positive	positive	positive	2 lines	multiple zones	multiple band
II	yellow	negative	positive	positive	1 line	single zone	1 band
III	yellow	positive	positive	positive	1 line	single zone	1 band
IV	yellow	positive	positive	-----	-----	multiple zones	-----
V	yellow	positive	positive	-----	-----	multiple zones	-----
VI	yellow	negative	negative	-----	-----	multiple zones	-----
VII	brown	negative	negative	-----	-----	multiple zones	-----

TABLE 2

<i>Amino Acid</i>	$\mu M$	<i>Est. No./Molecule</i>
Aspartic	41.2	10
Threonine	18.6	5
Serine	17.8	4
Glutamic	37.6	9
Proline	12.8	3
Glycine	18.4	5
Alanine	36.0	9
Valine	11.4	3
Methionine	4.4	1
Isoleucine	5.0	1
Leucine	7.0	2
Tyrosine	4.0	1
Phenylalanine	3.9	1

While further fractionation of the remaining allergenic fractions appears to be feasible, our interests are concerned with the structure and functional groups of an allergenic compound, and the method presented here is deemed sufficient for the preparation of two such compounds in workable quantities.

The method discussed here, using DEAE cellulose, recommends itself as a preliminary fractionation method. The flexibility of the procedures allows a clean fractionation under extremely mild conditions. These fractions may be refractionated on the ion exchanger to improve the resolution prior to carrying out other fractionation or test procedures. These procedures are easily scaled up for large production, which is not true of many of the currently used methods.

### *Summary*

DEAE cellulose has been used to recover seven distinct fractions from an aqueous extract of timothy pollen. Two of these fractions appear to be homogeneous compounds, one of which is a polypeptide and the other a polypeptide-carbohydrate complex.

### *Acknowledgement*

The investigation was supported by a research grant E-1573 from the National Institute of Allergy and Infectious Diseases, Public Health Service.

### *Literature Cited*

- Abramson, H. A. 1947. Chemical, physical and immunological properties of electrophoretically purified pollen extracts. *Ann. Allergy*, 5:19.
- Abramson, H. A., D. H. Moore, and H. H. Gettner. 1942. Electrophoretic and ultracentrifugal analysis of hay fever producing component of ragweed pollen extract. *J. Phys. Chem.*, 46:192.
- Bookman, R., and H. Wax. 1954. Fractionation of ragweed antigens by the use of ion exchange resins. *J. Allergy*, 25:12.
- Loveless, M. H., M. A. Wright, and A. Ryan. 1951. Allergenic fractions of low ragweed pollen II. Some immunologic, electrophoretic and chemical characteristics of diffusates. *J. Allergy*, 22:120.
- Moore, S., D. H. Spackman, and W. H. Stein. 1958. Chromatography of amino acids on sulfonated polystyrene resins. *Anal. Chem.*, 30:1185.
- Newell, J. M. 1943. Electrophoretic studies of the chemical fractionation of pollen extracts. *J. Allergy*, 14:444.
- Robbins, K. C., R. A. Hecht, S. A. Taub, and J. Shields. 1959. Fractionation of short ragweed pollen extracts on diethylaminoethyl cellulose. *J. Immunol.*, 82:477.
- Wilson, M. W., and B. H. Pringle. 1954. Experimental studies of the agar plate precipitin test of Ouchterlong. *J. Immunol.*, 73:232.

---

### *Errata*

The following corrections should be made in the "Abstracts of Papers To Be Presented at 1961 Meeting of Northwest Scientific Association," published in the November, 1961, issue of *Northwest Science*.

p. 163. The departmental affiliation of Mario Rabinowitz should read *Department of Physics*.

p. 165. The authors of the paper "Influence of Soil Type on the Mycorrhizae of Douglas-Fir" should read: *Ernest Wright and Denis P. Lavender, Forest Research Laboratory, Oregon State University, Corvallis, Oregon*.