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QW 2. 169

Immunochemistry. Pergamon Press 1966. Vol. 3, pp. 91-100. Printed in Great Britain

ALLERGENS FROM COMMON RYE GRASS POLLEN (*LOLIUM PERENNE*)—I

CHEMICAL COMPOSITION AND STRUCTURE

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(Received 7 July 1965)

Abstract—The amino-acid compositions of two prominent rye pollen allergens of the so-called 'major group' (I-B and I-C), showed many striking similarities. Molecular weights of 27,000 and 26,700, respectively, were calculated from these analyses. Both tryptic and chymotryptic 'fingerprints' of allergen I-B were very similar to the corresponding fingerprints of I-C, but showed marked differences from those of a further allergenic fraction, II-B, of rye allergenic group II.

Peptides containing specific amino acids, including cystine, were also located on the fingerprints of the group I allergens. The above results provided further substantiation for the classification of rye grass pollen allergens into the groups of isoallergens suggested by previous physical and immunological data.

INTRODUCTION

IN TWO previous papers,^(1,2) the isolation procedure for, and the physical characterization of, highly skin-active rye pollen allergens were described in detail, and a preliminary account of certain chemical and immunochemical properties was given. The high degree of purity of these substances was demonstrated by the techniques of starch gel electrophoresis, ultracentrifugation, immunodiffusion and N-terminal amino-acid analysis. This paper deals mainly with the amino-acid compositions of the so-called 'major' allergens (group I) and with aspects of structural similarity between these molecules. Earlier investigations into the chemical structure of rye pollen allergens (Johnson and Thorne;⁽³⁾ Feinstein⁽⁴⁾) have been extended and results are compared with those of other workers⁽⁵⁻¹¹⁾ studying Timothy, ragweed and alder pollens.

Rye pollen allergens were divided into two groups of 'isoallergens', a 'major' (I) and a 'minor' (II) group, on the basis of immunological and physical properties.^(1,2) The properties of the isoallergens in a given group were very similar, but were markedly different from those of the other group. The presence of comparable groups of isoallergens in both Timothy and ragweed pollens has been suggested from preliminary studies of Timothy pollen,⁽²⁾ and from the investigations of King and his collaborators⁽⁸⁻¹⁰⁾ into short ragweed pollen.

The major groups of allergens (I) were found to be allergenically active in all pollen-sensitive individuals and were shown to elicit the formation of normal '7S' antibody (IgG)⁽¹²⁾ in the rabbit. Rabbit antisera against whole rye pollen extract produced a single precipitin line when they were allowed to diffuse against any purified group I allergen or a mixture of these allergens. A similar result has been

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† Much of the work presented here was carried out while one of us (D. G. M.) was a visitor at the Laboratory of Molecular Biology, Cambridge, England.

obtained for corresponding ragweed allergens in gel diffusion experiments against anti-ragweed sera.⁽⁹⁾ Antigenic identity was thus established between isoallergens of the major group I within a given pollen, although it seems unlikely, from the immunodiffusion experiments of Wodehouse,⁽¹³⁾ that a similar identity (or partial identity) exists between group I allergens of grass and ragweed pollens. The minor group II allergens of rye, Timothy and ragweed pollens were skin-active only in certain pollen-sensitive individuals and, apparently, did not readily evoke the formation of IgG antibody in the rabbit.^(1,2,6,8)

Individual group I isoallergens of both rye and ragweed pollens were physically indistinguishable except in starch gel electrophoresis at alkaline pH: it seems possible that the group II isoallergens may be similarly related. From ultracentrifugal data, molecular weights of the group I rye allergens were found to be 32–34,000, and corresponding molecular weights for the ragweed allergens were found to be about 38,000 from physical and amino acid analytical studies. Group II allergens of rye, ragweed and Timothy were found to possess molecular weights ranging from 9,000–11,000.^(1,2,8,14)

The results given below show that the two prominent rye pollen allergens, I-B and I-C (earlier referred to as α and β ⁽¹⁾), possess very similar amino acid compositions and give almost identical tryptic and chymotryptic peptide 'fingerprints'. This establishes the close structural similarity of these group I rye allergens.

EXPERIMENTAL

Materials

Defatted rye grass pollen was generously given by Dr F. H. Milner of the Bencard Allergy Unit.

All chemicals were of 'Analar' grade unless otherwise stated. Fluorodinitrobenzene (FDNB) was obtained from B.D.H. Ltd., and 1-dimethyl-amino-naphthalene-5-sulphonyl chloride (DNS-Cl or 'dansyl' chloride) was a product of Mann Biochemical Corp. Nitrogen-free sulphuric acid, used in the Kjeldahl nitrogen determination, was obtained from B.D.H. 98–100% formic acid was also a product of B.D.H.: only freshly-opened bottles were used for critical oxidation experiments.

The crystalline enzymes trypsin and chymotrypsin were obtained from Worthington Biochemical Corp.

Methods

1. *N-terminal amino acid determination.* The FDNB⁽¹⁵⁾ and 'dansyl'⁽¹⁶⁾ methods of end-group analysis were performed as previously described.⁽¹⁾

2. *Nitrogen estimation.* Quantitative estimation of the nitrogen content of the allergens was made by the micro-Kjeldahl method (Pregl and Roth⁽¹⁷⁾) using the catalyst mixture of Ma and Zuazaga,⁽¹⁸⁾ and also by a micro-Dumas procedure.* All freeze-dried, salt-free samples (3–4 mg), were carefully dried to constant weight at 105° and stored in sealed containers over P₂O₅ prior to weighing to an accuracy of 0.002 mg. All estimations were made in duplicate.

* This estimation was performed by the Micro-Analytical Section of the Department of Chemistry, Cambridge.

3. *Amino acid analysis.* I-C (α and β) were hydrolysed in constant-boiling HCl for 24 hr at 105° to minimize conversion to I-B. The hydrolysates were then analysed by the method of analysis of a solution containing guanido-propionitrile. Analysis was performed on a Model 120, using a solution for the ninhydrin analyses and calculations.

'Half-cystine' was previously oxidized and determined spectrophotometrically. The amide compound material (about 10% later, a control was run) showed the short colour number of amino acid values. It was not extrapolated or zero times (0).

The number of amino acids on the basis of both allergens is also since the present. The isoallergens since physical methods the results of p 4. *Enzymic analysis.* Allergenic fraction and trypsin or 1 : 100. The results of each digest electrophoresis

Two methods

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2nd 1

3. *Amino acid analysis.* Accurately-weighed, dry samples of allergens I-B and I-C (α and β) of about 2 mg were hydrolysed with 0.5 ml of freshly-distilled, constant-boiling HCl (5.7 N). Hydrolysis tubes were evacuated before sealing to minimize conversion of tyrosine to chlorotyrosine. Hydrolysis times of 12, 24, 48 and 96 hr at $105^\circ \pm 2^\circ$ were used for samples of I-B and two 24 hr periods for the hydrolysis of I-C. Hydrochloric acid was immediately removed under vacuum and the hydrolysates were dissolved in 0.42 ml of citrate buffer at pH 2.2 and 0.20 ml of a solution containing $0.1 \mu\text{mole}$ each of β -2-thienyl-DL-alanine and L-2-amino-guanido-propionic acid, serving as internal markers in subsequent analysis. Analysis was performed on a Beckman-Spinco Automatic Amino Acid Analyser (Model 120), modified for accelerated eluent flow, using 0.30 ml of the above solution for both long (50 cm) and short (10 cm) columns. Calibration of the ninhydrin solution was performed both before and after each series of analyses and averages of the correction factors were used in subsequent calculations.

'Half-cystine' was determined as cysteic acid on separate samples of allergens previously oxidized with performic acid at -10° (cf. Hirs⁽¹⁹⁾). Tryptophan was determined spectrophotometrically by the method of Goodwin and Morton.⁽²⁰⁾ The amide content of I-B was estimated by hydrolysis of a known weight of dry material (about 0.8 mg) with 2.85 N HCl for 3 hr at 100° . Both this sample and, later, a control sample (i.e. one which had not been hydrolysed) were applied to the short column. The ammonia contents of both samples were found and the number of amide groups was calculated from the difference between these two values. It was necessary to correct the molar proportions of certain amino acids by extrapolation of the values obtained at different hydrolysis times to either infinite or zero times (e.g. Tristram and Smith⁽²¹⁾).

The numbers of residues of individual amino acids per molecule were calculated on the basis of a histidine content of 3. Histidine was selected as the standard for both allergens since virtually no losses of this amino acid occur on hydrolysis, and also since the analytical figures showed that very few histidine residues were present. The integral value for the number of histidines was 3.0 or 4.0 in both allergens since these gave molecular weights approximating to those found by physical methods (Table 2). A histidine content of 3 was further suggested from the results of peptide fingerprinting (see below).

4. *Enzymic hydrolysis and 'fingerprinting' of peptides.* Samples of freeze-dried allergenic fractions (about 2 mg) were dissolved in 0.5 ml of 1% NH_4HCO_3 and trypsin or chymotrypsin was added to give enzyme to allergen ratios of 1:50 or 1:100. The samples were incubated for 16 hr at 30° and freeze-dried. One-fifth of each digest was examined for the presence of insoluble 'core' material by electrophoresis at pH 3.5 followed by 'chlorine staining'.⁽²²⁾

Two methods of fingerprinting were used:

(i)^(23,24) *1st dimension:* Electrophoresis (50 min at 3 kV) in 1.24 M pyridine-0.05 M acetic acid adjusted to pH 6.5.

2nd dimension: Electrophoresis (65 min at 3 kV) in 0.06 M pyridine-1.0 M acetic acid adjusted to pH 3.5.

(ii)⁽²⁵⁾ 1st dimension: Chromatography in *n*-BuOH : CH₃COOH : H₂O : pyridine (30 : 6 : 24 : 20)—18 hr at room temperature.

2nd dimension: Electrophoresis (65 min at 3 kV) in pyridine acetate buffer, pH 3.5 (as above).

Whatman No. 3 paper was used for all experiments. After running in the first dimension, paper strips containing the peptides were cut out, stitched to another sheet of paper, and run in the second dimension with the electric field direction at right angles to that of first. Peptides were revealed by ninhydrin-cadmium acetate staining,⁽²⁶⁾ chlorine staining,⁽²²⁾ or by use of staining reagents for specific amino acids. Modified Sakaguchi, Pauly and α -nitroso- β -naphthol methods were used to detect arginine, histidine and tyrosine, respectively.⁽²⁷⁾ Tyrosine and tryptophan were also weakly detectable by the Pauly method.

5. *Applications of a 'diagonal fingerprinting procedure'*. The number of cystine residues per allergen molecule was determined by application of the method of Brown and Hartley⁽²⁸⁾ on tryptic digests of I-B in experiments at pH 2.0, 3.5 and 6.5. This involved an initial high voltage paper electrophoretic separation of peptides obtained from enzymic digests. Cystine-containing peptides were oxidized with performic acid in the vapour phase on the paper to yield pairs of cysteic-peptides. By electrophoresis in a second dimension, at the same pH, these cysteic-peptides were normally well resolved from other peptides on the diagonal line, mainly as a result of their newly acquired charge. However, at pH 2 other effects of the fission must be mainly responsible.

A vapour-phase reaction of hydroxylamine was also used in place of the performic oxidation in an attempt to detect peptides containing carbohydrate attached by glycosidic ester linkage (cf. Gottschalk *et al.*⁽²⁹⁾). After electrophoresis, at pH 6.5 or 3.5 (using the pyridine acetate buffer systems described in the previous section) of either a tryptic digest of allergen I-B, or a purified glycopeptide fraction obtained from this allergen,^(1,30) the separated peptides were exposed to hydroxylamine-water vapour at 37°: the treated peptides were then subjected to electrophoresis at the same pH in the second dimension. The resultant fingerprints were stained for either peptide^(22,26) or carbohydrate.⁽³¹⁾

RESULTS

1. Amino terminal analysis

Purified allergens I-B and I-C, and a mixed group I fraction, were all shown to possess isoleucine as the only unprotected N-terminal group, using both the FDNB and dansyl methods. The yield of Ile. was low in the dansyl method (approximately 10 per cent of the theoretical): a relatively low yield (not estimated) was also obtained using FDNB. This finding is discussed in the succeeding paper.⁽³⁰⁾ The N-terminal amino acids possessed by Sephadex fractions, eluted both before and after I-B or I-C in the purification procedure, were always absent from the highly purified allergenic fractions. Thus, alanine, possessed by fractions IS.1 and IS.1a; and Glu., Ala. and Asp., found in fractions IS.3 (see ref. 1 for nomenclature) were not observed in the purified major allergens. Allergen II-B possessed glutamic acid and alanine as the main N-terminal groups and aspartic acid and isoleucine as minor groups.

2. Amino acid analysis

The results of amino acid analysis are given in Table 1: Comparison of the results of the two experiments.

Amino acid	No. of residues
Lys.	26.2 ± 0.3
His.	3.0
NH ₃	11 ± 2
Arg.	5.9 ± 0.3
Asp.	26.2 ± 0.3
Thr.	17.1 ± 0.3
Ser.	12.0 ± 0.3
Glu.	20.1 ± 0.3
Pro.	12.9 ± 0.3
Gly.	28.1 ± 0.3
Ala.	17.9 ± 0.3
$\frac{1}{2}$ Cys. ^e	6.2 ± 0.3
Val.	13.7 ± 0.3
Met.	2.0 ± 0.3
Ile.	10.1 ± 0.3
Leu.	9.1 ± 0.3
Tyr.	8.5 ± 0.3
Phe.	8.0 ± 0.3
Try. ^f	5.9 ± 0.3
Amide ^g	4.5 ± 0.3
Totals ^h	—

^a 12-, 24-, 48-hr. oxidized sample hydrolyses or, in

^b 2 × 24-hr. hydrolyses

^c Compositio

^d For I-B and

6 histidines and

King *et al.*⁽⁹⁾

^e Estimated a

^f Spectropho

^g From 3-hr

^h Excluding

comparison. I given in Tab insufficient p experiments.

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2. Amino acid compositions

The results of amino acid analyses of rye allergens I-B and I-C are presented in Table 1: King's results for corresponding ragweed allergens are included for

TABLE 1. AMINO ACID COMPOSITIONS OF POLLEN ALLERGENS

Amino acid	Allergen I-B ^a			Allergen I-C ^b			Ragweed Allergens ^c	
	No. of residues ^d	No. of residues to nearest integer	g/100 g total a.a.	No. of residues ^d	No. of residues to nearest integer	g/100 g total a.a.	No. of residues to nearest integer	g/100 g total a.a.
Lys.	26.2 ± 0.2	26	12.8	25.7 ± 0.6	26	12.9	18	6.1
His.	3.0	3	1.6	3.0	3	1.6	6	2.2
NH ₃	11 ± 2	—	—	15 ± 2	—	—	31	—
Arg.	5.9 ± 0.2	6	3.5	6.0 ± 0.1	6	3.5	16	6.5
Asp.	26.2 ± 0.2	26	11.6	26.2 ± 0.7	26	11.8	49	15.3
Thr.	17.1 ± 0.1	17	6.8	16.2 ± 0.3	16	6.5	17	4.8
Ser.	12.0 ± 0.1	12	4.2	10.8 ± 0.2	11	3.9	26	6.4
Glu.	20.1 ± 0.2	20	9.9	20.4 ± 0.2	20	9.9	25	8.6
Pro.	12.9 ± 0.3	13	5.0	14.1 ± 0.1	14	5.5	15	4.1
Gly.	28.1 ± 0.3	28	7.1	27.3 ± 0.6	27	6.9	37	6.5
Ala.	17.9 ± 0.1	18	5.4	18.5 ± 0.5	18	5.4	31	6.5
½ Cys. ^e	6.2 ± 0.2	6	2.4	5.6 ± 0.4	6	2.4	7	2.0
Val.	13.7 ± 0.4	14	5.5	13.8 ± 0.4	14	5.5	24	6.6
Met.	2.0 ± 0.1	2	1.0	2.2 ± 0.2	2	1.0	7	2.5
Ile.	10.1 ± 0.1	10	4.4	9.9 ± 0.2	10	4.4	20	6.2
Leu.	9.1 ± 0.2	9	4.0	11.0 ± 0.2	11	4.9	21	6.5
Tyr.	8.5 ± 0.1	9	5.5	8.5 ± 0.3	9	5.5	4	1.7
Phe.	8.0 ± 0.2	8	4.4	8.0 ± 0.2	8	4.5	12	4.7
Try. ^f	5.9 ± 0.2	6	4.1	5.9 ± 0.5	6	4.2	6	2.7
Amide ^g	4.5 ± 0.5	4	0.2	—	—	—	—	—
Totals ^h	—	233	99.2	—	233	100.3	341	100.1

^a 12-, 24-, 48- and 96-hr hydrolyses of native allergen: 2 × 24 hr hydrolyses on performic-oxidized samples. Compositions quoted based on averages of values obtained from these hydrolyses or, in certain cases, 'extrapolated values'.⁽²¹⁾

^b 2 × 24-hr hydrolyses of native allergen: 1 × 24-hr hydrolysis of oxidized sample. Preliminary amino acid compositions therefore based on average values.

^c Composition based on average of analyses of allergens IV-B and IV-C from King *et al.*⁽⁹⁾

^d For I-B and I-C; based on 3 histidines per molecule. For ragweed allergens; based on 6 histidines and 7 methionines per molecule (equivalent to mol. wt. of 37,800 according to King *et al.*⁽⁹⁾)

^e Estimated as cysteic acid on oxidized samples.

^f Spectrophotometric analysis.

^g From 3-hr hydrolysis in 2.85 N HCl at 100°.

^h Excluding amide.

comparison. Data calculated from these analyses and nitrogen determinations are given in Table 2. The analysis for I-C was less accurate than for I-B since insufficient purified I-C was available to perform an analytical time-series of experiments.

It was possible to make an estimate of the percentage carbohydrate in the group I rye allergens since the proportion of nitrogen in both the whole allergen molecules and in the protein moieties (calculated from the analytical data in Table 1) were

known. Thus minimal molecular weights of both I-B and I-C could be estimated by chemical methods alone. Approximate molecular weights were also calculated during the spectrophotometric determination of tryptophan, assuming tyrosine contents of 9.0 (Table 2).

TABLE 2. NITROGEN CONTENTS AND DERIVED DATA

	I-B	I-C ^a	Ragweed ^b allergens
N recovered from analyser column (%)	98 ± 3	99 ± 2	98 ± 1
N in allergen protein (%)	14.0 ± 0.1	14.0 ± 0.1	—
N in whole molecule (Kjeldahl) (%)	13.2 ± 0.3	—	17.1 ± 0.3
N in whole molecule (Dumas) (%)	13.3	—	—
Carbohydrate (%)	5.4 ± 1.0	5.4 ± 1.0	0.5 ± 0.6 ^c
Mol. wt. of protein part	25,560	25,240	} 37,800
Mol. wt. of whole molecule	27,020	26,680	
Mol. wt. (estimated from try. + tyr. content)	26,300	27,000	—
$\bar{M}_d$	0.723	0.717	0.72

^a Preliminary data based on 24-hr hydrolyses only (Table 1).

^b See notes *c*, *d* under Table 1.

^c Calculated as arabinose (spectrophotometric method).

^d Cohn and Edsall's⁽³²⁾ method.

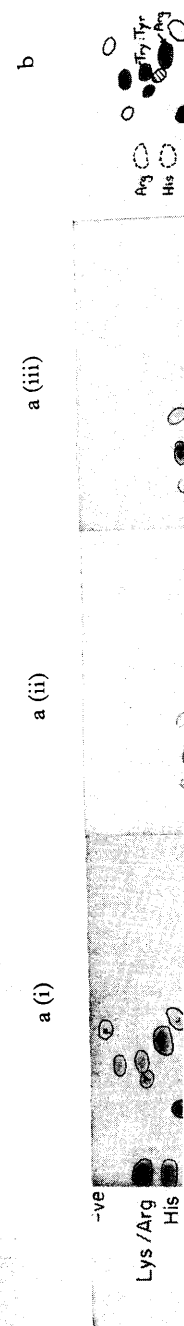
3. Fingerprinting methods

The best peptide separations were obtained using chromatography in the first dimension and electrophoresis in pyridine acetate buffer at pH 3.5 in the second, method (ii). Photographs of these ninhydrin-stained fingerprints are shown in Figs. 1 and 2. Figures 1(b) and 2(b) illustrate the apparent similarities and differences between peptides from enzymic digests of I-B and I-C. Peptides staining specifically for arginine, histidine, tyrosine and tryptophan⁽²⁷⁾ (and also cystine-containing peptides) are indicated on these figures, and Table 3 compares the numbers of such peptides with the numbers of the corresponding amino acids found by analysis.

In many cases it was possible to pick out partially overlapping peptides due to differences in the colours observed on reaction with ninhydrin-CdAc and also in the rates at which these colours developed (see Figs.). Since no insoluble 'core' material was detected in any of the digests, the fingerprints obtained were true representations of all peptides present in the allergens.

The figures show that very few significant peptide differences exist between allergens I-B and I-C. Certain small differences in peptide maps were invariably found for digests made on different occasions using the same allergen and enzyme: similar small variations were also found where the ionic strength, pH and composition of the developing solvents varied slightly. Of the very few peptide differences observed, it is considered that only those found consistently (Figs. 1(b) and 2(b)) are likely to be significant. Many peptide differences were observed between tryptic fingerprints of I-B (or I-C) and II-B using method (i), but fingerprints of I-B and I-C as obtained by this method were almost identical.

Cystine-peptides. Tryptic fingerprints of allergen I-B using the diagonal method with performic oxidation at pH 6.5–6.5 and at pH 3.5–3.5 are shown in Fig. 3.



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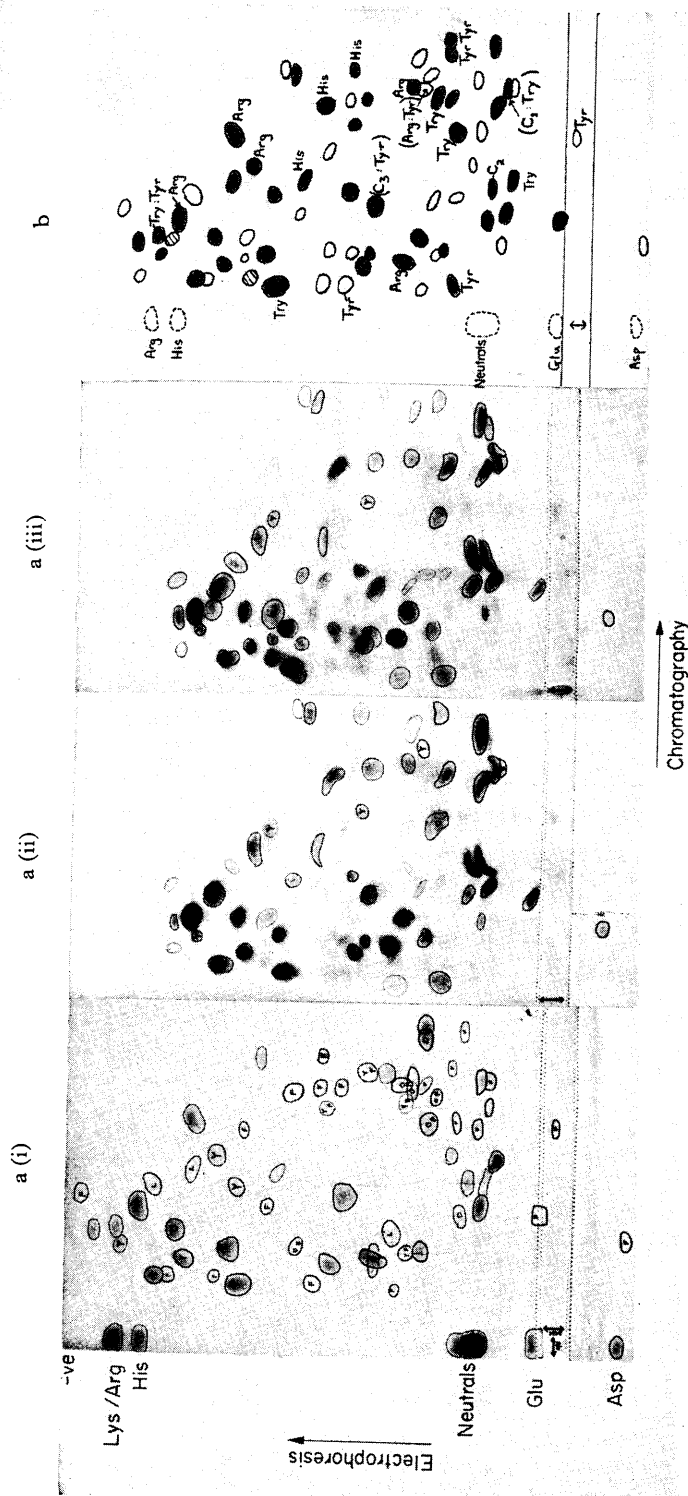


FIG. 1. Fingerprints for tryptic hydrolysates of allergens I-B and I-C, *a(i)* and *a(ii)* Allergen I-B. Enzyme to allergen ratios = 1:100 and 1:50, respectively.

a(iii) Allergen I-C. Enzyme to allergen ratio = 1:50. Staining with ninhydrin-CdAc for all Figs. a. Peptide colours Y—yellow, O—orange, R—red; F—faint colouration; D—dark staining; L—colour development late.

b. A diagrammatic comparison of the peptides in digests of I-B and I-C. Peptides shaded // found only in I-B and peptides shaded /// found only in I-C. The major areas of staining for both digests are shown in black: areas of weak staining are not shaded.

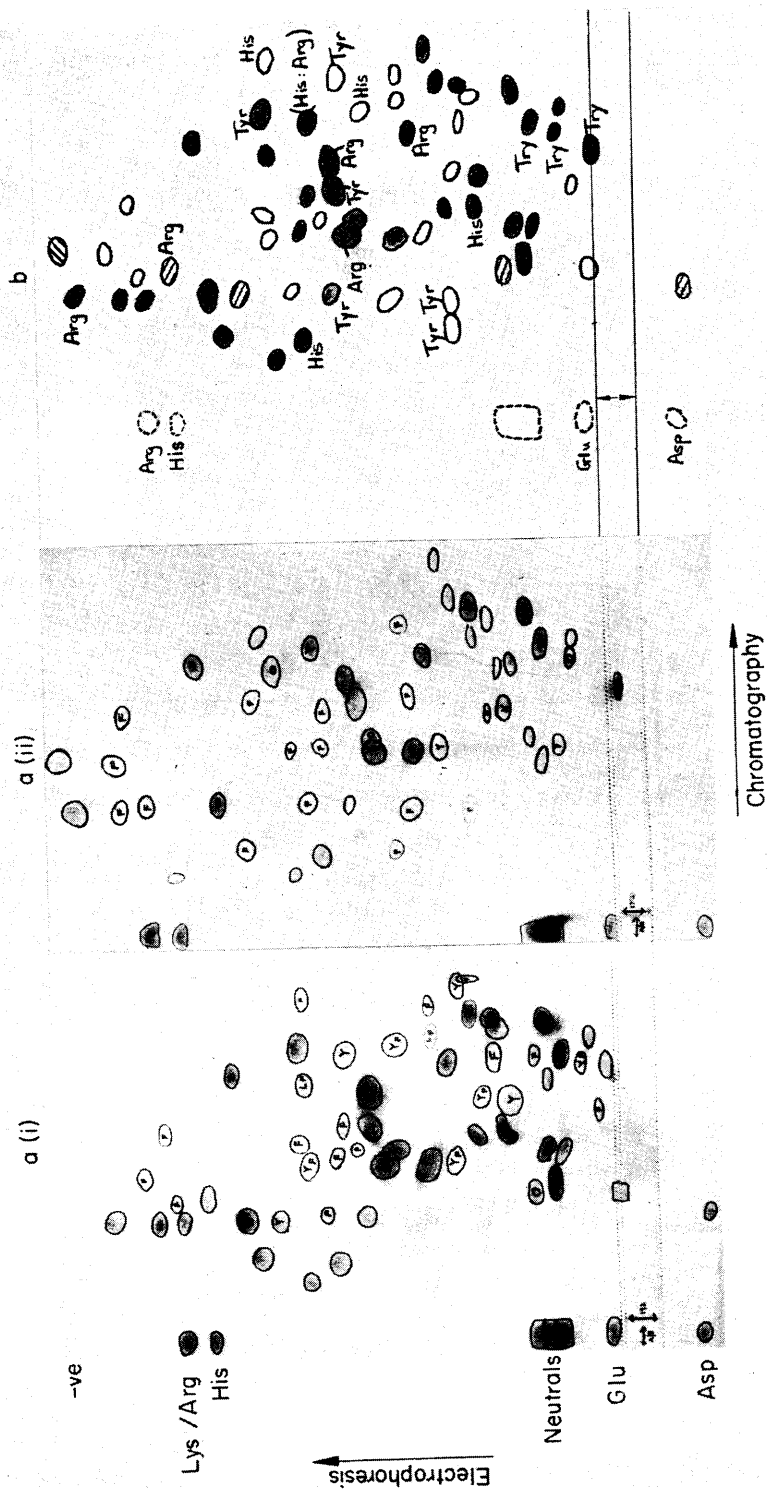


FIG. 2. Fingerprints for chymotryptic hydrolysates of allergens I-B and I-C, obtained using method (ii).
a(i) and *a(ii)* Allergens I-B and I-C, respectively. Enzyme to allergen ratios = 1:100. Staining as Fig. 1a.
b. A diagrammatic comparison of the peptides in digests of I-B and I-C (see legend to Fig. 1b for nomenclature).

2nd Dimension

FIG. 3
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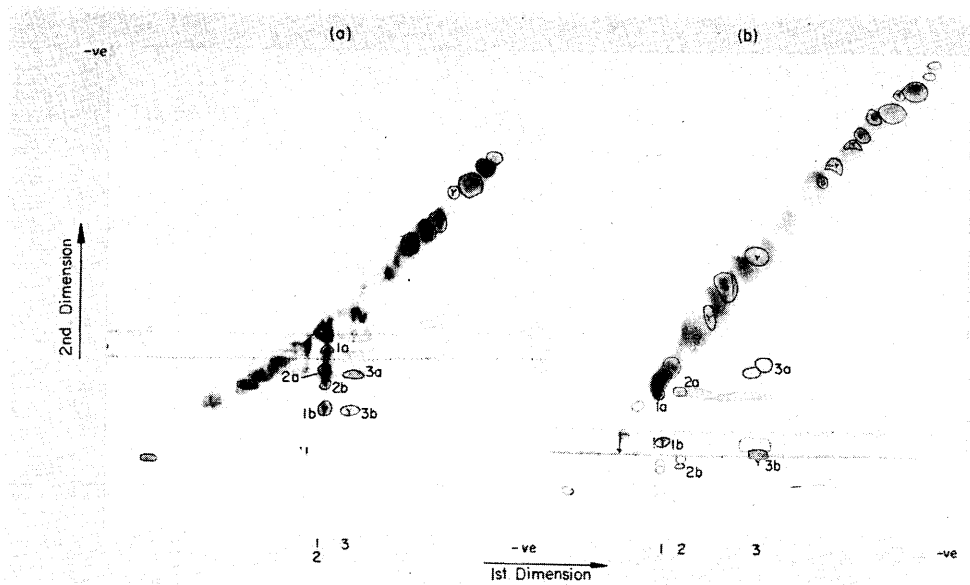


FIG. 3. Fingerprints for diagonals formed (a) at pH 6.5-6.5 and (b) pH 3.5-3.5 for tryptic hydrolysates of allergen I-B. Oxidation with performic acid (2 hr at room temperature, atmospheric pressure) after electrophoresis in the first dimension. Staining with ninhydrin-CdAc. Pairs of cysteic peptides are labelled 1a, b, etc.: 0 = origin.

TABLE 3. COMPARISON OF THE NUMBER OF PEPTIDES CONTAINING CERTAIN AMINO ACIDS WITH THE NUMBER OF SUCH RESIDUES FOUND BY AMINO ACID ANALYSIS

Digest	Histidine		Arginine		Tyrosine		Tryptophan		Cystine ^a	
	By analysis	From fingerprint	By analysis	From fingerprint	By analysis	From fingerprint	By analysis	From fingerprint	By analysis	From fingerprint
I-B _T	3	3	6	6	9	9	6	4	3	3
I-C _T	3	3	6	6	9	9	6	4	3	—
I-B _C	3	5	6	6	9	7	6	3	3	—
I-C _C	3	5	6	5	9	7	6	3	3	—

^a Cystine content in column 'by analysis' = $\frac{1}{2}$ (cysteic acid).
Subscripts T and C refer to tryptic and chymotryptic hydrolyses, respectively.

The apparent electrophoretic mobilities of cystine peptides were calculated relative to reference amino acids or peptides on these diagonal fingerprints at pHs 6.5 and 3.5 and referred back to the original tryptic fingerprints. Thus it was possible to locate the cystine peptides with reasonable certainty on the tryptic fingerprints shown in Fig. 1.

In the experiment at pH 6.5–6.5, two pairs of cysteine-peptides, 1a,b and 2a,b, were found in those peptides comprising the 'neutral band': one other pair, 3a,b, was derived from a weakly basic peptide. At pH 3.5–3.5, cystine-peptides 1 and 2 were just resolved from one another and individual cysteine-peptides could be correlated with those observed at pH 6.5–6.5 as shown in Fig. 3. Confirmation of the existence of three pairs of cysteine-peptides was obtained by experiments at pH 2.0–2.0 (1.40 M acetic acid–0.53 M formic acid). It therefore seems safe to predict the presence of three cystine residues in allergen I-B to account for the six half-cystine residues found by amino acid analysis, although proof by sequence determination would be desirable.

The glycopeptide linkage. The diagonal technique at pH 6.5–6.5, with hydroxylamine treatment at 37° for 18 hr, was used to examine glycopeptides in a whole tryptic digest of allergen I-B. A blurred picture was obtained, with at least six ninhydrin-staining areas located away from the diagonal. This suggests that several alkali-susceptible peptide bonds had been broken. To obtain a more positive indication of the effect of hydroxylamine on the glycopeptide linkage in allergen I-B, a purified glycopeptide fragment,^(1,30) was examined at pH 3.5–3.5. Reaction with hydroxylamine at 37° for 5 hr produced no new ninhydrin-staining spots, and left only a spot corresponding to the glycopeptide which stained strongly with both carbohydrate and chlorine stains. The mobility of this spot was identical both before and after hydroxylamine treatment, confirming that no peptide or glycopeptide bonds had been broken. This finding will be discussed in the succeeding paper.⁽³⁰⁾

DISCUSSION

In the present work, a preliminary investigation of the chemical composition and structure of the peptide moieties of two prominent rye allergens, I-B (α) and I-C (β), has been reported. The results provide a basis for future amino-acid sequence studies of these allergens. The great similarity of amino-acid composition between the two allergens is evident (Table 1); in particular, the quantities of all basic and acidic amino acids agreed within experimental error. Certain small differences may be attributed to inaccuracies in the analysis, particularly that for I-C. The only significant difference would appear to be in the proportion of leucine in the two allergens. Results showing close correlation between tryptic and chymotryptic fingerprints of I-B and I-C also suggest a close similarity of amino acid sequence between the two allergens. These chemical findings therefore correlate well with earlier results showing the similarity of their antigenic determinants and of several physical characteristics including molecular weight and ultraviolet absorption spectra. Conversely, the difference between fingerprints of II-B and the group I allergens is in conformity with both their immunological and physical differences.

On the alkaline storage in their stability, one group

The analyses (32,000) On the the analysis by pyrolysis

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Acknowledgements. Section facilities sacrificed the Beech and also their maintenance

On the basis of starch gel electrophoretic experiments at both acidic and alkaline pHs, and also the decrease in the I-B/I-C ratio with increasing time of storage of whole pollen, it has been suggested that I-B and I-C may differ solely in their amide composition. The analytical findings do not contradict this possibility, but to prove it would require an amide determination accurate to less than one group: this has not yet been feasible.

The molecular weights of group I allergens calculated from the amino-acid analyses (Table 2) showed fair agreement with those obtained from physical data (32,000 by sedimentation-diffusion analyses and 34,000 by the Archibald method). On the other hand, the partial specific volumes, $\bar{v}$, of I-B and I-C calculated from the analyses,⁽³²⁾ showed poor agreement with the value of 0.745 obtained previously by pycnometry; no explanation for this can yet be advanced.

A detailed comparison of the numbers of certain amino acids as identified by analysis with those appearing on the fingerprints (Table 3) is not justifiable due to variable and incomplete splitting of peptide bonds by the enzymes (especially by chymotrypsin), depending on the conditions of reaction. It is also possible that more than one residue of a particular amino acid occurs in certain peptides. However, since the numbers of histidine and arginine residues are small, and since the specificity of trypsin in hydrolysing peptide bonds adjacent to arginine and lysine is high, one might expect good agreement for both histidine and arginine. The agreement shown in these favourable cases is further evidence for the soundness of assuming a histidine content of 3.0 in calculating the analytical data given in Tables 1 and 2.

The amino acid compositions of group I allergens for the rye and ragweed pollens are markedly different (Table 1). King and his collaborators^(9,10,33) have reported that their ragweed allergens are very resistant to enzymic digestion by trypsin, chymotrypsin, papain and, to a lesser extent, by pepsin. Only bacterial enzyme preparations, such as subtilisin or nagarse, have been found to produce extensive disruption of the molecules. Since such marked differences both in chemical composition and in susceptibility towards enzymic action are seen between the major allergens (group I) of rye and ragweed pollens, it was surprising to observe such a striking similarity between their starch gel electrophoretic patterns at alkaline pH^(1,2) and also in the distribution of allergenic material within these patterns. However, antigenic differences have been observed between grass and ragweed pollens;⁽¹³⁾ and it is also known that grass pollen-sensitive individuals are not sensitive to ragweed pollen without prior exposure (and vice-versa⁽³⁴⁾). Such differences may now be partially explained in terms of differences in chemical composition and structure. The more relevant comparison of allergenic groupings lies in the future.

Acknowledgements—We are grateful to Dr F. Sanger, F.R.S., of the Protein Chemistry Section of the Laboratory of Molecular Biology, Cambridge for excellent laboratory facilities and to Dr L. F. Smith for many helpful discussions as well as his generous sacrifice of bench space. Defatted pollen samples were kindly given by Dr F. H. Milner of the Bencard Allergy Unit. We thank Mr G. Lee for performing the amino-acid analyses and also Drs T. P. King, P. S. Norman and J. T. Connell for permission to reproduce their data for ragweed allergens. One of us (D. G. M.) is indebted to D.S.I.R. for a maintenance grant.

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