Journal of Chromatography, 328 (1985) 425–431
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17 661

Note

Aqueous size-exclusion analysis of *Parietaria* pollen extracts

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(Received February 11th, 1985)

Analytical and purification problems are often encountered in pollen extract chemistry and biochemistry. The analysis and purification of pollens has been attempted with a variety of chromatographic methods, mainly gel filtration and ion exchange[1]. "Fingerprints" of pollen or concentration of biological activity are seldom obtained, as a consequence of the high heterogeneity of these pollen extracts. Thus, instrumental analysis needs to be accompanied by skin tests and techniques based on radioimmunoassays (RIA)[2].

Short ragweed (*Ambrosia elatior*) is widespread in the U.S.A. and its allergenic pollen has been purified by DEAE-cellulose chromatography[3]. The allergenic pollen of birch (*Betula alba*) is common in Europe and has been purified by gel filtration[4]. Two very common Graminaceae grass allergenic pollens have been purified: perennial rye grass (*Lolium perenne*) by gel filtration[5] and timothy (*Phleum pratense*) by combined gel filtration–ion exchange[6].

These techniques failed to give highly purified material when the purification of *Parietaria* species was attempted[7]. Therefore, a high-performance liquid chromatography (HPLC) study was performed in order both to provide a "fingerprint" of *Parietaria* allergenic extracts for commercial use in the Mediterranean area and to obtain highly purified allergenic extracts for further studies on their chemical composition.

EXPERIMENTAL

Parietaria pollen extracts

A 105-g amount of dry pollen was extracted with 2100 ml of 0.01 *M* phosphate buffer (pH 7.2) for 24 h at room temperature. The pollen grains were filtered and the extract was dialysed for 48 h against water, then filtered on a 0.45-μm Millipore membrane and lyophilized (2-ml aliquots).

HPLC analyses

HPLC analyses were performed by dissolving lyophilized pollen extracts in

0021-9673/85/$03.30

water containing $5 \cdot 10^{-3}$ *M* sodium azide (protein concentration 2 mg/ml) and injecting on a 10-μl Rheodyne loop. The instrument was a Varian 5000 HPLC isocratic system or equivalent, equipped with a Micropak TSK 2000SW (30 cm × 1/8 in. O.D. × 8 mm I.D.) size-exclusion column connected with a Micropak TSK 3000SW (30 cm × 1/8 in. O.D. × 8 mm I.D.) size-exclusion column. The detector was a Varian UV50 (280 nm) and the recorder was a Varian 9176 with a chart speed of 0.5 cm/min, 1 mV full-scale.

The solvent was doubly distilled water containing $5 \cdot 10^{-3}$ *M* sodium azide, filtered on 0.45-μm Micropak filters. The flow-rate was 0.9 ml/min.

Radio-allergo sorbent test (RAST) on the chromatographic fractions

Single chromatographic fractions of 75 μl were shaken with cyanogen bromide-activated paper discs[8] overnight at room temperature, then washed with 0.1 *M* phosphate buffer (pH 7.4). The discs were then treated with 50 μl of serum diluted 2:3 with the same buffer, shaken for 3 h at room temperature, washed with phosphate buffer (three 1-ml portions) and 50 μl of ^{125}I-labelled anti-IgE solution were added to each disc. After shaking overnight at room temperature the discs were rinsed three times (10 min) with 2.5 ml of 0.9% saline containing 0.5% of Tween 20. The radioactivity of the discs was determined and was proportional to the allergenic potency of the fraction[9].

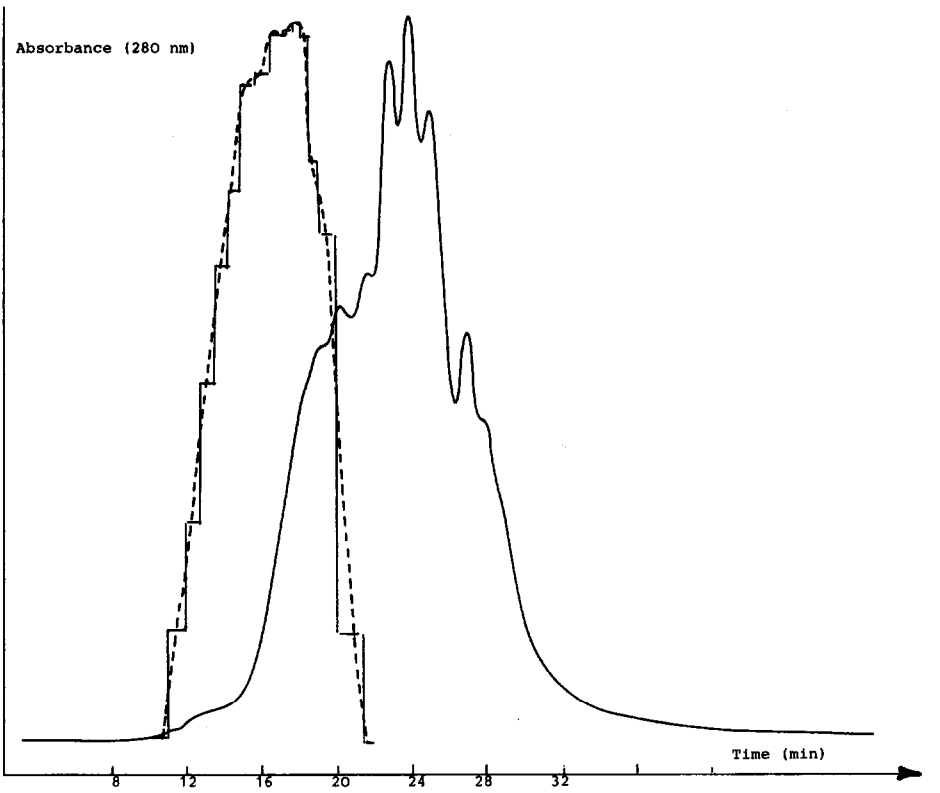

Fig. 1. *Parietaria officinalis* pollen extract from supplier A. ———, HPLC profiles; ---------, RAST profile.

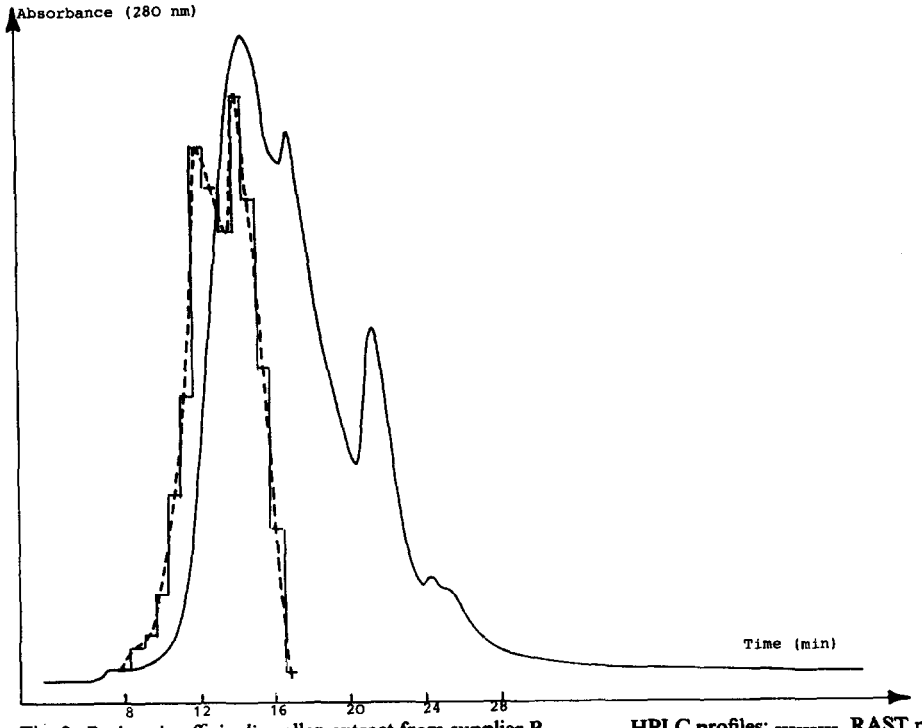

Fig. 2. *Parietaria officinalis* pollen extract from supplier B. ———, HPLC profiles; ---------, RAST profile.

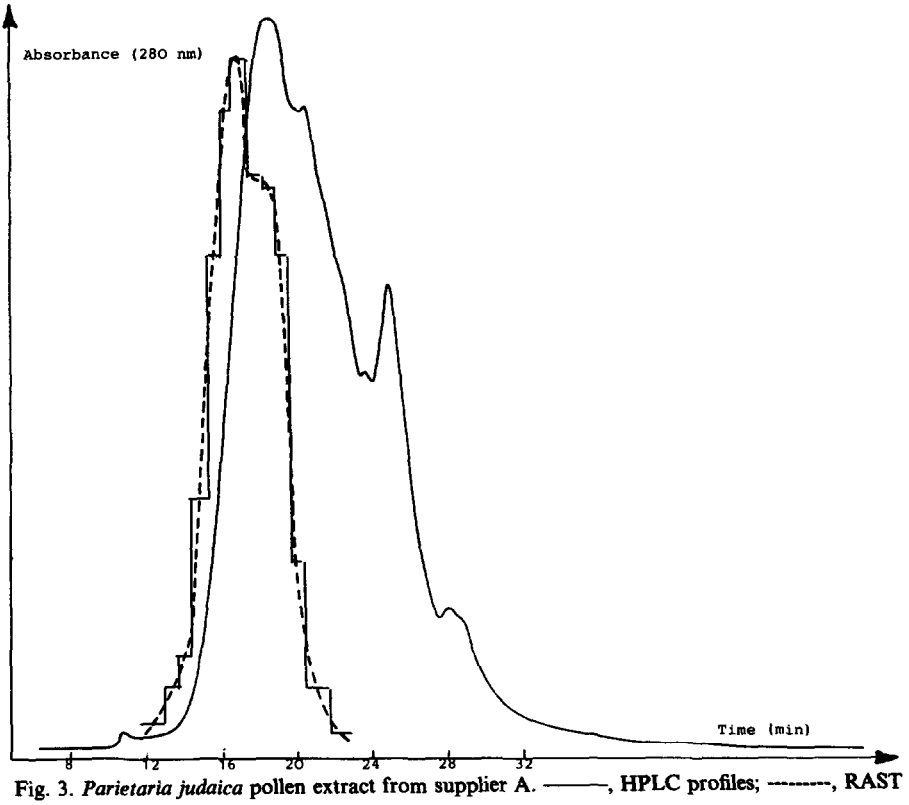

Fig. 3. *Parietaria judaica* pollen extract from supplier A. ———, HPLC profiles; ---------, RAST profile.

RESULTS AND DISCUSSION

A size-exclusion HPLC–RAST study of several pollen extracts was performed. They were two samples of commercial *Parietaria officinalis* pollen and two samples of *Parietaria judaica* pollen obtained from different suppliers (suppliers A and B, Figs. 1–4). The pollen extracts tested showed different elution profiles, whereas different samples of the same pollen extract exibited the same elution profile. Hence an approximate "fingerprint" of pollen extracts can be obtained.

All the fractions of the eluate of every pollen extract were submitted to RAST in order to test for any enrichment of the allergenic activity in some fractions of the eluate and to exclude any non-specific binding. The latter could be excluded as fractions containing only the solvent gave negative RAST.

Figs. 1–4 indicate that not all the elution peaks show immunological activity. This was in fact concentrated in 5–10 of the 50–70 collected fractions. Re-injection of these fractions after lyophilization showed elution peaks corresponding to part of the original total eluate.

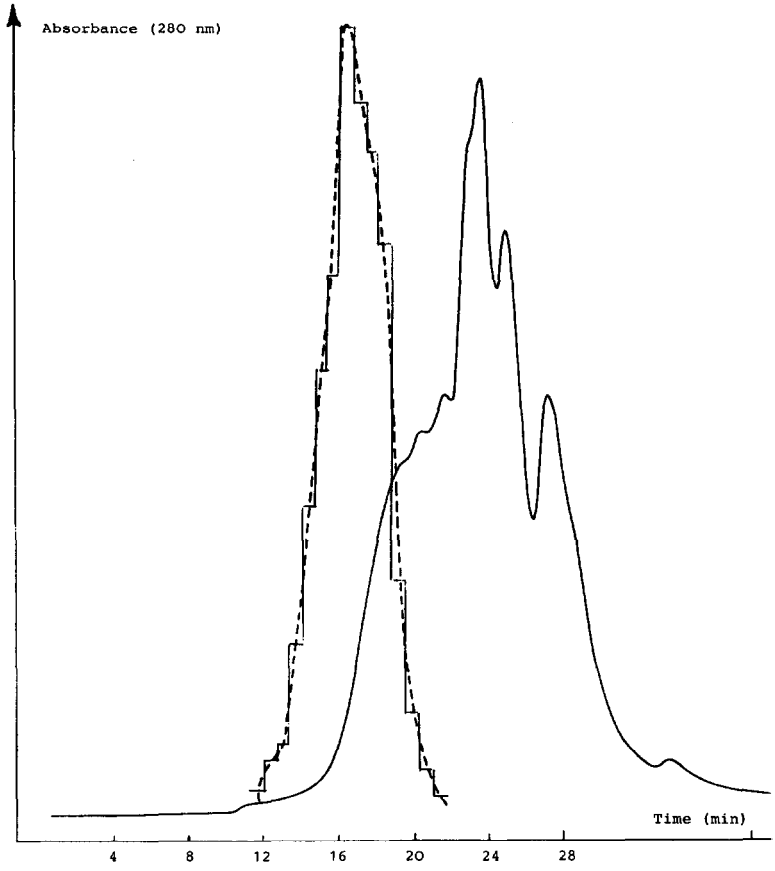

Fig. 4. *Parietaria judaica* pollen extract from supplier B. ———, HPLC profiles; ---------, RAST profile.

In *Parietaria* pollens the elution profiles of different species from the same collector showed greater similarity than the profiles of the same species from the second supplier. This could reflect the different procedures for the collection of pollen grains and the possible denaturation of some of the allergenic proteins in some instances.

A small amount of high-purity *Parietaria* pollen (98%) to be submitted to HPLC and RAST could be obtained from the supplier (see Fig. 5). This pollen (Fig. 6) gave a different eluate and a sharper RAST profile than the other samples tested and could be more suitable for further studies.

The appearance of some components after V_t (total permeation volume) sug-

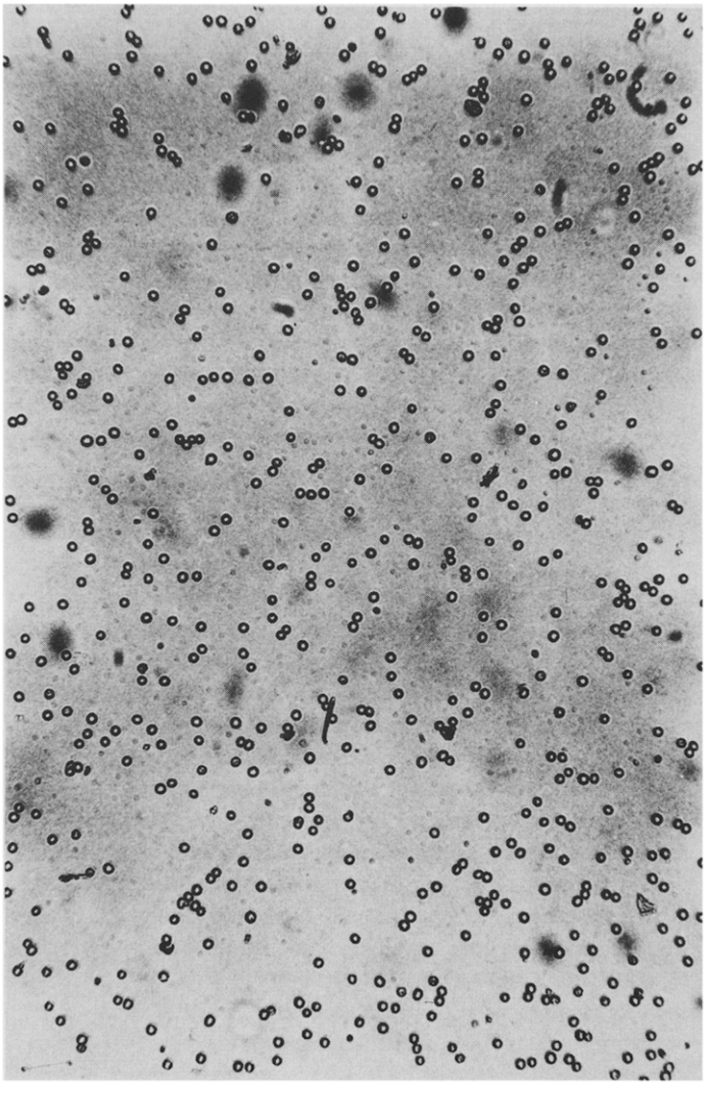

Fig. 5. Microphotograph of the high-purity *Parietaria officinalis* pollen extract.

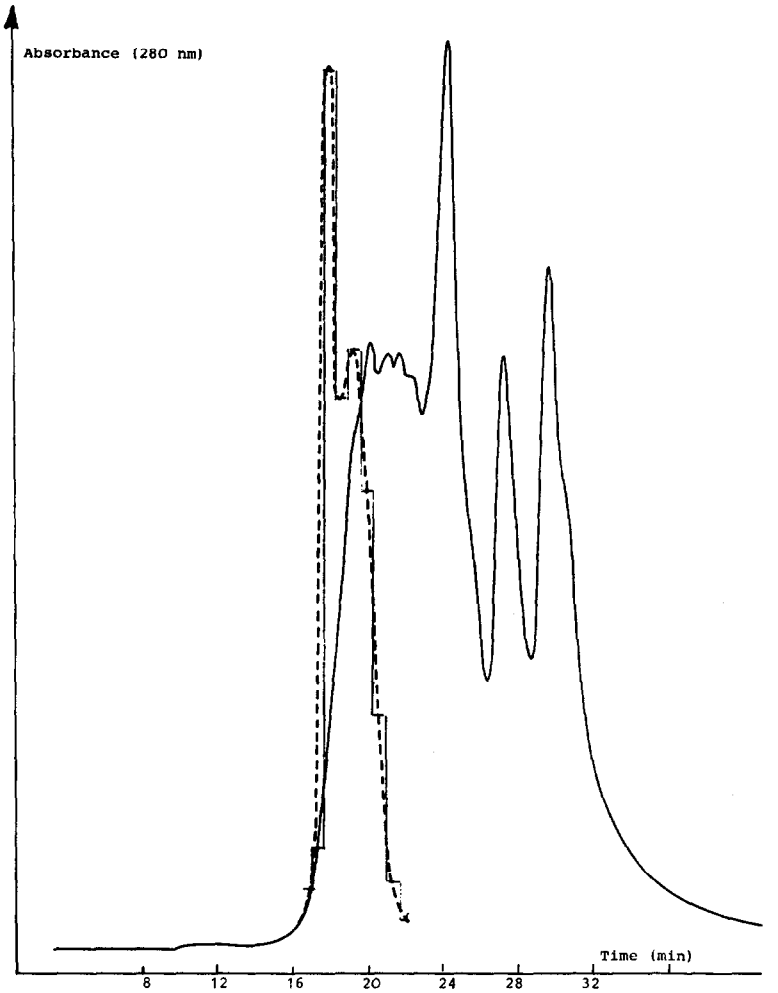

Fig. 6. High-purity *Parietaria officinalis* pollen extract. ———, HPLC profiles; --------, RAST profile.

gests that some absorption of the material on the column occurs. Probably some basic material is involved. However, RAST of these fractions was negative.

Attempts to enrich further the immunological activity were unsuccesful with the size exclusion technique. However, its efficiency in the concentration of the immunological activity in a small number of fractions, the possibility of repeated analyses and the recovery of allergenic material after the chromatography suggest that this technique is useful both for quality control and the enrichment of allergenic materials.

Further enrichment of the extracts for structural studies demands different purification techniques. These are currently under investigation.

ACKNOWLEDGEMENT

We thank Mr. E. Bolzacchini for his invaluable contribution in carrying on the experimental work.

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