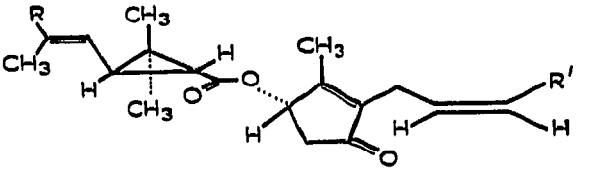
Notes

CHROM. 5873

Preparative scale separation of pyrethrins by liquid–liquid partition chromatography

Pure samples of the six insecticidally active esters of pyrethrum have proved extremely difficult to obtain directly from the natural extract, due to the close chemical similarity of the three esters within each series.



	R	*R'*
Pyrethrin I	CH_3	$-CH=CH_2$
Cinerin I	CH_3	$-CH_3$
Jasmolin I	CH_3	$-CH_2-CH_3$
Pyrethrin II	$CH_3-O-C(=O)-$	$-CH=CH_2$
Cinerin II	$CH_3-O-C(=O)-$	$-CH_3$
Jasmolin II	$CH_3-O-C(=O)-$	$-CH_2-CH_3$

The best methods so far available for obtaining gram quantities of cinerins I and II and pyrethrins I and II is by reconstitution from the acid chlorides of chrysanthemic or pyrethric acids and the relevant alcohol, the various components themselves being derived from the natural esters[1]. These methods have not been applied to the preparation of jasmolins. The early chromatographic separations on alumina[2,3] and charcoal[4] did not give complete resolution of cinerins and pyrethrins and failed to detect the presence of jasmolins. STAHL AND PFEIFLE[5] later demonstrated that a complete separation of the three esters in each series could be achieved by thin-layer chromatography using a continuous development technique.

Preparative gas chromatography (GC) has been used successfully to separate the three pyrethrin I esters[6] and jasmolin II has been purified by repeated chromatography on a silica gel/plaster of Paris column[7].

All six esters have been prepared by completely synthetic routes[8], primarily in order to confirm structure assignments, but these are complex, multi-stage processes involving, at certain stages, resolution of enantiomers in both acid and alcohol parts of the molecule.

No system has yet been developed for the preparative scale separation of all

six esters directly from pyrethrum extract. The following describes such a system using liquid–liquid partition chromatography at elevated pressures.

Experimental and results

Apparatus. The apparatus consisted of a pumping system comprising a variable stroke piston pump supplied by F. A. Hughes & Co., capable of operating up to pressures of 250 p.s.i. with a maximum pumping rate of 230 ml/h. Pulsation damping was effected by incorporation of a bourdon gauge into the pressure line.

The columns were of the variable bed type, constructed of glass with PTFE bed supports, supplied by Quickfit Instrumentation Ltd. These would operate up to a maximum pressure of 80 p.s.i. Two column configurations were used, the first employing a main column 450 × 25 mm with a precolumn 450 × 10 mm and the second, a main column 1,000 × 38 mm with a precolumn 450 × 25 mm. All columns were water-jacketed and maintained at 23°.

Sample injection was by means of a Chromatronix Inc. Type SV-8031 injection valve fitted with 0.5-ml and 1.0-ml sample loops for the smaller and larger columns, respectively. All connections were made using 1/16 in. O.D. PTFE tubing.

Chromatography. The chromatography was first carried out on the 450 × 25 mm column using nitromethane, supported on Celite, as the stationary phase, with mobile phases of hexane and, subsequently, carbon tetrachloride–hexane (1:3). The support was prepared by sieving Celite 545 (supplied by Koch-Light Laboratories Ltd.) through a 300-mesh screen and drying at 140°. The columns were packed using the tamping technique described by HOWARD AND MARTIN[9]. Small increments of the packing material slurried in hexane were added to the column and the bed was firmly compressed using a plunger comprising a perforated stainless-steel disc, closely fitting the bore of the column, welded at its centre to a long stainless-steel rod. When the packing was complete the top bed support was screwed firmly down and the stationary phase was added by passing nitromethane saturated with hexane until approximately two column volumes had been eluted. Hexane, saturated with nitromethane, was then pumped through the column until bleeding of the nitromethane had stopped. This coating procedure was found to give better separations and greater loading capacities than the more conventional method of precoating the support with the stationary phase before packing the column.

The precolumn was packed with 200-mesh Celite containing an equal weight of nitromethane. This column was by-passed during conditioning of the main column.

A concentrated solution of pyrethrins was obtained by cleaning up pyrethrum oleoresin on a column of silica gel (British Drug Houses Ltd.), the pyrethroid fraction being eluted with ether–hexane (1:3). 0.5 ml of this solution, containing approximately 130 mg total pyrethrins, was injected onto the top of the column, which was then developed with hexane saturated with nitromethane at a flow rate of 100 ml/h. Fractions of 4 ml were collected and analysed for pyrethroids by GC using the conditions described below. When elution of pyrethrin I was nearly complete, the mobile phase was changed to carbon tetrachloride–hexane (1:3), saturated with nitromethane.

The pyrethroid content of the tubes was plotted against fraction number and the resulting graph is shown in Fig. 1. The elution order for both the I and II series was jasmolin, cinerin, pyrethrin. A resolution of 1.03 was obtained between jasmolin I

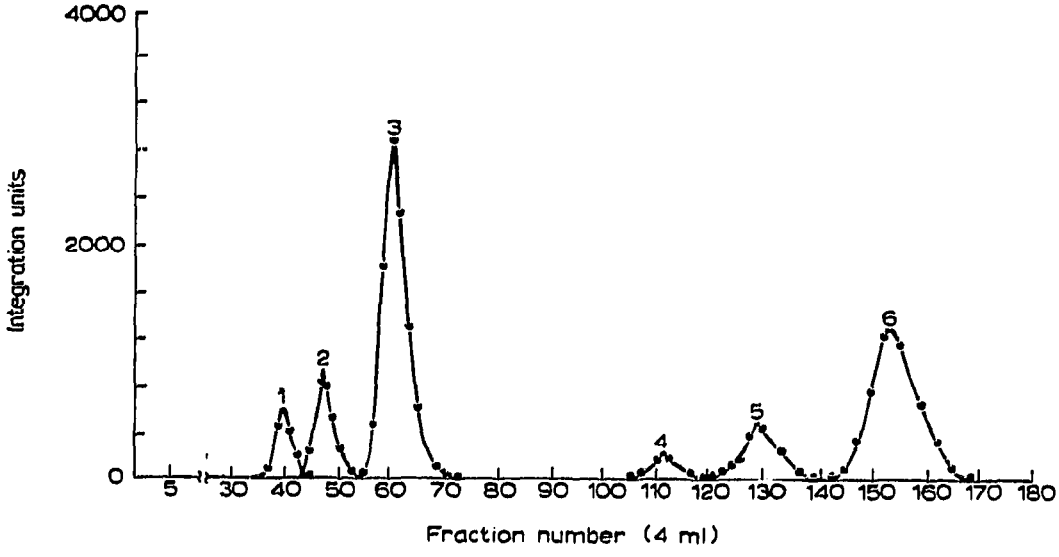


Fig. 1. Pyrethroid content of fractions collected from a 450 × 25 mm column analysed by gas chromatography. 1 = Jasmolin I; 2 = cinerin I; 3 = pyrethrin I; 4 = jasmolin II; 5 = cinerin II; 6 = pyrethrin II.

and cinerin I, the least resolved components, (calculated from the formula $R_s = 2\ (V_2 - V_1)/(W_2 + W_1)$, where V_1 and V_2 are the retention volumes and W_1 and W_2 the peak widths[10]).

The scale of the separation was increased by using a column 1,000 × 38 mm. Preparation of the Celite and the column packing procedure were carried out as described previously. Better columns were obtained in this case, however, by packing the column with the Celite slurried in nitromethane rather than hexane. The first mobile phase, hexane saturated with nitromethane, was then pumped through the column until stationary phase bleed had stopped. A solution of pyrethrum extract* containing approximately 270 mg total pyrethrins in 1 ml of hexane was injected

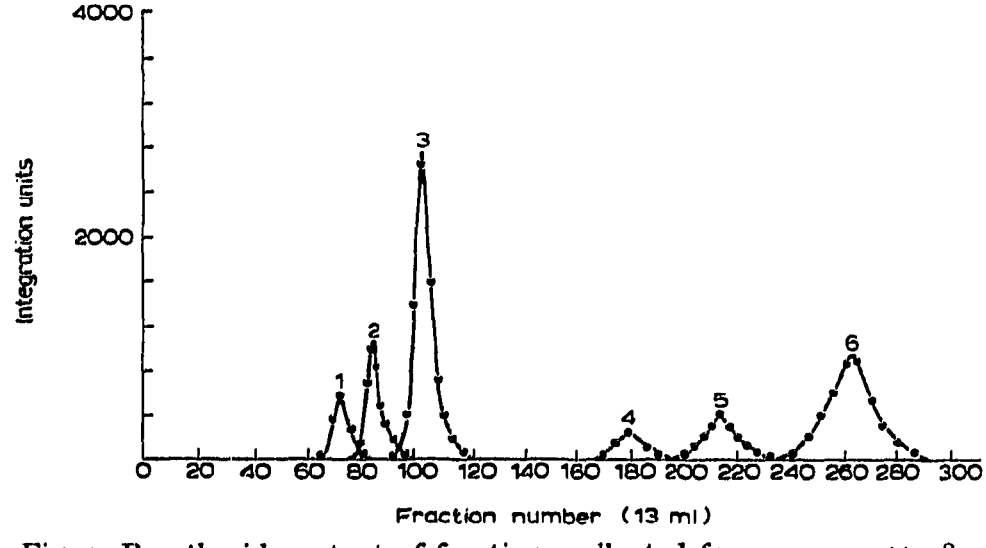


Fig. 2. Pyrethroid content of fractions collected from a 1,000 × 38 mm column analysed by gas chromatography. Assignments as in Fig. 1.

* Obtained by diluting Pale Concentrate with an equal volume of hexane. The author is grateful to the Pyrethrum Marketing Board of Kenya for supplying this material.

onto the column, which was developed, as before, with hexane and carbon tetrachloride–hexane (1:3), at a flow rate of 230 ml/h. Under these conditions the column inlet pressure was 25 p.s.i. Fractions of 13 ml were collected and analysed for pyrethroid content by GC. The results are shown in Fig. 2.

The separation of "pyrethrins I" obtained was not quite as good as with the smaller column, the resolution between cinerin I and jasmolin I reducing to 0.89. By careful selection of the fraction collector tubes, however, it was possible to obtain good recoveries, averaging about 60%, of the pure esters. A complete separation, with at least two blank tubes between each component, was obtained for the "pyrethrins II".

After elution of the pyrethroids, the column was regenerated by flushing with nitromethane, to remove any residual pyrethrum constituents, followed by hexane, saturated with nitromethane. The precolumn was bypassed during the regeneration process. A column has so far been used four times without noticeable reduction in efficiency.

The individual pyrethroids were initially identified by GC. The assignments

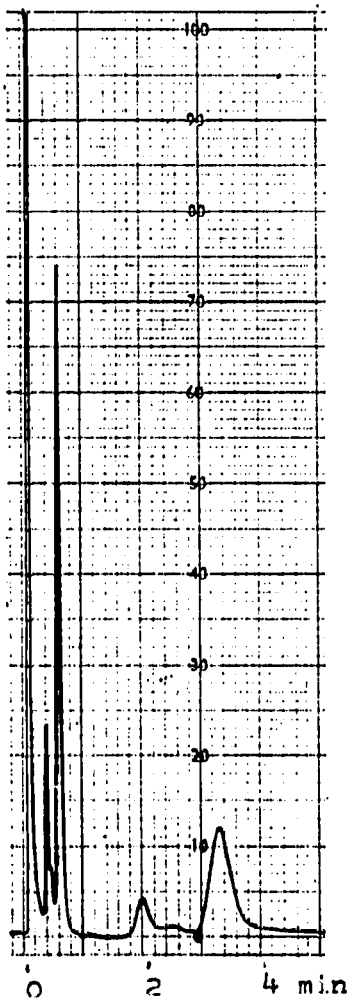


Fig. 3. Separation of pyrethrins by gas chromatography. Instrument: Pye Series 104 gas chromatograph fitted with flame ionization detector. Column: 1 ft. × ⅛ in. glass, packed with 1% OV-225 on Gas-Chrom Q, 100–120 mesh. Carrier: nitrogen at 100 ml/min. Temperatures: column, 192°; detector, 250°. Injection volume: 1 μl of solution containing 1% total pyrethrins.

were checked by comparing their NMR spectra, recorded in carbon tetrachloride solution on a Varian T60 NMR spectrometer, with published data[11]. This also gave a good indication of their purity with respect to non-pyrethroid contaminants. The spectra of cinerin I, pyrethrin I, cinerin II and pyrethrin II compared exactly, indicated a level of purity greater than 90%. The spectra of jasmolins I and II showed the presence of minor impurities giving signals between 0.3 and 1.0 p.p.m. from TMS.

GLC analysis of the fractions. Identification of the pyrethroids was performed by GC. In view of the large number of fractions collected, it was necessary to obtain conditions which would give rapid results. This was particularly important for the series II esters, especially pyrethrin II, where temperature limitations imposed by its thermal stability[12] have resulted in long retention times, generally in the order of 15–20 min[13]. This problem was overcome using a short (1 ft. × 1/8 in.) glass column packed with 1% OV-225 on 100–120 mesh Gas-Chrom Q, operated at a carrier flow rate of 100 ml/min. These conditions gave adequate resolution of cinerin II and jasmolin II, while performing a complete pyrethrum analysis in less than 4 min (Fig. 3).

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