CHROM. 3333

Separation of scopoletin from fluorescent compounds in roots of *Pisum Sativum* var. meteor by Sephadex gel filtration

Previous methods of separation of fluorescent compounds in plants have relied mainly on the use of paper chromatography and estimation directly on the paper by spot size measurement[1] or alternatively by elution from paper and estimation by fluorimetry[2]. Both methods were found by this author to be unsatisfactory. Methods based on spot area measurements proved unreliable due to fluctuations in the relationship between spot area and the intensity of fluorescence and were further complicated by the rather diffuse periphery of the spots.

Elution from paper was found to be quantitatively unsatisfactory with 5–30 % loss of fluorescence during 30 such separations. The loss increased with increasing concentration of scopoletin applied to the chromatograms.

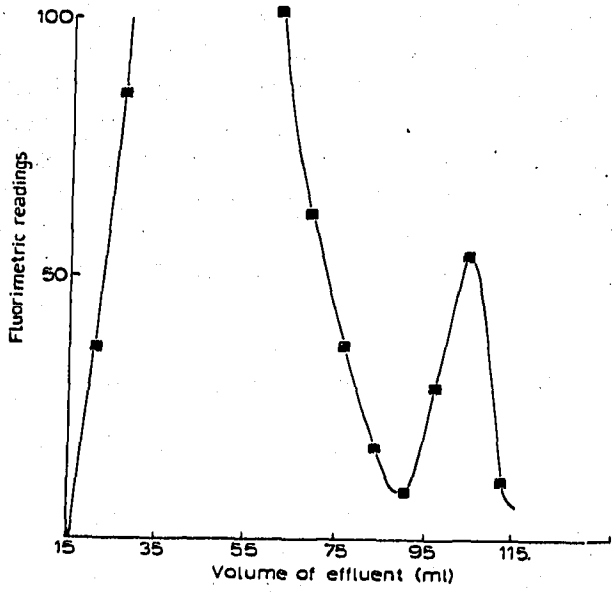
The technique described here is based on separation using sephadex gel filtration.

For the initial extraction, root systems from twenty plants of *Pisum Sativum* var. meteor, grown in culture solution for 8 days at 22° were removed, frozen, to prevent enzymatic conversion of scopoletin to the glycoside scopolin, for twelve hours and then macerated. Plants grown in vermiculite yield similar results. The ground material was oven dried at 80° and extracted with 2 % acetic acid at 80° for 4 h. The supernatant liquid was drawn off and the residue further extracted with 2 % acetic acid until no fluorescence remained. The supernatant was evaporated at 80° to dryness and taken up in 1 ml of distilled water (phthalate buffer pH 6.8 may equally well be used).

A twelve inch, half inch diameter quickfit column of Sephadex G-15 was prepared and allowed to settle for twelve hours prior to use. Columns allowed to stand longer (up to seven days) behaved not differently from those standing twelve hours. The extract was quantitatively transferred to the column. An extension column was added and distilled water used to wash the extract through the column. Seven ml fractions were collected and examined for fluorescence using an E.I.L. direct reading fluorimeter. The results are shown in Fig. 1. Band I began eluting after 15 cc had run through the column and continued to elute until 90 cc had run off. Band II began to emerge immediately and was collected in 21 cc between 91 and 112 cc. The length of time taken depends on how tightly the column is packed. In the procedure described, band II emerges after 2–3 h. Left on the column at the end of the run are two further bands which move but little. Band III, a blue fluorescing zone penetrates between 50 and 80 mm into the column after 125 cc have been eluted. Band IV, a yellow fluorescing zone penetrates up to 20 mm into the column.

Band III may be separated from the other fluorescing bands using Sephadex G-25. On this grade of Sephadex an incomplete separation of bands I and II was obtained, as shown in Fig. 2. Band IV again penetrates but little, reaching 50 mm into the column after 200 ml have been eluted.

Band I, being a rather diffuse band on G-15 was concentrated and run on Sephadex G-10, no further separation was effected. That this band was due to only one compound was confirmed using paper chromatography (Tab. I). Each fluorescent



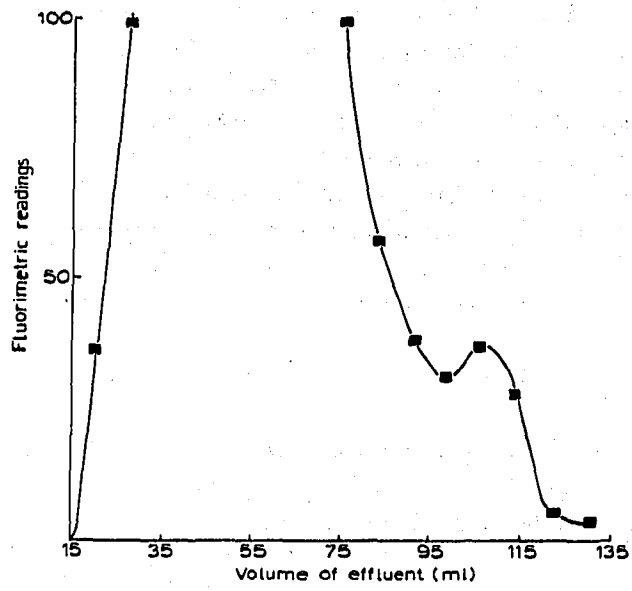


Fig. 1. Elution diagram for the separation of bands I and II on Sephadex G-15 columns.

Fig. 2. Elution diagram for bands I and II on Sephadex G-25 columns.

TABLE I

R_F VALUES OF SCOPOLETIN, SCOPOLIN, CHLOROGENIC ACID, CAFFEIC ACID AND ISOLATED COMPOUNDS

The solvent systems used are: (1) Isopropanol–acetic acid–water (5:0.1:95, v/v/v); (2) butanol–acetic acid–water (4:1:2, v/v/v); (3) 10% acetic acid; (4) benzene–acetic acid–water (6:7:3, v/v/v); (5) ethyl acetate–water (1:1, v/v).

Compound	*R_F values in solvent systems*				
	1	*2*	*3*	*4*	*5*
Band I	0.72	0.50	0.76	—	0.08
Band II	0.48	0.86	0.47	0.85	0.85
Band III	0.32	0.80	0.38	0.7	0.34
Band IV	0.80	0.69	—	—	—
Scopolin	0.74	0.47–0.63	0.75	—	0.09
Scopoletin	0.48	0.85	0.48	0.85	0.85
Caffeic acid	0.35	0.78–0.83	0.38	0.67	0.35
Chlorogenic acid	0.80	0.68	—	—	—

band was examined and an attempt made to identify each compound by paper chromatographic methods. Synthetic scopoletin[3] was used for reference. This was run alongside each band as well as in combination with the extracted bands. Other reference compounds used include scopolin, caffeic acid and chlorogenic acid.

Band II thus appears to be scopoletin. This was confirmed by means of excitation and emission spectra measurements.:

Emission spectra:	Band I	440–445 mμ.
	Band II	465 mμ.
	Scopoletin	465 mμ.
Excitation spectra:	Band II	max 263, 306, 350 mμ; min 274, 315 mμ.
	Scopoletin	max 262, 305, 355 mμ; min 274, 318 mμ.

It is further suggested on the basis of the chromatography evidence that band I corresponds to scopolin, band III to caffeic acid and band IV to chlorogenic acid.

The value of this technique in separating the scopoletin (band II) from the other fluorescent compounds was illustrated by evaporating 0.4 μg of synthetic scopoletin and 0.4 μg of caffeic acid in 2 % acetic acid to dryness, taking up in distilled water and running on Sephadex G-15, followed by fluorimetric estimation. The reading obtained for the scopoletin containing fraction was compared with a direct reading of an equivalent weight of scopoletin in the same volume of distilled water. Loss from 10 replicate experiments was at most 2 %.

Thanks are due to Dr. J. S. DAVIDSON for the preparation of scopoletin.

Department of Chemistry and Biology,
Barking Regional College of Technology,
Longbridge Road, Dagenham, Essex (Great Britain)

J. ROBERTS

1 H. D. FOWLER, *Nature*, 188 (1960) 1044.
2 T. MINAMIKAWA, T. AKAGAWA AND I. URITANI, *Plant Physiol.*, 38 (1963) 493.
3 H. D. BRAYMER, M. R. SHETLAR AND S. H. WENDER, *Biochim. Biophys. Acta*, 44 (1960) 163.

Received November 20th, 1967

J. Chromatog., 34 (1968) 112–114

CHROM. 3304

A device for streaking thin-layer chromatograms

Large-scale thin-layer chromatography often requires application of a sample in a band, or streak, along the starting line of the chromatogram. A device which applies a known quantity of sample solution in a uniform, homogeneous streak is described in this note. It is very easy and inexpensive to build, and has the great advantage of requiring no supervision while it is operating.

Fig. 1 shows the principle parts of the streaker. Basically, the device consists of a peristaltic pump with a moving delivery nozzle. The prototype was constructed with a children's "Erector Set" (a toy mechanical construction kit), with a few simple specially-machined parts. It is driven by a small electric motor equipped with a gear-reducer, to give a drive speed of 0.25 r.p.m. The motor drives a brass roller (1.25 cm in diameter). The tube containing the sample is squeezed between this drive roller and a freely rotating roller mounted on a movable shaft; pressure is supplied by an elastic band. As the pump tube is pulled and squeezed by the rollers, the sample solution is forced out through a polyethylene nozzle (a short length of 0.28 mm I.D. polyethylene tubing) attached to the 1.6 mm I.D. rubber pump tube by means of a tapered glass connector. The pump tube is clamped gently to a four-wheeled carriage which rolls on a metal track alongside the thin-layer plate. Obviously, the sample is