Notes

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The dry column chromatography of two isomeric abscisic acids

Crystalline *cis,trans*- and *trans,trans*-abscisic acids* have been separated by using dry column chromatography[1]. The solvent system used for development was benzene–ethyl acetate–acetic acid (50:5:2, v/v)[2]. The adsorbent used was silicic acid which was equilibrated with solvent prior to use. This was carried out on a kilogram scale as follows: 1 kg of Mallinckrodt 100 mesh silicic acid and 100 g of the solvent mixture were tumbled together in a 3 l roundbottom flask by a closed rotary film evaporator at atmospheric pressure. If an angle near the horizontal is used, friction is sufficient to keep the flask turning. The silicic acid was tumbled for at least 12 h before use. A cellulose dialysis bag makes the best column with this solvent system. Nylon tubing stretches and softens with this solvent, causing channeling and little if any separation is effected. The mixed abscisic acid isomers were first deposited on silicic acid in the ratio of 1 g abscisic acid to 5 g silicic acid by dissolving the abscisic acid in methanol, combining this with the silicic acid in a roundbottom flask and stripping off the methanol under vacuum. Gentle warming is required so that excessive splattering does not occur in the rotary film evaporator.

The cellulose dialysis bag was prepared for use by soaking it in water for about 15 min. Pieces, 1.1 m long, of 13/4 in. wide, flat tubing were used. One end of the softened tube was rubbed open, and then the rest of the tubing was opened completely by blowing dry nitrogen into it. One end of the tube was tied shut with a simple overhand knot and the outside of the nitrogen-inflated tube was wiped dry. The tube was allowed to stand and dry for 1 h before packing.

The column was packed with 300 g of equilibrated silicic acid in 100 g portions. After each addition of silicic acid the column was tamped by bouncing on a flat surface until the column was firm. Any pinhole breaks in the cellulose tubing caused by flexing can be patched with scotch tape.

Finally, 6 g of the silicic acid–abscisic acid mixture were placed on top of the column and slightly compacted by bouncing. The total length of the column was about 75 cm. The solvent mixture, 250 ml, was placed in a suitable separatory funnel which was then tightly stoppered and pressure equilibrated by momentarily opening the stopcock over a beaker until suckback occurred. The funnel was then mounted above the column with its tip extending about 3 cm below the top of the column, and the stopcock was opened to begin the development which required about 30 h.

When the development was complete, the top 18 cm was cut into cm sections with a sharp knife and the cellulose casing scraped clean. Each portion of silicic acid was eluted in a 42.5 mm diameter course porosity sintered glass funnel with 50 ml of

* From Reynolds Tobacco Company.

absolute methanol. The methanol solvent was stripped off in tared round-bottom flasks, on a rotary film evaporator under vacuum with gentle heating by a flow of hot tap water. The weight of each fraction was determined and then it was redissolved in methanol at the rate of 10 mg per ml. Ten microliters of each fraction were spotted onto a 20 cm Brinkmann MN Polygram Sil N-HR/UV_{254} thin-layer chromatography plate 1.5 cm from the bottom and 1 cm apart. A stream of air was blown over the plate to aid evaporation of the solvent and the spots were kept as small as possible. The plate was then developed to within 1 cm of the top of the plate, dried and redeveloped until the isomers were well resolved. Two repetitions were usually sufficient.

When this TLC plate was viewed under ultraviolet light the top cm of the column contained no abscisic acid. The next 5 cm contained 0.25 to 0.30 g of essentially pure crystalline *cis,trans*-abscisic acid, the optically inactive natural isomer. The next 4 cm contained about 0.25 g of *cis,trans*-abscisic acid, which was contaminated with a trace at an estimated 10% of the *trans* isomer. The next 2 cm contained 0.15 to 0.20 g of abscisic acid which appeared to be a 1 to 1 mixture of the isomer. The remaining fractions contained 0.25 to 0.30 g of *trans,trans*-abscisic acid, containing 10% or less of the *cis* isomer. The *cis* isomer after recrystallization from benzene had a melting point of 188°. The *trans* isomer, likewise crystallized from benzene melted at 143°.

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1 B. Loev and M. M. Goodman, *Chem. Ind.* (*London*), (1967) 2026.
2 B. V. Milborrow, personal communication.

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A thin-layer chromatographic separation of cerebrosides and related compounds

As a part of our current studies on cerebroside biosynthesis, a thin-layer chromatography system was desired that would separate a mixture containing cerebrosides and some likely metabolic precursers (*e.g.*, ceramide, psycosine, sphingosine, and fatty acid) from each other. This system could provide a rapid assay for the incorporation of fatty acid or glucose into cerebrosides or into any of the possible intermediates, while separating these products from the radioactive substrates. While numerous thin-layer chromatography systems have been developed that separate one or more of the lipids mentioned above[1–5], a search of the literature revealed no system that