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Note

Rapid preparative separation of natural products by centrifugal thin-layer chromatography

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Among the commonly used preparative-scale separation techniques, centrifugal chromatography has so far played only a minor role. However, attempts to increase the separation speed by acceleration of the flow-rate of the mobile phase using centrifugal force were reported many years ago by Hopf[1] and Caronna[2]. Numerous papers dealing with this topic have appeared and were reviewed by Deyl *et al.*[3]. An apparatus called a chromatofuge has been developed and its performance illustrated by the separation of four closely related purines: caffeine, theobromine, theophylline and xanthine[4]. Recently ,an instrument for preparative, centrifugally accelerated, radial, thin-layer chromatography (TLC), the Chromatotron, became commercially available. In this system, the TLC plate (rotor) is not horizontal but inclined, and thus allows more efficient collection of the eluate.

The efficiency of this method was demonstrated by Derguini *et al.*[5], who separated 100-mg amounts of *cis/trans* isomeric esters on silica with *n*-hexane–diethyl ether (99:1). The recovery of pure esters from the Chromatotron was 90% whereas it was only 80% in preparative liquid chromatography.

In connection with our work on the isolation of constituents of higher plants, we have evaluated the Chromatotron for its suitability to achieve rapid preparative separations of various classes of natural products. This paper describes the application of centrifugal TLC to the isolation of xanthones, triterpenes and saponins. Some advantages and limitations of the method are discussed.

EXPERIMENTAL

All of the separations were carried out on a Chromatotron Model 7924 (Harrison Research, Palo Alto, CA, U.S.A.). The rotors were coated with silica gel GF_{254} for TLC (E. Merck, Darmstadt, G.F.R.); the layer thickness was 2 mm (prepared from a slurry formed of 60 g of silica gel, 2.4 g of calcium sulphate hemihydrate and 120 ml of water). Solvent was delivered by the pump at a flow-rate of 4–6 ml/min. UV detection was carried out at 254 or 366 nm. Fractions of 2–4 ml were collected. In the separation of triterpenes, the fractions were monitored by TLC on pre-coated aluminium sheets (Merck) with chloroform–methanol (9:1); detection was effected with Godin reagent[6]. Prior to sample application, the plates were pre-washed with solvent to remove impurities from the silica gel.

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RESULTS AND DISCUSSION

The principle of the operation of the Chromatotron is simple, as shown in Fig. 1. The mixture to be separated is applied as a solution near the centre of a rotor coated with a thin-layer of adsorbent (layer thickness 1–4 mm). Elution with a solvent gives concentric bands of the components, which are spun off from the edge of the rotor together with solvent. A collection system brings the eluate to a single output tube. UV-active compounds can be observed directly through a quartz lid during the separation.

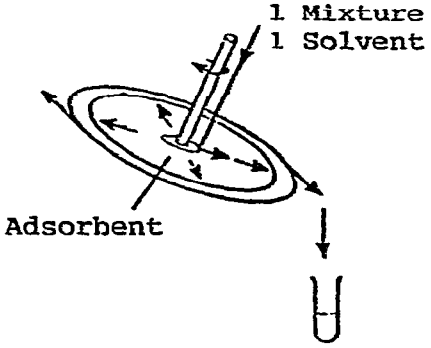
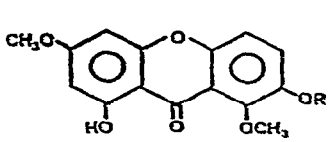


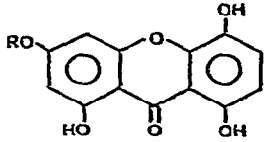
Fig. 1. Principle of centrifugal TLC.

Isolation of xanthone aglycones, which are strong inhibitors of monoamino oxidase[7], from American *Gentiana* species[8] was carried out by centrifugal TLC. A crude chloroform extract of *Gentiana detonsa* Fröl. (400 mg) chromatographed with chloroform as eluent furnished a first fraction of the least polar constituents (fatty acids, pigments, etc.), followed by decussatin (1) (7 mg) and gentiacaulein (2) (12 mg) within 20 min.

A mixture (120 mg) of bellidifolin (3) and desmethylbellidifolin (4) obtained after acid hydrolysis of a methanol extract of *Gentiana strictiflora* (Rydb.) A. Nels afforded pure compound 3 (55 mg) and pure compound 4 (48 mg) in less than 30 min. Chloroform with increasing amounts of methanol was used as the solvent.



1 : R = CH_3: decussatin
2 : R = H: gentiacaulein



3 : R = CH_3: bellidifolin
4 : R = H: desmethylbellidifolin

Separation of xanthone aglycones from *Gentiana* species was previously carried out[9] by polyamide open-column chromatography with methanol–water–acetic acid (90:5:5) and required at least one full day (column packing not included).

We also employed centrifugal TLC for the purification of saponin hydrolysates. A mixture (80 mg) of oleanolic acid and hederagenin could easily be separated. Chloroform–methanol (99.5:0.5) was used to obtain oleanolic acid, whereas for the

elution of the more polar hederagenin, we switched to chloroform–methanol (98:2). The separation was completed in less than 20 min and about 30 mg of each sapogenin was obtained in pure form.

From a column chromatographic fraction (359 mg) of a ginseng extract, it was possible to isolate 50 mg of ginsenoside Rg_1 and 75 mg of ginsenoside Rd with chloroform-methanol-water (100:30:3). However, no baseline separation could be achieved. Preliminary assays showed that centrifugal TLC is probably more suitable for the separation of less polar saponins possessing only one or two sugars, such as *Hedera* saponins[10].

Selection of the solvent is based on the TLC behaviour of the sample to be separated, but R_F values should be less than 0.5. We noticed that if the R_F values are higher than 0.5 on analytical TLC plates, and even with $\Delta R_F = 0.1$–0.2, the sample was eluted too quickly from the centrifugal TLC instrument and no separation occurred. One should start with a relatively weakly polar solvent systems (R_F *ca.* 0.1) and increase the polarity during the separation.

CONCLUSION

Centrifugal TLC is a simple and very rapid technique for the preparative separation or purification of natural products in the range from several milligrams to 0.5 g. It can replace preparative TLC (no scraping of bands to recover the sample) and, in some instances, column chromatography. The method is economical as the consumption of solvent is very small (generally less than 150 ml) and the coated rotors can be regenerated for re-use. However, the resolution is limited and cannot be compared with that in HPLC. There is also a restriction in the choice of stationary phases. Recently, a procedure has been developed for converting silica gel layers with starch binder into octadecyl reversed-phase layers[11]. The method is based on that described by Gilpin and Sisco[12] for the preparation of chemically bonded TLC plates. This will greatly increase the versatility of the technique and make it more suitable for the separation of polar substances. When used in combination with other chromatographic methods, preparative centrifugal TLC can be of great help for the isolation of natural products.

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