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## Note

### Separation of non-polar compounds by droplet counter-current chromatography

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Droplet counter-current chromatography (DCCC) is a widely used technique for the separation or enrichment of natural compounds<sup>1</sup>. Because of the requirement to form droplets, especially water-containing solvent systems have been developed, although some studies with non-aqueous solvent systems have been made<sup>2–4</sup>. During our investigations on non-polar compounds from anise fruits we found that a non-aqueous solvent system of *n*-hexane and methanol can be used in DCCC. This binary solvent system is easy to handle and can be used even in the descending and the ascending modes. Its capability is shown by the separation of an extract from anise fruits and additionally by the separations of some synthetic mixtures containing non-polar compounds with very similar  $R_F$  values in thin-layer chromatography (TLC).

#### EXPERIMENTAL

##### *Apparatus*

For DCCC a DCCC-A instrument (Rikakikai, Eyela Tokyo, Japan), 300 columns (2 mm I.D.), an LKB 2138 Uvicord S detector, operating at 254 nm (range 0.2), an LKB 7000 Ultracrac fraction collector, and a two-channel recorder LKB 2210 (speed 0.2 mm/min, 100 mV) were used. For gas chromatography (GC) a Perkin-Elmer F 22 chromatograph, a fused-silica-OV-1-CB column (50 m × 0.25 mm I.D., 0.35 μm) (Chrompack, Frankfurt, F.R.G.) and a carrier gas (nitrogen) flow-rate of 1 ml/min, 1.5 bar at 60°C were employed. The temperature of the injection port was 300°C, that of the oven was 60°C raised at 6°C/min to 220°C. Flame ionization detection (FID): air flow-rate 350 ml/min, 3 bar; hydrogen flow-rate 35 ml/min, 2 bar; attenuation 8, range 1; split 1:35; recorder PE-56, 2.5 mV, 10 mm/min.

##### *Reagents*

Anethole (Merck, Darmstadt, F.R.G.), safrole (Roth, Karlsruhe, F.R.G.), anisaldehyde (Merck), myristicine (isolated from the essential oil of nutmeg), foeniculine (isolated from the essential oil of star anise fruits) and pseudoisoeugenyl-(2-methylbutyrate) (isolated from the essential oil of anise fruits) were used. The solvents *n*-hexane and methanol (Merck, pure grade) were distilled once.

#### *Preparation of solvent system*

*n*-Hexane and methanol were mixed in a separating funnel and shaken intensively. After 1 h the two phases were separated and used for the DCCC experiments.

#### *Preparation of the anise extract*

Spanish anise fruits were extracted by percolation with light petroleum (b.p. 50–70°C). After evaporation of the solvent the crude extract was treated by steam distillation under atmospheric pressure. The distillate obtained was investigated by GC. A 1- $\mu$ l volume of a solution of the distilled oil (1% in *n*-hexane) was injected. Retention times (min) and contents (%) were: sesquiterpenes (10.88, 1.5); anethole (11.15, 87); anisaldehyde (13.80, 0.5); pseudoisoeugenyl-(2-methylbutyrate) (23.23, 3).

#### *Preparation of sample solutions for DCCC*

A 102-mg amount of the anise extract was diluted in 18 ml of a mixture of the upper and lower phases (ratio 1:1) of the solvent system. A 50-mg amount of each pair of the synthetic mixtures (ratio 1:1) was prepared in the same manner: I, pseudoisoeugenyl 2-methylbutyrate/anisaldehyde; II, myristicine/safrole; III, foeniculine/anethole; IV, foeniculine/safrole.

#### *DCCC separation procedures*

The upper phase of the solvent system was used as the stationary phase. An 18-ml volume of sample solution was injected into the sample reservoir. The lower phase of the solvent system was used as the mobile phase.

*Separation of the anise extract.* The flow-rate of the mobile phase was 20 ml/h; fractions of 7 ml were collected.

*Separation of the synthetic mixtures.* The flow-rate was 16 ml/h; fractions of 5 ml were collected.

#### *TLC analysis procedure*

The conditions were as follows: silica gel 60 (aluminium foils, Merck); toluene; anisaldehyde-sulphuric acid reagent (0.5 ml anisaldehyde, 10 ml CH<sub>3</sub>COOH, 85 ml methanol, 5 ml H<sub>2</sub>SO<sub>4</sub>), 100°C.

## RESULTS

First we investigated an extract from the fruits of anise. The fractionation of this extract was carried out using the solvent system *n*-hexane-methanol and the descending mode. Fig. 1 shows the result. The extract contained mainly anisaldehyde, pseudoisoeugenyl-(2-methylbutyrate), anethole and some sesquiterpenes. By using the present solvent system the separation of all these compounds was possible. Additionally, we obtained some fractions containing a not yet identified compound (D-1). The thin-layer chromatogram (Fig. 1) of this extract yielded  $R_F$  values from 0.20 to 0.95 for the diverse compounds.

We were interested in the capability of the solvent system for the separation of compounds with more similar  $R_F$  values. For the synthetic mixtures, four pairs (I–IV)

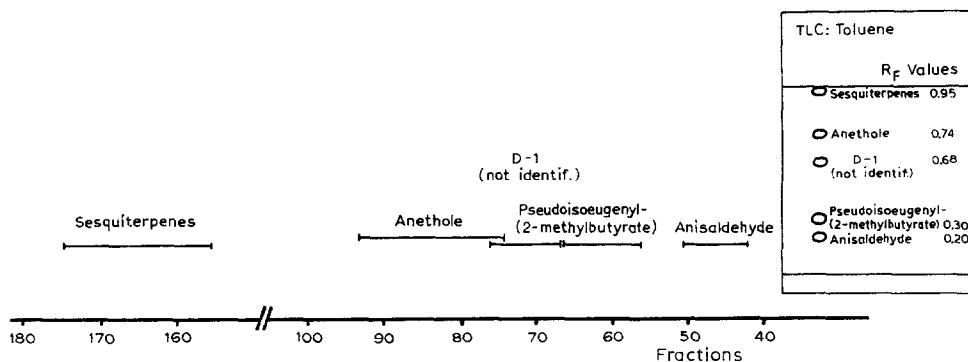


Fig. 1. DCCC elution chromatogram of an extract from the fruits of anise (102 mg) monitored at 254 nm, and TLC of this extract (silica gel, toluene).

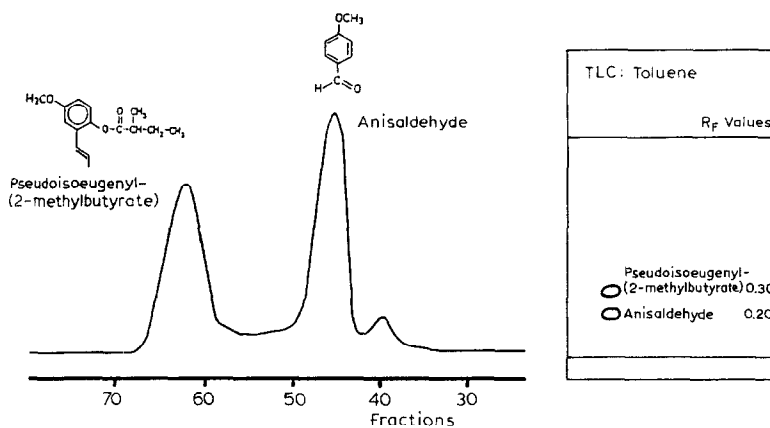


Fig. 2. DCCC elution chromatogram of a mixture of pseudoisoeugenol-(2-methylbutyrate) and anisaldehyde (50 mg, 1:1) monitored at 254 nm, and TLC of this mixture (silica gel, toluene).

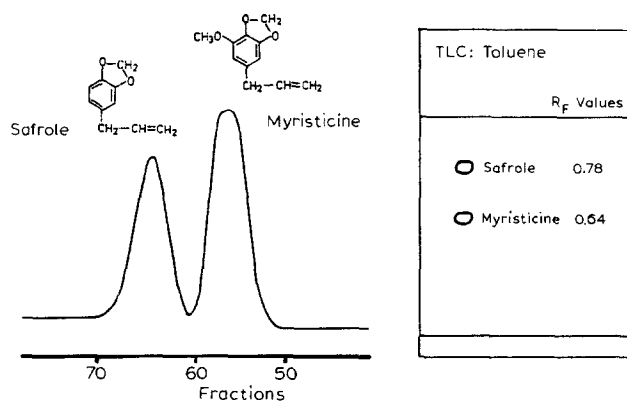


Fig. 3. DCCC elution chromatogram of a mixture of myristicine and safrole (50 mg, 1:1) monitored at 254 nm, and TLC of this mixture (silica gel, toluene).

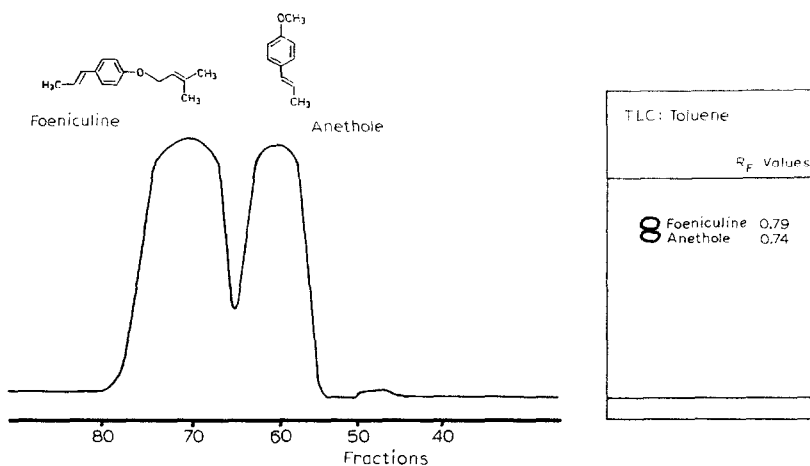


Fig. 4. DCCC elution chromatogram of a mixture of foeniculine and anethole (50 mg, 1:1) monitored at 254 nm, and TLC of this mixture (silica gel, toluene).

of non-polar substances were used, some of them (\*) occurring naturally in the fruits of anise: I, pseudoisoeugenyl-(2-methylbutyrate)(\*)/anisaldehyde(\*); II, myristicine/safrole; III, foeniculine/anethole(\*) and IV, foeniculine/safrole. From each pair, 50 mg were taken for the DCCC separations. The ratio of the compounds in each pair was 1:1. The separations of these samples were also carried out by using the solvent system *n*-hexane-methanol in the descending mode. Fig. 2 shows the result for the first pair. The differences in their  $R_F$  values in TLC with toluene as the solvent is 0.1. In the DCCC experiment a very good baseline separation was obtained. The second pair (Fig. 3) shows a greater difference in its  $R_F$  values of about 0.24. The DCCC separation was not as good as that of the first pair, but a baseline separation was achieved. The components of the third pair show very little differences in their  $R_F$

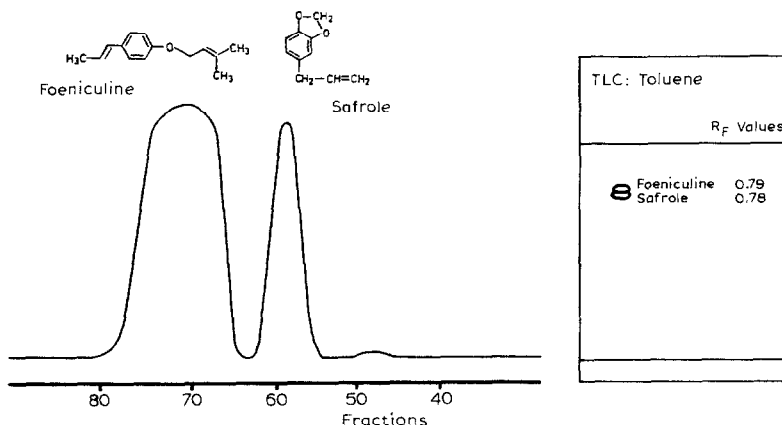


Fig. 5. DCCC elution chromatogram of a mixture of foeniculine and safrole (50 mg, 1:1) monitored at 254 nm, and TLC of this mixture (silica gel, toluene).

values. In toluene the difference is only about 0.05. However, even this pair was separated sufficiently with DCCC and the present solvent system (Fig. 4). The compounds of the fourth pair show the most similar  $R_F$  values in TLC, the difference being only about 0.01. The capability of the present solvent system was demonstrated by a sufficient separation of the components of this pair (Fig. 5).

#### CONCLUSIONS

A solvent system suitable in DCCC in the non-aqueous mode for the separation of non-polar compounds is presented. This binary solvent system containing *n*-hexane and methanol forms droplets sufficiently. Its capability is shown by the separation of several compounds from an extract from the fruits of anise. Some synthetic mixtures of compounds with very similar  $R_F$  values (TLC, toluene) can be separated quantitatively.

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