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Note

Water-free solvent system for droplet counter-current chromatography and its suitability for the separation of non-polar substances

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With the development of new designs of apparatus, liquid–liquid chromatographic methods[1] have made a come-back. Of these methods, droplet counter-current chromatography[2] (DCCC) has been widely used and has proved successful in the separation of many natural products. Up till now, the limitations of the method have been that only relatively polar substances can be separated and also that there is a danger of hydrolysis occurring in the aqueous solvent systems, especially when relatively long separation times of between 1 day and 1 week are employed. Water-free solvent systems have been difficult to establish because they need to meet the main condition for DCCC: the formation of suitable droplets having more or less the diameter of the tubes. Using as a guide the work by Hecker[3] on partition chromatography, we have recently developed a water-free system that satisfies this condition.

Preliminary experiments[4] have shown the system to be suitable for the separation of non-polar valepotriates and of the essential oil of *Echinacea angustifolia*. However, these results need to be confirmed by the separation of a complex, but nevertheless well defined, mixture of natural compounds. We therefore selected the essential oil of chamomile (*Matricaria chamomilla*), the components of which (Fig. 1) can be qualitatively and quantitatively examined by gas chromatography (GC)[5].

MATERIALS AND METHODS

Apparatus

A DCC-A instrument (Tokyo Rikakikai Co., Tokyo, Japan) with 300 columns (0.2 mm I.D.) was employed. The pressure was 4 kg/cm^2 and each fraction consisted of 120 drops collected at a rate of 4 drops per min.

Essential oil of Matricaria chamomilla

Flowers of *M. chamomilla* (from Argentina) were distilled in 10-g portions, according to the European Pharmacopoeia. The essential oil from each distillation was collected in 2 ml of pentane, the solution was dried, and the pentane was evaporated under nitrogen.

Gas chromatography

The total essential oil and the combined fractions were chromatographed using

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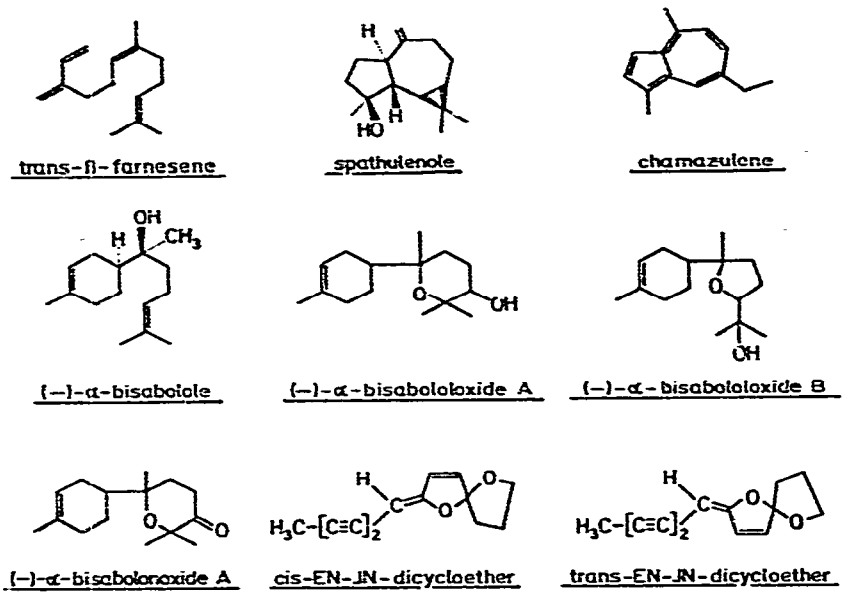

Fig. 1. Known components of the essential oil of *M. chamomilla*.

a Varian chromatograph fitted with a Methyl-Silicon (fused-silica capillary) 19091-60050 column (Hewlett-Packard, Avondale, PA, U.S.A.) of length 50 m. The following conditions were employed: injection temperature, 230°C; detection temperature, 250°C; temperature programmed to 140°C at 4°C/min; pressure, 1.5 bar; sensitivity, $36 \cdot 10^{-11}$; injection volume, 0.2 μl. For the total essential oil, guajazulene was used as internal standard. The purity of the combined fractions was calculated from the peak areas.

Thin-layer chromatography

Each fraction was examined by thin-layer chromatography (TLC) on silica gel 60 G (E. Merck, Darmstadt, G.F.R.) using 10 × 6.6 cm plates. For conditions, see ref. 5. Identical fractions were combined.

RESULTS AND DISCUSSION

The essential oil of *M. chamomilla* was found to have the constitution shown in Table I.

After analysis by TLC, the essential oil was split into ten fractions which were examined by GC. The main components are listed in Table II. As can be seen, four fractions have compounds with a purity greater than 90%. This means after just one run, four of the seven known constituents of the mixture can be isolated almost pure. The amounts of these compounds, starting from 0.75 g of essential oil (*i.e.* farnese, 24.3 mg; chamazulene, 40.5 mg; spathulenole, 15 mg; bisaboloxide B, 36.2 mg) would be enough to enable the necessary spectra for structural elucidation to be run.

If the purity is insufficient for this purpose, a second run can be performed with

TABLE I

COMPOSITION OF ESSENTIAL OIL OF *MATRICARIA CHAMOMILLA*

Guajazulene as internal standard.

Constituent	*Content (mg per 100 ml)*
Farnesene	12
Bisabololoxide B	35
Bisabolole	6
Spathulenole	1.2
Bisabololoxide A	7.8
Chamazulene	6.1
cis-EN-IN-Dicycloether	6.4

either the same or a different solvent system. After pre-screening on a silica plate, according to the method of Hostettmann[2], we chose the system *n*-hexane–ethylacetate–nitromethane–methanol (9:1.5:4.5:1.5, v/v), with the upper phase as mobile phase. Fractions g + h (90–104) from the first run (0.1568 g) gave the fractions shown in Table III.

The essential oil obtained from steam distillation of chamomile always contains fatty acids. After a DCCC separation using 750 mg of volatile oil, 613 mg were recovered from the upper phase; over 90% of the fatty acids (*ca.* 130 mg) remained in the lower phase together with only a small amount of the volatile oil.

TABLE II

COMBINED FRACTIONS OF THE FIRST DCCC RUN

n-Hexane–ethyl acetate–nitromethane–methanol (9:2:2:3) as eluent; upper phase as mobile phase.

Fraction		*Weight (mg)*	*Main component(s)*	*Purity according to GC peak area (%)*
a	(1–21)	153	Farnesene	26
b	(22–38)	24.3	Farnesene	96
c	(39–55)	9.8	Farnesene	58
d	(56–72)	40.5	Chamazulene	96
e	(73–84)	15.0	Spathulenole	93
f	(85–89)	8.7	Bisabolole, bisabololoxide B, spathulenole	–
g	(90–98)	80.0	Bisabololoxide B	69
			Bisabolole	30
h	(99–104)	76.8	Bisabololoxide B	80
			Bisabolole	10
i	(105–106)	17.4	Bisabololoxide B	98
j	(107–109)	18.8	Bisabololoxide B	86
k	(110–113)	12.4	Mixture + bisabololoxide A	5
l	(114–123)	13.9	Mixture EN–IN–dicycloether	–
m	(124–282)	142	Mixture	–

TABLE III

FRACTIONS OF THE SECOND DCCC RUN OF FRACTIONS g AND h

n-Hexane–ethyl acetate–nitromethane–methanol (9:1.5:4.5:1.5) as eluent; upper phase as mobile phase.

Fraction		*Weight (mg)*	*Main component(s)*	*Purity according to GC peak area (%)*
0	(0–71)		–	–
A	(72–75)	0.6	Bisabolole	100
B	(76–78)	2.0	Bisabolole	53
			Bisaboloxide B	10
C	(79–84)	6.2	Bisabolole	78
			Bisabololoxide B	22
D	(85–89)	16.6	Bisabololoxide B	98
			Bisabolole	2
E	(90–93)	15.9	Bisabololoxide B	100
F	(94–102)	8.7	Bisabololoxide B	94
G	(103–113)	1.8	Bisabololoxide A	80

The constituents of the volatile oil showed a somewhat different elution pattern with DCCC from that obtained with GC. DCCC gives a separation according to the polarity of the components, *i.e.* farnesene is eluted first followed by chamazulene and spathulenole, with the oxygenated bisaboloids being eluted last. In GC, farnesene has the shortest retention time, followed by bisabololoxide B, bisabolole, bisabololoxide A, chamazulene and finally *cis–trans*-EN-IN-dicycloether.

On the basis of these results, DCCC with a water-free solvent system appears to be an appropriate method for the separation of apolar substances.

REFERENCES

1 Y. Ito and R. L. Bowman, *J. Chromatogr. Sci.*, 8 (1970) 315–323.
2 K. Hostettmann, *Planta Med.*, 39 (1980) 1–19.
3 E. Hecker, *Verteilungsverfahren im Laboratorium*. Verlag Chemie, Weinheim, 1955.
4 H. Becker, W. Ch. Hsieh and Ch. D. Vereiis, *Supplement Chromatographie*, G.I.T., Darmstadt, 1981, pp. 34–40.
5 J. Reichling and H. Becker, *Deut. Apoth.-Ztg.*, 117 (1977) 275.