Journal of Chromatography, 446 (1988) 157–161
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1368

ISOLATION OF PARTHENOLIDE BY DROPLET COUNTER-CURRENT CHROMATOGRAPHY

Á. KÉRY*, Gy. TURIÁK and P. TÉTÉNYI
Research Institute for Medicinal Plants, Budakalász (Hungary)

SUMMARY

The advantages and potential of droplet counter-current chromatography in the semi-preparative- and preparative-scale isolation of parthenolide and related sesquiterpene lactones was studied. Two systems, benzene–chloroform–methanol–water and chloroform–trichloroethylene–acetonitrile–methanol–water, were found to be suitable for the isolation of sesquiterpene lactones from Asteraceae plants.

INTRODUCTION

Plant species in the Asteraceae (Compositae) family characteristically contain sesquiterpene lactones as biologically active secondary metabolites, which occur only sporadically in other Angiosperm families[1]. Reports dealing with their isolation and structural elucidation have increased dramatically in recent years. As a result, the number of the currently known naturally occurring sesquiterpene lactones is around 1500 (ref. 2). Many of the newly isolated sesquiterpene lactones have an intensely bitter taste and are frequently the genuine bitter principles and possibly also the physiologically active agents of some drugs, and new information about their biological properties is accumulating. The substances have proved to be insecticides, stomachics, vermifuges, vertebrate and fish poisons, allergens, etc.

Many sesquiterpene lactones exhibit antitumour and cytotoxic activity, effecting selective alkylation on enzymes controlling cell division. Antibacterial and antimalarial activities of these compounds are also well known[3–5].

A variety of chromatographic methods are available for the analytical separation of sesquiterpene lactones[6–9]. The isolation of these compounds on even a moderate scale has been largely limited to the use of common column chromatography, combined with time-consuming preparative thin-layer chromatography (TLC). Droplet counter-current chromatography (DCCC) has previously been applied to the separation of a number of classes of polar and semipolar compounds, including various glycosides, peptides and alkaloids[10]. There have been some promising attempts to use DCCC for separating germacranolide (*Zoegea leptaurea* L.) and guaianolide (*Centaurea behen* L.) types of sesquiterpene lactones[11,12]. It therefore

* Present address: Institute of Pharmacognosy, Semmelweis Medical University, Üllői út 26.III, 1085 Budapest, Hungary.

0021-9673/88/$03.50

seemed worthwhile to apply this powerful technique to the preparative-scale separation of sesquiterpene lactones.

The major problems associated with the chromatography of these compounds are the presence of structurally closely related molecules within one plant species and the instability of certain sesquiterpene lactone groups during adsorption chromatography. Secondary reactions, induced by the column material, *e.g.*, isomerization of double bonds, cyclization and dimerization, may occur during prolonged adsorption. Moreover, some sesquiterpene lactones form artefacts during chromatography on silica gel (*e.g.*, cyclodecadiene ketone is converted into guaianolide on contact with silica gel for 1 week)[1]. The utility of DCCC for the preparative-scale isolation of parthenolide (germacranolide) from samples of various Asteraceae plants (*Tanacetum vulgare* L., *Chrysanthemun parthenium* Bernh.) was investigated in this work and the method is described in this paper.

EXPERIMENTAL

Droplet counter-current chromatography

DCCC was carried out using a Model A DCC chromatograph (Tokyo Rikakikai, Tokyo, Japan); the instrument was fitted with 300 glass capillaries (400 × 2 mm I.D.), connected in series. A mobile-phase flow-rate of *ca.* 10–12 ml/h was maintained throughout the experiments. Individual fractions of 2.5 ml were collected by means of an automatic fraction collector (Multirac Model 2111; LKB, Stockholm, Sweden). Two solvent systems were used: (1) benzene–chloroform–methanol–water (5:5:7:2) (BCMW), ratio of upper layer to lower layer = 9.6:10.4) and (2) chloroform–trichloroethylene–acetonitrile–methanol–water (2:4:2:5:2) (CTAMW), ratio of upper layer to lower layer = 7.9:7.1.

Direct comparison of the TLC separation results obtained by developing silica gel (Kieselgel 60 DC Alufolien, 0.2 mm; Merck, Darmstadt, F.R.G.) and reversed-phase silica gel (Kieselgel RP-18 F 254 S; Merck) plates with the layers formed by the two solvent systems were used to judge which layers gave the better separation of parthenolide from other compounds in plant samples. The results suggested that the organic (lower) layers were more promising as mobile phases. The aqueous (upper) layers require longer elution times and hence more solvent.

Analysis

Partition of the constituents in crude extracts between the upper and the lower phases was studied by distributing the same amount (30 mg) of samples between a 1:1 ratio of the upper and lower layers of the BCMW and CTAMW systems. After equilibration for 1 h at 25°C with stirring and subsequent separation, the two phases were evaporated to dryness under reduced pressure and the ratios (milligrams in upper layer to milligrams in lower layer) were calculated.

The procedure was followed by quantitative TLC densitometry of the characteristic constituents (parthenolide, artemorin). Aliquots of each sesquiterpene lactone fraction were applied to thin-layer plates, coated with HPTLC silica (Kieselgel 60 HP-TLC-Alufoline; Merck) and the layers were developed with benzene–*n*-hexene–acetonitrile (30:20:21).

The amounts and relative amounts of the sesquiterpene lactone components

were determined with parthenolide and artemorin standards by means of densitometry, using a Shimadzu dual-wavelength thin-layer chromatoscanner, with built-in recorder and integrator (Simadzu, Kyoto, Japan). Sulphuric acid and phosphomolybdic acid were used as developing reagents with heating at 100°C for 10 min, and the densitometer was set at 520 and 600 nm, respectively.

Plant samples

The parthenolide sources used were Asteraceae plant extracts. Dried, powdered aerial plant parts (500 g) were extracted with 3 × 4 l of chloroform at room temperature for 5 h each. The chloroform extracts were then mixed and evaporated to dryness at 40°C under reduced pressure. The residues were dissolved in 100 ml of ethanol (96%, v/v)–water (1:1), then lead acetate solution (4%, w/w) was added drop by drop to precipitate the ballast substances. After 10 h the solution was centrifuged until transparent. The clear liquid was concentrated (40 ml) under vacuum and extracted with 4 × 50 ml of chloroform. The chloroform extracts were then mixed and dried (with anhydrous sodium sulphate), filtered and evaporated to dryness under reduced pressure. The crude samples were checked for parthenolide and other sesquiterpene lactones by TLC and IR spectroscopy in chloroform (Spectromom 2000; MOM, Budapest, Hungary) in a 0.4-mm cell. The total lactone and parthenolide contents were compared with a parthenolide standard by means of densitometry and IR spectroscopy, respectively.

Prior to model DCCC experiments, 1 g of the crude samples was dissolved in a 1:1 mixture of the phases to be used in chromatography. Parthenolide was identified by standard procedures, and the identity was confirmed by spectroscopic techniques (UV, IR, ^{1}H NMR and mass spectrometry) and comparison with authentic samples[13,14].

RESULTS AND DISCUSSION

When crude extracts of sesquiterpene lactones containing parthenolide were partitioned between the upper and lower phases of the BCMW and CTAMW systems, the distribution ratios showed clearly that the components of the plant extracts differ in polarity.

Having subjected the sample to DCCC using the BCMW and CTAMW systems with the upper layers as stationary phases and the organic layers as mobile phases, parthenolide was eluted with the first 130 (95–125) ml and 90 (55–85) ml of the mobile phases, respectively. All compounds were eluted with 250 ml of mobile phases in both systems (Fig. 1). The separation of parthenolide was regarded as satisfactory with the BCMW and CTAMW systems with the organic layers as mobile phases, as they formed distinct zones with very little overlap. For the separation of other compounds (minor sesquiterpene lactones) the systems were also sufficiently discriminatory, although the elution of more polar compounds was delayed. Better separation was achieved with the newly developed CTAMW system.

When the same systems were tried in the alternative mode, *i.e.*, with the organic layers as stationary phases, the more polar compounds were eluted within a reasonable time and with a reasonable volume of solvents, but the elution of parthenolide was very time consuming, as large elution volumes were required.

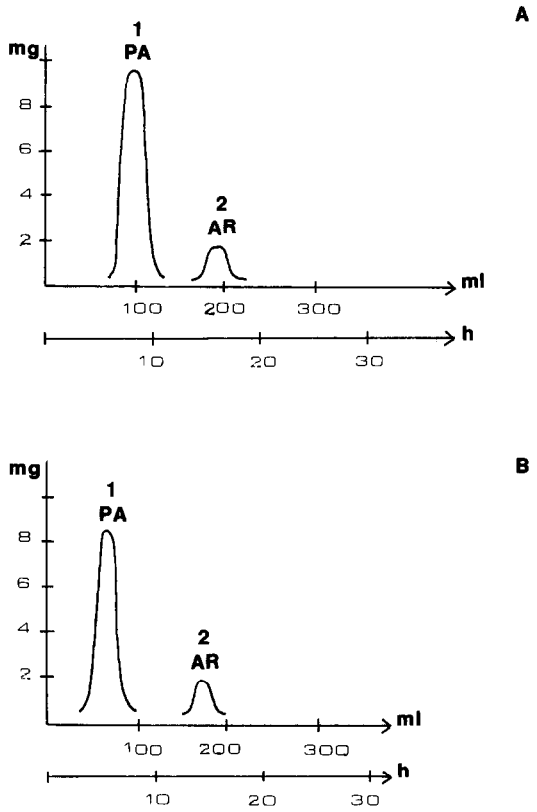

Fig. 1. DCCC of sesquiterpene lactones using (A) benzene–chloroform–methanol–water (5:5:7:2) and (B) chloroform–trichloroethylene–acetonitrile–methanol–water (2:4:2:5:2) as solvent systems. The amount in mg per tube is plotted against the volume (ml) of mobile (organic) phase. Peaks: 1 = parthenolide (PA); 2 = artemorin (AR).

In order to test the reproducibility and usefulness of the two systems for preparative work, the same and larger amounts (2 g) of crude extracts were subjected to DCCC, using BCMW and CTAMW with the organic layers as mobile phases. The various zones were eluted in the same fractions, but the amount of contaminants in each zone increased then 2 g of samples were introduced. The results indicate that 1.7 g of these sesquiterpene lactone mixtures (rich in parthenolide) represents close to the limit of the capacity for these systems. However, considering the relatively large amount of parthenolide separated in a single chromatographic step (180–300 mg), the minor production of artefacts during the isolation procedure and the relatively modest amounts of solvents needed for the separations, the results can be regarded as satisfactory.

The results reported here indicate that the solvent system benzene–chloroform–methanol–water (5:5:7:2) and particularly the newly developed chloroform–trichloroethylene–acetonitrile–methanol–water (2:4:2:5:2) system, give satisfactory separations of germacranolides (parthenolide, etc.) by DCCC. Our experience suggests that the lower (organic) layers of the two solvent systems should

be used as the mobile phase for the less polar parthenolide and related compounds (artemorin, etc.), whereas the upper layers of the systems may be preferable for the more polar sesquiterpene lactones.

REFERENCES

1 N. H. Fisher, E. J. Olivier and H. D. Fisher, in W. Herz, H. Grisebach and G. W. Kirby (Editors), *Progress in the Chemistry of Organic Natural Products: the Biogenesis and Chemistry of Sesquiterpene Lactones*, Springer-Verlag, Vienna, New York, 1979, pp. 48–388.
2 J. Harmatha and I. Nawrot, *Biochem. Syst. Ecol.*, 12 (1984) 95.
3 E. Rodriquez, G. H. N. Towers and J. C. Mitchell,*Phytochemistry*, 15 (1976) 1573.
4 S. M. Kupchan, J. W. Ashmore and A. T. Sneden, *J. Pharm. Sci.*, 67 (1978) 865.
5 K. H. Lee, T. Ibuka, R. Y. Wu and T. A. Geissman, *Phytochemistry*, 16 (1977) 1177.
6 B. Drodz and E. Bloszyk, *Planta Med.*, 33 (1978) 379.
7 A. K. Picman, R. L. Ranieri, G. H. N. Towers and J. Lam, *J. Chromatogr.*, 189 (1980) 187.
8 B. Marchand, H. Behl and E. Rodriquez, *J. Chromatogr.*, 265 (1983) 97.
9 H. Pysalo, E.-L. Seppä and K.-G. Widén, *J. Chromatogr.*, 190 (1980) 466.
10 K. Hostettmann, M. Hostettmann-Kaldas and O. Sticher, *J. Chromatogr.*, 186 (1979) 529.
11 Á. Kéry, *4th Danube Symposium on Chromatography, Bratislava (1983)*, Abstract Volume, p. 24.
12 Á. Kéry, A. M. Husain and A. A. Al-Jeboory, *Advances in Liquid Chromatography, Szeged (1984)*, Abstract Volume, p. 50.
13 M. Soucek, V. Herout and F. Sorm, *Collect. Czech. Chem. Commun.*, 26 (1961) 803.
14 H. Yoshioka, W. Renold, N. H. Fischer, A. Higo and T. J. Mabry, *Phytochemistry*, 9 (1970) 823.