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Thin-layer Chromatography of Plant Extracts

III. Countercurrent Separation of Plant Extracts

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Plant extracts can be divided into fractions, suitable for thin-layer chromatography, with a simple manual countercurrent separation, using only about three separatory funnels.

Lipophilic extracts were separated using immiscible phases obtained by mixing water, methanol and one or more of the following solvents: benzene, cyclohexane and diethyl ether. Extracts, intermediate in polarity, were partitioned between ether and water. The material in a water fraction so obtained was divided into two fractions, one extractable with a mixture of chloroform and ethanol from a water solution, half saturated with sodium sulfate, and one residual fraction.

The present study is concerned with the feasibility of using countercurrent separation in order to obtain fractions of plant extracts, suitable for thin-layer chromatography.

Countercurrent technique has been reviewed by Hecker,[1] by Jübermann,[2] and by Metzsch,[3] and procedures useful particularly for the present purpose have been described by O'Keeffe *et al.*[4] and by Hultin.[5] In these papers rapid manual procedures are given, for which only a few separatory funnels are needed.

A two-phase system obtained by mixing benzene and 80 % ethanol has been used previously for lipophilic substances.[6] In the present work a mixture of benzene, methanol and water 2:1:1 was found useful for the separation of a lipophilic plant extract into two fractions. These fractions were analyzed by thin-layer chromatography, using several solvent mixtures. The results are shown in Fig. 1. It is evident that several commonly-used solvent mixtures for thin-layer chromatography produce reasonably well separated groups of spots on the chromatograms, indicating that this countercurrent separation was efficacious. The solvent system cyclohexane, ether, methanol and water 9:6:10:5 was also quite efficient.

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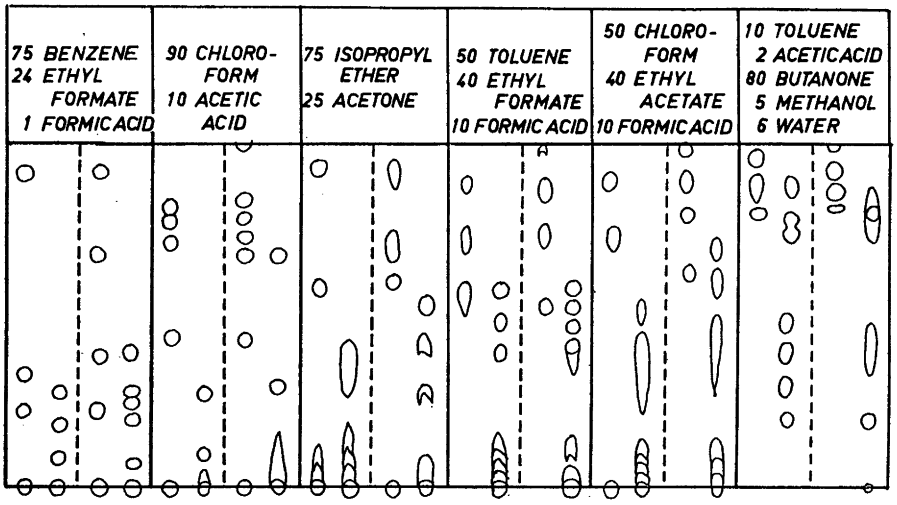

Fig. 1. Thin-layer chromatograms showing the efficaciousness of simple manual countercurrent separations for fractionating a lipophilic *Mortonia scabrella* extract. Fractions obtained using a solvent system consisting of benzene, methanol and water are spotted on the left half of the chromatoplates, and fractions obtained using a solvent system consisting of cyclohexane, ether, methanol and water are spotted on the right half. The less polar fractions of each sample are to the left of the more polar fractions. The six chromatograms were developed with solvent mixtures of the volume composition listed above each drawing, and were visualized with iodine vapor.

The primary separation of a plant extract into one lipophilic and one hydrophilic fraction is normally done with either chloroform and water or with ether and water. In the course of this work, the system consisting of ether and water has been used predominantly. It is evident from Fig. 2 that the separation of the fractions is satisfactory for thin-layer chromatography.

A mixture of chloroform and ethanol (3:2 v/v) has been suggested [7,8] and extensively used for extracting glycosides from a water solution, half saturated with sodium sulfate, and this procedure has been tried here for dividing a hydrophilic preparation into two fractions which, after they have been recovered and spotted for thin-layer chromatograms, give fairly well separated groups of spots, if suitable solvent mixtures are used for the development, as shown in Fig. 3.

Most of the various systems of immiscible solvents mentioned here are rather flexible. The volume ratio between their two phases can without inconvenience be changed over a wide range, *e.g.*, from 1:3 to 3:1. The composition of the two phases of solvent mixtures with at least three components can be gradually changed, usually also over a large interval. Thus, the various degrees of polarity between fractions obtained in countercurrent separation can be chosen almost at will. For routine analyses by thinlayer chromatography one must find at least one suitable solvent mixture for the development of each chromatogram. As shown in Figs. 1—3 one can expect often to find more than one.

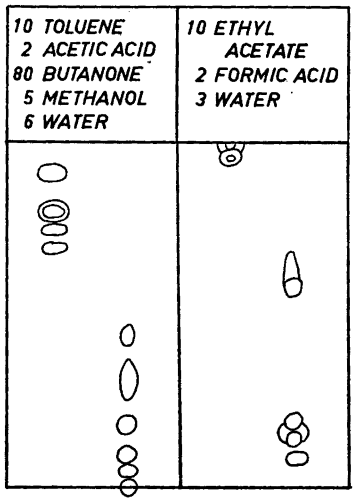

Fig. 2. Thin-layer chromatograms showing the efficaciousness of simple manual countercurrent separations. A methanol extract of Senna leaves, *Cassia* sp. was countercurrent separated with ether and water. The two fractions were spotted on Silica Gel G chromatoplates, the less polar fraction to the left, and developed with solvent mixtures of the volume composition listed above each drawing. Spots were visualized in one experiment with a 1 % solution of potassium hydroxide in ethanol, and in another experiment with a 1 % solution of diphenylboric acid β-aminoethyl ester in methanol.

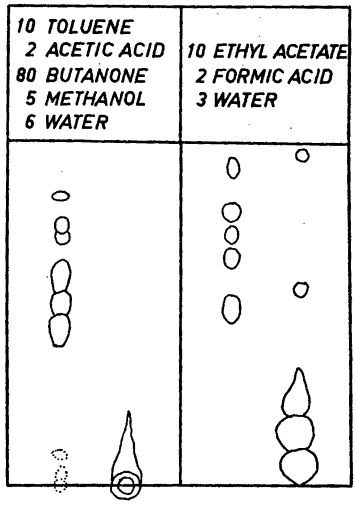

Fig. 3. Thin-layer chromatograms showing the efficaciousness of simple liquid-liquid extractions and showing how the relatively more and less polar parts of a rather hydrophilic fraction of a *Feijoa sellowiana* extract are developed by each of two different solvent mixtures of the volume composition listed above each drawing. The most polar fraction is spotted to the right.

For the best results a thin-layer chromatographic procedure should correspond to a fraction so closely that almost nothing remains at the starting point, and almost nothing follows the front. This work concerns only the efficiency of the separation as such, and it can be seen from Fig. 1 that if two adjacent fractions are spotted on several chromatoplates for development with each of several solvent mixtures in a series with increasing dielectric constant, it may be possible to select one or a few solvent mixtures by which even the fastest moving component of the more hydrophilic fraction does not move completely with the front.

EXPERIMENTAL

Plant material. Green parts of *Feijoa sellowiana* Berg (Myrtaceae) and *Mortonia scabrella* A. Grey (Celastraceae) were collected in Boyce Thompson Southwestern Arboretum. Leaves of *Nerium oleander* L. (Apocynaceae) were collected in Los Angeles. Senna leaves, *Cassia* sp. (Leguminosae) were obtained as a powdered, commercial product from Horton & Converse, Los Angeles, Calif.

Countercurrent separation of a lipophilic plant extract. Air-dried leaves of *Mortonia scabrella* (a species which contains particularly large amounts of lipophilic substances) which had been defatted with pentane in a Soxhlet apparatus were extracted with the cyclohexane and acetone azeotrope [9] and the extracted material recovered by evaporating the solvent mixture in a rotating evaporator in vacuum. One sample was fractionated by countercurrent separation using 3 separatory funnels and equal volumes of the two phases from a mixture of benzene, methanol, and water 2:1:1 v/v, and another sample was similarly fractionated, using equal volumes of the two phases from a mixture of cyclohexane, ether, methanol and water 9:6:10:5 v/v. The material in the various fractions was recovered by evaporation and spotted on Silica Gel G chromatoplates. The result obtained after development with various solvent mixtures for thin-layer chromatography is shown in Fig. 1.

Countercurrent separation of a plant extract intermediate in polarity. Senna leaves, *Cassia* sp. were extracted with methanol and the extracted material countercurrent separated into two fractions using equal volumes of the two phases obtained from a mixture of diethyl ether and water. The two fractions were spotted on Silica Gel G chromatoplates. The result obtained after development with two different solvent mixtures is shown in Fig. 2.

Fractionation of a hydrophilic plant extract. Air-dried leaves of *Feijoa sellowiana*, after having been extracted with pentane and with moist ether,[10] were extracted with methanol. The methanol extract was evaporated and countercurrent separated into two fractions using the two phases obtained from a mixture of ether and water, one volume of the ether phase and three volumes of the water phase. The water phases were pooled, concentrated in vacuum, half saturated with sodium sulfate and extracted in a separatory funnel three times, each time with an equal volume of a mixture of chloroform and ethanol 3:2 v/v. These extracts were washed, successively, in another separatory funnel with one portion of a half saturated solution of sodium sulfate in water, the volume of which was 1/10 of that of the solution extracted. The extracts were pooled, dried with anhydrous sodium sulfate and evaporated. The remaining water phases were pooled, evaporated almost but not entirely to dryness, and the organic material was extracted with a mixture of equal volumes of acetone and methanol. The two fractions were spotted on Silica Gel G chromatoplates. The result obtained after development with two different solvent mixtures for thin-layer chromatography is shown on Fig. 3.

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