Thin-layer Chromatography of Plant Extracts

II. Sequential Extraction of Plant Material

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Extraction of plant material with a series of solvents of increasing dielectric constant provides only a rough and partial separation of the substances. A large proportion of the constituents appear in several extracts, and each fraction includes substances with polar properties so different that in thin-layer chromatography some substances remain at the starting-point even if others are moving close to the front.

It is not possible to separate all components of a plant extract in one thinlayer chromatographic procedure. Nevertheless, a few thin-layer chromatographic analyses have been published in which the components of one class of substances have been separated directly from a crude extract.^{1,2} When a general analysis is needed, preliminary fractionation should be run so that a series of completely resolved thin-layer chromatograms can be produced. The fractionation method should be efficacious so that overlapping between adjacent fractions is minimized.

In general plant analysis fractionation has been accomplished by using a series of solvents with increasing polarity, e.g., petroleum ether, diethyl ether, chloroform, ethanol, and water.^{3,4} This study concerns evaluation of sequential extraction for producing fractions suitable for thin-layer chromatography.

In the course of the present investigation, material from various plant species was extracted with several solvents in the order of increasing polarity, selected from a group comprising pentane, diethyl ether, chloroform, wet ether, tetrahydrofuran, 95 % ethanol, methanol, acetone-ether 1:1, and water.

Examples are shown in Figs. 1 and 2. Most of the substances appear in several extracts, and most of the fractions include substances with polar properties so different that in thin-layer chromatography some constituents

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remain at the starting-point while others are moving close to the front. So much overlapping occurs in sequential extraction that it should be considered as only a rough or preliminary fractionation.

EXPERIMENTAL

Standard extraction of plant material.^{3,4} Ten grams of the leaves of Nerium oleander L. (Apocynaceae) dried at 50°C and ground, were extracted in a Soxhlet apparatus with the following series of solvents: pentane, ether, chloroform and 95 % ethanol; and then extracted with water at room temperature. The solvents were removed in a rotating vacuum evaporator. Thin-layer chromatographic analyses of the extracts were made, using silica gel G as the adsorbent. The chromatoplates were dried and sprayed with 20 % antimony pentachloride in carbon tetrachloride, then heated to about 120°C for visualizing the spots. The results are shown in Fig. 1.

85 PENTANE 15. t-AMYL ALCOHOL		90 CHLOROFORM 10 ACETIC ACID			50 TOLUENE 40 ETHYL FORMATE 10 FORMIC ACID			10 TOLUENE 2 ACETIC ACID 80 BUTANONE 5 METHANOL 6 WATER			50 ETHYL ACETATE 30 BUTANONE 10 FORMIC ACID 10 WATER	
P	E	P	E	С	E	C	95	С	95	W	95	W
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Fig. 1. Thin-layer chromatograms showing overlapping in sequential extraction. Dried leaves of Nerium oleander were extracted with the following series of solvents: pentane (P), ether (E), chloroform (C), 95 % ethanol (95), and water (W). The extracts were analyzed by thin-layer chromatography. The chromatograms were developed with solvent mixtures of the volume composition listed above each drawing, and were visualized with antimony pentachloride.

Sequential extraction with another series of solvents. Ten grams of air-dried, ground root-bark of Karwinskia humboldtiana (Zucc.) Roem & Schult. (Rhamnaceae) were extracted with the following series of solvents: pentane, moist ether ⁵ and tetrahydrofuran; and then with water-acetone 1:1. The thin-layer chromatographic analyses of these extracts are shown in Fig. 2.

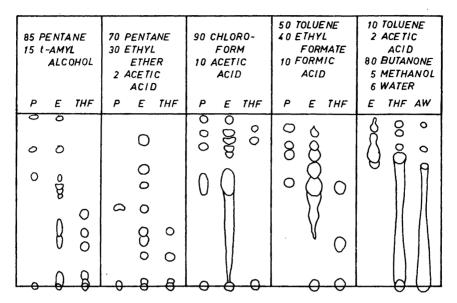


Fig. 2. Thin-layer chromatograms showing overlapping in sequential extraction. Air-dried root bark of Karwinskia humboldtiana was extracted with the following series of solvents: pentane (P), moist ether (E), tetrahydrofuran (THF) and a mixture of acetone and water (AW). The extracts were analyzed by thin-layer chromatography, using silica gel G as the adsorbent, and solvent mixtures for the development as indicated above the drawings. Spots were visualized with antimony pentachloride.

Acknowledgements. This work was supported by U.S. Army Contract No. DA 118-108-CML-6613(A) CPI-15190. The Karwinskia humboldtiana was collected in the Boyce Thompson Southwestern Arboretum.

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Received November 13, 1964.