

Thin-layer Chromatography of Plant Extracts

IV. Alkaloids

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Solvent mixtures previously used for developing thin-layer chromatograms of various groups of alkaloids have now been tested for other alkaloids and other plant material alkaloid fractions than those for which they were originally intended. Some were selected and arranged in order of increasing dielectric constant on a logarithmic scale for general use in the silica gel thin-layer chromatographic analysis of preparations containing alkaloids of corresponding polarity.

Three new solvent mixtures have been developed: one acidic mixture of intermediate polarity consisting of chloroform, acetic acid, and methanol, 60:5:35, one alkaline mixture of intermediate polarity consisting of butanol, chloroform, and ammonia, 50:10:4, and one acidic mixture of high polarity consisting of ethanol, formic acid, water, and formamide, 50:10:10:30.

The R_F values for an alkaloid usually differ considerably if alkaline solvents and if acid solvents of the same dielectric constant are used for developing the chromatogram.

Our previous studies on thin-layer chromatographic analysis of plant extracts were restricted to alkaloid-free plants.¹⁻³

Thin-layer chromatographic analysis of alkaloids is usually done with media comprising an organic base, particularly diethyl amine,⁴ whereas for the development of thin-layer chromatograms of substances other than alkaloids, neutral or acid media are used.

Thin-layer chromatographic analysis of alkaloids and related substances has been reviewed recently by Waldi,⁵ Stahl,⁶ and Randerath.⁷ The standard procedures for isolating alkaloids from a plant extract^{8,9} have been reviewed by Seka.¹⁰

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EXTRACT FRACTIONING

A simple countercurrent extraction using a few separatory funnels as described earlier gives a well-defined separation of a plant extract.^{3,11} If such a separation is carried out at a pH of about 3 with equal volumes of ether and water, neutral and acid lipophilic substances go mostly into the ether phase, and the alkaloids go into the water phase with hardly any exceptions other than colchicine, the pK_A value of which is as small as 1.70, and a not yet identified passionflower alkaloid, the partition ratio of which is 1 at pH about 2.5.³³

The various portions of the water phase are pooled, made less acid and concentrated in vacuum. Sodium hydroxide is added to bring the pH value to about 8, and alkaloids with no quaternary nitrogen are extracted with ether and thus separated from quaternary alkaloids and other constituents. The various portions of the ether phase are pooled and dried with anhydrous sodium sulfate, and the ether is evaporated.

Quaternary alkaloids present in the original extract remain in the water phase and can be extracted with chloroform-ethanol (v/v 3:2), but glucosides and other hydrophilic substances are extracted simultaneously.¹²⁻¹⁴

THIN-LAYER CHROMATOGRAPHY

A large number of solvent mixtures suitable for the development of regular silica gel G plates spotted with alkaloids, alkaloid mixtures and plant extract fractions containing alkaloids and related substances have been published. Those, found to be of interest in routine procedures for plant analysis, have been selected from Stahl's monograph and from other sources¹⁵⁻¹⁷ and arranged in order of increasing dielectric constant on a logarithmic scale in Table 1. One of the highly polar solvent mixtures listed has been used extensively in paper chromatography¹⁶ but does not seem to have been used earlier for thin-layer chromatographic analysis of alkaloids, but a similar mixture has been used for flavon glucosides.¹⁸ Table 1 also includes three new solvent mixtures.

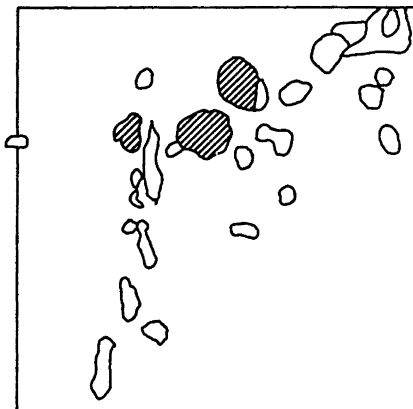
The visualization of alkaloids was done with an iodoplatinic acid reagent and with a modification of the Thies and Reuter modification of Dragendorff's reagent.¹⁹⁻²² The sensitivity and the specificity of the reaction with the latter reagent could be increased by subsequent spraying with dilute sulfuric acid.^{20,23}

Several solvent mixtures have been suggested for the two-dimensional thin-layer chromatography of simple indole derivatives.^{24,25} For pyridindole alkaloids of intermediate polarity, good two-dimensional chromatograms were obtained in the present study using the following solvent mixtures: methyl acetate, isopropanol, and 25 % ammonia, 45:35:20, and acetic acid, butanol, and water, 15:60:25 (Fig. 1). Mixtures of bisbenzyltetrahydroisoquinoline alkaloid derivatives were separated with the solvent mixtures benzene-methanol, 80:20, and chloroform-acetone-diethyl amine, 50:40:10.

Table 1. A selection of solvents and solvent mixtures for thin-layer chromatography of alkaloids on silica gel chromatoplates, listed in order of increasing dielectric constant ϵ on a logarithmic scale. For mixtures, volumes of the components are given, and also page references to Stahl's monograph.⁶ New solvent mixtures suggested here are designated by an asterisk*. Abbreviations: Acone acetone, Amm ammonia, Bzne benzene, BuOH butanol, Chlf chloroform, cHxne cyclohexane, DEA diethyl amine, EtOAc ethyl acetate, EtOH ethanol, HOAc acetic acid, HCOOH formic acid, iPrOH isopropanol, MeOAc methyl acetate, MeOH methanol, w water; S&K Stahl and Kaldewey.

	ϵ	ϵ	Solvent mixtures for thin-layer chromatography
cHxne	2.0	2	
Bzne	2.3		90 cHxne + 10 DEA (292) (Waldi IV)
		3	70 cHxne + 20 Chlf + 10 HOAc (294) 70 Bzne + 20 EtOAc + 10 DEA (292) (Waldi V) 50 cHxne + 40 Chlf + 10 DEA (292) (Waldi III)
DEA	3.8	4	
Chlf	4.7	5	90 Chlf + 10 DEA (292) (Waldi II)
EtOAc	6.0	6	
HOAc	6.2		
MeOAc	6.7		
		8	80 Bzne + 20 MeOH (294) (Neubauer & Mothes)
		10	50 Chlf + 40 Acone + 10 DEA (292) (Waldi I)
		15	60 Chlf + 5 HOAc + 35 MeOH *
BuOH	17.1		
iPrOH	18.3		10 Chlf + 50 BuOH + 4 Amm (conc.)*
Acone	20.7	20	45 MeOAc + 35 iPrOH + 20 Amm (25 %) (304) (S&K) 25 Chlf + 10 HOAc + 65 MeOH (294) 1 HAc + 4 BuOH + 1 w (342, 406, 412) 40 Chlf + 40 MeOH + 20 Amm (17 %)
EtOH	24.3	30	15 HOAc + 60 BuOH + 25 w (Partridge; ¹⁶ Smith ¹⁷)
MeOH	32.6		
		40	
		50	
HCOOH	58.5	60	50 EtOH + 10 HCOOH + 10 w + 30 Foam *
w	78.5	80	
		100	
Foam	109		

Fig. 1. Two-dimensional thin-layer chromatographic analysis of a commercial preparation of harmalol, using a new combination of previously known solvent mixtures. A solution of the preparation was spotted in the lower left corner and developed, first (upwards in the figure) with a mixture of the following volume composition: 45 methyl acetate, 35 isopropyl alcohol and 20 ammonia (25 %) and then (in the direction from left to right in the figure) after partial drying in a vacuum desiccator, with 15 acetic acid, 60 butanol and 25 water. The spots were viewed in UV light. Shaded areas in the figure represent spots with particularly strong fluorescence. The capability of spots to give fluorescence disappears rather rapidly. In one experiment 50 spots with fluorescence were counted.



EXPERIMENTAL

Material. Parts of the following species, and the following substances of plant origin were used in this work: *Artemisia tridentata* Nutt. (leaves collected in the San Bernardino Mountains), *Datura meteloides* A. DC. (seeds collected in the Mojave Desert) and *Simmondsia chinensis* (Link) C. K. Schneid. (leaves collected in Tonto National Forest);²⁶⁻²⁸ preparations of ayahuasca²⁹ and curare from Peru; commercial preparations of harmol, norharmaline iodomethylate, harmalol (from L. Light & Co., Colnbrook, England), betaine, choline, harmaline, harman, harmine, isotetrandrine, narcotine, sparteine, and strychnine; and preparations of chondrocurine, curine, and isochondrodendrine and their methylated and demethylated derivatives, made up in this laboratory.^{11,30-31}

Ether-soluble fraction of alkaloids. Ten grams of ground plant material were defatted, if necessary, in a Soxhlet apparatus with pentane and then extracted with methanol. The methanol extract was evaporated and the residue countercurrent separated with ether-water 3:1 at pH 3. The water phases were pooled and made slightly alkaline (pH 8) with sodium hydroxide. The aqueous mixture was extracted with an equal volume of ether three times. The combined ether phases were dried with anhydrous sodium sulfate and evaporated.

Quaternary fraction of alkaloids. The slightly alkaline water phase remaining from the ether extraction was half-saturated with sodium sulfate and was then extracted three times with an equal volume of chloroform-ethanol 3:2. These portions were then washed with a tenth volume of water half saturated with sodium sulfate, then pooled, dried with sodium sulfate and evaporated.

New solvent mixtures for the development of thin-layer chromatograms. Chloroform-acetic acid-methanol, 60:5:35, gave good results in the separation of pyridindole (harmala) alkaloids. Chloroform-butanol-ammonia (conc.), 10:50:4, was efficient in separating the bisbenzyltetrahydroisoquinoline alkaloids. Ethanol-formic acid-water-formamide, 50:10:10:30, was useful for quaternary compounds. After development the plates were sprayed with a water solution containing 0.5 % hydrochloric acid and 0.25 % sodium nitrite and warmed to dryness.³² This destroyed the formamide.

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