Although this separation was carried out with trace concentrations of Ca(II) and Mg(II), the method has been applied equally successfully to macro amounts (0.1 *M*) of these elements. The method should also be readily adaptable to the analysis of natural waters containing reasonable amounts of sodium chloride as well as such other ions as carbonates and sulfates.

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Notes

Chromatographic separation of *Rauwolfia serpentina* and opium alkaloids on thin layers of alumina

The chromatographic separation of alkaloids constitutes a problem that has been studied extensively. This is due above all to the wide distribution of these substances in natural materials, in which they mostly occur in low concentration accompanied by large amounts of impurities. Use has been made of paper chromatography for the separation of alkaloids, but one of the drawbacks of this method is that separation is only obtained after 5 to 12 h[1]. Moreover, the separation is not so clear specially in the case of *Rauwolfia* alkaloids. These alkaloids are located by means of ultra violet light, and ajmaline becomes visible only after spraying the developed chromatogram with sodium acetate solution[1]. In studies on the composition of complexes or crude products as regards their individual basic constituents, specially in the case of *Rauwolfia* alkaloids a need was felt for a suitable and quick method for their chromatographic analysis.

We have succeeded in achieving further progress in the chromatography of *Rauwolfia* and opium alkaloids by applying the method described by MOTTIER AND POTTERAT[2] for the separation of some synthetic fat-soluble pigments.

Materials and methods

0.5 % solutions of the following alkaloids were prepared: reserpine, serpentinine, serpentine, ajmaline, ajmalicine, morphine, narcotine, codeine and papaverine.

The *Rauwolfia* alkaloid solutions were dissolved in chloroform, narcotine in acetone, codeine, papaverine and morphine in ethyl alcohol.

Aluminium oxide for chromatography (E. Merck & Co., according to Brockmann) was used as the adsorption medium for the *Rauwolfia* alkaloids and aluminium oxide anhyd. (E. Merck & Co.) for the opium alkaloids.

Detection was carried out by means of the modified Dragendorff's reagent. Spraying has to be done very carefully and from a distance to avoid the absorbent layer being blown away by the aerosol stream.

Preparation of chromatographic plates with a thin layer of alumina

Plates were prepared according to the method of DAVIDEK *et al.*[3] by applying alumina in a dry state to glass plates of 90 × 350 mm. The alumina was subsequently smoothed by means of a roller made from a glass rod. The plates thus prepared are ready to be used directly for the chromatographic separation.

Experimental arrangement

The solution of the individual alkaloid or of a mixture is applied at a distance of about 2–3 cm from the edge of the plates. After allowing the solvent to dry, the plate is placed in an inclined position (at an angle of 20–30°) in the chromatographic chamber together with the solvent. A glass solvent trough 18 in. long × 6 in. wide × 5 in. high is used as the chromatographic chamber. Development with the solvent is carried out by the ascending technique until the solvent has reached the end (about 40–60 min). The plate is then removed from the chamber and allowed to dry. Finally it is sprayed very carefully with Dragendorff's reagent.

Discussion

Model experiments were performed with some of the *Rauwolfia* and opium alkaloids. The dependence of the R_F values of the individual substances on the solvent system as well as the best solvent system for their separation were studied.

Table I shows the R_F values obtained for *Rauwolfia* alkaloids using different solvent mixtures. It has been found that ethyl alcohol is essential for the separation of serpentine and ajmaline. On the other hand reserpine and ajmalicine can only be separated in the absence of alcohol. Consequently two chromatograms must be run

TABLE I

SEPARATION OF *Rauwolfia* ALKALOIDS BY THIN-LAYER CHROMATOGRAPHY

No.	*Alkaloids*	*Solvents*	*R_F values*	*Remarks*
1	Serpentine	Chloroform–acetone (85:15)	0.024	Serpentine and ajmaline gave only one spot
	Ajmaline			
	Reserpine		0.60	
	Ajmalicine		0.77	
2	Serpentine	Absolute ethyl alcohol	0.75	
	Serpentinine		0.86	
	Ajmaline		0.87	
3	Serpentine	Chloroform–ethyl	0.34	
	Ajmaline	alcohol–acetone (90:5:5)	0.51	
	Serpentinine		0.73	
	Reserpine		0.89	

TABLE II

SEPARATION OF OPIUM ALKALOIDS BY THIN-LAYER CHROMATOGRAPHY

No.	*Alkaloids*	*Solvents*	R_F *values*
1	Morphine	Dry acetone	0.033
	Codeine		0.77
	Papaverine		0.89
	Narcotine		0.92
2	Morphine	Chloroform	0.034
	Codeine		0.38
	Narcotine		0.77
	Papaverine		0.88
3	Morphine	Benzene–chloroform–	0.06
	Codeine	–acetone (70:15:15)	0.33
	Papaverine		0.73
	Narcotine		0.77

with the solvents chloroform–ethyl alcohol–acetone (90:5:5) and chloroform–acetone (85:15) to obtain a complete separation of all the alkaloids.

Table II shows the data obtained for opium alkaloids. In this case chloroform, acetone or a mixture of benzene–chloroform–acetone (70:15:15) were found to be most suitable for the separation of the opium alkaloids morphine, codeine, narcotine and papaverine. If chloroform alone is used as the irrigating solvent the spot of

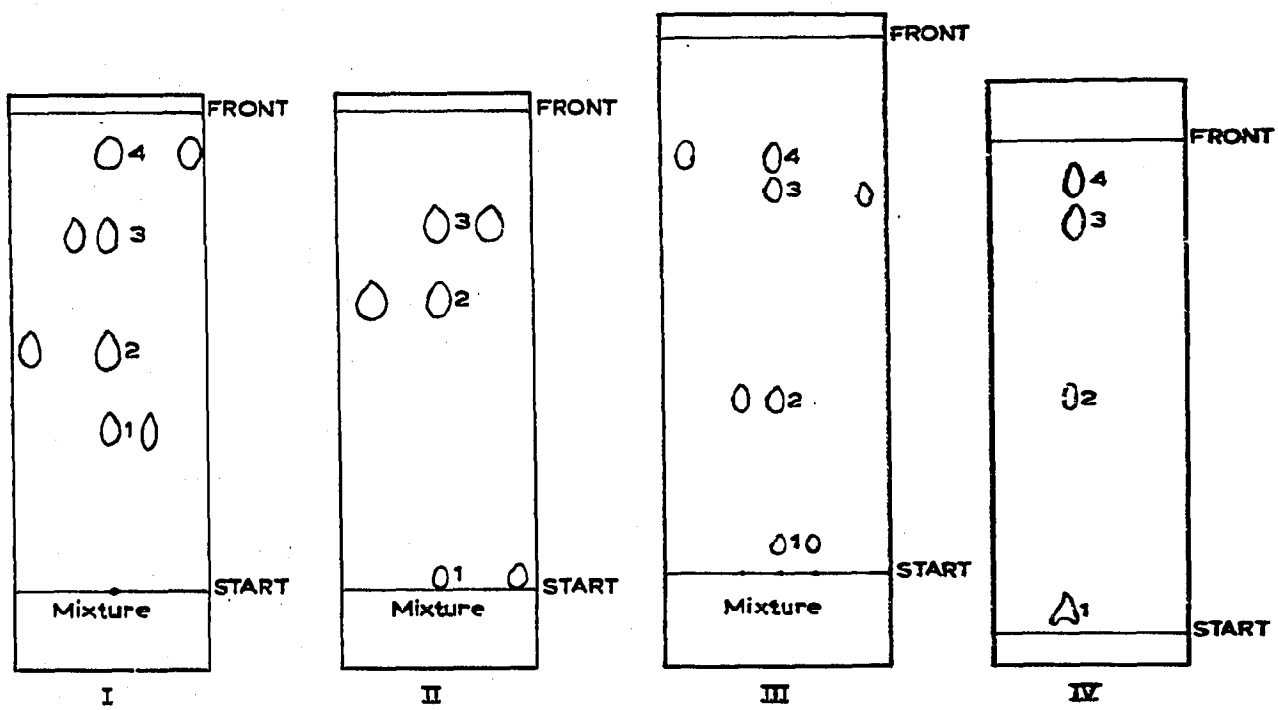

Fig. 1. I. Chromatogram of *Rauwolfia serpentina* alkaloids. Solvent: chloroform–ethyl alcohol–acetone (90:5:5). 1 = serpentine; 2 = serpentinine; 3 = ajmaline; 4 = reserpine. II. Chromatogram of *Rauwolfia serpentina* alkaloids. Solvent: chloroform–acetone (85:15). 1 = serpentine; 2 = reserpine; 3 = ajmalicine. III. Chromatogram of opium alkaloids. Solvent: benzene–chloroform–acetone (70:15:15). 1 = morphine; 2 = codeine; 3 = papaverine; 4 = narcotine. IV. Chromatogram of opium alkaloids. Solvent: pure chloroform. 1 = morphine; 2 = codeine; 3 = narcotine; 4 = papaverine.

papaverine is located at the end of the chromatogram, while with acetone or a mixture of benzene, chloroform and acetone narcotine is found at the top.

Typical chromatograms obtained in this way are shown in Fig. 1, I–IV.

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Use of sequentially applied location reagents and multiple transparent overlays in thin-layer chromatography*

Studies now in progress in this laboratory, utilizing thin-layer chromatography for the separation of tissue lipids[1], have demonstrated a need for a simple, rapid method for permanently recording the positions and total areas of separated lipid classes on the developed chromatoplate prior to quantitation. The separated lipids have differed widely in amount under various experimental conditions, producing a degree of variability as well as interference in the final separations that is not completely standardized by comparison with known lipids. This has necessitated the frequent use of multiple, sequentially applied location reagents. Difficulties have occurred when spots previously demonstrated would be hidden by location reagents applied later in the sequence. It became impossible to relate these sequentially visualized areas unless a permanent record was made after the use of each location reagent. Recent notes by GETZ AND LAWSON[2] and HILTON AND HALL[3] have suggested the feasibility of utilizing standard office photocopiers. The overlay technique described in this communication requires no special equipment and has the added feature that it permits the superpositioning of the series of overlays outlined during sequential spot localization. Direct comparisons can thus be made of the various spots developed with the individual location reagents, positive identifications can be made, and—most important—any incomplete separations can be rapidly determined.

The developed chromatoplate, after being sprayed with the first location reagent, is placed under a plate of either clear glass or plastic. This plate is elevated from the surface of the table enough to allow the chromatoplate to be slipped under-

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