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THIN-LAYER CHROMATOGRAPHIC STUDY OF ISOQUINOLINE ALKALOIDS WITH THE BN-CHAMBER

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SUMMARY

Continuous solvent flow thin-layer chromatography with horizontal development using the BN-chamber was successful in the separation of isoquinoline alkaloids on thin layers of silica gel HF_{254} with acidic and basic solvent systems. Attempts to replace silica gel by other adsorbents did not have a desirable effect. Quantities as low as 1 μg of pure alkaloids were applied and detected by fluorescence under long-wave UV light. The quantity applied was under the detection limit of Dragendorff and iodoplatinate reagents. This procedure is limited to lesser quantities (a maximum of 5 μg of alkaloid per spot) owing to the overlapping of the various spots.

INTRODUCTION

Any classification of alkaloids is difficult because an alkaloidal drug may contain several different alkaloids which in addition frequently do not show the same pharmacological activity. It is for this reason that a classification based on the chemical structures of the alkaloids is often recommended by certain authors[1–5] and has been adopted in this study. The alkaloids which were examined in the course of this investigation contain the "isoquinoline nucleus" and are therefore classified under one common group.

Brenner and Niederwieser[6] were the first to construct and use a new type of chamber (BN-chamber) for continuous solvent flow thin-layer chromatography (TLC) with horizontal development. This chamber has since been improved and the scope of its applications widened. Its principle and mode of operation have already been described[6,7]. The chamber is extremely useful for substances which are not resolved in a normal 10–18 cm run by ascending TLC. As far as the authors are aware, this is the first report on the TLC separation of nine isoquinoline alkaloids (excluding one non-alkaloidal isoquinoline base) with the help of the BN-chamber.

EXPERIMENTAL AND RESULTS

Solvents

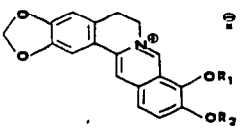
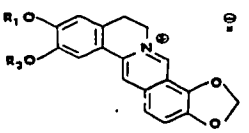
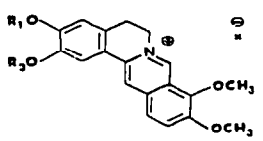
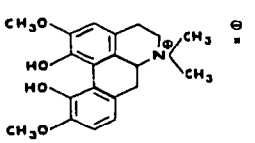
The solvents used in this study were reagent grade and were obtained either from Fisher Scientific, Montreal, Canada, or J. T. Baker Chemicals, Montreal, Canada. Absolute ethanol (British and U.S.A. pharmacopoeia grade) was a product of Canadian Industrial Alcohols and Chemicals, Corbyville, Canada.

Alkaloids

The list of the substances chromatographed is given in Table I, together with their chemical structures. Their identity and purity were confirmed by ultraviolet spectrophotometry and by infrared and nuclear magnetic resonance spectroscopy.

TABLE I

CHEMICAL STRUCTURES OF THE ISOQUINOLINE ALKALOIDS

Name	*Chemical structure*
Berberine, $R_1 = R_2 = CH_3$ Berberrubine, $R_1 = H$; $R_2 = CH_3$ Thalifendine, $R_1 = CH_3$; $R_2 = H$	
Tetradehydrocheilanthifoline, $R_1 = H$; $R_2 = CH_3$ Coptisine, $R_1 + R_2 = CH_2$	
Demethyleneberberine, $R_1 = R_2 = H$ Columbamine, $R_1 = CH_3$; $R_2 = H$ Jatrorrhizine, $R_1 = H$; $R_2 = CH_3$ Palmatine, $R_1 = R_2 = CH_3$	
Magnoflorine	

Berberine sulphate was a product of S. B. Penick, New York, N.Y., U.S.A., and was chromatographically pure. Berberrubine chloride was prepared by heating berberine chloride in an inert atmosphere[8]. Berberine was converted into demethyleneberberine (non-alkaloidal isoquinoline base) by demethylenation with sulphuric acid in the presence of phloroglucinol[9]. Columbamine, jatrorrhizine and palmatine chlorides were synthesized, purified and characterized according to the methods already described[10,11]. Tetradehydrocheilanthifoline and coptisine chlorides were isolated and identified from the rhizome of *Coptis groenlandica*[12,13].

Dyes

Nile blue A and crystal violet were obtained from Harleco (Hartman-Leddon, Philadelphia, Pa., U.S.A.) and Matheson, Coleman & Bell, East Rutherford, N.J., U.S.A., respectively.

Chromatographic plates

The glass plates, 20 × 20 cm, were coated with silica gel HF_{254} (E. Merck, Darmstadt, G.F.R.) using the apparatus and procedure described by Stahl[7]. The slurry of silica gel for five plates was prepared by shaking 25 g of adsorbent with 80 ml of distilled water in an erlenmeyer flask for 2 min. The thin liquid suspension was poured into the spreader and spread to a thickness of 0.25 mm. The silica gel layers were allowed to dry at room temperature (20–22°) and then activated by heating for 45 min at 120°.

Standard alkaloid and dye solutions

Standard solutions of the alkaloids (0.1 %) were prepared in absolute methanol–water (1:1). Reference dye solutions (0.1 %) were made in methanol.

Application of samples and dyes

One μl (containing 1 μg) each of the alkaloid and dye solutions were applied to the starting line placed at a distance of 1.5 cm from the edge of the plate. The solutions

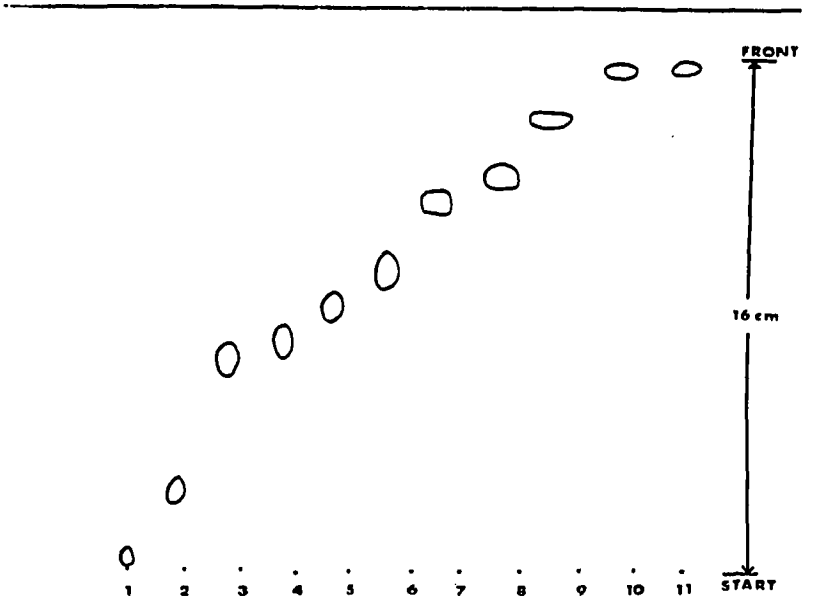


Fig. 1. Separation of isoquinoline alkaloids on thin layers of silica gel HF_{254} using methyl acetate–methanol–29 % ammonium hydroxide (67:25:8), solvent system S_1. 1 = Demethyleneberberine iodide; 2 = magnoflorine iodide; 3 = columbamine chloride; 4 = tetradehydrocheilanthifoline chloride; 5 = jatrorrhizine chloride; 6 = palmatine chloride; 7 = coptisine chloride; 8 = berberine sulphate; 9 = thalifendine chloride; 10 = berberrubine chloride; 11 = Nile blue A. Detection by fluorescence in long-wave UV light.

were applied with a 10-μl graduated micropipette. The distance between neighbouring starting points was 1.5 cm.

Development

The chromatograms were developed by the continuous solvent flow horizontal technique. A considerable variety of solvent systems was examined, but only the following two (labelled S_1 and S_2) gave satisfactory results: S_1, (basic system) methyl acetate–methanol–29% ammonium hydroxide (67:25:8), and S_2, (acidic system) ethanol–chloroform–glacial acetic acid (67:30:3). These solvent mixtures were freshly prepared every day.

The BN-chamber was placed on a cork ring approximately 12 cm in diameter during development with system S_1[6,7]. No such support was used when the chromatograms were developed with system S_2. Cold water at 10° with an approximate flow of 500–600 ml/min was circulated through the cooling block when system S_1 was employed. The development of chromatograms with system S_2 was carried out without water cooling. Nile blue A and crystal violet were used as test dyes for systems S_1 and S_2, respectively. The dye was applied on one of the extreme starting points of the chromatogram for the purpose of checking migration. The chromatogram was removed from the chamber when the dye reached the edge of the cover plate. The development time in each of the two different solvent systems was approximately 4 h. The chromatograms were dried at 60° for 15 min after development. Figs. 1 and 2

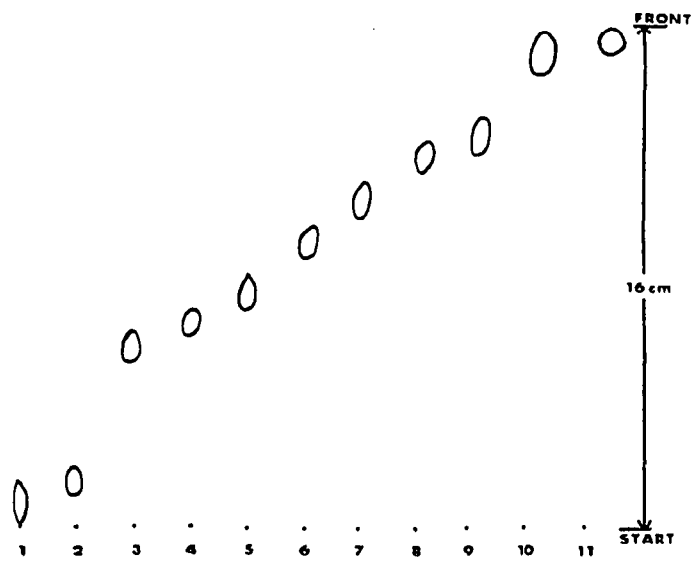


Fig. 2. Separation of isoquinoline alkaloids on thin layers of silica gel HF_{254} using ethanol–chloroform–glacial acetic acid (67:30:3), solvent system S_2. 1 = Demethyleneberberine iodide; 2 = magnoflorine iodide; 3 = coptisine chloride; 4 = tetradehydrocheilanthifoline chloride; 5 = palmatine iodide; 6 = columbamine chloride; 7 = berberine sulphate; 8 = jatrorrhizine chloride; 9 = berberrubine chloride; 10 = thalifendine chloride; 11 = crystal violet. Detection by fluorescence in long-wave UV light.

TABLE II

FLUORESCENCE OF THE ALKALOIDS USING TWO DIFFERENT SOLVENT SYSTEMS

No.	*Alkaloids*	*Fluorescence after development with solvent system S_1 (basic)*	*Fluorescence after development with solvent system S_2 (acidic)*
1	Demethyleneberberine iodide (non-alkaloidal isoquinoline base)	Yellow	Yellow
2	Magnoflorine iodide	Blue	Blue
3	Columbamine chloride	Yellow	Light yellow
4	Tetradehydrocheilanthifoline chloride	Golden yellow	Golden yellow
5	Jatrorrhizine chloride	Yellow	Light yellow
6	Palmatine chloride	Green	Olive green
7	Coptisine chloride	Bright yellow	Bright yellow
8	Berberine sulphate	Green	Olive green
9	Thalifendine chloride	Greenish blue	Olive green
10	Berberrubine chloride	Olive green	Greenish yellow

illustrate the separation of the alkaloids using the solvent systems S_1 and S_2, respectively.

Detection of the alkaloids

The quantity of the alkaloids (1 μg) applied on the silica gel layers fell below the limit of detection of both commonly used Dragendorff and iodoplatinate reagents. These alkaloids, however, yield typical fluorescence colours in long-wave UV light (365 nm). Table II shows the fluorescence of the detected alkaloids obtained after development with two different solvent systems.

TABLE III

$hR_{St.}$ VALUES OF THE ALKALOIDS OBTAINED WITH TWO DIFFERENT SOLVENT SYSTEMS

No.	*Alkaloids*	*$hR_{St.}$ value relative to berberrubine chloride in solvent system S_1 (basic)*	*$hR_{St.}$ value relative to thalifendine chloride in solvent system S_2 (acidic)*
1	Demethyleneberberine iodide (non-alkaloidal isoquinoline base)	0	0
2	Magnoflorine iodide	17	5
3	Columbamine chloride	44	57
4	Tetradehydrocheilanthifoline chloride	46	39
5	Jatrorrhizine chloride	54	79
6	Palmatine chloride	61	45
7	Coptisine chloride	75	32
8	Berberine sulphate	80	68
9	Thalifendine chloride	90	100
10	Berberrubine chloride	100	84

hR_{St} values

Since there is no definite solvent front in continuous flow chromatography, it is difficult to evaluate R_F values of the compounds concerned. Therefore, the migration distances of the alkaloids were compared with that of a reference substance chromatographed simultaneously and belonging to a similar compound class. Berberrubine chloride was used as the reference substance in system S_1 and thalifendine chloride in system S_2. The hR_{St} values of the alkaloids using two different solvent systems are given in Table III.

DISCUSSION

It is evident that continuous solvent flow TLC with horizontal development is able to play an important role in the analysis of polar isoquinoline alkaloids and other compounds having similar chemical structures. In the present study, the migration behaviour of the alkaloids was examined using basic and acidic solvent systems on thin layers of silica gel. Other adsorbents such as basic and acidic alumina (Merck), cellulose (Macherey, Nagel & Co., Düren, G.F.R.) were tried in place of silica gel but they failed to give a good separation.

One advantage of this technique is that quantities as low as 1 μg of pure alkaloids can be detected by fluorescence under long-wave UV light. The present method is not suitable, however, if larger quantities of the compounds (maximum of 5 μg of alkaloid per spot) are applied on silica gel layers. This causes overlapping of the various spots.

In the basic solvent system S_1, columbamine (hR_{St} 44) and tetradehydrocheilanthifoline (hR_{St} 46) are not resolved from each other. Moreover in the same system, the following pairs of alkaloids have close hR_{St} values: tetradehydrocheilanthifoline (hR_{St} 46) and jatrorrhizine (hR_{St} 54), jatrorrhizine (hR_{St} 54) and palmatine (hR_{St} 61), as well as coptisine (hR_{St} 75) and berberine (hR_{St} 80). The same alkaloids are very well separated from each other in the acidic solvent system S_2. Similarly, in the acidic solvent system S_2 demethyleneberberine (hR_{St} 0) and magnoflorine (hR_{St} 5), coptisine (hR_{St} 32) and tetradehydrocheilanthifoline (hR_{St} 39), as well as jatrorrhizine (hR_{St} 79) and berberrubine (hR_{St} 84) have close hR_{St} values. These alkaloids are very well resolved from each other in the basic solvent system S_1.

ACKNOWLEDGEMENT

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