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Note

Circular thin-layer chromatography of quaternary alkaloids

Quaternary protoberberine alkaloids in plant material

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Because of the high polarity of quaternary alkaloids, special problems are ecountered by their thin-layer chromagraphic (TLC) analysis. In a previous paper[1] we described the use of highly polar solvent systems consisting of solutions of inorganic salts of various molarity and methanol for the TLC of quaternary alkaloids. Similar solvents have also been reported by other investigators[2].

In order to facilitate the isolation of the alkaloids from the silica gel layer after their separation, without interference from the inorganic salt used, we report here the use of a solvent system containing a volatile salt (ammonium carbonate). Circular TLC was applied in order to achieve a better separation than with ordinary one-dimensional TLC.

EXPERIMENTAL

Isolation of the alkaloids from plant material

The quaternary alkaloids were extracted from the roots of *Jatrorrhiza palmata* with 70% ethanol. The extract was concentrated under reduced pressure, extracted with diethyl ether to remove neutral ether-soluble compounds and the residue of the ethanolic extract was dissolved in aqueous ethanol. This solution was used for circular TLC.

Because of the low percentage of quaternary alkaloids in the stem of *Chasmanthera dependens*, the alkaloids were extracted with methanol and isolated by precipitation with Mayer's reagent, the precipitate being treated with Amberlite IRA 400 ion-exchange resin, as described elsewhere[3].

Circular TLC

Merck TLC sheets (20 × 20 cm) pre-coated with silica gel (Kieselgel 60 F_{254}, 0.2 mm) were used for the analysis in combination with a circular desiccator-type chromatography tank, about 3 cm high and 25 cm in diameter. The silica layer of the sheet was placed downwards towards the solvent. The solvent was brought to the centre of the thin-layer plate by means of a cotton wick. Depending on the thickness of the wick, the speed of development of the chromatogram with the mobile phase

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could be controlled. The solvent system was isopropanol–1 *M* ammonium carbonate–dioxane (15:5:1). The alkaloids were applied as a thin circular line 2 cm from the centre of the plate. Detection was as follows: under UV light of 254 nm, jatrorrhizine brownish, palmatine orange and columbamine brownish; under UV light of 366 nm, jatrorrhizine orange-brown, palmatine yellow and columbamine green-brown; and in daylight, jatrorrhizine orange, palmatine yellow, and columbamine green-yellow.

Isolation of the alkaloids from the thinlayer after separation

The silica gel containing the alkaloid was scraped off, extracted with methanol–10% ammonia solution (9:1) in a small chromatographic tube and the extract was evaporated to dryness on a water-bath. During the last part of the procedure the ammonium carbonate evaporated.

RESULTS AND DISCUSSION

Circular chromatograms of the alkaloids from the roots of *Jatrorrhiza palmata* (jatrorrhizine, palmatine and columbamine) and from the stem of *Chasmanthera dependens* (jatrorrhizine and palmatine) are shown in Fig. 1. Columbamine, which was found in another sample of *Chasmanthera dependens*[3], could not be detected in the sample investigated here. For comparison, chromatograms obtained by one-dimensional TLC using the same TLC sheets and solvent system (reference substances and alkaloids from the roots of *Jatrorrhiza palmata*) are shown in Fig. 2.

In a previous study[1] we used methanol–aqueous ammonium nitrate solutions

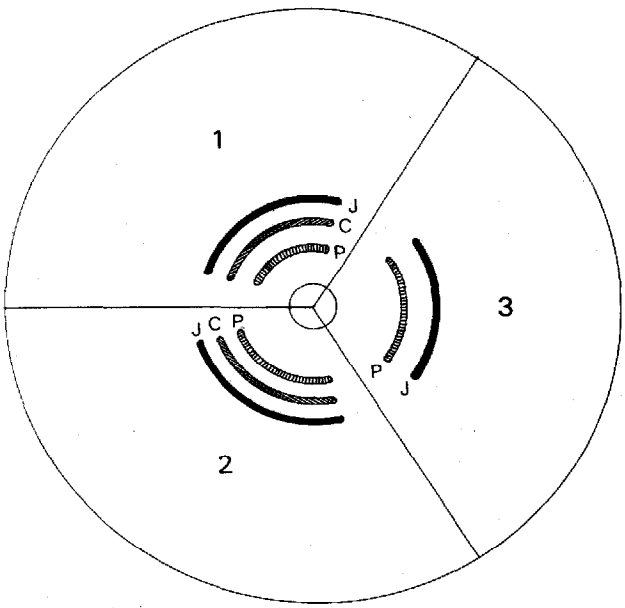

Fig. 1. Circular TLC of jatrorrhizine (J), columbamine (C) and palmatine (P). 1 = Reference substances; 2 = alkaloids from the roots of *Jatrorrhiza palmata;* 3 = alkaloids from the stem of *Chasmanthera dependens.*

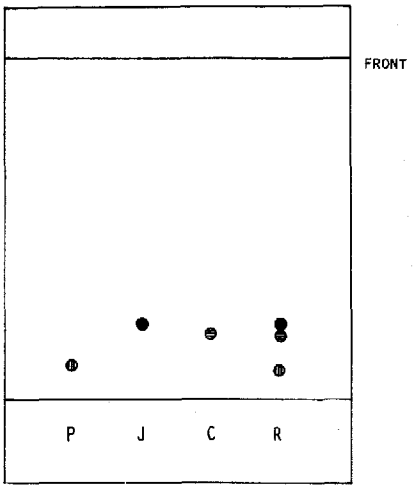

Fig. 2. TLC of jatrorrhizine (J), columbamine (C) and palmatine (P) reference substances and from the roots of *Jatrorrhiza palmata* (R).

for the separation of various quaternary alkaloids. Other salts were also found to be suitable and other polar solvents, such as acetone, tetrahydrofuran, isopropanol and dioxane could also be used. The solvent system isopropanol–1 *M* ammonium carbonate–dioxane (15:5:1) was found to be most suitable for the separation of the protoberberine-type alkaloids, allowing a complete separation of the major components, columbamine, jatrorhizine, palmatine and berberine. This solvent system also proved to be suitable for preparative TLC. On evaporation of the solvent after extraction of the alkaloids from the silica gel the salt volatilized completely.

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