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Note

A simple thin-layer chromatographic method for separation of cinchona alkaloids

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Sri Lanka has long been associated with the cinchona industry[1,2], and cinchona bark samples sent by exporters to our laboratory for analysis showed a wide variation in both total-alkaloid and quinine contents when examined by spectroscopic methods[3]. It therefore appeared desirable to devise a simple technique, suitable for repetitive application, which would demonstrate the distribution of alkaloids in the various specimens of cinchona.

Many authors have established conditions for separating cinchona alkaloids by thin-layer chromatography (TLC)[4–9], but none of these were completely satisfactory for our purposes. A suitable method devised in this laboratory is described below.

EXPERIMENTAL

The TLC plates (20 × 20 cm) were coated with a 0.3-mm layer of a slurry of silica gel G (20 g) and silica gel GF_{254} (20 g) with water (65 ml) and 0.1 *N* sodium hydroxide (18 ml), and then dried at 110° for 1.5 h.

After application of the alkaloid mixture (2 mg) in ethanol, each plate was developed in one direction with chloroform–methanol–17% ammonia (24:6:0.05); the plates were then dried, turned through 90° and developed in the second direction with diethyl ether–diethylamine (17:1). This second development was repeated in order to improve the separation. Finally, the plates were completely dried, and the positions of the fluorescent spots determined under UV radiation after spraying with a modified Dragendorff reagent[10] and then with potassium iodoplatinate solution to obtain distinct spots.

RESULTS AND DISCUSSION

Preliminary experiments carried out with benzene–diethyl ether–diethylamine solvent systems containing different amounts of each component showed that the best separation was achieved with diethyl ether–diethylamine (17:1); double development further improved the separation. This solvent system separated the total-cinchona-alkaloid mixture into eight components, and when it was combined with the chloroform–methanol–17% ammonia (24:6:0.05) solvent in two-dimension TLC the

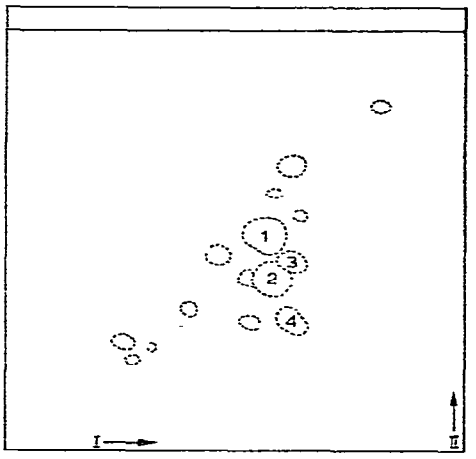

Fig. 1. Thin-layer chromatogram showing the positions of cinchona alkaloids. I, first dimension [development with chloroform–methanol–17% ammonia (24:6:0.05, v/v)]; II, second dimension [development with diethyl ether–diethylamine (17:1, v/v)]. 1 = cinchonine; 2 = cinchonidine; 3 = quinidine; 4 = quinine.

mixture was resolved into fourteen components (see Fig. 1). The spots were visible under UV radiation, and, for visualisation, Dragendorff reagent was first sprayed; this gave orange spots, but the colour soon faded and the spots were not distinct. When the plates were over-sprayed with potassium iodoplatinate solution, the spots were distinctly visible and the colour persisted for over 2 hours. Spraying with potassium iodoplatinate solution alone, however, was not very satisfactory, and it was necessary to use both spray reagents.

We are currently using this method to study several cinchona varieties submitted by the trade for analysis. A study to enable us to obtain an idea of the geographical locations of the best quality cinchona types is also in progress; in this work, the method has served as a valuable and rapid screening technique.

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