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SHORT COMMUNICATION

Thin-layer chromatography and high voltage electrophoresis of quaternary alkaloids from *Fagara* species

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Because of their physical properties quaternary alkaloids can be separated chromatographically by partitioning between immiscible solvents on a cellulose support using either paper (Kuck, Abonico & others, 1967) or cellulose thin-layers (Calderwood & Fish, 1966). However, the minimum time needed is some 3–4 h including a 1 h equilibration period.

Electrophoresis at low voltages was suggested for the separation of quaternary alkaloids (Marini-Bettolo & Coch Frugoni, 1958) but the time required was also about 3 h. With the introduction of high voltage electrophoresis, the possibility of shortening the separation time became apparent, for example, the separation of tertiary ergot alkaloids was reduced to 45 min (Agurell, 1965). We wished to effect rapid separation of the quaternary bases of the genus *Fagara* (Rutaceae) and have compared the results of thin-layer chromatographic separations with those achieved by electrophoresis.

Experimental

Alkaloids. Solutions of salts (iodide or chloride) 1% in ethanol of the following alkaloids were used: candicine, coryneine, tembetarine, magnoflorine, *N*-methylcorydine, *N*-methylisocorydine, laurifoline, xanthoplanine, palmatine, berberine, chelerythrine, nitidine.

Thin-layer chromatography. Plates with cellulose (Whatman Chromedia CC41), spread to 0·25 mm and dried at 40°, were developed with (1) 0·1N hydrochloric acid, (2) n-butanol saturated with 2N hydrochloric acid, or (3) n-butanol–pyridine–water (6:4:3). The plates were equilibrated for 1 h before development for which times were 35, 150 and 120 min respectively.

High voltage electrophoresis. Of the several buffer solutions tried, the Britton–Robinson barbitone buffer, pH 6·8–8·0, gave best results with the alkaloids examined.

A Camag high voltage electrophoresis cell was used and the potential gradient was varied between 75 and 125 V/cm, the cell being cooled by water at a flow rate of 2 litres/min. Electrophoresis was for 20 min. Platinum electrodes were necessary since the alkaline barbitone buffer corrodes the usual electrodes. Chromatography papers, Whatman No. 1, 40 × 20 cm, were saturated with the buffer solution and the excess removed by pressing between layers of absorbent paper.

Alkaloid detection. The thin-layer chromatograms and the electropherograms were examined under ultraviolet light (366 nm), after exposure to ammonia vapour; they were then sprayed with iodoplatinate reagent.

Table 1. *Thin-layer chromatographic characteristics of the quaternary alkaloids from* Fagara *species*

Alkaloids	Rf values* Solvents 1	2	3	Fluorescence after NH_3	Colour with iodoplatinate reagent
Candicine..	0·92	0·45	0·47	—	Purple
Coryneine	0·88	0·28	0·64	—	Pale blue
Tembetarine	0·85	0·55	0·57	—	Green
Magnoflorine	0·24	0·30	0·24	Blue	Purple
N-Methylisocorydine ..	0·77	0·53	0·49	Blue	Purple
N-Methylcorydine ..	0·74	0·57	0·65	Blue	Purple
Laurifoline	0·11	0·19	0·42	Blue	Purple
Xanthoplanine	0·34	0·42	0·63	Blue	Purple
Palmatine..	0·15	0·28	0·45	Yellow	Brown
Berberine	0·13	0·36	0·49	Lime green	Brown
Chelerythrine	0·07	0·21	0·92	Yellow	Brown
Nitidine	0·00	0·00	0·40	Green	Brown

* Mean values of between 10–20 runs.

Results and discussion

The separation of the twelve alkaloids obtained by thin-layer chromatography using solvents 1–3 is shown in Table 1. By using these systems together with fluorescence (after exposure to ammonia vapour), and also the colours produced with iodo-

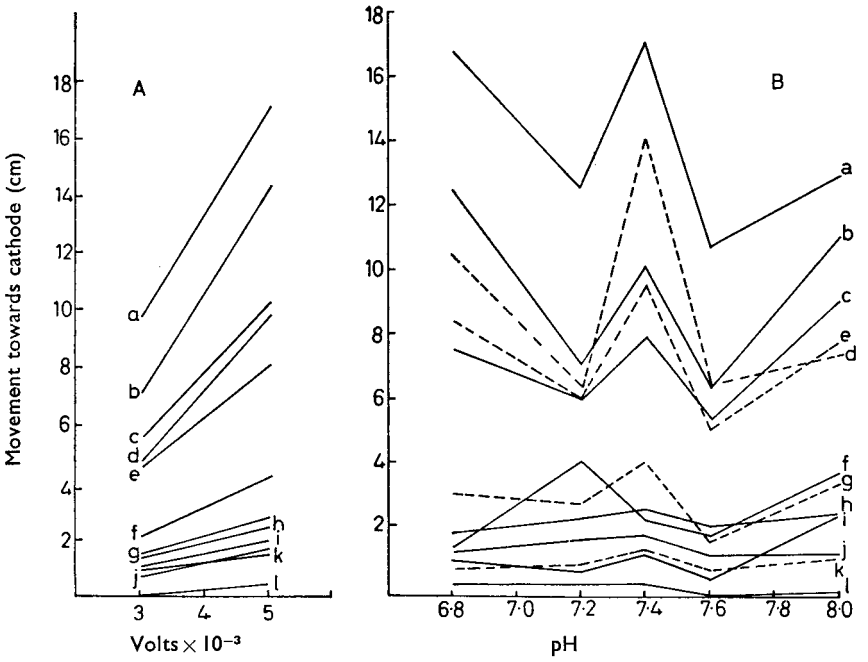

FIG. 1. A. Electrophoretic movement of alkaloids at different voltages; 20 min; pH 7·4. The alkaloids are: a; candicine; b, coryneine; c, tembetarine; d, *N*-methylisocorydine; e, *N*-methylcorydine; f, xanthoplanine; g, palmatine; h, berberine; i, chelerythrine; j, laurifoline; k, magnoflorine and 1, nitidine.

B. Electrophoretic movement of alkaloids in buffered conditions at different pH values; 20 min; 5000 V. The alkaloids are: a, candicine; b, tembetarine; c, *N*-methylisocorydine; d, *N*-methylcorydine; e, coryneine; f, berberine; g, xanthoplanine; h, palmatine; i, magnoflorine; j, chelerythrine; k, laurifoline; l, nitidine.

platinate reagent, it is possible to identify all twelve alkaloids. A disadvantage is the time taken for separation; with solvent 2 this was 210 min. However, compared with paper chromatography (Kuck & others, 1967), the use of cellulose thin-layers, using solvent 3, is advantageous since candicine, tembetarine, *N*-methylisocorydine and nitidine were more easily identified.

The separation of the twelve alkaloids by high voltage electrophoresis is shown in Fig. 1A and B. Fig. 1A indicates the advantage of using the highest possible voltage, the alkaloids moving greatest distances at 5,000 V. All the alkaloids were reasonably well separated at pH 7·4 (Fig. 1B), but laurifoline, magnoflorine, berberine, palmatine, and the benzophenanthridines, chelerythrine and nitidine were best separated at pH 8·0. The principal advantage of this method is the rapidity (20 min), with which the separations can be achieved.

The influence of hydroxyl groups on mobility of the alkaloids is shown by the fact that coryneine with two hydroxyl groups, has a lower mobility than candicine with one hydroxyl group. In the aporphine series, also, the mobility of magnoflorine (two hydroxyl groups) is very much less than that of either *N*-methylcorydine (one hydroxyl) or *N*-methylisocorydine (one hydroxyl) and the mobility of laurifoline (two hydroxyl groups) less than that of xanthoplanine, and this alkaloid, with one hydroxyl group at position 9, has a lower mobility than either *N*-methylcorydine or *N*-methylisocorydine, each with one hydroxyl group at carbons 1 and 11 respectively.

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REFERENCES

AGURELL, S. (1965). *Acta pharm. sueica*, **2**, 372.

BRITTON, H. T. S. & ROBINSON, R. A. (1931). *J. chem. Soc.*, 1456–1462.

CALDERWOOD, J. M. & FISH, F. (1966). *J. Pharm. Pharmac.*, **18**, *Suppl.*, 119S–125S.

KUCK, A. M., ALBONICO, S. M., DEULOFEU, V. & ESCALENTE, M. G. (1967). *Phytochem.*, **6**, 1541–1550.

MARINI-BETTOLO, G. B. & COCH FRUGONI, J. A. (1958). *J. Chromatog.*, **1**, 182–185.