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Note

Thin-layer chromatography of some quaternary alkaloids and alkaloid N-oxides

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For the analysis of quaternary alkaloids, paper chromatography (PC) is usually employed. For example, two-dimensional PC has been successfully used in the separation of the components of naturally occurring complex mixtures of curare alkaloids^{1,2}. The chromatography of these quaternary and bisquaternary alkaloids has been reviewed by Marini-Bettolo and his co-workers^{3,4}. Paper electrophoresis has also been applied to the separation of such alkaloids⁵. However, thin-layer chromatography (TLC) of quaternary alkaloids and alkaloid N-oxides gives rise to some problems; such separations can only be made by use of very polar solvent systems. The classical solvent systems of butanol, water and acetic or formic acid may be used in connection with thin layers of silica gel. The disadvantage of such systems is the rather long development time of the chromatograms and the long saturation time of the chromatography tanks (24 h). The bisquaternary curare alkaloids have very low R_F values in such solvent systems. Other TLC systems were therefore required for the analysis of this type of alkaloid.

We found that the solvent systems described by Jane⁶ for high-speed liquid chromatography of drugs of abuse on silica gel columns were also useful for TLC separations of quaternary alkaloids and alkaloid N-oxides. The solvent systems were aqueous methanol solutions of ammonium nitrate of various concentrations.

EXPERIMENTAL

Merck TLC aluminium sheets (20 × 20 cm) precoated with silica gel 60 F₂₅₄ (layer thickness, 0.25 mm; Art. 5554) were used for the analysis summarized in Table I, and Merck TLC plates (5 × 20 cm) precoated with silica gel 60 F₂₅₄ (layer thickness, 0.25 mm; Art. 5714) were used for the analysis summarized in Tables II and III. The plates were stored in the original packing and were not activated before use.

The working conditions were: temperature, 22°; relative humidity, 23%. The plates were developed in normal chromatography chambers (the insides of which were covered with filter paper), which were saturated with the solvent system for 1 h before use. The plates were developed over a distance of 10 cm. Solvent systems: 1 = methanol-0.2 M NH₄NO₃⁻ (3:2); 2 = methanol-2 M NH₄OH-1 M NH₄NO₃ (7:2:1) (see also Tables I-III). Methanol (Baker Analyzed Reagent, Cat. No. 8045) was obtained from J. T. Baker, Deventer, The Netherlands.

RESULTS AND DISCUSSION

The R_F values of some tertiary and quaternary alkaloids and alkaloid N-oxides, obtained by means of TLC on silica gel layers using aqueous methanol solutions of ammonium nitrate and ammonium nitrate with ammonia, are summarized in Table I. It is to be noted that the N-oxides have R_F values higher than those of the corresponding tertiary alkaloids, which is opposite to the usual TLC behaviour of these compounds on silica gel with less polar organic solvents as the mobile phase.

The effect of changes in the solvent systems 1 and 2 on the R_F values of some alkaloids are summarized in Tables II and III. When ammonium nitrate in system 1 was replaced by ammonium chloride or sodium chloride no major changes in the R_F values were observed. No great changes in the R_F values of the quaternary alkaloids and the alkaloid N-oxides were observed when calcium or magnesium chloride were used instead of ammonium nitrate (Table II). However, the R_F values of the tertiary alkaloids increased considerably when magnesium chloride was used. Moreover, a kind of bearding of the tertiary alkaloids was observed, resulting in unsymmetrical bell-sized spots on the chromatogram. Use of a 0.1 M solution of calcium chloride gave approximately the same R_F values for the quaternary alkaloids as use of 0.2 M solutions of ammonium salts. When ammonium acetate, ammonium hydrogen carbonate or ammonium carbonate were used instead of ammonium nitrate, the R_F values

TABLE I

R_F VALUES OF SOME QUATERNARY AND TERTIARY ALKALOIDS AND ALKALOID N-OXIDES IN THE TLC SYSTEMS METHANOL-0.2 M NH_4NO_3 (3:2), 1, AND METHANOL-2 M NH_4OH -1 M NH_4NO_3 (7:2:1), 2, ON SILICA GEL

Compound	System	
	1	2
Dihydrotoxiferine	27	24
C-Alkaloid H	23	22
Caracurine V methiodide	16	14
Bisnordihydrotoxiferine N-oxide	24	74
Bisnordihydrotoxiferine di-N-oxide	29	79
Bisnor-C-alkaloid H N-oxide	14	65
Bisnor-C-alkaloid H di-N-oxide	20	75
Caracurine V N-oxide	12	45
Caracurine V di-N-oxide	17	72
Bisnordihydrotoxiferine	15	58
Bisnor-C-alkaloid H	5	50
Caracurine V	3	27
Alcuronium	33	43
Tubocurarine	20	40
Macusine B	60	57
Melinonine A	49	48
Antirrhine methochloride	51	51
Strychnine methochloride	31	30
Strychnine N-oxide	33	72
Strychnine	19	63
Serpentine	54	66
Alstonine	57	66

TABLE II

INFLUENCE OF DIFFERENT SALTS, SALT CONCENTRATION AND DIFFERENT SOLVENT RATIOS ON THE R_F VALUES OF SOME QUATERNARY AND TERTIARY ALKALOIDS AND ALKALOID N-OXIDES AS OBSERVED IN TLC ON SILICA GEL

Salt	Molarity of salt solution	Ratio of methanol to salt solution	Compound	Alstonine	Dihydro-toxiferine	Alcuronium	Meltrontine A iodide	Strychnine N-oxide	Strychnine	Carvacurine V
NH ₄ Cl	0.2	3:2	57	27	37	51	30	28	4	
NH ₄ NO ₃	0.2	3:2	61	26	34	49	40	25	3	
NaCl	0.2	3:2	59	24	37	48	49	29	4	
MgCl ₂	0.2	3:2	63	34	44	54	33	38	36	
CaCl ₂	0.2	3:2	64	33	44	55	33	40	38	
CuCl ₂	0.1	3:2	57	26	34	48	28	26	20	
NH ₄ NO ₃ [*]	0.2	3:2	47	9	14	30	46	16	0	
NH ₄ NO ₃ ^{**}	0.2	3:2	59	10	14	32	49	27	9	
NH ₄ OOCCH ₃	0.2	3:2	54	14	24	40	34	21	2	
NH ₄ OH	0.2	3:2	10	1	0	3	60	30	3	
(NH ₄) ₂ CO ₃	0.2	3:2	38	5	7	25	45	16	1	
NH ₄ HCO ₃	0.2	3:2	36	4	4	21	42	13	0	
0.1 M NH ₄ OOCCH ₃ - 0.1 M CH ₃ COOH	0.2	3:2	54	11	14	43	33	39	4	
NH ₄ Cl	0.2	2:3	55	24	32	48	30	28	0	
NH ₄ Cl	0.2	1:4	26	10	16	35	21	25	0	
NH ₄ Cl	0.2	4:1	51	13	23	38	23	17	5	
NH ₄ Cl	1	3:2	64	45	53	59	32	50	44	
NH ₄ NO ₃	1	3:2	65	47	54	60	32	50	45	

* Before development the plate was exposed to ammonia vapours for 30 min.

** The plate was first developed with ethyl acetate-isopropanol-25% ammonia (9:7:1) and then allowed to dry in the open air for 30 min, before development in the solvent system mentioned.

TABLE III
 INFLUENCE OF THE AMMONIA CONCENTRATION AND SOLVENT RATIO ON THE R_f VALUES OF SOME QUATERNARY AND TER-
 TIARY ALKALOIDS AND ALKALOID N-OXIDES IN METHANOL-AQUEOUS AMMONIA-AMMONIUM NITRATE SOLUTIONS, AS OB-
 SERVED IN TLC ON SILICA GEL

<i>Solution</i>	<i>Total molarity of aqueous solution</i>	<i>Ratio of methanol to salt solution to ammonia solution</i>	<i>Compound</i>	<i>Alstonine</i>	<i>Dihydro- toxicferine</i>	<i>Aleurostium</i>	<i>Melinonine A iodide</i>	<i>Strychnine N-oxide</i>	<i>Strychnine</i>	<i>Carac- citrine V</i>
1 M NH_4NO_3 -2 M NH_4OH	1.67	7:1:2	68	29	37	44	66	58	28	
1 M NH_4Cl -2 M NH_4OH	1.67	7:1:2	68	24	40	47	66	57	29	
1 M NaCl -2 M NH_4OH	1.67	7:1:2	69	23	40	49	67	58	28	
0.1 M NH_4NO_3 -2 M NH_4OH	1.37	7:1:2	52	0	1	11	68	60	31	
1 M NH_4NO_3 -0.1 M NH_4OH	0.4	7:1:2	56	11	19	36	47	22	3	
1 M NH_4NO_3 -1 M NH_4OH	1	7:1:2	66	28	37	47	63	48	17	
1 M NH_4NO_3 -1 M NH_4OH	1	5:2:3	75	51	64	66	76	56	22	
1 M NH_4NO_3 -1 M NH_4OH	1	5:1:4	75	40	58	61	75	59	26	
1 M NH_4NO_3 -1 M NH_4OH	1	5:4:1	73	53	66	67	69	32	12	
1 M NH_4NO_3 -1 M NH_4OH	1	27:1:2	28	1	3	11	32	24	3	
1 M NH_4NO_3 -1 M NH_4OH	1	2:3:5	81	76	79	79	89	65	7	
1 M NH_4NO_3 -1 M NH_4OH	1	3:3:4	84	73	83	83	89	63	14	

of the quaternary alkaloids, and to a lesser extent those of the tertiary alkaloids, decreased, whereas the values of the N-oxides were little affected. When the plates were saturated with ammonia vapour, or first run with the solvent system ethyl acetate-isopropanol-25% ammonia (9:7:1), in which system only the tertiary alkaloids move, a considerable decrease in the R_F values of the quaternary alkaloids was observed. In a weakly acidic solvent system (0.1 M ammonium acetate-0.1 M acetic acid) the R_F values of the tertiary alkaloids increased compared with a pure ammonium acetate system, whereas the quaternary alkaloids were not affected. Apparently the pH of the mobile phase affects the R_F values of the alkaloids. An increase of the ionic strength to 1 M increased particularly the R_F values of the tertiary and quaternary alkaloids. Variations of the ratio of methanol to water showed that the highest R_F values were obtained with a ratio of 2:3 or 3:2.

For solvent system 2 consisting of ammonium nitrate and ammonia in water-methanol, it was found that ammonium nitrate could be replaced by other salts without major changes in the R_F values. A decrease in the ionic strength of the salt led to a decrease in the R_F values of the quaternary alkaloids (Table III). A decrease in the ammonia concentration affected especially the tertiary alkaloids, giving lower R_F values. Decreasing the concentration of methanol resulted in an increase in the R_F values of the quaternary alkaloids. However, the dimeric alkaloid caracurine V gave a decreased R_F value under these conditions.

As mentioned by Jane⁶, the choice of solvent system is not straightforward. The kind of solvent systems mentioned above seems to offer new possibilities for the separation of alkaloids by means of TLC, in particular for both quaternary and bis-quaternary alkaloids and alkaloid N-oxides. More extensive studies of TLC of a series of other alkaloids will be made in an attempt to make the results of alkaloid separations more predictable.

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