Detection of pyrrolizidine alkaloids on thin-layer chromatograms

For the detection of pyrrolizidine alkaloids on paper or thin-layer chromatograms several reagents have been used, and these vary in sensitivity and specificity.

Exposure to iodine[1,2] is the least specific, giving brown spots with many classes of compounds, though it has the advantages that subsequent treatment with another reagent, or isolation of the compound from the plate, are possible. Other reagents are general for organic bases. "Platinum iodide" is excellent for pyrrolizidine alkaloids on paper chromatograms[3] but is less effective on thin-layer chromatograms. Dragendorff's reagent has been used for thin-layer chromatograms of pyrrolizidine alkaloids[4] but it also is non-specific and its sensitivity is limited by the pale colour of its spots.

The method described here is lengthy and less convenient than those mentioned above, but it has the advantages of being more sensitive, and specific for alkaloids having an unsaturated pyrrolizidine ring. It is an extension of the method described by DANN[5] for the detection of N-oxides of pyrrolizidine alkaloids. After chromatography the alkaloids on the plate are converted to N-oxides by a hydrogen peroxide spray followed by heating. For the detection of N-oxides, this stage is omitted. Treatment with acetic anhydride then converts the N-oxides to pyrroles, which with Ehrlich reagent (4-dimethylaminobenzaldehyde) give blue or mauve spots. The oxidation stage cannot be applied to paper chromatograms.

Only alkaloids having an unsaturated (3-pyrroline) ring in the basic moiety will respond to this procedure. Compounds of this type other than pyrrolizidine alkaloids would seem to be rare in nature. Certain indole and pyrrole derivatives give strong red or mauve colours with Ehrlich's reagent. However, these appear to be suppressed after the oxidation and acetic anhydride treatments.

The method, with modifications, has been applied to the estimation of pyrrolizidine alkaloids in solution, and a full discussion wil appear elsewhere[6].

Experimental

Reagents

Chemicals were analytical grade where available.

30 % hydrogen peroxide. "100 volumes" solution, in which was dissolved sodium pyrophosphate, 2–4 mg per ml.

Peroxide anhydride. As an alternative to the above reagent: "100 volume" hydrogen peroxide, with phosphate as above, diglyme (diethylene glycol dimethyl ether), and acetic anhydride, 1:1:2 by volume, mixed in that order. This reagent was prepared just before use and any remaining unused was washed away immediately, to avoid dangerous organic peroxide formation.

Reagents containing hydrogen peroxide were kept from contact with skin and with combustible materials, such as wood and paper. A sheet of polythene in a fume cupboard formed a suitable background for all spraying operations, and eye protection was worn.

Acetic anhydride[5]. Acetic anhydride, light petroleum (b.p. 80–100°) and benzene, 1:4:5 by volume.

Ehrlich's reagent. Dimethylaminobenzaldehyde (1 g) was dissolved in absolute ethanol (70 ml), carbitol (diethylene glycol monoethyl ether; 30 ml), and hydrochloric acid (1.5 ml). The carbitol could be replaced by ethanol with slightly inferior results (weaker spots, stronger background colour).

Procedure

The alkaloids, applied as methanol solutions, were chromatographed on Kieselgel G (Merck) plates (0.25 mm layers). The usual solvent system was chloroform–acetone–ethanol–0.88 ammonia (5:3:1:1, by volume), and runs of 10 cm were usual. The non-aqueous phase from *n*-butanol–acetic acid–water (4:1:5 by volume) was also used, especially for N-oxides and for compounds such as chlororetronecine, which were not stable as free bases.

Plates were air-dried, sprayed lightly with 30 % aqueous hydrogen peroxide, and heated at 90–100° for about 15 min. A small oven, preferably in a fume cupboard was suitable for this purpose. However, similar results could be obtained using a boiling water bath, kept in a fume cupboard. The plates were placed horizontally over the steam, coated sides up, after first being carefully warmed to avoid cracking the glass. After cooling, the plates were sprayed with acetic anhydride reagent, and heated as before for 15 min. This caused some alkaloids to appear as weak brown spots, fluorescent when viewed in ultraviolet light[5]. However, much greater sensitivity was achieved by spraying with Ehrlich's reagent, and heating the plates for a further 5–15 min. The alkaloids then appeared as blue spots against a pale yellow or nearly colourless background. The spots were almost permanent, though the background darkened with keeping. They could not be eluted from the plate.

The first two sprays could be combined into one, with a saving in time but a slight reduction in sensitivity. The dried plates were sprayed with fresh "peroxide-anhydride" reagent and heated as before for 15 min before applying the Ehrlich reagent spray.

Results

The method was first tested on a chromatogram of retrorsine N-oxide (Table I), the oxidation stage being omitted. The use of Ehrlich's reagent greatly improved the sensitivity of detection after using Dann's reagent, bringing the limit down to 0.1 μg.

TABLE I

THIN-LAYER CHROMATOGRAPHY OF RETRORSINE N-OXIDE (ISATIDINE)

Solvent system: *n*-butanol–acetic acid–water.
(A) Visible (brown) spots after spraying with acetic anhydride reagent and heating for 15 min. (B) Yellow fluorescent spots visible under long wavelength ultraviolet illumination after treatment A. (C) Blue spots visible after treatment A followed by Ehrlich's reagent and heating for 10–15 min.

Amount applied	*Relative strengths of spots (R_F 0.20)* *		
(μg)	*A*	*B*	*C*
26	+ +	+ + +	+ + +
12.8	+	+ +	+ + +
6.4	+	+ +	+ +
3.2	+	+	+ +
1.6	—	trace	+
0.8	—	trace	+
0.4	—	faint trace	+
0.2	—	—	+
0.1	—	—	trace

* + Means a distinct spot; — means no spot visible.

TABLE II

THIN-LAYER CHROMATOGRAPHY OF RETRORSINE

Solvent system: chloroform–acetone–ethanol–ammonia.

Amount applied (*μg*)	*Relative strengths of spots* (R_F *0.35*) *shown by reagents*			
	Platinum iodide spray	*Exposure to iodine vapour*	*Modified Dragendorff reagent*	*Present method*
128	+ + +	+ + +	+ + +	+ + + +
64	+ +	+ +	+ + +	+ + +
32	+ +	+ +	+ +	+ +
16	+	+	+	+ +
8	+	+	+	+
4	trace	+	trace	+
2	—	trace	faint trace	+
1	—	—	—	+
0.5	—	—	—	+
0.25	—	—	—	trace

TABLE III

THIN-LAYER CHROMATOGRAPHY OF PYRROLIZIDINE ALKALOIDS AND OTHER BASES

Solvent systems: chloroform–acetone–ethanol–ammonia.
Detection: by the method described in the text.

Group	*Compound*	R_F*	*Colour of spot*
I	Retrorsine	0.35	blue
	Diacetylretrorsine	0.70	blue
	Monocrotaline	0.43	blue
	Senecionine	0.63	blue
	Anacrotine	0.33	blue
	Lasiocarpine	0.74	blue
	Heliotrine	0.33	blue
	Supinine	0.28	mauve
II	Rosmarinine	0.33	blue
III	Retronecine	0.09	blue
	Heliotridine	0.05	blue
IV	Platynecine	0.00	weak yellow-brown
	Retronecanol	—	none
V	Strigosine	—	none
VI	Brucine	—	none
	Strychnine	—	none
	Arecoline	0.78	weak yellow-brown
	Quinine	—	none
	Nicotine	—	none
VII	Benzylamine	—	none
	Pyrrolidine	—	none
VIII	Indole	0.80	very weak brown
	Pyrrole	—	none

* R_F values varied slightly with different batches of solvent and adsorbent. Hence these values should be regarded as relative, not absolute.

Using retrorsine alkaloid, a comparison was made of four methods of detection, similar plates being treated with different reagents (Table II). The present method (including oxidation stage) was superior to the others, probably because of the good contrast of blue spots against yellow background, and the limit of detection was nearly as good as that for retrorsine N-oxide.

For other alkaloids (Table III) the limits of detection were not determined, but ester alkaloids having unsaturated pyrrolizidine basic moieties (group I) all gave well defined blue or mauve spots in the range 1–20 μg. Rosmarinine (II) also gave a blue spot, which appeared after a longer heating time. Though this alkaloid has a saturated base, it is possible that dehydration of the hydroxylated ring occurs. Unsaturated pyrrolizidine amino alcohols (III) also gave blue spots, which needed slightly longer than the esters to reach maximum intensity. The saturated amino alcohols (IV) and esters (V) gave either no colour or weak brown spots. Some unrelated alkaloids (VI) and bases (VII) were tested. These either gave no colour, or weak brown spots which faded after prolonged heating, whereas they gave strong spots with the other reagents mentioned in Table II. The strong colours normally given by Ehrlich's reagent with indole and pyrrole (VIII) were almost completely suppressed after the treatment with hydrogen peroxide.

By running a duplicate chromatogram, but omitting the oxidation stage, only unsaturated pyrrolizidine N-oxides and certain indoles and pyrroles (where present) could be detected. A chromatogram treated only with Ehrlich's reagent showed only indole and pyrrole derivatives. Thus unsaturated pyrrolizidine alkaloids could be distinguished from their N-oxides and from other constituents of a mixture, as well as from other bases.

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