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Note

The use of π-acceptors for detection of alkaloids on thin layers

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Many reagents are available for the detection of alkaloids on paper and thin-layer chromatograms[1], the most popular being Dragendorff's reagent, which gives one colour with all alkaloids irrespective of their structure. It is therefore desirable to examine other possible reagents. Electron acceptors have proved of value in thin-layer chromatography (TLC) for the detection and identification of many electron-rich aromatic and heterocyclic compounds, including some of biological interest[2–11]. This factor, in addition to the high sensitivity attained in the assay of alkaloids with π-acceptors[12], stimulated the investigation of these reagents for the detection of alkaloids in TLC.

EXPERIMENTAL

The alkaloids used were of pharmaceutical grade (DAB 7), obtained from various manufacturers. 7,7,8,8-Tetracyanoquinodimethane (TCNQ) (Fluka, Buchs, Switzerland), 2,4,7-trinitrofluorenone (TNF) (Fluka), 2,4,5,7-tetranitrofluorenone (TetNF) (Fluka), 2,3-dichloro-5,6-dicyanoquinone (DDQ) (Merck, Darmstadt, G.F.R.) and 2,4-dinitrofluorobenzene (DNFB) (Merck) were sufficiently pure to be used as purchased. Tetracyanoethylene (Merck, "for synthesis") was purified according to published procodures[13].

Pre-coated silica gel G (0.25 mm) plates (Merck) with a fluorescent indicator were used without prior activation. The alkaloids were applied in chloroform solutions (bases) or in methanol–water (7:3) (salts) at a concentration of 5 mg/ml (2–5 μl).

The developing solvent was acetone–toluene–methanol–ammonia (45:40:10:5). Spray reagents were used at a concentration of 0.2% in acetonitrile.

RESULTS AND DISCUSSION

The colours produced by reaction of the alkaloids with the π-acceptors are given in Table I. TCNE gave no distinct colours with most of the alkaloids examined. In general, the order of decreasing sensitivity and wider applicability is TCNQ >

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TABLE I

COLOURS OF TLC SPOTS OF ALKALOIDS WITH π-ACCEPTORS

Colours: Bl = blue; Br = brown; Gn = green; Go = gold; Gy = grey; nc = no colour; Or = orange; R = red; V = violet; W = white; Y = yellow. White background unless otherwise stated.

Alkaloid	*TCNQ**	*TNF*		*TetNF after heating*	*DDQ***	*DNFB*
		In the cold	*After heating*			
Atropine	Y-W	nc	V	Gn-Gy***	Or	Y
Scopolamine	Gn-Y	nc	Gy	Gy***	nc	Y
Homatropine	Go	nc	V	V-Gy***	Or	Y
Homatropine·MeBr	Go	nc	R	V***	nc	Y-W
Atropine·$MeNO_3$	Y-Or	nc	R	V***	nc	Y-W
Tropine	Go	nc	V-Gy	V***	Gn	Y-W
Pilocarpine	Go	nc	Gy	V-Gy***	V	Y
Ephedrine	Gn-Y	nc	Gy	faint***	nc	Y-Or
Cocaine	Go	nc	V-Gy	V***	nc	Or
Morphine	Bl	Or-Br	Or	Or	Gn	Or
Codeine	Y-Go	Or-Br	Y	Gy	V-Br	Or
Papaverine	Y-Go	Or	Y	Br	V	Or
Quinine	Y-Go	Y	V-Br	V	V	Or
Brucine	Go	V	V	Gy	V	Or
Strychnine	Y	Y	Y	Or	Or	Or
Veratrine	Go	Or	Or	Gy	Or	Or
Reserpine	Gn	Gy	Gy	Or	Gn	Or-R
Ergotamine	Y-Gn	Gy-Br	Gy-Br	Gy	Br	Or-R

* Colours given were on a pale bluish green background.
** Pale violet background.
*** No colour in the cold.

TNF, TetNF > DDQ > DNFB. The detection limits with TCNQ ranged from 0.5 to 10 μg per 50 mm^2 (Table II).

Alkaloids seem to interact with TCNQ on thin layers by complex reactions, which are probably different from reactions in solution[12]. In the latter instance, the predominant chromogen was shown to be the blue-coloured $TCNQ^-$ radical ion[12]. The various shades of colours produced on the chromatograms (Table I) imply that other interactions also took place. These interactions could involve additional π-complex formation with the aromatic rings in some alkaloids, possible dimerization of the cationic or anionic radial ions formed from the ionization reaction, oxidation–reduction reactions and the formation of zwitterionic resonance structures[10].

Alkaloids lacking large aromatic nuclei such as the tropane family, pilocarpine and ephedrine did not produce colours with TNF and TetNF in the cold (Table I). This result may indicate that these reagents interact mainly by π-complex formation. Thus, only polynuclear alkaloids would react in the cold to a sufficient extent for colour development, and only after heating would alkaloids of simpler structure be revealed, possibly through other types of interactions.

This behaviour could be used with advantage in the preliminary assignments of the structures of alkaloids. The combination of the π-acceptor reagents with other general alkaloid reagents, such as Dragendorff and iodoplatinate reagents, could be a very powerful means of obtaining useful information on the class of alkaloid present.

TABLE II

$R_F \times 100$ VALUES AND DETECTION LIMITS IN THE TLC OF ALKALOIDS WITH TCNQ
Developing solvent: acetone–toluene–methanol–ammonia (45:40:10:5).

Alkaloid	$R_F \times 100$	*Detection limit (μg per 50 mm²)*
Atropine	44	5
Scopolamine	65	8
Homatropine	43	10
Homatropine·MeBr	9	5
Atropine·$MeNO_3$	17	10
Tropine	27	2
Pilocarpine	58	5
Ephedrine	80	0.5
Cocaine	88	3
Morphine	34	2
Codeine	50	4
Papavarine	78	8
Quinine	58	5
Brucine	42	5
Strychnine	57	8
Veratrine (two spots)	49 83	8
Reserpine	91	10
Ergotamine	70	8

The rapid development of colours at room temperature with non-corrosive reagents, the variation of colour shades with many alkaloids depending on their structure and the sensitivity and stability of colours suggest obvious uses of these π-acceptor reagents to supplement existing methods for the detection of alkaloids on chromatograms.

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