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Note

Chloranil, a sensitive detection reagent for pyrrolizidine alkaloids on thin-layer chromatograms

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For the detection of pyrrolizidine alkaloids on thin-layer chromatographic (TLC) plates, Mattocks[1] developed a reagent specific for hepatotoxic alkaloids with an unsaturated (3-pyrroline) ring in the basic moiety.

The alkaloids that are usually isolated as free bases after reduction of their corresponding N-oxides, which are often abundant in plant material, have to be converted into the N-oxides again after TLC separation of the free bases, by treatment with hydrogen peroxide, prior to further derivatization. The N-oxides are treated with acetic anhydride for their conversion into pyrroles, which give coloured compounds with Ehrlich reagent. Although the detection limit for retrorsine is low (0.25 μg), the complete detection method is lengthy and the conditions are critical. Another disadvantage of the method is that several hazardous chemicals are required. Because we experienced problems with the reagent, we discontinued investigations with this detection technique.

A second frequently used method for the detection of pyrrolizidine alkaloids involves the use of Dragendorff's reagent[2–7]. This reagent, however, is not only non-specific towards the different groups of alkaloids that can be detected, but also gives positive results with common plant sterols, triterpenes[8], α- and γ-pyrones and several other compounds[9], especially when used in combination with sodium nitrite as intensifier spray.

Chloranil is used as a spray for the detection of capsaicin and hydroquinone derivatives. It yields blue or red-brown spots with the former compounds after exposure to ammonia vapour[10]. A further use has been described for the detection of alkaloids that form charge-transfer complexes with π-acceptors such as chloranil[11].

In this paper we describe the use of chloranil as a sensitive and highly convenient spray reagent for the detection of pyrrolizidine alkaloids on TLC plates.

EXPERIMENTAL

Materials

Pyrrolizidine alkaloid extracts from *Echium vulgare*, *Omphalodes verna*, *Heliotropium europaeum*, *Symphytum officinale*, *Cynoglossum officinale*, *C. nervosum* and *Amsinckia intermedia* were derived according to Pedersen[5]. All reagents and

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solvents were of analytical-reagent grade. Lycopsamine, acetyllycopsamine (or diastereoisomers) and symphytine were purified from *Symphytum officinale* alkaloid extracts by means of ion-pair high-performance liquid chromatography on a Waters Prep LC/System 500 A under conditions described previously for TLC[12]

Detection reagents

Dragendorff's reagent in a modification according to Munier (not diluted) was used[13]. The intensifier spray was a 10% (w/v) solution of sodium nitrite[8].

Chloranil was used as a 1% (w/v) solution in toluene or methylene chloride. After spraying, the plates were heated at 105°C for 15 min. Sulphuric acid (2 *N*) was used as an intensifier spray.

Thin-layer chromatography

Ion-pair system. Silica gel thin-layer plates (pre-coated, Schleicher & Schüll, Dassel, G.F.R.) were impregnated with a 0.15 *M* solution of lithium chloride in methanol for 5 sec by dipping, followed by blotting and drying at 105°C. The plates were stored in a desiccator. Pyrrolizidine alkaloids were separated as ion pairs with chloroform–methanol (75:25) as eluent.

Straight-phase system. Pyrrolizidine alkaloids were separated on pre-coated silica gel TLC plates using chloroform–methanol–ammonia (85:14:1) as the eluent (see *Isolation and mass spectrometry of a main alkaloid from Omphalodes verna*).

Determination of responses to detection reagents

For determination of the responses to chloranil, amounts of 5 μg of several alkaloids in appropriate solvents were spotted on to silica gel thin-layer plates.

Minimal detectable amounts of lycopsamine, acetyllycopsamine (or diastereoisomers), symphytine, brucine and harmaline were determined by spotting decreasing amounts of the alkaloids on TLC plates.

The spots were revealed with Dragendorff and chloranil reagents followed by spraying with intensifier sprays.

Isolation and mass spectrometry of a main alkaloid from Omphalodes verna

Bands of an extract from *Omphalodes verna* in chloroform were applied to preparative TLC plates (20 × 20 cm, 1 mm silica gel layer; Merck, Darmstadt, G.F.R.) by means of a Camag Chromatocharger. After development of the plates with the straight-phase system, the borders of the plate were sprayed with Dragendorff reagent and the partially located main band was scraped off and powdered. The alkaloid was stripped from the silica gel by elution with methanol. Mass spectrometry of the alkaloid was performed on a Finnigan 3300 quadrupole mass spectrometer equipped with a 6110 data system with an electron energy of 70 eV and an ionizing current of 100 μA.

RESULTS AND DISCUSSION

Several alkaloids with different structural groups show a positive response to chloranil on TLC plates after spraying with a 1% (w/v) solution of chloranil in toluene. When methylene chloride was used as the solvent for chloranil, however, a considerable

decrease in the colour response occurred; toluene was therefore used as the solvent for chloranil throughout.

From Table I it can be seen that, depending on the circumstances during the detection procedure, several colours could appear even for a single compound. This phenomenon might be a possible aid in qualitative evaluations when screening for natural products.

TABLE I

COLOURS AND RESPONSES (RELATIVE TO BACKGROUND) OF SEVERAL ALKALOIDS (5 μg) DETECTED WITH CHLORANIL, IMMEDIATELY AFTER SPRAYING (A), FOLLOWED BY HEATING FOR 15 MIN AT 105°C (B) AND SUBSEQUENTLY SPRAYING WITH 2 *N* SULPHURIC ACID (C)

Order of increasing response: −, (±), ±, +, ++, +++, ++++.

Alkaloid	*A*		*B*		*C*	
	Colour	*Response*	*Colour*	*Response*	*Colour*	*Response*
Papaverine	Light brown	±	Grey	++	Grey	+++
Cinchonine	Brown	±	Brown	±	Brown	±
Theophylline	—	—	—	—	—	—
Emetine	Brown	+	Brown	+	Ochre	++
Scopolamine	—	—	—	—	—	—
Solanidine	Brown	+	Brown	±	Purple	±
Brucine	Blue	+	Blue	+	Purple	++
Caffeine	—	—	—	—	—	—
Narceine·HCl	Brown	±	White	+	Purple	+
Morphine	Red	+	Yellow	+	Red	++
Tropine	Purple	+	Purple	+	Brown	+
Quinine	Brown	±	Brown	±	Purple	±
Narcotine	—	—	Brown	(±)	Brown	(±)
Atropine	Brown	±	Purple	+	Red	+
Hyoscyamine	Brown	±	Purple	+	Purple	+
Strychnine	Brown	±	Purple	+	Purple	+
Codeine	Brown	±	Purple	+	Purple	+
Cephaline·HCl	Brown	±	Brown	+	Brown	++
Aconitine	Brown	±	Brown	±	Brown	±
Capsaicine	Brown	±	White	+	Brown	+
Nicotine	Purple/brown	++	Red/brown	+	Red	+
Berberine·HCl	Yellow	+	Yellow	++	Ochre	+++
Ephedrine	Yellow	±	Purple	+	Purple	
Piperidine	Blue	++	Grey	+	Grey	+
Harmaline	Green/black	+++	Black	++++	Black	++++
Symphytine	Green/brown	++	Brown	+++	Grey/brown	++++

* Yellow-coloured alkaloid.

Both harmaline and symphytine were highly sensitive to the reagent. From a comparison of the minimal detectable amounts of the pyrrolizidine alkaloids tested visible after spraying with chloranil and its intensifier and with Dragendorff's reagent and its intensifier, it could be concluded that a slightly improved detection limit could be achieved for acetyllycopsamine and symphytine by using chloranil (Table II). The use of sulphuric acid as the intensifier for chloranil did not give rise to a higher response with the alkaloids, but changed the background of the plate from purple

TABLE II

MINIMAL DETECTABLE AMOUNTS OF BRUCINE, LYCOPSAMINE, ACETYLLYCOPSAMINE AND SYMPHYTINE WITH DRAGENDORFF REAGENT AND CHLORANIL AFTER USE OF THEIR RESPECTIVE INTENSIFIER SPRAYS

Alkaloid	*Minimal detectable amount (μg)*	
	Dragendorff + nitrite	*Chloranil + acid*
Brucine	0.40	0.40
Harmaline	0.04	0.04
Lycopsamine	2.0	2.0
Acetyllycopsamine	0.20	0.15
Symphytine	0.20	0.15

to a very light brown, which led to an increase in the relative response due to enhanced contrast.

Other advantages of chloranil over Mattocks' and Dragendorff's reagents are that the method is very convenient because of the ease of preparation of the spray and that the spots remain visible on the TLC plates for more than a year. Further, chloranil is far more specific than Dragendorff's reagent.

The mass spectrum of the main alkaloid originating from *Omphalodes verna*, which was purified by means of preparative TLC, showed a base peak at *m/e* 124. The appearance of this peak together with a peak at *m/e* 83 (75%) indicates a monoester pyrrolizidine alkaloid with a (±)-trachelanthamidine or (±)-isoretronecanol nucleus[14,15]. These necines lack the 1,2-double bond in the basic moiety and could therefore be considered as non-hepatotoxic even when esterified at the C9 position[1]. After TLC separation of the original alkaloid extract derived from *Omphalodes verna*, a main and two minor spots appeared after spraying with Dragendorff's reagent. Chloranil, however, failed to give any coloured spots under all conditions tested.

Comparison of the alkaloid patterns obtained by TLC separation of extracts (ion-pair system) from *Echium vulgare*, *Heliotropium europaeum*, *Symphytum officinale*, *Amsinckia intermedia*, *Cynoglossum nervosum* and *C. officinale* and detection with Dragendorff's reagent and chloranil did not show any differences. These plants are known to contain pyrrolizidine alkaloids with necines having the double bond in the 1,2-position and with esterification of the primary hydroxyl group at C9[16]. Hence chloranil might be useful as a reagent for hepatotoxic pyrrolizidine alkaloids. This, together with the ease of preparation, the use of non-hazardous chemicals, the low detection limit and the stability of the spots, should be of great value in the detection of most pyrrolizidine alkaloids.

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