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CHROMOGENIC REACTIONS OF *RAUWOLFIA* ALKALOIDS AFTER SEPARATION BY THIN-LAYER CHROMATOGRAPHY

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SUMMARY

Forty-one *Rauwolfia* alkaloids were tested using 11 chromogenic reagents. Ferric chloride–perchloric acid reagent was preferred for general use. The dihydroindole alkaloids comprised the most readily definable group but aricine, 10, 11-dimethoxyheteroyohimbines, sarpagine and *ar*-substituted 18-hydroxy-yohimbines and their esters could be distinguished using various reagents.

INTRODUCTION

The genus *Rauwolfia* has yielded a large number of indole alkaloids[1] and several workers have reported systems of thin-layer chromatographic (TLC) separation mainly on silica gel layers for these closely-related compounds[2–4]. As an aid to identification and as a supplement to the information gained from R_F values, the use of chromogenic reagents has also been reported[2,4,5]. The results cited in earlier literature were considered inconclusive and therefore 41 *Rauwolfia* alkaloids have been tested using 11 chromogenic reagents sprayed on to silica gel thin layers after chromatographic separation. The common chemical structures of *Rauwolfia* alkaloids are shown in Fig. 1.

METHODS

Chromatographic separation techniques and systems were described in an earlier communication[3].

MATERIALS

Silica gel G (Merck, Darmstadt, G.F.R.) and Kieselgel GF (Merck) were used for the thin layers. The plates (20 × 20 cm with layers 250 μm thick) were activated (60 min) at 110° and stored in a desiccator. Plates were used within 72 h of preparation.

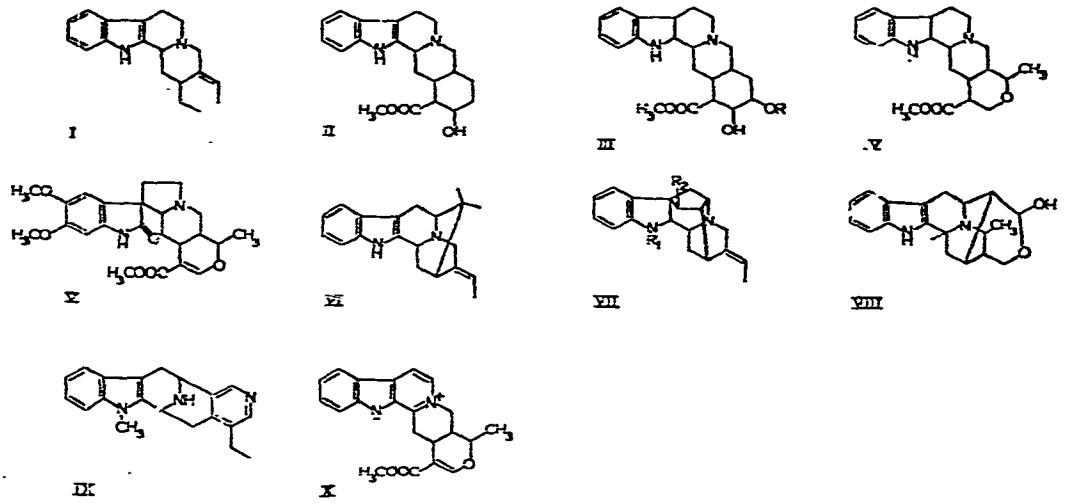

Fig. 1. Common chemical structures of *Rauwolfia* alkaloids. I, E-*seco* indole; II, yohimbine; III, 18-hydroxy-yohimbine (R = H) and esters (R = trimethoxybenzoic or trimethoxycinnamic); IV, heteroyohimbine; V, oxindole; VI, sarpagan; VII, dihydroindole (R_1 = H or CH_3; R_2 = OH or = O); VIII, peraksine; IX, suaveoline; X, anhydronium bases.

Developing solvents

The following solvent systems were used: (1) xylene–isooctane–ethyl acetate–diethyl ether (15:45:5:40); (2) *n*-butanol–ethyl acetate–ethylene dichloride(10:30:60); (3) acetone–petroleum ether (b.p. 40–60°)–carbon tetrachloride–isooctane (35:30:20:15); (4) methanol–methylethyl ketone–*n*-heptane (8.4:33.6:58); (5) acetone–*n*-butanol–isooctane (33.6:8.4:58); (6) acetone–petroleum ether (b.p. 40–60°)–glacial acetic acid (45:45:10); (7) acetone–methanol–glacial acetic acid (70:25:5); (8) *n*-butanol–glacial acetic acid–water (4:1:1).

Chromogenic reagents

(1) Ferric chloride–perchloric acid reagent: 5% ferric chloride in 35% (v/v) aqueous perchloric acid solution; (2) ceric sulphate reagent: 1% ceric sulphate in 10% (w/w) sulphuric acid solution; (3) Van Urk's reagent: 1% *p*-dimethylaminobenzaldehyde in 50% (v/v) concentrated hydrochloric acid in absolute alcohol; (4) vanillin reagent: 1% vanillin in 50% hydrochloric acid in methanol; (5) Frohde's reagent: 1% ammonium molybdate in 50% (w/w) sulphuric acid solution; (6) ammonium vanadate reagent: 1% ammonium vanadate in 50% nitric acid solution; (7) phosphomolybdic reagent: 0.5% phosphomolybdic acid in 50% nitric acid solution; (8) Van Urk–Salkowski reagent: solution A, Van Urk reagent; solution B, Salkowski reagent (2.03 g ferric chloride hexahydrate in 500 ml water and 300 ml concentrated sulphuric acid); the reagent was used as a mixture of solutions A and B in the proportion 1:3; (9) ninhydrin reagent: 1% ninhydrin in 25% (v/v) concentrated sulphuric acid in ethanol; (10) hydroxylamine–ferric chloride reagent: 1% hydroxylamine in 5% chloride in 50% sulphuric acid; (11) cinnamaldehyde reagent: 1% cinnamic aldehyde in methanol. After spraying plates were allowed to dry by standing in an atmosphere of hydrochloric acid.

Reference alkaloid solutions

Solutions (0.1%) of alkaloids in methanol were freshly prepared and 5–10-μl quantities applied to thin-layer plates prior to development.

After development the plates were dried for 5 min with a hot-air blower. Spray reagents were applied to the dried plates and the spray reagent solvent removed by heating using a hot-air dryer for 5 min and subsequent heating in an oven at 100° for 5–10 min or as otherwise directed until maximum colour had developed. The resultant chromogenic reactions are recorded in Table I.

RESULTS AND DISCUSSION

Previous work in our laboratories[6–9] has shown that the alkaloids of the *Rauwolfia* species form five main groups *viz.*, (a) E-*seco* indole (I), (b) yohimbine (II) and 18-hydroxy-yohimbine esters (III), (c) heteroyohimbine (IV) and derived oxindoles (V), (d) sarpagan (VI), and (e) dihydroindole (VII) together with a small number of trace alkaloids of different indole structure *e.g.* peraksine (VIII), suaveoline (IX), and the anhydronium bases, *e.g.* serpentine (X). It was therefore important to discover whether the groups could be differentiated on the basis of chromogenic reactions.

Ferric chloride–perchloric acid reagent

Perchloric acid and an oxidising agent has been used to yield coloured complexes in the assay of the dihydroindole alkaloid ajmaline[10].

Using ferric chloride–perchloric acid reagent, the dihydroindole group was clearly differentiated. The N_a-demethyl alkaloids *e.g.* norajmaline, nortetraphyllicine, produced an orange colour whilst the N_a-methyl dihydroindoles *e.g.* ajmaline, tetraphyllicine, yielded a deep red or crimson colour. Dihydroindole alkaloids having methoxy substitution of the aromatic ring *e.g.* purpeline, seredamine, yielded purple or violet colours but it was not possible to differentiate *ar*-substituted N_a-methyl and demethyl forms. The colour reactions were well-defined in the range 0.5–50 μg but the reagent quenched ultraviolet fluorescence.

E-*seco* indole alkaloids and *ar*-methoxy substituted 18-hydroxy-yohimbine esters yielded a greenish-brown reaction but the non-substituted esters gave a greyish colour. Such colours were difficult to detect below the 1-μg level. Sarpagine, a 10-hydroxy-sarpagan, produced a characteristic steel-grey colour detectable at 0.5 μg but other sarpagans only gave weak greyish colours which were difficult to detect below the 1-μg quantity. Other yohimbine and heteroyohimbine alkaloids yielded greyish-brown or brown colours which were equally difficult to detect and of little diagnostic value. Except for serpentine, which yielded a pronounced blue-violet fluorescence (366 nm wavelength), all fluorescences were quenched by the reagent.

Ceric sulphate–sulphuric acid reagent

The use of ceric ammonium sulphate reagent in the identification of the indole alkaloids of *Catharanthus* species was discussed by Farnsworth *et al.*[11].

With ceric sulphate reagent, as with the previous reagent, the dihydroindole group is defined clearly in the 0.5–50 μg range, the colour produced being indicative of the structure of the individual alkaloid *i.e.* N_a-methyl compounds give crimson, the N_a-demethyl compounds pink and the *ar*-substituted compounds purple. The

TABLE I

CHROMOGENIC REACTIONS OF *RAUWOLFIA* ALKALOIDS

	Ferric chloride–perchloric acid	*Ceric sulphate*	*Van Urk*	*Vanillin*	*Frohde*
E-Seco indoles					
Corynantheol	Green-brown	Green-brown	Pink	Violet-blue	Green
Corynantheal	Green-brown	Green-brown	Pink	Violet-blue	Yellow-green
Geissoschizol	Green-brown	Green-brown	Pink	Violet-blue	Yellow-green
Yohimbines					
Yohimbine	Grey	Grey-brown	Pink	Violet-blue	Brown-green
α-Yohimbine	Grey	Grey-brown	Pink	Violet-blue	Brown
ψ-Yohimbine	Grey	Grey-brown	Pink	Violet-blue	Brown
11-Methoxyyohimbine	Brown	Brown	Brown	Mauve	Yellow
17-Acetylyohimbine	Grey	Grey	Pink	Violet-blue	Grey
17,18-Diacetylyohimbine	Brown	Grey	Pink	Violet-blue	Brown
18-Hydroxy-yohimbine	Grey	Grey	Pink	Violet-blue	Brown-grey
18-Hydroxy-yohimbine esters					
Reserpine	Green-brown	Green-brown	Brown	Mauve	Yellow-green
Renoxidine	Green-brown	Green-brown	Brown	Red-violet	Green
Rescinnamine	Green-brown	Green-brown	Brown	Red-violet	Yellow-green
Deserpidine	Grey	Grey	Pink	Blue-violet	Yellow-green
Methyl deserpidate	Grey	Grey	Pink	Violet-blue	Yellow-green
Heteroyohimbines					
Ajmalicine	Grey-brown	Brown	Pink	Violet-blue	Green
Tetrahydroalstonine	Grey-brown	Grey-brown	Pink	Violet-blue	Yellowish-green
Aricine	Brown	Brown	Purple	Red-violet	Green
10,11-Dimethoxy-ajmalicine	Brown	Blue-brown	Brown	Grey	Orange
Reserpiline	Blue-brown	Violet	Brown	Grey	Pink
Isoreserpiline	Blue-brown	Violet-brown	Brown	Grey	Pink
Oxindoles					
Carapanaubine	Buff	Buff	Brown	Brown	Green
Isocarapanaubine	Pale pink	Buff	Brown	Brown	Green
Rauvoxine	Buff	Buff	Brown	Brown	Green
Rauvoxinine	Buff	Buff	Brown	Brown	Green
Isoreserpiline–ψ-indoxyl	Pale pink	Buff	Brown	Yellow	Yellow-green
Sarpagans					
Sarpagine	Steel grey	Violet	Pink	Pale violet	Violet
Normacusine B	Grey	Grey	Pink	Violet-blue	Green
Normacusine B-O-methyl	Grey	Grey	Pink	Violet-blue	Green
Dihydroindoles					
Norajmaline	Orange	Pink	Pale green	Pale green	Yellow
Nortetraphyllicine	Orange	Pink	Pale green	Pale green	Yellow
Norpurpeline	Violet	Purple	Blue	Grey	Grey
Endolobine	Purple	Purple	Blue	Grey	Grey
Ajmaline	Crimson	Crimson	Green	Pink	Red
Tetraphyllicine	Red	Crimson	Green	Pink	Red
Ajmalidine	Crimson	Crimson	Green	Pink	Red
Purpeline	Blue-violet	Purple	Blue	Grey	Grey
Seredamine	Violet	Purple	Blue	Grey	Grey
Miscellaneous types					
Peraksine	Grey	Brown	Pink	Violet-blue	Yellow-green
Suaveoline	Grey	Grey	Pink	Violet-blue	Green
Serpentine	Buff	Buff	—	Yellow	Yellow

Ammonium vanadate	*Phosphomolybdic acid*	*Van Urk–Salkowski*	*Ninhydrin*	*Hydroxylamine–ferric chloride*	*Cinnamaldehyde*
Green	Yellow-green	Brown	Yellow-brown	Yellow-brown	Blue-grey
Green	Yellow-green	Brown	Yellow-brown	Yellow-brown	Blue-grey
Green	Yellow-green	Brown	Brown	Yellow-brown	Blue-grey
Brown	Yellow	Green	Grey	Yellow	Blue-grey
Brown	Yellow-green	Green	Grey	Yellow	Blue-grey
Brown	Yellow-green	Green	Grey	Yellow	Blue-grey
Greenish-grey	Yellow	Yellow-green	Yellow-green	Brown	Blue-grey
Grey	Yellow-green	Grey	Brown	Yellow	Blue-grey
Grey	Yellow-green	Grey	Grey	Yellow	Blue-grey
Grey	Yellow-green	Grey	Grey	Yellow	Blue-grey
Brown-green	Yellow-green	Yellow-green	Yellow-green	Yellow-green	Pink-red-purple
Yellow-green	Yellow-green	Yellow-green	Yellow-green	Brown	Pink-red-purple
Brown-green	Yellow	Yellow-green	Yellow-green	Yellow-green	Pink-red-purple
Green	Yellow	Grey	Grey	Yellow	Pink-red-purple
Green	Yellow	Grey	Grey	Yellow	Pink-red-purple
Green	Yellow-green	Brown	Yellow-green	Yellow-green	Blue-grey
Green	Yellow	Brown	Yellow-green	Brown	Blue-grey
Olive-green	Green	Yellow-green	Grey	Yellow-brown	Purple
Brown	Yellow-green	Grey	Brown	Brown	Blue-grey
Brown	Yellow-green	Grey	Brown	Brown	Grey-brown
Brown	Yellow-green	Grey	Brown	Brown	Grey-brown
Buff	Brown	Brown	Yellow-brown	Brown	Grey-brown
Buff	Yellow	Pink	Buff	Brown	Grey-brown
Buff	Yellow	Pink	Buff	Brown	Grey-brown
Buff	Yellow	Brown	Buff	Brown	Grey-brown
Yellow	Brown	Brown	Brown	Yellow	Grey-brown
Brown	Yellow-brown	Pale violet	Pale green	Brown	Violet
Grey	Yellow	Green	Grey	Yellow	Blue-grey
Grey	Yellow	Green	Grey	Yellow	Blue-grey
Yellow	Orange	Brown	Pink	Orange	Yellow
Yellow	Orange	Brown	Yellow	Orange	Yellow
Brown	Yellow-brown	Grey	Grey	Pale violet	Yellow
Brown	Violet-brown	Purple	Grey	Pale violet	Red-purple
Red	Pink	Red	Pink	Pink	Blue-grey
Red	Pink	Red	Pink	Pink	Blue-grey
Red	Pink	Pink	Pink	Pink	Blue-grey
Grey	Yellow-brown	Yellow-brown	Grey	Pale violet	Blue-grey
Yellow-brown	Violet-brown	Grey	Pale violet	Pale violet	Purple
Grey	Yellow-green	Yellow-green	Grey	Yellow	Blue-grey
Grey	Yellow-green	Yellow-green	Grey	Yellow	Blue-grey
Buff	Buff	Buff	Brown	Buff	—

colours produced fade but can be regenerated by respraying. The blue-violet fluorescences (366 nm wavelength) were still apparent after spraying and drying.

Alkaloids of the indole type (yohimbines, heteroyohimbines and sarpagans) normally yielded grey or brown colour reactions but substitution of the *ar*-ring resulted in different colour reactions. Thus reserpine, renoxidine (reserpine N-oxide) and rescinnamine (all 11-methoxy-substituted) yielded a greenish-brown colour; the 10,11-dimethoxy-substituted heteroyohimbines, 10,11-dimethoxy-ajmalicine, reserpiline and isoreserpiline yielded blue-brown, violet and violet-brown, respectively; sarpagine (10-hydroxy-sarpagan) gave a violet colour whereas aricine, a 10-methoxy-heteroyohimbine, produced a brown colour. The oxindole alkaloids yielded a buff colouration as did the anhydronium base serpentine. The E-*seco* group, although not *ar*-substituted gave a greenish-brown reaction.

The reagent was satisfactory throughout the 0.5–50 μg range and ultraviolet fluorescences were apparent after spraying and drying except for the oxindoles and Ψ-indoxyl.

Van Urk reagent

The reaction between *p*-dimethylaminobenzaldehyde, and the indole nucleus has been exploited for the colorimetric assay of yohimbine[12] and reserpine[13].

With Van Urk's reagent, the unsubstituted indole alkaloids usually produced a pink colour although the methoxy-substituted 18-hydroxy-yohimbines and heteroyohimbines and the oxindoles gave a brown colour reaction. Aricine, the 10-methoxy-heteroyohimbine, was atypical and yielded a purple colouration.

The N_a-demethyl dihydroindole group of alkaloids with unsubstituted *ar*-rings yielded pale green and the corresponding N_a-methyl compounds gave green colours. The *ar*-substituted compounds whether N_a-demethyl or N_a-methyl yielded blue colour reactions.

In general the reagent was effective over the 0.5–50 μg range. Ultraviolet fluorescences were quenched for dihydroindole, oxindole and sarpagan compounds.

Vanillin reagent

The red colour produced when an indole nucleus reacts with vanillin was employed by Banes[14] for the assay of reserpine.

Using the spray reagent the indole alkaloids of the yohimbine, heteroyohimbine, E-*seco* and sarpagan types gave violet-blue colour reactions except for the dimethoxyheteroyohimbines (grey) and the related oxindoles (brown).

The dihydroindole group was distinct; the N_a-methyl compounds of the ajmaline type yielded pink colours, the N_a-demethyl compounds pale green and the mono-*ar*-substituted compounds grey.

The reagent was effective over the 0.5–50 μg range. The reagent quenched the ultraviolet fluorescence of dihydroindoles, oxindoles and sarpagans.

Frohde's reagent (ammonium molybdate reagent)

The unsubstituted indoles and mono-substituted indoles (E-*seco* heteroyohimbines, 18-hydroxy-yohimbines, heteroyohimbines and *ar*-unsubstituted sarpagans) yielded green chromogenic reactions as did the oxindoles. The *ar*-unsubstituted yohimbines however produced brown reactions and the dimethoxy-hetero-

yohimbines reserpiline and isoreserpiline gave characteristic pink reactions. Sarpagine was characterised by a violet colour.

The dihydroindole alkaloids behaved quite differently; the *ar*-substituted compounds yielded grey colours, the N_a-methyl compounds red and the N_a-demethyl compounds yellow.

The reagent yielded satisfactory results in the 0.5–50 μg range. Ultraviolet fluorescence was quenched for *ar*-unsubstituted N_a-methyl dihydroindoles and sarpagans.

Ammonium vanadate reagent

Vanadate reagent reactions have been employed to yield colour complexes for the assay of at least seven *Rauwolfia* alkaloids[15,16].

In general the colours produced resembled those obtained using Frohde's reagent and the reagent was effective in the 0.5–50 μg range. Ultraviolet fluorescences after spraying were weak or not apparent except for serpentine which yielded a strong blue-violet fluorescence.

Phosphomolybdic reagent

With this reagent the indole alkaloids normally yielded yellow-green or yellow-brown colour reactions.

The dihydroindole alkaloids were again clearly differentiated, the *ar*-substituted compounds yielding yellow-brown, the N_a-methyl compounds pink and the N_a-demethyl alkaloids orange chromogenic reactions.

The reactions were readily detectable in the 1.5–50 μg range. Ultraviolet fluorescences after spraying were weak or absent except for serpentine.

Van Urk–Salkowski reagent

Yohimbines and sarpagans produced green colours although the 10-hydroxy-sarpagan sarpagine yielded violet and the esterified yohimbines yellow-green (11-methoxy-substituted) or grey (*ar*-unsubstituted). The dimethoxy-substituted heteroyohimbines *e.g.* reserpiline, isoreserpiline, also gave grey colours but the 10-methoxy relative, aricine, yielded yellow-green. The dihydroindoles were not so clearly differentiated as, although the N_a-methyl type yielded red colours, the N_a-demethyl compounds gave brown and the *ar*-substituted compounds grey. All other alkaloids tested yielded brown colours with this reagent.

The reactions were readily detected in the 1–50 μg range but at 0.5 μg colours were difficult to differentiate. Ultraviolet fluorescences after spraying were only easily detectable for 18-hydroxy-yohimbines and their esters (blue-green) and serpentine (intense blue-violet).

Ninhydrin reagent

Ninhydrin (triketohydrindene hydrate) has been widely used as a spray reagent for the detection of amino acids[17]. As alkaloids are amino acid-derived it seemed reasonable to investigate the possibilities of this reagent with *Rauwolfia* alkaloids.

Most E-*seco* heteroyohimbine, yohimbine, 18-hydroxy-yohimbine and hetero-

yohimbine alkaloids yielded a yellow-green, yellow-brown or grey colour although the dimethoxy-*ar*-substituted heteroyohimbines yielded a definite brown colour.

The sarpagans could not be clearly differentiated yielding a pale green (sarpagine) or grey colouration and similarly the oxindoles produced a non-definitive buff colour. Although the brown or buff colours could be detected at 0.5-μg amounts, the yellow or grey colours were difficult to differentiate below 1 μg.

The dihydroindoles were more clearly separated, the ajmalidine group (ajmaline, norajmaline, ajmalidine and tetraphyllicine) yielding pink, nortetraphyllicine yellow, seredamine (12-methoxy-tetraphyllicine) pale violet and purpeline, norpurpeline and endolobine grey.

Except for serpentine, ultraviolet fluorescence colours were either not apparent or weak after application of ninhydrin reagent.

Hydroxylamine–ferric chloride reagent

This reagent was employed by Vincent and Schwal[18] for the quantitative determination of reserpine or rescinnamine.

The dihydroindoles were clearly defined, the N_a-demethyl compounds giving an orange reaction, the N_a-methyl compounds pink and the *ar*-substituted alkaloids pale violet colours.

The oxindoles produced brown colour reactions and the sarpagans yellow colours although sarpagine also yielded a brown colour. These reactions were readily identifiable in the 0.5–50 μg range.

The remaining indole alkaloids gave insignificant yellowish, yellowish-green or yellowish-brown chromogenic reactions which were not easily detectable below 1 μg alkaloid.

The ultraviolet fluorescences after spraying were only conspicuous for the 18-hydroxy-yohimbine ester alkaloids (blue-green) the heteroyohimbines (blue-green) and serpentine (intense blue-violet).

Cinnamaldehyde reagent

The use of cinnamic aldehyde reagent as a spray reagent was recommended by Kaiser and Popelak[19].

Applying this method to TLC a reasonable range of colours was obtained. Greyish-brown colours indicated dimethoxyheteroyohimbines and derived oxindoles although isoreserpiline–Ψ-indoxyl yielded an orange colour. An intense yellow colour was obtained with the N_a-demethyl-dihydroindoles norajmaline and nortetraphyllicine. Pink changing to red-purple indicated the 18-hydroxy-yohimbine ester alkaloids, violet sarpagine and purple aricine or *ar*-substituted dihydroindoles. Blue-grey colours were obtained with N_a-methyl-dihydroindoles, E-*seco* indole alkaloids, *ar*-unsubstituted sarpagans, yohimbines and the heteroyohimbines ajmalicine and tetrahydroalstonine. Serpentine yielded no reaction.

Considerable care was needed in the detection of colours produced by less than 5 μg alkaloid.

Ultraviolet fluorescence colours after spraying were only detected for 18-hydroxy-yohimbine ester alkaloids, yohimbines, heteroyohimbines and serpentine.

CONCLUSIONS

The oxidising reactions are particularly useful and, in our experience, the best general reagents are ferric chloride–perchloric acid, ammonium vanadate and ceric sulphate–sulphuric acid reagents although the last named yields less stable colours.

The dihydroindole alkaloids comprise the most readily definable group and it is possible to differentiate the *ar*-substituted forms as well as the *ar*-unsubstituted N_a-demethyl and N_a-methyl alkaloids.

The indole alkaloids of the E-*seco* heteroyohimbine, yohimbine, 18-hydroxy-yohimbine, heteroyohimbine and sarpagan types are not so readily differentiated as groups although certain alkaloids do behave atypically. Thus aricine can be identified using Van Urk reagent, 10,11-dimethoxyheteroyohimbines employing vanillin or Frohde reagents, sarpagine using ferric chloride–perchloric acid, Frohde, Van Urk–Salkowski, ninhydrin or cinnamaldehyde reagents, and *ar*-substituted 18-hydroxy-yohimbine esters using ferric chloride–perchloric acid, ceric sulphate, Van Urk, Van Urk–Salkowski, ninhydrin or cinnamaldehyde reagents. Chromogenic reactions can only be reproducible if the solvent systems are driven off completely from the adsorbent layers. Normally drying at 105° for 1 h will remove the solvents but, in our experience, solvent systems containing diethylamine cannot successfully be driven off and therefore interfere with chromogenic reagents. Although the fluorescences of *Rauwolfia* alkaloids viewed under screened ultraviolet light are useful in differentiating the alkaloids before spraying with chromogenic reagents[4], no advantage was obtained by viewing after spraying.

Experience is an important factor in assessing the significance of the chromogenic reactions and combination of the colour reactions with fluorogenic reactions under screened ultraviolet light[4] and TLC data[3,4] yields a useful tool in the characterisation and identification of the *Rauwolfia* alkaloids.

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