Journal of Chromatography, 298 (1984) 289–307
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,799

IDENTIFICATION OF *TABERNAEMONTANA* ALKALOIDS BY MEANS OF THIN-LAYER CHROMATOGRAPHY AND CHROMOGENIC REACTIONS*

T. A. VAN BEEK*, R. VERPOORTE and A. BAERHEIM SVENDSEN
Department of Pharmacognosy, Subfaculty of Pharmacy, University of Leiden, Gorlaeus Laboratories, P.O. Box 9502, 2300 RA Leiden (The Netherlands)
(Received April 4th, 1984)

SUMMARY

A method for the identification of 100 *Tabernaemontana* alkaloids by means of their hR_F values in four solvents and their chromogenic reactions with three spray reagents is described.

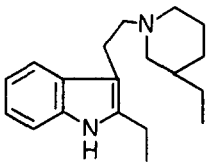
INTRODUCTION

Thin-layer chromatography (TLC) in combination with different spray reagents is a powerful tool used as a standard control technique during the isolation, separation and purification of non-volatile natural products. For the positive identification of natural products, however, it is usually used only for known substances and then only when reference material is available (coTLC). It has been little used in the identification of known compounds without reference samples, or of unidentified compounds, but its possible application is probably underestimated. Especially when reactions with different selective spray reagents or the fluorescent properties of the compound are taken into account, many compounds or groups of compounds can be easily distinguished from one another. It is often possible to predict the presence of a certain group in the molecule responsible for the reaction. If two compounds give the same colour reaction it is usually possible to differentiate between them with TLC owing to differences in their polarity and thus to identify them by means of the hR_F values.

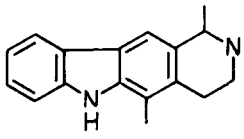
For the large group of the indole alkaloids (*ca.* 2500 representatives) only a few investigations have been carried out giving hR_F values and/or chromogenic behaviour for the purpose of identification. Farnsworth *et al.*[1] gave the hR_F values and chromogenic responses with the ceric-ammonium sulphate reagent for 63 *Catharanthus* alkaloids. Kasymov *et al.*[2] and Vachnadze *et al.*[3] carried out similar investigations for *Vinca* alkaloids partly dealing with the same alkaloids as Farnsworth. Court and Iwu[4] published the chromogenic behaviour of 41 *Rauwolfia* alkaloids with

* Part 10 in the series Pharmacognostical studies of *Tabernaemontana* species, for part 9 see ref. 14.

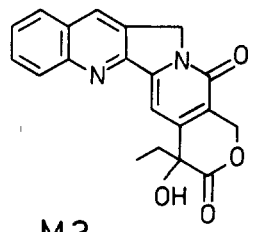
0021-9673/84/$03.00



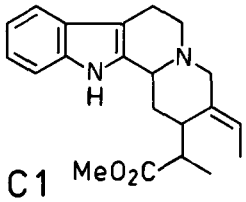
M1



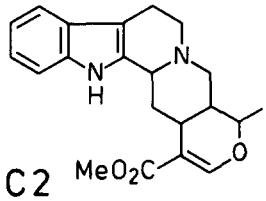
M2



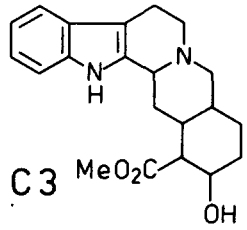
M3



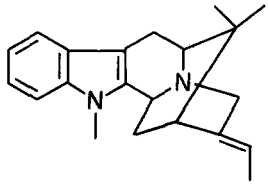
C1



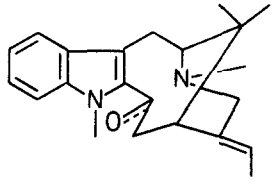
C2



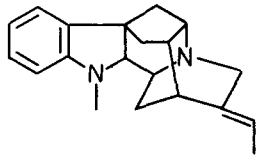
C3



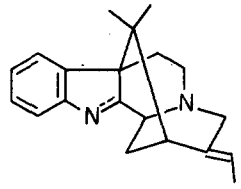
C4



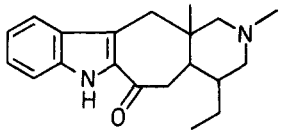
C5



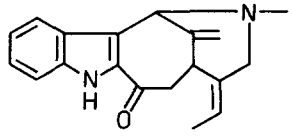
C6



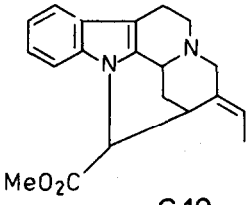
C7



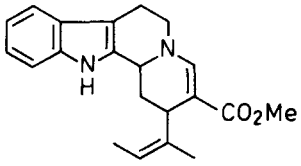
C8



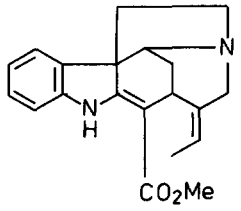
C9



C10



V1



S1

Fig. 1.

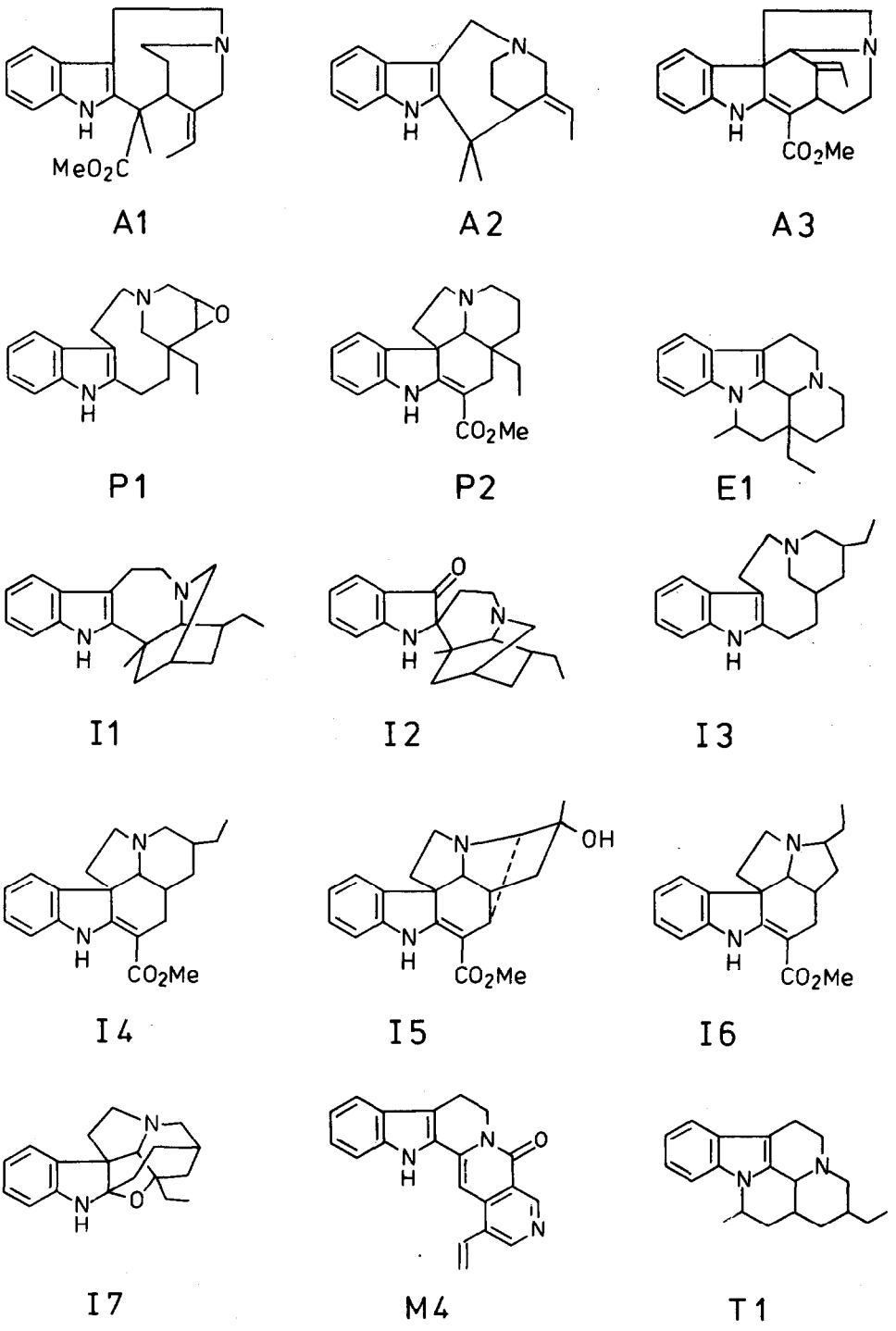


Fig. 1. Basic skeletons of the various groups of *Tabernaemontana* alkaloids.

eleven different spray reagents. Court and Timmins[5] published the hR_F values of 24 *Rauwolfia* alkaloids in ten different solvent systems. Shellard and Lala[6] published the hR_F values of some *Mitragyna* alkaloids. Phillipson and Bisset[7] studied some *Strychnos* alkaloids and discussed the relation between the structure and retention. Phillipson and Hemingway[8] gave the hR_F values and chromogenic responses of a series of *Uncaria* alkaloids. For a more exhaustive review of all TLC investigations on indole alkaloids see "Chromatography of Alkaloids"[9]. No such investigation has been published of alkaloids isolated from *Tabernaemontana* species. The genus belongs to the family of the Apocynaceae and comprises *ca.* 100 species. About 250 different indole alkaloids have been isolated so far from the genus. Although some of the *T.* alkaloids also occur in the genera *Rauwolfia* and *Catharanthus*, most of them are different and restricted to the genera *Tabernaemontana, Tabernanthe, Voacanga* and *Stemmadenia.* The basic skeletons of the various groups of *Tabernaemontana* alkaloids are given in Fig. 1. The code of the different skeleton types is based on biosynthetical grounds and has been published elsewhere[10]. The most frequently occurring groups are those of the monomeric I1 and C5 type and of the dimeric C5-I1 type. In the present publication the hR_F values in four solvent systems and the chromogenic responses with three spray reagents for 100 different alkaloids belonging to the various subgroups of the genus *T.* are described. The data obtained may be of value for the identification of these and similar alkaloids especially when reference material is not available.

MATERIALS AND METHODS

Ready-made silica gel PF_{254} (Merck, type 5715) plates were used. The plates (20 × 20 cm with layers 0.25-mm thick) were stored under normal laboratory conditions. The following solvent systems were used: S1, cyclohexane–chloroform–diethylamine (6:3:1); S2, toluene–absolute ethanol, containing 1.74% g/v ammonia gas (19:1)*; prior to development the plates were stored for 20 min in an atmosphere of ammonia**; S3, chloroform-methanol (9:1); S4, ethyl acetate–2-propanol–26% g/v ammonia (17:2:1).

All solvents were distilled before use or were of p.a. quality, except for the absolute ethanol which was used as such.

Glass developing chambers (internal size 21.7 × 5.7 × 20.7 cm) lined with filter paper were used. In each instance, 60 ml of the appropriate solvent were placed in the chamber and equilibrated for exactly 30 min prior to the introduction of the plate. A fresh charge of solvent was used for the development of each plate. The alkaloids used in this study were either isolated in our laboratories from various *Tabernaemontana, Alstonia* or *Strychnos* species, purchased from Roth-Chemische Fabrik-Karlsruhe, or were gifts from other research laboratories.

On every plate nineteen different alkaloids in amounts of 5–20 μg were applied as stripes (7 × 1.5 mm) 2.0 cm from the bottom of the plate. The alkaloids were chromatographed as free bases, dissolved in chloroform, ethanol, or a mixture of the two. The plates were developed for a distance of 15–17 cm. The temperature during

* Prepared by bubbling dry ammonia through absolute ethanol cooled to 0°C for 30 min.

** Prepared by placing five 10-ml flasks filled with 26% g/v ammonia in a sealed glass developing chamber.

the experiments was 22 ± 2°C and the relative humidity 25 ± 5%. Following the chromatographic development the plates were air-dried for 1 h. Then the alkaloids were detected by means of their quenching of 254-nm light and by their possible fluorescence under 366-nm light. The centre of each spot, for purposes of hR_F calculation, was taken as the approximate centre of maximum quenching of 254-nm light. The hR_F was calculated as:

$$hR_F = 100 \times \frac{\text{distance of alkaloid's spot from point of sample application (mm)}}{\text{distance of solvent front from point of sample application (mm)}}$$

Three reference alkaloids (voacangine, tacamine and vobasine) were spotted on each plate and served as indicators for defects in the plates or other abnormal chromatographic phenomena. Each of the 100 *T.* alkaloids was chromatographed at least twice in each solvent system (S1–S4). The hR_F values given in Table I are the average values of all the experiments. After detection at 254 nm the plates were sprayed with one of three detection reagents: D1, 3.25% $FeCl_3$ in 35% $HClO_4$ (FCPA reagent); D2, 1% $CeSO_4$ in 10% H_2SO_4 (CSSA reagent); D3, 0.2% 7,7,8,8-tetracyanoquinodimethane in acetonitrile (TCNQ reagent)[11].

Approximately 4–5 ml of spray reagent were applied evenly to each plate. Any colours or colour changes were noted, and 5 min after spraying with D1 or D2 the plates were heated with a hot air blower (air temperature 220°C). Any colour changes during the heating (short, intermediate and prolonged) were noted. After spraying and heating, any possible fluorescence (254 and 366 nm) was noted. Also any colour changes after heating and after one day were noted. The plates sprayed with D3 were not heated, and in this case colours were noted after 10 min. Plates developed with S1 were not used because the residual diethylamine could not be adequately removed without possible decomposition of the alkaloids and interfered with the chromatogenic reactions. Only plates developed with system S3 were sprayed with D3 to eliminate any possible interference from any residual ammonia or diethylamine from the solvent systems S1, S2 and S4.

RESULTS AND DISCUSSION

Choice of solvents

The following reasons led to the choice of the four solvent systems used in our investigations.

(1) The systems used should possess a high discriminating power. Therefore, one diethylamine-containing system, one water-free ammonia-containing system, one ammonia- and water-containing system and one neutral system were used. Different solvents were used to enhance the discriminating effect. Although many other combinations are possible, experience taught that the systems mentioned work well for indole alkaloids and have very different separation characteristics.

(2) The solvents should give a reasonably short development time of the TLC plate. Therefore butanol, acids or high concentrations of water were not used.

(3) The system should be easy to prepare. Therefore, no more than three components were used. Also no component should occur in a concentration lower than 5% to enhance reproducibility.

TABLE I

hR_F VALUES OF *TABERNAEMONTANA* ALKALOIDS

Alkaloid	*Biosynthetical group*	*hR_F value in solvent**			
		S1	*S2*	*S3*	*S4*
Geissoschizine	C1	1	16; 20**	30 t	66 t
Isositsirikine	C1	16	9	16 t	45
16-Epi-isositsirikine	C1	5	7	14 t	38
Tetrahydro-alstonine	C2	45	51	69	75
Reserpiline	C2	25	23	53	57
Isoreserpiline	C2	32	32	67	70
Serpentine	C2	0	1	0	4 f
Yohimbine	C3	16	14	30 t	54
Akuammidine	C4	7	10	22	52
Normacusine-B	C4	6	10	7 t	49
Pericyclivine	C4	11	21	37	61
16-Epi-affinine	C5	16	14	25 t	57
Dregamine	C5	34	25	44	60
Perivine	C5	17	19	23	52
Tabernaemontanine	C5	38	30	55	68
Anhydro-vobasindiol	C5	23	20	18 t	53
Vobasine	C5	35	25	48	62
Vobasinol	C5	4	11	20 t	52
Akuammiline	C7	24	15	34	38
Desacetyl-akuammiline	C7	12	9	23	27
Picraline	C7	8	11	30	36
Methuenine	C8	36	25	22 t	51
Isomethuenine	C8	21	12	7 t	25
Pleiocarpamine	C10	33	23	28 t	43
Vallesiachotamine	V1	19 f	23***	62***	66
Isovallesiachotamine	V1	19 f	25***	65***	66
Akuammicine	S1	35	26	21 t	45
12-Hydroxy-akuammicine	S1	2	8	13 t	31
Akuammicine N_4-oxide	S1	0	3	11	4
Norfluorocurarine	S1	26	22	22	41
Stemmadenine	A1	13	10	10 t	43
Apparicine	A2	34	28	19 t	58
16-Hydroxy-16,22-dihydroapparicine	A2	25	19	10 t	48
Vallesamine	A2	21	13	12 t	47
O-Acetyl-vallesamine	A2	33	27	34 t	58
Tubotaiwine	A3	35	23	21 t	40
Tubotaiwine N_4-oxide	A3	0	2	8	3
Voaphylline	P1	39	52	66	74
12-Methoxy-voaphylline	P1	50	55	69	75
Voaphylline-hydroxy-indolenine	P1	38	19	6	62
Voaphyllinediol	P1	3	8	7 t	50
Lochnericine	P2	48	47	70	70
Pachysiphine	P2	48	47	70	70
Tabersonine	P2	55	60	70	73
Vincamine	E1	42	32	39	61
12-Methoxy-14,15-dehydrovincamine	E1	45	38	59	65
Conopharyngine	I1	39	37	68	71
3*R*-Hydroxy-conopharyngine	I1	31	30	65	59
19*S*-Hydroxy-conopharyngine	I1	27	23	61	61
Conopharyngine-hydroxy-indolenine	I1	44	27	68	66

TABLE I (*continued*)

Alkaloid	*Biosynthetical group*	*hR_F value in solvent**			
		S1	*S2*	*S3*	*S4*
3*R*/*S*-Hydroxy-conopharyngine-hydroxy-indolenine	I1	35	20	66	50
Coronaridine	I1	51	57	71	76
11-Hydroxy-coronaridine	I1	4	12	42	65
3-(2'-Oxopropyl)coronaridine	I1	43	45	68	69
19*S*-Heyneanine	I1	38	33	62	68
19*R*-Heyneanine (19-epi-h.)	I1	35	29	56	67
Ibogaine	I1	38	54	19 t	75
Ibogaline	I1	31	35	17 t	70
Ibogamine	I1	44	58	20 t	76
19*R*-Iboxygaine (19-epi-i.)	I1	17	22	12 t	60
Tabernanthine	I1	35	52	19 t	75
Voacangine	I1	47	54	70	75
Isovoacangine	I1	44	52	70	75
3*R*/*S*-Hydroxy-isovoacangine	I1	35	41	66	67
19*S*-Voacristine	I1	34	31	62	67
19*R*-Voacristine (19-epi-v.)	I1	30	28	55	67
20*R*-15,20-Dihydro-cleavamine	I3	45	61	36 t	76
(−)-Velbanamine	I3	31	38	19 t	74
(+)-Isovelbanamine	I3	22	28	26 t	70
20*R*-1,2-Dehydro-ψ-aspidospermidine	I4	56	45	39 t	69
20*S*-Hydroxy-1,2-dehydro-ψ-aspidospermidine	I4	28	21	22 t	59
20*R*-Pandoline	I4	50	46	69	72
20*S*-Pandoline (20-epi-p.)	I4	33	33	54	69
20*R*-ψ-Vincadifformine	I4	58	61	69	75
Pandine	I5	33	28	51	68
Dichomine	I7	31	19	15 t	30
Tacamine	T1	30	22	30 t	46
16,17-Anhydro-tacamine	T1	37	33	46	55
16*R*-Descarbomethoxy-tacamine	T1	5	8	11 t	30
16*S*-Descarbomethoxy-tacamine	T1	16	15	15 t	42
16-Epi-tacamine	T1	4	7	16 t	24
19*S*-Hydroxy-tacamine	T1	3	2	7 t	12
Tacamonine	T1	25	27	42	45
17-Hydroxy-tacamonine	T1	8	10	28	31
Angustine	M4	12	13	46	62
Conoduramine	C5-I1	35	31	44 t	71
11-Demethyl-conoduramine	C5-I1	19	19	32 t	67
3*R*/*S*-Hydroxy-conoduramine	C5-I1	28	28	40 t	59
Conodurine	C5-I1	50	39	55	71
3*R*/*S*-Hydroxy-conodurine	C5-I1	44	36	54	67
Tabernaelegantine A	C5-I1	54	49	61	74
Tabernaelegantine B	C5-I1	36	37	45 t	73
Tabernaelegantine D	C5-I1	35	30	36 t	67
Tabernamine	C5-I1	37	32	9 t	69
Voacamidine	C5-I1	44	32	34 t	69
Voacamine	C5-I1	47	34	45	71
3*R*/*S*-Hydroxy-voacamine	C5-I1	42	30	44	64
Voacorine	C5-I1	36	24	40	64
Vobparicine	C5-A2	17	14	12 t	51
Vobtusine	P2-P2	41	38	41 t	68

* hR_F is ±2 for normal-shaped spots and ±3 for spots showing tailing; f = fronting, t = tailing.
** Probably rotamers.
*** Values may be interchanged.

(4) No excessively volatile components such as diethyl ether should be used in order to prevent changes in composition of the solvent during the development which would lower the reproducibility.

(5) No excessively non-volatile or slightly volatile components such as DMSO or DMF should be used in order to prevent long saturation times.

hR_F values

The general conclusion can be drawn that *Tabernaemontana* alkaloids of the C (corynanthean), V (vallesiachotaman), S (strychnan), E (eburnan), A (aspidospermatan) and T (tacaman) classes are more polar than alkaloids of the P (plumeran) and I (ibogan) classes, while the dimeric indole alkaloids are of intermediate polarity. However, any further discussion of the hR_F values will be limited to a comparison of the alkaloids within the individual classes.

C class. All of the alkaloids can be easily separated from one another by means of one or more of the solvents. Alkaloids possessing a free hydroxy group, a free N_bH, a quaternary N_b or a methoxy substitution in the aromatic ring, have lower hR_F values than similar alkaloids without these groups.

I class. Most of the alkaloids can be easily separated from one another in two or more of the solvents except for some closely related alkaloids of group I1, *i.e.* coronaridine, voacangine (10-methoxy-coronaridine) and isovoacangine (11-methoxy-coronaridine). A good separation of these alkaloids can only be accomplished with system S1. An easy distinction between alkaloids of group I1 possessing a 16-carbomethoxy group (*i.e.* coronaridine, voacangine, isovoacangine, voacristine) and corresponding alkaloids lacking such a group (ibogamine, ibogaine, tabernanthine, ibogaline, iboxygaine) can be made with the neutral system S3. This is due to the different pK_a value of the alkaloids which is of little or no importance in the alkaline solvents. The pK_a is *ca.* 8 for alkaloids possessing a 16-carbomethoxy group and *ca.* 6 for alkaloids lacking such a group[12]. In I1 type alkaloids differing only in the aromatic substitution pattern the hR_F value decreases in the order no substitution, 10-methoxy, 11-methoxy and 10,11-dimethoxy substitution. This is similar to the TLC behaviour of methoxy substituted hetero-yohimbine alkaloids as observed by Phillipson and Shellard[13]. As expected, the introduction of a polar hydroxy group lowers the hR_F value in all cases.

Dimeric alkaloids of the C5-I1 type. Although these alkaloids are very similar, they can be satisfactorily separated in one or two of the solvents used.

Alkaloids of the V, S, A, P, T and M groups. These groups are too small for a discussion of each group. The separation of the individual alkaloids in these groups gives no problems except for the isomeric pair lochnericine/pachysiphine, which cannot be separated in any of the four systems used. If necessary, however, these compounds can be separated by means of the solvent 1,2-dichloroethane–ethyl acetate–*n*-propanol (6:3:1) (hR_F for lochnericine and pachysiphine 57 and 59, respectively).

Fluorescent properties of T. alkaloids

Many alkaloids of the C class exhibit fluorescence under 366 nm light. All acylindole alkaloids studied in this investigation have a weak but characteristic blue fluorescence; the quaternary alkaloid serpentine possesses an intense blue fluorescence, and reserpiline, isoreserpiline, tetrahydroalstonine and yohimbine have vary-

ing fluorescent properties. Alkaloids of the A1, A2, P1, E1, I1, I3, I5, I7, T1 and C5-I1 groups do not possess distinct fluorescent properties.

Ferric chloride–perchloric acid (FCPA) spray reagent

Alkaloids of the C class do not yield distinct colours. Most colours are grey or grey-black, and no distinction between closely related isomers is therefore possible. Exceptions are anhydrovobasindiol, akuammiline, desacetylakuammiline, picraline and pleiocarpamine, which give orange or purple-pink colours. Alkaloids of the A class can be divided into two groups: those having an α-methylene-indoline chromophore (A3 type) which give a blue colour, and alkaloids having a different chromophore (A1, A2 type) which all give some kind of purple-black colour. Similarly alkaloids of the P class can be divided into two groups: those having an α-methylene-indoline chromophore (P2 type) which again yield a blue colour, and alkaloids having a different chromophore which give orange or purple colours depending on the aromatic substitution pattern or certain functional groups in the aliphatic part of the molecule. The many alkaloids of the I1 group, having the same skeleton and differing only in the position and number of substituents, can be easily distinguished from each other with this reagent. In most cases the colours change on heating, making an even better differentiation possible. All the possible aromatic substitutions yield different colours, thus making a clear differentiation between the isomeric voacangine (10-methoxy-coronaridine) and isovoacangine (11-methoxy-coronaridine) possible, whereas such a differentiation by means of mass spectrometry (MS) or protonnuclear magnetic resonance spectroscopy (NMR) is impossible or very difficult. Not only are changes in the aromatic moiety important, but also the presence or absence of a carbomethoxy function at the 16-position or a hydroxy function at the 3-position, influence the colour markedly. In the I3 group one can observe that both the aromatic and the aliphatic parts of the molecule play an important role in determining the eventual colour. The only difference between velbanamine and isovelbamine lies in the relative configuration of the 20-hydroxy group. They give, however, different colours. Alkaloids of the ψ-aspidospermidine group (I4 type) are unusual, because they do not give any colour at all even on prolonged heating. Alkaloids of the I4 and I5 type having an α-methylene-indoline chromophore give blue colours upon heating. Dichomine (I7 type) yields a distinct orange colour resembling the colour of picraline. This fact suggests that an indoline chromophore with an oxygen substitution at C_2 might be responsible for this behaviour. All the tacamine alkaloids of the T1 type and vincamine (E1 type) give upon prolonged heating greenish-black colours, resembling the colours given by the isositsirikines. The tacamonines give an unusual light green colour with the reagent on prolonged heating. The many closely related dimeric alkaloids of the C5-I1 type give, similar to the alkaloids of the I1 type, many distinct colours depending on variations in the aromatic substitution pattern or the absence/presence of certain functional groups in the aliphatic part. Especially in combination with the hR_F data a positive discrimination between the alkaloids mentioned is more clear with this simple colour reaction than with sophisticated MS and proton NMR techniques. Discrimination between isomers is impossible by means of MS, whereas a high-resolution apparatus and reference spectra are needed in the case of proton NMR.

TABLE II

CHROMOGENIC REACTIONS AND FLUORESCENT PROPERTIES OF *TABERNAEMONTANA* ALKALOIDS

Abbreviations: bk = black; bl = blue; br = brown; col = colour; da = dark; flu = fluorescence; gn = green; go = gold; gy = grey; imm = immediate; int = intense; li = light; or = orange; pa = pale; pi = pink; pu = purple; r = red; va = vague; wh = white; y = yellow; Δ0 = no heating; Δ1 = short heating (5–10 sec); Δ2 = intermediate heating (15–20 sec); Δ3 = prolonged heating (40–50 sec).

Alkaloid	*Group*	*Ferric chloride–perchloric acid*
Geissoschizine	C1	gy-br(Δ2), gy-gn(Δ3)
Isositsirikine	C1	gy-br(Δ2), gn-bk(Δ3)
16-Epi-isositsirikine	C1	gy-br(Δ2), gn-bk(Δ3)
Tetrahydro-alstonine	C2	gy(Δ2), va gy-br(Δ3)
Reserpiline	C2	va pi → gy(Δ0), gy(Δ1,Δ2), va or-br(Δ3)
Isoreserpiline	C2	va pi → gy(Δ0), gy(Δ1,Δ2), va or-br(Δ3)
Serpentine	C2	no col. (Δ3)
Yohimbine	C3	gy(Δ2), gn-bk(Δ3), gn-bl (1 day)
Akuammidine	C4	gy-bl(Δ2), gy-bk(Δ3)
Normacusine-B	C4	gy(Δ2), va br-gy(Δ3)
Pericyclivine	C4	gy-bk(Δ1-Δ3)
16-Epi-affinine	C5	gy(Δ2), bk-gn(Δ3)
Dregamine	C5	gn-bk(Δ3)
Perivine	C5	gn-bk(Δ3)
Tabernaemontanine	C5	gn-bk(Δ3)
Anhydrovobasindiol	C5	or-br(Δ3)
Vobasine	C5	gn-bk(Δ3)
Vobasinol	C5	pu-bk(Δ2), gy-bk(Δ3)
Akuammiline	C7	pu-pi(Δ3, 1 day)
Desacetyl-akuammiline	C7	pu-pi(Δ3, 1 day)
Picraline	C7	or (Δ2), int. or (Δ3), pi (1 day)
Methuenine	C8	br(Δ3)
Isomethuenine	C8	br(Δ3)
Pleiocarpamine	C10	pi(Δ3)
Vallesiachotamine	V1	li br-or(Δ0,Δ1), br-or(Δ2), va br(Δ3); pa br flu under 366 nm after spraying
Isovallesiachotamine	V1	li br-or(Δ0,Δ1), br-or(Δ2), va br(Δ3); pa br flu under 366 nm after spraying

Ceric sulphate–sulphuric acid	*Tetracyano-quinodimethane*	*Fluorescence (366 nm) before spraying*
No col(Δ0-Δ3), or-y (1 day)	li bl-gn	–
y(Δ0-Δ2) int y(Δ3), y(1 day); int y flu with int bl rim at both 254 and 366 nm after spraying	bl-gn	–
y(Δ0-Δ2), int y(Δ3), y (1 day); int y flu with int bl rim at both 254 and 366 nm after spraying	bl-gn	–
li y(Δ3); va y-br flu with bl rim at both 254 and 366 nm after spraying	va gn centre with br rim, bl-gn (30 min)	gy-wh
li or-br(Δ0-Δ2), y-gn(Δ3); pa or flu at 366 nm after spraying	gn	br-y
li or-br(Δ0-Δ2), y-gn(Δ3); pa or flu at 366 nm after spraying	gn	br-y
No col (Δ3); int bl flu at both 254 and 366 nm after spraying	or	int bl
va pi(Δ0), fades within 10 sec, li bl(Δ3); va y-br flu with bl rim at both 254 and 366 nm after spraying	pa li br	bl-gy
li pu-pi(Δ0-Δ2), fades on further heating	No col	–
pu-pi(Δ0) fades within 1 min, va br-gy(Δ3)	pa li br-y	–
pu-pi(Δ0) fades within 1 min, va br-gy(Δ3)	No col	–
va br-gy(Δ3)	va gn	bl
wh-pu → wh(Δ0), wh(Δ1,Δ2), y-br(Δ3); br-y flu at 366 nm after spraying	li br-y	bl
y-br(Δ3); br-y flu at 366 nm after spraying	bl	bl
wh-pu → wh(Δ0), wh(Δ1,Δ2), y-br(Δ3); br-y flu at 366 nm after spraying	li br-y	bl
li or (2 min,Δ0), li orΔ1,Δ2), va y-br(Δ3)	li br-y	–
wh-pu → wh(Δ0), wh(Δ1,Δ2), y(Δ3); br-y flu at 366 nm after spraying	li br-y	bl
y centre with pu rim(Δ0), y(Δ1), int y(Δ2,Δ3); pi flu under 366 nm after spraying	pa li br	–
No col(Δ0-Δ3)	No col	–
pi-pu, fades quickly(Δ0), bl-gy(Δ1), gy(Δ2), va gy (Δ3); or-br flu under 366 nm after spraying	No col	–
pi-pu(Δ0), bl(Δ1,Δ2), bl-gn(Δ3), pi (1 day)	No col	–
li go(Δ0), or-y(Δ3); da centre with li br rim under 366 nm after spraying	li br	bl
li go(Δ0), or-y(Δ3); da centre with li br rim under 366 nm after spraying	li br	bl
pu, fades quickly (Δ0), va br centre with y rim(Δ2), y-br(Δ3), or-br (1 day); weak y-pu flu under 366 nm after spraying	bl	–
da y(Δ0-Δ3, 1 day); y-br flu under 366 nm after spraying	va br-gn	–
da y(Δ0-Δ3, 1 day); y-br flu under 366 nm after spraying	va br-gn	–

(Continued on p. 300)

TABLE II (*continued*)

Alkaloid	*Group*	*Ferric chloride–perchloric acid*
Akuammicine	S1	va go(Δ0), va go-bl(Δ1), bl(Δ2), int bl(Δ3), bl (1 day)
12-Hydroxy-akuammicine	S1	va pa gy-go(Δ0,Δ1), bl(Δ2,Δ3)
Akuammicine-N_4-oxide	S1	bl(Δ2,Δ3, 1 day)
Norfluorocurarine	S1	va go(Δ0-Δ2), bl-gn(Δ3) → y(1 min) → y-gn (15 min), y (1 day)
Stemmadenine	A1	pu-br(Δ2), pu(Δ3, 1 day)
Apparicine	A2	pu-gy(Δ2), gy-bk(Δ3)
16-Hydroxy-16,22-dihydro-apparicine	A2	pu-gy(Δ2), gy-bk(Δ3)
Vallesamine	A2	gy(Δ2), da bl-gn(Δ3), bk-pu (1 day)
O-Acetyl-vallesamine	A2	gy(Δ2), da bl-gn(Δ3), bk-pu (1 day)
Tubotaiwine	A3	va pa or(Δ0,Δ1), bl(Δ2), da bl(Δ3), br-or (30 min), or (1 hr, 1 day)
Tubotaiwine N_4-oxide	A3	bl(Δ2,Δ3), or (1 hr, 1 day)
Voaphylline	P1	col varies from or-br to pu-pi (both Δ2,Δ3) depending on the solvent chosen and the exact time of drying
12-Methoxy-voaphylline	P1	pu(Δ2,Δ3)
Voaphylline-hydroxy-indolenine	P1	br(Δ2,Δ3)
Voaphyllinediol	P1	or-br(Δ2,Δ3)
Lochnericine	P2	bl-gy(Δ2), bl(Δ3)
Pachysiphine	P2	bl-gy(Δ2), bl(Δ3)
Tabersonine	P2	br-go(Δ2), bl-gn → bl(Δ3) after 5 min y centre, after 15 min y
Vincamine	E1	bl-gy(Δ3)
12-Methoxy-14,15-dehydro-vincamine	E1	bl-gy(Δ2), gy(Δ3)
Conopharyngine	I1	pu (imm) → br(5 sec) → gn(30 sec)(Δ0), gn(Δ1-Δ3)
3*R*-Hydroxy-conopharyngine	I1	pu(Δ0-Δ2), br(Δ3)
19*S*-Hydroxy-conopharyngine	I1	br(imm) → gn(30 sec)(Δ0), gn(Δ1-Δ3)
Conopharyngine-hydroxy-indolenine	I1	da y(Δ0-Δ2), va or (Δ3)
3*R*/*S*-Hydroxy-conopharyngine-hydroxy-indolenine	I1	y (Δ0,Δ1), y-or(Δ2,Δ3)
Coronaridine	I1	gy(Δ2,Δ3)
11-Hydroxy-coronaridine	I1	da go(imm), fades quickly (Δ0), gy-bk(Δ2,Δ3)
3-(2′-Oxopropyl)-coronaridine	I1	gy-bk(Δ1,Δ2), gn-bl-bk(Δ3), bk-bl (1 day)
19*S*-Heyneanine	I1	gy(Δ2,Δ3)
19*R*-Heyneanine	I1	gy(Δ2,Δ3)
Ibogaine	I1	va go-br(imm), fades within 5 min(Δ0), va br(Δ2,Δ3)

Ceric sulphate–sulphuric acid	*Tetracyano-quinodimethane*	*Fluorescence (366 nm) before spraying*
da bl(imm) → wh-y centre with bl rim (30 sec) → br-go (5 min)(Δ0); go flu under 366 nm after spraying	No col	bl-wh
or-go(imm) → pu(15 sec)(Δ0), pu(Δ1,Δ2), li br-go(Δ3); pa wh flu under 366 nm after spraying	bl	wh-bl
da bl(imm) → wh-y centre with bl rim (30 sec) → br-go (5 min)(Δ0); go flu under 366 nm after spraying	No col	bl-wh
bl(imm) → y → go(Δ0), go(Δ1-Δ3)	pa y-br	wh
pu, fades (Δ0), va gy(Δ3)	bl	–
va pu-br(imm) → gy-br (5 min)(Δ0), gy-br(Δ1-Δ3)	or-br-pi	–
va pu-br(imm) → gy-br (5 min)(Δ0), gy-br(Δ1-Δ3)	or-br-pi	–
li pu-br, fades(Δ0), va br-pu(Δ3)	or-br-pi	–
li pu-br, fades(Δ0), br(Δ3)	or-br-pi	–
da bl(imm) → gy(2 min), br-go-gn(5 min)(Δ0), va gn (Δ2,Δ3); br flu under 366 nm after spraying	li br	–
da bl(imm) → gy(2 min), br-go-gn(5 min)(Δ0), va gn (Δ2,Δ3); br flu under 366 nm after spraying	li br	–
pu-pi, fades(Δ0), va gn(Δ3)	or	–
bl → pu-gy(Δ0), va br(Δ3)	va bl-gn	–
pu, after 1 min wh centre, fades within 5 min(Δ0), va br(Δ2,Δ3)	va li br-gn	–
int pu, fades within 1 min(Δ0), bl-gn(Δ3)	br-gn	–
bl(imm) → bl-pu(10 sec), fades within 5 min(Δ0), go-br(Δ2,Δ3); pa wh-bl flu under 366 nm after spraying	va li br	bl-wh
bl(imm) → bl-pu(10 sec), fades within 5 min(Δ0), go-br (Δ2,Δ3); pa wh-bl flu under 366 nm after spraying	va li br	bl-wh
bl-pu, fades within 30 sec(Δ0), after 2 min wh-br centre with pi rim	bl	bl
y(Δ3), int. y flu with int. bl rim under both 254 and 366 nm after spraying	No col	–
bl(imm) → y(5 min)(Δ0), br-y(Δ1-Δ2), va br(Δ3); or-br flu after spraying	No col	–
gn(Δ0-Δ3)	bl-gn	–
pu → y centre with pu rim (3 min)(Δ0), go (Δ1-Δ3)	or-br	–
gn(Δ0-Δ3)	gn	
or with wh rim (Δ0-Δ2), br (Δ3)	No col	–
No col	No col	–
pa bl(Δ0), fades quickly	va y-br	–
br(Δ0-Δ2), br-y(Δ3)	bl	–
No col(Δ0-Δ3)	or-br	–
pa bl(Δ0), fades quickly	y-br	–
pa bl(Δ0), fades quickly	bl-gn	–
pu(imm), fades(Δ0)	va y-br	–

(Continued on p. 302)

TABLE II (*continued*)

Alkaloid	*Group*	*Ferric chloride–perchloric acid*
Ibogaline	I1	pu(imm), fades within 5 min(Δ0), li br-or(Δ2,Δ3)
Ibogamine	I1	No col. (Δ0-Δ2), pa br(Δ3)
19*R*-Iboxygaine	I1	or(imm) → or-pi (5 min)(Δ0), or-pi(Δ1,Δ2) br(Δ3)
Tabernanthine	I1	pa pu(imm), fades within 3 sec (Δ0), gy(Δ2), gn-br(Δ3)
Voacangine	I1	pu(Δ2), gy(Δ3)
Isovoacangine	I1	go(Δ0-Δ2), gn-go(Δ3)
3*R*/*S*-Hydroxy-isovoacangine	I1	bl, fades within 3 sec (Δ0), li bl(Δ1), bl-gy (Δ2), gy(Δ3)
19*S*-Voacristine	I1	pu(Δ2), gy-gn-pu(Δ3)
19*R*-Voacristine	I1	pu(Δ2), gy-gn-pu(Δ3)
20*R*-15,20-Dihydro-cleavamine	I3	bl-gy(Δ3)
(−)-Velbanamine	I3	r(Δ2,Δ3), pu-or (1 day)
(+)-Isovelbanamine	I3	va y(Δ0-Δ2), gy-br(Δ3), or-br (1 day)
20*R*-1,2-Dehydro-ψ-aspidospermidine	I4	No col (Δ0-Δ3)
20*S*-Hydroxy-1,2-dehydro-ψ-aspidospermidine	I4	No col (Δ0-Δ3)
20*R*-Pandoline	I4	va li gy-pu(Δ0,Δ1), bl(Δ2,Δ3)
20*S*-Pandoline	I4	va li gy-pu(Δ0,Δ1), bl(Δ2,Δ3)
20*R*-ψ-Vincadifformine	I4	va li gy-pu(Δ0,Δ1), bl(Δ2,Δ3)
Pandine	I5	bl(Δ2,Δ3) after 5 min y centre
Dichomine	I7	or (Δ3)
Tacamine	T1	gn-bk(Δ3)
16-Epi-tacamine	T1	gn-bk(Δ3)
16*R*-Descarbomethoxy-tacamine	T1	br-gn-gy(Δ3)
16*S*-Descarbomethoxy-tacamine	T1	br-gn-gy(Δ3)
16,17-Anhydro-tacamine	T1	gy(Δ2), gn-gy(Δ3)
19*S*-Hydroxy-tacamine	T1	gn-bk(Δ3)
Tacamonine	T1	li gn(Δ3)
17-Hydroxy-tacamonine	T1	li gn(Δ3)
Angustine	M4	y coloured compound, y(Δ1), int.y(Δ2), va gn(Δ3), y (1 day)
Conoduramine	C5-I1	go(Δ0), int. go(Δ1,Δ2) gn-go(Δ3)
3*R*/*S*-Hydroxy-conoduramine	C5-I1	go(Δ1), da bl(Δ2), gy-bk(Δ3)
11-Demethyl-conoduramine	C5-I1	va pu(Δ1,Δ2), gy-bl(Δ3)
Conodurine	C5-I1	da gn(Δ0), bl-gn(Δ1), va br-gn(Δ2), va go(Δ3)
3*R*/*S*-Hydroxy-conodurine	C5-I1	li bl(Δ0,Δ1), bl(Δ2), gy(Δ3)
Tabernaelegantine A	C5-I1	bl(imm) → bl-gn(10 sec) → gn(20 sec)(Δ0), gn(Δ1,Δ2) da gn-go(Δ3)

Ceric sulphate–sulphuric acid	*Tetracyano-quinodimethane*	*Fluorescence (366 nm) before spraying*
pu (imm), fades within 3 sec(Δ0)	va y-br	–
pu (imm), fades (Δ0)	va y-br	–
va pu, fades quickly(Δ0)	y-br	–
pa pu (imm), fades quickly (Δ0), va go(Δ3); va y-bl flu under 366 nm after spraying	va y-br	bl
pu, fades (Δ0), bl-gn(Δ3)	gn-br	–
go(Δ0-Δ3)	bl-gn-gy	–
bl(Δ0), fades quickly	pa or	–
pu, fades quickly (Δ0), bl-gn(Δ3)	gn-y-br	–
pu, fades quickly (Δ0), bl-gn(Δ3)	gn-y-br	–
pu(Δ0), fades quickly	da bl	pa bl
pu(Δ0), fades within 5 min	va li or-br	–
pu with wh centre(Δ0), fades within 5 min	bl	–
int or(Δ0), fades within 5 min	va y-br	–
or(Δ0-Δ2), fades on further heating	No col	–
da bl, fades within 30 sec (Δ0)	bl-gn	bl
li bl fades within 30 sec(Δ0)	bl-gn	bl
li bl fades within 30 sec (Δ0)	va li gn	bl
bl(imm) → bl with y rim (30 sec) → y (2 min)(Δ0), y(Δ1-Δ2), go-y(Δ3)	va y-br	–
int. or → li bl centre with or rim (5 min)(Δ0), li bl centre with or rim(Δ1-Δ3)	bl	–
li y(Δ0-Δ2), y(Δ3), or-y (1 day); int. y flu with int. bl rim under both 254 and 366 nm after spraying	No col	–
li y(Δ0-Δ2), y(Δ3), or-y (1 day); int. y flu with int. bl rim under both 254 and 366 nm after spraying	No col	–
li y(Δ0-Δ3), pu(1 day); int. y fly with int. bl rim under both 254 and 366 nm after spraying	No col	–
li y(Δ0-Δ3), pu (1 day); int. y flu with int.bl rim under both 254 and 366 nm after spraying	No col	–
y(Δ0-Δ3), or-pu (1 day); int. y-br flu with int. bl rim under both 254 and 366 nm after spraying	No col	–
li y(Δ0-Δ2), y(Δ3), or-y (1 day); int. y flu with int. bl rim under both 254 and 366 nm after spraying	No col	–
No col (Δ0-Δ3), or (1 day); bl flu under 366 nm after spraying	No col	–
No col(Δ0-Δ3), or (1 day); bl flu under 366 nm after spraying	No col	–
y coloured compound, y(Δ0-Δ3, 1 day)	y-li br	int.wh
go(imm) → y, fades(Δ0), go-y(Δ2,Δ3)	gn	–
bl, fades within 10 sec (Δ0), va gn-br(Δ3)	gn-br	–
pu → va pu(Δ0), gy-pu(Δ1-Δ3)	gn	–
da gn, fades within 3 sec(Δ0)	br-gn	–
bl, fades within 10 sec(Δ0)	br-gn	–
da gn, fades within 10 sec(Δ0), gn(Δ2,Δ3)	gn-li br	–

(Continued on p. 304)

TABLE II (*continued*)

Alkaloid	*Group*	*Ferric chloride–perchloric acid*
Tabernaelegantine B	C5-I1	go(Δ0,Δ1), int go(Δ2), da gn-br centre with gn-bl rim(Δ3), gn-bl (1 h)
Tabernaelegantine D	C5-I1	go(Δ0,Δ1), int. go(Δ2), da gn-br centre with gn-bl rim(Δ3), gn-bl (1 h)
Tabernamine	C5-I1	va li bl-gn(Δ0), no col.(Δ1,Δ2), li br(Δ3)
Voacamidine	C5-I1	go-br(Δ0), br-gn-gy(Δ1), da bl(Δ2,Δ3)
Voacamine	C5-I1	go(Δ2), bl-gn → gn centre with br rim(Δ3)
3*R*/*S*-Hydroxy-voacamine	C5-I1	bl-gy(Δ2), gy(Δ3)
Voacorine	C5-I1	go(Δ2), bl-gn → gn centre with br rim(Δ3)
Vobparicine	C5-A2	gy(Δ2), gn-bk(Δ3)
Vobtusine	P2-P2	da bl(Δ0-Δ3, 1 day)

Ceric sulphate–sulphuric acid (CSSA) spray reagent

The variation in colour given by alkaloids of the C class is greater with this reagent than with the FCPA spray reagent. Therefore, it is more difficult to draw any definite conclusions about a specific structural part of the molecule giving a particular colour. Also many different fluorescences under 366 nm light were apparent after spraying. Most characteristic was the intense yellow centre surrounded by an intense blue rim given by the isositsirikines. Only a few other indole alkaloids investigated here (the tacamines and the vincamines) give such a response. These alkaloids can be easily detected in an extract after one chromatographic run and spraying with the CSSA reagent. The vallesiachotamines give an immediate dark yellow colour, which is stable on heating. No other alkaloid gives this reaction. Norfluorocurarine (S1 type) and the alkaloids of the A3 and P2 types, which have the α-methylene-indoline chromophore in common, all give a blue colour which fades quickly. Alkaloids of the A1, A2 and P1 types, which have an indole chromophore in common, give some kind of purple colour. Vincamine, as mentioned before, gives the same reaction as the isositsirikines. The reason for this similar and characteristic behaviour is unclear. The structures have little in common. The reaction is not given by 12-methoxy-14,15-dehydrovincamine. This selective and intense fluorescence may be useful in a quantitative determination of vincamine in biological fluids. The CSSA reagent is of less use than the FCPA reagent for the I1 type of alkaloids. The colours are less distinct, less variable, fade quickly and in most cases do not change on heating. The velbanamines and the dihydrocleavamines (I3 type) can be easily distinguished from the corresponding ψ-aspidospermidines with this reagent. Alkaloids of the former type give a purple colour, whereas the latter type give an orange colour. An intense orange colour is also given by dichomine, the only representative of the I7 type of alkaloids. With the CSSA reagent dichomine can be more sensitively detected than with the FCPA reagent or 254 nm detection. Alkaloids of the I4 and I5 type give, as expected, a blue colour with the CSSA reagent (α-methylene-indoline chromophore). The six tacamines investigated here give the same yellow colour and the same specific fluorescence as the vincamines and isositsirikines after spraying. The tacamonines

Ceric sulphate–sulphuric acid	*Tetracyano-quinodimethane*	*Fluorescence (366 nm) before spraying*
gn-br(imm) → y centre with gy rim (15 sec), fades within 2 min(Δ0), y-go(Δ3)	gn	–
gn-br(imm) → go-y(15 sec), fades within 2 min(Δ0), y-go-gn(Δ3)	gn	–
No col.(Δ0-Δ3)	li y-br	–
bl-pu(imm) → go(5 sec), fades within 15 sec, br-go(Δ2,Δ3)	or-br	–
pu, fades within 30 sec (Δ0), gn-br-y(Δ3)	gn	–
bl(imm) → bl-pu (5 sec), fades within 15 sec (Δ0), gy(Δ3)	li br-y	–
pu, fades within 30 sec(Δ0), gn-br-y(Δ3)	gn-br	–
br-go(Δ0), fades upon heating	y-li br	–
da bl(Δ0-Δ3), pu-gy (1 day)	y-gn	bl

(16-carbonyl) do not show this kind of behaviour, which again stresses certain specific structural requirements. The tacamines are unusual in giving all kinds of beautiful colours one day after spraying with the CSSA reagent. The yellow, orange, pink or purple colours persist for many days or even for weeks. Like the I1 type of alkaloids the colours of the dimeric C5-I1 type of alkaloids are less distinct and fade quickly. Thus in order to obtain useful information, one has to observe quickly and carefully.

Tetracyanoquinodimethane (TCNQ) spray reagent

Less variation in colours is observed with this reagent than with the FCPA or CSSA reagents. The colours most commonly observed are blue, green and light brown. In some cases no colour is found. The colours are usually less intense than with the two other reagents. The C1 type alkaloids yield a blue-green colour, and the C2 type alkaloids, except for the quaternary serpentine, give green colours. Most of the C4, C5, C7 and C8 types of alkaloid give a weak brown colouration, although there are some exceptions. Alkaloids of the V, S, A and P classes give various colours. Most of the I1 type of alkaloid give yellow-brown colours, but the 3-hydroxy derivatives give a more orange and 11-hydroxy-coronaridine a blue colour. Quite remarkable is the ability of the reagent to distinguish between 19*R*- and 19*S*-heyneanine, something which neither of the two oxidizing reagents is capable of. This fact demonstrates clearly the different capabilities of this reagent when compared with the other two. The most common colour given by the alkaloids of the groups I3, I4, I5 and I7 is a bluish colour. Alkaloids of the chemically similar E and T class do not give colours at all with the TCNQ reagent at the concentration used. Many of the dimeric alkaloids give greenish colours. In the C5-I1 group it is not possible to distinguish from each other the alkaloids with different attachments between the two monomeric halves.

CONCLUSIONS

The best chromogenic reagent of the three reported here seems to be the FCPA

spray reagent. It gives many different colours with the alkaloids investigated, especially when the TLC plates are heated with a hot air blower and the colour changes are observed after the heating is stopped. The colours are stable for at least 10 sec, and most of them even longer. Changes in the aromatic (indole) part of the alkaloid have a greater influence on the colour than changes in the aliphatic part. Thus, the reagent is more valuable with the isomeric I1 type alkaloids than with the isomeric C class alkaloids. The C1, C5 and T1 types of alkaloid can be easily distinguished; they all give greenish-black colours after prolonged heating. The C5 group (acylindole alkaloids) can be distinguished from the other two groups by means of the blue fluorescence before spraying. Also all alkaloids that possess an α-methyleneindoline chromophore (many of the S1, A3, P2 (including vobtusine), I4 and I5 types) comprise a definable group, all of them giving a more or less intense blue colour on heating. This is not observed for other alkaloids and is thus characteristic for this group. The alkaloids that have a N_1–C_2–O system (picraline and dichomine) give a very clear, pure orange colour after prolonged heating.

The CSSA reagent is certainly useful, but it has some drawbacks compared with the FCPA reagent: many colours can be observed for only a few seconds, after which they tend to fade quickly; other colours are less distinct; in only a few cases are changes of colour seen on heating. An advantage of the CSSA reagent over the FCPA reagent is that many alkaloids still exhibit some fluorescence after spraying and heating. This can give extra information: many alkaloids of the C1, E1 and T1 types give a highly characteristic yellow-blue fluorescence after spraying. Alkaloids with an α-methylene-indoline chromophore yield a similar blue colour with this reagent as with the FCPA reagent. Vallesiachotamine and isovallesiachtamine give an intense yellow colour. The CSSA reagent is particularly useful for detecting alkaloids with an "isolated" indole chromophore (*i.e.* cleavamines, velbanamines and voaphyllines) and the related alkaloids with an indolenine chromophore (*i.e.* ψ-1,2-dehydroaspidospermidines). Alkaloids of the latter group give orange colours, alkaloids of the former group purple colours. The information acquired with the CSSA spray reagent is most valuable in conjunction with the information already obtained with the FCPA reagent. The colours given by the π-acceptor TCNQ are usually less distinct and intense than with the other two reagents. The variation in colours is smaller and no clearly definable groups or chromophores can be deduced from the results. Also taking into account the potential toxicity of this reagent and its solvent acetonitrile, the FCPA and CSSA spray reagents should be recommended for a general TLC detection of *Tabernaemontana* alkaloids.

ACKNOWLEDGEMENTS

We thank Prof. H. Achenbach, Dr. P. D. Bakana, Prof. A. Cavé, Prof. J. F. Cicció, Prof. M. Damak, Prof. B. Danieli, Prof. C. Djerassi, Dr. P. J. Houghton, Prof. D. G. I. Kingston, Prof. M. E. Kuehne, Dr. G. Massiot, Dr. P. Perera, Prof. J. Poisson, Mr B. Richard, Prof. S. Takano, Mr P. Thepenier and Dr. C. Tibell for their kind gifts of reference substances. Financial support by the "Fonds Harold Quintus Bosz" and the "Van Leersum Fonds" for studies of *Tabernaemontana* species is gratefully acknowledged.

REFERENCES

1 N. R. Farnsworth, R. N. Blomster, D. Damratoski, W. A. Meer and L. V. Cammarato, *Lloydia*, 27 (1964) 302.
2 Sh. Z. Kasymov, Kh. N. Aripov, T. T. Shakirov and S. Yu. Yunusov, *Khim. Prir. Soedin.*, 3 (1967) 352.
3 V. Yu. Vachnadze, V. M. Malikov, Kh. T. Il'yasova, K. S. Mudzhiri and S. Yu. Yunusov, *Khim. Prir. Soedin.*, 9 (1973) 72.
4 W. E. Court and M. M. Iwu, *J. Chromatogr.*, 187 (1980) 199.
5 W. E. Court and P. Timmins, *Planta Med.*, 27 (1975) 319.
6 E. J. Shellard and P. K. Lala, *Planta Med.*, 33 (1978) 63.
7 J. D. Phillipson and N. G. Bisset, *J. Chromatogr.*, 48 (1970) 493.
8 J. D. Phillipson and S. R. Hemingway, *J. Chromatogr.*, 105 (1975) 163.
9 A. Baerheim Svendsen and R. Verpoorte, *Chromatography of alkaloids, Journal of Chromatography Library Vol. 23A*, Elsevier, Amsterdam, Oxford, New York, 1983.
10 T. A. van Beek, R. Verpoorte, A. Baerheim Svendsen, A. J. M. Leeuwenberg and N. G. Bisset, *J. Ethnopharmacol.*, 10 (1984) 1.
11 G. Rücker and A. Taha, *J. Chromatogr.*, 132 (1977) 165.
12 M. Shamma and H. E. Soyster, *Experientia*, 20 (1964) 36.
13 J. D. Phillipson and E. J. Shellard, *J. Chromatogr.*, 31 (1967) 427.
14 T. A. van Beek, R. Verpoorte and A. Baerheim Svendsen, *Tetrahedron Lett.*, 25 (1984) 2057.