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CHROMATOGRAPHIC AND SPECTROSCOPIC METHODS FOR THE IDENTIFICATION OF ALKALOIDS FROM HERBARIUM SAMPLES OF THE GENUS *UNCARIA*

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SUMMARY

A combination of thin-layer chromatography, gas–liquid chromatography, ultraviolet spectroscopy and mass spectrometry techniques for the alkaloid screening of herbarium samples of the genus *Uncaria* (Rubiaceae) is described. Some sixty alkaloids are distinguished by the screening procedure, and they represent heteroyohimbine, oxindole, roxburghine, simple β-carboline, pyridino-indolo-quinolizidinone and gambirtannine types.

INTRODUCTION

Several members of the genus *Uncaria* (Rubiaceae) have yielded alkaloids[1] but the majority of species have not been investigated chemically. In the case of the closely related genus *Mitragyna*, alkaloids have been isolated from each of the known species[1]. Although *Uncaria* is the larger of these two genera, the fact that not all the species have been investigated for alkaloids can in part be attributed to the confusion which existed in the genus and hence to the uncertain botanical identification of many individual collections. *Uncaria* has now been revised and from the 120 names which appear in the Kew Index, some 39 species are recognised[2]. Several hundred small samples of herbarium material representing all 39 species have been made available to us for alkaloid screening. In the past, alkaloid screening procedures have utilised simple extractions followed by chemical tests which involved colour or precipitation reactions, but the development of sensitive chromatographic and spectroscopic techniques now ensures that much more detailed information can be obtained about the alkaloids present even in relatively small amounts of plant material. Thin-layer chromatography (TLC)[3–6] and gas–liquid chromatography (GLC)[7] have been used to separate and identify many of the oxindole and heteroyohimbine alkaloids found in *Mitragyna* species. Tentative chromatographic identifications made during screening procedures may be confirmed by ultraviolet (UV) spectroscopy[8] and mass spectrometry (MS)[9–11] since the spectra of many of these alkaloids have been reported. This communication describes the analytical procedures which were developed for and

used in the identification of some sixty alkaloids, including all the types formerly known to be present in either *Uncaria* or *Mitragyna* species.

METHODS

Thin-layer chromatography

20 × 20 cm plates were spread with 0.25 mm of silica gel G/GF_{254} (2:1) (Merck, Darmstadt, G.F.R.) and activated at 100° for 45 min. The following solvent systems were used: (A) chloroform–acetone (5:4); (B) chloroform–ethanol (95:5); (C) ether–ethyl acetate (1:1); (D) ethyl acetate–isopropanol–conc. ammonia (100:2:1); (E) ethyl acetate–isopropanol–conc. ammonia (80:15:5); (F) ethyl acetate–isopropanol–conc. ammonia (60:35:5); (G) chloroform–methanol (6:1); (H) methanol–diethylamine (96:8). The solvent systems used were selected from A to H; A–E were used for the separation of tertiary alkaloids, and E to H for the separation of N_4-oxides. The solvent front was allowed to run at least 15 cm.

The alkaloid spots were visualised by examining the plates in UV light (254 and 365 nm) and then spraying with reagents which distinguished between the different structural types. Plates sprayed with Dragendorff's reagent were oversprayed with 0.2 *M* $FeCl_3$ in 35% $HClO_4$ and heated first in a hot air current and then in an oven at 100°. Other plates were sprayed either with Ehrlich's reagent (1% 4-dimethylaminobenzaldehyde in 96% ethanol, followed by exposure to HCl vapour) or with 2% ceric sulphate in 2 *N* H_2SO_4. The hR_F values and the colours given by the various alkaloids in response to UV light and to the chromogenic reagents are given in Tables I and II, respectively.

Gas–liquid chromatography

Glass columns ($^1/_4$ in. I.D.) packed with 5% SE-52 on Varaport 30 (80–100 mesh) were used for GLC separations on either a Perkin-Elmer F 11 or a Hewlett-Packard 5700 A instrument fitted with flame ionisation detectors. With the former an 0.5-m column was used at an oven temperature of 230° and with the latter a 2-ft. column at 240° and a carrier gas (nitrogen) flow-rate of 60 ml/min in each case. Both

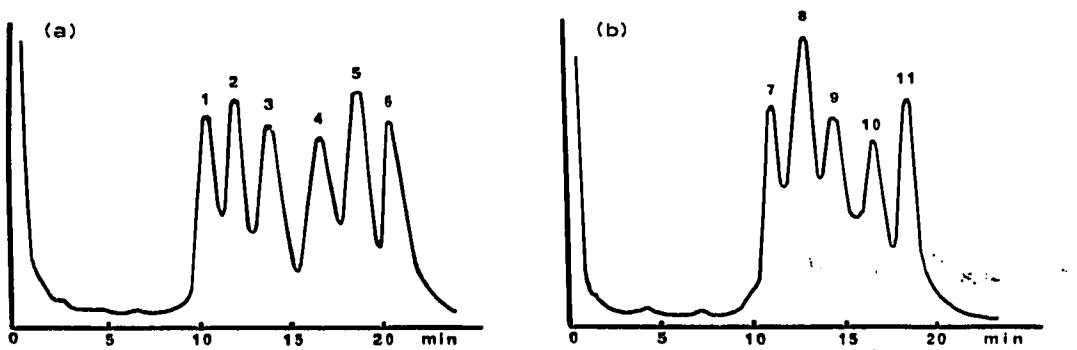


Fig. 1. GLC separations of some of the alkaloids included in the screening. (a) Heteroyohimbines; (b) oxindoles. 1 = Isoajmalicine; 2 = hirsutine; 3 = akuammigine; 4 = corynantheidine; 5 = dihydrocorynantheine; 6 = ajmalicine; 7 = rhynchophylline; 8 = pteropodine; 9 = mitraphylline; 10 = rotundifoline; 11 = ciliaphylline.

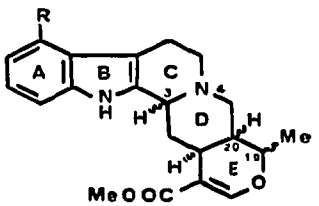
systems gave similar results. The alkaloids were dissolved in methanol and 1–2 μl were injected. The R_f values are given in Table III and sample separations are illustrated in Fig. 1.

UV spectroscopy

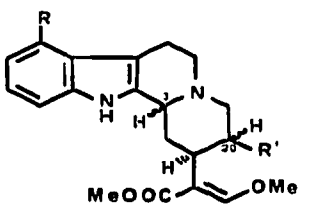
The UV spectra of the alkaloids were determined in either methanol or ethanol solutions using a double-beam recording spectrophotometer (Unicam SP 800 or Perkin-Elmer 402). The bathochromic shift in the absorption maxima of phenolic alkaloids was observed on addition of three drops of 1 *N* KOH to the solution and the reversibility of the shift ascertained by the addition of six drops of 1 *N* HCl. The wavelengths of the absorption maxima of the types of alkaloids investigated are given in Table IV.

Mass spectrometry

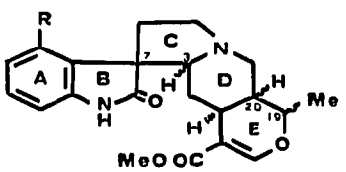
The mass spectra were determined on an AEI MS 902 spectrometer at 70 eV and with inlet temperatures between 210° and 230° for most of the alkaloids. An inlet temperature of 250° was used for pyridino-indolo-quinolizidinones. Differences in



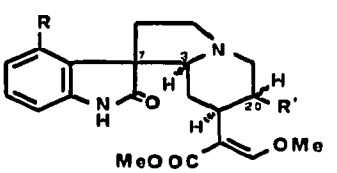
I Pentacyclic heteroyohimbine (R = H, OH or OMe)



II Tetracyclic heteroyohimbine (R = H, OH or OMe R' = Et or vinyl)



III Pentacyclic oxindole (R = H, OH or OMe)

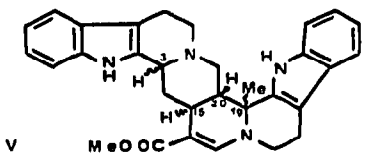


IV Tetracyclic oxindole (R = H, OH or OMe R' = Et or vinyl)

I – IV can exist as isomers defined as :-

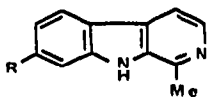
	C-3H	C-20H
allo	α	α
epiallo	β	α
normal	α	β
pseudo	β	β

III and IV can exist as A or B isomers in which the lactam carbonyl can lie below (A) or above (B) the plane of the C–D rings.

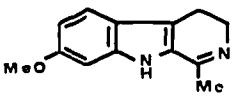


V

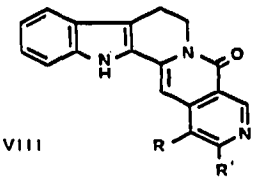
		C-3H	C-19H
Roxburghine	C	α	α
"	D	β	α
"	E	β	β



VI Harmane (R=H)
Harmine (R=OMe)

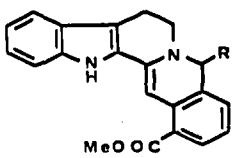


VII Harmaline

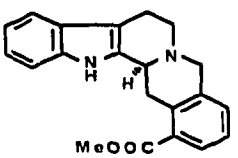


VIII

Angustine (R = $CH=CH_2$; R'=H)
Angustoline (R = CH(OH)Me; R'=H)
Angustidine (R = H; R'= Me)



IX Gambirtannine (R = H_2)
Oxogambirtannine (R = O)



X Dihydrogambirtannine

relative abundance of the fragment ions of some of the alkaloids can occur at different operating temperatures and according to the length of time the compound is in the spectrometer. The most important fragment ions and their percentage relative abundances for typical spectra of a number of alkaloids are listed in Table V.

RESULTS AND DISCUSSION

The hR_F values, colour reactions with selected spray reagents, R_t values, UV spectra and mass spectra of some sixty alkaloids (structures I–X) for which the genus *Uncaria* was screened are listed in Tables I–V.

Some of the TLC systems chosen for examining alkaloid extracts of herbarium samples of *Uncaria* were selected from those described for the separation of *Mitragyna* alkaloids[3–6], although other systems were used. In particular, the use of diethylamine in solvent systems for routine screening procedures was avoided since it is essential for the most sensitive determination of alkaloids that this solvent is removed from the plates prior to spraying with Dragendorff's reagent. Systems D to F, which include ammonia in the solvent mixtures, were found to be practical alternatives to

TABLE I

hR_F VALUES OF ALKALOIDS INCLUDED IN THE SCREENING

Alkaloid	*Solvent system*							
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*
Pentacyclic heteroyohimbines (I)								
Ajmalicine (*normal*, R = H, C-19 Me ∝)	72	60	62	71	88			
Isoajmalicine (*pseudo*, R = H, C-19 Me ∝)	17	27	7	23	79			
Mitrajavine (*pseudo*, R = OMe, C-19 Me ∝)	15	23	8	26	78			
Tetrahydroalstonine (*allo*, R = H, C-19 Me ∝)	83	72	77	81	90			
Akuammigine (*epiallo*, R = H, C-19 Me ∝)	50	45	34	50	85			
4-R akuammigine N-oxide	0	0	0	0	24	38	50	70
19-*epi*-Ajmalicine (*normal*, R = H, C-19 Me β)	78	63	68	75	88			
3-Iso-19-*epi*-ajmalicine (*pseudo*, R = H, C-19 Me β)	20	39	—	27	—			
Rauniticine (*allo*, R = H, C-19 Me β)	43	25	35	60	88			
Tetracyclic heteroyohimbines (II)								
Dihydrocorynantheine (*normal*, R = H, R′ = Et)	69	44	60	76	86			
Gambirine (*normal*, R = OH, R′ = Et)	44	14	48	63	80			
Speciogynine (*normal*, R = OMe, R′ = Et)	62	40	58	77	86			
Hirsutine (*pseudo*, R = H, R′ = Et)	10	11	4	19	74			
Hirsuteine (*pseudo*, R = H, R′ = vinyl)	16	15	8	25	76			
Mitraciliatine (*pseudo*, R = OMe, R′ = Et)	10	10	4	18	74			
Corynantheidine (*allo*, R = H, R′ = Et)	80	60	75	80	88			
Mitragynine (*allo*, R = OMe, R′ = Et)	76	58	72	80	87			
Isocorynantheidine (*epiallo*, R = H, R′ = Et)	33	21	27	56	82			
Speciociliatine (*epiallo*, R = OMe, R′ = Et)	22	16	18	56	82			
Pentacyclic oxindoles (III)								
Isomitraphylline (*normal* A, R = H, C-19 Me ∝)	68	45	45	60	74			
Isomitraphylline N-oxide	0	0	0	0	18	24	32	69
Javaphylline (*normal* A, R = OMe, C-19 Me ∝)	29	22	18	42	76			
Mitraphylline (*normal* B, R = H, C-19 Me ∝)	51	37	16	37	66			
Mitraphylline N-oxide	0	0	0	0	2	3	6	42
Isopteropodine (*allo* A, R = H, C-19 Me ∝)	72	47	56	69	77			
Isopteropodine N-oxide	0	0	0	0	24	35	44	71
Pteropodine (*allo* B, R = H, C-19 Me ∝)	68	47	50	65	77			
Pteropodine N-oxide	0	0	0	0	6	6	9	54
Speciophylline (*epiallo* A, R = H, C-19 Me ∝)	29	26	8	19	59			
Speciophylline N-oxide	0	0	0	0	2	3	5	53
Uncarine F (*epiallo* B, R = H, C-19 Me ∝)	60	38	38	52	77			
Uncarine F N-oxide	0	0	0	0	21	29	38	69
Uncarine A, (*normal* A, R = H, C-19 Me β)	70	45	49	65	76			
Uncarine B (*normal* B, R = H, C-19 Me β)	62	41	26	47	71			
Tetracyclic oxindoles (IV)								
Isorhynchophylline (*normal* A, R = H, R′ = Et)	70	40	56	66	78			
anti-Isorhynchophylline N-oxide	0	0	0	0	21	38	42	75
Isocorynoxeine (*normal* A, R = H, R′ = vinyl)	70	42	57	69	77			
Rotundifoline (*normal* A, R = OH, R′ = Et)	73	48	58	70	77			
Rhynchociline (*normal* A, R = OMe, R′ = Et)	9	18	12	41	74			
Rhynchophylline (*normal* B, R = H, R′ = Et)	35	22	11	29	66			
Rhynchophylline N-oxide	0	0	0	0	2	3	13	57
Corynoxeine (*normal* B, R = H, R′ = vinyl)	43	22	15	35	65			
Isorotundifoline (*normal* B, R = OH, R′ = Et)	48	25	34	54	71			
Ciliaphylline (*normal* B, R = OMe, R′ = Et)	27	11	19	27	63			

(Continued on p. 168)

TABLE I *(continued)*

Alkaloid	*Solvent system*							
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*
Corynoxine (*allo* A, R = H, R′ = Et)	73	45	63	71	78			
Corynoxine B (*allo* B, R = H, R′ = Et)	48	25	35	55	78			
Speciofoline (*epiallo* A, R = OH, R′ = Et)[22]	67	42	46	62	75			
Roxburghine C (V)	82	33	67	77	90			
Roxburghine D (V)	53	33	45	66	89			
Roxburghine E (V)	17	20	6	29	78			
Dimeric indole alkaloid[23]	35	14	17	59	87			
Harmane (VI)	29	11	16	37	72			
Harmine (VI)	17	6	7	26	67			
Harmaline (VII)	0	0	0	12	47			
Angustine (VIII)	57	30	33	57	82			
Angustidine (VIII)	44	20	17	39	74			
Angustoline (VIII)	18	7	5	19	56			
Gambirtannine (IX)	87	85	81	85	90			
Dihydrogambirtannine (X)	83	71	74	78	89			
Oxogambirtannine (IX)	83	79	70	78	87			

many of the diethylamine systems previously described. Combinations of the TLC systems used enables all sixty alkaloids (I–X), included in the screening, to be separated (Table I). For all solvent systems used, the correlations between hR_F values and the structural and stereochemical variations of the heteroyohimbine alkaloids were in agreement with those previously reported[4]. These can be briefly summarised as:

(1) For alkaloids of a single structure type, the hR_F values for the four configurations (I, II) are generally in the order *allo* > *normal* > *epiallo* > *pseudo*.

(2) *Ar*-methoxy substitution results in slightly lower hR_F values in comparison to the corresponding *ar*-unsubstituted analogues.

(3) C-20 vinyl E-*seco* heteroyohimbines (II, R′ = vinyl) have hR_F values slightly higher than the corresponding C-20 ethyl analogues (II, R′ = Et).

The TLC systems selected gave results similar to those previously described for the separation of oxindole alkaloids (III, IV)[5,6]. Alkaloids possessing configurations in which the N_4 lone-pair electrons and the amide carbonyl are *syn* are more strongly adsorbed than those in which the configuration is *anti*. Separations of alkaloids which differ in having *cis* or *trans* D/E ring junctions were obtained for the oxindole alkaloids although the separations were not as distinct as those achieved for the heteroyohimbines. When compared with previous findings[6], differences in order of mobility were noted for some of the closed E ring oxindoles (III) having the A configuration. Isomitraphylline (III, *normal* A, C-19 Me α) is now found to be consistently more strongly adsorbed than uncarine A (III, *normal* A, C-19 Me β) and isopteropodine (III, *allo* A, C-19 Me α). Since the N_4 lone-pair electrons probably take little part in the adsorption of these three isomers[5,6] and since the amide carbonyl has a similar environment in all three isomers, it is possible that the differences in adsorption may be due to the varying availability of the pyran oxygen or the carbomethoxy group.

TABLE II

DETECTION OF ALKALOIDS ON SILICA GEL LAYERS

Alkaloid	*UV*		*$FeCl_3$/$HClO_4$*	*Ehrlich's reagent*	*$Ce(SO_4)_2$/H_2SO_4*
	*365 nm ft**	*254 nm*			
Pentacyclic heteroyohimbines					
Ajmalicine	y/w	q	g → br	pu	—
Isoajmalicine	y/w	q	g → br	pu	—
Mitrajavine	or/y	q	g → br	pu	—
Tetrahydroalstonine	y/w	q	g → br	pu	—
Akuammigine	y/w	q	g → br	pu	—
4-R akuammigine N-oxide	—	q	g → br	pu	—
19-*epi*-Ajmalicine	y/w	q	g → br	pu	—
3-Iso-19-*epi*-ajmalicine	y/w	q	g → br	pu	—
Rauniticine	y/w	q	g → br	pu	—
Tetracyclic heteroyohimbines					
Dihydrocorynantheine	b/w	q	b/g → br	pu	—
Gambirine	—	q	grey before heating → br	g	br
Speciogynine	y	q	g → br	pu	—
Hirsutine	b/w	q	g → br	pu	—
Hirsuteine	b/w	q	g → br	pu	—
Mitraciliatine	y	q	g → br	pu	—
Corynantheidine	b/w	q	b/g → br	pu	—
Mitragynine	y	q	g → br	pu	—
Isocorynantheidine	b/w	q	g → br	pu	—
Speciociliatine	y	q	g → br	pu	—
Pentacyclic oxindoles					
Isomitraphylline	—	q	p	—	—
Isomitraphylline N-oxide	—	q	p	—	—
Javaphylline	—	q	p	—	—
Mitraphylline	—	q	p	—	—
Mitraphylline N-oxide	—	q	p	—	—
Isopteropodine	—	q	gr → p	—	—
Isopteropodine N-oxide	—	q	gr → p	—	—
Pteropodine	—	q	gr → p	—	—
Pteropodine N-oxide	—	q	gr → p	—	—
Speciophylline	—	q	gr → p	—	—
Speciophylline N-oxide	—	q	gr → p	—	—
Uncarine F	—	q	gr → p	—	—
Uncarine F N-oxide	—	q	gr → p	—	—
Uncarine A	—	q	p	—	—
Uncarine B	—	q	p	—	—
Tetracyclic oxindoles					
Isorhynchophylline	—	q	b → p**	—	—
anti-Isorhynchophylline N-oxide	—	q	b → p	—	—
Isocorynoxeine	—	q	b → p**	—	—
Rotundifoline	—	q	b → pu → br	p	transient br
Rhynchociline	—	q	b → pu	p	—
Rhynchophylline	—	q	b → p**	—	—
Rhynchophylline N-oxide	—	q	b → p	—	—

(Continued on p. 170)

TABLE II *(continued)*

Alkaloid	*UV*		$FeCl_3/HClO_4$	*Ehrlich's reagent*	$Ce(SO_4)_2/H_2SO_4$
	*365 nm fl**	*254 nm*			
Corynoxeine	—	q	b → p**	—	—
Isorotundifoline	—	q	b → pu → br	p	transient br
Ciliaphylline	—	q	b → pu	p	—
Corynoxine	—	q	b → p**	—	—
Corynoxine B	—	q	b → p**	—	—
Speciofoline	—	q	b → pu → br	p	transient br
Roxburghine C	—	q	g → br	pu	r
Roxburghine D	—	q	g → br	pu	r
Roxburghine E	—	q	g → br	pu	or/r
Dimeric indole alkaloid[23]	—	q	g → br	pu	—
Harmane	b	b fl.	p/pu	—	—
Harmine	b	b fl.	pu → br	—	—
Harmaline	b	y/w fl.	pu → br	y	or
Angustine***	y	y fl.	transient gr	y	—
Angustidine***	y	y fl.	transient gr	y	—
Angustoline***	y	y fl.	transient gr	y	y
Gambirtannine	y/or	q	g	y	—
Dihydrogambirtannine	—	q	pu → g	p	—
Oxogambirtannine***	y	y fl.	gr → br	y	br

* Key to abbreviations: b = blue; br = brown; fl = fluorescence; g = grey; gr = green; or = orange; p = pink; pu = purple; q = fluorescence quenching; r = red; w = white; y = yellow.

** The rate of change of colour of the unsubstituted tetracyclic oxindoles on prolonged heating is slower for rhynchophylline and isorhynchophylline than for the corynoxines or the corynoxeines.

*** These alkaloids give yellow spots on spraying with Dragendorff's reagent, rather than orange.

Spray reagents which differentiated between the various types of alkaloids proved particularly useful in their identifications (Table II). The majority of the alkaloids gave orange spots with Dragendorff's reagent and the plates could then be oversprayed with 0.2 *M* ferric chloride in 35% perchloric acid. When this reagent was previously used for the quantitative estimation of *Mitragyna* alkaloids[12] on TLC plates, it was reported that oxindole alkaloids gave pink spots and that heteroyohimbines gave brown spots on heating at 120° for 1 h. During the present investigation it was found that a wider range of colours resulted if less heat was applied, *e.g.* E-*seco* oxindoles (IV) give intermediate blue colours whereas their closed E ring analogues (III) give either a greenish or no intermediate colour; on prolonged heating pink spots are obtained with both types of oxindole alkaloids. Although less pronounced, the heteroyohimbines (I, II) develop characteristic grey colours before forming the more stable browns. Ehrlich's reagent was used because it reacts selectively with indole alkaloids and with *ar*-substituted oxindoles enabling them to be distinguished from the unsubstituted oxindoles in mixtures. The roxburghines (V) are readily recognised by their distinctive brick-red colour with ceric sulphate[13] and the pyridino-indolo-quinolizidinones (VIII) by their yellow fluorescence, their yellow colour with Dragendorff's reagent and their greenish colours with ferric chloride in perchloric acid.

The GLC system chosen was considered to be the most satisfactory of several

TABLE III

R_t VALUES OF ALKALOIDS INCLUDED IN THE SCREENING

Alkaloid	R_t *(min)*	*Alkaloid*	R_t *(min)*
Pentacyclic heteroyohimbines		Uncarine A	13.5
Ajmalicine	20.6	Uncarine B	13.5
Isoajmalicine	10.7		
Mitrajavine	19.5	*Tetracyclic oxindoles*	
Tetrahydroalstonine	17.5	Isorhynchophylline	10.9
Akuammigine	14.3	*anti*-Isorhynchophylline N-oxide	10.9
4-R akuammigine N-oxide	14.3	Isocorynoxeine	10.3
19-*epi*-Ajmalicine	19.5	Rotundifoline	16.0
3-Iso-19-*epi*-ajmalicine	12.7	Rhynchociline	17.9
Rauniticine	15.5	Rhynchophylline	10.9
		Rhynchophylline N-oxide	10.9
Tetracyclic heteroyohimbines		Corynoxeine	10.3
Dihydrocorynantheine	18.8	Isorotundifoline	16.0
Gambirine	—	Ciliaphylline	17.9
Speciogynine	37.7	Corynoxine	10.2
Hirsutine	12.2	Corynoxine B	10.2
Hirsuteine	11.8	Speciofoline	15.5
Mitraciliatine	22.3		
Corynantheidine	16.9	Roxburghine C	—
Mitragynine	33.2	Roxburghine D	—
Isocorynantheidine	16.7	Roxburghine E	—
Speciociliatine	32.1	Dimeric indole alkaloid[23]	—
Pentacyclic oxindoles		Harmane	0.6
Isomitraphylline	14.0	Harmine	1.5
Isomitraphylline N-oxide	14.0	Harmaline	1.4
Javaphylline	23.2		
Mitraphylline	14.0	Angustine	—
Mitraphylline N-oxide	14.0	Angustidine	—
Isopteropodine	12.4	Angustoline	—
Isopteropodine N-oxide	12.4		
Pteropodine	12.4	Gambirtannine	—
Pteropodine N-oxide	12.4	Dihydrogambirtannine	20.8
Speciophylline	12.4	Oxogambirtannine	—
Speciophylline N-oxide	12.4		
Uncarine F	12.4		
Uncarine F N-oxide	12.4		

methods tried, including the one previously described for the separation of *Mitragyna* heteroyohimbines[7]. The R_t values for the alkaloids included in the screening procedure are given in Table III. The effects of substitution and stereochemical differences on the R_t values of the heteroyohimbines are the same as those previously described[7], *i.e. pseudo* < *epiallo* < *allo* < *normal*; the introduction of one methoxy group into the indole nucleus results in an increase in R_t value. For the oxindole alkaloids, it was observed that the *normal* A and B isomeric pairs of either structural type (III or IV) have the same R_t value, different from that which is common to the four A and B *allo*/*epiallo* isomers. No separations could be obtained by altering the chromatographic conditions and it is likely that these alkaloids undergo thermal isomerisation during GLC and run as a single compound. Thermal isomerisation of such alkaloids

about the C-3/C-7 bond is well known[1]. The 9-hydroxyoxindole alkaloids rotundifoline (IV, *normal* A, R = OH, R′ = Et) and isorotundifoline (IV, *normal* B, R = OH, R′ = Et) were detected as a single peak under the GLC conditions although the corresponding heteroyohimbine alkaloid gambirine (II, *normal*, R = OH, R′ = Et) was not detected. This suggests that the former pair of alkaloids may be chromatographed as the non-phenolic isomer (rotundifoline) in which the C-9 hydroxyl is intramolecularly hydrogen-bonded to N_4.

For both pentacyclic (III) and tetracyclic (IV) oxindole alkaloids, the *allo* and *epiallo* isomers were eluted before the *normal* isomers. Substitution at C-9 has a marked effect on R_f values with a progressive increase 9-H < 9-OH < 9-OMe. The E ring plays some part in determining the position of equilibrium between the SE-52 and the carrier nitrogen; for a given configuration the E-*seco* compounds are eluted before the E-cyclic ones and the presence of a C-20 vinyl rather than an ethyl substituent in the E-*seco* alkaloids slightly reduces the R_f value as does the change of the C-19 methyl configuration from α to β in the E cyclic series (observed for the *normal* isomers). N_4-Oxides of the oxindole and heteroyohimbine alkaloids have the same R_f values as the parent tertiary alkaloids and it is highly likely that they decompose at column temperatures.

The combination of GLC with TLC is particularly useful for the analysis of plant extracts containing oxindole alkaloids since the number of GLC peaks is less than the number of TLC spots and hence the structural types of the alkaloids present are more readily determined, *e.g.* if the *allo* and *epiallo* closed E ring oxindole isomers (III, C-19 Me α) are present in an extract together with their N-oxides, then eight TLC spots would be observed but only one GLC peak (*cf.* Tables I and III). The GLC results at 230–240° are further simplified in comparison to the TLC separations since the roxburghines and pyridino-indolo-quinolizidinones were not detected and the simple β-carbolines have very short retention times.

Although TLC, using different chromogenic reagents, and GLC are powerful analytical methods for the detection of these alkaloids, identification must still be considered to be of a tentative nature. Further confirmation of identity can be obtained by elution of alkaloids from TLC plates for UV spectroscopic examination. The routine use of UV spectroscopy enables the alkaloids to be assigned to a particular group (see Table IV). The unsubstituted heteroyohimbines of the pentacyclic and tetracyclic types and their 9-methoxy analogues exhibit absorption due to the summation of the indole and the conjugated ester vinyl-ether chromophores. The only 9-hydroxyheteroyohimbine, gambirine, in addition exhibits a bathochromic shift with alkali which is reversible on the addition of acid. The unsubstituted oxindoles of the pentacyclic and tetracyclic types have a common UV absorption due to the summation of the oxindole and ester vinyl-ether chromophores. 9-Methoxy- and 9-hydroxy-oxindoles differ from the unsubstituted oxindole alkaloids in their UV absorption. A bathochromic shift with alkali is not observed if the 9-hydroxyl is hydrogen-bonded to the N_4 lone-pair electrons, *e.g.* rotundifoline[14] (IV, *normal* A, R = OH, R′ = Et). The roxburghines[13], pyridino-indolo-quinolizidinones[15] and the simple β-carbolines[7] also give characteristic UV spectra.

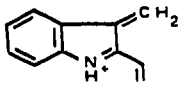
Conclusions drawn from TLC, GLC and UV data during an alkaloidal screening programme may be confirmed by MS. The tentative identifications of *Uncaria* alkaloids made on the basis of chromatographic and UV spectroscopic

TABLE IV

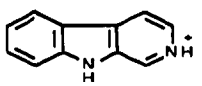
UV SPECTRAL DATA ON ALKALOIDS INCLUDED IN THE SCREENING

Chromophore type	*Example*	*Solvent*	$\lambda_{max.}$ *(nm)*	$\lambda_{min.}$ *(nm)*
9-H heteroyohimbine	dihydrocorynantheine	MeOH	225, 245sh, 283, 291	
9-OH heteroyohimbine	gambirine	MeOH	226, 248sh, 286, 296	
		MeOH/KOH	228, 248sh, 296, 307	
		MeOH/HCl	225, 248sh, 286, 296	
9-OMe heteroyohimbine	mitragynine	MeOH	225, 247sh, 285, 293	
9-H oxindole	isorhynchophylline	MeOH	211, 243, 284	224
9-OH oxindole (non-phenolic)	rotundifoline	MeOH	222, 242sh, 288–296	
		MeOH/KOH	222, 242sh, 288–296	
9-OH oxindole	isorotundifoline	MeOH	220, 242sh, 286–295	235
		MeOH/KOH	231, 295–307	
		MeOH/HCl	229, 255sh, 286–297	235
9-OMe oxindole	ciliaphylline	MeOH	220, 243, 285–293	234
Roxburghine	roxburghine D	MeOH	224, 285, 292	
Harmane		MeOH	211, 235, 239, 250, 282, 289, 336, 349	
Harmine		MeOH	210, 240, 302, 327, 338	
Harmaline		MeOH	216, 228, 261, 341, 352, 380	
Angustine		MeOH	233, 257sh, 293, 306, 383, 400	
Angustidine		MeOH	243, 253, 289, 301, 373, 391	
Angustoline		MeOH	227, 254sh, 293, 303, 380, 399	
Gambirtannine		MeOH	213, 235, 257sh, 311, 345sh, 395	
Dihydrogambirtannine		MeOH	224, 294, 301	
Oxogambirtannine		MeOH	221, 257, 300, 366, 383	

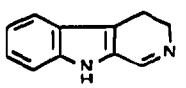
evidence were almost invariably confirmed by MS of eluted alkaloids. *Ar*-unsubstituted closed E ring alkaloids of the ajmalicine-type (I) give strong M^+ (*m/e* 352) together with unusually strong $M^+ - 1$ ions due to the loss of the C-3 hydrogen. Fragments which contain the indole nucleus occur at *m/e* 156, 169, 170, 184, and 225 (XI–XV, resp.)[9–11] (see Table V). Substitution of the aromatic nucleus by hydroxyl or methoxyl groups would result in the presence of such fragments 16 or 30 mass units (MU) higher and the M^+ and $M^+ - 1$ ions would be similarly increased. Although it has been concluded that the differences between C-19 Me epimers (I) cannot be distinguished by MS[16], the configurational isomers *normal*, *allo*, *epiallo* and *pseudo* (I) can be differentiated on the basis of the relative intensities of the ions at *m/e* 156, 184, 223, and 251. Compounds of the *normal* and *pseudo* configurations exhibit a fragment ion at *m/e* 184 which is considerably more abundant than those at *m/e* 169 and 170, while the fragments at *m/e* 209 and 225 are of moderate abundance accompanied by smaller amounts of ions exhibiting peaks at *m/e* 223 and 251. In contrast, the *allo* and *epiallo* isomers exhibit a *m/e* 184 fragment ion of similar or lower abundance than the *m/e* 169 and 170 ions, while the ions at *m/e* 209 and 225



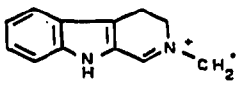
XI m/e 156



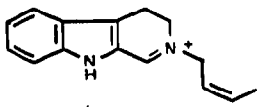
XII m/e 169



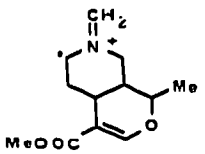
XIII m/e 170



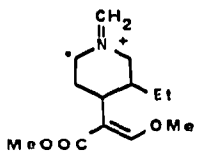
XIV m/e 184



XV m/e 225



XVI m/e 223



XVII m/e 239

are overshadowed by the abundant *m/e* 223 and 251 fragments[9]. Hence MS can be used to distinguish *cis* D/E from *trans* D/E isomers in the closed E ring heteroyohimbine series (I).

The MS of the unsubstituted tetracyclic heteroyohimbines (II) give a M^+ at *m/e* 368, a strong $M^+ - 1$, fragments indicating loss of Me, Et and OMe, and ions XI–XV[9–11]. In the *ar*-unsubstituted compounds, the intensity of the ions at *m/e* 156, 169 and 170 is significantly less when the C-20 ethyl group is axial (*i.e.*, *allo* and *epiallo*) than where it is equatorial (*i.e.*, *normal* and *pseudo*). The configurational isomers of the *ar*-unsubstituted and of the 9-methoxy tetracyclic heteroyohimbines can be distinguished by comparing the ratio of the peaks at *m/e* 225 and 239, which has been reported to be 2:1 for the *normal*, 3:1 for the *pseudo*, 0.5:1 for the *allo*, and 0.4:1 for the *epiallo* configurations[9,10]. Although in practice some doubt may arise in distinguishing *normal* from *pseudo* or *allo* from *epiallo* by MS, it should be noted that each member of these pairs would have been distinguished by TLC and GLC.

The oxindole alkaloids can readily be distinguished from the heteroyohimbines since they do not produce $M^+ - 1$ fragments. The pentacyclic oxindole alkaloids (III) show a prominent peak at *m/e* 223 (XVI) derived from the alicyclic portion of the molecule, together with an ion of 15 MU lower due to the loss of the C-19 methyl and thus they are distinguished from the E-*seco* oxindole-alkaloids (IV), which give rise to an ion at *m/e* 239 (XVII) with subsequent loss of Me, Et and OMe. Both the pentacyclic and tetracyclic oxindole alkaloids fragment to give ions derived from the oxindole moiety having *m/e* 130, 144, 145, 146, and 159. Again *ar*-substitution is readily recognised since these ions and the M^+ will appear at 16 or 30 MU higher, depending upon whether the substituent is a hydroxyl or a methoxyl group. It has been reported that the relative abundance of any of these ions cannot be related to the stereochemistry of the oxindole alkaloids[9,10]. During our screening work, the

TABLE V

MASS SPECTRA OF ALKALOIDS DETERMINED DURING THE SCREENING

Alkaloid	M^+ (%)	*Fragment ions (%)*
Ajmalicine	352 (100)	351 (73), 337 (13), 251 (8), 225 (8), 223 (2), 209 (12), 184 (47), 170 (12), 169 (15), 156 (58)
Isoajmalicine	352 (100)	351 (66), 337 (13), 251 (<5), 225 (13), 223 (13), 209 (20), 184 (47), 170 (27), 169 (15), 156 (93)
Tetrahydroalstonine	352 (100)	351 (78), 337 (37), 251 (17), 225 (13), 223 (39), 209 (15), 184 (18), 170 (18), 169 (30), 156 (62)
Akuammigine	352 (100)	351 (60), 337 (80), 251 (38), 225 (2), 223 (54), 209 (19), 184 (21), 170 (18), 169 (29), 156 (60)
19-*epi*-Ajmalicine	352 (100)	351 (70), 337 (15), 251 (3), 225 (8), 223 (10), 209 (7), 184 (30), 170 (21), 169 (23), 156 (70)
3-Iso-19-*epi*-ajmalicine	352 (95)	351 (70), 337 (5), 251 (3), 225 (11), 223 (10), 209 (10), 184 (45), 170 (41), 169 (44), 156 (100)
Dihydrocorynantheine	368 (100)	367 (90), 353 (55), 251 (10), 239 (14), 225 (24), 213 (25), 184 (70), 170 (39), 169 (25), 156 (30)
Gambirine	384 (90)	383 (80), 369 (60), 267 (9), 255 (11), 241 (21), 213 (10), 200 (100), 186 (39), 185 (32), 172 (30)
Speciogynine	398 (100)	397 (91), 383 (20), 281 (13), 269 (17), 255 (38), 214 (98), 213 (23), 200 (100), 199 (38), 186 (53)
Hirsutine	368 (100)	367 (68), 353 (100), 311 (19), 251 (13), 239 (13), 225 (28), 197 (17), 184 (70), 170 (27), 169 (28), 156 (29)
Hirsuteine	366 (89)	365 (69), 351 (100), 335 (36), 237 (22), 223 (51), 184 (78), 170 (53), 169 (42), 156 (53)
Isomitraphylline	368 (64)	351 (5), 337 (7), 223 (100), 222 (10), 208 (11), 146 (6), 145 (5), 144 (7), 130 (11), 69 (23)
Isomitraphylline N-oxide	384 (37)	368 (53), 367 (14), 223 (100), 208 (20), 159 (36), 146 (17), 145 (17), 144 (23), 130 (40), 69 (>100)
Mitraphylline	368 (40)	351 (3), 337 (4), 223 (100), 222 (13), 208 (11), 146 (6), 145 (10), 144 (6), 130 (11), 69 (27)
Mitraphylline N-oxide	384 (13)	368 (60), 366 (10), 239 (9), 223 (100), 208 (11), 159 (35), 146 (14), 145 (8), 144 (16), 130 (32), 69 (100)
Isopteropodine	368 (100)	351 (8), 337 (8), 223 (77), 222 (24), 208 (19), 180 (19), 146 (6), 145 (9), 144 (8), 130 (14), 69 (41)
Isopteropodine N-oxide	384 (100)	368 (51), 367 (47), 239 (42), 223 (73), 208 (26), 201 (69), 180 (39), 159 (58), 146 (34), 145 (35), 144 (61), 130 (95), 69 (>100)
Pteropodine	368 (100)	351 (6), 337 (8), 223 (86), 222 (33), 208 (25), 180 (21), 146 (8), 145 (11), 144 (11), 130 (19), 69 (40)
Pteropodine N-oxide	384 (50)	368 (80), 367 (14), 239 (54), 223 (100), 208 (36), 180 (36), 159 (45), 146 (23), 145 (23), 144 (40), 130 (70), 69 (>100)
Speciophylline	368 (100)	351 (7), 337 (8), 223 (78), 222 (28), 208 (25), 180 (11), 146 (9), 145 (12), 144 (12), 130 (21), 69 (78)
Speciophylline N-oxide	384 (3.5)	368 (95), 223 (100), 208 (32), 180 (30), 159 (16), 146 (12), 145 (13), 144 (16), 130 (28), 69 (62)
Uncarine F	368 (100)	351 (8), 337 (10), 223 (70), 222 (20), 208 (15), 180 (13), 146 (5), 145 (7), 144 (6), 130 (11), 69 (45)
Uncarine F N-oxide	384 (45)	368 (47), 367 (100), 223 (50), 213 (80), 208 (28), 180 (13), 159 (31), 146 (20), 145 (19), 144 (32), 130 (50), 69 (45)
Uncarine A	368 (50)	351 (5), 337 (6), 223 (100), 222 (12), 208 (13), 146 (8), 145 (11), 144 (10), 130 (19), 69 (41)
Uncarine B	368 (85)	351 (6), 337 (8), 223 (100), 222 (13), 208 (16), 146 (6), 145 (7), 144 (8), 130 (15), 69 (35)

(Continued on p. 176)

TABLE V *(continued)*

Alkaloid	*M⁺ (%)*	*Fragment ions (%)*
Isorhynchophylline	384 (100)	369 (5), 367 (6), 355 (5), 353 (9), 239 (80), 238 (38), 224 (29), 210 (17), 208 (21), 146 (6), 145 (9), 144 (10), 130 (17), 69 (85)
anti-Isorhynchophylline N-oxide	400 (10)	384 (100), 382 (23), 239 (88), 238 (42), 224 (35), 210 (21), 208 (27), 144 (27), 130 (37), 69 (>100)
Isocorynoxeine	382 (100)	367 (9), 365 (5), 351 (18), 237 (15), 236 (20), 222 (23), 208 (15), 206 (19), 159 (18), 146 (17), 145 (20), 144 (30), 130 (52), 108 (56), 69 (46)
Rotundifoline	400 (100)	385 (5), 383 (4), 371 (4), 369 (10), 239 (100), 238 (28), 224 (30), 210 (16), 208 (19), 162 (10), 161 (7), 160 (11), 146 (21), 69 (67)
Rhynchophylline	384 (100)	369 (7), 367 (6), 355 (5), 353 (10), 239 (95), 238 (43), 224 (31), 210 (19), 208 (24), 146 (8), 145 (12), 144 (13), 130 (20), 69 (>100)
Rhynchophylline N-oxide	400 (4)	384 (100), 382 (36), 239 (75), 238 (39), 224 (32), 210 (25), 208 (25), 146 (16), 145 (18), 144 (21), 130 (32), 69 (89)
Corynoxeine	382 (100)	367 (8), 365 (5), 351 (14), 237 (6), 236 (9), 222 (7), 206 (7), 192 (6), 146 (3), 145 (3), 144 (5), 130 (8), 108 (30), 69 (15)
Isorotundifoline	400 (100)	385 (6), 383 (4), 371 (1), 369 (11), 239 (91), 238 (32), 224 (35), 210 (18), 208 (21), 162 (10), 161 (8), 160 (12), 146 (23), 69 (>100)
Corynoxine	384 (100)	369 (9), 367 (9), 355 (5), 353 (12), 239 (90), 238 (42), 224 (35), 210 (20), 208 (26), 146 (8), 145 (11), 144 (17), 130 (25), 69 (>100)
Corynoxine B	384 (100)	369 (9), 367 (9), 355 (5), 353 (10), 239 (84), 238 (39), 224 (30), 210 (17), 208 (24), 146 (7), 145 (8), 144 (12), 130 (20), 69 (85)
Speciofoline	400 (100)	385 (8), 383 (4), 371 (4), 369 (10), 239 (68), 238 (23), 224 (37), 210 (13), 208 (18), 162 (7), 161 (6), 160 (11), 146 (21), 69 (64)
Roxburghine D	492 (72)	491 (17), 477 (24), 461 (10), 362 (14), 336 (21), 321 (67), 308 (12), 307 (12), 293 (10), 279 (17), 221 (14), 197 (16), 184 (100), 171 (36), 156 (24), 144 (17), 143 (14), 130 (14), 69 (17)
Harmane	182 (100)	181 (33), 155 (13), 154 (23), 139 (17)
Angustine	313 (100)	
Angustoline	331 (100)	316 (68), 314 (41), 313 (91)

routine use of MS has led to the observation that although this appears to be true for the tetracyclic oxindoles (IV), a relationship does exist for the pentacyclic oxindoles (III). When the spectra are determined at 230° and run under standard conditions, the *allo*/*epiallo* isomers of the pteropodine type have a M^+ at *m/e* 368 of greater intensity than the ion at *m/e* 223 (XV), whereas the *normal* isomers, the mitraphyllines and uncarines A and B, give spectra in which the intensity of the M^+ at 368 is less than that of the *m/e* 223 fragment. C-20 vinyl analogues of the E *seco* heteroyohimbine (II) and the oxindole alkaloids (III) are difficult to distinguish chromatographically, particularly when mixtures of alkaloids are present. Such compounds are readily detected by MS since the M^+ occurs at 2 MU lower than for the

C-20 ethyl compounds, *e.g.* hirsuteine, M^+ 366; corynoxeine, M^+ 382. The N-oxides of the heteroyohimbines (I, II) and the oxindoles (III, IV) give M^+ ions of varying abundance, together with ions at $M^+ - 16$ corresponding to the parent tertiary bases and other fragments which are characteristic of the latter[17–19].

Confirmation of the identity of other alkaloids included in the screening procedure can also be obtained by MS. The roxburghines (V) give spectra having M^+ at *m/e* 492 together with a number of characteristic fragment ions[13]. The abundance of the M^+ of the pyridino-indolo-quinolizidinones (VIII) has been found to be temperature dependent, being more intense at 250° than at 230°; due to the highly unsaturated nature of these alkaloids few fragment ions exist[15,20]. Gambirtannine (IX) also shows little fragmentation of the M^+ (*m/e* 330) although a prominent $M^+ - 1$ ion is present[21].

The combination of TLC, UV detection and chromogenic reagents allows the tentative identification of any of the sixty alkaloids selected for the screening of *Uncaria* species. GLC enables a further, though more simplified, chromatographic identification to be obtained. The chromophore types of the separated alkaloids eluted from TLC plates are determined by UV spectroscopy and more definitive identification of the alkaloids can be made by MS. These methods have been used for the investigation of some 400 small samples of herbarium material representing all 39 species of *Uncaria*. These results will be published separately.

The development of these analytical procedures has enabled the identification of alkaloids in every species of this pantropical genus and for some species collections made over a wide geographical range have been analysed. Naturally herbaria guard their collections jealously and indeed would cease to exist if they allowed their specimens to be squandered for chemical research. However, herbaria often hold material in excess of their requirements and this work demonstrates that with sensitive analytical techniques now available information on alkaloids may be obtained from small quantities of plant material. The methods could also be applied to the detection of other secondary metabolites and similar investigations into other genera may yield the detailed information required for chemosystematics.

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