Journal of Chromatography, 244 (1982) 91–98
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,909

SEPARATION OF HETEROYOHIMBINE AND OXINDOLE ALKALOIDS BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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(Received March 17th, 1982)

SUMMARY

The separation of nine heteroyohimbine and eight oxindole alkaloids has been investigated by using reversed-phase high-performance liquid chromatography. The behaviour of these seventeen alkaloids has been compared with the separations obtained on silica gel thin-layer chromatography plates. Whereas the sequence of separation on adsorbent layers can be rationalised, at a molecular level, by assessing the ability of nitrogen lone-pair electrons to hydrogen bond with silanol hydroxyl groups, no clear pattern emerges from the results of the reversed-phase high-performance liquid chromatographic separations. Although some alkaloids exhibit the expected reversed-phase behaviour, other alkaloids follow the same sequence of elution as found on adsorption systems.

INTRODUCTION

Thin-layer chromatography (TLC) has proved to be invaluable for the separation of heteroyohimbine- (I, II)[1,2] and their corresponding oxindole- (III, IV)[2–4] type alkaloids. Heteroyohimbine alkaloids may be separated by gas–liquid chromatography (GLC)[5] but the corresponding oxindoles undergo thermal isomerisation and thus the usefulness of this separation technique is limited. A combination of TLC and GLC techniques has been utilised, with UV spectroscopy and mass spectrometry (MS), for the identification of these alkaloid-types obtained from herbarium specimens of *Uncaria* species[6]. The application of these combined chromatographic and spectroscopic techniques to extracts obtained from a large number of herbarium specimens resulted in an assessment of the alkaloids present in all 34 species of this pantropical genus[7]. The occurrence of alkaloids was related to the botanical revision of the genus which had resulted in a drastic reduction of the 120 specific names listed in the Kew Index. A number of heteroyohimbine- (I, II) and oxindole- (III, IV) type

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0021-9673/82/0000–0000/$02.75

alkaloids have been separated by high-performance liquid chromatographic (HPLC) techniques using silica gel adsorbent columns[8] and limited use has been made of reversed-phase HPLC separations[9,10]. The present communication describes the separation of a number of heteroyohimbine- and oxindole-type alkaloids by the technique of reversed-phase HPLC and discusses the molecular mechanisms involved.

MATERIALS AND METHODS

Alkaloids

Nine heteroyohimbine- and eight oxindole-type alkaloids were obtained from Thai species of *Uncaria*. The alkaloids were identified by a combination of chromatographic (TLC, GLC, HPLC) and spectroscopic techniques (UV, MS, ^{1}H nuclear magnetic resonance) with authentic samples[11].

Apparatus

An Altex isocratic liquid chromatograph (Model 330) fitted with a valve loop injector (Reodyne 7125) (10 μl) was used. The UV detector was set at 254 nm and a flat-bed recorder (Kipp and Zonen) was fitted.

Packing material and column

A stainless-steel tube, 25 cm × 4 mm (I.D.) was packed with 5 μm Spherisorb ODS (Jones Chromatography) using the slurry technique and a Jones HPLC packing instrument.

Operation procedure

The instrument was operated at laboratory temperature (approximately 20°C) using a flow-rate of 2 ml/min and at pressures between 1000 and 3000 p.s.i.

RESULTS AND DISCUSSION

Nine heteroyohimbine (I, II) and eight oxindole (III, IV) alkaloids were investigated. Their structures and the retention times, t_R, obtained are given in Table I. The results clearly indicate that although the nine heteroyohimbines (I, II) were readily separated by solvents a and b, there was no useful separation for pentacyclic oxindole alkaloids (III) although two tetracyclic oxindole alkaloids were well separated. The failure to separate, in particular, the four diastereoisomeric alkaloids isopteropodine, pteropodine, speciophylline and uncarine F in solvent system a on Spherisorb ODS is in marked contrast to the separations reported previously on Corasil C_{18} utilising the same solvent system[9]. Although slight separation was obtained for these four alkaloids using solvent system c in the present work, the separation is in no way comparable to those described in a previous literature report[9].

The separation of alkaloids of types I–IV on silica gel TLC systems has been explained mainly in terms of lone pair N-4 electrons bonding to silanol hydroxyl groups[2]. Steric factors which contribute to hindrance of N-4 lone-pair electrons, result in reduced adsorption and it is therefore possible to explain the sequence of R_F values for heteroyohimbine alkaloids (I, II) as *allo* > *normal* > *epiallo* > *pseudo*[2]. The same explanation has been offered in order to explain the behaviour of heteroyohimbine alkaloids on HPLC silica gel systems[8]. It might be anticipated that on reversed-phase HPLC systems, the sequence of separation would be reversed. However, the present results indicate that this does not necessarily follow.

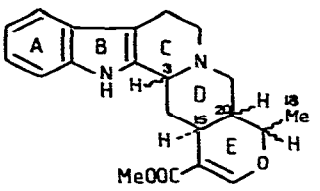
Table II contrasts the separation of the two pentacyclic heteroyohimbine al-

TABLE I

STRUCTURES OF HETEROYOHIMBINE- AND OXINDOLE-TYPE ALKALOIDS AND t_R VALUES OBTAINED ON REVERSED-PHASE HPLC

Spherisorb ODS (5 μm), for conditions see Materials and methods. The alkaloids I–IV can exist as diastereoisomers defined as *allo* (C-3 H α, C-20 H α), *epiallo* (C-3 H β, C-20 H α), *normal* (C-3 H α, C-20 H β), *pseudo* (C-3 H β, C-20 H β); III and IV can exist as either A or B isomers in which the lactam carbonyl lies below (A) or above (B) the plane of the C–D rings. Solvent systems used: a = methanol–water (80:20); b = methanol–water–conc. ammonium hydroxide (80:20:1); c = acetonitrile–1 % ammonium carbonate (60:40); d = methanol–1 % ammonium carbonate (80:20).

Alkaloid	t_R *(min) in solvent system*			
	a	*b*	*c*	*d*
Pentacyclic heteroyohimbines (I)				
V, Tetrahydroalstonine (*allo*, C-18 Me α)	5.1	4.8	6.2	3.6
VI, Rauniticine (*allo*, C-18 Me β)	10.9	8.6	10.0	10.0
VII, Akuammigine (*epiallo*, C-18 Me α)	5.8	4.6	6.5	4.2
VIII, 3-Iso-rauniticine (*epiallo*, C-18 Me α)	1.0	1.0	–	–
IX, Ajmalicine (*normal*, C-18 Me α)	4.3	3.4	5.2	2.9
X, 3-Iso-ajmalicine (*pseudo*, C-18 Me α)	2.0	2.0	–	–
XI, 19-Epi-3-isoajmalicine (*pseudo*, C-18 Me β)	1.6	1.6	–	–
Tetracyclic heteroyohimbines (II)				
XII, Dihydrocorynantheine (*normal*)	7.7	3.9	–	3.7
XIII, Hirsutine (*pseudo*)	12.7	4.9	–	–
Pentacyclic oxindoles (III)				
XIV, Isopteropodine (*allo*, A)	3.8	3.8	3.7	2.9
XV, Pteropodine (*allo*, B)	3.8	3.2	2.8	2.3
XVI, Speciophylline (*epiallo*, A)	3.7	3.0	2.8	2.2
XVII, Uncarine F (*epiallo*, B)	3.7	3.0	2.7	2.2
XVIII, Mitraphylline (*normal* A)	3.4	3.2	3.0	2.4
XIX, Isomitraphylline (*normal*, B)	3.7	3.0	2.7	2.0
Tetracyclic oxindoles (IV)				
XX, Isorhynchophylline (*normal*, A)	3.0	3.0	3.3	3.3
XXI, Rhynchophylline (*normal*, B)	6.2	4.0	5.1	4.2



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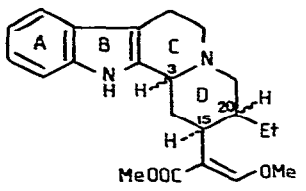

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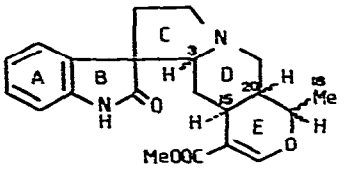

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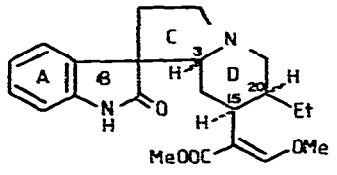

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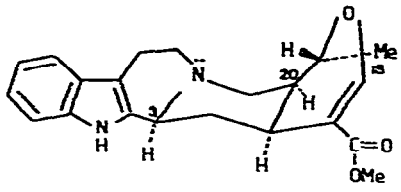
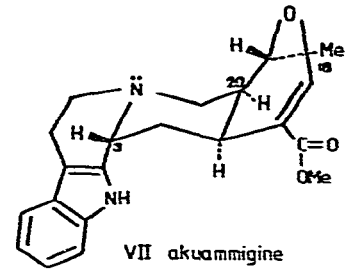
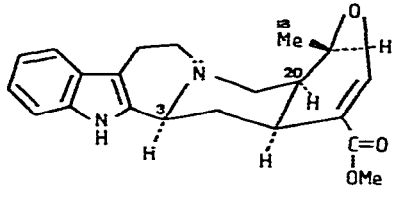
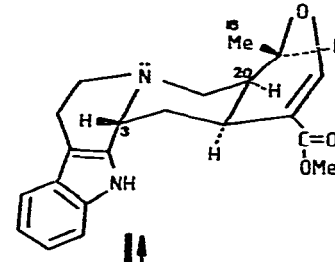


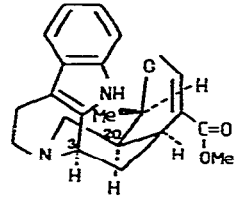
V tetrahydroalstonine



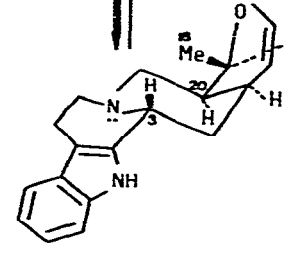
VII akuammigine



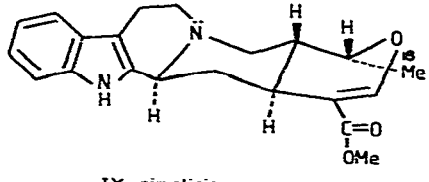




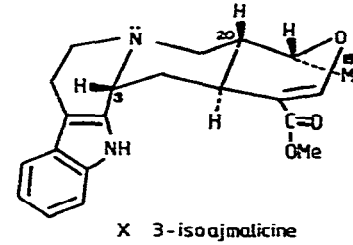
VI rauniticine



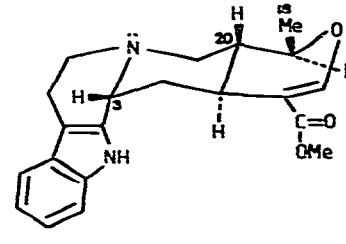
VIII 3-isorauniticine



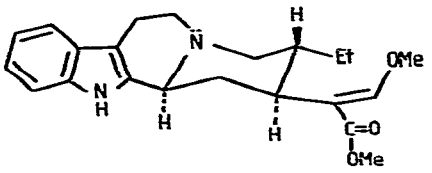
IX ajmalicine



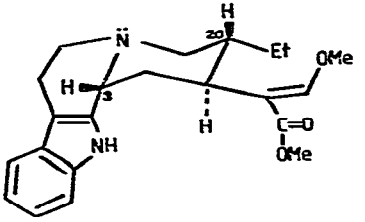
X 3-isoajmalicine



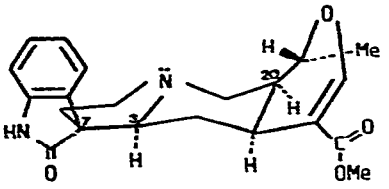
XI 19-epi-3-isoajmalicine



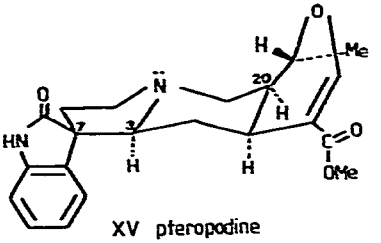
XII dihydrocorynantheine



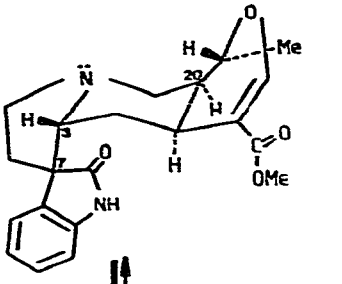
XIII hirsutine

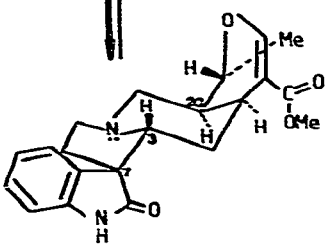


XIV isopteropodine

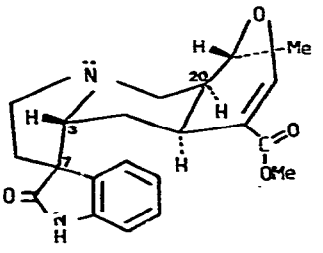


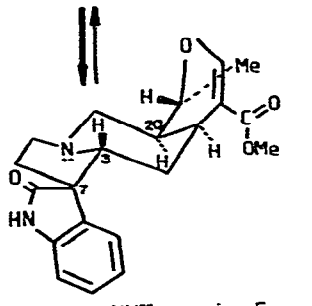
XV pteropodine



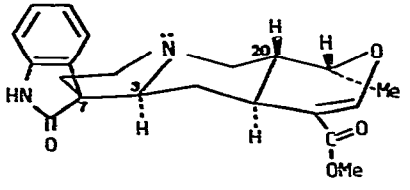


XVI speciophylline

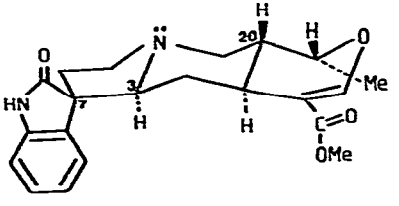




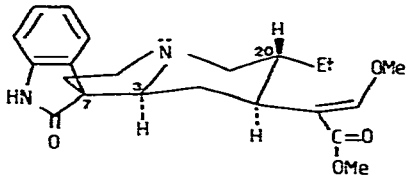
XVII uncarine F



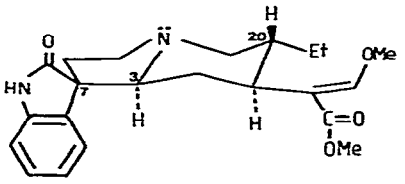
XVIII isomitraphylline



XIX mitraphylline



XX isorhynchophylline



XXI rhynchophylline

kaloids tetrahydroalstonine (I, *allo*, C-18 Me α) and ajmalicine (I, *normal*, C-18 Me α) on reversed-phase HPLC and on silica gel TLC systems. Comparison of the t_R values of tetrahydroalstonine (V) and ajmalicine (IX) (Table II) shows that tetrahydroalstonine is more retained than is ajmalicine. This behaviour is in agreement with that described previously for reversed-phase HPLC separations[10] and is the reverse of that found on silica gel TLC adsorption systems. 3-Isoajmalicine (X) is retained less on reversed-phase HPLC using solvent systems a and b (Table I) than ajmalicine (IX) and again, this is the anticipated reverse behaviour in comparison with adsorption separations on silica gel TLC systems[6]. Although the t_R values for 3-isoajmalicine (X) and 19-*epi*-3-isoajmalicine (XI) are close (Table I), the order of separation is also reversed to that on silica gel TLC systems[6].

However, when the t_R values for the *allo–epiallo* pair of diastereoisomers, tetrahydroalstonine (V) and akuammigine (VII) (Table I) are compared with their R_F values for silica gel TLC systems[6], the sequence, somewhat surprisingly, is the same. Furthermore, in contrast to the behaviour of the *pseudo* isomers 3-iso-ajmalicine (X) and 19-*epi*-3-isoajmalicine (XI), the change in configuration at C-19 for the *allo–epiallo* diastereoisomers results in a marked change of behaviour. Rauniticine (VI), the C-19 epimer of tetrahydroalstonine (V) is more strongly retained on reversed-phase HPLC (Table I, systems a–d) whereas 3-isorauniticine (VIII), C-19 epimer of akuammigine (VII) is rapidly eluted. In contrast, it should be noted that 3-isorauniticine (VIII) is strongly retained on silica gel TLC systems[12]. It is possible that the anomalous behaviour of the alkaloids VI, VII and VIII can be due to conformational changes in the C–D rings due to inversion of N-4.

The tetracyclic diastereoisomeric alkaloids (II) dihydrocorynantheine (XII, *normal*) and hirsutine (XIII, *pseudo*) which separate well on reversed-phase HPLC (Table I) show a sequence of elution which is the same as for adsorption TLC. This behaviour contrasts with that of the corresponding pentacyclic heteroyohimbine alkaloids (I) ajmalicine (VI, *normal*) and 3-isoajmalicine (VII, *pseudo*). It is difficult to offer any rational explanation for these contrasting sets of behaviour.

TABLE II

t_R VALUES ON REVERSED-PHASE HPLC AND hR_F VALUES ON SILICA GEL OF TETRAHYDROALSTONINE (V) AND AJMALICINE (IX)

Alkaloid	t_R *(min)*		hR_F***
	*Present results**	*Literature***	
Tetrahydroalstonine (V)	5.1	14.65	54
Ajmalicine (IX)	4.3	7.90	36

* Spherisorb ODS, methanol–water (80:20).

** LiChrosorb RP-8, acetonitrile–0.01 *M* ammonium carbonate (47:53)[10].

*** Silica gel, chloroform–methanol (98:2)[12].

The oxindole alkaloids (III, IV) are generally well separated by silica gel TLC systems. The sequence of elution can also be related to the availability of the N-4 lone-pair electrons to bond to silanol hydroxyl groups. Furthermore, when N-4 lone-pair electrons are *cis* to the oxindole carbonyl, binding to silica gel is stronger than for the corresponding *trans* isomer. Although the present investigation failed to produce a satisfactory separation for the four *allo–epiallo* diastereoisomeric pentacyclic oxindole alkaloids isopteropodine (XIV), pteropodine (XV), speciophylline (XVI) and uncarine F (XVII), a comparison can be made between reversed-phase HPLC literature t_R values and hR_F values reported for silica gel TLC systems (Table III). The *allo* B isomer pteropodine (XV) is only slightly more adsorbed on TLC than the A isomer isopteropodine (XIV) but the literature t_R values[9] for reversed-phase HPLC separation indicates a wide separation on the same sequence and not reversed as might be anticipated. The anomalous behaviour of the *epiallo* A isomer speciophylline (XVI) and the *epiallo* B isomer, uncarine F (XVII) on silica gel TLC systems has been explained in terms of inversion of N-4 for the preferred conformations in comparison to those of the *allo* isomers[3]. The sequence of elution on reversed-phase HPLC for speciophylline (XVI) and uncarine F (XVIII) is the same as that obtained on silica gel TLC systems (Table III).

The behaviour of the two normal pentacyclic oxindoles (III), isomitraphylline (XVIII), mitraphylline (XIX) and the two normal tetracyclic oxindoles rhynchophylline (XX), isorhynchophylline (XXI) on reversed-phase HPLC and silica gel TLC systems is compared in Table IV. Both sets of isomers separate well on reversed-phase HPLC and on TLC. The present results indicate that isomitraphylline (XVIII) is less retained than mitraphylline (XIX) on a reversed-phase HPLC and follows the same sequence of separation as adsorption on silica gel TLC systems (Table IV). However, the t_R values found in the present work are reversed in sequence from those reported previously[9]. Similarly, the t_R values of the tetracyclic oxindoles isorhynchophylline (XX) and rhynchophylline (XXI) follow the same sequence as adsorption on TLC and the order of t_R values in the present work is the same as those reported previously[9].

Although the mechanism of reversed-phase HPLC is not completely understood it might be expected that the sequence of elution for any series of compounds would be in the reverse order to that observed for silica gel systems. The present results for some seventeen closely related compounds indicates that the mechanism of

TABLE III

t_R VALUES ON REVERSED-PHASE HPLC AND hR_F VALUES ON SILICA GEL OF THE PENTACYCLIC *allo–epiallo* OXINDOLE ALKALOIDS OF THE PTEROPODINE-TYPE

Alkaloid	t_R *(min)*		hR_F***
	*Present results**	*Literature***	
Isopteropodine (XIV)	3.8	0.8	72
Pteropodine (XV)	3.8	2.3	68
Speciophylline (XVI)	3.7	3.0	29
Uncarine F (XVII)	3.7	1.8	60

* Spherisorb ODS, methanol–water (80:20).

** Corasil C_{18}, methanol–water (80:20)[9].

*** Silica gel, chloroform–acetone (5:4)[6].

TABLE IV

t_R VALUES ON REVERSED-PHASE HPLC AND hR_F VALUES ON SILICA GEL OF THE *NORMAL* OXINDOLE ALKALOIDS ISOMITRAPHYLLINE, MITRAPHYLLINE, ISORHYNCHOPHYLLINE, RHYNCHOPHYLLINE

Alkaloid	t_R *(min)*		hR_F***
	*Present results**	*Literature***	
Isomitraphylline (XVIII)	3.4	6.2	68
Mitraphylline (XIX)	4.7	3.2	51
Isorhynchophylline (XX)	3.0	2.7	70
Rhynchophylline (XXI)	6.2	3.6	35

* Spherisorb ODS, methanol–water (80:20).
** Corasil C_{18}, methanol–water (80:20)[9].
*** Silica gel, chloroform–acetone (5:4)[6].

separation is complex. The silica gel adsorption behaviour can be explained in relationship to molecular structures and in particular to availability of N-4 lone-pair electrons for bonding to silanol hydroxyl groups. The behaviour of seventeen alkaloids falls into distinct sequences on TLC adsorption systems but when compared with reversed-phase HPLC systems, some alkaloids exhibit the anticipated reverse order sequence of elution whereas other alkaloids do not. The reversed-phase packing material, Spherisorb ODS, is formed by bonding octadecyl silyl groups ($C_{18}H_{37}Si$–) to silica gel. It is possible that a proportion of residual silanol hydroxyl groups remain on reversed-phase packings[13] and hence retention on such stationary phases would probably be by a combination of adsorption and partition mechanisms[14]. It has been envisaged that hydrocarbon chains extending from the surface of silica gel particles would allow for non-polar interactions to take place[15,16]. If sufficient hydrocarbon chains were present, any residual silanol hydroxyl groups would be sterically hindered and adsorption to N-4 lone-pair electrons of these alkaloids could not take place. The present study indicates that adsorption does take place to some considerable extent with some of these alkaloids when a reversed-phase support is used but that some of these compounds appear to separate by a reversed-phase mechanism. No simple correlation appears to exist for reversed-phase HPLC as there does for these compounds separated by silica gel TLC systems.

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