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Gas-liquid chromatography of heteroyohimbine alkaloids: the effect of methoxy substitution and of configuration

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The gas–liquid chromatographic retention times of heteroyohimbine alkaloids on a 1% SE-30 column are in the order pseudo < epiallo < allo < normal. The introduction of one methoxy group into the indole nucleus doubles the retention time while two methoxy groups increase it by a factor of four. Binding of the indole nucleus reinforced by the suitably orientated lone pair electrons of the basic nitrogen to the liquid phase of the column in the important conformers of the various configurations, is used to explain the results.

AMONG the heteroyohimbine alkaloids stereochemical features have been shown to influence their behaviour on thin-layer chromatograms (Phillipson & Shellard, 1967).

The present work describes the use of gas–liquid chromatography for the separation and characterization of heteroyohimbine alkaloids of known stereochemistry (Wenkert & Bringi, 1959; Wenkert, Wickberg & Leicht, 1961a, b; Shamma & Moss, 1961; Joshi, Raymond-Hamet & Taylor, 1963; Beckett, Shellard & Tackie, 1965; Lee, Trager & Beckett, 1967; Trager, Lee & Beckett, unpublished observations). The retention times of these alkaloids are interpreted in terms of stereochemical, conformational and electronic factors.

Experimental

Alkaloids. Tetrahydroalstonine, aricine, reserpinine, tetraphylline, isoreserpinine, reserpiline, rauniticine, raunitidine, corynantheidine, mitragynine (Smith Kline and French Laboratories, Philadelphia, U.S.A.); raumitorine and epi-3-rauvanine (Dr. J. Poisson); rauvanine (Dr. M. M. Janot); isoraunitidine (Dr. M. Shamma); dihydrocorynantheine (S. B. Penick & Co.); speciogynine, speciociliatine, mitraciliatine and paynantheine (Dr. J. D. Phillipson); akuammigine, hirsutine and mitrajavine (Dr. E. J. Shellard); 3-isocorynantheidine and iso-paynantheine were prepared from corynantheidine and paynantheine respectively (unpublished).

APPARATUS

A Perkin Elmer Model F.11 Gas Chromatograph with hydrogen flame ionization detector was used under the following conditions: 1% S.E.30 on Gas-Chrom. P, 80–100 mesh, acid washed and treated with dimethyl dichlorosilane, oven temperature 215°, injection temperature 330°, hydrogen pressure 20 lb/inch^2, air pressure 25 lb/inch^2, nitrogen pressure 10 lb/inch^2, column length 1 metre. The packed column was conditioned at 190° under continuous nitrogen flow for four days.

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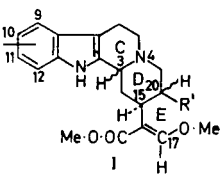
ALKALOIDAL SOLUTIONS

0·1% Solutions of the alkaloids in ethyl acetate were used and 3 μl injected until a constant retention time was obtained. The 0·1% solution was diluted to 0·05 and 0·025% and injections made to check whether retention times changed with dilution. The retention times of all the alkaloids were determined relative to ajmalicine which was maintained at constant retention time throughout the experiment by suitable adjustment of the nitrogen gas flow through the column.

Results and discussion

The retention times of the heteroyohimbine alkaloids possessing the closed ring E are shown in Table 1 and those of the open ring E in Table 2.

The alkaloids are of two main types: (I) heteroyohimbine alkaloid with open ring E (I) in which R=H or OMe and R'=Et or $-CH=CH_2$ and (2) heteroyohimbine alkaloids with closed ring E (II) in which R=H, mono or di-OMe groups.



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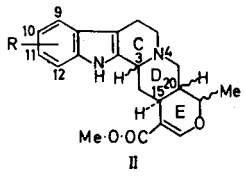

II

The alkaloids of type I have the same stereochemistry at C-15 and about the double bond; four diastereoisomers are then possible as follows (Trager, Lee & Beckett, 1967):

Configuration	C-3H	C-15H	C-20H
Normal	α	α	β
Pseudo	β	α	β
Allo	α	α	α
Epiallo	β	α	α

The closed ring E compounds have an extra asymmetric centre at C-19 and the methyl group is designated α or β respectively when below or above ring E.

Tables 1 and 2 show that the retention times of these alkaloids are influenced principally by (*a*) the introduction of methoxy substituents into the indole nucleus and (*b*) the overall geometry of the molecule, i.e., whether normal, pseudo, allo or epiallo. Differences in the location of the methoxy substituents have only a slight influence on retention times [Table 1: cf. compounds 9 with 8 (Rt 20·3, 19·7) and 2 with 3 (Rt 22·5, 22·7)]; this effect is also shown in simple indoles where retention times (at 105° on 1% SE-30, N_2 7 lb/inch^2, H_2 20 lb/inch^2, air 20 lb/inch^2; injection temp. 160°) are: indole 3·1, 4-OMe indole 9·3, 5-OMe indole 10·1 and 5-OHMe indole 10·3. Table 1 shows that one methoxy group approximately doubles the retention time of the compound with none (cf. 1 with 2, 6 with 7, 11 with 12, 15 with 16) whilst two methoxy groups

TABLE 1. RETENTION TIMES AND CONFIGURATIONS OF CLOSED RING E HETERO-*YOHIMBINE ALKALOIDS*

Alkaloid	Configuration[1] R		Configuration of C-19-Me	Retention time (min)	pKa[2]
1 Ajmalicine	Normal	H	α	10·5	6·31
2 Tetraphylline	Normal	11-OMe	α	22·5	6·39
3 Raumitorine	Normal	10-OMe	β	22·7	
4 Rauvanine	Normal	10,11-di-OMe	β	40·1	
5 Rauniticine	Allo	H	β	7·1	6·24
6 Tetrahydroalstonine	Allo	H	α	8·9	5·83
7 Raunitidine	Allo	11-OMe	β	16·3	6·20
8 Aricine	Allo	10-OMe	α	19·7	5·75
9 Reserpinine	Allo	11-OMe	α	20·3	6·01
10 Iso-reserpiline	Allo	10,11-di-OMe	α	34·0	6·07
11 Akuammigine	Epiallo	H	α	7·1	
12 Iso-reserpinine	Epiallo	11-OMe	α	15·6	6·49
13 Iso-raunitidine	Epiallo	11-OMe	β	17·9	6·42
14 Reserpiline	Epiallo	10,11-di-OMe	α	26·3	6·20
15 Iso-ajmalicine	Pseudo	H	α	5·3	
16 Mitrajavine	Pseudo	9-OMe	α	9·9	
17 Epirauvanine	Pseudo	10,11-di-OMe	β	23·6	

[1] Wenkert & others (1961a, b); Shamma & Moss (1961).
[2] Moss (1962).

TABLE 2. RETENTION TIMES AND CONFIGURATION OF OPEN RING E HETEROYOHIMBINE ALKALOIDS

Alkaloid	Configuration[1] R		R′	Retention time (min)	pKa[2]
18 Dihydrocorynantheine	Normal	H	-Et	9·9	7·47
19 Speciogynine	Normal	9-OMe	-Et	21·3	7·40
20 Paynantheine	Normal	9-OMe	$-CH=CH_2$	21·0	7·42
21 Corynantheidine	Allo	H	-Et	8·7	7·15
22 Mitragynine	Allo	9-OMe	-Et	16·7	7·06
23 3-Isocorynantheidine	Epiallo	H	-Et	4·2	7·45
24 Speciociliatine	Epiallo	9-OMe	-Et	16·3	7·44
25 Hirsutine	Pseudo	H	-Et	6·3	7·89
26 Mitraciliatine	Pseudo	9-OMe	-Et	12·1	7·95
27 Isopaynantheine	Pseudo	9-OMe	$-CH=CH_2$	12·0	

[1] Tamelin, Aldrich & Katz (1956); Wenkert & Bringi (1959); Joshi & others (1963); Bartlett, Sklar & others (1962); Shamma & Moss (1962); Weisbach, Kirkpatrick & others (1965); Lee & others (1967); Trager, Phillipson & Beckett (1968); Trager, Lee & others (1967).
[2] Beckett & Morton (1967).

increase it by a factor of 4 (cf. 1 with 4, 5 with 10, 11 with 14, 15 with 17).

Because of conformational changes (see later) the introduction of one methoxy group into the epiallo open ring E alkaloids has a greater effect than the other substitution. The change from open ring E to closed ring E compounds with an α-C-19-methyl group produces minor effects on retention time (Tables 1 and 2: cf. 18 with 1, 21 with 6, 25 with 15, 19 with 2) provided of course that major conformational changes are also not involved (see below). Replacement of the C-20 ethyl group by the C-20 vinyl group in the open ring E alkaloids has little effect on the retention time (Table 2: cf. 19 with 20, 26 with 27).

THE EFFECT OF CONFIGURATION ON RETENTION TIMES

From Tables 1 and 2 it can be seen that there is a progressive increase in retention time in the order pseudo < epiallo < allo < normal except

that the retention times of the epiallo open ring E compounds are apparently anomalous; the retention time of the non-methoxy compound (23) showing a fall compared with the pseudo compound (25) (Rt 4·2, 6·3) while that of the monomethoxy compound (24), instead of being less, is similar to the corresponding allo compound (22) (Rt 16·3, 16·7).

It has been established (Trager, Lee & Beckett, 1967), that in the open ring E alkaloids, the normal, pseudo and allo configurations exist almost exclusively in the conformations shown in Fig. 1. In non-polar solvents, the epiallo configuration exists as an equilibrium between DI and DIII conformations (see Fig. 1) with DI predominating.

In the closed ring E alkaloids with the C-19-α-methyl group, the normal, pseudo and allo configurations exist almost exclusively in conformations analagous to those of the open ring E alkaloids for rings A, B, C, D (see Fig. 2), whereas the epiallo configuration exists as an equilibrium between

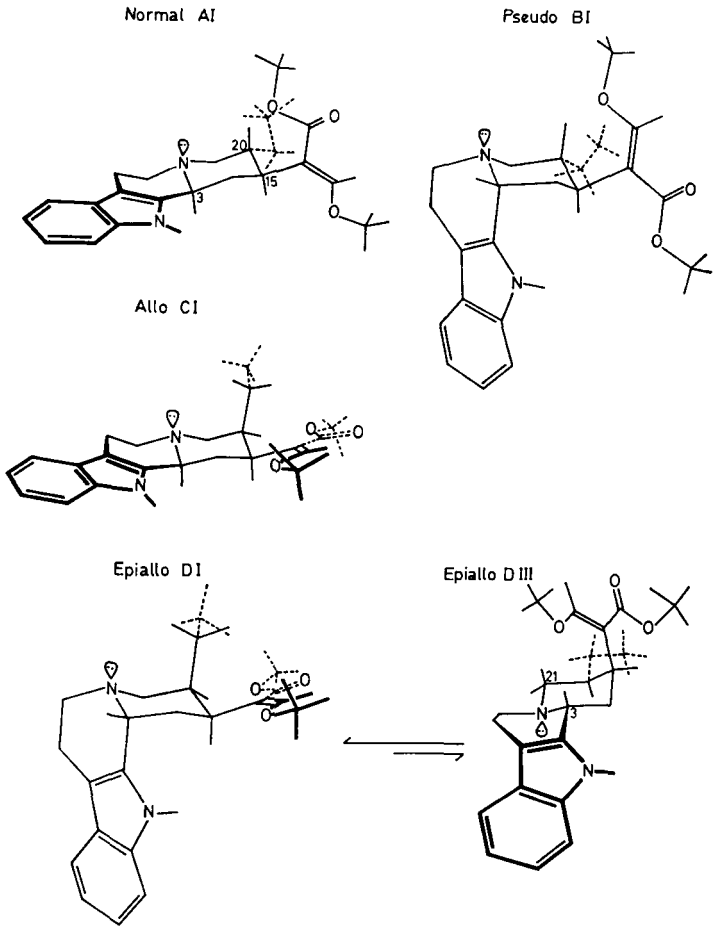

FIG. 1. The preferred conformations of open ring E alkaloids (Trager & others, 1967) AI, normal; BI, pseudo; CI, allo; DI and DIII epiallo.

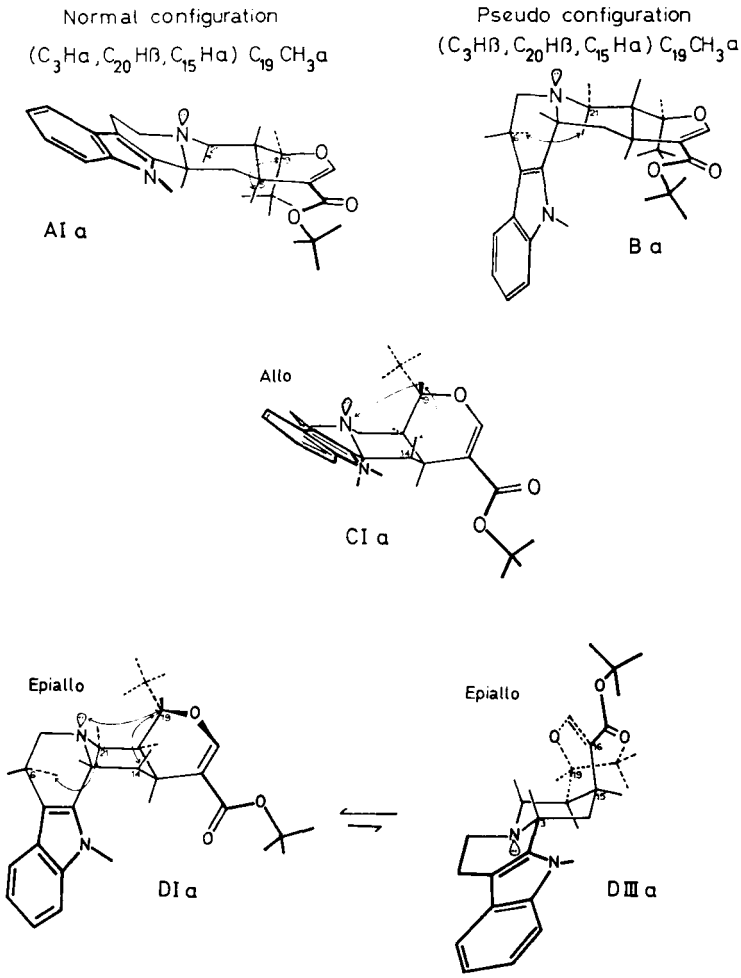

FIG. 2. The preferred conformations of closed E-ring $C_{19}-CH_3\alpha$ heteroyohimbine alkaloids (Trager, Lee & Beckett, unpublished observations) AIα, normal; Bα, pseudo; CIα, allo; DIα and DIIIα, epiallo.

DIα and DIIIα in roughly equal amounts. Models suggest that the change from C-19-α-methyl to C-19-β-methyl does not alter the preferred conformation of the normal and pseudo configurations (Fig. 3). Such a change in C-19 geometry, however, would be expected to cause the allo configuration to exist as an equilibrium mixture of about equal contributions from CIβ and CIIIβ (Fig. 3). This change in the C-19-methyl would also result in the epiallo equilibrium between DIβ and DIIIβ being displaced more in the direction of DIIIβ (Fig. 3).

INTERPRETATION OF RETENTION TIMES IN TERMS OF PHYSICO-ORGANIC CHARACTERISTICS

We first consider the gas–liquid partition characteristics of those

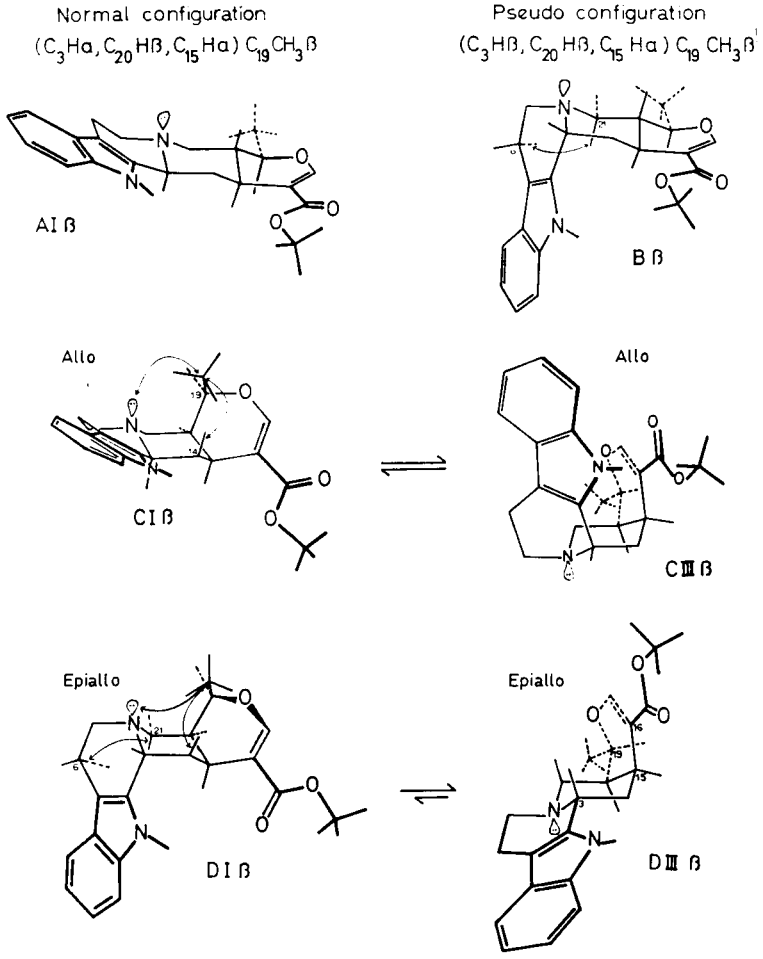

FIG. 3. The preferred conformations of closed ring E C-19-Meβ heteroyohimbine alkaloids (Trager, Lee & Beckett, unpublished observations). AIβ, normal; Bβ, pseudo; CIβ and CIIIβ allo; DIβ and DIIIβ, epiallo.

configurations which exist substantially as one conformer, i.e., normal, pseudo and allo configurations of open ring E alkaloids and closed ring E alkaloids with the C-19-α-methyl group.

Since the change from the open to the closed ring E makes only small changes in the retention times of these alkaloids (Tables 1 and 2: cf. 18 with 1, 25 with 15, 21 with 6), the ring E probably plays little part in the equilibrium between stationary phase and carrier gas despite the variety of conformations it adopts in these various alkaloids (see Figs 1 and 2).

On the other hand, methoxy groups in the indole nucleus displace the equilibrium in favour of the stationary phase in a consistent manner and play a major role. In the normal isomers, rings A, B, C and D are in

the same plane whilst in the pseudo isomers (see Figs 1 and 2) ring D is at right angles to the co-planar A, B and C. Such a change reduces the retention times significantly (Table 2: cf. 18 with 25, 19 with 26, 20 with 27; Table 1: cf. 1 with 15, 4 with 17), although this effect is not so great as that introduced by methoxy groups. In the normal configuration, the lone pair of the basic nitrogen atom is suitably orientated to reinforce the binding of the indole nucleus to a surface whereas its direction in the pseudo isomer (Figs 1 and 2) is in the plane of rings A, B and C rather than at right angles to this plane.

We suggest (*a*) that displacement of the equilibrium in favour of the SE-30 stationary phase is strongest in those isomers in which the binding of the indole nucleus to the stationary phase is reinforced by the optimally orientated lone pair of the basic nitrogen atom and (*b*) that introduction of the methoxy groups into the indole nucleus has an even greater effect than the optimally orientated lone pair. Changes in configuration and conformation, in so far as they may influence the closeness of approach to the stationary phase may then be used to interpret the results obtained for the other heteroyohimbine alkaloids.

In the open ring E allo alkaloids, the axial C-20-ethyl group (Fig. 1) constitutes more of a barrier to nitrogen lone-pair reinforcement than does the equatorial orientation in the corresponding normal alkaloids; retention times are thus reduced (Table 2: cf. 21 with 18, 22 with 19); the change from normal to allo geometry in the closed ring E alkaloids with the C-19-α-methyl group (Table 1: cf. 1 with 6, 2 with 9) has the same effect. This steric factor has less influence than the change in orientation of the lone pair relative to the plane of the indole nucleus. Thus allo compounds in the open ring E and C-19-α-methyl closed ring E series have shorter retention times than their corresponding normal isomers but longer than the corresponding pseudo compounds.

When more than one conformation is present in major amounts in the equilibrium mixtures for a particular configuration, as in the open ring E and closed ring E epiallo alkaloids and in the closed ring E allo alkaloids with C-19-β-methyl groups, changes in conformation may produce apparent anomalies to the above generalizations.

The retention time of the epiallo open ring E alkaloid containing a methoxy group (24) is longer than that of a corresponding pseudo alkaloid (26; Rt 16·3, 12·1) but shorter when this group is absent (cf. 23 with 25; Rt 4·2, 6·3). This may be explained by the substantial contribution of DIII in the methoxy epiallo configuration in which the nitrogen lone pair can reinforce the inherent binding of the methoxy substituted indole nucleus at a surface. In the absence of this strongly binding methoxy group, DI (Fig. 1), will be the preferred epiallo conformation; this conformation is similar to the pseudo BI conformation but possesses an axial C-20-ethyl rather than the equitorial one in BI. Despite the fact that in BI and DI the lone pair is not correctly orientated to substantially reinforce the indole nucleus binding, it will be expected that an axial ethyl will reduce binding more than the equitorial one.

Consideration of the conformation of normal ring E (Fig. 1, AI) and the

normal closed ring E alkaloids with C-19-methyl-α (Fig. 2, AIα) or β (Fig. 3, AIβ) indicates that corresponding alkaloids of these series should have the same retention times; results are in accord with this view (Tables 1 and 2; cf. 18 with 1, 19 with 2 and 3). Also, the closed ring E alkaloids with C-19-methyl α or β in the normal series should have similar retention times to those of the allo series with C-19-α-methyl since this group represents the same steric hindrance to the nitrogen lone pair, in the conformations AIα, AIβ and CIα (Figs 2 and 3); results are in accord with this deduction (Table 1: cf. 1 with 6, 2 with 3 and 9). Corresponding allo alkaloids with C-19-methyl-β, because of the conformational equilibrium between CIβ and CIIIβ (Fig 3), should have shorter retention times than the C-19-methyl-α isomers since in CIIIβ the nitrogen lone pair orientation cannot reinforce the binding of the indole nucleus; results support this conclusion (Table 1: cf. 5 with 6, 7 with 9). In the closed ring E epiallo configurations, the greater contribution of DIIIβ in the C-19-β-methyl isomer (13) with the nitrogen lone pair suitably orientated to the indole nucleus to reinforce binding (Fig. 3) should have a slightly longer retention time than the corresponding C-19-α-methyl isomer (12) in which DIIIα (Fig. 2) is less important in the equilibrium mixtures (Table 1: cf. 13 with 12). Also a consideration of the conformational contributions of CIβ and CIIIβ in the allo C-19-β-methyl configuration and the DIα and DIIIα conformation in the epiallo C-19-α-methyl configuration leads to the conclusion that allo-β and epiallo-α alkaloids should have similar retention times and this is borne out by results (Table 1: cf. 5 with 11, 7 with 12).

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