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The influence of stereochemistry on pK_a, rate of quaternization and partition coefficients of corynantheidine-type alkaloids

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In alkaloids of the corynantheidine group, the rate of quaternization at N-4 and the pK_a values give a measure of the degree of steric hindrance at this site due to the axial hydrogen at C-3 and the ethyl group at C-20. These hinder the availability of the lone pair of electrons on N-4 to electrophiles. Partition coefficients indicate that lipid solubility is associated with planarity of the molecules; this explains why the more planar isomers (*allo* and *normal*) are more highly metabolized in microsomes than the less planar isomers (*pseudo* and *epiallo*).

Stereochemical features in corynantheidine-type alkaloids have been shown to influence the rate of metabolism in liver microsomes (Beckett & Morton, 1967); with methoxy substitution in the indole nucleus they have been used to explain the behaviour of these alkaloids on thin layers and in gas-liquid chromatography (Phillipson & Shellard, 1967; Beckett & Dwuma-Badu, 1968). The stereospecificity of biological receptors is well known (Beckett, 1959) for compounds such as analgesics, antibiotics and alkaloids.

The stereochemistry of some corynantheidine-type alkaloids has now been re-examined in the light of their pK_a values, rates of quaternization, partition coefficients and metabolism by liver microsomes.

EXPERIMENTAL

Materials

Mitragynine and corynantheidine (Smith, Kline and French Laboratories, Philadelphia, U.S.A.); speciogynine and speciociliatine (Dr. J. D. Phillipson); mitraciliatine (Professor A. N. Tackie); hirsutine (Professor E. J. Shellard); dihydrocorynantheine (S. B. Penick and Co.); isocorynantheidine was prepared from corynantheidine (unpublished).

Apparatus

A Cambridge Conductance bridge with a conventional conductivity cell having black platinized electrodes with large surface area (about 2 cm^2) was used for the rate studies. The cell was maintained at 25° ± 0·5°.

For the partition coefficients, a special glass stoppered test tube (50 ml capacity) was used. A thermostatically controlled water bath adjusted to 37° ± 0·5° fitted with a shaking device, which shook the tubes gently (60–80 strokes/min) along the XY plane was employed to attain equilibrium conditions. The pH values of the aqueous solutions were measured using Dynacap pH meter.

Method

(1) The methyl iodide for the rate experiments was distilled and treated with mercury to remove iodine present as impurity. Methanol, spectral grade (BDH), was distilled twice. The alkaloid (5 mg; 1·25 m mol) was added to methanol (10 ml) and the conductivity measured until it remained constant; methyl iodide (0·5 ml) (about five hundred fold excess) was then added rapidly from a microburette, a stop clock being started simultaneously. The conductivity was measured at one minute intervals for 15 min and then at 5 min intervals for a further 75 min. Finally a reading was made after three or four days to give an "infinity" reading.

(2) For the partition experiment, the Sorensen phosphate buffer was freshly prepared and its pH adjusted to 7·4 with either of the components using a pH meter; samples (0·25, 0·5, 0·75 and 1 mg) of the alkaloids were placed in a 10 ml volumetric flask and heptane (spectroscopic grade), previously saturated with phosphate buffer pH 7·4 for 24 h, was added. These solutions were shaken vigorously on a mechanical shaker until the alkaloids dissolved. The ultraviolet spectrum of each solution was determined using buffer saturated heptane in the reference cell and a calibration curve constructed using the ultraviolet peak for each alkaloid (280–292 nm). Owing to the insolubility of these alkaloids in the aqueous buffer phase (heptane saturated) very small quantities 0·05, 0·075 and 0·1 mg were weighed, each was made to volume in a volumetric flask and shaken for 15 h at 37° to dissolve and the ultraviolet calibration curve made similarly in the buffer phase pH 7·4 as above.

The partition coefficients of the alkaloids were determined by adding 10 ml of a solution of 1 mg of alkaloid in 30 ml of heptane to 10 ml of buffer pH 7·4. The mixture was shaken at 37° for 24 h. The phases were separated and the concentration of alkaloid in each was determined. The final pH of the aqueous phase was checked with a pH meter. The initial concentration of the alkaloid in heptane was checked as described above using the ultraviolet calibration curve. The determination was repeated on a second 10 ml portion of the solution of the alkaloid in heptane.

RESULTS AND DISCUSSION

The pK_a and the total percentage metabolism in rabbit liver microsomes recorded in Tables 1 and 2 were obtained from Beckett & Morton (1967). Fig. 1 shows a graph of conductance against time for the quaternization experiments on corynantheidine-type alkaloids. The conductivity of the solution, G_t, reflects the concentration of the

Table 1. pK_a *and rate of quaternization of cornyantheidine-type alkaloids*

	Alkaloid	Configuration[2]	R (in I)	Rate $K \times 10^{-4}$	pK_a[1]
1.	Speciogynine	*Normal*	OMe	0·89	7·40
2.	Dihydrocorynantheine	*Normal*	H	1·06	7·47
3.	Mitraciliatine	*Pseudo*	OMe	3·40	7·95
4.	Hirsutine	*Pseudo*	H	4·70	7·89
5.	Mitragynine	*Allo*	OMe	0·14	7·06
6.	Corynantheidine	*Allo*	H	0·16	7·15
7.	Speciociliatine	*Epiallo*	OMe	0·82	7·44
8.	Isocorynantheidine	*Epiallo*	H	0·65	7·45

[1] Beckett & Morton (1967).

[2] Tamelin, Aldrich & Katz (1956); Wenkert & Bringi (1959; Joshi, Raymond-Hamet & Taylor, (1963); Bartlett, Sklar & others (1962); Weisbach, & others (1965); Lee & others (1967); Trager & others (1967; 1968).

Table 2. *Partition coefficients in heptane/buffer* pH 7·4 *and percentage of total metabolism in rabbit liver microsomes of corynantheidine-type alkaloids*

Alkaloid	Configuration[2]	R (in I)	Partition $\frac{C_h}{C_b}$ Coefficient K_p	Relative % metabolism[1] by microsomes
1. Speciogynine	*Normal*	9-OMe	14·5	89
2. Dihydrocorynantheine	*Normal*	H	6·9	15·8	41
3. Mitraciliatine	*Pseudo*	9-OMe	4·8	24·2	25
4. Hirsutine	*Pseudo*	H	2·7	12·4	21
5. Mitragynine	*Allo*	9-OMe	68·0	103·7	69
6. Corynantheidine	*Allo*	H	23·1	34·7	33
7. Speciociliatine	*Epiallo*	9-OMe	4·0	9·0	42
8. Isocorynantheidine	*Epiallo*	H	2·6	6·0	23

[1,2] See refs at foot of Table 1.

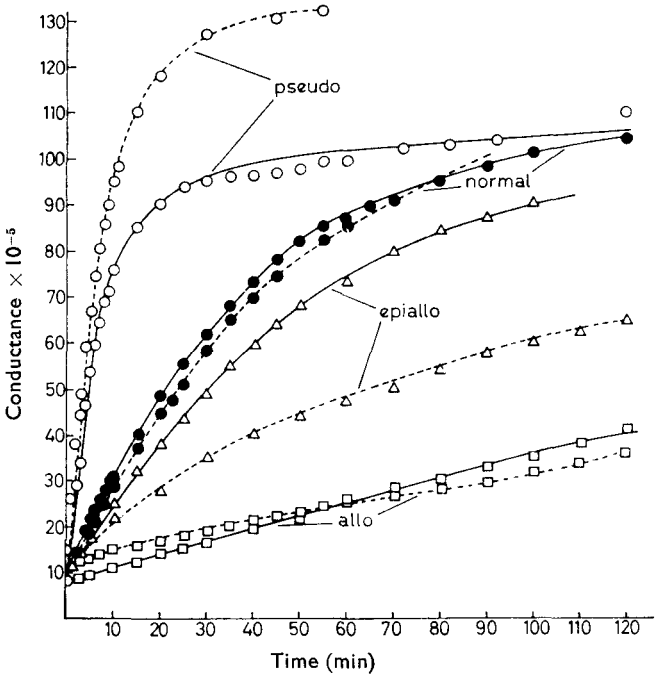


FIG. 1. The influence of stereochemistry on the rate of quaternization of corynantheidine-type alkaloids with methyl iodide. ○, *Pseudo.* ●, *Normal.* △, *Epiallo.* □, *Allo.* ———Methoxy alkaloids. - - - - - - Non-methoxy alkaloids.

methiodide at any time, t. Thus the value of $(G\infty - G_t)$, where $G\infty$ is the conductivity after complete quaternization, is a measure of the concentration of unquaternized alkaloid. The large excess of methyl iodide allows a first order treatment:

$$\ln (G_\infty - G_t) = \ln G\infty - kt \qquad (1)$$

where k is the pseudo first order rate constant. To permit comparison, the same excess of methyl iodide was used for each alkaloid, and the same quantity of alkaloid used in each case (Moss, 1962; Shamma & Moss, 1962). The *normal, allo* and *epiallo* alkaloids gave a linear plot of $\ln (G_\infty - G_t)$ versus t, but for the *pseudo* compounds the plot began to curve after some 7 min; the rate constants, k, are calculated therefore for the first 7 min. only.

For the calculation of partition coefficient values shown in Table 2, equation (2) was used:

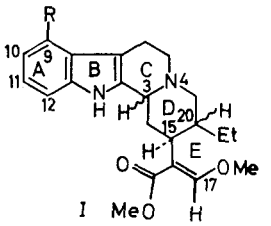
$$Kp = C_h/C_b \ (1 - \alpha) \qquad (2)$$

Where kp is the true partition coefficient, C_h is the total concentration in the organic (heptane) phase, C_b is the total concentration in the buffer phase and α is the degree of dissociation of the alkaloid. For bases, the correction factor $1/(1 - \alpha)$ is given by:

$$1/(1 - \alpha) = 1 + \text{antilog } (pK_a - pH)$$

where the pH value is that of the buffer phase after equilibrium.

The corynantheidine-type alkaloids have the basic structure (I) in which R = H or OMe. There are three asymmetric centres C-3, C-15 and C-20, the stereochemistry at C-15 and about the double bond is the same in all the isomers (Wenkert & Bringi,



R = H or O Me

1959; Weisbach, Kirkpatrick & others, 1956; Trager, Lee & Beckett, 1967) therefore four diastereoisomers are possible as follows:

Configuration	C-3H	C-15H	C-20H
normal	α	α	β
pseudo	β	α	β
allo	α	α	α
epiallo	β	α	α

Conformational analysis supported by physico-chemical data has shown that the *normal* and *allo* compounds exist to the extent of at least 95% in conformations AI and CI in which rings A, B, C and D are coplanar but with a C-20 ethyl group equatorial in the former compound and axial in the latter (Fig, 2). The *pseudo* isomer exists at least 95% in conformation BI in which ring D is at right angles to the coplanar rings A, B, and C with the C-20 ethyl group equatorial; on the other hand the *epiallo* isomer exists in an equilibrium between the non-planar conformation (DI), with a C-20 ethyl group axial, and the approximately planar DIII, with an equatorial C-20 group: conformer DI predominates (Trager, Lee & Beckett, 1967).

pKa and rate of quaternization

Table 1 shows that there are no significant differences in the pK_a values of the non-methoxylated and the methoxylated isomers of the same configuration. Although there are slight differences in the rates of quaternization (Table 1, cf cpd 1 with 2; 3 with 4; 5 with 6; 7 with 8); these are not as marked as the differences in the rate and pK_a values due to changes in configuration.

The conductivity curves (Fig. 1) and the pK_a values (Table 1) indicate that the *pseudo* and *normal* isomers, in that order, are the strongest bases and quaternize more quickly than the other compounds examined. The rate of quaternization is sensitive

to steric hindrance (Brown & Eldred, 1949) and two aspects deserve comment. In the preferred conformation of both the *pseudo* (BI) and *normal* (AI) isomers (Fig. 2) the bulky C-20 ethyl group is equatorial whereas in the other compounds, *allo* (CI) and *epiallo* (DI), it is axial and thus creates hindrance to attack by an electrophilic reagent at N-4. In addition, the *normal* series (AI) has the configuration at C-3 of a *trans*-quinolizidine and is more sterically hindered at N-4 than is the *pseudo* isomer (BI) which corresponds to a *cis*-quinolizidine.

The relationship between the *allo* (CI) and *epiallo* (DI) (see later) isomers are of the same kind as those between the *normal* (AI) and *pseudo* (BI) respectively (Fig. 2) and this provides an explanation of the more ready quaternization of the *epiallo* isomers compared to the *allo* isomers. Methoxy substitution in the *epiallo* series produces a basic strength and quaternization rate (Table 1 cf. cpd 7 with cpd 1) almost equivalent to that of *normal*, the compound with the C-20 equatorial ethyl group. This is probably due to the significant contribution of conformation DIII (*trans*-quinolizidine) in the *epiallo* configuration in which the C-20 ethyl group is now equatorial.

Thus the pK_a and the rate of quaternization are in accord with the conformations suggested for the corynantheidine type alkaloids by Trager, Lee & Beckett (1967).

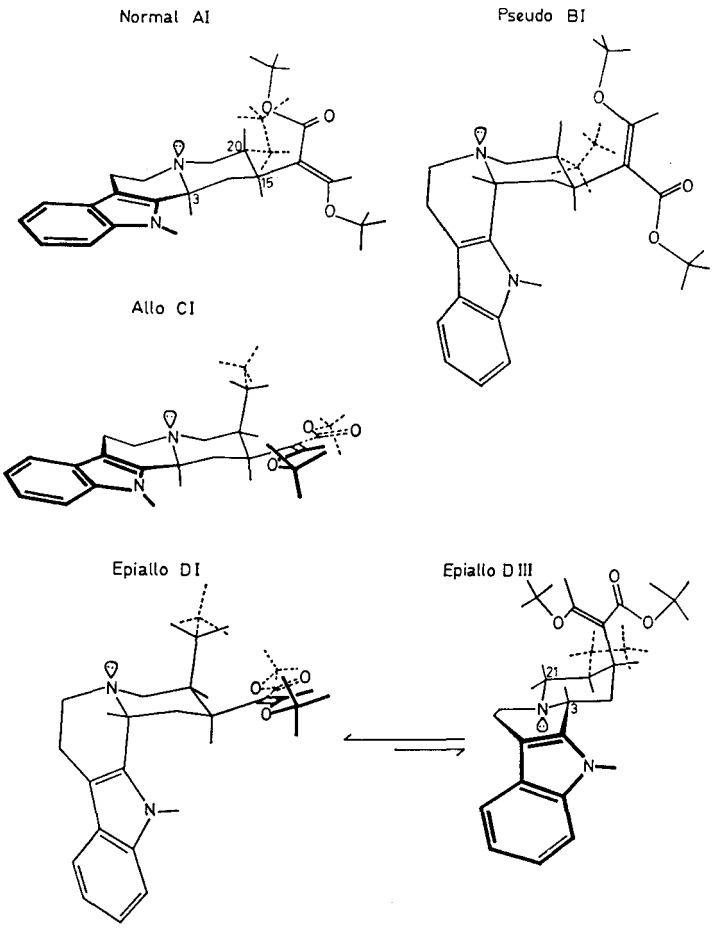


FIG. 2. The preferred conformations of Corynantheidine-type alkaloids (open E-ring); (Trager & others, 1967) AI, *normal*; BI, *pseudo*; CI, *allo*; DI and DIII *epiallo*.

Partition coefficients and metabolism in liver microsomes

It has been suggested by Mayer, Maickel & Brodie (1959) that the rates of entry of drugs into the cerebrospinal fluid (CSF) from plasma may be predicted from their partitition coefficients between heptane, benzene or chloroform and water at pH 7·4. These authors showed that drugs enter the CSF at rates which depend upon the lipid solubility of the unionized molecules and that rapid penetration is assured provided that there is a sufficient proportion of this form in the plasma. That lipid solubility is the physical property governing the passage of uncharged molecules across membrane barriers is supported by the work of Brodie, Kurz & Schanker (1960), Schanker (1959) and Kakemi, Arita & others (1967). It is also known that partition coefficients of molecules influence the rate of metabolism by liver microsomes (Ahmed, 1958; McMahon, 1961).

Partition coefficients for the corynantheidine-type alkaloids in the heptane–buffer pH 7·4 system and the metabolism by rabbit liver microsome of the isomers under identical conditions are shown in Table 2.

Each planar *normal* and *allo* isomer has a higher partition coefficient than its corresponding non-planar *pseudo* and *epiallo* isomer (Table 2 cf. cpd 1, 2, 5 and 6 with 3, 4, 7 and 8). Methoxy substitution increases the partition coefficients in all the isomers (Table 2 cf. cpd 1 with 2, 3 with 4, 5 with 6, 7 with 8). The change in the C-20 ethyl group from the *axial* to the equatorial position in the series *normal* and *allo* increases partition coefficient whereas the same change in the *pseudo* and *epiallo* compounds decreases it. This is probably because of the conformational equilibrium between DI and DIII in the *epiallo* compounds.

Each planar isomer is metabolized to a greater extent than its corresponding non-planar one whilst methoxy isomers are metabolized more than their non-methoxy analogues. This would be expected since the higher lipid solubility of the planar isomers favours metabolism. However, metabolism is also influenced by the affinity and activity of a molecule at a metabolic site as well as by its availability. Thus the change from a C-20 equatorial ethyl to a C-20 axial group in the planar compounds (Fig. 1, cf. AI with CI) reduces metabolism (Table 2, cf. 1, 2 with 5 and 6) despite the increase in partition coefficients; this is explicable in terms of the axial ethyl group constituting a steric hindrance to the reinforcement by the basic nitrogen lone pair to the binding of the flat indole nucleus to the metabolic site. The increase in retention times in gas-liquid chromatography, upon making this change, has been similarly explained (Beckett & Dwuma-Badu, 1968).

The change from the planar *normal* AI to the *pseudo* BI compounds, in which ring D is roughly at right angles to the rings A, B and C (Fig. 1), reduces not only the partition coefficients and the total metabolism but also produces a change in the route of metabolism (Beckett & Morton, 1967), thus emphasizing the importance of the site binding as well as the site availability of the molecules.

In contrast to the *pseudo* isomers (Table 2 cf. cpd 7 and 8 with 3 and 4), the *epiallo* isomers are not only metabolized by enol *O*-demethylation as are the planar *normal* and *allo* compounds (Table 2, cf. 1 and 5 with 7), but they are also metabolized to an extent greater than would be expected from their partition coefficients. This is in accord with the planar *epiallo* conformation DIII influencing both the route and amount of metabolism, a situation which exists only in this configuration since it has been established by Trager & others (1967) that the *normal*, *allo* and *pseudo* configurations exist to the extent of 95% in the conformations AI, CI and BI (Fig. 2) respectively.

The methoxy isomers have partition coefficients at least double those of their non-methoxy analogues in both planar (*normal* and *allo*) and non-planar *pseudo* isomers but with the metabolism results for the planar isomers, the methoxy group doubles the metabolism over their non-methoxy analogues while for the *pseudo* analogues only a small increase in the metabolism occurs. This is consistent with the change in metabolic route which results on the change from planar to non-planar isomers.

Only one of the isomers studied in Table 1 is active as an analgesic and antitussive agent; this is mitragynine (Table 1, *allo*) its activity could arise from its planarity which helps it to bind to a surface, from its pK_a (7·06) which ensures sufficient concentration in the plasma and from its higher lipid solubility allowing easier penetration to the CNS. The planar non-methoxylated alkaloid of *allo* configuration (Table 2 compound 6) may be inactive not only because of its less favourable partition characteristics but because of its reduced electron availability in the indole nucleus.

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