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SHORT COMMUNICATION

The *in vitro* metabolism of mitragyna alkaloids of corynantheidine structure

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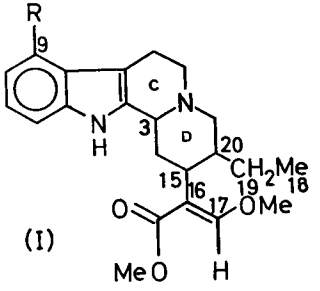
CORYNANTHEIDINE has the structure (I) and, apart from *cis-trans* geometry about the C(16)–(17) double bond, four diastereoisomeric pairs of enantiomorphs are possible.



(I)

Alkaloid (I; R=H)	Configuration	C_3H	$C_{20}H$	$C_{15}H$
Corynantheidine	allo	α	α	α
Dihydrocorynantheine	normal	α	β	α
Isocorynantheidine	epiallo	β	α	α
Hirsutine	pseudo	β	β	α

Isocorynantheidine (epiallo) was obtained by C_3H isomerisation of corynantheidine (unpublished observations), and hirsutine was extracted from *M. hirsuta* (Shellard, Beckett, Tantivatana, Phillipson and Lee, 1966); all compounds have $COOMe/OCH_3$ in the *trans* configuration (Lee, Trager & Beckett, 1966).

EXPERIMENTAL

Incubations of these four compounds with rabbit liver microsome preparations were carried out as previously described for the metabolism of oxindole compounds (Beckett & Morton, 1966). Formaldehyde production during the incubations was determined by the method of Cochin & Axelrod, (1959) and the metabolism of the alkaloids by all routes (expressed as a percentage of total alkaloid added to the mixtures) was determined by a method similar to that described by Kato, Chiesara & Vassanelli (1962). Thin-layer chromatography and the determination of partition coefficients were carried out as previously described for oxindole compounds (Beckett & Morton, 1966) and the pK'_a values were determined in water by a micro-technique (Jolliffe & Ahmad, unpublished).

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RESULTS AND DISCUSSION

The *in vitro* *O*-demethylation (and metabolism by all routes) of the corynantheidine-type alkaloids was influenced significantly by the stereochemistry of the alkaloids (see Table 1).

TABLE 1. ABSOLUTE CONFIGURATION, pK_a' VALUES AND PARTITION COEFFICIENTS, AND FORMALDEHYDE PRODUCTION AND PERCENTAGE METABOLISM OF CORYNANTHEIDINE-TYPE ALKALOIDS AFTER INCUBATION WITH RABBIT LIVER MICROSOME PREPARATIONS

Alkaloid	Formaldehyde production per hr per gram of liver (μ moles)*	Metabolism by *O*-demethylation (calculated from formaldehyde production) %	Metabolism by all routes†	Partition coefficient Heptane/phosphate buffer pH 7·6	pK_a'‡
Corynantheidine (allo)	0·99	26·0	32·6	332	7·15 ± 0·07
Dihydrocorynantheine (normal)	1·34	35·3	41·5	265	7·47 ± 0·07
Isocorynantheidine (epiallo)	0·58	15·3	23·9	199	7·45 ± 0·04
Hirsutine (pseudo)	0·04	1·1	21·4	249	7·89 ± 0·04

* 3·8μ moles of each alkaloid were added per gram of liver. The data presented represent the average from three animals, all results of which were within ± 10% of the recorded values.

† The metabolism by all routes was derived by difference from the concentration of unmetabolised alkaloid determined by a method similar to that of Kato & others (1962).

‡ The pK_a' values were determined in aqueous solution at 37° (±0·5°).

The results show that the figures for the metabolism by *O*-demethylation, as calculated from formaldehyde produced during the incubation of corynantheidine, isocorynantheidine and dihydrocorynantheine, were not dissimilar from the figures of the metabolism by all routes obtained by determination of unchanged alkaloid after incubation, indicating that *O*-demethylation of these alkaloids was the major metabolic reaction during incubations with rabbit liver microsome preparations. This was confirmed by thin-layer chromatography of n-butanol extracts from incubation mixtures of these alkaloids, because each alkaloid only gave one spot other than the parent alkaloid spot (Table 2). These spots of metabolites gave positive colours with Dragendorff's reagent but negative results with reagents which give colours with phenols. The production of formaldehyde in

TABLE 2. THIN-LAYER CHROMATOGRAPHIC RF VALUES OF CORYNANTHEIDINE-TYPE ALKALOIDS AND THEIR METABOLITES AFTER INCUBATION WITH RABBIT LIVER MICROSOME PREPARATIONS

Alkaloid	Rf values: solvent system*		
	I	II	III
Corynantheidine	0·90	0·74	0·83
Corynantheidine metabolite	0·73	0·51	0·57
O-Desmethylcorynantheidine	0·72	0·51	0·56
Dihydrocorynantheine	0·91	0·76	0·85
Dihydrocorynantheine metabolite	0·78	0·54	0·60
Isocorynantheidine	0·69	0·46	0·67
Isocorynantheidine metabolite	0·50	0·32	0·52
Hirsutine	0·56	0·31	0·51
Hirsutine metabolite	0·35	0·19	0·36

* I. Chloroform-ethanol (9:1). II. Benzene-ethyl acetate (2:3). III. Chloroform-acetone (3:4). Silica gel 'G' (Merck) was used. Chromatogram thickness 250 μ.

these incubations can arise only from oxidative demethylation of the enol-ether on C(17) (see I). Ring hydroxylation is contra-indicated because of the failure of the metabolites to give a colour with phenolic reagents. The *O*-demethylation to produce an OH on C(17) as the major metabolic reaction, under the above conditions, is further confirmed for corynantheidine because the corynantheidine metabolite had properties identical with an authentic sample of *O*-desmethyl corynantheidine (Weisbach, & others 1965) on thin-layer chromatograms in three solvent systems.

However, for the pseudo isomer, the 20-fold difference between the percentage metabolism calculated from formaldehyde production and the metabolism by all routes calculated from unchanged alkaloid remaining after incubation, indicated that a different metabolic pathway was involved for this alkaloid. Again one major route was indicated because only one spot other than the parent alkaloid was present on thin-layer chromatograms of n-butanol extracts from the incubation mixtures.

Although the allo, normal and epiallo alkaloids are metabolised by the same route, the formaldehyde production from the epiallo compound indicates that it is only metabolised to about half the extent of the other two alkaloids. There was no direct correlation between heptane-water partition coefficients and pK_a' values (Table 1) and the observed metabolic rates and routes of the alkaloids.

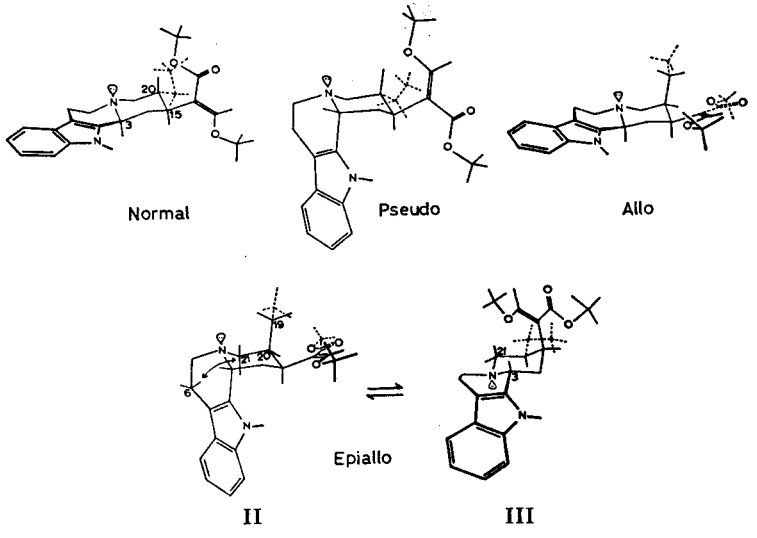

FIG. 1. Preferred conformations of normal, allo and pseudo configurations of corynantheidine-type alkaloids and conformational interchange between the two most important conformations of the epiallo configuration of corynantheidine-type alkaloids.

There was, however, some correlation between metabolism and the conformation of these compounds. It has been shown (Lee, Trager & Beckett, 1966) that the normal, pseudo and allo configurations exist at least to the extent of 95% in the conformations shown in Fig. 1, i.e. in the

normal and allo configurations, the indole nucleus is in the general plane of the piperidine ring (D) and in the pseudo configuration it is approximately at right angles to it. Thus, for the planar configurations, *O*-demethylation is the main metabolic route, whereas when the indole nucleus is approximately at right angles to the general plane of the piperidine ring, *O*-demethylation is prevented and another metabolic pathway adopted.

Analysis has shown that in the epiallo configuration in $CDCl_3$, approximately 75% exists in conformation II and 25% in conformation III (Fig. 1). Under aqueous conditions at pH 7·6 the alkaloid will be about 50% ionised. Ionisation and solvation of the protonated nitrogen would increase the steric size of this centre and tend to displace the conformation equilibrium slightly in the direction of III. Thus the epiallo configurations will probably exist in both planar III and non-planar II conformations in roughly equal amounts under the metabolic conditions. The reduced *O*-demethylation of this isomer (epiallo), as compared with those isomers (normal and allo) which exist almost entirely in planar conformation, may thus be explained.

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