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THE METABOLISM OF CORYNANTHEIDINE AND 9-METHOXYCORYNANTHEIDINE-TYPE ALKALOIDS BY LIVER MICROSOMES

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Abstract—The qualitative and quantitative metabolism by liver microsome preparations of corynantheidine-type alkaloids, with and without aromatic ring substitution, is influenced significantly by the stereochemistry of the alkaloids. Normal, allo and epiallo isomers are metabolized principally by *O*-demethylation, whereas the alkaloids possessing the pseudo configuration are metabolised by a different process to yield unidentified products. *O*-Dealkylation of the enol ether group occurs more readily in planar than in non-planar isomers, and increased metabolism is observed with alkaloids possessing aromatic methoxy substitution.

ALTHOUGH increasing interest is being shown in the pharmacological properties of indole alkaloids, few studies have been carried out on the biotransformation of these compounds.

The metabolism of reserpine[1, 2], strychnine[3, 4] and more recently the *Vinca* alkaloids[5] has been investigated, but no previous studies on the metabolism of corynantheidine-type indole alkaloids have been reported. The present studies were carried out† to determine the effects of the stereochemistry of the molecules on the qualitative and quantitative metabolism of these alkaloids by liver microsome preparations, as variations in pharmacological activity have been observed within this series of compounds, i.e. mitragynine possesses analgesic and anti-inflamatory properties, but speciogynine, its stereoisomer, is much less active (private communication). These differences may be due to variations in the rate of biotransformation of these alkaloids in the test animals.

METHODS

Incubations of the corynantheidine-type alkaloids with rat, guinea-pig and rabbit liver microsome preparations were carried out as previously described for the metabolism of oxindole derivatives.[7] Formaldehyde production during the incubation was determined by the method of Cochin and Axelrod (1959)[8], and the percentage overall metabolism (expressed as a percentage of total alkaloid added to the incubation mixtures) was determined by a method similar to that of Kato, Chiesara and Vassanelli (1962).[9] Thin-layer chromatography and the determination of partition coefficients

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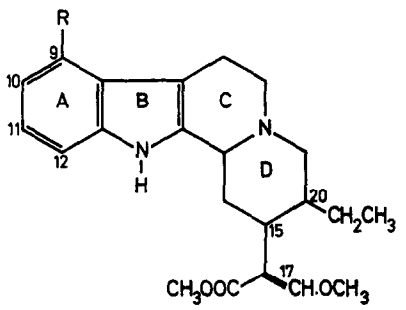
† A summary of some of these studies has been presented elsewhere.[6]

were carried out as previously described.[10] The p*Ka* values were determined in water at 37° (± 0·5°) by a micro-technique (Jolliffe and Ahmad, unpublished).

RESULTS

Under the described incubation conditions, the coryantheidine-type indole alkaloids (I), possessing the normal, allo and epiallo configurations were metabolised by rabbit



(I) R = H or OCH_3

liver microsome preparations, but not by those of rat or guinea-pig. The alkaloids possessing the pseudo configuration were metabolised by the liver microsomes of all three species.

O-demethylation of the enol ether group, i.e. at C_{17}, as calculated from the formaldehyde produced, was found to be the major metabolic reaction when allo, normal and epiallo isomers were incubated with rabbit liver microsome preparations, but another unidentified reaction occurred with the pseudo isomers (Table 1). These results were

TABLE 1. FORMALDEHYDE PRODUCTION AND TOTAL OVERALL METABOLISM OF CORYNANTHEIDINE-TYPE ALKALOIDS OF KNOWN CONFIGURATION AFTER INCUBATION WITH RABBIT LIVER MICROSOME PREPARATIONS

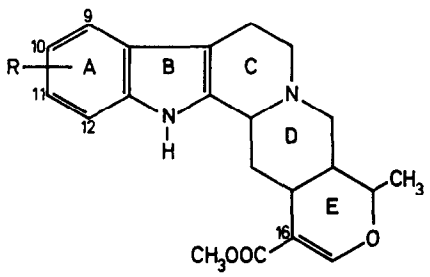
Alkaloid	R (I)	Configuration	Formaldehyde produced in 1 hr/g liver (μmole)*	Percentage metabolism by *O*-demethylation (calculated from formaldehyde production)	Total percentage overall metabolism
Speciogynine	OCH_3	normal	3·42	90·0	88·6
Mitraciliatine	OCH_3	pseudo	0·05	1·3	25·0
Mitragynine	OCH_3	allo	2·60	65·8	68·9
Speciociliatine	OCH_3	epiallo	1·44	37·9	41·9
Paynantheine (C_{20} Vinyl)	OCH_3	normal	3·01	79·2	77·8
Dihydrocorynantheine	H	normal	1·34	35·3	40·5
Hirsutine	H	pseudo	0·04	1·1	21·4
Corynantheidine	H	allo	0·99	26·0	32·6
iso-3-Corynantheidine	H	epiallo	0·58	15·3	22·9

* 3·8 μmole of each alkaloid were added per g liver.

The data presented, represent averages of results for three animals, all results being within ±10 per cent of the recorded values.

confirmed by thin-layer chromatographic examination of *n*-butanol extracts of the incubation medium, in which one metabolite giving a positive colour reaction with Dragendorff's reagent and negative results with reagents for detecting phenols, was formed from each alkaloid (Table 3). These metabolites possessed an u.v. absorption spectrum identical to that of the parent alkaloid and did not exhibit bathochromic shifts in the presence of sodium hydroxide. The standard reference enol *O*-demethylated metabolites have not been synthesised, except in the case of corynantheidine, and thus direct confirmation of the structure of the metabolites by comparison of physico-chemical properties has not been possible. The metabolite of corynantheidine was shown to be identical in R_f value, chemical colour reactions and ultraviolet absorption spectrum with *O*-desmethylcorynantheidine.[6]

Negligible formaldehyde production was observed during the incubation of some closed E-ring indole alkaloids of similar structure (II) with rabbit liver microsome



(II) R = H or OCH_3

preparations, irrespective of configuration and position of methoxyl substitution in the aromatic ring (Table 2).

TABLE 2. FORMALDEHYDE PRODUCTION AND TOTAL OVERALL METABOLISM OF CLOSED E-RING INDOLE ALKALOIDS OF KNOWN CONFIGURATION AFTER INCUBATION WITH RABBIT LIVER MICROSOME PREPARATIONS

Alkaloid	R (II)	Configuration	Formaldehyde produced in 1 hr/g liver (μmole)*	Percentage metabolism by *O*-demethylation (calculated from formaldehyde production)	Total percentage overall metabolism
Javacillin	OCH_3 (9)	pseudo	0·04	1·1	26·1
Tetraphylline	OCH_3 (10)	normal	0·04	1·1	69·5
Aricine	OCH_3 (10)	allo	0·02	0·6	57·4
Reserpinine	OCH_3 (11)	allo	0·04	1·1	62·0
Ajmalicine	H	normal	0·06	1·5	33·3
Tetrahydroalstonine	H	allo	0·04	1·1	65·8

* 3·8 μmole of each compound were added per g liver.

The data presented, represent averages of results for three animals, all results being within ±10 per cent of the recorded values.

Partition coefficent and p*Ka* values for the corynantheidine and 9-methoxycorynantheidine stereoisomers are recorded in Table 4.

DISCUSSION

As had been previously observed with methoxyoxindoles,[10] *O*-demethylation reactions occurred when corynantheidine-type alkaloids were incubated with liver

TABLE 3. THIN-LAYER CHROMATOGRAPHIC R_f VALUES OF METHOXYCORYNANTHEIDINE-TYPE ALKALOIDS AND THEIR METABOLITES AFTER INCUBATION WITH RABBIT LIVER MICROSOME PREPARATIONS

Alkaloid†	R_f values: Solvent system* (I)	(II)
Speciogynine	0·74	0·92
Speciogynine metabolite	0·50	0·83
Mitraciliatine	0·38	0·47
Mitracilatine metabolite	0·21	0·29
Mitragynine	0·71	0·91
Mitragynine metabolite	0·47	0·80
Speciociliatine	0·52	0·68
Speciociliatine metabolite	0·37	0·45

* (I) Chloroform–ethanol (19:1).
(II) Benzene–ethylacetate (2:3).

† The thin-layer chromatographic R_f values of the corynantheidine-type alkaloids and their metabolites have been previously presented.[6]

TABLE 4. PARTITION COEFFICIENT AND p*Ka* VALUES FOR CORYNANTHEIDINE-TYPE ALKALOID

Alkaloid	Configuration	Partition coefficient Heptane/phosphate buffer pH 7·6	p*Ka**
Speciogynine	normal	199	7·40 ± 0·03
Mitraciliatine	pseudo	225	7·95 ± 0·03
Mitragynine	allo	249	7·06 ± 0·03
Speciociliatine	epiallo	249	7·44 ± 0·04
Paynantheine	normal	265	7·42 ± 0·04
Dihydrocorynantheine	normal	265	7·47 ± 0·07
Hirsutine	pseudo	249	7·89 ± 0·05
Corynantheidine	allo	332	7·15 ± 0·07
Iso-3-Corynantheidine	epiallo	199	7·45 ± 0·04

* The p*Ka* values were determined in aqueous solution at 37° (±0·5°).

microsome preparations from rabbits, but not when incubated with those of rats or guinea-pigs. Metabolism of the normal, allo and epiallo isomers thus occurred with rabbit liver microsomes but not with those of rats or guinea-pigs whereas the pseudo isomers, which were metabolised by another as yet unidentified reaction, were metabolised by liver preparations of all three species.

Ring hydroxylation or *O*-demethylation of the aromatic methoxy group in the 9-methoxycorynantheidine isomers were contraindicted because of the failure of the metabolites to give a positive reaction with phenol detecting reagents, together with the absence of a bathochromic shift in the u.v. absorption spectra in the presence of sodium hydroxide.

O-demethylation was shown to be a minor pathway in the metabolism of the closed E-ring alkaloids, i.e. when the enol ether groups were not available for enzymic *O*-demethylation, negligible *O*-demethylation of the aromatic methoxy groups occurred.

The metabolites formed after incubation of the pseudo isomers with rabbit, rat or guinea-pig microsome preparations were not formed by *O*-demethylation reactions or by hydrolysis of the ester group at C_{16}, since the metabolites were not acidic and did not react to form a yellow complex (black under ultra-violet light) with acridine or a pink colour with 2,6-dichlorophenolindophenol. Their structures have not yet been elucidated.

Conformational analyses supported by physico-organic data[11] have shown that the normal, pseudo and allo configurations exist at least to the extent of 95 per cent in the conformations shown in Fig. 1, i.e. in the normal and allo configurations the

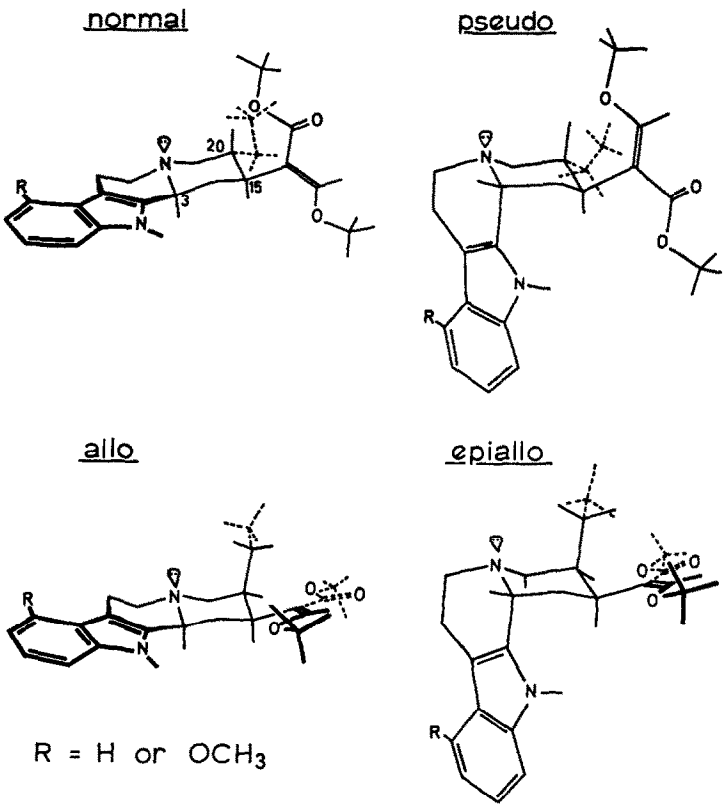


FIG. 1. Preferred conformations of corynantheidine-type alkaloids.

indole nucleus is in the general plane of the piperidine ring, but in the pseudo configuration it is approximately at right angles to it. Conformational analysis and NMR spectra in $CDCl_3$ indicate that, in the epiallo configuration, about 75 per cent exists in conformation DI and 25 per cent in conformation DIII (Fig. 2). Under

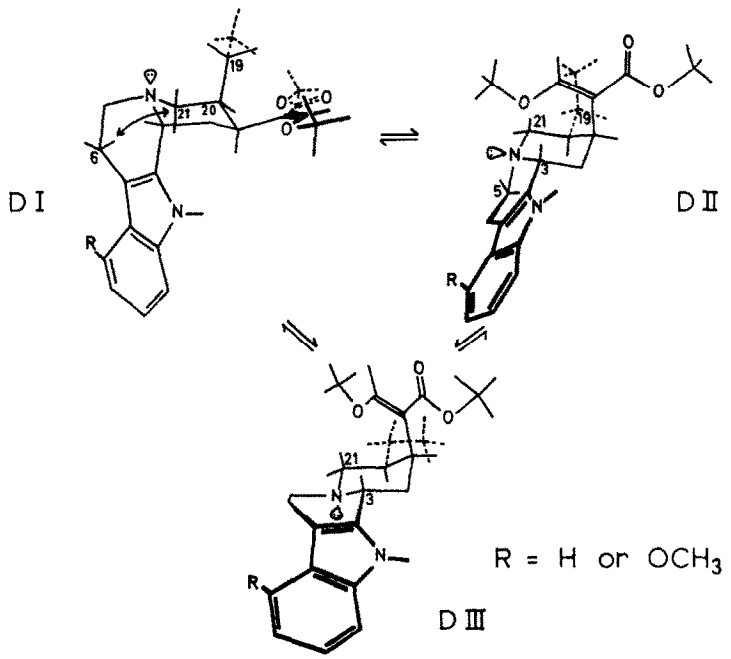


FIG. 2. Possible conformations of the epiallo isomers of corynantheidine-type alkaloids. (Approx. 75% DI and 25% D III in $CDCl_3$ solution.)

aqueous conditions at pH 7·6, these epiallo alkaloids will be about 50 per cent ionized. Ionization and solvation of the protonated nitrogen atom would increase the steric size of this centre and tend to displace the conformation equilibrium slightly in the direction of DIII. Corresponding conformational changes under aqueous conditions would not be expected in the normal, allo and pseudo conformations. Thus, the enol *O*-demethylation of the epiallo compounds, being intermediate between the negligible *O*-demethylation of the non-planar pseudo compounds and the figures obtained for the planar normal and allo compounds, are explicable in terms of the conformational equilibrium between the planar DIII and the non-planar DI in the epiallo configuration.

The increased *O*-demethylation observed with the alkaloids possessing the normal configuration, when compared with those of the allo configuration, may be due to the enol ether group being perpendicular to the plane of the indole nucleus in the normal configuration, but being parallel to the indole rings and therefore orientated further from the indole moeity in the allo configuration. The differences in *O*-demethylation between the corynantheidine-type isomers could not be explained by differences in the partition coefficients or p*Ka* values of the alkaloids (Table 4).

The corynantheidine-type alkaloids may be bound to the 'enzyme protein surfaces' by the flat phenyl ring of the indole nucleus, an association involving the interaction

of π electron orbitals. The increased total metabolism and *O*-demethylation of the methoxycorynantheidine-type alkaloids, when compared to that of the unsubstituted alkaloids, may be explained by the mesomeric effect of the methoxyl group on π orbitals of the indole nucleus, increasing the binding of the alkaloid to the protein surface.

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