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THE METABOLISM OF *N*-ALKYLOXINDOLES*

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Abstract—Dealkylation of *N*-alkyloxindoles after incubation with rat, rabbit and guinea-pig liver microsome preparations is described. The *N*-methyl group is dealkylated more readily than the *N*-ethyl or *N*-propyl groups.

Hydroxylation in the 5-position of the oxindole nucleus, as shown by physico-chemical comparison of the phenolic metabolites with standard synthetic hydroxyalkyloxindoles, and more overall metabolism occurred with *N*-propyloxindole, the compound with highest lipid solubility, than with *N*-methyl or *N*-ethyl-oxindole. The presence of an ethyl substituent in the 3-position of the oxindole nucleus increased the dealkylation of *N*-methyl-oxindole.

EARLY studies[1, 2] on microsomal dealkylation of compounds, demonstrated that oxidative *N*-dealkylation of amines is catalysed by the microsomal fraction from mammalian liver preparations and requires molecular oxygen and reduced nicotinamide adenine dinucleotide. Similar requirements have been reported for *in vitro* demethylation of amides such as *N*-methylcarbamates,[3] and more recently for demethylation of *N*-methylbarbiturates and *N'*-methylsulphanylureas.[4, 5]

It has been previously suggested[6] that microsomal enzymes are protected by a lipoidal barrier which is penetrated only by lipid-soluble compounds. Later studies[7] have shown that there is a correlation between the rate of enzymic demethylation and the lipid solubility of drug substrates both in microsomal preparations and the intact animal.

The present studies were designed to investigate the effects of increasing chain length of *N*-alkyl substituents on the rates of microsomal metabolism of oxindole.

METHODS

Incubation experiments

Incubations of the oxindoles with microsomal liver preparations from rats, guinea-pigs and rabbits which had been pretreated with sodium phenobarbitone, the extraction of metabolites and their separation and characterisation by thin-layer chromatography and physico-chemical techniques were carried out as previously described.[8] Formaldehyde production was measured by the method of Cochin and Axelrod[9] and the partition coefficients by a micro-technique previously described.[10]

Gas–liquid chromatography

A Perkin–Elmer F11 chromatograph equipped with a flame ionisation detector and a 0–5 mV Leeds and Northrup Speedomax G recorder Model S were employed.

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The chromatographic column was glass tubing ¼″ o.d., 2 m in length, and packed with 2% S.E. 30 on acid washed and dimethyl-dichlorosilane treated 80–100 mesh Chromosorb G; it was conditioned for 24 hr under the operating conditions: oven temperature, 150°; injection block temperature about 250°; hydrogen pressure 22 lb/in²; air pressure, 30 lb/in² and nitrogen pressure 20 lb/in². The protein-free incubation extracts after the *in vitro* metabolism of the *N*-substituted oxindole compounds were adjusted to pH 7·0 and twice extracted with equal volumes of diethyl ether A.R. The combined ether extracts were evaporated, at room temperature under reduced pressure, to 0·5 ml and 1 μl of the solution was injected on to the column using a 10 μl Hamilton microsyringe. The concentrations of *N*-alkyloxindole starting material, and oxindole formed during the incubations, were determined by comparing the peak heights with previously constructed calibration curves for the various compounds. The peak height: concentration relationship was linear over the range 0·005–0·1 μmole. Oxindole was used as a standard for determining the accurate and uniform functioning of the gas–liquid chromatographs and was injected onto the column between each determination of metabolites in the extracts.

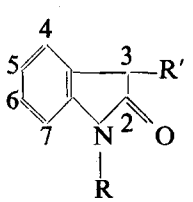
Experimental results were only accepted if the control oxindole peaks did not vary by more than 5 per cent during the experiment.

RESULTS AND DISCUSSION

The unchanged substrates and metabolites formed after the incubation of *N*-alkyloxindoles with rat, rabbit and guinea-pig liver microsome preparations were demonstrated on thin-layer chromatograms and qualitatively determined by gas–liquid chromatographic methods (see Tables 1–3).

As the amounts of phenolic oxindole metabolites could not be easily measured by gas–liquid chromatography under the described conditions due to their very long retention times, these were calculated by subtracting the percentages of unchanged substrates and other metabolites from 100 per cent (see Table 2).

Examination of the extracts by thin-layer chromatography showed that two metabolites were formed from each substrate when *N*-methyl, *N*-ethyl- and *N*-propyloxindole (I) were incubated with liver microsome preparations of rats, rabbits and guinea-pigs



(I) R = CH_3, C_2H_5, C_3H_7; R′ = H or C_2H_5

(see Table 1). One metabolite in each case (Metabolite A) was phenolic and identical in R_f values, chemical colour reactions and u.v. absorption spectrum with the appropriate standard synthetic 5-hydroxy derivatives; the second metabolite (Metabolite B) in each case was non-phenolic and was characterised as oxindole (see Table 1). The u.v. absorption spectra of 5-hydroxy-*N*-ethyloxindole and 5-hydroxy-*N*-propyloxindole as expected, were identical with the spectrum of 5-hydroxy-*N*-methyloxindole previously published.[8]

TABLE 1. THIN-LAYER CHROMATOGRAPHIC R_f VALUES AND CHEMICAL COLOUR REACTIONS OF SOME STANDARD OXINDOLES AND *N*-ALKYLOXINDOLES AND THEIR METABOLITES AFTER INCUBATIONS WITH RAT, GUINEA-PIG AND RABBIT LIVER MICROSOME PREPARATIONS

Compound	R_f values (Solvent system*) I	II	III	Colour reactions: Chloroimide reagent	Diazotised *p*-nitro-aniline reagent	Brentamine fast red reagent
N-methyloxindole	0·64	0·62	0·59	maroon	yellow	yellow
N-methyloxindole Metabolite A	0·47	0·39	0·40	purple	brown	maroon-red
N-methyloxindole Metabolite B	0·55	0·57	0·50	red	yellow	yellow
N-ethyloxindole	0·69	0·72	0·61	magenta	yellow	yellow
N-ethyloxindole Metabolite A	0·50	0·47	0·44	purple	brown	maroon-red
N-ethyloxindole Metabolite B	0·55	0·56	0·50	red	yellow	yellow
N-propyloxindole	0·76	0·78	0·67	magenta	yellow	yellow
N-propyloxindole Metabolite A	0·53	0·52	0·48	purple	brown	maroon-red
N-propyloxindole Metabolite B	0·55	0·57	0·51	red	yellow	yellow
3-Ethyl-*N*-methyloxindole	0·72	0·68	0·73	red	yellow	yellow
Metabolite A	0·60	0·65	0·69	maroon	yellow	yellow
Metabolite B	0·49	0·54	0·53	violet	brown	maroon-red
5-Hydroxy-*N*-methyl-oxindole	0·48	0·37	0·40	purple	brown	maroon-red
5-Hydroxy-*N*-ethyl-oxindole	0·51	0·47	0·44	purple	brown	maroon-red
5-Hydroxy-*N*-propyl-oxindole	0·53	0·51	0·48	purple	brown	maroon-red
Oxindole	0·55	0·56	0·50	red	yellow	yellow
3-Ethyloxindole	0·61	0·65	0·70	maroon	yellow	yellow

* Solvent systems.
Silica gel 'G' (E. Merck/Darmstadt)
I Chloroform: ethyl alcohol, 19:1 v/v
II Benzene: ethyl acetate, 2:3 v/v
III Chloroform: acetone, 5:4 v/v

TABLE 2. GAS–LIQUID CHROMATOGRAPHIC RETENTION TIMES, PARTITION COEFFICIENTS AND CONTENT OF INCUBATION MEDIA AFTER THE INCUBATION OF *N*-ALKYLOXINDOLES WITH RABBIT LIVER MICROSOME PREPARATIONS

Compound	Percentage content of incubation extract*: unchanged substrate	oxindole	calculated 5-hydroxylated derivative	Partition coefficient Ethylene dichloride/phosphate buffer pH 7·6	Retention times of parent compounds (min)
N-Methyloxindole	57·1	12·0	30·9	10·8	1·9
N-Ethyloxindole	50·1	8·3	41·6	19·6	2·4
N-Propyloxindole	32·8	3·2	64·0	31·4	3·0
Oxindole	—	—	—	—	4·2
3-Ethyl-*N*-methyloxindole	—	—	—	37·8	5·4

* 12·0 μmole of each compound were added to the incubation medium.
The data presented, represent the average results for three animals all results being within ± 10 per cent of the recorded values. The rentention times recorded apply only to the described operating conditions (see methods).

3-Ethyl-*N*-methyloxindole also gave rise to two metabolites; the major metabolite was non-phenolic and was characterised as 3-ethyloxindole. The minor metabolite was phenolic and possessed chemical colour reactions and had the u.v. absorption spectrum of a 5-hydroxylated oxindole derivative (see Table 1 and ref. 8). As synthetic 5-hydroxy-3-ethyl-*N*-methyloxindole was not available, direct comparison with the metabolite was not possible.

Measurement of formaldehyde produced during incubations with liver microsomes of all three species (Table 3) showed that *N*-demethylation occurred to a greater extent with 3-ethyl-*N*-methyloxindole than with *N*-methyloxindole; probably the higher lipid solubility of the former compound accounts for this difference. The major metabolite of *N*-methyloxindole was formed by 5-hydroxylation (see Tables 2 and 3).

TABLE 3. FORMALDEHYDE PRODUCTION DURING THE *N*-DEMETHYLATION OF OXINDOLE DERIVATIVES AFTER INCUBATION WITH THE LIVER MICROSOME PREPARATIONS OF RATS, RABBITS AND GUINEA-PIGS

Compound	Formaldehyde production in 1 h/g of liver* (μ mole)		
	Rabbit	Rat	Guinea-pig
N-Methyloxindole	1·18	0·76	0·89
3-Ethyl-*N*-methyloxindole	3·30	2·37	2·60

* 12 μmoles of each compound were added per g of liver. The data presented, represent the average results for four animals, all results being within $\pm$ 10 per cent of the recorded values.

Quantitative examination by gas–liquid chromatography of ether extracts of the incubation media after the incubation of *N*-alkyloxindoles with rabbit liver microsome preparations, showed that the overall metabolism and especially the 5-hydroxylation reactions occurred to a greater extent with *N*-propyloxindole than with *N*-methyl or *N*-ethyloxindole (see Table 2). These results may be explained by the higher lipid solubility of *N*-propyloxindole than that of the *N*-methyl and *N*-ethyl derivatives (see Table 2).

The formation of oxindole by *N*-dealkylation reactions occurred at a greater rate with *N*-methyloxindole than with *N*-ethyl or *N*-propyloxindole; thus size of alkyl group rather than lipid solubility of the parent compound plays the more important role in these dealkylations as is also observed in the case of dealkylation of *p*-nitrophenylalkoxyethers.[11]

In other types of compound,[12, 13] however, *N*-dealkylation is reported to occur more rapidly than does *N*-demethylation; such results have been explain in terms of a free radical mechanism in the microsome.

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