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THE METABOLISM OF NICOTINE INTO TWO OPTICALLY-ACTIVE STEREOISOMERS OF NICOTINE-1′-OXIDE BY ANIMAL TISSUES *IN VITRO* AND BY CIGARETTE SMOKERS

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Abstract—Nicotine-1′-oxide, prepared by the oxidation of (−)-nicotine with hydrogen peroxide, has been resolved into two optically active stereoisomers by fractional precipitation with ammonium reineckate. (−)-Nicotine is enzymically oxidized into both isomers of nicotine-1′-oxide *in vitro*. These enzyme systems are stereospecific since guinea-pig and rabbit liver form similar amounts of each stereoisomer while BALB/c mouse and hamster liver and guinea-pig lung synthesize chiefly *laevo*-rotatory nicotine-1′-oxide. Both stereoisomers of nicotine-1′-oxide were identified in the urine of cigarette smokers.

THE ABSORPTION and metabolism of nicotine after the smoking of tobacco were demonstrated by the identification of cotinine in the urine of smokers.[1] The distribution and fate of nicotine has been reviewed by McKennis[2] and cotinine was found to be a major metabolite in numerous species. Another metabolic oxidation product of nicotine is nicotine-1′-oxide which was identified in the liver, kidney and urine of the rabbit after administration of nicotine.[3] In *in vitro* experiments nicotine is converted into cotinine by a combination of the microsomal and soluble fraction of rabbit liver in the presence of NADPH and O_2,[4] and into nicotine-1′-oxide by a rabbit liver supernatant preparation.[5]

The present paper shows that nicotine-1′-oxide can be resolved into two isomers with different optical activities and demonstrates the *in vitro* formation of both stereoisomers by animal tissues and their presence in the urine of cigarette smokers. The numbering of the nicotine molecule used in this paper is shown in Fig. 1.

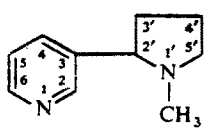

FIG. 1. Numbering of nicotine molecule.

MATERIALS

(−)-Nicotine, purchased from British Drug Houses Ltd., Poole, Dorset, England, was distilled under reduced pressure and stored in ampoules, a fresh ampoule being used for each experiment.

(−)-Cotinine was prepared by an oxidation of nicotine.[6] Nicotine-1′-oxide was prepared by the oxidation of nicotine with hydrogen peroxide and characterized as the dipicrate, m.p. 156° (lit.[7] m.p. 154–158°).

METHODS AND RESULTS

Chromatography. Paper chromatograms were developed on Whatman No. 1 or 3 MM paper with *n*-butanol:*n*-propanol: 2N NH_4OH (2:1:1) by the descending technique. Thin-layer chromatography (TLC) plates were coated with 0·25 mm of Silica gel $HF_{254\,+\,366}$ or aluminium oxide GF_{254} (type E) (E. Merck A.G., Darmstadt, Germany) and activated at 100° for 1 hr. Ascending chromatograms were developed with one of the solvent systems described in the legends to the tables.

Compounds on chromatograms were located as: (1) dark spots when viewed under ultraviolet light; (2) black spots on a pink background after spraying with iodoplatinate reagent;[8] (3) orange spots after spraying with potassium bismuth iodide.[9]

Semi-quantitative estimations of spot intensity depended on the precipitation of a black complex when nicotine-1′-oxide was treated with iodoplatinate. Compounds were located on paper or thin-layer chromatograms under ultraviolet light. The appropriate areas were removed from the chromatograms and treated with 4 ml dilute iodoplatinate solution (spray reagent[8] diluted 1 in 13). This solution has E_{max} at 494 mμ and formation of the black complex, which was removed by centrifugation, resulted in a decrease in absorption at this wavelength. The decrease produced by biologically formed nicotine-1′-oxide was compared with values obtained by adding known amounts of synthetic nicotine-1′-oxide.2 HCl to reaction mixtures or non-smoker's urine.

Resolution of nicotine-1′-oxide into two isomers

When either nicotine-1′-oxide or its dipicrate was examined by chromatography on Whatman No. 1 paper with *n*-butanol:*n*-propanol:2N NH_4OH (2:1:1) as the solvent system they were resolved into two compounds with R_f values of 0·51 and 0·41. Semi-quantitative estimations of spot intensity indicated that, in freshly prepared samples, the ratio of the amount of the faster running compound (R_f 0·51) to that of the slower running one (R_f 0·41) was approximately 2:1. These compounds, which were stereoisomers of nicotine-1′-oxide with different optical activities, were separated by fractional precipitation of the reineckates. After the addition of ammonium reineckate (14 m-moles in 50 ml methanol) to nicotine-1′-oxide (28 m-moles in 500 ml water) and stirring for 0·5 hr, the pink precipitate was removed by filtration. A further addition of ammonium reineckate (14 m-moles in 50 ml methanol) to the filtrate produced a second precipitate which was again removed after stirring for 0·5 hr. This procedure was repeated until a total of 56 m-moles of ammonium reineckate had been added; the final filtrate was kept overnight and the crystalline material that separated removed by filtration. Chromatography of the nicotine-1′-oxide reineckates as described previously caused the complex to split into the red reinecke acid (R_f 0·77) and nicotine-1′-oxide (R_f 0·51 or 0·41). Chromatograms of the various fractions showed that the first precipitate was the reineckate of nicotine-1′-oxide with R_f 0·52 and the final precipitate the reineckate of nicotine-1′-oxide with R_f 0·41. Intermediate fractions contained both isomers. The reineckates were dissolved in methanol containing 3% ammonia and the solutions filtered to remove an insoluble brown contaminant. The reineckates were

precipitated from the filtrates by addition of acetic acid and crystallized from aqueous methanol.

The absorption spectra of reineckates of colourless bases resemble that of ammonium reineckate between 360 and 600 mμ because the absorption is exclusively due to the reineckate moiety of the molecule. Spectrophotometric analysis of reineckates can therefore be used for molecular weight determinations.[10] The nicotine-1′-oxide reineckates and ammonium reineckate dissolved in methanol containing 3% (v/v) ammonia have an absorption maximum at 520 mμ and molecular weights were calculated by comparing $E^{1\%}_{1cm}$ values at this wavelength. Table 1 shows that after purification both isomers of nicotine-1′-oxide existed as monoreineckates.

TABLE 1. PROPERTIES OF NICOTINE-1′-OXIDE REINECKATES

Compound	1% E_{1cm}(520 mμ)	Mol. wt. Found	Mol. wt. Required	m.p.
Ammonium reineckate $(NH_4[Cr(NH_3)_2(SCN)_4]H_2O)$ Mol. wt. 354,4	2·97	—	—	—
Nicotine-1′-oxide (R_f 0·41) reineckate	2·07	509	—	136–140° (decomp.)
Nicotine-1′-oxide (R_f 0·51) reineckate	2·11	499	—	184–188° (decomp.)
Nicotine-1′-oxide monoreineckate $(C_{14}H_{21}CrN_8OS_4)$	—	—	497	—
Nicotine-1′-oxide direineckate $(C_{18}H_{28}Cr_2N_{14}OS_8)$	—	—	816	—

The nicotine-1′-oxides were recovered from the monoreineckates by elution from a column of aluminium oxide. The monoreineckates were dissolved in a small volume of methanol containing 3% (v/v) ammonia and applied to the column. On elution with chloroform:methanol (5:2) the nicotine-1′-oxides were eluted and the red reinecke acid remained at the top of the column.

The nicotine-1′-oxides were also recovered and the isomers separated by applying a solution of the reineckates in methanol containing 3% (v/v) ammonia as a strip to Whatman 3 MM chromatography paper. After development in *n*-butanol:*n*-propanol:2N NH_4OH (2:1:1) the nicotine-1′-oxide isomers which appeared as dark bands under ultraviolet light were eluted with methanol. After evaporating the eluates to dryness under reduced pressure at 30° and drying, the residues were washed with a small volume of acetone and crystallized from ethyl acetate.

Optical activities were determined in a Perkin–Elmer Model 141 polarimeter using micro cells (1 ml) with 10 cm light path. After crystallization from ethyl acetate, the solvent was decanted, the crystals dried with a stream of dry N_2 and immediately dissolved in methanol for determination of the optical activity. The concentration was determined by transferring 1 ml of the methanolic solution into a weighed flask fitted with an outlet and inlet. The solution was evaporated to dryness with a stream of dry N_2 and the flask closed and re-weighed. The faster running isomer (R_f 0·51) crystallized as colourless deliquescent prisms, m.p. 168° decomp., $[\alpha]_D^{22}$ + 65·1 (c 4·38 in methanol). The other isomer (R_f 0·41) also crystallized in colourless prisms, $[\alpha]_D^{22}$ − 76·0

(c 3·05 in methanol) but was considerably more deliquescent and the m.p. was not determined. For nicotine-1′-oxide lit. m.p. 172–173°;[11] 172–173°;[12] 154–155·2° and $[\alpha]_D^{21.5}$ + 65·3 (c 5·13 in methanol);[13] and $[\alpha]_D^{21}$ + 66 (c 5·0 in methanol).[5]

Identification of nicotine-1′-oxide isomers as metabolites of nicotine

(a) *By guinea-pig liver and lung* in vitro. Fresh tissue from male animals was homogenized in 3 vol. ice-cold KCl (1·15% w/v) using an Ultra-Turrax homogenizer. The homogenates were centrifuged at 5° in an Angle 50 centrifuge (Measuring and Scientific Equipment Ltd., London) for 20 min at 2650 g_{max} and the supernatant phase used for incubations with nicotine. Incubation mixtures contained Na_2HPO_4–NaH_2PO_4 buffer pH 7·5 (1·2 m-moles); nicotinamide (480 μmoles); glucose 6-phosphate (200 μmoles); NADP (4 μmoles); glucose 6-phosphate dehydrogenase (20 μg); nicotine (144 μmoles); and 4 ml homogenate supernatant in a total volume of 24 ml. Mixtures, with nicotine omitted, were pre-incubated with shaking at 37° for 10 min, the reaction was started by addition of the nicotine and the mixtures incubated for 2 hr. A similar mixture without incubation after the addition of nicotine or complete reaction mixtures in which the tissue had been boiled before incubation were used as controls. The reactions were stopped with 36% (w/v) trichloroacetic acid (2 ml) and the protein removed by centrifugation. The supernatant phase was made alkaline with 5 N NaOH (1·5 ml) and extracted with three portions of dichloromethane (10 ml). The aqueous phase was treated with glacial acetic acid (1·5 ml) and decolorising charcoal (800 mg) (May and Baker Ltd., Dagenham, England). The charcoal was removed by filtration, washed with water (40 ml) and the metabolites eluted with 3% (v/v) ammonia in methanol (20 ml). The eluate was evaporated at 35° under reduced pressure to approximately 0·2 ml and applied as a band to a silica gel $HF_{254\,+\,366}$ TLC plate which had also a sample of nicotine-1′-oxide applied as a marker. After development in *n*-butanol:ethanol:2N NH_4OH (4:1:1) the strip of silica gel corresponding to the position of nicotine-1′-oxide (R_f 0·15) was removed from the plate and shaken with methanol (14 ml). The mixture was filtered, the filtrate evaporated to dryness and the residue dissolved in methanol (0·2 ml) for chromatography. Table 2 shows that the

TABLE 2. CHROMATOGRAPHIC PROPERTIES OF NICOTINE-1′-OXIDE ISOMERS AND NICOTINE METABOLITES

Compound	R_f Chromatographic system 1	2	3	4	5
Nicotine-1′-oxide (*dextro*-rotatory)	0·33	0·59	0·34	0·51	0·15
Nicotine-1′-oxide (*laevo*-rotatory)	0·25	0·66	0·25	0·41	0·15
Guinea-pig liver metabolites	0·26, 0·35	0·59, 0·67	0·27, 0·34	0·42, 0·51	0·15
Guinea-pig lung metabolites	0·24, 0·32	0·58, 0·66	0·25, 0·33	0·42, 0·51	0·15
Metabolites in smoker's urine	0·26, 0·33	0·58, 0·66	0·24, 0·34	0·42, 0·51	0·15

Chromatographic systems: 1, 2 and 3 on TLC plates coated with $Al_2O_3GF_{254}$; 4 on Whatman No. 1 chromatography paper; 5 on TLC plates coated with silica gel $HF_{254\,+\,366}$. Solvent systems: 1, benzene:methanol (9:1); 2, chloroform:ethyl acetate-acetic acid (7:2:1); 3, *iso*-amyl alcohol: 5% (w/v) citric acid (9:1); 4, *n*-butanol:*n*-propanol:2N NH_4OH (2:1:1); 5, *n*-butanol:ethanol:2N NH_4OH (4:1:1).

Compounds were located as dark spots when chromatograms were viewed under ultraviolet light, as grey spots against a pink background when sprayed with iodoplatinate reagent, or as orange spots when sprayed with potassium bismuth iodide.

chromatographic properties of the nicotine metabolites synthesised by guinea-pig liver and lung were identical with those of the two isomers of nicotine-1′-oxide in chromatographic systems. Nicotine-1′-oxides formed either chemically or by guinea-pig tissues also had the same R_f values in system 5 although in this case the isomers were not separated. The relative intensity of the spots of each isomer formed by the two tissues showed that the liver synthesised similar amounts of each (Table 3) but that

TABLE 3. COMPARISON OF NICOTINE-1′-OXIDE ISOMERS FORMED BY LIVER HOMOGENATES FROM DIFFERENT SPECIES

Species	Nicotine-1′-oxide isomers	
	Laevo-rotatory	*Dextro*-rotatory
Albino guinea-pig	*	*
Albino rabbit	†	†
BALB/c mouse	*	‡
CB Cream Syrian hamster	†	‡

Incubation mixtures were as described in the text. Results represent the mean values of at least three experiments and indicate the approximate amounts of each isomer of nicotine-1′-oxide formed by 300 mg liver in 30 min. * = 100–150 μg; † = 50–100 μg; ‡ = 0–50 μg.

lung formed almost entirely the *laevo*-rotatory isomer with only trace amounts of the *dextro*-rotatory form. Under the same conditions liver formed approximately 10 times as much *laevo*-rotatory isomer and 50 times as much *dextro*-rotatory isomer as lung. No metabolites were seen if complete reaction mixtures were boiled before incubation. The identity of the metabolites was confirmed by two colour reactions. All substances listed in Table 2 appeared as black spots on a pink background when sprayed with iodoplatinate reagent or as orange spots when sprayed with potassium bismuth iodide.

(b) *By livers of other species* in vitro. The ability of livers from male animals of other species to form the two isomers of nicotine-1′-oxide was investigated by the same method used for guinea-pig tissues employing chromatographic system 4 (Table 2). Both isomers of nicotine-1′-oxide were identified in reaction mixtures after incubating nicotine with homogenates of liver from BALB/c mice, rabbits or hamsters. Considerable differences in the relative size of the spots of the two isomers and the intensity of colours after spraying chromatograms with colour reagents (as above) indicated that the amounts of each isomer formed varied in different species. For comparisons of spot intensities, 6 ml reaction mixtures, with concentrations of constituents as for guinea-pig liver, were used and incubations were for 30 min. After treatment with charcoal as before the eluates were applied directly to 3 MM paper and chromatograms developed in system 4 (Table 2). The results are shown in Table 3 and although the values are presented as semi-quantitative results they indicate that whereas guinea-pig and rabbit formed comparable amounts of each isomer, mouse and hamster synthesised chiefly *laevo*-rotatory nicotine-1′-oxide. The stereospecificity of the enzymic oxidation of nicotine to nicotine-1′-oxide by different sepecies and tissues is being studied by quantitative methods.

(c) *By cigarette smokers.* Specimens of urine were collected from male non-smokers or cigarette smokers between 16·00 and 19·00 hr. Several specimens from each group

(total volume 300–600 ml) were pooled, cooled to 5° and centrifuged at 2000 g_{max} for 15 min. The supernatant phases were adjusted to pH 3·0 with 40% (w/v) trichloroacetic acid and treated with decolorising charcoal (1 g/100 ml urine). The charcoal was removed by filtration and washed with water (300 ml) followed by ethanol (20 ml). The metabolites were eluted with 300 ml methanol containing 3% (v/v) ammonia which was then concentrated at 35° under reduced pressure to approximately 100 ml. This elutate was shaken with aluminium oxide (60 g) which adsorbed most of the coloured impurities present at this stage. The aluminium oxide was removed by filtration and washed with methanol (80 ml). The filtrate and washing were combined and concentrated to 0·6 ml at 35°. The concentrate was transferred to a 500 × 60 mm tube, small amounts of finely divided aluminium oxide removed by centrifugation and the supernatant phase applied as bands to silica gel HF_{234} + 366 TLC plates. A sample of nicotine-1′-oxide was also applied as a marker and the chromatograms developed in *n*-butanol:ethanol:2N NH_4OH (4:1:1). The marker (R_f 0·15) was located as a dark spot under ultraviolet light or after spraying with iodoplatinate reagent. The areas of silica gel corresponding to this location were scraped from the plates and shaken with methanol (20 ml) which was filtered. The filtrates were evaporated to dryness at 35°, the residues dissolved in methanol (0·2 ml) and applied as bands to aluminium oxide HF_{254} TLC plates which also had marker spots of both isomers of nicotine-1′-oxide. After development of chromatograms in chloroform:ethyl acetate:acetic acid (7:2:1), the aluminium oxide corresponding to the nicotine-1′-oxide position was scraped off and the metabolites eluted with methanol and concentrated as before for chromatography.

Table 2 shows that the urine of cigarette smokers contains two compounds which are chromatographically identical with the isomers of nicotine-1′-oxide in five chromatographic systems. In all experiments the amount of *dextro*-rotatory nicotine-1′-oxide (as judged by the relative size of the spots) exceeded that of the *laevo*-rotatory isomer. For semi-quantitative experiments, known amounts of nicotine-1′-oxide (mixed isomers) were added to non-smoker's urine, which was then subjected to the same procedure, and the spot intensities compared with those of smoker's urine. In a typical experiment the combined urine samples (340 ml) from four smokers, who had collectively smoked 60 cigarettes on the day of urine collection, contained approximately 80 μg of *laevo*-rotatory nicotine-1′-oxide and approximately 800 μg of the *dextro*-rotatory isomer.

Reaction of nicotine-1′-oxide with potassium dichromate and isatin

The treatment of amine oxides with chromate or dichromate may cause oxidation, demethylation, isomerization in which the oxygen atom is transferred from the nitrogen to the α-carbon (or 5′ position) atom or a combination of these reactions.[14] Treatment of nicotine-1′-oxide with potassium dichromate produced several reaction products which could be separated by TLC but their chromatographic properties varied, presumably because of their instability. The inclusion of isatin in the reaction mixture, however, resulted in the formation of a stable crimson complex which could be extracted into organic solvents. Isatin has been used to detect certain pyrrolidine derivatives after chromatography.[15]

Reaction mixtures contained nicotine-1′-oxide in 1 ml water, 2% (w/v) potassium dichromate (0·1 ml) and isatin reagent[3] (0·2 ml) and were heated at 100° for 15 min.

After cooling, the mixtures were shaken with ethyl acetate (6 ml) which was removed then shaken with N NaOH (0·2 ml). The ethyl acetate extracts contained a crimson and complex with absorption max. at 505 mμ. Similar reactions were carried out using nicotine-1′-oxide isolated from mixtures in which nicotine had been incubated with guinea-pig liver, or lung, or from the urine of cigarette smokers as described above. The ethyl acetate extracts were evaporated to dryness and the residues dissolved in methanol (0·1 ml) and examined by TLC. Table 4 shows that synthetic nicotine-1′-oxide formed a product with chromatographic properties identical with those of

TABLE 4. CHROMATOGRAPHIC PROPERTIES OF COMPLEX FORMED BY ISATIN AND POTASSIUM DICHROMATE OXIDATION PRODUCT OF NICOTINE-1′-OXIDE

Source of nicotine-1′-oxide	R_f Chromatographic systems 1	2	3
Synthetic	0·66	0·31	0·57
Nicotine + guinea-pig liver incubation	0·66	0·31	0·57
Nicotine + guinea-pig lung incubation	0·66	0·31	0·57
Cigarette smoker's urine	0·64	0·31	0·56

Complex was visible as a crimson spot.
Chromatographic system: 1 and 2 on TLC plates coated with silica gel HF_{254} + 366; 3, with $Al_2O_3GF_{254}$. Solvent systems: 1, acetone:benzene:ethanol: ammonia (40:50:5:3); 2 and 3, benzene:methanol (9:1).

products from nicotine-1′-oxide synthesised by guinea-pig tissues or isolated from the urine of smokers. Reaction mixtures containing 25 μg nicotine-1′-oxide produced a clearly visible crimson spot. This was absent if either nicotine-1′-oxide, potassium dichromate or isatin was omitted or if complete mixtures were not heated. By analogy with other amine-*N*-oxides[14] possible oxidation products are nornicotine, cotinine, desmethylcotinine, 3-(5′-hydroxy-1′-methylpyrrolid-2′-yl)pyridine and 3-(5′-hydroxypyrrolid-2′-yl)pyridine. However, reaction mixtures in which nornicotine, cotinine or desmethylcotinine were substituted for nicotine-1′-oxide did not form the crimson complex. Therefore, it seems probable that the complex is due to a reaction between isatin and 3-(5′-hydroxypyrrolid-2′-yl)pyridine. On the other hand, McCaldin[15] considers that a ring containing the —NH—CH_2—CH_2— sequence is required for the production of a coloured complex. The structure of the crimson complex formed between isatin and a potassium dichromate oxidation product of nicotine-1′-oxide has not been determined.

DISCUSSION

The factor or factors responsible for the carcinogenic effect of the smoking of tobacco on the human lung[16] has not been identified, but it is possible that nicotine or some of its metabolites play a part in this process. One of the metabolites of nicotine, nicotine-1′-oxide, might be considered as a possible carcinogen by analogy with the purine *N*-oxides which induce tumours when injected into rats.[17, 18] The rapid absorption, excretion and metabolism of nicotine have been demonstrated by Beckett and

Triggs,[19] who found that the maximum rate of nicotine excretion in urine occurred during the first 15 min after smoking two cigarettes and the maximum rate of cotinine excretion about 2 hr after smoking.

Nicotine is converted into cotinine *in vitro* by lung tissue of numerous species including man[20, 21] and to both isomers of nicotine-1′-oxide by guinea-pig lung. Hence these metabolites may be formed in the lungs of smokers by enzymic oxidation of absorbed nicotine. Alternatively, they could be transported to the lungs after formation in the liver, which is the principal site of nicotine metabolism.[20] The enzymic oxidation of nicotine to cotinine is catalyzed by a combination of the microsomal drug metabolizing system of rabbit liver, which requires NADPH and O_2, and the soluble liver fraction.[4] Since rabbit liver preparations oxidize nicotine to nicotine-1′-oxide in the presence of NADPH[5] this reaction is probably also catalyzed by the microsomal enzymes. The presence of nicotine in the urine of smokers indicates that it circulates in the body and would be available as a substrate for these enzyme systems. The formation of both cotinine and nicotine-1′-oxide are detoxication mechanisms since, when tested by subcutaneous injections into mice, nicotine is 50 times as toxic as cotinine[22] and 20 times as toxic as nicotine-1′-oxide.[23]

The oxidation of nicotine to nicotine-1′-oxide with hydrogen peroxide[7] produced two isomers which could be separated by chromatography. Since (−)-nicotine was used in all experiments the configuration at the asymmetric C atom of the pyrrolidine ring is fixed. However, the existence of two isomers due to different arrangements of groups at the quaternary nitrogen atom is possible, one isomer having the pyridyl and methyl groups *cis* to each other and the other having *N*-oxide oxygen *cis* to the pyridine ring. When the methyl and pyridyl groups are not *cis* to each other they offer the minimum of mutual steric hindrance; this suggests that nicotine-1′-oxide is likely to exist predominantly in this form.[12] In the present work, chromatograms of nicotine

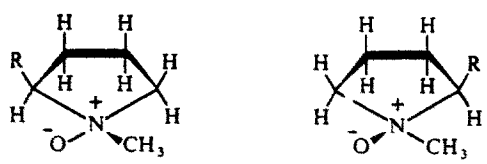

Fig. 2. Isomers of nicotine-1′-oxide.

oxidation products indicated, by reaction with iodoplatinate, that freshly prepared samples contained approximately twice as much *dextro*-rotatory as *laevo*-rotatory isomer, although quantitative experiments are needed to confirm this observation. Although the stereoisomers did not have equal and opposite optical activities, as would be predicted from the fact that they are not enantiomorphs, one was *dextro*-rotatory and one *laevo*-rotatory. Both isomers are fairly stable in solution but form deliquescent solids. The *laevo*-rotatory isomer is the more unstable and is probably the isomer with the methyl and pyridyl groups in *cis* positions.

When nicotine was oxidized enzymically both isomers of nicotine-1′-oxide were synthesised but the relative amounts of each varied with the species and with the tissue.

For example, whereas guinea-pig liver synthesised similar amounts of the two isomers, liver from BALB/c mice and guinea-pig lung formed almost exclusively the *laevo*-rotatory isomer. Although the *dextro*-rotatory isomer predominated in smokers' urine this may not reflect the amounts synthesized by various organs, since *in vivo* the isomers may show stereospecificity during further metabolism or reactions with cell constituents before excretion. Stereospecificity is also demonstrated in the oxidation of ethylbenzene into optically active isomers of methylphenyl-carbinol by rat-liver microsomes in which 80·9 per cent of the product was the *dextro* isomer. The stereospecificity was reduced when the microsomal enzymes were induced by pretreatment of the rats with phenobarbital.[24] This suggests that many conditions, such as species, strain, age, sex, hormone regulation, nutritional status and diet, which are known to affect these enzymes[25] may also change their stereospecificity.

Other drugs which are converted to *N*-oxides by liver microsomes are chlorpromazine,[26,27] imipramine[28] and dimethylaniline.[29] In these compounds the N atom is in the side-chain of the molecule and diphenhydramine is also metabolized by this route by the Rhesus monkey.[30] However, the formation of *N*-oxides of tremorine by rabbit liver slices,[31] chlorcyclizine by men and rats,[32] guanethidine by rats,[33] perazine by men[34] and nicotine by men and animal tissues suggests that *N*-oxidation of ring *N*-atoms is a general route for the conversion of nitrogenous foreign compounds into water-soluble metabolites.

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