J. Pharm. Pharmacol. 1996, 48: 1320–1326
Received 15 April, 1996
Accepted 15 April, 1996

The Failure of Substrate pKa to Influence the Microsomal Formation of Amides from *N*-Benzylamines: the Microsomal Metabolism of *N*-Benzyl Pyrrolidine, *N*-Benzyl Carbazole and *N*-Acetyl-*N*-benzyl-4-methylaniline

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Abstract

The in-vitro hepatic microsomal metabolism of *N*-benzylpyrrolidine (NBP), *N*-benzylcarbazole (NBC) and *N*-acetyl-*N*-benzyl-4-methylaniline (NANBMA) has been studied, using hamster microsomal preparations, to establish whether the corresponding amide is formed.

Amide formation was not observed with any of the substrates utilized, although several metabolic products were detected by HPLC with UV detection. These included the oxidative debenzylation products (for all substrates), ring hydroxylated products (for NBC) and a lactam metabolite (for NBP).

The results support the concept that the metabolic conversion of benzylic amines to the corresponding amide involves an *N*-oxidative step.

The in-vitro metabolism of substituted *N*-benzylanilines by hepatic microsomal preparations from various species has previously been studied to establish the formation of the corresponding amides (Gooderham & Gorrod 1985, 1986; Gorrod & Gooderham 1985; Ulgen 1992; Ulgen et al 1994). Several mechanisms have been proposed for the metabolic formation of amides (Ulgen et al 1994). One mechanism suggested was that initial oxygen attack results in hydroxylation of the benzylic carbon atom to form a carbinolamine intermediate which is further oxidized to give the amide (Fig. 1, route a). As the stability of carbinolamines is related to the pKa of the constituent nitrogen, it was thought that *N*-benzylcarbazole (NBC), with a very low pKa, might form a stable carbinolamine. If amide formation utilizes route a, then metabolism of NBC might give *N*-benzoylcarbazole (NBZC) by further oxidation of the carbinolamine. It is considered that formation of an *N*-oxide metabolite from NBC is not possible because the lone pair of electrons of the nitrogen in NBC is not available because of the delocalizing influence of the aromatic ring system (Gorrod & Temple 1976). The lack of *N*-oxidation would presumably promote aliphatic carbon oxidation, to afford the carbinolamine and hence the amide (NBZC).

In contrast with carbazole, with a very low pKa, pyrrolidine is a highly stable five-membered saturated heterocyclic base with a pKa of 11.3 (Albert 1968). Introduction of a benzyl group on the nitrogen of this ring produces a strongly basic substrate, *N*-benzylpyrrolidine (NBP). The rationale for selecting NBP was to ascertain whether the increased pKa of the nitrogen would enable the formation of a carbonyl structure on the benzylic carbon to give N-benzoylpyrrolidine, NBZP. A secondary consideration was to confirm the formation of the alicyclic α-oxo metabolite (*N*-benzyl-2-pyrrolidone, NBZPO) described previously (Ho & Castagnoli 1980). Although metabolism of NBP was previously studied in conjunction with [$Na^{14}CN$] as a trapping agent for iminium ions formed during metabolic incubation (Ho & Castagnoli 1980), no report has been made on the formation of a benzylic amide. The most common metabolite of pyrrolidine-containing drugs is the pyrrolidone derivative (Michaelis et al 1970; Oelschlager & Al Shaik 1985; Whittlesea & Gorrod 1993; Gorrod & Aislaitner 1994).

Another route proposed for the formation of an amide from a secondary aniline requires an initial oxygenation step on the constituent nitrogen, giving an *N*-hydroxy compound which could be further oxidized to a nitrone, then conversion of the nitrone to the corresponding amide via an oxaziridine intermediate (Fig. 1, route b). Previous studies on the in-vitro hepatic metabolism of *N*-benzyl-4-methylaniline (NBMA)

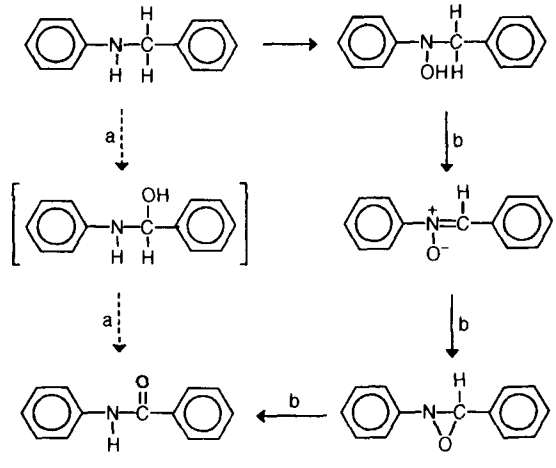

FIG. 1. Proposed mechanisms for the metabolic production of amides from *N*-benzylsubstituted anilines.

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showed that this was the best substrate for the formation of the corresponding amide and nitrone (Gooderham & Gorrod 1985; Gorrod & Gooderham 1985; Gooderham & Gorrod 1986; Ulgen 1992; Ulgen & Gorrod 1992; Ulgen et al 1994) in a number of species. A metabolic study of *N*-acetyl-*N*-benzyl-4-methylaniline (NANBMA), the acetyl derivative of NBMA, was, therefore, performed in-vitro to establish whether the corresponding diamide, *N*-acetyl-*N*-benzoyl-4-methylaniline (NANBZMA) is formed. The aim was to inhibit initial *N*-oxidation by introducing the acetyl group into the substrate molecule which, because of the delocalizing effect of the carbonyl group, would lower the pKa and prevent oxygenation of the constituent nitrogen. This new substrate having a lower pKa than NBMA would be expected to stabilize the carbinolamine and to have greater lipophilicity compared with NBMA. If the corresponding diamide is detected, it would mean that the *N*-oxidative route proposed above is probably not involved in the formation of amides. In contrast, a negative result for amide formation from NANBMA would support the concept that metabolic amide formation requires initial *N*-oxidation. From our previous experiments on the metabolism of other benzylic anilines, it is predicted that a number of other metabolic products, i.e. the hydroxymethyl derivative, debenzylated amine and the corresponding aldehyde will be produced from this substrate. Other metabolites would probably be hydrolysis products which would interfere with diamide formation. Microsomal hydrolysis of amides is, however, known to be inhibited by sodium fluoride (Sertkaya & Gorrod 1987); to minimize the risk of deacetylation this metabolic experiment was performed in the presence of sodium fluoride. The deacetylation of arylamides is influenced by the physicochemical properties of the amide (Sertkaya & Gorrod 1988). The results of the above experiments are the subject of this communication.

Materials and Methods

Pyrrolidine, carbazole, *N*-benzyl-2-pyrrolidinone (NB2PO), 4-methylaniline (*p*-toluidine), acetyl and benzoyl chlorides were purchased from Aldrich (UK). Sodium fluoride was obtained from Hopkins & Williams (UK). Sodium amide was obtained from Sigma (UK). NBMA was synthesized as reported elsewhere (Ulgen 1992). *N*-Acetyl-4-methylaniline (NAMA) was prepared by acetylation of *p*-toluidine with acetyl chloride. *N*-Benzoyl-4-methylaniline (NBZMA) was prepared by benzoylation of *p*-toluidine. All chromatographic solvents were obtained from Merck. The HPLC column (μ-Bondapak C18 5μm (25 cm × 4·6 mm i.d.) was purchased from Phase Separations (Deeside, UK). The guard column packing material (Whatman pellicular ODS) was purchased from Whatman (Maidstone, Kent, UK). The HPLC chromatograph consisted of an isocratic system comprising one LCD analytical ConstaMetric 3200 solvent delivery system, a Rheodyne syringe-loading sample-injector valve (model 7125) fitted with a 20-μL sample loop, a Milton Roy SpectroMonitor-3100 variable wavelength UV detector and a Milton Roy electronic integrator. Glucose-6-phosphate dehydrogenase was purchased from the Boehringer Mannheim (London, UK). Nicotinamide adenine dinucleotide phosphate mono sodium salt (NADP) and glucose-6-phosphate disodium salt were obtained from Sigma.

Preparation of NBP, NBC and NANBMA and their potential amide metabolites NBZP, NBZC and NANBZMA

NBP. Equimolar amounts of pyrrolidine (1·98 g, 0·028 mol) and benzyl chloride (3·54 g, 0·028 mol) were reacted to prepare NBP. An oil was formed which immediately solidified. The material was treated with sodium bicarbonate solution and extracted with diethyl ether (3 × 12 mL). Evaporation of the ether under vacuum at 20°C yielded a crude oil which was shown by TLC and HPLC analysis to contain several impurities. Column chromatography on 0·063-0·200-mm (70-230 mesh ASTM) silica gel 60 (E. Merck, Darmstadt, Germany) using methanol-chloroform (50:50, v/v) as eluent afforded NBP as a pure compound (yield 75%). Elemental analysis, found: C, 81·93; H, 9·38; N, 8·68%; calc. for $C_{11}H_{15}N$: C, 81·00; H, 9·38; N, 8·63%. EI mass spectral analysis showed a molecular ion peak and a fragmentation pattern characteristic of the proposed structure. Major fragments (% relative abundance): 161·96 (10·5), 160·87 (98·6), 159·8 (97·1), 90·89 (59·9), 91·98 (18), 84 (90·6), 69·9 (100). Maximum UV absorption: 205 nm (in MeOH).

NBC. To prepare NBC, carbazole (5 g, 0·03 mol) and potassium hydroxide (66% aqueous solution; 20 mL) were added to equimolar amounts of benzyl bromide (5·13 g, 0·03 mol) in the presence of potassium iodide in acetone (40 mL) and heated under reflux for 3 h. The hot reaction mixture was poured into water and the solid obtained recrystallized from absolute ethanol (m.p. 119°C, lit.118–120°C; yield 65%) (Weast & Astle 1979). Major EI mass spectral fragments (% relative abundance): 91 (95), 139 (1·3), 166 (7), 167 (2·4), 180 (4·8), 257 (100). Maximum UV absorption: 235, 254 and 290 nm (in MeOH).

NANBMA. For the preparation of NANBMA, NBMA (6.6 g, 0.0334 mol) and acetyl chloride (2·62 g, 0·0334 mol) in benzene (20 mL) were heated under reflux for 1 h at 120°C. The benzene was removed by evaporation and the residue added to a solution of sodium carbonate. The mixture was extracted with diethyl ether (3 × 12 mL). The organic extracts were combined and the solvent evaporated. The acetylated amine was isolated using column chromatography on silica gel 60 (see above) with petroleum ether (b.p. 40–60°C)-acetone (50:50, v/v) as eluent. The crude product was further purified by preparative TLC using petroleum ether (b.p. 40–60°C)-chloroform (25:75, v/v) as eluent (yield 80%). Found: C, 79·47; H, 7·16; N, 5·82%; calc. for $C_{16}H_{17}NO$: C, 80·30; H, 7·16; N, 5·85%. ^{1}H NMR ($CDCl_3$) (δ ppm, number of protons, signal origin): 1·87 (s, 3H, $-COCH_3$, 2·33 (s, 3H, H_3C-Ar), 4·88 (s, 2H, N-CH_2), 6·88 (d, 2H, protons *ortho* to *N*), 7·13 (d, 2H, protons meta to *N*) and 7·25 (m, 5H, $-CH_2-C_6H_5$). The IR spectrum showed characteristic amide stretching at 1650 cm^{-1}. EI mass spectral analysis showed a molecular ion peak and characteristic fragmentation pattern corresponding to the proposed amide. Major fragments (% relative abundance): 91 (76), 96 (32), 97 (73), 239 (100), 240 (6.5) Maximum UV absorption: 209 nm (in MeOH).

NBZP. The method employed for the preparation of NBZP was benzoylation of pyrrolidine (1·98 g, 0·028 mol) with benzoyl chloride (3·93 g, 0·028 mol) in the presence of NaOH solution (10 mL, 2 N) (Braun & Beschke 1906). Extraction with diethyl

ether (3 × 20 mL) and evaporation of the solvent afforded an oily liquid. The oil was purified by column chromatography on silica gel 60 with petroleum ether (b.p. 40–60°C)-acetone (10:90, v/v) as eluent (yield 75%). Elemental analysis gave C, 75·39; H, 7·47; N, 7·99%; calc. for $C_{11}H_{13}NO$: C, 73·12; H, 7·59; N, 8·01%. EI mass spectral analysis showed a molecular ion peak and characteristic fragmentation pattern corresponding to the proposed amide. Major fragments (% relative abundance): 70 (4·3), 77 (63·8), 105 (100), 174 (16·1), 174 (16·1), 175 (38·4), 176 (8·48). Maximum UV absorption: 200 nm (in MeOH).

NBZC. NBZC was prepared by the method of Stevens and Tucker (1923) from carbazole (5 g, 0·03 mol) and benzoyl chloride (4·22 g, 0.03 mol); the product was recrystallized from ethanol (yield 91·5%; m.p. 97°C, lit. 98°C (Stevens and Tucker 1923)). EI mass spectral analysis showed a molecular ion peak and a characteristic fragmentation pattern corresponding to the proposed amide. Major fragments (% relative abundance): 77 (4·6), 105 (100), 166 (2·6), 167 (1·2), 271 (38·7). Maximum UV absorption: 220 and 274 nm (in MeOH).

NANBZMA. To prepare NANBZMA, NAMA (2.984 g, 0.02 mol) was dissolved in toluene (20 mL) and sodium amide (0·975 g, 0·025 mol) added. The mixture was heated under reflux until evolution of ammonia could no longer be detected by litmus paper. Benzoyl chloride (2·81 g, 0·02 mol in 10 mL toluene) was then added and the mixture heated under reflux for 6 h at 80°C. TLC analysis showed a new product which gave a yellow colour with Dragendorff's reagent (specific for a tertiary nitrogen) together with unreacted secondary amide and some impurities. The toluene was removed by evaporation under vacuum and the oily residue added to a solution of sodium carbonate (10%, 50 mL) and extracted with dichloromethane (DCM; 50 mL). The DCM was evaporated and the tertiary amide was isolated as an oil by column chromatography on silica gel 60 (see above) with petroleum ether (b.p. 40–60°C)-acetone (50:50, v/v) as eluent (yield 80%). Found: C, 77.11; H, 6·12; N, 5·84%; calc. for $C_{16}H_{15}NO_2$: C, 75·87; H, 5·97; N, 5·53%. ^{1}H NMR (CH_3OH) (δ ppm, number of protons, signal origin): 2·311 (s, 3H, $-COCH_3$), 2·345 (s, 3H, H3C-Ar), 7·09 (d, 2H, *ortho* to the methyl), 7·179 (d, 2H, *meta* to the methyl) and 7·312 to 7·611 (m, 5H, $-CO-C_6H_5$). EI-Mass spectral analysis showed a molecular ion peak and fragmentation pattern characteristic of the proposed amide. Major fragments (% relative abundance): 77 (76), 86 (57), 105 (100), 149 (45), 191 (10), 211 (66), 212 (15), 253 (25), 254 (5). The UV spectrum of this compound was also consistent with the proposed structure of this compound ($\lambda_{max} = 209$ nm (in MeOH)). The IR spectrum showed characteristic amide stretching at 1650 cm^{-1}.

Analytical procedures for the detection and identification of NBP, NBC, NANBMA and their potential metabolites

The separation techniques used were based on TLC and isocratic HPLC. TLC was performed on commercial glass-backed plates coated with a 0·25 mm layer of silica gel GF_{254} (E. Merck) with suitable solvent systems. Table 1 shows TLC R_F and HPLC R_t values of the three substrates and their potential metabolites, using a variety of solvent systems. After development the plates were examined under UV light (254 nm) and then sprayed with diazotized sulphanilic acid reagent (specific for phenolic compounds) then sodium carbonate. The reaction products were eluted under isocratic conditions with the mobile phase acetonitrile-0·2 M phosphate buffer of suitable composition and at a flow rate of 1–1·5 mL min^{-1} (Table 1). The metabolic products were detected by their absorbance at 254 nm. Retention times of compounds under these conditions are given in Table 1.

Incubation and extraction procedures. Hepatic washed hamster microsomal preparations were prepared at 0°C using the calcium chloride precipitation method of Schenkman & Cinti (1978). Incubations were performed in a shaking water bath at 37°C using a standard co-factor solution at pH 7·4 . Co-factors consisting of NADP (2 μmol), glucose-6-phosphate (10 μmol), glucose-6-phosphate dehydrogenase (1 unit), $MgCl_2$ (20 μmol) prepared in phosphate buffer (0·2 M, pH 7·4; 2 mL) were pre-incubated for 5 min before addition of microsomes (1 mL) equivalent to 0·5 g original liver and substrate (5 μmol in

Table 1. Chromatographic properties of benzylic amines and some of their potential metabolites.

Compound	R_F × 100 values (TLC)	Retention times (min) (HPLC)
Benzaldehyde		3 (A3) : 12 (A1) : 5 (A2)
N-Benzylcarbazole (NBC)	56 (S1) : 72 (S2)	22 (A3)
N-Benzoylcarbazole (NBZC)	63 (S1) : 64 (S2)	18 (A3)
Carbazole	25 (S1) : 52 (S2)	6·5 (A3)
N-Benzylpyrrolidine (NBP)	15 (S3) : 25 (S4)	6 (A1)
N-Benzoylpyrrolidine (NBZP)	58 (S3) : 80 (S4)	15 (A1)
N-Benzyl-2-pyrrolidinone (NB2PO)	48 (S3) : 67 (S4)	16 (A1)
N-Acetyl-*N*-benzyl-4-methylaniline (NANBMA)	14 (S5) : 29 (S6)	17 (A2)
N-Acetyl-*N*-benzoyl-4-methylaniline (NANBZMA)	26 (S5) : 45 (S6)	13 (A2)
N-Benzoyl-4-methylaniline (BZMA)	21 (S5) : 39 (S6)	12 (A2)
N-Benzyl-4-methylaniline (NBMA)	62 (S5) : 68 (S6)	29 (A2)
N-Acetyl-4-methylaniline (NAMA)	2 (S5) : 6 (S6)	4 (A2)

Solvent systems: S1 = petroleum ether-acetone (90:10, v/v), S2 = benzene-ethyl acetate (90:10, v/v), S3 = petroleum ether-acetone (10:90, v/v), S4 = methanol- chloroform (50:50,v/v), S5 = petroleum ether-chloroform (50:50, v/v), S6 = petroleum ether-chloroform (25:75, v/v), A1 = acetonitrile-0.02 M phosphate buffer (30:70, v/v), total pH 3, flow rate 1 mL min^{-1}, A2 = acetonitrile-0.02 M phosphate buffer (40:60, v/v), total pH 7, flow rate 1·5 mL min^{-1}, A3 = acetonitrile-0·02 M phosphate buffer (50:50, v/v), total pH 6·5, flow rate 1 mL min^{-1}.

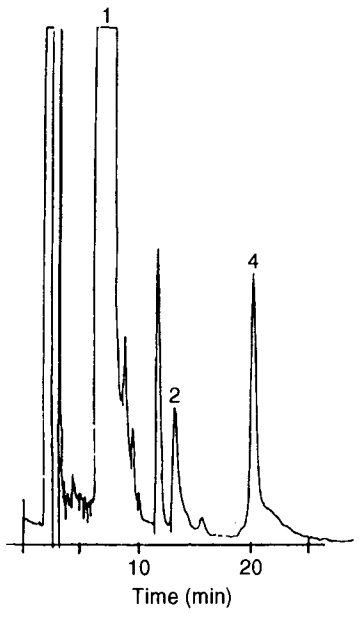

FIG. 2. HPLC chromatogram obtained after extraction from male hamster microsomal incubation mixture with NBP as substrate. 1. NBP, 2. benzaldehyde, 4. NB2PO.

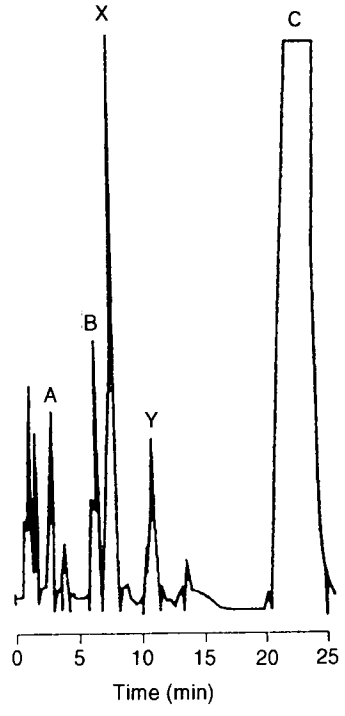

FIG. 3. HPLC chromatogram obtained after extraction from male hamster microsomal incubation mixture with NBC as substrate. A. benzaldehyde, B. carbazole, C. NBC, X and Y phenolic metabolites.

50 μmol methanol per flask). Metabolic reactions were stopped by extraction with DCM (2 × 5 mL). The DCM extracts were evaporated to dryness with a stream of N_2. Dry organic residues were reconstituted in 200 μL methanol for HPLC analysis and 100 μL methanol for TLC analysis. In the experiments in which NANBMA were used as substrate, NaF (100 μmol) was added to each flask.

Results

N-Benzylpyrrolidine

Examination of extracts obtained after in-vitro metabolism of NBP failed to show the formation of the benzylic amide, NBZP. Benzaldehyde and the lactam, *N*-benzyl-2-pyrrolidinone (NB2PO) were, however, observed by HPLC (Fig. 2) and further confirmed by use of a Rapiscan UV detector. Drugs bearing the pyrrolidine ring, e.g. nicotine and prolintane, are known to be metabolized to lactam derivatives (Oelschlager & Al Shaik 1985; Whittlesea & Gorrod 1993). NBP has previously been shown to produce NB2PO as a metabolite detectable by GLC (Ho & Castagnoli 1980).

N-Benzylcarbazole

After the incubation of NBC with hamster microsomal preparations four metabolites were detected. These metabolites were recognized by use of HPLC, UV, TLC and EIMS-MS (Ulgen et al 1994). An HPLC chromatogram of an organic extract obtained after in-vitro metabolism of NBC by hepatic hamster microsomes is shown in Fig. 3. The results from EIMS-MS showed that the two uncharacterized metabolites are ring-hydroxylated products of carbazole (Ulgen et al 1994). Further confirmation of the formation of *N*-dealkylated metabolites of NBC i.e. carbazole and benzaldehyde was achieved using a Rapiscan UV detector connected to HPLC (Ulgen et al 1994).

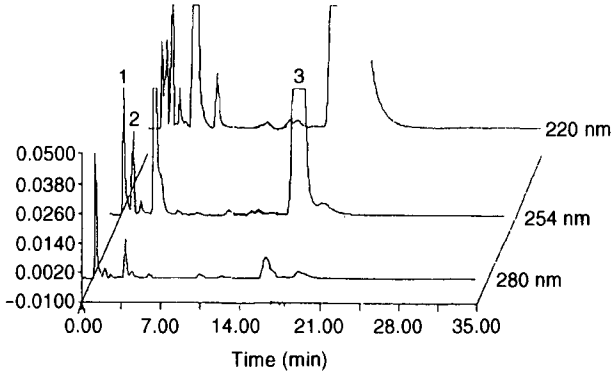

FIG. 4. HPLC chromatograms obtained at different detector wavelengths (220, 254 and 280 nm) after extraction from male hamster microsomal incubation mixture with NANBMA as substrate. 1. NAMA, 2. benzaldehyde, 3. NANBMA.

N-Acetyl-N-benzyl-4-methylaniline

After metabolism of NANBMA, the diamide, NANBZMA was not detected, although four metabolic products were observed by use of HPLC (Fig. 4). Two were confirmed as NAMA and benzaldehyde by use of HPLC (Fig. 4) and UV. The structure of the other two metabolites remains to be elucidated. One metabolite is probably the corresponding 4-hydroxymethyl compound as has been observed for the parent secondary amine (Ulgen & Gorrod 1992).

Fig. 5a, b, c shows established metabolic pathways for the substrates studied.

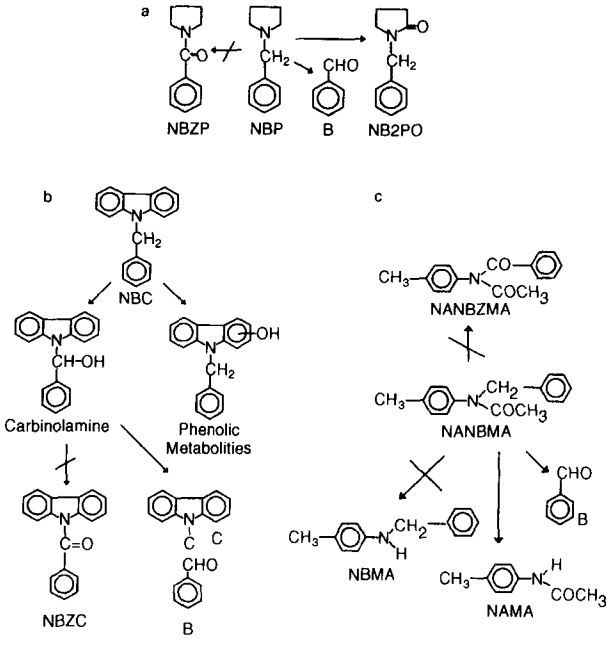

FIG. 5. Metabolic reactions involved in the biotransformation of NBP (a), NBC (b) and NANBMA (c).

Discussion

This work with the benzylic amines, NBP, NBC and NANBMA as substrates clearly indicates that the pKa of the constituent nitrogen is not an important controlling factor in the formation of amides from benzylic amines. Table 2 summarizes the metabolic reactions observed after the metabolic oxidation of these *N*-benzylic amines. The in-vitro microsomal metabolism of the amines NBP and NBC, which were chosen to examine the effect of their extreme pKa characteristics on benzylic amide production, did not indicate the formation of the corresponding amide or carbinolamine derivatives. Previous studies on *N*-benzylpyrrolidine have also shown that the major primary metabolite produced by rabbit liver microsomes was *N*-benzyl-2-pyrrolidone (Ho & Castagnoli 1980). The failure to detect oxidation of the α-benzylic carbon is also in agreement with published data (Ho & Castagnoli 1980). In this respect this substrate is similar to the related *N*-benzylpiperidine for which it has been shown that whereas α-C-oxidation of the alicyclic ring occurred to give the 2-piperidone, α-C-oxidation of the benzylic carbon did not occur (Masumato et al 1991). This is in contrast with the open-chain benzylic amine; *N*-benzyldipropylamine (NBDP), for which evidence was obtained (Masumato et al 1991) by the cyanide trapping technique (Gorrod & Aislaitner 1994) for the formation of iminium ions derived from α-carbon oxidation of both the propyl and benzyl substituents (Masumato et al 1991), although the oxidation of the benzyl substituent occurred to less than 5% of the *N*-propyl α-oxidation. Similarly, two isomeric cyano adducts were observed when nicotine was metabolized in the presence of cyanide (Nguyen et al 1976; Nguyen et al 1979); one metabolite was derived from oxidation of the α-carbon of the pyrrolidine ring, the other by oxidation of the *N*-methyl group. As it is now generally believed that α-carbonyl formation from *N*-alicyclic and *N*-alkyl compounds proceeds via an iminium ion species which react with water (Fig. 6), it is clear that iminium ion formation is sensitive to minor structural differences. In general it seems that although an *N*-alkyl group can be metabolized to an iminium ion, the presence of an α aromatic ring, as in benzyl compounds, prevents this reaction. With *N*-benzyldipropylamine bond cleavage predominates, compared with *N*-benzylpiperidine. This might be because of a greater contribution of electrons to the constituent nitrogen, as reflected in the pKa (*N*-benzylpiperidine pKa = 8·72; NBDP pKa = 9·52) which in turn alters the electron density on the benzylic α-carbon enabling oxidation to proceed. If this proposal is correct then *N*-(2,4,6-trimethylbenzyl)piperidine would be expected to be oxidized at the benzylic carbon; experiments are in hand to verify this prediction. An alternative pathway to *N*-benzylpyrrolidine-2-one formation, involving rearrangement of an *N*-benzylpyrrolidine-*N*-oxide, has recently been described (Oelschlager & Schmidt 1992) but no evidence for the formation of the benzylic carbonyl compound was produced.

No carbinolamine or carbonyl compound was formed from NBC; it was thought that this α-hydroxybenzyl compound would be stable, because of the low basicity of NBC. It has previously been shown that *N*-hydroxymethylcarbazole is a metabolite of *N*-methylcarbazole (Gorrod & Temple 1976) and it appears again that substitution of the methyl group with a phenyl group inhibits benzylic oxidation. A study of the metabolism of a series of *N*-alkylcarbazoles has recently been undertaken (Hollenberg & Wurster 1994). Unique in this series *N*-ethylcarbazole yielded an α-C-hydroxy product.

The metabolic experiment with NANBMA as a substrate did not show the presence of the deacetylated hydrolysis product, NBMA, indicating that fluoride inhibited this hydrolytic reaction. This was desirable as the parent amine, NBMA does form an amide (Gooderham & Gorrod 1985; Gorrod & Gooderham 1985; Gooderham & Gorrod 1986; Ulgen 1992; Ulgen et al 1994) which might be produced via an intermediate

Table 2. A summary of metabolic reactions observed after the metabolic oxidation of benzylic amines.

Substrate	Debenzylated substrate	Benzaldehyde	Benzylic amide	Cyclic lactam	Uncharacterized metabolites
NBC	+	+	−	N.A.	Ring hydroxylation +
NBP	−	+	−	+	Ring hydroxylation +
NANBMA	+	+	−	N.A.	Methyl hydroxylation +

+ = metabolite detected by HPLC. − = metabolite not detected by HPLC. N.A. = not applicable. Pyrrolidine could not be detected by chromatographic methods.

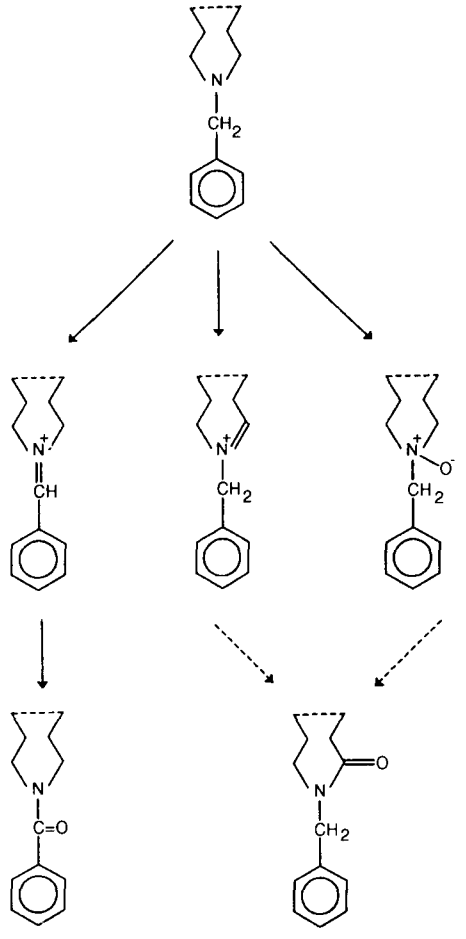

FIG. 6. Possible metabolic intermediates involved in the biotransformation of *N*-benzylalkylamines: solid line, *N*-benzyl-*N*,*N*-dialkylamine; dashed line, *N*-benzyl-*N*,*N*-cycloamine.

N-oxidation product. It was thought that introduction of a metabolically stable acetyl group into the substrate would delocalize the nitrogen lone pair electrons and thereby prevent *N*-oxidation, as there are no reports that tertiary amides can form *N*-oxides. As acetylation prevented oxidation of the α-benzylic carbon to the diamide this might be taken as evidence

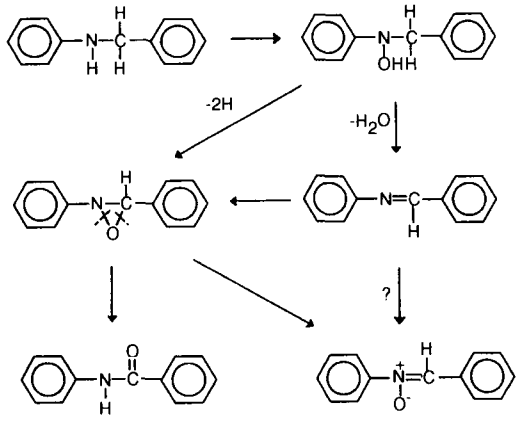

FIG. 7. Alternative metabolic pathways for the formation of amides and nitrones from *N*-benzylanilines.

of the involvement of an *N*-oxidation step in amide-formation from NBMA. α-*C*-Benzylic oxidation must, however, have occurred as the debenzylated products *N*-acetyl-4-methylaniline (NAMA) and benzaldehyde were identified as metabolites. This clearly supports the concept that α-*C*-benzylic oxidation leads to debenzylation whereas *N*-oxidation leads to amide formation. As *N*-oxidation of *N*-benzylanilines produces the *N*-hydroxy derivatives (Gooderham & Gorrod 1985) which are further oxidized to the corresponding nitrones (Gooderham & Gorrod 1985), we have previously examined the possibility that amides are produced via this route (Ulgen & Gorrod 1994). We suggested that nitrones could rearrange to oxaziridines that would yield amides after ring cleavage. Metabolic studies with diaryl nitrones failed, however, to yield amides as metabolites (Ulgen & Gorrod 1994) if the incubation and subsequent analytical procedures were performed in the dark. If the nitrones were exposed to light, amides were produced as reaction products. This suggests that amides are produced from secondary hydroxylamines by a route not involving nitrone formation. Two alternative pathways (Fig. 7) are possible, one would be direct conversion to an oxaziridine by dehydrogenation and rearrangements to the amide. A second pathway could be loss of water to form the corresponding imine, which could be oxidized to an oxaziridine and thence to the amide or nitrone as discussed previously (Gorrod & Ulgen 1994). One of the unknown metabolites observed during the metabolism of NANBMA could be the 4-hydroxymethyl compound because in addition to our study (Ulgen & Gorrod 1992) Daly et al (1967) observed the formation of an analogous compound from *N*-acetyl-4-methylaniline (NAMA).

In conclusion, the results might be summarized as follows: NBC does not form a benzoyl derivative because it cannot form an intermediate *N*-oxidation product. NBP does not form a benzoyl derivative as any intermediate iminium ion or *N*-oxide reacts or rearranges to the 2-pyrrolidone rather than to a benzylic carbon oxidation product. NANBMA does not form a diamide as *N*-oxygenation cannot proceed because of the delocalization of the lone pair of electrons on the nitrogen. We conclude that the experiments described support the concept that the metabolic conversion of benzylic amines to the corresponding amide involves an *N*-oxidative step.

Acknowledgement

The authors thank King's College London Mass Spectrometry Services for EI mass spectral analysis.

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