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THE FORMATION AND METABOLISM OF *N*-HYDROXYMETHYL COMPOUNDS—III

THE METABOLIC CONVERSION OF *N*-METHYL AND *N,N*,-DIMETHYLBENZAMIDES TO *N*-HYDROXYMETHYL COMPOUNDS*

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Abstract—The stability of metabolically-generated *N*-(hydroxymethyl) compounds was investigated using a series of *N*-methylbenzamides as model substrates. *N*-(Hydroxymethyl)-benzamide was characterized as a major metabolite of *N*-methylbenzamide *in vitro*, and was also identified as a urinary metabolite of *N*-methylbenzamide. N-(Hydroxymethyl) compounds were also found as metabolites of 4-chloro-*N*-methylbenzamide and 4-*t*-butyl-*N*-methylbenzamide *in vitro*. Thus substitution in the 4-position of the phenyl ring of derivatives of *N*-(hydroxymethyl)-benzamide did not affect their stability sufficiently to cause degradation to formaldehyde under the conditions used. *N*-(Hydroxymethyl)-*N*-methylbenzamide was identified as a metabolite of *N,N*-dimethylbenzamide *in vitro*. However, *N*-(hydroxymethyl)-*N*-methylbenzamide was less stable than *N*-(hydroxymethyl)-benzamide under alkaline conditions. Furthermore, *N*-(hydroxymethyl)-*N*-methylbenzamide, unlike *N*-(hydroxymethyl)-benzamide and its 4-substituted derivatives, was positive in the colorimetric assay for formaldehyde, presumably because of its degradation to produce formaldehyde. Thus substitution on the nitrogen atom which bears the methyl group in *N*-methylbenzamide markedly affected the stability of the *N*-methylol produced during oxidative metabolism. *N*-Formylbenzamide was identified as a metabolite of *N*-methylbenzamide in suspensions of mouse hepatocytes and also *in vivo*. The mechanism for its production probably involves the generation of *N*-(hydroxymethyl)-benzamide.

N-(Hydroxymethyl) or *N*-methylol derivatives (**A**) of various amines and amides can be synthesized by reaction of the appropriate amine or amide with formaldehyde [1]. The reaction is readily reversible in aqueous media [2]. It is therefore not surprising that *N*-(hydroxymethyl) compounds produced during the oxidative *N*-demethylation of *N*-methyl-containing xenobiotics have been considered to be transient species which rapidly degrade to produce the *N*-desmethyl compound (**B**) and formaldehyde (**C**) [3, 4].

$$R_2N{-}CH_3 \xrightarrow{Fe^{3+}\rightarrow[O]} \underset{\mathbf{A}}{R_2N{-}CH_2OH} \longrightarrow \underset{\mathbf{B}}{R_2NH} + \underset{\mathbf{C}}{CH_2O}$$

However, it was shown as early as 1953, in one of the first studies of metabolic *N*-demethylation *in vitro*, that the *N*-(hydroxymethyl) derivative of 3-methyl-4-methylaminoazobenzene had sufficient stability to undergo further chemical reaction with glutathione to form a methylene thioether [5]. Although this conclusion was tentative, there have since been many *N*-(hydroxymethyl) intermediates isolated during the metabolism of *N*-methyl-containing compounds. The evidence pertaining to the formation and properties of these intermediates has been recently summarized [6].

A study of the conditions under which *N*-(hydroxymethyl) compounds or alternatively formaldehyde are formed as metabolites of xenobiotics bearing *N*-methyl groups is toxicologically relevant, as such carbinolamines are capable of the aminomethylation of nucleophiles such as glutathione [7, 8] or may act as transport forms of formaldehyde [9], a carcinogen [10]. In an attempt to define those characteristics of a *N*-methyl-containing compound which predispose it to form a stable *N*-(hydroxymethyl) intermediate during metabolism, we have investigated the stability of both synthetic *N*-(hydroxymethyl) compounds and those produced during metabolism [6, 11–14]. Synthetic *N*-(hydroxymethyl) compounds have widely differing stabilities [15] but appear to be relatively stable when the *N*-(hydroxymethyl) group is in an electron-deficient environment [16–18]. An example of such a stable *N*-methylol is *N*-(hydroxymethyl)-

* For No. II in this series see [24].

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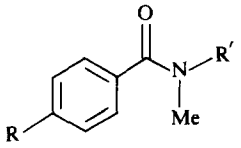
Abbreviations used: BSTFA, *N,O*-bis-(trimethylsilyl)-trifluorocetamide; HPLC: high pressure liquid chromatography, GC–MS: gas chromatography–mass spectrometry.



	R	R'
A	H	H
B	Cl	H
C	*t*-Bu	H
D	H	Me

Fig. 1. Derivatives of *N*-methylbenzamide used in this study.

benzamide, which can be readily synthesized [19] and has a half-life of 21.1 hr in Earl's buffer, pH 7.4, at 37° [6].

In this study we wished to test the hypothesis that the metabolism of *N*-methyl compounds which have the *N*-methyl moiety in an electron-deficient environment leads to stable *N*-(hydroxymethyl) derivatives and not to formaldehyde. As model compounds, we chose *N*-methylbenzamide [the methyl analogue of *N*-(hydroxymethyl)-benzamide], its 4-chloro and 4-*t*-butyl derivatives, and *N,N*-dimethylbenzamide (Fig. 1, **A–D**). These derivatives of *N*-methylbenzamide possess substituents which influence the electron density in the amide portion of the molecule [20], and thus may affect the stability of metabolites obtained by hydroxylation of the carbon atom of the *N*-methyl moiety.

MATERIALS AND METHODS

Chemicals. Glucose-6-phosphate, glucose-6-phosphate dehydrogenase and NADP were purchased from Sigma Chemical Co. (Poole, U.K.). All *N*-methyl-substituted benzamides and their respective *N*-desmethyl derivatives were prepared by reaction of the appropriate benzoyl chloride with the corresponding amine by the Schotten–Baumann technique [21]. All *N*-(hydroxymethyl)-benzamides were prepared by treatment of the corresponding benzamide with excess aqueous formaldehyde (37%) in boiling tetrahydrofuran in the presence of potassium carbonate.

Animals. Male Balb c mice (20–25 g) were used for all experiments.

In vitro *metabolism.* Liver fractions and hepatocytes were prepared according to the methods described in [22] and [23], respectively. Incubations using liver fractions were performed in Earl's buffer, pH 7.4 (2.5 ml) in the presence of 5 mM $MgCl_2$ and sufficient glucose-6-phosphate, glucose-6-phosphate dehydrogenase and NADP to yield 1 mM NADPH. Reactions were started by the addition of substrate in a volume of not more than 0.1 ml of the solvent indicated below. Substrate concentrations used were: *N*-methylbenzamides 10 mM (methanol), except in the case of incubations with isolated hepatocytes where a concentration of 1 mM (acetone) was used; *N,N*-dimethylbenzamide 5 mM (acetone). Microsomes were added to give concentrations equivalent to 1 g and 0.7 g wet liver weight/2.5 ml in the case of incubations containing *N*-methylbenzamide and *N,N*-dimethylbenzamide, respectively. Supernatant (9000 g) was added to give a concentration equivalent to 0.25 g wet liver weight/2.5 ml. Incubations with hepatocytes were performed as described previously [24].

At the end of the incubation period, reactions were stopped by immersing the incubation mixtures in ice. For HPLC analysis 0.15 ml of the appropriate internal standard (see below) was added and the mixtures were prepared as described previously [24]. For colorimetric analysis of formaldehyde equivalents formed during microsomal metabolism, microsomes were removed by centrifugation at 27,000 *g* for 20 min. Then to 2 ml of the supernatant from duplicate incubations, either 0.25 ml Earl's buffer, pH 7.4, or 0.25 ml 1 M NaOH (to hydrolyse stable carbinolamines) was added. The mixtures were left at room temperature for 5 min and were then assayed for formaldehyde according to the method of Nash [25].

In vivo *metabolism.* *N*-Methylbenzamide was injected into mice i.p. in 0.1 ml of 10% DMSO/arachis oil to give a dose of 200 mg/kg. Urine was collected and prepared for analysis as described previously [24].

HPLC analysis. Separation of metabolites was performed using an Altex 100A pump, a Waters radial compression unit equipped with a C_{18}, 5 μm reverse phase column and a Pye LC–UV detector ($\lambda = 254$ nm). The conditions used for the separation of standard compounds were: (a) *N,N*-dimethylbenzamide, *N*-methylbenzamide, *N*-(hydroxymethyl)-benzamide and benzamide—mobile phase 30% methanol–water, flow rate 2 ml/min, internal standard 4-nitrobenzamide 0.2 mg/ml in methanol; (b) 4-chloro-*N*-methylbenzamide, 4-chloro-*N*-(hydroxymethyl)-benzamide and 4-chlorobenzamide—mobile phase 50% methanol–water, flow rate 1.5 ml/min, internal standard 4-nitrobenzamide 0.2 mg/ml in methanol; (c) 4-*t*-butyl-*N*-methylbenzamide, 4-*t*-butyl-*N*-(hydroxymethyl)-benzamide and 4-*t*-butylbenzamide—mobile phase 57% methanol–water, flow rate 2 ml/min, internal standard 4-chlorobenzamide 0.2 mg/ml in methanol.

Isolation and characterization of metabolites. HPLC eluates corresponding to peaks with the retention times of authentic standards were collected from the column and evaporated to dryness under a stream of nitrogen at room temperature. The residues were dissolved in acetone (0.025 ml) and subjected to mass spectral analysis. Samples were either admitted directly to the mass spectrometer under chemical ionization conditions (as described previously [24]) or where derivatized and analyzed by electron impact GC–MS.

Derivatization of isolated metabolites. Metabolites and standard compounds, chromatographically isolated, were dissolved in pyridine (0.05 ml), and BSTFA (0.05 ml) was added. The mixtures were heated at 60° for 15 min and subjected to GC–MS analysis.

GC–MS analysis. GC–MS analysis was performed

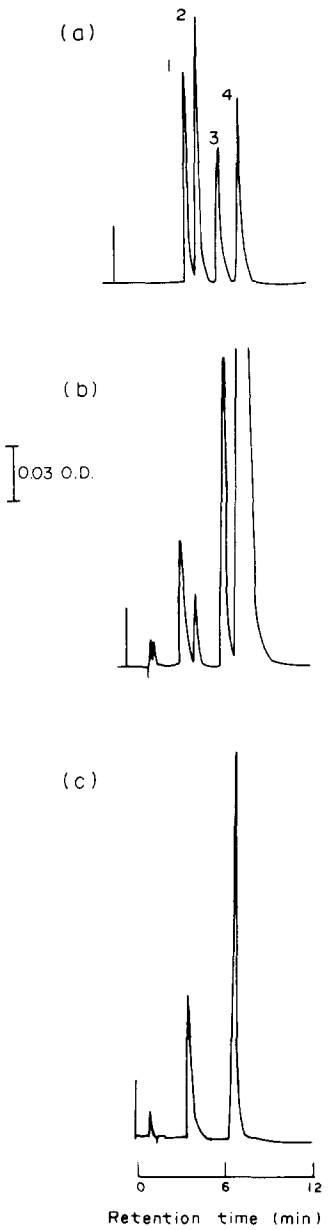


Fig. 2. High pressure liquid chromatograms of extracts of (a) a mixture of the following reference compounds: 1, *N*-hydroxymethylbenzamide; 2, benzamide; 3, internal standard; 4, *N*-methylbenzamide; (b) a mixture of *N*-methylbenzamide (10 mM) with microsomes and a NADPH-generating system, incubated for 30 min at 37°; and (c) as incubation mixture (b) but omitting *N*-methylbenzamide.

using a Pye 204 gas chromatograph equipped with a fused silica SE52 capillary column linked to a VG 7070 mass spectrometer. The injector temperature was 250°, the column temperature was 100° initially increasing by 32°/min for 5 min. A 1:10 split of carrier gas (helium) was used, the mass spectrometer source temperature was 220°, and an electron energy of 70 eV was used.

RESULTS

The incubation of *N*-methylbenzamide with microsomes and an NADPH-generating system produced a metabolite with a retention time identical to that of *N*-(hydroxymethyl)-benzamide (1, Fig. 2). The identity of this metabolite was confirmed by comparison with authentic *N*-(hydroxymethyl)-benzamide using GC–MS analysis after derivatization with BSTFA (Fig. 3). 4-Chloro-*N*-methylbenzamide and 4-*t*-butyl-*N*-methylbenzamide were likewise metabolized to their respective *N*-(hydroxymethyl) derivatives in microsomes. These metabolites were characterized by comparison of their retention times with those of authentic standards and, in the case of 4-chloro-*N*-(hydroxymethyl)-benzamide, mass spectrometry (not shown). The extent of biotransformation of *N*-methylbenzamide and its 4-chloro and 4-*t*-butyl derivatives to their respective *N*-methylols and *N*-desmethyl derivatives in microsomal and 9000 *g* supernatant fractions of liver is shown in Table 1.

A chromatogram of an extract of the products of metabolism of *N*-methylbenzamide by isolated hepatocytes is shown in Fig. 4. *N*-(hydroxymethyl)-benzamide (1, Fig. 4), benzamide (2, Fig. 4) and *N*-formylbenzamide (5, Fig. 4) were identified as metabolites of *N*-methylbenzamide (4, Fig. 4) on the basis of their chromatographic retention times. These metabolites were also detected in the urine of mice which had received *N*-methylbenzamide, 200 mg/kg i.p. (Fig. 5). 4-Chloro-*N*-methylbenzamide and 4-*t*-butyl-*N*-methylbenazmide were also metabolized to their *N*-(hydroxymethyl) derivatives by isolated hepatocytes and the extent of their biotransformation compared to that of *N*-methylbenzamide is shown in Table 2.

On incubation with microsomes, *N,N,*-dimethylbenzamide (4, Fig. 6) produced a metabolite (3, Fig. 6) with a retention time identical to that of *N*-methylbenzamide and another metabolite (1, Fig. 6). The identity of metabolite 3 (Fig. 6) was confirmed as *N*-methylbenzamide by mass spectrometry. Metabolite 1 (Fig. 6) decomposed upon alkaline hydrolysis with a corresponding increase in the amount of *N*-methylbenzamide present in the mixture. The time course of production of metabolites 1 and 3 (Fig. 6) is shown in Fig. 7, and indicates that the amount of metabolite 1 first increased and then decreased. The decrease was presumably due to chemical degradation or further metabolism of metabolite 1. Metabolite 1 was derivatized using BSTFA and subjected to GC–MS analysis. The mass spectrum obtained indicated that metabolite 1 was *N*-(hydroxymethyl)-*N*-methylbenzamide [*m/z* 237, M^+ for $C_6H_5CON(CH_3)CH_2OSi(CH_3)_3$, 61.2%; *m/z* 222 $[C_6H_5CON(CH_3)CH_2OSi(CH_3)_2]^+$, 37.5%; *m/z* 192 $[C_6H_5C(OSi(CH_3)_2)=NCH_3]^+$, 40.7%; *m/z* 105, $C_6H_5CO^+$, 100%]. Attempts to synthesize *N*-hydroxymethyl-*N*-methylbenzamide were not successful.

Formaldehyde, or a precursor which reacted like formaldehyde with Nash reagent, was generated during the microsomal metabolism of *N,N*-dimethylbenzamide and the amount of Nash-positive species in the incubation mixture increased after alkaline hydrolysis (Table 3). In the case of *N*-methylbenzamide no formaldehyde could be detected in the metabolism mixture unless alkaline hydrolysis was

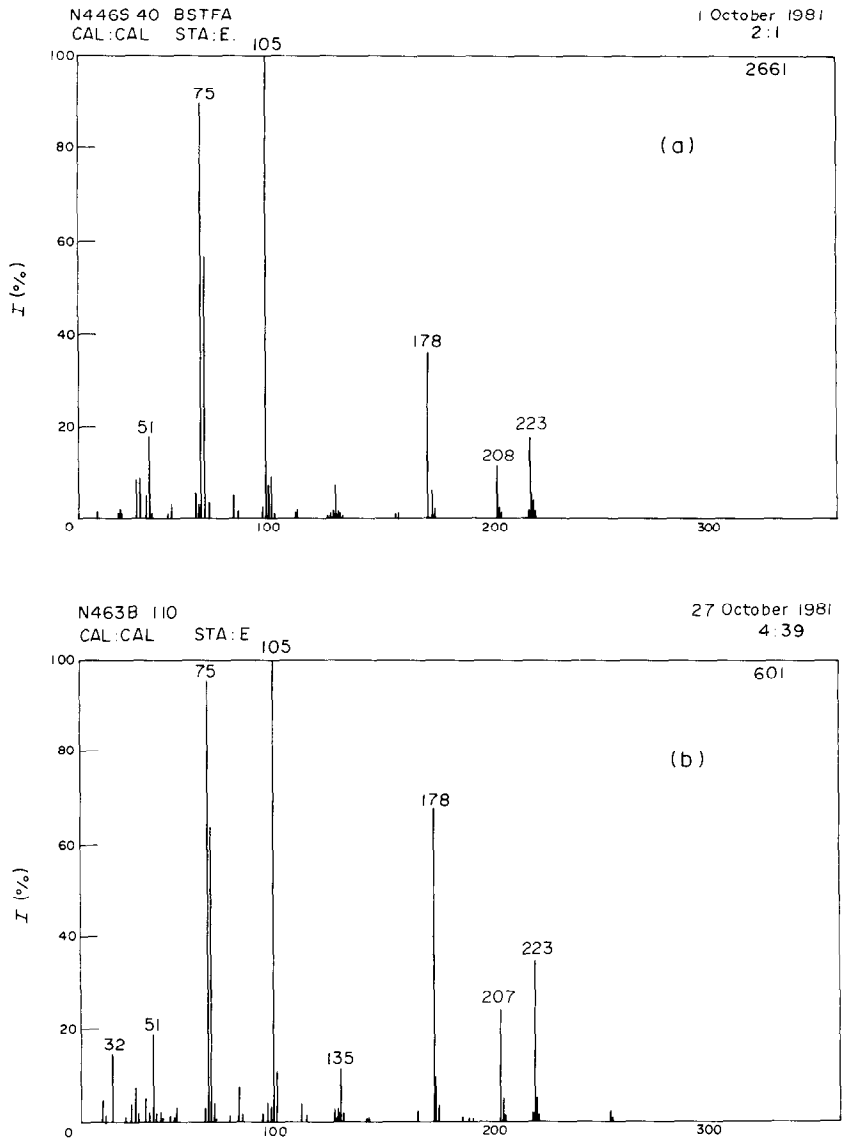


Fig. 3. Mass spectra of a peak (Rt = 8.8 min) produced during the GC–MS analysis of authentic *N*-hydroxymethylbenzamide (a) and an *in vitro* metabolite of *N*-methylbenzamide with an identical retention time on HPLC (b), after derivatization with BSTFA.

Table 1. The extent of *N*-demethylation of 4-substituted *N*-methylbenzamides (10 mM) in microsomal and 9000 *g* supernatant fractions of liver

	% Metabolism* to			
	N-(hydroxymethyl) derivative		*N*-desmethyl derivative	
Substrate	microsomes	9000 *g* supernatant	microsomes	9000 *g* supernatant
N-Methylbenzamide	2.8	2.9	0	0
4-Chloro-*N*-methylbenzamide	1.7	1.7	0	0
4-*t*-Butyl-*N*-methylbenzamide	0.6	0.5	0	0.2

* Mixtures contained either microsomes equivalent to 1 g wet liver weight or 9000 *g* supernatant equivalent to 0.25 g wet liver weight and sufficient cofactors to generate 1 mM NADPH. Mixtures were incubated at 37° for 30 min.

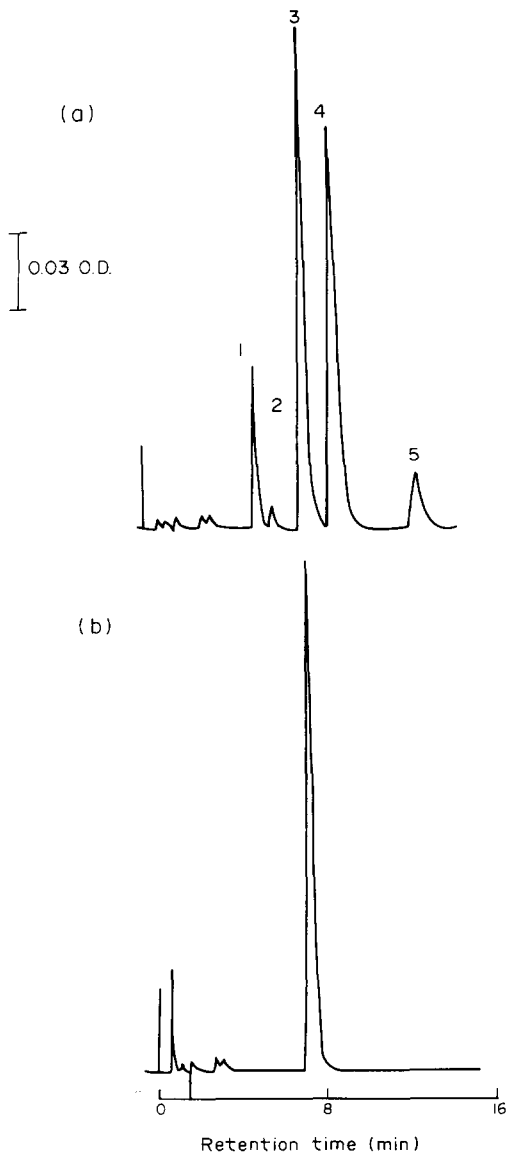


Fig. 4. High pressure liquid chromatograms of (a) an extract of a mixture of *N*-methylbenzamide (1 mM) with hepatocytes incubated at 37° for 90 min; and (b) an extract of an incubation of mixture omitting *N*-methylbenzamide. (1, *N*-hydroxymethylbenzamide; 2, benzamide; 3, internal standard; 4, *N*-methylbenzamide; 5, *N*-formylbenzamide).

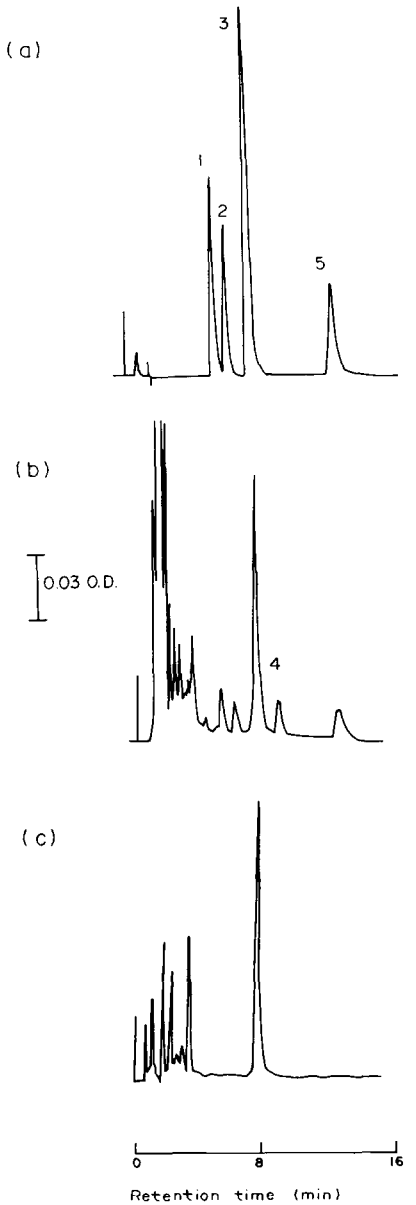


Fig. 5. High pressure liquid chromatograms of extracts of (a) a mixture of reference compounds (for identification see legend to Fig. 4); (b) urine from mice which had received *N*-methylbenzamide (4) 200 mg/kg i.p.; and (c) urine from mice which had received vehicle only.

Table 2. The metabolism of 4-substituted *N*-methylbenzamides (1 mM) by isolated hepatocytes

Substrate	% Metabolism* to *N*-(hydroxymethyl) derivative	*N*-desmethyl derivative	*N*-formyl derivative
N-Methylbenzamide	10.5	2.5	2.2
4-Chloro-*N*-methylbenzamide	8.6	6.9	n.d.
4-*t*-Butyl-*N*-methylbenzamide	†	†	n.d.

* After 90 min incubation at 37° with 5×10^5 cells/ml.
† Below 0.1%.
n.d., Not determined.

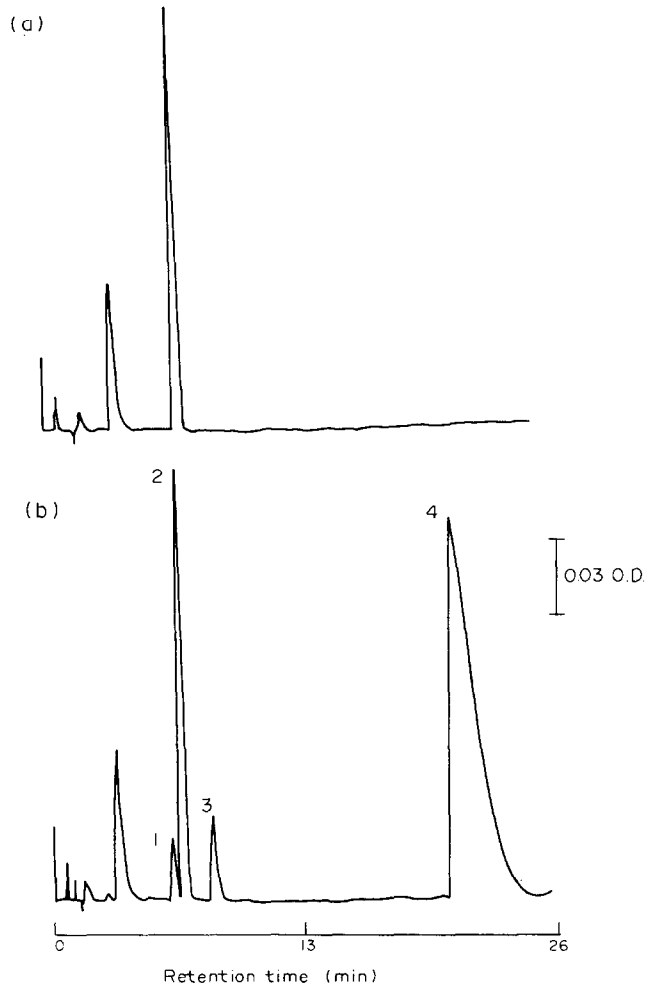


Fig. 6. High pressure liquid chromatograms of (a) an extract of a mixture of microsomes and a NADPH-generating system incubated for 30 min at 37°; (b) an extract of an identical incubation mixture containing *N,N*-dimethylbenzamide (5 mM). (1, Unidentified metabolite; 2, internal standard; 3, *N*-methylbenzamide; 4, *N,N*-dimethylbenzamide.)

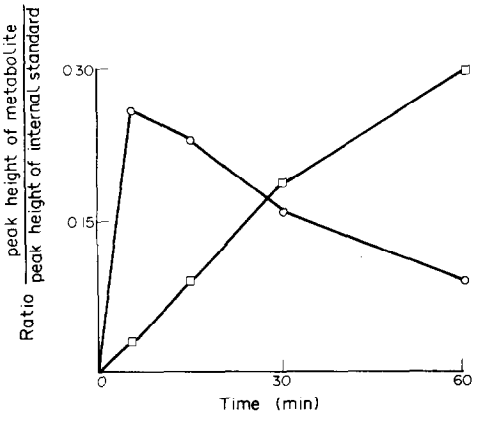


Fig. 7. Time course of production of *N*-methylbenzamide (□) and an unidentified metabolite (○) during the metabolism of *N,N*-dimethylbenzamide (5 mM) in microsomes.

performed prior to the Nash reaction (Table 3). It was conceivable that the differences in the production of Nash-positive species during the metabolism of *N*-methylbenzamide and *N,N*-dimethylbenzamide were due to the different stabilities of their respective *N*-(hydroxymethyl) compounds formed during metabolism. This was confirmed by an investigation of the stability of authentic *N*-(hydroxymethyl)-benzamide and metabolically-generated *N*-(hydroxymethyl)-*N*-methylbenzamide in various buffer systems at 37° (Fig. 8). The results show that under alkaline conditions *N*-(hydroxymethyl)-benzamide is more stable than *N*-(hydroxymethyl)-*N*-methylbenzamide.

In all *in vitro* incubations, no other metabolites were identified when the pH of the incubation mixtures were adjusted to pH 2 and re-extracted after the normal extraction process had been performed. This implies the absence of phenolic metabolites which may have been ionized at pH 7.4 and thus

Table 3. Formaldehyde equivalents generated during the microsomal metabolism of *N,N*-dimethylbenzamide (5 mM) and *N*-methylbenzamide (10 mM), measured using the Nash reagent before and after alkaline hydrolysis

Substrate	Formaldehyde equivalents (μM) measured Before alkaline hydrolysis	After alkaline hydrolysis
N,N-dimethylbenzamide (5 mM)	195	349
N-Methylbenzamide (10 mM)	0	90

Results are from one experiment, representative of three.

may not have been removed during the first extraction. When the pH of the urine of mice which had received *N*-methylbenzamide was adjusted to pH 2 and re-extracted, the liquid chromatogram of the extract showed two major peaks which were not extracted from control urine adjusted to pH 2. The chemical ionization mass spectrum of one of these metabolites suggested that it may be hippuric acid [*m/z* 180, MH^+, 100%; *m/z* 135, $(MH-COOH)^+$, 13%; *m/z* 105, $C_6H_5CO^+$ 66%]. Benzoic acid can be converted to hippuric acid in animals [26] and thus it is possible that this metabolite was formed *in vivo* by enzymic hydrolysis of the amide moiety followed by conjugation with endogenous glycine.

DISCUSSION

Although there are many examples in the literature of the biotransformation of *N*-methyl-containing xenobiotics to characterizable metabolites which possess *N*-(hydroxymethyl) groups (summarized in [6]), the conditions and structural requirements for their formation have not been studied. We report here that *N*-(hydroxymethyl)-benzamide, a stable compound which can be readily synthesized from formaldehyde and benzamide, is indeed a major metabolite *in vitro* and a minor metabolite *in vivo* of *N*-methylbenzamide. 4-Substitution in the phenyl ring of *N*-(hydroxymethyl)-benzamide alters its rate of breakdown to produce formaldehyde [6, 19]. However, 4-chloro-*N*-(hydroxymethyl)-benzamide and its 4-*t*-butyl derivatives both have half-lives in Earl's buffer, pH 7.4, at 37° of greater than 9 hr [6]. Thus these compounds would be expected to be sufficiently stable to be isolated using the conditions described in this study. This was confirmed by the characterization of *N*-methylols during the metabolism *in vitro* of 4-chloro-*N*-methylbenzamide and its 4-*t*-butyl derivative. Therefore although 4-substitution in the phenyl ring of *N*-methylbenzamides does result in quantitative differences in their metabolism (Tables 1 and 2), it does not alter the stability of the respective *N*-methylol derivatives sufficiently to cause qualitative differences in metabolism, i.e. to affect whether a stable *N*-(hydroxymethyl) compound or formaldehyde is produced.

N-(Hydroxymethyl)-benzamide and its 4-chloro and 5-*t*-butyl derivatives, like *N*-(hydroxymethyl)-formamide [15], are so stable at pH 7.4 that they do

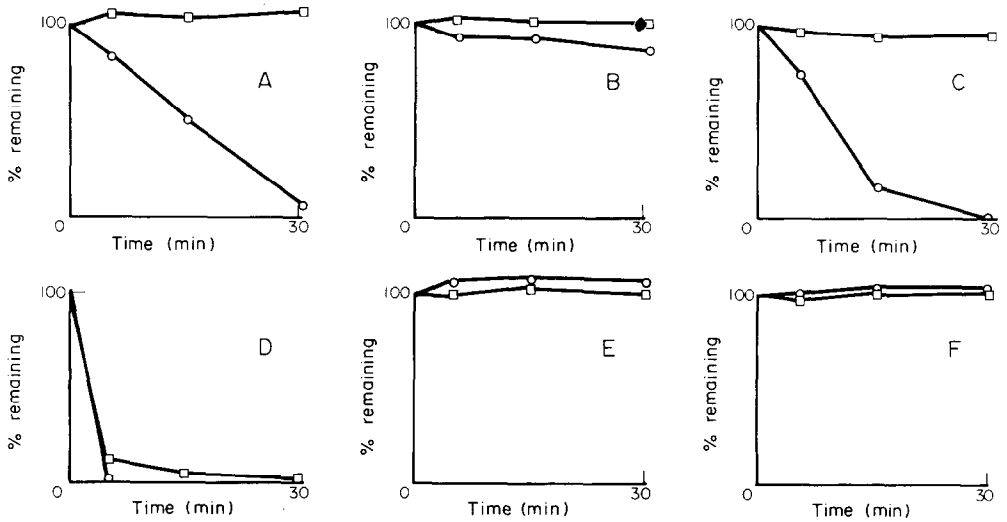


Fig. 8. The stability of authentic *N*-(hydroxymethyl)-benzamide (□) and metabolically-generated *N*-(hydroxymethyl)-*N*-methylbenzamide. (○) at 37° in the following buffer system: A—Earl's, pH 7.4; B—0.01 M Tris, pH 7.4; C—0.01 M Tris, pH 9; D—0.01 Tris, pH 12; E—acetate, pH 5; F—acetate, pH 2.8.

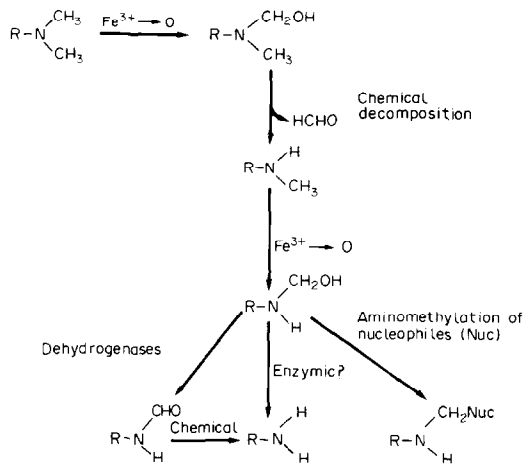
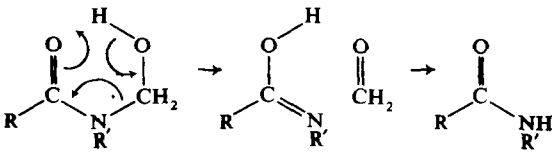


Fig. 9. Proposed scheme for the metabolism of the methyl groups of *N*-methyl-substituted benzamides.

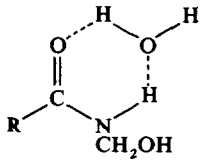
not react in the Nash assay. This assay detects formaldehyde and is used routinely to measure metabolic *N*-demethylation. Thus, assessment of the *N*-demethylation of these compounds by means of the Nash reaction, without a confirmatory chromatographic analysis, would produce erroneous results.

N-Formylbenzamide (5, Fig. 4 and 5), as well as *N*-(hydroxymethyl)-benzamide (1, Figs, 4 and 5) and benzamide (2, Figs. 4 and 5), were identified as metabolites of *N*-methylbenzamide (4, Figs. 4 and 5) in hepatocytes and *in vivo* on the basis of chromatographic retention times. We have shown previously that *N*-(hydroxymethyl)-benzamide can be metabolized *in vitro* to *N*-formylbenzamide by pyrazole-sensitive dehydrogenases [24]. Unlike *N*-(hydroxymethyl)-benzamide, *N*-formylbenzamide is an unstable species and degrades rapidly to produce benzamide. Thus it seems probable that the route whereby *N*-formylbenzamide and benzamide are produced *in vivo* involves the production and further metabolism of *N*-(hydroxymethyl)-benzamide (see Fig. 9).

Surprisingly, another substituent on the nitrogen atom of *N*-methylbenzamide markedly affected the stability of the *N*-methylol produced during *N*-demethylation. The *N*-(hydroxymethyl) compound produced during the metabolism of *N,N*-dimethylbenzamide was an unstable species and degraded partly to produce formaldehyde under the conditions used for *in vitro* metabolism studies. *N*-(Hydroxymethyl)-*N*-methylbenzamide, unlike *N*-(hydroxymethyl)-benzamide, produced a positive response in the Nash test prior to its hydrolysis by alkali (Table 3). Therefore, in the metabolic scheme proposed for the metabolism of the *N*-methyl groups in *N,N*-dimethylbenzamide (Fig. 9), the first *N*-demethylation leads to formaldehyde liberated from the degradation of an unstable *N*-methylol whereas the second *N*-demethylation yields a stable *N*-methylol. That the *N*-(hydroxymethyl)-*N*-methyl derivative is less stable than the *N*-(hydroxymethyl) derivative may be due to the electron-donating effect of the second methyl moiety which would oppose the electron-withdrawing effect of the carbonyl group. Alternatively the lack of an amide hydrogen atom in *N*-(hydroxymethyl)-*N*-methylbenzamide may prevent solvent participation in hydrogen bonding and this may account for the difference in stabilities. Tanka *et al.* [27] have suggested that intramolecular hydrogen bonding of *N*-(hydroxymethyl)-*N*-phenylureas (R = C_6H_5NH) leads to their degradation as described below and this may also apply to *N*-(hydroxymethyl)-benzamides (R = C_6H_5).



These workers suggested that the greater stability of the *N*-(hydroxymethyl) derivative (**I**) as opposed to the *N*-(hydroxymethyl)-*N*-methyl derivative (**II**) may be due to the fact that the *N*-hydroxymethyl group in **I** can be held in a configuration *trans* to the carbonyl group by a water molecule.



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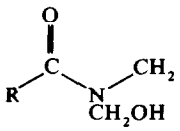

II

In the case of **II** this hydrogen bonding is not possible due to the lack of an amide hydrogen atom, and consequently the formation of the cyclic transition state leading to breakdown of the molecule is more probable. Another possibility is that lack of the amide hydrogen atom in **II** may prevent tautomerism to the enol form of the molecule, which would prevent the generation of the transition state and subsequent degradation. Whatever the reason, it is conceivable that *N*-methylols of different stabilities may be generated during the successive *N*-demethylations of other *N*-methyl-containing substrates.

Like *N*-methylols produced during successive *N*-demethylations of the same substrate, *N*-methylols generated during the metabolism of different types of *N*-methyl-containing compound may have varying stabilities [15]. Furthermore, different types of *N*-methylols may undergo degradation by different pH-dependent mechanisms. For example, it has been shown that *N*-methylols derived from *N*-methylmelamines are more stable in basic rather than acidic solution [28], whereas *N*-methylolamides such as *N*-(hydroxymethyl)-benzamide [6, 19] and *N*-(hydroxymethyl)-nitrosamines [29] are more stable under acidic conditions. This varying stability of *N*-methylols may have toxicological implications as either the intact *N*-methylol [7, 8] or its degradation products, formaldehyde [2] and in certain cases the *N*-des-(hydroxymethyl) species [30–32], are capable of reacting with biological nucleophiles. It is therefore conceivable that particular types of *N*-methyl may be responsible for the antitumour activity observed with certain *N*-methyl-containing com-

pounds, e.g. *N*-methylmelamines [33], 1-aryl-3,3-dimethyltriazenes [34] and *N*-methylformamide [35]. Similarly the generation of *N*-methylols of a particular stability or reactivity may contribute to the toxicity and carcinogenicity of other *N*-methyl-containing compounds such as *N,N*-dimethylnitrosamine [36], azoxymethane [37] and *N,N*-dimethylaminoazobenzene [38]. This hypothesis and the possible involvement of *N*-formyl compounds produced during *N*-demethylation in the pharmacological and toxicological effects of *N*-methyl-containing compounds are currently under investigation in these laboratories.

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