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

THE OXIDATIVE *N*-DEMETHYLATION OF *N*-DIMETHYL DERIVATIVES OF ARYLAMINES, ARYLTRIAZENES, ARYLFORMAMIDINES AND ARYLUREAS INCLUDING THE HERBICIDE MONURON

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Abstract—The metabolism of the *N*-methyl moieties of aryldimethylamines and *N*-methyl compounds of the general formula Aryl-*X*-N(Me)$_2$, where *X* is either —N=N— (3-aryl-1,1-dimethyltriazenes), —NHCO— (*N'*-aryl-*N*,*N*-dimethylureas) or —N=CH— (*N'*-aryl-*N*,*N*-dimethylformamidines) was studied using mouse liver microsomes. Products of microsomal metabolism were reincubated with mouse liver homogenate devoid of microsomes and assayed colourimetrically for formaldehyde. This allows metabolically generated formaldehyde to be distinguished from formaldehyde precursors. Whereas the *N*-methyl moieties of the aryldimethyltriazenes, formamidines and amines were metabolised to formaldehyde, the aryldimethylureas formed stable formaldehyde precursors upon metabolism. The products of metabolism of one such aryldimethylurea, the herbicide monuron (*N'*-(4-chlorophenyl)-*N*,*N*-dimethylurea) were investigated using a high pressure liquid chromatographic method. Two metabolites were found on incubation of monuron with microsomes, one of which was identified as the *N*-desmethyl compound by mass spectrometry. The other product showed chromatographic properties similar to 4-chlorophenylurea but resembled the monomethylaryl urea on mass spectral analysis. It is concluded that this metabolite is likely to be *N'*-(4-chlorophenyl)-*N*-hydroxymethyl-*N*-methylurea. A urinary product of conjugative metabolism obtained after the administration of monuron to mice also gave the mass spectrum of the monomethyl compound after deconjugation which suggests that a conjugated *N*-hydroxymethyl compound may have been formed *in vivo*.

The oxidative *N*-demethylation of drugs which contain *N*-methyl groups (A in Fig. 1) is a metabolic pathway which is ubiquitous in xenobiochemistry. The pathway is considered to be initiated by the hydroxylation of the methyl carbon to form an N-hydroxymethyl or carbinolamine compound (Fig. 1, B). These *N*-hydroxymethyl compounds are generally thought to be unstable and to decompose to yield the *N*-desmethyl compound (Fig. 1, C) and formaldehyde [1]. It is probable that for the majority of *N*-methyl containing xenobiotics this reaction sequence occurs in the liver, an organ which is able to detoxify the formaldehyde, a species which might otherwise be toxic to peripheral cells [2].

Some xenobiotics do, however, undergo oxidative *N*-demethylation to yield relatively stable *N*-hydroxymethyl compounds, which are sometimes identified as their conjugates [3–11]. This is not altogether surprising given that the reaction between certain amines or amides and formaldehyde can give rise to characterisable, synthetic *N*-hydroxymethyl compounds of varying stability [12]. However, only certain amines and amides undergo this reaction, a fact which may be pertinent to the observation that relatively few *N*-hydroxymethyl compounds have been isolated after the metabolism of their progenitor *N*-methyl compounds.

We wished to determine those molecular features of *N*-methyl containing xenobiotics which might predispose them to form characterisable *N*-hydroxymethyl compounds and to estimate the stability of the latter. This was considered to be important for two reasons. Firstly, if stability was such that either the inherently reactive *N*-hydroxymethyl compound or the formaldehyde formed on its breakdown were available extrahepatically, such compounds may present a potential toxicological hazard to the host. Secondly, certain *N*-methyl containing antitumour drugs have been shown to form relatively stable *N*-hydroxymethyl compounds which have been implicated in their cytotoxicity [13]. Thus, an understanding of the molecular features which give rise to such compounds may be useful in predicting the structures of novel agents to be screened as potential antineoplastic drugs.

In this paper we report on the results obtained from a colourimetric assay [14] which distinguishes between free formaldehyde and its precursors such as *N*-hydroxymethyl compounds, formed from the *in vitro* metabolism of certain model compounds

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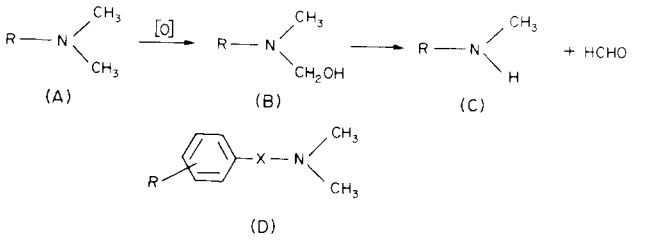


Fig. 1. Metabolism of *N,N*-dimethyl compounds.

which contain the *N*-methyl group. In addition, selected compounds have been subjected to HPLC (high pressure liquid chromatographic) analysis after their metabolism *in vitro*, in order to examine in more detail whether stable *N*-hydroxymethyl compounds had been formed. The model compounds where *X* equals —N=N— (triazenes), *X* equals —NHCO— (ureas), *X* equals —N=CH— (formamidines), or where *X* was absent (amines). Our rationale for choosing these particular types of compound was based primarily on previous studies of cytotoxic dimethylaryltriazenes where synthetic carbinolamines were shown to be relatively stable compounds [15], one of which was isolated as a conjugate in the urine of rats given a dimethylaryltriazene [11]. The ureas and formamidines are structurally similar to the aryltriazenes but the nitrogen bearing the methyl groupings is placed in different electronic and steric environments. The arylamines were chosen as simpler models and were considered appropriate to this study as evidence had been presented which suggests that a substituted arylamine, 3'-methyl-4-(methylamino)-azobenzene (MAB) may be metabolised to a carbinolamine [16].

MATERIALS AND METHODS

Animals and compounds. Male BALBc mice (20–25 g) were used for all metabolism experiments. The aryl dimethylamines (Ia–e Table 1) used in this study were obtained commercially. The 3-aryl-1,1-dimethyltriazenes (IIa–e) were prepared by treatment of the appropriate aryldiazonium salt with aqueous dimethylamine according to published procedures [17]. Condensation of anilines with dimethylformamide dimethylacetal, generally by published methods [18], furnished the formamidines (IIIa–f). IIIa, b, d, e were isolated as their tosylate salts.

Ureas (IVa, b, e, f, g) in addition to *N'*-(4-chlorophenyl)-*N*-methylurea (VI) and 4-chlorophenylurea (VII) were prepared by addition of a solution of the corresponding aryl isocyanate in diethyl ether or tetrahydrofuran to a large excess of ethereal dimethylamine. Melting points were consistent with published values [19–22].

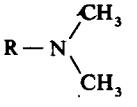
N'-(4-Cyanophenyl)-*N,N*-dimethylurea (IVc): 4-cyanobenzoic acid (5.88 g) was treated with boiling thionyl chloride (40 ml) and DMF (100 μl) for 2 hr. Evaporation of excess reagent gave the crude benzoyl chloride which, in acetone (50 ml), was added to 20% w/v aqueous sodium azide (80 ml). The mixture was stirred for 10 min, then extracted with ether (2 × 100 ml). The combined extracts were dried (Na_2SO_4), filtered and the solvents evaporated to give the crude benzoyl azide (ν_{max} (Nujol): 2220, 2180, 1690 cm^{-1}). This material, in dry toluene (100 ml), was stirred at reflux for 1 hr. The resulting solution of the isocyanate, from this Curtius reaction, was added to a tenfold excess of ethereal dimethylamine (300 ml). After 17 hr at ambient temperature, the solid was isolated and recrystallised from aqueous methanol to give the previously unreported *N'*-(4-cyanophenyl)-*N,N*-dimethylurea (4.90 g; 65%) as pale buff needles, MPt 152–153°. (Found: C 63.61%, H 5.63%; N 22.9%. Calculated for $C_{10}H_{11}N_3O$: C 63.48%; H 5.86%; N 22.21%.) ν_{max} (Nujol): 3410, 2260, 1730, 1600 cm^{-1}. $\delta((CD_3)_2SO)$: 7.75 (4H) s aryl-H, 7.1 (1H) br NH, 3.40 (6H) s $N(CH_3)_2$. *N*-(4-Trifluoromethylphenyl)-*N,N*-dimethylurea [23] was prepared similarly (from 4-trifluoromethylbenzoyl chloride) in 53% yield.

Assay for formaldehyde and its precursors. The metabolism of the *N*-methyl compounds was investigated essentially as described in [14]. In this assay substrates are incubated with hepatic microsomes for 30 min. At the end of the incubation period microsomes are precipitated by centrifugation and an aliquot of the supernatant is incubated with liver homogenate freed from microsomes. Metabolically generated formaldehyde is oxidised by the formaldehyde dehydrogenases which are abundant in mitochondria and liver cell cytosol, but virtually absent in microsomes [24]. Formaldehyde precursors, however, are not metabolised by these enzymes, and are detectable analytically as formaldehyde at the end of the incubation. Both formaldehyde and formaldehyde precursors were quantified by the colourimetric method according to Nash [25].

Substrate concentrations in the microsomal incubations varied between 0.5 and 5 mM according to the degree of *N*-demethylation of the substrates. Concentrations were used which gave substantial absorbance readings (>0.3 absorbance units) for Nash positive species at the end of the microsomal incubations. Control incubations were carried out without cofactors, and without substrate in the microsomal incubations. Substrates were also incubated with microsome free homogenate and NAD in the presence and absence of formaldehyde, to ensure that the substrates did not inhibit the removal of formaldehyde by aldehyde dehydrogenases, and

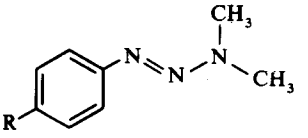
Table 1. *N,N*-Dimethyl compounds used in this study

Aryldimethylamines I



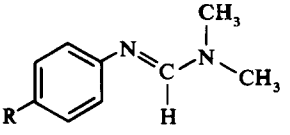
Ia: R = phenyl
b: R = 4-methylphenyl
c: R = cyanophenyl
d: R = pyridin-2-yl
e: R = pyridin-4-yl

Aryldimethyltriazenes II



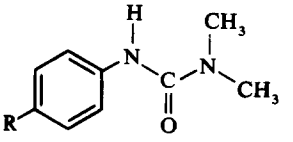
IIa: R = H
b: R = CF_3
c: R = Cl
d: R = $COCH_3$
e: R = CO_2CH_3

Aryldimethylformamidines III



IIIa: R = H
b: R = CH_3
c: R = CN
d: R = CF_3
e: R = Cl
f: R = SO_2NH_2

Aryldimethylureas IV



IVa: R = H
b: R = CH_3
c: R = CN
d: R = CF_3
e: R = Cl
f: R = Br
g: R = OCH_3

that the substrates themselves were not demethylated to Nash positive species by the microsome-free homogenate.

Incubation mixtures were deproteinized either by the addition of 20% trichloroacetic acid as described in [14], or in the case of incubations with acid labile triazenes as substrates with 0.6 ml of a 20% zinc sulphate solution followed by 0.6 ml of a saturated barium hydroxide solution. It is noteworthy to point out difficulties in the application of the colourimetric assay for formaldehyde to studies of the metabolism of the dimethyltriazenes. We found that the triazene derivatives IIa, d and e (Table 1) on reaction with Nash reagent formed species which absorbed at 412 nm, and thus superimposed the absorption of the chromophore produced from formaldehyde and Nash reagent. Interpretation was possible except in the case of IIe by using control incubations with triazenes and defining the conditions under which this led to high absorbance readings at 412 nm.

Metabolism of monuron. Metabolic incubations were performed in a final volume of 2.5 ml of Earls buffer (pH = 7.4) in which the final concentration of monuron was 1 mM. Cofactors were added to give a concentration of NADPH 1 mM and $MgCl_2$ 5 mM. Hepatocytes were prepared according to the method described in [26].

HPLC analysis of monuron and its metabolites. Metabolic incubates or urine samples were prepared for analysis by the addition of an equal volume of cold methanol containing internal standard (*N,N*-dimethyl-*N'*phenylurea), centrifuged and injected onto the HPLC column. Separation of the metabolites was performed on a 4.6 × 150 mm Ultrasphere ODS column (C_{18} reverse phase) using a linear gradient elution system from 10% methanol/water to 100% methanol over twenty minutes, a mobile phase flow rate of 1 ml/minute and a u.v. detection system (λ = 247 nm).

Chemical ionisation mass spectra. Chemical ionisation conditions were used as it was found that fewer contaminating peaks were seen in the spectra compared to those seen using electron impact. The mass spectra were determined on a VG 7070 mass spectrometer using isobutane as reagent gas. Spectra were run at a scan rate of 1 second/decade and were processed using a VG 2035 data system.

Mass numbers and percentage intensities of the major fragments in the mass spectra of compounds and metabolites referred to in the results section are as follows.

N'-(4-Chlorophenyl)-*N*-methylurea: m/z 185 (^{35}Cl-MH$^+$, 100%); m/z 187 (^{37}Cl-MH$^+$, 34.4%); m/z 168 (4.4%); m/z 151 (9.2%), m/z 127 (Cl-C_6H_4-NH_2^+, 13.2%). Metabolite with retention time identical with that of *N'*-(4-chlorophenyl)-*N*-methylurea on HPLC analysis : m/z 185 (100%); m/z 187 (36.2%); m/z 168 (6.6%); m/z 151 (8.7%); m/z 127 (14.2%). 4-Chlorophenylurea: m/z 171 (^{35}Cl-MH$^+$, 100%); m/z 173 (^{37}Cl-MH$^+$, 33.7%); m/z 127 (^{35}Cl-$C_6H_4NH_2^+$, 21.2%). Metabolite with retention time identical with that of 4-chlorophenylurea on HPLC analysis: m/z 185 (100%); m/z 187 (33.9%); m/z 168 (4.3%); m/z 151 (13.9%); m/z 127 (11.7%).

Deconjugation of urine samples. Urine samples were collected from mice in metabowl cages (Jencons, U.K.) after the injection of monuron 200 mg/kg i.p. as a suspension in 10% DMSO/arachis oil.

Enzymatic hydrolysis of urine samples was performed using 0.2 ml of urine diluted to 2 ml with acetate buffer (pH = 5) containing either β-glucuronidase (5000 u) or sulfatase (150 u sulfatase and

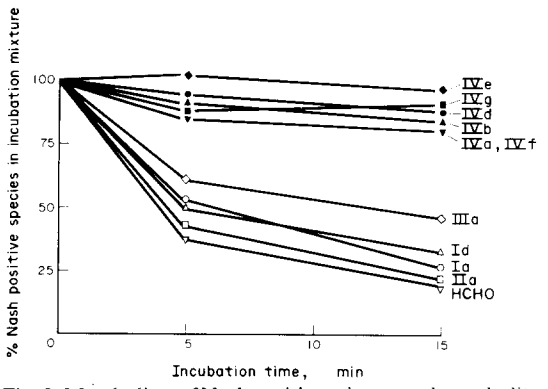


Fig. 2. Metabolism of Nash positive microsomal metabolites of dimethylaniline (Ia), 2-dimethylaminopyridine (Id), phenyldimethyltriazene (IIa), phenyldimethylformamidine (IIIa) and aryldimethylureas (IV a, b, d, e, g) by mouse liver homogenate free from microsomes, ∇ indicates disappearance of formaldehyde. Values are the mean of at least three experiments. Details of incubation conditions under Materials and Methods and in reference [14]. Results are expressed as percentage of the Nash positive species generated during the microsomal incubation after subtraction of control values obtained with incubations devoid of cofactors.

5000 u glucuronidase) (Sigma, U.K.). The samples were incubated at 37° for 17 hr and prepared for HPLC analysis as described above.

RESULTS

A number of *N*-methyl containing molecules (Table 1) were incubated with mouse liver microsomes and underwent oxidative *N*-demethylation to metabolites which gave a positive reaction with Nash reagent and were thus characterised as free formaldehyde or precursors of formaldehyde. Three compounds were not metabolized to species forming 3,5-diacetyl-2,6-dimethyl-1,4-dihydropyridine, the coloured chromophore in the Nash reaction: 4-dimethylaminopyridine (Ie), *N'*-(4-sulphonamidophenyl)-*N*,*N*-dimethylformamidine (IIIf) and *N'*-(4-cyanophenyl)-*N*,*N*-dimethylurea (IVc).

In order to test whether the species which gave a positive Nash reaction was free formaldehyde or a stable precursor of formaldehyde such as the *N*-hydroxymethyl metabolite B (Fig. 1), an aliquot of the microsomal incubate was reincubated with microsome-free liver homogenate as a source of formaldehyde oxidising enzymes. After 5 and 15 min the incubate was tested for residual Nash-positive species. Figure 2 shows the amount of such species obtained on incubation of compounds Ia, Id, IIa, IIIa, IVa–g, and formaldehyde, with microsome free liver homogenate. The Nash-positive metabolites of the 4-substituted derivatives of dimethylaniline Ib, Ic, phenyldimethyltriazenes IIb–d and phenyldimethylformamidine IIIb–e on exposure to the formaldehyde dehydrogenases in the microsome free liver homogenate behaved in essentially the same way as their unsubstituted congeners Ia, IIa, and IIIa and are not included in Fig. 2. The 4-substituted derivatives of phenyldimethylurea IVa–g formed metabolites which reacted with Nash reagent but were not substrates of formaldehyde metabolizing enzymes (Fig. 2). This result led us to conclude that the aryldimethylureas IV are metabolised to stable precursors of formaldehyde. In order to test if these precursors of formaldehyde were *N*-hydroxymethyl compounds, we subjected one derivative in this series, IVe, the herbicide monuron, to a more detailed metabolism study. Figure 3 shows the high pressure liquid chromatogram of a sample of the incubation mixture of monuron with mouse liver microsomes compared with a chromatogram of a solution containing reference compounds. Two metabolites were observed. On mass spectral investigation, one metabolite was identified as the *N*-demethylated derivative of IVe, *N'*-(4-chlorophenyl)-*N*-methylurea (VI, Fig. 4). The other metabolite in the chromatogram (Fig. 3) of the

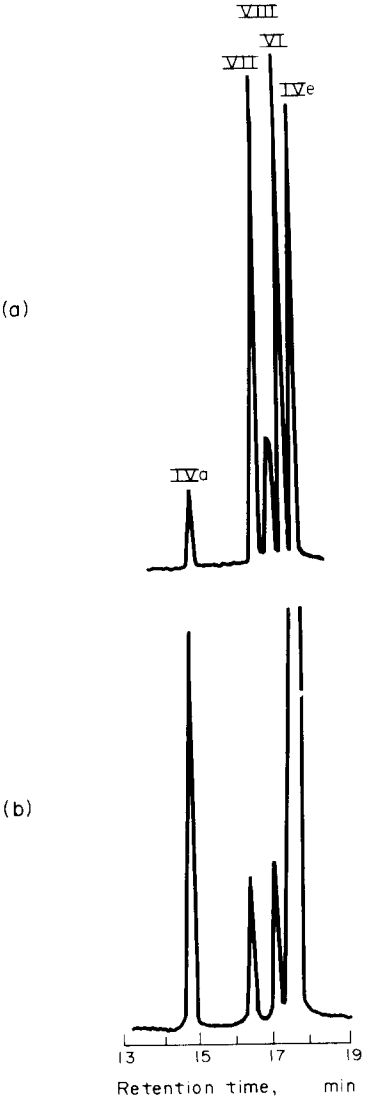


Fig. 3. High pressure liquid chromatogram of (a) a mixture of monuron (IVe), *N'*-(4-chlorophenyl)-*N*-methylurea (VI), 4-chloroaniline (VIII), 4-chlorophenylurea (VII) which were considered as possible metabolites and internal standard *N'*-phenyl-*N*,*N*-dimethylurea (IVa). (b) An extract of an incubation mixture of monuron (IVe) with mouse liver microsomes fortified with an NADPH generating system.

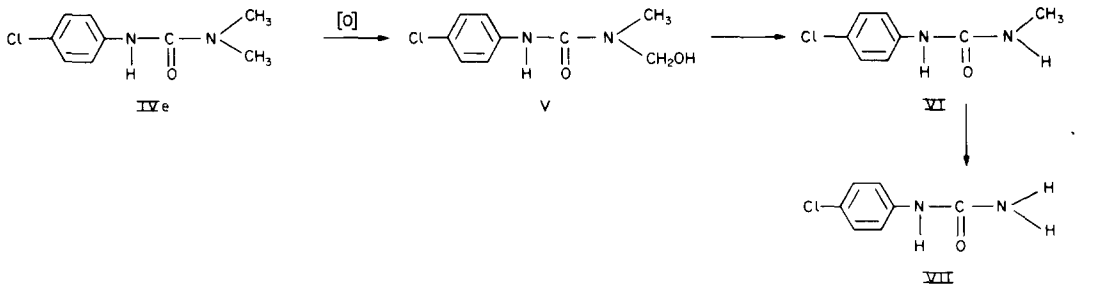


Fig. 4. Metabolism of monuron.

microsomal incubate had a retention volume similar to that of 4-chlorophenylurea (VII, Fig. 4). However, on treatment with acid or on heating the sample this metabolite decomposed with a corresponding increase in the amount of *N'*-(4-chlorophenyl)-*N*-methylurea VI. The chemical ionization mass spectrum of this unstable metabolite was identical with a synthetic sample of *N'*-(4-chlorophenyl)-*N*-methylurea. This suggested to us the possibility that the hydroxymethyl compound V (Fig. 4) had indeed been formed. This metabolite was also found on incubation of monuron with whole liver homogenate, 9000 g supernatant and isolated mouse hepatocytes. The major urinary metabolite of monuron was 4-chlorophenylurea VII, as identified by mass spectral investigation of the eluent obtained on high pressure liquid chromatography of a urine sample. After incubation of urine samples with a mixture of β-glucuronidase/sulfatase a metabolite was identified which on HPLC analysis (Fig. 5) and mass spectral comparison was identical with *N'*-(4-chlorophenyl)-*N*-methylurea VI.

DISCUSSION

There is no doubt that some xenobiotics containing *N*-methyl moieties are metabolised to identifiable *N*-hydroxymethyl compounds. Such species have been identified as metabolites of a number of *N*-methyl-amides [6, 7, 9], *N*-methyltriazenes [10, 11] and *N*-methylcarbazole [4]. The antineoplastic agents hexamethylmelamine and pentamethylmelamine were metabolized to formaldehyde precursors [14] and *N*-hydroxymethylpentamethylmelamine has been identified as the major *in vitro* metabolite of hexamethylmelamine [3]. Mueller and Miller [16] presented indirect evidence for the presence of an *N*-hydroxymethyl metabolite of the arylamine derivative 3'-methyl-4-(methylamino)-azobenzene (MAB). Their interpretation was based on the metabolic production of formaldehyde and on the ability of glutathione to react with a metabolic intermediate to yield a water soluble azo dye which could be hydrolyzed subsequently to the water insoluble *N*-demethylated aminoazo dye. It may be relevant that in all of these cases the *N*-methyl group is attached to molecules of electronegative character.

We investigated the *in vitro* metabolism of arylamine derivatives Ia–e (Table 1), one of which has the strongly electron-withdrawing cyano group in the 4-position of the aryl moiety (Ic) and which is

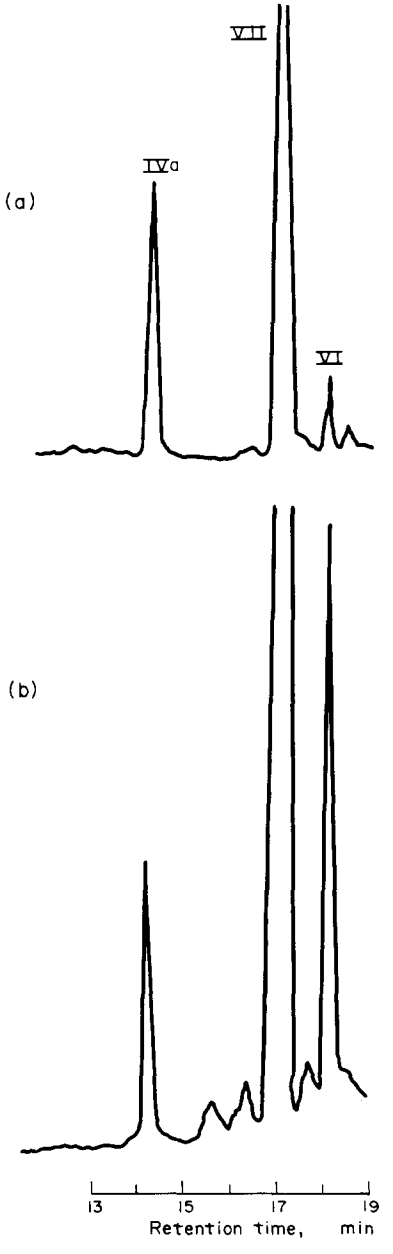


Fig. 5. High pressure liquid chromatogram of (a) an extract of urine of mouse after administration of 200 mg/kg monuron i.p., (b) an extract of a sample of the same urine after incubation with glucuronidase/sulfatase. For the explanation of the identifying numbers see legend to Fig. 3.

mutagen like MAB [27], but could not find evidence for the metabolic generation of formaldehyde precursors of measurable stability. The yellow colour of substituted aminoazobenzene derivatives such as MAB interfered with the colourimetric assay and prevented investigation of the metabolism of these compounds. Changing the arylamine structure I (Table 1) by introducing a —N=N— or a —N=CH— moiety into the molecule between the *N,N*-dimethyl and the aryl portions of I, leads to the triazenes II and the formamidines III which were metabolised to species which, like the metabolites of the *N,N*-dimethylarylamines I, behaved biochemically like formaldehyde (Fig. 2). The failure of the assay to detect stable *N*-hydroxymethyl metabolites of dimethyltriazenes is puzzling, in view of the reported isolation of a glucuronide of an *N*-hydroxymethyl-*N*-methyltriazene as a urinary metabolite of an aryl dimethyltriazene in the rat [11]. This may be due to species differences in metabolism, or may reflect a lack of conjugation in this microsomal system which could stabilise any *N*-hydroxymethyl metabolites when produced. Whatever the explanation the difficulties involved in predicting the metabolic behaviour of compounds *in vivo* from results obtained using *in vitro* model systems must be stressed.

The only compounds tested in this study which formed stable formaldehyde precursors were the urea derivatives IV (Table 1). These molecules differ from the *N,N*-dimethylarylamines I in that they contain an —NH—CO— structure inserted between the aryl ring and the *N,N*-dimethylamino group which reduces the electron density at the nitrogen atom bearing the *N*-methyl group. Evidence that the stable formaldehyde precursors were *N*-hydroxymethyl compounds was corroborated by the results of the chromatographic and mass spectral investigation of an *in vitro* metabolite of *N'*-(4-chlorophenyl)-*N,N*-dimethylurea (IVe, monuron). This metabolite behaved chromatographically like 4-chlorophenylurea (VII, Fig. 4) but its mass spectrum identified it as *N'*-(4-chlorophenyl)-*N*-methylurea (VI, Fig. 4). We suggest, therefore, that it is *N'*-(4-chlorophenyl)-*N*-hydroxymethyl-*N*-methylurea (V, Fig. 4). The glucoside conjugate of this compound (V) was reported as a product of the metabolism of monuron in cotton plants [28], but our results are the first indication that this *N*-hydroxymethyl metabolite of monuron is generated in animals. That a urinary metabolite of monuron on enzymatic deconjugation was identified as the monomethyl compound VI (Fig. 5) is also compatible with the suggestion that its precursor was the carbinolamine V. It is conceivable that V after hydrolysis of its glucuronide or sulphate derivatives decomposes on incubation at 37° in acetate buffer (pH = 5) to the monomethyl derivative VI. Alternatively this precursor may be a conjugate of VI linked to the conjugating species via either of the two nitrogen atoms.

All derivatives of IVa, except IVc, irrespective of the electron-withdrawing (IVd, e, f) or electron-donating (IVb, g) nature of the substitute formed metabolites which were formaldehyde precursors but not substrates of formaldehyde metabolising enzymes (Fig. 2). It therefore appears that the nature of the substitute in the aryl ring of IV does not determine whether metabolism leads to a stable carbinolamine or directly to formaldehyde, likewise the variation of substituents in the 4-position of the aryl moieties of the aryldimethyltriazenes II and the aryldimethylformamidines III does not change the instability of their metabolic *N*-hydroxymethyl intermediates. It is likely that several physicochemical factors influence the equilibrium which the carbinolamine B (Fig. 1) maintains with formaldehyde [29] and its is possible that of all the factors involved the electronic environment of the *N*-hydroxymethyl moiety as influenced by para substitute in the aryl ring is only of minor importance.

We have recently investigated the stability of the *N*-hydroxymethyl derivative of formamide, $OHC\text{-}NHCH_2OH$, and have found that it is surprisingly stable and does not react as a formaldehyde precursor unless treated with strong alkali [30]. Similarly, *N*-hydroxymethylbenzamide, ($C_6H_5\text{-}CO\text{-}NHCH_2OH$) has been reported to be very stable at physiological pH [31], and we have evidence that *N*-hydroxymethylbenzamide is indeed a metabolite of *N*-methylbenzamide ($C_6H_5\text{-}CO\text{-}NH\text{-}CH_3$). These recent findings (to be presented in a future report) and the results discussed above underline the fact that the oxidative metabolism of the *N*-methyl moiety in xenobiotic molecules is more complex than simply a pathway leading to the *N*-desmethyl compound and formaldehyde. Depending on as yet unknown factors associated with the structure of the molecule metabolites with *N*-hydroxymethyl groups can be generated with widely different stabilities under physiological conditions. Those of intermediate stability may decompose to liberate formaldehyde at extrahepatic sites or may participate in endogenous metabolic pathways as 'active formaldehyde' [32], or may aminomethylate biologically important targets. A recent report of the metabolism of MAB suggests that further reactions of a conjugated methylol may be relevant to the carcinogenicity of MAB [33] and we would suggest that the putative carbinolamines formed from other *N*-methyl containing carcinogens, such as dimethylnitrosamine, may similarly contribute to their toxicology, a hypothesis actively under investigation by us. Finally, it is also pertinent to stress the inadequacy of the colourimetric determination of formaldehyde when used to assess the metabolic *N*-demethylation of those substrates forming very stable *N*-hydroxymethyl compounds which do not react in the Nash test for formaldehyde.

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