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THE FORMATION OF A NOVEL MERCAPTURIC ACID DURING THE METABOLISM OF AN *N*-METHYL AROMATIC AMINE, 4-CYANO-*N*,*N*-DIMETHYLANILINE

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As part of a collaborative study on the genotoxicity of aromatic amines[1] we have recently investigated the metabolism of 4-cyano-*N*,*N*-dimethylaniline (CDA) in rats and mice. We now wish to report the formation of a sulphur containing metabolite, which indicates an important role of glutathione in the metabolism of an aromatic amine *N*-methyl group.

Materials and Methods: 4-Cyano-*N*,*N*-dimethyl-[^{14}C]aniline was given orally (18.5 mg kg^{-1}) to five male Fischer rats and four male ICI mice. The urine was collected on ice over 24 h. To obtain larger quantities of metabolites, a further four male rats were given four daily oral doses (65 mg kg^{-1}) of [^{14}C]CDA. All urine samples were quantitated by liquid scintillation counting, and analysed by TLC (SiO_2, ethyl acetate/formic acid/water, 70/4/4) and HPLC using acetonitrile/water gradients with a constant 0.5% v/v acetic acid. The eluant from the ODS column was monitored at 245 nm and radiochemically (Berthold ^{14}C radioflow detector). Urine (up to 1 ml) was injected directly onto the HPLC, and the metabolite was isolated by collection into liquid nitrogen-cooled flasks. A sample (ca. 3 μg) isolated by HPLC was analysed directly by fast atom bombardment mass spectrometry (FABMS)[2] (VG 7070E spectrometer). Samples for NMR (Brucker 360 MHz) were further purified by freeze-drying, taking up in methanol and passing down the HPLC for a second time. An authentic sample of the metabolite was synthesised from 4-cyanoaniline, formaldehyde and *N*-acetylcysteine in ethanol/water. The product was purified by HPLC.

Results: Elimination of radioactivity was rapid: 86.3 (rats) and 77.6% (mice) of the dose within 24 h. The novel metabolite, which is the subject of this paper, was formed in large amounts in both species (15% of the dose in rats, 33% in mice). The NMR and MS of the isolated metabolite are shown as Figs. 1 and 2. The combined data from the two spectroscopic analyses coupled with the similarity to the spectra and chromatographic properties of the synthetic material confirm the structure of the metabolite as *N*-acetyl-*S*-(4-cyano-anilinomethyl)cysteine.

Discussion: *N*-Demethylation has long been recognised as an important biotransformation of *N*-methyl aromatic amines[3]. Normally intermediates of this process are not isolated as they are highly reactive, although in a few cases, where the nitrogen is also bonded to an electron withdrawing group, *N*-methylols or their sulphate or glucuronide conjugates have been detected in animals[4]. *N*-Methylols have been shown to be capable of reacting at pH 7 *in vitro* with cysteine and glutathione, but there have, to date, been only two reports of the identification of thio-ether conjugates of *N*-methylols *in vivo*. Ketterer and co-workers have shown that, during the metabolism of dimethylaminoazobenzene by rats, *N*-methyl glutathione conjugates are excreted in the bile[5,6]. These metabolites were too unstable to be isolated and characterised by spectroscopic techniques. The *N*-methyl conjugate of CDA described here is unusual in that it is stable enough to survive the several enzymic steps that convert the glutathione function to an *N*-acetyl cysteine function. The isolation of this novel metabolite in high yields (15-33% of the dose) indicates that, during *N*-demethylation, an electrophilic intermediate with sufficient stability to react with cellular nucleophiles must be produced in large quantities. These three examples suggest that *N*-demethylation, widely regarded as an innocous biotransformation, may initiate cytotoxic and genotoxic responses.

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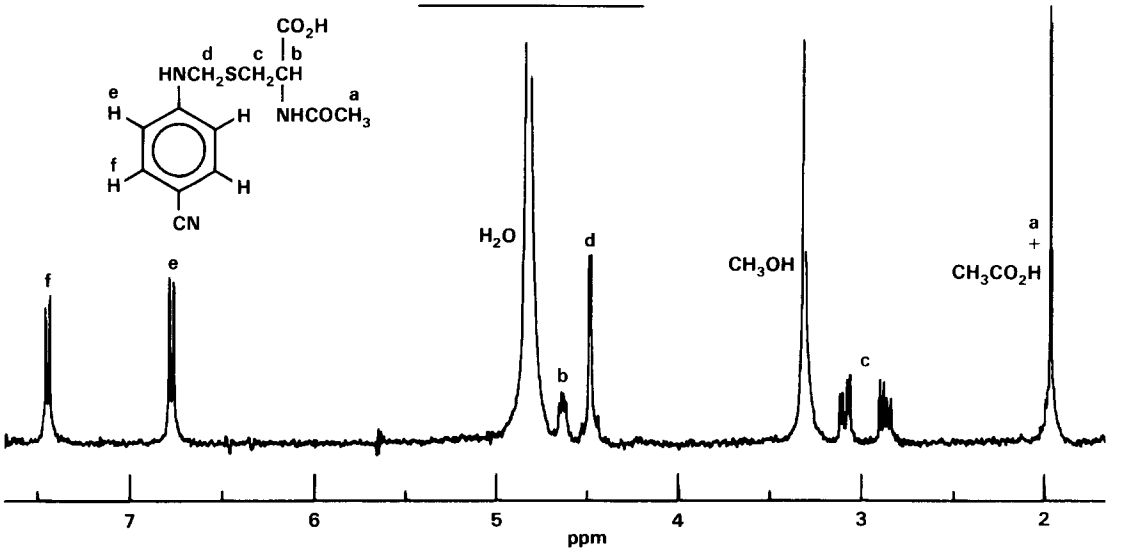

Fig 1 NMR spectrum of novel CDA metabolite in CD_3OD

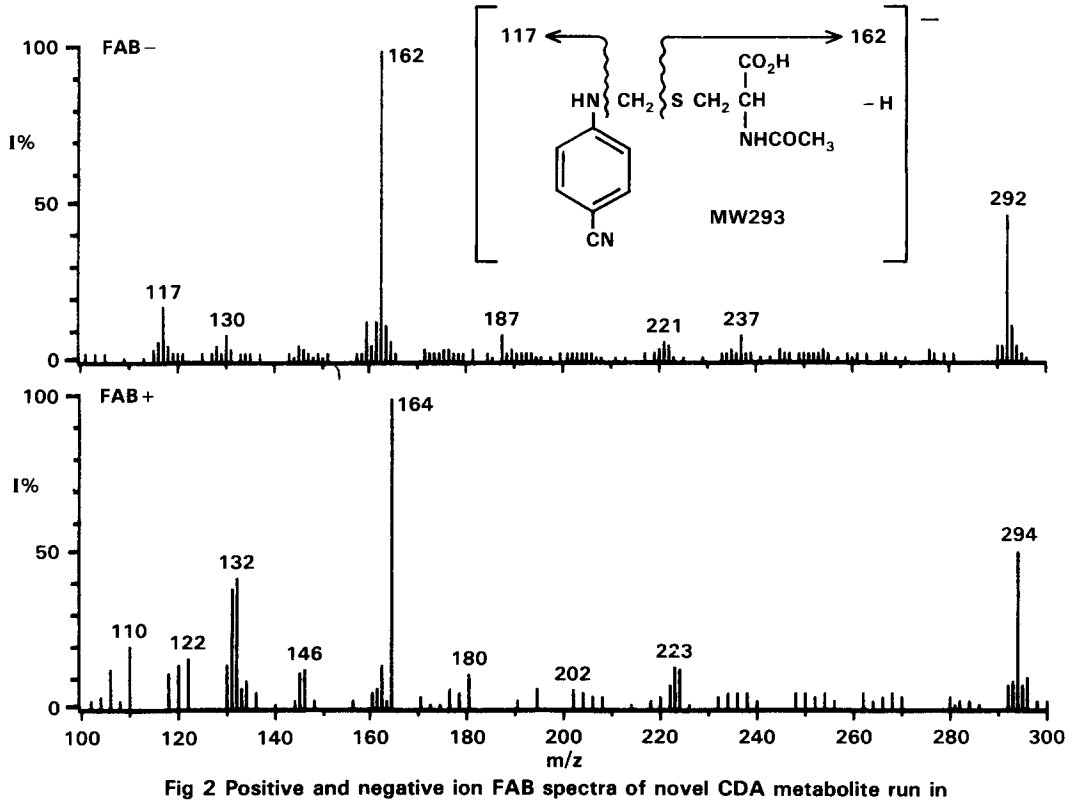

Fig 2 Positive and negative ion FAB spectra of novel CDA metabolite run in glycerol, employing xenon as bombarding gas at 8KV energy