METABOLIC C- AND N-OXIDATION AND N-METHYLATION OF [2,6^{14}C]PYRIDINE IN VIVO: DETERMINATION OF URINARY METABOLITES BY H.P.L.C.

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A considerable number of drug molecules in use today contain nitrogen heteroaromatic ring systems within their structures. However, a systematic study of the comparative roles of C- and N-oxidation and particularly N-methylation in the metabolism of such heterocycles is patently lacking. In order to establish basic ideas on metabolic options open to such drugs, determination of complete metabolic profiles of simple heterocycles would be of predictive value. These compounds may undergo metabolic reactions at the heteroatom (e.g. N-methylation, N-oxidation) and at ring carbons (C-oxidation) (see recent review by Damani and Crooks 1981).

In this study, several potential *in vivo* metabolites of pyridine have been synthesised and characterised by standard chemical methods. The diverse chemical and physical properties of these compounds precluded the use of a single h.p.l.c. column for their analysis. The use of a Partisil 10 ODS column (0.5 cm x 25 cm) with phosphate buffer (0.067M, pH 7.4, 4.2 ml/min) as eluant readily effects the separation of pyridine (Rt = 22.4 min) and its four oxidation products, pyridine N-oxide (Rt = 6.6 min), 3-hydroxypyridine (Rt = 5.4 min), 2-pyridone (Rt = 4.4 min) and 4-pyridone (Rt = 3.4 min). However, the very polar water soluble N-methylpyridinium ion eluted in the void volume. This metabolite was assayed using a Partisil 10 SCX column (0.5 cm x 25 cm) with a methanol : ammonium acetate (0.3M, pH 3.7) mixture (30 : 70 ^{v}/v, 1.5 ml/min) as the eluant. Under these conditions both N-methylpyridinium ion (Rt = 11.2 min) and pyridine (protonated at pH 3.7, Rt = 5.5 min) are well separated from the four oxidation products, which elute early.

Urine samples* from a rat, guinea pig, hamster and rabbit administered [2,6^{14}C] pyridine (7 mg/Kg; ∿10 μCi) were analysed directly by h.p.l.c. on the two columns described above. Eluant fractions were collected at appropriate time intervals and the ^{14}C determined by liquid scintillation counting using Luma gel (4 ml) as the scintillant. Radiochromatograms were constructed, and the radioactivity associated with each metabolite expressed as a percentage of the total injected onto the column.

The N-methylpyridinium ion (∿ 30%) and the N-oxide (∿ 65%) accounted for almost all the ^{14}C in hamster urine, confirming early reports that these were important urinary metabolites in certain species (Damani *et al* 1978; D'Souza *et al* 1980). In the rat, guinea pig and rabbit, 4-pyridone was also formed, accounting for about 20-30% of urinary radioactivity, whereas 2-pyridone was a minor (∿ 1-2%) metabolite in all the species studied. Free 3-hydroxypyridine represented about 3-5% of ^{14}C in rat, guinea pig and rabbit urine. In the rat and rabbit, where pyridine N-oxide, N-methylpyridinium ion and 4-pyridone accounted for less than 40% of ^{14}C in urine, it is very probable that 3-hydroxypyridine is present in a conjugated form. In addition there was a suggestion of the presence in the urine of these two species of other, as yet unidentified, metabolites.

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