A HIGH-PERFORMANCE LIQUID-CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF IN VITRO METABOLITES OF METYRAPONE

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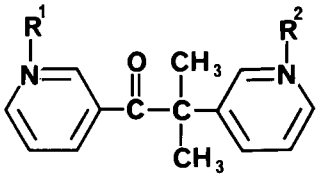

(I) : R^1= **O** , R^2 = nothing

(II) : R^1= nothing, R^2 = **O**

(III) : R^1=R^2 = **O**

Metyrapone : R^1=R^2 = nothing

Metyrapone, a diuretic and a diagnostic agent in pituitary function determination, is used in vitro as an inhibitor of cytochrome P_{450} dependent monooxygenation (Hildebrandt, 1971), but its mechanism of inhibition is not fully understood. Until recently, the only known metabolite of metyrapone was the alcohol, 2-methyl-1,2-bis(3-pyridyl)-propan-1-ol (Metyrapol) (Kahl, 1970; Hollands & Johnson 1973). Oxidation at pyridyl nitrogen appears to be a general cytochrome P_{450} mediated metabolic route for several 3-substituted pyridines (Damani et al 1978). This has led us and others (Kahl et al 1978) to reinvestigate the metabolism of metyrapone.

In this study the N-oxides (I), (II) and (III) were synthesised by peracid oxidation of metyrapone and isolation of products by preparative t.l.c. Metyrapol and its three N-oxides were obtained by selective borohydride reduction of the carbonyl group of metyrapone and of its N-oxides, I, II and III. Structures of all synthetic products were confirmed by spectroscopic methods.

Metyrapone N-oxides appear to be thermolabile (Kahl et al 1978), thus precluding the use of g.l.c. as a direct method of analysis. H.p.l.c. is an alternative method of analysis which has not been used extensively for the quantitation of N-oxygenated metabolites (Patterson et al 1978). We have carried out the h.p.l.c. analysis of in vitro metabolites of metyrapone on a reverse phase octadecylsilica column (10x0.5cm) (Hypersil) using a 0.05M phosphate buffer, pH 7.4, containing acetonitrile (2.5-50%) at a flow rate of 1.0ml per min (40 bar). Preliminary in vitro metabolic studies were carried out using the hepatic soluble fraction, the soluble plus microsomal fraction and the microsomal fraction from various animal species. Metyrapone is metabolised to metyrapol when the enzyme source is the soluble or soluble plus microsomal fraction. Metyrapone N-oxides (I) and (II) are only formed in the presence of microsomes and a NADPH-regenerating system. The di-N-oxide (III) and the N-oxides of metyrapol were not detected.

Thus a rapid and sensitive method of analysis of in vitro N-oxide metabolites of metyrapone has been developed. The formation of these metabolites may be of relevance in understanding the cytochrome P_{450} inhibitory properties of metyrapone.

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