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N-GLUCOSIDES: NEW PHASE-II METABOLITES OF TWO BARBITURATES

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ABSTRACT

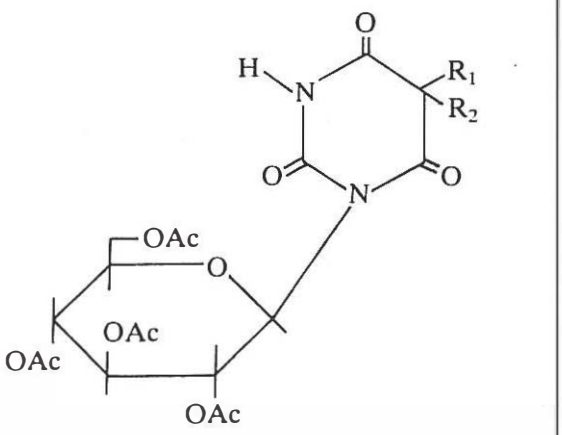
The new phase-II N-glucoside metabolites of butobarbitone and pentobarbitone are identified and quantified in human urine after single doses of these drugs. The identification and quantification of these metabolites were carried out by mass spectroscopy technique (accurate mass measurement and high voltage scanning).

It was found that the butobarbitone N-glucoside comprised 26% of the dose with t1/2= 44h, while the pentobarbitone N-glucoside was 43% with t1/2= 40h.

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INTRODUCTION

In previous investigations of the human metabolism of the hypnotics butobarbitone and pentobarbitone, less than 60% of the ingested drug has been considered as phase-1 metabolites. The phase-1 metabolites namely 3′-hydroxy, 3′-aldehyde, 3′-carboxy and 4′-hydroxy derivatives of these drugs were previously identified and quantified.[1,2]



	R_1	R_2
Compound I	Et	-Bu
Compound III	C_2D_5	-Bu
Compound II	Et	$CH(CH3)C_3H_7$

The identification of the N-glucosides as major metabolites of the barbiturates in man has been a recent discovery.[3,4] Similar studies on N-glucosidation of other drugs such as sulfonamides have also been carried out.[6] As it has been suggested that the major metabolites of amylobarbitone, phenobarbitone and sulphonamides, are the N-glucosides, therefore it was decided to investigate the conjugation of butobarbitone and pentobarbitane in the present study.[3,4,5,6] It was also important to develop a highly specific technique for the study of these conjugates.

To investigate the presence of the N-glucosides of these two drugs, it was necessary to synthesize these conjugates. Butobarbitone N-glucoside tetra-O-acetate (I), Pentobarbitone N-glucoside tetra-O-acetate (II) and butobarbitane-d_5-N-glucoside tetra-O-acetate (III) which was used as internal standard were synthesized. The complete synthesis procedures will be published soon.

MATERIALS AND METHODS

Mass spectral studies

The synthetic compounds I, II and III, after methylation with diazomethan, were studied by ZAB-IF VG-micromass spectrometer using the accurate mass measurement technique.

The ions peaks which were used for the quantification measurements of compounds I, II and the refer-

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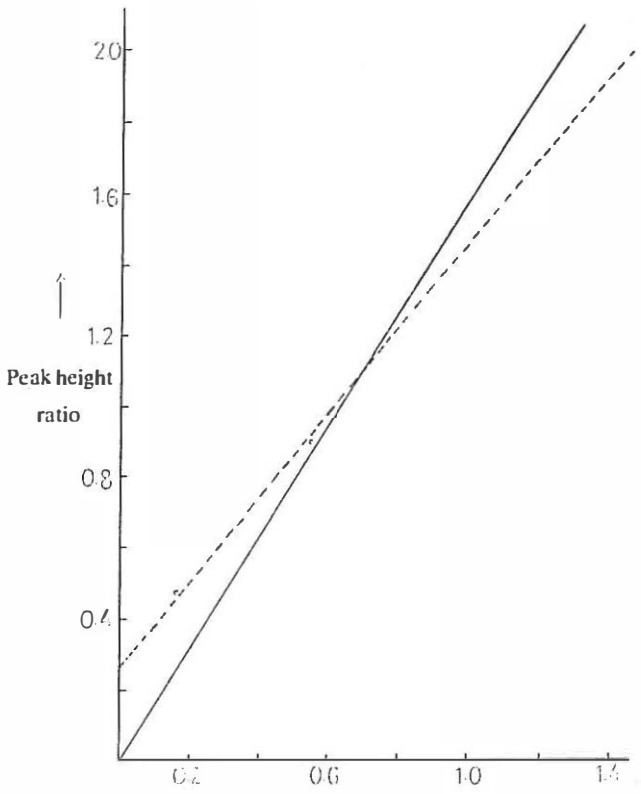


Fig.1. Calibration curve of butobarbitone run (the N-glucoside measurements).

ence compound III were [(M/Z 321 and363), (M/Z 335 and 377) and (M/Z 326 and 368)] respectively.

The mass spectral technique of identification and quantification of these metabolites showed a high degree of reproducibility and specificity.

Urine sample preparation for mass spectral analysis

Two healthy male volunteers (47 and 27 yrs of age) who had not taken any drug during the previous four weeks ingested therapeutic dose of butobarbitone or pentobarbitone (100 mg) before retiring. After collection of the metabolic urine every 8h for the first two days or 12h for the second two days, aliquotes (10 ml) were freeze-dried after adding a known amount (0.3-1.0ml) of compound III chloroform solution. The residue was taken up in methanol (2 ml) and methylated with the etheral diazomethane (2 ml, 10 min.) After evaporation, 5 ml of acetic anhydride and 5 ml of pyridine were added. The mixture was allowed to react overnight (20°C).

After evaporation, the residue was extracted with chloroform (2 ml) and the filtrate was used for mass spectral analysis.

Table I. Summary of the single-doses of butobarbitone and pentobarbitone N-glucoside measurements

metabolite	amount (mg)	%of dose	corre lation	t1/2 (h)
Butobarbitone N-methyl N-glucoside tetraacetate	75	29	-0.95	44
pentobarbitone-N-methyl N-glucoside tetraacetate	106.5	23	-0.90	40

Calibration curves

The system was calibrated by using blank urine to which known amounts of compounds I and III or II and III had been added and methylated with diazomethane. The plot of peak height ratio against the concentration (mg/dl) for both N-conjugates were linear (Figure 1). The (K) values which were obtained from these curves were used for measure of the metabolite concentration.

RESULTS AND DISCUSSION

The urinary excretion of the butobarbitone and pentobarbitone N-glucosides in man was studied after single dose of 100 mg of butobarbitone and pentobarbitone.

It would be more convincing if this study had been carried out on a larger number of volunteers which would have given statistically significant figures for the N-glucoside formation, but due to ethical and technical problems, these drugs have only been studied on two volunteers.

In this study, butobarbitone and pentobarbitone metabolism have been studied in two single-dose runs, and the system was calibrated using compound III: 5-batyl-5 (ethyl-d_5) barbituric acid-N-(2, 3, 4, 6- tetra- O- acetyl- β- D- glucopyranoside) as an internal standard. The mass spectral analysis of the urine samples and the calibration curve were carried out as described before.

The calibration curve showed a linear relationship when the peak height ratio (P.R.) was plotted against the concentrations of compound I: the synthetic butobarbitone-N- (2, 3, 4, 6- tetra- O- acetyl- B-D-glucopyranoside) (mg/dl) in the blank urine samples (Figure 1).

The quantitative results of the urinary excretion of butobarbitone and pentobarbitone N-glucoside are summarised in Table I.

It is clear that the N-glucoside is a major metabolite (29% of the dose) which is consistent with the published figures of 29% for amylobarbitone.[3] Plotting the rate of excretion of the derivatised butobarbitone and pentobarbitone N-glucoside (mg/h) against time (h)

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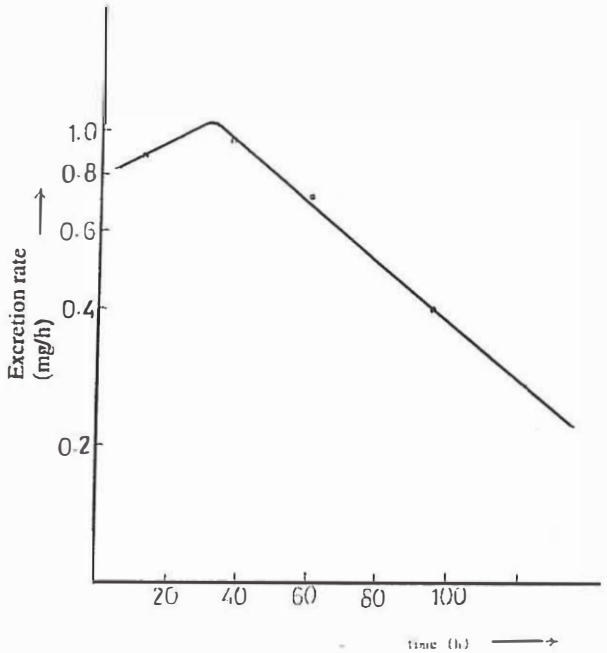


Fig.2. Plot of rate of excretion (mg/h) of butobarbitone N-glucoside against time (h) following a single-dose (100 mg) of butobarbitone.

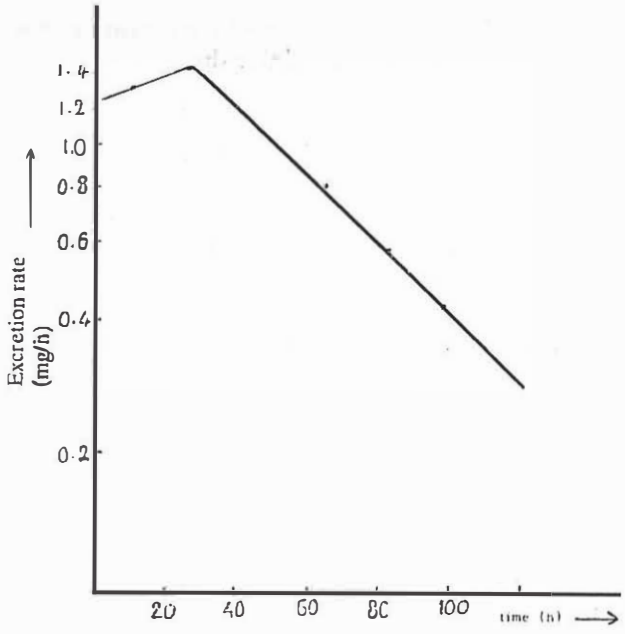


Fig.3. Plot of rate of excretion (mg/h) of pentobarbitone N-glucoside against time (h) following a single dose (100 mg) of pentobarbitone.

gave a straight line in Figures 2 and 3 respectively.

The half-life of butobarbitone and pentobarbitone N-glucosides (44 and 40h) which are similar to those for the other metabolites, suggesting that, as with the phase-1 metabolites, the rate of excretion of the N-glucoside is mainly determined by its rate of formation.

Isolation of urinary butobarbitone N-glucoside

In order to obtain supporting evidence for the structure of butobarbitone N-glucoside as a urinary metabolite of butobarbitone, a careful isolation and purification of the N-glucoside from the metabolic urine was carried out, using column chromatography and P.L.C. purification. The isolated butobarbitone N-glucoside was acetylated, and examined by N.M.R. and mass spectroscopy.

The comparison between the N.M.R. spectra of the isolated urinary butobarbitone N-glucoside and the corresponding synthetic material, has showed all the appropriate peaks in both spectra, but the N.M.R. spectrum of the isolated N-glucoside also showed the presence of impurities.

The mass spectra of the urinary and the synthetic butobarbitone N-glucoside (Figure 4) showed a convincing similarity, with all the key peaks that were present in both spectra.

The ions of M/Z 321 and 363, which were used in the quantification of urinary butobarbitone N-glucoside, using the mass spectral technique were present in the spectrum of the purified urinary N-glucoside as well as in the synthetic material.

From the above N.M.R. and mass spectral evidence, it is clear that the metabolite, which we have measured using the mass spectral technique, is the butobarbitone N-glucoside.

Finally by present work and similar previous works on other drugs, it was clear that N-glucosidation is a

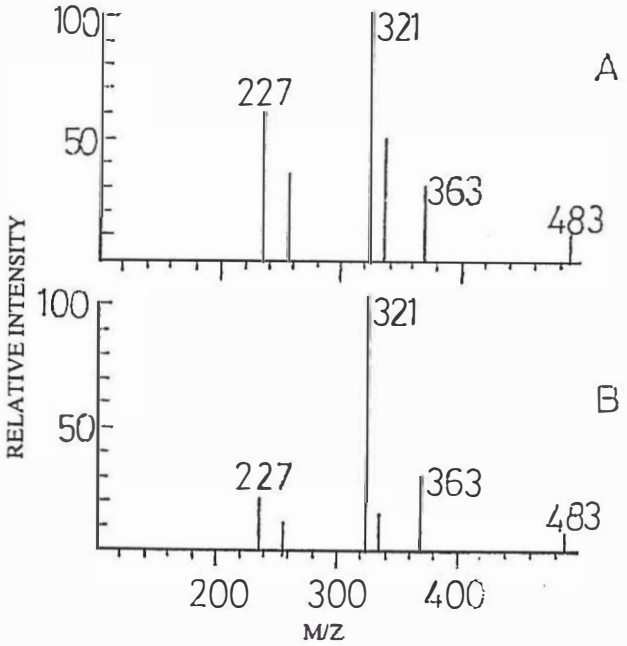


Fig.4. Electron ionisation mass spectra of:
A. Methylated and acetylated urinary butobarbitone N-glucoside
B. Synthetic butobarbitone- N- methyl- N'- (2, 3, 4, 6- tetra- O- acetyl- β- D- glucopyranoside.

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new route of metabolism not only for barbiturates but also for other similar N-containing drugs.

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