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Mass Spectrometric Determination of Butobarbitone and its Metabolites in Man

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Abstract—The urinary excretion of butobarbitone and its metabolites has been studied quantitatively using combined gas chromatography-mass fragmentography, tuned to ions at *m/e* 169 and 184. After a single oral dose (200 mg) to healthy male volunteers, unchanged drug (7 to 9%), 3'-hydroxybutobarbitone (22 to 27%), 3'-oxobutobarbitone (14 to 18%) and the 3'-carboxylic acid (4 to 8%), were found. In all cases, the maximum rate of excretion was in or near the third 12 hour urine.

Introduction

THE HUMAN metabolism of butobarbitone has not been investigated since the early controversy over the excretion of unchanged butobarbitone.[1,2] Studies in the dog,[3] the rat[4] and the mouse[5] have shown that 3'-hydroxybutobarbitone is excreted in all these species. Ledvina and Kael[4] have shown, in addition, that the 3'-carboxylic acid is present in rat urine.

Thus, we considered it probable that the above metabolites would be formed during the human metabolism of butobarbitone. We further considered 3'-oxobutobarbitone a likely metabolite, following from the observations of Tsukamoto *et al.*[6] in connection with quinalbarbitone. Accordingly these three metabolites were synthesized as reference compounds.

The quantification of the excretion products of butobarbitone was carried out by mass fragmentography. This technique has been described by a number of authors[7–9] and has been used in the study of barbiturate metabolism by Draffan, Clare and Williams.[10]

Experimental

Butobarbitone was supplied by May and Baker Ltd.

5-(3'-Hydroxybutyl)-5-ethylbarbituric acid

Diethyl ethylmalonate (63 g) was added (20°C) to a solution of sodium (0.42 g) in dry ethanol (50 ml); methyl vinyl ketone (23.3 g) was added dropwise (40 min), with stirring (10 ± 2 °C). Acidification, ether extraction and distillation yielded diethyl ethyl-3'-oxobutylmalonate (b.p. 97 to 99 °$C_{0.35\,mm}$, 56.2 g). Reduction of this Michael adduct (46.2 g) with sodium borohydride (5.0 g) in ethanol (250 ml, 96%) yielded the 3'-hydroxy analogue. Cyclization of the crude reduction product (45.9 g) with urea (11 g) in dry methanol (150 ml) containing dissolved sodium (5.5 g) at 120 to 130 °C (1.5 h) furnished 5-(3'-hydroxybutyl-5-ethylbarbituric acid (24.9 g), m.p. 153 to 5 °C; (lit. m.p. 152 to 3 °C).

5-(3'-Oxobutyl)-5-ethylbarbituric acid

Oxidation of the 3'-ol with chromium trioxide in acetone furnished 5-(3'-oxobutyl)-5-ethylbarbituric acid, m.p. 158 to 9 °C. Found: C, 53.0; H, 6.1; N, 12.8. $C_{10}H_{14}N_2O_4$ requires C, 53.1; H, 6.2; N, 12.4%.

5-(3'-Carboxypropyl)-5-ethylbarbituric acid

Diethyl ethylmalonate (10.3 g) was added dropwise to a stirred solution of sodium (1.84 g) in dry ethanol (50 ml); 5-bromopent-1-ene (10 g) was added dropwise to the stirred, refluxing solution. After heating under reflux (90 min), work-up in the usual way and distillation, diethyl ethylpent-4-enylmalonate (9.3 g, b.p. 100 to 104 °C c. 0.5 mm) was obtained. Base-catalysed cyclization with urea yielded 5-(pent-4'-enyl)-5-ethylbarbituric acid (6.34 g, m.p. 125 to 7 °C). Found: C, 59.5; H, 6.8; N, 12.4. $C_{11}H_{16}N_2O_3$ requires C, 58.9; H, 7.2; N, 12.5%. Lemieux oxidation of the pentenylbarbituric acid (3.21 g) in water (250 ml), aqueous potassium carbonate (0.1 M, 7 ml) and aqueous sodium periodate (20 g in 300 ml), to which was added potassium permanganate (0.32 g) in aqueous potassium carbonate (0.1 M, 125 ml), was carried out. Acidification, decolourisation (sodium bisulphite), extraction with ether and crystallization from ethanol yielded *5-(3'-carboxypropyl)-5-ethylbarbituric acid* (1.14 g, m.p. 225 to 8 °C). Found: C, 49.4; H, 5.9; N, 11.3. $C_{10}H_{14}N_2O_5$ requires C, 49.6; H, 5.8; N, 11.6%.

All compounds showed n.m.r., i.r. and mass spectra consistent with the assigned structures.

Metabolism Studies

Two healthy male volunteers each took butobarbitone tablets B.P. (2 × 100 mg, Evans Medical Ltd) before retiring, and urine was collected in 12 h batches and stored at 0 °C until extraction took place. Extraction and methylation of aliquots (50 ml) of each batch was carried out as previously described,[11] after addition of a known volume (typically 0.5 ml) of a methanolic solution of pentobarbitone (25.5 mg/100 ml) as internal standard. The methylated product after

removal of solvent was taken up in ethanol (0.75 ml) and, without delay, c. 0.2μl of the extract injected into the Finnigan 1015 gas chromatograph—mass spectrometer, operating with a 9 ft 2% QF-1 column, at an oven temperature of 190 °C, injection port 265 °C, separator and transfer line at 150 °C and ionization current of 250 μA at 70 eV; 5 injections of each sample were made. The 4 channel peak selector was set up with two channels tuned to *m/e* 169 and two to *m/e* 184. These fragment ions were chosen for the reasons outlined by Draffan *et al.*[10]

Calibration curves were plotted using results from blank urine to which a standard amount of the pentobarbitone solution, and varying amounts of a solution of known weights of butobarbitone and the three synthetic metabolites, had been added. The relative amounts of butobarbitone and metabolites in the synthetic mixtures were chosen so that all experimental measurements fell within the calibration area.

An internal standard was used to minimise errors caused by variations in the extraction technique, and to eliminate the need for accurate volume measurement prior to, and during, injection into the g.c.-m.s. system. Pentobarbitone was chosen as standard as (a) its gas chromatographic peak came in a suitable region of the metabolite chromatogram, (b) its extraction and methylation behaviour will be similar to those of butobarbitone and its metabolites, and (c) it gives similar response at *m/e* 169 and 184 to those of the butobarbitone group, being a typical 5-ethylbarbituric acid.[12]

Results

An initial mass fragmentographic examination of a methylated urine extract from a volunteer who had taken 200 mg butobarbitone, monitoring ions at *m/e* 169 and 184, revealed the presence of four major components giving rise to significant ions at these *m/e* values. A control urine from the same volunteer, extracted and methylated in an identical manner, showed no ions of these *m/e* values on g.c.-mass fragmentography.

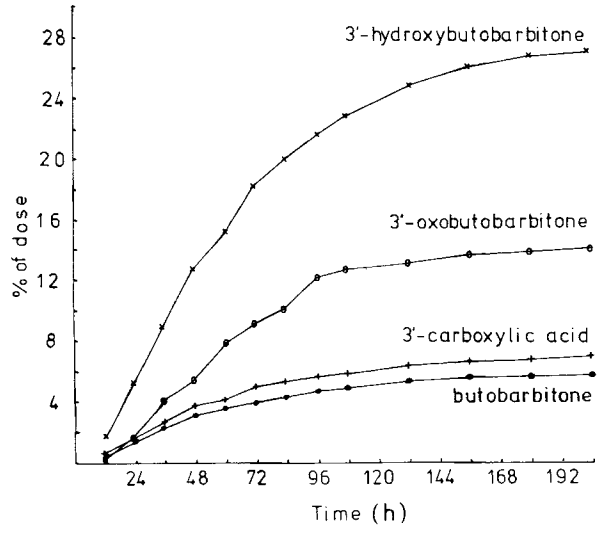

FIG. 1. Cumulative urinary excretion of butobarbitone and its metabolites (volunteer 1).

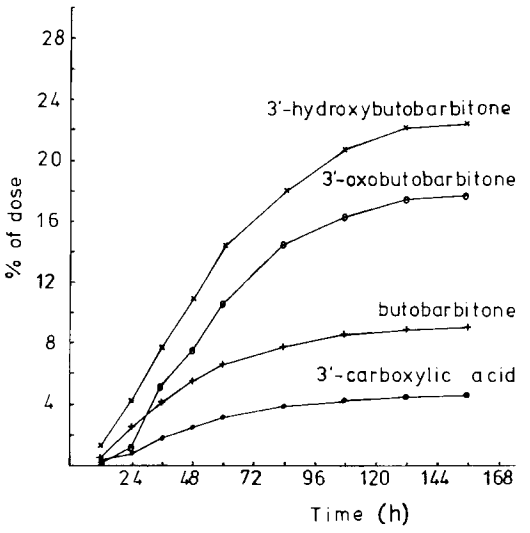

FIG. 2. Cumulative urinary excretion of butobarbitone and its metabolites (volunteer 2).

The identity of these four components was confirmed by monitoring ions at the *m/e* values listed in parenthesis below, and comparing the chromatograms with methylated butobarbitone (relative retention 1.0, *m/e* 97, 112, 126), 3'-hydroxybutobarbitone (relative retention 2.6, *m/e* 97, 112, 126), 3'-oxobutobarbitone (relative retention 3.1, *m/e* 43, 97, 112, 126) and the 3'-carboxylic acid (relative retention 3.9, *m/e* 97, 112, 126, 211, 212).

The results of the quantitative studies are represented in Figs. 1 and 2. The vertical axis corresponds to the cumulative percentage of the ingested butobarbitone which is excreted in the urine in the stated form. The overall picture, expressed as a percentage of ingested butobarbitone, is summarized in Table 1.

TABLE 1. Percentage of butobarbitone excreted

	Volunteer 1	Volunteer 2
Unchanged butobarbitone	7.0	9.0
3'-hydroxybutobarbitone	27.0	22.4
3'-oxobutobarbitone	14.1	17.7
3'-carboxylic acid	5.7	4.6
Total recovery	53.8	53.7

Discussion

During our attempts to quantify these four components, two problems were encountered, one associated with the operation of the quadrupole spectrometer and the other with the chemical stability of one of the metabolites.

Since our early model of peak selector does not operate on an integrating principle, but merely records the instantaneous peak height at a particular *m/e*

value, it is important that the instrumental resolution be adjusted to give relatively broad peaks, so that a small drift of the operating conditions (say 0.1 a.m.u.) does not give rise to a significant change in the apparent peak height. The *m/e* setting was checked at intervals throughout the analysis.

The results for the 3′-oxo metabolite were more erratic than those of the other metabolites. This may well be related to the lability of the 3′-oxobutyl side-chain, due to the possibility of a retro-Michael reaction, as has been suggested for the analogous metabolite of pentobarbitone.[13] Certainly, re-examination of the ethanolic solution of the methylated urinary metabolite extract after storage for a few days at 20 °C gave significantly lower results for the 3′-oxo metabolite, although an ethanolic solution of the methylated metabolites themselves appeared quite stable, in the absence of the natural urine components. Storage of the methylated urinary metabolite extract in the dry state at 0 °C for a few days preserved the integrity of the metabolites. A control experiment on a urine sample extracted immediately after collection, and again after storage for several days at 0 °C, showed that all metabolites were stable under these conditions for up to a week.

Excretion of unchanged butobarbitone reaches a shallow maximum about 24 h after ingestion; the maxima for the three metabolites occur about the 24 to 36 h fraction. The total amount excreted in both volunteers amounts to about 54% of the ingested dose, a recovery consistent with the observations reported by other workers on closely related barbiturates.[14,15]

The great advantage of the gas chromatographic-mass fragmentographic approach to drug metabolism studies of this type over the conventional gas chromatographic approach is that it enables meaningful results to be obtained, even when gas chromatographic conditions are such that the drug metabolites are not chromatographically resolved from the other urine components, providing these unresolved components do not produce significant ions at the *m/e* values being monitored. This possibility must be checked by a control run on blank urine.

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