Butobarbitone metabolism in man: identification of 3′-ketobutobarbitone

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After single 200 mg oral doses of butobarbitone (I) in man, 5·4% was excreted in urine unchanged and 28·2% as the 3′-hydroxy metabolite (II) in six days. A new metabolite, the ketone (III), was detected in urine following oral administration both of I and of II. Oxidation of the hydroxy to the ketone metabolite was demonstrated *in vitro* with the 9000g fraction of rat liver. Use was made of selective ion monitoring g.c.-m.s. methods in metabolite detection.

Butobarbitone (I) was originally reported by Maynert (1952) to be metabolized to 3′-hydroxybutobarbitone (II) in the dog. In the rat, II was confirmed to be the major metabolite and a further product, tentatively identified as a carboxylic acid (IV), was also isolated (Ledvina & Kacl, 1965). Quantitative studies of the metabolism of butobarbitone in man appeared to be lacking and an investigation based on methods developed in the estimation of 3′-hydroxyamylobarbitone in body fluids (Grove & Toseland, 1970; Draffan, Clare & Williams, 1973) is now described. As well as unchanged drug (I) and metabolite (II) a further metabolite a 3′-keto derivative (III) was identified after oral administration of the drug.
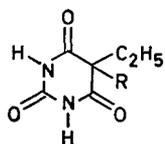
I, $R = -CH_2CH_2CH_2CH_3$

II, $R = -CH_2CH_2CH(OH)CH_3$

III, $R = -CH_2CH_2COCH_3$

IV, $R = -CH_2CH_2CH_2COOH$

METHODS

Determination of I and II in urine

Six healthy adult volunteers took an oral dose of 200 mg butobarbitone (Soneryl, May & Baker Ltd.). Complete urine collections were made for the ensuing six days. A further volunteer took an oral dose of 50 mg 3′-hydroxybutobarbitone, urine collection being made for three days.

Gas-liquid chromatography (g.l.c.) with flame ionization detection was used in the determination of I and II. Urines were filtered through columns of florisil and aliquots (10 ml) of the eluates were acidified (pH 2–3) and extracted with ether for the measurement of I by the method of Berry (1973). In the measurement of II, a procedure was adopted analogous to that described for 3′-hydroxyamylobarbitone (Grove & Toseland, 1970) in which the florisil eluates are saturated with ammonium sulphate and made basic (pH 8·9) before ether extraction.

Synthesis of III

Oxidation of II with chromium trioxide in dilute sulphuric acid gave the ketone III.

Recrystallization of III from aqueous ethanol afforded colourless prisms, p.m. 159–161° (Kofler block, uncorrected).

Thin-layer chromatography (t.l.c.)

Silica gel G (Merck, 0·25 mm layer) plates were used with the solvent systems A, ethyl acetate–methanol–ammonia (85:10:5) and B, benzene–chloroform–ethanol–acetone (80:10:5:5). Barbiturates were located with 1% mercurous nitrate or 1% mercurous sulphate oversprayed with 10% diphenylcarbazone in chloroform.

R_F values in system A were: I, 0·68; II, 0·18; III, 0·25 and in system B: I, 0·5; II, 0·19; III, 0·34.

Gas chromatography-mass spectrometry (g.c.-m.s.)

Combined g.c.-m.s. was effected with a Varian 1400 gas chromatograph coupled via a silicone membrane separator to an AEI MS 12 mass spectrometer. A 6 ft × $\frac{1}{8}$ inch glass column packed with 3% OV-1 on Gas-Chrom Q (100–120 mesh) was used with on-column injection and helium as carrier gas. Instrument settings for single ion monitoring and for scanning of barbiturate spectra were as previously described (Draffan & others, 1973).

Derivatives were prepared before g.c.-m.s. Diazomethane in ether–methanol (10:1) was used in methylation. O-Methyloximes were prepared with excess O-methyl hydroxylamine hydrochloride in pyridine at room temperature (20°) for 6 h, the reaction mixture being used directly for g.c.-m.s.

Relative retention times at 180° for the NN'-dimethyl derivatives of I, II, III and 3'-hydroxypentobarbitone were 1·0, 1·88, 1·65 and 2·4 respectively; $\%\Sigma_{40}$ values at m/e 169 and 23 eV were 39·5, 22·0, 18·0, and 19·2 respectively.

Rat liver preparations

Rat liver 9000g and microsomal fractions were prepared using standard procedures. Incubation of 3'-hydroxybutobarbitone was for 30 min at 37° at 1 mM substrate and 1·5 mg ml⁻¹ protein concentration with appropriate cofactors (Kuntzman, Ikeda & others, 1967) in 3 ml volumes of complete medium. Conversion of the hydroxy to the ketone metabolite was monitored in 0·05 ml aliquots by extraction into ether (1 ml) at pH 5, methylation and single ion monitoring g.c.-m.s.

RESULTS AND DISCUSSION

After oral administration of 200 mg butobarbitone, 5·4% of the dose was recovered unchanged in the urine and 28·2% as the anticipated 3'-hydroxy metabolite (II) in six days (Table 1). Several pathways of metabolism of 5-alkyl barbiturates are well established (Parke, 1971). These include ω-oxidation to acids, ω-1 oxidation to alcohols which may be further oxidized to ketones, conjugation of alcohols and barbiturate ring scission. The fate of ingested metabolite II was also studied in a volunteer who took a 50 mg oral dose. In three days 56·8% was recovered unchanged in the urine (47·7% in 12 h, 9·1% in the ensuing 60 h), in marked contrast to a similar study with 3'-hydroxyamylobarbitone in which the dose was totally excreted at the glomerular filtration rate (Grove & Toseland, 1971). There was no evidence of conjugation of II, as judged by acid hydrolysis (Balasubramian, Lucas & others, 1970) which did not increase the extraction yield. However, t.l.c. of ether extracts of urine, obtained at both acidic and mildly alkaline pH, showed both II and a second

Table 1. *Fraction of the dose excreted in urine during six days as butobarbitone (I) and 3'-hydroxybutobarbitone (II) following a single 200 mg oral dose of butobarbitone.*

Subject	Sex	Age	Wt (kg)	% of dose in urine I	II
1	M	35	73	3·1	32·0
2	F	29	57	6·1	38·6
3	F	26	70	7·6	39·0
4	F	19	48	8·0	16·4
5	F	18	46	2·9	19·9
6	M	19	67	4·5	23·4
				x̄ 5·4	x̄ 28·2

less polar component which also gave barbiturate staining reactions. In relating this new metabolite to I and II without extensive sample purification, advantage was taken of the favourable behaviour of *N*-methylated barbiturates in ion monitoring g.c.-m.s.

Extracts of urine were methylated with diazomethane and screened by g.c.-m.s. with the mass spectrometer focused either at m/e 169 or 184. These fragment ions are abundant in the spectra of barbiturates with an alkyl side chain (R) and a γ-hydrogen available for McLafferty rearrangement (Gilbert, Millard & Powell, 1970).

Fig. 1 (a) shows the total ion current chromatogram from a methylated extract of urine after butobarbitone ingestion. Figs 1 (b) and 1 (c) are the corresponding chromatograms monitoring m/e 169 and 184 respectively. Peaks 1 and 2 are *NN'*-dimethyl I and II. The unidentified barbiturate component recognized by t.l.c. following ingestion of II was eluted and methylated and shown to be coincident with peak 3. In its mass spectrum*, m/e 239 and 226 suggested probable M-15 and M-28 fragments and a molecular weight of 254, 2 mass units less than *NN'*-dimethyl II. This, together with a fragment ion at m/e 211, tentatively assigned to loss of 43 ($COCH_3$), suggested a 3'-ketone. The presence of a ketone was confirmed by treatment of the methylated urine extract with *O*-methyl hydroxylamine hydrochloride which resulted in a shift of component 3 in the chromatogram due to *O*-methylketox-
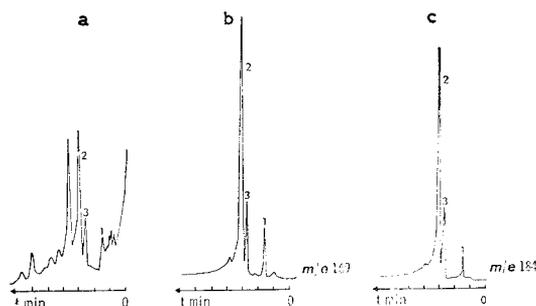

Fig. 1. Total ion current (a) and single ion chromatograms (b and c) from a methylated extract of urine voided 72–84 h after the ingestion of a single 200 mg oral dose of butobarbitone. Peaks 1, 2 and 3 are *NN'*-dimethyl I, II and III respectively.

* Mass spectrum of *NN'*-dimethyl (III); m/e values with relative intensities in parentheses. Only peaks with relative intensities $\geqslant$ 2 are included above m/e 100 and > 5 below m/e 100: 239 (2), 226 (14), 211 (9), 186 (2), 185 (20), 184 (100), 183 (18), 170 (8), 169 (87), 156 (6), 155 (3), 126 (13), 112 (13), 97 (11), 81 (6), 71 (8), 69 (11), 58 (10), 55 (20), 43 (54), 41 (12).

ime formation. The spectrum of this derivative contained an abundant fragment at m/e 100, consistent with fission γ-to the ketoxime $[CH_2CH_2C(NOCH_3)CH_3]^+$.

m/e 184 m/e 169
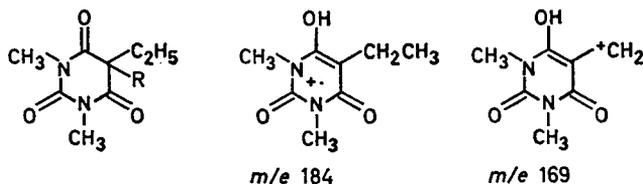
Establishment of the new metabolite as III, 3'-ketobutobarbitone, was obtained by synthesis of the ketone from hydroxybutobarbitone by chromium trioxide-sulphuric acid oxidation. Comparison of t.l.c. behaviour, mass spectra and g.l.c. retention times as the dimethyl and *O*-methyl-ketoxime derivatives confirmed the identity of the synthetic material with metabolite III.

Oxidation of hydroxypentobarbitone to the 3'-ketone has been shown to be stereo-specific and effected by the soluble, non-microsomal enzymes of the liver (Kuntzman & others, 1967). Consistent with this observation, conversion of $(\pm)$-II to III was obtained on incubation with the 9000 g fraction of rat liver, while no further oxidation was observed on incubation of II with the microsomal fraction.

After ingestion of metabolite II, the ketone III was detected in urine both by t.l.c. and by ion monitoring g.c.-m.s.   This metabolite was also present in urine from each of the subjects who took butobarbitone and in one case of butobarbitone overdosage where it was readily detectable by t.l.c.   The acid (IV) (Ledvina & Kacl, 1965) was not identified.   Its formation from I, possibly via an ω-hydroxy intermediate, but not from II would be predicted (Parke, 1971).   The limited screening of urine follow-ing dosage of I was here directed toward the recognition of II and III and was based on extraction at mildly alkaline pH, unfavourable for the recovery of a carboxylic acid. We do not therefore exclude the possibility of IV as a metabolite of butobarbitone in man.

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