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Metabolism of heptabarbitone

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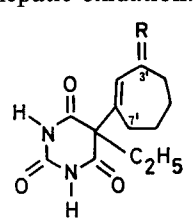
The human metabolism of 5-ethyl-5-cycloheptenylbarbituric acid, heptabarbitone, Medomin, taken orally, has been studied. Comparison of g.c., and g.c.-m.s. data, from derivatized urine extracts, with data from authentic synthetic samples, has shown that 5-(3′-oxocycloheptenyl)-5-ethylbarbituric acid (II) and 5-(3′-hydroxycycloheptenyl)-5-ethylbarbituric acid (III) are major metabolites of heptabarbitone. In addition, similar data have been obtained for a third metabolite. There was no evidence for the presence of the parent drug in the urine of the two volunteers. The rate of urinary excretion of the three metabolites is briefly discussed.

In previous studies of the metabolism of the hypnotic, heptabarbitone, the only identified metabolite was 5-(3′-oxocycloheptenyl)-5-ethylbarbituric acid, in amounts up to 45% of the administered dose (e.g., Bernhard & Bickel, 1957). Excretion in the urine was reported to be rapid, with a half-life of the drug in the rat of 6–8 h.

The closely related drugs, hexobarbitone (1,5-dimethyl-5-cyclohexenylbarbituric acid) and cyclobarbitone (5-cyclohexenyl-5-ethylbarbituric acid), have been studied in detail, and the results have been reviewed by Bush & Sanders (1967) and Bush & Weller (1972). The main route of detoxification involves oxidation at the allylic 3′-position of the cyclohexenyl ring to either a hydroxy or a keto group. Although Bush, Butler & Dickison (1953) claim to have isolated two different ketonic metabolites from the urine of dogs to which hexobarbitone had been administered, neither of these has physical data in accordance with those of synthetic 6′-oxohexobarbitone, as prepared by Tsukamoto, Yoshimura & Toki (1956). Thus, there does not appear to be any clear evidence for metabolism of the cyclohexenyl barbiturates by hepatic oxidation at the 6′-position; the alternative allylic position (i.e. 3′) presumably suffers less steric hindrance.

On the basis of our knowledge of the metabolism of these other barbiturates, we should expect to find both the 3′-oxo and the 3′-hydroxy derivatives of heptabarbitone. In addition, due to the greater conformational mobility of the seven-membered ring, as compared with the cyclohexenyl ring, it would not be altogether unexpected to find that the 7′-position also underwent hepatic oxidation.

I R=H,H
II R=O
III R=H,OH



MATERIALS AND METHODS

Materials

Heptabarbitone (I) was kindly donated by Geigy (U.K.) Ltd. 5-(3′-Oxocycloheptenyl)-5-ethylbarbituric acid (II) was prepared by chromium trioxide oxidation

of heptabarbitone. Reduction of (II) by sodium borohydride afforded (±)-(3′-hydroxycycloheptenyl)-5-ethylbarbituric acid (III).*

Methods

G.l.c. A Pye Series 104 chromatograph, with inlet heater (about 250°) and flame ionization detector (column temperature) was used. Extracts were examined on a 9 ft glass column packed with 3% QF 1 on Diatomite CQ, programmed at 200° for 5 min, then 6° min^{-1} to 250°.

G.l.c.—mass spectrometry. A Finnigan Model 1015 instrument, fitted with a 9 ft glass column packed with 1% QF 1 on Gas-Chrom Q (100/120 mesh) was used. The carrier gas was helium at an inlet pressure of about 20 p.s.i.; the column was programmed at 190° for 5 min, then 4° min^{-1} to 245°. The injection heater was held at about 260° and the separator at about 190°; the glass line to the mass spectrometer was maintained near 150°. The mass spectrometer was operated with an ionizing current of 250 mA, at 90 eV.

Metabolism sequence. Heptabarbitone (300 or 400 mg; Medomin tablets) was taken before retiring; urine was collected in 8 h batches for three days. Aliquots (100 ml) of each batch, and of blank urine, and of blank urine to which weighed amounts (about 2·5 mg%) of drug and synthetic metabolites had been added, were worked up and derivitized in the usual way (Gilbert, Millard & others, 1973). Before injection of the silylated extracts into the Finnigan, excess reagents were removed (Rotovap) and the residue taken up in dry pyridine (0·2 ml).

RESULTS AND DISCUSSION

Examination of the derivitized urine extracts consistently showed three major metabolite peaks. The first of these (retention time 12·8 min) had g.c. and g.c.-m.s. properties in both derivitization states identical with those of synthetic 5-(3′-hydroxycycloheptenyl)-5-ethylbarbituric acid. The metabolite peak of longest retention time (16·8 min) was present at the same position in both methylated and methylated/silylated extracts. Its properties corresponded to those of methylated 5-(3′-oxocycloheptenyl)-5-ethylbarbituric acid (II).

In addition, a third metabolite peak was present in the g.c. trace of the methylated extract, at Rt 13·4 min. As the position of this peak is shifted on silylation (12·1 min), it follows that this metabolite must contain a free hydroxyl group. That the metabolite is of barbiturate origin is indicated by the presence of the *m/e* 169 ion, plus other typical barbiturate-derived ions (see e.g. Gilbert, Millard & Powell, 1970), in the mass spectra of this component in both derivitization states. Further, the presence of a strong peak at *m/e* 183 (for cycloheptenyl-OTMS), plus a molecular ion at *m/e* 366, and an (M^+-15) ion at 351, constitutes strong evidence that this metabolite is isomeric with the 3′-hydroxy derivative previously identified. Yet more evidence for this conclusion was obtained by subjecting a portion of a first 8 h urine extract to oxidation by chromium trioxide in ice-cold acetone (Jones oxidation). G.c.-m.s. examination of the product revealed only two barbiturate-derived peaks of closely similar retention times (about 17 min). Both components gave mass spectra

* Details of the preparation and analytical and spectral properties of these compounds are deposited with the British Library, Boston Spa, Yorkshire, U.K., under Reference No. SUP 90006 (6 pages).

closely similar to that of authentic 3′-oxoheptabarbitone (II), with a significant peak at *m/e* 109 (for the cycloheptenoyl ion).

The evidence thus indicates that this third metabolite is a hydroxyheptabarbitone. Although confirmation of structure must await evidence from synthesis (work is continuing along this line), it would seem most likely that this metabolite arises through hepatic hydroxylation at the other allylic position—i.e. the 7′-position in the cycloheptenyl ring.

Values for the concentration of the urinary metabolites have been obtained from the g.c. traces by comparison with traces from extracts of blank urine to which known amounts of the synthetic metabolites had been added. The values for the unidentified metabolite are based on the assumption that its response, both in the extraction process, and towards the flame ionization detector, will be similar to that of the 3′-hydroxy isomer. The results are summarized in Table 1.

Table 1. *Excretion of heptabarbitone metabolites, expressed as a percentage of initial dose of heptabarbitone.*

Time (h)	Volunteer G			Volunteer M		
	3′ oxo	3′ OH	x′ OH	3′ oxo	3′ OH	x′ OH
0– 8	2·0	3·0	3·2	2·2	5·8	4·0
8–16	1·8	3·7	3·1	0·9	4·7	2·4
16–24	2·7	6·5	5·0	0·85	4·4	2·2
24–32	0·9	2·8	2·1	0·15	1·7	0·8
32–40	0·8	2·8	1·9	0·02	0·8	0·4
40–48	0·3	1·8	1·2	—	0·5	0·2
48–56	0·03	0·9	0·6	—	0·3	0·2
56–64	—	—	—	—	—	—
Total	8·5	21·5	17·1	4·1	18·2	10·3

As can be seen, for Volunteer M, the excretion rate of the three metabolites steadily decreases from the initial values; the two hydroxylated metabolites decrease in concentration at similar rates, but excretion of the 3′-ketone falls off rather more rapidly, over 50% of this metabolite being excreted in the first 8 h.

Although the results for Volunteer G are distorted by the exceptionally high liquid throughput in the third 8 h period, there is some evidence that the rate of excretion of the metabolites is not following the steadily decreasing pattern observed for Volunteer M, but that the rates are rising to a shallow maximum reached in about the third urine batch. This is in line with the pattern observed in the study of the metabolism of nealbarbitone (Gilbert & others, 1973).

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