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A study of the human metabolism of secbutobarbitone

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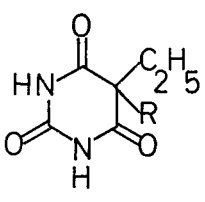
The urinary excretion of secbutobarbitone (I) and its metabolites has been studied quantitatively using combined gas chromatography-mass spectrometry. After a single oral dose was given to healthy male volunteers, unchanged drug (5–9%), 2′-hydroxysecbutobarbitone (II, 1·7–3·2%), 2′-oxosecbutobarbitone (III, less than 1%), and the carboxylic acid (IV, 24–34%) were found. The kinetics of the excretion process were studied.

Secbutobarbitone was first introduced into the British Pharmacopoeia in 1973, but the drug had been in clinical use for a number of years. No account of its metabolism in man has been published, but Maynert & Losin (1956) have reported the isolation of 28–35% of the terminal acid (IV) and 3–5% of unchanged secbutobarbitone from the urine of dogs treated with the drug.

It seemed to us that (ω-1) oxidation of the larger side-chain is likely to occur, in addition to the reported ω oxidation. Accordingly, the 2′-hydroxy- (II), 2′-oxo- (III) and terminal acid (IV) derivatives of secbutobarbitone were all synthesized.

Quantification of the excretion products of the drug was carried out by the mass fragmentography method (Gilbert & Powell, 1974).



I R = —CH(CH$_3$).CH$_2$CH$_3$

II R = —CH(CH$_3$OH).CH.CH$_3$

III R = —CH(CH$_3$O).C.CH$_3$

IV R = —CH(CH$_3$).CH$_2$CO$_2$H

V R = —CH(CH$_3$).CH$_2$CH=CH$_2$

VI R = —CH$_2$CH$_2$CH$_2$CH$_3$

VII R = —CH$_2$CH$_2$CH(OH).CH$_3$

VIII R = —CH$_2$(CH$_2$)$_4$CH$_3$

IX R = —CH(CH$_3$).CH$_2$CH$_2$CO$_2$H

X R = —CH(CH$_3$).CH$_2$CH$_2$CH$_3$

XI R = —CH(CH$_3$).CH$_2$CH(OH).CH$_3$

MATERIALS AND METHODS

Secbutobarbitone was supplied by May and Baker Limited. The diastereoisomeric (±)-5-ethyl-5-(2′-hydroxy-1′-methylpropyl)barbituric acids (II) were synthesized separately from *cis*- and *trans*-but-2-ene via the bromohydrin and malonic ester derivatives. Jones oxidation of either of the diastereoisomeric pairs gave rise to the (±)-2′-oxo metabolite (III). The terminal acid was prepared by a method similar to that used by Wood & Horning (1953) for the corresponding derivative of pentobarbitone. Conversion of pent-4-en-2-ol to the bromo-derivative, reaction of this

bromide with ethyl ethylmalonate, followed by condensation with urea furnished 5-ethyl-5-(1′-methylbut-3′-enyl)barbituric acid (V). Lemieux oxidation of the unsaturated side-chain yielded (±)-5-ethyl-5-(2′-carboxy-1′-methylethyl)barbituric acid (IV). This acid had m.p. 219–222°, in close agreement with the acid (m.p. 218–219°) isolated by Maynert & Losin (1956) from the urine of dogs; this acid was not synthesized by these workers.

The preparative, spectral and analytical data for all these compounds are deposited with the British Library, Boston Spa, Yorkshire, U.K., under Reference No. SUP 90017 and occupy 8 pages.

Metabolism studies

Healthy male volunteers took secbutobarbitone as powder (J.T., 100 mg) or the sodium salt as capsules (J.G., J.P., 200 mg) before retiring, and the urine was collected at intervals and stored at 0° until extraction. After addition of a known volume of a methanolic solution of 3′-hydroxybutobarbitone as internal standard to aliquots (50 ml) of each urine batch, extraction and methylation were carried out as previously described (Gilbert, Millard & others, 1974a). The methylated product, after removal of solvent, was taken up in methanol (0·5 ml), and triplicate injections each of *ca* 0·15 μl, were made into the Finnigan 1015 gas chromatograph–mass spectrometer, using conditions previously described (Gilbert & Powell, 1974). All four channels of the peak selector were tuned to *m/e* 183, a particularly prominent fragment ion in the mass spectra of the alcohol and ketone metabolites. 3′-Hydroxybutobarbitone was used as the internal standard on the grounds of suitable retention characteristics, and common fragment ions. The preliminary investigation carried out with volunteer J.T. did not employ an internal standard, but was based on a comparison of peak heights from injections of a standard extract with those of the unknown extracts; this experiment used *m/e* 169 and 184 for quantification. The close correspondence of the three sets of results lends support to the reliability of the two methods employed.

RESULTS AND DISCUSSION

G.c.–m.s. examination of the methylated extract of urine from two volunteers (J.T. and J.P.), monitoring ions at *m/e* 169 and 184, showed the presence of two major and two minor components giving rise to significant ions at these *m/e* values. The identity of the two major components as unchanged secbutobarbitone (I, relative retention 1·0), and the 2′-carboxylic acid (IV, relative retention 3·6) was confirmed by comparison of their mass spectra with those of authentic samples. The two minor components were identified by monitoring ions at *m/e* 97, 126, 169, 183 and 184, and comparing the relative intensities of these ions with those from authentic methylated 2′-hydroxysecbutobarbitone (II, relative retention 2·1), and 2′-oxosecbutobarbitone (III, relative retention 1·6). The relationship between these last two metabolites was confirmed by Jones oxidation of an extract of urine, followed by methylation, when the component giving rise to a peak at relative retention 2·1 on g.c.–m.s. disappeared, and the peak at relative retention 1·6 was enhanced in intensity. Although the diastereoisomeric alcohols (II) were separately synthesized, both racemates had the same g.c. retention times under our conditions, and they showed indistinguishable mass spectra. Therefore, we were unable to determine which of the isomeric alcohols were present in the urine extracts.

The choice of fragment ions on which to base the quantification of the three metabolites and the unchanged drug, presented some difficulties. The ions previously used (*m/e* 169 and 184) were not very satisfactory for the hydroxy- (II) and oxo- (III) metabolites, because of the low relative abundance of these ions in their mass spectra; this fact, coupled with the low concentrations of these two metabolites in the extracts, made quantitation difficult. The fragment ion of *m/e* 183 was the ion of greatest abundance above *m/e* 100 for both of these metabolites, and was a significant, though less abundant, ion for the parent barbiturate (I), the acid metabolite (IV), and the internal standard (VII). Although next to an ion of high relative abundance (*m/e* 184), and therefore likely to be particularly sensitive to instrumental resolution and drift, the 183 ion was found to be suitable for quantitation in practice. The results for the drug and its acid metabolite were more consistent than for the hydroxy and oxo metabolites even though, in the first two, the ion at *m/e* 184 was much more abundant than that at *m/e* 183.

Detailed results for one of the volunteers (J.P.) are given in Table 1. A summary of the three sets of results for secbutobarbitone (I) shows that the difference in dose levels and in the form in which the drug was administered had no significant effect on the metabolism. A comparison of the results for secbutobarbitone (I) with those previously published for butobarbitone (VI) (Gilbert & Powell, 1974; Gilbert, Natunen & others, 1974b), also included in Table 2, discloses an interesting change in the predominant site of oxidative attack of the barbiturate side-chain. The major metabolite of secbutobarbitone is the acid (IV), arising from ω-oxidation of the larger substituent; this product accounts for some 29% of the ingested dose, whereas (ω-1) oxidation to the 2′-hydroxy (II) or the 2′-oxo (III) derivatives, accounts for less than 3% of the dose. In contrast, ω-oxidation of the larger substituent in butobarbitone (VI) accounts for only 5% of the dose, whilst (ω-1) oxidation accounts for over 40%. In both cases, about 8% of the dose was excreted as unchanged drug.

A comparison of first order rate constants shows unchanged secbutobarbitone to be excreted at a slightly lower rate (average k = 0·0015) than unchanged butobarbitone (average k = 0·0019): no zero order component is present in any of the cases studied. The overall metabolism and excretion of the hydroxy, keto and acid metabolites also occurs at a slower rate for secbutobarbitone (average of the sum of the first order rate constants = 0·00667) than for butobarbitone (average of the sum of the

Table 1. *Excretion of secbutobarbitone and its metabolites in subject J.P.*

Time	8h	16h	24h	32h	40h	48h	60h	72h	84h	96h
Secbutobarbitone ..	0·14	0·84	1·92	0·37	0·45	0·74	0·61	0·38	0·34	0·91
2′-oxo (III)	tr	tr	tr	tr	tr	0·02	0·03	0·03	0·03	0·05
2′-hydroxy (II)	0·06	0·16	0·19	0·18	0·23	0·19	0·37	0·37	0·22	0·20
Terminal acid (IV) ..	0·95	2·75	2·36	1·93	2·7	2·3	3·4	3·12	2·47	2·34

Time	108h	120h	132h	144h	156h	168h	180h	192h	204h	Total
Secbutobarbitone ..	0·28	0·50	0·12	0·21	0·07	0·27	0·06	0·06	0·06	8·33
2′-oxo (III)	0·03	0·04	0·03	0·05	0·03	tr	tr	tr	tr	0·34
2′-hydroxy (II)	0·24	0·15	0·14	0·15	0·11	0·09	0·07	0·04	0·05	3·21
Terminal acid (IV) ..	2·45	1·72	1·29	1·09	0·82	0·90	0·6	0·45	0·5	34·15

Secbutobarbitone R = $CHMeCH_2Me$
2′-Oxo metabolite R = $CHMeCOMe$
2′-Hydroxy metabolite R = $CHMeCHOHMe$
Terminal acid metabolite R = $CHMeCH_2COOH$

Table 2. *Comparison of excretion of butobarbitone and secbutobarbitone and its metabolites in man.*

		Butobarbitone		Secbutobarbitone		
		J.G.	J.P.	J.T.	J.G.	J.P.
Parent barbiturate	Recovery	9·0%	7·0%	5·4%	9·5%	8·3%
	First order k	0·0022	0·0016	0·0014	0·0019	0·0013
	Lag time	7·7h	6·8h	—	7·2h	7·8h
Oxo metabolite (ω-1 oxidation)	Recovery	17·7%	14·1%	0·7%	0·5%	0·5%
	First order k	0·0035	0·0027	0·00013	—	—
	Lag time	11·8h	9·8h	—	—	—
Hydroxy metabolite (ω-1 oxidation)	Recovery	22·4%	27·0%	2·3%	1·7%	3·2%
	First order k	0·0053	0·0061	0·0004	0·00034	0·00064
	Lag time	7·0h	6·4h	—	6·6h	5·3h
Terminal acid metabolite (ω oxidation)	Recovery	4·6%	5·7%	24·1%	28·0%	34·2%
	First order k	0·0011	0·0012	0·0058	0·0058	0·0068
	Lag time	6·1h	4·9h	—	6·8h	5·5h

first order rate constants = 0·00895). Some zero order component enters into the metabolism and/or excretion of these metabolites in both barbiturates, usually over the first two or three days. In one individual, no zero order component was found for the acid metabolite of either butobarbitone or secbutobarbitone. The sum of the first order excretion rate constants for the metabolites and the unchanged drug gives a measure of the rate of elimination of the drug from the body, from which an estimate of the biological half-life of the drug may be obtained. For butobarbitone, this estimate is 59 h (57–60) and for secbutobarbitone 85 h (79–90). Thus the branching of the four carbon chain appears to prolong the residence time of the drug in the body.

Extensive work on the oxidative metabolism of saturated alkyl chains has been carried out by Maynert and his co-workers. Using 5-ethyl-5-hexylbarbituric acid (VIII) administered to dogs, Maynert (1965) was able to show that ω-oxidation accounted for 4% and (ω-1) oxidation for 24% of the dose. (ω-2), or β-oxidation, to give 5-(3′-carboxypropyl)-5-ethylbarbituric acid (IX) in 16% yield was suggested by Maynert, but not positively proven. This metabolite would have the same structure as the acid metabolite derived from butobarbitone, and the properties reported by Maynert are in agreement with those of the synthetic compound prepared by us (Gilbert & Powell, 1974).

A similar picture is found in pentobarbitone (X), where (ω-1) oxidation predominates; 47–51% of the dose was recovered as one or other of the diastereoisomeric alcohols (XI) (Maynert & Dawson, 1952). No ketone corresponding to these alcohols has so far been reported, but this is probably due to the facile degradation of the 3′-oxo side-chain under mildly alkaline conditions (Carol & Philip, 1971). The terminal acid arising from ω-oxidation has been reported by Titus & Weiss (1955), who quote a recovery of 4·5%; their recovery of the 3′-ols (XI) was 62%, and of unchanged pentobarbitone was 0·8%.

From the work cited above, it is obvious that for alkyl barbiturates containing chains of four or more carbon atoms, the (ω-1) carbon atom is the principal site of oxidative metabolic attack, a mixture of hydroxy and ketonic metabolites being produced. Attack on the ω-carbon atom to give the carboxylic acid is a minor route, involving about 5% of the drug. Compounds with a chain length of six or

more carbon atoms may also be susceptible to β-oxidation (ω-2); this does not appear to occur unless the (ω-2) carbon atom is separated from the barbiturate ring by at least two saturated carbon atoms. Where the chain length falls to three carbon atoms, (ω-1) oxidation involves attack of the carbon atom next but one to the barbiturate ring. This is a site characteristically resistant to metabolic attack (cf. barbitone), and ω-oxidation becomes the preferred metabolic route. It is possible that attack of the penultimate carbon atom of secbutobarbitone is inhibited for steric reasons; this question would be answered by a comparative study of the human metabolism of 5-ethyl-5-n-propylbarbituric acid.

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