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Metabolism of quinalbarbitone

J. N. T. GILBERT, W. L. HETHERINGTON, J. W. POWELL AND W. B. WHALLEY

Department of Pharmaceutical Chemistry, The School of Pharmacy, Brunswick Square, London WC1N 1AX, U.K.

The human metabolism of (±)-5-allyl-5-(1′-methylbutyl) barbituric acid (I), quinalbarbitone, taken orally, has been studied. Comparison of g.c. and g.c.-m.s. data from derivatized extracts of urine with similar data from synthetic samples confirmed the presence of the two diastereoisomeric forms of 5-allyl-5-(3′-hydroxy-1′-methylbutyl)barbituric acid (II), 5-allyl-5-(3′-oxo-1′-methylbutyl)barbituric acid (III), 5-allyl-5-(1′-methyl-3′-carboxypropyl)barbituric acid (IV), and 5-(2′,3′-dihydroxypropyl)-5-(1′-methylbutyl)barbituric acid (V) in the urine. There was no evidence for the urinary excretion of unchanged drug. The rate of excretion of these metabolites has been examined in some detail, and rate-limited kinetics shown to apply for excretion of the acid (IV) and the diol (V).

The first major study of the human metabolism of quinalbarbitone (I) (Waddell, 1965) showed the presence of the diol (V), and two diastereoisomeric forms of the 3′-ol (II), as the major urinary metabolites. He also provided evidence for the presence of the des-allyl compound VI, and the 5-hydroxy derivative of VI (i.e. VII); but as these two metabolites were only identified by means of paper chromatography, some doubt must remain about their chemical nature. Tsukamoto, Yoshimura, & Ide (1963), studying the metabolism of quinalbarbitone in rabbits, found both the 3′-ol (II) and the terminal carboxylic acid (IV), but no trace of the diol. Later work by Ide, Yoshimura & Tsukamoto (1967) on the action of rabbit liver homogenate on quinalbarbitone showed a little of the 3′-oxo derivative (III) to be formed, in addition to the 3′-ol (II) and the acid (IV).

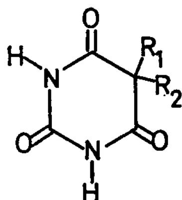
This paper describes an investigation of the metabolism of orally-administered therapeutic doses of quinalbarbitone in healthy volunteers.

MATERIALS

5-Allyl-5-(3′-hydroxy-1′-methylbutyl)barbituric acid (II) was synthesized by the condensation of pent-3-en-2-one with diethyl allylmalonate, reduction of the resulting ketone by sodium borohydride, and cyclization with urea. Fractional crystallization of the resulting diastereoisomeric racemates yielded pure racemate A. Racemate B of II was obtained, contaminated with a little racemate A, by fractional crystallization from the mother-liquors. Racemate A (m.p. 176–177°) has a similar infrared spectrum to Waddell's "hydroxysecobarbital F", whilst racemate B (m.p. 164-167°) corresponds to his "hydroxysecobarbital S" (Waddell, 1965). Oxidation of II with chromium trioxide/acetone yielded the 3′-oxo metabolite (III). The terminal acid (IV) was prepared by a modification of the method used by Tsukamoto & others (1963) for the preparation of the propyl analogue. Condensation of (±)-5-bromohex-1-ene with diethyl malonate followed by ozonolysis, and cyclization with urea, afforded 5-(1′-methyl-3′-carboxypropyl)barbituric acid. Treatment with allyl bromide in aqueous ethanolic sodium hydroxide yielded IV. The diol V was obtained by acid

hydrolysis of epoxy-quinalbarbitone, this being the product of the reaction of 3-chloroperbenzoic acid on quinalbarbitone. The synthetic diol is almost certainly a mixture of the four possible stereoisomers. 5-(1′-Methylbutyl)barbituric acid (VI) and 5-hydroxy-5-(1′-methylbutyl)barbituric acid (VII) were prepared by standard reactions.

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 90012, 6 pages.



	R_1	R_2
I	$-CH(CH_3)-CH_2-CH_2-CH_3$	$-CH_2-CH=CH_2$
II	$-CH(CH_3)-CH_2-CH(OH)-CH_3$	$-CH_2-CH=CH_2$
III	$-CH(CH_3)-CH_2-C(=O)-CH_3$	$-CH_2-CH=CH_2$
IV	$-CH(CH_3)-CH_2-CH_2-COOH$	$-CH_2-CH=CH_2$
V	$-CH(CH_3)-CH_2-CH_2-CH_3$	$-CH_2-CH(OH)-CH_2(OH)$
VI	$-CH(CH_3)-CH_2-CH_2-CH_3$	$-H$
VII	$-CH(CH_3)-CH_2-CH_2-CH_3$	$-OH$
VIII	$-CH_2-CH_2-CH_2-COOH$	$-C_2H_5$
IX	$-CH_2-CH_2-CH(OH)-CH_3$	$-C_2H_5$
X	$-CH_2-C[CH_3]_3$	$-CH_2-CH(OH)-CH_2(OH)$
XI	$-CH_2-C[CH_3]_3$	$-CH_2-CH=CH_2$

Methods

Urine was collected in batches, after quinalbarbitone sodium (4 or 6 × 50 mg Seconal Sodium tablets, Lilly) had been taken orally in the late evening. Samples were worked up and derivatized as previously described (Gilbert, Millard & others, 1974). Gas chromatographic analysis was carried out on both QF1 and OV17 columns as before. Peaks were quantified with reference to calibration curves from

similar runs on extracts from blank urine to which known amounts of synthetic metabolites had been added.

The g.c.-m.s. analysis was carried out on a Finnigan 1015 instrument fitted with a 3% QFI column, under conditions previously described (Gilbert & Powell, 1974).

RESULTS AND DISCUSSION

Using the g.c. method, the 16 h urine of each of the three male and three female volunteers was examined. The results are summarized in Table 1. As can be seen, the diol V is the major metabolite in all but one case; the acid IV comes next, and the products of ω–1 hydroxylation (II) are the least important metabolites. On this small sample of volunteers, no significant metabolic differences between the sexes are apparent. The close similarity of the metabolic patterns shown by this group of volunteers gives confidence that the volunteer used for the extended metabolic study described below is a "typical" case.

The results of the extended study, obtained by the g.c. method, are summarised in Table 2. With the exception of the acid metabolite, the maximum rate of excretion is during the second 12 h period. The data of Table 2 were then examined using the

Table 1. *Urinary excretion of quinalbarbitone metabolites in the first 16 h after administration of a single oral dose (300 mg to males; 200 mg to females) (Recoveries in % dose).*

Subject	Wt(kg)	Dose (mg kg^{-1})	Acid (IV)	Mono-ol (II)*	Diol (V)	Total
I	70	2·9	4·4	4·4	4·2	13·0
II	57	3·5	2·9	2·4	3·8	9·1
III	51	3·9	2·7	2·5	4·3	9·5
Mean ♀	59	3·4	3·3	3·1	4·1	10·5
IV	76	3·9	3·2	2·6	4·5	10·3
V	76	3·9	3·6	3·4	4·1	11·1
VI	88	3·4	3·4	2·5	3·6	9·5
Mean ♂	80	3·7	3·4	2·8	4·1	10·3
Overall Mean	70	3·5	3·4	3·0	4·1	10·4

*The figures refer to the total mono-ols excreted. In all cases the metabolic product was *ca* 20% of racemate A and 80% of racemate B.

Table 2. *Urinary excretion of metabolites after a single oral dose of quinalbarbitone sodium (300 mg, 3·4 mg kg^{-1}) to a healthy male volunteer (Recoveries in % dose).*

Time (h)	Acid (IV)	Mono-ol (II)	Diol (V)
0–12	2·3	1·1	1·7
12–24	2·3	2·1	3·9
24–36	1·9	1·7	3·2
36–48	1·4	1·4	3·5
48–60	1·2	0·8	2·1
60–72	0·7	0·6	1·5
72–84	0·5	0·4	0·8
84–96	0·4	0·3	0·6
96–108	0·3	0·2	0·3
Total	11·0	8·6	17·6

Table 3. *Computer-derived parameters for urinary excretion of the metabolites of quinalbarbitone, and cognate barbiturates.* Coefficients of variation are indicated in parentheses.

	Lag Time (h)	Michaelis-Menten kinetics		1st Order rate constant (x 10^3)	Extrapolated Recovery (%)
		Period	Max. Rate		
Quinalbarbitone					
Acid (IV)	1·7	0–48 h	1·0 (20%)	4·8 (7·3%)	44·6
Mono-ol (II)	6·5	No	—	4·0 (10%)	
Diol (V)	7·3	0–48 h	0·60 (16%)	8·2 (14%)	
Butobarbitone†					
(Case 1)					
Acid (VIII)	4·9	No	—	1·2 (14%)	59·9
Mono-ol (IX)	6·4	0–96 h	0·84 (35%)	6·1 (9·3%)	
(Case 2)					
Acid (VIII)	6·1	0–60 h	0·057 (14%)	1·1 (10%)	68·5
Mono-ol (IX)	7·0	0–60 h	0·27 (9·4%)	5·3 (9·4%)	
Nealbarbitone*					
Diol (X)	3·7	0–84 h	0·80 (33%)	14 (10%)	45·2

† Results taken from Gilbert, Natunen & others (1974).
* Computed from results published by Gilbert, Millard & others (1974).

Saunders computer program (Gilbert & others, 1974). The results of this numerical analysis are summarised in Table 3, alongside comparative results.

It is interesting that, of the three metabolites of quinalbarbitone shown here, only the acid (IV) and the diol (V) show rate-limited kinetics. In the case of the isomeric barbiturate, nealbarbitone (XI), where the only detected metabolite is the diol (X), there is again a zero-order element to the process. As we are only looking at urine concentrations, it is not possible to say whether the limited-rate step is a metabolic or an excretion process, although the former would seem more probable. If, as suggested by, *inter alia*, Maynert, Foreman & Watabe (1970) diols are formed from olefins in the liver via epoxide intermediates, it could, in principle, be either the epoxidation or the hydrolysis step which can be kinetically saturated; again the former would seem more probable. Clearly, these points could be clarified, in principle, by feeding firstly the diol, and secondly the epoxide, to the same human volunteers, and investigating their rate of urinary excretion.

The excretion of quinalbarbitone acid (IV) appears to follow Michaelis-Menten kinetics in the early stages. With butobarbitone, production of the terminal acid (VIII) followed first order kinetics with one volunteer, but Michaelis-Menten kinetics with the other volunteer, so no generalizations can be drawn at this stage.

Although the quoted lag time is very sensitive to the way in which the cumulative excretion results are extrapolated back to zero concentration, it does relate to the physical delay which occurs between ingestion of a drug, and the start of excretion of the drug or its metabolite. Amongst the processes which contribute to this delay must be included (a) absorption of the drug; (b) the onset of metabolic change; (c) distribution of the drug or metabolite into other body compartments; and (d) the excretion characteristics of the substance. The shorter lag time for the acid metabolite (IV) could be related to its greater polarity, compared with the other metabolites. This should reduce the tendency for this metabolite to be distributed in the fat depots, for example, and so encourage rapid excretion. A study of the distribution of directly-administered labelled metabolites in animals would help to clarify the situation.

The excretion of the mono-ols (II) appears to follow first order kinetics. This

observation contrasts with the results of the butobarbitone analysis, where the rate-limited pattern is followed for the ω-1 hydroxylated product (IX). Clearly, analysis of further barbiturate excretion patterns will be needed before useful generalizations emerge. It is already clear, however, that it cannot be taken for granted that barbiturate elimination necessarily follows a simple first order rate. This is especially so when high dosages are involved, as is clear from the recent pharmacokinetic study of amylobarbitone metabolism in dogs by Garrett, Bres & others (1974).

The confirmation of identity of the metabolites of quinalbarbitone, and search for further urinary metabolites, was extended using the g.c.-m.s. method. In the case of the methylated mono-ol (II) and the methylated acid (IV), mass spectra were obtained using the Finnigan 1015 which were compared with those obtained from authentic samples, and the identity of these two metabolites was thereby confirmed. Similarly after silylation of the methylated urine extracts, the diol V gave a satisfactory mass spectrum, similar to that from synthetic material.

Using the mass fragmentography approach as used in the butobarbitone study (Gilbert & Powell, 1974), the derivatized extracts were searched for any other metabolites showing typical barbiturate fragments. No response was obtained for unchanged drug in the urine. However, a positive response was shown at *m/e* values 138, 169, 179, 181, 196 and 237 at the appropriate retention time, and in the appropriate intensity ratios for the 3′-oxo metabolite (III), and this is taken as positive evidence for the presence of this metabolite in the urine, even though it was not possible to obtain a satisfactory mass spectrum for this compound, due to its lack of separation from other urinary components. Preliminary quantification of the 3′-oxo metabolite indicates that it accounts for *ca* 3% of the ingested dose, over 96 h. The identification of the two, barely-separated, diastereoisomeric racemates of the mono-ol (II) was further corroborated by g.c.-m.s. examination of a methylated urine extract which had been subjected to Jones oxidation before analysis. All response for the isomers of II decreased to zero, whilst the peak assigned to the ketone III increased significantly. A search was also carried out for the des-allyl compound VI mentioned by Waddell (1965), but with negative results. It would seem probable that Waddell's spot on the paper chromatogram was due to 5-allylbarbituric acid, arising from the facile retro-Michael reaction which takes place when 3′-oxo metabolites come into contact with fluid of pH >7 (Carroll & Philip, 1971). No trace was found of the 5-hydroxy metabolite (VII) mentioned by Waddell.

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