J. Pharm. Pharmac., 1974, **26**, 119–122 *Received September* 13, 1973

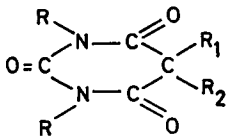
Metabolism of nealbarbitone

J. N. T. GILBERT, B. J. MILLARD, J. W. POWELL, W. B. WHALLEY AND B. J. WILKINS

Department of Pharmaceutical Chemistry, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX, U.K.

The human metabolism of 5-allyl-5-neopentylbarbituric acid (nealbarbitone, Censedal), taken orally, has been studied. Comparison of the gas chromatography—mass spectra of extracts from urine with the spectra of model compounds showed nealbarbitone diol (III) to be the only urinary metabolite present in significant amount (about 30–40% of dose); the rate of excretion of diol reached a maximum about 36 h after ingestion of a single 300 mg dose. Treatment of nealbarbitone with a model oxidase of the Udenfriend type resulted only in oxidation of the allyl side-chain to the 2-oxopropyl derivative, in low yield.

Although the metabolism of barbiturates has been widely reported (e.g. see Foreign Compound Metabolism in Mammals, Vol. 1, pp. 152–155), there appears to be no published account of investigations of the metabolism of nealbarbitone. A number of papers has been published on the metabolism of the closely-related drug quinalbarbitone (II) (e.g. Tsukamoto, Yoshimura & Ide, 1963, 1967; Cochin & Daly, 1963; Waddell, 1965), about which both Cochin & Daly (1963) and Waddell (1965) report 3-hydroxylation of the 1-methylbutyl side-chain and loss of the allyl substituent in man. Waddell (1965) also reports isolation of the diol, formed by dihydroxylation of the allyl group. In nealbarbitone, the allyl group might be expected to undergo similar oxidation, or possibly to be lost metabolically, but the neopentyl side-chain is likely to resist enzymic attack.



R = H in the parent compound, CH_3 in the methylated derivative.

I $R_1 = -CH_2CH=CH_2$ $R_2 = -CH_2C[CH_3]_3$
II $R_1 = -CH_2CH=CH_2$ $R_2 = -C(Me)H[CH_2]_2CH_3$
III $R_1 = -CH_2CH(OH)CH_2OH$ $R_2 = -CH_2C[CH_3]_3$

MATERIALS AND METHODS

Materials

Nealbarbitone (I) was kindly donated by May and Baker Limited. (±)-5-(2′,3′-Dihydroxypropyl)-5-neopentylbarbituric acid (III) was prepared from nealbarbitone by treatment with *m*-chloroperbenzoic acid, followed by acid hydrolysis of the resulting epoxide.

The following new compounds were also prepared: (±)-5-(2′-hydroxypropyl)-5-neopentylbarbituric acid, and its *NN*′-dimethyl derivative; 1,3-dimethyl-5-(2′-oxopro-

pyl)-5-neopentylbarbituric acid; 5-neopentylbarbituric acid; and 5-carboxymethyl-5-neopentylbarbituric acid, and its 1,3-dimethyl derivative.*

Methods

Gas-chromatography and gas chromatography-mass spectrometry. A Pye Series 104 chromatograph, fitted with an inlet heater maintained *ca* 50° above column temperature, and one of the following columns, was used: (a) A 5 ft glass column packed with 3% OV17 on Diatomite CQ, 100–120 mesh, temperature programmed at 185° for 5 min, then 6° min^{-1} to 265°. (b) A 9 ft glass column packed with 3% QF1 on Diatomite CQ, 100–120 mesh, programmed at 170° for 5 min, then 6° min^{-1} to 250°.

The column effluent was connected to a flame ionization detector for straight g.c., or to an AEI MS 902 as previously described (Gilbert, Millard & Powell, 1970) for g.c.-m.s.

Urine was collected in three batches (0–12, 12–24 and 24–48 h) after nealbarbitone had been taken orally (240 mg, Subjects A and B) and 12 h fractions for five days, plus a single 12 h fraction on the eighth day, after administration of a single oral dose (300 mg, Subject C). Portions of each urine fraction, a portion of blank urine from each volunteer and a portion of each blank, to which known amounts of potential metabolites had been added before extraction, were worked up as follows: To urine (100 ml) was added sodium dihydrogen phosphate (10 g, giving a pH of *ca* 5), and sodium sulphate (decahydrate, 30 g), and the solution extracted with ethyl acetate (redistilled, 150, 100 and 100 ml). Each organic extract was separately washed with water (10 ml), all washings being added to the aqueous layer. The combined organic extract was further washed with water (10 ml), dried (Na_2SO_4) and evaporated to dryness (Rotovap). The residue was taken up in methanol (5 ml), ethereal diazomethane (*ca* 5 ml) added, the solution allowed to stand at room temperature (*ca* 15 min), then evaporated to dryness (Rotovap). The methylated residue was dissolved in ethanol (2·0 ml), and this solution divided into two equal portions. One portion was used directly for g.c. and the second portion was evaporated to dryness, then taken up in dry pyridine (0·6 ml), hexamethyldisilazane (0·3 ml) and trimethylchlorosilane (0·1 ml). After standing for at least 1 h, the supernatant was used for g.c. and g.c.-m.s. examination.

RESULTS AND DISCUSSION

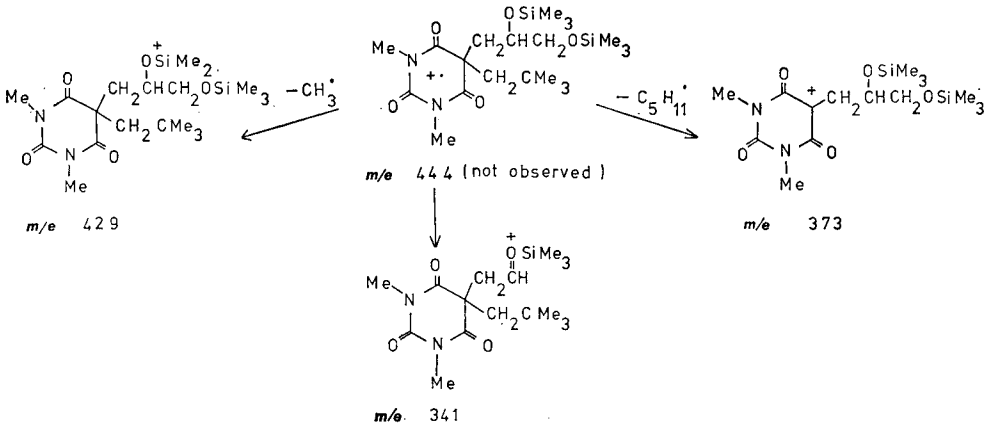
Human metabolism

The g.c. examination of each extract, in each derivatization state, was carried out on both the QF1 and the OV17 columns to reduce the possibility of drug metabolite peaks being obscured by peaks associated with blank urine.

A peak of appropriate retention time for derivatized nealbarbitone diol (III) was produced in all chromatograms of silylated extracts. The identity of this metabolite was confirmed by means of the g.c.-mass spectrum. The fragmentation pathways are given in Scheme I.

The excretion of (III) was quantified on the QF1 column by comparison with the corresponding peak from blank urine to which synthetic diol (III, 1·03 mg %) had been added before work-up. A preliminary experiment over 48 h showed an unexpectedly

* The preparative, spectral and analytical data for all of these compounds are deposited with the British Library, Boston Spa, Yorkshire, U.K., under Reference No. SUP 90 0 06 (6 pages).



Scheme I

high level of (III) at the end of this period (A and B, Table 1). A more extensive study showed excretion as the diol to be at a maximum rate at the end of the second day (C, Table 1); detectable amounts of the metabolite were still being excreted on the eighth day.

Examination of a urine fraction for the presence of metabolite conjugates by re-extraction after treatment of a previously extracted portion with hydrochloric acid (2N) gave no positive results.

In the present work* there was no evidence of excretion of nealbarbitone as (a) unchanged drug, (b) 5-carboxymethyl-5-neopentylbarbituric acid and (c) (±)-5-(2′,3′-epoxypropyl)-5-neopentylbarbituric acid, in amounts greater than 0·1 mg per 100 ml urine and as (d) 5-neopentylbarbituric acid in amounts greater than 1·5 mg %. The observation (c) is of interest in connection with the suggestion by Maynert, Foreman & Watanabe (1970) that epoxides are obligatory intermediates in the metabolism of olefins to glycols. Clearly, if nealbarbitone epoxide is an intermediate in the formation of the diol, it is completely hydrated to the more polar diol before excretion. Observation (d) points to the conclusion that deallylation is not a major route of elimination of nealbarbitone in contrast to the results of Cochin & Daly (1963) and Waddell (1965) for quinalbarbitone (II).

Table 1. *Excretion of nealbarbitone diol expressed as percentage of initial dose of nealbarbitone.*

Time		0–12 h	12–24 h		24–48 h
Subject A		1·8	8·8		11·5
Subject B		4·7	7·0		12·4
Time	0–12 h	12–24 h	24–36 h	36–48 h	48–60 h
Subject C	3·6	5·4	5·8	6·1	5·1
Time	60–72 h	72–84 h	84–96 h	96–108 h	108–120 h
Subject C	3·8	2·5	2·1	1·0	1·0

* On a preliminary run, unchanged nealbarbitone (*ca* 5% of dose) was detected in the urine of a volunteer who had taken 5 × 60 mg drug over 11 h.

Model oxidase system

In an experiment to compare the *in vivo* metabolism of nealbarbitone with the drug's behaviour with a model oxidase of the Udenfriend type (Udenfriend, Clark & others, 1954), the only isolated product (by t.l.c., and by g.c.) was shown to be identical with synthetic 5-(2'-oxopropyl)-5-neopentyl-barbituric acid by mixed m.p., infrared, ultraviolet and nmr. That neither the epoxide, nor the corresponding secondary alcohol are intermediates in this oxidation is indicated by the observation that both of these compounds were recovered unchanged when submitted to the oxidase system. Clearly, the Udenfriend system is not an appropriate model for the hepatic enzymes involved in the metabolism of nealbarbitone.

Acknowledgements

The authors wish to thank the Science Research Council for a grant to purchase the MS 902; and Smith Kline and French for a grant to purchase g.c. equipment.

REFERENCES

COCHIN, J. & DALY, J. W. (1963). *J. Pharmac. exp. Ther.*, **139**, 154–159.
GILBERT, J. N. T., MILLARD, B. J. & POWELL, J. W. (1970). *J. Pharm. Pharmac.*, **22**, 897–901.
MAYNERT, E. W., FOREMAN, R. L. & WATABE, T. (1970). *J. biol. Chem.*, **245**, 5234–5238.
TSUKAMOTO, H., YOSHIMURA, H. & IDE, H. (1963). *Chem. Pharm. Bull. Tokyo*, **11**, 9–13.
TSUKAMOTO, H., YOSHIMURA, H. & IDE, H. (1967). *Ibid.*, **15**, 411–419.
UDENFRIEND, S., CLARK, C. T., AXELROD, J. & BRODIE, B. B. (1954). *J. biol. Chem.*, **208**, 731–739.
WADDELL, W. J. (1965). *J. Pharmac. exp. Ther.*, **149**, 23–28.