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The synthesis and urinary estimation of *N*-hydroxyaprobarbitone

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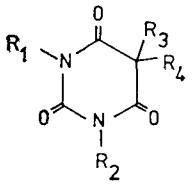
N-Hydroxyaprobarbitone (I) has been synthesized by oxidation of aprobarbitone, characterized, and a method developed for its estimation in urine. It has shown to be no more than a minor metabolite (<4%) in the human metabolism of aprobarbitone.

In view of recent reports (Tang, Inaba & Kalow, 1975, 1977a,b) that *N*-hydroxy-derivatives are major metabolites of amylobarbitone and pentobarbitone in man, it seemed desirable to develop a synthetic route to these derivatives, in order to investigate their properties more fully, and to confirm unequivocally their involvement in metabolic processes. As a study of the human metabolism of aprobarbitone was taking place in these laboratories, this drug was the chosen substrate for development of the synthetic route towards *N*-hydroxybarbiturates.



	R_1	R_2	R_3	R_4
I	H	OH	$-CH_2-CH=CH_2$	$-CH(CH_3)_2$
II	CH_3	OCH_3	,,	,,
III	H	$OCOCH_3$	,,	,,
IV	H	H	$-CH_2-CH(OH)-CH_2OH$	,,
V	H	H	$-CH_2-C(=O)-CH_3$	$-CH_2-CH(CH_3)_2$

* Correspondence.

METHODS

Aprobarbitone (100 mg) was taken orally in a gelatine capsule by a healthy male volunteer before retiring, and urine was collected in 8 h batches for the following 3 days. Aliquots of each batch were extracted, derivatized, and examined on the Finnigan 1015 g.c.-quadrupole mass spectrometer using the previously described techniques (Gilbert & Powell, 1976). Aprobarbitone was quantified using quinalbarbitone as internal standard, the mass spectrometer being tuned to *m/e* 195. Aprobarbitone diol was determined in relation to quinalbarbitone diol (Gilbert, Hetherington & others, 1975); the methylated extract was treated with dichlorodimethylsilane (Gilbert & Powell, unpublished), and examined at *m/e* 131. *N*-Hydroxyaprobarbitone was quantified by examination of the peak at *m/e* 211, using 5-(2′-oxopropyl)-5-(2′-methylpropyl)barbituric acid (V, Gilbert, Johnson & Powell, 1977) as internal standard.

RESULTS

Unchanged aprobarbitone excreted in the urine during the three days following ingestion of a 100 mg dose accounted for 9% of the drug. The diol (IV) accounted for a further 12% of the dose. By using multiple ion monitoring at *m/e* 169, 181, 195 and 211, a peak occurred on all channels at a relative retention time of 2·1 (methylated aprobarbitone =1·00), using a 9 ft, 2% OV 210 column operating at 210°. The responses on these channels were in intensity ratios similar to those obtained by treatment of urine in which synthetic *N*-hydroxyaprobarbitone (I) had been dissolved, and at the appropriate retention time. By means of a calibration curve derived from urine doped with known amounts of (I), and of (V) as internal standard, the *N*-hydroxyaprobarbitone in metabolic urine was estimated to account for 3·6% of the ingested aprobarbitone.

DISCUSSION

The preparation of hydroxamic acids from secondary amides by treatment of the *N*-trimethylsilyl derivatives with a diperoxo complex of molybdenum (Matlin & Sammes, 1972) suggested a possible route for the preparation of the required *N*-hydroxy derivatives of barbiturates†. The barbiturate was first silylated under rather vigorous conditions; it is then treated with the bis-*NN*-dimethylformamide complex of diperoxomolybdenum^{VI} (one equivalent; excess of the complex gives rise to the 1,3-dihydroxy derivative of the barbiturate in poor yield) in dichloromethane overnight. Separation of the required *N*-hydroxy derivative from aprobarbitone by differential extraction with ethyl acetate at controlled pH proved satisfactory, due to the higher acidity of the product, as compared with the parent drug.

The structure of *N*-hydroxyaprobarbitone (I) was confirmed by the following evidence. (a) A positive reddish colour reaction, as expected for a hydroxamic acid, was obtained with ferric chloride. (b) The ultraviolet spectrum was consistent with the formulation of the oxidation product as a 1,5,5-trisubstituted barbiturate. (c) The elemental analysis was consistent with mono oxidation. (d) The accurate mass measurement of the molecular ion confirmed the molecular formula. The EI mass spectrum of *N*-hydroxyaprobarbitone was significantly different from that of aprobarbitone whereas Tang & others (1975, 1977a) found the CI mass spectra of their *N*-hydroxy barbiturates to be identical to those of the parent drug. (e) Methylation with diazomethane clearly yielded an *N,O*-dimethyl derivative, as shown by both nmr and mass spectral evidence. It is unexpected that Tang & others (1977a) found the N–OH group of amylobarbitone and pentobarbitone derivatives resistant to methylation. (f) Acetylation was shown by nmr and ms to give a mono-*O*-acetyl derivative. No difficulty was found in obtaining a characteristic mass spectrum for the acetate.

A satisfactory linear calibration curve was obtained for estimation of *N*-hydroxyaprobarbitone in urine, using the mass spectral fragment at *m/e* 211, for the concentration range 3–30 mg litre^{-1}. Using this technique, the *N*-hydroxylated metabolite of aprobarbitone was shown to be present to an extent of 3·6% of the dose, in the three days following administration of the drug. Thus it is clear that this metabolic route is no more than a minor one, in the case of this drug, unless the hydroxamic acid undergoes further changes within the body. Some 75% of the dose still remains unaccounted for.

The kinetics of excretion of drug and metabolite, and the consequences of these observations, will be reported elsewhere. Meanwhile, it is clear that the concept of *N*-hydroxylation does not solve all the remaining problems involved in the metabolic fate of barbiturates in humans.

Materials. Aprobarbitone (5-allyl-5-isopropylbarbituric acid, m.p. 141·5–143·5°) was kindly donated by Hoffmann La Roche Ltd. Aprobarbitone (200 mg) was heated under reflux (3 h) with hexamethyldisilazane (HMDS, 6 ml) and trimethylchlorosilane (TMCS, 3 ml) in the presence of sulphuric acid (2 drops, conc., added after the other reagents). Evaporation under reduced pressure yielded the bis-trimethylsilyl derivative as a gum; this was taken up in dry methylene dichloride (5 ml). The supernatant was decanted from any inorganic residue, and treated with freshly prepared and recrystallized diperoxo-oxobisdimethylformamidomolybdenum^{VI} (MoO_5·2DMF, 380 mg; see Mimoun, Roche & Sajus, 1969) overnight. The yellow solution was treated with saturated aqueous EDTA (6 ml, pH 9·5) to break the hydroxamic acid/molybdenum complex (Matlin & Sammes, 1972). Extraction with ethyl acetate removed unchanged aprobarbitone; after adjustment of the pH of the residue to 6–7, re-extraction with ethyl acetate, evaporation of solvent, and crystallization from methylenedichloride yielded (±)-1-*hydroxy-5-allyl-5-isopropylbarbituric acid* (I) as a hygroscopic solid (m.p. 126–7°C, 42 mg). Found: C, 52·8; H, 6·4; N, 12·0; *m/e* 226·0954. $C_{10}H_{14}N_2O_4$ requires C, 53·1; H, 6·2; N, 12·4%; *m/e* 226·0953. *m/e* 226 (6), 211 (6) ($M^{+\cdot}$–$CH_3^{\cdot}$), 209 (5) ($M^{+\cdot}$–$OH^{\cdot}$), 184 (23), 183 (57), 167 (33), 141 (7), 140 (24), 124 (15); 113 (7), 109 (15), 43 (64), 41 (100). λ max (N/100 aq. NaOH) 225 nm (ϵ=8900). The nmr spectrum ($CDCl_3$) showed the allyl and isopropyl substituents to be unchanged.

Treatment of a methanolic solution of (I) with ethereal diazomethane yielded (±)-1-methoxy-3-methyl-5-allyl-5-isopropylbarbituric acid (II) as a gum. Nmr ($CDCl_3$): δ 3·35 (s, 3H, N–CH_3) and δ 3·95 (s, 3H, O–CH_3). *m/e* 254 (4), 253 (5), 241 (21), 223 (6), 211 (9), 199 (11), 195 (11), 181 (11), 169 (11), 123 (11), 112 (10), 111 (14), 110 (10), 109 (20), 57 (100), 43 (100).

Acetylation of *N*-hydroxyaprobarbitone (I, 5 mg) with acetic anhydride (0·5 ml) and pyridine (0·5 ml) yielded the acetate (III) as a gum; nmr ($CDCl_3$) 2·2 (s, 3H, acetyl); *m/e* 268 (5·5), 225 (35) ($M^{+\cdot}$–$CH_3CO^{\cdot}$), 209 (14) ($M^{+\cdot}$–CH_3COOH), 184 (31), 183 (50), 167 (28), 140 (13), 109 (12), 43 (100).

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