N-Hydroxyamylobarbitone—a metabolite of amylobarbitone?

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In view of the published reports of evidence for the occurrence of major amounts of *N*-hydroxylated metabolites of barbiturates in urine (Tang, Inaba & Kalow, 1975, 1977a, b), and of the reported physiological dangers of *N*-hydroxy compounds (Weisburger & Weisburger, 1973), we have synthesized authentic *N*-hydroxyamylobarbitone, and checked on its occurence as a urinary metabolite in man.

N-Hydroxyamylobarbitone was synthesized by an adaptation of the published method for *N*-hydroxyaprobarbitone (Gilbert, Nelmes & Powell, 1978), using the analogous hexamethylphosphoramide complex of diperoxomolybdenum^{VI} (Matlin & Sammes, 1972). It was purified by preparative t.l.c. (silica gel GF 254, acetone) and showed a single spot on analytical t.l.c. Soft crystals, decompose >200°. Found *m/e* 242·1268, $C_{11}H_{18}N_2O_4$ requires 242·1266; *m/e* 242 (2) $M^{+\cdot}$, 227 (3) $M^{+\cdot} - CH_3\cdot$, 225 (4) $M^{+\cdot} - OH\cdot$, 172 (100) $M^{+\cdot} - C_5H_{10}$, 157 (46), 129 (48). On treatment with ethereal diazomethane, a gum was formed, showing signals in the nmr at δ 3·32 (*N*-methyl) and δ 4·0 (*O*-methyl). The methylated product was subjected to g.c.-ms (Finnigan 1015), using a 9 ft OV 210 column at 210°; it gave a single peak at relative retention time 1·6 (*NN'*-dimethylamylobarbitone = 1·00), and showed the following major mass spectral fragments: *m/e* 270 (1) $M^{+\cdot}$, 225 (7) $M^{+\cdot} - CH_3\cdot$, 242 (7) $M^{+\cdot} - C_2H_4$, 239 (6) $M^{+\cdot} - OCH_3\cdot$, 227 (6), 216 (14), 201 (22), 200 (100) $M^{+\cdot} - C_5H_{10}$, 185 (26), 171 (21), 170 (22), 169 (26), 155 (21), 143 (94), 126 (23).

When synthetic *N*-hydroxyamylobarbitone was added (1·5 μg ml^{-1}) to the urine of a volunteer who has ingested 200 mg sodium amylobarbitone during the previous 24 h, and the urine extracted with ethyl acetate then derivatized (Gilbert & Powell, 1976), a sharp peak was observed on the single ion monitor trace (*m/e* 200) at the appropriate retention time. Urine from the same batch to which no synthetic *N*-hydroxyamylobarbitone had been added showed only baseline noise (<5% of peak height from spiked urine) and contained unchanged amylobarbitone (0·6 μg ml^{-1}). 3′-hydroxyamylobarbitone (24 μg ml^{-1}) and amylobarbitone acid (Baldeo, Gilbert & Powell, 1977; 3·4 μg ml^{-1}). The same experiment was repeated on a second batch of urine from the same volunteer, and on urine from a separate volunteer. In all cases, the search was carried out at *m/e* 185 and *m/e* 169 in addition to *m/e* 200; the results were never positive.

In a further set of experiments, aliquots of the 12–24 h urine of 10 volunteers who had ingested amylobarbitone sodium (200 mg), were extracted with ether, and the methylated extracts examined on a Varian 1400 g.l.c. fitted with a 6 ft glass column packed with 3% OV 17 on Gas Chrom Q. Using an N_2 flow rate of 20 ml/min, an oven temperature of 185°, and an injector temperature of 220°, the retention time of methylated *N*-hydroxyamylobarbitone was 2·8 min, and the limit of its detection when extracted from blank urine was 0·5 μg ml^{-1}. The concentration of *N*-hydroxylamyobarbitone in all urine samples was below this detection limit.

The methylated *N*-hydroxyamylobarbitone was also examined by g.c.-ms in the CI mode with methane as reagent gas, using a Finnigan 4000 operating at a source temperature of 220°C; the g.c. conditions were as described above but using CH_4 instead of N_2 as carrier gas. The spectrum showed the following ions; *m/e* 311 (5), 299 (7), 271 (100, MH^+), 269 (11), 255 (12), 241 (41), 141 (13). This spectrum is entirely consistent with the structure of methylated *N*-hydroxyamylobarbitone and establishes that this derivative can be readily gas chromatographed under the above conditions without decomposition.

There is thus no doubt that the excretion of free *N*-hydroxyamylobarbitone is not significant in the metabolism of amylobarbitone in these 12 persons.

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