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HEXOBARBITAL-1',2'-EPOXIDE: A POSSIBLE INTERMEDIATE IN THE METABOLISM OF HEXOBARBITAL

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Hexobarbital (1,5-dimethyl-5-(1'-cyclohexenyl)-barbituric acid) is one of the most widely used test compound in drug metabolism studies where changes in sleeping time are correlated with changes in oxidative drug metabolism enzyme activity. The metabolism of hexobarbital in several species has recently been reviewed[1,2]. The major urinary metabolites are 3'-allylic-oxidation products, whereas nitrogen-demethylated products and barbiturate ring cleavage products appear to be of minor significance. Generally 3'-ketohexobarbital is thought to be formed by cytoplasmatic dehydrogenation of 3'-hydroxyhexobarbital, the latter compound resulting from the interaction between hexobarbital and cytochrome P-450 containing mixed function oxidases. However, for non-cyclic olefin-substituted barbiturates like quinal[3]- and nealbarbitone[4], evidence was found for the presence of 2',3'-dihydroxy metabolites in rat urine, probably formed by chemical or enzymatic hydrolysis of more or less stable 2',3'-epoxides, thus indicating the presence of an epoxide-diol pathway. The question arose whether a similar pathway could also exist for cylic olefin-substituted barbiturates. Hexobarbital was chosen as a model compound.

In this preliminary communication the identification of hexobarbital-1',2'-epoxide in the urine of rats treated with hexobarbital is reported. In addition evidence was obtained, by studying the metabolic fate of the epoxide itself, that an epoxide pathway may be an important route in the overall metabolic fate of hexobarbital.

Materials and Methods

Hexobarbital-1',2'-epoxide was prepared by treating hexobarbital with m-chloroperbenzoic acid; the detailed procedure will be published elsewhere*.

* Both an α- and a β-epoxide are formed upon peracid oxidation of hexobarbital, the chemical and physical properties of which are not expected to differ appreciably. The synthetic epoxide appears homogeneous on TLC, HPLC, GLC, H-nmr and mass spectra. Neither this epoxide nor hexobarbital itself induce chemical mutagenicity in the Ames Salmonella / microsome test[6].

α- And β-3'-hydroxyhexobarbital and 3'-ketohexobarbital were synthesized according to previously described procedures[5]. These compounds served as reference material for studies concerning stability, extraction behaviour from rat urine, gas chromatography and gas chromatography-mass spectrometry. A GCV-Pye Unicam gas chromatograph, with a nitrogen-selective detector and a LKB-2091-2130 (EI/CI) gas chromatograph-mass spectrometer-computer system were used, equipped with OV-17 packed or capillary SCOT-columns. Gas chromatograms were run under isothermal conditions, at 210° for packed or 170° for capillary columns. 70 eV mass spectra were recorded continuously at 2 sec/scan with a 0.5 sec interscan delay. CI mass spectra were obtained with isobutane as a reactant gas. With male Wistar rats (body-weight ca 200 g, starved for 24 h) two series of experiments were performed: a) intraperitoneal injection of 20 mg of hexobarbital in 0.8 ml of propylene glycol; b) intraperitoneal injection of 20 mg hexobarbital-1',2'-epoxide in 0.8 ml of propylene glycol; in addition a control experiment was performed with an intraperitoneal injection of 0.8 ml propylene glycol. Urine was collected for 8 or 24 hours.

Results and Discussion

After extraction of the urine, obtained from the rats treated with hexobarbital, with dichloromethane/pentane (80:20) the gas chromatogram showed at least three peaks with mass spectral characteristics, similar to those of hexobarbital. A small one, with the shortest retention time, had a retention time and a mass spectrum (Table 1,I) identical to hexobarbital.

Table 1.
Most abundant m/e-values at 70 eV (relative intensities %) and relative retention times (min).

ref. number	*name*	*relative retention time*	$M^{+\cdot}$	*other m/e-values*
I	*hexobarbital (HB)*	*1.00*	*236(15)*	*221(100) 81(48) 167(38)*
II	*3'-hydroxy-HB*	*1.45*	*252(2)*	*97(100) 167(38) 138(4)*
III	*3'-keto-HB*	*3.37*	*250(75)*	*95(100) 235(35) 207(32)*
IV	*1',2'-epoxy-HB*	*1.37*	*252(30)*	*97(100) 167(68) 127(41)*

The two other peaks, which were substantially larger, had retention times and mass spectra (both electron impact and chemical ionisation) identical to synthetic 3'-hydroxyhexobarbital (Table 1, II) and synthetic 3'-ketohexobarbital (Table 1, III) respectively. The same urine extract was further, more thoroughly, studied with mass chromatography, resulting in the positive identification of a small quantity of hexobarbital-1',2'-epoxide.

In Fig. 1 the mass spectrum of synthetic hexobarbital-1',2'-epoxide is shown. In Fig. 2 a typical urinary mass chromatogram of three specific m/e-values of the epoxide is shown.

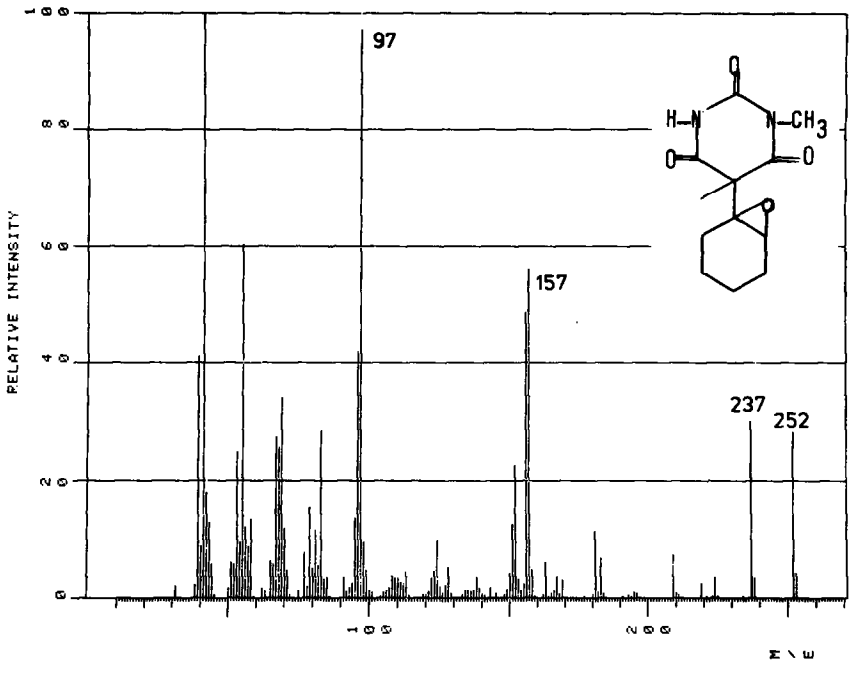

Fig. 1. Mass spectrum of hexobarbital-1',2'-epoxide at 70 eV.

The retention time at which the three characteristic masses appeared simultaneously corresponded exactly to the retention time of synthetic hexobarbital-1',2'-epoxide, thus confirming the presence of this epoxide in the urinary extract. In the second series of experiments urine of rats, treated with hexobarbital-1',2'-epoxide, was extracted and investigated in the same way as described before. Interestingly, again 3'-hydroxy- and 3'-ketohexobarbital (Table 1, II and III) were found to be the most abundant metabolites of this epoxide. Besides some unchanged epoxide, two other metabolites were found. Their mass spectra are very similar to those of two 3'-hydroxyhexobarbital-1',2'-epoxides, obtained by synthesis. Work on their definite structure assignment is in progress. The expected dihydroxyderivative could not be identified nor synthesized, possible due to its lack of stability.

The identification of hexobarbital-epoxide as a new metabolite of hexobarbital and the indentification of 3'-hydroxy- and 3'-ketohexobarbital as the major metabolites of this epoxide suggest that the epoxide pathway may be of importance in the formation of 3'-hydroxy- and 3'-ketohexobarbital, the major metabolites of hexobarbital.

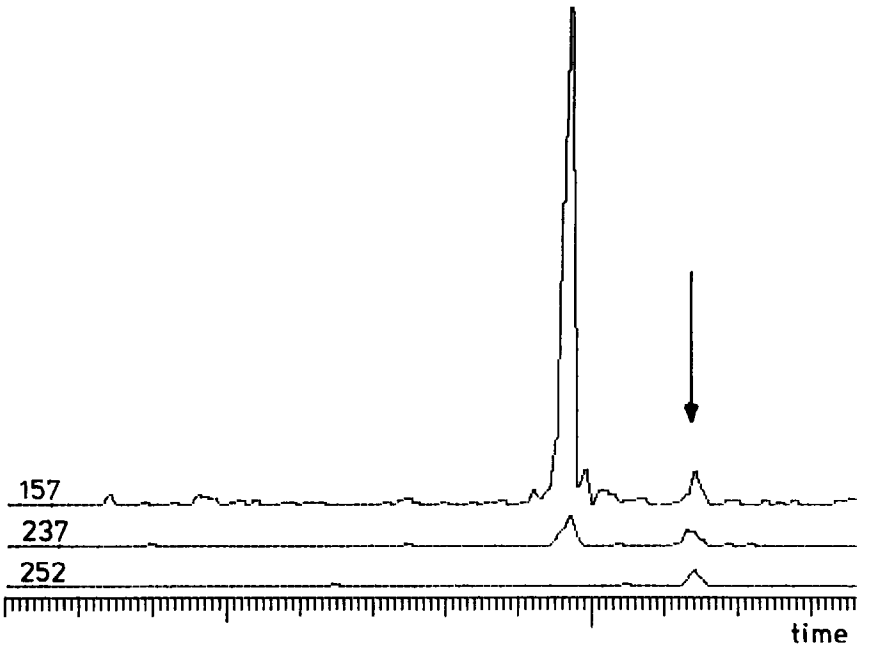

Fig. 2. Mass chromatogram with three typical m/e-values of a rat urinary extract.

On the other hand it cannot be excluded at the present stage that the epoxide pathway is only an alternative metabolic pathway and is of minor importance in the actual metabolism of hexobarbital. To define the extent of occurrence of both possibilities in the metabolism of hexobarbital and similar compounds further research is in progress.

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