Technical Papers

Metabolic Oxidation of Phenobarbital to *p*-Hydroxyphenobarbital

Thomas C. Butler

Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill

Numerous aromatic compounds have been found to undergo oxidation to phenolic products in the mammalian organism. This type of reaction might be expected to occur in the phenyl group of phenobarbital (5-ethyl-5-phenyl barbituric acid), but it has not hitherto been described. This report (*1*) concerns the discovery in dog urine of a product of the metabolism of phenobarbital and its identification as the *p*-hydroxy derivative of phenobarbital.

The compound as present in urine is largely conjugated and can be released by acid hydrolysis. An equal volume of concentrated hydrochloric acid is added to urine, and the mixture is refluxed for 3 hr. Phenobarbital and *p*-hydroxyphenobarbital are stable under these conditions. Both compounds are extracted from the acid urine with ether, and their separation and purification are accomplished by a systematic procedure of partitions between ether and buffers and benzene and buffers and finally by crystallization from water. The physical properties on which the isolation procedures are based are shown in Table 1. From urine collected for 3 wk from a dog receiving daily doses of phenobarbital, there were isolated in this way 1.54 g of *p*-hydroxyphenobarbital and 0.29 g of unchanged phenobarbital.

The structural identification of the urinary product was established by synthesis. The synthesis of *p*-hydroxyphenobarbital, which has not previously been described, was carried out by the following procedure. *p*-Nitrophenobarbital was prepared by the method of Pierce and Rising (*2*), and the position of the nitro group was confirmed by their procedure of hydrolysis and oxidation to *p*-nitrobenzoic acid. *p*-Nitrophenobarbital was converted to the phenolic derivative by catalytic reduction with hydrogen, diazotization of the amine, and hydrolysis of the diazonium salt. *p*-Hydroxyphenobarbital crystallizes from water with 1 mole of water. It melts at 222° to 223°C, corrected. The ultraviolet absorption spectrum as it is influenced by *p*H is indicative of three dissociations, corresponding to the loss of the protons from the two nitrogen atoms and the phenolic group. Identity of the urinary product with the synthetic compound was demonstrated by the method of mixed melting points. Analysis of hydrate (urinary origin): C, 54.01, 54.20; H, 5.09, 4.95; N, 10.42, 10.52 percent. Calculated for $C_{12}H_{14}O_5N_2$: C, 54.12; H, 5.30; N, 10.52 percent.

Table 1. Physical properties of phenobarbital and *p*-hydroxyphenobarbital furnishing the basis for the isolation procedures.

Compound	Partition coefficient of acid form		pK_1'*
	Ether/water	Benzene/water	
Phenobarbital	60	1	7.23
p-Hydroxyphenobarbital	5	0	7.30

* At 38°C and total ionic strength of 0.1.

The other isomeric hydroxyphenobarbitals have not yet been found in urine, but a search for traces of these compounds continues. The conjugated form or forms in which *p*-hydroxyphenobarbital is excreted are still unidentified. Conjugation might be expected to occur with both glucuronic acid and sulfuric acid.

In doses as high as 1 g/kg, *p*-hydroxyphenobarbital is not anesthetic in mice. It appears that oxidation of phenobarbital to the phenolic derivative is a mechanism of major importance in the pharmacologic inactivation of the drug.

References and Notes

1. This investigation was supported in part by a research grant (B-384) from the National Institute of Neurological Diseases and Blindness, National Institutes of Health, U.S. Public Health Service.
2. A. E. Pierce and M. M. Rising, *J. Am. Chem. Soc.* **58**, 1361 (1936).

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Determination of Isotopic Carbon in Organic Compounds

K. E. Wilzbach and W. Y. Sykes

Chemistry Division, Argonne National Laboratory, Lemont, Illinois

The determination of isotopic carbon in organic compounds has been simplified by development of a procedure in which the sample is heated with copper oxide in a sealed tube and the carbon dioxide produced is isolated by fractional condensation in vacuum. The method is applicable to a wide variety of compounds and yields results that are reproducible to 1 percent and agree with values obtained by a more elaborate procedure (*1*) based on Pregl combustion. Operations required to obtain a sample of gas for isotopic analysis can be performed in 30 min without elaborate equipment. The similarity of the procedure to the zinc fusion technique (*2*) for tritium assay renders it particularly attractive where research with tritium, as well as with C^{14}, is anticipated.

A convenient system for application of this procedure to the determination of C^{14} is shown in Fig. 1. In combustion tube *A*, made from a 17-cm length of 11-mm OD Pyrex 1720 glass tubing (*3*) by drawing out one end to form a break tip, are placed 0.75 g of