ETHYL(3-HYDROXYISOAMYL)BARIURIC ACID AS THE PRINCIPAL METABOLITE OF AMYTAL*

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An earlier study (1) from this laboratory proved conclusively that Amytal, 5-ethyl-5-isoamylbarbituric acid (I), is not detoxified by simple hydrolysis of the barbituric acid ring and indicated that the alkyl side chain (or chains) must suffer alteration in vivo. Recently (2), it was discovered that the isomeric drug, pentobarbital, is excreted as the diastereoisomers of 5-ethyl-5-(3-hydroxy-1-methylbutyl)barbituric acid. This finding, reflecting an unusual pattern of biological oxidation, initiated further work to identify the end-products of Amytal. The present report describes the isolation, proof of structure, and quantitative determination in the urine of dogs of the principal metabolite of the drug.

From extracts of urine of dogs after the administration of Amytal it was possible to isolate only one metabolite of the drug, but this was obtained in relatively good yield (35 per cent). The compound had an elementary composition corresponding to Amytal with 1 additional oxygen atom. It had the characteristic ultraviolet absorption spectrum of 5,5-dialkylbarbituric acids. The presence of a hydroxyl group was indicated by the infra-red spectrum and proved by the preparation of a crystalline acetate. The metabolite was optically inactive.

Although there are six different monohydroxy derivatives of Amytal, it was reasoned that 5-ethyl-5-(3-hydroxyisoamyl)barbituric acid (II) was the most likely structure for the metabolite. Special consideration of this isomer was based largely on two facts. First, ethyl groups attached to the barbituric acid ring are very stable in vivo (3, 4). Secondly, primary alcohols resulting from o oxidation are rapidly oxidized to acids (5, 6), whereas secondary or tertiary alcohols from non-terminal carbon oxidation appear to be fairly stable in vivo, as judged from the metabolites of pentobarbital and hydrolapachol (discussed below). The surmise was proved to be correct by synthesis.

The metabolite of Amytal was synthesized by two different methods. The first procedure involved the addition of water to 5-ethyl-5-(3-methyl-
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2-butenyl)barbituric acid (III). The second method was based on the greater propensity for oxidation of tertiary carbon atoms. Treatment of Amytal with chromic acid in glacial acetic acid gave the 3-hydroxyisoamyl derivative in 30 per cent yield. A by-product of the reaction was 5-carboxymethyl-5-ethylbarbituric acid; this substance became the only isolable product if the reaction time or temperature was increased. The oxidation of an isoamyl chain to the 3-hydroxy derivative by chromic acid has been reported for hydrolapachol by Fieser (7).

The quantitative determination of the metabolite in urine was made by the isotope dilution technique. Amytal labeled with N15 (1) was dissolved in 1 equivalent of sodium hydroxide and administered to two dogs by stomach tube in a dose of 45 mg. per kilo. In accordance with previous data (1), the animals excreted 69 and 76 per cent of the administered isotope in the urine in 48 hours. In order to guard against coprecipitation of other labeled metabolites, the products from the dilution experiments were analyzed in the mass spectrophotometer not only as the alcohol but
also as the acetyl derivative. The results revealed that, in the urine of both dogs, 67 per cent of the excreted isotope was in the form of 5-ethyl-5-(3-hydroxyisoamyl)barbituric acid. This high yield signifies that the metabolic fate of Amytal is now known more completely than that of any other barbiturate, except barbital, which is excreted completely unchanged (3, 8).

The present work provides the second example of the metabolic conversion of an isoamyl chain to the corresponding 3-hydroxy derivative. The first demonstration of this particular oxidative pattern was by Fieser et al. (9), who found that hydrolapachol was excreted by man as hydroxy-hydrolapachol. It is still too early to predict whether this pattern is general for the isoamyl group attached to a carbocyclic or heterocyclic ring. Other compounds containing the isoamyl chain which have been studied for elucidation of their metabolic fate are isoamylmalonic acid (10) and γ-methylvaleric acid (11). Even with acyclic substances like these, present evidence is not sufficient to exclude the possibility of oxidation at the tertiary carbon atom of the isoamyl group.

EXPERIMENTAL

Isolation of Metabolite—Amytal in a dose of 45 mg. per kilo was administered orally once a week to four dogs weighing 9 to 16 kilos until a total of 6.0 gm. of drug had been given. The 48 hour urines were brought to pH 6.5 and maintained at this pH during 48 hours of continuous extraction with ether. The ether extracts were combined, evaporated to a volume of 600 ml., and extracted ten times with 150 ml. portions of water. The aqueous extracts were combined and distilled in vacuo until the volume was 25 ml. On standing in the refrigerator the solution deposited 1.56 gm. of light brown needles; m.p. 180–185°. Evaporation of the filtrate at reduced pressure yielded an additional 0.56 gm. of crystals with the same melting point. The product was decolorized with charcoal and recrystallized three times from water. The pure compound melted at 187–188°.

Analysis—C_{12}H_{18}O_{4}N_{2}. Calculated. C 54.49, H 7.48

Ultraviolet Spectrum—\( \epsilon = 7700 \) (0.5 N NaOH, 255 μm)

Infra-Red Spectrum—Strong absorption band at 2.8 μ

Acetyl Derivative—The metabolite (100 mg.) and 3 ml. of acetic anhydride

1 All melting points were taken with a calibrated Fisher-Johns apparatus. The elementary analyses were carried out by Mr. Joseph F. Alicino. The infra-red spectrum was determined at the Lilly Research Laboratories through the courtesy of Dr. E. C. Kleiderer and Mr. Thomas V. Parke. Thanks are due to Dr. David Rittenberg and his staff in the Department of Biochemistry, Columbia University, for the mass spectrometric determinations of N°18.
were heated on a steam bath for 12 hours. Water was added and the aqueous acetic acid removed in vacuo. The crystalline residue was dissolved in ethanol and precipitated with water; m.p. 169-180°. After recrystallization from aqueous ethanol and water, the product melted at 185-186°; yield, 70 mg.

Analysis—C_{12}H_{20}O_{3}N_{2}. Calculated. C 54.91, H 7.09
Found. " 54.90, " 6.77

The melting point of the acetate was depressed after admixture with the metabolite.

Isotope Dilution Experiments—The labeled Amytal was described in a previous report (1); it contained 15.3 atoms per cent excess N^{15}. Both dogs were females; they were not fed on the day of the administration of drug and were given an essentially nitrogen-free diet consisting of sucrose and corn oil during the 2nd day in order to prevent dilution of the isotope by exogenous nitrogen. The 48 hour urine samples were diluted to 600 ml., and a 200 ml. aliquot was used for each dilution experiment. Both natural and synthetic metabolites were employed as the diluent; the usual amount was 300 mg. The metabolite was reisolated from urine by continuous extraction with ether and evaporation of the solvent. Recrystallization of the residue was continued until the melting point was appropriate and the isotopic composition was constant (±1 per cent). The purified metabolite was then converted to the acetate and again analyzed in the mass spectrophotometer. In every case the isotopic composition of the acetate was identical with that of the metabolite.

Synthesis of 5-Ethyl-5-(3-methyl-2-butenyl)barbituric Acid—This compound had not been reported in the chemical literature when the present work was done but has since been described by Walton, Doczi, and King (12). No experimental details are required here, because the procedure, yields, and physical constants for intermediate compounds and the final product reported by Walton and coworkers match ours perfectly.

Synthesis of 5-Ethyl-5-(3-hydroxyisovalyl)barbituric Acid—A solution of 500 mg. of 5-ethyl-5-(3-methyl-2-butenyl)barbituric acid in 1.75 ml. of concentrated sulfuric acid was allowed to stand at room temperature for half an hour. During this time the original light yellow solution turned dark orange. It was then poured over 7.5 gm. of cracked ice to yield a sticky precipitate which largely disappeared by the time all of the ice had melted. The product was heated for a few minutes on a steam bath to complete the dissolution of oily material. After cooling to room temperature and neutralization to pH 6, the solution deposited 300 mg. of light yellow crystals; m.p. 170-175°. Extraction of the filtrate five times with an equal volume of ether yielded an additional 75 mg. of solid; m.p. 175-185°. After two recrystallizations from water the product melted at
187-188°. There was no depression of the melting point when the compound was mixed with the metabolite of Amytal.

**Chromic Acid Oxidation of Amytal**—In a round bottomed flask were placed 1.35 gm. of Amytal, 8.0 gm. of chromic acid, and 75 ml. of acetic acid. The mixture was stirred with a Hershberg stirrer for half an hour at 20° and then poured into 250 ml. of ether. The ether solution was extracted with small portions of water until it was colorless and then evaporated to a thick, light colored oil. Trituration of the oil with 10 ml. of ether yielded a white solid which was collected by filtration and washed with 3 ml. of ether; m.p. 178-186°; yield, 373 mg. After one recrystallization from water the product melted at 187-188° and did not depress the melting point of the Amytal metabolite.

Evaporation of the filtrates and trituration with 5 ml. of ether yielded 73 mg. of a white solid melting at 295-298°. After recrystallization from water the product melted at 304-305°.

Analysis—$\text{C}_9\text{H}_9\text{O}_4\text{N}_2$. Calculated. C 44.85, H 4.71. Found. " 44.50, " 4.87.

This compound is apparently 5-carboxymethyl-5-ethylbarbituric acid. Staudinger (13) gives the melting point as 280-281°. This discrepancy can be explained on the basis of the rate of heating in the determination of the melting point. Very slow heating leads to decomposition and, consequently, even lower melting points than that recorded in the patent.

The ethyl ester was prepared by adding dry hydrogen chloride to a solution of 500 mg. of the acid in 25 ml. of absolute ethanol and heating under a reflux for 15 hours. Evaporation of the solvent gave a light brown solid; m.p. 141-150°. After three recrystallizations from 50 per cent ethanol, the product melted at 176-177°; yield, 353 mg.


**SUMMARY**

A metabolite of Amytal isolated from the urine of dogs was identified as 5-ethyl-5-(3-hydroxyisooamyl)barbituric acid. The compound was synthesized by the addition of water to 5-ethyl-5-(3-methyl-2-butenyl)barbituric acid and by the oxidation of Amytal with chromic acid. Isotope dilution experiments demonstrated that, after an anesthetic dose of labeled Amytal in dogs, 67 per cent of the excreted isotope was present as this alcoholic end-product.

**BIBLIOGRAPHY**

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