ETHYL(3-HYDROXY-\(n\)-BUTYL)BARBITURIC ACID AS A METABOLITE OF NEONAL*

BY E. W. MAYNERT

WITH THE TECHNICAL ASSISTANCE OF JANE M. DAWSON AND ELIZABETH WASHBURN

(From the Department of Pharmacology, College of Physicians and Surgeons, Columbia University, New York, New York)

(Received for publication, August 1, 1951)

Earlier studies (1, 2) from this laboratory indicated that in the dog the alkyl side chains of pentobarbital and Amytal do not undergo \(\omega\) oxidation but are subject to oxidation at the \(\gamma\)-carbon atom of the amyl chains. Dogs excrete pentobarbital as two optically active diastereoisomers of ethyl(3-hydroxy-1-methylbutyl)barbituric acid and Amytal as ethyl(3-hydroxy-isoamyl)barbituric acid. In order to learn whether this unusual oxidative pattern is general for a straight chain of 4 carbon atoms attached to a barbituric acid ring the metabolic fate of Neonal, 5-\(n\)-butyl-5-ethylbarbituric acid, was investigated.

The procedure used for the isolation of other barbiturate metabolites yielded only one end-product of Neonal. Elementary analyses, ultraviolet and infra-red absorption spectra, and a positive iodoform test characterized the substance as 5-ethyl-5-(3-hydroxybutyl)barbituric acid (IV). Inasmuch as the compound was optically inactive, it was decided to confirm the structure by synthesis.

4-Bromobutene-1 (I) was prepared by the method of Linstead and Rydon (3) which involves the addition of allyl magnesium bromide to trioxymethylene and subsequent treatment of the unsaturated alcohol with phosphorus tribromide in pyridine. The bromide was caused to react with ethyl ethylmalonate to yield 3-butenylethylmalonic ester (II). The disubstituted ester was condensed with urea to form 5-(3-butenyl)-5-ethylbarbituric acid (III). Treatment of the latter substance with sulfuric acid and then water led to the desired 5-ethyl-5-(3-hydroxybutyl)barbituric acid (IV). This compound proved to be identical with the metabolite of Neonal.

It is now evident that, in the metabolism of substances containing alkyl chains, non-terminal as well as \(\omega\) oxidation must be considered. Previous work on the metabolic fate of compounds containing alkyl groups has been largely confined to carboxylic acids, in which \(\beta\) oxidation occurs with facility. Few substances containing alkyl chains attached to carbocyclic

* Studies on barbiturates, VII. This investigation was supported by a research grant from the National Institutes of Health, United States Public Health Service.
or heterocyclic rings have been investigated. At present, it would appear that \( \omega \) oxidation is more general than non-terminal oxidation. Indeed, \( \omega \) oxidation is known to occur with the 1-methylbutyl group when attached to the thiobarbituric acid ring (4) and to the \( n \)-butyl group when attached to a phenyl ring (5). However, in most studies concerned with metabolic fate, only a small portion of the administered substance has been accounted for in excreta. It is possible that both patterns of biological oxidations are involved in the metabolic alteration of many compounds.

From the information available it is not clear whether non-terminal oxidation of barbiturates should be regarded as \( \gamma \) or \( \omega \)-I oxidation or whether the pattern is non-specific. Studies of the metabolic fate of barbituric acid derivatives with longer side chains should answer this question and also afford information on the generalness of non-terminal oxidation. The mechanism by which the hydroxyl group is introduced into an alkyl chain is also obscure and remains a problem for future investigation.

**EXPERIMENTAL**

Isolation of Metabolite—Neonal sodium in a dose of 50 mg. per kilo was administered orally biweekly to four dogs weighing 10 to 17 kilos until a total of 7.2 gm. of drug had been given. The 48 hour urines were adjusted to pH 6.5 and extracted continuously with ether for 48 hours. The ether

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\begin{align*}
\text{CH}_2=\text{CHCH}_2\text{CH}_2\text{Br} & + \text{CH}_3\text{CH}_2\text{CH}(&\text{COOCH}_2\text{H})_2 \rightarrow \text{NaOEt} \\
\text{CH}_2=\text{CHCH}_2\text{CH}_2\text{C}(\text{COOCH}_2\text{H})_2 & \\
\text{CH}_3\text{CH}_2 & \\
\text{OH} & \\
\text{CH}_3\text{CH}_2\text{CHCH}_3\text{CH}_2 & \text{CO—NH} \\
\text{CH}_3\text{CH}_2 & \text{CO—NH} \\
\text{CH}_3\text{CH}_2 & \text{CO—NH} \\
\text{CH}_3\text{CH}_2 & \text{CO—NH} \\
\text{CH}_3\text{CH}_2 & \text{CO—NH} \\
\text{CH}_3\text{CH}_2 & \text{CO—NH}
\end{align*}
\]

\[
\begin{align*}
\text{CH}_2=\text{CHCH}_2\text{CH}_2\text{CH}_2\text{CO—NH} & + \text{H}_2\text{SO}_4 \rightarrow \text{H}_2\text{O} \\
\text{CH}_2=\text{CHCH}_2\text{CH}_2 & \text{CO—NH} \\
\text{CH}_3\text{CH}_2 & \text{CO—NH} \\
\text{CH}_3\text{CH}_2 & \text{CO—NH} \\
\text{CH}_3\text{CH}_2 & \text{CO—NH} \\
\text{CH}_3\text{CH}_2 & \text{CO—NH}
\end{align*}
\]

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. D. L. Tabern of the Abbott Laboratories for a generous sample of Neonal sodium.
extracts were combined (volume, 900 ml.) and extracted ten times with 200 ml. portions of water. The aqueous extract was distilled in vacuo until the volume was 5 ml. After standing in the refrigerator for several weeks the solution deposited 385 mg. of a light brown solid; m.p. 134–145°. Further evaporation of the filtrate yielded two additional crops of crystals melting at 142–146°; weight, 415 mg. The three batches were combined, decolorized with charcoal, and recrystallized from water. After one recrystallization the compound was pure; m.p. 152–153°.

Analysis—C_{16}H_{18}O_{3}N_{2}. Calculated. C 52.51, H 7.07
Found. " 52.78, " 6.85

Ultraviolet Spectrum—ε = 7960 (0.5 N NaOH, 255 μm)

Infra-Red Spectrum—Strong absorption band at 2.8 μ

Acetyl Derivative—3 ml. of acetic anhydride and 100 mg. of the metabolite were heated overnight on a steam bath. Water was added and the aqueous acetic acid removed in vacuo to leave a colorless oil. Trituration with 1 ml. of water caused the oil to crystallize; m.p. 130–132°. After one recrystallization from water, the product melted at 135–136°; yield, 85 mg.

Analysis—C_{18}H_{19}O_{4}N_{2}. Calculated. C 53.55, H 6.66
Found. " 53.46, " 6.60

Iodoform Test—The test was made on the pure metabolite by the method of Shriner and Fuson (6). The iodoform was isolated and purified, and its melting point checked against an authentic sample.

4-Bromobutene-1—This substance was prepared satisfactorily from the corresponding alcohol by the method of Linstead and Rydon (3). The yields reported for the synthesis of 4-hydroxybutene-1 could be duplicated only if the ether extracts of the crude alcohol were dried with sodium sulfate and then calcium sulfate instead of potassium hydroxide pellets alone.

Ethyl 3-Butenylethylmalonate—To a clear solution of sodium ethoxide prepared from 10.4 gm. of sodium and 225 ml. of anhydrous alcohol were added successively 88 gm. of ethylmalonic ester and 61 gm. of 4 bromobutene-1. The reaction mixture was heated under a reflux for 8 hours and allowed to stand overnight. The alcohol was evaporated and 175 ml. of water were added. The organic layer was separated and distilled in vacuo. The distillate consisted of 51 gm. of unchanged ethylmalonic ester and 29 gm. of the desired product; b.p. 123–124° (15 mm.); n^23_D 1.4341. The presence of the large amount of starting material suggests that insufficient time had been allowed for the reaction to go to completion. Inasmuch as enough 3-butenylethylmalonic ester was obtained for the necessary experiments, this point was not investigated.

5-(3-Butenyl)-5-ethylbarbituric Acid—20 gm. of the disubstituted malonic ester and 6.0 gm. of urea were added to a solution of sodium ethoxide
prepared from 2.9 gm. of sodium and 58 ml. of absolute alcohol. The reaction mixture was stirred and heated under a reflux for 48 hours. The solvent was evaporated in vacuo and 70 ml. of water were added. The cloudy solution was extracted four times with 15 ml. portions of ether to remove unchanged ester (2.4 gm.). The alkaline solution was freed of ether by aeration and acidified with hydrochloric acid to precipitate a yellow oil which crystallized on standing in the refrigerator; yield, 15 gm.; m.p. 60–75°. After recrystallization from water, the compound melted at 87–88°.

Analysis—C_{16}H_{16}O_{5}N_{2}. Calculated. C 57.14, H 6.66
Found. " 57.13, " 6.71

This compound was difficult to recrystallize because of a great propensity to separate as an oil. Water was the best of a large number of solvents and mixtures of solvents which were tested. However, sublimation in vacuo was a superior method of purification.

5-Ethyl-5-(3-hydroxybutyl)barbituric Acid—A solution of 500 mg. of butenylethylbarbituric acid in 1.3 ml. of concentrated sulfuric acid was allowed to stand at room temperature for 11 hours. The dark orange liquid was poured over 8 gm. of cracked ice. A precipitate appeared but redissolved before all the ice had melted. The solution was neutralized to pH 6 and extracted nine times with an equal volume of ether. Evaporation of the solvent yielded a colorless oil which was recrystallized from a minimum amount of water; m.p. 152–153°; yield, 260 mg. The melting point of a mixture of the compound with the metabolite of Neonal was also 152–153°.

The acetate of 5-ethyl-5-(3-hydroxybutyl)barbituric acid was prepared. This substance (m.p. 135–136°) did not depress the melting point of the acetate of the Neonal metabolite.

SUMMARY

A metabolite of Neonal was isolated from the urine of dogs and characterized as 5-ethyl-5-(3-hydroxybutyl)barbituric acid. A synthesis of this compound is reported.

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E. W. Maynert, Jane M. Dawson and With the technical assistance of Jane M. Dawson and Elizabeth Washburn


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