The Reactions of Some Barbituric Acid Derivatives in Concentrated Sulfuric Acid

BY E. W. MAYNERT AND ELIZABETH WASHBURN

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Certain dialkylbarbituric acids are subject to dealkylation in sulfuric acid. The reaction appears to be dependent upon resonance in the barbituric acid ring and the relative stabilities of the dislodged carbonium ions. Appropriately substituted thiobarbituric acids rearrange in sulfuric acid to yield 2-alkylthio derivatives. A vinyl group attached to the barbituric acid ring is cleaved, whereas an allyl group may be readily converted to the 2-hydroxypropyl derivative. The acid ring is cleaved, whereas an allyl group may be readily converted to the 2-hydroxypropyl derivative. The acid ring is transformed into the dilactone of bis-(2-hydroxypropyl)-malonic acid.

In connection with the proof of structure of the metabolites of pentobarbital it was discovered that the alcoholic metabolites (I) reacted upon standing for a week in concentrated sulfuric acid at room temperature to yield 5-ethylbarbituric acid (II).1

\[ \text{I} \quad \text{II} \]

\[ \begin{array}{c}
\text{CH}_3\text{CCH}_2\text{CH}_2\text{C} \equiv \text{N} \\
\text{CH}_3\text{CH}_2
\end{array} \]

To define the scope of this rather surprising dealkylation, the behavior under similar experimental conditions of a number of barbituric acid derivatives and related compounds was investigated. The results are summarized in Table I; individual experiments in the table are referred to in the text by appropriate letters given in parentheses after the compound name.

5,5-Disubstituted barbituric acids containing two primary alkyl groups (a,b) or a phenyl and a primary alkyl group (c) were stable in sulfuric acid. In contrast, dialkylbarbituric acids containing a secondary group (d,e) suffered the loss of the secondary group. Likewise, benzylethylbarbituric acid (f) was converted to ethylbarbituric acid. The importance of a long reaction time at room temperature was alluded to by the dealkylation of ethylisopropylbarbituric acid (e), inasmuch as Loubriel2 has reported that this compound is stable in sulfuric acid.

The cleavage of secondary chains from the 5-position of the barbituric acid ring was confined to disubstituted compounds. Monoalkyl derivatives like 5-isopropylbarbituric acid (g) and 5-(1-methylbutyl)-barbituric acid (h) were recovered unchanged.

Participation of the barbituric acid ring in caus-

1. Studies on Barbiturates, VIII. This investigation was supported by a research grant from The National Institutes of Health, Public Health Service.


The structure of the product, 5-n-butyl-2-isopropyl-thiobarbituric acid (VIII), was proved by synthesis from the sodium salt of 5-n-butyl-2-thiobarbituric acid and benzyl bromide according to the method used by Lee for the preparation of the 5-isopropyl-2-allylthio derivative.

5-Ethyl-5-benzyl- and 5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acids (n,o) also rearranged to give 2-alkylthio derivatives. Preliminary identification of the products was based on ultraviolet spectra characteristic of 2,5-dialkythiobarbituric acids (Fig. 1). The structure of the 2-benzylthio derivative was confirmed by synthesis from the sodium salt of 5-ethyl-2-thiobarbituric acid and benzyl bromide. Whether isomerization of the 1-methylbutyl group occurred during the rearrangement was not determined, but it was suggested by the difficulties encountered in the purification of the product.

5-Ethyl-2-thiobarbituric acid was also a product of the reaction of the 5-benzyl-5-ethyl-2-thio derivative in sulfuric acid. In accordance with the experience with ordinary dialkylbarbituric acids containing two primary alkyl groups, 5-ethyl-5-isopropyl-2-thiobarbituric acid (p) was stable in sulfuric acid. In contrast to ordinary dialkylbarbituric acids, 5-n-butyl-2-thiobarbituric acid (VIII) was found to be stable in sulfuric acid.

Certain barbituric acid derivatives having alkyl groups in the 5-position can be readily converted to thiobarbituric acid derivatives (VIII), which were proved by synthesis from the sodium salt of 5-n-butyl-2-thiobarbituric acid and isopropyl bromide according to the method used by Lee for the preparation of the 5-isopropyl-2-allylthio derivative.

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ted to alcohols by dissolution in concentrated sulfuric acid followed by treatment with water. Loubriel and Maynert have reported some alcohols derived in this manner from the allyl, the 3-methyl-2-butenyl and the 3-butenyl groups. The hydration of such double bonds is faster than the dealkylations discussed above. This is illustrated nicely by the reaction of 5-allyl-5-(1-methylbutyl)-barbituric acid (IX), which after 10 minutes gives a 65% yield of 5-(2-hydroxypropyl)-5-(1-methylbutyl)-barbituric acid (X). On longer standing in sulfuric acid the yield of the alcohol decreases; presumably, dealkylation is a participating reaction, but it was not possible to isolate any 5-(2-hydroxypropyl)-barbituric acid.

It was of interest to study a barbituric acid containing the vinyl group to determine whether treatment with sulfuric acid and then water would yield an alcohol. The results were somewhat unexpected. The only isolable product from the reaction of 5-n-butyl-5-vinylbarbituric acid (XI) was 5-n-butyl-barbituric acid (XV). Since it is highly improbable that the vinyl group was ejected as a vinylcarbonium ion, it would appear that the reaction proceeds either via XIII or by the acid-catalyzed aldol reversal of XIV.

The action of sulfuric acid on 5-ethyl-5-(1-methyl-1-butenyl)-barbituric acid(s) resulted in the loss of the substituted vinyl group. Compared with the other dealkylations the reaction was quite fast; after 11 hours a 50% yield of ethylbarbituric acid was obtained. In contrast, 5-n-butyl-5-vinylbarbituric acid was recovered largely unchanged when subjected to the same conditions. The reason for the unusual stability of the double bond in the unsubstituted vinyl group is not clear, but, if the mechanism proposed above is correct, the rapidity of the removal of the 1-methylbutenyl group can be ascribed to the fact that the ejected carbonium ion is tertiary.

A barbiturate in which a substituted vinyl group was incorporated in a cyclic structure, 5-(1-cyclohexenyl)-5-ethylbarbituric acid (t) yielded only charcoal.

In the light of the stability of the barbituric acid ring in the compounds discussed above, it was surprising to find that 5,5-diallylbarbituric acid (XVI) was readily cleaved to give a practically quantitative yield of the dilactone of bis-(2-hydroxypropyl)-malonic acid (XVII).

![Fig. 1.—The ultraviolet spectra of selected barbituric acid derivatives: thio derivatives in 0.1 N sodium hydroxide; others in 0.5 N sodium hydroxide.](image)

The product melted at 103-105° and was probably identical with the compound melting at 105° obtained by Leuchs and Lemcke through the action of fuming hydrobromic acid on diallylmalonic acid and its diethyl ester; however, this question was not investigated.

The data available at present are insufficient for a thorough understanding of the mechanism of the formation of the spiran lactone from diallylbarbituric acid. However, the following facts appear to be germane. First, when the reaction is carried out in 96% sulfuric acid, treatment with water results in an immediate precipitation of the lactone as a crystalline solid. Under similar conditions but with 100% sulfuric acid, no lactone separates from the aqueous solution and only a small amount of the compound can be isolated by extraction. Secondly, the lactone can be obtained from diallylbarbituric acid by treatment with hot 48% hydrobromic acid. Under the same conditions ethylisopropylbarbituric acid is stable (vide infra). These results suggest that the lactone is formed via the intermediates XVIII and XIX and that the conversion of XIX to the lactone is facilitated by the presence of water in the reaction mixture. All attempts to isolate other recognizable products from 100% sulfuric acid resulted in failure.

The effect of temperature on the reactions of barbituric acid derivatives was studied in only a few isolated instances. The results were that experiments b, d and e, which were conducted at room temperature for 2 weeks were duplicated exactly by heating at 100° for 1 hour.

A few experiments were done to compare the action of Lewis acids with sulfuric acid on the dealkylation of barbiturates. Boron trifluoride in ether (45%) had no effect on ethylisopropylbarbituric acid during heating for 70 hours at 100°. However, aluminum chloride reacted readily with ethyl-(1-methylbutyl)-barbituric acid in boiling toluene to give a 66% yield of ethylbarbituric acid. In contrast with sulfuric acid, aluminum chloride also caused the dealkylation of barbiturates containing two primary alkyl groups; the product from ethylisopropylbarbituric acid appeared to consist of a mixture of monoalkyl derivatives, but only isoamylnonylbarbituric acid was isolated in pure form.

The treatment of ethyl-(1-methylbutyl)-thiobarbituric acid with aluminum chloride in toluene gave an 80% yield of 5-ethyl-2-thiobarbituric acid. Apparently the toluene reacted as an effective recipient for the dislodged alkyl group; no 2-alkylothio derivatives were detected in the reaction mixture. The literature on the preparation of ethylthiobarbituric acid is contradictory. Earlier workers reported yields of about 45% by usual methods, but others like Lee were able to obtain yields of only 1%. This question was not investigated, but if the later investigators are correct, the dealkylation described above may afford a convenient synthetic route.

Heyl and Cope have reported that hot 45% hydrobromic acid converts 5-n-butyl-5-(1-ethoxy-2-hydroxyethyl)-barbituric acid (XX) to n-butylbarbituric acid (XXI). In order to determine whether this reaction represents a special case of the more general dealkylation discussed above, the action of hydrobromic acid on ethylisopropylbarbituric acid was studied. After heating under reflux for 10 hours the dialkybarbituric acid was recovered unchanged.

Experimental  

Starting Materials.—Most of the disubstituted barbituric acids used in this study are sold commercially as drugs and were obtained by purchase or donation from the manufacturers. All reactions were carried out on the free acids. The other compounds were synthesized according to published methods. The only discrepancy worthy of notation was in the melting point of 5-isobutyl-5-(2-hydroxopropyl)-barbituric acid. It was found that the melting point of this compound depended markedly upon the rate of heating. Values as low as 225°, as high as 231°, were obtained; Loublriel reported the melting point as 225-218°.

New compounds prepared during this investigation are listed in Table II. The products were isolated by pouring the reaction mixtures (usually dark brown) into about 3 to 6 volumes of ice-water. All products except ethylbarbituric acid, which is very soluble in water, separated as crystalline solids from the aqueous sulfuric acid and were collected by filtration and washed with water. The yields were increased by extraction of the filtrates with ether; continuous extraction with ether was usually employed for the isolation of ethylbarbituric acid. The crude products were recrystallized as indicated in the table.

The dilactone of bis-(2-hydroxypropyl)-malonic acid (XVII) was differentiated from diallylmalonic acid, which has the same elementary analysis, by its neutrality in solution. Its solubility properties were identical with those described by Fittig and Hjelt; no isomers could be separated from it by the method of Leuchs and Lemcke.

Anal. Calcd. for C_{10}H_{10}O: C, 58.80; H, 6.52. Found: C, 58.70; H, 6.58.

An Analysis of the Literature of Barbituric Acids in Sulfuric Acid

February 5, 1933

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Anal. Calcd. for C_{10}H_{10}O: C, 58.80; H, 6.52. Found: C, 58.70; H, 6.58.

(8) H. Leuchs and H. Lemcke, Ber., 47, 2573 (1914).
(11) D. Heyl and A. C. Cope, This Journal, 65, 569 (1943).
(12) All melting point determinations were made with a calibrated Fisher-Johns apparatus.
(13) Carbon and hydrogen micro-analyses by Mr. Joseph F. Ali-Cino.
The biological synthesis of peptide bonds through the agency of proteases has been suggested and deserves an192 open question the concept that the specificity of the proteases is sharp enough to mediate the formation of sufficiently unique end-products. Only not must the enzyme or other system (templet select from a variety of biologically available junior peptide and amino acid fragments, but it would seem that proteinsynthetic agents from different sources must necessarily exhibit some differences in their abilities to catalyze reactions from the same substrate(s). This latter type of specificity, of the many kinds that may be considered, is the principal subject of this paper.

Evidence for enzyme-controlled specificity in peptide bond synthesis has been offered. Chymotrypsin was found to catalyze the coupling of benzoyltyrosine and glycaminide, whereas no reaction was recorded with papain-cysteine. The experimental details available indicate that each

Table II

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<td>5-(2-Acetoxypropyl)-5-(1-methylbutyln)-</td>
<td>150-160</td>
<td>Water</td>
<td>C_{12}H_{14}N_{2}O_{4}</td>
<td>59.35</td>
<td>59.26</td>
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<td>5-Benzyl-5-ethyl-2-thio-</td>
<td>193-194</td>
<td>Alcohol</td>
<td>C_{12}H_{14}N_{2}O_{4}</td>
<td>59.55</td>
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<td>5-n-Butyl-2-isopropyl-2-thio-</td>
<td>207-238</td>
<td>Alcohol</td>
<td>C_{12}H_{14}N_{2}O_{4}</td>
<td>54.51</td>
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<td>5-n-Butyl-5-isopropyl-2-thio-</td>
<td>172-183</td>
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<td>C_{12}H_{14}N_{2}O_{4}</td>
<td>54.51</td>
<td>55.05</td>
<td>7.48</td>
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<tr>
<td>5</td>
<td>5-Ethyl-2-amylthio-</td>
<td>258-270</td>
<td>Alcohol</td>
<td>C_{12}H_{14}N_{2}O_{4}</td>
<td>54.51</td>
<td>54.54</td>
<td>7.48</td>
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<td>6</td>
<td>5-Ethyl-2-benzylthio-</td>
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<td>Alcohol</td>
<td>C_{12}H_{14}N_{2}O_{4}</td>
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<td>59.93</td>
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<td>5-(2-Hydroxypropyl)-5-(1-methylbutyln)-</td>
<td>215-216</td>
<td>Aqu. alcohol</td>
<td>C_{12}H_{14}N_{2}O_{4}</td>
<td>59.24</td>
<td>58.68</td>
<td>7.87</td>
<td>7.70</td>
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* Kjeldahl nitrogen. The structure of the amyl group was not determined. Melting points as high as this were rarely obtained. Nevertheless, crude products with melting points as low as 165-185° gave better than 90% yields of pure acetate (compound 1) when treated with acetic anhydride.

Reaction of 5-Ethyl-5-(1-methylbutyl)-2-thiobarbituric Acid with Aluminum Chloride.—Six grams of anhydrous aluminum chloride was added to a solution of 2.00 g. of at 190-192°. By reworking the filtrates an 80% yield was obtained. In the consideration of any hypothesis of specificity are considered.

Instances of protease-controlled specificity in the synthesis of different substituted peptides from the same substrates, benzoylphenyllalanine and glycaminide, are presented. Similar enzyme-controlled specificities, when benzoyltryptophan was the acid component or alaninanilide was the aminoid reactant, were observed. The compounds obtained from the acid component or alaninanilide were limiting equilibrium has been presented.6

Acid with Aluminum Chloride.—Six grams of anhydrous and aqueous alcohol; after two recrystallizations it melted to 70-75°. and bibliography.

175-193°. The product was recrystallized from alcohol and aqueous alcohol; after two recrystallizations it melted at 190-192°. By reworking the filtrates an 80% yield was obtained. Further recrystallization of 5-ethyl-2-thiobarbituric acid raised the melting point to 195-196° but it still retained a very light brown color; Wheeler and Janssen report the melting point as 190-192°.

Study of the information available has thrown open to question the concept that the specificity of the proteases is sharp enough to mediate the formation of sufficiently unique end-products. Not only must the enzyme or other system (templet select from a variety of biologically available junior peptide and amino acid fragments, but it would seem that proteosynthetic agents from different sources must necessarily exhibit some differences in their abilities to catalyze reactions from the same substrate(s). This latter type of specificity, of the many kinds that may be considered, is the principal subject of this paper.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF IOWA STATE COLLEGE]

**Enzymic Synthesis of Peptide Bonds. V. Instances of Protease-Controlled Specificity in the Synthesis of Acylamino Acid Anilides and Acylpeptide Anilides**

**BY FRANK JANSSEN, MILTON WINITZ AND SIDNEY W. FOX**

Received September 3, 1952

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(4) Author to whom inquiries should be addressed.

(5) M. Bergmann and H. Fraenkel-Conrat, **J. Biol. Chem.**, 119, 707 (1937), and bibliography.


(7) S. W. Fox, C. W. Pettinga, J. S. Halverson and H. Wax, **Arch. Biochem.**, 21, 21 (1940).


