[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY]

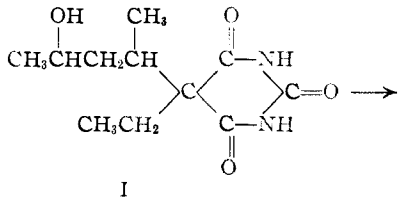
The Reactions of Some Barbituric Acid Derivatives in Concentrated Sulfuric Acid[1]

BY E. W. MAYNERT AND ELIZABETH WASHBURN

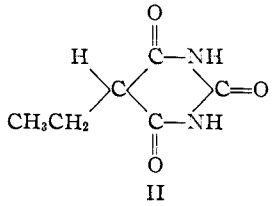
RECEIVED AUGUST 27, 1952

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. Diallylbarbituric acid is transformed into the dilactone of bis-(2-hydroxypropyl)-malonic acid. Possible mechanisms for these reactions are discussed, and a few experiments with hydrobromic acid and representative Lewis acids are described.

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).[2]



I



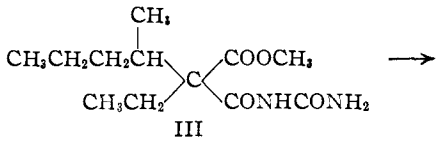
II

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.

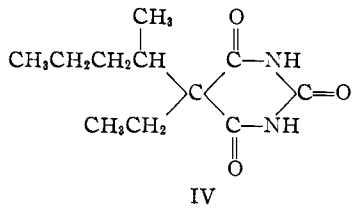
5,5-Disubstituted barbituric acids containing two primary alkyl groups (a,b) or a phenyl and a primary alkyl group (c) were stable in sulfuric acid. In contrast, dialkylbarbituric acids containing a secondary group (d,e) suffered the loss of the secondary group. Likewise, benzylethylbarbituric acid (f) 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 Loubriel[3] 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 causing dealkylation was indicated by study of some theoretical hydrolytic products of a 5,5-dialkylbarbituric acid containing a secondary chain. Ethyl-(1-methylbutyl)-malonuric acid (i) and the similarly substituted malonic acid (j) and malonamide (k) were stable. The methyl ester of the malonuric acid (III) reacted in 24 hours to give a 40% yield of the corresponding barbituric acid (IV). This experiment provided further evidence that under the conditions employed dealkylation was slow.

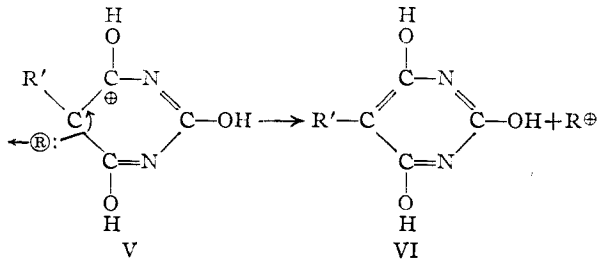


III



IV

In the compounds mentioned so far, dealkylation appears to depend upon two factors: the relative stability of alkyl carbonium ions and the gain in resonance energy which results from loss of an alkyl group. For purposes of discussion, barbituric acids and their acyclic derivatives will be regarded as monoacid bases in sulfuric acid. It is possible that this may not be an accurate representation inasmuch as Hammett[4] has pointed out that some nitrogen compounds appear to add two protons in sulfuric acid. Consideration of the protonated form of a disubstituted barbituric acid (V) and its dealkylation product (VI) reveals that loss of an alkyl group would lead to a considerable increase in resonance energy. Dealkylation of the protonated forms of the related acyclic structures would not be



V VI

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

(2) E. W. Maynert and J. M. Dawson, *J. Biol. Chem.*, **195**, 389 (1952).

(3) J. W. Loubriel, THIS JOURNAL, **56**, 1968 (1934).

(4) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p. 48.

TABLE I

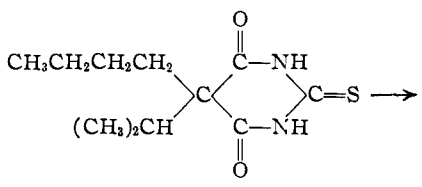
THE REACTIONS OF BARBITURIC ACID DERIVATIVES IN CONCENTRATED SULFURIC ACID AT ROOM TEMPERATURE

Experiment	Compound[a]	Weight, g.	Sulfuric acid, ml.	Time, days	Product[a]	Solvent for recrystallization	Yield or recovery, %
a	5,5-Diethyl-	1.00	5.0	13	Unchanged	Water	85
b	5-Ethyl-5-isoamyl-	1.00	5.0	14	Unchanged	Water	85
c	5-Ethyl-5-phenyl-	1.00	5.0	21	Unchanged	Water	90
d	5-Ethyl-5-(1-methylbutyl)-	1.00	5.0	13	5-Ethyl-	Alcohol	65
e	5-Ethyl-5-isopropyl-	1.00	5.0	14	5-Ethyl-	Alcohol	60
f	5-Benzyl-5-ethyl-	1.00	5.0	14	5-Ethyl-	Alcohol	65
g	5-Isopropyl-[b]	2.00	10.0	14	Unchanged	Water	70
h	5-(1-Methylbutyl)-[b]	1.00	5.0	90	Unchanged	Water	60
i	Ethyl-(1-methylbutyl)-malonuric acid	1.00	5.0	21	Unchanged	Pptn. from $CHCl_3$ with ligr.	60
j	Ethyl-(1-methylbutyl)-malonic acid	1.00	5.0	21	Unchanged	Water	90
k	Ethyl-(1-methylbutyl)-malonamide	0.25	1.2	18	Unchanged	Water	65
l	Methyl ethyl-(1-methylbutyl)-malonurate (III)	0.10	0.2	1	5-Ethyl,5-(1-methylbutyl)-	Water	40
m	5-*n*-Butyl-5-isopropyl-2-thio-(VII)	1.00	5.0	14	5-*n*-Butyl-2-isopropylthio-(VIII)	Alcohol	75
n	5-Benzyl-5-ethyl-2-thio-	0.80	3.0	12	5-Ethyl-2-benzylthio-	Alcohol	15
					5-Ethyl-2-thio-	Alcohol	15
o	5-Ethyl-5-(1-methylbutyl)-2-thio-	2.00	10.0	14	5-Ethyl-2-amylthio-[c]	Alcohol	55[d]
p	5-Ethyl-5-isoamyl-2-thio-	2.00	10.0	14	Unchanged	Aq. alcohol	90
q	5-Allyl-5-(1-methylbutyl)-(IX)	2.00	8.0	10 min.	5-(2-Hydroxypropyl)-5-(1-methylbutyl)-(X)	Aq. alcohol	65
r	5-*n*-Butyl-5-vinyl-(XI)[e]	0.30	1.3	14	5-Butyl-	Alcohol	45
s	5-Ethyl-5-(1-methyl-1-butenyl)-	2.00	10.0	11 hr.	5-Ethyl-	Alcohol	50
t	5-(1-Cyclohexenyl)-5-ethyl	2.00	10.0	4	Charcoal		
u	5,5-Diallyl-(XVI)	2.00	10.0	14	Dilactone of bis-(2-hydroxypropyl)-malonic acid (XVII)	Alc. or water	85

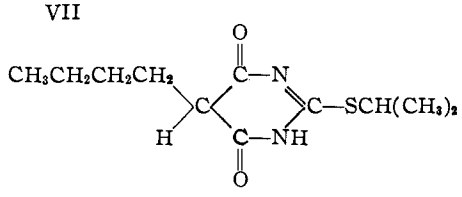
[a] Unless otherwise specified, the compound is a barbituric acid substituted as indicated. [b] Kindly supplied by Mr. W. A. Lott of the Squibb Institute for Medical Research. [c] The structure of the amyl group was not determined. [d] Crude yield with m.p. 230–250°; an apparently pure isomer, m.p. 268–270°, was isolated in 15% yield. [e] Kindly supplied by Dr. A. C. Cope of Massachusetts Institute of Technology.

expected to result in a comparable gain in resonance. The conversion of V to VI requires that the carbonium ion have sufficient stability to depart from the electron pair. Under the experimental conditions employed, only the more stable carbonium ions like benzyl or secondary alkyl were removed.

The behavior of 5,5-dialkyl-2-thiobarbituric acids under similar experimental conditions was examined to determine whether a carbonium ion ejected from the 5-position would alkylate the sulfur. This possibility was realized with 5-*n*-butyl 5-isopropyl-2-thiobarbituric acid (VII).



VII



VIII

The structure of the product, 5-*n*-butyl-2-isopropylthiobarbituric acid (VIII),[5] was proved by synthesis from the sodium salt of 5-*n*-butyl-2-thiobarbituric acid and isopropyl bromide according to the method used by Lee[6] for the preparation of the 5-isopropyl-2-allylthio derivative.

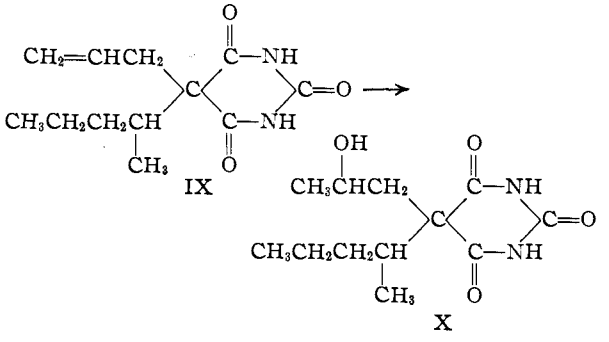
5-Ethyl-5-benzyl- and 5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acids (n,o) also rearranged to give 2-alkylthio derivatives. Preliminary identification of the products was based on ultraviolet spectra characteristic of 2,5-dialkylthiobarbituric acids (Fig. 1). The structure of the 2-benzylthio derivative 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-isoamyl-2-thiobarbituric acid (p) was stable in sulfuric acid.

Certain barbituric acid derivatives having alkenyl groups in the 5-position can be readily conver-

(5) Probably 5-*n*-butyl-4,6-dihydroxy-2-isopropylthiopyrimidine is a more appropriate name. However, Lee[6] has designated similar compounds as 2-alkylthiobarbituric acids.

(6) J. Lee, THIS JOURNAL, 60, 993 (1938).

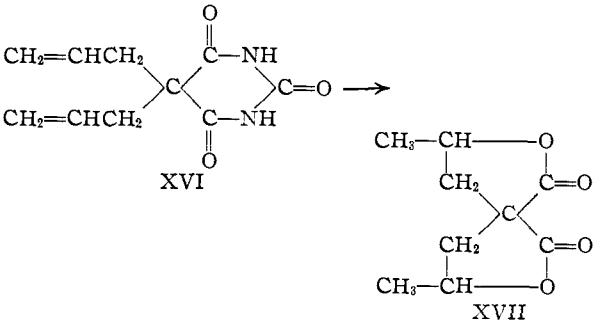
ted to alcohols by dissolution in concentrated sulfuric acid followed by treatment with water. Loubriel[3] and Maynert[7] have reported some alcohols derived in this manner from the allyl, the 3-methyl-2-butenyl and the 3-butenyl groups. The hydration of such double bonds is faster than the dealkylations 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*-butylbarbituric 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).



The reaction was relatively fast; after 11 hours a 35% yield of this spiranolactone was obtained.

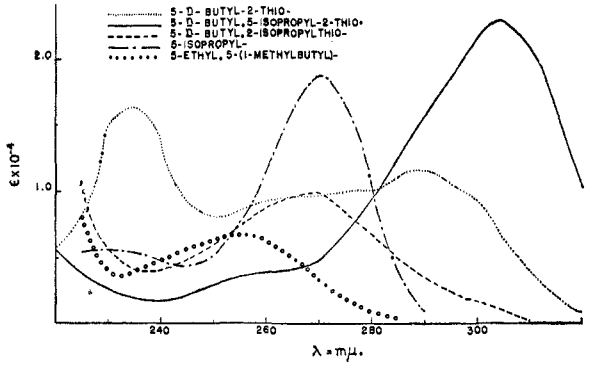
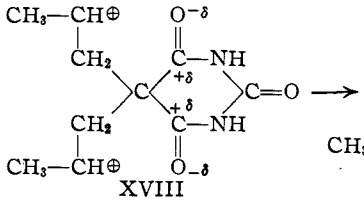


Fig. 1.—The ultraviolet spectra of selected barbituric acid derivatives: thio derivatives in 0.1 *N* sodium hydroxide; others in 0.5 *N* sodium hydroxide.

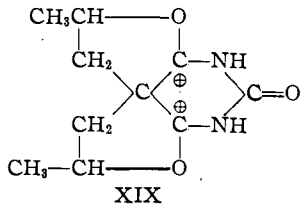
(7) E. W. Maynert, *J. Biol. Chem.*, **195**, 397, 403 (1952).

The product melted at 103–105° and was probably identical with the compound melting at 106° obtained by Leuchs and Lemcke[8] 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 spiranolactone 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



XVIII



XIX

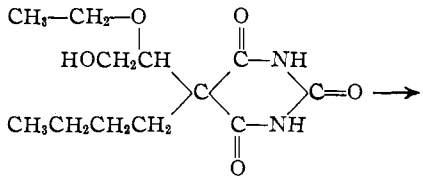
in the reaction mixture. All attempts to isolate other recognizable products from 100% sulfuric acid resulted in failure.

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.

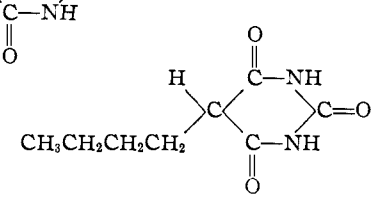
A few experiments were done to compare the action of Lewis acids with sulfuric acid on the dealkylation of barbiturates. Boron trifluoride in ether (45%) had no effect on ethylisopropylbarbituric acid during heating for 70 hours at 100°. However, aluminum chloride reacted readily with ethyl-(1-methylbutyl)-barbituric 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 ethylisoamylbarbituric acid appeared to consist of a mixture of monoalkyl derivatives, but only isoamylbarbituric 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-alkylthio derivatives were detected in the reaction mixture. The literature on the preparation of ethylthiobarbituric acid is contradictory. Earlier workers[9,10] reported yields of about 45% by usual methods, but others like Lee[6] 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[11] have reported that hot 48% 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



XX



XXI

general dealkylation discussed above, the action of hydrobromic acid on ethylisopropylbarbituric acid was studied. After heating under reflux for 10 hours the dialkylbarbituric acid was recovered unchanged.

Experimental[12,13]

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-hydroxypropyl)-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 251°, were obtained; Loubriel[3] reported the melting point as 216–218°.

New compounds prepared during this investigation are listed in Table II.

Reactions in Sulfuric Acid.—The experimental conditions are summarized in Table I. 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[14]; no isomers could be separated from it by the method of Leuchs and Lemcke.[8]

Anal. Calcd. for $C_9H_{12}O_4$: C, 58.70; H, 6.52. Found: C, 58.70; H, 6.56.

(8) H. Leuchs and H. Lemcke, *Ber.*, **47**, 2573 (1914).

(9) H. L. Wheeler and G. S. Jamieson, *Am. Chem. J.*, **32**, 342 (1904).

(10) A. Einhorn and H. von Diesbach, *Ann.*, **359**, 171 (1908).

(11) D. Heyl and A. C. Cope, THIS JOURNAL, **65**, 669 (1943).

(12) All melting point determinations were made with a calibrated Fisher–Johns apparatus.

(13) Carbon and hydrogen micro-analyses by Mr. Joseph F. Alicino.

(14) R. Fittig and E. Hjelt, *Ann.*, **216**, 52 (1883).

TABLE II

BARBITURIC ACID DERIVATIVES

No.	Compound	M.p., °C.	Solvent for recrystallization	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found[a]
1	5-(2-Acetoxypropyl)-5-(1-methylbutyl)-	159–160	Water	$C_{14}H_{22}N_2O_5$	56.36	56.26	7.43	7.12		
2	5-Benzyl-5-ethyl-2-thio-	193–194	Alcohol	$C_{13}H_{14}N_2O_2S$	59.53	59.72	5.38	5.33		
3	5-*n*-Butyl-2-isopropyl-thio-	257–258	Alcohol	$C_{11}H_{18}N_2O_2S$	54.51	54.92	7.48	7.83	11.56	11.3
4	5-*n*-Butyl-5-isopropyl-2-thio-	152–153	Alcohol	$C_{11}H_{18}N_2O_2S$	54.51	55.05	7.48	7.03	11.56	11.3
5	5-Ethyl-2-amylthio-[b]	268–270	Alcohol	$C_{11}H_{18}N_2O_2S$	54.51	54.54	7.48	7.40	11.56	11.7
6	5-Ethyl-2-benzylthio-	258–260	Alcohol	$C_{13}H_{14}N_2O_2S$	59.53	59.93	5.38	5.78		
7	5-(2-Hydroxypropyl)-5-(1-methylbutyl)-	215–216[c]	Aq. alcohol	$C_{12}H_{20}N_2O_4$	56.24	56.68	7.87	7.70	10.94	10.8

[a] Kjeldahl nitrogen. [b] The structure of the amyl group was not determined. [c] 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 the barbituric acid in 30 ml. of hot toluene and heated under reflux for 18 hours. The mixture was poured into 300 ml. of water and the phases were separated. The aqueous phase was extracted with a minimal amount of toluene to remove black oily droplets, adjusted to *p*H 1 and extracted continuously with ether for 24 hours. Evaporation of the ether gave 1.45 g. of a light brown crystalline solid, m.p. 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 Jamieson[9] and Einhorn[10] report the melting point as 190–192°.

NEW YORK, N. Y.

[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[1-3]

BY FRANK JANSSEN, MILTON WINITZ AND SIDNEY W. FOX[4]

RECEIVED SEPTEMBER 3, 1952

Instances of protease-controlled specificity in the synthesis of different substituted peptides from the same substrates, benzoylphenylalanine and glycinanilide, 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 ficin-catalyzed reactions were predominantly transamidation products whereas those from the chymotrypsin-catalyzed reactions were coupling products. Benzoylphenylalanine and glycinanilide yielded in the presence of papain a mixture of benzoylphenylalaninanilide and benzoylphenyllalanylglycylglycinanilide. Theoretical implications of these instances of specificity are considered.

The biological synthesis of peptide bonds through the agency of proteases has been suggested[5] and deserves as rigorous an evaluation as it is possible to obtain. In the consideration of any hypothesis of the biosynthesis of peptide bonds, the discernible problems which must be solved include those of the energetics and of the specificity of synthesis. The thermodynamic feasibility of the mechanism involving reversal of hydrolysis has been frequently discussed; a hypothetical means for elimination of the restriction of an exponentially unfavorable limiting equilibrium has been presented.[6]

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.[7-9] Not only must the enzyme or other system (templet?[9,10]) 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.

Evidence for enzyme-controlled specificity in peptide bond synthesis has been offered.[11] Chymotrypsin was found to catalyze the coupling of benzoyltyrosine and glycinanilide whereas no reaction was recorded with papain-cysteine. The experimental details available indicate that each

(1) Paper IV. S. W. Fox and M. Winitz, *Arch. Biochem. Biophys.*, **35**, 419 (1952).

(2) Journal Paper No. J-2140 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 1111. This project has been supported by the National Cancer Institute of the National Institutes of Health, Public Health Service, and by the Rockefeller Foundation.

(3) Presented in part at the Twelfth International Congress of Pure and Applied Chemistry, New York City, September 12, 1951. Some of the work is described in the Ph.D. thesis of Milton Winitz, 1951, and in the M.S. thesis of Frank Janssen, 1952.

(4) Author to whom inquiries should be addressed.

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

(6) S. W. Fox, *Proc. Natl. Acad. Sci.*, **37**, 291 (1951).

(7) S. W. Fox, C. W. Pettinga, J. S. Halverson and H. Wax, *Arch. Biochem.*, **25**, 21 (1950).

(8) P. C. Caldwell and C. Hinshelwood, *J. Chem. Soc.*, 3156 (1950).

(9) F. Haurowitz, "Chemistry and Biology of Proteins," Academic Press, Inc., New York, N. Y., 1950, p. 348.

(10) A. Claude, *Adv. Prot. Chem.*, **5**, 423 (1949).

(11) J. S. Fruton, *Cold Spring Harbor Symp. Quant. Biol.*, **6**, 55 (1938).