yielded tetrahydroxystearic acids with melting points of 152 and 173°. The oleic acid present has been proven to be 9-octadecanoic acid.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

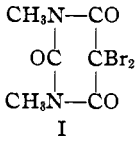
# Researches on Pyrimidines. CLI. The Constitution of Dibarbituric Acid[1]

BY ROLLIN D. HOTCHKISS AND TREAT B. JOHNSON

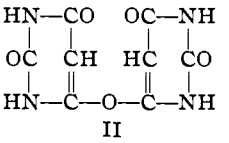
When A. von Baeyer reported in 1864 the preparation of barbituric acid, he described, among its other properties, its conversion by heat into an insoluble new acid, bibarbituric acid.[2] He also reported the isolation of several salts and two bromine derivatives of this new pyrimidine. In the seventy years which have elapsed since that time, barbituric acid and its derivatives have been studied extensively. Dibarbituric acid, however, has received only passing mention by investigators who were dealing with other substances.[3,4] The investigation reported in this paper is concerned with the determination of the structure of this difficultly soluble pyrimidine compound.

Von Baeyer postulated that dibarbituric acid can be represented by the formula $C_8H_6N_4O_5$, corresponding to the union of two molecules of barbituric acid with loss of one molecule of water. This empirical formula is supported by the results now obtained. The literature does not record any attempt to give a structural expression, except that of Conrad and Guthzeit,[3a] who proposed the formulation III.

On treatment with dimethyl sulfate and potassium hydroxide, dibarbituric acid yields a trimethyl and a tetramethyl derivative. When the latter is warmed with bromine water it undergoes bromination and cleavage into two moles of 1,3-dimethyl-5,5-dibromobarbituric acid (I). This fact indicates that the dibarbituric acid molecule contains two intact barbituric acid nuclei united through a linkage not involving the nitrogen atoms. A di-enol anhydride, as represented by

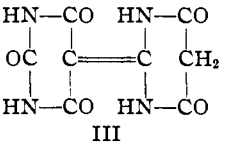


I

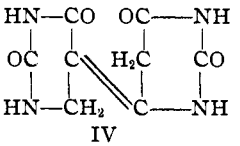


II

formula II would not have the stability toward hydrolysis that dibarbituric acid does, nor would it give *enol* reactions and form salts when all four of the cyclic nitrogen positions have been methylated. The barbituric acid nuclei must, therefore, be linked through carbon atoms, and, since the linkage is easily cleaved by bromine water under the conditions mentioned above, it is probably a double bond. It seems possible, therefore, to exclude all other formulas for dibarbituric acid except III and IV, and their respective tautomeric modifications.

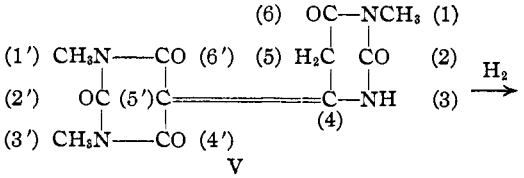


III

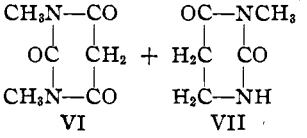


IV

Definite experimental evidence rendering it possible to decide between formulas III and IV was obtained through the hydrogenolysis of N-trimethyldibarbituric acid. Repeated attempts to reduce dibarbituric acid and its N-methyl homologs by a variety of methods failed to result in the preparation of dihydro derivatives. At high temperatures and pressures, however, catalytic reduction of the N-trimethyl derivatives in dry dioxane solution brought about a smooth hydrogenolysis. On the basis of the results obtained from this re-



V



VI VII

(1) From a thesis presented by Rollin D. Hotchkiss to the Graduate Faculty of Yale University in June, 1935, as partial fulfilment of the requirements for the degree of Doctor of Philosophy.

(2) A. von Baeyer, *Ann.*, **130**, 145 (1864). Throughout this paper the name dibarbituric acid will be used in conformity with modern nomenclature.

(3) (a) M. Conrad and M. Guthzeit, *Ber.*, **15**, 2846 (1882); (b) C. Matignon, *Ann. chim. phys.*, [6] **28**, 292 (1893); (c) H. Biltz and H. Wittek, *Ber.*, **54**, 1035 (1921); (d) H. Biltz and T. Kohler, *ibid.*, **56**, 2482 (1923).

(4) It seems possible that insoluble products observed by Wood and Anderson, *J. Chem. Soc.*, **95**, 979 (1909), and Grimaux, *Bull. soc. chim.*, **31**, 146 (1879), might be impure dibarbituric acid, although these authors did not recognize them as such.

action, the trimethyldibarbituric acid can be represented by formula V, and consequently the course of the reaction may be interpreted as expressed above. The pyrimidine VI is a known compound (1,3-dimethylbarbituric acid), and its identity was readily established. The pyrimidine VII is the previously unknown 1-methyl-4,5-dihydrouracil and its structure was established by synthesis from methyl isocyanate and β-alanine, essentially according to the method of E. Fischer.[5] The general applicability of this reaction as adapted to aliphatic isocyanates was substantiated by the preparation of the known compound, 1-methyl-4-phenyl-4,5-dihydrouracil, by combining methyl isocyanate with β-phenyl-β-alanine in an analogous manner.

To adhere to a formula of the type III, one must assume that reduction of one of the five carbonyl groups of the pyrimidine molecule occurred and that the extremely small concentration (under the conditions used, not more than 0.15%) of water produced brought about hydrolytic cleavage of the double bond. Such an assumption appears to the authors improbable, and quite unnecessary. Cleavage of carbon–carbon linkages by hydrogen has been observed previously in the use of the high-temperature, high-pressure Adkins reduction technique.[6] We feel justified, therefore, in accepting the hydrogenolysis as formulated above as a proof of the constitution of the trimethyl derivative (V), which we may now call 1,1′,3′-trimethyldibarbituric acid V, and consequently of dibarbituric acid itself (IV). It is to be expected that enolic forms play a part in the reactivity of these substances in solution, as for example in their reaction with ferric chloride and in salt formation.

Von Baeyer obtained a yellow substance which he considered to be dibromodibarbituric acid hydrobromide, when dibarbituric acid was treated with water and bromine in the cold. The present authors have observed the formation of a yellow product only when liquid bromine has been allowed to come into contact with crystals of the usual bromination product, dibromodibarbituric acid. The yellow product has the properties that one would expect a mixture would have, containing free bromine and the dibromo acid. Furthermore, von Baeyer's supposition that dibromodibarbituric acid reacts with concentrated hydrobromic acid to form the yellow dibromo hydrobromide, appears to be incorrect. It was found that hydrobromic acid merely reduces this "active bromine compound," free bromine is liberated, and a debrominated acid is produced.

(5) First used with organic isocyanates by E. Fischer and H. Leuchs, *Ber.*, **35**, 3796 (1902). See also Johnson and Livak, THIS JOURNAL, **58**, 299 (1936).

(6) See for example, J. M. Sprague and H. Adkins, *ibid.*, **56**, 2669 (1934); R. Connor and H. Adkins, *ibid.*, **54**, 4678 (1932).

## Experimental Part

All melting points reported in this paper were determined in a Kullmann copper block using a standardized thermometer. Nitrogen was determined by half-micro Kjeldahl technique.

**Preparation of Dibarbituric Acid, $C_8H_6O_5N_4$.**—Baeyer's method of preparation by heating barbituric acid in glycerol[1] was followed except that the ammonium, rather than the potassium, salt was used in order to obtain a more readily filterable product. Inasmuch as von Baeyer gave very few details, the conditions that proved most satisfactory after many trials will be given here: 15 g. of finely powdered anhydrous barbituric acid was mixed in a large test-tube with 15 g. of glycerol that had been dried by heating to 170°. The tube was inserted in a sulfuric acid bath at 125° and the temperature of the bath was raised in forty-five minutes to 150°, maintained at that point for ninety minutes, raised in fifteen minutes to 170°, at which temperature it was kept for two hours, then gradually decreased to 150° during ninety minutes. The contents of the tube were occasionally stirred during this time as they became more and more solid, and at the end of the process had a uniform pale orange color. The tube was removed from the bath, cooled and its contents were dissolved in about 3200 cc. of boiling water containing a small excess of ammonium hydroxide. The boiling solution was treated with Norite and filtered, using a heated funnel. The filtrate was allowed to stand overnight at room temperature, whereupon the bulky semi-crystalline mass that had separated was filtered off by suction and washed with cold water. The moist salt was redissolved in 3300 cc. of boiling water containing an excess of ammonium hydroxide. The clear yellow solution was treated again with Norite in several portions and filtered hot through a heated filter into a mechanically stirred solution containing 30 cc. of concentrated hydrochloric acid and about 30 cc. of water. The precipitate was allowed to settle out, separated by decantation and filtration on a suction filter, washed well with water and alcohol and dried at 100°. The yield of dibarbituric acid was 9.5–10 g. or 67–72% of the theoretical amount, as a fine, soft, white powder.

*Anal.* Calcd. for $C_8H_6O_5N_4$: C, 40.32; H, 2.54; N, 23.53; mol. wt., 238.1. Found: C, 40.18, 40.21; H, 2.60, 2.72; N, 23.46, 23.52; mol. wt., 238.0, 239.2.

A less pure, yellowish product may be obtained with saving of time if the first ammoniacal solution is filtered directly into an excess of acid. The use of anhydrous oxalic acid in place of glycerol[3d] does not increase the yield of pure substance.

The solubility of dibarbituric acid in 100 g. of water is about 0.27 g. near the boiling temperature, and about 0.14 g. at 25°. A neutral or weakly acidic solution at room

temperature, upon addition of a drop of 5% ferric chloride solution, forms a suspension of a precipitate that appears brownish by transmitted light and fluorescent purple by reflected light. This test is sensitive and highly characteristic of dibarbituric acid and its salts. When dilute hydrochloric acid is added dropwise to an ammoniacal suspension of ammonium dibarbiturate containing sodium nitrite, a violet-colored solution results. When this solution is made acidic its color changes to yellow, indicating according to Hantzsch and Robison,[7] the formation of the true isonitroso derivative. Dibarbituric acid was recovered unchanged after being boiled for two hours with 6 *N* hydrochloric acid; twenty hours with hydriodic acid, red phosphorus and glacial acetic acid; and from five to seventy hours with from 2–20% potassium hydroxide solutions.

When treated with an excess of bromine in a small amount of water, dibarbituric acid is changed into a yellow product that steadily loses weight when preserved over red phosphorus, until it contains less than 2.6 atoms of bromine per molecule of original acid. The yellow substance is rapidly decolorized by sodium bisulfite solution. Dibromodibarbituric acid reacts with concentrated hydrobromic acid in acetic acid to form free bromine and a colorless reduction product which, unlike the dibromo acid, does not liberate iodine from potassium iodide. The amount of water of crystallization observed in crystalline dibromodibarbituric acid by von Baeyer corresponds to one mole of water, not as stated by him, to two moles.

**1,1',3'-Trimethyldibarbituric Acid.**—Dibarbituric acid (2.0 g.) was warmed in a mixture of 3 cc. of 60% potassium hydroxide solution and 20 cc. of water, then cooled to 25°. During four minutes, 6 cc. of dimethyl sulfate was added in portions so that the temperature did not rise appreciably, while the mixture was stirred mechanically. In the course of thirty minutes, 3 cc. of 60% potassium hydroxide solution was added at the rate of about two drops per minute. At the end of this time, or upon warming, precipitation of fine needles of potassium trimethyldibarbiturate occurred. The mixture was cooled in an ice-bath for two hours, then filtered without washing. The product on the filter was dissolved in 30 cc. of hot water, treated with Norite and filtered. The filtrate was shaken well with 3 cc. of concentrated hydrochloric acid. Rapid crystallization ensued and the mixture became semi-solid. After it had stood in an ice-bath for a short time, the suspension was filtered, and the product on the filter was washed with alcohol, then recrystallized by dissolving in hot 50% ethyl alcohol, filtering and adding 0.5 cc. of concentrated hydrochloric acid to the filtrate. After a period of one hour in the ice-bath, the colorless needles were filtered off and washed with alcohol. The yield was 1 g. or 40% of trimethyldibarbituric acid monohydrate.

*Anal.* Calcd. for $C_{11}H_{12}O_5N_4 \cdot H_2O$: $H_2O$, 6.04; mol. wt., 298.2. Found: $H_2O$, 5.96; mol. wt., 300.8, 300.8.

On drying for one hour in a vacuum over phosphorus pentoxide at 100°, anhydrous trimethyldibarbituric acid, m. p. 286–287° (corrected) was obtained.

*Anal.* Calcd. for $C_{11}H_{12}O_5N_4$: N, 20.00; mol. wt., 280.1. Found: N, 19.95, 20.20; mol. wt., 282.9.

(7) A. Hantzsch and R. Robison, *Ber.*, **43**, 67 (1910).

The substance is moderately soluble in water, ethyl alcohol and dioxane. When its water solution is treated with bromine, either at room temperature or at the boiling point, there is formed a bromo derivative that crystallizes out of the cooled solution in colorless needles. After recrystallization from 20% ethyl alcohol, this substance melts at 225–227° (corrected) with decomposition. Trimethyldibarbituric acid gives with ferric chloride, a clear, deep purple solution from which are slowly deposited yellow crystals that become jet black in the desiccator.

Tetramethyldibarbituric acid is present in the filtrate from the potassium trimethyldibarbiturate and from the first recrystallization, and may be recovered by a method to be described below.

**1,1',3,3'-Tetramethyldibarbituric Acid.**—Four grams of dibarbituric acid was dissolved by warming in 35 cc. of water and 4.5 cc. of 60% potassium hydroxide solution, and the solution was cooled to room temperature. To the stirred mixture, 6 cc. of dimethyl sulfate was added in 0.5-cc. portions during twenty minutes, then 18 cc. more was added at once. A 60% potassium hydroxide solution was run in at the rate of two drops per minute to keep the mixture practically neutral as the dimethyl sulfate slowly reacted. Needles of potassium trimethyldibarbiturate separated out during the process but gradually were converted to granular crystals of the tetramethyldibarbiturate. When 24 cc. of potassium hydroxide solution had been added, stirring was stopped and the alkaline solution was left in the icebox overnight. The somewhat pinkish potassium tetramethyldibarbiturate was filtered off, dissolved in 30 cc. of boiling water and filtered hot after treating with Norite. To the filtrate was added 3 cc. of concentrated hydrochloric acid, with thorough mixing. Compact colorless crystals of tetramethyldibarbituric acid separated out slowly. The cooled mixture was filtered and the product on the filter was washed with alcohol. It weighed 3 g. after drying at 100°. This is a yield of 60% of the theoretical amount. After recrystallization from water (adding small amounts of hydrochloric acid to the cooled filtrates to decrease the solubility), pure tetramethyldibarbituric acid was obtained, as colorless transparent prisms, m. p. 271.5–272.5° (corrected). Dried at 100° in a vacuum over phosphorus pentoxide, it gave the following analytical results.

*Anal.* Calcd. for $C_{12}H_{14}O_5N_4$: N, 19.05. Found: N, 18.88, 19.17.

This pyrimidine is very soluble in water, methyl and ethyl alcohols, acetic acid, acetone and chloroform. Its water solution gives a clear red color with ferric chloride.

A considerable amount of tetramethyldibarbituric acid is retained in the filtrates from the preparation and recrystallization. It can best be recovered by making the solution strongly alkaline with potassium hydroxide, evaporating it to about one-third volume and cooling. The potassium salt, made insoluble by the excess of alkali, separates, and is worked up as already indicated. Recrystallization of the potassium salt from dilute alcohol or acetone gives compact colorless crystals.

*Anal.* Calcd. for $C_{12}H_{13}O_5N_4K$: N, 16.87. Found: N, 16.86, 16.76.

Tetramethyldibarbituric acid, boiled in hydrochloric acid solution, suffers partial decomposition; on evapora-

tion, and sublimation of the residue, there are obtained long colorless needles, melting at 122.5–123° (corrected). The melting point of a mixture of the sublimate with synthetic 1,3-dimethylbarbituric acid was 122–123°. The synthetic material was prepared by the method of Biltz and Hamburger[8] from 1,3-dimethyl-5,5-dibromobarbituric acid. The latter was prepared by methylating barbituric acid with an excess of dimethyl sulfate,[9] and adding bromine to the acidified reaction mixture.

Tetramethyldibarbituric acid treated in water solution with a small excess of bromine, reacts in the cold without forming a permanent precipitate. When heated, however, more bromine is decolorized, and if an excess is provided, a colorless crystalline substance begins to precipitate. This product, recrystallized from alcohol, melts at 171–173° (corrected). It melts at the same temperature when mixed with synthetic 1,3-dimethyl-5,5-dibromobarbituric acid prepared as indicated above.

*Anal.* Calcd. for $C_6H_6N_2O_3Br_2$: N, 8.93. Found: N, 8.82, 8.84.

When 114 mg. (0.000388 mole) of analytically pure tetramethyldibarbituric acid was treated in hot solution with an excess of bromine water, there was obtained 186 mg. (0.000593 mole) of dimethyldibromobarbituric acid, melting at 170–173° without recrystallization.

**Hydrogenolysis of 1,1',3'-Trimethyldibarbituric Acid.**—Pure 1,1',3'-trimethyldibarbituric acid was recrystallized three times from hot water and dried at 100° in a vacuum over phosphorus pentoxide for three and one-half hours. Of the dry product, 4.78 g. was placed in a copper bomb with 200 cc. of 1,4-dioxane that had been distilled over sodium. About 4 g. of Raney nickel catalyst (preserved under dry dioxane) was added and the bomb was heated to 150° under a pressure of 130–140 atmospheres of hydrogen. After six hours of heating at 150°, the bomb was cooled and opened. The dioxane solution was filtered, then evaporated under reduced pressure to a volume of 3–4 cc. When cooled it deposited clusters of prismatic needles; these were filtered off and washed with dioxane until colorless. Of the 0.3 g. of product, 0.21 g. was recrystallized from 0.5 cc. of 95% alcohol, filtered off, washed with a few drops of alcohol and dried. There was obtained 0.1 g. of colorless needles, m. p. 129–131° (corrected). The m. p. of a mixture with synthetic 1-methyl-4,5-dihydrouracil was the same. This reduction product did not decolorize bromine water.

*Anal.* Calcd. for $C_5H_8N_2O_2$: N, 21.87. Found: N, 21.97, 21.95.

The filtrate from the hydrouracil was evaporated to dryness and the brown residue was recrystallized from absolute ethyl alcohol. There was obtained 0.48 g. of colorless needles which after recrystallization melted at 122–123° (corrected) both alone and in mixture with authentic 1,3-dimethylbarbituric acid of the same melting point, prepared as mentioned in the preceding section. This substance gave upon bromination 1,3-dimethyl-5,5-dibromobarbituric acid melting at 171–172° (corrected) alone or when mixed with a known sample of this substance prepared as in the preceding section. The filtrate from the dimethylbarbituric acid contained over a gram more of this substance in crude form.

When an attempt was made to reduce trimethyldibarbituric acid as above but at 75° instead of 150°, the reduction mixture gave a considerable amount of unchanged acid and some crystals closely resembling those of the 1-methyl-4,5-dihydrouracil.

**Synthesis of 1-Methyl-4,5-dihydrouracil.**—Beta-alanine hydrochloride was prepared from succinimide according to the directions of Hale and Honan.[10] Methyl isocyanate was prepared by the method of Slotta and Lorenz.[11] In 1.2 cc. of water, 1.70 g. of β-alanine hydrochloride was dissolved. Enough 60% potassium hydroxide (about 0.5 cc.) was added to make the mixture just basic. The solution was cooled in an ice-bath, and shaken with 1.5 g. of methyl isocyanate until the latter had all dissolved. It was then warmed, 2 cc. of concentrated hydrochloric acid added, and the acidic solution was evaporated at 100° until nearly dry. The pasty residue was extracted with eight 3-cc. portions of boiling dioxane, stirring well, and decanting. The extracts on evaporation to 1 cc. gave 0.62 g. of colorless product which was recrystallized from 95% alcohol, giving colorless, prismatic needles. These melted at 129.5–131° (corrected).

*Anal.* Calcd. for $C_5H_8N_2O_2$: N, 21.87. Found: N, 21.81, 21.87.

1-Methyl-4,5-dihydrouracil is readily soluble in water, ethyl alcohol and dioxane.

In a similar manner, β-phenyl-β-alanine was allowed to react with methyl isocyanate, except that the less soluble hydrouracil formed in this case precipitated out of the acidic solution after short warming. The product was in the form of colorless needles; after recrystallization, they melted at 149–151° (corrected). This substance gave no melting point depression when mixed with a sample of 1-methyl-4-phenyl-4,5-dihydrouracil, prepared according to the methylation procedure of Evans and Johnson.[12]

## Summary

The constitution of dibarbituric acid has been established by a study of its N-methyl homologs.

Evidence has been adduced to show that the dibromodibarbituric acid hydrobromide reported by von Baeyer is not a definite compound.

A study of the pharmacological action of dibarbituric acid and its derivatives is now in progress in this Laboratory.

NEW HAVEN, CONN. RECEIVED JANUARY 10, 1936

(8) H. Biltz and T. Hamburger, *Ber.*, **49**, 635 (1913).

(9) J. Herzig, *Z. physiol. Chem.*, **117**, 13 (1921).

(10) W. J. Hale and E. M. Honan, THIS JOURNAL, **41**, 770 (1919).

(11) K. H. Slotta and L. Lorenz, *Ber.*, **58**, 1320 (1925).

(12) J. Evans and T. B. Johnson, THIS JOURNAL, **52**, 4993 (1930).