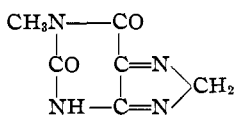
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

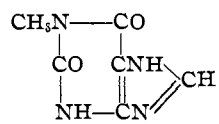
Desmotropism of Xanthine Derivatives[1]

BY TREAT B. JOHNSON AND JOSEPH C. AMBELANG[2]

The conclusion of vanVeen[3] that *toxoflavin*[4] is to be assigned the empirical formula $C_6H_6O_2N_4$ and represented structurally by formula (I), or an isomer of a monomethylxanthine (II) or (III), has stimulated a renewed activity by this Laboratory in the study of new purines, and the relationship of such constructions to the field of chemotherapy for infectious disease.[5] If vanVeen's postulation is correct, then *toxoflavin* (I) assumes a position of immediate scientific interest to workers in the field of purines, and presents the first chemical evidence in support of the desmotropic formulation for purine (IV) to be recorded in the chemical literature.[6]



I



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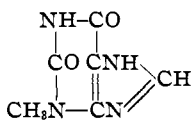

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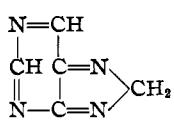

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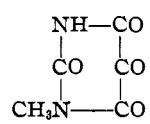


V

(1) Researches on Pyrimidines, CLXIII.

(2) Sterling Professorship of Chemistry Research Assistant, 1938–39.

(3) VanVeen and Mertens, *Proc. Akad. Wetenschappen Amsterdam*, **36**, 666 (1933); *Rec. trav. chim.*, **53**, 257, 398 (1934); vanVeen and Baars, *Proc. Akad. Wetenschappen Amsterdam*, **40**, 1 (1937); *Rec. trav. chim.*, **57**, 248 (1938).

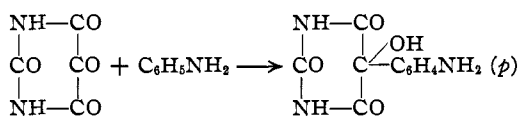
(4) A name given by vanVeen and Mertens to the prosthetic group of a natural pigment formed in the culture of *Bacterium bongkrek*. This substance (toxoflavin) formed by the action of *Bacterium cocovenenans* is the cause of the well-known bongkrek and semaji-poisonings met with in Java.

(5) VanVeen seems to have demonstrated conclusively that *toxoflavin* is a pyrimidine compound closely related structurally to alloxan. It is impossible, however, to conclude from the present experimental evidence whether the methyl group functioning in its formula occupies position 1 or 3 in the supposed purine molecule (I).

(6) "Harnsäure und Xanthine," H. Biltz, *J. prakt. Chem.*, **145**, 83 (1936). "The 4,5-double bond in xanthines does not exhibit the same unsaturation value as that in the uric acids. Conjugation with the 8,9-double bond in a xanthine, or with the 7,8-double bond in an isoxanthine, bestows on the imidazole ring of the purine an aromatic character [Biltz and Sauer, *Ber.*, **64**, 752 (1931)], thereby destroying the influence of unsaturation exercised by the double bond. This aromatic system exhibits, as might be expected, an influence favorable for substitution in the 8-position of the purine molecule."

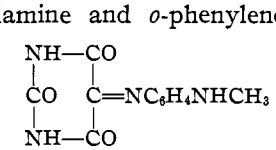
The purpose of this communication is to draw attention to specific reactions which characterize the differences in behavior of the pyrimidine alloxan toward cyclic and acyclic mono- and dibasic amines. The interesting facts that *toxoflavin* gives the murexide reaction, and when oxidized with potassium chlorate and hydrochloric acid yields methylalloxan (V),[6] suggest the possibility of new heterocyclic condensations being brought about by interaction of alloxan with aliphatic diamines. The authors are not aware that the action of such diamines on alloxan has been investigated previously.

The condensation reactions of aromatic amines with alloxan can be divided into two distinct groups as follows: 1, condensations involving addition of alloxan to the nucleus of the aromatic amine used giving derivatives of dialuric acid (VI). This characteristic behavior was first described by Pellizzari[7] and applies to monoamines of the aniline type or its nitrogen substituted derivatives. The same type of reaction applies when phenols are allowed to react with alloxan.

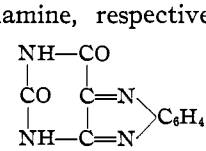


VI

2, Condensations of the anil type which result by interaction of alloxan with diaminobenzene derivatives as illustrated by the formation of the two anils (VII) and (VIII) from *o*-methylphenylenediamine and *o*-phenylenediamine, respectively.[8]



VII



VIII
(Alloxazine)

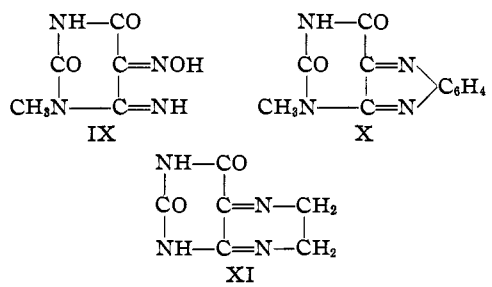
No anils of either type (VII or VIII) obtained by the action of aliphatic amines on alloxan have been described thus far. This pyrimidine (alloxan) functions as a strong oxidizing agent when allowed to react in aqueous solution with acyclic

(7) Pellizzari, *Gazz. chim. ital.*, **17**, 412 (1887).

(8) (a) Piloty and Finkh, *Ann.*, **333**, 41 (1904); (b) Mohlau and Litter, *J. prakt. Chem.*, **73**, 453 (1907); (c) Kuhling and Kaselitz, *Ber.*, **39**, 1319 (1916); (d) also recent work of Rudy and Cramer, *ibid.*, **72**, 227 (1939).

monoamines leading to complicated molecular changes with formation of the corresponding amine salts of purpuric acid, which finally undergo conversion to murexide.[8a] Strecker[9] first disclosed this remarkable behavior of alloxan in a study of its action on α-amino acids, and observed that such acids are oxidized by alloxan to the corresponding aldehydes with formation of murexide.[10]

The authors are now able to report that the reactivity of alloxan toward methylenediamine and ethylenediamine has been investigated, and that neither diamine was found to be productive of any changes of immediate biochemical interest. Methylenediamine showed no tendency to react with alloxan to form xanthine or any desmotropic modification of a purine (IV) corresponding to the constitution proposed for toxoflavin (I). Also ethylenediamine failed to react with alloxan in a manner corresponding to that of *o*-phenylenediamine with formation of a cyclic anil (XI).



IX X

XI

A description of the reactions revealed in this study is given in the experimental part of this paper. It is also of especial interest to note here that the imido-oxime derivative of methylalloxan (IX), which is characterized by the same anil type of unsaturation as is expressed in van-Veen's proposed formula for toxoflavin (I), does not react with *o*-phenylenediamine to form *methylalloxazine* (X). VanVeen and Baars[3] report that *toxoflavin* is not methylated further by the action of diazomethane or methyl iodide and undergoes complete destruction by energetic action.

Experimental Part

Attempts at Condensation of Alloxan with Methylenediamine.—The methylenediamine dihydrochloride used in this research was prepared according to the method described by Knudsen.[11]

(9) Strecker, *Ann.*, **123**, 363 (1862).

(10) Leucine = isovaleraldehyde; alanine = acetaldehyde; glycine = formaldehyde. See also Hurtley and Wootten, *J. Chem. Soc.*, **99**, 288 (1911).

(11) Knudsen, *Ber.*, **47**, 2698 (1914).

1. In Glacial Acetic Acid Solution.—One gram (0.009 mole) of pulverized methylenediamine dihydrochloride was added to an acetic acid solution (20 cc.) of 0.8 g. (0.01 mole) of fused sodium acetate. After standing for three hours with occasional shaking, the mixture was refluxed a few minutes and allowed to stand until cold. The acid solution was then filtered to remove undissolved sodium chloride and mixed with a solution of 0.3 g. (0.0028 mole) of alloxan anhydride in 10 cc. of glacial acetic acid. A deep red color developed immediately. After four days of standing the acid solution was heated at 90–100° for one and one-half hours, when the red coloration became somewhat lighter. On cooling, a small amount of crystalline material separated. It was washed carefully with dry ether and dried *in vacuo* over concentrated sulfuric acid; yield 0.12 g. This showed no sharp m. p. and slowly decomposed when heated above 253°. It was identified as alloxan. This experiment was repeated but without securing any evidence of the formation of a purine condensation product. The recovered alloxan was highly discolored, due apparently to admixture of a small amount of murexide.

Anal. Calcd. for $C_4H_2O_4N_2$: N, 20.00. Found: N, 19.73.

2.—No formation of a purine was observed by interaction of methylenediamine dihydrochloride with alloxan in aqueous solution in the presence of sodium acetate. The solution took on a deep red color due to slow formation of murexide, and the odor of formaldehyde was detected easily.

3.—Attempts also were made to bring about a condensation in absolute alcohol solution. In an acidified solution (with hydrochloric acid) ammonium chloride was precipitated, and formaldehyde generated. Alloxan alcoholate was used also for condensation with methylenediamine but without success. Interaction of the diamine with alloxan in pyridine solution led to the production of a black tar. It is of interest to note here that methylenediamine dihydrochloride as well as trioxymethylene reduce Fehling's solution after gentle warming of the alkaline solution.

Condensation of Alloxan with Ethylenediamine. 1. In Acid Solution.—One and six-tenths grams (0.01 mole) of alloxan monohydrate dissolved in 10 cc. of water was added to a solution of 0.75 g. (0.012 mole) of ethylenediamine in 7 cc. of 6 *N* hydrochloric acid (0.042 mole). The characteristic murexide coloration did not develop and a small quantity of colorless crystals finally separated on standing. The yield was only 0.5 g. and this was not increased by warming the solution. This reaction product contained chlorine and was dried for analysis *in vacuo* over concentrated sulfuric acid; m. p. 225–230° with decomposition. The crystals were colored slightly pink, indicating the possible admixture of a trace of murexide. The results of analysis indicated a constitution conforming to that of a hydrochloride of alloxan-[ethylenediamine-anil-hydrate]-5.

Anal. Calcd. for $C_6H_{10}O_4N_4 \cdot HCl$: C, 30.1; H, 4.6; N, 23.4. Calcd. for $C_6H_{10}O_4N_4 \cdot HCl \cdot H_2O$: C, 29.1; H, 4.4; N, 22.7. Found: C, 29.7; H, 3.80; N, 22.74.

The filtrate from the preceding experiment was diluted with acetone until no further precipitation of solids took place (1.44 g.). This substance was identified as the di-

hydrochloride of ethylenediamine equivalent to 87% of the diamine originally used.

Anal. Calcd. for $C_2H_{10}N_2Cl_2$: N, 21.1. Found: N, 21.2.

2. In Alcohol Solution.—Alloxan anhydride (0.4 g. or 0.0028 mole) was dissolved in 15 cc. of absolute alcohol, and dry hydrogen chloride gas bubbled into the solution until it was decolorized to a pale yellow. After cooling in a refrigerator (two days) and finally filtering, 0.2 g. (0.0033 mole) of ethylenediamine in 10 cc. of absolute alcohol was added. A colorless, granular precipitate deposited, and took on a pink color on exposure to the air. It was separated, washed with absolute alcohol and ether and then dried *in vacuo* over concentrated sulfuric acid. The yield was only 0.5 g. The compound was free from chlorine. It was very soluble in water and attempts to purify it by precipitation from aqueous solution with acetone or pyridine, or by diluting an acetic acid solution with chloroform, were unsuccessful. A specimen dried to constant weight at 100° over phosphoric anhydride melted at about 214° with decomposition.

Anal. Calcd. for alloxan-[ethylenediamine-anilhydrate]-5, $C_6H_{10}O_4N_4$: C, 35.64; H, 4.9; N, 27.71. Found: C, 35.27, 36.2; H, 4.28, 4.53; N, 26.89, 28.1.

Two-tenths gram of the above condensation product was dissolved in 11 cc. of 0.1 *N* hydrochloric acid and combined with 22 cc. of water containing 0.3 g. of picric acid. After concentrating the solution *in vacuo*, and cooling, the picrate of ethylenediamine separated. This salt was purified by crystallization from water and melted at 227–235° with decomposition. When mixed with pure ethylenediamine picrate, no depression of the melting point was observed.

Anal. Calcd. for $C_{14}H_{14}O_{14}N_8$: N, 21.62. Found: N, 21.82.

Ethylenediamine gives an intense crimson coloration when added to an aqueous solution of alloxan. The dihydrochloride of the amine produces no coloration under the same experimental conditions. Furthermore, the presence of ethylenediamine dihydrochloride did not interfere with the formation of *alloxazine* by interaction of *o*-phenylenediamine dihydrochloride with alloxan in aqueous solution.

Interaction of *o*-Phenylenediamine Dihydrochloride with Methylalloxan-4-imino-5-oxime.—Toxoflavin and methylalloxan[12] react with *o*-phenylenediamine dihydrochloride in aqueous solution on warming to give methylalloxazine $C_{11}H_{18}O_2N_4$ (X). The above oxime derivative (IX)[13] does not interact with the *o*-diamine in a similar manner to form this alloxazine derivative. Combination in boiling water solution leads to the formation of an amorphous, brown substance which is soluble in hot acetic acid, and is reprecipitated in an amorphous condition by addition of water. We were unable to isolate any product of crystalline character from the reaction mixture.

Summary

1. Attention is called to the characteristic differences in chemical behavior of the pyrimidine alloxan toward aromatic and aliphatic amines.

2. Methylenediamine and alloxan do not interact to form xanthine or any tautomeric modification of this purine.

3. Ethylenediamine shows a different behavior toward alloxan than its aromatic analog *o*-phenylenediamine, and does not interact to form the corresponding aliphatic alloxazine analog. A simple addition product is formed without cyclization.

4. The oximido-imide (4,5) derivative of methylalloxan does not interact with *o*-phenylenediamine to form an alloxazine.

(12) VanVeen and Baars, *Rec. trav. chim.*, **57**, 259, 261 (1938).

(13) Traube, *Ber.*, **33**, 3048 (1900).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

Synthesis of α-Aminopelargonic Acid

BY TREAT B. JOHNSON

At the urgent request of a group of workers in experimental medicine the author consented to undertake the synthesis of this unknown α-amino acid (I). Polypeptide combinations of this amino acid are believed to occur in the degradation products of the proteins of beet roots and in leaves of *Pelargonium roseum;* and several

$$CH_3CH_2CH_2CH_2CH_2CH_2CH_2\underset{\displaystyle NH_2}{\underset{|}{C}}HCOOH$$

I

dipeptide derivatives containing this amino acid have been described previously by Hopwood and Weizmann[1] in 1911.

The fact that pelargonic acid itself is known to influence certain physiological changes of interest to medicine, lends interest in the possible physiological behavior of the unknown amino acid and its derivatives. The fatty acid is characterized, for example, by its physical capacity as a surface-active substance and may act, depending upon the percentage present, as an accelerator or an

(1) Hopwood and Weizmann, *J. Chem. Soc.*, **99**, 1577 (1911).