[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Pteridines. XI. The Structure of Wieland's "Bis-alloxazine"[1]

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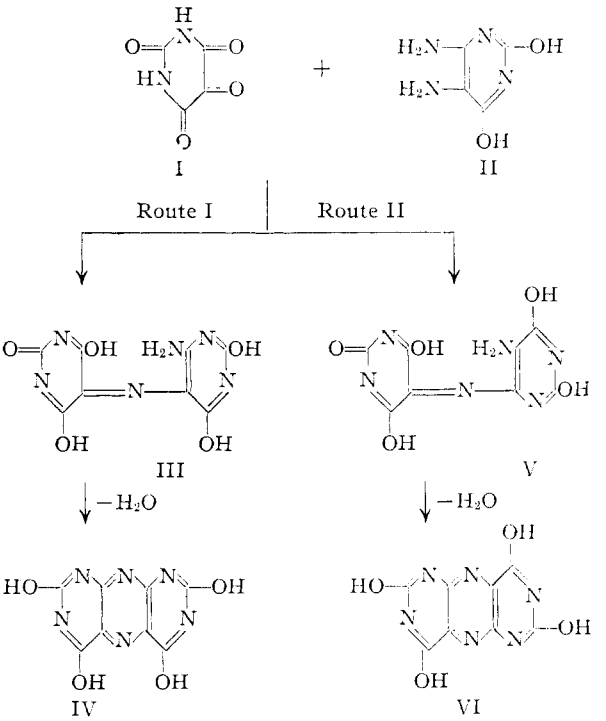
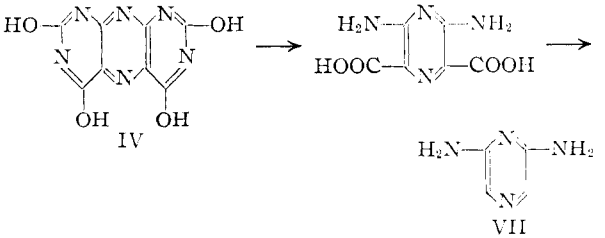
The product of the reaction between 5,6-diamino-2,4-dihydroxypyrimidine sulfate and alloxan has been shown to be 2,4,5,7-tetrahydroxypyrimido(5,4-g)pteridine (IV), in agreement with the original postulate of Wieland.[7] The reaction between alloxan and 5,6-diaminopyrimidines in dilute acid solution appears to be general and leads by way of a colored intermediate (corresponding in structure to III) to derivatives of pyrimido(5,4-g)pteridine. This conclusion is supported by several independent syntheses of derivatives of this ring system. The formation of the colored intermediate serves as a sensitive qualitative test either for alloxan or for the 5,6-diaminopyrimidine.

The reaction of alloxan with aromatic 1,2-diamines has been widely used to prepare a host of heterocyclic compounds.[2] The first reported use of a 5,6-diaminopyrimidine in this reaction was by Sachs and Meyerheim,[3] who described the condensation of alloxan with 1,3-dimethyl-5,6-diamino-2,4-(1H,3H)pyrimidinedione in neutral solution to give 1,3-dimethyl-2,4(1H,3H)diketo-7-hydroxy-6-pteridinecarbonylurea. Subsequently a number of condensations of alloxan with 5,6-diaminopyrimidines have been carried out,[4-10] principally in acidic solution[11]; it has been assumed that tricyclic structures similar to IV or VI have been formed.

Wieland and co-workers[7] showed that the reaction between alloxan and 5,6-diamino-2,4-dihydroxypyrimidine involved a purple-bronze intermediate III which was isolated and characterized and which was readily transformed by heating in basic solution into a final product for which structure IV was proposed, and for which the name "bis-alloxazine" was suggested. It was recognized that the reaction might have proceeded by way of the isomeric intermediate V to VI, but structure IV was preferred for the final product because of the known greater reactivity of the 5-amino group in the 5,6-diaminopyrimidine and the 5-carbonyl group in alloxan. No direct proof was offered, however, to support this assignment of structure. Other workers either have not indicated which isomer was produced,[4,5,10] or have assigned the formulation IV[7-9] or VI[6] to the products without experimental verification.

It has now been found that the reaction of alloxan with 5,6-diamino-2,4-dihydroxypyrimidine gives 2,4,5,7-tetrahydroxypyrimido(5,4-g)pteridine (IV),[12] in support of the original postulate of Wieland that the condensation reaction proceeds by route I. Thus, IV was cleaved with strong aqueous sodium hydroxide by the method of Weijlard, Tishler and Erickson[13] to give a diaminopyrazine-





(1) This investigation was supported in part by a research grant (C-2031 ET) from the National Cancer Institute of the National Institutes of Health, Public Health Service.

(2) For example, see O. Kuhling, *Ber.*, **24**, 2363 (1891); **27**, 2116 (1894); **28**, 1968 (1895); R. Kuhn and F. Weygand, *ibid.*, **67**, 1409, 1459 (1934); **68**, 1282 (1935); H. Rudy and K. F. Kramer, *ibid.*, **71B**, 1234 (1938); H. Rudy and O. Majer, *ibid.*, **71B**, 1243, 1323 (1938); **72B**, 933 (1939); A. McCoubrey and W. Webster, *J. Chem. Soc.*, 1719 (1948).

(3) F. Sachs and G. Meyerheim, *Ber.*, **41**, 3957 (1908).

(4) R. Robinson and M. L. Tomlinson, *J. Chem. Soc.*, 467 (1935).

(5) R. Kuhn and A. H. Cook, *Ber.*, **70B**, 761 (1937).

(6) B. Hepner, I. Kelner, A. Simonberg and H. Kaltman, *Cong. chim. ind. Compt. rend.*, **17**, I, 228 (1937).

(7) H. Wieland, A. Tartter and R. Purrmann, *Ann.*, **545**, 209 (1940).

(8) H. S. Forrest, R. Hull, H. J. Rodda and A. R. Todd, *J. Chem. Soc.*, 3 (1951).

(9) E. M. Gal, *Experientia*, **7**, 261 (1951).

(10) H. Brederick, I. Hennig, W. Pfleiderer and O. Deschler, *Ber.*, **86**, 845 (1953).

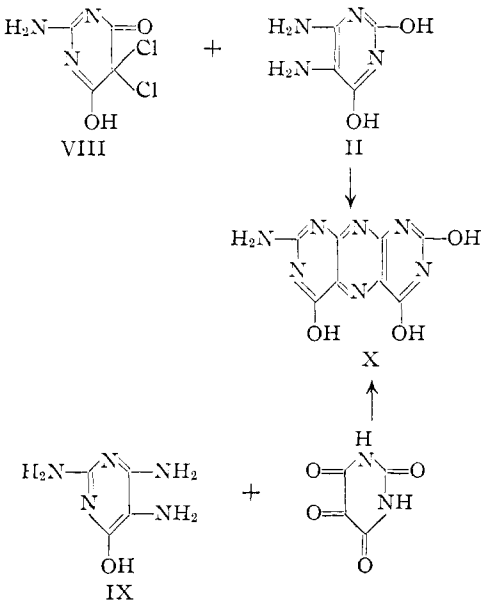
(11) Experiments in this Laboratory have shown that the condensation products of alloxan and 5,6-diaminopyrimidines in basic solution differ from the products obtained in acidic solution. Similar results have been demonstrated recently in the condensation of alloxan and o-phenylenediamine (R. B. Barlow, H. R. Ing and I. M. Lewis, *J. Chem. Soc.*, 3242 (1951); F. E. King and J. W. Clark-Lewis, *ibid.*, 3379 (1951)). This phase of the problem is being investigated further.

(12) Various names have been suggested for these compounds. Robinson (ref. 4) referred to his product as a "bis-pyrimidazine"; Wieland (ref. 7) proposed the name "bis-alloxazine"; Todd (ref. 8) employed the systematic name 1,3,6,8,9,10-hexa-aza-anthracene and also suggested "diazaisoalloxazine" for a 9-alkyl derivative; and Gal (ref. 9), who apparently believed he had prepared members of this series for the first time, proposed the name "7,9-diaza-alloxazine" and "pyrimido(4,5-b)pteridine." We believe the shortened systematic name "pyrimido(5,4-g)pteridine" to be advantageous.

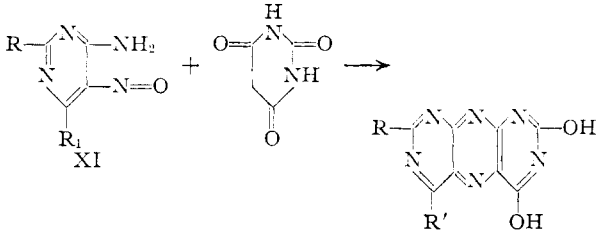
(13) J. Weijlard, M. Tishler and A. E. Erickson, THIS JOURNAL, **67**, 802 (1945).

dicarboxylic acid which was not characterized but which was decarboxylated directly to 2,6-diaminopyrazine (VII).

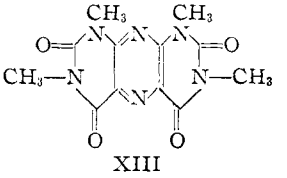
Further evidence in favor of a pyrimido(5,4-g)pteridine structure for the reaction product of a 5,6-diaminopyrimidine and alloxan was found in the observations that (1) the condensation of 5,5-dibromo- or 5,5-dichlorobarbituric acid with 5,6-diamino-2,4-dihydroxypyrimidine gave a product identical with the alloxan condensation product IV, and (2) the condensation of 2-amino-4-hydroxy-6-keto-5,5-dichlorodihydropyrimidine (VIII) with 5,6-diamino-2,4-dihydroxypyrimidine (II) yielded a product X identical with that obtained from 2,5,6-triamino-4-hydroxypyrimidine (IX) and alloxan. These results are in agreement with the recent confirmation by Wilson[14] that the reaction between a 5,6-diaminopyrimidine and an acid chloride, ester or acid occurs exclusively with the 5-amino group.



Furthermore, Timmis[15,16] recently has reported the synthesis of a number of pyrimido(5,4-g)pteridines by an independent and unequivocal method involving the condensation of barbituric acid with 6-amino-5-nitrosopyrimidines (XI). Using his method, we have prepared 2,4,5,7-tetrahydroxypyrimido(5,4-g)pteridine (IV) and 2,4-diamino-5,7-dihydroxypyrimido(5,4-g)pteridine (XII). By comparison of infrared spectra (Nujol mull), it was shown that a sample of IV, prepared by Timmis's method, was identical with the "bis-alloxazine" prepared by Wieland's method and that XII was identical with the product of the condensation of 2,4,5,6-tetraminopyrimidine and alloxan. It thus seems a valid generalization that the tricyclic structures obtained from 5,6-diaminopyrimidines and alloxan are derivatives of pyrimido(5,4-g)pteridine.[16a]



Methylation of IV with methyl iodide and potassium carbonate in acetone gave 1,3,6,8-tetramethyl-2,4,5,7(1H,3H,6H,8H)pyrimido(5,4-g)pteridinetetrone (XIII), m.p. 403–404°, apparently identical with the product, m.p. 403°, obtained by Timmis[16] from the condensation of 1,3-dimethyl-6-amino-5-nitroso-2,4(1H,3H)pyrimidinedione and dimethylbarbituric acid. Bredereck[10] recently has reported the preparation of a "tetramethyl-bis-alloxazine," m.p. 390°, by the treatment of 1,3-dimethyl-6-amino-5-acetylamino-2,4(1H,3H)pyrimidinedione with sulfuric acid, but no decision was made as to whether the product was a derivative of IV or VI. In all probability the product was identical with XIII.[17]



A recent patent[18] claims the synthesis of XIII by the condensation of 1,3-dimethyl-5,6-diamino-2,4-(1H,3H)pyrimidinedione with dimethylalloxan, followed by heating the resulting reaction mixture with 0.5 *N* sodium hydroxide for two hours. However, the product was reported to melt at 206–207° and is obviously not identical with XIII. We have found that when XIII is heated at reflux with 1 *N* sodium hydroxide for three hours, 2,6-bis-(methylamino)-N-methyl-N′-methylpyrazine-3,5-dicarboxamide (XIV), m.p. 231–232.5°, is formed in 81% yield. The lability of XIII to basic hydrolysis also has been noted by Bredereck,[10] who stated that mild alkaline hydrolysis of XIII gave a pteridine derivative which was not identified. Hence, the above reaction product, m.p. 206–207°, is probably either N,1,3-trimethyl-7-methylamino-2,4-(1H,3H)-pteridinedione-6-carboxamide or a mixture of hydrolysis products.

The reaction between 5,6-diaminopyrimidines and alloxan to give colored intermediate condensation products (corresponding in structure to III) has been observed by a number of workers,[3,6,7] and serves as a sensitive qualitative test for the presence of either reactant. This reaction has been of particular value in our work with the pteridines because of the intense color given by a trace of most 5,6-diaminopyrimidines and alloxan in 0.5 *N* hydro-

(14) W. Wilson, *J. Chem. Soc.*, 1157 (1948).

(15) G. M. Timmis, *Nature*, **164**, 139 (1949).

(16) G. M. Timmis, U. S. Patent 2,581,889 (Jan. 8, 1952); *C. A.*, **46**, 7594 (1952).

(16a) NOTE ADDED IN PROOF.—Dr. G. H. Hitchings and Dr. E. A. Falco of the Wellcome Research Laboratories have communicated the information that they also have prepared IV by the methods of Wieland and Timmis and found the two products to be identical by analysis and comparison of ultraviolet absorption spectra.

(17) We will report later on an unequivocal synthesis of the isomeric "bis-alloxazine" VI. The tetramethyl derivative of VI melts at 358–360° and thus provides final confirmation of the above structural assignments.

(18) O. DeGarmo, U. S. Patent 2,561,324 (July 24, 1951); *C. A.* **46**, 1595 (1952).

chloric acid. Although the color is much less intense with pyrimidines lacking an enolizable group in either the 2- or 4-positions, this test has been found to be more convenient and reliable than the nitric acid method employed by Traube.[19]

Experimental[20]

The 2,4,5,7-Tetrahydroxypyrimido(5,4-g)pteridine (IV). Method A.—This compound was prepared by the method of Wieland[7] from 5,6-diamino-2,4-dihydroxypyrimidine sulfate and alloxan.

Method B.—This compound was also prepared by the method of Timmis[15,16] from 6-amino-2,4-dihydroxy-5-nitrosopyrimidine and barbituric acid.

Method C.—A mixture of 2.0 g. of 5,6-diamino-2,4-dihydroxypyrimidine sulfate, 2.5 g. of 5,5-dichlorobarbituric acid and 75 ml. of glacial acetic acid was heated under reflux for two hours. A deep red color appeared at the beginning of the reaction which gradually lightened to orange as heating proceeded. The cooled reaction mixture was filtered to give a red-orange solid which was washed thoroughly with acetone and then suspended in 100 ml. of boiling water made alkaline to *p*H 10 with ammonium hydroxide. The color of the suspended solid rapidly changed from orange to yellow. After heating the alkaline mixture for several minutes, the *p*H was adjusted to 5 with acetic acid and the yellow solid collected by filtration; yield 1.5 g. (58%). Comparable results were obtained using 5,5-dibromobarbituric acid.

The products from methods A, B and C were identical as shown by a comparison of their infrared spectra (Nujol mull).

2,6-Diaminopyrazine (VII).—Ten grams of 2,4,5,7-tetrahydroxypyrimido(5,4-g)pteridine was added to 65 ml. of 15% sodium hydroxide solution and the mixture was heated in a steel autoclave at 170° for three hours. Addition of 18% hydrochloric acid to the cooled bomb contents caused copious evolution of carbon dioxide and the separation of 2,6-diamino-3,5-pyrazinedicarboxylic acid as a dark red solid; yield 7.1 g. (89.5%). Two grams of this material was added to 30 ml. of 80% sulfuric acid and the solution heated for 30 minutes at 195–200°. The black reaction mixture was cooled, added to 75 g. of ice, and the resulting solution made strongly alkaline with sodium hydroxide. It was then extracted for 20 hours with peroxide-free ether, the ether extract dried over sodium sulfate, and the ether removed by evaporation. Recrystallization of the residue from methylene chloride–petroleum ether gave 0.23 g. of yellow needles, m.p. 133–136° dec. The reported decomposition point of 2,6-diaminopyrazine is 136°.[21] The low yield of diaminopyrazine obtained by this degradation is not surprising in view of its known instability.

2-Amino-4,5,7-trihydroxypyrimido(5,4-g)pteridine (X). Method A.—A solution of 5.0 g. of freshly recrystallized 2,5,6-triamino-4-hydroxypyrimidine bisulfite (0.024 mole) in 12 ml. of boiling water was added to a solution of 4.0 g. (0.025 mole) of alloxan monohydrate in 6 ml. of water. Immediately upon mixing a deep purple color appeared with the simultaneous separation of a purple solid. After cooling to 0°, the reaction mixture was filtered and the solid washed with a small amount of ice-cold water followed by acetone; yield 4.5 g. (71%). The microcrystalline half-condensation product (corresponding in structure to III) turned orange upon heating above 300°, indicating ring closure to 2-amino-4,5,7-trihydroxypyrimido(5,4-g)pteridine; it was dried for analysis at 140° *in vacuo* over phosphorus pentoxide for ten hours.

Anal. Calcd. for $C_8H_7N_7O_4$: C, 36.2; H, 2.7; N, 37.0. Found: C, 36.2; H, 2.8; N, 37.0.

Ring closure to 2-amino-4,5,7-trihydroxypyrimido(5,4-g)pteridine was effected by boiling 0.4 g. of the above half-condensation product in 10 ml. of 0.1 *N* sodium hydroxide for a period of ten minutes. During this time, the color lightened from a deep cherry-red to yellow. After treatment with Norit, the filtrate was acidified to *p*H 6 with hydrochloric acid and the precipitated solid collected by filtration and washed with water followed by acetone to give 0.4 g. (quantitative). The product was purified by dissolving in dilute sodium hydroxide, treating with Norit and filtering into boiling dilute hydrochloric acid and was obtained as a light yellow microcrystalline solid which darkened slightly without decomposition upon heating to 360°.

Anal. Calcd. for $C_8H_5N_7O_3$: C, 38.9; H, 2.0; N, 39.7. Found: C, 38.6; H, 2.2; N, 39.8.

Method B.—A mixture of 1.0 g. (0.0048 mole) of 5,6-diamino-2,4-dihydroxypyrimidine sulfate, 1.15 g. (0.0058 mole) of 2-amino-4-hydroxy-6-keto-5,5-dichlorodihydropyrimidine[22] and 50 ml. of glacial acetic acid was heated under reflux for three hours. The usual deep red color characteristic of the intermediate half-condensation product appeared, but on further heating it faded rapidly to yellow with the simultaneous separation of a yellow solid. The reaction mixture was cooled, filtered and the solid washed well with water followed by acetone. The solid was then suspended in 100 ml. of boiling 0.1 *N* hydrochloric acid, the *p*H adjusted to 10 with ammonium hydroxide, the solution heated for a few minutes and then reacidified to *p*H 5 with hydrochloric acid; yield 1.04 g. (87.5%). The product was identical with the product obtained by method A as shown by a comparison of infrared spectra (Nujol mull).

2,4-Diamino-5,7-dihydroxypyrimido(5,4-g)pteridine (XII). Method A.—To a solution of 3.0 g. (0.0135 mole) of 2,4,5,6-tetraminopyrimidine bisulfite in 50 ml. of 0.5 *N* hydrochloric acid was added a solution of 2.5 g. (0.0156 mole) of alloxan monohydrate in 10 ml. of water. The deep purple color of the half-condensation product appeared immediately upon mixing, but after a few minutes lightened to orange with the simultaneous separation of an orange solid. After adjusting the *p*H to 9 with dilute sodium hydroxide, the reaction mixture was boiled for ten minutes, reacidified to *p*H 6 with hydrochloric acid, cooled to 0° and filtered. The orange solid was thoroughly washed with water followed by acetone; yield 2.2 g. (66%). The product was purified by suspending in boiling 0.1 *N* hydrochloric acid, filtering and then resuspending in boiling water and filtering. An orange microcrystalline solid was obtained which did not darken upon heating to 360°.

Anal. Calcd. for $C_8H_6N_8O_2$: C, 39.0; H, 2.5; N, 45.5. Found: C, 39.0; H, 2.5; N, 45.6.

Method B.—This compound was also prepared by the method of Timmis[15,16] from 2,6-diamino-4-hydroxy-5-nitrosopyrimidine and barbituric acid. It was identical with the product obtained by method A as shown by a comparison of infrared spectra (Nujol mull).

1,3,6,8-Tetramethyl-2,4,5,7(1H,3H,6H,8H)pyrimido(5,4-g)-pteridinetetrone (XIII).—A mixture of 5 g. of 2,4,5,7-tetrahydroxypyrimido(5,4-g)pteridine (IV), 45 ml. of methyl iodide, 50 g. of potassium carbonate and 150 ml. of acetone was heated under reflux for 24 hours. Addition of 100 ml. of water followed by filtration gave a green-yellow solid which was placed in a Soxhlet cup and extracted with chloroform for 48 hours. A light yellow solid separated from the chloroform extract which was collected by filtration, dried and sublimed at 280° (0.1 mm.) to give 1.2 g. of a white crystalline solid, m.p. 403–404°. An additional 0.5 g. of pure product was obtained by sublimation of the chloroform-insoluble residue; total yield 1.7 g. (28%).

Anal. Calcd. for $C_{12}H_{12}N_8O_4$: C, 47.4; H, 4.0; N, 27.6. Found: C, 47.5; H, 3.9; N, 27.9.

2,6-Bis-(methylamine)-N-methyl-N′-methylpyrazine-3,5-dicarboxamide (XIV).—A mixture of 1.0 g. of XIII and 30 ml. of 1 *N* sodium hydroxide was heated under reflux for 2.5 hours, 15 ml. of ethanol added and heating continued for an additional 30 minutes. Cooling caused the separation of yellow crystals which were collected by filtration and washed with cold ethanol; yield 0.67 g. (81%), m.p. 230–232°. Sublimation at 180° (0.6 mm.) gave dense yellow crystals, m.p. 231–232.5°.

Anal. Calcd. for $C_{10}H_{16}N_8O_2$: C, 47.6; H, 6.4; N, 33.3. Found: C, 47.3; H, 6.1; N, 33.3.

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(19) W. Traube, *Ber.*, **33**, 1371 (1900).

(20) The microanalyses were performed by Mrs. Lucy Chang, Mrs. Esther Pett and Mr. Joseph Nemeth. The infrared spectra were determined by Mr. James Brader.

(21) K. H. Schaaf and P. E. Spoerri, THIS JOURNAL, **71**, 2043 (1949).

(22) R. B. Angier, J. H. Mowat, J. H. Boothe, C. W. Waller, J. Semb, B. L. Hutchings, E. L. R. Stokstad and Y. SubbaRow, *ibid.*, **70**, 1274 (1948).