established by mixture melting points, and by comparison of their infrared and ultraviolet spectra with those of authentic samples.

Reconstitution of Rescinnamine.—3,4,5-Trimethoxycinnamic acid[16] (2 g.) was converted to the acid chloride by refluxing for 2.5 hours with thionyl chloride (5 ml.) in benzene (100 ml.). The excess thionyl chloride and benzene were removed *in vacuo* yielding a crystalline residue. Methyl reserpate (1.0 g.) and dry pyridine (50 ml.) was added to the crude acid chloride and the stoppered mixture was agitated on an automatic shaker for 16 hours. At the end of this time, ice (50 g.) was added to decompose the excess acid chloride. The solution was filtered and evaporated to dryness *in vacuo* with the aid of several small additional portions of benzene. The resulting tan colored resin was dissolved in chloroform (100 ml.) and washed successively with equal volumes of dilute hydrochloric acid, dilute aqueous potassium hydroxide and water. The chloroform layer was then taken to dryness and the resulting resinous material was crystallized from benzene (20 ml.) yielding needles (1.10 g.). After several recrystallizations from acetone–water, the sample melted at 237–238° (vac.), $[\alpha]^{24}D$ −95 ± 2 (*c* 1.0 in $CHCl_3$).

(16) K. H. Slotta and H. Heller, *Ber.*, **63**, 3029 (1930).

Acknowledgment.—We wish to express our thanks to C. Stimmel and I. Znak in the Riker Analytical Department for the optical rotations, equivalent weight determinations and spectral data.

LOS ANGELES, CALIFORNIA

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS, AND THE WELLCOME RESEARCH LABORATORIES]

Pyrimidopteridines by Oxidative Self-condensation of Aminopyrimidines[1,2]

BY E. C. TAYLOR, JR.,[3] HARVEY M. LOUX, ELVIRA A. FALCO AND GEORGE H. HITCHINGS

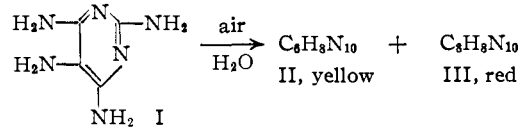
RECEIVED NOVEMBER 15, 1954

The extremely insoluble, highly fluorescent and deeply colored substances commonly encountered as by-products during the course of reactions involving 4,5-diaminopyrimidines and formerly believed to be amorphous decomposition products of the latter have been found to be pyrimido[5,4-g]- and pyrimido[4,5-g]pteridines formed by oxidative self-condensation of the diaminopyrimidine in the presence of air. A number of examples of the reaction have been given which illustrate its scope and limitations and a mechanism for the conversion has been advanced. The potassium ferricyanide oxidation product of 5-aminouracil (XIII) has been shown to be 2,4,6,8-tetrahydroxypyrimido[4,5-g]pteridine (XII) rather than XIV ("diuracilpyridazine") as previously reported.

4,5-Diaminopyrimidines are commonly used intermediates for the synthesis of purines, pteridines and related condensed pyrimidine systems. The formation of extremely insoluble, highly fluorescent and deeply-colored by-products during these reactions, particularly when carried out in alkaline solution, has been observed frequently, and it has been assumed that these substances were amorphous decomposition products of the diaminopyrimidines. The present paper presents evidence to show that these substances are instead pyrimidopteridines formed by oxidative self-condensation of the diaminopyrimidine in the presence of air.

This investigation was initiated by the observation that a fluorescent, insoluble and deeply-colored substance was formed as a by-product during the course of a synthesis which involved 2,4,5,6-tetraminopyrimidine (I). Trials with various combinations of the components of the original reaction mixture demonstrated that none of the other components was involved and that the product in question must have originated from the tetraminopyrimidine. This conclusion was confirmed by the observation that the same product was formed in 60% yield (based on I) by passing a slow stream of air through a warm aqueous solution of I.

An examination of the ultraviolet absorption spectrum of the new substance showed the presence of intense absorption bands in both the near and far ultraviolet of a character which suggested a relationship to the "bis-alloxazine" of Wieland.[4,5] When the substance was recrystallized from glacial acetic acid, a separation into two isomeric compounds with the empirical formula $C_8H_8N_{10}$ was achieved. Both components were isolated from the recrystallization as their yellow acetates; the major component, which was the more soluble, was obtained from its acetate as a yellow solid (II)



which imparted a strong blue fluorescence to aqueous solutions, while the minor component was obtained from its acetate as a dark red crystalline solid (III) which imparted a greenish-yellow fluorescence to aqueous solutions.

Deamination of the yellow isomer II with sodium nitrite in dilute hydrochloric acid gave a product, $C_8H_4N_6O_4$, which proved to be identical with an authentic sample of "bis-alloxazine," as shown by comparison of both ultraviolet and infrared absorption spectra. "Bis-alloxazine" was first prepared by Wieland in 1940[4] by the condensation of alloxan (V) with 4,5-diaminouracil (VI), and its structure was established as 2,4,5,7-tetrahydroxypyrimido[5,4-g]-pteridine (IV) both by an unequivocal synthesis due to Timmis[6] from barbituric acid (VII) and 6-amino-2,4-dihydroxy-5-nitrosopyrimidine (VIII) and by Taylor, Cain and Loux[5] by

(1) This work 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, to the University of Illinois.

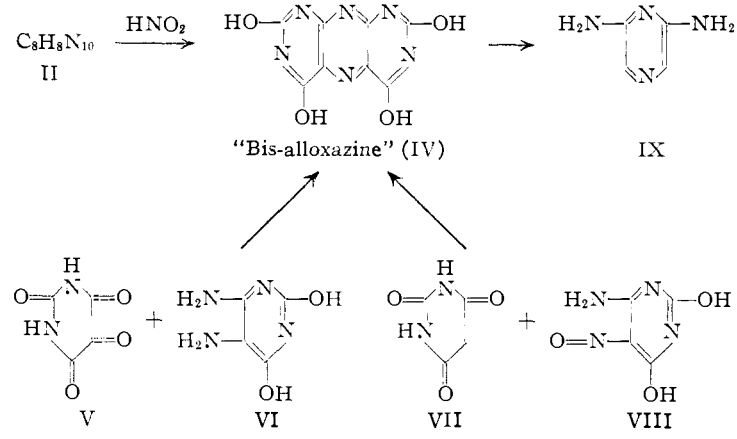
(2) Presented before the Division of Organic Chemistry at the 126th Meeting of the American Chemical Society, September 12–17, 1954, New York City.

(3) Frick Chemical Laboratory, Princeton University, Princeton, New Jersey.

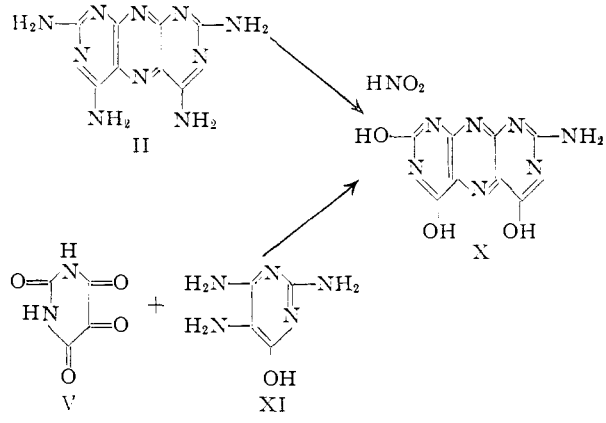
(4) H. Wieland, A. Tartter and R. Purrmann, *Ann.*, **545**, 209 (1940).

(5) E. C. Taylor, Jr., C. K. Cain and H. M. Loux, THIS JOURNAL **76**, 1874 (1954).

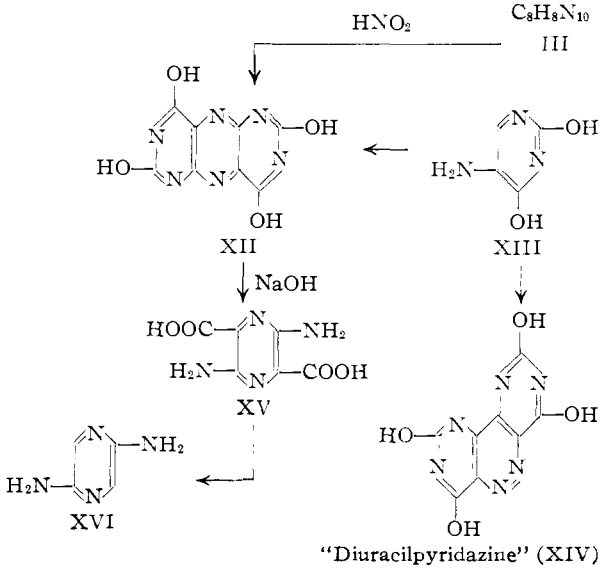
(6) G. M. Timmis, *Nature*, **164**, 139 (1949).



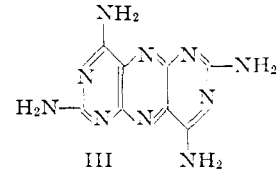
degradation to 2,6-diaminopyrazine (IX). Thus, the yellow isomer II must be 2,4,5,7-tetraaminopyrimido[5,4-g]pteridine.



Confirmatory evidence for this structural assignment was obtained by partial deamination of II with only the theoretical amount of sodium nitrite. The product of the deamination was identical with the condensation product of alloxan (V) with 2,4,5-triamino-6-hydroxypyrimidine (XI) and therefore must be 2-amino-4,5,7-trihydroxypyrimido[5,4-g]-pteridine (X).[5]



Deamination of the less soluble red isomer III also gave a compound, $C_8H_4N_6O_4$, which, however, was not identical with the deaminated product IV of II. Examination of the ultraviolet absorption spectrum of this deaminated product revealed that it was very probably identical with the potassium ferricyanide oxidation product of 5-aminouracil (XIII), which had been described as "diuracilpyridazine" and assigned[7] structure XIV by analogy with the oxidation of isobarbituric acid to 4,4'-diisobarbituric acid.[8] The identity of the deaminated product of the red isomer with "diuracilpyridazine" was confirmed subsequently by comparison of infrared spectra. The structure of the latter compound was established unequivocally as 2,4,6,8-tetrahydroxypyrimido[4,5-g]pteridine (XII) (the long-missing isomer of "bis-alloxazine") by cleavage with sodium hydroxide to 2,5-diaminopyrazine-3,6-dicarboxylic acid (XV), which was decarboxylated by repeated vacuum sublimation to give the known[9] 2,5-diaminopyrazine (XVI). Acetylation of XVI gave the known[9] 2,5-diacetamidopyrazine. Thus, the red isomer must be 2,4,6,8-tetraminopyrimido[4,5-g]pteridine (III), and the two isomeric tetraminopyrimidopteridines arose by oxidative self-condensation of 2,4,5,6-tetraminopyrimidine (I).



Subsequent investigations have shown that the oxidative self-condensation of 4,5-diaminopyrimidines to pyrimidopteridines with air is a general reaction, although both the reaction conditions and the nature of the substituent groups on the pyrimidine ring have a marked effect on the course of the reaction. Thus, 4,5-diaminouracil (VI) was converted into a mixture of 2,4,5,7-tetrahydroxypyrimido(5,4-g)pteridine ("bis-alloxazine") (IV) and 2,4,6,8-tetrahydroxypyrimido(4,5-g)pteridine ("diuracilpyridazine") (XII) when a slow current of air was passed through a solution of the pyrimidine in water or in 1% sodium hydroxide. On the other hand, only IV was formed when an ammoniacal solution of VI was warmed on a steam-bath overnight, or when a current of air was passed through the warm ammoniacal solution. By contrast, 2,4,5-triamino-6-hydroxypyrimidine (XI) was converted into a mixture of 2,7-diamino-4,5-dihydroxypyrimido(5,4-g)pteridine and 2,6-diamino-4,8-dihydroxypyrimido(4,5-g)pteridine by each of the above reaction conditions, while 2-mercapto-4,5,6-triaminopyrimidine was unaffected in neutral solution, and was converted only to 2,7-dimercapto-4,5-diaminopyrimido(5,4-g)pteridine by air oxidation in dilute sodium hydroxide solution. 2-Mercapto-

(7) O. Baudisch and D. Davidson, *J. Biol. Chem.*, **71**, 497 (1927).
(8) O. Baudisch and D. Davidson, *ibid.*, **64**, 619 (1925).
(9) D. M. Sharefkin and P. E. Spoerri, THIS JOURNAL, **73**, 1637 (1951).

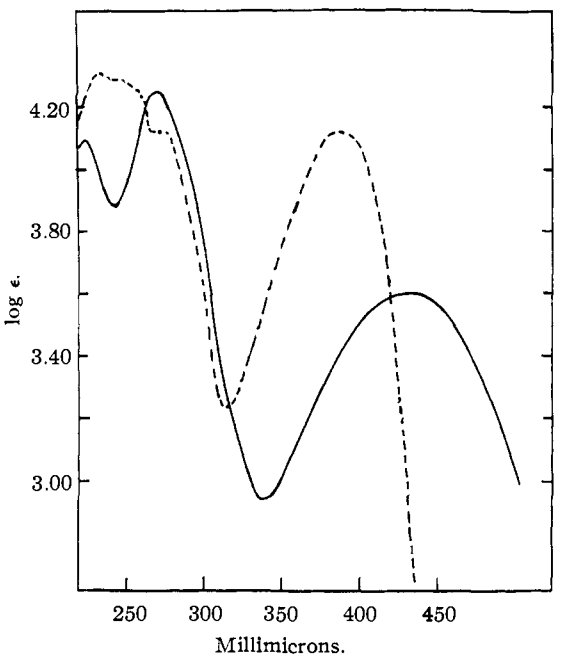


Fig. 1.———, 2,4,5,7-tetrahydroxypyrimido[5,4-g]pteridine (IV); ——, 2,4,6,8-tetrahydroxypyrimido[4,5-g]-pteridine (XII); spectra determined in 0.1 *N* sodium hydroxide.

6-hydroxy-4,5-diaminopyrimidine was also unaffected by air oxidation in neutral solution and was converted in alkaline solution into a compound which, by analogy with the previous results, is probably 2,7-dimercapto-4,5-dihydroxypyrimido-(5,4-g)pteridine, although the wave length of maximum absorption is only 345 mμ (*vide infra*). 6-Hydroxy-4,5-diaminopyrimidine and 4,5-diaminopyrimidine were unaffected by air both in neutral and in alkaline solution.

It was pointed out in the foregoing discussion that potassium ferricyanide oxidation of 5-aminouracil (XIII) gives XII in high yield. In view of the mild conditions now shown to be sufficient for the oxidative self-condensation of 4,5-diaminopyrimidines to pyrimidopteridines, the air oxidation of XIII was attempted under a variety of conditions. In no instance could pyrimidopteridine formation be detected. 2,4-Dihydroxy-6-aminopyrimidine (6-aminouracil) was unaffected both by air and by potassium ferricyanide.

Except for those cases where the isomers formed were separated and identified individually (see Experimental), the above results were based on an examination of the ultraviolet absorption spectrum of the reaction product which had been freed of unreacted diaminopyrimidine by extraction with dilute hydrochloric acid. An absorption maximum at 380–390 mμ indicated the presence of the 5,4-g isomer, while the presence or absence of a sharply defined shoulder at 430–450 mμ indicated the presence or absence of the 4,5-g isomer. In all cases investigated, this method provided direct and apparently unequivocal qualitative evidence for the presence or absence of each isomer in the reaction product. The method is illustrated graphically with the isomers IV and XII in Figs. 1 and 2.

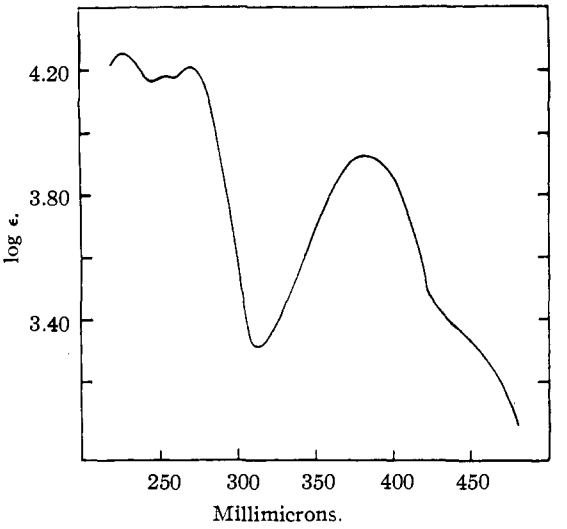


Fig. 2.—Spectrum of IV (3 parts) and XII (1 part) in 0.1 *N* sodium hydroxide. The same spectrum is given by the product of air oxidation of VI in neutral or 1% sodium hydroxide solution.

Our concept of the course of this reaction is outlined in Fig. 3, which, for purposes of illustration, depicts the oxidative self-condensation of diaminouracil (VI) to "bis-alloxazine" (IV) and "diuracilpyridazine" (XII). We envisage the initial step in this conversion as the oxidation of VI to the pyrimidinequinone-imine XVII, which may undergo hydrolysis under the reaction conditions to give the quinone XVIII (or XIX). Subsequent condensation of either XVII or XVIII (XIX) with unchanged VI can give either or both of the two anils XX and XXI, depending on which amino group in VI condenses with the imino or keto group of XVII or XVIII (XIX), respectively. Ring closure of the anils either by direct loss of ammonia or by preliminary hydrolysis to XXII and XXIII followed by dehydration would lead directly to the observed products IV and XII.

This mechanism is consistent with the observation that oxidative self-condensation fails with those 4,5-diaminopyrimidines which lack an enolizable group in the 2-position, since in such cases oxidation to a quinone-imine (XVII) would be precluded. It is also consistent with the observation that, in all cases thus far examined of oxidative self-condensation of 4,5-diaminopyrimidines, the 5,4-g isomer (corresponding to IV) either predominates or is formed to the exclusion of the 4,5-g isomer (corresponding to XII), since it would be expected that the imino group of XVII, or the keto group of XVIII (XIX), would react preferentially, if not exclusively, with the more reactive 5-amino group of the 4,5-diaminopyrimidine.

The methylation of IV with methyl iodide and potassium carbonate in acetone to give 1,3,6,8-tetramethyl-2,4,5,7(1H,3H,6H,8H)pyrimido(5,4-g)-pteridinetetrone (XXIV), m.p. 403–404°, has been described.[5] A similar methylation of the isomeric tetrahydroxypyrimido(4,5-g)pteridine (XII) now has been carried out to give 1,3,5,7-tetramethyl-2,4,6,8(1H,3H,5H,7H)pyrimido(4,5-g)pteridinetetrone (XXV), m.p. 358–360°. XXV also proved

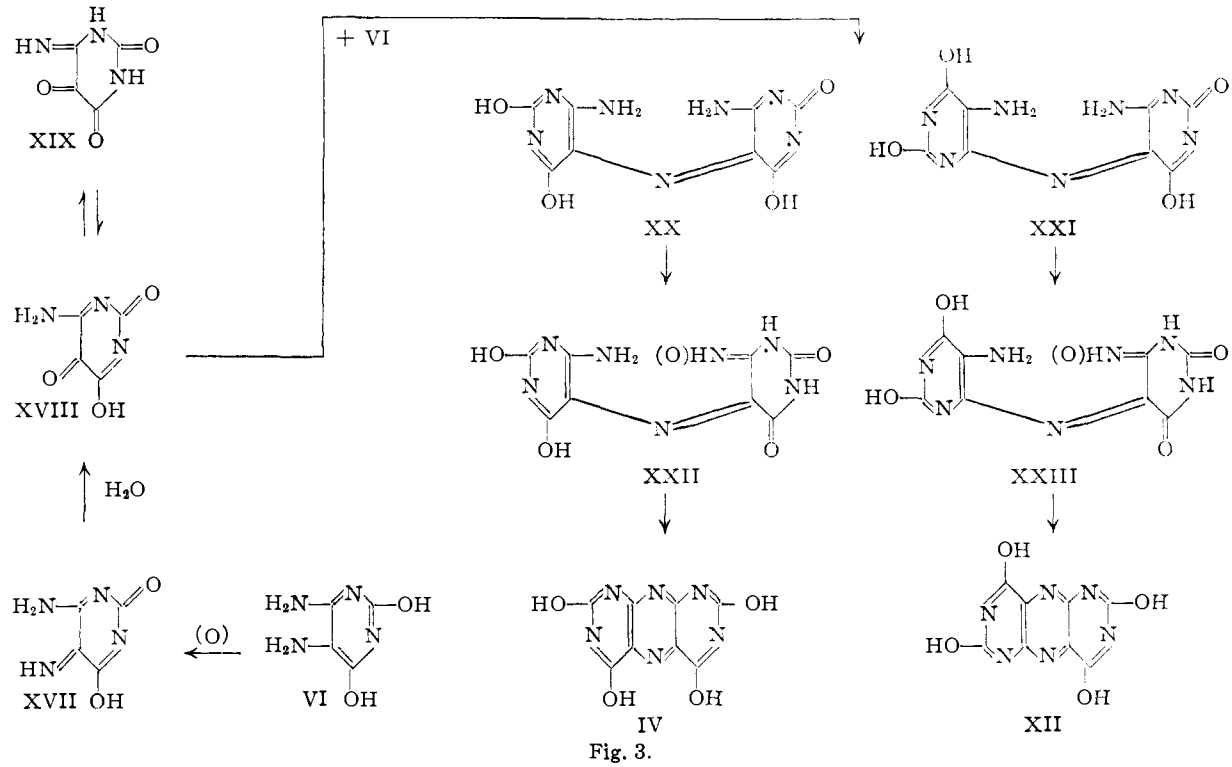
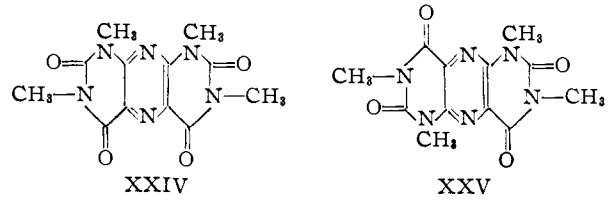


Fig. 3.

to be labile to alkaline ring cleavage,[5] for heating with 1 *N* sodium hydroxide for three hours gave 2,5-bis-(methylamino)-N-methyl-N′-methylpyrazine-3,6-dicarboxamide and (presumably) products of further hydrolysis.



XXIV XXV

Since the structures of the tetramethyl derivatives XXIV and XXV are known with certainty, it is now possible to identify a number of products whose reported structures are either in doubt or in error. Thus, Bredereck has reported[10] the condensation of the monohydrochloride of 1,3-dimethyl-4,5-diamino-2,6(1H,3H)pyrimidinedione (XXVI) with 1,3-dimethylalloxan (XXVII) in aqueous solution to give a product, m.p. 390°, which he indicated could be either XXIV or XXV. He obtained the same product by the action of 2 *N* sulfuric acid on XXVI or its monoacetyl derivative. This product can now be assigned structure XXIV with certainty.[11]

Similarly, the discrepancy in the melting point of XXIV (reported m.p. 403°), unequivocally prepared by Timmis[12] in 1952 from the reaction of 1,3-dimethylbarbituric acid with 1,3-dimethyl-4-amino-5-nitroso-2,6(1H,3H)pyrimidione (XXVIII) with the compound (m.p. 390°) obtained by Blicke and Godt[13] from the action of aqueous hydrochloric acid on XXVI, as well as (apparently) the agreement in melting point of this compound with that reported by Bredereck (ref. 10), led the former authors to assign structure XXV to their product. This structural assignment is incorrect, since XXV has now been shown to melt at 358–360°, and thus the product obtained by Blicke and Godt is also XXIV.

The recent claim by DeGarmo[14] to the synthesis of XXIV (reported m.p. 206–207°) by the condensation of dimethylalloxan (XXVII) with XXVI, followed by treatment of the resulting reaction mixture with hot 0.5 *N* sodium hydroxide for two hours, is now confirmed as incorrect, and his product must be either N,1,3-trimethyl-7-methylamino-2,4(1H,3H)pteridinedione-6-carboxamide (XXIX) or a mixture of hydrolysis products, as previously suggested.[15]

Experimental

Preparation of the Isomeric Tetraminopyrimidopteridines (II and III).—Twenty grams of 2,4,5,6-tetraminopyrimidine sulfate was converted to the free base I by the careful addition of 0.5 *N* sodium hydroxide to a suspension of the sulfate in 100 ml. of water, and the resulting solution was warmed on a steam-bath for 48 hours while running in a slow current of air. Ammonia was continuously evolved from the reaction mixture during this time. The mixture was then cooled and the orange solid (6.6 g.) removed by filtration, washed well with water and dried.

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

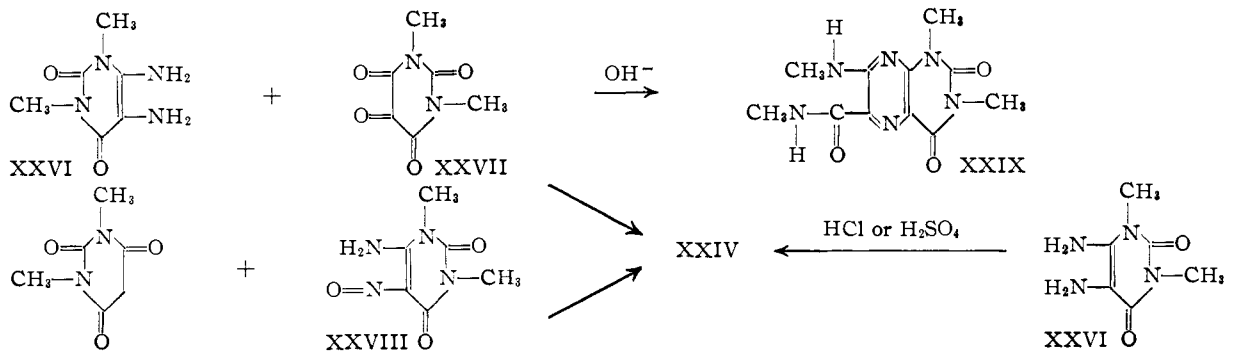
(11) NOTE ADDED IN PROOF.—Bredereck (H. Bredereck and W. Pfleiderer, *Ber.*, **87**, 1268 (1954)) has recently confirmed this structural assignment by demonstrating the identity of his product (m.p. 390°) with XXIV prepared by the method of Timmis (ref. 12).

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

(13) F. F. Blicke and H. C. Godt, Jr., THIS JOURNAL, **76**, 2798 (1954).

(14) O. DeGarmo, U. S. Patent 2,561,324; *C. A.*, **46**, 1595 (1952).

(15) NOTE ADDED IN PROOF.—The structure XXIX for this product recently has been shown to be correct (ref. 11).



Isolation of 2,4,5,7-Tetraminopyrimido(5,4-g)pteridine (II).—The dry orange solid prepared as described above was boiled with one liter of glacial acetic acid. Filtration of the hot mixture gave 5.1 g. of the acetate of II which gave II as a bright yellow micro-crystalline solid upon solution in 0.1 *N* hydrochloric acid followed by precipitation with dilute ammonium hydroxide. It did not darken on heating to 360°.

Anal. Calcd. for $C_8H_8N_{10}\cdot{}^1/_2H_2O$: C, 37.9; H, 3.6; N, 55.3. Found: C, 37.7; H, 3.5; N, 56.0.

Isolation of 2,4,6,8-Tetraminopyrimido(4,5-g)pteridine (III).—The glacial acetic acid filtrate above was concentrated to about 500 ml. and then allowed to stand overnight. The yellow-orange acetate of III which separated (1.1 g.) was dissolved in dilute acetic acid. Addition of dilute ammonium hydroxide gave a dark red flocculent solid (III) which changed to a dark red microcrystalline solid on standing in solution. The solid was separated by filtration, dried *in vacuo* at 140° and then allowed to come to equilibrium with the atmosphere. It did not darken on heating to 360°.

Anal. Calcd. for $C_8H_8N_{10}\cdot{}^1/_2H_2O$: C, 37.9; H, 3.6; N, 55.3. Found: C, 37.5; H, 3.6; N, 55.4.

Deamination of 2,4,5,7-Tetraminopyrimido(5,4-g)pteridine (II) to "Bis-Alloxazine" (IV).—To a solution of 250 mg. of II in 40 ml. of 1 *N* hydrochloric acid was added 1.5 g. of sodium nitrite dissolved in 25 ml. of water, and the reaction mixture was heated on a steam-bath for three hours. The yellow solid which had separated was removed by filtration, washed well with water and purified by solution in 10% ammonium hydroxide followed by precipitation with dilute hydrochloric acid.

Anal. Calcd. for $C_8H_4N_6O_4\cdot H_2O$: C, 36.1; H, 2.3; N, 31.6. Found: C, 36.0; H, 2.2; N, 32.4.

The product was shown to be identical with an authentic sample of 2,4,5,7-tetrahydroxypyrimido(5,4-g)pteridine ("bis-alloxazine") (IV)[4,5] by comparison of ultraviolet and infrared absorption spectra.

Deamination of 2,4,5,7-Tetrahydroxypyrimido(5,4-g)pteridine (II) to 2-Amino-4,5,7-Trihydroxypyrimido(5,4-g)pteridine (X).—When the deamination as described above was carried out using only the theoretical amount of sodium nitrite (275 mg.) and the reaction mixture worked up as described, a product was obtained which was shown by comparison of ultraviolet and infrared absorption spectra to be identical with an authentic sample of 2-amino-4,5,7-trihydroxypyrimido(5,4-g)pteridine (X), prepared by the condensation of alloxan with 2,4,5-triamino-6-hydroxypyrimidine.[5]

Deamination of 2,4,6,8-Tetraminopyrimido(4,5-g)pteridine (III) to 2,4,6,8-Tetrahydroxypyrimido(4,5-g)pteridine (XII).—To a solution of 50 mg. of III in 20 ml. of 1 *N* hydrochloric acid was added a solution of 150 mg. of sodium nitrite in 5 ml. of water, and the resulting reaction mixture was warmed on a steam-bath for three hours. An additional 150 mg. of sodium nitrite was added and the mixture warmed an additional 12 hours. The yellow-orange precipitate which had formed was filtered off (30 mg.) and purified by solution in 200 ml. of 10% ammonium hydroxide followed by precipitation with dilute hydrochloric acid. The product was obtained in the form of a micro-crystalline yellow-orange solid which did not darken on heating to 360°.

Anal. Calcd. for $C_8H_4N_6O_4\cdot H_2O$: C, 36.1; H, 2.3; N, 31.7. Found: C, 35.8; H, 2.8; N, 31.9.

By comparison of ultraviolet and infrared spectra, this product was shown to be identical with "diuracilpyridazine" prepared by the potassium ferricyanide oxidation of 5-aminouracil (XIII) according to the directions of Baudisch and Davidson.[7]

Alkaline Cleavage of XII to 2,5-Diaminopyrazine-3,6-dicarboxylic Acid (XV).—A mixture of 7.0 g. of 2,4,6,8-tetrahydroxypyrimido(4,5-g)pteridine ("diuracilpyridazine") (XII),[7] 40 ml. of water and 5 ml. of sodium hydroxide was placed in a small steel bomb and heated at 170° for three hours. The cooled reaction mixture was filtered, the collected brown solid digested 30 minutes with 0.1 *N* sodium hydroxide and the suspension filtered. Acidification of the combined filtrates with 6 *N* hydrochloric acid gave 3.0 g. (63%) of 2,5-diaminopyrazine-3,6-dicarboxylic acid, which was purified by solution in dilute sodium hydroxide followed by precipitation with dilute hydrochloric acid. The product was obtained as a microcrystalline red solid which decomposed slowly in the neighborhood of 220°.

Anal. Calcd. for $C_6H_6N_4O_4$: C, 36.4; H, 3.1; N, 28.3. Found: C, 36.5; H, 3.2; N, 28.2.

Sublimation of XV at 200° (0.5 mm.) gave 2,5-diaminopyrazine (XVI) as a crystalline yellow solid, m.p. (in a sealed, evacuated tube) 223–224° dec.; 2,5-diaminopyrazine is reported to decompose at 215°.[9] Acetylation of our preparation of XVI gave 2,5-diacetamidopyrazine, m.p. 365–366°. The reported melting point for 2,5-diacetamidopyrazine is 365°.[9]

Oxidative Self-condensations of 4,5-Diaminopyrimidines.—The directions given below for the air oxidation of 4,5-diaminouracil (VI) are representative of all the air oxidations carried out, whether in 1% sodium hydroxide, ammoniacal or neutral solution (see Discussion).

A suspension of 10 g. of diaminouracil sulfate (VI) in 200 ml. of water was carefully adjusted to *p*H 7 with dilute sodium hydroxide and then heated on a steam-bath for 48 hours. A slow stream of air was passed through the solution throughout this period. The brown solid which had formed was filtered off and digested for 15 minutes with 1 *N* hydrochloric acid to remove unreacted diaminouracil. The ultraviolet absorption spectrum of the acid-insoluble residue in 0.1 *N* sodium hydroxide showed a maximum at 365 mμ (indicating the presence of 2,4,5,7-tetrahydroxypyrimido(5,4-g)pteridine (IV)) and a clearly-defined shoulder at 435 mμ indicating the presence of 2,4,6,8-tetrahydroxypyrimido-(4,5-g)pteridine (XII).

1,3,5,7-Tetramethyl-2,4,6,8(1H,3H,5H,7H)pyrimido-(4,5-g)pteridinetetrone (XXV).—A mixture of 10 g. of 2,4,-6,8-tetrahydroxypyrimido(4,5-g)pteridine (XII), 100 g. of potassium carbonate, 300 ml. of dry acetone and 135 ml. of methyl iodide was heated under reflux with stirring for 24 hours. The reaction mixture was then diluted to twice its volume with water and filtered. The collected reddish-brown solid was washed with dilute ammonium hydroxide followed by water and then sublimed at 200° (0.5 mm.) to give 2.30 g. (19%) of dense yellow crystals of XXV, m.p. 358–360°.

Anal. Calcd. for $C_{12}H_{12}N_6O_4$: C, 47.4; H, 4.0; N, 27.6. Found: C, 47.9; H, 3.6; N, 27.9.

2,5-Bis-(methylamino)-N-methyl-N'-methyl-3,6-pyrazinedicarboxamide.—A suspension of 1.0 g. of XXV, 30 ml. of 1 *N* sodium hydroxide and 5 ml. of ethanol was heated under reflux for three hours to give a red solution. Cooling gave a red crystalline solid which was removed by

filtration, washed with aqueous ethanol (5%) and sublimed at 140° (0.7 mm.) to give 0.28 g. (34%) of 2,5-bis-(methylamino)-N-methyl-N'-methyl-3,6-pyrazinedicarboxamide, m.p. 253–254°. No other pure compound could be isolated from the hydrolysis mixture.

Anal. Calcd. for $C_{10}H_{16}N_6O_2$: C, 47.6; H, 6.4; N, 33.3. Found: C, 47.7; H, 6.4; N, 33.1.

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Spiro-1'-benzenesulfonylpiperidine-4',5-barbituric Acid and Related Derivatives of Isonipecotic Acid

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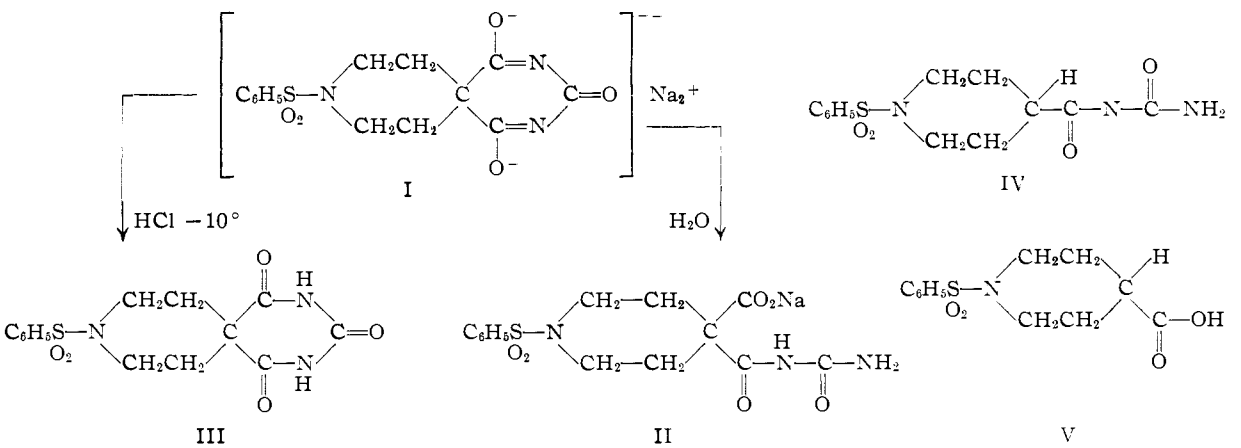
RECEIVED OCTOBER 21, 1954

A derivative of spiropiperidine-4',5-barbituric acid has been synthesized. The piperidine ring has the same effect as the cyclopentane ring in increasing both the ease of formation of the barbituric acid and the cleavage of the barbituric acid ring by aqueous alkali. Some isonipecotic acid derivatives have been prepared and subjected to pharmacological examination.

The instability of spirocyclopentane-1,5'-barbituric acid toward cold aqueous alkali has been described in a previous report.[1] This behavior, aside from the possible pharmacological interest, seemed to afford justification for the synthesis and study of a spiropiperidine derivative III which contains the same number of carbon atoms in the derived ring.

N-Benzenesulfonyldiethanolamine dibenzenesulfonate was prepared from benzenesulfonyl chloride and diethanolamine in a yield of 80%. This by condensation with sodiomalonic ester in benzene gave the needed 4,4-dicarboxyethyl-1-benzenesulfonylpiperidine (83%), which reacted with urea to give sodium spiro-1'-benzenesulfonylpiperidine-4',5-barbiturate (I) in nearly quantitative yield. The last reaction



took place with great ease at 40°. This is in agreement with previously reported cases[1] of the effect of rings in facilitating the reaction. By quickly mixing the salt with a freezing mixture of hydrochloric acid and ice the desired spiro acid III was obtained.

The spiro acid by standing in aqueous alkaline solution was hydrolyzed first to the salt II, the acid of which could be isolated after standing one hour. Further standing for eleven days converted II to the ureide of 1-benzenesulfonylisonipecotic acid (IV). This was hydrolyzed to 1-benzenesulfonylisonipecotic acid (V) when refluxed with sodium hydroxide in a solution of alcohol and water. The identical acid also was made from 1-benzenesulfonylpiperidine-4,4-dicarboxylic acid through the loss of carbon dioxide by heat.

The acid chloride of V when heated gradually with urea gave the identical ureide IV. The acid chloride also reacted smoothly to yield the amide and the esters, but a mixture of products was obtained by its action with hydrazine. The methyl ester was found to be much superior as a reagent for the synthesis of 1-benzenesulfonylisonipecotylhydrazine (Table I).

The above amide gave 56% inhibition[2] at 0.2 mg./cc. in the *in vitro* tuberculosis test, no hypnosis at 400–900 mg./kg. in rats, and 20% protection by the electro shock and no protection by the metrazol method at 400 mg./kg. *per os*. The hydrazide at 0.2 mg./cc. gave 68% inhibition in the tuberculosis test. In mice it was ineffective against influenza virus, MM virus, streptococcus pyogenes, typhoid, klebsiella pneumoniae and pseudomonas aeruginosa. The ureide at 0.2 mg./cc. gave no inhibition in the tuberculosis test, no hypnosis at 400–900 mg./kg. by mouth in rats and 20% protection by the electroshock but no protection by the metrazol method at 400 mg./kg. *per os*. The spirobarbituric acid III by vein in rats produced convulsions in-

(1) G. S. Skinner, George Limperos and R. H. Pettebone, THIS JOURNAL, **72**, 1648 (1950).

(2) Pharmacological tests by Eli Lilly and Company.