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Pteridine Chemistry. II. The Action of Excess Nitrous Acid Upon Pteroylglutamic Acid and Derivatives

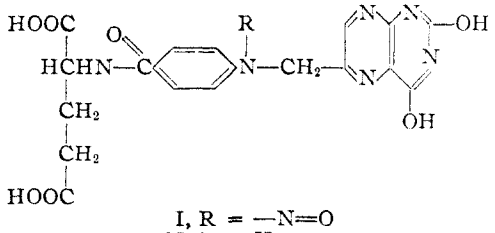
BY ROBERT B. ANGIER, JAMES H. BOOTHE, JOHN H. MOWAT, COY W. WALLER AND JOSEPH SEMB

A series of experiments have been carried out whose objective was the determination of the most desirable conditions for the deamination of pteroylglutamic acid to its corresponding 2-hydroxy derivative. The results demonstrate that the best procedure involved the use of excess nitrous acid in a solution of 6 *N* sulfuric acid and glacial acetic acid at 50°. The N^{10}-secondary amine of pteroylglutamic acid was simultaneously converted to the corresponding nitrosamine derivative. However, this nitroso group was easily removed by the use of phenol and concentrated hydrochloric acid. The whole procedure has been applied to several pteroic acid and pteridine derivatives.

The action of excess nitrous acid in strong mineral acids upon 2-aminopteridines has led to the destruction of the ring system in the case of xanthopeterin,[1] and to deamination in the 2-position in several simple pteridines.[2] Likewise, formylpteroic acid has been deaminated by the action of nitrous acid in a solution of hydrochloric and acetic acids.[3]

On the other hand it has been shown that pteroylglutamic acid in cold hydrochloric acid solution reacts quantitatively with one mole of nitrous acid to form N^{10}-nitrosopteroylglutamic acid.[4]

In this Laboratory it was found that excess nitrous acid in a mixture of mineral acid and acetic acid produced simultaneous deamination of the 2-position and nitrosation of the 10-position in pteroylgutamic acid (PGA). The resulting product, N-[4-{[(2,4-dihydroxy-6-pteridyl)-methyl]-N-nitrosoamino}-benzoyl]-glutamic acid (I) crystallized from the reaction mixture as an almost pure, white crystalline material. The nitroso group was readily removed by the action of phenol and concentrated hydrochloric acid to give directly a yellow crystalline product, N-[4-{[(2,4-dihydroxy-6-pteridyl)-methyl]-amino}-benzoyl]-glutamic acid (II) (commonly referred to as 2-hydroxypteroylglutamic acid). When assayed with *Streptococcus faecalis* R II showed a growth-stimulating activity of 0.3 to 7% (PGA 100%), the exact value depending upon the method of preparation of I. In order to check on the microbiological activity of II it was then prepared by direct synthesis from 2,4-dihydroxy-5,6-diaminopyrimidine, *p*-aminobenzoylglutamic acid and 1,1,3-tribromoacetone by the procedure of Hultquist and Dreisbach.[5] After purification this material had physical properties and ultraviolet absorption spectra which were identical with those of II prepared by the deamination procedure. The product prepared by direct synthesis had a growth-stimulating activity of 0.02% (PGA 100%) when assayed with *S. f.* R.



I, R = —N=O
II, R = H

The low microbiological activity of 2-hydroxypteroylglutamic acid (II) provided a convenient method for determining the extent to which PGA was deaminated and thus it permitted the determination of the best conditions for this reaction. PGA was deaminated under varying conditions and the resulting nitroso derivatives were then denitrosated. *S. f.* R assays on the final products gave directly the per cent. of unreacted PGA and indicated the extent of the reaction.

The results of a number of these reactions have been presented in Table I. They may be summarized as follows: (1) The best conditions for the reaction involve the use of a solution of 6 *N* sulfuric acid and glacial acetic acid at 50°; this gives almost 100% deamination (the use of 4 *N* hydrochloric acid instead of 6 *N* sulfuric acid is only slightly less effective). (2) The presence of acetic acid is necessary in order to give a satisfactory deamination reaction. This is particularly evident at 25° but it is also true at 50°. (3) Hypophosphorous acid does not cause reductive deamination of PGA.

TABLE I

Acids used	Temp., °C.	Product: Assay with *S. f.* R, %	Product: Chemical assay, %[a]
H_2SO_4 (10 cc.), acetic acid (25 cc.)	25	5.4	82
H_2SO_4 (10 cc.)	25	78.5	83
H_2SO_4 (10 cc.), 50% H_3PO_2 (20 cc.)	25	98	88
H_2SO_4 (10 cc.), 50% H_3PO_2 (20 cc.), acetic acid (20 cc.)	25	6.9	86
H_2SO_4 (10 cc.), hydrochloric acid (25 cc.)	25	77	83
H_2SO_4 (10 cc.), acetic acid (25 cc.)	50	0.33	81
H_2SO_4 (10 cc.)	50	14	84
H_2SO_4 (20 cc.)	50	15	83
H_2SO_4 (10 cc.), 50% H_3PO_2 (20 cc.)	50	20	86
Hydrochloric acid (30 cc.) acetic acid (25 cc.)	50	1.4	85

[a] B. L. Hutchings, *et al.*, *J. Biol. Chem.*, **168**, 705 (1947).

General Procedure.—Fifty cc. of water and 3 g. of pteroylglutamic acid were used in each experiment. The PGA was dissolved in the acidic solution and cooled to 10°. To this was added in portions 7 g. of solid sodium nitrite. This solution was then brought to the indicated temperature and the reaction allowed to proceed. At 25° the reaction time was between 2.5 and 3 hours. At 50° the reaction time was one hour. The product crystallized directly out of the reaction mixture in each experiment except in the case of the sulfuric acid–hypophosphorous acid solution at 25° where no deamination occurred. In this case it was necessary to add several volumes of water to obtain the product.

(1) C. Schopf and A. Kottler, *Ann.*, **539**, 134 (1939).

(2) H. Wieland, *et al.*, *ibid.*, **507**, 245 (1933); E. C. Taylor, Jr., and C. K. Cain, THIS JOURNAL, **71**, 2538 (1949).

(3) D. E. Wolf, *et al.*, *ibid.*, **69**, 2758 (1947).

(4) D. B. Cosulich and J. M. Smith, Jr., *ibid.*, **71**, 3574 (1949).

(5) M. E. Hultquist and P. F. Dreisbach, U. S. Patent 2,443,165; June 8, 1948.

At 50° complete solution occurred in all experiments before the product began to crystallize. At 25° in dilute sulfuric acid alone the nitrosopteroylglutamic acid which formed precipitated out and did not redissolve. It was at first thought that this low solubility might be the cause of the poor deamination. However, the sulfuric acid–hypophosphorous acid solution completely dissolved the solid and still no appreciable deamination occurred. In addition it will be noted that even at 50° in dilute sulfuric acid in which complete solution did occur the reaction did not go to completion.

Using the preferred method of deamination as described above pteroyl-α-glutamylglutamic acid[6] and 7-methylpteroylglutamic acid[7] were converted to their respective 2-hydroxy derivatives. By similar methods 2-amino-4-hydroxy-6-methylpteridine and 2-amino-4-hydroxy-7-methylpteridine were also deaminated. Attempts to deaminate pteroic acid under these conditions were unsuccessful probably due to the extreme insolubility of the nitrosopteroic acid which was formed. However, by starting with formylpteroic acid[3] this difficulty was avoided since the deamination then occurred before the formyl group could be hydrolyzed. The hydrolysis did occur, however, and 2-hydroxy-N^{10}-nitrosopteroic acid was obtained.

During the course of this work II was oxidized with an alkaline permanganate solution to produce 2,4-dihydroxy-6-pteridinecarboxylic acid.

Ultraviolet Absorption Data.—In Figs. 1 and 2 are given the ultraviolet absorption spectra of compounds I and II at different hydrogen ion concentrations. The spectrum of II in 0.1 *N* hydrochloric acid is almost identical with that of PGA. In 0.1 *N* sodium hydroxide the maximum which is present at 255 mμ in PGA has disappeared leaving only a shoulder and the maximum at 365 mμ has shifted to 370 mμ. The most striking difference, however, is seen in 0.1 *N* ammonium hydroxide solution. Whereas PGA has exactly the same absorption spectrum in 0.1 *N* ammonium hydroxide that it has in 0.1 *N* sodium hydroxide II exhibits a marked difference in its spectra in these same solvents. This same change in spectra has also been seen with the nitroso derivative (II) as well as with 2,4-dihydroxy-6-methylpteridine and 2,4-dihydroxy-7-methylpteridine. Again the corresponding 2-amino pteridines do not show this difference. This suggests that the oxygen in the 2-position in these compounds exists in the keto form even in 0.1 *N* ammonium hydroxide but is forced into the enol form in 0.1 *N* sodium hydroxide.

Experimental[8]

N-[4-{[(2,4-Dihydroxy-6-pteridyl)-methyl]-N-nitroso-amino}-benzoyl]-glutamic Acid (I).—Pteroylglutamic acid (9.0 g.) was partially dissolved in a solution of 150 cc. of water, 75 cc. of acetic acid and 30 cc. of concentrated sulfuric acid. This was cooled to 10° and 20 g. of solid sodium nitrite was added in portions. A clear solution was obtained. The solution was heated to 50° and an additional 7 g. of sodium nitrite was added. After a short time the product began to crystallize. The temperature was kept between 40 and 50° for one hour and the mixture then cooled to 10° for several hours. The crystalline product was collected, washed with water and dried; yield 7.1 g. (93%).

A portion of this material (1.1 g.) was recrystallized once from 100 cc. of water and a second time from 80 cc. of water to give a white crystalline product; yield 0.6 g.; m.p., decomposes at 205–215°. This product gave a strongly positive Lieberman nitrosamine test.

Anal. Calcd. for $C_{19}H_{17}N_7O_8$: C, 48.41; H, 3.63; N, 20.81. Found: C, 47.99; H, 3.93; N, 20.71.

In 0.1 *N* soduim hydroxide I showed *E* (1%, 1 cm.) maxima of 605 at 260 mμ and 140 at 372.5 mμ; in 0.1 *N*

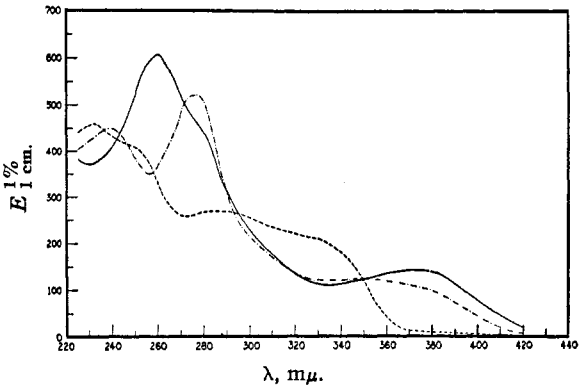


Fig. 1.—Ultraviolet absorption spectra of 2-hydroxy-N^{10}-nitrosopteroylglutamic acid (I): —, 0.1 *N* sodium hydroxide; —.—.—, 0.1 *N* ammonium hydroxide; — — — —, 0.1 *N* hydrochloric acid.

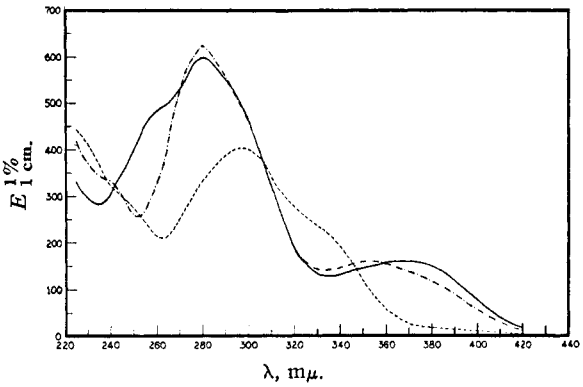


Fig. 2.—Ultraviolet absorption spectra of 2-hydroxypteroylglutamic acid (II): —, 0.1 *N* sodium hydroxide; —.—.—, 0.1 *N* ammonium hydroxide; — — —, 0.1 *N* hydrochloric acid.

ammonium hydroxide the maxima were 451 at 240 mμ, 520 at 275–277.5 mμ and 122 at 350–355 mμ; in 0.1 *N* hydrochloric acid the maxima were 459 at 232.5 mμ and 271 at 287.5 mμ.

N-[4-{[(2,4-Dihydroxy-6-pteridyl)-methyl]-amino}-benzoyl]-glutamic Acid (II).—Three grams of I was suspended and partially dissolved in 15 cc. of concentrated hydrochloric acid. To this was added 2 g. of phenol and the mixture allowed to react for 15 minutes with occasional stirring. It was then treated with 1.5 g. of Norite, filtered and the filter cake was washed with 5 cc. of concentrated hydrochloric acid. The filtrates were combined and poured into 200 cc. of water preheated to 60°. Upon cooling the product crystallized. The solid was collected, washed with water, several times with ethanol and with ether. The ethanol removes the nitrosophenol and some phenol oxidation products; yield 2.3 g. (82%); chem. assay[9] 82.5%.

The product was suspended in 150 cc. of water at 70° and magnesium oxide was added to *p*H 8–9. This was treated with 1.5 g. of Norite and filtered hot. The filtrate at 50° was acidified to *p*H 1.5 and the product crystallized. After cooling the solid was collected, dissolved in 10 cc. of concentrated hydrochloric acid, treated with one gram of Norite and filtered. The filter cake was washed with 5 cc. of concentrated hydrochloric acid and the combined filtrates were added to 150 cc. of water at 60°. After cooling the product was collected and crystallized again from a hydrochloric acid solution to give yellow needle-like crystals; yield 1.3 g.; m.p. decomposes at 220–224°[10]; chem. assay 93%[9]; *S.f.* R. assay 0.33%.

(6) J. H. Mowat, *et al.*, THIS JOURNAL, **70**, 1096 (1948).

(7) J. H. Boothe, *et al.*, *ibid.*, in press.

(8) All melting points are corrected for the exposed stem of the thermometer.

(9) B. L. Hutchings, *et al.*, *J. Biol. Chem.*, **168**, 705 (1947).

(10) The decomposition range on this compound as well as the other 2-hydroxypteroic acid derivatives is not a very reliable means of identification.

In 0.1 *N* sodium hydroxide II showed *E* (1%, 1 cm.) maxima of 595 at 280 mμ and 162 at 370 mμ; in 0.1 *N* ammonium hydroxide the maxima were 625 at 280 mμ and 159 at 355 mμ; in 0.1 *N* hydrochloric acid the maximum was 403 at 297.5 mμ.

It was not possible to obtain completely satisfactory microanalyses on this compound (II). Therefore, it was converted to its diethyl ester.

Diethyl Ester of II.—II (0.5 g.) and 0.5 g. of *p*-toluenesulfonic acid were dissolved in 10 cc. of hot absolute ethanol and heated to reflux for 15 minutes. After cooling several hours the crystalline product was collected, washed and dried. It was then redissolved in 10 cc. of ethanol containing 0.1 g. of *p*-toluenesulfonic acid and heated to reflux for 10 minutes The solution was treated with Norite, filtered and the filtrate was cooled to give 0.3 g. of crystalline product. This was crystallized twice from 25-cc. portions of ethanol; yield 0.25 g. of light yellow needle-like crystals; m.p. 203–207°[10]; chem. assay 98%.

Anal. Calcd. for $C_{23}H_{26}N_6O_7$: C, 55.41; H, 5.26; N, 16.87. Found: C, 55.15; H, 5.61; N, 16.64.

N-[4-{[(2,4-Dihydroxy-6-pteridyl)-methyl]-N-nitroso-amino}-benzoyl]-α-glutamylglutamic Acid (III).—Pteroyl-α-glutamylglutamic acid (6.6 g.) was converted to the corresponding 2-hydroxy-N^{10}-nitroso derivative (III) by using the procedure described above for preparing I; yield 6.2 g. (90%). This was recrystallized once from 230 cc. of water; yield 5.4 g. A sample of this was recrystallized two more times from water to give a white crystalline product; m.p. decomposes at 180–184°.[10]

Anal. Calcd. for $C_{24}H_{24}N_8O_{11}$: C, 47.99; H, 4.03; N, 18.67. Found: C, 47.86; H, 4.40; N, 19.19.

N-[4-{[(2,4-Dihydroxy-6-pteridyl)-methyl]-amino}-benzoyl]-α-glutamylglutamic Acid (IV).—III (3.9 g.) was suspended in 12 cc. of concentrated hydrochloric acid and 2 cc. of phenol was added. This was stirred for 15 minutes, treated with 2 g. of Norite and filtered. The filter cake was washed with 3 cc. of concentrated hydrochloric acid. The combined filtrates were then poured into 150 cc. of water heated to 60° and the solution was cooled. The solid was collected on a funnel and washed with water. It was then suspended in 100 cc. of ethanol which dissolved most of it. To this was added 500 cc. of ether and the mixture was cooled several hours. The product was collected and dried; yield 1.6 g. This was dissolved in 100 cc. of a dilute solution of sodium hydroxide. The solution was acidified to *p*H 2.0 and heated to 60°. Magnesium oxide was added to *p*H 8–9, 1.0 g. of Norite was added and the mixture was filtered. The filtrate at 50° was acidified to *p*H 1.7 and upon cooling the product crystallized. It was cooled well and the solid was collected. The wet cake was dissolved in 5 cc. of concentrated hydrochloric acid, treated with 0.6 g. of Norite and filtered. The filter cake was washed with 2 cc. of hydrochloric acid and the combined filtrates were added to 70 cc. of warm water. Upon cooling the product crystallized; yield 0.9 g.

A portion of this (0.4 g.) was recrystallized twice from 25-cc. portions of water; small yellow needle-like crystals; yield 0.3 g.; m.p., decomposes at 179–189°[10]; chem. assay 95%.[9]

Anal. Calcd. for $C_{24}H_{25}N_7O_{10}$: C, 50.43; H, 4.41; N, 17.17. Found: C, 50.26; H, 5.00; N, 17.03.

In 0.1 *N* sodium hydroxide IV showed *E* (1%, 1 cm.) maxima of 453 at 285 mμ and 133 at 370 mμ; in 0.1 *N* hydrochloric acid it showed a maximum of 318 at 297.5 mμ.

N-[4-{[(2,4-Dihydroxy-7-methyl-6-pteridyl)-methyl]-amino}-benzoyl]-glutamic Acid (V).—7-Methylpteroylglutamic acid[7] (3.0 g.) was converted to the corresponding 2-hydroxy-N^{10}-nitroso derivative by the method described above for the preparation of I. The nitroso compound was then denitrosated and the resulting product purified in exactly the same manner described above for the preparation of II; yield 0.75 g. of yellow crystalline material; chem. assay 96%.[9]

Anal. Calcd. for $C_{20}H_{20}N_6O_7 \cdot H_2O$: C, 50.62; H, 4.68; N, 17.72. Found: C, 50.28; H, 4.99; N, 17.81.

In 0.1 *N* sodium hydroxide V showed maxima at 282.5 mμ and 357.5 mμ. In 0.1 *N* hydrochloric acid it had a maximum at 302.5 mμ.

2,4-Dihydroxy-7-methylpteridine (VI).[11]—Three grams of 2-amino-4-hydroxy-7-methylpteridine was dissolved in a solution of 50 cc. of water, 25 cc. of concentrated hydrochloric acid and 25 cc. of acetic acid by warming. This was cooled to 10° and 15 g. of sodium nitrite was added in portions with shaking. After 10 minutes the solution was heated to 50° for 30 minutes and then to 75° for 15 minutes. It was then cooled in the refrigerator overnight. The product was collected, washed with water, acetone and ether, and dried; yield 1.0 g. This was recrystallized twice from 100- and 80-cc. portions of acetic acid; yield 0.4 g.; m.p., decomposes slowly above 300°.

Anal. Calcd. for $C_7H_6N_4O_2$: C, 47.19; H, 3.42; N, 31.43. Found: C, 47.56; H, 4.0; N, 31.57.

In 0.1 *N* sodium hydroxide VI showed *E* (1%, 1 cm.) maxima of 749 at 250–252.5 mμ and 388 at 360 mμ; in 0.1 *N* ammonium hydroxide VI showed maxima of 695 at 237.5 mμ, 531 at 272.5 mμ and 371 at 347.5 mμ; in 0.1 *N* hydrochloric acid VI showed one maximum of 555 at 325 mμ.

2,4-Dihydroxy-6-methylpteridine (VII).—Three grams of 2-amino-4-hydroxy-6-methylpteridine was treated just as described above for the 7-methyl isomer. To the resulting solution at room temperature was added 210 cc. of a 10 *N* sodium hydroxide solution. This was cooled in a refrigerator overnight to give a crystalline sodium salt which was collected and redissolved in 35 cc. of water. The resulting solution was treated with Norite and filtered. To the filtrate was added 30 cc. of a 10 *N* sodium hydroxide solution. This was cooled to give a crystalline product which was collected and dissolved in 30 cc. of water. The solution was filtered and the filtrate was acidified with acetic acid. Upon cooling a white crystalline product was obtained; yield 0.45 g.; m.p. sinters at 272–274° and decomposes at 310–320°.

Anal. Calcd. for $C_7H_6N_4O_2$: C, 47.19; H, 3.42; N, 31.43. Found: C, 47.39; H, 3.89; N, 31.40.

In 0.1 *N* sodium hydroxide VII showed *E* (1%, 1 cm.) maxima of 841 at 255 mμ and 318 at 367.5 mμ; in 0.1 *N* ammonium hydroxide VII showed maxima of 661 at 237.5 mμ, 650 at 270 mμ and 309 at 352.5 mμ; in 0.1 *N* hydrochloric acid VII showed maxima of 622 at 230 mμ and 449 at 332.5 mμ.

4-{[(2,4-Dihydroxy-6-pteridyl)-methyl]-amino}-benzoic Acid (VIII).—N^{10}-Formylpteroic acid[3] (0.5 g.) was added to a solution of 100 cc. of water, 50 cc. of acetic acid and 20 cc. of sulfuric acid previously cooled to 10°. To this was added 3 g. of sodium nitrite. The formylpteroic acid only partly dissolved. This was heated to 55° to give a clear solution. After about 10 minutes a solid began to appear. After two hours at room temperature this was cooled a short while and the product was collected; yield 0.4 g. This gave a positive Lieberman nitrosamine test.

In 0.1 *N* sodium hydroxide this compound showed maxima at 260 mμ and 372.5 mμ; in 0.1 *N* ammonium hydroxide it showed maxima at 237.5 mμ, 275 mμ and 352.5 mμ; in 0.1 *N* hydrochloric acid it showed maxima at 235 mμ and at 290 mμ.

This nitroso derivative was then suspended in 5 cc. of concentrated hydrochloric acid and 0.5 cc. of phenol was added. After stirring for 15 minutes this was poured into 60 cc. of warm water. A yellow precipitate formed immediately. After cooling this was collected, washed with water, ethanol and ether, and dried; yield 0.35 g.

A portion of this product (0.25 g.) was recrystallized as its sodium salt from 7 cc. of a dilute sodium hydroxide solution. The product was collected, redissolved in 10 cc. of very dilute sodium hydroxide and the solution was poured into 70 cc. of hot 1 *N* hydrochloric acid; yield of product 90 mg.; m.p. decomposes at 290–295°.[10]

Anal. Calcd. for $C_{14}H_{11}N_5O_4 \cdot H_2O$: C, 50.76; H, 3.96; N, 21.14. Found: C, 50.22; H, 4.20; N, 21.14.

In 0.1 *N* sodium hydroxide this compound showed *E* (1%, 1 cm.) maxima of 750 at 277.5 mμ and 201 at 370–372.5 mμ.

2,4-Dihydroxy-6-pteridinecarboxylic Acid.—Two grams of II was dissolved in a solution of 3 cc. of 10 *N* sodium hydroxide in 100 cc. of water. This was oxidized at 90° by adding solid potassium permanganate until the permanganate color persisted. The excess color was destroyed with

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

ethanol and the manganese dioxide was filtered off. The filtrate was acidified and cooled overnight. The crystalline product was collected, washed with water, acetone and ether, and dried; yield 0.7 g. This was dissolved in 15 cc. of warm dilute sodium hydroxide solution, treated with Norite and filtered; 15 cc. of a 10 *N* sodium hydroxide solution was added and the whole solution was cooled well. The crystalline product was collected and recrystallized in the same manner. The product was collected, redissolved in about 60 cc. of water by heating. The hot solution was acidified with hydrochloric acid and cooled to give a white crystalline product; yield 0.4 g.; dried three hours at 140° in a drying pistol over phosphorus pentoxide; m.p., does not melt below 300°.

Anal. Calcd. for $C_7H_4N_4O_4$: C, 40.3; H, 1.92; N, 26.9. Found: C, 40.6; H, 2.64; N, 27.2.

In 0.1 *N* sodium hydroxide this compound showed *E* (1%, 1 cm.) maxima of 935 at 267.5 mμ and 391 at 370 mμ; in 0.1 *N* hydrochloric acid the maxima were 471 at 237.5 mμ, 569 at 265 mμ and 469 at 330 mμ.

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PEARL RIVER, N. Y. RECEIVED APRIL 12, 1951

[CONTRIBUTION FROM THE WELLCOME RESEARCH LABORATORIES]

Studies on Condensed Pyrimidine Systems. IX. The Synthesis of Some 6-Substituted Purines

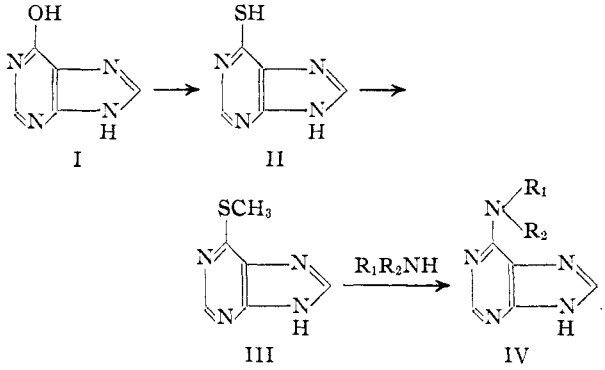
BY GERTRUDE B. ELION, ELIZABETH BURGI AND GEORGE H. HITCHINGS

A variety of 6-aminopurines has been prepared by the reaction of 6-methylmercaptopurine with aliphatic and aromatic amines. The treatment of hypoxanthine with phosphorus pentasulfide under specified conditions leads to the formation of 6-mercaptopurine in satisfactory yields. Some modifications and improvements in the preparation of hypoxanthine from 4-amino-6-hydroxy-2-mercaptopyrimidine are described.

In connection with the program of preparing compounds which might act as antagonists for the purine and pyrimidine portions of nucleic acid[1,2,3] it was decided to synthesize a number of purines in which the amino group of adenine had been substituted by various aliphatic, aromatic and heterocyclic amines. No such purines have been unequivocally synthesized previously, although Bredereck[4] has reported the picrate of an N-methyladenine made *via* the methylation of adenosine with dimethyl sulfate, at *p*H 13–14.

The chlorination of hypoxanthine with phosphoryl chloride either in the presence or absence of dimethylaniline was unsatisfactory. However, it was found that the direct replacement of oxygen by sulfur which had been successful with hydantoins[5] and pyrimidines[6,7] could likewise be applied to purines. The treatment of hypoxanthine (I) with phosphorus pentasulfide led to 6-mercaptopurine (II). Methylation of the latter with methyl iodide or dimethyl sulfate resulted in the formation of III. When dimethyl sulfate was used, spectrophotometric examination of the mother liquors revealed that at least two purines other than 6-mercapto- and 6-methylmercaptopurine were present. These by-products, presumably N-methylpurines, were partially separated from the mother liquor residues by fractional crystallization but were not obtained in a pure state.

The replacement of mercapto or methylmercapto groups by amino groups is well known in the pyrimidine series[8,9] but has rarely been successful with purines. Such a replacement has been reported, with 2-hydroxy-8-methylmercaptopurine and methylamine[10] but failure has been reported in attempts to replace the 2-methylmercapto group of 2-methylmercaptoadenine.[11]



When 6-mercaptopurine (II) was heated with aqueous ethylamine at 140° for 15 hours in a sealed tube a considerable amount of hydrogen sulfide was formed, and some 6-ethylaminopurine was shown to be present in the reaction mixture by spectrophotometric measurements; with aniline at 180° for seven hours, 90% of II was recovered. On the other hand, the replacement of the 6-methylmercapto group by amines was found to proceed smoothly when III was heated in a sealed tube with primary alkylamines, dimethylamine, morpholine, aniline and *p*-chloroaniline. Diethylamine did not react with III at 130° but when the temperature was raised to 150°, 6-diethylaminopurine (IV, $R_1 = R_2 = C_2H_5$) was obtained. With hydrazine and

(1) G. H. Hitchings, E. A. Falco and M. B. Sherwood, *Science*, **102**, 251 (1945).

(2) G. H. Hitchings, G. B. Elion, E. A. Falco and H. VanderWerff, Abstracts, American Chemical Society, New York, N. Y., 3 C (1947).

(3) G. H. Hitchings, G. B. Elion, E. A. Falco, P. B. Russell, M. B. Sherwood and H. VanderWerff, *J. Biol. Chem.*, **183**, 1 (1950).

(4) H. Bredereck, H. Haas and A. Martini, *Ber.*, **81**, 307 (1948).

(5) H. R. Henze and P. E. Smith, THIS JOURNAL, **65**, 1090 (1943).

(6) H. C. Carrington, *J. Chem. Soc.*, 124 (1944).

(7) G. B. Elion and G. H. Hitchings, THIS JOURNAL, **69**, 2138 (1947).

(8) P. B. Russell, G. B. Elion, E. A. Falco and G. H. Hitchings, *ibid.*, **71**, 2279 (1949).

(9) T. B. Johnson and C. O. Johns, *Am. Chem. J.*, **35**, 175 (1905).

(10) C. O. Johns, *J. Biol. Chem.*, **21**, 319 (1915).

(11) K. J. M. Andrews, N. Anand, A. R. Todd and A. Topham, *J. Chem. Soc.*, 2490 (1949).