[CONTRIBUTION FROM SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

A Synthesis of Isoguanine Labeled with Isotopic Nitrogen[1]

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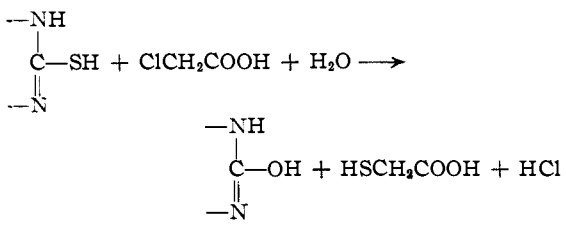
The synthesis of isoguanine (2-oxyadenine) (VI) from uric acid described by Fischer[3] is not adaptable for the introduction of an isotopic label into the purine ring. A synthesis has now been developed which depends upon the starting materials, malononitrile and thiourea. Since these may be readily prepared with isotopic labels, the introduction of isotopic nitrogen or carbon into several positions of the pyrimidine moiety is made possible.

Ammonia, containing an excess of N^{15}, was condensed with cyanogen bromide[4] to give an excellent yield of cyanamide and a quantitative recovery of ammonium bromide. Labeled thiourea resulted from the treatment of the cyanamide with hydrogen sulfide.[5]

$$CNBr + 2\overset{*}{N}H_3 \longrightarrow C\overset{*}{N}H_2 + \overset{*}{N}H_4Br$$

$$H\overset{*}{N}{=}C{=}N^*H + H_2S \longrightarrow N^*H_2{-}\overset{\overset{S}{\|}}{C}{-}N^*H_2$$

It was first proposed to prepare 2-thioladenine (thioisoguanine) (X) by the procedure of Traube[6] and to convert this to isoguanine by the technique of Wheeler and Liddle.[7]



However, this reaction led to the formation of the intermediate carboxymethylthioisoguanine (XIII), the carboxymethylthiol group of which proved to be so unusually resistant to acid hydrolysis that isoguanine was not obtained therefrom. Traube's synthesis of thioisoguanine (X) involves the initial condensation of thiourea and malononitrile to give a 2-thiolpyrimidine (I). The necessity for the conversion of a 2-thiol to a 2-hydroxy group could not be avoided by a substitution of urea for thiourea since the former is not readily condensed with malonitrile.

In the case of the 2-thiolpyrimidine (I), treatment with chloroacetic acid also resulted in the formation of a carboxymethylthiol ether (II) which was sufficiently stable to permit its isolation. In the case of this carboxymethylthiol derivative, the hydrolysis to the desired 2-hydroxy derivative (III) could be achieved in very satisfactory yield, as was the case with the corresponding 2-methylthiol derivative.[8] It was therefore possible to complete the synthesis of isoguanine (VI) after the 2-hydroxyl group had been thus introduced.

The three diaminopyrimidines (I, II and III) involved in this work were converted to the 5-nitroso intermediates (VII, XI and IV) with nitrous acid and reduction to the triaminopyrimidines (VIII, XII and V) was accomplished with sodium hydrosulfite.

The three triaminopyrimidines showed different reactivities toward formic acid. With XII, the closure of the imidazole ring to the purine (XIII) was accomplished by refluxing XII with formic acid in the presence of sodium formate, whereas only the intermediate 5-formylamino compound (IX) resulted from similar treatment of VIII. The triamino intermediate (V) did not appear to be appreciably affected under these conditions and isoguanine (VI) was not obtained by this method.

To obtain isoguanine from V, a method for the formation of the imidazole ring was devised that appears to have general applicability. When the sulfate of 2-hydroxy-4,5,6-triaminopyrimidine (V) was heated with a dilute solution of formic acid in anhydrous formamide at 160° in a sealed tube a 93% yield of isoguanine (VI) was easily afforded. Optimal results were achieved on heating for three hours, whereas prolonged treatment caused marked destruction. Singly, neither formamide nor formic acid yielded the purine under these conditions. This method was also applied successfully to VIII and XII, as well as to 2,4,5,6-tetraminopyrimidine (XIV).

The conversion of 2-thioladenine (X) to adenine, accomplished by the action of Raney nickel, constitutes a new synthesis of that purine.

Isoguanine is found in nature as the aglycone of the nucleoside, crotonoside, of the croton bean (*Croton tiglium* L.).[9,10] A sample of natural isoguanine, which was kindly furnished us by Dr. J. R. Spies, made possible a detailed comparison between the synthetic and the natural products. When a comparison of the ultraviolet spectra of

(1) Presented before the Division of Organic Chemistry at the 113th Meeting of the American Chemical Society, Chicago, April, 1948.

(2) The authors gratefully acknowledge the assistance of the Office of Naval Research, the James Foundation of New York, Inc., and Lord and Taylor, New York.

(3) E. Fischer, *Ber.*, **30**, 2226 (1897).

(4) K. Bloch and R. Schoenheimer, *J. Biol. Chem.*, **138**, 167 (1941).

(5) A. A. Plentl and R. Schoenheimer, *ibid.*, **153**, 203 (1944).

(6) W. Traube, *Ann.*, **331**, 64 (1904).

(7) H. L. Wheeler and L. M. Liddle, *Am. Chem. J.*, **40**, 547 (1908).

(8) H. L. Wheeler and G. S. Jamieson, *Am. Chem. J.*, **32**, 342 (1904).

(9) J. R. Spies, THIS JOURNAL, **61**, 350 (1939).

(10) E. Cherbuliez and K. Bernhard, *Helv. Chim. Acta*, **15**, 464, 978 (1932).

0.00002 M solutions of natural and synthetic isoguanine is made, certain differences are manifest, although there is a general agreement (Fig. 1).

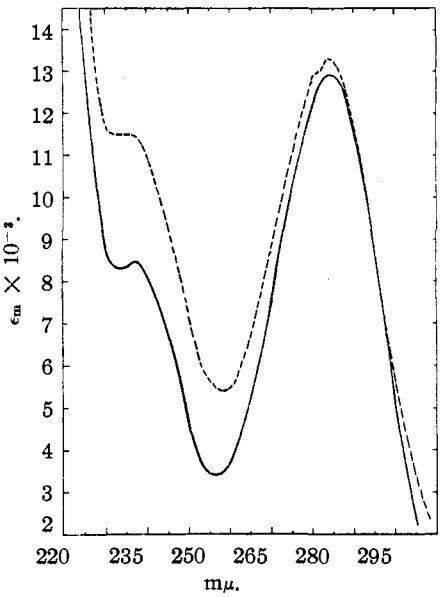


Fig. 1.—Ultraviolet absorption spectra of isoguanine, *p*H 9.8: —, synthetic; ----, natural (from croton beans).

The spectra, determined in a solution of *p*H 9.7, are a plot of molecular extinction coefficient, ϵ_m, against wave length in mμ. Whereas elementary analyses of each sample fail to reveal any gross impurities, an examination of the compounds by the Craig counter-current distribution technique[11,12,13] reveals that the synthetic product is more than 99.5% homogeneous, while the natural contains considerable impurity possessing a higher distribution constant (Fig. 2). Assuming the absorption of the impurity to be the same as that of isoguanine it may be calculated that the impurity represents about 5.5% of the sample.[14] The circles represent actual optical density values from which the distribution coefficients were calculated,[11] K = 0.79 (for the synthetic) and K = 0.77 (for the natural). These values are identical within experimental error. The triangles represent values calculated for a homogeneous substance with this K.

The discrepancies in the absorption curves (Fig. 1) due to the impurity in the natural product, are almost completely eliminated when the absorption spectra (Fig. 3) of samples from tube 5 (the "maximum" tube) of each distribution are compared.

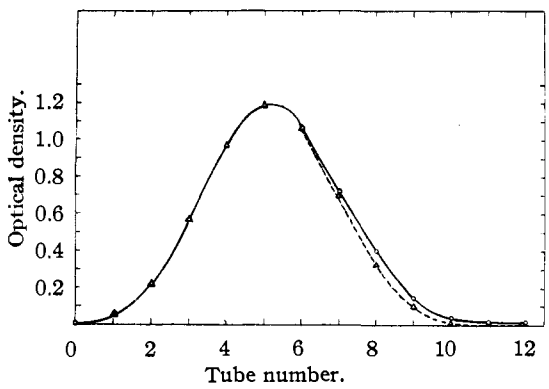


Fig. 2.—Counter-current distribution pattern of natural isoguanine in 5:3-*n*-butanol:isopropanol-1 M phosphate buffer. *p*H 9.8: —O—, observed values; --Δ--, calculated values for a homogeneous substance with K = 0.77.

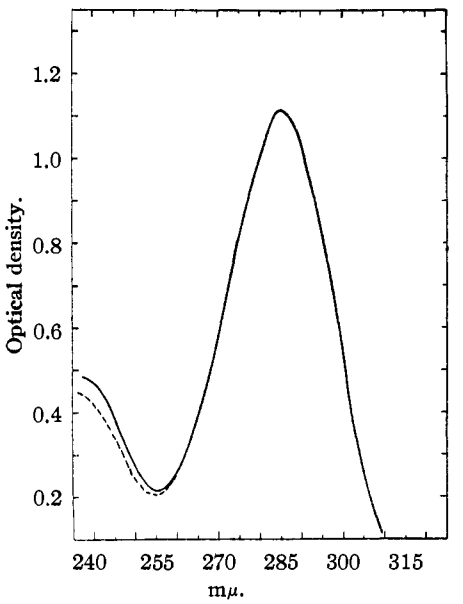


Fig. 3.—Ultraviolet absorption spectra of isoguanine, *p*H 9.8 after a 12-plate counter-current distribution in 5:3-*n*-butanol:isopropanol-1 M phosphate buffer, *p*H 9.8. —, synthetic; ---, natural.

Experimental

Cyanamide.[4]—Nine grams of ammonium nitrate (containing about 30 atom per cent. excess N^{15} in the ammonium radical) was dissolved in 25 ml. of water, and the ammonia was liberated by refluxing for two hours with 15 ml. of 12 N potassium hydroxide and was aerated through a sodium hydroxide drying tower into a tube containing 65 ml. of freshly prepared anhydrous ethanol kept at −70°. Freshly distilled cyanogen bromide (6.0 g.) in 45 ml. of dry ether was added and the tube was securely stoppered and kept at room temperature for about sixteen hours. The isotopic ammonium bromide that had formed was collected, the filtrate was concentrated to dryness *in vacuo* below 40°, the residue was taken up in absolute ether and the undissolved ammonium bromide was combined with the first crop to yield a total recovery of 5.05 g. (93%). In succeeding runs, 97, 100 and 98% recoveries of ammonium bromide were obtained.

Anal. Calcd. for NH_4Br: Br, 81.3.[15] Found: Br, 81.4.

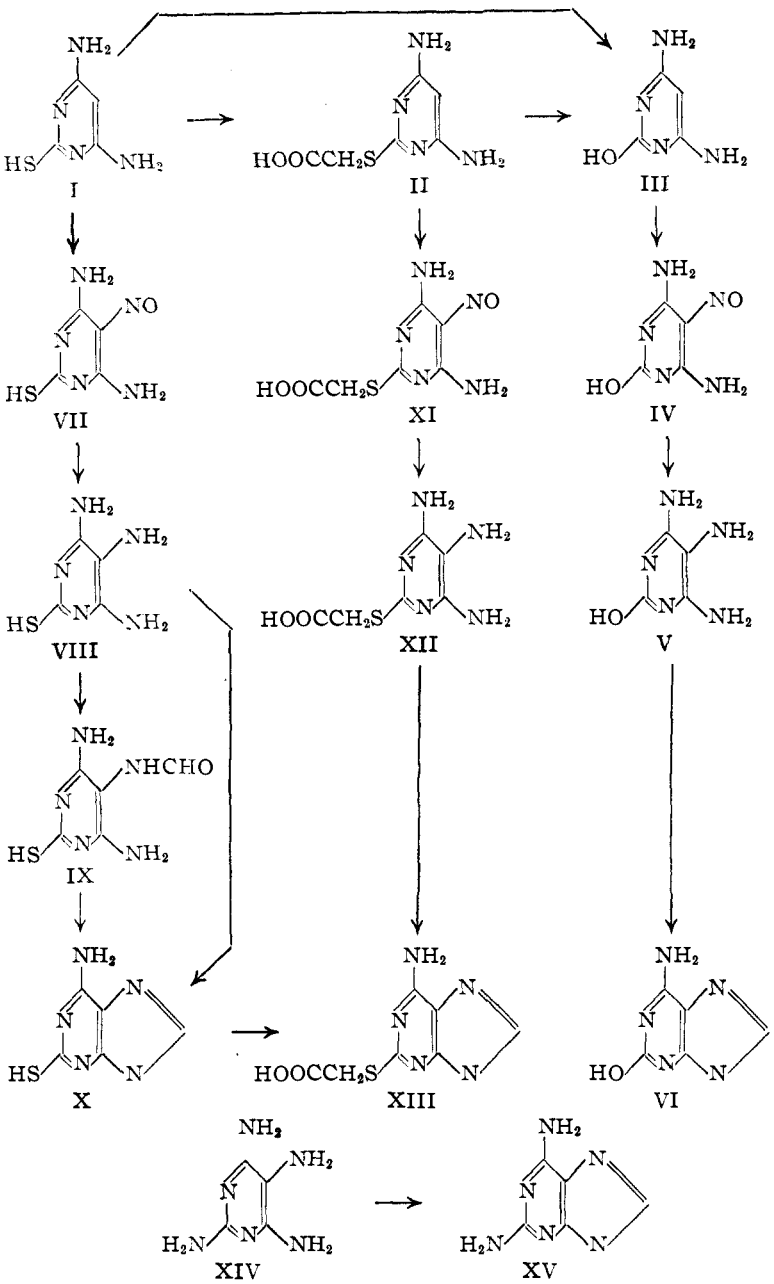
(11) B. Williamson and L. C. Craig, *J. Biol. Chem.*, **168**, 687 (1946).

(12) J. F. Tinker and G. B. Brown, *ibid.*, **173**, 585 (1948).

(13) L. F. Cavalieri, A. Bendich, J. F. Tinker and G. B. Brown, submitted for publication.

(14) A sample of the free base, subsequently received from Dr. Spies, proved to be homogeneous within experimental error.

(15) Corrected for isotope content.



The ether filtrate was concentrated to dryness and 2.18 g. of cyanamide was obtained (93%). Succeeding runs resulted in yields of 87, 92 and 96%. The crystalline product was stored in a desiccator in the refrigerator.

Thiourea.—This was prepared from isotopic cyanamide and hydrogen sulfide[5] in yields of 91 and 92%. The product was shown to contain 15.0 atom per cent. excess N^{15}.[16]

4,6-Diamino-2-thiolpyrimidine (I).—The following modification of Traube's synthesis[6] was used. To 60.0 g. of thiourea (0.79 mole) in 400 ml. of absolute ethanol containing 18.0 g. sodium (0.783 mole) was added 50.0 g. malononitrile (0.76 mole) and the mixture was refluxed for two hours. Complete solution was effected by adding 3 volumes of water and the solution was neutralized with glacial acetic acid to yield 74.0 g. of white needles (70%) which could be used without further purification. A sample, recrystallized from water, was dried at room temperature.

Anal. Calcd. for $C_4H_6N_4S$: N, 39.4. Found: N, 39.5.

4,6-Diamino-5-nitroso-2-thiolpyrimidine (VII).—23.3 g. of diaminothiolpyrimidine (I) was dissolved in 430 ml. glacial acetic acid and 85 ml. of water was added. The solution was stirred mechanically at 2 to 5° and 21 g. of sodium nitrite in 43 ml. of water was added in two portions and the stirring continued for three hours. The green precipitate was collected and was washed several times with cold water and dried; yield 25.0 g. (89%). The washed, moist derivative could be reduced directly.

4,5,6-Triamino-2-thiolpyrimidine (VIII).—Reduction of the nitroso compound with ammonium sulfide[6] invariably gave poor yields of the triamine. This may perhaps be due to the general instability in neutral or in alkaline solution[13] of aminopyrimidines of this type.

To 8.55 g. of the nitroso compound (VII) (0.05 mole) suspended by continuous stirring in 375 ml. of water, 25.1 g. of $Na_2S_2O_4 \cdot 2H_2O$ (0.12 mole) was added. The mixture was boiled for three minutes, during which time the green color was completely bleached; 50 ml. of 50% sulfuric acid (V/V) was cautiously added and the hot mixture was filtered through a layer of Norite. Upon cooling, 6.0 g. (47%) of long white needles separated. These were recrystallized from 2 *N* sulfuric acid.

Anal. Calcd. for $C_4H_7N_5S \cdot H_2SO_4$: N, 27.4. Found: N, 27.3.

The ultraviolet absorption spectrum was determined on a Beckman Model DU spectrophotometer. A solution of 6.50 mg. per l. in water showed maxima at 245 mμ (ϵ_m = 13,900) and 295 mμ (ϵ_m = 11,900) and minima at 227 mμ (ϵ_m = 8,900) and 259 mμ (ϵ_m = 7,800).

4,6-Diamino-5-formamido-2-thiolpyrimidine (IX).—8.26 g. of 4,5,6-triamino-2-thiolpyrimidine (VIII) sulfate was refluxed for seven hours with 83 ml. of 90% formic acid containing 9.3 g. of anhydrous sodium formate. The solution was concentrated to dryness under reduced pressure and the residue, upon recrystallization from 200 ml. of water, gave 4.91 g. of white needles (80%). This was dried over phosphorus pentoxide at room temperature.

Anal. Calcd. for $C_5H_7ON_5S \cdot H_2O$: N, 34.5. Found: N, 34.7.

This compound appeared to be homogeneous when examined by the countercurrent distribution technique,[11,12] K = 0.08.[17] The ultraviolet absorption spectrum, 6.40 mg. per liter in 0.1 *M* phosphate buffer, *p*H 6.5, revealed three maxima at 245 mμ (ϵ_m = 16,800), 266 mμ (ϵ_m = 13,700), 295 mμ (ϵ_m = 14,400).

6-Amino-2-thiolpurine (Thioladenine or Thioisoguanine) (X).—According to Traube's original directions,[6] ring closure of the above formylamine (IX) was accomplished by high temperature dehydration of the potassium salt. In our hands, the over-all yields obtained thus were much lower than those obtained by the dehydration of

(16) The isotope determinations were carried out by Dr. Stephen Friedland.

(17) In *n*-butanol–1 *M* potassium phosphate buffer, *p*H 6.5.

the free pyrimidine. An attempt was also made to effect ring closure by heating the formyl derivative in 2 *N* sulfuric acid solution at 100° for one and one-half hours, but this treatment caused considerable destruction. Heating at 160° *in vacuo* for a few hours resulted in the loss of the molecule of water of crystallization, but not in ring closure. However, when 4.89 g. of 4,6-diamino-5-formamido-2-thiolpyrimidine (IX) was heated at 237° under 1 mm. pressure for four hours, it lost about 22% in weight; 3.80 g. of the resulting pale yellow residue gave 2.76 g. of pale yellow needles on recrystallization from 5% sulfuric acid. After a second recrystallization, colorless needles were obtained which were dried at 100° *in vacuo* over phosphorus pentoxide.

Anal. Calcd. for $(C_5H_5N_5S)_2 \cdot H_2SO_4 \cdot H_2O$: N, 31.0. Found: N, 30.8.

Alternatively, the purine could be obtained directly from the triaminothiolpyrimidine (VIII) thus avoiding the isolation of the formamido intermediate: 1.38 g. of 4,5,6-triamino-2-thiolpyrimidine sulfate (0.0054 mole) was heated for three hours at 160° in a bomb tube containing 30 ml. of anhydrous formamide and 520 mg. of 98% formic acid (0.01 mole). The pale yellow crystalline deposit that resulted on chilling the contents of the tube amounted to 1.01 g. (quantitative yield) after washing with water, alcohol and ether, and drying *in vacuo* over phosphorus pentoxide. Almost colorless needles of the sulfate (0.77 g.) were obtained on recrystallization of 0.85 g. from 5% sulfuric acid.

Anal. Calcd. for $(C_5H_5N_5S)_2 \cdot H_2SO_4 \cdot H_2O$: N, 31.0. Found: N, 31.0.

On examination by counter-current distribution, the compound appeared to be completely homogeneous, K = 0.48.[17] The absorption, determined on a solution of 6.80 mg. per l. in 0.1 *M* potassium phosphate buffer, *p*H 6.38, showed maxima at 230 mμ (ϵ_m = 9,640) and 285 mμ (ϵ_m = 12,600) with a shoulder at 255–262 (ϵ_m = 7,300).

Adenine from (X).—1.6 g. of thioisoguanine (X) sulfate was suspended in 25 ml. of water, was neutralized with sodium carbonate, and 25 ml. of 1% sodium carbonate solution was added. After the addition of several grams of Raney nickel,[18] the mixture was refluxed for two and one-half hours and filtered. Upon prolonged chilling, 0.34 g. of a yellow solid separated which was crystallized from water, and dried *in vacuo* over phosphorus pentoxide at 100°.

Anal. Calcd. for $C_5H_5N_5$: N, 52.0. Found: N, 52.1.

The distribution pattern was that of a homogeneous substance, K = 2.38,[12,17] and the absorption spectrum was that of adenine.

4,6-Diamino-2-carboxymethylthiolpyrimidine (II).—When an attempt was made to convert 4,6-diamino-2-thiolpyrimidine to the corresponding diamino-2-hydroxy compound by adapting the chloroacetic acid desulfurization method of Wheeler and Liddle,[7] the intermediate carboxymethyl thioether (II) separated.

Ten grams of 4,6-diamino-2-thiolpyrimidine and an equal weight of chloroacetic acid were placed in 140 ml. of cold water and the mixture was refluxed with constant stirring for one hour. The copious white precipitate, which began to separate soon after the boiling started, was removed after chilling and amounted to 11.0 g. (79%). Colorless needles were obtained upon recrystallization from water.

Anal. Calcd. for $C_6H_8O_2N_4S$: N, 28.0. Found: N, 27.6.

Hexagonal prisms resulted upon crystallization from 2 *N* sulfuric acid. These were dried at room temperature over phosphorus pentoxide *in vacuo*.

Anal. Calcd. for $(C_6H_8O_2N_4S)_2 \cdot H_2SO_4 \cdot H_2O$: C, 27.91; H, 3.90. Found: C, 27.87; H, 4.37.[19]

4,5,6-Triamino-2-carboxymethylthiolpyrimidine (XII).—6.3 g. of 4,6-diamino-2-carboxymethylthiolpyrimidine was suspended in 140 ml. of glacial acetic acid, 35 ml. of water was added, and to the mixture kept at 5°, 5.8 g. of sodium nitrite in 21 ml. of water was added with mechanical stirring. After three hours at 5°, the brick-red mixture was cooled overnight in the refrigerator, collected, and washed with three portions of chilled water. The moist nitroso derivative was suspended in 400 ml. of water, 27 g. of sodium hydrosulfite was added, and the mixture was boiled for three minutes whereupon the color was bleached. Norite was added, and upon chilling the filtrate 3.0 g. of yellow-orange needles was obtained.

Anal. Calcd. for $C_6H_9O_2N_5S$: C, 33.50; H, 4.22. Found: C, 34.01; H, 5.31.[19]

The absorption spectrum, 13.2 mg. per l. in 0.1 *M* phosphate, *p*H 6.50, showed maxima at 217 mμ (ϵ_m = 25,500) and 273 mμ (ϵ_m = 8,680) with a minimum at 251 mμ (ϵ_m = 6,000).

6-Amino-2-carboxymethylthiolpurine (XIII).—(a) 400 mg. of the triamine (XII) was refluxed for seven hours with 7 ml. of 98% formic acid containing 0.7 g. of anhydrous sodium acetate. The yellow-orange residue which resulted after evaporation to dryness under reduced pressure yielded 171 mg. of white needles upon recrystallization from 5% sulfuric acid. An analytical sample was dried at room temperature *in vacuo* over phosphorus pentoxide.

Anal. Calcd. for $(C_7H_6O_2N_5S)_2 \cdot H_2SO_4 \cdot 2H_2O$: C, 28.86; H, 3.11; N, 24.05; S, 16.51. Found: C, 29.11; H, 3.66; N, 23.98; S, 16.50.[19]

This purine showed a distribution coefficient K = 0.20.[17]

The absorption spectrum, 4.60 mg. of free base per l. in 0.1 *M* phosphate buffer, *p*H 6.50 showed maxima at 232 mμ (ϵ_m = 22,000) and 275 mμ (ϵ_m = 12,500) and minima 217 mμ (ϵ_m = 13,400) and 251 mμ (ϵ_m = 8,800).

Upon refluxing this substance with 2 *N* sulfuric acid for two hours, the compound was recovered unchanged.

(b) 30.1 mg. of 2-thioladenine (X) and 30 mg. of chloroacetic acid in 1 ml. of water were heated in a sealed tube for one hour at 100°; 27.4 mg. of the free base was obtained on chilling the neutralized solution. An analytical sample of the sulfate was obtained on recrystallization from sulfuric acid as above. The ultraviolet spectrum was identical with that above.

(c) 3.18 g. of XII was suspended in 30 ml. of formamide containing 0.8 ml. of 98% formic acid and heated in a sealed tube for three hours at 160°. After chilling, 1.66 g. (50%) of a pale yellow crystalline deposit was collected and recrystallized from 5% sulfuric acid. The ultraviolet spectrum coincided with that above. A distribution constant of 0.22 was found.[17]

2-Hydroxy-4,6-diaminopyrimidine (III).—(a) 300 mg. of 4,6-diamino-2-carboxymethylthiolpyrimidine (II) was refluxed for two hours with 10 ml. of 2 *N* sulfuric acid; 1 ml. of 2.5 *N* hydrochloric acid was then added and refluxing continued for thirty minutes; 128 mg. of needles deposited on cooling which gave 94 mg. of elongated white rectangles upon recrystallization from 2 *N* sulfuric acid.

Anal. Calcd. for $(C_4H_6N_4O)_2 \cdot H_2SO_4$: N, 32.0. Found: N, 32.4.

(b) To 5.40 g. of 4,6-diamino-2-thiolpyrimidine (I) and 5.5 g. of chloroacetic acid was added 75 ml. of boiling water, and the solution was refluxed for 1.25 hours. Very little solid separated. Without cooling, 9.5 ml. of 18 *N* sulfuric acid was added and the refluxing continued for an additional hour. An odor reminiscent of thioglycolic acid was noted throughout this period. Norite was added and upon cooling the filtrate, 5.02 g. (77% yield) of white elongated prisms deposited.

Anal. Calcd. for $(C_4H_6N_4O)_2 \cdot H_2SO_4$: N, 32.0. Found: N, 31.8.

The hydrochloride was obtained upon recrystallization from 2 *N* hydrochloric acid.

(18) A. A. Pavlic and H. Adkins, THIS JOURNAL, **68**, 1471 (1946).

(19) Performed by Dr. A. Elek, Rockefeller Institute for Medical Research.

Anal. Calcd. for $C_4H_6N_4O \cdot HCl$: N, 34.5. Found: N, 34.5.

4,5,6-Triamino-2-hydroxypyrimidine (V).—(a) 15.3 g. of the diamine (III) sulfate was very finely pulverized and suspended in a mixture of 110 ml. of glacial acetic acid and 110 ml. of water. The mixture was kept at about 5° and 11.0 g. of sodium nitrite in 25 ml. of water was added with constant stirring. The carmine red-colored precipitate was collected after two hours and washed with three small portions of chilled water. The moist precipitate was suspended in 400 ml. of water, 45 g. of sodium hydrosulfite[20] was added and the mixture boiled for three minutes, during which time the substance was bleached; 53 ml. of 18 *N* sulfuric acid was added carefully, the mixture boiled for a few minutes and filtered after Norite treatment to yield, on chilling, 12.0 g. (58% yield) of white needles, which were recrystallized from 2 *N* sulfuric acid.

Anal. Calcd. for $C_4H_7ON_5 \cdot H_2SO_4$: N, 29.3. Found: N, 28.9.

(b) Five grams of the sulfate of III (0.029 mole), dissolved in 670 ml. of boiling water, was treated with 3.0 g. of sodium nitrite (0.044 mole) dissolved in 50 ml. of water. After five minutes, an equal volume of crushed ice was added and the deposit of the red nitroso compound (IV) was collected, washed with cold water and dried to yield 3.8 g. (86%). This was suspended in 180 ml. of water, 5.5 g. of sodium hydrosulfite added and the mixture boiled for three minutes; 23 ml. of 18 *N* sulfuric acid was cautiously added and the mixture rapidly filtered after Norite treatment; on cooling, 3.91 g. (67%) of white needles was collected. An analytical sample was recrystallized from 2 *N* sulfuric acid and dried at 130° *in vacuo* over phosphorus pentoxide. The distribution constant was found to be 0.14.[17]

Anal. Calcd. for $C_4H_7ON_5 \cdot H_2SO_4$: N, 29.3. Found: N, 29.2.

The absorption spectrum, 7.41 mg. per l. in 0.1 *M* phosphate buffer, *p*H 6.5, showed a maximum at 282.5 mμ (ϵ_m = 17,900) and a minimum at 252.5 mμ (ϵ_m = 2,600).

Isoguanine (2-Hydroxyadenine) (VI).—Various schemes for the formylation and ring closure of the hydroxytriamine (V) to the purine (VI) were attempted. However, when formamide and formic acid were used together, an almost quantitative yield of the desired product was secured; 2.84 g. of 4,5,6-triamino-2-hydroxypyrimidine sulfate (0.012 mole), 35 ml. of formamide and 0.85 ml. of 98% formic acid (0.022 mole) were heated in a bomb tube at 160° for three hours. Upon cooling 1.67 g. (93%) of the crystalline free base (VI) deposited. (Ammonium sulfate precipitated from the filtrate on the addition of alcohol.) White, prismatic needles of the monohydrate were obtained on crystallization from 5% sulfuric acid. The substance did not lose its water of crystallization upon heating at 130° *in vacuo* for three hours. This property of isoguanine has been previously described.[3,10]

Anal. Calcd. for $(C_5H_5N_5O)_2 \cdot H_2SO_4 \cdot H_2O$: N, 33.5. Found: N, 33.4.

Isotopic Isoguanine.—Isoguanine sulfate prepared from thiourea containing 15.0 atom per cent. excess N^{15} was shown[15] to contain 5.4 atom per cent. excess N^{15} (expected 6.0).

Anal. Calcd. for $(C_5H_5ON_5)_2 \cdot H_2SO_4 \cdot H_2O$: S, 7.65.[15] Found: S, 7.65.

This sample was compared with a sample isolated from croton beans by Dr. J. R. Spies. The spectra of 2 × 10^{-5} *M* solutions in 0.1 *M* phosphate buffer of *p*H 9.8 are given in Fig. 1; the maxima for the synthetic sample are found at 237.5 mμ (ϵ_m = 8,450) and 285 mμ (ϵ_m = 12,900) and minima at 233 mμ (ϵ_m = 8,250) and 257 mμ (ϵ_m = 3,400). In the case of the natural sample, the lower wave length maximum and minimum are obscured by a shoulder from 232–237 mμ (ϵ_m = 11,500) and the higher wave length maximum is found at 284 mμ (ϵ_m = 13,300) with a small shoulder at 280 mμ.

To determine the degree of homogeneity of these specimens, each was subjected to 12-plate counter-current distribution analysis[11,12] in 5:3-*n*-butanol:isopropanol-1 *M* phosphate buffer, *p*H 9.8, and measurements of the optical density at 285 mμ were made on each tube. The distribution pattern for the synthetic sample corresponds with that of a homogeneous compound (containing less than 0.5% ultraviolet absorbing impurity), K = 0.79. The pattern for the natural product is given by the solid line in Fig. 2 (K = 0.77). The two K values are identical within experimental error. The broken line represents the theoretical distribution pattern for a homogeneous substance with K = 0.77. Assuming the absorption of the impurity to be the same as that of the isoguanine, the pattern indicates the presence of 5.5% contamination in the natural sample. The spectra (Fig. 3) of the buffer–alcohol solutions obtained in tube 5 of each distribution are nearly identical (slit width 0.7 mm., sensitivity maximal).

Xanthine from Isoguanine.—A sample of synthetic isoguanine was converted to xanthine according to the directions of Spies,[9] but a poor yield (6%) was obtained. Distribution coefficient, K = 0.45.[12,17]

Anal. Calcd. for $C_5H_4O_2N_4$: N, 36.9. Found: N, 36.7.

2,6-Diaminopurine (XV).—1.33 g. of 2,4,5,6-tetraaminopyrimidine sulfate (XIV)[21,22] was heated at 160° for three hours in a sealed tube with 15 ml. of formamide and 0.4 ml. of 98% formic acid. Upon cooling, 1.53 g. of yellow crystals was deposited. These were recrystallized from 5% sulfuric acid to yield white needles. The product was dried at 140° *in vacuo* over phosphorus pentoxide.

Anal. Calcd. for $(C_5H_6N_6)_2 \cdot H_2SO_4 \cdot H_2O$: S, 7.70. Found: S, 7.67.

The compound appeared homogeneous when examined by counter-current distribution, K = 1.21.[17] The absorption spectrum, 4.68 mg. per l. in phosphate buffer, *p*H 6.49, showed maxima at 247 mμ (ϵ_m = 7,000) and at 280 mμ (ϵ_m = 8,820) and minima at 235 mμ (ϵ_m = 6,300) and at 261 mμ (ϵ_m = 5,360).

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Summary

Syntheses of 2-thioladenine, 2-carboxymethylthioladenine, 2,6-diaminopurine, adenine and isoguanine are described. Isoguanine has been synthesized containing an excess of isotopic nitrogen in the 1 and 3 positions. A rigorous comparison of the synthetic product with natural isoguanine has been carried out.

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