[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, OREGON STATE COLLEGE]

Purines. VI. The Preparation of Certain 6-Substituted- and 6,9-Disubstituted Purines[1]

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The preparation of the plant growth factor, kinetin, and analogous 6-alkylamino- and 6-arylamino-purines has been carried out through the use of 6-chloropurine and suitable amines. The method compares favorably with that used by Miller in the first reported synthesis of kinetin. A study was also made of the cyclization of various 6-alkylamino- and 6-arylamino-4,5-diaminopyrimidines. These diamines were synthesized starting from 4-amino-6-chloro-5-nitropyrimidine. Whenever possible, their cyclization led to the production of 9-substituted adenines and attempts to prepare analogs of kinetin by this method therefore were unsuccessful. The proof of the structure of these 9-substituted adenines was based upon their conversion to the corresponding hypoxanthines and from their lack of acidic characteristics.

The recent discovery of kinetin[2] has focused attention on other analogs of adenine as possible cytokinsetic agents. Several of these have been prepared by Strong and co-workers[3] who employed 6-mercaptopurine as the starting material. For the past several years this laboratory has been investigating the chloropyrimidines[4] as intermediates for the preparation of potentially biologically active purines, azapurines, and pteridines. In view of the importance of Strong's work it was felt that a preparation of kinetin analogs[5] using chloropyrimidines or purines as intermediates was much in order.

The starting material for these studies was 4,6-pyrimidinedione.[6] The nitration of this intermediate by the method of Boon[7] at 15–20° was found to give more reproducible yields when the temperature was increased to 35–40° and a small excess of fuming nitric acid was added to the mixture toward the end of the reaction.

Chlorination of the 5-nitro-4,6-pyrimidinedione was followed by monoammonolysis to give 4-amino-6-chloro-5-nitropyrimidine[7] which still retained a rather reactive chloro substituent. This intermediate reacted readily in boiling *n*-butanol with aniline, benzylamine, morpholine, and methylamine to give the corresponding substituted pyrimidines. Reduction of the nitro substituent then was carried out with hydrogen using Raney nickel as the catalyst. All the 4,5-diamino intermediates were isolated as their sulfate salts with the exception of the 6-morpholino-4,5-diaminopyrimidine, which had to be isolated in crude form as the free base. The cyclization of this pyrimidine was affected by formylation in 90% formic acid, followed by cyclization in boiling formamide. The ultraviolet absorption spectrum and melting point of the resulting 6-morpholinopurine were identical to those reported by Elion.[8]

The cyclization of 6-anilino-4,5-diaminopyrimidine using various cyclization procedures was studied to establish the comparative activity of the anilino substituent. While the 4-amino nitrogen was expected to be more reactive in cyclization reactions than the 6-anilino nitrogen, the converse proved to be true in all cases studied. The cyclizations were carried out (1) by boiling the sulfate salt in formamide, (2) by forming the formyl derivative and converting it to the purine either in boiling formamide or by heating in the absence of a solvent, and (3) by thioformylating and cyclizing with sodium dithioformate. In all instances 6-amino-9-phenylpurine (9-phenyladenine) was formed. The presence of a free amino group was established by its easy conversion to 9-phenyl-6-purinone (9-phenylhypoxanthine) by the nitrous acid method of Fischer.[9] Furthermore, the nine position of the cyclization product was occupied as judged by the purine's lack of solubility in base, since an unsubstituted imidazole ring should impart acidic characteristics to the compound.

The formyl intermediate was found to have a very indistinct melting point, being converted by heat into the purine and finally melting sharply at the melting point of 6-amino-9-phenylpurine (9-

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(2) Miller, Skoog, Okumura, Van Saltza, and Strong, *J. Am. Chem. Soc.*, **77**, 2662 (1955).

(3) Okumura, Von Saltza, Strong, Miller, and Skoog, *Chem. Eng. News*, **33**, 3298 (1955); Abstracts, Minneapolis meeting A.C.S. 26A (1955); Baker, Joseph, and Shant, *J. Org. Chem.*, **19**, 631 (1954).

(4) Robins, Dille, and Christensen, *J. Org. Chem.*, **19**, 930 (1954); Dille, Sutherland, and Christensen, *J. Org. Chem.*, **20**, 171 (1955); Dille and Christensen, *J. Am. Chem. Soc.*, **76**, 5087 (1954); Daly and Christensen, *J. Am. Chem. Soc.*, **78**, 225 (1956).

(5) These analogs are being submitted to Dr. David Greenberg, School of Medicine, University of California, and to Dr. K. S. Pilcher, School of Science, Oregon State College, for screening purposes. Tests will be made for carcinostatic and virus inhibitory activities.

(6) Hull, *J. Chem. Soc.*, 2214 (1951).

(7) Boon, Jones, and Ramage, *J. Chem. Soc.*, 96 (1951).

(8) Elion, Burgi, and Hitchings, *J. Am. Chem. Soc.*, **74**, 411 (1952).

(9) Fischer, *Ann.*, **215**, 253 (1882).

phenyladenine). The reaction of the 6-anilino-4,5-diaminopyrimidine with sodium dithioformate was unusual in that the thioformamido derivative could not be isolated. Instead the purine was formed, even under the mild conditions used for the thioformylation.

The cyclizations of 6-benzylamino-4,5-diaminopyrimidine and 4,5-diamino-6-methylaminopyrimidine were carried out by boiling their sulfate salts in formamide. In both cases a 9-substituted adenine was formed. This was determined for 6-amino-9-benzylpurine (9-benzyladenine) by its easy conversion to the corresponding 9-substituted hypoxanthine and by its insolubility in base. In this cyclization the presence of a small amount of the other isomer was indicated since extraction of the crude material with aqueous base yielded a product with a much sharper melting point. The amount formed, if any, was not sufficient for isolation and characterization. The 6-amino-9-methylpurine (9-methyladenine) prepared from the 4,5-diamino-6-methylaminopyrimidine had identical properties to those reported by Gulland[10] and Fischer.[11]

Another approach to the synthesis of analogs of kinetin may be made *via* the aminolysis of 6-chloropurine. This chloro substituent is quite reactive as judged by its ammonolysis in *n*-butanol. On this basis, it appears possible to synthesize the various 6-substituted purines without recourse to sealed tube reactions.

Reactions of amines with 6-chloropurine that yield 6-morpholino-, 6-furfurylamino- (kinetin), 6-anilino-, and 6-benzylamino-purines are here reported. The properties of the morpholino- and anilino-purines were identical to those of the compounds as reported by Elion.[8] The furfurylaminopurine was identical to that made by Miller.[2]

EXPERIMENTAL

5-Nitro-4,6-pyrimidinedione. 4,6-Pyrimidinedione[6] (11.2 g.) was added with stirring at 15–20° to a mixture of fuming (93%) nitric acid (20.4 g.) and acetic acid (36 g.). The mixture then was stirred for 20 minutes while the temperature was slowly raised to 35–40°. Fuming nitric acid (2–8 g.) then was added isothermally until the highly exothermic reaction occurred: immediately the pasty, frothy reaction mixture was poured onto ice. The pink product after standing several hours was removed by filtration. Yield, 11 g. (70%). The product was sufficiently pure for the subsequent chlorination.

General procedure for the aminolysis of 4-amino-6-chloro-5-nitropyrimidine. One gram of 4-amino-6-chloro-5-nitropyrimidine[7] was added to 20 ml. of *n*-butanol containing 2 g. of the amine. After refluxing for 1½ hours, the solution was cooled, filtered, and recrystallized. The results of these experiments are given in Table I.

General procedure for the reduction of 4-amino-5-nitro-6 substituted pyrimidines. The pyrimidine (2 g.) was suspended in 150 ml. of methanol and the product then was hydrogenated at 40 p.s.i. using a Raney nickel catalyst (3 g.). On completion of the reduction the Raney nickel[12] was removed and washed with a small amount of methanol. The combined solution then was made strongly acid with dilute sulfuric acid. After cooling, the sulfate salt of the diamine was collected. In Table II are given the results of these experiments.

General procedure for aminolysis of 6-chloropurine. 6-Chloropurine[13] (0.5 g.) was refluxed for 2 hours with 10 ml. of *n*-butanol that contained one gram of the appropriate amine. The results of these experiments are reported in Table III.

6-Morpholinopurine. 4,5-Diamino-6-morpholinopyrimidine (0.6 g.) was dissolved in 15 ml. of 90% formic acid and the mixture was refluxed for 15 minutes. The excess formic acid was removed by means of a hot air fan and the residue was boiled with 15 ml. of formamide for 20 minutes. The mixture then was diluted with 25 ml. of water and cooled overnight. Recrystallization from a water-ethanol (1:1) mixture gave 0.38 g. (60%) of product, m.p. 302–304° dec.

Anal. Calc'd for $C_9H_{11}N_5O$: C, 52.7; H, 5.40. Found: C, 52.8; H, 5.64.

6-Amino-9-phenylpurine (9-phenyladenine). Method A. 6-Anilino-4,5-diaminopyrimidine sulfate (1 g.) was boiled with 10 ml. of formamide for 20 minutes. After dilution with 40 ml. of water and cooling overnight, the yellow precipitate was removed by filtration and recrystallized from a water-ethanol (1:1) mixture to yield 0.5 g. (71%). M.p. 235–238°.

Anal. Calc'd for $C_{11}H_9N_5$: C, 62.6; H, 4.30. Found: C, 62.5; H, 4.38.

Method B. 6-Anilino-4,5-diaminopyrimidine sulfate (0.8 g. was refluxed in 25 ml. of 98% formic acid for 15 minutes, cooled, and diluted with 25 ml. of water. The excess formic acid was removed using a hot air fan and the residue then was dissolved in 5 ml. of water; the solution was adjusted to *p*H 8–9 with concentrated ammonium hydroxide. After cooling 0.32 g. (52%) of 4-amino-6-anilino-5-formamidopyrimidine was obtained which was recrystallized by dissolving in 6 *N* acetic acid, treating with Norit, and reprecipitating with ammonium hydroxide. The compound softened at about 195° and finally melted at 235–238°.

Anal. Calc'd for $C_{11}H_{11}N_5O$: C, 57.6; H, 4.84. Found: C, 57.5; H, 5.08.

The above formamidopyrimidine (100 mg.) was boiled with 4 ml. of formamide for ten minutes. Upon dilution with 10 ml. of water and cooling overnight, 70 mg. (78%) of 6-amino-9-phenylpurine was obtained, m.p. 232–236° dec.

Method C. 6-Anilino-4,5-diaminopyrimidine sulfate (1 g.) was dissolved in 300 ml. of water and the solution was brought to neutrality with aqueous sodium hydroxide. Sodium dithioformate (2 g.) was added and the mixture was allowed to stand for two days at room temperature. Upon concentration to one-half volume and cooling 0.58 g. (79%) of 6-amino-9-phenylpurine was obtained. Recrystallization from ethanol-water (1:1) gave a product, m.p. 235–238° dec.

9-Phenyl-6-purinone (9-phenylhypoxanthine). 6-Amino-9-phenylpurine (0.5 g.) was dissolved in 20 ml. of water containing 2 g. of concentrated sulfuric acid. The solution was cooled to 70–80° and an aqueous solution of sodium nitrite (1 g. in 5 ml. of water) was added slowly with stirring. The mixture was boiled for 3–5 minutes and then was cooled and filtered. The crude product was dissolved in 2 *N* sodium hydroxide, treated with Norit, filtered, and acidified with acetic acid. Yield, 0.26 g. (56%); m.p. 306–308° dec.

Anal. Calc'd for $C_{11}H_8N_4O$: C, 62.3; H, 3.80. Found: C, 62.1; H, 4.05.

6-Amino-9-benzylpurine (9-benzyladenine). One gram of 6-benzylamino-4,5-diaminopyrimidine was boiled in 10 ml. of formamide for 20 minutes, diluted with water, concentrated before a hot air fan, rediluted with water, and cooled

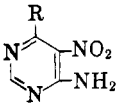
(10) Gulland and Ensor, *J. Chem. Soc.*, 765 (1936).

(11) Fischer, *Ber.*, **30**, 2226 (1897).

(12) Adkins and Covert, *J. Am. Chem. Soc.*, **54**, 4116 (1932).

(13) Bendich, Russell, and Fox, *J. Am. Chem. Soc.*, 76, 6073 (1954).

TABLE I

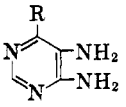


PRODUCTS FROM AMINOLYSIS OF 4-AMINO-6-CHLORO-5-NITROPYRIMIDINE

R	Yield, %	M.P., °C.	Empirical Formula	Analyses C Calc'd	C Found	H Calc'd	H Found
Morpholino[a]	79	176–179	$C_6H_{11}N_5O_3$	42.7	42.8	4.92	5.06
Anilino[b]	85	212–214	$C_{10}H_9N_5O_2$	51.9	52.0	3.92	4.06
Benzylamino[b]	94	191–194	$C_{11}H_{11}N_5O_2$	53.9	54.1	4.52	4.23
Methylamino[c]	72	241–245	$C_5H_7N_5O_2$	35.5	35.7	4.17	4.32

[a] Recrystallized from *n*-butanol. [b] Recrystallized from dioxane-water. [c] Reaction was carried out in H_2O. No recrystallization was required.

TABLE II

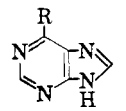


PRODUCTS FROM REDUCTION OF 4-AMINO-5-NITRO-6-SUBSTITUTED PYRIMIDINES

R	Yield, %	M.P., °C.	Empirical Formula	Analyses C Calc'd	C Found	H Calc'd	H Found
Morpholino[a]	—	—	$C_8H_{13}N_5O$	48.6	—	6.70	—
Anilino	79	>300	$C_{10}H_{11}N_5 \cdot H_2SO_4$	40.1	40.3	4.40	4.52
Benzylamino	64	>300	$C_{11}H_{13}N_5 \cdot H_2SO_4$	42.2	42.4	4.83	4.85
Methylamino	89	>300	$C_5H_9N_5 \cdot H_2SO_4$	25.3	25.3	4.67	4.74

[a] 6-Morpholino-4,5-diaminopyrimidine could not be isolated as the sulfate and was obtained in crude form as the free base by concentrating the methanolic solution to dryness.

TABLE III



PRODUCTS FROM AMINOLYSIS OF 6-CHLOROPURINE

R	Yield, %	M.P., °C.	Empirical Formula	Analyses C Calc'd	C Found	H Calc'd	H Found
Morpholino[a]	63	301–303[c]	$C_9H_{11}N_5O$	52.8	52.8	5.40	5.52
Anilino[a]	64	279–282[c]	$C_{11}H_9N_5$	62.7	62.6	4.30	4.45
Benzylamino[a]	80	216–218	$C_{12}H_{11}N_5$	63.8	63.7	4.89	4.83
Furfurylamino[b]	72	265–266[d]	$C_{10}H_9N_5O$	55.9	56.1	4.22	4.40

[a] Recrystallized from ethanol-water (1:1). [b] Recrystallized from absolute ethanol. [c] Reported previously by Elion.[8] [d] Reported previously by Miller.[2]

overnight. Recrystallization from ethanol-water (1:1) gave 0.43 g. of product, m.p. 192–210°. Extraction of this with 20 ml. of warm 1 *N* sodium hydroxide left a residue of 0.37 g. (53.5%), m.p. 224–225° dec.

TABLE IV

ULTRAVIOLET ABSORPTION SPECTRA OF PURINES AT *p*H 6

	λ_{max} mμ	E × 10^4	λ_{min} mμ	E × 10^3
6-Morpholinopurine	282	1.89	238	2.16
6-Anilinopurine	290	1.40	243	2.38
6-Benzylaminopurine	268	1.62	233	1.98
6-Furfurylaminopurine	266	1.88	234	3.32
6-Amino-9-phenylpurine	260	1.41	241	9.56
9-Phenyl-6-purinone	227	2.01		
6-Amino-9-benzylpurine	260	1.42	233	2.14
9-Benzyl-6-purinone	247	1.68	229	6.42
6-Amino-9-methylpurine	261	1.31	229	2.22

Anal. Calc'd for $C_{12}H_{11}N_5$: C, 64.0; H, 4.92. Found: C, 63.8; H, 5.00.

9-Benzyl-6-purinone (9-benzylhypoxanthine). 6-Amino-9-benzylpurine was converted to the corresponding purinone by the method described for the synthesis of 9-phenyl-6-purinone. Yield, 0.21 g. (42%); m.p. 254–258°.

Anal. Calc'd for $C_{12}H_{10}N_5O$: C, 63.6; H, 4.46. Found: C, 63.5; H, 4.54.

6-Amino-9-methylpurine (9-methyladenine). One gram of 4,5-diamino-6-methylaminopyrimidine sulfate was boiled for 15 minutes in 10 ml. of formamide. The mixture was diluted with 20 ml. of water and then was concentrated before a hot air fan to yield 0.48 g. (76%). Recrystallization from water gave a product, m.p. 300° (with sublimation).

Anal. Calc'd for $C_6H_7N_5$: C, 48.3; H, 4.73. Found: C, 48.4; H, 4.80.

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