THE SYNTHESIS OF CERTAIN 6- AND 9- SUBSTITUTED PURINES OF BIOLOGICAL INTEREST

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DEDICATION

To Doctor Bert E. Christensen whose help and encouragement has made this and previous work possible.

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THE SYNTHESIS OF CERTAIN 6- AND 9- SUBSTITUTED PURINES OF BIOLOGICAL INTEREST

INTRODUCTION

In the course of studies on the development of plant embryos in vitro, it was discovered by Van Overbeek (25, pp. 350-351), that an extract of coconut milk provided certain factors which were essential for the growth and development of very young Datura embryos; the older embryos being able to develop on a medium containing only dextrose, salts, vitamins, and purines.

Further work has revealed a variety of plant sources (5, p. 332 and 26, p. 221) which may provide this factor, and its use in studies of plant tissues has become widespread. Duhamet (8, pp. 770-771), for example, has revealed this factor as the first to show activity in stimulating the growth of plant tumor tissue, as was confirmed by Nickell's work (21, pp. 225-228) on plant virus tumor tissue. Furthermore it (15, pp. 16-24) has been clearly demonstrated that the coconut milk factor is primarily concerned with cell division, since tobacco pith tissue receiving optimum amounts of indole acetic acid underwent little development other than cell enlargement until a small amount of coconut milk extract was added. This addition caused very active

cell division and concomitant growth. Using an assay method which measured the amount of cell division occurring in excised tobacco callus tissue or carrot root tissue, Mauney et al (18, pp. 485-497) were able to obtain a four thousand three hundred and fifty fold purification of the active factor from coconut milk extract. However, other workers (23, pp. 401-410) found indications of the presence of a number of growth factors and identification appears difficult. Miller et al (19, p. 1392) then found that four year old preparations of deoxynucleic acid from herring sperm exhibited cell division activity, as did fresh preparations which had been autoclaved in an acidic medium.

Isolation of this factor (now christened "kinetin") from deoxynucleic acid (20, pp. 2662-2663) showed it to be a nitrogenous compound with properties similar to adenine. Acid hydrolysis yielded adenine and an aldehydic compound which gave the 2,4-dinitrophenylhydrazone of levulinic acid in poor yield. As judged from acidic characteristics and the formation of a silver salt, the nine position of this purine was unsubstituted; it was suspected that kinetin was a 6-alkylaminopurine.

Using the method developed by Elion and Hitchings (9, p. 412), 6-furfurylaminopurine was synthesised and it proved to be identical in physical and

biological properties to the naturally occurring kinetin (20, pp. 2662-2663).

Interest in the study of the biological potentialities of this material may be expected to be paralleled by interest in the properties of structurally similar compounds. The literature reveals a number of analogs (7, pp. 311-312, 9, p. 412, and 17, p. 655) of kinetin, however, biological testing of these analogs has been very limited (10, p. 652).

The synthetic procedure used to prepare most of these analogs of adenine involves the treatment of hypoxanthine with phosphorous pentasulfide to give 6-mercaptopurine, which is then converted to 6-methylmercaptopurine via methylation with methyl iodide (9, pp. 412-414). Sealed tube reactions with various amines (9, pp. 412-413) afford a route to a number of 6-alkylamino and 6-arylaminopurines.

A more straight forward procedure for their synthesis would involve the aminolysis of 6-chloropurine. The chlorination of hypoxanthine was recently reported by Bendich (4, pp. 6075-6076). This chlorine substituent appeared to be quite reactive as judged by its ammonolysis (4, p. 6076) in n-butanol at reflux temperature. On this basis, it appears possible to synthesize the various 6-substituted purines without recourse

to sealed tube reactions.

Hypoxanthine was synthesized; starting with the condensation of thiourea and ethyl cyanoacetate, followed by nitrosation and reduction of the nitroso group to 4,5-diamino-6-hydroxy-2-thiopyrimidine (24, pp. 71-76) which was then cyclized in formamide (22, p. 265) to yield 2- thiohypoxanthine. The final step was the removal of the mercapto group with twenty-five percent nitric acid (24, pp. 78-79).

Reactions of amines with 6-chloropurine yielded the 6-morpholino, 6-furfurylamine (kinetin), 6-anilino and 6-benzylaminopurines. The properties of the morpholino and anilinopurines were identical to those of the compounds as reported by Elion (9, p. 412). The furfurylaminopurine was identical to that made by Miller (20, pp. 2662-2663).

In order to avoid the above procedure leading to 6-chloropurine, the direct cyclization of 6-chloro-4,5-diaminopyrimidine was attempted.

Previous work (22, pp. 264-265) led only to the production of hypoxanthine, cyclization having been accompanied by hydrolysis. However, on the basis of the mild conditions needed to carry out thioformylation and cyclization of diaminopyrimidines (1, pp. 383-386 and 2, pp. 386-387) with sodium dithioformate, the attempt was made to use this method for the preparation of 6-

chloropurine.

The required diamine, 6-chloro-4,5-diaminopyrimidine, was synthesized; starting with the condensation of ethyl formate and malondiamide as described by Hull (14, p. 2214). The nitration of the resulting 4,6-dihydroxyprimidine by the method of Boon (6, p. 99) was unsuccessful as the starting material was recovered, while continued increase of the temperature led to a sudden and violent reaction. It was then discovered that holding the temperature at thirty-five to forty degrees, while adding a small excess of fuming nitric acid led to a controllable nitration which gave fairly reproducible yields.

Chlorination of the 4,6-dihydroxy-5-nitropyrimidine was followed by mono-ammonolysis to give 4-amino-6-chloro-5-nitropyrimidine (6, p. 99). Reduction of the nitro group with zinc dust (22, p. 265) completed the synthesis of 6-chloro-4,5-diaminopyrimidine.

The attempt to thioformylate the above diamine was unsuccessful; the starting material being recovered unchanged. No further effort was made to prepare 6-chloropurine by cyclization.

Another approach to the synthesis of analogs of kinetin, which was explored, was the cyclization of corresponding 6-alkylamino or 6-arylamino-4,5-diaminopyrimidines.

Todd and coworkers (2, pp. 318-322, 3, pp. 383-386, 16, pp. 852-855 and 17, pp. 652-656) had indicated that 6-aminopurines bearing the alkyl substituent in the nine position would result. One explanation being advanced that the amino group exists predominately in the unreactive imino form. However, it was reported that in some cases (2, pp. 318-322 and 17, p. 655) the 6-alkylaminopurine was obtained along with the usual isomer. These were the cyclizations of 4-amino-5-thioformamido-6-triacetyl-d-xylosidaminopyrimidines in boiling pyridine. These workers postulated that in this instance chelation had occurred between the acetyl group of the number two sugar carbon and the alkylamino group of the pyrimidine. When carried out in methanolic sodium methoxide (16, p. 854), the cyclization took place in the normal manner yielding the 9-xylosidoadenine; the acetyl groups presumably being removed before cyclization.

Still, it appeared worthwhile to investigate the possibility of obtaining kinetin analogs by this procedure. The synthesis of the 6-alkylamino or arylamino-4,5-diaminopyrimidines was attempted by aminolysis of 6-chloro-4,5-diaminopyrimidine (22, p. 265) in n-butanol. This chlorine proved to be quite unreactive and all attempts to replace it with morpholine failed. 4-amino-6-chloro-5-nitropyrimidine (6, pp. 99), as expected, had a much more reactive chloro substituent.

It reacted readily in boiling n-butanol with aniline, benzylamine, morpholine, and methylamine to give the corresponding pyrimidines. Reduction of the nitro group was then carried out with hydrogen using raney nickle as the catalyst. The resulting 4,5-diamino compounds were isolated as the sulfate salts, except in the case 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 effected by formylation in ninety percent formic acid, followed by cyclization in boiling formamide. The ultraviolet absorbtion spectrum and melting point of the resulting 6-morpholinopurine were identical to those reported by Elion (9, p. 412).

In order to investigate the possibility of forming different cyclization products by varying the method of cyclization, 6-anilino-4,5-diaminopyrimidine was chosen since the 4-amino nitrogen might be expected to be more reactive than the 6-anilino nitrogen. The converse proved to be true in all cases. 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 cases, 9-phenyladenine was formed, as judged by

its easy conversion to 9-phenylhypoxanthine by the nitrous acid method of Fisher (12, pp. 309-310) which indicated a free amino substituent. 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 would 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 9-phenyladenine. The reaction of the pyrimidine 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-diamino-pyrimidine and 4,5-diamino-6-methylaminopyrimidine were carried out by boiling their sulfate salts in formamide. In both cases the 9-substituted adenine formed. This was determined for 9-benzyladenine by its easy conversion to the corresponding hypoxanthine and by its insolubility in base. In this cyclization the presence of a small amount of the other isomer was indicated, for extraction of the product with aqueous base resulted in a much sharper melting point. The amount formed, if any, was not sufficient for isolation and characterization. The 9-methyladenine formed from the 4,5-diamino-6-

methylaminopyrimidine had identical properties to those reported by Gulland (13, p. 766) and Fischer (11, pp. 225-2251).

The ultraviolet absorbtion spectra has been determined for the purines and appears in Table IV.

EXPERIMENTAL

Nitration of 4,6-dihydroxpyrimidine.[1] 4,6-dihydroxypyrimidine (11.2 grams) (14, p. 2214) was added with stirring at 15-20° centigrade to a mixture of fuming (93%) nitric acid (20.4 grams) and glacial acetic acid (36 grams). The mixture was then stirred for twenty minutes while the temperature was slowly raised to 35-40° centigrade. Fuming nitric acid (2-8 grams) was then added isothermally until the highly exothermic reaction occurred. Immediately the pasty, frothy reaction mixture was poured on ice. The pink product was filtered off after standing. Yield 11 grams (70%). It was sufficiently pure without recrystallization for the subsequent chlorination.

Aminolysis of 4-amino-6-chloro-5-nitropyrimidine. One gram of 4-amino-6-chloro-5-nitropyrimidine (6, p. 99) was added to 20 milliliters of n-butanol containing 2 grams of the amine. After refluxing for one and one-half hours, the solution was cooled and filtered. The crude pyrimidine was then recrystallized as indicated in Table I. Yields and analytical data are also reported in Table I.

Reduction of nitropyrimidines. Two grams of the pyrimidine was suspended in 150 milliliters of

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1. Modification of the procedure of Boon et al (6, p. 99)

methanol and was then hydrogenated at forty pounds per square inch using a raney nickle catalyst (3 grams). On completion of the hydrogenation, the raney nickle was filtered off and washed with a small amount of methanol. The solution was then made strongly acid with dilute sulfuric acid. After cooling, the sulfate salt of the diamine was filtered off. Yields and analytical data is reproduced in Table II.

Aminolysis of 6-chloropurine. One-half gram of 6-chloropurine (4, pp. 6075-6076) was refluxed for 2 hours with 10 milliliters of n-butanol that contained one gram of the amine. Recrystallization solvents, yields and analytical data are given in Table III.

6-morpholinopurine.[2] A. 4,5-diamino-6-morpholinopyrimidine (.6 gram) was dissolved in 15 milliliters of 90% formic acid and refluxed for fifteen minutes. The excess formic acid was removed before a fan and the residue was boiled with 15 milliliters of formamide for twenty minutes. The mixture was diluted with 25 milliliters of water and cooled overnight. Recrystallization from a 50% water-ethanol mixture gave .38 grams (60%). Melting point 302-304°C dec. Analytical: Calcd. C, 52.7; H, 5.40. Found C, 52.8; H, 5.64.

B. 6-morpholinopurine was also prepared from 6-chloropurine by the general method given above.

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2. Previously reported (9, p. 412)

9-phenyladenine. A. 6-anilino-4,5-diamino-pyrimidine sulfate (1 gram) was boiled with 10 milliliters of formamide for twenty minutes. After dilution with 40 milliliters of water and cooling overnight, the yellow precipitate was filtered off and recrystallized from a 50% water-ethanol mixture to afford .5 gram (71%). Melting point 235-238°C dec. Analytical: Calcd. C, 62.6; H, 4.30. Found C, 62.5; H, 4.38.

B. 6-anilino-4,5-diaminopyrimidine sulfate (.8 gram) was refluxed in 25 milliliters of 98% formic acid for fifteen minutes, cooled, and diluted with 25 milliliters of water. The excess formic acid was removed in front of a fan and the residue was then taken up in 5 milliliters of water and the pH adjusted to 8-9 with concentrated ammonium hydroxide. After cooling .32 gram (52%) of 4-amino-6-anilino-5-formamidopyrimidine was obtained which was recrystallized by dissolving in 6 normal acetic acid, treating with norite, and reprecipitating with ammonium hydroxide. The compound softened on heating at about 195° centigrade and finally melted at 235-238° centigrade. Analytical: Calcd. C, 57.6; H, 4.84. Found C, 57.5; H, 5.08.

This formamidopyrimidine (100 milligrams) was boiled with 4 milliliters of formamide for ten minutes. Upon dilution with 10 milliliters of water and cooling overnight, 70 milligrams (78%) of 9-phenyladenine was

obtained. Melting point 232-236°C dec.

C. 6-anilino-4,5-diaminopyrimidine sulfate (1 gram) was dissolved in 300 milliliters of water and the solution brought to neutrality with aqueous sodium hydroxide. Two grams of sodium dithioformate was added and the mixture allowed to stand for two days at room temperature. Upon concentration to one-half volume and cooling .58 grams (79%) of 9-phenyladenine was obtained. Recrystallization from 50% ethanol-water gave a melting point of 235-238° centigrade.

9-phenylhypoxanthine. One-half gram of 9-phenyladenine was dissolved in 20 milliliters of water containing 2 grams of concentrated sulfuric acid. The solution was cooled to 70-80° centigrade and an aqueous solution of sodium nitrate (1 gram in 5 milliliters of water) was added slowly with stirring. The mixture was boiled for 3-5 minutes and then cooled and filtered. The crude hypoxanthine was dissolved in 2 normal sodium hydroxide, treated with norite, filtered and acidified with acetic acid. Yield .26 gram (56%). Melting point 306-308°C dec. Analytical: Calcd. C, 62.3; H, 3.80; Found C, 62.1; H, 4.05.

9-benzyladenine. One gram of 6-benzylamino-4,5-diaminopyrimidine was boiled in 10 milliliters of formamide for twenty minutes, diluted with water, concentrated before a hot air fan, rediluted with water and

cooled overnight. Recrystallization from 50% ethanol-water gave .43 gram. Melting point 192-210°C. Extraction of this with 20 milliliters of warm 1 normal sodium hydroxide left a residue of .37 grams (53.5%). Melting point 224-225°C dec. Analytical: Calcd. C, 64.0; H, 4.92. Found C, 63.8; H, 5.00.

9-benzylhypoxanthine. 9-benzyladenine was converted to the corresponding hypoxanthine by the method described in the synthesis of 9-phenylhypoxanthine. Yield .21 gram (42%). Melting point 254-258°C. Analytical: Calcd. C, 63.6; H, 4.46. Found C, 63.5; H, 4.54.

9-methyladenine.[3] One gram of 4,5-diamino-6-methylaminopyrimidine sulfate was boiled for fifteen minutes in ten milliliters of formamide. The mixture was diluted with 20 milliliters of water and then concentrated before a hot air fan to yield .48 grams (76%). Recrystallization from water gave a melting point of 300° centigrade with sublimation. Analytical: Calcd. C, 48.3; H, 4.73. Found C, 48.4; H, 4.80.

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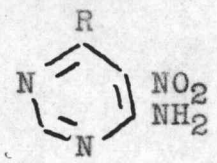
3. Previously reported (11, pp. 2250-2251)

TABLE I



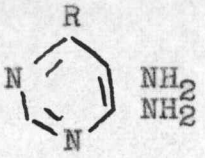
R	Yield	Melting Point	Empirical Formula	Carbon % Calcd.	Carbon % Found	Hydrogen % Calcd.	Hydrogen % Found
Morpholino[a]	79	176-179	$C_6H_{11}N_5O_3$	42.7	42.8	4.92	5.06
Anilino[b]	85	212-214	$C_{10}H_9N_5O_2$	51.9	52.0	3.92	4.06
Benzylamino[b]	94	191-194	$C_{11}H_{11}N_5O_2$	53.9	54.1	4.52	4.23
Methylamino[c]	72	241-245	$C_5H_7N_5O_2$	35.5	35.7	4.17	4.32

a - recrystallized n-butanol

b - recrystallized dioxane-water

c - reaction was carried out in H_2O. No recrystallization was required.

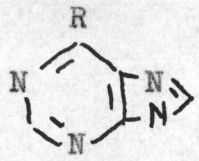
TABLE II



R	Yield	Melting	Empirical Formula	Carbon % Calcd.	Carbon % Found	Hydrogen % Calcd.	Hydrogen % 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-morphilino-4,5-aminopyrimidine 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



R	Yield	Melting Point	Empirical Formula	Carbon % Calcd.	Carbon % Found	Hydrogen % Calcd.	Hydrogen % 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 50% ethanol-water

b - recrystallized from absolute ethanol.

c - reported previously by Elion (9, p. 412)

d - reported previously by Miller (20, pp. 2662-2663)

TABLE IV
Ultraviolet Absorbtion Spectra
(pH 6)

	Maximum	ε	Minimum	ε
6-morpholinopurine	282	$1.89 \cdot 10^4$	238	$2.16 \cdot 10^3$
6-anilinopurine	290	$1.40 \cdot 10^4$	243	$2.38 \cdot 10^3$
6-benzylaminopurine	268	$1.62 \cdot 10^4$	233	$1.98 \cdot 10^3$
6-furfurylaminopurine	266	$1.88 \cdot 10^4$	234	$3.32 \cdot 10^3$
9-phenyladenine	260	$1.41 \cdot 10^4$	241	$9.56 \cdot 10^3$
9-phenylhypoxanthine	227	$2.01 \cdot 10^4$		
9-benzyladenine	260	$1.42 \cdot 10^4$	233	$2.14 \cdot 10^3$
9-benzylhypoxanthine	247	$1.68 \cdot 10^4$	229	$6.42 \cdot 10^3$
9-methyladenine	261	$1.31 \cdot 10^4$	229	$2.22 \cdot 10^3$

SUMMARY

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-chloro-purine 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 were therefore unsuccessful.

The proof of the structure of these 9-substituted adenines was obtained from their conversion to the corresponding hypoxanthines and from their lack of acidic characteristics.

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