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A SYNTHESIS OF 1-METHYL, N^2-ALKYLGUANINES

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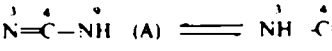
Abstract--An unambiguous one step synthesis of the titled compounds is described through a reduction and cyclization of the appropriate 5-nitrosopyrimidine IX (R, a–d).

ON THE basis of results obtained from the oxidation of different hydroxy- and aminopurines by xanthine oxidase (XO), Bergmann *et al.*[1,2] have concluded, that the participation of the tautomeric form B of the partial structure of the purine ring



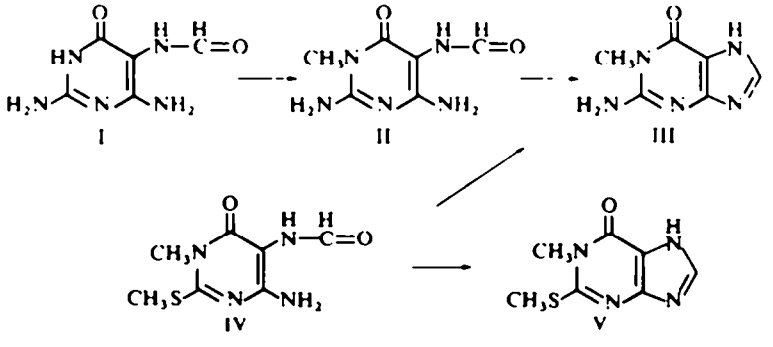
is necessary for the enzymatic process which probably involves chelation with an appropriate receptor in the active center. Furthermore it has been shown, that N^2-dimethylguanine is oxidized at position 8.

If the above assumption is correct, N^2-dimethylguanine methylated at either N^1 or N^3 should not be attacked by XO, as compounds of this type cannot form an "active" tautomer.

In view of the above suggestion concerning the action of XO, we wish to report, that we have completed a facile synthesis of 1-methyl, N^2-dialkylguanines (Xa–d). This group of compounds which has hitherto been unavailable, may prove to be of value in studies with this enzyme.

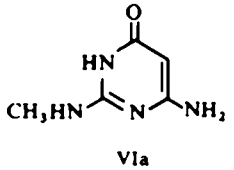
A number of unsuccessful, or only partially successful attempts at the synthesis of this type compounds have been reported in the literature. Thus, direct methylation of guanine by a number of methylating agents has been found to give a mixture of isomers bearing the Me group in position 1, 7, or 9.[3]

An unambiguous synthesis of 1-methylguanine (III) has been carried out[4] by methylation of I, giving rise to II followed by cyclization with formamide to give III.

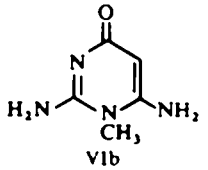


Elion[5] has found that by ring closure of IV with formamide, III is formed as a minor by-product only, apparently by the reaction of the 2-methylmercapto group with ammonia generated during the heating with formamide. Upon heating of V[5] with aqueous dimethylamine in a closed vessel at 150°, only the appropriate xanthine has been isolated.[6]

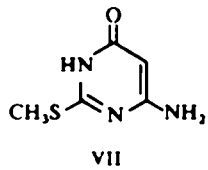
On the other hand, the synthesis of N^2-monomethylguanine has been carried out with only partial success.[7] The condensation of methylguanidine and ethyl cyanoacetate affords VIa and VIb, obviously as a result of a Dimroth rearrangement under alkaline condensation.[8]



VIa



VIb



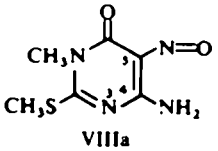
VII

An attempted substitution of 2-methylthio-4-aminopyrimidine-6-one (VII), under rather drastic conditions has been reported by Roth *et al.*[7] Boiling VII with the appropriate amine lead usually to no reaction. In some cases either a mixture was obtained or a complete discomposition of the compound was observed.

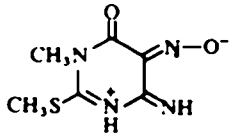
Until recently, the direct methylation of N^2-dimethylguanine was not investigated.

The route chosen by us for an unambiguous synthesis of compound X was based on the following considerations:

a. It has been observed,[10] that if the condensation of S-methylthiouronium sulfate with nitrosodiomalondialdehyde is carried out in piperidine, the isolated product is 2-piperidino-5-nitrosopyrimidine. The use of 1-ethylpiperidine affords the desired substance, i.e. 2-methylthio-5-nitrosopyrimidine.
b. Introduction of a nitroso group is reported to make VII reactive to different nucleophiles.[11]
c. N^3-methylation of guanine increases the electrophylicity of the pyrimidine ring and stabilizes the negative charge of the imidazole ring.[12]
d. The reactivity of the pyrimidine ring increases by quaternization of the ring-N in the position 3, and as a result, the adjacent C atom is converted into a reactive center of the molecule.[12] Atom N^3 in VIII being a member of an amidine group can be quaternized by the proton gained from N^4 with the contribution of a 5-nitroso group.



VIIIa



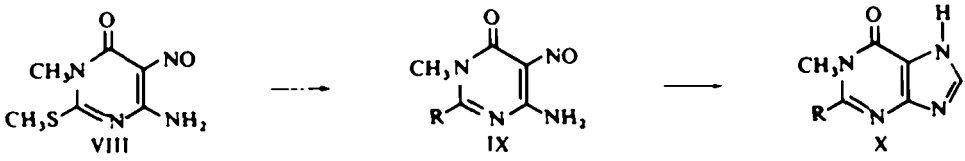
VIIIb

The above discussed data let us believe that the introduction of a nitroso group—a powerful electron-withdrawing center—into position 5, would accentuate the electron-deficiency in the pyrimidine ring (VIIIa–b) similar to the same effect observed in the imidazole moiety in 3-methylguanine.[12] We chose therefore 4-amino-5-nitroso-1-methyl-2-methylthiopyrimidine-6-one (VIII)[9] for starting material. The nitroso

group indeed caused a considerable increase in the reactivity. The nucleophylic displacement of the 2-methylmercapto group was carried out under mild conditions by boiling VIII with the appropriate amine in an ethanolic solution. (Use of an aquous solution did not lead to the desired product.)

The rapid displacement was evidenced by the evolution of methyl mercaptan, which was observed by a filter paper treated with lead acetate (yellow spot). As the evolution of the gas ceased (about 20–30 min), the product was collected from the chilled reaction mixture and in most cases it was pure according to chromatographic data.

We did not observe any difference in the reaction of 2-methylthio-4-amino-5-nitroso-pyrimidine-6-one and its 1-methyl homolog.



R: a = dimethylamino
b = pyrrolidino
c = piperidino
d = morpholino

R: a–d as in IX

The reduction of the nitroso group IX (R, a–d) and cyclization to the imidazole ring was carried out under mild conditions in a one step reaction. Thus the dissolved

TABLE 1. AMOUNTS OF STARTING MATERIALS FOR THE PREPARATION OF 1-METHYL-2-ALKYLAMINO-4-AMINO-5-NITROSO PYRIMIDINE-6-ONE (IX; R, a–d) AND YIELDS OBTAINED

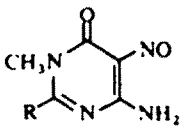
R	Start. mat.[a] g	Amine[b] ml	Amine[b] %	Yield %
Xa	2·0	60	25	62
Xb	4·0	6·9	8·6	85
Xc	4·0	7·6	9·8	65
Xd	4·0	6·5	8·1	61

[a] Compound VIII. [b] Amines in 96% ethanol.

compound IX in 98–100% formic acid, sodium dithionite was added at room temperature. The colour of the solution turned to yellow within a few minutes and the reaction mixture boiled for 8 hr. The products were obtained in a high purity in a yield of 65–90%.

This smooth one step reduction-cyclization was studied with four compounds. Thus, N^2-dimethylamino-4-amino-5-nitrosopyrimidine-6-one gave N^2-dimethylguanine, which was identical in all respects with an authentical sample prepared by the method of Elion *et al.*[13]

TABLE 2. PHYSICAL PROPERTIES AND ANALYTICAL DATA OF 1-METHYL-2-ALKYLAMINO-4-AMINO-5-NITROSO PYRIMIDINE-6-ONE (IX)



R	M.p.[a] °C	Formula	C %		H %		N %		λ_{max} (mμ) 0·1N HCl	R_f[b]
			Calcd.	Found	Calcd.	Found	Calcd.	Found		
Dimethylamino-[c]	258/9	$C_7H_{11}N_5O_2$	42·6	42·7	5·5	5·7	35·5	35·7	225 275 325	0·82
Pyrrolidino-	237/8	$C_8H_{13}N_5O_2$	48·4	48·4	5·8	6·0	31·4	31·8	226 270 325	0·83
Piperidino-	182/3	$C_{10}H_{15}N_5O_2$	50·6	50·2	6·3	6·5	29·5	29·4	230 270 330	0·89
Morpholino-	227	$C_8H_{13}N_5O_3$ $\frac{1}{4}H_2O$	44·4	44·5	5·7	5·9	28·7	28·4	226 275 335	0·78

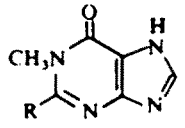
[a] All m.ps were taken on a Thomas–Hover m.p. apparatus and are uncorrected

[b] Solvent: Isopropanol DMF 25% ammonia = 65:25:10; all R_f values refer to theophilline (R_f = 0·75).

[c] Ref 5.

In this new synthetic procedure the isolation of the reduction product is eliminated. The formylation of the 5-amino group and the following dehydration-cyclization is carried out in the same reaction mixture.

TABLE 3 SPECTROPHOTOMETRIC AND CHROMATOGRAPHIC DATA OF 1-METHYL-N^2-ALKYLAMINOGUANINES

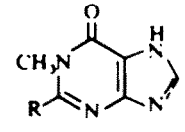


R	0·1N HCl		0·1N NaOH		R_f in Solvent[a]			Fluorescence[b]
	λ_{max} (mμ)	log ε max	λ_{max} (mμ)	log ε max	A	B	C	
Dimethylamino-	265	4·22	223	4·06	0·73	0·79	0·78	Blue
			270	3·80				
Pyrrolidino	265	4·10	228	4·41	0·75	0·83	0·79	Violet
			269	4·11				
Piperidine	267	3·94	225	4·49	0·78	0·84	0·82	Violet
			269	4·30				
Morpholino-	265	4·11	225	4·34	0·75	0·79	0·77	Violet
			271	4·13				

[a] Solvent A: Ethanol–Acetic Acid–Water = 85:5:10;
B: Isopropanol–DMF–25% Ammonia = 65:25:10;
C: Ethanol–DMF–Water = 60:20:20;
All R_f values refer to theophylline (R_f = 0·75).

[b] Under a Minerallight lamp, emitting light about 254 mμ.

TABLE 4 PHYSICAL PROPERTIES AND ANALYTICAL DATA OF 1-METHYL-N^2 ALKYLAMINOGUANINES



R	M.p.[a] °C	Carbon		Hydrogen		Nitrogen		Yield %
		Calcd.	Found	Calcd.	Found	Calcd.	Found	
Dimethylamino-	280	49·7	49·4	5·7	5·8	36·3	36·7	82
Pyrrolidino-	250	54·8	54·9	5·9	6·2	32·0	32·0	65
Piperidino-	246	56·6	56·3	6·4	6·6	30·0	30·0	72
Morpholino-	276	51·1	51·0	5·5	5·6	29·8	29·3	90

[a] All m.ps are uncorrected.

EXPERIMENTAL

Absorption spectra were measured in a CF 4 "OPTICA" spectrophotometer.

Paper chromatograms were developed by the descending method using Whatman paper No. 1.

Starting material. Known methods were used for the preparation of 1-methyl-2-methylthio-4-amino-5-nitrosopyrimidine-6-one.[9]

General procedure. Preparation of IX (R, a–d). The quantity of compound VIII given in Table 1 was heated under reflux for 30 min with the appropriate amine in 96% ethanolic soln. The product crystallized on

chilling and was collected by filtration. Only X (R = d) had to be recrystallized from boiling water. All other substances were chromatographically pure and were used without any further purification in the next step. The physical properties and analytical data of IX (R, a–d) obtained are given in Table 2.

1-Methyl-N²-alkylaminoguanines. (X; R = a–d). To the appropriate nitroso-pyrimidine IX (1 g) in 80 ml 98–100% formic acid 2 equivs of $Na_2S_2O_4 \cdot 2H_2O$ were added at room temp. After 5 min stirring by means of a magnetic stirrer, the discoloured soln was heated under reflux for 8 hr. After cooling, the amorphous S was separated by filtration and the filtrate was then evaporated to dryness under reduced press. The residue was dissolved in 1N KOH with gentle heating, filtered and the soln was adjusted to pH 4 with glacial AcOH. Samples for analyses were recrystallized from water and dried at 100°/1mm over P_2O_5. The physical properties and analytical data of the 1-methyl-N²-alkylaminoguanines obtained are given in Tables 3 and 4.

2-Dimethylamino-4-amino-5-nitroso-6-hydroxypyrimidine.[9] This compound was synthetized by a modification of the method described.[7] 2-Methylthio-4-amino-5-nitrosopyrimidine-6-one[14] (25 g) was suspended in aqueous dimethylamine (25%; 300 ml) and refluxed for 20 min. After chilling the reaction mixture, the product was isolated by filtration. One recrystallization from boiling water afforded 15 g (60% yield) pure product, m.p. 259°. It was identical with a sample prepared by the method of Roth *et al.*[7]

N²-Dimethylguanine.[13] To 2-Dimethylamino-4-amino-5-nitrosopyrimidine-6-one (6 g) dissolved in 98–100% formic acid (150 ml) $Na_2S_2O_4 \cdot 2H_2O$ (13·8 g) was added at room temp. After the colour of the nitroso-compound disappeared the reaction mixture was boiled under reflux for 8 hr. After chilling, the S was removed by filtration. The formic acid was evaporated under reduced press on a water-bath. The residue in hot 1N KOH was reprecipitated by glac. ACOH. The solid was filtered to yield 4·6 g of product, m.p. 300°. The spectral data was identical with those given by Elion.[13] A small sample was recrystallized from boiling water for analysis. (Found: C, 46·9; H, 5·3; N, 39·4. Calc. for $C_7H_9N_5O$: C, 46·9; H, 5·0; N, 39·1%.)

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