Nitrosaminopurines and Nucleosides, Synthesis and Biological Activity†

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The growth inhibitory activity of 6-(*N*-nitrosohydroxylamino)purine (**1**) prompted the synthesis of new nitroso- and (methylnitrosamino)purine and nucleoside derivatives. Oxidation or nitrosation of some (hydroxylamino)purines led to their nitroso or nitrosohydroxylamino derivatives. Several (methylnitrosamino)-purines have been obtained in good yields by the unusual reaction of nitrous acid on α-methylhydrazino-purines. Among them 2-, 6-, and 8-(methylnitrosamino)purine (**15**, **19**, **22**), the 3-oxide of **19** (**16**), and 2-(methylnitrosamino)hypoxanthine (**23**) were obtained. Nitrosation of (methylamino)purines also gave (methylnitrosamino)purines, though this reaction is less effective. Screening tests of some of the new nitroso derivatives against mouse leukemia have thus far shown no inhibitory activity.

Screening studies on 6-(*N*-nitrosohydroxylamino)purine (**1**)[1] showed a marked inhibitory activity against several solid tumors and mouse leukemia. We have prepared several nitroso derivatives, mainly (*N*-methylnitrosamino)- and (*N*-nitrosohydroxylamino)purines and nucleosides in order to investigate their biological activities.

Some antibiotics obtained from *Streptomyces* contain the *N*-nitroso function; thus, alanosine,[2] an amino acid [L-2-amino-3-(nitrosohydroxylamino)propionic acid], has antiviral and antitumor properties. Synthetic derivatives of 1-methyl-1-nitrosourea have been extensively investigated;[3] some of them have been found to inhibit growth.[4]

It is known that many aliphatic and aromatic *N*-nitrosamino derivatives are among the potent oncogenic compounds.[5] It has been postulated that although *N*-nitrosamino derivatives are not found widely in nature, there exists the possibility that they can be formed *in vivo*.[5,6]

Purine *N*-oxide derivatives such as guanine and xanthine 3-oxide exert oncogenic activity and could be considered as potential oncogens of endogenous origin.[7] Another possible source of endogenous oncogens could be found in substituted (*N*-nitrosamino)purines. The secondary amines, 6-(methylamino)purine[8,9] and 2-(methylamino)hypoxanthine,[10] are present as minor bases of transfer RNA and also DNA. More recently, other minor components of transfer RNA, purines and pyrimidines containing secondary amines, have been isolated.[11] These bases could be nitrosated *in vivo*, thus forming (methylnitrosamino)purines that could show oncogenic effects. In order to study the potential biological activities of nitrosaminopurines, we have synthesized 6-(methylnitrosamino)purine, its 3-oxide, 2-(methylnitrosamino)hypoxanthine, and other related derivatives.

It is known from the studies of the Millers[12] and the Weisburgers[13] that, in order to be oncogenic, N-substituted aromatic amines have to undergo metabolic conversion to hydroxylamino derivatives. Purines containing secondary amines could conceivably be oxidized *in vivo* to N-substituted hydroxylaminopurine derivatives. When applied to 6-(methylamino)purine and 2-(methylamino)hypoxanthine, this oxidation would correspond to their conversion to 6-(methylhydroxylamino)purine and 2-(methylhydroxylamino)hypoxanthine. The former compound was previously reported[14] and is currently being assayed for oncogenicity. The only (*N*-nitrosamino)purine derivative thus far assayed, 6-(*N*-nitrosohydroxylamino)purine, was found devoid of oncogenic activity.

It was found that the 3-oxide of 6-(hydroxylamino)purine[15] possesses a greater activity against mouse leukemia‡ than the parent compound, 6-(hydroxylamino)purine,[16] and that the 9-ribosyl derivative of the latter had a greater chemotherapeutic index than the base.[17] We have prepared the 3-oxide and the 9-ribosyl derivative of 6-(*N*-nitrosohydroxylamino)-purine to study their potential growth inhibitory activity.

Synthetic Studies

It was found by Robins that nitrosation of aminopurines such as 6,8-diaminopurine gives diazonium and nitropurine derivatives.[18] Later Shapiro reported that nitrous acid treatment of DNA yields 2-nitrohypoxanthine;[19] on the other hand in our studies and those of Montgomery, nitrosation of hydrazinopyrimidines or purines affords either azido or tetrazolo derivatives.[1,20,21] We previously reported the oxidation and nitrosation of 6-(hydroxylamino)purine[16] to yield respectively 6-nitrosopurine and 6-(*N*-nitrosohydroxylamino)purine (**1**).[1]

α-Methylhydrazines were generally prepared by reaction of an excess of dilute ethanolic methylhydrazine and the purine containing the appropriate leaving group (halogeno, methylmercapto, methylsulfonyl) at reflux temperature (Table I). Exceptionally, 6-(α-methylhydrazino)purine 3-oxide (**5**) was prepared at 25° to avoid reduction of the *N*-oxide function and 2-fluoro-6-(α-methylhydrazino)purine (**9**) at 5° from 6-chloro-2-fluoropurine (**8**). When the latter compound **8** was treated at reflux temperature, substitution at C_2 also occurred forming 2,6-di(α-methylhydrazino)-purine (**10**).

We have found a convenient new route for the preparation of methylnitrosamino derivatives in the nitrosation of α-methylhydrazino compounds. We observed that when 6-(α-methylhydrazino)purine (**17**) was treated with 1–3 equiv of $NaNO_2$ in acid solution, 6-(methylnitrosamino)purine (**19**) was formed in about 1 hr in good yield. Similar results were obtained with 2-(α-methylhydrazino)hypoxanthine (**14**) and other (α-methylhydrazino)purines (Table II).

We also found that other methylhydrazines undergo the same nitrosation reaction. In this manner, when 2-methylsemicarbazide (α-methylhydrazinocarbamide) (**28**) was treated with $NaNO_2$ and HCl, nitrosomethylurea (**29**) was readily formed. Likewise, α-methylhydrazino-*p*-nitrobenzene (**30**) and 2-(α-methylhydrazino)-5-nitropyridine (**33**) [from 2-chloro-5-nitropyridine (**32**) and CH_3NHNH_2] were converted

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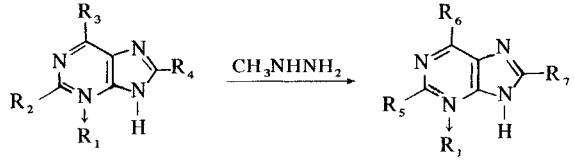
‡Unpublished data from J. H. Burchenal.

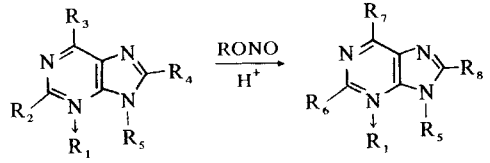
Table I. Synthesis of (α-Methylhydrazino)purines



Starting material	R_1	R_2	R_3	R_4	Amount, mmol	Ratio[a] $MeNHNH_2$: purine	Reaction time, hr	Temp, °C	Reaction product	Yield, %	Mp, °C	R_1	R_5	R_6	R_7	Formula	Analyses[b]
2[c]		F	H	H	5	8	2	80	**3**	62	236		CH_3NNH_2	H	H	$C_6H_8N_6$	C, H, N
4[d]	O	H	Cl	H	30	5	72	25	**5**	55	240 dec	O	H	CH_3NNH_2	H	$C_6H_8N_6O$	C, H, N
6		H	H	SCH_3	30	3.5	20	80	**7**	74	266		H	H	CH_3NNH_2	$C_6H_8N_6 \cdot 0.5H_2O$	C, H, N
8[c]		F	Cl	H	2	10	18	5	**9**	75	270		F	CH_3NNH_2	H	$C_6H_7N_6F \cdot {}^2/_3H_2O$	C, H, N, F
8[c]		F	Cl	H	5	10	3	80	**10**	83	298		CH_3NNH_2	CH_3NNH_2	H	$C_7H_{12}N_8 \cdot H_2O$	C, H, N
11[c,e]		F	SH	H	12	3	18	80	**12**	45	310 dec		CH_3NNH_2	SH	H	$C_6H_8N_6S$	C, H, N, S
13[f]		CH_3SO_2	OH	H	5	6	12	80	**14**	70	>300		CH_3NNH_2	OH	H	$C_6H_9N_6O \cdot H_2O$	C, H, N

[a] A 20% methylhydrazine ethanolic solution was used. [b] Analytical samples dried at 25° *in vacuo*. [c] J. A. Montgomery and K. Hewson, *J. Amer. Chem. Soc.*, 82, 463 (1960). [d] *Cf.* ref 32. [e] A. Giner-Sorolla and J. H. Burchenal. *J. Med. Chem.*, **14**, 816 (1971). [f] M. Ikehara, A. Yamazaki, and T. Fujieda, *Chem. Pharm. Bull.*, **10**, 1075 (1962).

Table II. Synthesis of (Methylnitrosamino)purines



Starting material	R_1	R_2	R_3	R_4	R_5	Amount, mmol	Ratio $NaNO_2$: purine	Acid[a]	Reaction time, hr	Temp, °C	Yield, %	Reaction product	Mp, °C	R_1	R_5	R_6	R_7	R_8	Formula	Analyses[b]
3		CH_3NNH_2	H	H	H	5.0	4	A	1	5	37	**15**	>300		H	CH_3NNO	H	H	$C_6H_6N_6O \cdot {}^2/_3H_2O$	C, H, N
5	O	H	CH_3NNH_2	H	H	1.5	20	A	1.5	5	27	**16**	214 exptl	O	H	H	CH_3NNO	H	$C_6H_6N_6O_2 \cdot H_2O$	C, H, N
17[c]		H	CH_3NNH_2	H	H	10.0	3	A	1	5	72	**19**	252 dec		H	H	CH_3NNO	H	$C_6H_6N_6O$	C, H, N
18		H	CH_3NH	H	H	1.0	18	B	7	5	69									
20		H	CH_3NH	H	Ribosyl	2.0	4	B	24	5	39	**21**	113 eff		Ribosyl	H	CH_3NNO	H	$C_{11}H_{14}N_6O_5$	C, H, N
7		H	H	CH_3NNH_2	H	5.3	8	A	1	5	71	**22**	232 dec		H	H	H	CH_3NNO	$C_6H_6N_6O \cdot 0.5H_2O$	C, H, N
14		CH_3NNH_2	OH	H	H	1.0	7	A	1	5	20	**23**[d]	>300		H	CH_3NNO	OH	H	$C_6H_6N_6O_2 \cdot 1{}^1/_3H_2O$	C, H, N
24[d]		CH_3NH	CH_3NH	H	H	3.0	4	B	2	25	64	**25**	265 dec		H	CH_3NH	CH_3NNO	H	$C_7H_9N_7O \cdot H_2O$	
26[c]		NH_2	CH_3NNH_2	H	H	3.0	5	B	4	5	34	**27**	>300		H	OH	CH_3NNO	H	$C_6H_6N_6O_2 \cdot {}^1/_3H_2O$	C, H, N

[a] A, 2 *N* HCl; B, 50% aqueous AcOH. [b] Samples were dried at 25° *in vacuo*. Compound **16**, H: calcd, 3.80; found, 3.21. **22**, H: calcd, 3.77; found, 3.26. **23**, H: calcd, 4.00; found, 3.16. **38**, N: calcd, 38.44; found, 36.85. [c] *Cf.* ref 14. [d] Attempts to prepare 2-(methylnitrosamino)hypoxanthine (**23**) from 2-(methylamino)hypoxanthine (1.2 mmol) [G. H. Hitchings and G. B. Elion, U. S. Patent 2,697,709 (1954); *Chem. Abstr.*, **50**, 1933 (1956)] in 50% aqueous AcOH (20 ml) and $NaNO_2$ (20 mmol) at 25° for 8 hr resulted in a mixture of **23** and starting material in about a 2:3 ratio.

to (methylnitrosamino)-*p*-nitrobenzene (**31**) and 2-(methylnitrosamino)-5-nitropyridine (**34**), respectively, by NO_2^- treatment.

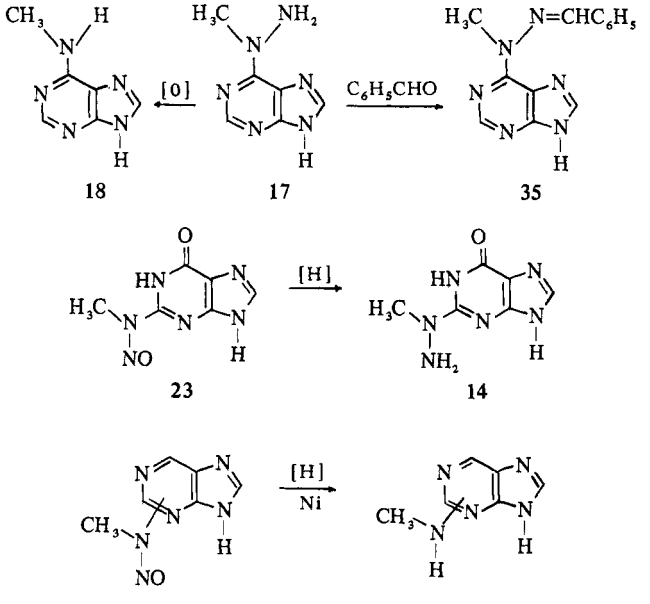
Therefore, it appears that the nitrosation of methylhydrazines to yield methylnitrosamines is a reaction of general application which in most cases occurs very rapidly, presumably by the formation of an intermediate diazonium salt which undergoes a displacement by NO, or, less likely, by an elimination of NH_3 from the α-methylhydrazino group and subsequent nitrosation of the resulting methylamino derivative with little or no excess of nitrosating agent. The nitrosation of (methylamino)purines has also been used as a confirmatory and alternate route to (methylnitrosamino)purines (Table II). It is a much slower reaction (about 5 hr) that requires a larger amount of $NaNO_2$ (10–20 equiv) and in some cases fails to take place.

The preliminary studies on nitrosation of 6-(methylamino)purine (**18**) were made by Dunn and Smith,[8] but they did not isolate and identify the reaction product. Jones and Walker[22] isolated and identified 6-(methylnitrosamino)purine (**19**) and also observed that the uv spectrum reported by Dunn and Smith was erroneous. They attributed the error to decomposition at pH 1 and 13 to yield **18**. A synthesis of **19** was later reported by Shapiro and Shiuey.[23]

There is also the possibility that the methylnitrosamino function is formed by oxidation of the NH_2. It is known that aromatic amines are usually oxidized to nitroso derivatives with peroxy acids.[24] We found that at 25°, peroxyacetic acid oxidation of the methylhydrazino derivatives gave a mixture of methylnitrosamino and methylamino derivatives; at 85°, however, the latter is exclusively formed, possibly by hydrolysis of the nitroso group (Scheme I). On the other hand, oxidation of **17** with $FeCl_3$ gave exclusively **18**. In previous work, upon reaction of $FeCl_3$ with 6-hydrazinopurine, 6-chloropurine was obtained though in low yield, presumably by formation of a diazonium chloride which underwent a Sandmeyer type of reaction.[16]

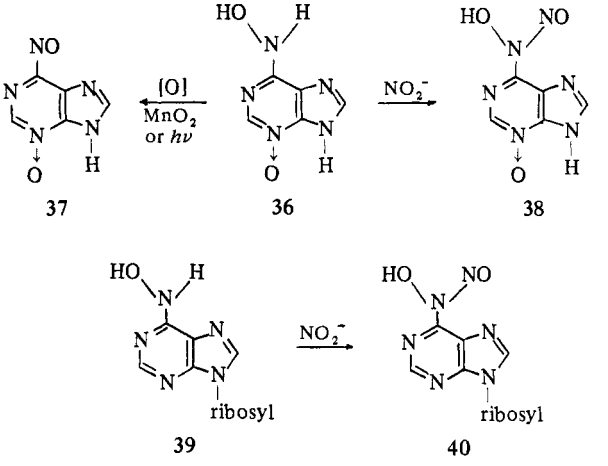
Reduction of (methylnitrosamino)purines with Raney Ni gave the corresponding (methylamino)purine. This behavior was also reported in the reduction of aromatic methylnitrosamines.[25] Exceptionally, Raney Ni reduction of 2-(methylnitrosamino)hypoxanthine (**23**) gave 2-(α-methylhydrazino)hypoxanthine (**14**)[26] (Scheme I) and in the case

Scheme I



18 17 35 23 14

Scheme II



37 36 38 39 40

of 6-(methylnitrosamino)purine 3-oxide (**16**) a stepwise reduction took place, namely, first into **19** then upon further treatment to **18**.

Benzaldehyde reacted with **17** to give α-benzylidino-β-methyl-β-6-purinylhydrazine (**35**) thus confirming its structure (Scheme I). (α-Methylhydrazino)purines (*cf.* ref 14) were mistakenly assigned a (β-methylhydrazino)purine formulation.

In contrast to the reaction of (α-methylhydrazino)purines with NO_2^- which in most cases required at least 1 hr to reach completion, the nitrosation of 6-(hydroxylamino)purine[16] and its 3-oxide[15] (**36**) occurred instantly (Scheme II). Oxidation of **36** with MnO_2 gave 6-nitrosopurine 3-oxide (**37**); this compound was also spontaneously formed from **36** by long exposure to diffuse light. Prolonged reaction of 9-β-D-ribofuranosyl-6-(hydroxylamino)purine (**39**)[27] with ethyl nitrite led to the corresponding 6-(*N*-nitrosohydroxylamino) derivative **40** in low yield.

Experimental Section§

(α-Methylhydrazino)purines. The data concerning the synthesis of these compounds are listed on Table I. Analytical samples were obtained by recrystallization with aqueous EtOH.

(Methylnitrosamino)purines. Their preparation is given in Table II, some of the uv spectra in Table III. All the described nitroso derivatives gave a positive Liebermann test.[28] Analytical samples were obtained by repeated washing with H_2O and EtOH and drying *in vacuo* at 25°.

Reaction of 2-Methylsemicarbazide (28) with Nitrous Acid. A solution of **28**[29] (1.50 g, 20 mmol) in 2 *N* H_2SO_4 (15 ml) was treated at 5° with $NaNO_2$ (2.8 g, 40 mmol) in H_2O (5 ml). After 1 hr of stirring at 5°, the resulting crystalline product was filtered and washed with cold H_2O to yield 1.08 g (57%) of a product which was identified as nitrosomethylurea (**29**) by ir and mixture melting point.

Reaction of (α-Methylhydrazino)-*p*-nitrobenzene (30) with Nitrous Acid. A solution of recrystallized (α-methylhydrazino)-*p*-nitrobenzene[30] (**30**) (1.25 g, 7 mmol) in 2 *N* HCl (20 ml) was treated with $NaNO_2$ (3.84 g, 56 mmol) in H_2O at 5° (6 ml) to yield 1.11 g (88%) of (methylnitrosamino)-*p*-nitrobenzene (**31**), orange needles, mp 97–99°. *Anal.* ($C_7H_7N_3O_3$) C, H, N.

2-(α-Methylhydrazino)-5-nitropyridine (33). A solution of 2-

§ Uv absorption spectra were determined with Beckman recording spectrophotometers, DBG and DU. Ascending paper chromatography was run on Whatman No. 1 paper in the solvent systems: concentrated aqueous NH_3–H_2O–isopropyl alcohol (10:20:70); 1-butanol–H_2O–formic acid (50:25:25); and 1 *M* ammonium acetate–EtOH (30:70). The determination of melting points was made with a Thomas-Hoover and Mel-Temp melting point apparatus and were corrected. Analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich. When analyses are indicated by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.

Table III. Uv Spectral Properties of Some (Methylnitrosamino)purines

pH	λ max, nm (ε × 10^{-3})
	2-(Methylnitrosamino)purine (15)
5	241.5 (9.7), 285.5 (11.9)
13	257.5 (11.1), 282 (10.3)
	6-(Methylnitrosamino)purine 3-Oxide (16)
1	246 (6.4), 288 (9.0), 323 (11.7)
7	241 (11.6), 322 (10.5)
13	246 (14.4), 327 (12.1)
	6-(Methylnitrosamino)purine (19)[a]
1	216 (10.6), 252 (5.7), 295 (10.7)
7	216 (11.4), 262 (6.8), 300 (10.3)
13	225 (12.9), 310 (7.4)
	9-β-D-Ribofuranosyl-6-(methylnitrosamino)purine (21)
3	221 (12.3), 265 (5.7), 295 (9.7)
7	224 (12.9), 265 (5.7), 295 (9.7)
13	227 (12.9), 265 (5.7), 295 (9.7)
	8-(Methylnitrosamino)purine (22)
1	300 (15.6)
7	296.5 (11.7)
13	302 (14.8)
	2-(Methylnitrosamino)hypoxanthine (23)
1	254 (9.1), 287.5 (4.9)
7	242.5 (9.4), 261 (7.7)
13	245 (10.0), 263 (6.6), 318.5 (2.5)

[a] See ref 23.

chloro-5-nitropyridine (32, 3 g, 17 mmol)# in 10% ethanolic solution of methylhydrazine (75 ml) was refluxed for 3 hr. The bright orange precipitate was filtered and washed with EtOH and dried to yield 2.51 g (88%) of 2-(α-methylhydrazino)-5-nitropyridine (33), mp 175°. *Anal.* ($C_6H_8N_4O_2$) C, H, N.

Nitrosation of 2-(α-Methylhydrazino)-5-nitropyridine (33). A solution of 33 (1.0 g, 6 mmol) in 2 *N* HCl (15 ml) was cooled to 5°. Treatment with $NaNO_2$ (1.66 g, 24 mmol) in H_2O (3 ml) at 5° gave 0.98 of orange needles (90%) of 2-(methylnitrosamino)-5-nitropyridine (34), mp 105–107° eff. *Anal.* ($C_6H_6N_4O_3$) C, H, N.

Reaction of 6-(α-Methylhydrazino)purine (17) with Benzaldehyde. Benzaldehyde (3 ml) was added to a hot solution of 17[14] (0.65 g, 4 mmol) in EtOH (100 ml) and the mixture refluxed for 5 hr. The solution was evaporated to dryness *in vacuo* and washed with EtOH to yield 0.82 g (81%) of a colorless crystalline product, mp 250°. Repeated recrystallization from EtOH gave colorless prisms: mp 252°; λ max in H_2O (pH 5.5) 330 nm, λ min 229 nm. *Anal.* ($C_{13}H_{11}N_6$) C, H, N.

Reaction of 17 with Peroxyacetic Acid. A solution of 17[14] (0.5 g, 3 mmol) in glacial AcOH (5 ml) and 30% H_2O_2 (1.5 ml) was kept at 25° for 3 days. The resulting crystalline precipitate was collected by filtration and identified as 6-(methylnitrosamino)purine (19), mp 252° dec (0.12 g, 23%). From the filtrate upon evaporation to dryness under reduced pressure, 6-(methylamino)purine (18) chromatographically homogeneous (0.30 g, 67%) was obtained.

Treatment of 17 with $FeCl_3$. Finely divided 17 (0.5 g, 3 mmol) was added with stirring to a 2 *M* $FeCl_3$ aqueous solution (5 ml). An effervescence occurred and a deep blue solution appeared. After extraction with ether (liquid–liquid extractor) for 24 hr, 6-(methylamino)purine (18), 0.16 g (38%), was isolated from the ethereal extracts.

Treatment of (Methylnitrosamino)purines with Raney Nickel. A suspension or solution of each of the methylnitrosamino compounds (*ca.* 20 mg) in H_2O (10 ml) was boiled with Raney Ni (*ca.* 100 mg) for 1–2 hr. In each case, solutions of the corresponding (methylamino)purines were obtained and identified by uv spectra (pH 1, 6.8, and 11) and paper chromatography. Exceptionally, 2-(methylnitrosamino)hypoxanthine (23) was reduced to 2-(α-methylhydrazino)hypoxanthine (14) (Scheme I). In the case of 6-(methylnitrosamino)purine 3-oxide (16), a stepwise reduction occurred; 6-(methylnitrosamino)purine (19) was obtained first, and then upon further Ni treatment, 6-(methylamino)purine (18) was formed.

6-Nitrosopurine 3-Oxide (37). Method A. A suspension of 6-(hydroxylamino)purine 3-oxide[15] (36, 0.25 g, 1.5 mmol) in H_2O (200 ml) was stirred at 25°. Active MnO_2[31] (0.75 g) was added and the stirring was continued for 18 hr at 25°. The suspension was filtered and the filtrate evaporated to dryness *in vacuo* below 25°. The red residue was taken up with H_2O (3 ml) and the pH of the suspension adjusted to 6 with solid NaAcO. The precipitate was collected by filtration, washed with cold H_2O, and dried to yield 103 mg of red crystals (46%), mp >300°. This compound was identified as the free base of the previously described bis Na salt derivative.[32]

Method B. After being kept 30 months in a transparent clear vial exposed to diffuse light, a sample of analytically pure 6-(hydroxylamino)purine 3-oxide (36) was found to have changed color to scarlet red. Its uv and R_f values in three different solvent systems (single spot) showed that this compound was identical with the compound obtained by method A (37).[32] *Anal.* ($C_5H_3N_5O_2$) C, H, N.

6-(*N*-Nitrosohydroxylamino)purine 3-Oxide (38). A solution of $NaNO_2$ (75 mg, 1.1 mol) in H_2O (0.1 ml) was added slowly to a suspension of 36 (154 mg, 0.9 mmol) in 2 *N* HCl (2.0 ml) and and stirred at 5° for 1 hr. A yellow crystalline precipitate appeared instantly; it was collected by filtration, washed with a little cold H_2O, and dried to yield 96 mg (53%) of yellow needles, mp 140° eff, that turned orange and red on exposure to air. This compound, when treated with Raney Ni as indicated above, gave a solution containing exclusively adenine. *Anal.* ($C_5H_4N_6O_3 \cdot 1.25H_2O$) C, H; N: calcd, 38.44; found, 36.85.

9-β-D-Ribofuranosyl-6-(*N*-nitrosohydroxylamino)purine (40). Glacial AcOH (2 ml) was added to a suspension of 9-β-D-ribofuranosyl-6-(hydroxylamino)purine[27] (39) (1 g, 3.5 mmol) in 70% aqueous EtOH (40 ml) and ethyl nitrite (6 ml) was slowly added at 5° with stirring. After 24 hr of stirring at 5°, the precipitate was collected by filtration to yield a light cream solid (0.12 g, 11%), mp 180° eff. *Anal.* ($C_{10}H_{12}N_6O_6 \cdot {}^2/_3H_2O$) C, H, N.

When 39 was treated with 2 *N* HCl and $NaNO_2$ in the usual nitrosation reaction, hydrolysis occurred and the desired 40 could not be isolated.

Biological Activity. In mouse leukemia L1210 with 6-(*N*-nitrosohydroxylamino)purine (1) at 50 mg/kg qd × 5 (1 dose in each of five consecutive days) survivals up to 26 days (*vs.* 8 days for controls) were obtained.

Screening data on several mouse leukemias with the other new derivatives mentioned here show negative results. Thus, the 9-ribosyl derivative of 6-(*N*-nitrosohydroxylamino)purine (40) at 300 mg/kg (qd × 5) against mouse leukemia L1210 resistant to 6-mercaptopurine gave no increase in survival time. As a comparison, in the same screening experiment, 9-β-D-ribofuranosyl-6-(hydroxylamino)purine (39) was used at 200 mg/kg (qd × 5); all ten animals survived after 40 days (*vs.* 7.8 days for controls), thus indicating that introduction of the NO group caused a complete loss of activity of the resulting (nitrosohydroxylamino)ribosyl derivative 40. This fact may be attributed to the instability of the derivative. The 3-oxide of 6-(*N*-nitrosohydroxylamino)purine (38) and 6-(methylnitrosamino)purine (19), when administered at 50, 100, and 200 mg (qd × 5), against mouse leukemia P815 had no effect. Screening data of 2-(α-methylhydrazino)-6-mercaptopurine (12), at dosages from 50 to 400 mg/kg (qd × 5), against mouse leukemia P815 also showed negative results.

Oncogenesis assays have been made on 6-(*N*-nitrosohydroxylamino)purine (1) and were negative. The dosage and technique for these assays have been previously described.[33,34]

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Synthesis and Biological Activity of the Hypothalamic LH- and FSH-Releasing Decapeptide

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The decapeptide <Glu-His-Trp-Ser(Bzl)-Tyr(Bzl)-Gly-Leu-Arg(NO_2)-Pro-Gly-NH_2 has been synthesized by solution methods in a stepwise fashion and by the solid-phase method. All protected intermediates were purified by silica gel chromatography. Removal of the blocking groups with liquid HF and a two-stage purification on Sephadex G-25 and G-15 yielded a homogeneous synthetic product with the same chromatographic properties and the same ability to release LH and FSH, from rat pituitaries *in vitro*, as the naturally occurring hormone.

The release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary is under control of the hypothalamus which secretes a controlling substance designated LH-releasing hormone (LH-RH)-FSH-releasing hormone (FSH-RH).[1] Evidence has been presented that both LH and FSH are under control of the same hypothalamic releasing hormone.[2,3] The structure of this hormone, LH-RH/FSH-RH, was described as the decapeptide <Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (I) for the porcine species.[4]

In order to provide independent confirmation of the validity of structure I for the hormone, we synthesized[5] this decapeptide and attained as well a second goal of providing a convenient route to substantial amounts of decapeptide for the more extensive studies which are required to ascertain its biological role. Several syntheses have been reported[6-11] independently of ours, with yields which are very low or not stated.

For the synthesis of I, the desired triprotected decapeptide <Glu-His-Trp-Ser(Bzl)-Tyr(Bzl)-Gly-Leu-Arg(NO_2)-Pro-Gly-NH_2 (X) was synthesized by solution and by solid-phase methods.

In one approach, a stepwise method of synthesis was employed, starting from glycinamide and using mainly *tert*-butyloxycarbonyl (Boc) amino acid active esters.[12] The Boc group was removed from intermediate peptides without affecting side-chain protecting groups by employing trifluoroacetic acid (TFA)-CH_2Cl_2 (1:1),[13] except for tryptophyl peptides, in which case 1% mercaptoethanol was added. All intermediates were purified by column chromatography on silica gel and the structure was corroborated from their nuclear magnetic resonance spectra. The yields at each step were usually high (70–95%) and the overall yield of X was 10% based on glycinamide.

The triprotected decapeptide X was also assembled by the solid-phase method[14] employing 4 *M* HCl–dioxane for the removal of Boc groups.[15] An aliquot of the peptide-resin was retained as octapeptide-resin VIIIa, and the remainder of the material was converted to the decapeptide-resin Xa. The completed Xa was ammonolyzed and purified by chromatography on silica gel yielding triprotected decapeptide X in good yield (35–40%),† identical with X prepared by the stepwise method. Thus, the solid-phase method appeared to be a convenient method for obtaining X rapidly and in good yield. Ammonolysis of VIIIa and chromatography of the crude product led to pure octapeptide VIII of comparable quality to the octapeptide made by the stepwise method.

A fragment-condensation method was also employed for making the octapeptide VIII. This method was of interest in order to develop flexible synthetic routes for analogs involving amino acid substitutions in either of the fragments being condensed. The synthesis of VIII was accomplished by a condensation involving the C-terminal tetrapeptide IV

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†From early chromatographic fractions the faster moving methyl ester precursor of X was isolated, thus indicating some transesterification in NH_3-MeOH. The ester structure was confirmed by nmr, which shows sharp and distinct methyl protons, and by conversion of the ester to X by ammonolysis.