Synthesis of Some Nitrosaminopurine and Hydroxyaminopurine Derivatives (1)

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Secondary and tertiary amines exist in several hypermodified bases in nucleic acids (2-4). We prepared some nitrosaminopurines corresponding to methylaminopurines occurring in DNA and tRNA by their interaction with nitrous acid in a variety of *p*Hs and temperature conditions (5). In the present work, the synthesis of nitrosamino derivatives from the naturally occurring (2-4) N^6-(Δ^2-isopentenyl)adenine and its 9-ribosyl derivative is reported. An improved synthesis of the previously reported N^2-nitroso-N^2-methylguanine (4) is also described. These nitrosamino derivatives may show, as most of the known nitrosamines (6,7), carcinogenic properties.

It is known that aromatic secondary amines undergo metabolic oxidation to *N*-hydroxy derivatives which are carcinogens (8,9). The secondary amines existing in nucleic acids could conceivably be oxidized *in vivo* to potentially carcinogenic *N*-hydroxy derivatives. We reported the synthesis of 6-methyl-hydroxylaminopurine (10); we have now prepared the N^2-hydroxy derivative of the naturally occurring N^2-methylguanine.

Treatment of N^6-(Δ^2-isopentenyl)adenine (I) and its 9-ribosyl derivative (II) with sodium nitrite in acetic acid gave N^6-nitroso-(Δ^2-isopentenyl)adenine (III) and N^6-nitroso-(Δ^2-isopentenyl)adenosine (IV). N^2-methylguanine (V) and nitrous acid afforded N^2-nitroso-N^2-methylguanine (VI). Reaction of *N*-methylhydroxylamine with 2-chloro-hypoxanthine (VII) yielded N^2-hydroxy-N^2-methylguanine (VIII).

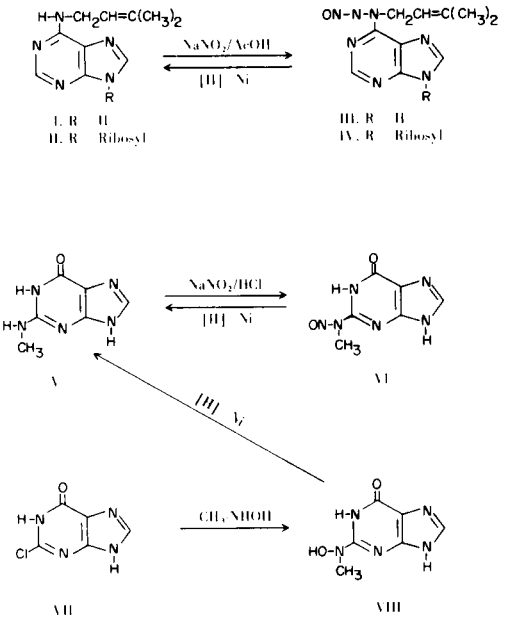
EXPERIMENTAL

Ultraviolet absorption spectra were determined with a Cary recording spectrophotometer, Model 11. Ascending paper chromatography was run on Whatman No. 1 paper on the following solvent systems: concentrated aqueous ammonia-water-isopropyl alcohol (10:20:70); 1-butanol-water-acetic acid (50:25:25); and 1 *M* ammonium acetate-ethanol (30:70). The determination of melting points was carried out with Mel-Temp and Thomas-Hoover melting point apparatus and the temperatures were corrected. Analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Michigan.

N^6-Nitroso-(Δ^2-isopentenyl)adenine (III).

A solution of N^6-(Δ^2-isopentenyl)adenine (I, 0.40 g., 2 mmoles) in 50% acetic acid (5 ml.) was cooled to 5° in an ice and water bath. A solution of sodium nitrite (0.55 g., 8 mmoles) in water (1.5 ml.) was added dropwise. The reaction was kept at 5° for 1 hour and 3 hours at 25°. The resulting precipitate was filtered, washed repeatedly with water and then with ethanol. After drying *in vacuo*, 0.41 g. (90%) of light yellow crystals were obtained, m.p. 220° dec.

Anal. Calcd. for $C_{10}H_{12}N_6O$: C, 51.71; H, 5.21; N, 36,18. Found: C, 51.46; H, 5.29; N, 36.25.



N^6-Nitroso-(Δ^2-isopentenyl)adenosine (IV).

To a solution of N^6-(Δ^2-isopentenyl)adenosine (II, 0.33 g., 1 mmole) in water (3 ml.) containing 50% acetic acid (2 ml.), sodium nitrite (0.80 g.) in water (3 ml.) was added dropwise and stirred for 18 hours at 5°. The resulting precipitate was filtered, washed repeatedly with cold water and ether. After drying *in vacuo*, yielded 0.17 g. (43%) of yellow crystals; m.p. 92° dec.

Anal. Calcd. for $C_{15}H_{20}N_6O_5 \cdot 2\ H_2O$: C, 44.99; H, 6.04; N, 20.99. Found: C, 45.29; H, 5.50; N, 21.20.

N^2-Methyl-N^2-nitrosoguanine (VI).

A solution of N^2-methylguanine (V, 0.33 g., 2 mmoles) in 2 *N* hydrochloric acid (5 ml.) was cooled to 5°. Sodium nitrite (0.55 g.) in water (1.5 ml.) was added dropwise and the reaction stirred at 5° for 1 hour and 3 hours at 25°. The precipitate was filtered, thoroughly washed with cold water and then with ethanol, dried *in vacuo* to yield 0.18 g. (51%) of light yellow

Table I

Absorption spectra λ max nm (ϵ x 10^3)

III	206 (14.2)	265 (6.28)	302 (8.26)	(ethanol)
IV	215 (12.0)	294 (8.15)		(ethanol)
VI	255 (9.71)	292 (6.93)		(*p*H 1)
	242 (12.4)	270 (7.33)		(*p*H 8.4)
VIII	252 (7.60)			(*p*H 1)
	255 (6.51)			(*p*H 6.6)

crystals. The mixed m.p., uv spectra at different *p*Hs and paper chromatography (3 solvents) were indistinguishable from a sample of N^2-methyl-N^2-nitrosoguanine previously prepared by nitrosation of N^2-methylhydrazinoguanine (5).

N^2-Methyl-N^2-hydroxyguanine (VIII).

A solution of 2-chlorohypoxanthine (11) (VII, 0.50 g., 3 mmoles) in an ethanolic solution of methylhydroxylamine (100 ml.) (made from 5.5 g. of methylhydroxylamine hydrochloride in 100 ml. of ethanol and neutralized with a 0.1 *N* ethanolic solution of potassium hydroxide) was refluxed for 3 hours. The solution was evaporated to dryness and the residue suspended in ethanol, filtered and washed with ethanol. The precipitate was dried *in vacuo* to yield 0.26 g. (48%) of white crystals, m.p. 250° dec.

Anal. Calcd. for $C_6H_7N_5O_2$: C, 39.77; H, 3.89; N, 38.66. Found: C, 39.72; H, 3.86; N, 38.80.

Treatment of (Methylnitrosamino)purines (III, IV, VI) and N^2-Methyl-N^2-hydroxyguanine (VIII) with Raney nickel.

A suspension of each of the above compounds (25 mg.) in water (10 ml.) was boiled with Raney nickel (100 mg.) for 1 hour. From the reaction solutions, the corresponding methylaminopurines (I, II, V) were identified by uv absorption spectra (at *p*H 6 and 13) and paper chromatography (single spot in 3 different solvents).

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