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Reaction of nitrous acid with alkylaminopurines

Nitrous acid deaminates adenine, guanine, and cytosine derivatives, converting them to the corresponding hypoxanthine, xanthine, and uracil derivatives, respectively[1]. These reactions occur at the nucleic acid level as well, and are responsible for the mutagenic action of nitrous acid[2]. Recently, investigators have used the reaction of nitrous acid with tRNA to probe the structure and biological activity of tRNA[3-6]. A number of unusual bases are present in tRNA, however, among which are the alkylaminopurines, N^6-methyladenine and N^2-methylguanine[7]. These substances are known to react with nitrous acid[8,9], but the products of these reactions have not been identified and characterized. We wish to report here that the product of this type of reaction is the *N*-nitrosoalkylaminopurine.

To a solution of 1.39 g (7.8 mmoles) of N^6-methyladenine (6-methylaminopurine, Cyclo Chemical Corp., Los Angeles, Calif.) in 120 ml of 5 M acetic acid was added 9.2 g (134 mmoles) of sodium nitrite, over a period of 25 min. The reaction flask was loosely stoppered and stirred for 30 min at 25°, and then cooled to 0°. The precipitate which formed was filtered, washed with cold water, and dried under vacuum at room temperature. The crude yield of 1.44 g was recrystallized from tetrahydrofuran–water (1:1, v/v), keeping the temperature at 40°, or lower. This gave 0.94 g (57 %) of N^6-nitroso-N^6-methyladenine (I) as a very pale yellow powder.

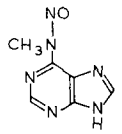
The compound had m.p. 235–250° (decomp.); ultraviolet max. in H_2O, pH 1, 216, 252, and 295 mμ (ε 10 600, 5700 and 10 700, respectively); ultraviolet max. in H_2O, pH 5, 216, 262, and 300 mμ (ε 11 400, 6800, and 10 300, respectively); ultraviolet max. in H_2O, pH 12, 225 and 310 mμ (ε 12 900 and 7400, respectively); infrared absorption (KBr) at 3.25, 3.40, 3.60, 3.80 (shoulder), 6.19, 6.40, 6.70, 6.86, 7.04, 7.26, 7.42, 7.79, 8.18, 8.68, 8.80, 9.90, 10.90, 11.06, and 12.50 μ; R_F (thin-layer chromatography on Avicel microcrystalline cellulose) 0.94 in methanol–chloroform–water (4:1:4, by vol.). The R_F of N^6-methyladenine in this solvent was 0.82. (Found: C, 40.75; H, 3.43; N, 46.82. $C_6H_6N_6O$ requires C, 40.48; H, 3.40; N, 47.22 %.)

As the naturally occurring N^2-methylguanine is not commercially available, the reaction of nitrous acid with the homologue, N^2-ethylguanine (readily prepared by lithium aluminium hydride reduction of acetylguanine[10]) was used instead. To a suspension of 289 mg (1.61 mmoles) of N^2-ethylguanine in 58 ml of 5 M acetic acid was added 2.22 g (32.2 mmoles) of sodium nitrite, over 15 min. The reaction flask was loosely stoppered and allowed to stand for 60 min at 25°. The N^2-ethylguanine gradually went into solution. The product was isolated and recrystallized in the same way as in the N^6-methyladenine reaction. This produced 233 mg (69 %) of N^2-nitroso-N^2-ethylguanine as a pale yellow powder.

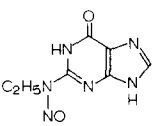
The product had no m.p. (decomp. above 210°); ultraviolet max. in H_2O, pH 1, 256 and 293 mμ (ε 11 900 and 9800, respectively); ultraviolet max. in H_2O, pH 5, 260 and 309 mμ (ε 10 500 and 7900, respectively); ultraviolet max. in H_2O, pH 9, 243 mμ (ε 16 000), shoulders at 265 and 295 mμ (ε 10 700 and 5000, respectively); ultraviolet max. in H_2O, pH 13, 245 and 310 mμ, shoulder at 265 mμ; infrared absorption (KBr) at 3.12, 3.28, 3.60, 3.80, 3.92, 5.80, 6.20, 6.41, 6.81, 7.06, 7.25, 7.70, 7.95, 8.35, 8.68, 9.10, 10.10, 10.34, 11.60, 12.28, 12.84, 13.40, and 13.62 μ; R_F (thin-

layer chromatography on Avicel microcrystalline cellulose) 0.93 in methanol–chloroform–water (6:10:1, by vol.); the R_F of N^2-ethylguanine in that solvent was 0.67. (Found: C, 39.97; H, 3.67; N, 40.64. $C_7H_8N_6O_2$ requires C, 40.31; H, 3.88; N, 40.40 %.)

The structures, I and II, were assigned to the products for



I



II

the following reasons: The analyses indicated that a hydrogen had been replaced by a nitroso group in each case. A strong band was seen in the infrared at 6.86 μ for I and 6.81 μ for II; N-Nitroso compounds have been reported to have a strong absorption at about that wavelength[11]. Compounds I and II were found to decompose to N^6-methyladenine and N^2-ethylguanine, respectively, on standing in acidic solution. The decompositions went to completion within 4 h in 1.0 M HCl, and within 48 h in 0.1 M HCl. Reduction of II by $NaBH_4$, $Na_2S_2O_4$, or $Zn(OH)_2$ and NaOH led to the formation of a single product with the R_F of N^2-ethylguanine. Upon recrystallization of I or II from hot mixed aqueous-organic solvents, some decomposition was also observed. These properties are consistent with the formulation of I and II as N-nitroso, rather than C-nitroso, compounds. The remaining question was the point of attachment of the N-nitroso groups.

Attack by nitrous acid upon a ring nitrogen of N^6-methyladenine or N^6-ethylguanine was unlikely, as hypoxanthine, xanthine, and N^2-dimethylguanine show no appreciable reaction with nitrous acid[1,12]. Spectrophotometric titration revealed pK_a values of approx. 2.0 and 8.5 for I, and 2.2, 6.0, and 10.8 for II. Thus I can form a monoanion, as can adenine, and II can form a dianion, as can guanine[13]. This suggests that I is unsubstituted upon its imidazole nitrogens, and that II is unsubstituted at N-1 and upon its imidazole nitrogens. Guanine derivatives with no substitution at N-1 and with one or no substituent on the amino group are known to react with glyoxal. This produces a characteristic shift in the ultraviolet spectrum[14]. When II was warmed for 5.5 h with an excess of glyoxal (50°, pH 4), no change in the ultraviolet was observed, nor was any new product detected upon analysis by thin-layer chromatography. All of these results are consistent with the structures assigned to I and II. Earlier workers had argued that an N-nitroso structure was unlikely for the reaction product of N^2-methylguanine and nitrous acid, because the product possessed a pK_a between 2 and 3 (ref. 9). This argument was apparently based upon the assumption that the amino group is the site of protonation in guanine derivatives, and that an N-nitrosamine could not accept a proton. However, this reasoning is invalid, as the site of protonation in guanine derivatives is at N-7 (ref. 13).

The formation of N-nitroso derivatives of N^6-methyladenine and N^2-methylguanine is analogous to the well known reaction of secondary aromatic amines with nitrous acid. Certain amino-substituted guanines, however, do not react with nitrous acid. Thus, neoguanosine (N^2-ribosylguanine)[15] and guanine propionate (N^2-α-carboxyethylguanine)[16] are not affected by that reagent. It is possible that the presence of the additional electron-withdrawing groups in these compounds renders

to N–N bond of a nitroso derivative unstable, even under the reaction conditions. It can be expected, however, that modification of tRNA by nitrous acid will convert the monoalkylaminopurines present to their *N*-nitroso derivatives. As Compounds I and II were found to be stable in 1 M NaOH for 24 h, it is possible that *N*-nitroso compounds could be detected after hydrolysis of the modified tRNA. It should be noted that N^6-methyladenine occurs in the DNA of a number of bacterial species[17], and the formation of I would be expected upon treatment of such DNA with nitrous acid.

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Biosynthesis of purine nucleotides in chick liver and intestine

During a study on the origin of deoxyribose of purine deoxyribonucleotides we have found a differential *in vivo* incorporation of ^3H from [$^3H_6$]glucose and ^{14}C from [^{14}C]glycine into ribose-bound purines as compared to deoxyribose-bound purines of chick liver and intestinal nucleic acids[1]. This observation has raised the question as to whether there are basic differences in the manner by which the purine moiety of ribonucleotides and deoxyribonucleotides are formed.

Two reactions are responsible for the synthesis of purine nucleotides: the *de novo* synthesis of purine nucleotides from formate and glycine and the pathway which utilizes preformed purine bases. In the latter pathway, the reaction is catalyzed by a purine nucleotide pyrophosphorylase or a purine nucleotide phosphorylase (*e.g.* EC 2.4.2.1). General opinion[2] holds that the *de novo* route is the primary pathway and