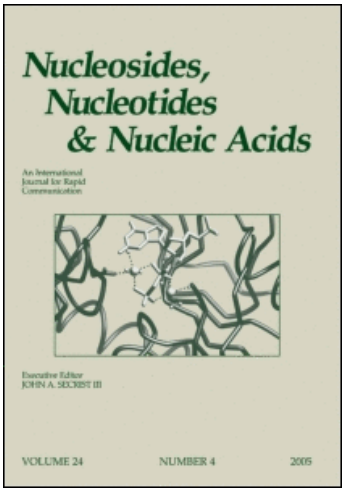

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BROMINE-INDUCED SELECTIVE N-MONODEMETHYLATION OF N^6,N^6-DIMETHYL-2',3'-O-ISOPROPYLIDENEADENOSINE

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ABSTRACT: Bromination of the title compound 1 with bromine in phosphate buffer has led to 8-bromo-N^6,N^6-dimethyl-2',3'-O-isopropylideneadenosine (2) and 2',3'-O-isopropylidene-N^6-methyladenosine (3). Under similar conditions, compound 2 gave 8-bromo-2',3'-O-isopropylidene-N^6-methyladenosine (4). The transformations 1 ⟶ 3 and 2 ⟶ 4 represent biomimetic models of *in vivo* N^6-demethylation of antibiotic puromycin.

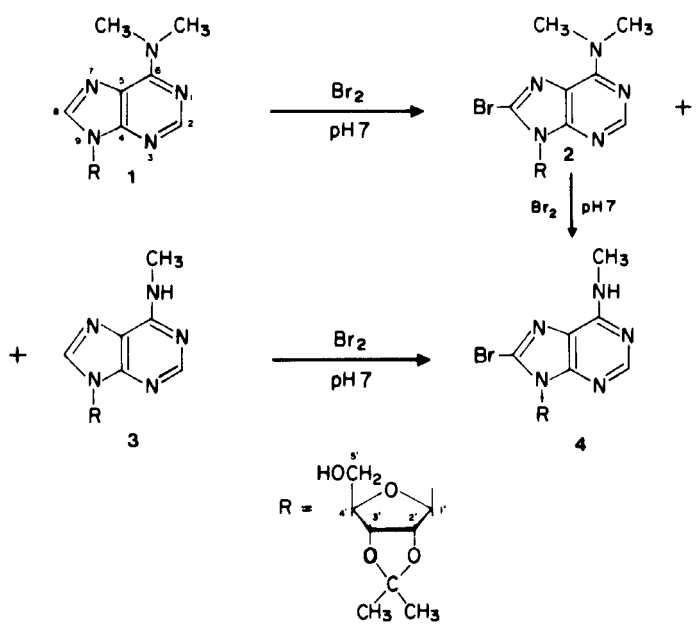
Enzymic N^6-monodemethylation is an important step in the metabolism of the antibiotic puromycin[1,2]. In search of biomimetic models of such a transformation, we have recently described[3] N^6-monodealkylation of N^6,N^6-dialkyl-2',3',5'-tri-O-acyladenosines (I) by oxidation with RuO_4 in CCl_4. A similar but much less selective dealkylation resulted[4] from the treatment of compounds I with $KMnO_4$ in 50% acetic acid. The loss of selectivity can be readily explained by a high reactivity of $KMnO_4$.

It is clear that milder methods must be sought in order to achieve a maximum selectivity under conditions, i.e., pH, temperature, aqueous medium and oxydation potential of the reagent, which mimic, as closely as possible, the enzyme-catalyzed N-dealkylation. The present work describes one such procedure.

N^6,N^6-Dimethyl-2',3'-O-isopropylideneadenosine (1)[5], when treated with bromine in phosphate buffer (pH 7) in aqueous dioxane for 17 h at 40°C, gave the expected 8-bromo derivative 2 (30%) accompanied by 2',3'-O-isopropylidene-N^6-methyladenosine (3, 15%). Although, in this

instance, N-demethylation occurred prior to the bromination, compound 2 was readily demethylated to give derivative 4 in 40% yield which was identical with the product obtained by bromination of 3 (Scheme 1). These results show that N-methyl groups in N^6,N^6-dimethyladenosine are susceptible to a selective N-demethylation with oxidizing agents milder[6] than RuO_4. The effect of pH seems to be of crucial importance. Thus, bromination of N^6,N^6-dimethyladenosine in acetate buffer (pH 4)[7] gave 8-bromo-N^6,N^6-dimethyladenosine as the only product.

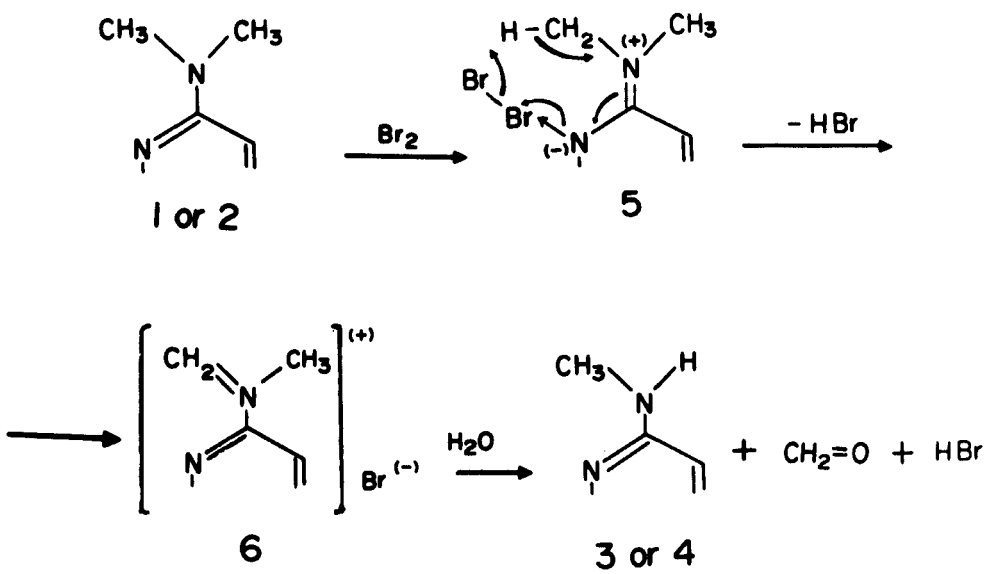
The structures of compounds 2-4 were confirmed by UV, NMR and electron-impact mass spectra. It is of interest that the presence of bromine atom in 2 did not change the fragmentation pattern of the base residue relative to that found in the parent nucleosides[8]. Thus, ion b + H - NCH_3 is present in both 2 and 3. Compounds 2 and 3 gave distinct M and M-15 peaks; the latter is characteristic for 2',3'-O-isopropylidene nucleosides[9,10]. The bromo derivatives 2 and 4 afforded the expected isotopic "doubling" of appropriate fragments. However, ions corresponding to M-$(CH_3)_2COOH$, observed in the pyrimidine (uracil) derivatives[9,10], were not found.



SCHEME 1

A possible mechanism of N-demethylation is shown in Scheme 2. Thus, the reaction could be initiated by a formation of adduct 5, as anticipated, e.g., in bromine-induced cleavage of aliphatic ethers[11] or amines[12]. Structure 5 then appears as the most likely because of the known[13,14] nucleophilic character of N-1 in purine nucleosides. Adduct 5 then collapses to give quarternary salt 6 which, in turn, is hydrolyzed to product 3 or 4. However, it is recognized that mechanism of oxidative cleavage of amines with bromine still remains to be established[12]; therefore, Scheme 2 describes only one possibility.

Although the mechanism of biological demethylation of puromycin aminonucleoside has not been investigated in detail, the formation of dealkylated products accompanied by the corresponding aldehyde has been observed in case of a variety of N-alkylated xenobiotics[15,16]. In this respect, the bromine-induced N-demethylation may more closely simulate the in vivo situation than oxidation with RuO_4 leading to the corresponding N-formyl derivatives[3]. In other aspects, the mechanisms of the enzyme- and bromine-induced demethylation are different. Nevertheless, the fact that a complex enzymic transformation may be mimicked by a simple chemical reaction in solution under conditions close to physiological is of interest.



SCHEME 2

The results reported herein and previously[3] indicate that selective N^6-monodemethylation of N^6,N^6-dimethyladenosine can be effected by agents of different oxidation potential and reaction mechanism.

EXPERIMENTAL SECTION

Melting points were taken on a Thomas Hoover capillary melting point apparatus and they are uncorrected. NMR spectra were determined with an FX 100 Fourier transform NMR spectrometer (JEOL Ltd., Tokyo, Japan) in $CDCl_3$ as solvent and $Si(CH_3)_4$ as an internal reference. Electron-impact mass spectra were obtained with a JMS D-100 mass spectrometer (JEOL Ltd., Tokyo, Japan).

Thin-layer chromatography (TLC) was performed as described[3]. For column chromatography silica gel 60, 70 - 230 mesh ASTM (Merck) was used.

Bromination of N^6,N^6-Dimethyl-2',3'-O-isopropylideneadenosine (1). Bromine (0.5 g, 2.8 mmol) was added to a stirred suspension of compound 1 (0.8 g, 2.3 mmol)[5] in 50% aqueous dioxane containing $Na_2HPO_4.H_2O$ (1.92 g, 7.2 mmol). The mixture was heated to 40°C for 17 h and the clear solution was, after cooling, extracted with chloroform. The organic layer was washed with 2 N $Na_2S_2O_3$ and water, it was dried ($MgSO_4$) and evaporated. The residue was chromatographed on a silica gel (15 g) column by using chloroform as an eluent. Compound 2 was eluted first, 0.31 g (31%); mp. 73-74°; UV_{max} (ethanol) 277 nm (ε 15,500); NMR ($CDCl_3$ + D_2O) δ, 8.20 (s, 1, H_2), 6.09 (d, 1, $H_{1'}$, $J_{1',2'}$ 4.9 Hz), 5.31 (t, 1, $H_{2'}$), 5.07 (d, 1, $H_{3'}$), 4.52 (s, 1, $H_{4'}$), 3.86 (d of q, 2, $H_{5'}$), 3.48 (s, 6, $N(CH_3)_2$), 1.67 and 1.38 (2s, 6, $(CH_3)_2C$). In the absence of D_2O, signals of δ, 6.67 and 6.56 (apparent 2d, 1, OH) were present. Mass spectrum, m/e (relative intensity) 415, 413 (10.1, 9.6, M), 400, 398 (5.0, 5.3, M - CH_3), 327, 325 (2.8, 3.8, M - CH_3COCH_3 - CH_2O), 385, 383 (6.0, 6.2, M - CH_2O), 326, 324 (7.7, 7.7, M - 89), 312, 310 (9.1, 12.5, M - 103), 272, 270 (12.7, 13.2, b-CHOH), 244, 242 (23.6, 31.5, b + 2H, m/e 242 is overlapped with $b^{81}Br$), 243, 241 (100.0, 96.6, b + H), 240 (8.4, $b^{79}Br$), 228, 226 (21.2, 21.6, b + H - CH_3), 215, 213 (8.6, 9.3, b + 2H - NCH_3), 214, 212 (91.5, 92.7, b + H

- NCH_3). Anal. Calcd. for $C_{15}H_{20}BrN_5O_4.1/7\ H_2O$: C, 43.22; H, 4.91; N, 16.80. Found: C, 43.36, H, 4.80, N, 16.52.

Elution with chloroform - methanol (95 : 5) afforded N-demethylated product 3, 0.13 g (14%), mp. 88 - 90° (foaming); UV_{max} (ethanol) 267 nm (ε 11,000); NMR ($CDCl_3$ + D_2O) δ, 8.34 (s, 1, H_8), 7.76 (s, 1, H_2), 5.84 (d, 1, $H_{1'}$, $J_{1',2'}$, 4.4 Hz), 5.16 (apparent qt, 2, $H_{2'}$ and $H_{3'}$), 4.54 (s, 1, $H_{4'}$), 3.87 (d of q, 2, $H_{5'}$), 3.19 (s, 3, NCH_3), 1.65 and 1.38 (2s, 6, $(CH_3)_2C$). In the absence of D_2O, signals of δ, 6.80, 6.69 (apparent 2d, 1, OH) and 6.04 (broad d, 1, NH) were present. Mass spectrum, m/e (relative intensity) 321 (12.1, M), 306 (6.5, M - CH_3), 291 (12.1, M - CH_2O), 263 (4.6, M - CH_3COCH_3), 233 (10.6, M - CH_3COCH_3 - CH_2O), 232 (44.9, M - 89), 218 (21.0, M - 103), 178 (63.0, b-CHOH), 150 (35.5, b + 2H), 149 (100.0, b + H), 148 (18.6, b), 119 (7.9, b + H - NCH_3). Anal. Calcd. for $C_{14}H_{19}N_5O_4.2/3H_2O$: C, 50.44; H, 6.15; N, 21.01. Found: C, 50.39; H, 6.01; N, 20.99.

8-Bromo-2',3'-O-isopropylidene-N^6-methyladenosine (4). A. Bromination of compound 3. The reaction was performed as mentioned above with compound 3 (0.16 g, 0.5 mmol). Column chromatography on silica gel (chloroform) gave product 4 which was crystallized from chloroform - petroleum ether, 56 mg (28%), mp. 173.5 - 174.5° UV_{max} (ethanol) 270 nm (ε 17,000); NMR ($CDCl_3$ + D_2O) δ, 8.30 (s, 1, H_2), 6.09 (d, 1, $H_{1'}$, $J_{1',2'}$ 4.9 Hz), 5.27 (t, 1, $H_{2'}$), 5.07 (d, 1, $H_{3'}$), 4.52 (s, 1, $H_{4'}$), 3.86 (d of q, 2, $H_{5'}$), 3.18 (s, 3, NCH_3), 1.67 and 1.38 (2s, 6, $(CH_3)_2C$). In the absence of D_2O, signals δ, 6.53, 6.42 (apparent 2d, 1, OH), 5.66 (poorly resolved d, 1, NH) and 3.19 (d, 3, CH_3NH, J_{CH_3NH} 5.1 Hz) were present. It was not possible to obtain M or M - 15 ions in the mass spectrum of 4 by a direct probe technique but fragments of m/e 229 and 227 (100.0, 100.0, b + H) were present. Anal. Calcd. for $C_{14}H_{18}BrN_5O_4.6/7\ H_2O$: C, 40.44; H, 4.62; N, 16.84. Found: C, 40.56; H, 4.35; N, 16.55.

B. N-Demethylation of compound 2. The reaction was performed as described above with bromo derivative 2 (0.083 g, 0.6 mmol). The crude product was chromatographed on a loose layer of silica gel which was developed in chloroform - methanol (95 : 5). Elution of the major UV absorbing band with the same solvent gave compound 4 which was

crystallized from chloroform - petroleum ether, 34 mg (43%), mp. 173.5 - 174.5°, mixed mp. with a sample prepared by method A was undepressed. NMR spectra of both products were identical.

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REFERENCES AND FOOTNOTES

1. Nagasawa, H. T.: Swingle, K. F.; Alexander, C. S. Biochem. Pharmacol. 1967, 16, 2211.
2. Kmetec, E.; Tirpack, A. Biochem. Pharmacol. 1970, 19, 1493.
3. Endo, T.; Źemlićka, J. J. Org. Chem. 1979, 44, 3652.
4. Kato, T.; Ogawa, S.; Ito, I. Tetrahedron Lett. 1981, 22, 3205.
5. Kato, T.; Źemlićka, J. J. Org. Chem. 1980, 45, 4006.
6. Thus, unlike bromine, RuO_4 rapidly oxidizes benzene ring: Djerassi, C.; Engle, R. R. J. Am. Chem. Soc. 1953, 75, 3838.
7. Ikehara, M.; Morisawa, H. Chem. Pharm. Bull. 1971, 19, 2593.
8. Shaw, S. J.; Desiderio, D. M.; Tsuboyama, K.; McCloskey, J. A. J. Am. Chem. Soc. 1970, 92, 2510.
9. Dolhun, J. J.; Wiebers, J. L. Org. Mass Spectrom. 1970, 3, 669.
10. Brown, P.; Pettit, G. R.; Robins, R. K. Org. Mass Spectrom. 1969, 2, 521.
11. Deno, N. C.; Potter, N. H. J. Am. Chem. Soc. 1967, 89, 3550.
12. Deno, N. C.; Fruit, R. E., Jr. J. Am. Chem. Soc. 1968, 90, 3502.
13. "Organic Chemistry of Nucleic Acids", (Kochetkov, N. K.; Budovskii, E. I., eds.), Plenum Press, New York, 1971, Part A, pp. 149-152.
14. Ref. 13, Part B, pp. 335-336.

15. Gescher, A.; Hickman, J. A.; Stevens, M. F. G. Biochem. Pharmacol. 1979, 28, 3235.
16. McMahon, R. E.; Culp, H. W.; Occolowitz, J.C. J. Am. Chem. Soc. 1969, 91, 3389.