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Methylation of Purine 2′-Deoxynucleosides with Trimethyl Phosphate

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Synopsis. Trimethyl phosphate was found to methylate deoxyadenosine (N-1) and deoxyguanosine (N-1, N-7, and O-6) in a homogeneous aqueous phase at 37 and 60 °C, giving the corresponding methyl derivatives.

The alkylation of nucleic acids, especially deoxyribonucleic acid (DNA) *in vivo*, is considered to be closely related to carcinogenesis or mutagenesis, and various alkylating agents have been employed for alkylation studies of nucleic acids and their components.[1-8]

Methylation reactions of nucleic acid-bases,[9] pyrimidine 2′-deoxynucleosides,[10] and ribonucleosides[11] were carried out successfully in a homogeneous aqueous phase using trimethyl phosphate (TMP) as a methylating agent.

In this paper, we wish to describe the reactions of deoxyadenosine (**1**) and deoxyguanosine (**4**) with TMP in an aqueous phase.

The reactions were carried out at 37 and 60 °C by stirring a mixture of 2′-deoxynucleoside and TMP in water at an appropriate pH (7—10 for **1** and 10—11 for **4**). The products were conveniently identified by comparison of their R_f values and UV spectra with those of the authentic samples, their yields being determined by means of UV spectra. The results are summarized in Table 1.

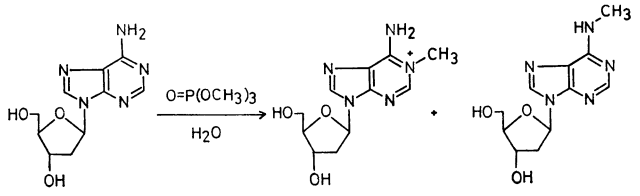
TABLE 1. METHYLATION OF PURINE 2′-DEOXYNUCLEOSIDES (dNu) WITH TRIMETHYL PHOSPHATE (TMP)[a]

dNu[b]	Temp °C	Mole ratio (TMP)/(dNu)	pH	Product[b]	UV-yield/% 24 h	48 h	72 h
dA (**1**)	37	15	7	1-Methyl-dA (**2**)	4	8	10
				N^6-Methyl-dA (**3**)	0	0	3
	37	60	7	**2**	5	9	13
				3	0	1	1
	37	15	10	**2**	3	3	3
				3	3	7	11
	37	60	10	**2**	4	7	8
				3	3	6	9
	60	15	7	**2**	38	52	58
				3	0	5	7
	60	15	10	**2**	5	6	7
				3	32	48	54
				Unknown A	2	3	3
				Unknown B	1	1	3
dG (**4**)	37	15	10	1-Methyl-dG (**5**)	27	35	32
				Imidazole ring opened 7-methyl-dG (**6**)	7	15	16
				Imidazole ring opened 1,7-dimethyl-dG (**7**)	7	15	25
				O^6-Methyl-dG (**8**)	2	3	9
	37	15	11	**5**	32	48	41
				6	11	14	17
				7	6	12	24
				8	3	3	6
	37	60	11	**5**	42	43	44
				6	16	9	10
				7	14	30	37
				8	3	3	2

a) Reaction size: deoxyadenosine (0.25 mmol)+TMP (3.8 or 15.0 mmol)+H_2O (2.5 ml); deoxyguanosine (0.05 mmol)+TMP (0.7 or 3.0 mmol)+H_2O (0.5 ml). b) dA and dG refer to deoxyadenosine and deoxyguanosine, respectively.

Compound **1** and adenosine are generally alkylated with methyl iodide[1] or diazomethane[6] at the N-1 position. Singer *et al.* reported the formation of 1-ethyl- and 7-ethyladenosine in the reaction of adenosine with diethyl sulfate.[3]

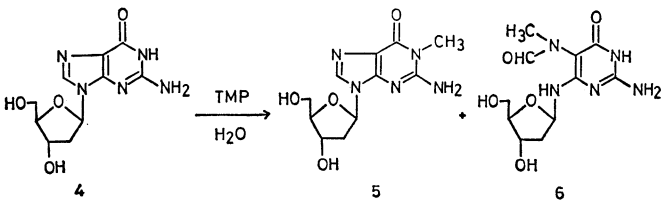
TMP also alkylates **1** at the N-1 position to give 1-methyldeoxyadenosine (**2**) and N^6-methyldeoxyadenosine (**3**) which arose from **2** by the Dimroth rearrangement under alkaline conditions. Two minor products obtained in the reaction at 60 °C, pH 10, were considered to be 5′ (or 3′)-*O*-methylated products found also in methylation of pyrimidine 2′-deoxynucleosides under similar conditions.[10]



1 2 3

In deoxyguanosine (**4**), the N-1 and N-7 positions are subjected to alkylation in preference to the other positions by alkylating agents.[1,2,4,7,8]

In the present method with TMP, **4** was methylated at the N-1, N-7, and O-6 positions giving 1-methyl- (**5**), imidazole ring opened 7-methyl- (**6**), imidazole ring opened 1,7-dimethyl- (**7**), and O^6-methyldeoxyguanosines (**8**). Identification of **5**, **6**, and **7** was based on comparison of their R_f values and UV spectra with those of the authentic samples, **8** being tentatively assigned through its UV spectra and characterestic fluorescence under UV light.



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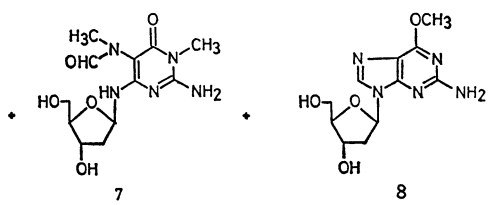

7 8

Thus, methylation of the N-1 position of **4** was found to be accelerated by the increase of pH of the reaction medium, the reactivities of the N-1 position of **1** and the N-7 position of **4** being independent of pH.

Occurrence of O^6-methylation of **4** may be worth remarking, since O^6-alkylation of guanine moiety in DNA seems to be related closely to the mutagenecity and the carcinogenecity through atypical base-pairing.[12]

Experimental

Melting points are uncorrected. UV spectra were recorded on a Hitachi 3T spectrometer, and NMR spectra on a Hitachi Perkin-Elmer R-20 spectrometer with a dilute solution in deuterioxide and sodium 3-(trimethylsilyl)propionate-d_4 as an internal standard. Thin-layer chromatography was performed on silica gel [GF_{254}(type 60), Merck] or cellulose (13254, Eastman) using the following solvents; A: chloroform–methanol, 5: 1, B: 2-propanol–water, 7: 3. Column chromatography was carried out using silica gel (Merck, Art. 7734, 70—230 mesh).

Commercial deoxyadenosine (**1**) and deoxyguanosine (**4**) were used without further purification. Trimethyl phosphate (TMP) was distilled prior to use.

Methylation of Deoxyadenosine (1). A mixture of **1** (125 mg, 0.5 mmol) and TMP (7.5 or 30 mmol) in water (5 ml) was stirred at 37 or 60 °C at an appropriate pH maintained throughout the reaction by occasional addition of 2 M sodium hydroxide. At an appropriate reaction time, 4 μl of the reaction mixture was spotted on silica gel TLC plate, which was developed immediately using solvent A. Two UV-absorbing products (**2** and **3**) were observed (R_f; **1**: 0.29, **2**: 0.01, **3**: 0.41). In the reaction at 60 °C, pH 10, additional two spots (Unknown A and B) appeared (R_f; A: 0.54, B: 0.60). Each product was identified by a comparison of its R_f and UV spectrum with those of the authentic sample and the yield was calculated from its UV spectrum in a similar way to that reported.[9] The results are summarized in Table 1.

The authentic sample of 1-methyldeoxyadenosine was prepared according to the procedure of Jones and Robins.[1]

N^6-Methyldeoxyadenosine was isolated as follows.

A mixture of **1** (1.0 g, 4.0 mmol) and TMP (8.4 g, 60.0 mmol) in water (10 ml, pH 10, NaOH) was stirred at 60 °C for 48 h. After the reaction mixture had been neutralized by concentrated hydrochloric acid, the solvent was removed by evaporation. The residue was purified by silica gel column chromatography (2.5×50 cm). Elution with chloroform afforded unchanged TMP, **3** being obtained by subsequent elution with chloroform–methanol (7: 1) (349 mg, 33%); mp 200—201 °C (lit,[1] 206—208 °C); UV λ_{max} (H_2O) nm: pH 1, 262.0, pH 7, 265.0, pH 13, 265.0 (lit,[1] pH 1, 261.0, pH 7, 265.0, pH 11, 265.0).

Methylation of Deoxyguanosine (4). The reaction of **4** (0.05 mmol) with TMP (0.7 or 3.0 mmol) in water (0.5 ml) at 37 °C, pH 10 or 11 afforded four UV-absorbing products (**5**, **6**, **7**, and **8**) on cellulose TLC which was developed using solvent B (R_f; **4**: 0.46, **5**: 0.67, **6**: 0.59, **7**: 0.73, **8**: 0.83). At an appropriate reaction time, the yield of each product was calculated in a similar way to that mentioned above. The UV spectrum of each product was as follows; **5**: λ_{max} (H_2O) nm: pH 1, 257.0, 280.0 (shoulder), pH 7, 255.5, 271.0 (shoulder), pH 13, 255.5, 272.0 (shoulder) (lit,[2] pH 1, 257.0, pH 11, 254.0); **6**: λ_{max} (H_2O) nm: pH 1, 272.0, pH 7, 273.0, pH 13, 266.0 (lit,[13] pH 1, 270.5, pH 11, 265.0); **7**: λ_{max} (H_2O) nm: pH 1, 272.0, pH 7, 273.0, pH 13, 273.0 (lit,[4] pH 1, 272.0, pH 13, 273.0); **8**: λ_{max} (H_2O) nm: pH 1, 241.0, 286.0, pH 7, 249.0, 279.0 (lit,[7] pH 1, 244.0, 286.0 pH 7, 248.0, 277.0, pH 13, 247.0, 278.0). The R_f and UV spectrum of each product agreed with those of the authentic sample or reported values. Products and their distribution are summarized in Table 1.

The authentic sample of **5** was prepared from the reaction of **4** with trimethylsulfonium hydroxide,[14] and that of **6** was given by the alkaline treatment of 7-methyldeoxyguanosine prepared according to the procedure of Jones and Robins.[1]

Compound **7** was prepared as follows. A mixture of **5** (57 mg, 0.2 mmol) and TMP (0.84 g, 6.0 mmol) in water (2 ml, pH 10, NaOH) was stirred at 60 °C for 30 h. The TLC of the reaction mixture showed only one UV-absorbing spot whose aqueous extract had λ_{max} at 273.0 nm. After the reaction mixture had been neutralized with concentrated hydrochloric acid, unchanged TMP was removed by extraction with chloroform. Evaporation of the water layer gave the residue which was washed with acetone several times. The acetone solution was concentrated and poured into a large excess of ether, giving white hydroscopic precipitate of **7** (31 mg, 49%); NMR (DMSO-d_6) δ=2.65 and 2.71 (0.8 H+2.2 H, two s, $N(CHO)CH_3$), 3.05 (3H, s, $N\text{-}CH_3$), 5.05—5.60 (1H, complex m, 1′-CH), and 7.55 and 7.62 (0.25H +0.75H, two s, $N(CHO)CH_3$); UV λ_{max} (H_2O) nm: pH 1, 272.0, pH 7, 273.0, pH 13, 273.0.

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