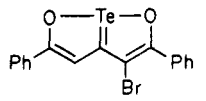
sulfide, diphenyl disulfide, and diphenyl diselenide, respectively, as well as reduction products **6** and **11**. Diphenyl telluride is oxidized to diphenyl telluroxide with **7**, but diphenyl selenide is unreactive toward **7**. The pertellurane dibromides and dichlorides do not react with olefins in CH_2Cl_2 after 48 h in the dark at ambient temperature, although irradiation leads to small amounts of allylic halides. Compounds **9** and **10** are reduced with hydrazine to give **11**[18] and **12**[19] in 71% and 89% yields,



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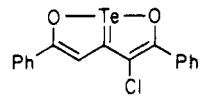

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respectively. All of the pertelluranes were susceptible to decomposition by hydrolysis.

Attempts to induce oxidative additions of iodine to **6** failed, as did attempts to iodinate the pentalene ring of **7** and **8**. This is somewhat surprising in view of the traditional stability of $RTeI_3$ and R_2TeI_2 compounds.[20]

Acknowledgment. We thank Dr. Thomas H. Whitesides and Dr. William N. Washburn for many helpful discussions.

Registry No. **5**, 87761-66-8; **6**, 87761-67-9; **7**, 87761-68-0; **8**, 87761-69-1; **9**, 87761-70-4; **10**, 87761-71-5; **11**, 87761-72-6; **12**, 87761-73-7; PhCOCl, 98-88-4; ethylmercaptan, 75-08-1; benzenethiol, 108-98-5; benzeneselenol, 645-96-5; diethyl disulfide, 110-81-6; diphenyl disulfide, 882-33-7; diphenyl diselenide, 1666-13-3; diphenyl telluride, 1202-36-4; diphenyl telluroxide, 51786-98-2; hydrazine, 302-01-2.

Supplementary Material Available: Tables of positional and thermal parameters (Tables I and II for **6**, Tables III and IV for **9**) and a table of bond angles about Te for **9** (Table V) (5 pages). Ordering information is given on any current masthead page.

(18) mp 166–167.5 °C; ^1H NMR ($CDCl_3$) δ 8.20 (s, 1 H), 8.10 (m, 2 H), 7.90 (m, 2 H), 7.50 (m, 6 H); IR (KBr) 1500 cm^{-1}; UV (CH_2Cl_2) λ_{max} 426 nm (log ϵ 4.28); FDMS, m/e 456 ($C_{17}H_{11}BrO_2{}^{130}Te$). Anal. Calcd for $C_{17}H_{11}BrO_2Te$: C, 44.9; H, 2.4; Te, 28.1. Found: C, 44.7; H, 2.4; Te, 27.3.

(19) mp 150–152.5 °C; ^1H NMR ($CDCl_3$) δ 8.20 (s, 1 H), 8.02 (m, 4 H), 7.50 (m, 6 H); IR (KBr) 1525 cm^{-1}; UV (CH_2Cl_2) λ_{max} 427 nm (log ϵ 4.505); FDMS, m/e 412 ($C_{17}H_{11}ClO_2{}^{130}Te$). Anal. Calcd for $C_{17}H_{11}ClO_2Te$: C, 49.8; H, 2.7. Found: C, 49.7; H, 2.8.

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Chemical Modification of Deoxyribonucleic Acids: A Direct Study by Carbon-13 Nuclear Magnetic Resonance Spectroscopy

Ching-jer Chang,* Jose DaSilva Gomes, and Stephen R. Byrn

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907

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The chemical modifications of salmon sperm and salmon testes DNAs with 90% ^{13}C-enriched methyl methanesulfonate have been directly studied by ^{13}C NMR and ^{31}P NMR. This direct stable isotope approach eliminates all tedious degradation and separation processes for determining the reactive sites and product distribution in studying the in vitro interaction between biological macromolecules and bioactive compounds. Seven methylated products, 7-methyldeoxyguanosine, 1-methyldeoxyadenosine, 3-methyldeoxycytidine, 1-methyldeoxyguanosine, 3-methylthymidine, methyl phosphodiester, and methyl phosphotriester were determined by comparison with model compounds and extensive study of the chemical properties of the methylated DNA. The relative specificity of the methylation reactions may correlate with the DNA conformation.

Chemical modification of polynucleotides and nucleic acids[1,2] is one of the promising approaches for studying the structure and function of nucleic acids.[3-14] It is evident that the success of this approach relies upon the accurate determination of the reaction specificity, sites of modification, and product distribution. Although the radioisotope-labeling method has generally been used because of its ultrahigh sensitivity, this approach, at best, can merely detect the degree of overall modification. In order to obtain structural information on a covalent interaction, it is necessary to carry out a series of enzymatic and acid-catalyzed hydrolyses followed by chromatography or electrophoresis under acidic or alkaline conditions to separate all modified bases and nucleosides, using radioactivity, fluorescence, or ultraviolet detector.[15,16] This

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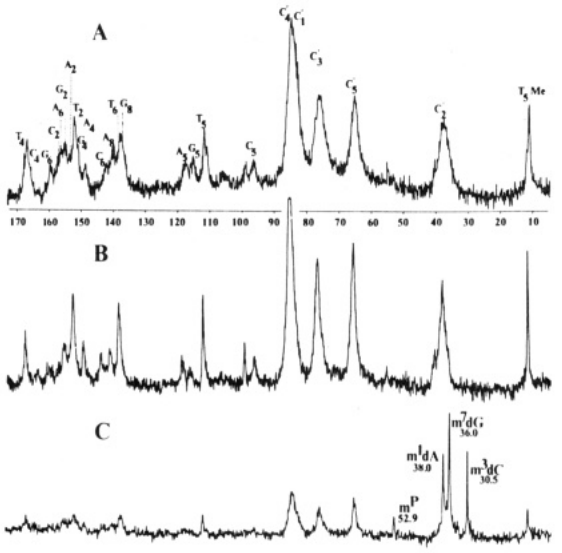
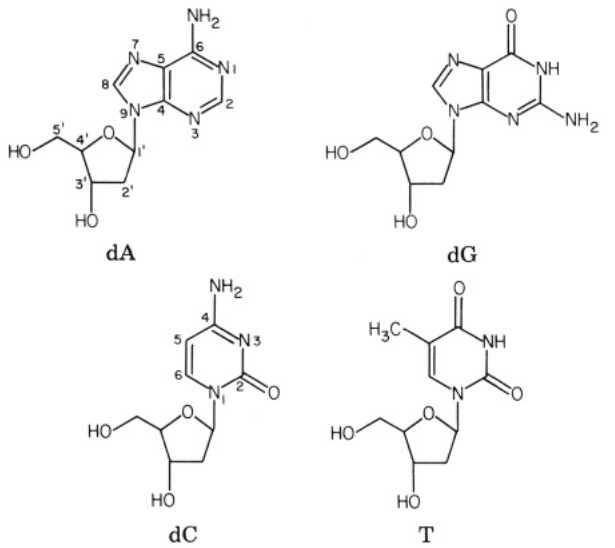


Figure 1. Proton-decoupled ^{13}C NMR spectra of DNA in 1.5 mL of D_2O at pD 7.4 and 6 °C. The spectra were measured on JEOL PFT-100 at 25.15 MHz and a repetition time of 2 s (23-μs pulse), using 10-mm sample tubes: (A) unmodified salmon sperm DNA (180 000 scans; 200 mg/1.5 mL), (B) salmon sperm DNA reacted with MeMS for 12 h (212 000 scans; 200 mg/1.5 mL), (C) salmon sperm DNA reacted with 90% ^{13}C-enriched MeMS for 3 h (82 000 scans; 130 mg/1.5 mL). m^3dC, 3-methyldeoxycytidine; m^7dG, 7-methyldeoxyguanosine; m^1dA, 1-methyldeoxyadenosine; mP, phosphomethyl group.

indirect method is not only tedious but also may lead to erroneous conclusions due to secondary chemical alteration and/or decomposition. It is therefore important to develop more direct methods to determine the chemical modifications of biological macromolecules.[17,18] Here, we report the application of nuclear magnetic resonance (NMR) spectroscopy in the direct study of chemical modification of deoxyribonucleic acids.



Results and Discussion

Both salmon sperm DNA and salmon testes DNA were purified by conventional enzymic digestions (bovine pancreatic ribonuclease A and *Streptomyces griseus* protease VI, phenol extraction, ethanol precipitation)[19,20] and extensive dialysis (Spectrapor 2 membrane, M_r cutoff 14 000). The natural-abundance ^{13}C NMR spectrum of salmon sperm DNA was measured, and the chemical shift assignments (Figure 1A) were primarily derived by comparison to those of the corresponding nucleosides and nucleotides.[21,22] The observed line widths for salmon sperm DNA are approximately one-half of those for double-stranded calf thymus DNA of 140 base pairs isolated from enzymatic degradation of chromatin.[23] The ^{13}C line widths and solubility in water indicate that this DNA is relatively small. We thus determined the average molecular weight by comparing with DNA molecular weight markers with use of agarose electrophoresis. We also measured the double-helical character by digesting with S_1-endonuclease from *Aspergillus oryzae* and then separating by hydroxyapatite column.[24,25] The low molecular weight (0.096×10^6) and degree of double strandedness (22%) have clearly showed that this DNA was highly denatured. This DNA was utilized in the initial studies mainly due to its high solubility in water, which permitted us to make quick evaluation of the feasibility of this direct ^{13}C NMR approach.

Modification of the Salmon Sperm DNA. The first modification was carried out by treating 202 mg of salmon sperm DNA with 50 μL (0.6 mmol) of methyl methanesulfonate (MeMS) in 6.47 mL of water at room temperature and pH 7.00 ± 0.01. The pH was controlled by a pH stat. After 3 h the reaction mixture was dialyzed, lyophilized, and subjected to NMR analysis. The ^{13}C natural abundance spectrum of the unmodified DNA and that of methylated DNA are shown in Figures 1A and 1B. No methyl carbon signal is clearly observable in Figure 1B due to the low degree of reaction. Some of the methyl signals may even be obscured by the C-2′ signal of deoxyribose. The limited solubility, high molecular weight of DNA, and inherent NMR sensitivity of ^{13}C nucleus resulted in poor signal-to-noise ratio of the ^{13}C natural abundance signals. This difficulty can be overcome by using 90% ^{13}C-enriched MeMS, which could be readily prepared from the reaction of ^{13}C-enriched methanol with methanesulfonic anhydride.[26] Since the natural abundance of ^{13}C nucleus is only 1.1%, any possible spectral interference from ^{13}C natural abundance signal of DNA, as shown in Figure 1B, can be greatly reduced (Figure 1C). Furthermore, this method also permits us to detect the early-stage modification or reduce the amount of modifying agent, which may be critical since a biological event often takes place after exposure to a limited amount of active agent.

Determination of Sites of Modification: Nitrogen Methylation. Figure 1C distinctly displays four methyl carbon signals at 30.5, 36.0, 38.0, and 52.9 ppm, whose resonance designations can be tentatively assigned on the basis of ^{13}C NMR studies of methylated deoxyribonucleosides and related ribonucleosides[17] and ribonucleotides (Table I).[27,28] All peaks below 40 ppm could

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Table I. Methyl Carbon Chemical Shifts of Model Compounds[a, b]

compd	δ	compd	δ
m^1dA	38.0	m^1A	37.8
m^6dA	27.1	m^6A	27.3
m^1dG	28.4	m^1G	28.5
m^6dG	53.3	m^6G	53.7
m^7dG	35.5	m^7G	35.5
m^3dC	30.9	m^3C	30.5
m^3T	27.6	m^3U	27.7
m^4T	54.1	m^4U	54.9
		m^3A	39.7

[a] Chang, C.-j.; Ashworth, D. J.; Chern, L.-J.; DaSilva Gomes, J.; Lee, C.-G.; Mou, P. W.; Narayan, R. *Org. Magn. Reson.*, in press. [b] Abbreviations: m^1dA, 1-methyldeoxyadenosine; m^6dA, N^6-methyldeoxyadenosine; m^1dG, 1-methyldeoxyguanosine; m^6dG, O^6-methyldeoxyguanosine; m^7dG, 7-methyldeoxyguanosine; m^3dC, 3-methyldeoxycytidine; m^3T, 3-methylthymidine; m^1A, 1-methyladenosine; m^6A, N^6-methyladenosine; m^1G, 1-methylguanosine; m^6G, O^6-methylguanosine; m^7G, 7-methylguanosine; m^3C, 3-methylcytidine; m^3U, 3-methyluridine; m^4U, O^4-methyluridine; m^3A, 3-methyladenosine.

be ascribed to the nitrogen-methyl resonance signals. The strongest signal at 36.0 ppm was assigned to the methyl carbon resonance of 7-methyldeoxyguanosine (m^7dG, 35.5 ppm). The other most prominent peaks at 38.0 and 30.5 ppm are identified as the methyl resonance signals of 1-methyldeoxyadenosine (m^1dA, 38.0 ppm) and 3-methyldeoxycytidine (m^3dC, 30.9 ppm), respectively.

In a preliminary study on the reaction of the core particles of rat liver DNA, the signal at 30.7 ppm was assigned to the methyl resonance peak of 3-methyldeoxyadenosine (m^3dA)[29] From an extensive comparison of the chemical shifts of methylribonucleosides with those of methyldeoxyribonucleosides, it becomes apparent that the methyl carbon shifts of the bases are not significantly perturbed by the variation at the ribose (Table I). In order to resolve this discrepancy, we have prepared 3-methyladenosine (m^3A) by modifying the procedures initially established by Saito and Fujii.[30-32] The methyl carbon of m^3A resonates at 39.7 ppm, which clearly indicates that the peak at 30 ppm should not be ascribed to the carbon resonance of m^3dA.

At higher degree of modification, several other *N*-methyl carbon signals are also detected (Figure 2C). The signals at 28.3 and 27.9 ppm were initially assigned to resonance signals of 1-methyldeoxyguanosine (m^1dG, 28.,4 ppm) and 3-methylthymidine (m^3T, 27.6 ppm), respectively. Nevertheless, their chemical shifts are too close to make unequivocal assignments of the specific resonances. To verify the resonance designations, we studied the reactions of poly(uridylic–guanylic acid), containing 1:1 and 10:1 molar ratio of uridine to guanosine with methyl methanesulfonate.[33] By comparing with the change of the product ratio, we have confirmed our initial assignment. A weak

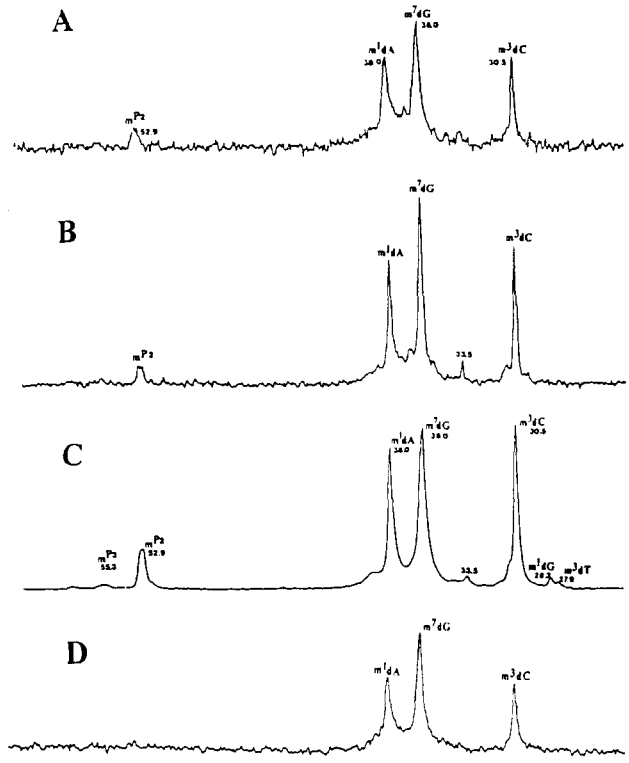

Figure 2. Methyl-carbon region of proton-decoupled ^{13}C NMR spectra of salmon sperm DNA after reacting with ^{13}C-enriched methyl methanesulfonate for (A) 3 h, (B) 6 h, (C) 12 h, and (D) 3 h; DNA was pretreated with alkaline phosphatase. m^3T, 3-methylthymidine; m^1dG, 1-methyldeoxyguanosine.

peak at 39.3 ppm, partially overlaped with the signal of unmodified DNA, might be attributed to the methyl carbon of m^3dA. An accurate assessment of its contribution (<2%) to the total products was not attempted. Another small signal at 33.5 ppm is assigned to the methyl resonance of 7-methylguanine (m^7Gua) on the basis of the chemical-transformation studies of modified DNA discussed in a later section.

Determination of Sites of Modification: Phosphate Methylation. The most downfield signal at 52.9 ppm (Figure 2A) is ascribed to the oxygen-methyl signal. A similar peak (53.3 ppm) in methylated Torula yeast RNA was previously designated to the methyl carbon resonance of methyl phosphate.[17] The ^{13}C–^{31}P two-bond coupling (6 Hz) allowed us to distinguish it from O^6-methylguanosine (m^6G, 53.7 ppm). But the broadness of the DNA signal prevented us from unambiguously measuring this coupling. In order to clearly establish the nature of the phosphoester adduct, we treated salmon sperm DNA with alkaline phosphatase to cleave all terminal phosphate groups. This DNA sample was then reacted with methyl methanesulfonate for 3 h. The ^{13}C NMR spectrum of this product (Figure 2D) showed the absence of the carbon signal at 52.9 ppm. This experiment not only differentiates the methyl phosphate from O^6-methyldeoxyguanosine (m^6dG, 53.3 ppm) but also suggests that only the terminal monophosphate group of DNA was significantly modified.

In parallel with ^{13}C NMR studies we also used ^{31}P NMR in the analysis of the salmon sperm DNA samples. All signals from −3.8 to −5.0 ppm were attributable to the ^{31}P resonances of random-coil polynucleotide (Figure 3A).[34] The most downfield peak at −2.8 ppm could be assigned to the terminal phosphate group, which was absent in the

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(31) We have not been able to repeat the original synthesis of m^3dA[30] and measure the ^{13}C chemical shift of the methyl carbon. In a private communication, Professor Fujii also indicated the failure of measuring the ^{13}C spectrum of this compound.
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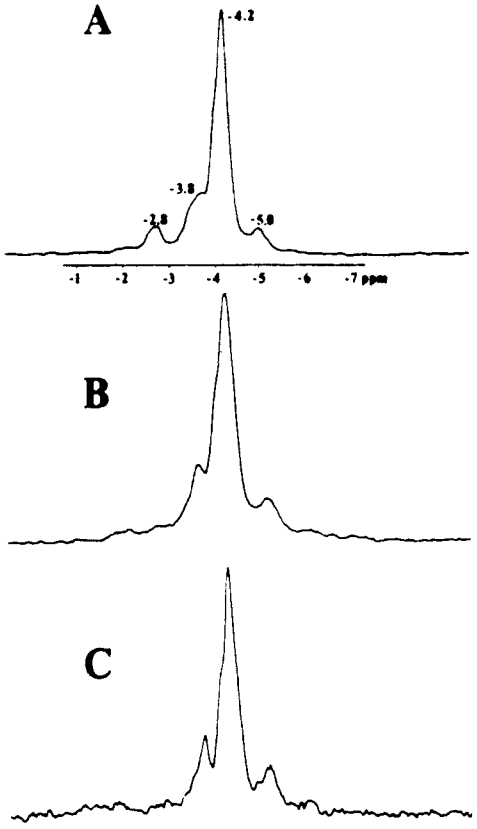

Figure 3. Proton-decoupled ^{31}P NMR spectra of salmon sperm DNA in 1.5 mL of D_2O at pH 7.4 and 20 °C: (A) unmodified DNA (5000 scans; 60 mg/1.5 mL), (B) alkaline phosphatase digested DNA (8600 scans; 60 mg/1.5 mL), (C) unmodified DNA reacted with 90% ^{13}C-enriched MeMS for 12 h (8700 scans; 60 mg/1.5 mL).

spectrum of DNA after alkaline phosphatase treatment (Figure 3B). Methylation of this phosphomonoester caused it to overlap with the phosphodiester signals,[35] confirming that the most downfield ^{13}C signal at 52.9 ppm was ascribed to the resonance methyl phosphodiester (m^{P2}). At a higher level of methylation (Figure 2C), a weak downfield ^{13}C peak at 55.3 ppm could be designated to the carbon resonance of methyl phosphotrieser (m^{P3}) as a result of methylation of the internal phosphate groups. This assignment is based on a direct comparison of the chemical shift to the methyl carbon shift of $dT_p(Me)dT$ (55.8 ppm).

Chemical Transformation of Modified DNA. The most frequently used method to analyze modified DNA was to degrade it into nucleosides by endonucleases and exonuclease under weak alkaline condition (ca. pH 8.2). In order to test the effect of these conditions on methylated DNA, we carefully examined the possible chemical transformation of methylated DNA in mild alkaline solution. It will provide us with some insight into the chemical properties of the modified DNA treated with MeMS for 3 h.

Methylated DNA was incubated in deuterium oxide solution at pD 8.6 and at room temperature for a period of 32, 56, and 80 h. A new signal at 33.5 ppm was observed after 32 h (Figure 4B). The appearance of this peak was at the expense of the peak of m^7dG (Figures 4B and 4C), indicating a specific chemical transformation from m^7dG. We simultaneously monitored the hydrolysis of m^7dG [NCH_3, $\delta(^1H)$ 4.1, $\delta(^{13}C)$ 35.5] into its aglycone 7-methylguanine, m^7Gua [NCH_3, $\delta(^1H)$ 3.9, $\delta(^{13}C)$ 33.5],

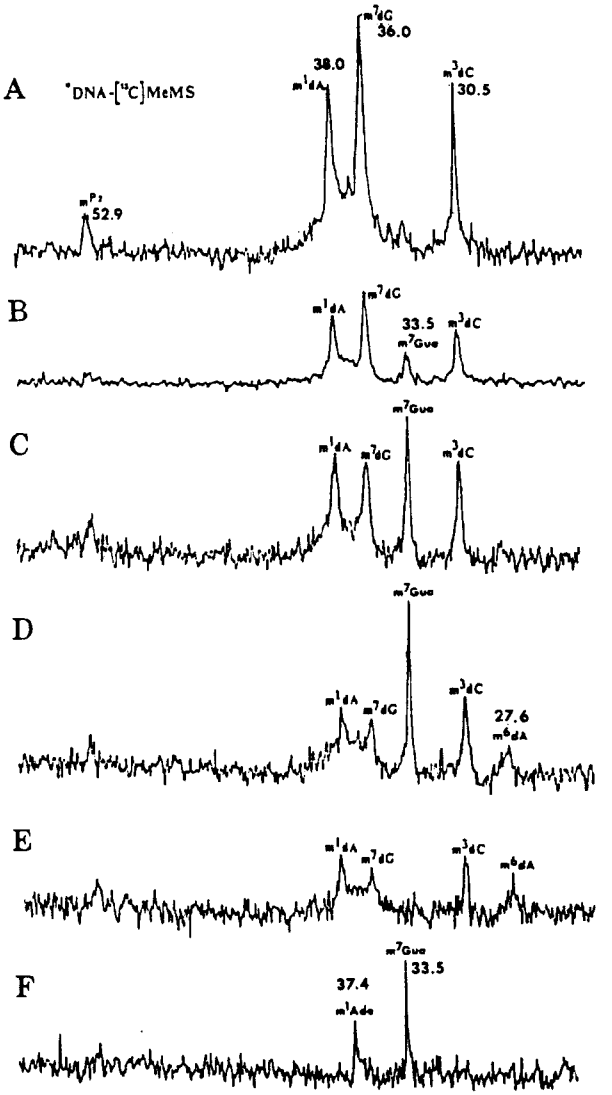

Figure 4. Methyl-carbon region of proton-decoupled ^{13}C NMR spectra of salmon sperm DNA after reacting with ^{13}C-enriched methyl methanesulfonate for 3 h: (A) before incubation; (B–F) after incubation in D_2O solution at pD 8.6 for (B) 32 h, (C) 56 h, (D) 80 h, (E) after dialysis of sample D, and F) dialysate of sample D.

under the same conditions by ^1H and ^{13}C NMR. Therefore, we could conclude that m^7dG was exclusively converted into m^7Gua at intact polynucleotide level. It is interesting to note that a similar modification of single-stranded or double-stranded DNA by dimethyl sulfate has been used in nucleic acid sequencing for detection of guanine.[36] It has been proposed by Maxam and Gilbert[37] that dimethyl sulfate specifically methylates the N-7 position of guanine, which leads to the opening between C-8 and N-9 in a base-catalyzed reaction, and piperidine then displaces the ring-opened 7-methylguanine from its sugar. However, the structure of the ring-opened 7-methylguanosine has not been rigorously established.[38,39] Box et al. recently observed two methyl carbon signals at

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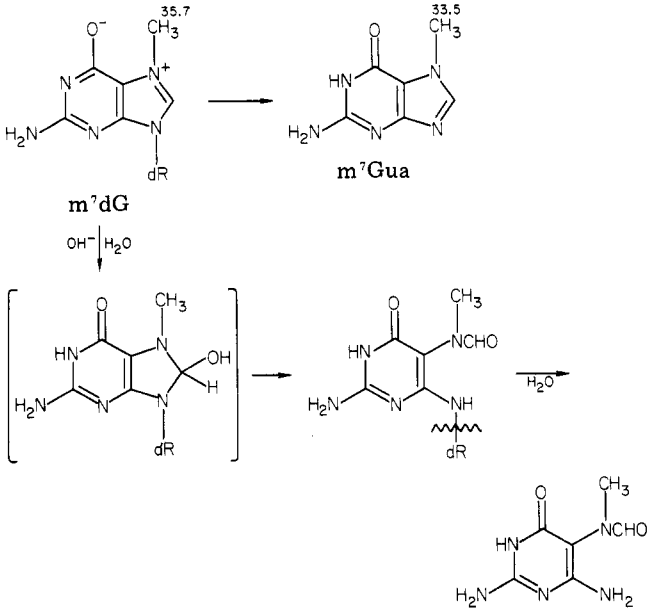


30.6 and 29.2 ppm for the ring-opened products of 7-methylguanosine at pH 9.0.[40] Only one signal at 33.5 ppm was detected in the methylated salmon sperm DNA (Figure 4C). Chetsanga et al. very recently also reported that the ring-opened 7-methylguanine residue in DNA was hardly released by chemical hydrolysis in vitro.[39] In order to induce the formation of the imidazole ring-opened product in polynucleotide, Boiteux and Laval treated methylated poly(dG-dC) with 0.2 N sodium hydroxide solution.[41] All these results exclude the alternative pathway of imidazole ring breakage in weak alkaline solution as proposed by Maxam and Gilbert.[37] Recently, Beranek et al. reported that the ring-opened 7-methylguanine product was detected as a major adduct in rat liver DNA after treatment with *N,N*-dimethylnitrosamine and 1,2-dimethylhydrazine.[42] The formation of this product in vivo may be enzymatically mediated.

At pD 8.6, 1-methyldeoxyadenosine [NCH_3, $\delta(^1H)$ 3.91, $\delta(^{13}C)$ 38.0] was slowly transformed ($t_{1/2}$ = 12 days) into two new products [A, NCH_3, $\delta(^1H)$ 3.14, $\delta(^{13}C)$ 27.9; B, NCH_3, $\delta(^1H)$ 4.0, $\delta(^{13}C)$ 37.4]. Jones and Robins[43] observed the conversion of m¹dA into 6-methyldeoxyadenosine (m⁶dA) in alkaline solution (0.2 N NaOH) and into 1-methyladenine (m¹Ade) in neutral solution. On the other hand, Lawley and Brookes[44] detected the transformation of m¹dA into m⁶dA, not m¹Ade, at neutral condition. Comparison with authentic samples showed that the products A and B were m⁶dA and m¹Ade, respectively. After incubation of the methylated DNA at pD 8.6 for 80 h, the new peak at 27.6 ppm (Figure 4D) could be assigned to the resonance of m⁶dA. The detection of m⁶dA in the methylated DNA by Krepinsky et al. could therefore result from the artificial conversion from m¹dA at pH 7.8.[29] Another obscure peak at 37.4 ppm may be derived from the methyl signal of m¹Ade. Its assignment could not be ascertained because of the overlapping with the C-2 signal of deoxyribose of DNA. We thus carried out a dialysis experiment. The ^{13}C spectrum of the dialysate definitely

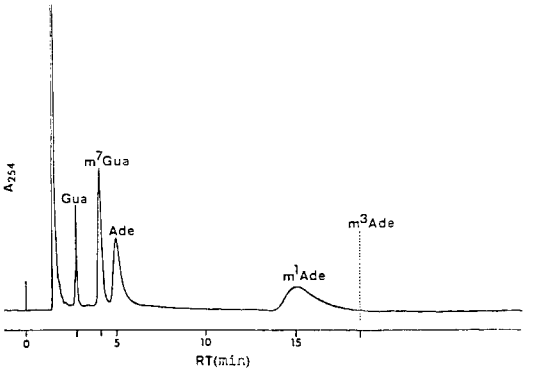
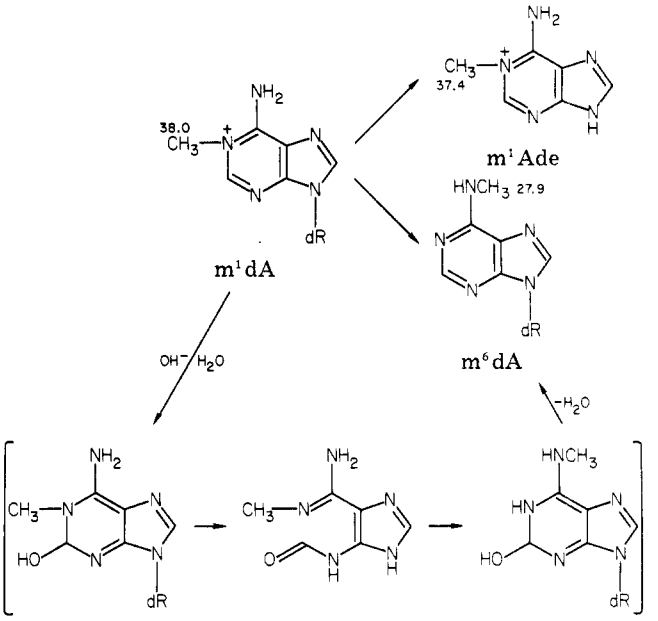
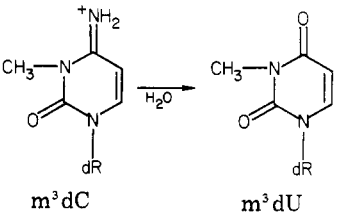


Figure 5. High-performance liquid chromatogram of the dialysate of methylated salmon sperm DNA after alkaline degradation at pD 8.6 for 80 h (sample F in Figure 4). Column, Waters RCM-CP_{18}; solvent, 0.004 M KH_2PO_4 (pH 5.1) and 8.5% CH_3CN; flow rate, 3.5 mL/min.

showed the presence of two aglycons, m¹Ade and m⁷Gua (Figure 4F). Meanwhile the spectrum of the undialyzable



fraction (Figure 4E) unequivocally indicated that the signal at 27.6 ppm was not ascribed to the product of glycosyl bond cleavage and that the stability of the signal was at 30.5 ppm. The model study of 3-methyldeoxycytidine [NCH_3, $\delta(^1H)$ 3.4, $\delta(^{13}C)$ 30.5] also showed a very slow conversion ($t_{1/2}$ = 26 days) to the transamination product, 3-methyldeoxyuridine, m³dU [NCH_3, $\delta(^1H)$ 3.3, $\delta(^{13}C)$ 28.4].



Consequently, the peak at 30.5 ppm undoubtedly could not be assigned to the methyl resonance signal of m³dA as concluded by Krepinsky et al.[29] since it was known that m³dA would readily converted to m³Ade.[30]

However, we could not completely rule out the possibility that the resonance signal of m³Ade might be accidently identical with the signal of m¹Ade or m⁷Gua because the methyl carbon resonance of m³Ade could not be measured due to its insolubility. We therefore analyzed

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Table II. Distribution of Methyl Products from Methylation of Salmon Sperm DNA with Methyl Methanesulfonate[a]

rctn time, h	% rctn[b]	m¹dA	m⁷dG	m³dC	m¹dG	m³T	mP2	mP3
3	2.5	26.2	44.5	23.0			6.3	
6	5.1	24.1	44.5	24.9			6.5	
12	14.8	22.6	41.6	24.0	1.5	1.0	8.1	1.0

[a] For abbreviations for compounds, see Table I. [b] Calculated from ^{14}C incorporation.

the dialysate by HPLC as shown in Figure 5. It unambiguously showed the absence of m³Ade and the depurination process of unmodified deoxyguanosine and deoxyadenosine as well.

Product Distribution. All of the product distributions were calculated from the integration curves and are summarized in Table II. It was assumed that the difference in spin–lattice relaxation time (T_1) for all methyl carbons was small under the measuring conditions (2-s repetition time for 90° pulses) on the basis of the previous T_1 measurement of methylated Torula yeast RNA.[17] Generally, a repetition time of greater than $5T_1$ is required for complete relaxation of excited nuclei.[45] Furthermore, by measuring the ^{13}C spectra at different repetition time (1, 2, and 5 s) to evaluate the actual experimental error, we confirmed that the repetition time of 2 s was sufficient.

Modification of Salmon Testes DNA. Single-stranded salmon sperm DNA was used in the initial studies of chemical modification of DNA mainly because of its higher solubility in water. This not only allowed us to efficiently evaluate the feasibility of this direct NMR approach but also to conduct a series of chemical and biochemical transformations for providing a firm basis for spectral interpretation and understanding the chemical properties of the modified DNA. Nevertheless, this denatured DNA would not afford much insight on the modification of double-stranded DNA. We therefore studied the modification of highly double-stranded (ds) and more native salmon testes DNA (65% ds) and calf thymus DNA (80% ds) to establish the effect of tertiary structure on the chemical modification of DNA. However, the NMR experiments were seriously hampered by the limited solubility even after moderate stirring (<30 mg/mL, salmon testes DNA; 5 mg/mL, calf thymus DNA) and the high viscosity of the aqueous solution. We were unable to measure the natural-abundance ^{13}C spectra of these native DNAs and the spectrum of modified calf thymus DNA. The ^{13}C spectrum of modified salmon testes DNA was initially measured by using a 20-mm wide-bore probe (37.5 MHz, ~8 mL of solution) and then a 10-mm probe (25 MHz, 2 mL of solution). The reduction of the viscosity of the solution and the decreasing line width of signals were discernible, suggesting a slow denaturation resulting from the initial stirring and the instability of this DNA in aqueous solution.

Our results showed that the reactivities of salmon sperm and salmon testes DNAs were considerably different. The degree of modification after 6-h reaction for salmon sperm DNA was 5%. On the other hand, salmon testes DNA required 78-h reaction to reach the same degree of modification. The spectral interpretation for methylated salmon testes DNA was based on the same arguments used for the analysis of the methylation products of salmon

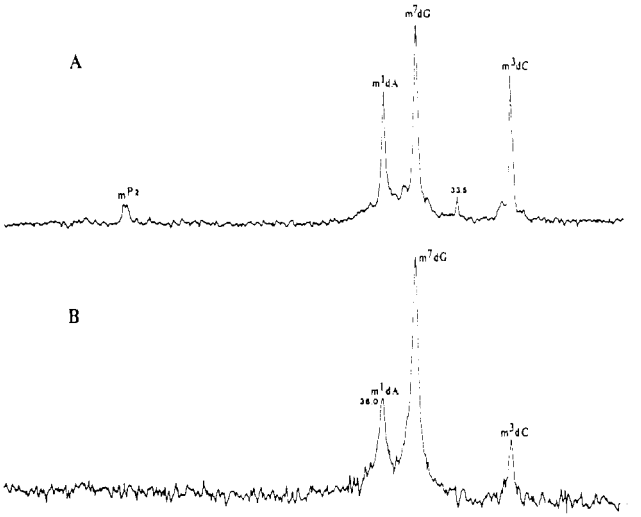

Figure 6. Methyl-carbon region of proton-decoupled ^{13}C NMR spectra of (A) salmon sperm DNA after reacting with ^{13}C-enriched methyl methanesulfonate for 6 h (5.1% reaction) and (B) salmon testes DNA after reacting with ^{13}C-enriched methyl methaensulfonate for 78 h (5.0% reaction).

sperm DNA. Methylation occurred at the N-1 position of deoxyadenosine (38.0 ppm, 14.2%), the N-7 position of deoxyguanosine (36.0 ppm, 72.1%), and the N-3 position of deoxycytidine (30.5 ppm, 13.7%) (Figure 6B). The most remarkable difference between the spectrum of salmon testes DNA (Figure 6B) and that of salmon sperm DNA (Figure 6A) was that the peak at 52.9 ppm corresponding to phosphomethyl signal was absent in the spectrum of methylated salmon testes DNA, again supporting that only the terminal phosphate group of polynucleotide could be significantly modified by methyl methanesulfonate. These results also reflects the difference of average molecular weight of the two DNAs (0.092×10^6 for salmon sperm DNA and 7.4×10^6 for salmon testes DNA). Another important difference between these two DNAs is their degree of double strandedness (22% for salmon sperm DNA, 65% for salmon testes DNA).

At the same degree of modification (5%), we could distinctly observe the difference of the product distribution. The m¹dA/m⁷dG and m³dC/m⁷dG ratios were much smaller in the methylated salmon testes DNA than in the methylated salmon sperm DNA (Table III). This may be primarily ascribed to that both the N-1 position of deoxyadenosine and the N-3 position of deoxycytidine are involved in base pairing, thus becoming less accessible for nucleophilic attack. Indeed, there is good correlation between the ratio of double strandedness (0.34) and the ratio of (m¹A + m³dC)/m⁷dA (0.35) for these two DNAs (Table III), implicating the potential use of a chemical method for determing the relative degree of double-helical character by NMR spectroscopy. Further experiments are necessary before a firm conclusion can be drawn.

Sensitivity Limitation. The inherent low sensitivity of NMR spectroscopy and the low degree of chemical modification will make the direct study of chemically modified in vivo samples difficult. It will not be directly applicable to the analysis of high molecular weight nucleic acids due to the limited solubility in aqueous solution. This difficulty can be overcome by reducing the size of molecule by using sonication[46] or restriction enzymes[20,29] or by measuring the ^{13}C signals at the solid state by using combined techniques of high-power proton decoupling,

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Table III. Product Distribution of Methylated DNAs

DNA source	rctn[b] time, h	% rctn	m^1dA	m^3dC	m^7dG	$(m^1dA + m^3dC)/m^7dG$	% ds[c]
salmon sperm	6	5.1	24.1	24.9	44.5	1.10	22
salmon testes	78	5.0	14.2	13.7	72.1	0.39	65

[a] For abbreviations for compounds, see Table I. [b] Calculated from ^{14}C incorporation. [c] ds, double strandedness.

^{13}C–^1H cross-polarization, and magic-angle spinning.[47] Another difficulty associated with the ^{13}C NMR method is the interference of naturally occurring ^{13}C signals from unmodified nucleic acids. This can be minimized by detecting other low natural-abundance nuclei (^2H, 0.015%; ^{15}N, 0.37%). Our unpublished result indicated that this requires the degradation of the ^2H-labeled nucleic acids into small components by enzymic hydrolysis to reduce the line width of ^2H resonance signals.[48]

Conclusions

We have demonstrated that the approach described here provides a useful method for the direct study of the chemical modification of nucleic acids, including the determination of the sites of reaction and product distribution. This method, which is nondestructive, has advantages over the degradation and separation process because it is less cumbersome and avoids the formation of artifacts. It can be used in the studies of the in vitro mechanism of action of chemical mutagens,[4,49] chemical carcinogens,[4,50] and anticancer agents.[51] This approach may permit us to probe conformational variations of nucleic acids by a chemical method.[52] We have showed that the NMR method may pave a better way for understanding the basic mechanisms of nucleic acid sequencing by chemical methods.[37] In addition, these direct results have provided us a reliable basis for developing other more sensitive methods, including liquid chromatography[53] and mass spectrometry,[54] for in vivo study.

Experimental Section

Materials. 2′-Deoxyadenosine, 2′-deoxycytidine, and thymidine were obtained from Sigma Chemical Co. 2′-Deoxyguanosine was purchased from Boehringer Mannheim Biochemicals. N^6-Methyl-2′-deoxyadenosine, adenine, 1-methyladenine, 3-methyladenine, 7-methyladenine, guanine, 3-methylguanine, and 7-methylguanine were obtained from Vega Biochemicals.

Table IV. Characterization of Salmon Deoxyribonucleic Acids

	salmon sperm DNA	salmon testes DNA
dA, %	26.2	29.4
dG, %	20.3	22.0
T, %	30.7	27.9
dC, %	22.8	20.6
% double strandedness	22	65
M_r	92 000	7.4×10^6

Deoxyribonucleic acid from salmon sperm was obtained from ICN Co. Deoxyribonucleic acids from salmon testes and calf thymus were purchased from Sigma Chemical Co. Deoxyribonucleic acid molecular weight markers were purchased from Boehringer Mannheim Biochemicals. Ribonuclease A from bovine pancreas, alkaline phosphatase type III from *Escherichia coli*, phosphodiesterase I from *Crotalus atorx* venom, S_1-nuclease from *Aspergillus oxyzase*, and protease type III from *Streptomyces griseus* were obtained from Sigma Chemical Co.

^1H NMR. The spectra of deoxynucleosides were measured at ambient temperature in Me_2SO-d_6 solutions, using a deuterium lock, on a Varian FT-80 NMR spectrometer observing ^1H at 79.54 MHz. A spectral width of 1000 Hz, acquisition time of 4.1 s, pulse width of 25 μs, and pulse delay of 6 s were usually used. The free-induction decay was accumulated with an 8K data point memory. The chemical shifts were determined with the center line of the Me_2SO-d_6 signal as the reference at 2.51 ppm, after calibration with Me_4Si.

^{13}C NMR. The spectra of most deoxynucleosides were recorded at ambient temperature in Me_2SO-d_6 solution, using a deuterium lock on a JEOL PFT-100 spectrometer observing at 25.15 MHz. The spectrometer is interfaced with a JEOL EC-100 computer with a 20K memory. A repetition time of 4 s and pulse width of 10 μs (45°) were generally employed. The ^{13}C NMR proton-decoupled spectrum of 7-methyl-2′-deoxyguanosine was measured on a Nicolet NTC-150 spectrometer observing at 37.73 MHz. The proton-coupled spectrum was not measured due to the instability of this modified deoxynucleoside. The ^{13}C NMR spectrum of O^6-methyl-2′-deoxyguanosine was measured on a Varian XL-200 NMR spectrometer observing at 50.3 MHz. The chemical shifts of the signals in the ^{13}C NMR spectra of deoxynucleosides were obtained with the center line of the Me_2SO-d_6 signal, at 39.6 ppm downfield from Me_4Si, as a reference line.

The ^{13}C NMR proton-decoupled spectra of DNA samples were measured at 6 °C on a JEOL PFT-100 spectrometer, a Nicolet NTC-200 spectrometer observing at 50.31 MHz, or a Varian XL-200 spectrometer, using repetition times of 2 s and 90° pulse angles. The NMR samples were prepared in D_2O and the pD values adjusted to 7.40 with NaOD. The chemical shifts of the deoxyribonucleic acid signals were determined with dioxane as internal reference at 66.6 ppm downfield from Me_4Si, ^{13}C NMR sample tubes had outside diameters of 10 mm (JEOL PFT-100), 12 mm and 20 mm (NTC-200 and NTC-150), and 16 mm (Varian XL-200).

^{31}P NMR. The spectra were measured on a Varian FT-80 NMR spectrometer or a NTC-200 spectrometer, utilizing 5-mm and 12-mm probe sizes, respectively. The chemical shifts of the deoxyribonucleic acid samples were determined with trimethyl phosphate as an external reference at 0 ppm.

Other Spectroscopies. UV spectra were recorded on a Cary-17 spectrometer; IR spectra were measured on a Beckman IR-33 spectrometer; and chemical ionization spectra were obtained

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(53) (a) DaSilva Gomes, J. "Interactions of Mutagens with Deoxyribonucleic Acids", Ph.D. Thesis, Purdue University, West Lafayette, IN, 1981. All modified and unmodified nucleosides could be separated on a Wahtman Partisil PXS 5/20 ODS (C_{18} reverse-phase) column eluted with a solution of 10 mM $NH_4H_2PO_4$, 3 mM tetramethylammonium chloride, and 3% acetonitrile at pH 6.53. (b) DaSilva Gomes, J.; Chang, C.-j. *Anal. Biochem.* **1983**, *129*, 387–391.

(54) (a) Ashworth, D. J.; Chang, C.-j.; Unger, S. E.; Cooks, R. G. *J. Org. Chem.* **1981**, *46*, 4770–4774. (b) Ashworth, D. J. "Quantitative Analysis of Nucleic Acid Alkylation in vitro and in Cell Culture", Ph.D. Thesis, Purdue University, West Lafayette, IN, 1982. A high-pressure liquid chromatographic system capable of separating 15 modified and unmodified nucleosides was developed; in conjunction with chemical-ionization mass spectrometry, the quantitation of methylated products of modified calf thymus DNA was achieved at μg level, using synthetic $^2H_3$-methyl-labeled nucleosides as internal references.

Table V. ^{1}H NMR Data of Deoxynucleosides and Some of Their Methylated Products[a, b]

	H_2	H_5	H_6	H_8	H_1	$NCH_3(O)$
dT			7.7 d (1.1)		6.2 t (6.9)	
m^3dT			7.8 d (1.1)		6.2 t (6.8)	3.2 s
dC		5.7 d (7.8)	7.8 d (7.8)		6.2 t (6.7)	
m^3dC		6.2 d (7.9)	8.2 d (7.9)		6.2 t (5.6)	3.4 s
dA	8.2 s			8.3 s	6.4 d (6.4), d (7.9)	
m^1dA	8.7 s			8.7 s	6.4 d (6.0)	3.8 s
dG				7.9 s	6.1 d (6.2), d (7.4)	
m^1dG				7.9 s	6.1 d (6.5), d (7.6)	3.4 s
m^7dG				9.3 s	6.2 d (7.6)	4.0 s
m^6dG				8.1 s	6.2 d (6.2), d (7.5)	(4.0) s

[a] Chemical shifts in ppm downfield from Me_4Si. [b] Coupling constants in hertz.

on a DuPont 21-492B mass spectrometer, utilizing isobutane as ionization gas.

pH Maintenance. In general, alkylations of deoxyribonucleic acids were carried out at pH 7.00 in 10–30-mL vessels, and the pH was maintained constant by using a Radiometer TTA 3 pH-stat titrator with a 2.5-mL autoburet ABU-12. Solution of sodium hydroxide (0.1 N) was used as titrants.

Thin-Layer Chromatography. Thin-layer chromatography in silica gel plates, type Polygram Sil G/UV254 (Brinkmann Instr. Inc.), was used to monitor the course of the methylation of the deoxynucleosides, utilizing different solvent systems.

High-Performance Liquid Chromatography (HPLC). HPLC separations were carried out on a Waters system equipped with a U6K injector, a 6000A solvent delivery unit, and a 440 dual-wavelength UV detector, monitoring efluent absorbances at 254 and 280 nm.

DNA Purification.[19,20] A DNA sample was dissolved in a minimal volume (v, mL) of saline–citrate buffer solution, pH 7.00. The DNA solution was then incubated for 30 min at 37 °C with ribonuclease A. The solution was then incubated for 60 min at the same temperature with protease VI. The enzymes were used in the ratio of 50 mg of ribonuclease and 100 mg of protease per gram of DNA. Following the two incubations, the solution was treated with v mL of water-saturated phenol. The mixture was shaken 30–60 times/min at 4 °C for 25 min. To the emulsion was added v mL of chloroform–isoamyl alcohol (24:1). The sample was shaken for another 2–5 min. The resulting emulsion was centrifuged at 8000 rpm for 15 min, on a Sorval RC2-B centrifuge refrigerated at 4 °C. After separation of the two phases, the aqueous layer was carefully removed, avoiding disturbance of the interphase. To the aqueous phase was added $2v$ mL of ice cold 95% ethanol. The solution was then allowed to stand at −15 °C for 6 h. The DNA was separated by centrifugation at 10000g for 20 min, and after the supernatant was decanted the sample was washed with cold absolute ethanol followed by ether. After drying of the DNA sample at room temperature in a vacuum desiccator, the purity of the DNA was checked by UV spectroscopy.

Average Molecular Weight of DNA.[55] Deoxyribonucleic acid samples from salmon sperm and salmon testes were characterized in terms of their molecular weight by gel electrophoresis. Agarose gels of different strengths (2% for salmon testes DNA and 3% for salmon sperm DNA) were prepared by Tris/borate buffer [0.089 M Tris, 0.89 M boric acid (pH 8.5) and 2.5 mM Na_2EDTA]. The gels were run in a 12-hole vertical tube apparatus in the same buffer solution. Sample sizes were also adjusted to give the best visualization. The DNA samples were dissolved in a buffer solution containing 10% of sucrose and were layered on the top of each gel. The amounts of DNA used were 6 μg of salmon sperm DNA and 3 μg of salmon testes DNA. The gels were run at constant current (50 V) for different periods of time (6 h for salmon sperm DNA and 9 h for salmon testes DNA). As a reference we utilized an hydrolysate of λ-DNA with restriction endonuclease Hind III with molecular weight bands at 15.58×10^6, 6.36×10^6, 4.38×10^6, 2.86×10^6, 1.49×10^6, 1.31×10^6, 0.36×10^6, and 0.092×10^6. Each band was visualized with an acetate buffer solution (pH 4.7) containing 0.2% of methylene blue, after acidification of the gels with 1 M acetic acid (10–15 min). Excess stain was removed by continuously washing the gels with distilled water (1–2 h). The molecular weights of the salmon sperm and of the salmon testes DNA used were calculated from linear relationships of log M_r vs. log R_f obtained for the corresponding reference gels (Table IV).

Double-Strandedness of DNA. (a) S_1-Endonuclease Digestion.[24] Samples of deoxyribonucleic acid (0.38 mg of salmon testes DNA and 0.45 mg of salmon sperm DNA) were treated with 1100 units of S_1-endonuclease for selective degradation of single-stranded fragments at 35 °C in 2 mL of a pH 4.5 buffer solution (0.03 M sodium acetate, 0.05M sodium chloride, 0.001 M zinc sulfate, 5% glycerol) for 30 min. The samples were frozen at −20 °C until further analysis.

(b) Hydroxylapatite Column Chromatography.[25] The endonuclease-treated DNA samples (94 μg of salmon testes DNA and 112 μg of salmon sperm DNA) were applied to an hydroxylapatite column (15 g of dry Bio-Gel HTP (Bio-Rad) packed with 90 mL of a 0.25 M sodium phosphate, 8 M urea buffer solution at pH 7.0 in a glass column eluted at medium pressure), and the single- and double-stranded fractions were separated, utilizing a step gradient elution procedure described by Miyazawa and Thomas. The column was eluted with a 0.25 M sodium phosphate buffer solution (pH 7.0) containing 8 M urea, then with 75 mL of a 0.14 M sodium phosphate buffer solution (pH 7.0), and finally with a 0.48 M sodium phosphate buffer solution (pH 7.0). The single- and double-stranded fractions were collected and quantitated by UV spectrometry. The results were indicated in Table IV.

Base Composition of DNA. Deoxyribonucleic acid samples from salmon sperm, salmon testes, and calf thymus were enzymatically degraded to the nucleoside level and analyzed by high-performance liquid chromatography.

(a) Enzymatic Degradation of DNA. The deoxyribonucleic acids (1 mg) were enzymatically degraded by treatment with 0.2 unit of phosphodiesterase I and 3 units of alkaline phosphatase type III, in 1 mL of Tris buffer at pH 7.2. The incubations were carried out at 37 °C, and their progress was monitored by reverse-phase HPLC (RP-HPLC) for periods up to 6 h. The samples were filtered through 2-μM Milipore filters, diluted to the appropriate concentration with Tris buffer (pH 7.2) and analyzed immediately to prevent degradation and/or rearrangement of unstable products.

(b) RP-HPLC Analysis of Degraded DNA. HPLC analysis of enzymatically degraded DNA samples were carried out with a Whatman Partisil ODS column eluted at 1.2 mL/min with a solution of 5 mM KH_2PO_4, 5 mM pentanesulfonic acid, and 7% methanol at pH 7.0. A step gradient was utilized for elution of the protein fraction (30% methanol/water, 1.5 mL/min). The column was allowed to reequilibrate with the initial solvent for periods between 30 and 45 min at 2 mL/min to establish reproductility of elution times. Quantitations were carried out from calibration curves prepared for each component. The percentages of four major nucleosides were shown in Table IV. Five minor nucleosides (deoxyuridine, deoxyinosine, 5-methyldeoxycytidine, and two unidentified components) were not included in the calculation of the base composition.

Preparation of Model Compounds. 1-Methyldeoxyadenosine,[43] 1-methyldeoxyguanosine,[56] 7-methyldeoxyguanosine,[43]

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Table VI. Carbon-13 NMR Chemical Shifts[a] and ^{13}C-^{1}H Coupling Constants[b] of 2'-Deoxynucleosides and Their Methylated Products

C no.	dT	m^3dT	dC	m^3dC
2	150.9 d, 7.9 (H_6) d, 1.8 (H_1')	150.7 d, 7.6 (H_6) d, 2.4 (H_1') q, 2.4 (NCH_3)	155.8 d, 7.3 (H_6) d, 1.8 (H_1')	147.8 d, 7.4 (H_6) d, <2 (H_1') q, 3.7 (NCH_3)
4	164.2 d, 11.1 (H_6) q, 3.7 (CH_3)	163.0 m	165.9 d, 9.8 (H_6) d, 2.4 (H_5)	159.2 d, 10.4 (H_6) q, 2.4 (NCH_3)
5	109.9 q, 6.1 (CH_3) d, 1.2 (H_6)	108.4 q, 6.1 (CH_3) d, 1.2 (H_6)	94.7 d, 181.3 (H_5)	94.4 d, 181.3 (H_5)
6	136.6 d, 180.1 (H_6) m	134.6 d, 179.8 (H_6) q, 6.1 (CH_3) d, 3.6 (H_1')	141.5 d, 186.8 (H_6) d, 2.4 (H_1') d, 1.8 (H_5)	141.8 d, 187.7 (H_6) d, 3.0 (H_5) d, 3.0 (H_1')
C_5-CH_3	12.6 q, 128.5 (CH_3) d, 4.0 (H_6)	13.0 q, 128.8 (CH_3) d, 4.3 (H_6)		
C_1'	84.3 d, 168.5 (H_1')	85.0 d, 169.1 (H_1')	85.4 d, 172.1 (H_1')	87.4 d, 163.1 (H_1')
C_2'	39.9 d, 133.1 (H_2')	39.8 d, 133.7 (H_2')	40.7 d, 134.0 (H_2')	39.9 d, 134.0 (H_2')
C_3'	70.8 d, 149.5 (H_3')	70.4 d, 149.5 (H_3')	70.8 d, 148.9 (H_3')	69.9 d, 150.2 (H_3')
C_4'	87.6 d, 147.1 (H_4')	87.5 d, 148.3 (H_4')	87.5 d, 148.3 (H_4')	88.4 d, 148.3 (H_1')
C_5'	61.7 t, 141.3 (H_5', H_5'')	61.4 t, 139.8 (H_5', H_5'')	61.7 t, 140.4 (H_5', H_5'')	60.9 t, 142.2 (H_5', H_5'')
N-CH_3		27.6 q, 141.6 (CH_3)		30.9 q, 144.0 (CH_3)

C no.	dA	m^1dA	dG	m^1dG	m^7dG	m^6dG
C_2	152.9 d, 199.6 (H_2)	148.0 d, 217.9 (H_2) q, 3.9 (NCH_3)	154.0 s	154.4 q, 2.4 (NCH_3)	159.7	159.8
C_4	149.2 d, 12.2 (H_2) d, 4.6 (H_8) d, 3.1 (H_1')	146.6 d, 12.2 (H_2) d, 5.0 (H_8) d, 2.8 (H_1')	151.3 d, 4.9 (H_8) d, 2.4 (H_1')	149.2 d, 4.8 (H_8) d, 2.3 (H_1')	149.3	153.8
C_5	119.6 d, 11.0 (H_8) d, 3.0	119.1 d, 11.6 (H_8)	116.9 d, 11.3 (H_8)	115.9 d, 11.3 (H_8)	107.9	114.0
C_6	156.4 d, 11.0 (H_2)	150.8 d, 9.6 (H_2) d, 2.4 (NCH_3)	157.5 s	156.8 q, 2.4 (NCH_3)	100.3	160.7
C_8	140.2 d, 213.0 (H_8) d, 3.7 (H_1')	142.7 d, 217.9 (H_8) d, 3.9 (H_1')	136.2 d, 213.0 (H_8) d, 2.9 (H_1')	136.0 d, 213.7 (H_8) d, 4.1 (H_1')	134.2	137.8
C_1'	84.6 d, 166.0 (H_1')	84.2 d, 166.6 (H_1')	83.2 d, 166.6 (H_1')	82.8 d, 164.2 (H_1')	85.0	82.9
C_2'	40.0 t, 133.4 (H_2')	39.8 t, 133.4 (H_2')	40.0 t, 133.4 (H_2')	39.9 t, 133.2 (H_2')	40.0	40.3
C_3'	71.4 d, 149.5 (H_3')	70.6 d, 145.9 (H_3')	71.2 d, 148.9 (H_3')	71.0 d, 148.3 (H_3')	70.0	70.8
C_4'	88.4 d, 147.7 (H_4')	88.3 d, 149.3 (H_4')	87.9 d, 148.3 (H_4')	87.8 d, 147.1 (H_4')	88.6	87.7
C_5'	62.3 t, 140.5 (H_5', H_5'')	61.5 t, 141.0 (H_5', H_5'')	62.1 t, 140.4 (H_5', H_5'')	62.0 t, 140.1 (H_5', H_5'')	61.2	61.7
NCH_3- (OCH_3)		38.0 q, 142.0 (CH_3) d, 2.6 (H_2)		28.4 q, 140.7 (CH_3)	35.5	(53.3)

[a] The data for each carbon resonance are shown in the following order: chemical shift, multiplicity, and coupling constants (coupling nucleus). [b] In hertz; d, t, q, m, denote doublet, triplet, quartet, multiplet.

O^6-methyldeoxyguanosine,[57] and 3-methyldeoxycytidine[58] were synthesized by the reported procedures. All compounds were characterized by comparing with the known physical properties and further substantiated by ^1H NMR (Table V) and ^{13}C NMR (Table VI) analyses, using the similar approaches in the analyses of methylated nucleotides.[27,28]

3-Methylthymidine. A mixture of 2'-deoxythymidine (0.256 g, 1.02 mmol) and potassium carbonate (0.250 g, 1.52 mmol) was slurried in 3 mL of dry Me_2SO. Methyl methanesulfonate (0.194 g, 1.76 mmol) in dry Me_2SO solution (1 mL) was added to the slurry. The mixture was stirred for 3.5 h at room temperature. The course of the reaction was monitored by thin-layer chromatography, using silica gel plates and a chloroform/methanol/ammonia (8:8:0.3) eluent mixture (R_f values: dT = 0.72, m^3dT = 0.76). The reaction mixture was finally treated with Celite and filtered with suction through a scintered glass filter. The filtrate (one spot on TLC) was then lyophilized and yielded 0.270 g of

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the product. The sample was washed with ethyl ether to remove residual amounts of Me_2SO and then dried in a vacuum desicator: final yield, 0.257 g (95%); mp 128 °C (lit.[59] mp 132–134 °C); UV (H_2O) δ_{max} 267 nm, δ_{min} 235 nm. The NMR results are given in Tables V and VI.

Synthesis of [^{13}C]Methyl Methanesulfonate (MeMS). ^{13}C-labeled methyl methanesulfonate was prepared from the reaction of ^{13}C-labeled methanol (90% ^{13}C enriched, KOR Isotopes) and methanesulfonic anhydride as described in our previous publication.[17] [^{14}C]Methyl methanesufonate was obtained from Amersham Corp. with specific activity of 50 mCi/mmol and purified by distillation under vacuum after mixing with ^{13}C-labeled methyl methanesulfonate.

Methylation of Salmon Sperm DNA with [^{13}C]Methyl Methanesulfonate. (a) Three- and Six-Hour Reactions. An aqueous solution of salmon sperm DNA (0.202 g, 0.60 mmol) was treated at room temperature with a mixture of ^{13}C- and ^{14}C-labeled methyl methanesulfonate (50 μL, 0.60 mmol, 0.08 μCi). The pH of the reaction mixture was maintained at 7.00 ± 0.01 with an automatic titrator, using a 0.1 N sodium hydroxide solution. An aliquot of the reaction mixture (3.5 mL) was withdrawn after 3 h. The sample was dialyzed and its progress was monitored by radioactivity measurements. The methylated DNA sample was finally lyophilized. Six hours after the start of the methylation reaction, the remaining solution was treated following the same procedure, and the product was lyophilized.

(b) Twelve-Hour Reaction. An aqueous solution of salmon sperm DNA (0.250 g, 0.74 mmol) was treated at room temperature for 12 h with a mixture of ^{13}C- and ^{14}C-labeled methyl methanesulfonate (65 μL, 0.74 mmol, 2 μCi). The pH of the reaction was maintained at 7.00 ± 0.02. The sample was worked up as described above.

Chemical Transformation of Methylated Salmon Sperm DNA in Alkaline Solution. (a) ^{13}C NMR Study. A deuterium oxide solution of methylated salmon sperm DNA (3-h reaction with ^{13}C-enriched MeMS) was adjusted to pD 8.6 and allowed to stand at room temperature for a period of 32 h. The base treatment was quenched by readjusting the pD to 7.4, and the ^{13}C NMR spectrum was measured. This treatment was repeated, and ^{13}C NMR spectra were measured after incubating for 56 and 80 h. The final ^{13}C NMR sample was dialyzed against neutral water and then lyophilized.

(b) Reverse-Phase High-Performance Liquid Chromatographic Analysis of Dialysate. The dialysate was redissolved in 5 mL of double-distilled water and was analyzed by reverse-phase HPLC on a radial-compressed C_{18} (10 μm) column from Waters, Inc. eluted at 2 mL/min with a solution containing 0.004 M KH_2PO_4 (pH 5.1) and 8.5% CH_3CN. Preliminary analysis of a pure mixture containing adenine, guanine, 1-methyladenine, 3-methyladenine, and 7-methylguanine was also carried out for identification purposes.

Stability of Mononucleoside at pD 8.6. The decomposition of m^7dG, m^1dA, and m^3dC (5 mg/0.5 mL) in a weak alkaline solution (pD 8.6) was examined by ^{1}H NMR. Integration data of the aromatic as well as the *N*-methyl proton signals were obtained in order to study the course of the degradations taking place in each case, as a function of time.

Methylation of Alkaline Phosphatase Treated Salmon Sperm DNA with [^{13}C]Methyl Methanesulfonate. (a) Alkaline Phosphatase Treatment. Purified salmon sperm DNA (0.400 g, 1.2 mmol) in 5 mL of 0.1 N Tris buffer pH 8.0 was incubated for 1 h at 37 °C with 90 units of alkaline phosphatase type III. The product of this incubation was then extensively dialyzed initially against 100 mL of Tris buffer, pH 7.2 (2 × 3 h) and neutral water (3 × 3 h) and then lyophilized.

(b) Methylation with ^{13}C-Enriched MeMS. An aqueous solution (5 mL) of the alkaline phosphatase treated DNA (0.130 g, 0.39 mmol) was reacted at room temperature with ^{13}C- and ^{14}C-labeled MeMS (30 μL, 0.35 mmol, 6 μCi) for a period of 3 h. The reaction conditions and the product isolation were carried out as previously described.

Methylation of Salmon Testes DNA with [^{13}C]Methyl Methanesulfonate. An aqueous solution (10 mL) of salmon testes DNA (0.100 g, 0.30 mmol) was treated at room temperature for 78 h with a mixture of ^{13}C- and ^{14}C-labeled MeMS (26 μL, 3.30 mmol, 4 μCi). The pH of the reaction mixture was maintained at 7.0 ± 0.1. The sample was worked up as described above.

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Registry No. m^7dG, 28074-91-1; m^1dA, 60192-55-4; m^3dC, 5040-21-1; m^1dG, 5132-79-6; mP^2, 813-78-5; mP^3, 512-56-1; m^3T, 958-74-7; dT, 50-89-5; methyl methanesulfonate, 66-27-3; dC, 951-77-9; dA, 958-09-8; dG, 961-07-9; m^6dG, 964-21-6; m^6dA, 2002-35-9; m^1Ade, 5142-22-3; m^3dU, 24514-32-7.

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Polystyrene-Bound Phenylseleninic Acid: Catalytic Oxidations of Olefins, Ketones, and Aromatic Systems

Richard T. Taylor* and Lawrence A. Flood

Chemistry Department, Miami University, Oxford, Ohio 45056

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A new synthesis of polystyrene-bound phenylseleninic acid, from reaction of mercurated polystyrene and selenium dioxide, is described. A triphasic system of the polymer (in catalytic amounts), aqueous hydrogen peroxide, and dichloromethane is shown to be an effective medium for the conversion of olefins into trans diols and ketones into esters. A biphasic system of the polymer and *tert*-butyl hydroperoxide in refluxing chloroform effects the selective oxidation of benzylic alcohols to the carbonyl species. In a similar catalytic system hydroxy aromatic compounds can be converted into quinones. Conversion of 1,5-dihydroxynaphthalene into juglone can be realized in 70% yield.

The use of organoselenium reagents in organic synthesis has been developed to a great extent over the past several years.[1] While such reagents have been shown to be extremely versatile, they are also moderately expensive and quite toxic. The fact that the phenylselenyl group is the most frequently encountered organic moiety suggested that these reagents could be incorporated into polystyrenes. The use of functionalized polymers in this context would provide significant advantages,[2] including decreased vol-

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