Methylation Study of Ribonucleosides, Deoxyribonucleosides, and 2'-*O*-Methylribonucleosides with Trimethylsulphonium Hydroxide and Trimethylsulphonium Iodide. Influence of the 2'-Hydroxy-groups on the Reactivity of the Base Moieties of Ribonucleosides

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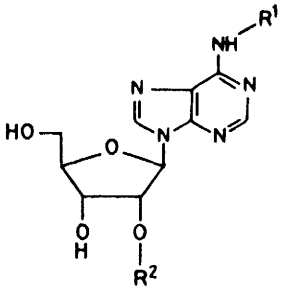
Methylations of the naturally occuring ribonucleoside (1), deoxyribonucleoside (2), and 2'-*O*-methylribonucleoside (3) were carried out using trimethylsulphonium hydroxide (Me_3SOH) and trimethylsulphonium iodide (Me_3SI). The base moiety of (2) and (3) are more reactive than the corresponding base moiety of (1). The sites and extent of methylation of (2) are considerably different from those of (1), but are almost identical with those of (3). The reactivities of (1)—(3) are discussed in connection to an intramolecular interaction of the 2'-OH groups with the base moiety of (1). The methylating characteristics of Me_3SOH and Me_3SI are also described. The kinetics indicate an S_N2 mechanism for methylation of nucleosides by Me_3S^+ ions.

ALKYLATION of nucleic acids and their components has been the subject of many chemical and biological studies. The most interesting aspects of the problem have arisen from the discovery of various kinds of methylated ribonucleosides from RNA,[1] and from studies of mutagenic and carcinogenic effects which were observed in living systems upon administration of alkylating agents.[2]

Most of these investigations dealt with ribonucleosides, and deoxyribonucleosides or their phosphates and polymers. By contrast few studies have been concerned with 2'-*O*-methylribonucleosides although they are present in high concentrations in the loop regions of tRNA and the 5'-terminus of mRNA.[3]

In this paper we describe methylation of the above mentioned three kinds of naturally occurring-nucleosides using trimethylsulphonium hydroxide (Me_3SOH) and trimethylsulphonium iodide (Me_3SI). These reagents are of particular interest since they may be considered as



Abbreviations used are: Ado, adenosine ($R^1 = R^2 = H$); 2'-MeAdo, $O^{2'}$-methyladenosine ($R^1 = H$, $R^2 = Me$); N^6,2'-Me_2Ado, N^6,$O^{2'}$-dimethyladenosine ($R^1 = R^2 = Me$); dAdo: deoxyadenosine; Guo, guanosine; Ino, inosine; Cyd, cytidine; Urd, uridine; 3-MeUrd, 3-methyluridine; dThd, thymidine. Similar abbreviations for other methyl nucleosides.

analogues of *S*-adenosylmethionine, a methyl-group donor in the biomethylation of nucleic acids.[4] Methylation of various kinds of functional groups with Me_3SOH has been reported previously.[5]

Further, we show for the first time the influence of the 2'-OH groups on the reactivity of the base moiety of ribonucleosides. Although the formation of intramolecular hydrogen-bonds between the 2'-OH groups and the base moiety has been suggested by n.m.r.[6] and u.v.[7] spectroscopic studies, as well as alkaline and enzymatic hydrolysis of RNA and oligonucleotides,[8] the effect of the interaction on the chemical reactivity of the base moiety has not yet been demonstrated. One way to shed light on this problem is to compare the reactivity of ribonucleosides with those of deoxyribonucleosides and 2'-*O*-methylribonucleosides, which lack the 2'-OH group. The difference in reactivity between these three kinds of nucleosides, if any, may provide information on important aspects on the nature of nucleic acids.

EXPERIMENTAL

U.v. spectra were measured with a Hitachi 3T spectrometer. N.m.r. spectra were recorded on a JEOL PS-100 using dilute solution in D_2O, with sodium [$^2H_4$]-3-(trimethylsilyl)propionate as internal standard. Electron-impact mass spectra were obtained using a JEOL 01SG-2 spectrometer at 75 eV and 80—120 °C ion-source temperature. Molecular weights (M.W.) were measured using a Knouer vapour-pressure osmometer using methanol as a solvent.

pK_a Values of nucleosides were measured by a u.v. spectroscopic method, using a Tokyo Denki MG-28 pH-meter with an expansion accessory, and aqueous sodium hydroxide and aqueous perchloric acid.

Materials.—Ribonucleosides and deoxyribonucleosides were commercial products. Authentic methyl nucleosides, imidazole-ring-opened derivatives of 7-MeGuo and 7-MedGuo, and 7-methylguanine were prepared according to literature methods.[9-12] Me_3SI was synthesized quantitatively by the reaction of dimethyl sulphide and a slight excess of methyl iodide at room temperature, using acetone as a solvent. The preparation of Me_3SOH from Me_3SI has been previously reported.[5]

Chromatographic Systems.—Dry-packed column chromatography was performed using silica gel (Merck, art 7734, 70—230 mesh). Thin layer chromatography (t.l.c.) was carried out mainly using the following solvent systems. Silica gel [Merck, GF_{254} (type 60)]: solvent A [chloroform–methanol (17 : 3 v/v)] for products of Ado, dAdo, Urd and dUrd; B [chloroform–methanol (15 : 1 v/v)] for products of 2'-MeUrd; C [chloroform–methanol (8 : 1 v/v)] for

products of 2′-MeAdo; D [chloroform–methanol (5 : 1 v/v)] for products of Cyd, dCyd and 2′-MeCyd; cellulose (Eastman chromagram sheet 13254): solvent E [propanol–concentrated ammonium hydroxide (3 : 1 v/v)] for products of Guo and dGuo.

General Procedure for Methylation Reactions and Analysis of the Reaction Mixtures.—A mixture of the nucleoside and a methanolic solution of Me_3SOH in a round-bottom flask was concentrated using a rotatory evaporator. The residue was heated in dimethylformamide (DMF) at 70 °C for 1 h with magnetic stirring. Similarly, a mixture of a nucleoside and Me_3SI was heated in DMF at 70—85 °C for 1—3.5 h. The product distribution in the reaction mixture was determined by u.v. spectroscopy in a manner similar to that described previously.[12] Representative results are summarized in Tables 1 and 2. The course of some methylation reactions are shown in Figures 1—3.

All isolated known compounds had n.m.r. and u.v. spectra (at pH 1, 7, and 13) and m.p.s which were consistent with the assigned structures or literature values. Known compounds which were not isolated were identified by comparison of their mobilities (R_F) in t.l.c., and comparison of the u.v. spectra of the aqueous extracts of the spots with those of authentic samples.

Since 2′-*O*-methyl- and 3′-*O*-methyl-nucleosides were always eluted in the same fraction in the column chromatography of a reaction mixture, their ratio was determined conveniently from the areas of the corresponding methoxy-groups in the n.m.r. spectrum of the mixture. The mixture was resolved into pure compounds by ion-exchange chromatography (Dowex 1 × 2, OH^- form, 100—200 mesh) according to the method employed by Gin and Dekker.[13] The following reactions are typical.

Methylation of Urd, dThd, Guo, dGuo, and Ino with 10% *Excess of* Me_3SOH. Each of these nucleosides (1.00 mmol) was allowed to react with the reagent (1.10 mmol) in DMF (4 ml) according to the general methylation procedure. 3-MeUrd, 3-MedThd, 1-MedGuo, and 1-MeIno were isolated easily in yields of 72, 90, 70, and 63%, respectively, by direct recrystallization, or column chromatography of the concentrated reaction mixtures. 1-Methylguanosine was obtained as pure needle-like crystals by ion-exchange chromatography of the concentrated reaction mixture [Dowex 1 × 2, OH^- form, 100—200 mesh, 1.5 × 50 cm, H_2O–NH_4OH (pH 10—11)], (0.15 g, 53%), m.p. 226—227 °C (methanol) (lit.,[14] 225—227 °C).

Methylation of Ado with Me_3SOH. A mixture of Ado (1.00 g, 3.75 mmol) and the reagent (7.50 mmol) in DMF (15 ml) was processed by the general methylation procedure. The reaction mixture was concentrated and applied to a silica gel column (1.7 cm × 80 cm, solvent A) to give a mixture of N^6,2′-Me_2Ado and N^6,3′-Me_2Ado (ratio 3 : 1) from the first fraction (0.11 g, 10%). The second fraction contained a mixture of 2′-MeAdo and 3′-MeAdo (2.7 : 1),

TABLE 1

Methylation of nucleosides with trimethylsulphonium hydroxide (Me_3SOH) [a]

Run	Nucleoside	Site and extent (%) [b] of methylation: Base	Site and extent (%) [b] of methylation: Sugar	Product (u.v.-spectroscopic yield, %) [c]
1	Ado	N-1, 6-NH_2 (38)	2′(3′)-OH (48)	1-MeAdo (4), N^6-MeAdo (12), 2′-MeAdo (19), 3′-MeAdo (7), 2′,N^6-Me_2Ado (18), 3′,N^6-Me_2Ado (4)
2	dAdo	N-1, 6-NH_2 (75)	3′(5′)-OH (trace)	1-MedAdo (6), N^6-MedAdo (32), N^6N^6-Me_2dAdo [d] (19)
3	2′-MeAdo	N-1, 6-NH_2 (70)	3′-OH (6)	1,2′-Me_2Ado (6), 2′,N^6-Me_2Ado (32), 2′,3′-Me_2Ado (6), 2′,N^6N^6-Me_3Ado [e] (16)
4	Guo	N-1, O-6 (67)	2′(3′)-OH (45)	1-MeGuo (31), 2′-MeGuo (11), 3′-MeGuo (4), 1,2′-Me_2Guo [f] (23), 1,3′-Me_2Guo [f] (7), O^6-MeGuo (6), 7-MeGuo (trace)
5	dGuo	N-1, O-6, 2-NH_2 (91)	3′(5′)-OH (15)	1-MedGuo (61), 3′(or 5′)-MedGuo (4), 1,N^2-Me_2dGuo (7), 1,3′(or 5′)-Me_2dGuo (11), O^6-MedGuo (5), 7-MedGuo (trace)
6	Cyd	N-3 (13)	2′(3′)-OH (35)	3-MeCyd (13), 2′-MeCyd (28), 3′-MeCyd (7)
7	dCyd	N-3, 4-NH_2 (42)	3′(or 5′)-OH (10)	3-MedCyd (25), N^4-MedCyd (17), 3′(or 5′)-MedCyd (10)
8	2′-MeCyd	N-3, 4-NH_2 (55)	3′-OH (8)	3,2′-Me_2Cyd (34), N^4,2′-Me_2Cyd (21), 2′,3′-Me_2Cyd (8)
9	Urd, dUrd, and dThd [a]	N-3 (87—95)	X′-OH (trace)	3-MeUrd (87), 3-MedUrd (90), 3-MedThd (95), respectively
10	2′-MeUrd [a]	N-3 (97)	3′(5′)-OH trace)	3,2′-Me_2Urd (97)
11	Ino [a]	N-1 (85)	2′(3′)-OH (trace)	1-MeIno (85)

[a] Reaction conditions: nucleoside–Me_3SOH–DMF = 1.0 mmol–2.0 mmol–4 ml; reaction temperature, 70 °C; reaction time, 1 h. For methylation of Urd, dUrd, dThd, 2′-MeUrd and Ino, 10% equiv. excess of Me_3SOH was used. [b] For example, the extent of methylation on the adenine ring of dAdo was calculated as follows; 5% (1-MedAdo) + 32% (N^6-MedAdo) + 2 × 19% (N^6N^6-Me_2dAdo) = 75%. [c] Yields based on isolated amounts of products are described in the Experimental section. [d] N^6N^6-Me_2dAdo, m.p. 174—175 °C (acetone–n-hexane); $\lambda_{max.}$ 275 nm (ε 17 500) (pH 7); δ 3.05 (s, 6, NMe_2); *m/e* 279 (M^+) (Found: C, 51.9; H, 6.15; N, 25.45. Calc. for $C_{12}H_{17}N_5O_3$: C, 51.60; H, 6.14; N, 25.07%). [e] Compound was tentatively identified; $\lambda_{max.}$ 269, 275, and 276 nm at pH 1, 7, and 13, respectively; δ 3.50 (s, 3 H, OMe) and 3.04 (s, 6, NMe_2); *m/e* 309 (M^+). [f] 1,2′-Me_2Guo was hygroscopic; m.p. *ca.* 80 °C (acetone–methanol); δ 3.40 (s, 3 H, NMe), and 3.51 (s, 3, OMe); $\lambda_{max.}$ 258, 254, and 255 nm at pH 1, 7, and 13, respectively; *m/e* 311 (M^+, 2%), 166 (base + 2, 30), 165 (base + 1, 100) and 222 (3). 1,3′-Me_2Guo; m.p. 140—143 °C (acetone–diethyl ether); $\lambda_{max.}$ 258 (ε 9 000), 254 (11 000), and 255 (10 500) nm at pH 1, 7, and 13, respectively; δ 3.48 (s, 3, NMe) and 3.60 (s, 3, OMe); *m/e* 311 (M^+, 6%), 208 (10), 166 (base + 2, 25) and 165 (base + 1, 100) (Found: C, 44.85; H, 5.7; N, 21.75. Calc. for $C_{12}H_{17}N_5O_5 \cdot 0.5H_2O$: C, 44.99; H, 5.66; N, 21.86%).

(0.19 g, 18%). N^6-MeAdo was then eluted from the column (0.11 g, 11%), m.p. 219—220 °C (ethyl acetate) (lit.,[15] 219—221 °C).

The mixture of N^6,2′-Me_2Ado and N^6,3′-Me_2Ado was chromatographed on an ion-exchange resin column (1.5

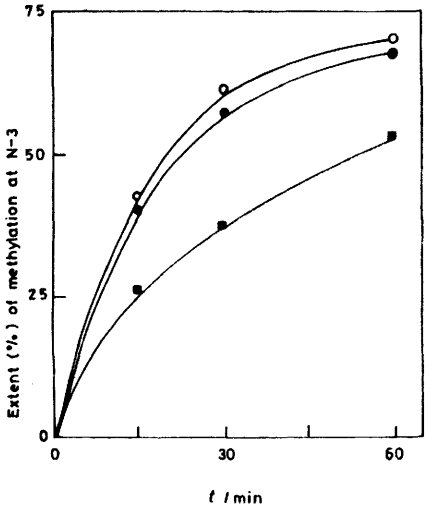


FIGURE 1 Methylation of Urd (■), 2′-MeUrd (●), and dUrd (○) (1.0 mmol) at the N-3 position with Me_3SOH (0.75 mmol) in DMF (4.0 ml) at 65 °C

cm × 30 cm) using water as a solvent. The fraction (375—600 ml) gave N^6,2′-Me_2Ado as crystals (0.06 g), m.p. 104—105 °C (acetone–n-hexane); the HCl salt had m.p. 178—180 °C (lit.,[16] 179—180 °C). The next fraction

TABLE 2

Methylation of nucleosides with trimethylsulphonium iodide (Me_3SI) and the second-order rate constants

Nucleoside	Products	Yield (%) [a]	$k \times 10^2$ (mol^{-1} min^{-1}) at 78 °C
Ado	1-MeAdo	88 (70)	2.6
dAdo	1-MedAdo	95 (81)	4.1
2′-MeAdo	1,2′-Me_2Ado	97 (73)	4.5
Guo	7-methylguanine	8 (5)	
	7-MeGuo	91 { 70 (54)	23.5
	ring-opened 7-MeGuo [b]	13	
dGuo	7-methylguanine	43 (32)	
	7-MedGuo	98 { 37 (11)	36.0
	ring-opened 7-MedGuo [c]	18	
Cyd	3-MeCyd	86 (72)	5.6
dCyd	3-MedCyd	95 (75)	9.5
2′-MeCyd	3,2′-Me_2Cyd	90 (67)	9.6
Urd, dUrd, dThd, and 2′-MeUrd	no reaction		

[a] A mixture of nucleoside (1.0 mmol) and Me_3SI (1.2 mmol) in DMF (4 ml) was heated at 85 °C for 3.5 h. Yields were obtained by a t.l.c.–u.v. method as mentioned in the Experimental section. Yields in parentheses were based on isolated amounts of products. [b] 2-Amino-5-(formylmethylamino)-4-(1-furanosylamino)pyrimidine-6(1*H*)-one. [c] A 2′-deoxyfuranosyl homologue of the compound described in *b*.

(765—975 ml) afforded N^6,3′-Me_2Ado (0.02 g), m.p. 189—190 °C (acetone–n-hexane); $\lambda_{max.}$ 262 nm (ε 15 900) (pH 1), 266 (15 300) (pH 7), and 266 (15 300) (pH 13); δ 2.95 (s,

* Peaks at *m/e* 192 (N^6,3′-Me_2Ado), 222 (1,2′-Me_2Guo), and 208 (1,3′-Me_2Guo) were assigned to H(base$^+$)–CH=CH(OH or OMe) ions (S. J. Shaw, D. M. Desiderio, K. Tsuboyama, and J. A. McCloskey, *J. Amer. Chem. Soc.*, 1970, **92**, 2510).

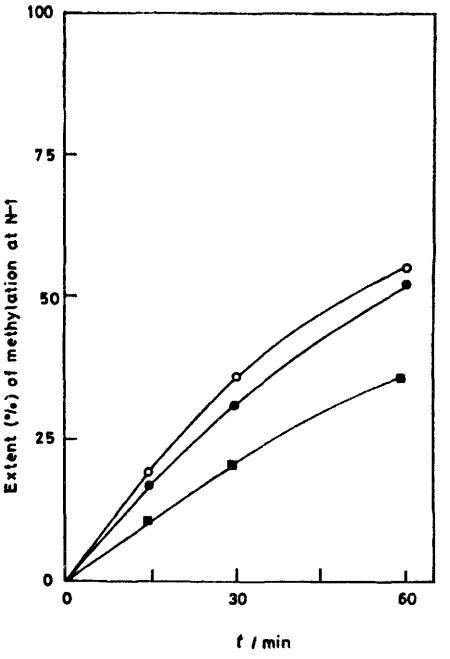


FIGURE 2 Methylation of Ado (■), dAdo (●), and 2′-MeAdo (○) (1.0 mmol) at the N-1 position with Me_3SI (2.0 mmol) in DMF (4.0 ml) at 70 °C

3H, NMe) and 3.63 (s, 3-H, OMe); *m/e* 295 (*M*, 30%), 192 (12),* 150 (base +2, 90) and 149 (base +1, 100) (Found: C, 48.75; H, 5.7; N, 24.0. Calc. for $C_{12}H_{17}N_5O_4$: C, 48.80; H, 5.80; N, 23.72%).

A mixture of 2′-MeAdo and 3′-MeAdo was similarly separated into the pure compounds; yields 0.09 g (9%) and 0.03 g (3%), respectively.

Methylation of dGuo with Me_3SOH. Deoxyguanosine

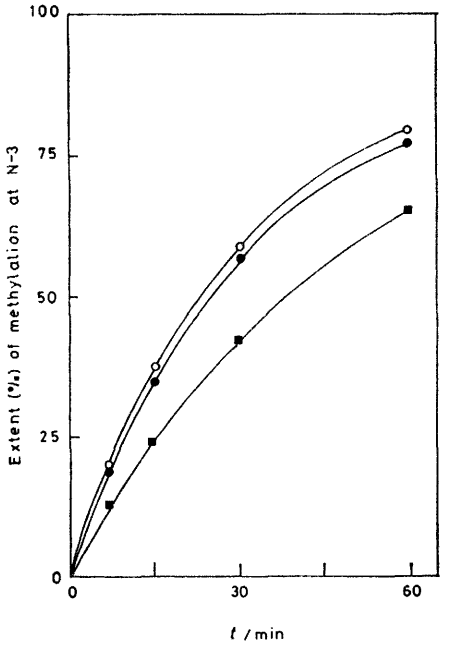


FIGURE 3 Methylation of Cyd (■), dCyd (●), and 2′-MeCyd (○) (1.0 mmol) at the N-3 position with Me_3SI (2.0 mmol) in DMF (4.0 ml) at 70 °C

(83 mg, 0.30 mmol) was allowed to react with Me_3SOH (0.61 mmol) in DMF (1.2 ml) at 70 °C for 1 h. The reaction mixture was concentrated and the residue was dissolved in a small amount of methanol. The solution, after standing overnight, gave crude 1-MedGuo, which was purified by anion-exchange chromatography, yield 19 mg (23%). The mother-liquor was applied to three silica gel preparative t.l.c. plates (20 × 20 cm, 15 g of support per plate). The loaded plates were developed with chloroform–methanol (6 : 1 v/v). Five major bands were scraped from the plates [bands 1—5 in order of increasing R_F values] and extracted with methanol. Band 1 (7 mg) was identified as 1-MedGuo by comparison of its u.v. and n.m.r. spectra, as well as m.p. and R_F values, with those of the authentic sample.

Bands 2—4 were assigned structures as follows.

Band 2, 1,N^2-Me_2dGuo (7 mg), m.p. 180 °C (decomposition), M.W. 294 (calc. 295); *m/e* 179 (base +1); $\lambda_{max.}$ 259 nm (ε 10 500) (pH 1), 257 (12 000) (pH 7), and 257 (12 200) (pH 13); δ 3.40 (s, 3-H, 2-NMe) and 3.51 (s, 3-H, 1-NMe); hydrolysis of band 2 with 1N-HCl gave 1,N^2-dimethylguanine.

Band 3, 3′(or 5′)-MedGuo (5 mg), m.p. 238—241 °C, M.W. 279 (calc. 281); *m/e* 151 (base +1); $\lambda_{max.}$ 256 nm (ε 12 000) (pH 1), 253 (13 100) (pH 7), and 256—270 (11 500) (pH 13); δ 3.43 [s, 3 H, 3′(or 5′)-OMe]; hydrolysis of band 3 with 1N-HCl provided guanine.

Band 4, 1,3′(or 5′)-Me_2dGuo (7 mg), m.p. 135—137 °C, M.W. 295 (calc. 295); *m/e* 165 (base +1); $\lambda_{max.}$ 259 nm (ε 10 500) (pH 1), 256 (12 500) (pH 7) and 256 (12 500) (pH 13); δ 3.41 [s, 3 H, 1-NMe or 3′(or 5′)-OMe] and 3.48 [s, 3 H, 3′(or 5′)-OMe or 1-NMe]; hydrolysis of band 4 with 1N-HCl afforded 1-methylguanine.

Band 5, O^6-MedGuo (6 mg), m.p. 125 °C (lit.,[17] 121—123 °C).

Methylation of dCyd with Me_3SOH. The reaction mixture from dCyd (0.43 g, 1.9 mmol) and Me_3SOH (5.2 mmol) in DMF (8 ml) was subjected to silica gel preparative t.l.c. using solvent C to give N^4-MedCyd (0.023 g, 5%), m.p. 193—194 °C (EtOH) (lit.,[18] 191—193 °C) and 3′(or 5′)-MedCyd (0.025 g, 6%). The HCl salt of the latter compound was prepared by the usual procedure, m.p. 183 °C (ethanol–diethyl ether); $\lambda_{max.}$ 280 nm (ε 13 000) (pH 1), 272 (8 500) (pH 7), and 274 (8 500) (pH 13) (Found: C, 43.5; H, 5.85; N, 15.0. Calc. for $C_{10}H_{15}N_3O_4$·HCl: C, 43.24; H, 5.80; N, 15.13%).

Methylation of Cyd with Me_3SI. Cytidine (0.48 g, 1.9 mmol) was reacted with Me_3SI (0.46 g, 2.3 mmol) in DMF (8 ml) at 85 °C for 3.5 h. The resulting solution was concentrated, dissolved in ethanol (5 ml), and poured into n-hexane (150 ml). The oily residue which separated was mixed with acetone (100 ml). The precipitate was recrystallized from acetone containing a small amount of methanol to give the hydriodide salt of 3-MeCyd (0.52 g, 72%), m.p. 179—180 °C; $\lambda_{max.}$ 278 nm (ε 11 300) (pH 1), 278 (11 000) (pH 7) and 265 (9 000) (pH 13); δ 3.52 (s, 3 H, Me), 3.83—4.60 (m, 5 H, 2′-H, 3′-H, 4′-H, and 5′-H_2), 5.90 (d, 1 H, *J* 2.5 Hz, 1′-H), 6.35 (d, 1 H, 5-H), and 8.17 (d, 1 H, 6-H) (Found: C, 31.3; H, 4.1; N, 10.65. Calc. for $C_{10}H_{15}N_3O_5$·HI: C, 31.18; H, 4.18; N, 10.91%).

Kinetics of Reactions of Nucleosides with Me_3SI.—The n.m.r. spectrum of a mixture of a nucleoside (0.10 mmol) and Me_3SI (0.20 mmol) in [$^2H_7$]DMF (0.5 mmol) was recorded (at 78 °C) at 5—10-min intervals to measure the areas of the base ring protons of the starting nucleoside and the methylated nucleosides, as well as the methyl group of Me_3SI. A t.l.c. analysis of the reaction mixture was also carried out to obtain the yield of the methylated nucleosides, which agreed well with the n.m.r. values. The data were substituted in the second-order equation rate = k[nucleoside][Me_3SI] to calculate k (Table 2). Since Guo did not go into complete solution under the above conditions, it (0.10 mmol) was dissolved in DMF (2.0 ml) at 78 °C and mixed with Me_3SI (0.20 mmol). The combined yields of 7-MeGuo and its derivatives (see Table 2) were obtained from a t.l.c. analysis of the reaction mixture and considered as to the extent of methylation at the N-7 position of Guo. The data fitted excellently the second-order rate equation by assuming that Guo also consumed an equivalent amount of Me_3SI.

RESULTS

Methylation by Me_3SOH.—The analysis and isolation of products was carried out very easily since the reagent co-produced only water and dimethyl sulphide. Typical methylation reactions using 2 equiv. of Me_3SOH are summarized in Table 1. Reaction proceeded homogeneously in most cases and was usually complete within 30 min. Results of methylation in sugar and base moieties of the nucleosides are described separately.

Ribose and deoxyribose moieties. Hydroxy-groups of the ribose moiety, especially 2′-OH and 3′-OH, were methylated more easily than the hydroxy-groups of the deoxyribose moiety. For instance, with 2 equiv. of Me_3SOH, the total extent of *O*′-methylation in Cyd and dCyd were *ca.* 35 and 10%, respectively (Table 1, runs 6 and 7). The ratio of 2′-*O*-methylation to 3′-*O*-methylation in the ribose moiety was 3—4 : 1. With a larger excess of Me_3SOH, ribose and deoxyribose hydroxy-groups were multiply methylated to give complex product distributions.

Adenine ring. Many methylating agents have been shown to attack predominantly the N-1 position under neutral and alkaline conditions.[14,15,19] With 2 equiv. of Me_3SOH, Ado was transformed into a mixture of 1-MeAdo, N^6-MeAdo, N^6,2′-Me_2Ado and N^6,3′-Me_2Ado (Table 1, run 1). Here, N^6-MeAdo may be derived mainly by a Dimroth rearrangement [20] of 1-MeAdo, and a quantitative conversion was indeed observed when 1-MeAdo was treated with Me_3SOH at room temperature. The direct methylation of the adenine ring at the 6-NH_2, which is much less nucleophilic than the N-1 atom, may account for the small proportion of total N^6-methylated adenosines produced.

In contrast, the sites and extents of methylation in dAdo were considerably different from those of Ado, but were nearly identical with those of 2′-MeAdo (Table 1, runs 2 and 3); *e.g.* the product distributions of the 1-methyl, N^6-methyl and N^6N^6-dimethyl derivatives were: Ado; 4%, 34%, and trace; dAdo and 2′-MeAdo, 5—6%, 32%, and 16—19%, respectively.

Guanine ring. Guanine rings in nucleic acids have been established as the prime targets for the attack of various kinds of alkylating agents. Under neutral conditions, the most reactive site in the ring is N-7.[2,15] Under alkaline conditions, guanine rings of nucleosides or polymers are methylated at N-7 and N-1.[11,14] Diazomethane and its homologues have been shown to methylate the ring at O-6 in addition to N-1 and N-7.[18,21]

In contrast, Me_3SOH hardly attacked the N-7 positions of Guo and dGuo (Table 1, runs 4 and 5). Yields of O^6-MeGuo and O^6-MedGuo were also very small. The reagent,

however, easily converted Guo and dGuo into the corresponding 1-methyl derivatives as the chief products. In addition, dGuo was methylated at the 2-NH_2 group to give also 1,N^2-Me_2dGuo. The reaction of 1-MedGuo and Me_3SOH also afforded 1,N^2-Me_2dGuo (10%) along with 1,3′(or 5′)-Me_2dGuo (25%) under comparable conditions. Guo and 1-MeGuo, however, were not completely methylated at the 2-NH_2 group.

The difference in reactivity between the guanine rings of Guo and dGuo was also observed in the total extent of methylation in the rings. For instance, with 2 equiv. of reagent (Table 1, runs 4 and 5): dGuo, 91% {= 61% (N-1) +2 × 7% (N-1 and 2-NH_2) +11% [N-1 and 3′ (or 5′)-OH] +5% (O-6): Guo, 67% [= 31% (N-1) +30% [N-1 and 2′ (3′)-OH] +6% (O-6)}.

Cytosine ring. Cytidine was methylated by Me_3SOH at the N-3 position and 2′(3′)-OH groups (Table 1, run 6). Most other methylating agents react at the N-3 position under neutral and weakly alkaline conditions,[11,22] and the 2′(3′)-OH groups under strongly alkaline condition.[11,23]

Although Me_3SOH scarcely methylated Cyd at the 4-NH_2 group, the reagent converted dCyd and 2′-MeCyd into the corresponding N^4-methyl derivatives in good yields (Table 1, runs 7 and 8). Furthermore the cytosine rings of dCyd and 2′-MeCyd reacted with the reagent more easily than that of Cyd. 1-Methylcytosine also resembled dCyd in reactivity, giving 1,3-dimethyl- (40%) and 1,N^4-dimethyl- (28%) cytosines by treatment with 2 equiv. of Me_3SOH at 70 °C for 1 h.

Uracil and thymine rings. Urd, dUrd, dThd, and 2′-MeUrd were converted quantitatively into the corresponding 3-methyl derivatives by a 10% excess of Me_3SOH (Table 1, runs 9 and 10). With the increased amount of the reagent, methylation occurred not only at N-3 but also the sugar hydroxy-groups to give various products. However, unlike diazomethane,[17,24] Me_3SOH did not methylate uracil and thymine rings at O-2 and O-4 at all.

Figure 1 shows the course of the reactions of Urd, dUrd, and 2′-MeUrd with Me_2SOH. It is clear that dUrd and 2′-MeUrd were methylated at N-3 considerably faster than Urd. The methylation rate of dThd was almost identical with that of dUrd.

Methylation by Me_3SI.—Results of reactions are summarized in Table 2. Unlike Me_3SOH, Me_3SI did not methylate the sugar hydroxy-groups. The amide groups of the guanine and hypoxanthine rings (N^1H), and the imide groups of the uracil and thymine rings (N^3H) were also not substituted by methyl groups. Methylation took place exclusively at the nucleophilic sites; adenine ring (N-1), guanine ring (N-7), and cytosine ring (N-3).

The kinetics of the reactions were consistent with the second-order equation, rate = k[nucleoside][Me_3SI]. The values of k are listed in Table 2. Figures 2 and 3 illustrate typically the course of the methylation reactions of various adenine and cytosine nucleosides. It is apparent again that base moiety of deoxyribonucleosides is of comparable reactivity to the corresponding base moiety of 2′-*O*-methylribonucleosides, but are more reactive than those of ribonucleosides.

Although Me_3SI provided products similar to those obtained by methyl iodide,[14,15] the former was much more efficient than the latter, which is employed usually in large excess. Further, Me_3SI allowed reactions to be run at high temperature (70—100 °C) in order to obtain products in good yields within a relatively short time.

DISCUSSION

The methylation of nucleosides by Me_3SI took place only at the nucleophilic sites of the base moiety. In contrast, Me_3SOH was capable of methylating nucleosides not only at the nucleophilic sites but also at the amide and the imide groups, and the sugar hydroxy-groups. Possibly, Me_3S^+ ion attacked the nucleophilic sites directly, or OH^- ion from the reagent abstracted protons from the groups to activate them as strong nucleophiles.[5]

Although the reactivity of other methylating agents has already been mentioned briefly in the Results section in comparison with the present reactions, diazomethane may be of special interest since it is activated by abstraction of a proton from substrate or solvent to give the $Me-N_2^+$ ion, a similar form to the $Me-S^+(Me)_2$ ion. The methyldiazonium ion, however, must be short-lived and readily give rise to Me^+ and N_2 for an S_N1 reaction, attacking the more electronegative atom (O > N). By contrast, the Me_3S^+ ion is much more stable and may undergo an S_N2 reaction to attack the more polarizable and less electronegative atom (N > O). This agrees well with the low tendency of Me_3SOH and Me_3SI to methylate the O atoms of guanine, uracil, and thymine. Reactions using Me_3SI demonstrated S_N2 kinetics.

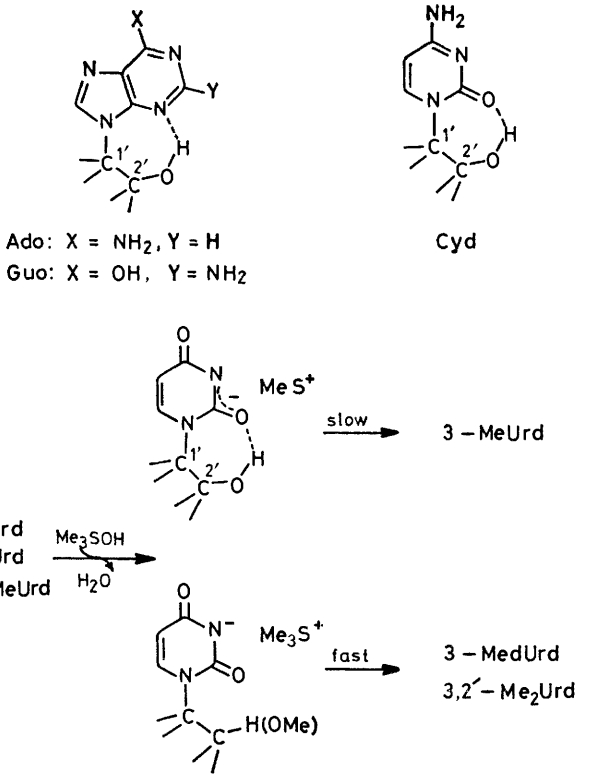
Meanwhile, the present methylation reactions revealed for the first time that the base moiety of deoxyribonucleosides was as reactive as the corresponding base moiety of 2′-*O*-methylribonucleosides, but was more reactive than those of ribonucleosides (extents of base methylation in Table 1, k values in Table 2, and Figures 1—3). In addition, the external amino-groups of deoxyribonucleosides and 2′-*O*-methylribonucleosides were substantially methylated whereas those groups of ribonucleosides underwent methylation to a limited extent (Table 1, runs 1—8) or to a much smaller extent even using 3—4 equiv. of Me_3SOH.

The reactivity of the base moiety is influenced clearly by the presence or absence of 2′-OH groups. The mechanism whereby 2′-OH groups affect the base reactivity is not immediately apparent. The stereochemistry of ribose moieties may not be relevant to the difference in the base reactivity between ribonucleosides and 2′-*O*-methylribonucleosides since 2′-*O*-methylation in ribonucleosides has been shown hardly to affect the ribose conformation (2′-*endo*).[25]

However, n.m.r.[6] and u.v.[7] spectra and hydrolysis of polynucleotides[8] have suggested the existence of intramolecular hydrogen-bonding between the 2′-OH groups of the sugar and the N-3 of the purine rings or the carbonyl groups at C-2 of the pyrimidine rings. It is plausible that these H-bonding interactions cause the base moiety of ribonucleosides to be less nucleophilic toward Me_3S^+ ions than the corresponding base moiety of deoxyribonucleosides and 2′-*O*-methylribonucleosides. The active forms of Urd, dUrd, and 2′-MeUrd are the anionic forms, which can be generated rapidly by Me_3SOH. The electron density at N-3 of the anionic form

of Urd may be decreased by H-bonding, and the site must then be less nucleophilic towards the Me_3S^+ ion than N-3 of the anionic forms of dUrd and 2′-MeUrd.

If the inductive effect of the electron-withdrawing 2′-OH groups in ribonucleosides were responsible for the decreased base reactivity, there should not be any significant differences in base reactivity between ribonucleosides and 2′-*O*-methylribonucleosides because



hydroxy- and methoxy-groups are almost similar in electron-withdrawing strength.[26]

Although no significant variations in pK_a values was observed in ribonucleosides, deoxyribonucleosides, and 2′-*O*-methylribonucleosides,* water has been known to break up intramolecular H-bonding.[27] In this respect it is noteworthy that 2′-*O*-methylation of single-stranded adenine-containing polynucleotides did cause the adenine rings to be more easily protonated; *e.g.* apparent pK_a values: poly(Ado), 5.85; poly(2′-MeAdo), 6.20.[28] The results might be ascribed to the decreased polarity around the nucleoside residues in the polymers. The reactivity of the above three kinds of nucleosides in nucleic acids is currently under investigation.

* Ado (3.6), dAdo (3.7), 2′-MeAdo (3.6), Guo (9.2), dGuo (9.3), 2′-MeGuo (9.2), Cyd (4.2), dCyd (4.3), 2′-MeCyd (4.3), Urd (9.2), dUrd (9.3), and 2′-MeUrd (9.3).

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