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Selective 2′-*O*-Methylation of Pyrimidine-Ribonucleosides by Trimethylsulfonium Hydroxide in the Presence of Mg^{2+} and Ca^{2+} Ions[1)]

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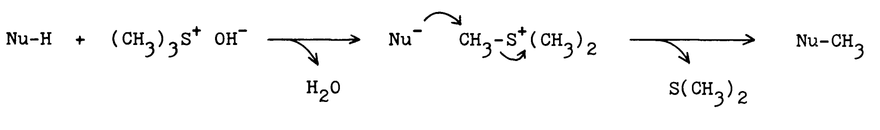
Synopsis. Reactions of various ribonucleosides with trimethylsulfonium hydroxide were investigated in the presence of Mg^{2+} and Ca^{2+} ions. The 2′-OH groups of pyrimidine-ribonucleosides were methylated selectively.

The 2′-OH group of a ribonucleoside is assumed to be similar in reactivity toward various acylating and alkylating reagents to the 2′-OH groups of other ribonucleosides.[2)] In this paper we wish to report that (a) the 2′-OH groups of pyrimidine-ribonucleosides were methylated selectively by trimethylsulfonium hydroxide [$(CH_3)_3S^+\ OH^-$, abbreviated as Me_3SOH hereafter] in the presence of Mg^{2+} and Ca^{2+} ions and (b) the selectivity was much higher than that observed in purine-ribonucleosides.



$$\text{Nu-H} + (CH_3)_3S^+\ OH^- \xrightarrow[H_2O]{} \text{Nu}^-\ \ CH_3\text{-}\overset{+}{S}(CH_3)_2 \xrightarrow[S(CH_3)_2]{} \text{Nu-CH}_3 \qquad (1)$$

Methylation reactions were carried out by stirring a mixture of ribonucleosides and 1.5—2 equivalents of Me_3SOH in the presence and absence of 0.25—1 equivalent of $Mg(acac)_2$ or $Ca(acac)_2$ (acac=acetylacetonato) in DMF at 70 °C for 1 h. The metal salts were soluble in the solvent and allowed the reactions to occur in homogeneous solutions. Me_3SOH is a SN2-type reagent which reacts with nucleophiles according to the general scheme of Eq. 1.[3)] Methylation of the acetylacetone ligand by Me_3SOH was negligible under the conditions used. Typical results are listed in Table 1. One may draw the following conclusions.

(i) In the absence of $Mg(acac)_2$ and $Ca(acac)_2$, methylation occurred rather randomly on the ribose and the purine or pyrimidine moieties of the starting nucleosides (Entries 1, 4, 6, and 8).

(ii) In the presence of $Mg(acac)_2$ and $Ca(acac)_2$, Ado gave chiefly a mixture of 2′-MeAdo and 3′-MeAdo in a ratio of ca. 2.5:1 (entries 2 and 3).[1)] The combined extents of 2′-*O*-methylation and 3′-*O*-methylation[4)], however, showed only slight increases to 39 and 19% from 31 and 11%, respectively, which were observed in the absence of the metal salts. The use of the metal salts did not offer any merits of 2′-*O*- and 3′-*O*-methylations of other purine-ribonucleosides such as Guo, *N*⁶-MeAdo and 1-MeGuo, either (the results are omitted in the Table).

(iii) By contrast, $Mg(acac)_2$ and $Ca(acac)_2$ allowed Me_3SOH to attack selectively and efficiently the 2′-OH groups of the pyrimidine-ribonucleosides such as Cyd, 3-MeUrd and 3-BenzylUrd (Entries 5 and 9—11). The yields of the 2′-*O*-methyl derivatives were better than those of the previous procedures.[5—8)] Urd gave 3,2′-Me_2Urd in a modest yield perhaps through 2′-*O*-methylation of the primary product, 3-MeUrd (Entry 7). The ratio of the 2′-*O*-methylation to the 3′-*O*-methylations were more than 10.

Many transition metal ions including Sn^{2+} and Cu^{2+} ions have been known to promote 2′-*O*- and 3′-*O*-methylations of both purine- and pyrimidine-nucleosides, affording the corresponding 2′-*O*-methyl and 3′-*O*-methyl derivatives with ratios of 2—5:1,[5—8)] and the effects have been attributed to the increased nucleophilicity of the 2′(3′)-*O*-atom upon their chelation with the metal ions.[9,10)] The same concept may be applied to an efficient 2′-*O*- and 3′-*O*-methylations of 2′,3′-*O*-(dibutylstannylene)uridine with methyl iodide.[11)] Although it has been unsettled why a combination of Me_3SOH and $Mg(acac)_2$ or $Ca(acac)_2$ permitted the pyrimidine-nucleosides to undergo selective 2′-*O*-methylation, yet the results would be ascribed most likely to the chelating property of the metal ions (to the nucleosides) which might be different from the transition metal ions. The mechanism of the 2′-*O*-methylation is now under investigation.

Experimental

Ado, Cyd, and Urd were the highest purities obtainable commercially. 3-MeUrd and authentic samples (2′-MeAdo, 3′-MeAdo, *N*⁶,2′-Me_2Ado, *N*⁶,3′-Me_2Ado, 2′-MeCyd, 3′-MeCyd, *N*⁴-MeCyd, 2′-MeUrd, 3,2′-Me_2Urd, and 3-MeUrd) were prepared previously.[8,13)] 3-BenzylUrd was synthesized by treating Urd (2.0 g, 8.2 mmol) in DMF (10 ml) with sodium hydride (60% in oil, 0.33 g, 8.2 mmol) followed by addition of benzyl bromide (1.7 g, 10 mmol) and refluxing the turbid mixture for 3 h. The reaction mixture was concentrated and applied to a silica-gel column. Elution with a mixture of chloroform and methanol (10-1 v/v) afforded 3-BenzylUrd, which was recrystallized from water; 2.4 g (88%), mp 179—181 °C, UV λ_{max} (H_2O) 261 nm (ε 7500) at pH 1 and 6, 261 nm (ε 7500) at pH 11.

Similarly, 2′-MeUrd was benzylated a the *N*-3 position to give 3-Benzyl, 2′-MeUrd in a 75% yield: Mp 156—158 °C; UV λ_{max} (H_2O) 260.5 nm (ε 10700) at pH 1 and 6, 262 nm (ε 10400) at pH 11; NMR ($CDCl_3$-CD_3OD, 2-1 v/v) δ=7.95 (d, 1, $J_{5,6}$=8.2 Hz), 7.35 (m, 5, C_6H_5), 5.88 (d, 2, $J_{1',2'}$=3.0 Hz), 5.77 (d, 2, $J_{5,6}$=8.2 Hz), 5.11 (s, 2, $CH_2C_6H_5$), 3.8—4.3 (m, 5, $C_{2'}H$, $C_{4'}H$, and $C_{5'}2H$), and 3.56 (s, 3, CH_3). Found: C,

Table 1. Methylation of Various Ribonucleosides with Me$_3$SOH in the Presence and Absence of Mg(acac)$_2$ and Ca(acac)$_2$[a]

Entry	Nucleoside	Metal ion	Products: TLC–UV Yields/% [Yields of Isolated Products][b]				
			2′-MeAdo	3′-MeAdo	N^6-MeAdo	N^6,2′-Me$_2$Ado	N^6,3′-Me$_2$Ado
1	Ado	none	19	7	18	12	4
2		Mg^{2+}	30[21]	13[3]	7	9	5
3		Ca^{2+}	28	12	8	10	3
			2′-MeCyd	3′-MeCyd	N^3-MeCyd		
4	Cyd	none	27	8	10		
5		Mg^{2+}	66[43]	5[2]	7		
			3,2′-Me$_2$Urd	3,3′-Me$_2$Urd	3-MeUrd		
6	Urd	none	45	12	23		
7		Mg^{2+}	35[21]	trace	40		
			3,2′-Me$_2$Urd	3,3′-Me$_2$Urd	3,2′,3′-Me$_3$Urd		
8	3-MeUrd	none	31	8	6		
9		Mg^{2+}	82[65]	trace	9		
10		Ca^{2+}	71	trace	8		
			3-Benzyl,2′-MeUrd				
11	3-BenzylUrd	Mg^{2+}	83[62]	c)			

a) Reaction conditions: nucleoside–Me$_3$SOH–Mg(acac)$_2$ or Ca(acac)$_2$–DMF=2 mmol–3.4 mmol–1 mmol–8 ml; reaction temperature and time: 70 °C and 1 h, respectively. b) The TLC–UV yields of the products in the reaction mixtures were determined in a manner similar to that mentioned previously (Ref. 13). The major products were isolated in the yields shown in the brackets. The isolated compounds had the following physical constants. 2′-MeAdo, mp 202—203 °C (from ethanol)(lit,[7] 202—203.5 °C); UV λ_{max} (H$_2$O) 259 nm (ε 14000) at pH 6. 3′-MeAdo, mp 176—177 °C (from ethanol)(lit,[7] 174—176 °C); UV λ_{max} (H$_2$O) 259 nm (ε 24500) at pH 6. N^6,2′-Me$_2$Ado, mp 104—105 °C (from acetone-hexane)(lit,[8] 103—105 °C); UV λ_{max} (H$_2$O) 265 nm (ε 14200) at pH 6. N^6,3′-Me$_2$Urd, mp 189—190 °C (from acetone-hexane)(lit,[8] 189—191 °C); UV λ_{max} (H$_2$O) 265 nm (ε 16500) at pH 6. 2′-MeCyd, mp 252—254 °C (from ethanol)(lit,[7] 251—254 °C); UV λ_{max} (H$_2$O) 271 nm (ε 8900) at pH 6. 3′-MeCyd, mp 209—210 °C (from ethanol)(lit,[7] 210—211 °C); UV λ_{max} (H$_2$O) 271 nm (ε 9000) at pH 6. 3,2′-MeUrd, mp 138—140 °C (from hexane)(lit,[14] 140—141 °C); UV λ_{max} (H$_2$O) 261 nm (ε 9800) at pH 6. 3-Benzyl,2′-MeUrd, mp as well as UV and NMR spectra were identical with those of the authentic sample (see the experimental section). c) The reaction of the Entry 11 gave 3-Benzyl,2′-MeUrd exclusively.

58.40; H, 6.05; N, 7.84%. Calcd for C$_{17}$H$_{20}$N$_2$O$_6$: C, 58.61; H, 5.78; N, 8.04%.

General Mathylation Procedure. A mixture of nucleoside (2.0 mmol) and 1.7 equivalents of a 0.7 M methanol solution (1 M=1 mol dm^{-3}) of Me$_3$SOH[3] was concentrated in the absence and presence of one equivalent of Mg(acac)$_2$ or Ca(acac)$_2$ below 20 °C. DMF (8 ml) was added to the resulting residue, and the mixture was stirred magnetically at 70 °C for 0.5—1 h. A product-distribution in the methylation reaction using authentic samples and isolation of major products were conducted in a manner similar to that mentioned previously.[8,13] A ratio, 2′-*O*-methyl nucleoside/3′-*O*-methyl isomer, was determined from the area ratio of the corresponding methoxyl groups in the NMR spectrum of the mixture which was separated from the reaction mixture by silica gel chromatography.

Optimal amounts of Me$_3$SOH and the M(acac)$_2$s were about 1.5—2 equivalents and 0.5 equivalent, respectively, to the starting nucleosides. The use of the excess M(acac)$_2$s did not improve overall yields of methylation. The optimal reaction temperature was 60—80 °C. At higher temperature, Me$_3$SOH underwent significantly decomposition to methanol and dimethyl sulfide.

References

1) Abbreviations used are: adenosine, Ado; cytidine, Cyd; guanosine, Guo; uridine, Urd; 3-benzyluridine, 3-BenzylUrd; N^6-methyladenosine, N^6-MeAdo; 2′-*O*-methyladenosine, 2′-MeAdo; 3′-*O*-methyladenosine, 3′-MeAdo; 1-methylguanosine, 1-MeGuo; 3-methyluridine, 3-MeUrd; 3,2′,-*O*-dimethyluridine, 3,2′-Me$_2$Urd; 3-benzyl-2′-*O*-methyluridine, 3-Benzyl-2′-MeUrd; Similar abbreviations for other methyl nucleosides.

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4) A combined extent of 2′(3′)-*O*-methylation=summation of TLC yields of 2′(3′)-*O*-methyl nucleosides.

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10) Cu^{2+} and other transition metal ions were less selective than Mg^{2+} and Ca^{2+} ions in methylating the 2′-OH

groups of ribonucleosides. For instance, when Ado and Cyd were subjected to methylation reactions using 0.5 equivalent of $Cu(acac)_2$ under the conditions similar to those of Entry 2 and 5 of Table 1, the former provided 2′-MeAdo and 3′-MeAdo in 41 and 37%, respectively, and the latter furnished 2′-MeCyd and 3′-MeCyd in 60 and 22%, respectively.8) A combination of diazomethane and tin(II) chloride converted Ado into 2′-MeAdo and 3′-MeAdo in 44 and 56%, respectively.6,7) No detectable methylation of Ado was observed with the systems of diazomethane and Mg^{2+} and Ca^{2+} ions.6)

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12) UV spectra were measured with a Hitachi 3T spectrometer. NMR spectra were recored on a Jeol PS-100 using dilute solution in $CDCl_3$ or a mixture of $CDCl_3$–CD_3OD (2–1 v/v) with tetramethylsilane as an internal standard.

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