Methylation of Adenosine and Related Nucleosides with Trimethylselenonium Hydroxide, and Regiospecific Effects of Copper(II) Ions

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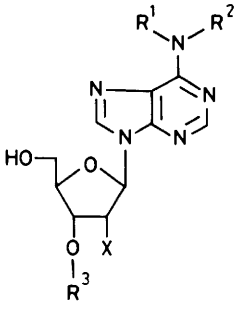
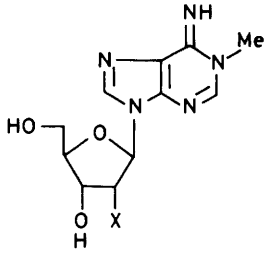
Methylation of adenosine, deoxyadenosine, 6-*N*-methyladenosine and 2′(3′)-*O*-methyladenosines with trimethylselenonium hydroxide was studied in the presence and absence of copper(II) acetylacetonate [$Cu(AA)_2$]. It was found that copper(II) ions promoted methylation of the 2′(3′)-OH groups of the ribonucleosides but suppressed methylation at the N-1 position of the adenine rings. The metal-ion effects are discussed in conjunction with a catalytic role for $Cu(AA)_2$ in the reactions.

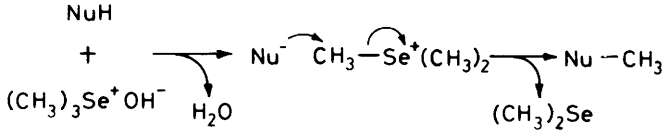
The co-ordination of metal ions to organic compounds often alters the reactivity of the ligands, either activating or deactivating the complexed sites.[1] Multifunctional compounds may, hence, be modified chemically at specific sites with the aid of appropriate metal ions. For instance, the amino groups of aminoglycoside–aminocyclitols were protected selectively by conventional blocking reagents *via* complexation of the antibiotics with transition-metal ions including cobalt(II), copper(II), and nickel (II) ions.[2] Copper(II) ions in amino acid-complexes, on the other hand, masked both the carbonyl and α-amino groups from most reagents, allowing only the side-chain to undergo electrophilic[3] and nucleophilic[4] reactions. Nucleic acids and their components have also been the subject of modification reactions in view of their interesting complexing properties with metal ions.[5,6] In particular, reactions of major nucleosides with various methylating agents have often been carried out in the presence of Lewis acids to give selective formation of the 2′(3′)-*O*-methyl derivatives,[7–9] which are minor but biologically important residues of RNA.[10] The metal-ion effects and the fate of the metal salts, however, have remained to be studied.





(**1**) $R^1 = R^3 = H, X = OH$
(**2**) $X = OH$
(**3**) $R^1 = Me, R^2 = R^3 = H, X = OH$
(**4**) $R^1 = R^3 = H, X = OMe$
(**5**) $R^1 = R^2 = H, R^3 = Me, X = OH$
(**6**) $R^1 = Me, R^2 = R^3 = H, X = OMe$
(**7**) $R^1 = R^3 = Me, R^2 = H, X = OH$
(**8**) $R^1 = R^2 = H, R^3 = Me, X = OMe$
(**9**) $R^1 = R^3 = X = H$
(**10**) $X = H$
(**11**) $R^1 = Me, R^2 = R^3 = X = H$
(**12**) $R^1 = R^2 = Me, R^3 = X = H$

In this paper we describe the regiospecific effects of copper(II) ions on the methylation of adenosine (**1**) and related nucleosides by means of trimethylselenonium hydroxide (TMSeH), $(CH_3)_3Se^+ OH^-$.[11] The most frequently investigated case of binding of copper ions to nucleosides has been that to adenosine (**1**),[5,12,13] and the complexation data were convenient for an analysis of metal-ion effects on the reactions. Copper(II) acetylacetonate [$Cu(AA)_2$] was chosen as the source of the metal ions due to its solubility in dimethylformamide (DMF), the solvent for the reactions. TMSeH, on the other hand, may be a suitable methylating agent because not only is it very active owing to the weak C–Se bond, in addition its co-products (water and dimethyl selenide) are unlikely to interfere with interactions between the nucleosides and $Cu(AA)_2$. A general scheme of methylation of a nucleophile (NuH) by the reagent is shown in Scheme 1.



Scheme 1.

Results and Discussion

Although kinetics of methylation reactions were not examined, all the nucleosides studied were transformed smoothly into the mono- and di-methyl derivatives by treatment with 1.3—3 mol equiv. of TMSeH in DMF at 60—80 °C for 20—40 min. The products were analysed by t.l.c., and the major products were isolated by column chromatography of the concentrated reaction mixtures. Typical product-distributions of the reactions are summarized in the Table. Methylation in the presence of $Cu(AA)_2$ was conducted similarly to exhibit the product-distributions in the Table. The conditions were optimized for the copper ion-assisted reactions; *viz.*, nucleoside–TMSeH–$Cu(AA)_2$–DMF = 1:2:0.5:4 mmol:mmol:mmol:ml, 70 °C, and 30 min (*vide infra*). Here, 6-*N*-methyladenine nucleosides (**3**), (**6**), (**7**), and (**11**) may be plausibly derived *via* Dimroth rearrangement[14] of the corresponding 1(*N*)-methyl isomers. Indeed, authentic 1(*N*)-methyladenosine (**2**) was converted quantitatively into the 6-*N*-methyl isomer (**3**) upon treatment with an equivalent of TMSeH in DMF at 25 °C for 2 h, where the reagent was inactive as a methylating agent but catalysed the rearrangement as an alkali. Direct methylation on the external 6-NH_2 group of (**1**), (**9**), *etc.* may be a minor event because the amino group is much less basic than the N-1 atom,[15] and TMSeH did not methylate the amino group of aniline under the conditions used for methylation of the nucleosides.

In the Table, one can see the following influences of $Cu(AA)_2$ on the reactivity of the nucleosides: (*a*) methylation on the adenine rings was inhibited and resulted in decreasing yields of the 1(*N*)(6-*N*)-methyladenine-nucleosides [entry 1, **3**—**5**], (*b*) methylation on the ribose 2′-OH and 3′-OH was enhanced considerably to give a mixture of 2′-*O*- and 3′-*O*-methyl-adenosines (**4**)—(**7**) in high yields (entries 1 and 2), and (*c*)

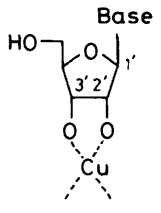
Table. Methylation of various adenine-nucleosides with TMSeH in the presence and absence of $Cu(AA)_2$ [a]

Entry	Nucleoside	Products	Product distribution (%) [b,c] $Cu(AA)_2$	Product distribution (%) [b,c] None	R_F [d]
1	Ado (**1**)	1-Methyl-Ado (**2**)	trace	7	0.1
		6-*N*-Methyl-Ado (**3**)	trace	13	0.33
		2′-*O*-Methyl-Ado (**4**)	54 (31)	19	0.40
		3′-*O*-Methyl-Ado (**5**)	37 (15)	5	0.40
		6-*N*, 2′-*O*-Dimethyl-Ado (**6**)	4	16	0.49
		6-*N*, 3′-*O*-Dimethyl-Ado (**7**)	3	7	0.49
		2′-*O*, 3′-*O*-Dimethyl-Ado (**8**)	trace	10	0.57
2	(**3**) [e]	(**6**)	37 (21)	9	
		(**7**)	33 (18)	3	
3	(**4**)	(**6**)	17	35 (18)	
		(**8**)	11	10 (7)	
4	(**5**)	(**7**)	9	30 (17)	
		(**8**)	23	26 (13)	
5	dAdo (**9**)	1-Methyl-dAdo (**10**)	10	7	0.1
		6-*N*-Methyl-dAdo (**11**)	20	36 (21)	0.36
		6-*N*, 6-*N*-Dimethyl-dAdo (**12**)	11	23 (14)	0.50
		Unknown	2	14	0.54

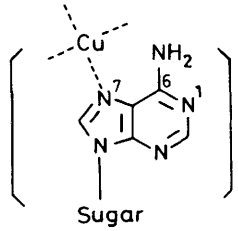
[a] Reaction conditions: nucleoside–TMSeH–$Cu(AA)_2$–DMF = 1:2:0.5:4 mmol:mmol:mmol:ml; temperature, 70 °C; *t*, 30 min. [b] The yields in parenthesis were based on isolated products. [c] M.p. (solvent of recrystallization) and $\lambda_{max.}$ (log ε) of the products isolated were: (**4**), 202—203 °C (EtOH) (lit.,[7] 202—203.5 °C), 259 nm (4.02); (**5**), 176—178 °C (EtOH) (lit.,[7] 177—178 °C), 259 nm (4.11); (**6**), 104—107.5 °C (acetone–n–hexane) (lit.,[9] 103—105 °C), 266 nm (4.13); (**7**), 189—192 °C (acetone–n–hexane) (lit.,[9] 189—191 °C), 266 nm (4.15); (**8**), 175—177 °C (ethyl acetate) [lit. (J. B. Jin and C. A. Dekker, *Biochemistry*, 1968, **7**, 1413), 177 °C], 261 nm (4.15); (**11**), 205—208 °C (MeOH) (lit.,[7] 206—208 °C), 265 nm (4.10); (**12**), 174—175 °C (acetone–n–hexane), 275 nm (4.24), δ 3.05 [s, 6, $N(CH_3)_2$] (Found: C, 51.4; H, 6.5; N, 24.5. $C_{12}H_{17}N_5O_3$ requires C, 51.60; H, 6.14; N, 25.08%). [d] Solvent for t.l.c. was chloroform–MeOH (17:3 v/v). [e] 2 Equiv. of $Cu(AA)_2$ were used with respect to (**3**); other conditions were the same as those mentioned in footnote *a*.

methylation ratios for the ribose ring (extent of methylation at the 2′-OH to that at the 3′-OH) approached values of 1:1 from 3—4:1 when the reactions were performed in the presence of $Cu(AA)_2$. The high reactivity of the 2′-OH group, which was observed in the absence of $Cu(AA)_2$, may be attributed to a hydrogen-bonding interaction of the 2′-OH with the 3′-OH and with N-3 of the adenine ring, making the 2′-OH group slightly more acidic than the 3′-OH group.[16–18]

Now, the aforementioned effects of $Cu(AA)_2$ can be rationalized by complex formation between the nucleosides and copper(II) ions. It has been reported that copper(II) ions bind to both the 2′-OH and the 3′-OH groups of adenosine (**1**) at pH > *ca.* 9.5 to give a complex having structure (**I**) and that the chelation shifts their pK_a values (12—13) down by approximately 2 pH-units.[12,13] Thus, the ribonucleosides (**1**) and (**3**)



(**I**)



(**II**)

probably underwent a ligand-exchange reaction with $Cu(AA)_2$ to afford complexes of type (**I**) while the reaction mixtures were alkaline due to the OH^- ions from TMSeH. The process facilitated ionization of both the ribose hydroxy groups, and the resulting oxyanions were readily attacked by $(CH_3)_3Se^+$ ions to provide the corresponding 2′(3′)-*O*-methylnucleosides in good yields. The small methylation ratios (2′-*O*-methylation:3′-*O*-methylation) are also consistent with the chelate structure, in which the reactivity of 2′-OH may be equalized with that of 3′-OH.

Unlike compounds (**1**) and (**3**), however, 2′(3′)-*O*-methyladenosines (**4**) and (**5**) and deoxyadenosine (**9**) did not exhibit a template effect of the copper ions (Table, entries 3—5). Perhaps the *O*′-methylnucleosides were too weak as ligands to produce complexes similar to (**I**), and thus the deoxyribonucleoside could not function as a chelating agent for copper ions.

Meanwhile, the alkaline reaction mixtures changed gradually to neutral solutions because the OH^- ions of TMSeH were consumed during the reactions. Copper(II) ions have been shown to co-ordinate to either N-1 or N-7 of adenosine (**1**) to give complexes of type (**II**).[5,12,13] The suppressed methylation at N-1 may thus be caused by the metal-ion binding because co-ordination to N-7 should reduce the basicity of N-1 through an inductive effect of the copper ion, and the metal ion on N-1 might block that position from attack of $(CH_3)_3Se^+$ ions. Indeed, when adenosine (**1**) (1 mmol) was allowed to react with trimethylselenonium iodide $[(CH_3)_3Se^+ I^-]$ (2 mmol) in DMF (4 ml) in the presence and absence of $Cu(AA)_2$ (0.5—1 mmol) at 70 °C for 30 min, compound (**2**) was obtained in 25—40 and 73—85% yield, respectively. Here, the reactions occurred in a neutral environment.

Interestingly, $Cu(AA)_2$ was recovered in 70—90% yield from the reaction mixtures which contained 0.5 mol equiv. of the metal complex with respect to the starting nucleosides. The consumption of 0.03—0.1 mol equiv. of $Cu(AA)_2$ in methylation reactions resulted in diminishing metal-ion effects as shown in the Figure. The good recovery of $Cu(AA)_2$ may suggest that the copper complex played a catalytic role in the methylation reactions as illustrated in Scheme 2; *i.e.*, (*a*) a nucleoside competed with an acetylacetone-ligand (AA) in co-ordinating to copper ions and (*b*) after methylation of the 2′-OH or 3′-OH group, the metal ions were detached from the nucleoside–copper chelate (**I**) and coupled again with the free AA to regenerate $Cu(AA)_2$.

An optimal amount of $Cu(AA)_2$ was about 0.5 mol equiv.

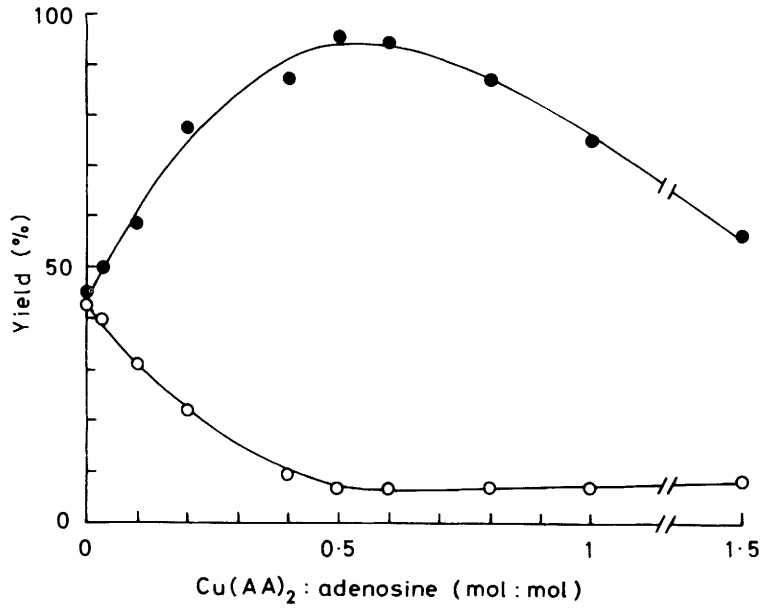
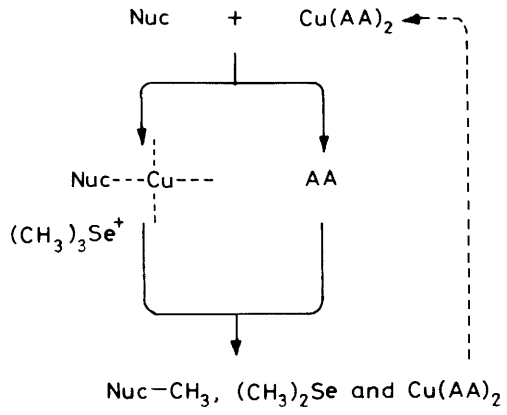


Figure. Effects of $Cu(AA)_2$ on the reaction of adenosine (**1**) (1 mmol) with TMSeH (2 mmol) in DMF (4 ml) at 70 °C for 30 min. ● and ○ are the sums of the yields of 2′(3′)-*O*-methyladenosines (**4**)—(**7**) and 1(*N*)(6-*N*)-methyladenosines (**2**), (**3**), (**6**), and (**7**), respectively



Scheme 2. Nuc = nucleoside, AA = acetylacetone ligand

with respect to the nucleosides (see the Figure). The use of excess of $Cu(AA)_2$ did not improve the overall extents of the methylation reactions, but rather increased the amounts of the starting nucleosides recovered. For instance, reaction of adenosine (**1**) using 1.5 mol equiv. of $Cu(AA)_2$ gave compounds (**1**) (34% recovery), (**4**) (31%), (**5**) (23%), and a mixture (2%) of (**6**) and (**7**) when the other conditions were similar to those of the reaction of the Table, entry 1. The inefficient methylation was ascribed to the side-reactions of TMSeH and $Cu(AA)_2$, which gave rise to 3-methylacetylacetone and possibly $Cu(AA)_2$.* The optimal reaction temperature was 60—80 °C. At higher temperatures, TMSeH underwent considerable thermal decomposition to methanol and dimethylselenide.

Experimental

Materials.—$Cu(AA)_2$ and nucleosides (**1**) and (**9**) were commercially available. Methylated nucleosides (**2**), (**3**), (**10**), and (**11**) were prepared according to the literature.[19] Other nucleosides (**4**)—(**8**) as well as TMSeH and $(CH_3)_3Se^+ I^-$ were obtained in our previous studies.[9,11,20] Dry-packed column chromatography was performed using silica gel (Merck, art 7734, 70—230 mesh). T.l.c. was carried out using silica gel [Merck, GF_{254} (type 60)].

General Methylation Procedure.—A mixture of nucleoside (5 mmol), a methanolic solution of TMSeH (0.39M; 5—10 mmol), and $Cu(AA)_2$ (0—5 mmol) was concentrated under reduced pressure below 30 °C. The resulting residue was dissolved in DMF (20 ml) and the solution was heated at 70 °C for 20—40 min and stirred magnetically under nitrogen. Major products were isolated by silica gel column chromatography using mainly chloroform–methanol mixtures as eluant with the volume ratios reported elsewhere.[20] The first fraction gave $Cu(AA)_2$ as green crystals in 70—90% recovery. The 2′(3′)-*O*-methylribonucleosides were eluted in the subsequent fraction; the yield ratio was determined conveniently from the peak area ratio of the corresponding methoxy groups in the n.m.r. spectrum of the mixture. The mixture was then resolved by means of ion-exchange chromatography (Dowex 1 × 2, 100—200 mesh; OH^- form) using water as eluant. Generally, the 2′-*O*-methylribonucleoside was eluted first followed by the $O^{3'}$-methyl isomer.

The product distribution of the methylation reaction was determined by addition of aqueous ammonium sulphide to the reaction mixture, removal of the precipitate by suction filtration, and work-up and analysis of the filtrate by a t.l.c.–u.v. spectroscopic method.[21]

All known and isolated products showed u.v. spectra at pH 1, 7, and 13 and n.m.r. spectra as well as m.p.s which agreed with those of the literature or authentic samples. Compounds which were not isolated were identified by comparison of their mobilities (R_F) in t.l.c. using several solvent systems and of u.v. spectra at pH 1, 7, and 13 of aqueous extracts of the spots with those of authentic samples. Results including product distributions, m.p.s, and R_F values are summarized in the Table.

* The reaction mixtures for entries 1 and 2 contained acetylacetone (3—6%), 3-methylacetylacetone (5—11%), and $Cu(OH)_2$ (10—25%). The organic compounds were identified and quantified by means of gas chromatography of the reaction mixtures.

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