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STUDIES ON CHEMICAL ALTERATIONS OF NUCLEIC ACIDS AND THEIR COMPONENTS—IX[1]

SYNTHESIS AND REACTION OF 1-AMINOADENOSINE AND RELATED COMPOUNDS

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Abstract—Adenosine and its related compounds reacted with hydroxylamine-O-esters to give the corresponding 1-amino derivatives. 1-Amino-adenines were rearranged in the presence of alkali to afford 5(4) - amino - 4(5) - (1,2,4 - triazol - 3 - yl) - imidazole. 1-Amino derivatives reacted with ethyl orthoformate to give 7H - s - triazolo[3,2 - i] - purine derivatives and with H_2S to give 1 - amino - 6 - mercaptopurines. All the 1-amino derivatives were readily deaminated to the parental adenines by treatment with $NaNO_2$.

INTRODUCTION

Our interest has recently been directed toward electrophilic amination reactions of nucleic acid bases because of the role that they may play in the chemical modification of deoxyribonucleic acid (DNA) for the chemical carcinogenesis by N- or O-substituted hydroxylamines.[2,3] Very little chemical data of this type of reaction toward nucleosides and nucleotides are available.[4,5] The reagents for this type of reactions may be comprised mainly of hydroxylamine derivatives which are substituted at the O atom of the molecule with an electron-withdrawing group such as acylmoiety of sulfuric acid, phosphoric acid, carboxylic acids, or dinitrophenol.[6-9] Our first choice of the reagent for this was hydroxylamine-O-sulfonic acid,[6] which readily reacts with guanine, adenine, uracil, and cytosine moieties of the nucleosides and nucleotides to afford their various N-amino or C-amino derivatives depending on the prevailing condition chosen for the reaction.[5,10-13] 2,4-Dinitrophenoxyamine[7] could sometimes take the place of hydroxylamine - O - sulfonic acid for more convenient synthesis of the products. The products so far obtained from the nucleosides are summarized in Table 1, including the results obtained in the present study.

Apart from the biological importance of this type of amination, this paper concerns the synthesis and reaction of 1-aminoadenosine and its related compounds. Although the protonated structure of these molecules may be written as $NH_2-N^+\!\!\diagup\!\!\diagdown$, they are distinguished from that of N-ylides by ready deprotonation from the neighbouring amino group ortho to the N-NH_2 function ($NH_2-N^+=C-NH_2 \rightarrow NH_2-N-C=NH$), so that, in fact, the p*Ka'* value† of 1-aminoadenosine (9·0) is considerably lower than that of N-aminopyridine (11·0)[12] ($NH_2-N^+\!\!\diagup\!\!\diagdown \rightarrow NH^--N^+\!\!\diagup\!\!\diagdown$). In addition, among the 1-substituted adenines, one may expect the amino derivatives to be distinguished from the alkyl and alkoxy derivatives by the chemical reactivity due to the nucleophilic character of the introduced N-amino group.

Table 1. Aminated products of nucleosides by treatment with hydroxylamine - O - sulfonic acid (HAOS) or 2,4 - dinitrophenoxyamine (DNPA) under various reaction conditions

Nucleoside	Reagent	Reaction medium[a]	Position of amination N-amino	C-amino
Guanosine	HAOS	pH 2–4		8[b]
	HAOS	pH > 9	1[c]	
	DNPA	DMF	(7)[d]	
Adenosine	HAOS	pH 2–7	1	
	DNPA	DMF	1	
Uridine	HAOS	pH 2–4		5[e]
	HAOS	pH > 9	3[f]	
Cytidine	HAOS	pH > 4	3[g]	
	DNPA	DMF	3[g]	

[a]pH Values are those in aqueous acetate or phosphate buffers. [b]Kawazoe and Huang, ref 10. [c]Broom and Robins, ref 5. [d]The structure has not been definitely confirmed yet. Huang and Kawazoe, unpublished data. [e]Maeda and Kawazoe, ref 11. [f]Maeda and Kawazoe, unpublished data. [g]Huang, Okamoto, Maeda and Kawazoe, ref 12.

RESULTS

Syntheses of 1-aminoadenosine derivatives. When adenosine was treated with hydroxylamine - O - sulfonic acid in phosphate buffer at around neutral pH at room temperature, 1-aminoadenosine (**1a**) was isolated from the mixture in a fairly good yield by paper chromatography. For synthetic purposes, **1a** was much more conveniently prepared by treating adenosine with 2,4-

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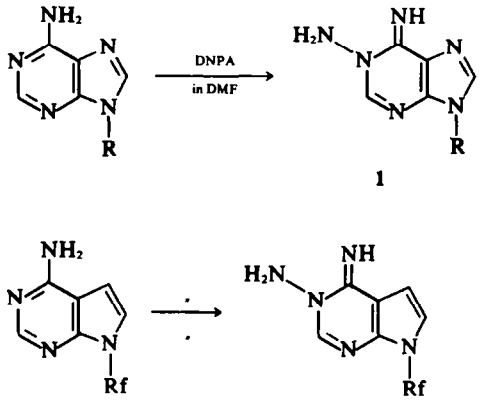
†Measured in D_2O using an ordinary pH-meter, no correction being made.

dinitrophenoxyamine in dimethylformamide. 1 - Amino - 9 - methyladenine (**1b**) and 1-aminoadenosine cyclic 3',5'-phosphate (1 - amino - cAMP) were readily prepared as in the case of **1a**. 1 - Aminotubercidin was also prepared in 95% yield, as formulated in Chart 1. This amination procedure seems, therefore, to be generally applicable to syntheses of 1-amino derivatives of adenosine and the related compounds. It is presumed that these aminations proceeded through electrophilic attack of the reagent involving the N–O heterolysis in uni- or bi-molecular mechanism.[5,8,9,12]



R: β-D-ribofuranosyl (**1a**), methyl (**1b**) or 3,5-cyclic-monophosphoryl-β-D-ribofuranosyl
Rf: β-D-ribofuranosyl

CHART 1

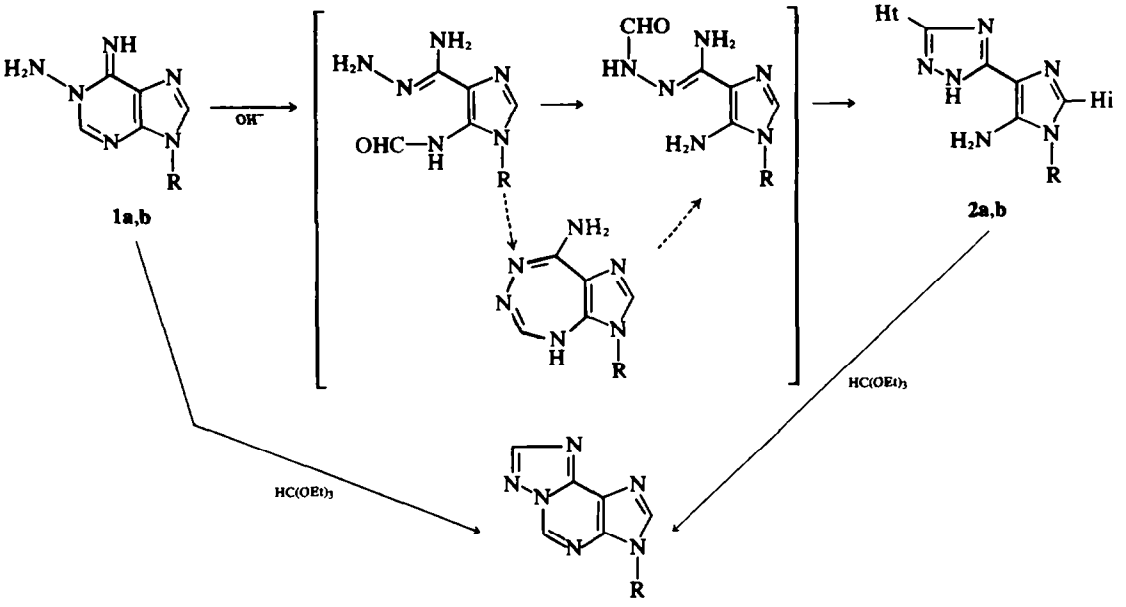
Alkaline rearrangement of 1-aminoadenine derivatives. 1-Aminoadenosine (**1a**) was rearranged in alkaline media to give the product **2a** in a quantitative yield. The reaction proceeded in aqueous solution at pH's higher than 7. At pH 11, for example, it was completed by heating at 60° for 2 h. 1 - Amino - 9 - methyladenine also gave the corresponding product (**2b**) under the same reaction condition as the above. The rearranged products (**2a, b**) were neither the normal Dimroth rearrangement products, i.e., 6-hydrazinopurine derivatives,[14] nor any degradative products obtained by treating the corresponding 6-hydrazino derivatives under the same reaction condition as that for the rearrangement. The products (**2a, b**) have the same elemental compositions as the corresponding starting materials and the molecular ion peak in the mass spectrum of **2b** appeared at 164 (*m/e*) which is the molecular weight **1b**. The products had two p*Ka* values, basic and acidic ones, respectively (3·8 and 10·3 for **2a**; 4·2 and 10·5 for **2b**). The NMR spectrum of **2b** measured in DMSO-d_6 showed a singlet due to N-Me protons (δ, 3·30), two singlets due to two C–H protons (7·45 and 8·05), a broad singlet due to NH_2 (5·70), and another broad singlet due to NH proton (14·1). It is worth pointing out that NH signal at δ 14·1 is too low a field for a normal basic NH proton. The NMR spectrum of **2a** showed the signals corresponding to those of **2b** and, in addition to these, signals for protons of the ribose moiety. Bratton–Marshall reaction of **2a, 2b** indicated the presence of NH_2 on the aromatic ring.[15] The reaction product obtained from **2a** showed a UV absorption maximum at 520 nm in pH 1 medium, which was very similar to that of 5 - aminoimidazole - 4 - carboxamide (AICA) derivatives.[16]

These data suggest that the rearranged products are 1-substituted 5 - amino - 4 - (1,2,4 - triazol - 3 - yl) - imidazole as formulated in Chart 2. In order to confirm the presence of a triazole ring in the molecule, H–D exchange reaction **2b** was examined. Thus, 1 - amino - 8 - deuterio - 9 - methyladenine (**1b**–D), which was prepared by amination of 8 - deuterio - 9 - methyladenine,[17] was rearranged to the deuterated derivative of **2b** (**2b**–D). Comparing the spectra of **2b** and **2b**–D, the signals at 7·45 and 8·05 were assigned to the hydrogen on the imidazole ring (Hi) and the one on the triazole ring (Ht), respectively. On the basis of this signal assignment of the NMR spectrum, the rates for H–D exchange of these two hydrogens were measured when **2b** was heated in NaOD–D_2O at 70°. Ht exchanged more readily with the solvent deuterium than Hi at pH' 8·2 or 9·4,* while, at pH' 12·8,* Hi exchanged quite readily but Ht resisted the exchange strongly. This characteristic of Hi and Ht coincides with that of N-substituted imidazole and N-unsubstituted triazole, respectively.

The structure proposed for the rearranged products (**2**) was finally confirmed by the following evidence. **2a** was hydrolysed in conc HCl at 37° for 2 days and the hydrolysate was purified by paper chromatography. The product was identical with 5(4) - amino - 4(5) - (1,2,4 - triazol - 3 - yl) - imidazole which was derived from 6-hydrazinopurine through several steps[18] (UVλ max (nm): pH 1, 244 and 260; pH 7, 265; pH 13, 262). In addition to this, the treatment of either **1b** or **2b** with ethyl orthoformate gave the same product, 7 - methyl - 7H - s - triazolo[3.2 - i] - purine (**3b**), as described in detail later.

The reaction mechanism appears to be as shown in Chart 2. Thus, the rearrangement started with alkaline hydrolytic cleavage of the 1,2-bond and CHO group thereby formed was transfered to N–NH_2 nitrogen, followed by the dehydrative ring-closure to yield the product **2**. In the course of the rearrangement of **1b** at pH' 11* at room temperature, one set of NMR signals, which may correspond to either one of the three intermediates shown in Chart 2, was observed in the spectrum of the mixture, overlapped with two other sets of signals due to the starting material and the product (**2b**). However, when the rearrangement was carried out at pH' 13,* the signals of the intermediate were observed but they soon changed to another set of signals due to the second intermediate. The latter was fairly stable at this pH but it changed to **2b** when the mixture was acidified to pH 11. In the later stage of the rearrangement, a small amount of formic acid was liberated, which may have been formed by hydrolysis of an N-formyl group in one of the intermediates. The structures of two intermediates thus detected in the NMR spectrum are still under investigation.

*pH' denotes the one of the D_2O soln measured by an ordinary pH-meter, no correction being made.



R: β-D-ribofuranosyl (**1a**, **2a**, **3a**) or methyl (**1b**, **2b**, **3b**)

CHART 2

Deamination of the N-*amino group introduced.* Distinguished from other 1-substituted adenines such as 1-alkyl and 1-alkoxy, 1-amino derivatives were readily deaminated by treatment with slight excess of sodium nitrite in aqueous acetic acid to give the starting adenines. It is of interest that the nitrosation took place on the N-amino group first and not on the C-amino group at position-6. This may be interpreted by comparison of NMR spectra of hydrochlorides of 1 - methyl - adenosine and **1a** measured in DMSO-d_6 at room temperature. Thus, the former showed two broad signals due to two NH protons separately, and the amino protons of the latter gave a similar pattern of signals to that of the former. This indicates that the cationic charge is distributed to the C-amino nitrogen of the salt of **1a** predominantly or at least to an appreciable extent, as seen in the salt of 1-methyladenosine. This suggests that the nitrosation took place on N-amino nitrogen, followed by liberation of N_2O to afford adenosine.[19]

Nucleophilic replacement of C(6)–NH_2 *with a sulfhydril group.* It is known that nucleophilic replacement of the 6-amino group of adenosine is facilitated to some extent by substitution at the position-1 by an alkyl group.[20] The 6-amino group in **1a** was readily replaced by a sulfhydril by treating it with hydrogen sulfide in dimethylformamide at 60°. 1 - Amino - 6 - mercaptopurine riboside (**4**) thus produced was readily deaminated to give 6-mercaptopurine riboside,[21] as formulated in Chart 3.

Reaction of 1-*aminoadenines with ethyl orthoformate.* When 1 - amino - 9 - methyladenine (**1b**) was refluxed in a mixture of ethyl orthoformate and acetic anhydride, the product **3b** was obtained in almost quantitative yield. The same product was formed by treating the alkaline rearranged product **2b** under the same reaction condition as shown in Chart 2. 1-Aminoadenosine (**1a**) was treated in a similar way, followed by acid-hydrolysis for unmasking the ribose moiety which had been masked by the ethoxymethylidene group. The UV and NMR spectra supported the structure of **3a**, although the product was too hygroscopic to be isolated pure enough for the elemental analysis.

The N-amino group may have a nucleophilic character and react with electrophiles such as aldehydes, acylating agents, etc.

DISCUSSION

It would appear that most of the carcinogens are converted metabolically to the ultimate forms of car-

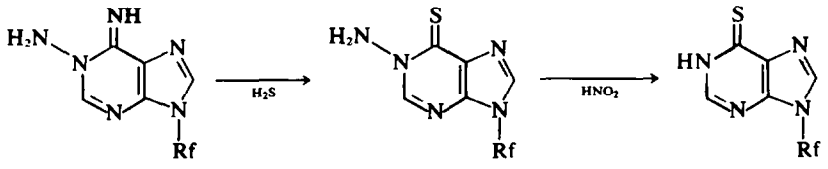


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CHART 3

cinogens that involve the electrophilic nature of reactivity under physiological conditions.[2] Although the kinetic study has not been done, this type of N-amination may be considered to proceed through a bimolecular electrophilic mechanism.[8] It may, therefore, be worth investigating whether or not this type of amination reaction is related to mutagenesis or carcinogenesis by arylhydroxylamines and hydroxylamine itself. Rosenkranz recently reported[22] that DNA was degraded by hydroxylamine - O - sulfonic acid under a physiological condition probably through modification of nucleic acid bases, although he used the *in vitro* experimental system. Further studies along this line seem worthwhile from the biological standpoint, and are now under investigation in our laboratory. Apart from the biological interest, N-amino derivatives of adenosine may be important intermediates for synthesis of 6-substituted adenine derivatives and some triazoloadenosines such as the aza-analogues of ethenoadenosine.[23]

EXPERIMENTAL

The experimental details of syntheses of **1a**, 1-aminoadenosine cyclic 3',5'-phosphate, 1-aminotubercidin, and deamination of **1a** are described in the preliminary communication.[12] Hydroxylamine - O - *sulfonic acid*[6] and *2,4-dinitrophenoxyamine*[7] were synthesized according to the reported preparative methods. TLC was carried out using Avicel-SF cellulose plates (purchased from Funakoshi Co., Tokyo) and appropriate solvent systems for elution. Solvent system A [i-PrOH : conc NH_4OH : H_2O = 7 : 1 : 2]; solvent system B [i-PrOH : 1% $(NH_4)_2SO_4$ aq = 2 : 1]; solvent system C [MeOH : conc HCl : H_2O = 7 : 2 : 1].

Reaction of adenosine with hydroxylamine - O - sulfonic acid. Adenosine (1·0 mmole) was dissolved in 30 ml H_2O containing 10 mmoles hydroxylamine - O - sulfonic acid and 25 mmoles AcOK, the pH of the solution being 4·5. The soln was kept at room temp for 6 days, when the pH of the soln was 4·2. The fact that **1a** was produced in 87% yield was indicated by TLC (solvent system A. R_f, 0·45 (R_f of adenosine, 0·60)). However, preparative purification of the product was tried without success because of a large quantity of the inorganic salts present in the mixture. Therefore, the alternative method using 2,4 - dinitrophenoxyamine was employed for preparation of **1a** on the large scale. See ref 12.

1 - *Amino* - 9 - *methyladenine* (**1b**). To a soln of 9-methyladenine (3 mmoles)[24] in 25 ml DMF containing 15 ml EtOH and 4·5 ml H_2O, 2,4-dinitrophenoxyamine (4·5 mmoles) in 5 ml DMF was added. After 4 days' standing at 37°, the mixture was treated as previously reported[12] to give **1b** in 86% yield, m.p. 275–276 (dec) (Found: C, 36·00; H, 4·47; N, 41·56. Calcd. for $C_6H_8N_6$ HCl: C, 35·91; H, 4·48; N, 41·89%); UVλ max (nm): pH 1, 259; pH 7, 250; pH 11, 259, 263(sh.) and 285(sh.); p*Ka'* in D_2O at 25°:* 9·7 (by NMR).

Alkaline rearrangement of 1-*aminoadenosine* (**1a**). The hydrochloride of **1a** (1·5 g) dissolved in 10 ml H_2O and adjusted to pH 11 with conc NaOH aq was heated at 60° for 2 h. On being kept at room temp, the product **2a** precipitated as a colorless solid, which was almost pure. The yield was 83%, m.p. 119–120° (dec) from H_2O. (Found: C, 40·41; H, 4·82; N, 28·10. Calcd. for $C_{10}H_{14}N_6O_4$ 5/6 H_2O (hygroscopic): C, 40·40; H, 5·27; N, 28·28%); UVλ max (nm): pH 1, 247 and 260(sh); pH 7, 260; pH 11, 250; p*Ka* in H_2O at 25°: 3·8 and 10·3 (by UV).

Alkaline rearrangement of 1 - *amino* - 9 - *methyladenine* (**1b**). When **1b** (200 mg) in 1·5 ml H_2O (pH 11) was heated at 60° for 2 h and cooled to room temp, 106 mg of dark green needles separated. The yield was 66%, m.p.: 260–261 (dec) from H_2O. (Found: C, 43·45; H, 4·93; N, 50·88. Calcd. for $C_6H_8N_6$: C, 43·89; H, 4·91; N, 51·20%); *m/e*: 164 (by mass spectrometry); UVλ max: pH 1, 241 and 258(sh); pH 7, 263; pH 11, 248; p*Ka* in H_2O at 25°: 4·2 and 10·5 (by UV).

1 - *Amino* - 6 - *mercaptopurine riboside* (**4**). The hydrochloride of **1a** (1·5 g; 4·7 mmoles) was dissolved in 30 ml DMF and placed in a reaction autoclave previously chilled with the dry ice–acetone mixture. Then, 60 ml H_2S-pyridine mixture, which was prepared by dissolving H_2S gas in 25 ml pyridine thoroughly chilled, was poured into the autoclave. The mixture was heated at 60° for 2 days. After the mixture was evaporated into dryness *in vacuo*, the residue was extracted with hot MeOH. The residue from the extract was recrystallized from H_2O to give **4** (270 mg) as fine needles, m.p.: 214–215° (dec). (Found: C, 38·93; H, 4·57; N, 21·86. Calcd. for $C_{10}H_{13}N_5O_4S$ 1/2 H_2O: C, 38·96; H, 4·54; N, 22·72%); UVλ max (nm): pH 1, 231(sh) and 319; pH 6, 230(sh) and 317; pH 13, 243(sh) and 316.

Deamination of **4** *to* 6-*mercaptopurine riboside.* To a soln of **4** (30 mg) in 3 ml H_2O and 5 ml AcOH, an equimolar amount of $NaNO_2$ in 1 ml H_2O was added with stirring and kept at room temp for one day. TLC (solvent system A or B) indicated that 90% of the product was 6-mercaptopurine riboside and 10% was presumed to be the disulfide derivative of the product. The mixture was recrystallized from H_2O to give 12 mg 6-mercaptopurine riboside as needles.[21]

Cyclization of 1 - *amino* - 9 - *methyladenine* (**1b**) *to* 7 - *methyl* - 7*H* - 1,2,4 - *triazolo*[3.2-i]*purine* (**3b**). **1b** hydrochloride (100 mg) was dissolved in a mixture of 20 ml ethyl orthoformate and 10 ml Ac_2O and refluxed for 20 min. TLC (solvent system A or B) indicated that **1b** was converted to the product **3b** in quantitative yield. The mixture was evaporated into dryness *in vacuo* and the residue was recrystallized from MeOH to give **3b** (68 mg) as needles. The yield was 79%, m.p.: 271°. (Found: C, 48·26; H, 3·46; N, 48·28. Calcd. for $C_7H_6N_6$: 48·27; H, 3·44; N, 48·27%). UVλ max (nm): pH 1, 276; pH 7, 281; pH 13, 282 (unstable).

Cyclization of 5 - *amino* - 1 - *methyl* - 4 - (1,2,4 - *triazol* - 3 - *yl*)*imidazole* (**2b**) *to* **3b**. The alkaline rearrangement product (**2b**; 43 mg) was dissolved in a mixture of 10 ml ethyl orthoformate and 5 ml Ac_2O and refluxed for 40 min. TLC (solvent system A, B or C) showed two spots; one was the product (**3b**) and the other unknown. The mixture was evaporated to dryness *in vacuo*. The residue was recrystallized from MeOH to give **3b** (18 mg) as needles. The yield was 39%.

Cyclization of 1-*aminoadenosine* (**1a**) *to* 7 - *β* - D - *ribofuranosyl* - 7H - 1,2,4 - triazolo[3.2 - i]*purine* (**3a**). **1a** hydrochloride (0·8 mmoles) was dissolved in a mixture of 30 ml ethyl orthoformate and 15 ml Ac_2O and refluxed for 1 h. TLC (solvent system B) indicated a quantitative yield of product; R_f: 0·90 (R_f of **1a**: 0·37). The mixture was evaporated and kept in 70% AcOH aq at room temp overnight in order to remove the ethoxymethylidene group from the ribose moiety. After the solvent was removed *in vacuo*, the residue was dissolved in MeOH and then, diluted with ethyl ether. On cooling, a crystalline material deposited but was too hygroscopic to be further purified by recrystallization. The structure was confirmed by NMR and UV spectroscopy; UVλ max (nm): pH 1, 279; pH 7, 278; pH 13, unstable.

*Measured in D_2O using an ordinary pH-meter, no correction being made.

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