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Studies on Chemical Alterations of Nucleic Acids and Their Components. X.[1] Syntheses and Reactivities of 3-Aminopyrimidine Nucleosides

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Treatment of pyrimidine nucleosides with hydroxylamine-O-esters (or hydroxylamine-O-aryl ethers) produced the corresponding 3-amino derivatives. 3-Aminouridine derivatives underwent deamination and acetylation to give uridines and 3-acetamidouridines, respectively. 3-Aminocytidine derivatives underwent, in addition to deamination, replacement with a sulfhydryl and cyclization with an orthoformate to give 3-amino-4-thiouridine and 1,2,4-triazolo[2,3-*c*]pyrimid-5(6H)-one derivatives, respectively. Proton magnetic resonance and ultraviolet data of 3-amino derivatives are tabulated.

Among chemical modifications of nucleic acids and their components, those involving electrophilic mechanisms seem to have drawn the attention of some chemists in the biological sciences, because the functional groups involved in the cell-constituent molecules are generally nucleophilic.[1,3–9] As one of a series of our studies along this line, the present study concerns N-amination of pyrimidine nucleosides with hydroxylamine-O-esters or -O-aryl ethers, which are representative electrophiles possibly to take part in the nucleic acid modification under physiological conditions. This type of reaction would appear to be related to the deoxyribonucleic acid (DNA) alterations *in vivo* induced by mutagenic hydroxylamine[10] and/or carcinogenic arylhydroxylamines.[3,9–11] Apart from such biological interest, this paper describes the reactivity of the N-amino derivatives thus prepared.

With regard to the direct amination of nucleosides, we have already reported on the preparation of 8-aminoguanosine,[6] 5-aminouridine,[7] 1-aminoadenosine,[5] and 3-aminocytidine.[5] Broom, *et al.* have described the preparation of 1-aminoguanosine and its related N-amino derivatives.[12] Recently, Rosenkranz reported that a certain change in ultraviolet (UV) absorption of DNA was produced when treated with hydroxylamine-O-sulfonic acid, although the detail has not yet been reported.[13]

Results and Discussion

Uridine reacted with hydroxylamine-O-sulfonic acid (HAOS) in alkaline media to give 3-aminouridine (I) in 35% yield. Since the reaction required the pH of the medium above

1) Part IX: G.-F. Huang, M. Maeda, T. Okamoto, and Y. Kawazoe, to be published. Part VIII: G.-F. Huang, T. Okamoto, M. Maeda, and Y. Kawazoe, *Chem. Pharm. Bull.* (Tokyo), **22**, 1938 (1974).
2) Location: *Tsukiji, Chuo-ku, Tokyo, 104, Japan.*
3) J.A. Miller and E.C. Miller, *Jerusalem Symp. Quantum Chem. Biochem.*, **1**, 237 (1969); J.A. Miller, *Cancer Res.*, **30**, 559 (1970) and literatures cited therein.
4) W.C.J. Ross, "Biological Alkylating Agents," Butterworth, London, 1962, pp. 1—200.
5) G.-F. Huang, T. Okamoto, M. Maeda, and Y. Kawazoe, *Tetrahedron Letters*, **1973**, 4541.
6) Y. Kawazoe and G.-F. Huang, *Chem. Pharm. Bull.* (Tokyo), **20**, 2073 (1972).
7) M. Maeda and Y. Kawazoe, *Tetrahedron Letters*, **1973**, 2751.
8) P.D. Lowley, *Mutation Res.*, **23**, 283 (1974).
9) E. Boyland, D. Manson, and R. Nery, *J. Chem. Soc.*, **1962**, 606.
10) E. Freese, "Chemical Mutagens," Vol. 1, Plenum Press, New York, 1971, p. 38.
11) E. Boyland and R. Nery, *J. Chem. Soc.*, **1962**, 5217.
12) A.D. Broom and R.K. Robins, *J. Org. Chem.*, **34**, 1025 (1969).
13) H.S. Resenkranz, *Chem.-Biol. Interactions*, **7**, 195 (1973).

the pK_a value of the substrate (9.5[14]), it is certain that the reaction proceeded through an electrophilic attack of the reagent on the anionic nitrogen atom in deprotonated uridine molecule. However, the yields of I, in strong alkaline conditions (pH>10), were not satisfactory because of high susceptibility of I to strong alkali. Compound (I) was more conveniently prepared by treating the sodium salt of uridine with 2,4-dinitrophenoxyamine (DNPA) in dimethylformamide than by the procedure using HAOS, the procedure being simpler and the yield much higher. Analoguously, 3-aminouracil derivatives were readily prepared by treatment of sodium salts of uracils with DNPA in dimethylformamide; 3-amino-1-methyluracil (II), 3-amino-2′,3′-O-isopropylideneuridine (III), and 3-amino-5-bromouridine (IV) from 1-methyluracil, 2′,3′-O-isopropylideneuridine, and 5-bromouridine, respectively. The

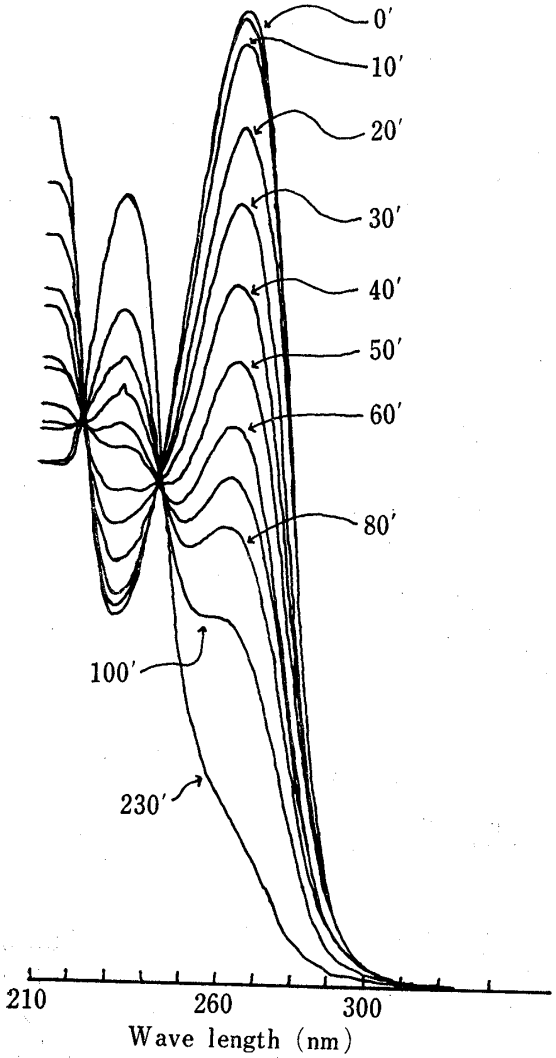


Fig. 1. Time-course of the UV Change in Alkaline Decomposition of 3-Aminouridine(I) in Aqueous 0.1N NaOH at 28°

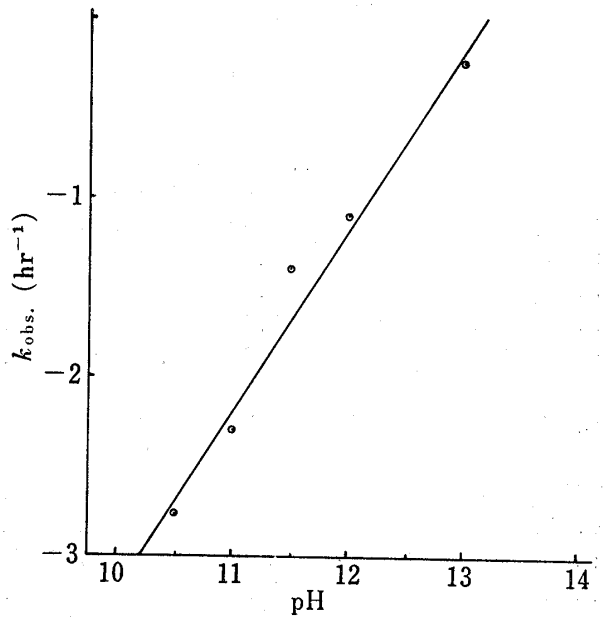


Fig. 2. pH-Dependence of Pseudo-first Order Rate for Decomposition of 3-Aminouridine (I) in Glycine Buffers (μ=1) at 37°

3-aminated uracils thus prepared were readily degraded in alkaline media above pH 10. In Fig. 1 is shown the time course of the UV spectral change of the 0.1N NaOH solution of II at 28°. The pH-dependence of the pseudo-first order rate for this alkaline degradation is given in Fig. 2. The products are assumed to undergo hydrolytic cleavage of the uracil ring as is known for 1, 3-disubstituted uracils such as 3-methyluridine.[15] Therefore, syntheses of 3-amino derivatives require the pH below 10 for the reaction medium.

3-Amino derivatives of cytidine and 1-methylcytosine (V and VI, respectively) were also prepared by treating with DNPA in dimethylformamide. While the amination of uracils proceeded *via* their conjugate bases, those of cytosine derivatives may involve an electrophilic attack on the basic nitrogen in the neutral form of molecules, as in the cases of alkylation

14) J. Wempen, R. Duschinsky, L. Kaplan, and J.J. Fox, *J. Am. Chem. Soc.*, **83**, 4755 (1961).
15) N.K. Kochetkov and E.I. Budovskii, "Organic Chemistry of Nucleic Acids," B, Plenum Press, London, 1972, p. 397.

with alkyl halides. The 3-aminated products were also susceptible to strong alkali as 3-aminouracils were. Thus, they were decomposed even around pH 10 at room temperature. The corresponding 3-aminouracil derivatives were thereby detected in their alkaline solutions, while 4-hydrazino derivatives, a type of the Dimroth rearrangement products,[16,17] were not. It can therefore be assumed that the degradation begins with deamination but not with a hydroxide anion attack on the C-2 of the cytosine ring.[18,19]

It is worth pointing out that 3-aminopyrimidines thus prepared showed UV absorptions very similar to those of the corresponding 1-alkyl derivatives as shown in Table II. This spectroscopic feature was true for 1-amino and 1-alkyl purine nucleosides and gave a clue for structural identification of the N-aminated products.[1,5]

TABLE I. PMR Data of Aminated Products and Their Derivatives Measured in 0.1 M DMSO-d_6 Solutions

Compound[a]	Chemical shift (δ)[b]							Coupling constant (Hz)		
	N–NH_2	C–NH_2	5-H	6-H	1′-H	N–Me	Other	J_{5-6}	$J_{1'-2'}$	J_{3-5}
3-NH_2-URf (I)	5.46		5.78	7.95	5.80			8.3	4.3	
3-NH_2-1-MeU (II)	5.45		5.70	7.61		3.33				
3-NH_2-isopURf (III)	5.45		5.79	7.81	5.88			8.0	2.4	
3-NH_2-5BrURf (IV)	5.54			8.53	5.79				3.5	
3-NH_2-CRf (V) HCl	5.65	9.16 10.24	6.37	8.33	5.77			7.9	3.2	
3-NH_2-1-MeC (VI) HCl	5.72	9.02 10.18	6.30	8.00		3.45		7.7		
VII			6.78[c]	7.81[c]		3.58	8.35(2-H[d])	7.6[c]		
VIII			6.85[c]	8.17[c]	6.08		8.37(2-H[d])	8.0[c]	3.9	
3-NH_2-4-thio-URf (IX)	6.65		6.56	7.86	5.81			7.8	3.7	
3-AcNH-1-MeU (X)	10.35		5.68	7.67		3.33	1.98($COCH_3$)	8.0		
1-MeU (XI)			5.50	7.57		3.24	11.15(NH)	8.0		1.2
1-MeC (XII)		6.85	5.63	7.52		3.21		8.0		

a) abbreviations I: 3-aminouridine, II: 3-amino-1-methyluracil, III: 3-amino-2′,3′-O-isopropylideneuridine, IV: 3-amino-5-bromouridine, V: 3-aminocytidine, VI: 3-amino-1-methylcytosine, VII: 6-methyl-1,2,4-triazolo[2, 3-*c*] pyrimid-5(6H)-one, VIII: 6-β-D-ribofuranosyl-1,2,4-triazolo[2,3-*c*]pyrimid-5-(6H)one, IX: 3-amino-4- thiouridine, X: 3-acetamino-1-methyluracil, XI: 1-methyluracil, XII: 1-methylcytosine

b) Chemical shifts (δ) were calibrated from the internal tetramethylsilane in ppm unit.

c) The numbering for these fused ring compounds followed that for the parent pyrimidine ring system.

d) See Chart 2 for the numbering.

Unlike 3-alkyl and 3-alkoxy derivatives, the introduced N-amino group in pyrimidines was readily deaminated by treatment with sodium nitrite in aqueous acetic acid to give the parent uracil and cytosine derivatives, respectively. This may constitute particular importance for synthetic chemistry because 3-amino compounds may be used as the reaction intermediates masked at the N-3 position. It is worth noting that the N-amino group of 3-aminocytosines was more susceptible to nitrous acid than the C-4 amino group. This suggests that more basic center of 3-aminocytosines is the C-amino nitrogen and that the salts of 3-aminocytosines carry more positive charge on the C-4 NH_2 than the N–NH_2, as formulated in Chart 3. This was evidenced by comparison of the nuclear magnetic resonance (NMR) spectra of the salts of 3-methyl and 3-amino cytosines measured in dimethylsulfoxide-d_6. Thus, the former

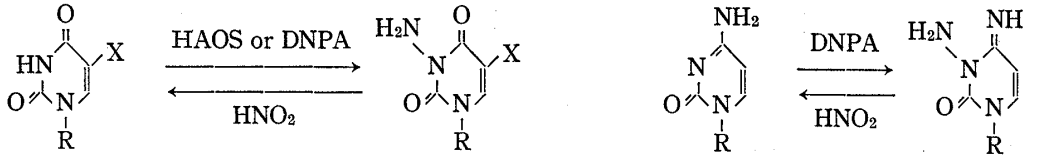
16) D.J. Brown, "Mechanism of Molecular Migrations," Vol. 1, Interscience Pub., New York, 1968, p. 209.
17) H. Mizuno, H. Okuyama, H. Hayatsu, and T. Ukita, *Chem. Pharm. Bull.* (Tokyo), **12**, 1240 (1964).
18) H. Siedel, *Biochim. Biophys. Acta*, **138**, 98 (1967).
19) I. Wempen, G.B. Brown, T. Ueda, and J.J. Fox, *Biochemistry*, **4**, 54 (1965).

Table II. UV Data of N-Aminated Products and Their Derivatives

Compound[a]	In H_2O			In 0.1N HCl		
	λ_{max} (nm)	($\varepsilon \times 10^{-3}$)	λ_{min} (nm)	λ_{max} (nm)	($\varepsilon \times 10^{-3}$)	λ_{min} (nm)
3-NH_2URf (I)	260	(8.4)	229	260	(8.6)	229
3-NH_2-1-MeU (II)	264	(8.0)	231	264	(8.2)	232
3-NH_2-isopURf (III)	260	(8.7)	228	260	(8.9)	229
3-NH_2-5-BrURf (IV)	277	(8.4)	243	279	(8.5)	243
3-NH_2-CRf(V)HCl	276	(11.8)	239	275	(11.9)	238
3-NH_2-1-MeC(VI)HCl	278	(10.9)	241	279	(11.3)	241
VII	267	(8.6)	217	271	(8.7)	217
VIII	268	(9.6)	219	267	(8.7)	219
3-NH_2-4-thio-URf (IX)	321	(20.2)	263	322	(20.3)	263
	243	(4.6)	232	244	(4.5)	232
3AcNH-1-MeU (X)	268	(9.2)	232			

a) abbreviation: See footnote (a) of Table I.

showed two broad signals due to two NH protons[20] (δ 9.17 and 9.77, respectively[21]). The amino protons of 3-aminocytidine (V) hydrochloride gave a very similar pattern of signals to that of the former (NH_2, δ 5.69 and two NH's, δ 9.16 and 10.24). This indicates that positive charge is distributed predominantly over the C-amino nitrogen (or at least to an appreciable extent) producing the double bond character in the bond between C-4 and 4-NH_2 nitrogen.



I, II, III and IV V and VI

I: R=β-D-ribofuranosyl, X=H
II: R=methyl, X=H
III: R=2,3-O-isopropylidene-β-D-ribofuranosyl, X=H
IV: R=β-D-ribofuranosyl, X=Br
V: R=β-D-ribofuranosyl
VI: R=methyl

Chart 1

Deamination of 3-aminouracils also took place by the action of bromine in aqueous media. Thus, the product from bromination of 3-aminouridine was not the 5-bromo derivative but uridine itself. It is assumed that the deamination may have proceeded through oxidation of N-amino group, followed by hydrolysis.[22]

One can expect that the amino group of 3-aminouracils possesses nucleophilic character, as seen in semicarbazides for example.[23] In fact, when 3-amino-1-methyluracil was treated with an excess of acetic acid at room temperature 3-acetamido-1-methyluracil (X) was produced in a good yield. The pK_a of 3-amino-1-methyluracil was determined to be 0.21 by UV spectro-

20) a) T.L.V. Ulbricht, *Tetrahedron Letters*, **1963**, 1027; b) H.T. Miles, *J. Am. Chem. Soc.*, **85**, 1007 (1963).
21) These values are those of 3-methylcytidine methosulfate.
22) The preparation of 3-amino-5-bromouridine (IV) was readily achieved by amination of 5-bromouridine with DNPA, as already described.
23) J. Zabicky, "The Chemistry of Amides," Interscience Pub., London, 1970, p. 187.

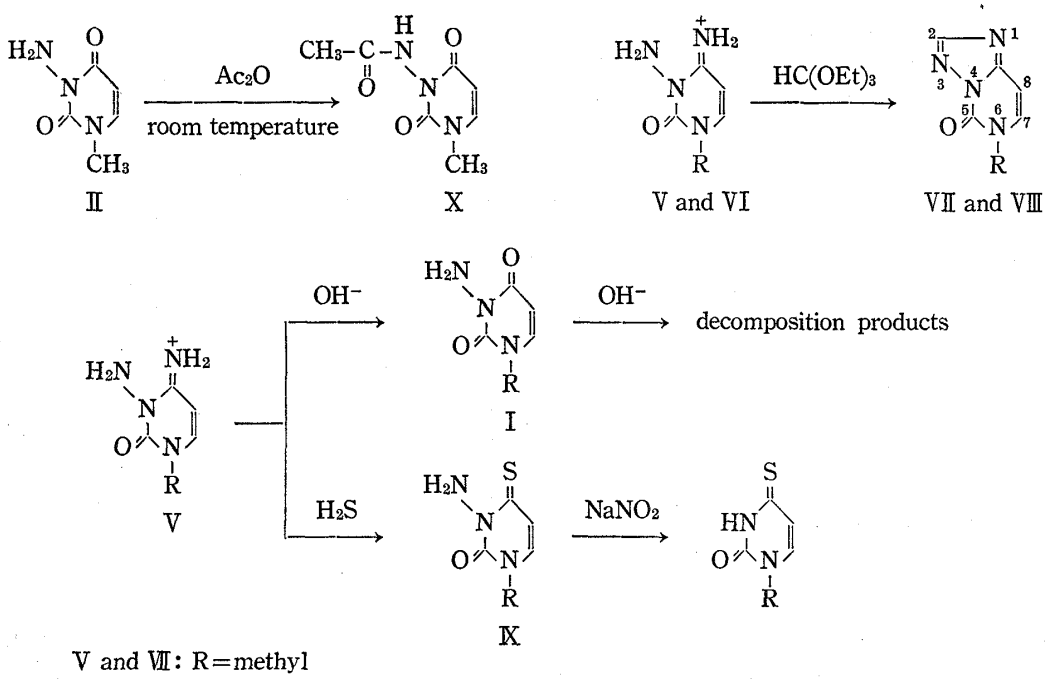


Chart 2

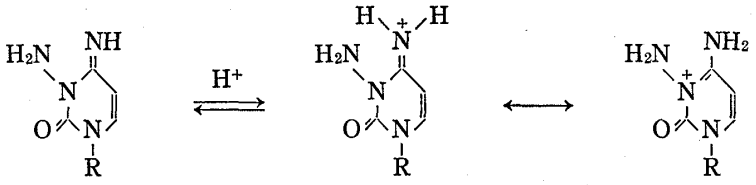


Chart 3

scopy.[24] This is considerably larger than that of 1-methyluracil (−3.40[25]), where protonation takes place in the oxygen atom at C-4. It is therefore assumed that the protonation in 3-aminouracils occurred in the N-amino nitrogen.

One of the reactivities of 3-aminocytosines seems to arise from the nucleophilic character of both N–NH_2 and C–NH_2 groups which are closely located. Thus, 3-amino-1-methylcytosine reacted with ethyl orthoformate in the presence of acetic anhydride to give a fused ring compound, 6-methyl-1,2,4-triazolo[2,3-*c*]pyrimid-5(6H)-one (VII), as formulated in Chart 2.[26] 3-Aminocytidine underwent this cyclization reaction under the same reaction condition. After the hydrolysis of the ethoxymethylidene group introduced to the 2′,3′-*cis*-diol of the ribose moiety, 6-β-D-ribofuranosyl-1,2,4-triazolo[2,3-*c*]pyrimid-5(6H)-one (VIII) was obtained in 92% yield. The structures of VII and VIII were supported by the NMR and analytical data. They are very weak bases, pK_a's being determined to be 0.14 and −0.24, respectively, by UV spectroscopy.[24] By treating 3-aminocytidine and 3-amino-1-methylcytosine with acetic anhydride without ethyl orthoformate, 6-β-D-ribofuranosyl-2-methyl-1,2,4-triazolo-[2,3-*c*]pyrimid-5(6H)-one and 2,6-dimethyl-1,2,4-triazolo[2,3-*c*]pyrimid-5(6H)-one were obtained, respectively, although the mono-, di, and tri- N-acetylated derivatives of the starting materials were yielded as by-products in both cases. The electronic structure and reactivity

24) The pK_a was measured in aqueous HCl solution at 23°.
25) A.R. Katritzky and A. Waring, *J. Chem. Soc.*, **1962**, 1540.
26) Analoguous reactions have been reported of some heterocyclic bases and nucleosides: ref. (1). G.L. Anderson, B.H. Rizkella, and A.D. Broom, *J. Org. Chem.*, **39**, 937 (1974); T. Tsuji, *Chem. Pharm. Bull.* (Tokyo), **22**, 471 (1974); T. Tsuji and T. Ueda, *ibid.*, **19**, 2530 (1971); T. Tsuji and Y. Kamo, *Chemistry Letters*, **1972**, 641.

of the new ring system thus obtained are now under investigation. The cyclization with cyanogen bromide was tried with V and VI[27] but failed to prepare the amino-substituted triazolopyrimidone derivatives.

3-Aminocytosines underwent the replacement reaction at the C-4 position. Thus, by treating 3-aminocytidine with hydrogen sulfide in a pyridine-dimethylformamide mixture, it was converted to 3-amino-4-thiouridine (IX), which was, in turn, deaminated by treatment with nitrous acid to give 4-thiouridine in a good yield.[28]

The NMR data for aminated compounds and their related compounds are summarized in Table I. The N-amino protons resonated at around δ 5.6 except for those of 3-amino-4-thiouridine (IX). Those of IX resonated in a lower field than those of the others. This downfield shift is assumed to be caused by anisotropy effect of 4-thio group.[29] This effect was also observed on 5-H of the pyrimidine ring. The chemical shift of 1'-H in VIII was 6.08 (δ), which is much lower than those of other series of nucleosides. This may indicate that the ring current of VIII is larger than the pyrimidine nucleosides examined here. The lower shift observed for 8-H of VII compared to the corresponding protons of the pyrimidine bases can be explained by the ring current effect of the triazole ring in addition to the electronic shielding effect. The UV data of the aminated derivatives prepared in the present study are summarized in Table II. As seen from the data, all the aminated compounds except for V and VI were not in the protonated form in the acidic condition indicated in the table.

Discussion on Mutagenic Mechanism of Hydroxylamine

The chemical modifications of the nucleic pyrimidine bases may be classified mainly into two (or three) categories of typical ionic reactions:[30] i) electrophilic substitution at the N-3 and/or substituent NH_2 and ii) series of reaction initiated by addition of nucleophiles to the C-6 (and direct substitution at the C-5 under certain particular reaction condition). As far as the modifications relating possibly to the DNA lesion *in vivo* are concerned, attention should be paid to the reaction (i), in addition to the photochemical and free radical processes. It would seem that the essential gene-material is protected *in vivo* from the attack of any kind of nucleophiles because it is surrounded by many nucleophiles involved in the cell-constituents under physiological conditions. Therefore, it appears that the reaction (ii) might not take part in the mutagenesis and/or carcinogenesis which are assumed to involve chemical alterations of the gene-material. As far as the evidence so far obtained is concerned, hydroxylamine is the only one example that appears to involve nucleophilic mechanism for its mutagenic activity among many popular chemical mutagens and carcinogens. The amination reaction described in this paper may suggest an alternative possibility for the molecular mechanism of hydroxylamine-mutagenesis, *i.e.*, an electrophilic substitution of the nucleic acid bases with amino group, as that with alkyl groups in mutagenesis of alkylating agents.

It may be of interest to note that the modified cytosine which Rosenkranz obtained by treating deoxycytidine with hydroxylamine-O-sulfonic acid under physiological condition seems to be 3-aminodeoxycytidine, since UV spectrum of 3-aminocytidine we recorded was superimposed with that reported in the literature.[13]

Experimental

All the melting points were measured with a Micro Melting Point Measurement Apparatus (Yanagimoto, Kyoto) and were uncorrected. Proton magnetic resonance (PMR) spectra were recorded with a JEOL-PS-

27) K.T. Potts and C. Hirsch, *J. Org. Chem.*, **33**, 143 (1968); K.T. Potts and R.M. Hushby, *J. Org. Chem.*, **31**, 3528 (1966).
28) T. Ueda, M. Imazawa, K. Miura, R. Iwata, and K. Odashima, *Tetrahedron Letters*, **1971**, 2507.
29) C.H. Wang, *Chem. Pharm. Bull.* (Tokyo), **21**, 2760 (1973).
30) N.E. Kochetkov and E.I. Budovskii, "Organic Chemistry of Necleic Acids," B, Plenum Press, London, 1972, p. 269.

Table III. Analytical Data and Melting Points of N-Aminated Products and Their Derivatives

Compound[a]	mp(°C)[b]	Formula	Aanlysis(%) Calcd. C	H	N	Found C	H	N
3-NH_2-URf (I)	83—84	$C_9H_{13}O_6N_3$	41.70	5.06	16.21	41.52	5.13	15.99
3-NH_2-1-MeU (II)	163—164	$C_5H_7ON_3$	42.55	5.00	29.79	42.60	5.01	30.01
3-NH_2iso-pURf (III)	160—161	$C_{12}H_{17}O_6N_3$	48.16	5.73	14.04	48.00	5.87	13.83
3-NH_2-5-BrURf (IV)	189—191(dec)	$C_9H_{12}O_6N_3Br$	31.96	3.57	12.42	32.06	3.53	12.17
3-NH_2-CRf(V)HCl	181—196(dec)	$C_9H_{15}O_5N_4Cl$	36.67	5.13	19.01	36.73	5.50	18.77
3-NH_2-1-MeC(VI)HCl	271—274(dec)	$C_5H_9ON_4Cl$	34.00	5.13	31.72	33.99	5.20	31.95
VII	208—210(sub)	$C_6H_6ON_4$	48.00	4.03	37.32	47.93	4.09	37.55
VIII	182—185	$C_{10}H_{12}O_5N_4$	44.78	4.51	20.89	44.73	4.61	20.38
3-NH_2-4-thio-URf (IX)	171—172	$C_9H_{13}O_5N_3S$	39.27	4.76	15.27	39.37	4.82	14.60
3-AcNH-1-MeU (X)	159—160(dec)	$C_7H_9O_3N_3$	45.90	4.95	22.94	45.65	5.05	22.73

a) abbreviation: See footnote (a) of Table I.
b) (dec): melt with decomposition. (sub): melt with sublimation.

100 spectrometer operating at 100 MHz with tetramethylsilane as the internal standard in the $CDCl_3$ and DMSO-d_6 solutions and with sodium dimethylsilapentane sulfonate in the D_2O solutions. UV spectra were recorded with a Cary-14 spectrometer using a 10 mm light path cell. Thin-layer chromatography (TLC) was carried out using Avicel-SF cellulose plates (purchased from Funakoshi Co., Tokyo) and appropriate solvent systems for elution. Uridine and cytidine were purchased from Kojin Co., Tokyo. 1-Methyluracil,[31] 1-methylcytosine,[31] 2′,3′-O-isopropylideneuridine,[32] and 5-bromouridine[33] were synthesized by authentic preparative methods. Hydroxylamine-O-sulfonic acid (HAOS)[34] and 2,3-dinitrophenoxyamine (DNPA)[35] were also prepared in our laboratory. All the other reagents used were purchased from Tokyo Kasei Co., Tokyo. The melting points and analytical data of the aminated products are shown in Table III.

3-Aminouridine (I)——Method A: HAOS (11.3 g; 0.1 mole) was added in small portions with vigorous stirring to a solution of 2.44 g of uridine (0.01 mole) and 20.7 g of K_2CO_3 (0.15 moles) in 60 ml of H_2O which has been chilled in ice water. Stirring was continued for 1 hr at room temperature and the reaction mixture was neutralized to pH 6 by addition of conc. HCl. The solution was evaporated to a half volume under reduced pressure and diluted with an equal volume of EtOH, when the inorganic material was precipitated. The precipitate was filtered off and the filtrate was evaporated to dryness under reduced pressure. The product was extracted from the residue several times with hot MeOH. The MeOH-extracts were combined and evaporated to dryness under reduced pressure. The residue was crystallized from EtOH–ether to afford 900 mg (35%) of white fine needles. They were recrystallized from MeOH.

Method B: DNPA (3.58 g; 0.18 moles) was added to a solution of uridine sodium salt (prepared from 3.66 g of uridine and 1.2 molar equivalents of sodium methoxide in MeOH) in 50 ml of dimethylformamide (DMF). The reaction mixture was kept standing at 37° for 4 days. After the solution was evaporated to dryness under reduced pressure, the residue was dissolved in 30 ml of H_2O and neutralized by addition of conc. HCl. The aqueous solution was thoroughly washed with ether. After the aqueous solution was evaporated to dryness under reduced pressure, the residue was recrystallized from EtOH–MeOH to give 3.1 g (80% yield) of the product (I).

3-Amino-1-methyluracil (II)——1-Methyluracil [sodium salt, prepatred from 1 g (7.94 mmoles) of 1-methyluracil and 318 mg (7.95 mmoles) of NaOH in MeOH] and 1.9 g (9.53 mmoles) of DNPA were dissolved in 20 ml of DMF and the solution was kept standing at 37° for 4 days. Then, the solution was evaporated to dryness under reduced pressure in a water bath below 60°. The brown red residue was dissolved in 20 ml of H_2O and made neutral by addition of 20% HCl. The unreacted DNPA and 2,4-dinitrophenol were removed by extraction with five 50-ml portions of ether. The product was extracted with five 70-ml portions of $CHCl_3$. The $CHCl_3$ extracts were combined, dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was recrystallized from EtOH–ether to afford 600 mg of the product (II) as pale brown fine needles (54% yield).

31) D.J. Brown, "The Pyrimidines," ed. by Weissberger, John Wiley and Sons, New York, 1962.
32) N.C. Yung and J.J. Fox, *J. Am. Chem. Soc.*, **83**, 3060 (1961).
33) T.K. Fukuhara and D.W. Visser, *J. Biol. Chem.*, **190**, 95 (1951).
34) H.J. Matsuguma and L.E. Audrieth, *Inorg. Synth.*, **5**, 122 (1957).
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3-Amino-2′,3′-O-isopropylideneuridine (III)——2′,3′-O-Isopropylideneuridine [sodium salt, prepared from 2.84 g (10 mmoles) of 2′,3′-O-isopropylideneuridine and 440 mg (11 mmoles) of NaOH in MeOH] and 2.39 g of DNPA were dissolved in 20 ml of DMF. After the solution was kept standing at 37° for one day, the solution was evaporated to dryness under reduced pressure to give red brown syrup. The residue was dissolved in 20 ml of H_2O and neutralized by addition of 20% HCl. This was thoroughly extracted with ether. The aqueous solution was evaporated to dryness under reduced pressure and the residue was recrystallized from EtOH–benzene to afford 2 g of brown crystalline material, which was recrystallized from EtOH to afford 1.5 g of the product (III) as light brown fine needles (50% yield).

3-Amino-5-bromouridine (IV)——Sodium salt of 5-bromouridine (prepared from 2 g of 5-bromouridine and a molar equivalent $NaOCH_3$ in MeOH) was dissolved in 30 ml of DMF. DNPA (1.48 g; 7.43 mmoles) in 12 ml of MeOH was added to this solution. After the dark red solution was kept standing at 37° for 4 days, the reaction mixture was evaporated to dryness under reduced pressure. The residue was dissolved in 20 ml of H_2O and neutralized by addition of dil. HCl. After removal of the unreacted DNPA and 2,4-dinitrophenol by extraction with ether, the aqueous solution was evaporated to dryness under reduced pressure and the residue was recrystallized from EtOH to afford 700 mg of the product (IV) as brown yellow fine needles (33% yield). The mother liquid was evaporated to a half volume and diluted with a small portion of ether to afford 500 mg of the product (IV).

3-Aminocytidine (V)——Cytidine (4.89 g; 20 mmoles) and 4.78 g (24 mmoles) of DNPA were dissolved in a mixture of 60 ml of DMF and 4 ml of MeOH. The reaction mixture was kept standing at 37° for 3 dasy. The solution was evaporated to dryness under reduced pressure and then, the residual dark oil was dissolved in 10 ml of H_2O. When the aqueous solution was made acidic by addition of conc. HCl, pale yellow crystalline material came out. The precipitates were filtered off and washed with small portion of H_2O. The filtrate and washing were combined and washed with five 100 ml portions of ether in order to remove the unreacted reagent and 2,4-dinitrophenol. The aqueous layer was evaporated to 40 ml under reduced pressure and 5 volumes of EtOH was added. Then, the solution was kept in a refrigerator to precipitate 5.1 g of brown needles of HCl salt of V (87% yield). The mother liquid was concentrated to 30 ml and diluted with 100 ml of EtOH to afford 0.5 g of the product.

3-Amino-1-methylcytosine (VI)——1-Methylcytosine (900 mg; 7.2 mmoles) and 1.72 g of DNPA (8.64 mmoles) were dissolved in 30 ml of DMF and 8 ml of MeOH. After the reaction mixture was kept at 37° for 2 days, it was evaporated to dryness under reduced pressure. The residue was dissolved in 10 ml of H_2O and acidified by addition of 20% HCl. The unreacted DNPA and 2,4-dinitrophenol were removed by extraction with ether. The aqueous solution was evaporated to dryness under reduced pressure and the residue was recrystallized from EtOH to afford 1 g of HCl salt of VI (79% yield) as pale yellow needles.

Deamination of 3-Aminopyrimidines——3-Aminocytidine (V), 3-aminouridine (I), or 3-amino-4-thiouridine (IX) was dissolved in a minimum amount of a mixture of H_2O and acetic acid (1 : 1 in volume), 1.2 molar equivalents of aqueous $NaNO_2$ was added in drops into the solution at room temperature or under ice cooling during 5 to 10 min with vigorous stirring. On addition of $NaNO_2$, N-amino group reacted readily with evolution of N_2O. After standing for 30 min at room temperature, the reaction mixture was worked up as usual procedure to afford cytidine, uridine, or 4-thiouridine.

Bromination of 3-Amino-2′,3′-O-isopropylideneuridine——To a solution of 500 mg (1.67 mmoles) of 3-amino-2′,3′-O-isopropylideneuridine in 10 ml of H_2O was added 533 mg (3.34 mmoles) of bromine. The reaction mixture was heated at 80° for 1 hr and the solution was evaporated to dryness under reduced pressure. The residue was crystallized from EtOH–ether. The crude product was identified to be a mixture of uridine and 5-bromouridine by TLC.

Acetylation of 3-Amino-1-methyluracil——3-Amino-1-methyluracil (141 mg; 1 mmole) was dissolved in 25 ml of acetic anhydride and the brownish yellow clear solution thus obtained was kept standing at room temperature for 6 days. The solution was evaporated to dryness under reduced pressure in a water bath below 40°. The residue was recrystallized from a mixture of ethyl acetate and petroleum benzene to afford 100 mg of X (71% yield). PMR spectrum supported the proposed structure.

6-Methyl-1,2,4-triazolo[2,3-*c*]pyrimid-5(6H)-one (VII)——A suspension of 3-amino-1-methylcytosine (VI) hydrochloride (746 mg; 5 mmoles) in 20 ml of ethyl orthoformate and 15 ml of acetic anhydride was refluxed for 10 hr. Then, the reaction mixture was evaporated to dryness under reduced pressure to afford crystalline material, which was recrystallized from EtOH–ether to afford 600 mg of VII as pale cream-colored prisms.

6-*β*-*b*-Ribofuranosyl-1,2,4-triazolo[2,3-*c*]pyrimid-5(6H)-one (VIII)——A mixture of 885 mg (3 mmoles) of 3-aminocytidine hydrochloride in 20 ml of ethyl orthoformate and 20 ml of acetic anhydride was refluxed for 10 hr. Then, the reaction mixture was evaporated to dryness under reduced pressure. The residue was dissolved in an appropriate amount of a mixture of EtOH and H_2O and the solution was heated at 80° for 2 hr to remove an ethoxymethylidene group substituted on the 2′,3′-*cis*-diol of the ribose moiety. The reaction was monitored by TLC, until the reaction had been completed. Then, the solution was evaporated to dryness under reduced pressure. The residue was recrystallized from EtOH to afford 710 mg of VIII (92% yield) as pale yellow prisms.

3-Amino-4-thiouridine (IX)——3-Aminocytidine hydrochloride (1.5 g; 5.1 mmoles) was dissolved in 30 ml of DMF and placed in a reaction autoclave previously chilled with a dry ice acetone mixture. Then, 50 ml of H_2S–pyridine mixture, which had been prepared by dissolving H_2S gas in 25 ml of pyridine thoroughly chilled, was poured into the autoclave. The mixture was heated at 55°—60° for 24 hr. After cooled, the H_2S was allowed to evaporate slowly, leaving a brownish solution. The solution was transferred into a round flask and evaporated to dryness under reduced pressure. The residue was recrystallized from 40 ml of a mixture of MeOH–EtOH (1 : 1 in volume) to afford 800 mg of IX (57% yield) as pale yellow needles.

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