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N→N Alkyl and Glycosyl Migration of Purines and Pyrimidines. II.[1] Glycosyl Migration of 3-Glycosyl-N^6-acyladenine Derivatives[2]

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The N-3→N-9 ribosyl (hydrogen halides or mercuric halides catalyzed) migration of N^6-acyl derivatives of 3-β-D-ribofuranosyladenine and its 5′-phosphate and 3-β-D-glucopyranosyladenine was found to occur by an intermolecular mechanism. Furthermore, formation of the 3- and 9-ribosyl derivatives was confirmed in the ribosylation reaction of N^6-benzoyladenine or its chloromercuri salt using milder conditions. These facts, contrary to the reports which described that the 9-glycosyl derivatives alone could be isolated, suggest that the reaction of N^6-benzoyladenine or its chloromercuri salt with glycosyl halides result in the initial formation of the 3-glycosyl derivatives followed by rapid conversion to the thermodynamically more stable 9-glycosyl derivatives in the presence of hydrogen halides or mercuric halides.

Glycosylation of N^6-acyladenine or its chloromercuri salts with sugar derivatives has widely been employed for the syntheses of the 9-substituted adenine nucleosides and nucleotides.[4] Alkylation of the same base with alkyl halides gave mainly the 3-alkyl-N^6-benzoyladenine.[5] Investigation on the difference in the product distribution of these two substitution reaction uncovered N-3→N-9 alkyl migration. The present paper describes the N-3→N-9 glycosyl migration of the derivatives of 3-isoadenosine,[6] its 5′-phosphate, and 3-isoglucosyladenine, and the properties of this migration. Furthermore, an effort was made to isolate the 3-riboside in the ribosylation reaction mixture of N^6-benzoyladenine or its chloromercuri salt, which has been reported to give only the 9-glycoside. Subsequently the relationship of glycosyl migration to glycosylation was clarified.

Glycosyl Migration

3-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl) adenine (Ia)[6] was benzoylated by treatment with benzoic anhydride. After chromatography on silica gel, carefully carried out within a few hours, 3-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-N^6-benzoyladenine (IIa) was obtained in 65% yield. When IIa was heated with hydrogen halide or mercuric halide in a solvent, the ribosyl migration occured. This migration reaction proceeded with greater ease than benzyl, allyl, or 3-methyl-2-butenyl migration just by heating in xylene or acetonitrile under reflux for 5—30 minutes. The product, 9-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-N^6-benzoyladenine (IIIa),

1) Part I: B. Shimizu and M. Miyaki, *Chem. Pharm. Bull.* (Tokyo), **18**, 579 3 (1970).
2) Preliminary communication was reported in *Chem. Ind.* (London), **1966**, 664.
3) Location: *2–58, 1–chome, Hiromachi, Shinagawa–ku, Tokyo.*
4) *a*) J. Davoll, B. Lythgoe and A.R. Todd, *J. Chem. Soc.*, **1948**, 967, 1685; *b*) J. Davoll and B.A. Lowy, *J. Am. Chem. Soc.*, **73**, 1650 (1951); *c*) J.J. Fox, N. Yung, J. Davoll and G.B. Brown, *ibid.*, **78**, 5060 (1957); *d*) T. Ukita and H. Hayatsu; *ibid.*, **84**, 1879 (1962); *e*) Y. Ishido and T. Sato, *Bull. Chem. Soc. Japan*, **34**, 347 (1961); *f*) J. Temple, R.L. Mckee and J.A. Montgomery; *J. Org. Chem.*, **28**, 2304 (1963); *g*) T. Nishimura, B. Shimizu and I. Iwai, *Chem. Pharm. Bull.* (Tokyo), **12**, 1471 (1964); T. Nishimura and B. Shimizu, *Agr. Biol. Chem.* (Tokyo), **28**, 224 (1964); *h*) M. Asai, M. Miyaki and B. Shimizu, *ibid.*, **31**, 319 (1967); *i*) B. Shimizu, M. Miyaki and K. Iwase, Japanese Patent 536100.
5) J.A. Montgomery and H.J. Thomas, *J. Heterocyclic Chem.*, **1**, 115 (1964).
6) *a*) N.J. Leonard and R.A. Laursen, *J. Am. Chem. Soc.*, **85**, 2026 (1963); *b*) *Idem, Biochemistry*, **4**, 354 (1965).

was isolated chromatographically on silica gel. This 9-ribosyl derivative (IIIa) was identical in its physical properties with the compound prepared by the reaction of N^6-benzoyladenine or its chloromercuric salt and 2,3,5-tri-O-benzoyl-D-ribofuranosyl halide. Debenzoylation of IIIa by sodium methoxide afforded 9-β-D-ribofuranosyladenine, its nuclear magnetic resonance (NMR) and ultraviolet (UV) spectra and melting point were identical with those of an authentic sample of β-adenosine (Chart 1). The 9-β-ribosyl derivative predominated in this migration reaction and the amount of 9-α-anomer depended on the catalyst used.

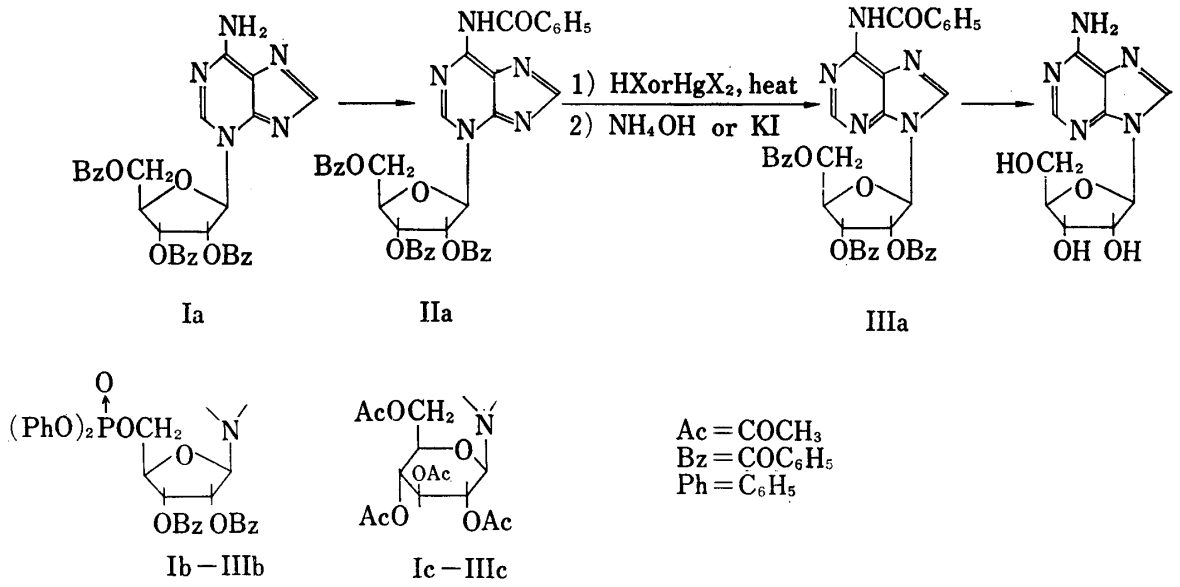


Chart 1

3-(2,3-Di-O-benzoyl-5-diphenylphosphoryl-β-D-ribofuranosyl-)N^6-benzoyladenine (IIb) was prepared in 64% yield from Ib by treatment with benzoic anhydride as in the case of the 3-ribofuranosyl derivative. Phosphoribosyl migration was observed when IIb was heated with mercuric bromide in acetonitrile under reflux for 30 min. The resulting 9-(2,3-di-O-benzoyl-5-diphenylphosphoryl-β-D-ribofuranosyl)-N^6-benzoyladenine (IIIb) was isolated in 54% yield.

To investigate the glucosyl migration, 3-glucopyranosyladenine was prepared. The reaction of adenine and 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl chloride in DMF at 90° for 20 hr and the following separation by chromatography afforded 3-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)adenine (Ic) and the corresponding 9-glucosyl isomer. Ic Thus obtained was then terated with benzoic anhydride to give 3-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-N^6-benzoyladenine (IIc) which was heated with mercuric bromide. Although the N-3→N-9 glucosyl migration was not observed on heating in acetonitrile under reflux (bp 82°), the migration took place in refluxing xylene (bp 137—140°) for 30 min with formation of 9-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-N^6-benzoyladenine (IIIc). The 9-glucoside (IIIc) was deacetylated by alkali to give 9-β-D-glucopyranosyladenine in 57% yield based on IIc. The slower rate of glucosyl migration as compared to that of the ribosyl or phosphoribosyl migration was assumed to be due to the lesser reactivity of the glucopyranosyl (six-membered) ring toward the nucleophilic displacement reaction than that of the ribofuranosyl (five-membered) ring. The glucosyl migration, of course, proceeded with greater ease than the alkyl migrations.

Nature of Glycosyl Migration

N-3→N-9 migration of benzyl, allyl and 3-methyl-2-butenyl groups catalyzed by hydrogen halide was intermolecular in nature,[1] and it appears reasonable to assume that the glycosyl migration catalyzed by hydrogen halide also proceeds intermolecularly. 3-Methyl-2-butenyl migration catalyzed by mercuric halide seems to proceed both by intra- and intermolecular

mechanisms depending on the temperature.[1] To establish whether the glycosyl migration by mercuric halide is intra- or intermolecular in nature, the migration was carried out in the presence of a different kind of purine base as follows: A mixture of IIa, N^6,N^6-dimethyladenine and mercuric bromide in a solvent was heated under the same conditions as in N-3→N-9 ribosyl migration. After chromatography, 3-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-N^6,N^6-dimethyladenine (11% yield) and its 9-substituted isomer (2.5%) were obtained along with IIIa (25%). An analogous reaction in the presence of N^2-acetylguanine afforded the 7- and 9-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-N^2-acetylguanine (23%) and IIIa (16%). This migration together with theophylline gave 7-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-theophylline (2.4%) and IIIa (40%). These results suggested the intermolecular nature of N-3→N-9 ribosyl migration of IIa caused by mercuric bromide.

When a solution of equimolar amounts of IIa, N^6-benzoyladenine-8-^{14}C (3.24×10^9 dpm/mole) and mercuric bromide in a mixture of DMA and xylene was heated under reflux for 10 min, there was isolated 9-ribosyl derivative which was found, after conversion to adenosine, to have 51% (1.66×10^9 dpm/mole) of the specific radioactivity of N^6-benzoyladenine-8-^{14}C. The radioactivity of the recovered N^6-benzoyladenine was reduced to 49% (1.58×10^9 dpm/mole) (Chart 2). This also suggests that the ribosyl migration is intermolecular. Mercuric bromide is presumed to act as an acid as in the case of intermolecular 3-methyl-2-butenyl migration by hydrogen halide, and this assumption may be supported by the fact that mercuric cyanide is less effective for the ribosyl migration in spite of its effectiveness for the intramolecular 3-methyl-2-butenyl migration.

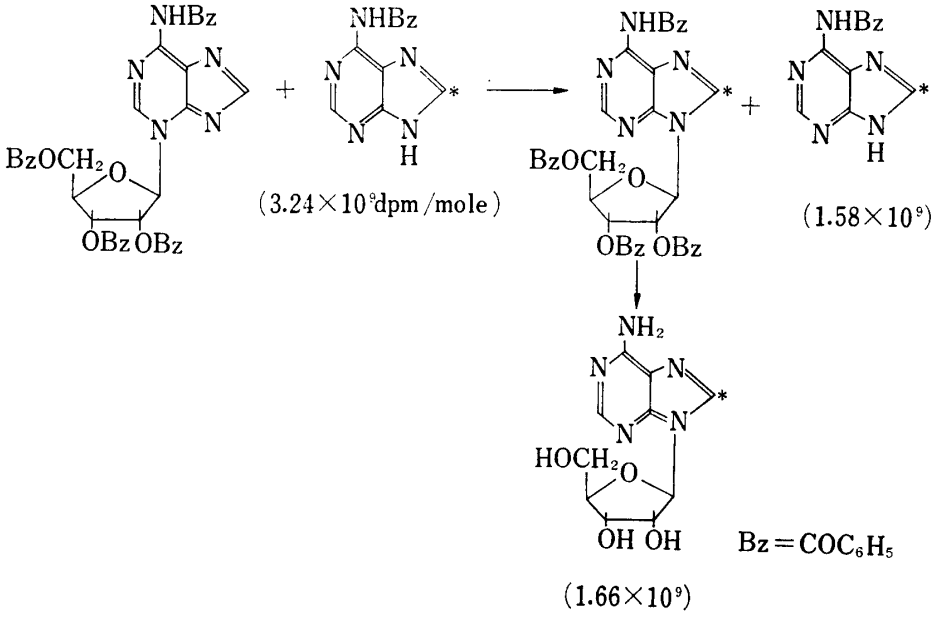


Chart 2

Benzoyl-3-isoadenosine (IIa) was converted into the 9-ribosyl isomer on being heated in the presence of various acid catalysts. Debenzoylation of the 9-riboside afforded mainly β-adenosine. α-Adenosine could be separated from the β-anomer by means of chromatography on Dowex 1 (OH form),[7] and each of the migration reaction mixtures was likewise chromatographed to determine the ratio of α-anomer to β-anomer. The amount of α-anomer was found to depend on the catalyst used, as indicated in Table I.

On the other hand, the condensation reaction of N^6-benzoyladenine and 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide was re-investigated to determine the possible presence of α-anomer in the reaction products which had been reported to be mainly 9-β-anomer. The products of the reaction at 100° for 2 hr in DMF were debenzoylated and separated by chro-

Table I. Yield of α- and β-Adenosine in Ribosyl Migration

Migration conditions			Adenosine	
Catalyst	Solvent	Time for reflux (min)	β:α	Over-all yield(%)
HBr	CH_3CN	15	80: 20	21
HCl	CH_3CN	30	87: 13	28
$HgBr_2$	X+DMF(12:1)	25	98.5:1.5	50
$HgCl_2$	X+DMF(20:1)	15	100: 0	59
$Hg(CN)_2$	X+DMA(10:1)	30	99.5:0.5	7
$Hg(CN)_2$	CH_3CN	120	100: 0	12

(X=xylene)

matography on Dowex 1 (OH). α-Adenosine was isolated together with β-adenosine and the ratio of β:α was 78:22, this value being analogous to that of the products obtained from ribosyl migration catalyzed by hydrogen bromide (80:20). The condensation reaction of chloromercuri-N^6-benzoyladenine and glycosyl halides has been reported to afford only the 9-β-glycosides analogously to the N-3 N-9 ribosyl migration catalyzed by mercuric halide.

Such a similarity in the relative yields of the α and β-anomers of the condensation and migration reactions suggested that the both reactions would proceed *via* a similar intermediate which regulates the ratio of anomers. According to this concept, correlation of the ribosylation to the ribosyl migration is proposed as shown in Chart 3. Contamination by the α-anomer in the condensation of N^6-benzoyladenine with ribosyl halide and in the migration catalyzed by hydrogen halide may be explained by the formation of a carbonium ion or an ion pair which can be attacked by the base from either the α or β positions. However the former is more hindered than the latter by the benzoyl group at position 2. In the condensation reaction of chloromercuri-N^6-benzoyladenine with ribosyl halide and in the ribosyl migration catalyzed by a mercuric compound an orthoester cation which can be attacked only from the β position may be the intermediate.

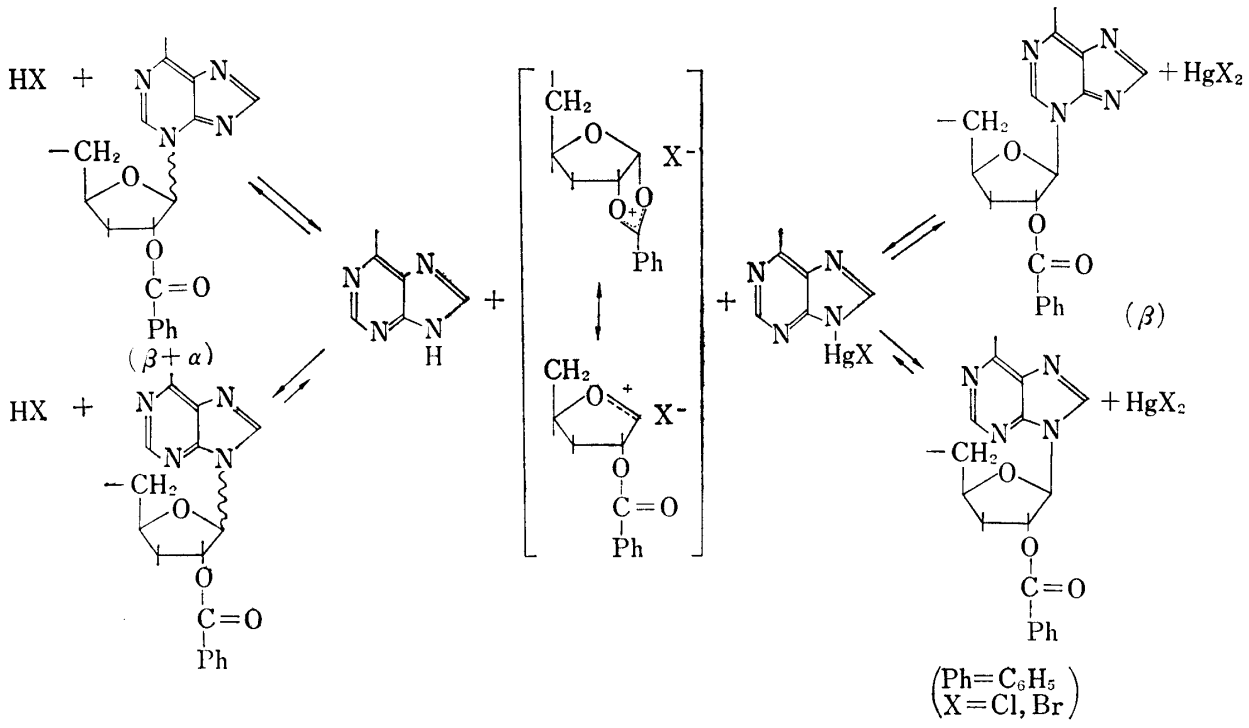


Chart 3

Isolation of 3-Riboside in Ribosylation of N^6-Benzoyladenine

If the correlation indicated in Chart 3 exists in the ribosylation and migration reaction, the condensation of N^6-benzoyladenine and ribosyl halide must result in the formation of the 3-riboside. Furthermore, the highest frontier electron density at N-3 in N^6-benzoyladenine indicated the initial attack at N-3 by the ribosyl halide. 3-Ribosyl-N^6-benzoyladenine formation was expected if the condensation reaction was carried out under milder conditions than those previously employed, *e.g.*, heating at 40°—60° for 20—40 hr, at 100° for 2 hr, or in refluxing xylene for 3 hr. Since the quantitative isolation of 3-ribosyl-N^6-benzoyladenine was considered difficult because of its unstability and due to its occurrence in small amounts, the yield of ribosylation products was determined as follows: N^6-benzoyladenine-8-^{14}C and 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide were heated at 50° in acetonitrile, the radioactive reaction mixture was diluted with pure cold IIa and IIIa, and then separated by chromatography on silica gel. The specific radioactivity of diluted compounds and the yield of 3- and 9-ribosides are shown in Table II. The 3-ribosyl derivative was isolated in a considerable amount, 12.6%, when the ribosylation reaction was stopped after 30 min. Prolonged heating decreased the ratio of the 3- to 9- riboside and the 3-riboside was hardly detected after 40 hr.

The ratio of 9-β- to 9-α-anomer was 87:13 in the ribosylation carried out at 50° for 30 min, but the α-anomer of 3-ribosyl derivative was hardly separated from the 3-β-isoadenosine by means of Dowex 1 (OH) column. Difference in the ratio of 9-β- to 9-α-anomer between the ribosylation at 50° (87:13) and that at 100° (78:22) appears not to be the experimental error. Whether anomerization of the 9-β-acylribofuranosyl derivative at high temperature occures or not remains to be investigated. In fact, anomerization was caused by hydrogen halides during the N-3→N-9 ribosyl migration in the ribosylation reaction.

Table II. Yield of 3- and 9-Ribosides, in the Reaction of N^6-Benzoyladenine-8-^{14}C and 2,3,5-Tri-O-benzoyl-D-ribofuranosyl Bromide

Conditions	Specific activity(dpm/mole) N^6-Benzoyladenine	Diluted riboside		Yield (%)	Ratio 9/3
20°, 5 min	6.35×10^9	3	1.64×10^8	2.7	0.85
		9	1.41×10^8	2.3	
50°, 30 min	3.24×10^9	3	3.62×10^8	12.6	1.21
		9	4.31×10^8	15.3	
50°, 7hr	6.35×10^9	3	6.49×10^7	1.0	12.4
		9	6.87×10^8	12.4	

Isolation of 3-Riboside in Ribosylation of Chloromercuri-N^6-benzoyladenine

Mercuric halide which catalyze the glycosyl migration seems to be formed in the glycosylation reaction of chloromercuri-N^6-benzoyladenine. Accordingly, the 3-glycoside formed must undergo a subsequent rapid migration giving rise to the 9-glycoside in the presence of a mercuric halide. To detect the 3-riboside, the condensation of chloromercuri-N^6-benzoyladenine and 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide was carried out under milder conditions and the reaction mixture was treated in a similar manner as described above for N^6-benzoyladenine. It was found that the 3-ribosyl derivative was also formed during the ribosylation at 50° for 30 min in 12% yield, as shown in Table III.

The difference in the reaction products between alkylation and glycosylation of N^6-acyladenine can be explained as follows: The reaction of the base with alkyl halide such as benzyl bromide gave mainly the 3-alkyl derivative because of the comparatively slow N-3→N-9 alkyl migration, whereas, the reaction with glycosyl halide afforded only the 9- glycoside because

7) C.A. Dekker, *J. Am. Chem. Soc.*, **87**, 4027 (1965).

Table III. Yield of 3- and 9-Ribosides in the Reaction of Chrolomercuri-N^6-benzoyladenine-8-^{14}C and 2,3,5-Tri-O-benzoyl-D-ribofuranosyl Bromide

Conditions	Specific activity (dpm/mole) N^6-Benzoyladenine-HgCl	Diluted riboside		yield (%)	Ratio 9/3
50°, 30 min	3.24×10^9	3	3.38×10^8	11.6	1.33
		9	4.62×10^8	16.4	

of the rapid N-3→N-9 glycosyl migration during glycosylation under the general reaction conditions. The O→N migration reaction[8] of pyrimidine and other heterocyclic derivatives rather seems to be in the same category as the N-3→N-9 migration. It may be reasonable to assume that the isolated intermediates in the reaction of purines of their chloromercuric salts with glycosyl halides are the 3-glycosides, while those of chloromercuric salts of pyrimidines are the O-glycosides. Corresponding to the chemical N-glycosylation, enzymic N-glycosylation of purines has been reported.[9] Nucleoside phosphorylase or pyrophosphorylase catalyses the reaction of purine bases with ribose 1-phosphate or 5-phosphoribosyl 1-phosphate to afford the 9-substituted nucleosides.[9] The only 3-substituted adenine, known to occur in nature, is triacanthine[10] which may be enzymically synthesized from adenine and 3-methyl-2-butenyl pyrophosphate. The other example of enzymic methylation of adenine with S-adenosylmethionine to give 3-methyladenine has been reported.[11] The remarkable difference in the rate of chemical N-3→N-9 migration reaction between glycosyl and 3-methyl-2-butenyl group may be of interest in connection with the mechanism of biological synthesis of the 9-glycosides and the 3-alkyl derivatives.

Experimental[12]

N^6-Benzoylation of 3-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)adenine (Ia)——Ia[6] (1 g) was heated with benzoic anhydride (0.6 g) in benzene (30 ml) under reflux for 6 hr and the solvent was evaporated *in vacuo*. The residue was chromatographed on silica gel (23 g) within 5 hr. After washig with benzene and benzene–$CHCl_3$, the column was eluted with $CHCl_3$ and the eluate was evaporated to dryness, giving the N^6-benzoyl derivative (IIa) (767 mg, 65%), amorphous. UV λ_{max}^{EtOH} mμ (ε): 231 (53000) and 307 (16200); $\lambda_{max}^{0.1NHCl/EtOH}$ mμ (ε): 231 (51100) and 304 (29100). $[\alpha]_D^{20}$:−76° (c=4.5, $CHCl_3$). *Anal.* Calcd. for $C_{38}H_{29}O_8N_5$: C, 66.76; H, 4.28; N, 10.25. Found: C, 66.95; H, 4.36; N, 10.00.

The benzoyl group at N^6 of IIa was extremely labile and debenzoylation occured even during chromatography on silica gel or when standing of the EtOH solution at room temperature.

Ribosyl Migration of IIa——A mixture of IIa (198 mg) and $HgBr_2$ (85 mg) in xylene (0.5 ml) was heated under reflux for 5 min and evaporated to dryness *in vacuo*. The residue was dissolved in $CHCl_3$ and the solution was washed with 30% KI and H_2O, dried and chromatographed on silica gel (3.5 g). The fraction eluted with benzene–$CHCl_3$ and $CHCl_3$ on evaporation of the solvent gave IIIa (112 mg, 62%), amorphous. UV λ_{max}^{EtOH} mμ (ε): 230.5 (47500) and 279 (21600); $\lambda_{max}^{0.1NHCl/EtOH}$ mμ (ε): 230.5 (48300) and 283 (23100). $[\alpha]_D^{25}$: −90° (c=0.37, $CHCl_3$). *Anal.* Calcd. for $C_{38}H_{29}O_8N_5$: C, 66.76; H, 4.28; N, 10.25. Found: C, 66.57; H, 4.30; N, 10.05. The fraction eluted with $CHCl_3$–MeOH (98:2) gave a substance (UV λ_{max}^{EtOH} mμ 277, 284, 329 and 340 (sh)) which exhibited the UV spectrum of 7-substituted adenine on debenzoylation. IIIa (105 mg) was heated in the presence of a catalytic amount of NaOMe in MeOH (15 ml) under reflux for 1 hr. After the solvent was evaporated, the residue was washed with ether and dissolved in H_2O (10 ml). Dowex-50 resin (H^+ form, 0.4 ml) was added to the aqueous solution and collected by filtration. Elution from the resi n with 1N NH_4OH and evaporation of the solvent at 0° gave 9-β-D-ribofuranosyladenine which was

8) *a*) G. Wagner and H. Pischel, *Arch. Pharm.*, **295**, 373 (1962) (Part 1), **300**, 737 (1967) (Part 23); *b*) T.L.V. Ulbricht and G.T. Rogers, *J. Chem. Soc.*, **1961**, 3345; **1965**, 6125, 6130; *c*) H.G. Gary and T.L.V. Ulbricht, *ibid.*, **1967**, 51; *d*) T. Ukita, H. Hayatsu and Y. Tomita, *Chem. Pharm. Bull.* (Tokyo), **11**, 1068 (1963).

9) A. Kornberg and I. Liebermann, *J. Biol. Chem.*, **215**, 417 (1955).

10) N.J. Leonard and J.A. Deyrup, *J. Am. Chem. Soc.*, **82**, 6202 (1960); **84**, 2148 (1962).

11) J. Axelrod and J. Daly, *Biochim. Biophys. Acta*, **61**, 855 (1962).

12) NMR Spectra were measured on Varian A60 Spectrometer.

recrystallized from H_2O (34 mg, 83%), mp 230°. UV $\lambda_{max}^{H_2O}$ mμ (ε): pH 1, 257 (14600); pH 7, 259.5 (15000); pH 13, 259.5 (15000). NMR: $H_{1'}=5.83$ ppm, $J_{1'-2'}=6$ cps, $N^6H_2=7.2$ ppm, $H_2,H_8=8.06$, 8.24 ppm (from TMS in DMSO-D_6). *Anal.* Calcd. for $C_{10}H_{13}O_4N_5$: $\frac{1}{2}H_2O$: C, 43.48; H, 5.07; N, 25.36. Found: C, 43.81; H, 5.53; N, 25.82. These values were similar to those of adenosine.

When the migration reaction was carried out in other solvents, the yields of adenosine were as follows: nitromethane, 52%; acetonitrile, 42%; DMF, 14%; DMSO, 10%.

N^6-Benzoylation of Ib——Ib[13] (200 mg) was heated with benzoic anhydride (100 mg) in refluxing benzene (20 ml) for 6 hr and the reaction mixture was evaporated to dryness. The residue was dissolved in $CHCl_3$ and the solution was chromatographed on silica gel (8 g) within 5 hr. The fraction eluted with $CHCl_3$–acetone (95:5) on evaporation of the solvent gave IIb (147 mg, 64%), amorphous UV λ_{max}^{EtOH} mμ: 232 and 308; $\lambda_{max}^{HCl/EtOH}$ mμ: 231, 285, 305 and 317 (sh).

Phosphoribosyl Migration of IIb——A mixture of IIb (140 mg) and $HgBr_2$ (62 mg) in CH_3CN (5 ml) was heated under reflux for 30 min and the solvent was evaporated. The $CHCl_3$ solution of the residue was washed with 30% KI and H_2O, dried and chromatographed on silica gel (3 g). The fraction eluted with benzene–acetone (90:10) on evaporation of the solvent gave IIIb (76 mg, 54%), amorphous. UV λ_{max}^{EtOH} mμ (ε): 230.5 (34500) and 279 (19200); $\lambda_{max}^{0.1NHCl/EtOH}$ mμ (ε): 230 (38500) and 283 (21600). $[\alpha]_D^{25}$: −58° ($c=0.4$, $CHCl_3$). *Anal.* Calcd. for $C_{43}H_{34}O_{10}N_5P$: C, 63.62; H, 4.19; N, 8.63; P, 3.82. Found: C, 63.42; H, 4.41; N, 8.38; P, 3.79.

Glucosylation of Adenine——A mixture of adenine (6.25 g) and 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl chloride[14] (18.3 g) in DMF (80 ml) was heated at 90° for 20 hr. After cooling, the reaction mixture was neutralized with NH_4OH at 0° and evaporated to dryness. The residue was extracted with $CHCl_3$ and the $CHCl_3$ solution was concentrated to 30 ml. Petroleum ether (100 ml) was added to the solution to give precipitates. The collected precipitates were dissolved in $CHCl_3$ and chromatographed on silica gel (150 g). Elution with EtOAc-MeOH (98:2) gave 9(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)adenine (1.20 g, 5.1%), amorphous. UV λ_{max}^{EtOH} 260 mμ: $\lambda_{max}^{HCl/EtOH}$ 257.5 mμ. NMR: $H_{1'}=6.17$ ppm ($J_{1'-2'}=9$ cps) from TMS in DMSO-d_6. *Anal.* Calcd. for $C_{19}H_{23}O_9N_5$: C, 49.03 H, 4.98; N, 15.05. Found: C, 48.61; H, 4.80; N, 14.70. Deacetylation of this compound by MeOH saturated with NH_3 gave 9-β-D-glucopyranosyladenine, mp 204—206°. Rechromatography of the fraction eluted with EtOAc–MeOH (95:5) gave Ic (1.58 g, 6.8%), amorphous. UV λ_{max}^{EtOH} 286 mμ: $\lambda_{max}^{HCl/EtOH}$ 279 mμ. NMR: $H_{1'}=6.40$ ppm ($J_{1'-2'}=8$ cps). *Anal.* Calcd. C, 48.50; H, 4.87; N, 14.53. Deacetylation of the compound (100 mg) by NH_3/MeOH gave 3-β-D-glucopyranosyladenine (32 mg, 50%), mp 280—283.° UV $\lambda_{max}^{H_2O}$ mμ (ε): pH 1, 276 (13100); pH 7, 279 (11900); pH 13, 280 (8800). *Anal.* Calcd. for $C_{11}H_{15}O_5N_5$: C, 44.44; H, 5.02; N, 23.59. Found: C, 44.04; H, 5.15; N, 23.16. A small amount (1.4%) of unknown derivative was also isolated by chromatography on silica gel, however it was not investigated further. [mp 217—221°; UV λ_{max}^{EtOH} 322 mμ: $\lambda_{max}^{HCl/EtOH}$mμ: 335 and 342. *Anal.* Found: C, 50.64; H, 5.49; N, 16.01].

N^6-Benzoylation of Ic——A solution of Ic (233 mg) and benzoic anhydride (170 mg) in benzene (10 ml) was refluxed for 4 hr and the solvent was removed. The residue was chromatographed on silica gel (6 g) within 5 hr. Elution with $CHCl_3$–MeOH (99:1) gave the N^6-benzoyl derivative (IIc) (115 mg, 40%), amorphous. UV λ_{max}^{EtOH} 305 mμ: $\lambda_{max}^{HCl/EtOH}$ mμ: 226, 305 and 315 (sh).

Glucosyl Migration of IIc——IIc (30 mg) was heated with $HgBr_2$ in CH_3CN (0.3 ml) under reflux for 40 min, however, TLC of the reaction mixture exhibited a single spot corresponding to IIc. The reaction mixture was evaporated to dryness *in vacuo*, dissolved in xylene (0.3 ml) and heated under reflux for 30 min. After removal of the solvent, the reaction product was extracted with $CHCl_3$. The $CHCl_3$ solution was washed with 30% KI and H_2O, dried and evaporated to dryness. The residue was treated with 0.05N NaOMe/MeOH and purified by chromatography on Dowex-1 (OH) (3 ml). The fraction eluted with H_2O (40—140 ml) gave 9-β-D-glucopyranosyladenine (4.5 mg, 57%), mp 203—205°, which was identical in all respects to the authentic sample.[4g,i]

Ribosyl Migration from IIa to N^6,N^6-Dimethyladenine——A mixture of IIa (890 mg), N^6,N^6-dimethyladenine (213 mg), $HgBr_2$ (469 mg), DMA (2 ml) and toluene (0.5 ml) was heated at 110—120° for 1 hr and evaporated to dryness. The residue was dissolved in $CHCl_3$ and the solution was washed with 30% KI and H_2O, dried, and chromatographed on silica gel. The fraction eluted with benzene–$CHCl_3$ (50:50) gave 9-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-N^6,N^6-dimethyladenine (20 mg, 2.5%), the fraction eluted with $CHCl_3$ gave IIIa (220 mg) and the fraction eluted with $CHCl_3$–MeOH (99:1) gave 3-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-N^6,N^6-dimethyladenine (86 mg, 11%). Each of these compounds was identified with the authentic sample[15] respectively in IR and UV spectra and *Rf* value of TLC.

Ribosyl Migration from IIa to N^2-Acetylguanine——A mixture of IIa (815 mg), N^2-Acetylguanine (230 mg), $HgBr_2$ (429 mg), DMA (5.5 ml) and toluene (1.2 ml) was heated at 110° for 1 hr. The reaction mixture was treated by the same manner as described above and chromatographed on silica gel (15 g). The

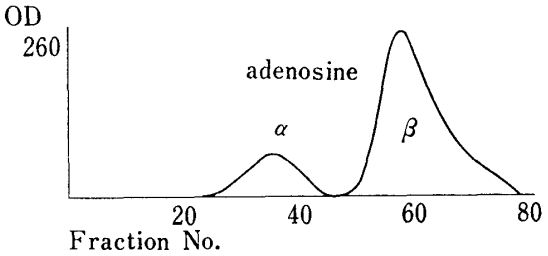
13) M. Asai, M. Miyaki and B. Shimizu, *Agr. Biol. Chem.*, 31, 319 (1967).
14) R.U. Lemieux, "Methods in Carbohydrate Chemistry" Vol. II, ed. by R.L. Whistler and M.L. Wolfrom, Academic Press Inc., New York and London, 1963, p. 223.

fraction eluted with $CHCl_3$ gave IIIa (130 mg, 16%) and that eluted with $CHCl_3$–MeOH (99:1) gave a mixture (1:1) of 7- and 9-(2,3,5-tri-O-benzoyl-D-ribofuranosyl)-N^2-acetylguanine (176 mg, 23%).

Ribosyl Migration from IIa to Theophylline——A mixture of IIa (300 mg), theophylline (395 mg), $HgBr_2$ (153 mg), DMF (2 ml) and toluene (1.3 ml) was refluxed for 30 min. The reaction mixture was treated by the same manner as described above and then chromatographed on silica gel (8 g). Debenzoylation of the product afforded from the benzene–$CHCl_3$ (50:50) and the $CHCl_3$ fractions gave 7-D-ribofuranosyltheophylline (33 mg, 2.4%), mp 190—192°. UV $\lambda_{max}^{H_2O}$ mμ: pH 1, 274.5; pH 7, 275; pH 13, 274.5. $[\alpha]_D^{25}$: +2.28° (c=1.0, H_2O). *Anal.* Calcd. for $C_{12}H_{16}O_6N_4$: C, 43.63; H, 5.49; N, 16.96. Found: C, 44.00; H, 5.06; N, 16.56. The fraction eluted with $CHCl_3$–MeOH (98:2) gave IIIa (120 mg, 40%).

Ribosyl Migration of IIa in the Presence of Labeled N^6-Benzoyladenine——A mixture of IIa (270 mg), N^6-benzoyladenine-8-^{14}C (3.24×10^9 dpm/mole, 94.4 mg), $HgBr_2$ (142 mg), DMF (0.6 ml) and xylene (0.8 ml) was heated under reflux for 20 min and evaporated to dryness *in vacuo*. The residue was dissolved in $CHCl_3$ and the solution was washed with 30% KI and H_2O. After removal of $CHCl_3$,the residue was dissolved in benzene (1.5 ml) and allowed to stand at room temperature over night. Precipitates appeared was collected by filtration and recrystallized from EtOH–H_2O to give N^6-benzoyladenine-8-^{14}C (74 mg), 1.58×10^9 dpm/mole. The filtrate and the mother liquor was chromatographed on silica gel (5 g). The fraction eluted with benzene–$CHCl_3$ (50:50) and $CHCl_3$ was treated with 0.1N NaOMe, and the resulting nucleoside was purified by chromatography on Dowex-1 (OH^-) to afford: 9-β-D-ribofuranosyladenine-8-^{14}C (55 mg, 52%), mp 234—234.5°, 1.66×10^9 dpm/mole.

Separation of α- and β-Anomer in Ribosyl Migration——The crude products by the ribosyl migration reaction induced by HgX_2 of HX (X=Br of Cl) were treated with 0.1N NaOMe/MeOH and applied to a column of Dowex-1 (OH^-). The column was eluted with H_2O–MeOH (90:10—70:30). The first fraction on recrystallization from aqueous EtOH gave α-adenosine,[15] mp 219—221°. UV $\lambda_{max}^{H_2O}$ mμ (ε): pH 1, 257 (14500); pH 7, 259 (14900); pH 13, 259.5 (15000). NMR: $H_{1'}=-153$ cps ($J_{1'-2'}=4.5$ cps), $H_{2'}H_8=-255$, -277 cps from dioxane in D_2O. *Anal.* Calcd. for $C_{10}H_{13}O_4N_5$: C, 44.94; H, 4.90; N, 26.21. Found: C, 44.79; H, 4.93; N, 26.50. The second fraction on recrystallization from H_2O gave β-adenosine. The amount of α-anomer depending on the catalyst is shown in Table I.



Isolation of α-Anomer in Ribosylation of N^6-Benzoyladenine (100° 2 hr)——N^6-Benzoyladenine (2.39 g) and 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide (prepared from 5.04 g of the acetate) were heated at 100° for 2 hr in DMF (100 ml). After cooling, NH_4OH was added to the reaction mixture and the solvent was evaporated and residue was chromatographed on silica gel (40 g). The column was washed with benzene and benzene–$CHCl_3$ (50:50), and eluted with $CHCl_3$. The first fraction on evaporation of the solvent gave 9-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-N^6-benzoyladenine (1.78 g, 26%), amorphous. UV λ_{max}^{EtOH} mμ (ε): 230.5 (48900) and 278 (22800); $\lambda_{max}^{0.1NHCl/EtOH}$ mμ (ε): 230.5 (48800) and 282.5 (23600). *Anal.* Calcd. for $C_{38}H_{29}O_8N_5$: C, 66.76; H, 4.28; N, 10.25. Found: C, 66.50; H, 4.27; N, 10.44. Debenzoylation of this compound gave β-adenosine. The second fraction gave 9-(2,3,5-tri-O-benzoyl-α-D-ribofuranosyl)-N^6-benzoyladenine (0.34 g, 5%), amorphous. UV λ_{max}^{EtOH} mμ (ε): 230.5 (50700) and 278.5 (21500); $\lambda_{max}^{0.1NHCl/EtOH}$ mμ (ε): 230.5 (52000) and 282.5 (23300). *Anal.* Found: C, 66.61; H, 4.20; N, 10.36. Debenzoylation of this compound gave α-adenosine.

Isolation of 3-Riboside in Ribosylation of N^6-Benzoyladenine——N^6-Benzoyladenine-8-^{14}C (3.24×10^9 dpm/mole, 48 mg) and 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide (prepared from 101 mg of the acetate) were heated at 50° for 30 min in CH_3CN (1.3 ml). The reaction was stopped by the addition of NH_4OH at 0° and the solvent was evaporated. A mixture of the residue and cold IIa (137 mg) and IIIa (137 mg) was extracted with benzene and the extract was chromatographed on silica gel (9 g). The fraction eluted with $CHCl_3$ was rechromatographed to give labeled IIIa: 4.13×10^8 dpm/mole. The fraction eluted with $CHCl_3$– acetone (98:2—95:5) was rechromatographed to give labeled IIa: 3.62×10^8 dpm/mole. The same

15) Part III: M. Miyaki and B. Shimizu, *Chem. Pharm. Bull.* (Tokyo), Submitted.

16) *a*) R.S. Wright, G.M. Tener and H.G. Khorana, *J. Am. Chem. Soc.*, **80**, 2004 (1958); *b*) L. Pichat, P. Dutay and Y. Lamore, *Compt. Rend.*, **259**, 2453 (1964); *c*) K. Onodera, S. Hirano and F. Matsuda, *Carbohyd. Res.*, **4**, 263 (1967).

reaction was carried out at 20° for 5 min and at 50° for 7 hr. Yields of the 3- and 9-ribosides were calculated from the following equation:

$$\frac{X}{100+X}=\frac{B}{A} \qquad \begin{pmatrix} A=\text{specific activity of } N^6\text{-benzoyladenine; } B=\text{specific} \\ \text{activity of the diluted 3- or 9-riboside; } X=\text{yield, \%} \end{pmatrix}$$

Isolation of α-Anomer in Ribosylation of N^6-Benzoyladenine (50°, 35 min)——A mixture of N^6-benzoyladenine (478 mg) and 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide (prepared from 1.01 g of the acetate) were heated at 50° for 35 min in a mixture of CH_3CN (5.5 ml), and DMA (1 ml), and treated with NH_4OH at 0° and evaporated to dryness. The residue was treated with NaOMe by usual manner and separated by chromatography on Dowex 1 (OH^-). The first fraction eluted with H_2O–MeOH (70:30) gave α-adenosine (7mg, 3%), the second fraction gave β-adenosine (113 mg, 21%) and the third fraction gave 3-isoadenosine (58 mg, 11%) whose anomers were not separated.

Chloromercuri-N^6-benzoyladenine-8-^{14}C——To a solution of N^6-benzoyladenine-8-^{14}C (3.24×10^9 dpm/mole, 100 mg) in H_2O (5 ml) containing 0.1N NaOH ($f=1.03$, 4.08 ml) was added a solution of $HgCl_2$ (111 mg) in EtOH (1 ml). The precipitates formed were collected by filtration, washed with H_2O–EtOH (90:10), EtOH and acetone, and dried (150 mg, 76%) Anal. Calcd. for $C_{12}H_8ON_5\cdot HgCl$: N, 14.76. Found: N, 15.04.

Isolation of 3-Riboside in Ribosylation of Chloromercuri-N^6-benzoyladenine——Chloromercuri-N^6-benzoyladenine-8-^{14}C (3.24×10^9 dpm/mole, 95 mg) and 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide (prepared from 101 mg of the acetate) were stirred in xylene (1.3 ml) at 50° for 30 min. The reaction mixture was worked up according to the procedure described in the case of ribosylation of N^6-benzoyladenine-8-^{14}C. The first fraction on rechromatography gave labeled IIIa (4.62×10^8 dpm/mole) and the second fraction gave labeled IIa (3.38×10^8 dpm/mole).

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