Purine Nucleosides. XXXI. The Directive Effect Which Certain Exocyclic Substituents at C-8 of Adenine Have on the Site of Ribosylation[1]

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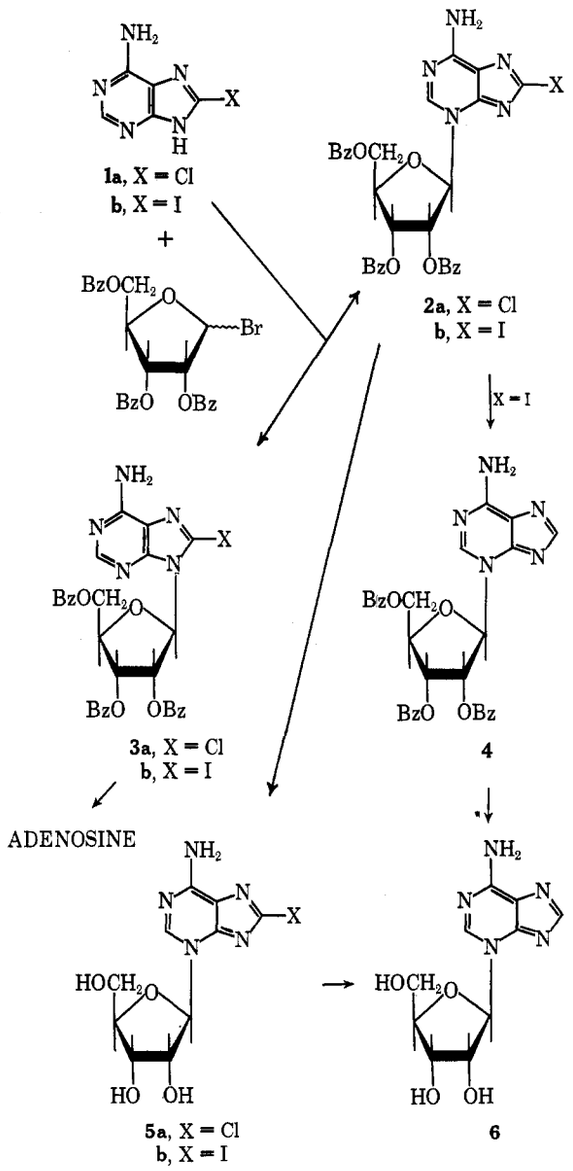
The extent to which different exocyclic substituents at the 8 position of adenine (H, Cl, I) influences the ratio of 3-ribosidation to 9-ribosidation has been studied. It has been established that the ratio can be correlated directly to the size of the group residing at the 8 position which indicates that steric considerations are probably the predominant factor.

It has been reported[2-5] that adenine is preferentially alkylated in the 3 position to yield predominantly 3-methyladenine. However, it has also been reported[6] that the "alkylation" of adenine with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide in acetonitrile furnished two isomers [3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)adenine (25%) and 9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)adenine (18%)]. It was proposed[6] that the bromo sugar was so reactive that the directive forces were less important, which accounted for the lack of specificity. An examination of space-filling molecular models (CPK) indicated that the insertion of a bulky group at C-8 of adenine would provide considerable steric hindrance to a large group attempting to enter at the 9 position. This prompted the present investigation in an effort to determine whether a steric effect would be observed in the ribosylation of 8-chloro- and 8-iodoadenine and if the effect could be correlated to the size of the 8 substituent.

8-Chloroadenine (**1a**) was stirred for 3 days with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide in dimethylformamide at 50° to provide starting material (**1a**, 32%) and two additional products, one a crystalline material (mp 231–232°) (22%) and the second a foam (12.5%). The pmr spectra of these compounds showed the H_2 signal to be at δ 8.68 and 8.99, respectively, as compared to δ 8.30 for 8-chloroadenine, *per se*. These spectra also showed all the characteristic peaks of a carbohydrate moiety in the proper proportion for a 1:1 adduct of carbohydrate to heterocyclic aglycon. Therefore, these two products were identified as nucleosides[7] of **1a** and on the basis of the work cited earlier they were tentatively assigned as the N-3 and N-9 isomers (**2a** and **3a**). The crystalline material, on treatment with methanolic ammonia, gave a product which was tentatively assigned as **5a** (mp 205° dec). The pmr spectrum of **5a** showed a singlet at δ 8.62 (H_2) and a doublet at 5.93 ($H_{1'}$). Dehalogenation with 10% palladium on charcoal and H_2 gas gave a good yield of nucleoside material (mp 213–215°). A comparison of the ultraviolet spectra of this nucleoside with that previously reported[6] for 3-β-D-ribofuranosyladenine (**6**) showed them to be identical and established



the structure of **2a** as 8-chloro-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)adenine and **5a** as 8-chloro-3-(β-D-ribofuranosyl)adenine. The pmr spectrum of **6** showed two singlets at δ 7.80 and 8.59. Comparing this spectrum with that of **5a** (singlet at δ 8.62) allowed us to unequivocally assign the downfield signal to H_2 and the signal at δ 7.80 to H_8. Treatment of the second product (**3a**) with methanolic ammonia, followed by 10% palladium on charcoal and H_2 gas, gave adenosine, as shown by a comparison of ultraviolet spectra and thin layer chromatographic properties with an authentic

(1) This work has been supported by Research Grant No. CA-11147, National Cancer Institute, National Institutes of Health, Public Health Service.

(2) J. W. Jones and R. K. Robins, *J. Amer. Chem. Soc.*, **84**, 1914 (1962).

(3) N. J. Leonard and J. A. Deyrup, *ibid.*, **84**, 2148 (1962).

(4) N. J. Leonard and T. Fujii, *ibid.*, **85**, 3719 (1963), and references cited therein.

(5) B. C. Pal, *Biochemistry*, **1**, 558 (1962).

(6) N. J. Leonard and R. A. Laursen, *ibid.*, **4**, 354 (1965).

(7) L. B. Townsend in "Synthetic Procedures in Nucleic Acid Chemistry," Vol. II, W. Werner Zorbach and R. Stuart Tipson, Ed., Interscience, New York, N. Y., 1972.

sample of adenosine. Thus, the structure of **3a** was established as 8-chloro-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)adenine. Further corroboration for these assignments was obtained by application of the $\Delta\delta$ rule.[8] Attempts to dehalogenate **2a** and **3a** directly were unsuccessful, presumably because of their insolubility in the solvents suitable for the reaction.

In an effort to increase the yield of the 3 isomer, we increased the size of the 8 substituent of adenine. 8-Iodoadenine was stirred with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide in dimethylformamide for 3 days at 50° to yield recovered 8-iodoadenine (**1b**, 31%) and two other products which by analogy to the reaction of 8-chloroadenine were tentatively assigned to be 8-iodo-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-adenine (**2b**, 30%) and 8-iodo-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)adenine (**3b**, 5.5%). In this case, **2b** was dehalogenated using 5% palladium on charcoal and H_2 gas to yield 3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)adenine (**4**). Removal of the blocking groups from the carbohydrate moiety with methanolic ammonia furnished 3-(β-D-ribofuranosyl)adenine (**6**). The above structure assignments were corroborated when the ultraviolet spectra and melting points of both nucleosides (**4** and **6**) agreed with those previously reported.[6] The structure of 8-iodo-tri-*O*-benzoyladenosine was established by dehalogenation with palladium on charcoal and H_2 gas to afford the previously reported 9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)adenine[6] and debenzoylation with methanolic ammonia to furnish adenosine (as established by ultraviolet spectra and tlc).

Therefore, the above data indicate that a substituent at position 8 of adenine has a marked influence on the site of ribosidation. The possibility that this influence is partially electronic cannot be discounted. However, if the primary electronic effect of the 8 substituent is inductive, one would expect 8-chloroadenine with the more electronegative chloro group (electronegativity 3.0) to give a higher ratio N-3 substitution to N-9 substitution than either 8-iodoadenine or adenine (electronegativities of H and I, 2.1 and 2.5, respectively). This would be due to a decrease in electron density in the imidazole ring relative to the pyrimidine ring. This is not the case, since the observed N-3:N-9 ratios for 8-iodo, 8-chloro, and 8-hydrogen are 5.5, 1.8, and 1.4, respectively. For an electronic rationale to be invoked would require that the resonance contribution by the 8-chloro group be of sufficient magnitude to more than compensate for the difference in electronegativity. On the other hand, an examination of space-filling molecular models (CPK) indicates that steric hindrance to attack at N-9 would result from the close proximity of a bulky 8 substituent and the large benzoyl group on the 2 position of the entering carbohydrate, while no such hindrance would be present for N-3 attack. This suggests that steric factors play the predominant role in determining the isomer ratios in these reactions.

Experimental Section

Melting points were determined with a Thomas–Hoover melting point apparatus and are uncorrected. Proton magnetic resonance spectra were measured with a Varian A-60 nmr spectrometer using tetramethylsilane as an internal standard. Ultraviolet spectra were determined with a Beckman DK-2 spectrophotometer and thin layer chromatography was run on SilicAR 7GF (Mallinckrodt). Elemental analyses were performed by Heterocyclic Chemical Corp., Harrisonville, Mo.

2,3,5-Tri-*O*-benzoyl-D-ribofuranosyl bromide was prepared by adding 20 ml of dichloromethane previously saturated at −30° with hydrogen bromide to a CH_2Cl_2 solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (2.7 g) also cooled to −30°. The solution was allowed to warm up to 0° and then evaporated to dryness *in vacuo*. The remaining traces of hydrogen bromide and acetic acid were removed by coevaporation with cold toluene (0°). The resulting syrup was used in the following reactions without further purification.

8-Chloro-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)adenine (2a) and 8-Chloro-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)adenine (3a).—To 8-chloroadenine[9] (1.0 g, 5.9 mmol) was added 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide prepared from 3.0 g (5.95 mmol) of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose and a catalytic amount of potassium iodide (2–5 mg) in 25 ml of dry dimethylformamide (AR grade, dried over 5 Å molecular sieves). The mixture was protected from moisture, stirred at 50° for 3 days, and then added dropwise with stirring to a mixture of ammonium hydroxide (28%) (1.5 ml) and water (400 ml). The solid was collected by filtration (3.5 g), dissolved in methanol (150 ml), and allowed to stand at room temperature for 16 hr. 8-Chloro-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)adenine (**2a**, 0.6 g) was collected by filtration and the methanol filtrate was evaporated to a volume of 15 ml and applied to three SilicAR 7GF preparative thick layer chromatography plates (20 × 40 cm, 3 mm thick). The plates were developed with a chloroform–acetone (4:1) mixture, the two uv-absorbing bands [**2a**, R_f 0.36 and 8-chloro-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)adenine (**3a**, R_f 0.56)] were removed and each nucleoside was eluted with hot methanol (50 ml). The fraction containing **2a** was evaporated to 10 ml on standing at room temperature to yield an additional 0.19 g of crystalline **2a** for a total yield of **2a** of 0.79 g (21%): mp 231–232°; uv λ_{max}, nm ($\epsilon \times 10^{-3}$), pH 1, 281 (24.5), 230.5 (51.5); pH 11, sh 318 (17.5), 306 (19.9), sh 285 (18.7), sh 279 (18.1), 237 (39.8); EtOH, sh 292 (16.2), 282.5 (17.5), sh 277 (16.2), 229 (54.0).

Anal. Calcd for $C_{31}H_{24}ClN_5O_7$ (**2a**): C, 60.84; H, 3.95; N, 11.40. Found: C, 60.82; H, 4.07; N, 11.18.

Evaporation *in vacuo* of the second methanol fraction yielded 0.45 g (12.5%) of **3a** as a foam, uv λ_{max}, nm ($\epsilon \times 10^{-3}$), pH 1, 261 (18.7), 233 (41.3).

Anal. Calcd for $C_{31}H_{24}ClN_5O_7 \cdot {}^1/_2H_2O$ (**3a**): C, 59.95; H, 4.06; N, 11.28. Found: C, 59.98; H, 4.10; N, 11.21.

Acidification (pH 4) of the aqueous filtrate from above resulted in the precipitation of unreacted 8-chloroadenine (**1a**, 0.32 g).

8-Iodo-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)adenine (2b) and 8-Iodo-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)adenine (3b).—2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl bromide, prepared from 1.9 g (3.8 mmol) of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose, and a catalytic amount of potassium iodide (2–5 mg) in dry dimethylformamide (25 ml) were added to 8-iodoadenine[10] (**1b**, 1.0 g, 3.8 mmol). The mixture was stirred at 50° for 3 days and the nucleosides (**2b**, R_f 0.69 and **3b**, R_f 0.78) were isolated as described above to yield 0.8 g (30%) of 8-iodo-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)adenine (**2b**): mp 233–234°; uv λ_{max}, nm ($\epsilon \times 10^{-3}$), pH 1, sh 296 (21.2), 288 (24.0), 232 (52.2); pH 11, sh 326 (18.4), 315 (19.8), 238.5 (36.0); EtOH, 298.5 (18.4), sh 286 (16.6), 230 (56.5).

Anal. Calcd for $C_{31}H_{24}IN_5O_7$ (**2b**): C, 52.77; H, 3.43; N, 9.97. Found: C, 52.39; H, 3.44; N, 9.76.

The yield of 8-iodo-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-adenine (**3b**) was 0.015 g (5.5%) (syrup): uv λ_{max}, nm ($\epsilon \times 10^{-3}$), pH 1, 262.5 (24.7), 233 (52.9).

Anal. Calcd for $C_{31}H_{24}IN_5O_7$ (**3b**): N, 9.97. Found: N, 9.70.

The yield of recovered 8-iodoadenine (**1b**) was 0.31 g (31%).

3-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)adenine (4).—8-Iodo-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)adenine (**2b**, 1.0 g) was suspended in a mixture of ethyl acetate (60 ml) and ethanol (40 ml). Sodium acetate (0.2 g) and 5% palladium on charcoal (0.5 g) were then added and the suspension was shaken on a

(8) K. R. Darnall and L. B. Townsend, *J. Heterocycl. Chem.*, **3**, 371 (1966).

(9) R. K. Robins, *J. Amer. Chem. Soc.*, **80**, 6671 (1958).

(10) R. T. Koda, J. A. Biles, and W. Wolf, *J. Pharm. Sci.*, **57**, 2056 (1968).

Parr hydrogenation apparatus under 40 psi of hydrogen gas at room temperature for 22 hr. The mixture was filtered through a celite bed and the filtrate was boiled down to a volume of 50 ml. The solution was allowed to stand at room temperature overnight to yield 0.44 g of **4**: mp 242–244° dec (reported[6] mp 246–247°); mixture melting point with an authentic sample[6] showed no depression; uv λ_{max}, nm ($\epsilon \times 10^{-3}$), pH 1, 276 (24.3), 230.5 (48.4); pH 11, sh 318 (14.8), 304 (17.4), 285 (18.0), 235 (37.4); EtOH, sh 294 (12.7), 279 (16.0), 229.5 (51.9).

Anal. Calcd for $C_{31}H_{25}N_5O_7$: C, 64.23; H, 4.34; N, 12.08. Found: C, 64.25; H, 4.24; N, 12.17.

3-(β-D-Ribofuranosyl)adenine (6). Method 1.—3-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)adenine (**4**, 0.18 g) was suspended in 15 ml of methanol, previously saturated at −5° with ammonia, in a sealed flask and allowed to stand at room temperature for 3 days. The resulting solution was evaporated to dryness *in vacuo* and the residue was extracted with ethyl ether (3 × 25 ml) leaving 0.08 g of solid. Recrystallization of this solid from a methanol–water mixture (about 3:1) furnished an analytical sample of **6**: mp 213–215° (reported[6] mp 210–211°); uv λ_{max}, nm ($\epsilon \times 10^{-3}$), pH 1, 274 (21.1); pH 11, 277 (15.0).

Anal. Calcd for $C_{10}H_{13}N_5O_4$: C, 44.91; H, 4.90; N, 26.19. Found: C, 44.89; H, 4.82; N, 26.12.

Method 2.—8-Chloro-3-(β-D-ribofuranosyl)adenine (**5a**, 0.1 g) was dissolved in water (5 ml) and then sodium acetate (0.1 g) and 10% palladium on charcoal (0.05 g) were added. The suspension was shaken on a Parr hydrogenation apparatus under 40 psi of hydrogen gas for 48 hr at room temperature and filtered through a celite bed, and the catalyst was washed with hot water (2.0 ml). The filtrate was evaporated to dryness *in vacuo* and the residue was recrystallized from methanol–water (5:1) to yield 0.04 g of product. Ultraviolet spectra and thin layer chromatography showed the product to be identical in all respects with that obtained by method 1, and a mixture melting point showed no depression.

8-Chloro-3-(β-D-ribofuranosyl)adenine (5a).—8-Chloro-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)adenine (**2a**, 0.25 g) was suspended in 50 ml of methanol, previously saturated at −5° with ammonia, in a sealed flask and then allowed to stand at room temperature for 4 days. The resulting solution was evaporated to dryness *in vacuo* and the residue was extracted with ethyl ether (3 × 25 ml). The remaining solid was dissolved in ethanol. The solution was cooled to 0° and the crystals which had formed after 18 hr were collected by filtration and washed with ethanol (2 ml) to yield 0.09 g of product which decomposes slowly above 205°; uv λ_{max}, nm ($\epsilon \times 10^{-3}$), pH 1, 279 (22.0); pH 11, 283 (17.8).

Anal. Calcd for $C_{10}H_{12}ClN_5O_4$: C, 39.79; H, 4.27; N, 23.15. Found: C, 39.52; H, 4.25; N, 22.93.

8-Iodo-3-(β-D-ribofuranosyl)adenine (5b).—8-Iodo-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)adenine (**2b**) (0.7 g) was suspended in methanol, saturated with ammonia at −5° (50 ml), in a sealed flask, and allowed to stand at room temperature for 4 days. The solution was evaporated to dryness *in vacuo* and the residue was extracted with ethyl ether (4 × 25 ml). The solid which remained was recrystallized from ethanol to yield 0.27 g of product: mp 202–205° dec; uv λ_{max}, nm ($\epsilon \times 10^{-3}$), pH 1, sh 295 (24.0), 287 (28.6), sh 227 (29.4); pH 11, 292.5 (19.1) 230 (15.9).

Anal. Calcd for $C_{10}H_{12}IN_5O_4$: C, 30.55; H, 3.08; N, 17.82. Found: C, 30.48; H, 3.21; N, 17.51.

8-Chloro-9-(β-D-ribofuranosyl)adenine.—8-Chloro-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)adenine (**3a**, 0.3 g) was added to methanol saturated with ammonia (saturated at −5°) (25 ml) in a sealed flask and allowed to stand for 4 days at room temperature. The solution was evaporated to dryness and the resulting syrup was extracted with ethyl ether (4 × 25 ml) to yield a solid. This solid was dissolved in ethanol and applied to a SilicAR 7GF thick layer plate (20 × 40 cm, 3 mm thick) and developed with ethyl acetate–ethanol (4:1). The band at R_f 0.53 was eluted with hot methanol (50 ml) and the eluent was allowed to evaporate to 25 ml on standing to yield 0.06 g of product: mp 209–211° (cloudy melt); uv λ_{max}, nm ($\epsilon \times 10^{-3}$), pH 1, 260 (19.3); H_2O, 262 (18.7); pH 11, 262 (19.8).

Anal. Calcd for $C_{10}H_{12}ClN_5O_4 \cdot {}^1/_2H_2O$: C, 38.56; H, 4.20; N, 22.48. Found: C, 38.85; H, 4.12; N, 22.53.

Adenosine. Method 1.—8-Chloro-9-(β-D-ribofuranosyl)-adenine (0.035 g) was dissolved in water (5 ml) containing sodium acetate (0.05 g), 10% palladium on charcoal (0.02 g) was added, and the suspension was shaken under 40 psi of hydrogen gas on a Parr hydrogenation apparatus for 40 hr. The mixture was filtered, the palladium on charcoal was washed with boiling water (5 ml), and the filtrate was evaporated to 3 ml on standing at room temperature to yield crystals (6 mg). A comparison of ultraviolet spectra and thin layer chromatographic properties with those of an authentic sample of adenosine showed them to be identical.

Method 2.—8-Iodo-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-adenine (**3b**, 0.010 g) was dissolved in a mixture of ethyl acetate (3 ml) and ethanol (2 ml) containing sodium acetate (5 mg), and 10% palladium on charcoal (8 mg) was added. The suspension was shaken under 40 psi of hydrogen as on a Parr hydrogenation apparatus for 24 hr and then filtered, and the catalyst was washed with boiling ethyl acetate. The filtrate was evaporated to dryness *in vacuo* and the solid residue was suspended in methanol saturated at −5° with ammonia and allowed to stand at room temperature for 3 days. The solution was evaporated to dryness. The residue was extracted with ethyl ether (4 × 10 ml) and the solid which remained was dissolved in hot water (2 ml) and allowed to stand at room temperature overnight to yield a small amount of crystalline product (yield not determined). Comparison of ultraviolet spectra and thin layer chromatographic properties with an authentic sample of adenosine showed this product to be identical with adenosine.

Registry No.—**2a**, 34388-76-6; **2b**, 34402-59-0; **3a**, 34388-77-7; **3b**, 34408-09-8; **4**, 28837-63-0; **5a**, 34408-11-2; **5b**, 34408-12-3; **6**, 2273-78-1; 8-chloro-9-(β-D-ribofuranosyl)adenine, 34408-14-5.