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Synthesis of Cyclic α-Amino Acids. IV. Syntheses of Adenine Nucleosides of 3-Amino-3-*C*-carboxy-3-deoxy-D-ribofuranose and 3-Amino-3-*C*-carboxy-3-deoxy-D-ribopyranose*[1]

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9-(3′-Amino-3′-*C*-carboxy-3′-deoxy-β-D-ribofuranosyl)adenine (I) and 9-(3′-amino-3′-*C*-carboxy-3′-deoxy-β-D-ribopyranosyl)adenine (II) have been synthesized. They are the first examples of nucleoside-derivatives which have an α-amino acid structure in their furanose or pyranose ring. A masked derivative (VII) of α-D-*erythro*-pentofuranos-3-ulose was converted into a hydantoin derivative (IX), which was acetolyzed and then treated with dry hydrogen chloride to give an acylglycosyl chloride (XI). Condensation of this derivative with chloromercuri-6-benzamidopurine followed by hydrolysis afforded I. Treatment of the hydantoin derivative (IX) with methanolic hydrogen chloride followed by hydrolysis gave methyl 3-amino-3-*C*-carboxy-3-deoxy-α-D-ribopyranoside (XVII), which has been found to be identical with one of the isomers of methyl 3-amino-3-*C*-carboxy-3-deoxy-α-D-pentopyranoside previously reported. The 1-*O*-acetyl-3-*N*-benzoyl-2,4-di-*O*-benzoyl derivative of ethyl ester of this acid (XVII) was fused with 6-benzamidopurine in the presence of *p*-toluenesulfonic acid and followed by hydrolysis to afford II. Structural proofs for the new nucleoside-derivatives were obtained from their ultraviolet, infrared and nuclear magnetic resonance spectra.

In a previous paper[1] we described the synthesis of a new kind of monosaccharide-derivatives which have an α-amino acid structure in a pyranose. Synthesis of nucleoside-related compounds[2] is in progress in this laboratoy. Interest in nucleoside chemistry has led us to synthesize new derivatives of nucleosides which have an α-amino acid structure in their carbohydrate moiety. Puromycin (III), an antibiotic purine derivative, exhibits carcinostatic and trypanocidal activities. As a biologically active moiety of puromycin, 9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)-6-dimethylaminopurine (IV) was demonstrated by Baker *et al.*[3] 3′-Amino-3′-deoxyadenosine[4] (V) was also found to be about twenty times as active as IV against the transplanted mammary adenocarcinoma of a C_3H mouse. Fukatsu and Umezawa[2] synthesized an adenosine nucleoside of 3-amino-3-deoxy-D-glucose and found it to have a weak anti-HeLa cell activity. As a result, we became interested in the synthesis of aminonucleoside-derivatives which have an α-amino acid structure in the ribofuranose or ribopyranose moiety. The present paper will present the synthesis of adenine nucleosides (I and II) of 3-amino-3-*C*-carboxy-3-deoxy-D-ribofuranose and of its pyranose-form.

The course of synthesis is summarized in Scheme 1.

Synthesis of 9-(3′-Amino-3′-*C*-carboxy-3′-deoxy-β-D-ribofuranosyl)adenine (I). The starting material, 5-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentos-3-ulose (VII) was prepared from 5-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-xylofuranose[5] (VI) by oxidation with Pfitzner-Moffatt reagent[6] in a good yield.

According to the Bucherer hydantoin synthesis, as reported by Hoyer,[7] VII was allowed to react with a mixture of potassium cyanide and ammonium carbonate under carbon dioxide atmosphere in an autoclave to afford 5-*O*-benzoyl-3-deoxy-1,2-*O*-isopropylidene-3-*C*-(spiro-5′-hydantoin)-α-D-pentofuranose (IX) as a major product, accompanied by the formation of 5-*O*-benzoyl-1,2-*O*-isopropylidene-

*[1] A part of this paper was presented at the 22nd Annual Meeting of the Chemical Society of Japan, Tokyo, April 4, 1969.

1) H. Yanagisawa, M. Kinoshita and S. Umezawa, This Bulletin, **42**, 1719 (1969).

2) S. Fukatsu and S. Umezawa, *ibid.*, **38**, 1443 (1965).

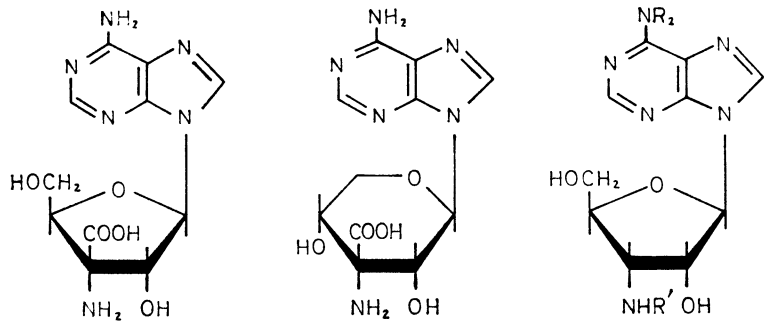
3) B. R. Baker, J. P. Joseph and J. H. William, *J. Amer. Chem. Soc.*, **77**, 1 (1955).

4) B. R. Baker, R. E. Schaub and H. M. Kissman, *ibid.*, **77**, 5911 (1955).

5) P. A. Levene and A. L. Raymond, *J. Biol. Chem.*, **102**, 317 (1933).

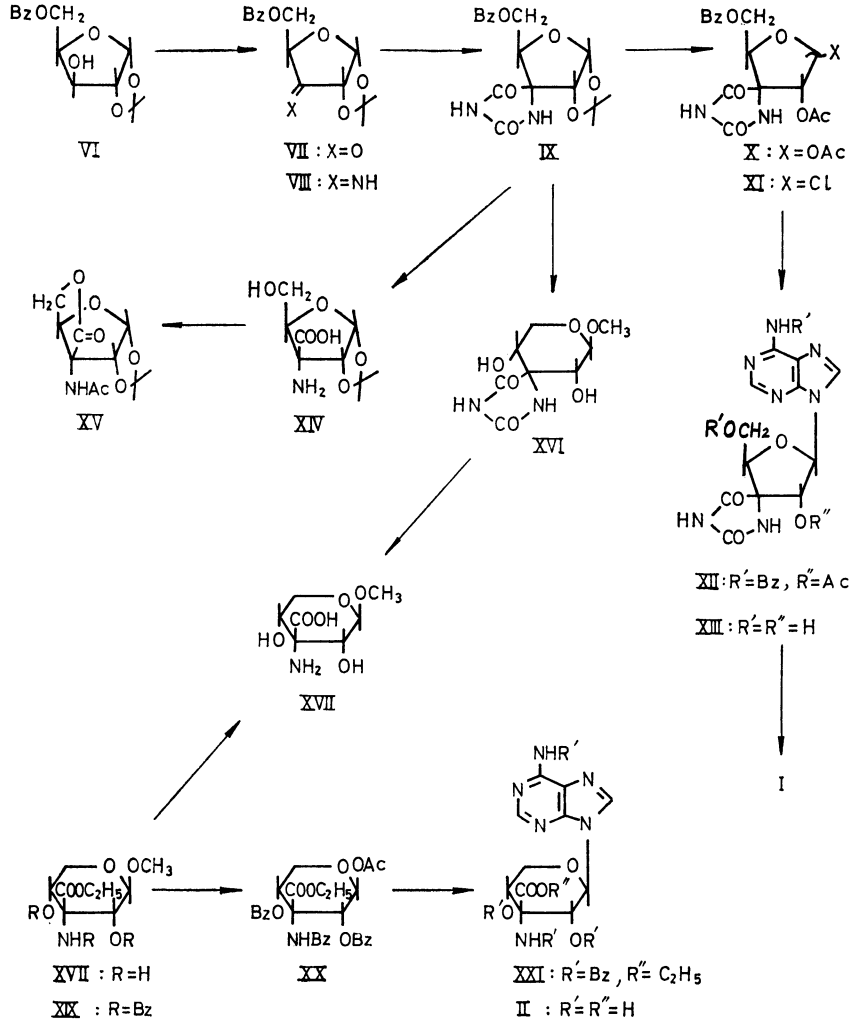
6) K. E. Pfitzner and J. G. Moffatt, *J. Amer. Chem. Soc.*, **85**, 3027 (1963); J. S. Brimacombe, J. G. H. Bryan, A. Husain, M. Stacey and M. S. Tolley, *Carbohyd. Res.*, **3**, 318 (1967).

7) H. L. Hoyer, *Chem. Ber.*, **83**, 491 (1950).



I II III (Puromycin): R = CH_3
R′ = CO·CH(NH_2) CH_2·C_6H_4·OCH_3-p
IV: R = CH_3, R′ = H
V: R = R′ = H

Chart 1



Scheme 1

α-D-*erythro*-pent-3-ulose imine (VIII). It is noteworthy that imine (VIII) was obtained as the main product by the usual Bucherer hydantoin synthesis which was carried out under atmospheric pressure without using carbon dioxide.

Hydrolysis of IX with barium hydroxide gave 3-amino-3-*C*-carboxy-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (XIV), which, on *N*-acetylation with acetic anhydride in methanol, afforded a γ-lactone (XV) showing an infrared absorption band at 1780 cm^{-1}. This lactone-formation indicates that XIV has a structure of 3-*C*-carboxy derivative of 3-amino-3-deoxy-D-ribose and XV is 3-acetamido-3-*C*-carboxy-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose-γ-lactone.

Acetolysis of the hydantoin derivative (IX) with a mixture of acetic anhydride and acetic acid in the presence of concentrated sulfuric acid gave a glassy product of 1,2-di-*O*-acetyl-5-*O*-benzoyl-3-deoxy-3-*C*-(spiro-5′-hydantoin)-D-pentofuranose (X). Its NMR spectrum showed it to be predominantly α-anomer. The pure α-anomer was obtained by crystallization in hot acetone-diisopropyl ether. Assignment of the α-configuration to the product was based on the coupling constant ($J_{1,2}$=4.1 Hz) shown in its NMR spectrum.

Treatment of X with hydrogen chloride in ether gave a syrupy product of 2-*O*-acetyl-5-*O*-benzoyl-1-chloro-1,3-dideoxy-3-*C*-(spiro-5′-hydantoin)-D-pentofuranose (XI). The coupling constant ($J_{1,2}$=4.2 Hz) observed in the NMR spectrum of XI showed that the glycosyl chloride was again α-anomer.

Reaction of the glycosyl chloride (XI) with chloromercuri-6-benzamidopurine produced a masked nucleoside, *i. e.* 9-[2′-*O*-acetyl-5′-*O*-benzoyl-3′-deoxy-3′-*C*-(spiro-5′-hydantoin)-β-D-pentofuranosyl]-6-benzamidopurine (XII). Hydrolysis of XII with 1.5 mol of methanolic barium hydroxide at room temperature gave the deacylated hydantoin-nucleoside, 9-[3′-deoxy-3′-*C*-(spiro-5′-hydantoin)-β-D-pentofuranosyl]adenine (XIII). Further treatment of XIII with excess amount of methanolic barium hydroxide under reflux afforded the desired nucleoside, 9-(3′-amino-3′-*C*-carboxy-3′-deoxy-β-D-ribofuranosyl)adenine (I).

The structures of I and XIII were indicated by their elemental analyses, negative rotations and ultraviolet spectra which showed the characteristic maximum of 9-substituted adenine at 259 mμ. The β-configuration of the products could be interpreted adequately in terms of Tipson's "*trans* rule"[8] as extended by Baker and his co-workers.[9]

The resonance for the anomeric proton of XIII at τ 4.00 appears as a doublet with a coupling constant ($J_{1',2'}$=7.3 Hz) unusually larger than a value of ≦2 Hz[10] which is expected for a β(*trans*) anomeric configuration. However, a similar observation was recently made with 3′-*C*-methyladenosine ($J_{1',2'}$=8.2 Hz) and adenosine ($J_{1',2'}$=6.0 Hz), and discussed by Nutt *et al.*[11] It may be attributed to the deformation of the furanose ring from its usual conformation by the increase in bulky group eclipsing interaction in the nucleosides.

Synthesis of 9-(3′-Amino-3′-*C*-carboxy-3′-deoxy-β-D-ribopyranosyl)adenine (II). In a previous paper,[1] the synthesis of four diastereomers of methyl (−)-3-amino-3-deoxy-3-*C*-ethoxycarbonyl-pentopyranoside from methyl β-D-xylopyranoside has been reported. An isomer (XVIII), which showed the largest R_f-value among the four isomers on thin-layer chromatography (TLC), was hydrolyzed to afford methyl (−)-3-amino-3-*C*-carboxy-3-deoxypentopyranoside, and its partial structure was deduced from NMR spectroscopy and chemical results.

Hydantoin derivative (IX) was treated with methanolic hydrogen chloride to give methyl 3-deoxy-3-*C*-(spiro-5′-hydantoin)-β-D-pentopyranoside (XVI). The coupling constant ($J_{1,2}$=8.5 Hz) in its NMR spectrum indicated that it is a β-anomer. Hydrolysis of XVI with barium hydroxide gave methyl 3-amino-3-*C*-carboxy-3-deoxy-β-D-riboside (XVII). It has been found that this riboside was identical with the methyl (−)-3-amino-3-*C*-carboxy-3-deoxypentopyranoside, their infrared spectra, optical rotations and R_f-values on paper chromatography being identical and mixed melting point showing no depression. Consequently, the structure of isomer (XVIII) was established as methyl 3-amino-3-deoxy-3-*C*-ethoxycarbonyl-β-D-ribopyranoside.

Benzoylation of XVIII yielded methyl 3-benzamido-2,4-di-*O*-benzoyl-3-deoxy-3-*C*-ethoxycarbonyl-β-D-ribopyranoside (XIX), which, on acetolysis, gave the desired product, 1-*O*-acetyl-3-benzamido-2,4-di-*O*-benzoyl-3-deoxy-3-*C*-ethoxycarbonyl-β-D-ribopyranose (XX). Since, in the NMR spectra of XIX and XX, the small coupling constants of $J_{1,2}$=1.6—2.0 Hz and $J_{4,5a}$=1.6—2.0 Hz and a long range coupling constants of $J_{2,4}$=1.2—1.5 Hz were observed, both XIX and XX seem to exist in a 1C conformation.

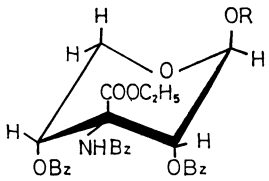
Attempts to convert XX into an acylglycosyl halide with hydrogen chloride in ether and with hydrogen bromide in acetic acid were unsuccessful. However, when the condensation reaction of XX and benzamidopurine was carried out by the fusion

8) R. S. Tipson, *J. Biol. Chem.*, **130**, 55 (1939); B. R. Baker, J. P. Joseph, R. E. Schaub and J. H. Williams, *J. Org. Chem.*, **19**, 1780 (1954); J. J. Fox and I. Wempen, *Advan. in Carbohyd. Chem.*, **14**, 283 (1959); J. A. Montgomery and H. J. Thomas, *ibid.*, **17**, 315 (1962).

9) B. R. Baker, Ciba Foundation Symposium, Chem. Biol. of Purines, p. 120 (1957).

10) R. U. Lemieux and D. R. Lineback, *Ann. Rev. Biochem.*, **32**, 155 (1963).

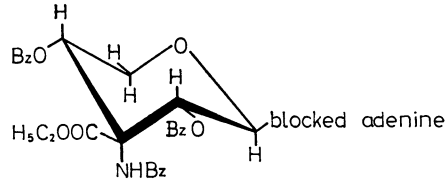
11) R. F. Nutt, M. J. Dickinson, F. W. Holly and E. Walton, *J. Org. Chem.*, **33**, 1789 (1968).



1C Conformer

XIX : R=CH_3

XX : R=Ac



C1 Conformer

XXI

Chart 2

procedure as reported by Sato *et al.*,[12] using *p*-toluenesulfonic acid as a catalyst, and subjecting the product to chromatography on silica gel, 9-(3′-benzamido-2′,4′-di-*O*-benzoyl-3′-deoxy-3′-*C*-ethoxycarbonyl-β-D-ribopyranosyl)-6-benzamidopurine (XXI) was obtained in a 16.6% yield.

The structure of XXI was indicated by elemental analysis, ultraviolet spectrum, negative rotation and the coupling constant ($J_{1',2'}$=9.0 Hz) in NMR spectrum. The coupling constant ($J_{4',5'}$=9.0 Hz) and the absence of a long range coupling between H-2 and H-4 protons showed that XXI may exist in a Cl conformation. The conversion of conformation between XIX (or XX) and XXI may be due to the change of bulkiness of the aglycones.

Hydrolysis of XXI with barium hydroxide in aqueous methanol followed by purification through an ion-exchange column gave a crystalline nucleoside, 9-(3′-amino-3′-*C*-carboxy-3′-deoxy-β-D-ribopyranosyl)adenine (II) in a 32.7% yield.

Experimental

Thin layer chromatography (TLC) was conducted by the use of silica gel (Daiichi Pure Chemicals Co., Inc.). The prepared plates were activated at 110°C. The spray reagents used were 10% sulfuric acid and 0.5% pyridine solution of ninhydrin. NMR spectra were taken with a Varian A-60D spectrometer at the frequency of 60 MHz in deuteriochloroform and deuteriodimethyl sulfoxide (DMSO-d_6) with tetramethylsilane as an internal standard. Melting point determinations for the samples showing higher melting points than 200°C were carried out on a micro hot stage. Unless noted otherwise, all concentrations were carried out in a rotary evaporator at reduced pressure under 40°C.

5-*O*-Benzoyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentos-3-ulose (VII). To a solution of 5-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-xylofuranose (VI)[5] (10.5 g, 0.0357 mol) in dimethyl sulfoxide (10.5 m*l*) and dry benzene (100 m*l*) containing pyridine (1 m*l*) and trifluoroacetic acid (0.5 m*l*), dicyclohexylcarbodiimide (10.5 g, 0.0509 mol) was added under cooling, and the solution was stirred for 18 hr at room temperature. Ether (100 m*l*) was then added and insoluble matter was filtered off. The organic layer was washed with water (20 m*l*×5) and dried over sodium sulfate. Removal of the solvent yielded a brown syrup containing crystals of dicyclohexylurea. Crystallization from boiling ether afforded a crystalline 5-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentos-3-ulose (VII); yield 6.44 g (61.8%), mp 96—97°C, $[\alpha]_D^{23}$ +135° (*c* 3.2, chloroform) [lit,[11] mp 98—99°C, $[\alpha]_D$ +136° (*c* 1, chloroform)].

5-*O*-Benzoyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentos-3-ulose Imine (VIII). A solution of potassium cyanide (100 mg) and ammonium carbonate (350 mg) in water (1 m*l*) was added to a solution of VII (200 mg) in ethanol (1 m*l*) and the mixture was stirred at 50°C for one hour. Evaporation of ethanol from the reaction mixture afforded VIII as needles; yield 120 mg (60.3%). An analytical sample was obtained by recrystallization from diisopropyl ether; mp 162°C, $[\alpha]_D^{20}$ +36° (*c* 0.8, ethanol), ν_{max}^{Nujol} 3400, 1735, 1697 (C=N) cm^{-1}; τ^{CDCl_3} 4.03 (d, C-1H, $J_{1,2}$=3.4 Hz), 8.43, 8.65 [s,s, >$C(CH_3)_2$].

Found: C, 61.54; H, 5.67; N, 5.18%. Calcd for $C_{15}H_{17}NO_5$: C, 61.85; H, 5.88; N, 4.81%.

5-*O*-Benzoyl-3-deoxy-1,2-*O*-isopropylidene-3-*C*-(spiro-5′-hydantoin)-α-D-pentofuranose (IX). A suspension of VII (5.00 g), potassium cyanide (4.45 g) and ammonium carbonate (8.25 g) in methanol (40 m*l*) was stirred under an initial carbon dioxide pressure of 50 kg/cm^2 for 5 hr at room temperature and for additional 5 hr at 55—60°C in an autoclave. The content was evaporated to remove methanol and water (30 m*l*) was added to the residue. The mixture was acidified (pH 1—2) with 3N hydrochloric acid (24 m*l*) and extracted with four 40-m*l* portions of ethyl acetate. Evaporation of the ethyl acetate layer afforded a yellow syrup (5.77 g). TLC (diisopropyl ether) of the syrup showed a main spot (R_f 0.22). Chromatography on silica gel (80 g) in diisopropyl ether-butanone (10 : 1) gave fractions containing a total yield of 2.41 g of crude IX; R_f 0.22. Recrystallization from hot ethyl acetate (15 m*l*) plus diisopropyl ether (15 m*l*) gave the pure hydantoin derivative IX (1.64 g) melting at 195—196°C. From the mother liquor a second crop (0.39 g) melting at 194—195°C was obtained: total yield 32.7%, $[\alpha]_D^{20}$ +34° (*c* 1.02, ethanol), ν_{max}^{KBr} 3345, 3275, 1787, 1739, and 1695 cm^{-1}: τ^{CDCl_3} 0.91 [s, N(3)-H], 3.57 [s, N(1)-H], 3.96 (d, C-1H, $J_{1,2}$=3.7 Hz), 8.44, and 8.64 [s,s >$C(CH_3)_2$].

Found: C, 56.07; H, 5.38; N, 7.47%. Calcd for $C_{17}H_{18}N_2O_7$: C, 56.35; H, 5.01; N, 7.73%.

12) T. Sato, T. Shimadachi and Y. Ishido, *Nippon Kagaku Zasshi*, **81**, 1440 (1960).

Other fractions of R_f 0.49 were collected and evaporated. The residue was crystallized from diisopropyl ether to afford imine (VIII); yield 1.02 g (13.4%), mp 162°C.

3-Acetamido-3-*C*-carboxy-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose-γ-lactone (XV). A mixture of the hydantoin derivative (IX) (362 mg), barium hydroxide octahydrate (3.15 g) and water (24 ml) was stirred at 125°C for 12 hr. The reaction mixture was heated with ammonium carbonate (1.92 g) for 10 min at 110°C and followed by additional heating for 20 min with 1.23 g of ammonium carbonate. The resulting mixture was filtered to remove barium carbonate and the filtrate was washed with ether. The aqueous layer was evaporated to afford a colorless powder of crude 3-amino-3-*C*-carboxy-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (XIV). A suspension of the crude XIV (430 mg) in methanol (5 ml) was treated with acetic anhydride (1 ml) for 3 hr at room temperature, after which the reaction mixture was evaporated. The residue was purified through a silica gel (10 g) column. Elution with ethyl acetate-benzene (1 : 1) afforded compound XV as prisms; yield 126 mg (50%). Recrystallization from acetone-diisopropyl ether; mp 147—148°C, $[\alpha]_D^{12.5}$ −59° (*c* 1.09, ethanol), ν_{max}^{KBr} 3265, 1780 (γ-lactone), 1657 and 1530 cm^{-1}; τ^{CDCl_3} 3.38 [s, N(1)–H], 4.00 (d, C–1H, $J_{1,2}$=4.0 Hz), 7.96 (s, CO–CH_3), 8.43 and 8.59 [s,s, >$CH(CH_3)_2$].

Found: C, 51.50; H, 5.79; N, 5.38%. Calcd for $C_{11}H_{15}NO_6$: C, 51.36; H, 5.88; N, 5.45%.

1,2-Di-*O*-acetyl-5-*O*-benzoyl-3-deoxy-3-*C*-(spiro-5′-hydantoin)-D-pentofuranose (X). To an ice-cooled solution of the hydantoin derivative (IX) (1.0 g) in acetic acid (7.5 ml) and acetic anhydride (7.5 ml) was added concentrated sulfuric acid (0.8 ml) dropwise and the solution was kept at room temperature for 22 hr. The reaction mixture was shaken with a mixture of 7% aqueous sodium acetate solution (40 ml), ice (10 g) and chloroform (20 ml) in a separating funnel. The aqueous layer was further extracted with three 10-ml portions of chloroform. The combined chloroform layers were dried over sodium sulfate and evaporated to give crude X (1.04 g, 92.7%) as a glassy solid, whose TLC (diisopropyl ether-butanone 3 : 1) showed a single spot having R_f-value of 0.43. A sample (490 mg) of the crude X was crystallized from hot diisopropyl ether-acetone to afford α-anomer of X (101 mg) as needles; mp 137—139°C; $[\alpha]_D^{20}$ +20° (*c* 0.88, ethanol); ν_{max}^{KBr} 3220, 1786, and 1725 cm^{-1}; τ^{CDCl_3} 3.39 (d, C–1H, $J_{1,2}$=4.1 Hz), 3.86 [s, N(1)–H], 4.71 (d, C-2H), 7.83 and 7.89 (s,s, $OCOCH_3$).

Found: C, 53.35; H, 4.63; N, 6.79%. Calcd for $C_{18}H_{18}N_2O_9$: C, 53.20; H, 4.47; N, 6.89%.

The NMR spectrum of the crude X showed that X existed mainly as an α-anomer.

2-*O*-Acetyl-5-*O*-benzoyl-1-chloro-1,3-dideoxy-3-*C*-(spiro-5′-hydantoin)-D-pentofuranose (XI). A mixture of X (500 mg), dry ether (19 ml) and acetyl chloride (0.3 ml) was saturated with dry hydrogen chloride under ice-salt cooling and allowed to stand for 38 hr in a refrigerator. The reaction mixture was evaporated at 30°C and the residual syrup was then dissolved in a small amount of absolute ether and again evaporated. This procedure was repeated three times to afford a syrup which was shown to consist of XI (R_f 0.40) by TLC using diisopropyl ether-butanone (3 : 1). The NMR signals for XI were observed at τ^{CDCl_3} 1.40 [s, N(3)–H], 3.39 (d, C–1H, $J_{1,2}$=4.2 Hz), 3.60 [s, N(1)–H], 4.72 (d, C–2H), and 7.84 (s, $OCOCH_3$).

6-Benzamido-9-[2′-*O*-acetyl-5′-*O*-benzoyl-3′-deoxy-3′-*C*-(spiro-5′-hydantoin)-β-D-pentofuranosyl]adenine (XII). A solution of XI (from 955 mg of X) in dry acetonitrile (35 ml) was stirred with chloromercuri-6-benzamidopurine (1.12 g) at 100—105°C for 2.5 hr. The reaction mixture was filtered while hot and insoluble matter was washed with acetonitrile (3×7 ml). The filtrate and washings were evaporated and the residue was chromatographed on silica gel (25 g). Elution with ethyl acetate-isopropyl alcohol (20 : 1) afforded the condensation product XII as a colorless powder, yield 442 mg (28.6%); $[\alpha]_D^{12.5}$ −32° (*c* 0.9, ethanol); λ_{max}^{MeOH} 230 and 280 mμ; ν_{max}^{KBr} 3280, and 1727 cm^{-1}.

9-[3′-Deoxy-3′-*C*-(spiro-5′-hydantoin)-β-D-pentofuranosyl]-adenine (XIII). To a solution of XII (2.22 g) in methanol (20 ml) was added 0.22N aqueous barium hydroxide solution (52 ml) and the solution was stirred for 24 hr at room temperature. Carbon dioxide gas was passed through the reaction mixture. The precipitate was filtered off and the filtrate evaporated. The residual colorless powder (2.16 g) was crystallized from water to yield crude XIII as pale-brown crytals; yield 472 mg (37%). A sample (300 mg) of the product was dissolved in hot water (10 ml). After removal of a small amount of insoluble matter by filtration, the filtrate was purified through an Amberlite IR-45 (OH^- type) column (1×18 cm). The fractions having an absorption peak at 260 mμ of UV-light were collected and concentrated. The resulting product (145 mg) was recrystallized from hot water to afford an analytical sample of XIII as colorless fine needles; mp 315—320°C (colored at 295°C), $[\alpha]_D^{25}$ −36° (*c* 0.77, dimethyl sufoxide), ν_{max}^{KBr} 1770, and 1725 cm^{-1}, $\lambda_{max}^{H_2O}$ 258.5 mμ (ε 20700), $\lambda_{max}^{0.1N\ HCl}$ 257 mμ (ε 20700) and $\lambda_{max}^{0.1N\ NaOH}$ 259.5 mμ (ε 19900); τ^{DMSO} 4.00 (d, C–1′H, $J_{1',2'}$=7.3 Hz), and 5.07 (d, C–2′H).

Found: C, 42.88; H, 4.54; N, 28.98%. Calcd for $C_{12}H_{13}N_7O_5$; C, 42.98; H, 3.91; N, 29.25%.

9-(3′-Amino-3′-*C*-carboxy-3′-deoxy-β-D-ribofuranosyl)-adenine (I). A mixture of XII (442 mg), methanol (5 ml), barium hydroxide octahydrate (1.24 g) and water (20 ml) was refluxed under stirring for 6.5 hr, after which ammonium carbonate (2.3 g) was added in portions and the mixture was heated for 30 min at 120°C. Barium carbonate was removed by filtration and washed with hot water. The filtrate and washings were collected and evaporated. The residual powder was purified through an Amberlite CG-50 column (pyridine type 1.2×20 cm) using buffer solutions, acetic acid-pyridine-water (2 : 3 : 1000 and 1 : 5 : 500). Fractions containing the product were pooled and concentrated. The residue (55 mg) was recrystallized from water to give an analytically pure sample of IV; mp 202—205°C (decomp.), $[\alpha]_D^{12.5}$ −30° (*c* 0.4, water), ν_{max}^{KBr} 3400, 3140, 1642, and 1600 cm^{-1}, $\lambda_{max}^{H_2O}$ 259 mμ (ε 14900), $\lambda_{max}^{0.1N\ HCl}$ 257 mμ (ε 14800), $\lambda_{max}^{0.1N\ NaOH}$ 260 mμ (ε 15600).

Found:[13] C, 42.71; H, 4.65; N, 27.34%. Calcd for $C_{11}H_{14}N_6O_5$: C, 42.58; H, 4.55; N, 27.09%.

Methyl 3-Deoxy-3-*C*-(spiro-5′-hydantoin)-β-D-pen-

13) The sample was dried over phosphorus pentoxide at 150°C/3 mmHg.

topyranoside (XVI). A suspension of the hydantoin derivative (IX) (640 mg) in 1N methanolic hydrogen chloride (25 ml) was refluxed for 35 hr, after which the reaction mixture was neutralized with basic lead carbonate and filtered. The filtrate was evaporated and the residue (510 mg) was recrystallized from methanol-ethyl acetate to afford XVI as colorless fine crystals; yield 130 mg(31%). An analytically pure sample was obtained by recrystallization from the same solvent as mentioned above; mp 288°C (decomp.), $[\alpha]_D^{12.5}$ −34° (*c* 2.48, water), ν_{max}^{KBr} 1755 (sh.), 1730, and 1717 cm^{-1}, τ^{D_2O} 5.56 (d, C-1H or C-2H, $J_{1,2}$=8.5 Hz), 6.29 (d, C-1H or C-2H) and 6.41 (s, OCH_3).

Found: C, 41.43; H, 5.28; N, 12.08%. Calcd for $C_8H_{12}N_2O_6$: C, 41.38; H, 5.21; N, 12.07%.

Methyl 3-Amino-3-*C*-carboxy-3-deoxy-β-D-ribopyranoside (XVII). A mixture of XVI (130 mg), barium hydroxide octahydrate (707 mg) and water (5 ml) was heated at 65—70°C for 20 hr, after which carbon dioxide gas was passed through the resulting mixture. The precipitate was removed by filtration and the filtrate evaporated. The residue (125 mg) was recrystallized from aqueous acetone to yield XVII as needles; yield 34 mg (29.4%), mp 250°C (decomp.), $[\alpha]_D^{15}$ −43° (*c* 1.0, water), R_f 0.13 (paper chromatography with *n*-butanol-acetic acid-water 4 : 1 : 1).

Methyl 3-Amino-3-deoxy-3-*C*-ethoxycarbonyl-β-D-ribopyranoside (XVIII). As reported in a previous paper,[1] periodate oxidation of methyl β-D-xylopyranoside followed by cyclization with ethyl nitroacetate and successive hydrogenation afforded a diastereomeric mixture of methyl (−)-3-amino-3-deoxy-3-*C*-ethoxycarbonylpentopyranoside, from which, an isomer (XVIII) of a R_f-value 0.73 on TLC could be obtained by column chromatography using ethyl acetate-ethanol (6 : 1). The amino acid which was obtained from XVIII by hydrolysis could also be described. Its partial structure could be described but a definite assignment of the configuration of the amino and carboxyl groups at C-3 could not be made. This amino acid[14] was now found to be identical with the above-mentioned amino acid XVII obtained from XVI by comparison of their melting points, infrared spectra and optical rotations.

Methyl 3-Benzamido-2,4-di-*O*-benzoyl-3-deoxy-3-ethoxycarbonyl-β-D-ribopyranoside (XIX). To a solution of XVIII (3.45 g) in dry pyridine (94 ml) was added benzoyl chloride (8.7 ml) under ice-cooling and kept for 18 hr at room temperature. The reaction mixture was poured into ice-water and extracted with four 50-ml portions of chloroform. The organic layer was washed with saturated aqueous potassium hydrogen sulfate and potassium hydrogencarbonate and evaporated to give a brown syrup. Purification through a silica gel column (200 g, 3.8×40 cm), eluting with *n*-hexane-butanone (5 : 1, 4 : 1 and 3 : 1) gave XIX (3.76 g, 46.4%) as a yellow syrup, which was crystallized by treatment with diisopropyl ether. Recrystallization from diisopropyl ether afforded an analytical sample; mp 135—136°C, $[\alpha]_D^{21}$ −64.3° (*c* 1.32, chloroform), ν_{max}^{KBr} 3415, 1745, 1724, 1673 cm^{-1}; τ^{CDCl_3} 2.98 (s, N-H), 3.72 (m, C-4H), 4.40 (d,d, C-2H, $J_{1,2}$=1.6 Hz, $J_{2,4}$=1.5 Hz), 5.00 (d, C-1H), 5.39 (d,d, C-5H, $J_{4,5}$=2.0 Hz, $J_{5a,5e}$=12.9 Hz), 5.61 (q, ester CH_2, J=7.3 Hz), 5.90 (d,d, C-5H, $J_{4,5}$=1.7 Hz), 6.56 (s, OCH_3) and 8.66 (t, ester CH_3).

Found: C, 66.06; H, 5.37; N, 2.80%. Calcd for $C_{30}H_{29}NO_9$: C, 65.80; H, 5.39; N, 2.56%.

1-*O*-Acetyl-3-benzamido-2,4-di-*O*-benzoyl-3-deoxy-3-*C*-ethoxycarbonyl-β-D-ribopyranose (XX). A mixture of XIX (500 mg), acetic acid (11 ml), acetic anhydride (11 ml) and concentrated sulfuric acid (0.7 ml) was stirred until the solid was dissolved completely. After standing for 2 days at room temperature, the resulting solution was treated with sodium acetate (3 g) under stirring for 30 min and evaporated. To the residue was added water (15 ml) and ether (15 ml) and the mixture was shaken. The separated ether-layer was washed with a 10% sodium hydrogencarbonate solution, dried over sodium sulfate and evaporated. The residual yellow syrup (585 mg), which was a mixture of two products of R_f-value 0.62 and 0.29 on TLC (*n*-hexane-butanone 3 : 1), was chromatographed on silica gel (20 g). Elution with the same solvent system gave two kinds of pale-yellow syrups; A 168 mg (R_f (0.62) and B 261 mg (R_f 0.29). Treatment of B with diisopropyl ether afforded XX as colorless needles; yield 186 mg (35.4%), mp 155—157°C, $[\alpha]_D^{21}$ −59° (*c* 1.16, chloroform), τ^{CDCl_3} 2.96 (s, N-H), 3.63 (d, C-1H, $J_{1,2}$=2.0 Hz), 3.72 (m, C-4H), 4.38 (d,d, C-2H, $J_{2,4}$=1.2 Hz), 5.25 (d,d, C-5H, $J_{4,5}$=2.0 Hz, $J_{5a,5e}$=13.1 Hz), 5.60 (q, ester CH_2, J=7.3 Hz), 5.81 (d,d, C-5H, $J_{4,5}$= 1.6 Hz), 7.85 (s, $COCH_3$) and 8.61 (t, ester CH_3).

Found: C, 65.12; H, 5.42; N, 2.62%. Calcd for $C_{31}H_{29}NO_{10}$: C, 64.69; H, 5.08; N, 2.43%.

An additional amount of XX (73 mg) was obtained from the syrup A by treatment with acetic anhydride, acetic acid and sulfuric acid as described above; total yield 49.2%.

6-Benzamido-9-(3′-benzamido-2′,4′-di-*O*-benzoyl-3′-deoxy-3′-*C*-ethoxycarbonyl-β-D-ribopyranosyl)-purine (XXI). A mixture of XX (362 mg) and 6-benzamidopurine (181 mg) was heated to cause it to melt in an oil bath (*ca.* 155°C). *p*-Toluenesulfonic acid (15 mg) was added to the melt and the mixture was heated for 30 min under reduced pressure (5 mmHg). After cooling, the solid mass was pulverized and extracted with ethanol. Evaporation of ethanol gave a glassy product (483 mg), which was then purified through a silica gel column (15 g, 1.2×29.5 cm) with chloroform-ethyl acetate mixtures (10 : 1, 8 : 1 and 3 : 1) to afford XXI as a colorless powder; yield 79 mg (16.6%). Since the product contained a small amount of insoluble matter in ethanol, the product was again treated with ethanol, filtered and the filtrate was concentrated to afford an analytically pure sample; mp 145—148°C, $[\alpha]_D^8$ −103°(*c* 0.46, ethanol), λ_{max}^{MeOH} 279 mμ (ε 20700), 230 mμ (ε 41300), τ^{CDCl_3} 2.86 (d, C-1′H or C-2′H, $J_{1',2'}$=9.1 Hz), 3.38 (d, C-1′H or C-2′H), 3.81 (d,d, C-4′H, $J_{4',5'_e}$=6.0 Hz, $J_{4',5'_a}$=9.0 Hz), 5.62 (q, ester CH_2, J=7.2 Hz), and 8.70 (t, ester CH_3).

Found: C, 64.55; H, 4.87; N, 10.38%. Calcd for $C_{41}H_{34}N_6O_9 \cdot C_2H_5OH$: C, 64.57; H, 4.91; N, 10.51%.

9-(3′-Amino-3′-*C*-carboxy-3′-deoxy-β-D-ribopyranosyl)adenine (II). To a solution of XXI (400 mg) in methanol (2.5 ml) was added 0.36N barium hydroxide solution (8.2 ml) and the mixture was kept for 2 days at 37°C. After evaporation of methanol, the residue was diluted with water and carbon dioxide gas was

14) Compound VIA-1 in Table 2 in the previous paper (Ref. 1).

passed through the solution for 30 min. Barium carbonnate was filtered off and the filtrate was washed with four 10-m*l* portions of ether. The aqueous layer was evaporated to afford a colorless powder (558 mg), which was purified through an Amberlite CG-50 pyridine type column (1×15 cm) by using buffer solutions, pyridine-acetic acid-water (1.5 : 1.0 : 1000 and 10 : 2.0 : 1000). Fractions containing the nucleoside was concentrated and the residue (249 mg) was recrystallized from water to yield colorless prisms of II (53 mg, 32.7%); mp 247°C (decomp.). $[\alpha]_D^{18}$ −20° (*c* 0.22, 0.1N HCl), $\lambda_{max}^{H_2O}$ 260 mμ (ε 15700), $\lambda_{max}^{0.1N\ HCl}$ 257 mμ (ε 14400) and $\lambda_{max}^{0.1N\ NaOH}$ 261 mμ (ε 13400).

Found:[13] C, 42.21; H, 4.75; N, 26.74%. Calcd for $C_{11}H_{14}N_6O_5$: C, 42.58; H, 4.55; N, 27.09%.