Studies on Antibiotics and Related Substances. XX. The Synthesis of Adenine Nucleosides of 3-Amino-3-deoxyglucose and 6-Amino-6-deoxyglucose

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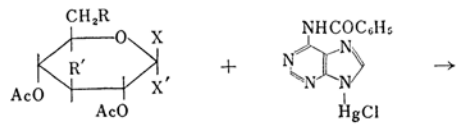
Several compounds obtained from streptomyces or fungi which contain purine or pyrimidine rings have shown an antibiotic or an antitumor property. The simplest examples are angustmycins,[1] nucleocidin,[2] cordycepin,[3] and nebularine.[4] Puromycin, an antibiotic purine derivative, belongs to the so-called aminonucleosides and exhibits carcinostatic and trypanocidal activities; its derivatives have been extensively synthesized by Baker and his coworkers.[5] Recently, 3'-amino-3'-deoxyadenosine[6] has been added to the group of aminonucleoside-antibiotics and shown to exhibit antitumor activity. We have also been interested in the aminonucleosides and have instituted a program aiming at the synthesis of nucleosides of rare aminosugars found in antibiotics. In this paper the syntheses of adenine nucleosides from chloromercuri-6-benzamidopurine and acetohalogeno derivatives of 3-amino-3-deoxy-D-glucose and 6-amino-6-deoxy-D-glucose, uncommon amino-sugars found as moieties of kanamycin, will be reported.

The required halogenoses, α-1-bromo- and β-1-chloro-derivatives of 2, 4, 6-tri-*O*-acetyl-3-carbobenzoxyamino-1, 3-dideoxy-D-glucopyranose and 2, 3, 4-tri-*O*-acetyl-6-carbobenzoxyamino-1, 6-dideoxy-D-glucopyranose, were synthesized by the methods previously reported.[7]



Ia: R=NHCbz, R'=OAc, X=Cl, X'=H
Ib: R=NHCbz, R'=OAc, X=H, X'=Br
VIa: R=OAc, R'=NHCbz, X=Cl, X'=H
VIb: R=OAc, R'=NHCbz, X=H, X'=Br

II

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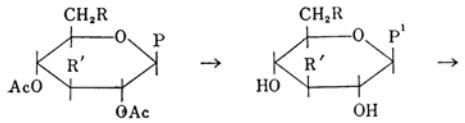
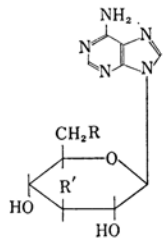
TABLE I. AMINONUCLEOSIDES AND THEIR BLOCKED DERIVATIVES

Aminonucleoside and derivative	Starting anomer of acetohalogenose	Yield* %	UV Absorption λ_{max}, mμ	$[\alpha]_D$**
III	Ia	84 (60)	280 (ε 23100) m	−6.6 (*c* 1.21) c
III	Ib	44	280 (ε 22500) m	−2.6 (*c* 1.35) c
IV	Ia	(55)	260 (ε 21500) m	+8.0 (*c* 1.33) m
IV	Ib	(81)	260	−0.8 (*c* 1.23) m
V	Ia	43	259 (ε 13300) w	+5.0 (*c* 1.40) w
V	Ib	48	259 (ε 13700) w	+3.9 (*c* 1.27) w
VII	VIa	53	280 (ε 20100) m	−7.9 (*c* 1.27) c
VII	VIb	37	280 (ε 21700) m	−14.1 (*c* 1.32) c
VIII	VIa	(67)	260 (ε 30600) mc	—
VIII	VIb	(38)	260 mc	—
IX	VIa	76	259 (ε 13600) w	+5.8 (*c* 1.56) w
IX	VIb	77	259 (ε 13700) w	+3.5 (*c* 1.43) w

* Figures in parentheses indicate yields of recrystallized products.
** Determined at room temperature. Exact value of specific rotation of VIII could not be c: Determined in chloroform., m: methanol, mc: methyl cellosolve, w: water



III: R=NHCbz, R′=OAc
VII: R=OAc, R′=NHCbz
IV: R=NHCbz, R′=OH
VIII: R=OH, R′=NHCbz
P=6-benzamidopurinyl-9
P¹=adenyl-9



V: R=NH₂, R′=OH
IX: R=OH, R′=NH₂

Chart A

The condensation of 2, 3, 4-tri-*O*-acetyl-6-carbobenzoxyamino-β-1-chloro-1, 6-dideoxy-glucopyranose (Ia) with chloromercuri-6-benzamidopurine gave 6-benzamido-9-(2′, 3′, 4′-tri-*O*-acetyl-6′-carbobenzoxyamino-6′-deoxy-β-D-glucopyranosyl)-purine (III in Chart A). The hydrolysis of III with methanolic ammonia gave 9-(6′-carbobenzoxyamino-6′-deoxy-β-D-glucopyranosyl)-adenine (IV). Finally, the hydrogenolysis of the carbobenzoxy derivative with a palladium catalyst gave 9-(6′-amino-6′-deoxy-β-D-glucopyranosyl)-adenine (V). The structure of V was indicated by its elemental analyses, its ultraviolet spectrum, which was similary to that of a 9-substituted adenine, and its slightly negative rotation. The β-configuration of the aminonucleoside was deduced from Tipson's "trans rule"[8] as extended by Baker and his coworkers.[9] The values of the specific rotation of the aminonucleoside was consistent with the theoretical presumption and the reported values[10] for 9-β-D-glucopyranosyladenine. Hydrolysis with weak acid gave only adenine, 6-amino-6-deoxy-D-glucose and the unchanged nucleoside.

When 2, 4, 6-tri-*O*-acetyl-3-carbobenzoxyamino-β-1-chloro-1, 3-dideoxy-D-glucose (VIa) was used instead of the blocked acetohalogeno 6-aminoglucose (Ia), 6-benzamido-9-(2, 4, 6-tri-*O*-acetyl-3-carbobenzoxyamino-3-deoxy-β-D-glucopyranosyl)-purine (VII) was obtained. This was converted to the desired nucleoside (IX) by deacetylation and hydrogenolysis.

Applications of exactly identical series of reactions to α-bromo-anomers of 2, 3, 4-tri-*O*-acetyl-6-carbobenzoxyamino-1, 6-dideoxy-D-glucopyranose and 2, 4, 6-tri-*O*-acetyl-3-carbobenzoxyamino-1, 3-dideoxy-D-glucopyranose furnished 9-(6-amino-6-deoxy-β-D-glucopyranosyl)-adenine and 9-(3-amino-3-deoxy-D-glucopyranosyl)-adenine respectively; they were identical

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, *Advances in Carbohydrate Chem.*, **14**, 283 (1959); J. A. Montgomery and H. J. Thomas, ibid., **17**, 315 (1962).

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

10) E. Fischer and B. Helferich, *Ber.*, **47**, 229 (1914); J. Davoll, B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 1946, 837. These workers reported $[\alpha]_D^{19}$ −10° (*c* 3.2, water) and $[\alpha]_D^{14}$ −9.7° (*c* 0.18, water) respectively.

FROM ANOMERS OF ACETOHALOGENOSES Ia AND Ib, VIa AND VIb

Formula	Analysis, %					
	Found			Calcd.		
	C	H	N	C	H	N
$C_{32}H_{32}N_6O_{11}$	58.46	4.92	12.29	58.15	4.89	12.70
$C_{19}H_{22}N_6O_6$	53.09	5.11	19.44	53.00	5.15	19.54
$C_{11}H_{16}N_6O_4$	43.98	5.47	28.45	44.56	5.44	28.38
$C_{32}H_{32}N_6O_{11}\cdot H_2O$	56.48	4.80	12.07	56.50	4.97	12.07
$C_{19}H_{22}N_6O_6$	53.00	5.15	19.47	53.24	5.29	19.54
$C_{11}H_{16}N_6O_4$	43.84	5.30	27.80	44.56	5.44	28.38

determined because this derivative showed exceptionary low solubility in ordinary solvents tested.

with the above-mentioned β-anomers of aminonucleosides V and IX. Thus, it has been shown that the α- and β-anomers of blocked acetohalogeno aminosugars (I, VI) both yield the same β-nucleosides (V, IX), a fact which can be interpreted adequately in terms of the extension of the "trans rule" made by Baker and his co-workers.[8]

The biological test of these compounds may be summarized as follows: compound IX possessed a weak anti-HeLa cell activity, but compound V was inactive in the antitumor tests. Both the compounds had a low toxicity.

Experimental

The data of yields, of ultraviolet absorption, and of specific rotation and the analyses of title compounds are summarized in Table I.

6-Benzamido-9-(2, 3, 4-tri-*O*-acetyl-6-carbobenzoxyamino-6-deoxy-β-D-glucopyranosyl)-purine (III). —The method used here was virtually analogous to that described by Baker and his co-workers.[11] A mixture of Hyflo Super Cel (diatomaceous earth) (2.56 g.), the chloromercuric salt (II) (2.16 g.) of 6-benzamidopurine,[12] and toluene (265 ml.) was stirred and heated, while about 20 ml. of toluene was distilled off to remove azeotropically any moisture present. After the mixture had then been refluxed for 30 min. and cooled to approximately 60°C, 2,3,4-tri-*O*-acetyl-6-carbobenzoxyamino-β-1-chloro-1,6-dideoxy-D-glucopyranose (Ia) (2.26 g.) was added. The mixture was stirred and refluxed for 10 hr. and then filtered hot. The filter-cake was thoroughly washed with hot ethanol. The filtrate and washings were combined and evaporated to dryness in vacuo. The residue was dissolved in chloroform, and the solution was shaken with a 30% aqueous potassium iodide solution. The chloroform layer was separated, dried over anhydrous sodium sulfate, and evaporated to dryness in vacuo, leaving a dark gum (2.8 g.).

The crude product was purified by chromatography on alumina. To a solution of the crude product (2.8 g.) in methanol, alumina (5 g., aluminum oxide, Merck) was added, and then the mixture was evaporated to dryness. The residue was placed on a column (1.0×30 cm.) of alumina (34 g.) and developed with cyclohexane-ethyl acetate (1 : 1); eighty-seven fractions were cut, containing 7 ml. each. The ultraviolet absorption spectra of the residue obtained from tube Nos. 5—50 showed a characteristic absorption at 280 mμ. The combined residues (2.49 g.) from the fractions were dissolved in a small quantity of methanol and filtered, and water was added to turbidity. After it had stood overnight, the solution deposited colorless crystals of the title compound (1.81 g.). The crystals had no definite melting point; a sample sintered from 96°C and became an opaque liquid. This compound was a 9-glucosyl derivative of adenine, as is shown by its ultraviolet absorption spectrum (Table I). In the infrared region, this compound in KBr showed absorptions at 3325 (NH), 1759 (C=O in OAc), 1718 (νC=C in Cbzo), 1608, 1589 (C=C), (C=N), 1219 (νC-O in OAc), 895 (shoulder, type 2b of pyranose ring), and 775, 739, 699 (phenyl) cm^{-1}.

The product was soluble in methanol, ethanol, benzene, dioxane or ethyl acetate, but practically insoluble in ether, ligroin, hexane, cyclohexane or water.

The same compound, III, was obtained by the condensation of the α-bromo-anomer (Ib) of 2,3,4-tri-*O*-acetyl-6-carbobenzoxy-amino-1,6-dideoxy-D-glucopyranose with 6-benzamidopurine (II). The product showed an infrared spectrum identical with that of the specimen prepared from Ia and 6-benzamidopurine. The yield, the ultraviolet absorption, and the specific rotation are shown in Table I

11) B. R. Baker, J. P. Joseph and R. E. Schaub, *J. Org. Chem.*, **19**, 1780 (1954).
12) J. Davoll and B. A. Lowy, *J. Am. Chem. Soc.*, **73**, 1650 (1951).

comparison with those of a sample of III obtained from Ia.

9-(6-Carbobenzoxyamino-6-deoxy-β-D-glucopyranosyl)-adenine (IV). — A solution of III (3.1 g.) in absolute methanol (190 ml.) was saturated with ammonia at 0°C, and then the solution was allowed to stand in a refrigerator overnight. The solution was evaporated to dryness in vacuo, and the residue was washed with water in portions to give a slightly brown solid (1.5 g.). Recrystallization from *n*-propanol gave colorless crystals (0.79 g.) of the title compound. The product sintered at about 127—128°C and decomposed at 180°C with the evolution of gas. The ultraviolet spectrum of the product in methanol showed characteristic absorptions (Table I.).

The compound was soluble in methanol or ethanol but almost insoluble in benzene, petroleum ether or water.

9-(6-Amino-6-deoxy-β-D-glucopyranosyl)-adenine (V).—A solution of IV (1.5 g.) in 25% aqueous methanol (40 ml.) was shaken with 5% Pd-C (1.0 g.) and hydrogen at room temperature for 14 hr. After filtration, the filtrate was evaporated up to dryness under reduced pressure to give a colorless powder (0.825 g.). To a solution of the crude product (0.2 g.) in water, a solution of picric acid (1.45 g.) in methanol (14.5 ml.) was added; this mixture was then allowed to stand in a refrigerator to give a picrate (0.447 g.). Recrystallization from water gave yellow needles (0.226 g.). The picrate sintered at 168°C, gradually darkened, and decomposed at 215°C.

Found: C, 36.90; H, 3.27; N, 21.57. Calcd. for $C_{23}H_{22}N_{12}O_{18}$ (754.4): C, 36.59; H, 2.94; N, 22.29%.

The picrate (0.226 g.) was dissolved in water (8.2 ml.) and stirred with Dowex 1X2 ion exchange resin (OH form) (200—400 mesh, 10 ml.); the filtrate was passed through a column (1.5×30 cm.) of Dowex 1X2 (OH form). The column was then developed with water, and those fractions which showed characteristic ultraviolet absorptions at 259 mμ were collected. The product obtained by evaporation to dryness under reduced pressure was a colorless powder (84 mg.); the product sintered at about 205°C, darkened, and decomposed at 211°C, with the evolution of gas. The ultraviolet spectrum of the product in water showed absorptions characteristic of 9-substituted adenine.

The product showed a single ninhydrin-positive spot of R_{fad} 0.36* upon the use of the descending method using an *n*-butanol - ethanol - water (10 : 5 : 7) solvent system and Toyo filter paper No. 52. The product was soluble in water, but nearly insoluble in methanol or ethanol.

A sample V was hydrolyzed with 1% acetic acid at room temperature for 5 days.

Chromatography on Toyo filter paper No. 50 by the ascending method using an *n*-butanol - acetic acid - water (4 : 2 : 1) solvent system showed two ninhydrin-positive spots (R_f 0.12, 0.25) of 6-amino-6-deoxy-D-glucose and unchanged nucleoside, and another spot of adenine (R_f 0.57), located by its slight opacity to the ultraviolet light of 2536 Å.

* R_f-Value relative to that of adenine taken as a unit.

6-Benzamido-9-(2,4,6-tri-*O*-acetyl-3-carbobenzoxyamino-3-deoxy-β-D-glucopyranosyl)-purine (VII) was prepared from 2,4,6-tri-*O*-acetyl-3-carbobenzoxyamino-1-β-chloro-1,3-dideoxy-D-glucopyranose (VIa) and 6-benzamidopurine (II) by a procedure analogous to that described in III; a sample purified by alumina chromatography and then by recrystallization from ethanol-water sintered at about 80°C.

The same compound, VII, was obtained in a similar condensation of the α-bromo anomer (VIb) of 2,4,6-tri-*O*-acetyl-3-carbobenzoxyamino-1,3-dideoxy-D-glucopyranose with 6-benzamidopurine (II). The product showed an infrared spectrum identical with that of the specimen prepared from VIa and 6-benzamidopurine. The yield, the ultraviolet absorption and the specific rotation are shown in Table I in comparison with those of a sample of VII obtained from VIa.

9-(3-Carbobenzoxyamino-3-deoxy-β-D-glucopyrnosyl)-adenine (VIII) was prepared from VII by a procedure analogous to that described in IV; a sample recrystallized from methanolic ammonia gradually darkened and decomposed at 233°C with the evolution of gas.

9-(3-Amino-3-deoxy-β-D-glucosyl)-adenine (IX) was prepared from VIII by a procedure analogous to that described in V. Picrate of IX: yield from a crude product, 84%; it gradually darkened and decomposed at 216—220°C.

Found: N, 21.55. Calcd. for $C_{23}H_{22}N_{12}O_{18}$ (754.4): N, 22.28%.

The yield of IX from its picrate was 92%. A sample purified with ion exchange resin Dowex 1X2 was recrystallized from water-ethanol; the product sintered a 170°C and decomposed at 191°C with the evolution of gas; the ultraviolet spectrum of the product in water showed the absorption characteristic of 9-substituted adenine. In the infrared region, this compound in KBr showed absorptions at 3320, 3180 (OH, NH), 1642, 1602 (C=N, C=C), 1058, 1043 (C-O), 890 cm^{-1}. (type 2b of the pyranose ring)

The product showed a single ninhydrin-positive spot of R_{fad} 0.63 by a method similar to that used for V. The paper chromatographic analysis of the acid-hydrolyzate of a sample of IX by the same procedure as that used for V showed two ninhydrin-positive spots of 3-amino-3-deoxy-D-glucose (R_f 0.16) and unchanged nucleoside (R_f 0.41) and a spot of adenine (R_f 0.57).

Bioassays.—It is interesting that IX shows antitumor activity. The minimum necessary concentration of IX for the anti-HeLa cell effect was 1 mg./ml. Preliminary tests indicated that IX possessed a weak antibacterial activity; IX completely inhibited the growth of Bacillus subtilis in the dilution of 1 : 1000. V showed almost no antitumor and antibacterial activity. V and IX had a low toxicity; V: LD_0 500 mg./kg. (mice, i. v.), IX: $LD_0 > 500$ mg./mg./kg. (mice, i. v.).

Summary

1) The subject amino-nucleoside have been synthesized from blocked acetohalogeno aminosugars and 6-benzamidopurine.

2) It has been shown that the α- and β-anomers of blocked acetohalogeno aminosugars (Ia, Ib and VIa, VIb) both yield the same β-nucleosides (V, IX).

3) 9-(3-Amino-3-deoxy-β-D-glucopyranosyl)-adenine was found to possess a weak antitumor activity.

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