PREPARATION OF NUCLEOSIDES VIA ISOPROPYLIDENE SUGAR DERIVATIVES

PART VI. SYNTHESIS OF 9-α- AND 9-β-D-ALLOFURANOSYLADENINES*

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ABSTRACT

2,3:5,6-Di-*O*-isopropylidene-D-allono-1,4-lactone was reduced with bis(2-butyl-3-methyl)borane to 2,3:5,6-di-*O*-isopropylidene-D-allofuranose. Reaction with thionyl chloride gave the halogenose, which was condensed with 6-benzamidochloromercuripurine. Both the α- and β-anomers resulted. Several crystalline derivatives were isolated during the stepwise removal of the blocking groups to yield 9-α- and 9-β-D-allofuranosyladenine. The anomeric configurations were established by degradation of 9-(2,3-*O*-isopropylidene-β-D-allofuranosyl)adenine to 9-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)adenine, and degradation of 9-(2,3-*O*-isopropylidene-α-D-allofuranosyl)adenine to 9-α-D-ribofuranosyladenine.

INTRODUCTION

For many years there has been interest in synthesis of hexofuranosyl nucleosides because of the structural similarity of these substances to ribose nucleosides and their potential value as metabolic inhibitors possessing antiviral or antitumor activity. 9-β-D-Allofuranosyladenine (**12**), which had been synthesised previously[2], is interesting in particular because of its close resemblance to adenosine and of the fact that in cytotoxicity studies it was an inhibitor of growth of human KB cells in tube dilution; this technique measures inhibition of growth by compounds acting as feedback inhibitors, competitive inhibitors, and cell poisons[3].

When benzoyl groups were used to block the 2,3,5, and 6 positions of allofuranose in the preparation of the adenine nucleoside, only the β-anomer **12** was

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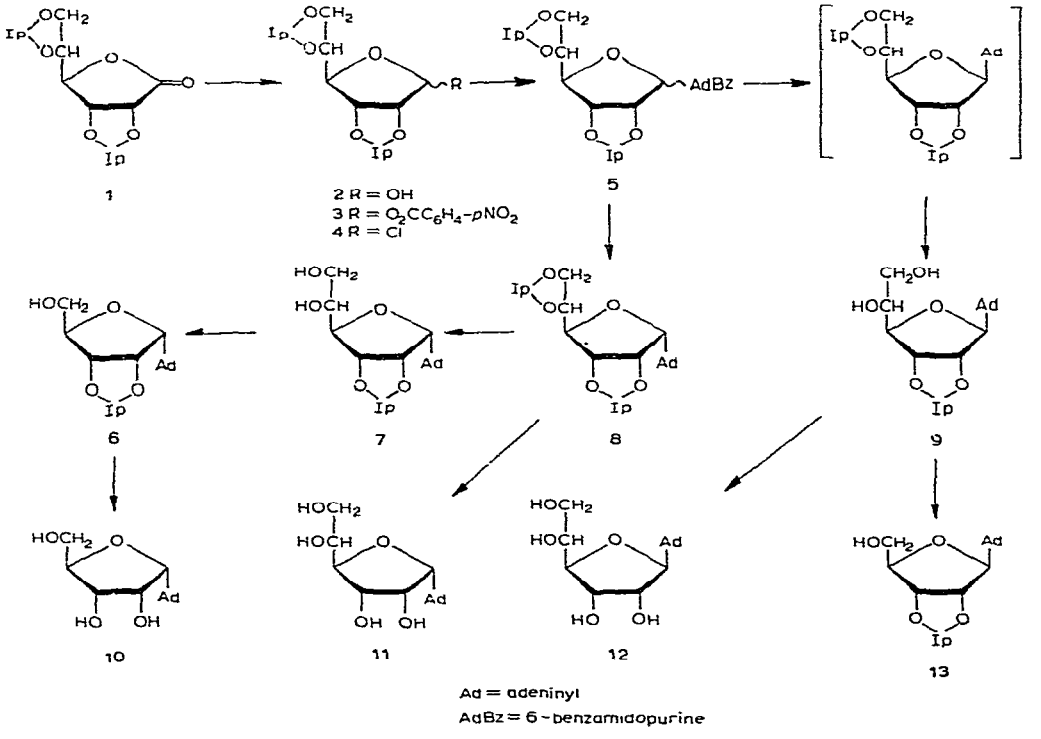
isolated[2], as was expected on the basis of the *trans* rule[4]. In previous work using isopropylidene blocking groups with other hexoses, only one anomer was isolated, although both were expected[5–7]. The work described in this communication shows that both anomers are formed when isopropylidene blocking groups are used in the preparation of 9-D-allofuranosyladenine.

DISCUSSION

The starting material was D-allono-1,4-lactone from which 2,3:5,6-di-*O*-isopropylidene-D-allono-1,4-lactone (**1**) was prepared by acetonation. Reduction of **1** with bis(2-butyl-3-methyl)borane yielded 2,3:5,6-di-*O*-isopropylidene-D-allofuranose (**2**), also characterized as the 1-*O*-*p*-nitrobenzoyl derivative **3**. From crystalline **2**, crystalline 2,3:5,6-di-*O*-isopropylidene-D-allofuranosyl chloride (**4**) was prepared with thionyl chloride in pyridine[6]. The halogenose **4** was coupled with 6-benzamidochloromercuripurine in xylene[8] to give a mixture of the α- and β-anomers of 6-benzamido-9-(2,3:5,6-di-*O*-isopropylidene-D-allofuranosyl)purine (**5**). Following removal of the *N*-benzoyl group with sodium methanolate, crystalline 9-(2,3:5,6-di-*O*-isopropylidene-α-D-allofuranosyl)adenine (**8**) was isolated in 9.8% yield (based on halogenose **4**), as well as a syrup containing 9-(2,3:5,6-di-*O*-isopropylidene-β-D-allofuranosyl)adenine. When treated with 70% aqueous acetic acid the syrup yielded 3.9% of crystalline 9-(2,3-*O*-isopropylidene-β-D-allofuranosyl)adenine (**9**). 9-(2,3-



O-Isopropylidene-α-D-allofuranosyl)adenine (**7**) was obtained in 72.8% yield by mild acid hydrolysis of **8**.

Configurations were established by degrading the anomers **7** and **9**. 9-(2,3-*O*-Isopropylidene-β-D-allofuranosyl)adenine (**9**) was cleaved with sodium periodate and the product reduced with sodium borohydride[7] to yield 9-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)adenine (**13**), which was identical with authentic isopropylideneadenosine[9]. From 9-(2,3-*O*-isopropylidene-α-D-allofuranosyl)adenine (**7**), a new compound, 9-(2,3-*O*-isopropylidene-α-D-ribofuranosyl)adenine (**6**), was similarly prepared. It was hydrolyzed with 25% aqueous acetic acid to 9-α-D-ribofuranosyladenine (**10**) which has been described by Wright *et al.*[10].

9-α-D-Allofuranosyladenine (**11**) was obtained by hydrolysis of 9-(2,3:5,6-di-*O*-isopropylidene-α-D-allofuranosyl)adenine (**8**) with 25% aqueous acetic acid. Similar hydrolysis of the crystalline 9-(2,3-*O*-isopropylidene-β-D-allofuranosyl)-adenine (**9**) yielded 9-β-D-allofuranosyladenine (**12**)[2].

It is of interest to note that the *cis*(α)-nucleoside was formed in greater yield than was the *trans*(β)-nucleoside (α:β ratio *ca.* 3:1). The importance of the 5,6-*O*-isopropylidene group in sterically inhibiting the approach of the purine nucleophile has been previously reported[5]. The preferred approach would be from the α-side, however additional opposing interaction from the 2,3-*O*-isopropylidene group has also been observed[11]. The combined effect may account for the small yield of nucleoside products obtained in comparison to the cases of D-mannose[2] and D-gulose[5].

EXPERIMENTAL

General methods. — Melting points were determined on a Kofler micro hot stage and correspond to corrected values. U.v. spectra were measured with a Perkin–Elmer Model 202 spectrograph. Optical rotations were determined in 1-dm tubes with a Perkin–Elmer Model 141 polarimeter. Thin-layer chromatography was carried out on Merk (Darmstadt) plates precoated with silica gel F-254, developed with 5% aqueous disodium hydrogen phosphate[12] (solvent A), 9:1 chloroform–methanol (solvent B), 43:7 butyl alcohol–water (solvent C), and 2:3 ethyl acetate–methanol (solvent D). Spots were located under a u.v. lamp with peak emission at 254 nm. The expression R_{Ad} refers to the ratio of the distance the nucleoside migrated to the distance which adenine migrated. Evaporations were carried out *in vacuo* at 45°. Elemental analyses were determined at Spang Microanalytical Laboratory, Ann Arbor, Michigan.

2,3:5,6-Di-O-isopropylidene-D-allono-1,4-lactone (**1**). — A suspension of D-allono-1,4-lactone (19.1 g, 0.107 mmole) in acetone (400 ml) containing conc. sulfuric acid (6.6 ml) was stirred until the lactone was dissolved, then kept for 16 h at room temperature. The solution was neutralized by vigorous stirring with granular sodium carbonate (50 g) for 8 h. The salts were removed by filtration, and the filtrate was evaporated to give a syrup to which toluene was added. A precipitate formed

almost immediately. The mixture was refrigerated overnight and filtered. The toluene filtrate was concentrated to a syrup which crystallized at room temperature. The crystals were washed with Skelly Solve B (15.6 g, 56.3% crude yield) and recrystallized from Skelly Solve B to give needles (14.5 g, 52.4% yield), m.p. 72–74°, $[\alpha]_D^{24}$ −77.8° (*c* 2, chloroform).

Anal. Calc. for $C_{12}H_{18}O_6$: C, 55.81; H, 6.97. Found: C, 55.69; H, 7.01.

2,3:5,6-Di-O-isopropylidene-D-allofuranose (**2**). — Compound **1** was reduced with bis(2-butyl-3-methyl)borane by a modification of the method previously used for such reductions[13]. To **1** (8.72 g, 33.7 mmoles) 1.25M bis(2-butyl-3-methyl)borane (75 mmoles) in tetrahydrofuran (60 ml) was added under nitrogen at room temperature. After the borane was added, the mixture was stirred until all the lactone was dissolved, then kept overnight at room temperature. Excess borane was decomposed with a few drops of water, and pyridine (10 ml) was added. The mixture was cooled in ice, stirred, and 30% hydrogen peroxide (10 ml) was added dropwise, allowing the reaction temperature to rise to 50°. The mixture was cooled in ice and filtered from precipitated salts. Anhydrous sodium sulfite was added portionwise to the stirred filtrate, until there was no further rise in temperature. The mixture was then cooled, filtered, and the filtrate was concentrated to a slurry which was extracted with chloroform. The extract was washed with water, dried, and concentrated to an oil. A small amount of Skelly Solve B was added and the mixture was refrigerated. The resulting crystalline mass was recrystallized from Skelly Solve B to yield irregular shaped crystals (7.34 g, 83.8%), m.p. 67–69°, $[\alpha]_D^{24}$ −26.6° (*c* 2, chloroform).

Anal. Calc. for $C_{12}H_{20}O_6$: C, 55.37; H, 7.69. Found: C, 55.49; H, 7.69.

2,3:5,6-Di-O-isopropylidene-1-O-p-nitrobenzoyl-D-allofuranose (**3**). — To the oil obtained from the reduction of **1** (436 mg), a solution of *p*-nitrobenzoyl chloride (558 mg) in dry pyridine (4.8 ml) was added. The reaction mixture was kept for 2 h at room temperature, and then mixed with ice–water, and extracted with chloroform. The chloroform layer was washed with M hydrochloric acid, water, 5% sodium hydrogen carbonate, and water, dried, and concentrated to a solid mass which was dissolved in a small amount of absolute ether and crystallized by adding Skelly Solve B. The product was recrystallized similarly to yield long needles (90 mg), m.p. 117.5–119°, $[\alpha]_D^{24}$ −42.6° (*c* 2, chloroform).

Anal. Calc. for $C_{19}H_{23}NO_9$: C, 55.74; H, 5.66; N, 3.42. Found: C, 55.84; H, 5.70; N, 3.42.

2,3:5,6-Di-O-isopropylidene-D-allofuranosyl chloride (**4**). — The method of preparation was essentially that used earlier for the preparation of the corresponding mannosyl derivative[6]. From 9.0 g (34.6 mmoles) of **2**, a yellow oil weighing 6.1 g was obtained. Distillation *in vacuo*, b.p. 0.1 mm 75–76°, yielded a clear, colorless oil. It crystallized spontaneously with evolution of heat when brought to room temperature, after being stored in the deep freezer (5.4 g, 55.9%), m.p. 39–42°. The compound is unstable at room temperature.

Anal. Calc. for $C_{12}H_{19}ClO_5$: C, 51.71; H, 6.87; Cl, 12.72. Found: C, 51.43; H, 6.94; Cl, 12.61.

6-Benzamido-9-(2,3:5,6-di-O-isopropylidene-D-allofuranosyl)purine (**5**). — Chloromercuri-6-benzamidopurine[8] (8.15 g, 17.2 mmoles) was coupled with 4.8 g (17.2 mmoles) of crystalline halogenose (**4**) in the presence of molecular sieve type 4A (8.15 g) and cadmium carbonate (3.73 g) in dry xylene (370 ml). The product was processed as described earlier for the corresponding mannosyl derivative[6], and yielded a syrup (7.6 g) which was suitable for the next step.

9-(2,3:5,6-Di-O-isopropylidene-α-D-allofuranosyl)adenine (**8**). — Syrup **5** (7.6 g) was dissolved in absolute methanol (33.4 ml) and M methanolic sodium methanolate (12.9 ml) was added. The mixture was kept for 24 h at room temperature, and then concentrated to a syrup which was partitioned between chloroform and water. The chloroform layer was washed with water until the wash was neutral and emulsion formation ceased, and then dried and concentrated. The resulting syrup, containing a fine precipitate, was dissolved in toluene (20 ml) and kept overnight at room temperature. The precipitate (726 mg) was filtered off, and the filtrate was kept for recovery of 9-(2,3-*O*-isopropylidene-β-D-allofuranosyl)adenine (**9**). The precipitate was recrystallized from abs. ethanol to yield square platelets (636 mg, 9.8% based on **4**), m.p. 268–269°, $[\alpha]_D^{24.5}$ −50.2° (*c* 1.5, pyridine); u.v. data: λ_{max} (abs. ethanol) 261 nm (ε 12,400).

Anal. Calc. for $C_{17}H_{23}N_5O_5$: C, 54.10; H, 6.14; N, 18.56. Found: C, 54.00; H, 6.13; N, 18.46.

9-(2,3-O-Isopropylidene-β-D-allofuranosyl)adenine (**9**). — The toluene filtrate which remained after the isolation of **8** was evaporated, and the residue was hydrolyzed in 70% aqueous acetic acid (70 ml) for 2.5 h at 50°. Following complete evaporation of the acetic acid, the product was partitioned between chloroform and water until emulsion formation ceased. The chloroform extract was again hydrolyzed with 70% acetic acid, and the procedure was repeated a second and third time. The combined aqueous extracts were evaporated and crystallized from methanol to yield 227 mg (3.9% based on **4**). Fine needles were obtained from methanol–water, m.p. 262–264°, $[\alpha]_D^{24.5}$ −108.7° (*c* 1.5, pyridine); u.v. data: λ_{max} (abs. ethanol) 261 nm (ε 12,500); R_{Ad}: 0.90 (solvent A), 1.5 (solvent B).

Anal. Calc. for $C_{14}H_{19}N_5O_5$: C, 49.85; H, 5.68; N, 20.76. Found: C, 49.75; H, 5.74; N, 20.61.

9-(2,3-O-Isopropylidene-α-D-allofuranosyl)adenine (**7**). — Hydrolysis of crystalline **8** (410 mg, 1.09 mmole) with 70% acetic acid (40 ml) was carried out as described for the preparation of **9**. Crystallization from abs. ethanol at room temperature gave irregular shaped crystals (267 mg, 72.8%) which upon recrystallization from 2-propanol yielded 185 mg (50.4%), m.p. 196–197.5°, $[\alpha]_D^{24.5}$ −19.8° (*c* 1.5, pyridine); u.v. data: λ_{max} (abs. EtOH) 260 nm (ε 12,800); R_{Ad}: 0.95 (solvent A), 1.23 (solvent B).

Anal. Calc. for $C_{14}H_{19}N_5O_5$: C, 49.85; H, 5.68; N, 20.76. Found: C, 49.55; H, 5.66; N, 20.64.

9-(2,3-O-Isopropylidene-β-D-ribofuranosyl)adenine (**13**). — The preparation of **13** was performed essentially as described for the preparation of 9-(2,3-*O*-isopropylidene-α-D-lyxofuranosyl)adenine[7]. From **9** (337.3 mg, 1.0 mmole) was obtained

200 mg (65.1%) of solid material which upon crystallization from ethanol yielded needles (152 mg, 49.5%) m.p. 220–222°. The mixed melting point with authentic isopropylidene-adenosine was undepressed, $[\alpha]_D^{24}$ −100.0° (*c* 2, pyridine); R_{Ad} : 0.82 (solvent A), 1.33 (solvent C); lit.[9]: $[\alpha]_D^{26}$ −99.8° (*c* 2, pyridine).

9-(2,3-O-Isopropylidene-α-D-ribofuranosyl)adenine (**6**). — The preparation of **6** was performed in the same way as that of **13**. From **7** (337.3 mg, 1.0 mmole), 148 mg (48.2%) of solid white material was obtained. Upon recrystallization from ethanol, fine needles (142 mg, 46.2%) resulted, m.p. 270–272°, $[\alpha]_D^{24}$ −13.7° (*c* 0.3, glacial acetic acid); u.v. data: λ_{max} (glacial acetic acid) 262 nm (ε 13,900), λ_{max} (0.1M hydrochloric acid) 258 nm (ε 15,300), λ_{max} (M hydrochloric acid) 258 nm (ε 15,200); R_{Ad} : 0.86 (solvent A), 1.31 (solvent C).

Anal. Calc. for $C_{13}H_{17}N_5O_4$: C, 50.81; H, 5.58; N, 22.79. Found: C, 51.09; H, 5.69; N, 22.18.

9-α-D-Ribofuranosyladenine (**10**). — The preparation of **10** from **6** was performed essentially as 9-α-D-lyxofuranosyladenine was prepared from its isopropylidene derivative[7]. From **6** (127 mg, 0.4 mmole), hydrolyzed with 25% aqueous acetic acid (4.7 ml) for 4 h at 100°, crystalline **10** (100 mg, 81%) was isolated. Recrystallization from methanol resulted in prisms (53 mg, 49.6%) m.p. 204–205°, $[\alpha]_D^{24}$ +28.0° (*c* 0.6, water); u.v. data: λ_{max} (water) 259 nm (ε 14,800), λ_{max} (M acetic acid) 257 nm (ε 14,500); R_{Ad} : 1.21 (solvent A), 0.92 (solvent C), and 1.07 (solvent D). Lit.[10]: m.p. 201°, $[\alpha]_D$ +24° (*c* 0.65, water); u.v. data: λ_{max} (at neutral pH) 259 nm (ε 14,500), λ_{max} (in acid) 257 nm.

Anal. Calc. for $C_{10}H_{13}N_5O_4$: C, 44.94; H, 4.90; N, 26.21. Found: C, 44.93; H, 4.79; N, 26.05.

9-α-D-Allofuranosyladenine (**11**). — The hydrolysis of the di-*O*-isopropylidene derivative (**8**) was carried out as described previously for the preparation of 9-α-D-lyxofuranosyladenine[7]. Compound **8** (74 mg, 0.2 mmoles) was hydrolyzed in 25% aqueous acetic acid (3.7 ml) for 4 h at 100° to give 22.5 mg (38.8%), isolated on crystallization from abs. ethanol. Recrystallization from water resulted in fine needles, m.p. 217–218°; $[\alpha]_D^{24}$ +33.9° (*c* 0.4, water); u.v. data: λ_{max} (water) 259 nm (ε 14,000); R_{Ad}: 1.29 (solvent A), 0.78 (solvent C), 0.91 (solvent D).

Anal. Calc. for $C_{11}H_{15}N_5O_5$: C, 44.44; H, 5.09; N, 23.56. Found: C, 44.02; H, 4.99; N, 23.04.

9-β-D-Allofuranosyladenine (**12**). — Hydrolysis of **9** (71.7 mg, 0.213 mmole) in 25% aqueous acetic acid (3.7 ml), as described for compound **11**, gave 41 mg (64.9%). When recrystallized from water, tiny needles resulted, m.p. 262-264°, $[\alpha]_D^{25}$ −54.7° (*c* 0.2, M hydrochloric acid); u.v. data: λ_{max} (water) 257 nm (ε 13,800), λ_{max} (M hydrochloric acid) 257 nm (ε 13,500); R_{Ad}: 1.29 (solvent A), 0.92 (solvent C), 0.94 (solvent D). Lit.[2]: m.p. 262–264°, $[\alpha]_D^{22}$ −57.2° (*c* 3.67, M hydrochloric acid); u.v. data: λ_{max} (water) 259 nm (ε 13,600); R_{Ad} : 1.39 (solvent A).

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