Note

Conversion of 2′,3′-*O*-isopropylideneadenosine into 9-(6-deoxy-β-D-allofuranosyl)-and 9-(6-deoxy-α-L-talofuranosyl)-adenines

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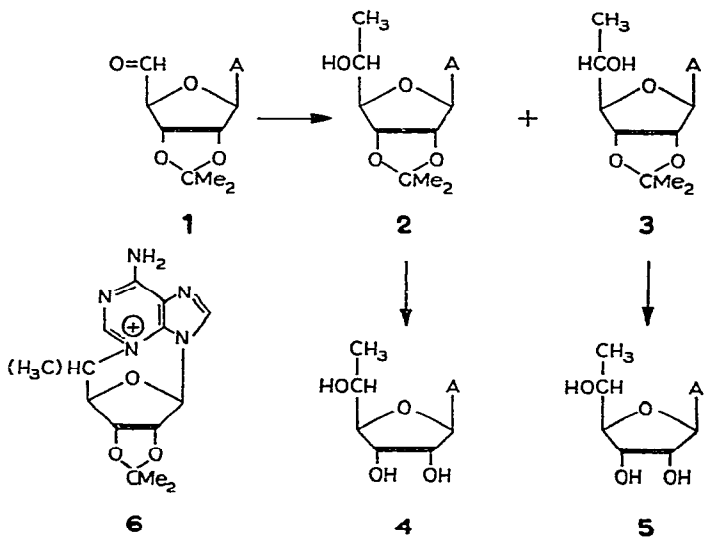
Syntheses of 5′-*C*-alkyl derivatives of adenosine, such as the two enantiomorphs of 5′-*C*-methyladenosine[1,2], 5′,5′-di-*C*-methyladenosine[3], and 5′-(hydroxymethyl)-5′-deoxyadenosine[4,5], have until recently been accomplished exclusively via condensation of an adenine derivative with 5-*C*-alkyl-D-ribofuranose derivatives. We have described a new and more direct route to 5′,5′-di-*C*-methyladenosine that hinges upon a Grignard reaction between the readily accessible 5′-methoxycarbonyl analog of 2′,3′-*O*-isopropylideneadenosine and methylmagnesium iodide[6]. We now report that reaction of the 5′-aldehyde analog of 2′,3′-*O*-isopropylideneadenosine with methylmagnesium iodide is likewise a feasible synthetic procedure, and provides a simplified route to the two enantiomorphic 5′-*C*-monomethyl derivatives of adenosine.

The sulfoxide–carbodiimide oxidation procedure of Pfitzner and Moffatt[7] converted 2′,3′-*O*-isopropylideneadenosine in 85% yield into a syrup considered to be the 5′-aldehyde analog **1**. The only other component detected was the starting material. This mixture was treated directly with methylmagnesium iodide in ether–*p*-dioxane–tetrahydrofuran under the conditions found suitable for reaction of the 5′-methoxycarbonyl analog of 2′,3′-*O*-isopropylideneadenosine[6]. Analysis by t.l.c. and paper electrophoresis showed slow disappearance of the aldehyde, accompanied by formation of three components having nucleosidic properties. The two major components were isolated crystalline in 11% and 7% yields, respectively, after chromatography on silica gel. Elemental analysis, together with u.v., i.r., and n.m.r. spectra indicated that both of these products were mono-*C*-methyl derivatives of 2′,3′-*O*-isopropylideneadenosine, and the high-resolution mass spectrum of each compound showed a peak at the *m/e* value expected for the molecular ion. N.m.r. decoupling experiments (irradiation at either the H-5′ or methyl frequencies) established that in both instances the methyl group was attached to C-5′ and the products were hence concluded to be the 2′,3′-isopropylidene ketals of 9-(6-deoxy-β-D-allofuranosyl)- and 9-(6-deoxy-α-L-talofuranosyl)adenines (**2** and **3** respectively).

Nucleoside 5′-aldehydes can undergo epimerization at the 4′-position under mild conditions[8]. However, three lines of evidence show that the two principal products of the Grignard reaction do in fact retain the C-4′ configuration of 2′,3′-*O*-isopropylideneadenosine. Firstly, a *trans* relationship between H-3′ and H-4′ is indi-

cated by the values (2.2 and 2.5 Hz) of $J_{3',4'}$. Secondly, the 5′-*O*-tosyl derivatives of both compounds, when heated gave rise to *p*-toluenesulfonate salts of products (**6**) having u.v.-spectral and t.l.c. properties similar to that of 3,5′-anhydro-2′-3′-*O*-isopropylideneadenosine *p*-toluenesulfonate. Furthermore under mildly basic conditions the anhydro derivatives **6** underwent the same u.v.- spectral transformations as 3,5′-anhydro-2′,3′-*O*-isopropylideneadenosine itself[9] due to cleavage of the N-1–C-2 bond[10]. Inversion at C-4′ of structures **2** or **3** would preclude their transformation into 3,5′-anhydronucleosides. Finally, the mass spectra of the two reaction products showed an appreciable peak at *m/e* 277, which results from transfer of the 5′-hydroxyl hydrogen to the adenine ring, together with loss of C-5′ and C-6′ as CH_3CHO. This result also argues that the compounds possess the configuration at C-4′ of structures **2** and **3** insofar as, among adenosine analogs, such fragmentation occurs to this extent only when the 5′-hydroxyl group and the adenine ring are *cis* to each other across the furanose ring[11].



Removal of the isopropylidene group of **2** and **3** by acidic treatment gave, respectively, 9-(6-deoxy-β-D-allofuranosyl)adenine (**4**) and 9-(6-deoxy-α-L-talofuranosyl)adenine (**5**) in high yield. The configuration at C-5′ of these compounds could be unequivocally assigned because of the large difference between their specific rotations, the values for which agreed substantially with those reported for these nucleosides previously prepared via condensation of blocked 5-*C*-methyl sugars with the purine moiety[1,2].

The Grignard reaction consistently produced ~50% more of **2** than of **3**. Grignard reactions of carbonyl compounds in diethyl ether solution are believed to proceed through a 6-centered, cyclic transition-state involving two molecules of

Grignard reagent[12]. A Corey–Pauling–Koltun molecular model of **1** showed that the 5′-aldehyde group can occupy two possible positions that maximize the distance of its oxygen atom from O-4′ as well as from O-3′, and which for this reason are presumably favored rotamers. Irrespective of which of these positions was examined, the purine ring interposed a greater degree of restraint upon assembly of the 6-centered transition state leading to the *α-talo* nucleoside **3** than upon the transition state that produced its *β-allo* enantiomorph **2**.

The Grignard reaction yielded a smaller proportion (5%) of a third product, isolated crystalline. Its n.m.r. spectrum showed signals corresponding to all of the non-exchangeable protons of 2′,3′-*O*-isopropylideneadenosine. Many fragments containing silicon were present in the mass spectrum including a prominent peak at *m/e* 352 corresponding to $C_{13}H_{18}N_5O_5Si$; the i.r. spectrum showed a strong band at 1020 cm^{-1} (absent for 2′,3′-*O*-isopropylideneadenosine) that could result from Si–O stretching. The compound is tentatively concluded to be a silicon-containing derivative of 2′,3′-*O*-isopropylideneadenosine, which arises from interaction of a product of the Grignard reaction with silica employed in the separation and analysis procedures.

EXPERIMENTAL

General. — Melting points (uncorrected) were determined by the capillary method. U.v. spectra were obtained in buffered aqueous solutions with a Cary Model 15 spectrophotometer, n.m.r. spectra in dimethyl sulfoxide-d_6 with a Varian HA-100 instrument with Me_4Si as external standard (concentric capillary tube), and i.r. spectra were determined for KBr discs with a Perkin–Elmer 137 instrument. Mass spectra were recorded at Battelle Memorial Institute, Columbus, Ohio, with an A.E.I. MS-9 spectrometer; data give *m/e* values, with relative intensities and assignments in parentheses. A Bendix automatic polarimeter 1169 was used to obtain specific rotations. Thin-layer chromatograms were run on Merck F-254 silica gel plates in (*A*) methanol–chloroform (1:9); (*B*) acetone–chloroform (1:1); (*C*) acetonitrile–diethyl ether (1:9); and (*D*) acetone–chloroform (3:7). Elemental analyses were made by Atlantic Microlabs, Atlanta, Georgia.

Oxidation[7] *of 2′,3′-O-isopropylideneadenosine to the 5′-aldehyde* (**1**). — 2′,3′-O-Isopropylideneadenosine (1.23 g) was dissolved in a mixture of dry dimethyl sulfoxide (4 ml), pyridine (0.32 ml), and trifluoroacetic acid (0.16 ml). A solution of *N,N′*-dicylohexylcarbodiimide (2.86 g) in dry dimethyl sulfoxide (3 ml) and dry benzene (1 ml) was added. After 24 h water (0.2 ml) was added, and 30 min later *N,N′*-dicyclohexylurea was filtered off and washed with dry dimethyl sulfoxide. Spectrophotometric assay revealed that more than 90% of the initial amount of adenosine derivatives was present. The filtrate was concentrated at 40° (0.03–0.05 torr) to a yellow glass. T.l.c. on Eastman cellulose sheets impregnated with 30% sodium bisulfite solution, (solvent ethanol–water, 7:3) indicated that ~85% of the 2′,3′-*O*-isopropylideneadenosine (R_F 0.95) had reacted to give one major product (R_F 0.84) that gave a positive reaction with aldehyde-specific spray reagents. The crude aldehyde was used immediately for the subsequent Grignard reaction.

9-(6-Deoxy-2,3-O-isopropylidene-β-D-allofuranosyl)adenine (**2**) *and 9-(6-deoxy-2,3-O-isopropylidene-α-L-talofuranosyl)adenine* (**3**). — The aldehyde **1** (prepared from 4.92 g of 2′,3′-*O*-isopropylideneadenosine) was dissolved in dry *p*-dioxane–tetrahydrofuran (1:1, 248 ml) and added dropwise with rapid stirring to a solution of methylmagnesium iodide (prepared from 10.86 g of magnesium and 26.8 ml of methyl iodide in 120 ml of ether), at 20° under nitrogen. A grey-white precipitate formed during the addition. Disappearance of aldehyde was monitored by electrophoresis of chloroform-soluble samples (obtained as described later) in 0.1M aqueous sodium bisulfite; the aldehyde moved toward the anode and the remaining ultraviolet-absorbing material towards the cathode. The suspension was stirred for 21 days and added to a mixture of 0.1M HCl (500 ml) and ice. Magnesium complexes were dissolved by addition of 2M HCl to pH 3.0 after which time concentrated ammonia was added to give pH 8.0. The reprecipitated magnesium salts were filtered off (Celite filter-aid), and the solution was concentrated to ~300 ml under diminished pressure and extracted with chloroform (6 × 400 ml). T.l.c. (system *A*) of the extract revealed three major u.v.-absorbing components having R_F 0.68 (unidentified product), 0.54 (**2** and **3**), and 0.48 (2′,3′-*O*-isopropylideneadenosine). T.l.c. with 10-fold repeated development in system *D* separated **2** and **3** and gave R_F values for the above four components of 0.73, 0.61 (compound **2**), 0.57 (**3**), and 0.50, respectively. The brown chloroform extracts were concentrated to a gum that was dissolved in ethyl acetate (12 ml); the solution was applied to a column (54 × 2.8 cm) of Merck silica gel 7734, which was eluted with ethyl acetate. This freed **2** and **3** from the above unidentified product having relatively high R_F, together with part of the 2′,3′-*O*-isopropylideneadenosine and much yellow material of unknown composition. Fractions containing **2**, **3**, and the remaining 2,3-*O*-isopropylideneadenosine were evaporated to a syrup and applied to ten 2-mm preparative-layer plates (20 × 20 cm). These were developed 12–14 times in system *C*, whereupon separation of **2** and **3** (R_F 0.59) from 2′;3′-*O*-isopropylideneadenosine (R_F 0.45) was achieved. The latter compound was identified by extraction of the zone with chloroform and ethanol, removal of volatiles, crystallization of the residue from ethyl acetate–light petroleum, and determination of the m.p. and i.r. spectrum. The total recovery of 2′,3′-*O*-isopropylideneadenosine was 7.2%. The zone containing **2** and **3** was extracted with chloroform (5 × 50 ml) and ethanol (4 × 50 ml). The extracts were filtered through Celite and then evaporated to a syrup, which was applied to five 2-mm preparative-layer plates. These were developed 13 times in system *D* in order to separate **2** and **3**. These compounds were extracted, and then dissolved in 50% aqueous methanol and applied to columns (43 × 0.9 cm) of Bio-Rad AG1-X2 (OH^-). Elution with the same solvent removed **2** and **3** respectively; silica and yellow material were retained on the column. Evaporation of the eluates gave **2** and **3** as white, amorphous powders. The yields, determined spectrophotometrically, were 10.9% and 7.2%, respectively.

9-(6-Deoxy-2,3-*O*-isopropylidene-β-D-allofuranosyl)adenine (**2**) crystallized from ethyl acetate–light petroleum as small cubes, m.p. 269–270°, $[\alpha]_D^{26}$ −91 ±2° (*c* 1, ethanol); i.r.: 3220, 3070, 1655, 1615, 1381, 1221, 1163 and 1094 cm^{-1}; u.v.:

λ_{max} 257 nm (ε 14.3×10^3) at pH 2; λ_{max} 259 nm, (ε 14.6×10^3) at pH 11; n.m.r.: δ 8.75 (s, 1, H-8), 8.57 (s, 1, H-2), 7.78 (s, 2, NH_2), 6.51 (d, 1, J 3.3 Hz, H-1'), 5.83 (d, 1, J 4.0 OH-5'), 5.69 (d of d, 1, J 3.3 and 6.2 Hz, H-2'), 5.43 (d of d, 1, J 2.2 and 6.3 Hz, H-3'), 4.35 (d of d, 1, J 2.2 and 4.8 Hz, H-4'), 4.15 (m, 1, J 4.8 and 6.0 Hz, H-5'), 1.91 and 1.69 (s, 3, CMe_2), and 1.41 (d, 3, J 6.0 Hz, Me); mass spectrum: *m/e* (relative intensity) given: 277 (9.50), 321 (2.86).

Anal. Calc. for $C_{14}H_{19}N_5O_4$: C, 52.32; H, 5.96; N, 21.80. Found: C, 52.17; H, 6.22; N, 21.57.

9-(6-Deoxy-2',3'-*O*-isopropylidene-α-L-talofuranosyl)adenine (**3**) formed crystals from ethyl acetate–light petroleum, m.p. 255°; i.r.: 3220, 3080, 1685, 1620, 1380, 1220, 1168 and 1085 cm^{-1}; u.v.: λ_{max} 257 nm (ε 14.3×10^3) at pH 2; λ_{max} 257 nm (ε 14.3×10^3) at pH 2; λ_{max} 259 nm (ε 14.6×10^3) at pH 11; 11; n.m.r.: δ 8.83 (s, 1, H-8), 8.59 (s, 1, H-2), 7.78 (s, 2, NH_2), 6.54 (d, 1, J 4.0 Hz, H-1'), 5.70 (d, 1, J 6.0 Hz, OH-5'), 5.62 (d of d, 1, J 3.8 and 6.0 Hz, H-2'), 5.32 (d of d, 1, J 2.5 and 6.0 Hz, H-3'), 4.4 (d of d, 1, J 2.5 and 4.0 Hz, H-4'), 4.22 (m, 1, J 4.0 and 6.0 Hz, H-5'), 1.93 and 1.69 (s, 3, CMe_2), and 1.49 (d, 3, J 6.0 Hz, Me); mass spectrum: *m/e* 277 (7.44); 321 (3.30).

Anal. Calc. for $C_{14}H_{19}N_5O_4$: C 52.32 H 5.96; N 21.80. Found: C 52.57; H 6.13; N 21.62%.

The product having R_F 0.68 in system *A* formed crystals from ethyl acetate–light petroleum m.p. 131–132°; i.r.: 3170 (sh), 1680, 1615 (sh), 1380, 1220, 1075, 1020 (sh) cm^{-1}; u.v.: λ_{max} 257 nm at pH 2 and259 n m at pH 11; n.m.r.: δ 8.75 (s, 1, (H-8) 8.62 (s, 1, H-2), 6.63 (d, 1, J 2.0 Hz, H-1'), 5.91 (d of d, 1, J 2.0 and 6.0 Hz, H-2'), 5.45 (d of d, 1, J 3.5 and 6.0 Hz, H-3'), 4.75 (d of d, 1, J 3.5 and 5.0 Hz, H-4'), 4.06 (d, 1, J 5.0, CH_2-5'), 3.6–4.6 (broad peak), 1.96 and 1.76 (s, CMe_2); mass spectrum: *m/e* 352 (20.0, $C_{13}H_{18}N_5O_5Si$); 306 (17.4, $C_{13}H_{16}N_5O_4$), 196 (1.0, $C_6H_{10}N_5OSi$), 175 (0.5, $C_6H_{11}O_4Si$), 165 (100, $C_6H_6N_5O$), 159 (0.5, $C_5H_7O_4Si$), 61 (89, CH_5OSi).

3,5'-Anhydro-5'-C-methyl-nucleosides (**6**). — A solution of compound **2** (4.6 mg) and *p*-toluenesulfonyl chloride (6.5 mg) in dry pyridine was kept for one day at room temperature, when t.l.c. indicated that conversion into the 5'-*p*-toluenesulfonate was complete. The product was isolated by p.l.c. in system *B* and was heated under reflux in *p*-dioxane (1 ml) for 1 h. The anhydronucleoside was isolated by t.l.c. in isopropanol–formic acid–water (7:1:2). It had λ_{max} 275 nm, λ_{min} 240 nm at pH 2 and pH 7.5, and λ_{max} 265 nm, λ_{min} 264 nm at pH 11. On acidification of the alkaline solution λ_{max} was 283 nm and λ_{min} 250 nm. These spectral properties are similar to those of 3,5'-anhydro-2',3'-*O*-isopropylideneadenosine[9]. The R_F values of **6** were almost identical with those of 3,5'-anhydro-2',3'-*O*-isopropylideneadenosine in three solvent systems. An anhydronucleoside (**6**) of the talofuranosyl derivative **3** was prepared in the same manner. It possessed R_F values and spectral characteristics indistinguishable from those of the anhydronucleoside prepared from **2**.

9-(6-Deoxy-β-D-allofuranosyl)adenine (**4**). — Compound **2** (62 mg) was dissolved in 70% aqueous acetic acid (20 ml, pH 2.5) containing acetone (6 ml) and the mixture

heated for 12 h at 100° (bath temp.). T.l.c. (system *A*) indicated that removal of the isopropylidene group was almost complete and that no detectable release of adenine had occurred. The solvents were removed *in vacuo*. A solution of the residue in methanol–water (3:7) was applied to a Bio-Rad 1-X2 (OH^-) column (42 × 0.8 cm). Unhydrolyzed **2** (5 mg) was eluted with 170 ml of methanol–water (3:7) after which time **4** was eluted with 210 ml of 50% aqueous methanol. Removal of volatiles *in vacuo* gave **4** as a white, amorphous solid (49.5 mg, 91%). Crystallization from acetone gave 30 mg, m.p. 134–135° (dec.) (reported[1], 135–142° for material crystallized from water), $[\alpha]_D^{26}$ −90 ±1° (*c* 0.8, water) [reported[1], $[\alpha]_D^{24.4}$ −74.2° (*c* 1.86, hydrochloride salt in water)]; i.r.: 3240, 3125, 1720, 1660, 1610, 1490, 1340, and 1075 cm^{-1}; u.v.: λ_{max} 257 nm, (ε 14.4 × 10^3) at pH 2; λ_{max} 259 nm (ε 14.6 × 10^3) at pH 11.

Anal. Calc. for $C_{11}H_{15}N_5O_4 \cdot C_3H_6O$: C, 49.55; H, 6.23; N, 20.69. Found: C, 49.43; H, 6.26; N, 20.60%.

9-(6-Deoxy-α-L-talofuranosyl)adenine (**5**). — Compound **3** (44.2 mg) was heated in aqueous acetic acid containing acetone, as described for the preparation of **4**. Compound **5** was freed of residual **3** with a Bio-Rad 1-X2 (OH^-) column, as described for **4**, and obtained as a white, amorphous powder (36.1 mg, 93%). Attempts at crystallization from acetone yielded a solvated gel; from ethanol or ethanol–petroleum ether it slowly formed small crystals (32 mg), m.p. 124–126° (dec), $[\alpha]_D^{25}$ −39 ±2° (*c* 1, water) [reported[2], $[\alpha]_D^{27}$ −35° (*c* 0.84, water)]; i.r.: 3170, 1640 (sh), 1480 (sh), 1335, 1130 and 1060 cm^{-1}; u.v.: λ_{max} 258 nm, (ε 14.3 × ^{3}10) at pH 2; λ_{max} 259 nm, (ε 14.6 × 10^3) at pH 11.

Anal. Calc. for $C_{11}H_{15}N_5O_4 \cdot 0.5C_2H_6O$: C, 47.36; H, 5.96; N, 23.03. Found: C, 47.23; H, 6.05; N, 23.24%.

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