PHOTOLYSIS OF AZIDO SUGAR DERIVATIVES. GENERATION OF 5'-ALDEHYDES FROM 5'-AZIDO DERIVATIVES OF ADENOSINE AND URIDINE*

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ABSTRACT

5'-Azido-5'-deoxy-2',3'-*O*-isopropylideneadenosine (**4**) [prepared by way of N^6-formyl-2',3'-*O*-isopropylidene-5'-*O*-*p*-tolylsulfonyladenosine (**2**)], upon photolysis and mild treatment with acid, gives the nucleoside 5'-aldehyde derivative **5**, characterized by reduction with borohydride to 2',3'-*O*-isopropylideneadenosine (**1**) and with borodeuteride to give the 5'-deuterated analog (**6**) of **1**. Similarly, photolysis of 5'-azido-2',3'-*O*-benzylidene-5'-deoxyuridine (**9**), followed by treatment with acid, gave the nucleoside 5'-aldehyde derivative **10**, characterized by borohydride reduction to 2',3'-*O*-benzylideneuridine (**7**) and borodeuteride reduction to the 5'-deuterated analog (**11**) of **7**. The position of deuterium labeling in **6** and **11** was verified by high-resolution n.m.r. and mass spectrometry.

INTRODUCTION

The application of photochemical techniques for effecting useful synthetic transformations in the carbohydrate field has been explored in this laboratory with a range of different reactions[1–4]. As shown with methyl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-α-D-glucopyranoside, photolysis of a primary azide in a solvent that is a poor hydrogen-donor, with subsequent mild hydrolysis of the resultant imino derivative, provides an excellent route to the corresponding ω-aldehyde[5,6]. The reaction is effective with unprotected glycosides as well as with acetylated ones[6,7], and has been exploited for preparation of 6-aldehydo analogs of starch[8,9] and cellulose[9,10], as well as with sugar derivatives of other configurations[7,9]. The reaction is also effective when aromatic aglycons are present[6,9]. When extended to secondary azides, the corresponding ketones are formed, but the yields are mediocre[7,11].

The successful photolytic conversion of a primary azide in an aryl glycoside system[6,9] into the corresponding aldehyde prompted an evaluation of the reaction in nucleoside systems, since the 5'-aldehyde analogs of nucleosides are of great

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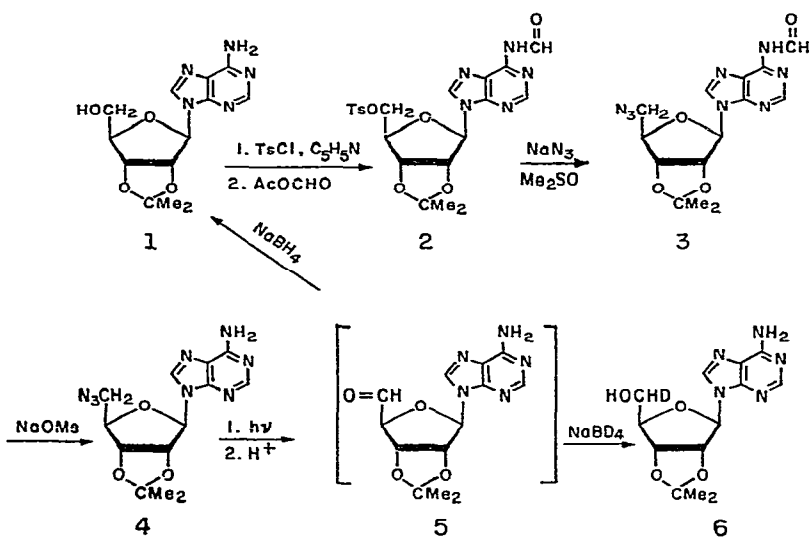
potential significance as reactive intermediates for structural modification of nucleosides to yield biologically important analogs, such as the 4′,5′-unsaturated nucleosides, polyoxins, and related structures.

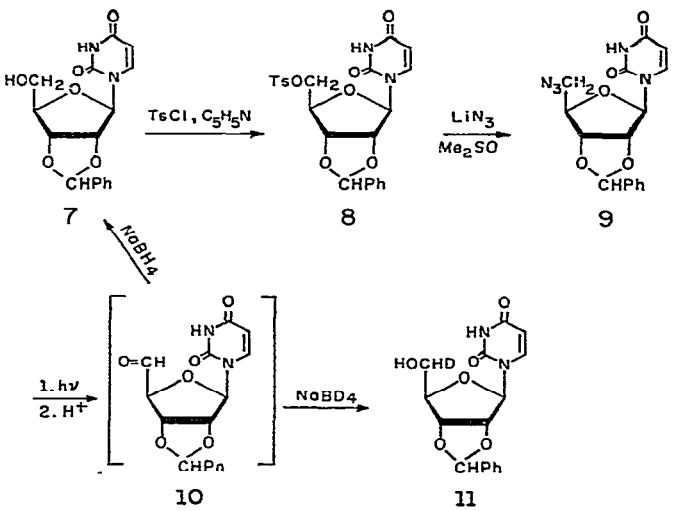
The present study on the photolysis of 5′-azido-5′-deoxy derivatives in the adenosine and uridine systems shows that conversion into the 5-′aldehydes can be effected readily without causing changes elsewhere in the nucleoside molecule. Reduction of these products with borodeuteride serves as a convenient characterizing reaction for the 5′-aldehydes and as a route for preparing nucleosides specifically deuterated at C-5′; specifically tritiated analogs could be obtained similarly.

DISCUSSION

Preparation of azido nucleoside derivatives. — The derivative selected for study in the adenosine series, namely, 5′-azido-5′-deoxy-2′,3′-*O*-isopropylideneadenosine (**4**), was prepared from 2′,3′-*O*-isopropylideneadenosine[12] (**1**) in three steps. Conversion of **1** into the crystalline N^6-formyl-5′-*O*-*p*-tolylsulfonyl derivative (**2**), essentially by the procedure of Jahn[13], proceeded practically quantitatively when the acetic–formic anhydride used was prepared by a new procedure[14]. Displacement of the sulfonate group in **2** by azide, by action of sodium azide in methyl sulfoxide, proceeded readily, to give the crystalline 5′-azide **3** in 89% yield, without significant interference by commonly encountered side-reactions[13,15] leading to anhydronucleoside salts. Deformylation of **3** by use of sodium methoxide gave the desired, crystalline azido nucleoside **4** in essentially quantitative yield. The intermediates **2**, **3**, and **4** were characterized in detail by elemental analyses, i.r. and u.v. spectra, and X-ray powder diffraction patterns (see Experimental section) and by n.m.r. (see Table I) and mass spectroscopy (see Table II).



In the pyrimidine nucleoside series, the 2′,3′-benzylidene acetal[16] (**7**) of uridine was 5′-*p*-toluenesulfonylated by the general procedure of Levene and Tipson[17] to give the crystalline 5′-*p*-toluenesulfonate (**8**) in 96% yield. Lithium azide in methyl sulfoxide converted **8** into the 5′-azide (**9**), obtained in 70% yield as a glass; the reagent used was more effective[18] than sodium azide, and interfering reactions were minimized. The three products, **7**, **8**, and **9**, were characterized in detail by the procedures used for the adenosine derivatives **2**, **3**, and **4** (see Experimental section and Tables I and II).



Photolysis of the azides **4** *and* **9**. — To effect the desired photolytic conversion of the azide, presumably[19–21] through formation of an intermediate nitrene (R–CH_2N:) that becomes stabilized by hydrogen migration to give an aldimine (R–CH=NH), a solvent (benzene) that is a poor hydrogen-donor was used in order to minimize a possible competing side-reaction that would lead to the corresponding primary amine. In view of the known susceptibility of the nucleoside bases to structural modification by short-wavelength radiation[22,23], a Corex filter was used to surround the medium-pressure, mercury arc lamp used as the light source; this filter excludes most of the radiation of wavelengths shorter than 260 nm. Under these conditions, it was found that photolysis of the azides **4** and **9** at room temperature under nitrogen was complete in 0.5–2.5 h, as evidenced by disappearance of the starting materials (t.l.c.) and the absence from the products of the characteristic i.r. absorption near 4.75 μm for the azide group. The u.v.-absorbing, methanol-soluble, and benzene-insoluble photolysis products were treated immediately with Amberlite IR-120 (H^+) resin at room temperature, by the general procedure established in earlier work[5,8,10], to hydrolyze the presumed aldimine product from the photolysis step. In each case, there was obtained an amorphous product that showed by t.l.c. a principal component

TABLE I

N.M.R.-SPECTRAL DATA[a] FOR COMPOUNDS 1–4, 6–9, AND 11

Compound	*Solvent*[b]	*Chemical shifts*[a] (τ)					
		H-1′	*H-2′*	*H-3′*	*H-4′*	*H-5′,5a′*	*Other*
2′,3′-*O*-Isopropylideneadenosine (1)	$(CD_3)_2SO$	3.78 d ($J_{1',2'}$ 3)	4.59 dd ($J_{2',3'}$ 6)	4.94 dd ($J_{3',4'}$ 2.5)	5.69 sx ($J_{4',5'}$ 5) (width 12 Hz)	6.32 d[c]	1.56 s, 1.72 s (H-2, H-8); 8.38 s, 8.61 s (CMe_2)
2′,3′-*O*-Isopropylideneadenosine-5′-*d* (6)	$(CD_3)_2SO$	3.78 d ($J_{1',2'}$ 3)	4.59 dd ($J_{2',3'}$ 6)	4.94 dd ($J_{3',4'}$ 2.5)	5.69 q (width 7 Hz)	6.32 m[d] (width 7 Hz)	1.56 s, 1.72 s (H-2, H-8); 8.38 s, 8.61 s (CMe_2)
2′,3′-*O*-Benzylideneuridine[e] (7)	$(CD_3)_2SO$	4.01 m (width ~1 Hz)	—4.85–5.20 m—		5.72 m (width 18 Hz)	6.27 m (width 8 Hz)	2.16 d, 2.25 d (H-6, $J_{5,6}$ 8); 2.4–2.7 (aryl); 3.85 s, 4.03 s, (PhC*H*); 4.25 d (H-5)
2′,3′-*O*-Benzylideneuridine-5′-*d*[e] (11)	$(CD_3)_2SO$	4.01 m (width ~1 Hz)	—4.85–5.20 m—		5.72 m (width 13 Hz)	6.27[d] (width 10 Hz)	2.16 d, 2.25 d (H-6, $J_{5,6}$ 8); 2.4–2.7 (aryl); 3.85 s, 4.03 s (PhC*H*); 4.25 d (H-5)
N^6-Formyl-2′,3′-*O*-isopropylidene-5′-*O*-*p*-tolylsulfonyladenosine (2)	$(CD_3)_2SO$	3.69 d ($J_{1',2'}$ 2)	4.60 dd ($J_{2',3'}$ 6)	4.98 dd ($J_{3',4'}$ 2.5)	—5.5–5.9 m—		−1.30 d (NH, *J* 10)[f]; 0.02 d (formyl)[g]; 1.41 s, 1.47 s (H-2, H-8); 2.41, 2.77 (4 protons, A_2B_2 of Ts); 7.64 s (aryl Me); 8.46 s, 8.68 s (CMe_2)
5′-Azido-5′-deoxy-N^6-formyl-2′-3′-*O*-isopropylideneadenosine (3)[i]	$CDCl_3$	3.84 d ($J_{1',2'}$ 2.5)	4.51 dd ($J_{2',3'}$ 6.0)	4.91 dd ($J_{3',4'}$ 4.0)	5.55 sx ($J_{4',5'}$ 5.5)	6.35 d	−1.32 d (NH, *J* 10)[f]; 0.16 d (formyl)[g]; 1.41 s, 1.49 s (*H*-2, H-8); 8.33 s, 8.56 s (CMe_2)

5′-Azido-5′-deoxy-2′,3′-*O*-isopropylideneadenosine (4)	$CDCl_3$	3.87d ($J_{1',2'}$ 2.5)	4.47dd ($J_{2',3'}$ 6)	4.92dd ($J_{3',4'}$ 3.5)	5.60sx ($J_{4',5'}$ 6)	6.42d	1.68s, 2.08s (H-2, H-8); 3.22s (NH_2)[f]; 8.39s, 8.59s (CMe_2)
2′,3′-*O*-Benzylidene-5′-*O*-*p*-tolylsulfonyluridine (8)[h]	$(CD_3)_2SO$	4.18d ($J_{1',2'}$2)	—4.8–5.2m—		—5.5–5.8m—		2.2–2.8m (aryl H, H-6); 3.92s, 4.03s (PhC*H*); 4.36d (*H*-5, $J_{5,6}$ 8); 7.61s (aryl Me)
5′-Azido-2′,3′-*O*-benzylidene-5′-deoxyuridine[h] (9)	$(CD_3)_2CO$	4.68d ($J_{1',2'}$ 2)	—5.25–5.80m—		6.12–6.38m	6.88–7.01m	2.88d, 2.92d (H-6, $J_{5,6}$ 10); 3.1–3.4 (aryl); 4.48s, 4.61s (PhC*H*); 4.88d (H-5)

[a]Measured at 60 MHz, unless otherwise noted. Peak multiplicities: d, doublet; dd, doublet of doublets; m, multiplet; q, quartet; s, singlet; sx, sextet. First-order couplings are given in Hz. [b]A small proportion of D_2O was present in the $(CD_3)_2SO$ and $(CD_3)_2CO$; spectra in $CDCl_3$ were measured before and after addition of D_2O. [c]Apparent doublet; satellite peaks of anticipated ABX system not observed. [d]Integrated intensity 1 proton. [e]A 4:6 mixture of diastereoisomers differing in configuration at the benzylic carbon atom, leading to doubling of the H-6 and PhCH signals [compare N. Baggett, A. B.Foster, J. M. Webber, D. Lipkin, and B. E. Phillips, *Chem. Ind.* (London), (1965) 136]. [f]Observed in dry solvent; disappears on deuteration. [g]Collapses to singlet when D_2O is added to the dry solvent. [h]As[e], but in 7:3 proportion. [i]Measured at 100 MHz.

TABLE II

MASS-SPECTRAL DATA FOR COMPOUNDS 1, 3, 4, 6–9, AND 11

Compound	*Mass-spectral peaks (relative intensities and probable assignments given in parentheses)*
2′,3′-*O*-Isopropylideneadenosine (1)	307 (0.9) $M^{\ddagger}$, 292 (5) $(M-CH_3)^+$, 277 (4) $(M-H_2CO)^{\ddagger}$, 262 (2) $(M-45)^+$, 249 (3) $(M-CH_3COCH_3)^{\ddagger}$, 232 (1) $(M-CH_3-CH_3COCH_3)^+$, 220 (1), 219 (6), 218 (29) $(C_9H_8N_5O_2)^+$, 204 (6), 202 (4), 190 (3), 178 (2) (base–$CH_2CH=\overset{+}{O}H$), 173 (2) (sugar moiety)$^+$, 165 (4), 164 (34) (base–$CH=\overset{+}{O}H$), 136 (27) (base, 2H)$^+$, 135 (100) (base, H)$^+$, 134 (5) (base)$^+$, 129 (2), 118 (4), 114 (3), 115 (3), 108 (15), 85 (5), 59 (30) $(CH_3-\overset{+}{C}(OH)CH_3)$
2′,3′-*O*-Isopropylideneadenosine-5′-*d* (6)	308 (0.8) $M^{\ddagger}$, 293 (4) $(M-CH_3)^+$, 277 (4) $(M-HDCO)^{\ddagger}$, 262 (2) $(M-46)^+$, 250 (3) $(M-CH_3COCH_3)^{\ddagger}$, 233 (1) $(M-15-CH_3CO_2H)^+$, 219 (8), 218 (40) $(C_9H_8N_5O_2)^+$, 204 (7), 202 (4), 178 (2) (base–$CH_2CH=\overset{+}{O}H$), 174 (2) (sugar moiety)$^+$, 164 (25) (base–$CH=\overset{+}{O}H$), 136 (30) (base, 2H)$^+$, 135 (100) (base, H$^+$), 134 (4) (base)$^+$, 129 (1), 119 (3), 116 (3), 115 (1.5), 108 (17), 85 (6), 59 (29) $[CH_3\overset{+}{C}(OH)CH_3]$
2′,3′-*O*-Benzylideneuridine (7)	332 (3) $M^{\ddagger}$, 331 (2) $(M-1)^+$, 301 (1) $(M-1-H_2CO)^+$, 255 (1) $(M-C_6H_5)^+$, 221 (9) (sugar moiety)$^+$, 220 (6), 219 (3), 195 (3), 192 (2), 180 (3), 179 (4), 175 (8), 167 (4), 145 (10), 141 (5) (base–$CH=\overset{+}{O}H$), 137 (9), 114 (23) $(C_4H_5N_2O_2, H)^{\ddagger}$, 113 (28) $(C_4H_5N_2O_2)^+$, 112 (28) $(C_4H_4N_2O_2)^{\ddagger}$, 107 (27), 106 (29) $(C_6H_5CHO)^{\ddagger}$, 105 (100) $(C_6H_5CO)^+$, 99 (15), 98 (12) $(C_4H_4NO_2)^+$, 91 (18), 79 (23), 77 (31) $(C_6H_5)^+$, 69 (90) $(C_3H_3NO)^{+\cdot}$, 68 (47), 57 (28) $(C_3H_5O)^+$, 51 (19)
2′,3′-*O*-Benzylideneuridine-5′-*d* (11)	333 (3) $M^{\ddagger}$, 332 (2) $(M-1)^+$, 301 (1) $(M-1-HDCO)^+$, 256 (1) $(M-C_6H_5)^+$, 222 (9) (sugar moiety)$^+$, 221 (7), 220 (4), 206 (3), 195 (4), 193 (2), 180 (2), 179 (3), 176 (8), 175 (2), 167 (3), 145 (8), 141 (4) (base–$CH=\overset{+}{O}H$), 137 (8), 115 (16) $(C_4H_5N_2O_2, D)^{\ddagger}$ 114 (4) $(C_4H_5N_2O_2, H)^{\ddagger}$, 113 (30) $(C_4H_5N_2O_2)^+$, 112 (29) $(C_4H_4N_2O_2)^{+\cdot}$, 106 (7) $(C_6H_5CHO)^{\ddagger}$, 105 (100) $(C_6H_5CO)^+$, 91 (11) $(C_7H_7)^+$, 79 (14), 77 (68) $(C_6H_5)^+$, 69 (64) $(C_3H_3NO)^{\ddagger}$, 68 (10), 57 (17) $(C_3H_5O)^+$, 51 (24)
5′-Azido-5′-deoxy-N^6-formyl-2′,3′-*O*-isopropylideneadenosine (3)	360 (0.5) $M^{\ddagger}$, 345 (2.5) $(M-CH_3)^+$, 332 (18) $(M-CHO)^{\ddagger}$, 304 (9) $(M-CH_2N_3)^+$, 302 (3) $(M-CH_3COCH_3)^{\ddagger}$, 274 (0.9), 246 (54), 232 (1), 218 (40) $(C_9H_8N_5O_2)^+$, 204 (54), 178 (15) (base–$CH_2CH=\overset{+}{O}H$), 164 (100) (N^6-formyl-base, H)$^+$, 136 (96) (base, 2H)$^+$, 135 (98) (base, H)$^+$, 112 (35), 108 (39), 70 (62), 43 (95) $(CH_3CO)^+$
5′-Azido-5′-deoxy-2′,3′-*O*-isopropylideneadenosine (4)	332 (0.4) $M^{\ddagger}$, 317 (3) $(M-CH_3)^+$, 304 (9) $(M-N_2)^{\ddagger}$, 288 (3), 277 (8), 274 (1) $(M-CH_3COCH_3)$, 262 (10), 246 (6), 128 (97) $(C_9H_8N_5O_2)^+$, 204 (32), 202 (11), 190 (9), 176 (22), 164 (23) (base–$CH=\overset{+}{O}H$), 148 (8), 136 (100) (base, 2H)$^+$, 135 (82) (base, H)$^+$, 118 (27), 85 (26), 70 (16), 59 (24), 43 (33) $(CH_3CO)^+$

TABLE II *Continued*

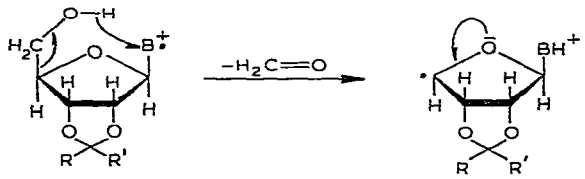
Compound	*Mass spectral peaks (relative intensities and probable assignments given in parentheses)*
2′,3′-*O*-Benzylidene-5′-*O*-*p*-tolylsulfonyluridine (8)	486 (1) $M^{\ddagger}$, 485 (0.3) $(M-1)^+$, 409 (0.5) $(M-C_6H_5)^+$ 396 (0.4), 375 (10) $(C_{19}H_{19}O_6S)^+$, 350 (2), 314 (7), 313 (11) 301 (0.5) $(C_{15}H_{13}N_2O_5)^+$, 281 (0.3), 269 (0.6), 244 (2), 241 (1), 239 (5), 209 (2), 195 (3), 193 (8), 172 (54) $(C_7H_8O_3S)^{\ddagger}$, 157 (6) $(base-CH_2CH=OH, 2H)^+$, 155 (4) $(base-CH_2\overset{+}{C}H=\overset{+}{O}H)$, 137 (3), 131 (5) $(base-CH=\overset{+}{O}H)$, 113 (6) $(base, 2H)^+$, 112 (12) $(base, H)^{\ddagger}$, 106 (98) $(C_6H_5CHO)^{\ddagger}$, 105 (100) $(C_6H_5CO)^+$, 91 (79) $(C_7H_7)^+$, 77 (96) $(C_6H_5)^+$, 65 (18) $(C_5H_5)^+$
5′-Azido-2′,3′-*O*-benzylidene-5′-deoxyuridine (9)	357 ($\ll$1) M^+, 356 (0.5) $(M-1)^+$, 329 (0.9) $(M-N_2)^{\ddagger}$, 279 (0.4), 235 (0.2), 218 (0.4), 140 (0.6), 122 (1), 112 (5) (base, H)‡, 106 (96) $(C_6H_5CHO)^{\ddagger}$, 105 (19) $(C_6H_5CO)^+$, 96 (5), 78 (13), 77 (100) $(C_6H_5)^+$

migrating as a single spot that gave a positive reducing-sugar test with aniline phthalate, a positive Schiff reaction indicative of the aldehyde group, and u.v. fluorescence behavior characteristic of a nucleoside; these data, and analogy with previous work[5–10], indicate that these reducing products were the aldehydes **5** and **10**, respectively. Both products showed by t.l.c. the presence of a minor, nonmigrating, u.v.-absorbing, nonreducing component; the possibility that the respective 5′-amino-5′-deoxy derivatives were present in these side-products cannot be excluded.

Characterization of the products. — The aldehyde derivatives **5** and **10** were not obtained crystalline; because of the ease with which such aldehydes can form a multitude of possible solvation products, oligomers, and elimination products, they were characterized by direct transformation into stable, readily identified compounds. In the preparation of 6-aldehydo derivatives by photolysis of 6-azido-6-deoxy derivatives of starch[8] and cellulose[10], it has already been shown that reduction of the aldehydo derivatives with borodeuteride followed by determination of the position and extent of deuterium incorporation in the reduced product provides a more convenient and accurate method for determining the aldehyde group than conventional derivatization through hydrazone derivatives. Accordingly, similar reduction procedures were used with the aldehydes **5** and **10**. Reduction of the crude aldehyde **5** with borohydride gave 2′,3′-*O*-isopropylideneadenosine (**1**), isolated crystalline in 54% yield (based on the starting azide **4**). Similarly, reduction of the aldehyde **10** gave crystalline 2′,3′-*O*-benzylideneuridine (**7**) in 58% yield based on the azide **9**. These yields of reduced products can be regarded as minimum values for the yields of the aldehydes from the azide precursors.

When the reductions were performed with borodeuteride, the corresponding 5′-monodeuterated analogs **6** and **11** of the nondeuterated nucleoside derivatives **1** and **7** were obtained. The position and extent of deuterium incorporation in these

products was established by n.m.r. and mass spectrometry. The n.m.r. spectra of the deuterated derivatives **6** and **11** were closely similar to those of the nondeuterated analogs **1** and **7** (for full details, see Table I), but the integrated intensity of two protons for the C-5′ protons in **1** and **7** was decreased to one proton for **6** and **11**; no replacement of other protons bonded to carbon could be detected by integration. Examination of the fine structure of the spectra was further concordant with replacement of one C-5′ proton by deuterium in the products **7** and **11**. Thus, the 6-line patterns observed for H-4′ in **1** became a 4-line pattern in **6** by loss of one of the H-4′, H-5′ couplings and its replacement by the much smaller H–D coupling. The changes anticipated were observed in the signals for the C-5′ protons, and the signals for H-3′, H-2′, and H-1′ were not affected. Likewise, the multiplet observed for H-4′ in compound **7** collapsed to a narrower multiplet in **11** through similar loss of one H-4′, H-5′ coupling; again, the patterns expected were observed for the C-5′ proton resonances, and there was no change in the H-3′, H-2′, and H-1′ signals. The mass spectra of the deuterated products **6** and **11** each showed a molecular-ion peak that was one mass-unit higher than from the nondeuterated analogs **1** and **7** (for full details of the mass spectra, see Table II). In addition, a number of key fragmentations, in which the deuterium-labeled intermediate exhibited a peak one *m/e* unit higher than the nondeuterated analog, served to pinpoint the deuterium label at C-5′. Of particular interest is a key fragmentation involving loss of C-5′ as formaldehyde from the nucleoside. In related nucleosides, the following fragmentation has been postulated[24] by use of the 5-OD derivative. This transformation is exhibited in **1** in forma-



tion, from the molecular ion (*m/e* 307) by loss of formaldehyde (30 daltons), of the fragment ion *m/e* 277; in the deuterated derivative **6**, the same ion (*m/e* 277) is formed from the molecular ion (*m/e* 308), evidently by loss of the deuterium atom in a monodeuterioformaldehyde (DHC=O, 31 daltons) fragment. In the pyrimidine derivative **7**, the principal fragmentation mode leads to an even-electron ion *m/e* 301, corresponding to loss of formaldehyde and the benzylic hydrogen atom; the parent M−1 ion is observed at *m/e* 331. The deuterated analog **11** likewise shows the *m/e* 301 fragment, but this ion is formed by loss of DHC=O (31 daltons) from the M−1 ion (*m/e* 332).

The foregoing transformations illustrate the applicability of the azide photolysis procedure[5–10] as a route for generating aldehyde functionality in the carbohydrate moiety of nucleosides and their derivatives, to provide intermediates useful for chain-extension reactions of various types[25,26], and for preparing 5′-tritiated nucleosides[27].

The reaction may be a useful alternative to chemical methods of oxidizing the hydroxymethyl group[26] in systems where functional groups sensitive to oxidants are present.

EXPERIMENTAL

General methods. — Unless otherwise indicated, solutions were evaporated under diminished pressure at 40°. Melting points were determined with a Thomas–Hoover "Unimelt" apparatus and are corrected. I.r. and u.v. spectra were recorded with a Perkin–Elmer Model 137 spectrophotometer and a Cary Model 14 recording spectrophotometer, respectively. N.m.r. spectra were recorded by using Varian A-60-A and HA-100 instruments, with tetramethylsilane as the internal standard: Chemical shifts are given on the τ scale. Optical rotations were measured with a Perkin–Elmer Model 141 recording polarimeter. Mass spectra were obtained with an AEI MS-9 double-focusing, high-resolution spectrometer at an ionization potential of 70 eV and an accelerating potential of 8 kV. A direct-insertion probe was employed for solids, at a temperature of 250°. Microanalyses were performed by W. N. Rond of this laboratory. X-Ray powder diffraction data give interplanar spacings in Å for CuKα radiation (camera diameter = 114.59 mm). Relative intensities were estimated visually: m, moderate; s, strong; v, very; w, weak. The three strongest lines are numbered (1, strongest). T.l.c. was performed on silica gel with "Baker-Flex" prepared sheets, type IB-F (J. T. Baker). Column chromatography was conducted with silica gel (No. 7734, E. Merck, Darmstadt, Germany). Photolyses were effected with a Hanovia 450-W, medium-pressure lamp (Cat. No. 679A36) contained in a quartz immersion-well. A filter of Corex tubing (1 mm, thickness) was employed, and the temperature of the solutions ranged from 15 to 20°.

Preparation of N^6-*formyl*-2′,3′-O-*isopropylidene*-5′-O-p-*tolylsulfonyladenosine* (**2**). — 2′,3′-*O*-Isopropylideneadenosine[12] (**1**, 9.80 g, 32.0 mmoles) was *p*-toluenesulfonylated, and the crude product[29] was immediately suspended in acid-free acetic–formic anhydride[14] (100 ml). The mixture was stirred overnight at ~25°, evaporated below 30°, and the residue triturated with methanol to give the crude, crystalline product; this procedure is essentially that of Jahn[13]. One recrystallization from 1:1 methanol–ethyl acetate gave pure **2**; yield 15.40 g (98% based on **1**), m.p. 160–163° (dec.) (lit.[13] m.p. 165°, sinters), R_F 0.75 (5:5:1 chloroform–acetone–cyclohexane); λ_{max}^{KBr} 2.91 (NH), 5.78, 5.82 (C=O), 6.15, 6.20, 6.80, 7.35, 8.15, 8.45, 9.22, 10.31, 11.44, 12.32, and 12.90 μm; X-ray powder diffraction data: 11.62 vw, 8.50 vs (1), 7.60 vw, 6.68 w, 5.72 vs (2), 5.29 w, 4.96 s (3), 4.63 m, 4.38 s, 4.11 s, 3.79 vw, 3.55 w, 3.41 m, and 3.20 s.

Anal. Calc. for $C_{21}H_{23}N_5O_7S$: C, 51.53; H, 4.74; N, 14.31; S, 6.54. Found: C, 51.62; H, 5.07; N, 14.45; S, 6.84.

5′-Azido-5′-deoxy-N^6-*formyl*-2′,3′-O-*isopropylideneadenosine* (**3**). — A solution of compound **2** (12.0 g, 22.9 mmoles) and sodium azide (6.5 g, 100 mmoles) in anhydrous methyl sulfoxide (100 ml) was heated for 30 min on a steam bath with exclusion of moisture. The resulting yellow solution was poured into water, and

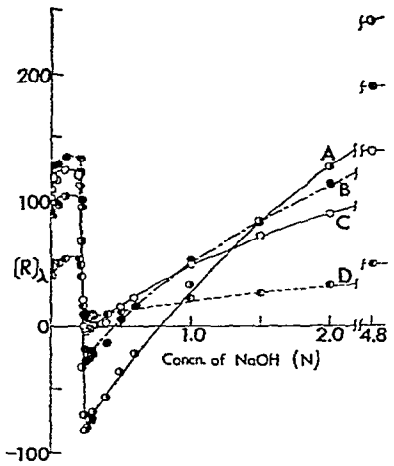


Fig. 2. Dependence of the reduced mean residue rotation ($[R]_\lambda$) of (1→3)-β-D-glucan on the concentration of sodium hydroxide at 25°. The wavelengths are (A) 240, (B) 265, (C) 300, and (D) 500 nm respectively.

In order to confirm the reversibility of the angle of rotation with the change of sodium hydroxide concentration, the values of $[R]_\lambda$ at the same alkali concentration were compared; one solution was prepared by dissolution of the glucan in an aqueous solution at a given concentration of alkali, the other solution was obtained by dilution of a more concentrated solution. The values obtained for both solutions were identical. The reversibility of the angle of rotation was shown for all the concentrations of alkali.

The results observed for the glucan solutions were also obtained with solutions of pachyman*, but not with solutions of amylose. In this case, the o.r.d. curves obtained for concentrations of alkali from 0.01 to 4.8M, where amylose molecules should be in the form of a random coil[7], were normal. Furthermore, the o.r.d. curve observed at pH 4.6, where the molecule takes the form of a deformed helix[7–12], was also normal.

The plot of reduced viscosity, (η_{sp}/c), against glucan concentration (c) was approximately linear in the range of glucan concentrations between 1–0.2 g/l at any concentrations of sodium hydroxide. When the intrinsic viscosity, {$[\eta]$ (dl/g)}, is plotted against the sodium hydroxide concentration (Fig. 3), the value of $[\eta]$ decreases with the increase of the alkali concentration from 0.005 to 0.19M. At higher concentrations, the viscosity falls abruptly between 0.19 and 0.22M, then rises sharply at 0.24M, and finally decreases gradually with the increase of the concentration.

Fig. 3 shows also the results of various determinations made on a 5 g/l solution of glucan at the rate of shear of 6000 sec^{-1}. The extinction angle (χ) of flow birefringence increases with the concentration of alkali under 0.19M; above this concentration, it showed a sharp increase, and at concentrations higher than 0.22M, it

*Pachyman is also a gel-forming (1→3)-β-D-glucan which was extracted from powdered Bukuryo (*Poria cocos*, supplied by Takeda Chemical Industries Ltd.) according to the method described by Saito *et al.*[2].

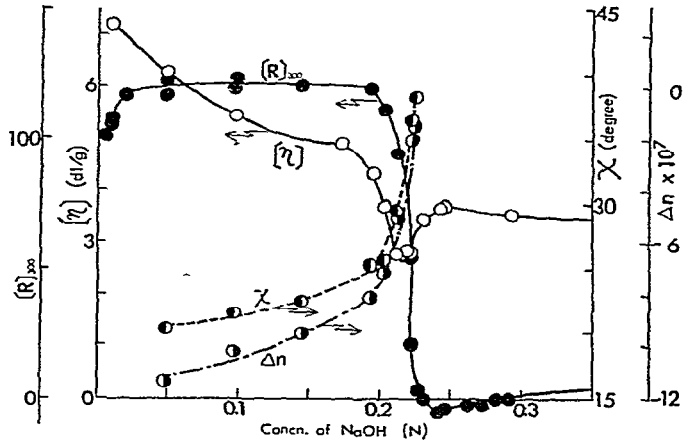


Fig. 3. Dependence of the intrinsic viscosity ([η]), the extinction angle (χ), the birefringence (Δn), and the reduced mean residue rotation at the wavelength of 300 nm, $([R]_{300})$ of (1→3)-β-D-glucan solutions on the concentration of sodium hydroxide. The values of χ and Δn were obtained on a 5 g/l glucan solution at the rate of shear of 6000 sec^{-1} and 30°.

could not be observed. The values of birefringence (Δn) shows changes similar to those of the extinction angle. The similarity of the relationship between χ and Δn and sodium hydroxide concentration (Fig. 3) was observed at rates of shear other than 6000 sec^{-1} and also at different concentrations of the glucan.

When sodium hydroxide was replaced by lithium hydroxide, a similar dependency of the o.r.d. and viscosity curves on alkali concentration was observed. However, the abrupt changes were observed at a concentration between 0.17 and 0.21M (Fig. 4)*.

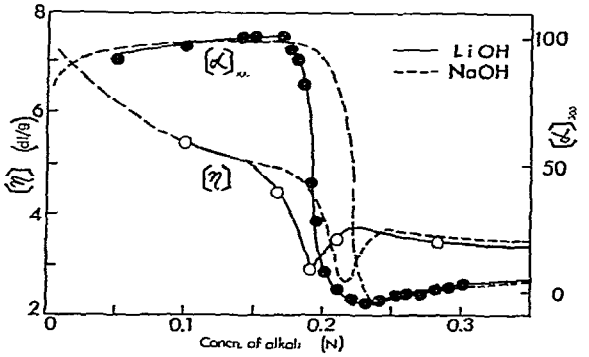


Fig. 4. Dependence of the intrinsic viscosity ([η]), and the specific rotation at the wavelength of 300 nm $([\alpha]_{300})$ of (1→3)-β-D-glucan solutions on the concentration of alkali.

*A similar shift of the viscosity-alkali concentration curve of amylose solutions, when sodium ions are replaced by lithium ions, was assumed by Erlander and Purvinas[13] on the basis of their experience with sodium and potassium ions.

sulfoxide (80 ml) was heated under nitrogen for 2.5 h on a steam bath. The resulting yellow solution was poured into water (600 ml), and the mixture was extracted with four 80-ml portions of chloroform. The combined extracts were washed twice with water, dried (magnesium sulfate), and evaporated, to give **9** as a glass, R_F 0.53 (25:25:6 chloroform–acetone–cyclohexane, detection by u.v. light). The product was freed of traces of slower-moving impurities on a column (5 × 40 cm) of silica gel by elution with 340 ml of the t.l.c. solvent mixture; the yield of chromatographically homogeneous, glassy **9** was 7.23 g (70%); $[\alpha]_D^{26}$ +6.5° (*c* 1.3, chloroform); λ_{max}^{EtOH} 258.0 nm (ε 10,200); λ_{max}^{KBr} 2.91 (NH), 3.16, 3.30, 3.42 (CH), 4.75 (N_3), 5.86–5.89 (broad, C=O), 6.82, 7.22, 7.84, 9.11, 9.15, 9.32, 13.12, and 14.32 μm; for n.m.r. and mass-spectral data, see Tables I and II.

Anal. Calc. for $C_{16}H_{15}N_5O_5$: C, 53.78; H, 4.23; N, 19.60. Found: C, 53.70; H, 4.24; N, 19.55.

Photolysis of 5′-azido-2′,3′-O-benzylidene-5′-deoxyuridine (9) to generate the aldehyde **10**. — By the general procedure used for the azide **4**, a solution of compound **9** (250 mg, 0.70 mmole) in dry benzene (180 ml) was photolyzed under nitrogen at 15–20°, to yield, after irradiation for 45 min and subsequent treatment with acidic ion-exchange resin, the aldehyde **10** as an off-white powder; yield 221 mg, R_F 0.35 (5:5:1 chloroform–acetone–cyclohexane); positive to u.v. light, aniline phthalate, and Schiff reagent. A minor, nonreducing, u.v.-absorbing side-product, which did not migrate on t.l.c., was also present. The n.m.r. spectrum of the product showed line-broadening similar to that observed with **5**.

Reduction of the aldehyde **10**. — *A. With borohydride to give 2′,3′-O-benzylideneuridine* (**7**). The aldehyde **10** (200 mg) in ethanol (50 ml) was reduced with sodium borohydride by the procedure used for the adenosine derivative **5**. After purification of the product on a column of silica gel with 13:1 ethyl acetate–methanol as eluant, there was obtained the pure uridine derivative **7**; yield 122 mg (64%), m.p. 187–188°; X-ray powder diffraction data: 10.00 m, 6.84 s (3), 5.89 vs (2), 4.94 s, 4.45 s, 4.09 vs (1), 3.92 m, 3.76 m, 3.44 s, 3.11 w, 3.01 vw, 2.91 m, and 2.80 w. The product was identical with an authentic sample of **7** by mixed m.p., i.r. and n.m.r. spectra, X-ray powder diffraction pattern, and t.l.c. in 3 solvent systems.

B. With borodeuteride to give 2′,3′-O-benzylideneuridine-5′-d (**11**). Reduction of the aldehyde **10** (200 mg) by the foregoing procedure, but with use of sodium borodeuteride (35 mg) as the reductant, gave the 5′-deuterated derivative **11** in comparable yield. The product was indistinguishable from **7** by mixed m.p., by X-ray diffraction pattern, and by t.l.c., but its n.m.r. and mass spectra (see Tables I and II, and Discussion section) indicated that one atom of deuterium had become incorporated at C-5′ per molecule of the product.

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