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NUCLEOSIDES. 140. STEREOSELECTIVITY OF THE REACTION OF 1-(2,3-ANHYDRO-5-O-BENZOYL- β -D-LYXOFURANOSYL)URACIL WITH AMMONIUM AZIDE. ISOLATION AND CHARACTERIZATION OF 2-AZIDO-XYLO-NUCLEOSIDE DERIVATIVES.¹

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Abstract: The composition of the products of reaction of 1-(2,3-anhydro-5-O-benzoyl- β -D-lyxofuranosyl)uracil (**1**) with NH_4N_3 was studied by a reverse-phase HPLC system which was found to separate the 3-azido-arabino **2** and 2-azido-xylo **3** isomers that were formed. The use of a 10:1 ratio of NH_4N_3 to **1** in refluxing EtOH was found to minimize ring opening at C-2 (7%). The higher stereoselectivity of ring opening produced by using a large excess of NH_4N_3 was suppressed by conducting the reaction in DMF. Preventing the escape of the NH_3 by-product only resulted in debenzoylation. The isolation of pure, crystalline **3** was achieved by reverse-phase preparative HPLC. Separation from the arabino isomer was also effected by debenzoylation and selective acetonide formation with the xylo isomer, which allowed facile isolation of the latter by normal phase chromatography. Hydrolysis of the acetonide **7** provided unprotected 2-azido-xylo nucleoside **6**, which was also obtained by NaOMe treatment of **3**. The mechanistic basis for the stereoselectivity of epoxide opening is discussed.

In 1976, we reported² a synthesis of 1-(3-acetamido-2-O-acetyl-5-O-benzoyl-3-deoxy- β -D-arabinofuranosyl)uracil (**4b**) (see Scheme I) by treatment of 1-(2,3-anhydro-5-O-benzoyl- β -D-lyxofuranosyl)uracil (**1**) with boron trifluoride etherate in acetonitrile followed by acetylation. At that time we also described an alternative route to **4b** involving opening of epoxide **1** by refluxing in ethanol with 1.65 equivalents of NH_4N_3 to provide pure 1-(3-azido-5-O-benzoyl-3-deoxy- β -D-arabinofuranosyl)uracil (**2**) after recrystallization of the crude product. Shortly thereafter, we reported³ that pure **2** could be obtained in 86% yield by treatment of **1** with 10 equivalents of NH_4N_3 , again after recrystallization of the reaction product.

Recently, this reaction was repeated by Hummel and Carty,⁴ who reported that the use of 2.0 equivalents of NH_4N_3 provided a crude product which, based on the ^1H NMR spectrum, consisted of 2 and another at C-2', and thus regioselectivity probably is not responsible for the product ratios observed.

An interesting result was obtained when DMF was used as the reaction solvent instead of EtOH. At 110°C in DMF, the reaction of 1 with both 1.65 and 10 equivalents of NH_4N_3 reached completion in less than two hours to provide almost identical ratios (3.6:1) of 2 to 3 in the product mixture (exp. 5 and 6).

Epoxide ring-opening under neutral and basic conditions has been interpreted⁶ as proceeding through a "borderline- $\text{S}_{\text{N}}2$ " transition state in which C-O bond breaking is more advanced than in regular $\text{S}_{\text{N}}2$ reactions. Ring-opening of 2,3-anhydro- β -D-lyxofuranosides, with few exceptions, occurs at C-3.⁷ It has been suggested⁸ that, although the steric environments of the epoxide ring carbons in the β -lyxosides are very similar, the nature of the substituents at C-1 and C-4 causes greater destabilization, via inductive electron withdrawal, of the transition state for nucleophilic reaction at C-2 than at C-3. The results reported for the reaction of 1 with NH_4N_3 would appear to be consistent with such a "borderline- $\text{S}_{\text{N}}2$ " transition state for epoxide cleavage, as all conditions employed in this and previous^{2,3,4} studies have resulted in predominant attack at C-3'.

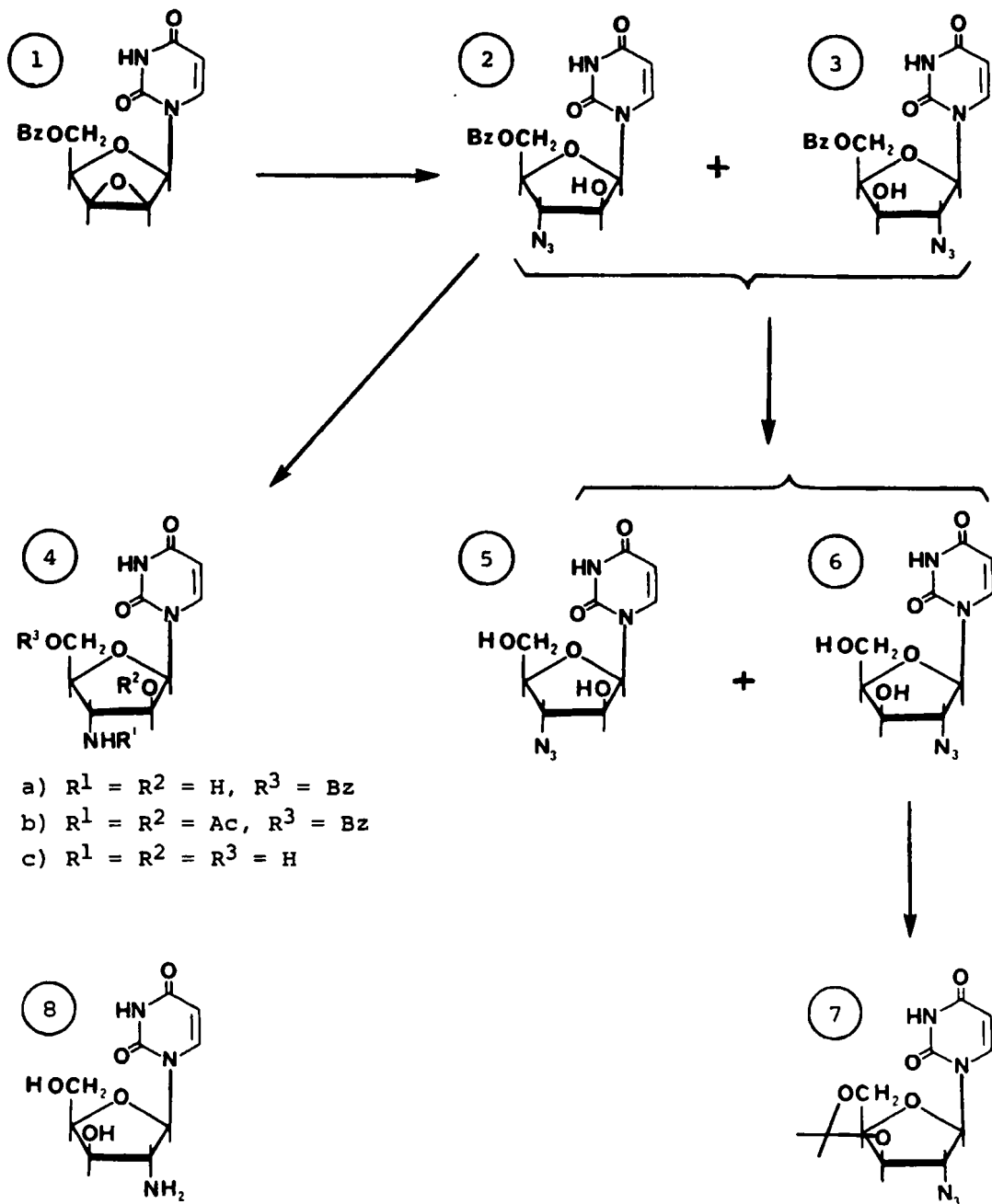
The increase in regioselectivity of epoxide cleavage observed with greater amounts of NH_4N_3 may be the result of an increase in the ionic strength of the solution, which is known⁹ to accelerate $\text{S}_{\text{N}}1$ reactions (the "salt effect"). It might also be argued that the greater electron deficiency at C-2' of 1 in the ground state could enhance the affinity of nucleophiles for this position and that this effect is suppressed when a large excess of nucleophile is present.

The reduced regioselectivity observed when DMF was used as the solvent, i.e., the absence of a dependence on reagent excess, may be due to a higher nucleophilicity of N_3^- in this solvent. Such a solvent effect is well precedented.¹⁰ For example, the reaction of 1-(2,3-anhydro- β -D-lyxofuranosyl)adenine with NaOBz has been reported¹¹ to occur in DMF but not in diethyleneglycol. Furthermore, superior nucleophiles are considerably less selective for C-3 attack in furanoside

epoxides. Thus, reaction of NaSCH_2Ph with methyl 2,3-anhydro- β -D-lyxofuranoside results in a 3:2 ratio of C-2 to C-3 attack,¹² whereas much less nucleophilic NH_3 affords predominantly the product from the expected C-3 attack.¹³ Enhancement of nucleophilicity probably was a factor contributing to the reversal in regioselectivity and would also be expected to favor an $\text{S}_{\text{N}}2$ mechanism. In conclusion, this study of the reaction of **1** with NH_4N_3 has revealed that both the amount of the nucleophile and the nature of the solvent used influence the regioselectivity of ring-opening, presumably by altering the relative expressions of the $\text{S}_{\text{N}}1$ and $\text{S}_{\text{N}}2$ components of the reaction.

As the synthesis of **3** and its debenzoylated analog **6** has not been previously reported, their isolation from the reaction mixture was desirable. Thus, the crude product obtained in experiment #3 which contained 21% of the minor product, was chromatographed on a silica gel column to yield a crystalline mixture of **2** and **3**. A substantial amount of pure **2** was selectively separated by fractional crystallization. The mother liquor was subjected to preparative reverse-phase HPLC, to provide pure 2'-azido xylo nucleoside in 9.7% yield overall from **1** (47% recovery). The product was crystallized and the IR and ^1H NMR spectral data as well as elemental analyses agreed with the structure of **3** for this compound. Its ^1H NMR spectrum showed that the doublet at δ 5.76 in $\text{DMSO}-d_6$ observed for the product mixture resulting from ring-opening of **1** was indeed the anomeric signal for **3**. Furthermore, **3** was found to co-migrate on the analytical HPLC with the major reaction product (retention time of 23.5 minutes).

The deprotected analog **6** was most conveniently isolated by acetonide formation. Thus, combined products from the reaction of **1** with NH_4N_3 , which consisted of 24% **3**, were freed of polar impurities by partitioning between CHCl_3 and H_2O , and then debenzoylated to yield a mixture of **5** and **6** (Scheme I). Treatment of the mixture with acetone in the presence of *p*-toluenesulfonic acid and 2,2-dimethoxypropane selectively formed the acetonide with the 3' and 5' hydroxyl groups of the xylo isomer **6** to give crystalline 1-(2-azido-2-deoxy-3,5-O-isopropylidene- β -D-xylofuranosyl)uracil (**7**) which was isolated in colorless crystalline form in 18% overall (77% recovery) yield from **1** after chromatographic purification. Compound **7** was deprotected with formic acid to yield 1-(2-azido-2-deoxy- β -D-xylofuranosyl)uracil (**6**)



Scheme 1

which was identical with material obtained by debenzoylation of pure 3. In addition to aiding isolation, the ability of 6 to yield an acetone provides further evidence for the 2'-azido-xylo configuration of 3 and 6.

EXPERIMENTAL SECTION

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. ^1H NMR spectra were recorded on a JEOL PFT-100 spectrophotometer in $\text{Me}_2\text{SO}-d_6$ or CDCl_3 using Me_4Si as an internal standard. Analytical TLC was performed on silica gel Analtech Uniplates developed with 9:1 CHCl_3 :MeOH and visualized using short wavelength UV light. Column chromatography was conducted under low pressure using flash grade silica gel (Merck #9385, 0.040–0.063 mm). Analytical HPLC was performed on a Waters C_{18} - μ Bondapak column (10 μ , 3.9 mm I.D. x 30 cm) using a Waters Model 6000A solvent delivery system, U6K injector and Model 440 UV detector (254 nm), and water:methanol (60:40 v/v; 1 mL HOAc added per gallon) as the mobile phase with a flow rate of 1 mL/min. Preparative HPLC was carried out on a Waters Prep LC 500A using a Prep Pak-500 C_{18} column. Elemental analyses were performed by Spang Microanalytical Laboratory.

Reactions of Ammonium Azide¹⁴ with 1 using Different Molar Ratios.

The quantities of reactants shown in Table 1 were stirred in 25 mL refluxing ethanol, using a wide bore condenser and no drying tube (to allow ammonia to escape easily). After the specified period of time, a sample was withdrawn, diluted with H_2O /MeOH, filtered and analyzed by analytical HPLC. Typical retention times: 5 and 6 - 3.6 min; 1 - 10.6 min; 2 - 17.8 min; 3 - 23.5 min.

Reaction in Sealed Tube and Characterization of Products (exp. #4).

Epoxide 1 and NH_4N_3 in absolute EtOH (12 mL) were heated in a sealed tube in a bomb for 24 h at 95–100°C. The resulting solution, which was analyzed by HPLC, was then evaporated *in vacuo* and twice dissolved in MeOH and evaporated to afford a syrup. This was chromatographed on silica gel using 12:1 CH_2Cl_2 :MeOH as the eluent to yield 2 and 3 (61:15) and a slower migrating, chromatographically homogeneous (TLC) component which, based on the ^1H NMR spectrum, was 62% 5³ and 38% 6. ^1H NMR (d_6 -DMSO) δ 3.66 (m, 2H, H-5',5''), 3.94–4.44 (m, 3H, H-2',3',4'), 4.85 (t, 0.4H, 5'-OH xylo, exch.), 5.26 (t, 0.6H, 5'-OH arabino, exch.), 5.59

Table 1. Composition of Products from Reaction of 1-(2,3-Anhydro-5-0-benzoyl-8-D-lyxofuranosyl)uracil and Ammonium Azide as Determined by HPLC.^a

Experiment No.	Amount of $\bar{1}$ (g)	Reaction Conditions			Solvent (mL)	Reaction time(h) (temperature °C)	Products			
		Amount of NH_4N_3^b (g)	Molar ratio $\text{NH}_4\text{N}_3/\bar{1}$				HPLC Peak Integration as % of Total			
							2	3	5	6
1	0.5	0.91	10.0		EtOH(25)	18 (reflux)	92	7	1	
2	1.0	0.3	1.65		EtOH(25)	22 (reflux)	56 ^h	17	1	
3	1.0	0.5 ^c	2.75		EtOH(25)	96 (reflux)	70	21	7	
4	0.5	0.15	1.65		EtOH(12)	24 ^d (95-100)	61(75) ^e	15(23) ^f	22 ^g	
5	0.25	0.075	1.65		DMF (8)	1.5 (110)	76	21	2	
6	0.25	0.45	10.0		DMF (8)	0.5 (110)	72	20	4	

^a For conditions, see Experimental

^b Reference 14

^c Last 0.2 g added during final 2 days (0.1 g/day)

^d In sealed tube in bomb (see Experimental)

^e Including 5

^f Including 6

^g 5.14% and 6.8% based on ¹H NMR integration after separation from 2 and 3

^h 25% of product is unreacted $\bar{1}$

(d, overlap with δ 5.67, total 1H, H-5 arabino), 5.67 (d, H-5, xylo), 5.76 (d, 0.4H, H-1' xylo and overlapping 0.4H, 3'-OH, xylo exch., $J_{1',2'} = 2.44$ Hz), 6.03 (d, 0.6H, H-1' arabino, $J_{1',2'} = 6.10$ Hz), 6.11 (d, 0.6H, 2'-OH arabino, exch.), 7.70 (d, overlap with δ 7.74, total 1H, H-6 arabino, $J_{5,6} = 8.24$ Hz), 7.74 (d, H-6 xylo, $J_{5,6} = 7.93$ Hz), 11.3 (m, 1H, NH exch.).

1-(2-Azido-5-O-benzoyl-2-deoxy- β -D-xylofuranosyl)uracil (3). The mixture obtained in experiment #3 as an ethanolic solution was evaporated to dryness, dissolved in CHCl_3 (35 mL) and extracted with water (2 x 25 mL), dried (Na_2SO_4) and evaporated to an oil. Chromatography on silica gel using CH_2Cl_2 :MeOH (20:1) as eluent yielded a mixture of 2 and 3 (4:1 by HPLC analysis; one spot on TLC) as a crystalline solid. This was dissolved in methanol, water added until slightly turbid and the solution allowed to stand to permit crystallization of pure 2 (0.36 g). Another crop of 2 was collected on cooling of the mother liquor. The filtrate was evaporated, dissolved in MeOH (14 mL) and water was then added (21 mL). This slightly turbid solution was filtered through a Millipore filter and then injected onto a preparative HPLC column using H_2O :MeOH (3:2) as the mobile phase. The band representing 3 was eluted with MeOH: H_2O (4:3), with considerable band broadening leading to overlap with 2. The elution was monitored by analytical HPLC and the fractions containing pure 3 were evaporated to yield a yellow syrup. The colored contaminants were removed by silica gel chromatography (20:1 CH_2Cl_2 :MeOH) to provide 3 as a colorless, chromatographically homogeneous syrup (0.11 g, 9.7%). This was triturated repeatedly with ether and the supernatants were treated with EtOH, concentrated *in vacuo* and cooled to yield white needles (18 mg): mp = 103-105°C. ^1H NMR (d_6 -DMSO) δ 4.35-4.59 (m, 5H, H-2',3',4',5',5''), 5.64, (d, 1H, H-5, $J_{5,6} = 7.9$ Hz), 5.83 (d, 1H, H-1', $J_{1',2'} = 1.8$ Hz), 6.06 (d, 1H, 3'-OH, $J_{3',\text{OH}} = 3.3$ Hz), 7.52-8.02 (m, 6H, H-6 and benzoyl); IR (KBr) 3500 (OH), 2120 (N_3) cm^{-1} .

Anal. Calcd. for $\text{C}_{16}\text{H}_{15}\text{N}_5\text{O}_6$: C, 51.48; H, 4.05; N, 18.76. Found: C, 51.40; H, 4.08; N, 18.60.

1-(2-Azido-2-deoxy-3,5-O-isopropylidene- β -D-xylofuranosyl)uracil (7). The product mixtures from a few reactions of 1 (total 1.9 g, 5.75 mmol)

with NH_4N_3 were combined. HPLC analysis showed this mixture to consist of 23.75% of 3, 68.0% of 2 and 6.5% of 5 and 6. It was dissolved in CHCl_3 (50 mL) and the solution extracted with H_2O (4 x 30 mL), dried (Na_2SO_4) and evaporated to yield a foam that was free of the polar material (including 5 and 6) by TLC. This was dissolved in dry MeOH (50 mL) and treated with NaOMe (6 mmol). After stirring for 5 h at room temperature, the reaction was neutralized with IR-50 resin (H^+), the resin washed with MeOH and the solutions evaporated, co-evaporated twice with water and then with absolute EtOH and MeOH/PhMe to yield a mixture of 5 and 6.

This was dissolved in anhydrous Me_2CO (300 mL), 2,2-dimethoxypropane (2 mL) and pTSH (50 mg) were added and then the solution was stirred at room temperature for 16 h. After stirring for 3 h with anhydrous potassium carbonate, the mixture was filtered, evaporated to dryness and partitioned between CHCl_3 (300 mL) and H_2O (300 mL). The aqueous layer was extracted with CHCl_3 (2 x 200 mL) and the combined CHCl_3 solutions dried (Na_2SO_4) and concentrated. Chromatography on silica gel using 2% EtOH in CHCl_3 as the eluent provided after evaporation and trituration with ether 0.33 g of acetone 7 as white crystals (18.3% yield from 1, 77% from 3): mp 211–212°C (slow dec. from 180°C); ^1H NMR (CDCl_3) δ 1.32 and 1.45 (2s, 6H, iPr), 4.09–4.24 (m, 5H, H-2',3',4',5',5''), 5.73 (d, 1H, H-5, $J_{5,6} = 8.24$ Hz), 5.84 (s, 1H, H-1'), 8.02 (d, 1H, H-6), 8.31 (br, s, 1H, NH, exch.); IR (KBr) 2120 (N_3) cm^{-1} .

Anal. Calcd. for $\text{C}_{12}\text{H}_{15}\text{N}_5\text{O}_5$: C, 46.60; H, 4.89; N, 22.64. Found: C, 46.64; H, 4.86; N, 22.74.

A compound isolated as a foam that eluted later from the column appeared according to the ^1H NMR in CDCl_3 to be an acetal. The spectrum was altered by addition of D_2O and, after evaporation of the solvent, the material was redissolved in d_6 -DMSO and the NMR indicated that conversion to nearly pure 5 had occurred.

1-(2-Azido-2-deoxy- β -D-xylofuranosyl)uracil (6).

A. The acetone 7 (40 mg, 0.13 mmol) was dissolved in conc. HCOOH (4 mL) and the solution stirred at room temperature for 2.5 h. After evaporation *in vacuo* and three co-evaporations with EtOH, the oil was treated with saturated NH_3/MeOH for 5 min. The solution was evaporated, the residue dissolved in water, re-evaporated and then twice dissolved

in water (20 mL) and lyophilized to provide **6** as a hygroscopic white powder (32 mg, 91%): mp <100°C. ^1H NMR (d_6 -DMSO) δ 3.69 (m, 2H, H-5',5''), 3.94-4.28 (m, 3H, H-2',3',4'), 4.85 (m, 1H, 5'-OH), 5.66 (d, 1H, H-5, $J_{5,6}$ = 7.6 Hz), 5.76 (d, 2H, H-1' and overlapping 3'-OH exch.), $J_{1',2'} = 2.44$ Hz), 7.74 (d, 1H, H-6), 11.3 (br, s, 1H, NH); IR (KBr) 3450 (OH), 2120 (N_3) cm^{-1} .

Anal. Calcd. for $\text{C}_9\text{H}_{11}\text{N}_5\text{O}_5 \cdot 1/4 \text{H}_2\text{O}$: C, 39.49; H, 4.23; N, 25.58. Found: C, 39.62; H, 4.22; N, 25.64.

B. Pure **3** (15 mg, 0.040 mmol) was dissolved in dry MeOH (1 mL) and 0.1 mmol NaOMe was added. After stirring for 5 h, the solution was neutralized with IRC-50 resin, which was washed with MeOH and the solutions filtered and evaporated. After co-evaporation with water and then EtOH, trituration with ether yielded resinous solid with ^1H NMR and R_f on TLC (8:1 CH_2Cl_2 :MeOH) identical with that of **6** obtained by Method A.

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