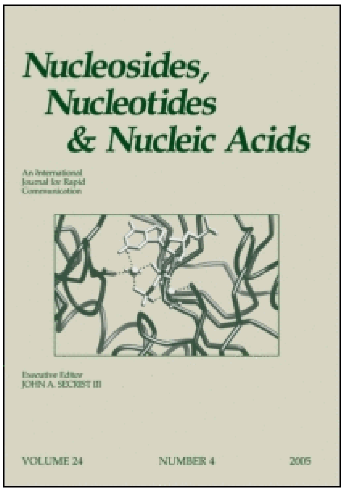

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Charles F. Hummel[a]; Robert P. Carty[a]
[a] Department of Biochemistry, Downstate Medical Center, SUNY, Brooklyn, New York

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REACTION OF AZIDE ION WITH 1-(2,3-ANHYDROLYXOFURANOSYL)URACIL. ISOLATION OF 1-(2-AMINO-2-DEOXY-β-D-XYLOFURANOSYL)URACIL AND 1-(3-AMINO-3-DEOXY-β-D-ARABINOFURANOSYL)URACIL.

Charles F. Hummel and Robert P. Carty
Department of Biochemistry, Downstate Medical
Center, SUNY, Brooklyn, New York 11203

Abstract: The reaction of the 2',3'-lyxoepoxide (1) with ammonium azide gives two products; namely, the 3'-arabino azide (2a) and in low yield 2'-xylo azide (3a). After debenzoylation and reduction the resulting mixture of amines was resolved by chromatography on a weak cation exchanger, Amberlite IRC-50, and afforded crystalline 1-(3-amino-3-deoxy-β-D-arabinofuranosyl)uracil (2c) and 1-(2-amino-2-deoxy-β-D-xylofuranosyl)uracil (3c) in the ratio of 4:1.

Our interest has focused on the synthesis of aminonucleosides and aminonucleotides as affinity labels for reaction with ribonuclease A[1]. During the course of this investigation the method of Reichman et al[2a] was used to prepare 1-(3-azido-3-deoxy-β-D-arabinofuranosyl)uracil (2b). [Chart I] After treatment of the 2',3'-lyxoepoxide (1) with ammonium azide, the resulting 2a was deblocked with sodium methoxide in methanol followed by catalytic hydrogenation with 10% Pd/C. This should have given 1-(3-amino-3-deoxy-β-D-arabinofuranosyl)uracil (2c).

Examination by NMR of the crude product derived from the reaction of epoxide (1) with ammonium azide showed that this product was composed of a mixture of isomers. The spectrum exhibited two different signals for the anomeric protons, ie, a doublet at δ6.12 and a singlet at δ5.83 in the ratio of 4:1. Therefore, we concluded that the product was really a mixture of 1-(3-azido-3-deoxy-5-O-benzoyl-β-D-arabinofuranosyl)uracil (2a) and 1-(2-azido-2-deoxy-5-O-benzoyl-β-D-xylofuranosyl)uracil (3a). Upon recrystallization of the crude product from absolute methanol, the singlet at δ5.83 attributed to the xylo isomer (3a) disappeared and

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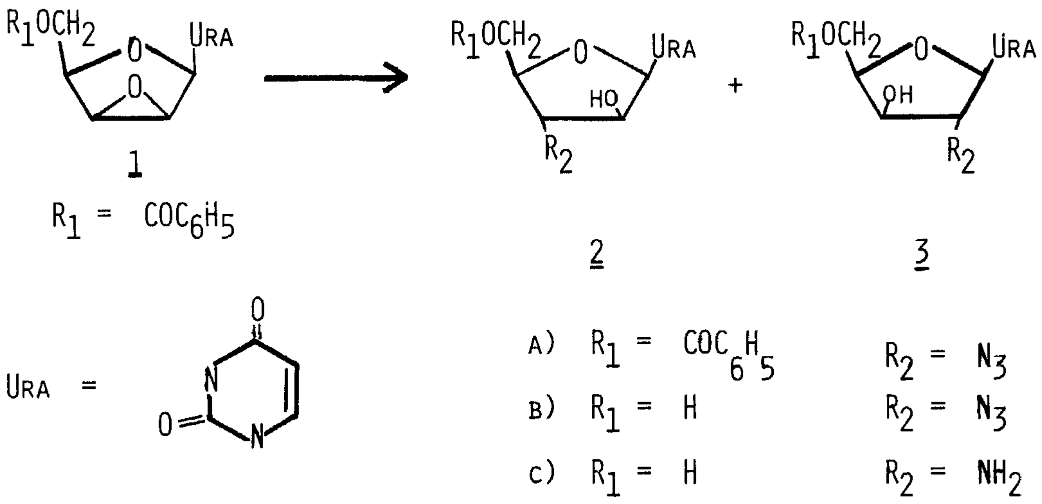
CHART I



only the doublet due to the arabino isomer (2a) was observed. However, the mother liquor from the recrystallization contained a considerable amount of 2a, as demonstrated by the NMR spectrum, which exhibited the two signals for the anomeric protons in the ratio of 2:3 for the arabino to xylo isomers.

Treatment of the mother liquor from the recrystallization of the crude azide with sodium methoxide followed by catalytic hydrogenation gave a 1:1 mixture of amines 2c and 3c. However, similar treatment of the recrystallized azide 2a produced only the 3'-aminoarabinosyl nucleoside 2c.

The nucleoside amines 2c and 3c were separated using Amberlite IRC-50 resin with 0.2M aqueous sodium phosphate at pH 6.36 as eluant. When the crude azide mixture (2a and 3a) was subjected to debenzoylation, catalytic hydrogenation and chromatography, the two isomeric amines 2c and 3c were isolated as crystalline solids in the ratio of 4:1.

These results are consistent with the reaction of other nucleoside epoxides with various nucleophiles including azide ion. The reaction of 1-(5-O-benzoyl-3-O-mesyl-β-D-arabinofuranosyl)uracil with sodium azide and ammonium chloride produced a 4:1 mixture of arabino and xylo isomers[3]. The intermediate was shown by TLC to be a 2',3'-lyxoepoxide which subsequently reacted with azide to afford the products.

In addition, the reaction of lithium azide with the 2',3'-lyxoepoxide derived from adenosine afforded a 10:1 mixture of arabino and xylo isomers which were separated and subsequently converted to the corresponding amines[4].

The identities of the two products 2c and 3c were established by NMR spectroscopy. The C-1 proton of the sugar moiety of 2' and 3'-amino nucleosides exhibits a characteristically different chemical shift and coupling constant.[5] In particular the signal for the anomeric proton of 2'-substituted xylo nucleosides resonates at a slightly lower field then the corresponding 3'-substituted arabino nucleosides. In addition the coupling constant ($J_{1',2'}$) for the anomeric proton is greater for a 3'-substituted arabino nucleoside than for the corresponding 2'-xylo isomer.

The NMR spectrum of 2c revealed a doublet at $\delta 6.09$ for the anomeric proton, while 3c exhibited a doublet at $\delta 5.85$ for its anomeric proton. Furthermore, the coupling constant ($J_{1',2'}$) for the anomeric proton in 2c is greater than that shown for 3c; ie, $J_{1',2'} = 6.0$ Hz vs $J_{1',2'} = 3.6$ Hz. As shown in Table I treatment of 2'-amino-2'-deoxyuridine with slightly more than one equivalent of HCl produced an upfield shift of the anomeric signal while a similar treatment of 3'-amino-3'-deoxyuridine produced no shift of the anomeric signal. Treatment of 3c with HCl produced an upfield shift of the anomeric signal while 2c showed no change in the position of the H-1' signal. The similarity of the behavior of 3c and 2c with the known 2'-amino and 3'-amino uridines strongly suggests that these products are the xylo and arabino substituted aminonucleosides.

In addition, the hydrochloride salt 3d exhibited identical physical properties (mp, IR, UV) to that reported by Wolfrom.[6] Finally the confirmation of the identity of 2a and 3c can be found by comparison of their physical properties with the literature values.[2,6]

These results clearly indicate that the reaction of the 2',3'-lyxoepoxide of uridine with ammonium azide gives not only a 3'-substituted arabino derivative but, in addition, a 2'-substituted xylo derivative, albeit, in lower yield. The 3'-azido arabino nucleoside (2a) can be separated from the 2'-azido xylo nucleoside (3a) by fractional crystall-

Table 1[a]	H-1'	H-1' + HCl	Δδ
1-(2-amino-2-deoxy-β-D-ribofuranosyl)uracil	6.01	6.15	.14
1-(2-amino-2-deoxy-β-D-xylofuranosyl)uracil (<u>3c</u>)	5.85	6.14	.29
1-(3-amino-3-deoxy-β-D-ribofuranosyl)uracil	5.73	5.73	0
1-(3-amino-3-deoxy-β-D-arabinofuranosyl) uracil (<u>2c</u>)	6.09	6.08	.01

(a) spectra were recorded using D_2O as solvent; 1.1 equivalents of 6N HCl in D_2O was added to the D_2O solution. The H-1' signals appeared as doublets.

ization. However, the mixture of amines (<u>2c</u> and <u>3c</u>) can only be separated by ion-exchange chromatography. The use of the weakly acidic ion-exchange resin, Amberlite IRC-50, may provide a facile, convenient analytical and preparative procedure for the purification of isomeric mixtures of amino nucleosides.

EXPERIMENTAL SECTION[8]

1-(3-Azido-5-O-benzoyl-3-deoxy-β-D-arabinofuranosyl)uracil (2a) and 1-(2-azido-5-O-benzoyl-2-deoxy-β-D-xylofuranosyl) uracil (2b).

Epoxide[9] (<u>1</u>) (7.27g) was added to absolute ethanol (150 mL) containing ammonium azide[10] (2.64g). This mixture was refluxed for 22 h and the solution was evaporated to give 8.16g (<u>2a</u> and <u>3a</u>), NMR ($CDCl_3$) δ 8.11 and 7.57 (m, 6, H-6 and benzoate), 6.12 (d, 0.8, $J_{1',2'}$ = 4 Hz, H-1' arabino), 5.83 (s, 0.2, H-1' xylo), 5.32 (d, 1, $J_{5,6}$ = 8 Hz, H-5), 4.79 (m, 4, H-2', 3', 4', OH), 4.20 (broad s, 2, H-5'). IR (KBr) 2100 cm^{-1} (N_3). Recrystallization from methanol afforded <u>2a</u> as a white crystalline solid, mp 150-152°C lit [2a] 154-156°C.

1-(3-Azido-3-deoxy-β-D-arabinofuranosyl)uracil (2b) and 1-(2-azido-2-deoxy-β-D-xylofuranosyl)uracil (3b).

The crude mixture of <u>2a</u> and <u>3a</u> (5.6g) was dissolved in methanol (100 mL) and treated with 1M sodium methoxide in methanol (15mL). The reaction mixture was stirred for 6 h at room temperature and then adjusted to pH 5-6 with Dowex 50X-2(H+) resin. The suspension was filtered, evaporated, co-evaporated 4x with water to remove traces of methyl benzoate and dried over P_2O_5 to give 3.90g of 2b and 3b as a white powder. NMR (DMSO-d_6) δ 11.25 (s, 1H, exchanges with D_2O, N-3), 7.79 (d,1,$J_{5,6}$ = 8Hz, H-6), 6.12 (d,0,8,$J_{1',2'}$ = 6 Hz, H-1' arabino) 5.70 (m, 2.2, OH, H-5, H-1' xylo; upon treatment with D_2O it resolves into a doublet $J_{5,6}$ = 8 Hz), 4.48 (q, which appears as a triplet after addition of D_2O, 2, OH, H-2') 4.00 (m, 4, H-3', 4',5'), IR (KBr) 2100 cm^{-1} (N_3).

1-(3-Amino-3-deoxy-β-D-arabinofuranosyl)uracil (2c) and 1-(2-amino-2-deoxy-β-D-xylofuranosyl)uracil (3c).

The mixture of 2b and 3b (3.0g) was dissolved in absolute methanol (50mL) and 10% Pd/C (1.0g) was added. The mixture was hydrogenated at room temperature in a Parr apparatus at 20 psi for 2.5 h. The suspension was filtered and the filtrate was concentrated *in vacuo* to a yellow syrup. The syrup was dissolved in water and centrifuged to remove particulate matter. The supernatant was transferred onto a column (37 x 5 cm) of Amberlite IRC-50 resin (particle size, 51 ± 13μ) and eluted with 0.2M aqueous sodium phosphate, pH 6.36. The column was monitored at 290 nm and each fraction contained 13.5 mL.

Fractions 62-84 were pooled and placed on a column (8 x 15 cm) of Dowex 50X-8 (200-400 mesh, ammonium form). Elution with 0.2N ammonium formate, pH 4.0 (4L); water (2L) and 1N ammonium hydroxide (2L) gave the product. The solution was evaporated to dryness, co-evaporated 3x with water and finally dried over P_2O_5 to give 0.515g of 3c as a white powder. Recrystallization from ethanol afforded the analytical sample, mp 173-175°C; NMR (DMSO-d_6) δ 7.75 (d,1, $J_{5,6}$ = 8 Hz, H-6), 5.57 (d, 1, $J_{5,6}$ = 8 Hz, H-5), 5.49 (d, 1, $J_{1',2'}$ = 4 Hz, H-1'), 4.05 (t, 1, H-2'), 3.50 (m, 4, H-3', 4', 5'); NMR (D_2O) δ 8.04 (d, 1, $J_{5,6}$ = 8.0 Hz, H-6), 6.00 (d, 1, $J_{5,6}$ = 8 Hz, H-5) 5.85 (d, 1, $J_{1',2'}$ = 3.6 Hz, H-1'; IR (KBr) 3.00, 3.10 (OH); 3.25, 3.40 (NH_2); 5.95, 6.05 (C=O). *Anal.* Cald for $C_9H_{13}N_3O_5$: C, 44.44; H, 5.39; N, 17.28. Found: C, 44.47; H, 5.31; N, 17.13.

Fractions 92-140 were pooled and chromatographed on a Dowex 50X-8 resin (200-400 mesh, ammonium form) as described above. The resulting material was evaporated and dried over

P_2O_5 to give 1.85g of 2c. Recrystallization from 95% ethanol afforded an analytical sample as white crystals, mp 212-214°C (dec), lit[7] 214-216°C (dec); NMR (D_2O) δ 7.67 (d, 1, $J_{5,6}$ = 8 Hz, H-6), 6.09 (d, 1, $J_{1',2'}$ = 6 Hz, H-1'), 5.72 (d, 1, $J_{5,6}$ = 8 Hz, H-5), (DMSO-d_6) δ 7.50 (d, 1, $J_{5,6}$ = 8 Hz, H-6), 6.02 (d, 1, $J_{1',2'}$ = 6 Hz, H-1'), 5.30 (d, 1, $J_{5,6}$ = 8 Hz, H-5), 4.10 (m, 3, OH, H-2'), 3.45 (m, 4, H-3', 4',5'); IR (KBr) 5.90, 6.05 (C=O); UV max (H_2O) pH 7, 262 nm (ε = 10,000), pH 2, 261 (ε = 10,200).

1-(2-Amino-2-deoxy-β-D-xylofuranosyl)uracil hydrochloride (3d).

2N Hydrochloric acid (1mL) was added to a solution of amine 3c (25mg) in methanol (2mL) and stirred at room temperature for 30 minutes. The mixture was evaporated to dryness, co-evaporated 3 x with water, 3 x with absolute ethanol and dried *in vacuo* over P_2O_5 to give 3d which was recrystallized from methanol-ether, mp 246-248°C (dec); lit[6a] 249-252°C (dec); NMR (D_2O δ 7.97 (d, 1, $J_{5,6}$ = 8 Hz, H-6), 6.14 (d, 1, $J_{1',2'}$ = 4 Hz, H-1'), 5.95 (d, 1, $J_{5,6}$ = 8 Hz, H-5). The IR and UV were virtually identical to that reported[6].

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8. NMR spectra were obtained on a Varian T-60A Spectrometer. IR spectra were recorded on a Perkin-Elmer Model 21 spectrophotometer. UV spectra were recorded on a Cary 14. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Microanalysis were performed by the Spang Microanalytical Laboratory, Eagle Harbor, Michigan. Solvents were purified and dried by standard methods. All evaporations of solvents were carried out at 35°C under reduced pressure. Thin-layer chromatography (TLC) was performed on silica gel 60 HF-254 [E. Merck A. G. Darmstadt, plates of 0.25 mm thickness]. The spots were visualized by ultraviolet light emitting radiation at 254 nm.

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