The Synthesis of O^2,2′-Anhydro-5,6-dihydro Nucleosides

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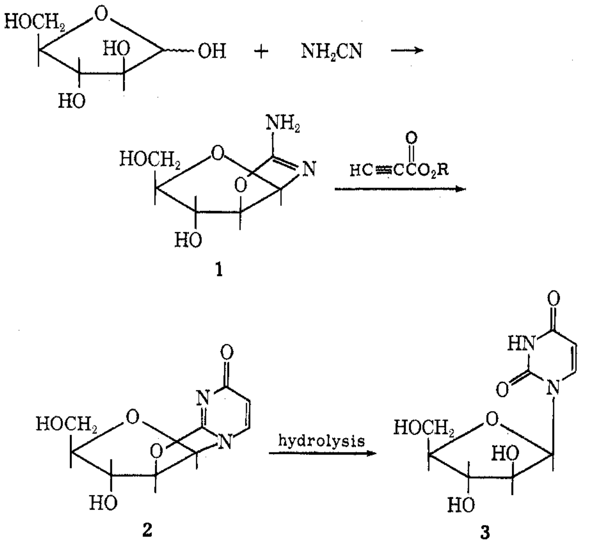
When 2-amino-β-D-arabinofurano[1′,2′:4,5]-2-oxazoline (1) was allowed to react with α,β-unsaturated esters, the previously unknown O^2,2′-anhydro-5,6-dihydrouridines were produced in moderate yield. The synthesis and spectral properties of three members of this class of compounds, O^2,2′-anhydro-5,6-dihydrouridine (**5a**), O^2,2′-anhydro-5-methyl-5,6-dihydrouridine (**5b**), and O^2,2′-anhydro-6-carbomethoxy-5,6-dihydrouridine (**5c**), are discussed.

Anhydro nucleosides have been studied extensively and are frequent intermediates along the synthetic pathway to novel nucleoside systems.[1] They also have been postulated[2] as naturally occurring intermediates in the biological conversion of the more common nucleosides to the less common ones and vice-versa. In contrast, there are very few reports of anhydrodihydro nucleosides in spite of the fact that dihydro nucleosides have been found to occur in some tRNAs.[3] In their synthesis of thio analogs of 5,6-dihydrouridine, Škarić, Gašpert, and Hohnjec reported the synthesis and spectral properties of O^2,5′-anhydro-2′,3′-O-isopropylidine-5,6-dihydrouridine.[4] To our knowledge no other reports of anhydrodihydro nucleosides have appeared. In this report, we describe the facile synthesis and spectral properties of several O^2,2′-anhydro-5,6-dihydrouridines, a potentially useful class of compounds in the study of dihydrouridines.

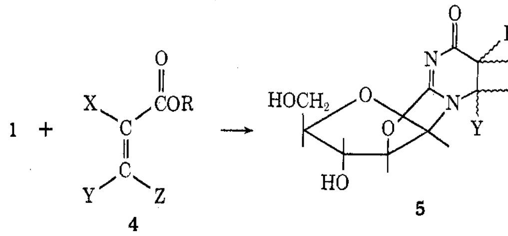
Recently, Sanchez and Orgel[5] described a novel, high yield synthesis of several arabino and ribo nucleosides. They found that the reaction of cyanamide with arabinose gave a good yield of 2-amino-β-D-arabinofurano[1′,2′:4,5]-2-oxazoline (1). The aminooxazoline **1** was in turn allowed to react with ethyl propiolate to give O^2,2′-anhydrouridine (2) in good yield (Scheme I).

SCHEME I



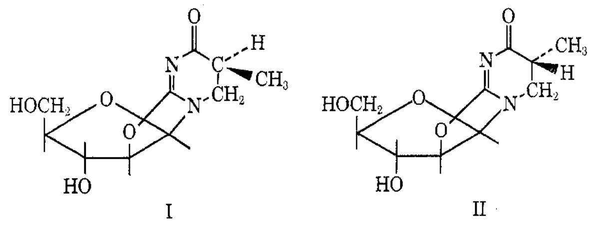
We found that a similar reaction took place when the aminooxazoline **1** was allowed to react with several activated olefins (Scheme II). When a solution of **1** was heated with methyl acrylate (**4a**), a glassy solid was obtained after removal of the solvent. Chromatography of the residue gave the desired O^2,2′-anhydro-5,6-dihydrouridine (**5a**) in 57% yield as white needles after recrystallization.

SCHEME II



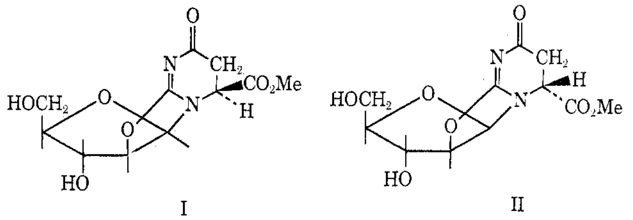
a, X = Y = Z = H; R = CH_3
b, X = CH_3; Y = Z = H; R = CH_3
c, X = H; Y = CO_2CH_3; Z = H; R = CH_3
d, X = Y = H; Z = $CO_2C_2H_5$; R = C_2H_5

The nmr spectrum of **5a** contained a typical A_2B_2 pattern with triplets centered at δ 2.45 and 3.57. The remainder of the spectrum was similar to that of O^2,2′-anhydrouridine. Likewise, when methyl methacrylate was allowed to react with the aminooxazoline, O^2,2′-anhydro-5-methyl-5,6-dihydrouridine (**5b**) was isolated in 36% yield. *A priori* a mixture of two diastereoisomers (epimeric at C-5) would be expected. Thin layer chromatography (tlc) (silica gel, 5% MeOH in $CHCl_3$, eight passes) indicated that the material was homogeneous. Likewise, the sharp melting point,



I II

5b



I II

5c

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[1] Leading reference: "The Chemistry of Nucleosides and Nucleotides," A. M. Michelson, Academic Press, New York, N. Y., 1963; J. J. Fox and I. Wempen, *Advan. Carbohyd. Chem.*, **14**, 283 (1959).

[2] (a) L. I. Pizer and S. S. Cohen, *J. Biol. Chem.*, **235**, 2387 (1960); (b) D. M. Brown, D. B. Parihar, C. B. Reese, and A. Todd, *J. Chem. Soc.*, 3035 (1958).

[3] R. W. Holley, G. A. Everett, J. T. Madison, and A. Zamir, *J. Biol. Chem.*, **240**, 2122 (1965).

[4] V. Škarić, B. Gašpert, and M. Hohnjec, *J. Chem. Soc. C*, 2444 (1970).

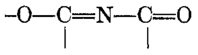
[5] R. A. Sanchez and L. E. Orgel, *J. Mol. Biol.*, **47**, 531 (1970).

196.5–198.0°, was consistent with a single component. However, the nmr spectrum showed two sets of peaks, ABC splitting, for the 6α and the 6β protons, indicating that both of the two possible epimers, **5b**-I and **5b**-II, were present but in unequal amounts. The possibility that the two sets of peaks were due to slowly interconverting conformers was eliminated because a large axial–axial coupling was observed for both components—a condition that could not exist in the case of conformers since one conformer would not have an axial proton in the 5 position.

The reaction of the aminooxazoline **1** with dimethyl fumarate gave a complex product mixture containing two major components. Chromatography on silica gel gave an oil which contained only the two major components. Treatment of this oil with hot methanol gave one of the isomers of **5c** as white needles. The configuration of the carbomethoxy group about C-6 has not been determined. All attempts to isolate the other major reaction product were unsuccessful.

When the aminooxazoline **1** was allowed to react with diethyl maleate, two major reaction products were observed by tlc. Chromatography on silica gel separated the major components from minor contaminants. The residual oil, after solvent removal, failed to crystallize from ethanol; however, when the oil was treated with hot methanol and allowed to stand at room temperature, white needles precipitated. This material was not the expected product **5d**, but the corresponding methyl ester **5c** as shown by ir, nmr, melting point, and mixture melting point. Apparently transesterification had occurred. More noteworthy is the fact that both the fumarate and the maleate add to the aminooxazoline **1** to give the same major product or, at least, a mixture of the two C-6 epimers. Very careful tlc (silica gel, 5% MeOH in $HCCl_3$ eight passes) of the analytically pure product from the reaction of **1** with **4c** and with **4d** indicated the presence of a small amount (~10–20%) of a second component with a similar R_f value. This minor component may well be the other epimer, but it could not be detected by nmr at 100 MHz. The mechanistic implications of this product distribution will be discussed later.

Spectral Properties. Infrared.—The ir spectrum of each of the anhydro-5,6-dihydrouridines **5a**, **5b**, and **5c** showed three absorptions which are apparently due to the system


$$-O-C{=}N-C{=}O$$

These frequencies and intensity of these absorptions are given in Table I. It should be noted that the only other anhydrodihydrouridine reported[4] in the literature O^2,5′-anhydro-5,6-dihydrouridine absorbed at 1681 and 1546 cm^{-1}. The relative intensities of the peaks at 1690 and 1605 cm^{-1} are unusual and apparently characteristic of this system.

Table I

Compd	Frequency, cm^{-1}		
5a	1690 (m)	1605 (vs)	1490 (sh)
5b	1690 (m)	1605 (vs)	1490 (sh)
5c	1685 (m)	1610 (vs)	1490 (sh)

Nmr Spectra.—Nmr spectra were measured at 60 and 100 MHz on samples dissolved in deuterium oxide or d_6-dimethyl sulfoxide and calibrated against internal tetramethylsilane.

Compound **5a** showed the expected signals for the arabinoside portion plus A_2B_2-type triplets for the cyclic methylenes of the dihydrouracil. The apparent vicinal coupling constant of 8.0 Hz indicated either a deceptively simple AA′BB′ system or a rapidly averaging AA′BB′ system. The parameters are listed in Table II. Compound **5b** gave an nmr spectrum that indicated it was a mixture. In addition to the typical signals for the arabinoside portion there were two sets of ABC patterns assigned to the uracil 5 and 6 hydrogens of two epimers **5b**-I and **5b**-II present in amounts of 55 and 45%. Both epimers showed a large diaxial vicinal coupling for the 5 hydrogen suggesting conformations having the 5 methyl exclusively equatorial in each. The parameters are listed in Table II.

Table II

Nmr Data Determined on Anhydrodihydro Nucleosides

Parameter	Shift, δ			
	5a[a]	**5b** major[b]	**5b** minor[b]	**5c**[a]
$\delta_{1'}$	5.88	6.07		6.04
$J_{1',2'}$	5.5	5.5		5.5
$\delta_{2'}$	5.08	5.32		5.23
$J_{2',3'}$	0.5[c]	0.5[c]		0.5[c]
$\delta_{3'}$	4.32	4.57		4.38
$J_{3',4'}$	1.0[c]	2.0		2.0
$\delta_{4'}$	4.03[d]	4.3[d]		4.09[e]
$J_{4',5'a}$	6.0	4.5		6.0
$J_{4',5'b}$				4.0
$\delta_{5'a}$	3.32[i]	3.59[f]		3.38[g]
$\delta_{5'b}$				3.49[g]
$J_{5'a,5b'}$				−12.5
$\delta_{5_{ax}}$	2.45[h]	2.82[i]	2.79[i]	2.65[g]
$J_{5_{ax},5_{eq}}$				−16.6
$J_{5_{ax},6_{ax}}$	8.0	12.5	12.5	8.6
$J_{5_{ax},6_{eq}}$	8.0	7.0	7.0	
$\delta_{5_{eq}}$	2.45[h]			2.84[g]
$J_{5_{eq},6_{ax}}$	8.0			4.4
$J_{5_{eq},6_{eq}}$	8.0			
$\delta_{6_{ax}}$	3.57[h]	3.40[j]	3.38[j]	4.80
$J_{6_{ax},6_{eq}}$		−12.0	−12.0	
$\delta_{6_{eq}}$	3.57[h]	3.87[j]	3.83[j]	

[a] 60-MHz spectrum, DMSO solvent, TMS reference. [b] 100-MHz spectrum, D_2O solvent, DSS reference. [c] Unresolved. Estimated from line width. [d] Apparent as two partly overlapped triplets of an A_2B subspectrum but probably actually a deceptive AA′B subspectrum. [e] Two four-line, partly overlapped X patterns of ABX subspectrum. [f] Apparent as a doublet of an A_2B subspectrum but probably actually a deceptive AA′B subspectrum. [g] Eight lines for the AB part of an ABX subspectrum. [h] Apparent as a triplet of an A_2B_2 system but probably actually a deceptive AA′BB′ system. [i] Two four-line partly overlapped C patterns of ABC system. [j] Eight lines for the AB part of an ABC system.

Compound **5c** gave an nmr spectrum that showed no evidence of a mixture. In addition to the usual signals for the arabinoside portion there was one ABX subspectrum attributed to the three uracil hydrogens. The parameters are listed in Table II.

To determine the conformation of the uracil portion the ABX subspectrum was analyzed[6] with the aid of the LAOCN program.[7] The spectrum was first factored by construction methods to determine the line number

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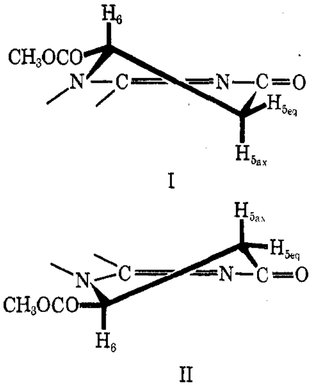
(6) G. Slomp, *Appl. Spectrosc. Rev.*, **2**, 263 (1969).

(7) S. Castellano and A. A. Bothner-By, *J. Chem. Phys.*, **41**, 3863 (1964).

assignments. The observed line frequencies were then fitted by iteration and the root mean square error of the fit was reduced from 0.393 to 0.069. The parameters are shown in Table II. The spectrum was judged to be a like-sign narrow-coupled case from the agreement of line intensities in the observed and calculated spectra. The magnitude of the 5_{ax},6 coupling constant indicates that these vicinal hydrogens are diaxial; hence the structure is either **5c**-I or **5c**-II and a choice cannot be made from these data.



5c

Circular Dichroism.—The CD spectrum of **5a**, **5b**, and **5c** were measured and the results are tabulated in Table III. Although the CD spectra showed what appeared to be characteristic differences, sufficient data to assign the absolute configuration was not available.

TABLE III

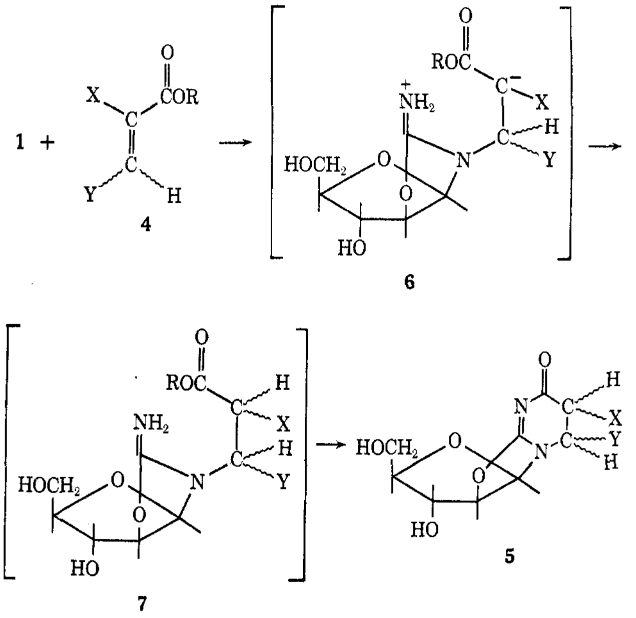
CIRCULAR DICHROISM SPECTRAL DATA

Compd	λ_{max}, mμ	$[\theta]_{max}$
5a	235	+11,800
5a	255	+11,400
5b	235	+23,700
5c	256.5	−9,350
5c	230	+48,500

Discussion

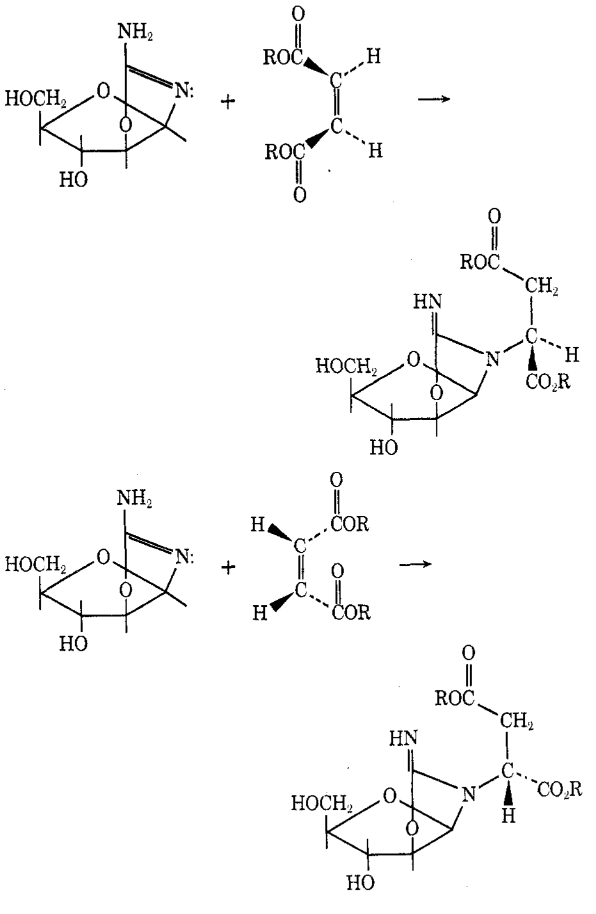
A mechanism which is consistent with the product formation and with the known mode of addition of the aminooxazoline **1** to activated triple bonds[5,8] is presented in Scheme III. The endocyclic nitrogen of the aminooxazoline **1** adds to the β carbon of the activated double bond in a Michael-type addition, followed by proton migration and ring closure to give the observed products. The anion generated at the 5 carbon in the intermediate **6** is protonated nonstereoselectively. This results in an almost equimolar mixture of the two epimers of **5b**. An alternate, but less likely, explanation for the formation of the epimeric mixture is the loss of the configurational identity of the product during work-up and isolation. The inverse mode of ring formation, *i.e.*, addition of the exocyclic amine to the ester carbonyl followed by ring closure, would also explain the observed products **5a–c**. This mechanism seems unlikely in view of the regiospecificity generally observed in the addition of amines to α,β-unsaturated esters.[9]

SCHEME III



The apparent stereoselectivity observed in the formation of **5c** and **5d** can be accounted for in one of several fashions. (1) The aminooxazoline **1** must approach the ester in a preferred fashion—from either the front or the back. The direction of approach is determined by the carboxyl group attached to the β carbon since the product ratio from the maleate and fumarate is the same. This is shown schematically in Scheme IV. (2) The initial adduct **7** (Scheme III)

SCHEME IV



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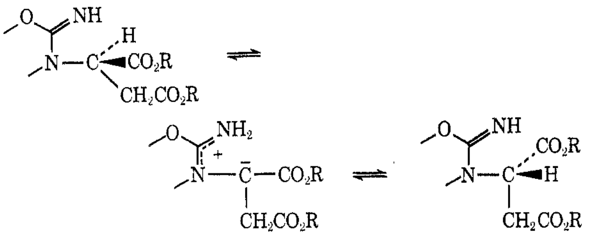
(8) C. M. Hall and A. J. Taylor, unpublished work.

(9) S. Patai and Z. Rapport in "The Chemistry of Alkenes," S. Patai, Ed., Interscience, New York, N. Y., 1964, Chapter 8.

undergoes epimerization to give an epimeric mixture in which one of the epimers predominates. Epimerization is followed by cyclization to give the observed distribution of diastereomers. Epimerization at C-6 might occur as shown in Scheme V.[10] (3) The product distribution of diastereomers may represent the equilibrium mixture which was achieved during the product isolation. It is not apparent from models that one diastereomer should be favored over the other.

SCHEME V



Experimental Section

O_2,2-Anhydro-5,6-dihydrouridine (5a). A mixture of 2-amino-β-D-arabinofurano[1′,2′:4,5]-2-oxazoline (1, 2.0 g, 0.0115 mol), methyl acrylate (4.0 g, 0.044 mol), and dimethylacetamide (70 ml) was heated at 75° for 2 hr, then allowed to stand for 18 hr at room temperature. Removal of the solvent left a glassy residue which failed to crystallize. The residue was chromatographed on a silica gel column (200 g). The column was eluted with 1 l. of 10% MeOH in CH_2Cl_2 and 2 l. of 15% MeOH in CH_2Cl_2. The majority of the product was eluted in the first 750 ml of 15% MeOH in CH_2Cl_2 eluate. Removal of the solvent left a solid which was recrystallized from absolute ethanol to give white needles (1.30 g, mp 177–179°). The total yield was 57%. The ir, nmr, and uv spectra were consistent with the proposed structure of O^2,2′-anhydro-5,6-dihydrouridine: the ir and nmr results are reported in Tables I and II; uv max (95% EtOH) 237 nm (ε 14,150), 273 (114, sh).

Anal. Calcd for $C_9H_{12}N_2O_5$: C, 47.37; H, 5.30; N, 12.28. Found: C, 47.40; H, 5.40; N, 12.04.

O^2,2′-Anhydro-5-methyl-5,6-dihydrouridine (5b). A mixture of the aminooxazoline 1 (2.0 g, 0.0115 mol), methyl methacrylate (4.0 g, 0.040 mol), and dimethylacetamide (70 ml) was heated at 70° for 3 hr and then it was allowed to stand at room temperature for 18 hr. Tlc (silica gel, 25% MeOH in $CHCl_3$) indicated that some aminooxazoline 1 was still present. An additional 4 g of methyl methacrylate was added, and the reaction mixture was heated an additional 8 hr at 75°. The solvent was removed to leave a glassy residue which was slurried in MeOH. An insoluble solid was collected by filtration (844 mg). This material was shown to be identical with the starting aminooxazoline 1 by tlc (silica gel 25% MeOH in $CHCl_3$). The residue from the filtrate was chromatographed on a silica gel column (100 g) using 10% MeOH in CH_2Cl_2 as the eluent. The desired product began to appear in the eluate after about 1.0 l. had been collected. The next 2 l. of eluate were combined and the solvent was removed to leave a crystalline solid (670 mg). This material was recrystallized from isopropyl alcohol to give a white solid in 36% yield (based on recovered 1, 582 mg, mp 196.5–198°). The nmr, ir, and uv spectra were consistent with the proposed structure **5b**: the ir and nmr results are reported in Tables I and II; uv (95% EtOH) 236 nm (ε 13,900), 273 (164, sh).

Anal. Calcd for $C_{10}H_{14}O_5N_2$: C, 49.58; H, 5.83; N, 11.57. Found: C, 49.72; H, 5.88; N, 11.51.

Reaction of 2-Amino-β-D-arabinofurano[1′,2′:4,5]-2-oxazoline (1) with Dimethyl Fumarate. A mixture of the aminooxazoline 1 (4.0 g, 0.023 mol), dimethyl fumarate (6.4 g, 0.044 mol), and dimethylacetamide (140 ml) was heated at 95–100° for 6 hr and then was allowed to stir at room temperature for 72 hr. Tlc (silica gel, 15% MeOH in CH_2Cl_2, uv or $KMnO_4$ solution) showed that considerable starting material remained. An additional 6.4 g of dimethyl fumarate was added and the mixture was heated for an additional 8 hr at 95–100°. Removal of the solvent left an oil which was chromatographed on a silica gel column (400 g). The column was eluted with 4 l. of 5% MeOH in CH_2Cl_2 followed by 1 l. of 15% MeOH in CH_2Cl_2 at which point a uv-absorbing material began to appear in the eluate (tlc, silica gel, 5% MeOH in $HCCl_3$). Ten 60-ml fractions were collected. Removal of the solvent from these fractions left an oil (1.99 g combined), which failed to crystallize. The oil was rechromatographed on silica gel (200 g) using 5% MeOH in CH_2Cl_2. After 6 l. of eluate had been eluted, 20 150-ml fractions were collected. Fractions 1–15 were combined and the residual oil crystallized from methanol to give white needles (225 mg, mp 185–186° dec). A second crop of 322 mg was also obtained (mp 185–186° dec; total yield 8%). The ir, uv, and nmr spectra were consistent with those expected for O^2,2′-anhydro-6-carbomethoxy-5,6-dihydrouridine (**5c**): the ir and nmr results are reported in Tables I and II (the $J_{5,6}$ indicated that the carbomethoxy group assumed an equatorial position, but the configuration about C-6 could not be determined with the available data); uv max (95% EtOH) 236 nm (ε 13,400), 269.5 (135, sh).

Anal. Calcd for $C_{11}H_{14}O_7N_2$: C, 46.15; H, 4.93; N, 9.79. Found: C, 46.47; H, 5.36; N, 10.21.

Reaction of 2-Amino-β-D-arabinofurano[1′,2′:4,5]-2-oxazoline with Diethyl Maleate. A mixture of the aminooxazoline 1 (4.0 g, 0.023 mol), diethyl maleate (7.60 g, 0.044 mol), and dimethylacetamide (140 ml) was heated at 95° for 6 hr and was stirred at room temperature for 18 hr. An additional 7.6 g of diethyl maleate was added and the reaction mixture was heated at 95° for 4 hr. Removal of the solvent left a viscous syrup. The excess diethyl maleate was separated by chromatography on silica gel (400 g, 2 l. of 5% MeOH in $CHCl_3$ followed by 2 l. of 15% MeOH in $CHCl_3$). The fractions which contained the reaction products (one minor and two major spots on tlc, 2% MeOH in $CHCl_3$) were combined and rechromatographed (silica gel, 200 g, MeOH in CH_2Cl_2). Tlc of the eluted fractions indicated that no significant degree of separation had been achieved. The fractions were combined and the solvent was removed to leave a syrup (3.52 g). This residue was dissolved in hot methanol and the resulting solution was allowed to stand for 18 hr in a draft to give colorless needles (395 mg, mp 183.5–184.5° dec). A portion of this material was recrystallized for analysis. Several additional crops were obtained from the mother liquor (887 mg, mp 186–187° dec), whose spectral properties were identical with those of the first crop. The total yield was 19%. The ir, nmr, and uv spectrum and elemental analysis indicated that this material was not the expected O^2,2′-anhydro-6-carboethoxy-5,6-dihydrouridine (**5d**), but the corresponding methyl ester: the ir and nmr results are reported in Tables I and II; uv max (95% EtOH) 236 nm (ε 14,350), 269 (180, sh). The melting point of an admixture of this material and the product obtained from the reaction of the aminooxazoline 1 and dimethyl fumarate was undepressed. Both materials had identical R_f values on tlc (silica gel, 2% MeOH in $CHCl_3$). Nmr showed no evidence for **5d** in the crystallized material.

Anal. Calcd for $C_{11}H_{14}N_2O_7$: C, 46.15; H, 4.93; N, 9.79. Found: C, 46.32; H, 4.95; N, 9.65.

Registry No.—**5a**, 35324-11-9; **5b**, 35324-12-0; **5c**, 35324-13-1.

Acknowledgment.—We are indebted to Dr. W. C. Krueger and his coworkers for the CD and uv measurements and to Mr. P. Meulman for measuring and interpreting the ir spectra.

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[10] We are indebted to the referee for first suggesting this possibility.