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Pyrimidine Nucleosides. IX. Facile Synthesis of 1-β-D-Lyxofuranosyluracil *via* 2,3′-Anhydrolyxosyl Intermediates[1,2]

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RECEIVED DECEMBER 3, 1960

1-β-D-Lyxofuranosyluracils were synthesized in high yield by simply refluxing 2,2′-anhydro-1-(3′-*O*-mesyl-β-D-arabinofuranosyl)-uracil derivatives in water.—The mechanism of the reaction has been established to proceed *via* 3′-*O*-mesylarabinofuranosyluracil (VII), and 2,3′-anhydrolyxofuranosyluracil (VIII) intermediates. The synthesis of a crystalline 2,3′-anhydro-1-(5′-*O*-mesyl-β-D-lyxofuranosyl)-uracil (VIIIa) is described.

Two of the four possible 1-β-D-aldopentofuranosylthymine isomers, namely those of the ribose and xylose configuration, were synthesized by the mercuri method[4] (*i.e.*, condensation of dithyminyl mercury with a suitably blocked aldopentosyl halide). A complementary method involving the inversion of the 2′-carbon of the sugar moiety through the formation of 2,2′-anhydro intermediates[5] was used in the synthesis of 1-β-D-arabinofuranosylthymine[6] and 1-β-D-lyxofuranosylthymine.[7] It is probable that condensation involving the halogenose of the lyxo or arabino configuration with dithyminyl mercury would have yielded the α-anomers.[8,9]

A recent paper[11] reported a versatile intermediate in the synthesis of various nucleoside derivatives of uracil. Trimesyloxyuridine (I, Fig. 1), obtained in near quantitative yield from uridine, was converted to both xylosyl- and arabinosyluracil by the action of sodium benzoate in N,N-dimethylformamide (DMF), followed by cleavage of the blocking groups. The present paper describes the synthesis of the fourth isomer, 1-β-D-lyxofuranosyluracil (VI), from this useful intermediate.

As reported previously,[11] treatment of I with an equivalent of sodium hydroxide under mild conditions gave 2,2′-anhydro-1-(3′,5′-di-*O*-mesyl-β-D-arabinosyl)-uracil (IIa) in high yield.

It was found that when IIa was simply refluxed in water for about 1 hour, the characteristic twin-peak ultraviolet spectrum of IIa (maxima at 247.5 and 224 mμ) gave way to a spectrum similar to that of uridine with a single maximum at about 260 mμ. Titration of the resulting solution indicated the evolution of about one equivalent of methylsulfonic acid. A crystalline monomesyloxypentosyluracil was isolated in good yield. This compound consumed rapidly one equivalent of metaperiodate, consistent with the presence of *cis* vicinal hydroxyl groups.[4] That this compound is 1-(5′-*O*-mesyl-β-D-lyxofuranosyl)-uracil (IIIa) is supported by the reactions shown in Fig. 1.

Compound IIIa readily formed in high yield an isopropylidene derivative, IV, in accordance with the presence of *cis* vicinal hydroxyls. The mesyloxy group of IV was smoothly replaced by warming with sodium benzoate in DMF[11,12] to yield the 5′-*O*-benzoyl compound V. Acid treatment of V removed the isopropylidene group to give IIIb, which upon saponification yielded a pentosyluracil, VI, that differed from the other three known 1-β-D-aldopentofuranosyluracils, the arabino,[5,11] ribo and xylo.[11] Treatment of compound VI with metaperiodate yielded a dialdehyde whose rotation of + 15° was nearly identical with the oxidation product given on similar treatment by the known 1-β-D-aldopentofuranosyluracils.[11] These data, supported by spectrophotometric, electrophoretic and polarimetric studies, permit the assignment of the 1-β-D-lyxofuranosyluracil structure VI to the product. Thus, there can be no question that IIa, on simply boiling in water, gave IIIa.

A more direct route to the synthesis of 1-β-D-lyxofuranosyluracil (VI; see Fig. 1) utilized the 5′-*O*-benzoyl derivative IIb as starting material. Refluxing an aqueous solution of IIb, which may be readily obtained in high yield from I,[11] gave VI in excellent yield *via* intermediate IIIb. Thus, uridine may be converted to 1-β-D-lyxofuranosyluracil in 60% yield *via* intermediates I, IIb and IIIb.

The reaction II to III (Fig. 2) was extended to the synthesis of the 5′-deoxy derivative IIIc, which was obtained in quantitative yield from the previously reported[11] IIc by refluxing in water. It is thereby shown that this conversion, II to III, occurs independently of the 5′-substituent.

The formation of lyxosyl derivatives III from II (Fig. 2) is unusual from several viewpoints. The reaction involves the removal under mild conditions of a secondary mesyloxy group. This mesyloxy group is replaced with net inversion by a hydroxyl group in an acid medium. The reaction proceeds stereospecifically to a 2′,3′-*cis* configuration. These unusual features prompted an investigation of its mechanism.

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, Public Health Service (Grant No. CY-3190) and from the Ann Dickler League.

(2) A preliminary report of this work has appeared.[3]

(3) R. Fecher, J. F. Codington and J. J. Fox, Abstracts, 137th Meeting, Am. Chem. Soc., 1960, p. 3-D.

(4) J. J. Fox, N. Yung, J. Davoll and G. B. Brown, *J. Am. Chem. Soc.*, **78**, 2117 (1956).

(5) D. M. Brown, A. Todd and S. Varadarajan, *J. Chem. Soc.*, 2388 (1956).

(6) J. J. Fox, N. Yung and A. Bendich, *J. Am. Chem. Soc.*, **79**, 2775 (1957).

(7) J. J. Fox, J. F. Codington, N. Yung, L. Kaplan and J. O. Lampen, *ibid.*, **80**, 5155 (1958).

(8) B. R. Baker, "Ciba Foundation Symposium, Chem. and Biol. of Purines," 1957, p. 120.

(9) A discussion of the synthesis of nucleosides is found in a recent review.[10]

(10) J. J. Fox and I. Wempen, *Advances in Carbohydrate Chem.*, **14**, 283 (1959).

(11) J. F. Codington, R. Fecher and J. J. Fox, *J. Am. Chem. Soc.*, **82**, 2794 (1960).

(12) E. G. Reist, L. Goodman and B. R. Baker, *ibid.*, **80**, 5775 (1958).

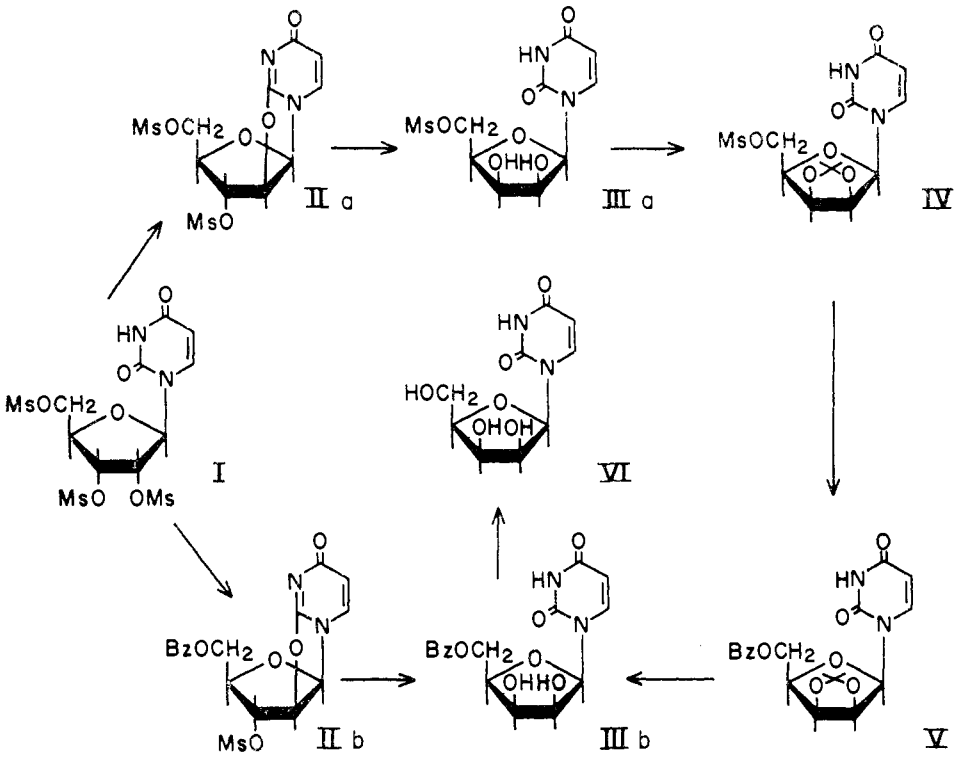

Fig. 1.

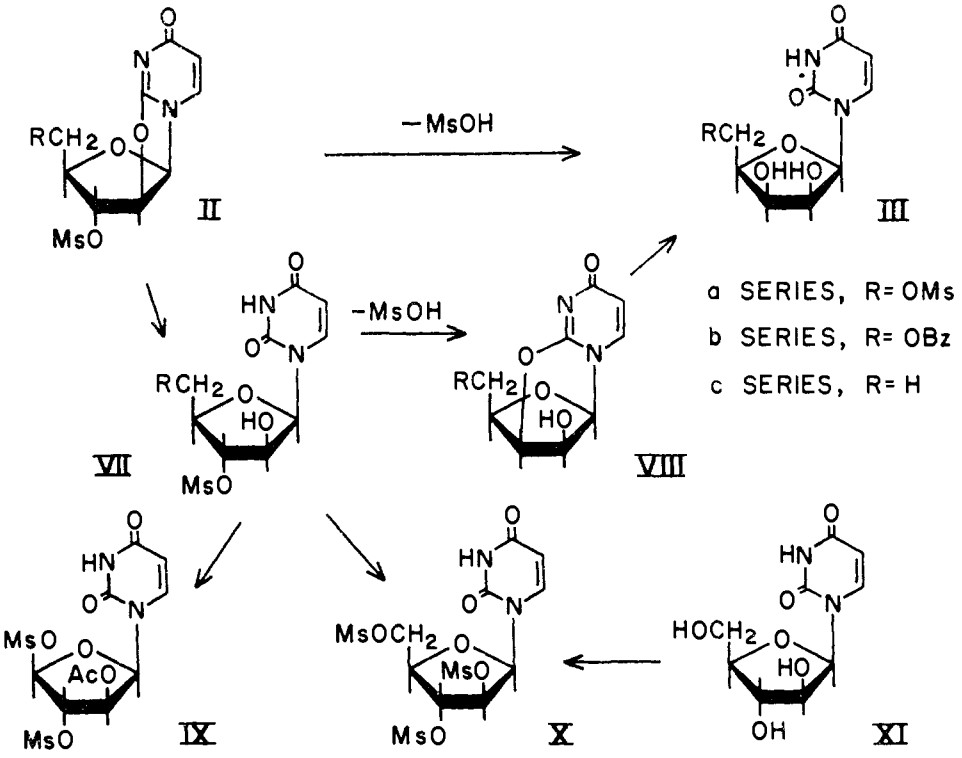

Fig. 2.

The data presented below establish the mechanism shown in Fig. 2 of II → VII → VIII → III. There is an initial cleavage of the 2,2′-anhydro group of II to give VII, a 3′-*O*-mesylarabinosyluracil derivative. Attack by the 2-carbonyl of the pyrimidine ring on C3′ of VII displaces the mesyloxy group with inversion to give VIII, a 2,3′-anhydrolyxofuranosyluracil derivative. Rupture of the 2,3′-anhydro bond of VIII under the acidic conditions of the reaction yields III.

It was possible to demonstrate the existence of the intermediate VIIIc (Fig. 2). It is clear that

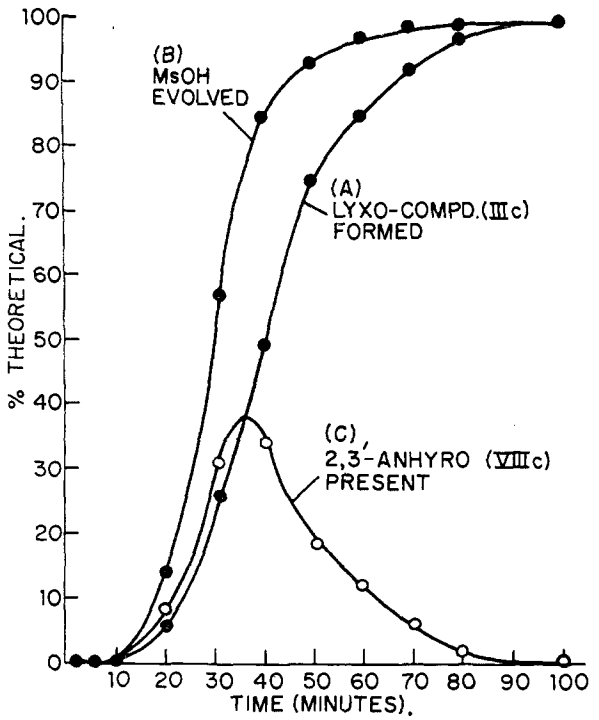

Fig. 3.—Studies of the reaction IIc to IIIc: curves (A) and (B) represent rate curves for the reaction; curve (C), the difference between curves (B) and (A), represents the existence of the intermediate VIIIc.

the amount of VIIIc present during the reaction of IIc to IIIc is due to the rate difference in the reactions VIIIc to IIIc and VIIc to VIIIc. An expression of the extent of the conversion of VIIc to VIIIc is given by the quantity of methylsulfonic acid evolved. An expression of the progress of the reaction VIIIc to IIIc is given by the amount of lyxo compound formed. Measurement of these quantities during the course of the reaction permitted the calculation of the molar concentration of VIIIc present. These measurements were made by the titration of aliquots, withdrawn periodically in the conversion of IIc to IIIc with: (1) alkali (methyl red indicator) to determine methylsulfonic acid formed and (2) sodium metaperiodate[4] to determine lyxo compound formed. The results are given as rate curves in Fig. 3.

It is clear from Fig. 3 that, at 100 minutes, curves (A) and (B) merge in accordance with the stoichiometry of the quantitative reaction IIc to IIIc. At earlier time intervals the rate curve for lyxo formation, (A), lags behind (B), the rate curve for the liberation of methylsulfonic acid. This lag can best be explained by the existence of a nonmesylated intermediate which is blocked at the 2'- or 3'-position so as to be unaffected by metaperiodate. It will be shown below that this intermediate is indeed VIIIc. The concentration of VIIIc as a function of time is given by curve (C) (Fig. 3) which is obtained by plotting the difference between curve (B) and curve (A) at various intervals during the course of the reaction. From curve (C) the maximum concentration of VIIIc is found to exist at 30 to 40 minutes after the beginning of the reaction.

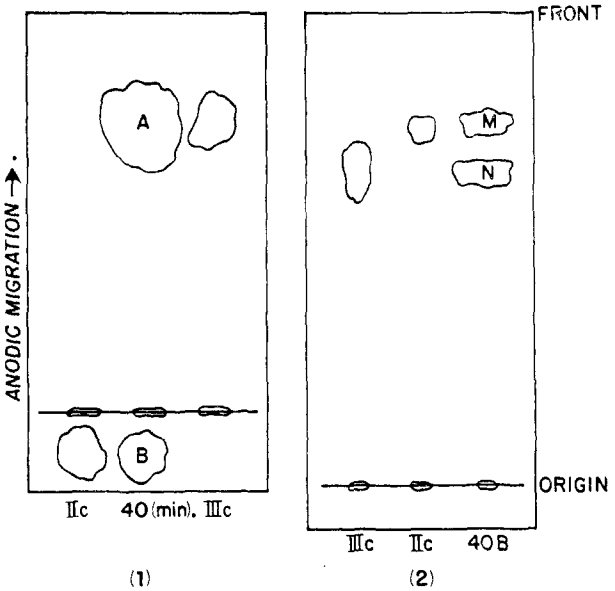

Fig. 4.—Studies of the reaction IIc to IIIc. Separation of the intermediates M and N present in the 40-minute aliquot by (1) ionophoresis (borate buffer, *p*H 6.0), followed by (2) paper chromatography (isopropyl alcohol–water, 1:1) of the eluted spot 40B of (1).

The paper electrophoretic separation of the 40-minute aliquot in borate buffer, *p*H 6.0,[11,13] as shown in Fig. 4 (1) effectively removed the lyxo product IIIc (spot A) from the other components (spot B). The eluted spot, 40-B (Fig. 4 (1)) was resolved by paper chromatography into two intermediates, M and N, as shown in Fig. 4 (2). By comparison of the properties of these spots (migration and spectral properties, after elution, as described below) with the properties of the starting material IIc, it was found that after 40 minutes no IIc was present in the reaction mixture. The identical migration behavior of N and IIIc required the prior use of ionophoresis (Fig. 4 (1)) in the separation.

The ultraviolet spectra of these intermediates after elution from the paper are given in Fig. 5, along with those of the other components of the reaction. The spectrum of the upper spot, M, is similar to that of the nucleoside IIIc but with a slight hypsochromic shift of its maximum, consistent with the presence of a mesyloxy group[14] (see Table I). The intermediate M has been shown to be of structure VIIc as shown in Fig. 2, as will be demonstrated below.

The lower spot N, as shown in Fig. 4 (2), has a spectrum similar to that of 2,2'-anhydrouridine derivatives, for example IIc (see Fig. 5). The spectrum exhibits two maxima, at 250 and 224 mμ. Although there is a marked spectral similarity between this intermediate and 2,2'-anhydro derivatives, it will be shown below that N is the 2,3' anhydronucleoside VIIIc. Indeed, it has been

(13) M. P. Gordon, O. M. Intrieri and G. B. Brown, *J. Am. Chem. Soc.*, **80**, 5161 (1958).

(14) The presence of mesyloxy groups in the sugar moiety has been found to affect the chomophoric pyrimidine group and cause hypsochromic shifts in the maxima. Table I gives the maxima and extent of the hypsochromic shift for a number of mesylated nucleoside derivatives.

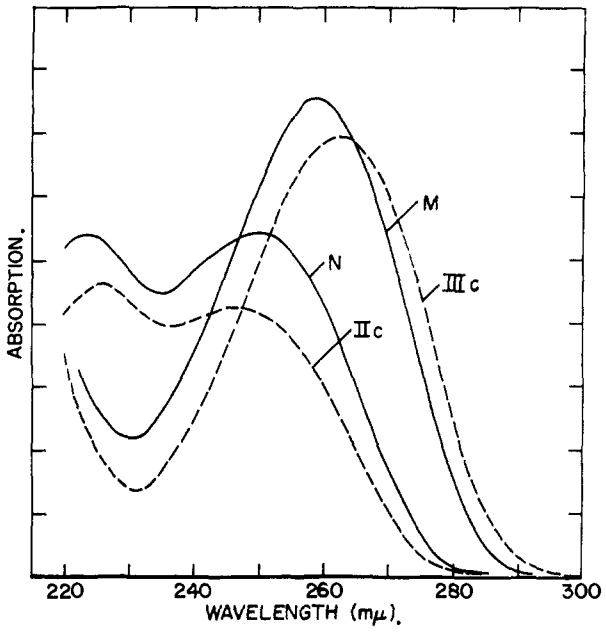

Fig. 5.—Studies of the reaction IIc to IIIc. Ultraviolet absorption properties of reaction intermediates M and N compared with those of IIc and IIIc.

found that spectra of 2,2′-anhydro- and 2,3′-anhydropentosyl derivatives of uracil have the same general characteristics (see Fig. 6).

TABLE I

SPECTRAL PROPERTIES IN THE ULTRAVIOLET OF MESYLOXY DERIVATIVES OF 1-β-D-ALDOPENTOFURANOSYLURACILS (IN WATER)

Nucleoside	λ_{max}, mμ	λ_{min}, mμ	Ratio (max./min.)	Hypsochromic shift (max.)
Pentosyluracils	262	230	4.8–5.0	0
5′-O-Ms-lyxo (IIIa)	261	230	4.9	1
3′-O-Ms-ribo[21]	259	229	4.8	3
3′,5′-Di-O-Ms-arab (VIIa)	260	229	4.4	2
2′-O-Ac-3′,5′-di-O-Ms-arab (IX)	257.5	228	4.2	4.5
2′,3′,5′-Tri-O-Ms-ribo (I)	256	227.5	3.5	6
2′,3′,5′-Tri-O-Ms-arab (X)	257	227.5	4.3	5
2,2′-Anhydropentosyluracils				
Arab[5]	250 (223)	235	1.3	0
3′,5′-di-O-Ms-arab (IIa)	247.5 (224)	234	1.2	2.5

2,3′-Anhydro derivatives of 1-β-D-aldopentofuranosyluracils had not been reported previously. Attempts to form compounds containing a 2,3′-anhydro bridge from 3′-tosyloxyuridine under various conditions[15] had been unsuccessful. A satisfactory explanation for this phenomenon, however, has not been forthcoming. Indeed, it has been particularly difficult to explain this fact in view of the ease and selectivity of formation of 2,3′-anhydro-1-(2′-deoxy-5′-O-mesyl-β-D-lyxosyl)-thymine from 3′,5′-di-O-mesylthymidine.[16]

The mechanism shown in Fig. 2 proposes the ready formation of a 2,3′-anhydro intermediate, VIII. Proof that VIII possesses a 2,3′-anhydro bond is described below. It was found that when a solution of IIa (Fig. 2) in acetone–water (1:1)

(15) D. M. Brown, D. B. Parihar, A. Todd and S. Varadarajan, *J. Chem. Soc.*, 3028 (1958).

(16) A. M. Michelson and A. R. Todd, *ibid.*, 816 (1955).

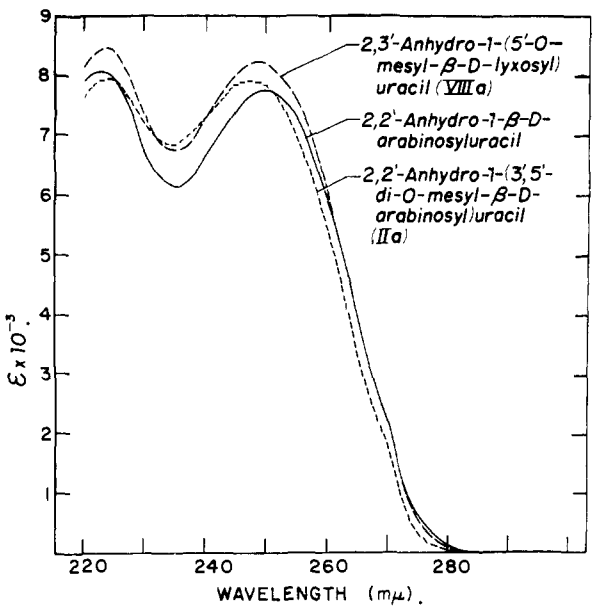

Fig. 6.—Ultraviolet absorption curves of 2,2′-anhydro- and 2,3′-anhydronucleosides in water.

was stirred with Dowex 50 (H^+ form) for 6 days at room temperature, cleavage of the 2,2′-anhydro bond was effected. Chromatographic separation in two solvent systems showed the product to be homogeneous and identical in migration behavior to a nucleoside intermediate observed in the course of the conversion of IIa to IIIa. This material was isolated as an amorphous solid, VIIa, which has resisted all attempts at crystallization. That this substance is 1-(3′,5′-di-O-mesyl-β-D-arabinosyl)-uracil (VIIa) is shown by the reactions described in Fig. 2.

Acetylation of VIIa by acetic anhydride afforded a 70% yield of crystalline 1-(2′-O-acetyl-3′,5′-di-O-mesyl-β-D-arabinosyl)-uracil (IX). The structure of IX, and therefore of VIIa, was established by mesylation of VIIa to a tri-O-mesyl nucleoside X. The physical properties, including optical rotation, of this compound X were identical with those of 1-(2′,3′,5′-tri-O-mesyl-β-D-arabinosyl)-uracil, prepared by the exhaustive mesylation of 1-β-D-arabinofuranosyluracil (XI). It is concluded, therefore, that VII has the arabino configuration,[17] and that the conversion of IIa to VIIa occurs in accordance with the well-documented "aryl-oxygen" fission observed in the cleavage of 2,2′-anhydronucleosides in acid media.[5,10] It should be noted that this proof of structure of VIIa constitutes firm chemical support for the previously reported[11] structure of IIa as a 2,2′-anhydronucleoside. Further evidence that compound VIIa is an intermediate in the reaction of IIa to IIIa was gained by its conversion in good yield to IIIa by refluxing in water.

It is inconceivable that VIIa could yield IIIa except *via* the 2,3′-anhydro intermediate VIIIa; and, indeed, it was found possible to convert VIIa

(17) A hypothesis involving a mesyloxy migration was suggested previously[3] for the conversion of II to III *via* a second 2,2′-anhydro intermediate. The demonstration of VII as a 3′-mesyloxyarabinosyl derivative rules out this hypothesis.

in good yield to VIIIa. Refluxing VIIa in dilute aqueous solution and periodically adjusting the *p*H with dilute alkali to *p*H 5 (to prevent cleavage of the anhydro bond) converted VIIa in 30 to 40% yield to the crystalline 5′-monomesyloxy derivative VIIIa plus some IIIa (due to anhydro cleavage); VIIIa could be readily converted to 1-(5′-*O*-mesyl-β-D-lyxofuranosyl)-uracil (IIIa) by refluxing in dilute acid.

The ultraviolet absorption spectrum of VIIIa is shown in Fig. 6. It exhibits two maxima, at 249 and 223 mμ. Such a twin-peaked spectrum is characteristic of 2,2′-anhydro derivatives and 2-alkoxyluridines.[18] The spectra of 2,2′-anhydro-1-(β-D-arabinofuranosyl)-uracil[5,19] and 2,2′-anhydro-1-(3′,5′-di-*O*-mesyl-β-D-arabinosyl)-uracil (IIa) are presented in Fig. 6 for comparison.

In contrast, it is to be noted that 2,5′-anhydro derivatives of 2′,3′-*O*-isopropylidene- and 2′,3′-di-*O*-acetyluridine are reported to have markedly different spectral properties,[18] a single maximum at 237–238 mμ. Observation of the vastly different spectral properties of 2,2′-anhydro and 2,5′-anhydro nucleosides led Brown and co-workers[18] tentatively to suggest that the 2,5′-anhydro derivative possessed a strained ring system. It would be consistent with this suggestion to conclude that in view of the spectral similarity between the 2,2′- and 2,3′-anhydro derivatives no significant difference in strain exists between the 6-membered ring of 2,3′-anhydro nucleosides and the 5-membered ring of their 2,2′-anhydro isomers.[20] This would be true only insofar as the strain effects electronic dislocations in the pyrimidine ring and thus affects the ultraviolet absorption spectra. Work designed to compare the relative stability of the 2,2′-anhydro and 2,3′-anhydro ring systems toward chemical reagents is underway in this Laboratory.[21]

Experimental[24,25]

1-(5′-O-Mesyl-β-D-lyxofuranosyl)-uracil (IIIa). Method A. From 2,2′-Anhydro-1-(3′,5′-di-*O*-mesyl-β-D-arabinosyl)-uracil (IIa).—A solution of 5.09 g. (0.013 mole) of IIa in 500 ml. of water was refluxed for 1 hour. The course of the reaction was observed spectrophotometrically, observing a shift of the characteristic spectrum of IIa (λ_{max}) 247.5, 224 mμ) to that of a nucleoside (λ_{max} 260 mμ).

The reaction flask was cooled, and the evolved acid was titrated. This required about one equivalent (methyl red) of sodium hydroxide.[26] The volume was reduced *in vacuo* to 25–50 ml. After refrigeration, the crystals were collected and washed with a little cold water, cold ethanol and ether. The material, m.p. 175.5–177° (dec., uncor.), weighed 1.68 g. An additional 0.66 g. was obtained from the mother liquor. The total yield was 55%. The material was crystallized from water (4 g. per 100 ml.) to yield colorless prisms, m.p. 169–170° dec. (sinters at 159°), $\alpha^{22}D$ + 87° ± 2° (water, *c* 0.4); periodate consumption, 0.98 mole/mole (within 4 minutes).

Anal. Calcd. for $C_{10}H_{14}N_2O_5S$: C, 37.2; H, 4.38; N, 8.69; S, 9.95. Found: C, 37.0; H, 4.43; N, 8.66; S, 10.1.

Method B. From 1-(3′,5′-Di-*O*-mesyl-β-D-arabinosyl)-uracil (VIIa).—Refluxing of VIIa in water (1 g./100 ml.) for about 1 hour afforded a 30–40% yield[26] of IIIa. The work-up and isolation of IIIa was the same as that described in method A. The product was shown to be identical (optical rotation, spectral properties and mixed melting point) with that obtained by method A.

Method C. From 2,3′-Anhydro-1-(5′-*O*-mesyl-β-D-lyxofuranosyl)-uracil (VIIIa).—A solution of 0.011 g. of VIIIa and 1.5 ml. of 0.021 *N* methylsulfonic acid was refluxed for 1 hour. The spectrum of the resulting solution was that of a nucleoside (λ_{max} 262 mμ, and λ_{min} 230 mμ). The migration of the product(s) was compared with that of a sample of IIIa, using the apparatus and techniques previously described[11] (paper electrophoresis, borate buffer, *p*H 6.0). The anodic migration (5 hours, 700 volts, 11.2 milliamp.) of the materials was found to be +12.1 cm. and −1.1 cm.[26] for the products of the reaction of VIIIa and +12.2 cm. for IIIa.

1-(2′,3′-*O*-Isopropylidene-5′-*O*-mesyl-β-D-lyxosyl)-uracil (IV).—Under anhydrous conditions a solution of 1.35 g. (0.0042 mole) of IIIa and 6.3 g. (0.037 mole) of *p*-tolylsulfonic acid in 140 ml. of acetone was stirred at room temperature. After 24 hours the reaction mixture was poured with stirring into 65 ml. of cold sodium bicarbonate solution (5%). The solution was neutralized with dilute acetic acid. Removal of the acetone from the solution *in vacuo* effected crystallization. After cooling, the product was collected and washed with a little cold water, cold ethanol and ether. A yield of 1.37 g. (90%), m.p. 222–224° (dec., uncor.), obtained. Recrystallization from acetone–water (1:1) gave colorless elongated prisms, m.p. 232–233° dec., $\alpha^{23}D$ +135 ±3° (acetone, *c* 0.6).

Anal. Calcd. for $C_{13}H_{18}N_2O_8S$: C, 43.1; H, 5.01; N, 7.73; S, 8.85. Found: C, 43.3; H, 5.02; N, 7.64; S, 8.85.

1-(5′-*O*-Benzoyl-2′,3′-*O*-isopropylidene-β-D-lyxosyl)-uracil (V).—A hot solution of 2.74 g. (0.019 mole) of sodium benzoate in 50 ml. of N,N-dimethylformamide was cooled to about 100°. After the addition of 1.37 g. (0.0038 mole) of IV, the solution was heated on a steam-bath for 3.5 hours. The solution was concentrated to a small volume *in vacuo*, and 50 ml. of cold water was added. After refrigeration the material was collected and washed with small amounts of cold water, cold ethanol and ether. A yield of 1.16 g. (79%) of colorless needles, m.p. 248.5–250°, was obtained. Crystallization from ethanol did not cause a change in the melting point, $\alpha^{27}D$ +169 ± 2° (acetone, *c* 0.5).

Anal. Calcd. for $C_{19}H_{20}N_2O_7$: C, 58.8; H, 5.19; N, 7.21. Found: C, 58.3; H, 5.26; N, 7.58.

1-(5′-*O*-Benzoyl-β-D-lyxofuranosyl)-uracil (IIIb). Method A.—A solution of 0.20 g. (0.00052 mole) of V in 5 ml. of 40% acetic acid was refluxed for 2 hours. The acetic acid was removed *in vacuo*, and the residual acid removed by repeated distillation with ethanol *in vacuo*. The residue was crystallized from 5 ml. of ethanol. On filtration, 0.12 g. (67%) of colorless needles, m.p. 228–230° (uncor.), was collected. Recrystallization gave needles melting at 238–240°, $\alpha^{28}D$ +57 ± 1° (DMF, *c* 0.4).

Anal. Calcd. for $C_{16}H_{16}N_2O_7$: C, 55.2; H, 4.63; N, 8.04. Found: C, 55.3; H, 4.64; N, 8.02.

Method B.—A mixture of 0.50 g. (0.0012 mole) of IIb and 150 ml. of water was refluxed for 4–5 hours. The course of the reaction was followed spectrophotometrically. Completion was indicated by the appearance of a spectrum characteristic of a monobenzoylated nucleoside (λ_{max} 262 and 232 mμ, λ_{min} 250 mμ, $\lambda_{232/262}$ 1.36 (ethanol–water (1:1))).

(18) D. M. Brown, A. R. Todd and S. Varadarajan, *J. Chem. Soc.*, 868 (1957).

(19) This compound was obtained through the courtesy of Dr. D. M. Brown of Cambridge University.

(20) Regarding the degree of "strain" in substituted 2-(2′,3′, or 5′)-anhydronucleosides, as reflected in their ultraviolet absorption spectra, consideration should be given to the nature and interraction of the substituents in the sugar moiety.

(21) A subsequent paper from this Laboratory will report on the reactions of 3′-*O*-mesyluridine and the synthesis of 2,3′-anhydroxylosyluracils.[22] A preliminary report of this work has appeared.[23]

(22) N. Yung and J. J. Fox, to be published.

(23) J. J. Fox and N. Yung, Abstracts, 138th Meeting, Am. Chem. Soc., 1960, p. 20-D.

(24) All melting points are corrected unless otherwise stated.

(25) Analytical determinations were performed by the Spang Microanalytical Laboratory, Ann Arbor, Mich.

(26) Refluxing IIa for a longer period results in the evolution of more than one equivalent of methylsulfonic acid. It has also been shown that a dilute acid solution of IIIa reacts further on refluxing with the loss of methylsulfonic acid to yield a non-mesylated nucleoside product(s). This material shows no anodic migration in borate buffer at *p*H 6.

After cooling, the product was filtered and washed with cold ethanol and ether. The material, m.p. 224–226° (uncor.), weighed 0.35 g. (83%). Crystallization from ethanol gave colorless needles, which upon mixing with a sample prepared by method A showed no depression of melting point.

1-β-D-Lyxofuranosyluracil (VI).—A solution of 0.30 g. (0.00086 mole) of IIIb and 0.00017 mole of sodium methoxide in 25 ml. of methanol was refluxed under anhydrous conditions for 1.5 hours. The methanol was removed *in vacuo*, and 25 ml. of water was added. After two extractions with 20-ml. portions of ether, the water was passed through a column of Dowex 50 (H^+ form). The effluent was concentrated *in vacuo*. By addition of ethanol and repeated evaporation *in vacuo*, crystallization was effected. The yield of product, m.p. 198–199° (uncor.), was nearly quantitative (0.22 g.). Crystallization from ethanol gave colorless needles, m.p. 203–204°, $\alpha^{22}D$ +95 ± 2° (water, *c* 0.4).[27] Addition of excess sodium metaperiodate to the solution gave a value $\alpha^{22}D$ +15 ± 2° within 3 minutes.

Anal. Calcd. for $C_9H_{12}N_2O_6$: C, 44.3; H, 4.95; N, 11.47. Found: C, 44.0; H, 4.79; N, 11.33.

1-(3′,5′-Di-*O*-mesyl-β-D-arabinosyl)-uracil (VIIa).—A solution of 1.0 g. (0.0026 mole) of 2,2′-anhydro-1-(3′,5′-di-*O*-mesyl-β-D-arabinosyl)-uracil (IIa) in 200 ml. of acetone–water (1:1) was stirred at room temperature with 3–4 g. of Dowex 50 (H^+ form). The course of the reaction was followed by paper chromatographic separation (isopropyl alcohol–water (7:3)) of a sample of the reaction mixture After 6 days the solution gave a single spot having a greater R_f value than the starting material (see below). After filtration of the resin the neutral solution was stirred at room temperature with activated charcoal. After removal of the charcoal the solution was carefully concentrated *in vacuo* to a small volume, maintaining the bath temperature below 40°. The flask was cooled, and the aqueous supernatant decanted from the residual gum. The gum was washed with a small amount of cold water, which was then decanted. The residual water was removed by azeotroping *in vacuo* with benzene. Evaporation *in vacuo* of an acetone solution gave a colorless amorphous solid. This gave a single spot upon chromatography in two different solvent systems; spectral properties in water: λ_{max} 260 mμ, λ_{min} 229 mμ, $\lambda_{ratio\ (max/min)}$ 4.4. The analytical values agreed with the presence of half of a mole of acetone.

Anal. Calcd. for $C_{11}H_{16}O_{10}N_2S_2 \cdot {}^1/_2\ (CH_3)_2CO$: C, 34.96; H, 4.46; S, 14.93; N, 6.52. Found: C, 34.63; H, 4.45; S, 15.29; N, 6.79.

RELATIVE R_f VALUES IN ISOPROPYL ALCOHOL–WATER (7:3) (DESCENDING PAPER CHROMATOGRAPHY AT 22–23°, 18 HOURS, SCHLEICHER AND SCHUELL PAPER, No. 597)

IIa	2,2′-Anhydro-1-(3′,5′-di-*O*-mesylarabinosyl)-uracil	0.66
VIIa	1-(3′,5′-Di-*O*-mesylarabinofuranosyl)-uracil	.80
VIIIa	2,3′-Anhydro-1-(5′-*O*-mesyllyxofuranosyl)-uracil	.64
IIIa	1-(5′-*O*-Mesyllyxofuranosyl)-uracil	.71
VI	1-β-D-Lyxofuranosyluracil	.60

1-(2′,3′,5′-Tri-*O*-mesyl-β-D-arabinosyl)-uracil (X). Method A.—Under anhydrous conditions 0.50 g. (0.0012 mole) of amorphous VIIa dissolved in 10 ml. of pyridine, was allowed to react in the cold with 0.5 ml. (0.007 mole) of methylsulfonyl chloride. After 15 hours, 1 ml. of water was added with stirring. The mixture was allowed to stand for 1 hour, then concentrated to a small volume *in vacuo*. Ice-water was added with stirring to give a volume of about 20 ml. A brown crystalline product, 0.50 g., m.p. 174–179°, was collected. Crystallization from 10 ml. of ethanol–water (1:1) gave 0.45 g. (81%) of pale brown needles, m.p. 179.5–180°, $\alpha^{26}D$ +71 ± 2° (dioxane, *c* 0.5). Admixture with a sample prepared by the exhaustive mesylation of 1-β-D-arabinofuranosyluracil (XI) (see method B below) gave no depression in melting point.

Method B.—Under anhydrous conditions a solution of 0.12 g. (0.00049 mole) of 1-β-D-arabinofuranosyluracil (XI)[11] in 2 ml. of dry pyridine was allowed to react in the cold with 0.25 ml. (0.0033 mole) of methylsulfonyl chloride. The procedure described above (method A) was followed. After one crystallization from ethanol–water (1:1), 0.16 g. (68%) of brown needles, m.p. 177–179°, was obtained. Recrystallization from ethanol–water (1:1) gave colorless needles melting at 181–181.5°, $\alpha^{20}D$ +71 ± 2° (dioxane, *c* 0.5).

Anal. Calcd. for $C_{12}H_{18}N_2O_{12}S_3$: C, 30.12; H, 3.79; N, 5.86; S, 20.10. Found: C, 30.65; H, 3.98; N, 5.68; S, 20.08.

1-(2′-*O*-Acetyl-3′,5′-di-*O*-mesyl-β-D-arabinosyl)-uracil (IX).—To a solution of 0.20 g. (0.00047 mole) of VIIa in 5.0 ml. of acetic anhydride was added 3 drops of dry pyridine, and the mixture warmed on a steam-bath for 2 hours under anhydrous conditions. The solution was concentrated *in vacuo*, and the remaining acetic anhydride decomposed by the addition with stirring of 25 ml. of an ice–water mixture. The acid was neutralized with $NaHCO_3$, and the mixture refrigerated overnight. The product was filtered and crystallized from 12 ml. of ethanol–water (1:1). The product was collected, washed with ethanol and dried with ether. The product weighed 0.16 g. (77%), m.p. 119–127°. Repeated crystallizations from absolute ethanol (0.4 g./100 ml.) gave the analytical sample, m.p. 126–127.5° (gum), $\alpha^{25}D$ +60 ± 1° (acetone–water (1:1), *c* 0.5).

Anal. Calcd. for $C_{13}H_{18}N_2O_{11}S_2$: C, 35.31; H, 4.10; N, 6.33; S, 14.50. Found: C, 35.49; H, 4.57; N, 6.30; S, 14.58.

2,3′-Anhydro-1-(5′-*O*-mesyl-β-D-lyxofuranosyl)-uracil (VIIIa).—A solution of 0.30 g. (0.0007 mole) of VIIa in 300 ml. of distilled water was refluxed for 10 minutes with methyl red as an internal indicator. After cooling, the solution was titrated with standard alkali to *p*H 5 (methyl red). Refluxing was continued, and the evolved acid neutralized periodically until one equivalent of methylsulfonic acid had been liberated in the reaction. This required about 3 hours. Upon concentration of the solution *in vacuo* to a small volume a colorless, crystalline material separated. The filtered product weighed 0.12 g., m.p. 188–191° (dec., uncor.). The filtrate was shown chromatographically to contain approximately equal amounts of VIIIa and IIIa. Crystallization from 5 ml. of ethanol–water (1:1) gave 0.08 g. of product (33%), m.p. 198–199° (dec., uncor.). Repeated crystallizations gave an analytical sample of colorless needles, m.p. 204–205° dec., $\alpha^{25}D$ −13 ± 1° (acetone–water (1:1), *c* 0.4).

Anal. Calcd. for $C_{10}H_{12}O_7N_2S$: C, 39.45; H, 3.98; N, 9.21; S, 10.55. Found: C, 39.66; H, 4.03; N, 9.40; S, 10.62.

1-(5′-Deoxy-β-D-lyxofuranosyl)-uracil (IIIc).—A solution of 1.0 g. (0.0035 mole) of 2,2′-anhydro-1-(5′-deoxy-3′-*O*-mesyl-β-D-lyxosyl)-uracil (IIc) in 150 ml. of water was refluxed for 2 hours. After cooling, the evolved acid required exactly one equivalent of sodium hydroxide for neutralization (methyl red). The water was removed *in vacuo*, and the residue dried by repeated distillations of small volumes of ethanol *in vacuo*. The product was separated from the sodium mesylate present by extraction with acetone. After removal of the acetone *in vacuo* the colorless residue was crystallized from ethanol. A yield of 0.78 g. (98%) of a colorless solid, m.p. 182–182.5° (uncor.), was obtained. Repeated crystallizations from ethanol produced colorless needles which melted at 190–191°, $\alpha^{25}D$ +66 ± 2° (water, *c* 0.8); periodate consumption, 0.97 mole/mole (within 5 minutes).

Anal. Calcd. for $C_9H_{12}N_2O_5$: C, 47.4; H, 5.30; N, 12.28. Found: C, 47.5; H, 5.06; N, 12.16.

Study of the Intermediates in the Conversion of 2,2′-Anhydro-1-(5′-deoxy-3′-*O*-mesyl-β-D-arabinosyl)-uracil (IIc) to 1-(5′-Deoxy-β-D-lyxofuranosyl)-uracil (IIIc).—A solution of 0.4011 g. of IIc in 40.0 ml. of distilled water was refluxed. From the time that complete solution was effected (which coincided with the beginning of boiling) 3-ml. aliquots of solution were withdrawn periodically during a period of 100 minutes. To minimize concentration changes, the aliquots were withdrawn through the condenser.

Each withdrawal was treated as follows: (1) 1.00 ml. was titrated with 0.00822 *N* sodium hydroxide (methyl red). (2) A 1.00-ml. aliquot was diluted with 5 ml. of water and let react with 2.00 ml. of 0.0375 *N* sodium metaperiodate for 10 minutes at room temperature. To the solution was

(27) A value for the specific rotation, $\alpha^{22}D$, of +89° was reported previously[11] for a sample of lesser purity.

then added 1 ml. of sodium bicarbonate solution (1%), 1 ml. of sodium borate solution (satd.), 0.4 g. of boric acid and 2 ml. of potassium iodide solution (2%). The iodine generated was titrated with 0.0200 *N* sodium arsenite (starch indicator). The results of the two determinations are presented in Fig. 3.

Separation of the Intermediates in the Reaction of IIc to IIIc.—In the above experiment the aliquot removed after 40 minutes of reflux was separated by paper electrophoresis in borate buffer, *p*H 6.0, using the apparatus and techniques previously described.[11] The anodic migrations (700 volts, 14 milliamp., 6.0 hours) of the components of the sample are: 40B, −2.5 cm.; 40A, +13.9 cm. These compared with the anodic migrations of 14.0 cm. for IIc and −2.0 cm. for IIIc. A reduced-scale overlay of these results is given in Fig. 4 (1).

Spot 40B was eluted with ethanol from the paper, the solution concentrated and separated into two components, spots M and N, using paper chromatography (isopropyl alcohol-water (7:3), Schleicher and Schuell No. 597 paper, descending technique, 18 hours). The results are shown in Fig. 4 (2).

Spots M and N were eluted from the paper, and the ultraviolet spectra of their solutions determined. The results are shown in Fig. 5.

Acknowledgments.—The authors wish to express their appreciation to Dr. Aaron Bendich for many useful discussions. They are grateful to Dr. George B. Brown for helpful suggestions and continued encouragement.

[CONTRIBUTION FROM THE LABORATORY OF THE CHILDREN'S CANCER RESEARCH FOUNDATION AND HARVARD MEDICAL SCHOOL, BOSTON 15, MASS.]

The Deuterium Exchange of Water-soluble Polypeptides and Proteins as Measured by Infrared Spectroscopy[1,2]

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RECEIVED DECEMBER 19, 1960

An infrared spectroscopic method is described which measures the rate of hydrogen → deuterium exchange of the secondary amide hydrogens of polypeptides and proteins. This method involves solution of the material in D_2O and the measurement of the time-dependent decrease in the intensity of the amide II absorption band around 1550 cm.$^{-1}$. To calibrate the measurements the deuterium exchange of poly-α,L-glutamic acid in helical and random conformations has been studied. It was found that the random coil conformation exchanges very rapidly (within ten minutes) whereas the helical conformation requires many hours for the complete exchange of the amide hydrogens for deuterium. Application has been made of this method to glutamic acid:lysine copolymers and to ten globular proteins from which estimates of the percentage hard-to-exchange amide hydrogens have been obtained. The hard-to-exchange amide hydrogens in these proteins range from less than 10% to about 60% of the backbone peptide hydrogens; thus there are proteins in which there are more hard-to-exchange amide hydrogens than the percentage helix, estimated from optical rotatory dispersion. Some suggestions are made as to the cause of the differences in the results obtained with the two methods.

Introduction

It has been known for some time that hydrogen atoms attached to nitrogen and oxygen in proteins are replaced by deuterium when such materials are dissolved in D_2O solutions. Indeed, several years ago we observed[3] that some of the amide hydrogens in bovine serum albumin and in ovalbumin exchanged rapidly with D_2O, whereas others required heating or alkaline treatment to effect exchange. On the basis of these observations the existence of two different types of amide groups in these proteins was postulated. We have now examined the rate of deuterium exchange for a series of water-soluble polypeptides of known composition and conformation and correlated these results with the conformation of these macromolecules as determined by optical rotation. In addition, we report new results on the rates and extent of deuterium exchange of a series of globular proteins. From these experiments we have determined the percentage "hard-to-exchange amide hydrogens" (HEAH) in these proteins.

In his work on the determination of the secondary structure of proteins, Linderstrøm-Lang and his co-workers[4] have examined the exchange reaction of insulin, ribonuclease and myoglobin with D_2O, using cryosublimation followed by density determination of the water removed to measure the amount of hydrogen → deuterium exchange. Some additional experiments on ribonuclease using similar techniques have recently been reported.[5] Haggis[6] has determined the exchange of deuterium for amino *and* amide hydrogen in certain proteins and nucleoproteins in the solid state using the infrared absorption band due to the NH stretch of such groups, which lies at 3300 cm.$^{-1}$. In addition, preliminary notes on the deuterium exchange of some polypeptides in non-aqueous systems have appeared.[7] Finally, mention should be made of the work of Fraser and MacRae[8] who studied hydrogen → deuterium exchange reactions in fibrous proteins in the hydrogen overtone region in the near infrared.

In our laboratory we have been particularly concerned with the determination of the structure of high molecular weight polypeptides in aqueous

(1) This is Polypeptides XXXIV. For the preceding paper in this series see L. Stryer and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 1411 (1961). Alternate address for E. R. Blout, Chemical Research Laboratory, Polaroid Corp., Cambridge 39, Mass.

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