Nucleosides. X. Anhydronucleosides and Related Compounds Derived from 2′,5′-Di-O-trityluridine[1-3]

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The di-tritylated uridine of Levene and Tipson is shown to be 2′,5′-di-O-trityluridine (VIII). The 3′-O-mesyl derivative of VIII is converted to a 2,3′-anhydro-xylosyluracil (VI) which leads to a sterospecific synthesis of 1-β-D-xylofuranosyluracil (XV) from uridine. The 3′,5′-O-isopropylidene derivative of XV is converted to a 2′-O-mesyl derivative (XVII) from which 2,2′-anhydro-lyxosyluracil (XIX) is prepared. Treatment of the 2′,5′-di-O-benzoate of 3′-O-mesyluridine with sodium benzoate in DMF affords 2,3′-anhydro-1-(2′,5′-di-O-benzoyl-β-D-xylosyl)-uracil (XXII) which, at its melting point, rearranges and resolidifies to 2,2′-anhydro-1-(3′,5′-di-O-benzoyl-β-D-arabinosyl)-uracil (XXIV). A mechanism for this unexpected intramolecular rearrangement proceeding via an orthoester intermediate is postulated.

In recent years, several studies have dealt with the formation of anhydro linkages between the 2-carbon atom of uracils and the 1-β-D-aldopentofuranosyl moiety of pyrimidine nucleosides.[4] Mono-sulfonyloxylated derivatives I, such as 2′-O-tosyl-5′-O-acetyluridine,[5] 2′-O-mesyl-5′-O-trityl-5-methylurine[6] and 2′-O-tosyl-5′-O-trityl-5-fluorouridine,[7] reacted readily with methanolic ammonia or, as in the latter case, with dilute alkali, to form 2,2′-anhydro-arabinonucleosides of general structure II. In the previous paper in this series[8] it was demonstrated that a 3′-O-mesyl derivative of 1-β-D-arabinofuranosyluracil (III) was converted readily into a 2,3′-anhydro-lyxosyl nucleoside (IV) by merely boiling III in water. In sharp contrast to the aforementioned studies, 3′-O-tosyl uridine (V)[9] failed to form a 2,3′-anhydro-xylosyl nucleoside (VI) by treatment with alkali or ammonia even under vigorous conditions.

Thus of the four possible anhydro nucleosides of 1-β-D-aldopentofuranosyluracil involving the 2′- or 3′-positions, two have been synthesized, namely II and IV.[10] Since anhydro nucleosides may serve as versatile chemical intermediates for the synthesis of new nucleoside analogs of possible use in biological studies or as potential anti-tumor agents, it was considered desirable to synthesize the remaining

anhydro nucleosides of uracil. This paper deals with the syntheses of 2,3′-anhydro-1-(β-D-xylofuranosyl)-uracil (VI) and 2,2′-anhydro-1-(β-D-lyxofuranosyl)-uracil (VII) and derivatives thereof.

It was suggested[12] that 3′-sulfonyloxyuridines might form 2,3′-anhydroxylosyl nucleosides under certain conditions, specifically by treatment with sodium benzoate in N,N-dimethylformamide (DMF).[13] For this purpose, a more accessible route to the synthesis of 3′-sulfonyloxyuridines was investigated. Levene and Tipson[16] had reported a di-O-trityluridine which they prepared from 5′-O-trityluridine. The position of the secondary O-trityl function (at C2′ or at C3′) in this nucleoside had not been determined by them. The proof of structure of VIII therefore was undertaken (see Fig. 2).

The di-O-trityluridine[16] (VIII) was synthesized directly from uridine in 32% yield.[17] Attempts to tosylate VIII with p-toluenesulfonyl chloride in pyridine (even at elevated temperatures) were

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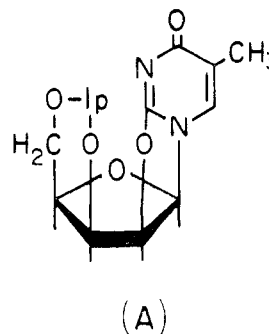
(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, Public Health Service (Grant No. CY-3190), the Ann Dickler League and the American Cancer Society.

(2) For a preliminary report see J. J. Fox and N. C. Yung, Abstr. Am. Chem. Soc., 138th Meeting, Division of Carbohydrate Chemistry, New York, N. Y., 1960, p. 20-D.

(3) Although the term "cyclonucleoside" has been employed for naming this class of compounds, the term "anhydronucleoside"[4] is more in keeping with carbohydrate nomenclature.

(4) For a general review, see J. J. Fox and I. Wempen, Adv. in Carbohydrate Chem., 14, 283 (1959).

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

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

(7) J. J. Fox, N. C. Yung, I. Wempen, R. Duschinsky and L. Kaplan, Abstr. Interna. Union Pure & Applied Chem. (Symposium on Natural Products), Australia, 1960, p. 66.

(8) R. Fecher, J. F. Codington and J. J. Fox, J. Am. Chem. Soc., 83, 1889 (1961).

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

(10) A 3′,5′-O-isopropylidene derivative of a 2,2′-anhydro-lyxosyl thymine (A) had been synthesized[11] from 1-(2′-O-mesyl-3′,5′-O-isopropylidene-β-D-xylosyl)-thymine. The free 2,2′-anhydro nucleoside was not isolated.

(11) J. J. Fox, J. F. Codington, N. C. Yung, L. Kaplan and J. O. Lampen, J. Am. Chem. Soc., 80, 5155 (1958).

(12) See ref. 4, p. 354.

(13) Reist, et al.,[14] have demonstrated that sodium benzoate in DMF is a powerful reagent for the nucleophilic displacement of secondary sulfonyloxy groups of certain carbohydrates. In our laboratory, this reagent has been effective in the formation of 2,2′-anhydro-arabinosyl-uracil derivatives from tri-O-mesyloxyuridine.[15]

(14) E. J. Reist, L. Goodman and B. R. Baker, J. Am. Chem. Soc., 80, 5775 (1958); E. J. Reist, R. R. Spencer and B. R. Baker, J. Org. Chem., 24, 1618 (1959).

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

(16) P. A. Levene and R. S. Tipson, J. Biol. Chem., 105, 419 (1934).

(17) The mother liquors of this reaction have been investigated. Mesylation of the mother liquor and then treatment with alkali and subsequent acid hydrolysis yielded 1-β-D-arabinofuranosyluracil. The formation of this latter nucleoside proves the presence of some 3′,5′-di-O-trityluridine in the original mother liquor since mesylation of the 2′-hydroxyl followed by alkali treatment should epimerize C2′ and subsequent de-tritylation with acid should yield 1-β-D-arabinofuranosyluracil.

Ms = methylsulfonyl
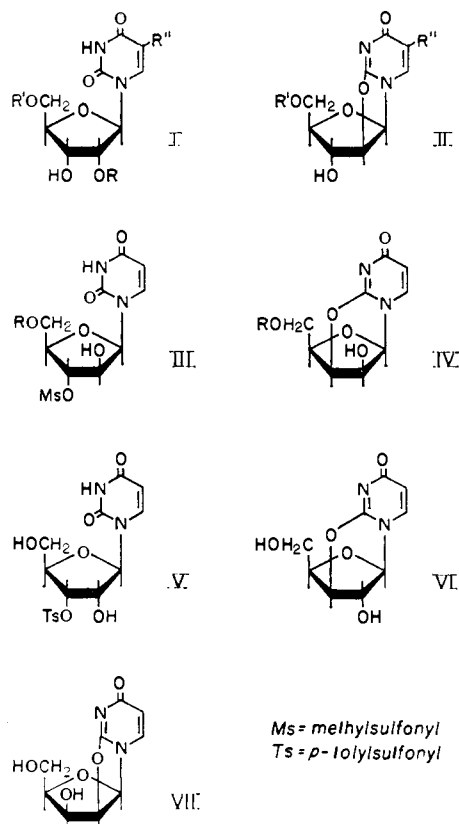Ts = p- tolylsulfonyl

Fig. 1.

Fig. 2.

unsuccessful, due, presumably, to steric hindrance imposed by the bulky trityl groups, and starting material was recovered unchanged. Mesylation of VIII, however, could be effected in high yields to give IX. De-tritylation of IX yielded a mono-O-mesyluridine (X) which was tosylated to di-O-tosyl-mono-O-mesyluridine (isolated as a glass). Treatment of this glass (XI) with sodium benzoate in acetamide for 30 minutes at 110–115° afforded 2,2'-anhydro-1-(5'-O-benzoyl-3'-O-mesyl-β-D-arabinosyl)-uracil (XII), a compound prepared previously[15] in this Laboratory by another route and whose structure was rigidly established.[8] These reactions (VIII → XII, Fig. 2) permit the assignment of the mesyloxy function only to position 3' in IX, X and XI and thereby establishes VIII as 2',5'-di-O-trityluridine.

3'-O-Mesyluridine (X) failed to react with one equivalent of dilute alkali for 30 minutes at 95°. Similarly, no spectral evidence to indicate anhydronucleoside formation was observed after boiling X for 3 hours in water. Treatment of X with sodium benzoate in DMF (100° for 6 hours) produced some darkening of the reaction mixture from which about 75% of starting material was recovered. Treatment of the filtrate of this reaction with warm alkali followed by paper ionophoresis (pH 6, borate buffer)[18] showed only traces of products other than starting material X. When the temperature of the reaction was raised to 135–140° for 15 minutes, intense darkening of the re-
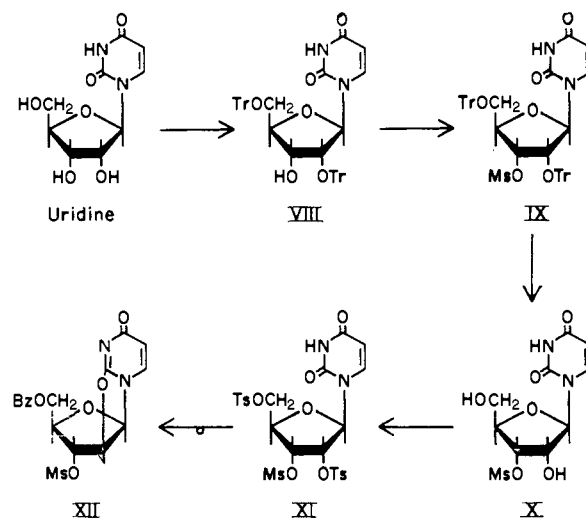
action mixture occurred.[19] Aside from starting material, only trace amounts of other products were found. The use of 3'-O-mesyluridine, as such, as an intermediate in the synthesis of 2,3'-anhydroxylosyluracil (VI) therefore was abandoned.

3'-O-Mesyl-2',5'-di-O-trityluridine (IX), however, reacted with sodium benzoate in DMF to form XIII (see Fig. 3), a crystalline compound

Tr = triphenylmethyl
Ip = isopropylidene

Fig. 3.

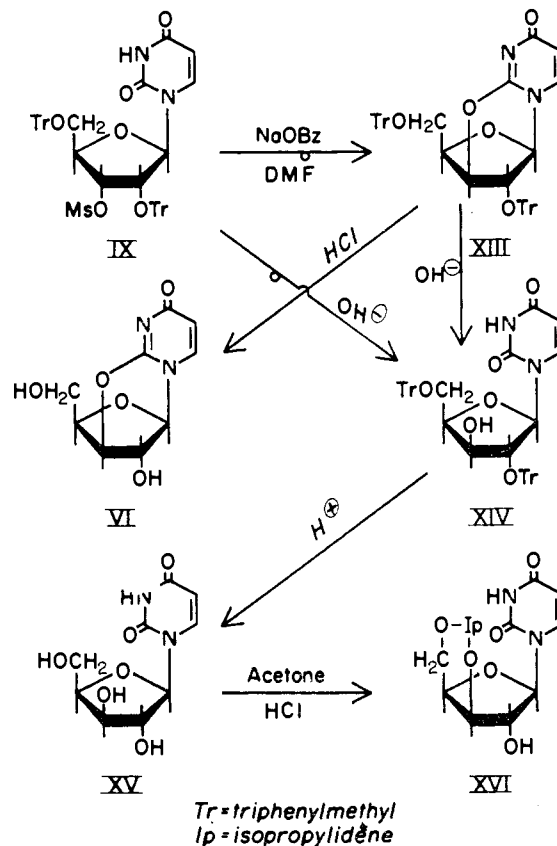(18) M. P. Gordon, O. M. Intrieri and G. B. Brown, J. Am. Chem. Soc., 80, 5161 (1958).

(19) It is noteworthy that treatment of uridine with sodium benzoate in DMF for 1 hour at 140° produced no darkening of the reaction mixture and starting material was recovered almost quantitatively.
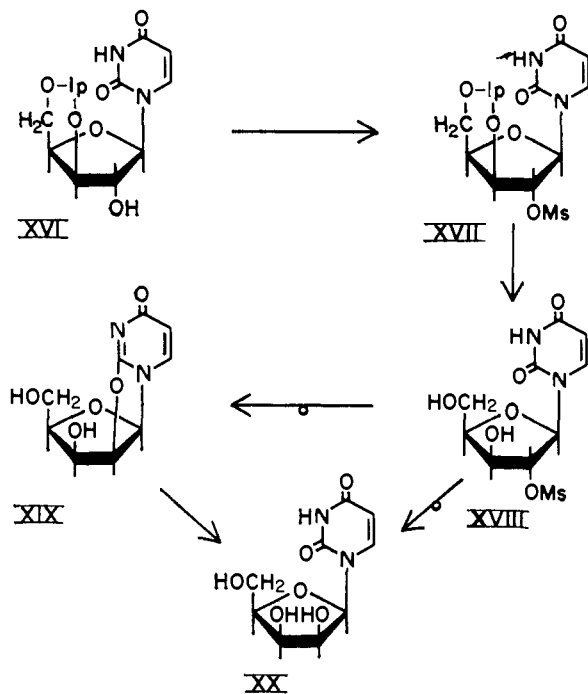
XVI   XVII

XIX   XVIII

XX

Fig. 4.

with analysis for a di-O-trityl-anhydro nucleoside. Since in IX and XIII the 2'- and 5'-positions are blocked by trityl functions, only the 2,3'-anhydro structure is possible. Indeed, rupture of the anhydro bond by heating XIII in dilute alkali (attack at C2) yielded a crystalline monohydroxy-di-O-tritylated nucleoside (XIV) in quantitative yield which, upon de-tritylation with acid, formed *exclusively* 1-β-D-xylofuranosyluracil (XV), a compound synthesized previously in this Laboratory by another route.[15] De-tritylation of XIII with ethanolic hydrogen chloride afforded crystalline 2,3'-anhydro-1-(β-D-xylofuranosyl)-uracil (VI). The ultraviolet absorption spectrum of VI differed from that of 1-β-D-aldopentofuranosyluracils and was generally similar to the spectrum exhibited by anhydronucleosides, such as II[5] (R', R'' = H, see Fig. 1) and IV[8] (R = Ms). Refluxing of the anhydronucleoside VI in dilute hydrochloric acid for 3 hours yielded, in addition to XV, two other minor components. One of these was characterized as uracil.[20] The formation of uracil under these conditions was not altogether unexpected since it had been observed previously[6,11,21] that treatment of certain anhydronucleosides with dilute acid at reflux temperatures for several hours liberated small amounts of aglycon. The other minor component did not exhibit anodic migration in paper electrophoresis (borate buffer, pH 6) but migrated (borate buffer, pH 9.2)[18] similarly to 1-β-D-arabinofuranosyluracil. The characterization of this component is under investigation.

It was found that treatment of the mono-O-mesyl-di-O-trityluridine (IX) with two equivalents

(20) Treatment of 1-β-D-xylofuranosyluracil (XV) with hydrochloric acid at reflux temperature for 4 hours did not produce uracil and XV was recovered.

(21) A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, 816 (1955).

of alkali in 80% ethanol at reflux temperature for ~100 hours afforded di-O-trityl-xylosyluracil (XIV) directly in almost quantitative yield. In light of the previously-described reaction of IX with sodium benzoate in DMF, it is likely that the 2,3'-anhydronucleoside XIII was an intermediate in the synthesis of XIV by alkaline treatment of IX.

1-β-D-Xylofuranosyluracil (XV) readily formed the 3',5'-O-isopropylidine derivative XVI[22] in acetone in quantitative yield simply by treatment with a few drops of concentrated hydrochloric acid or with gaseous hydrogen chloride. In fact, treatment of uridine in acetone with hydrogen chloride afforded a simpler procedure for the synthesis of crude 2',3'-O-isopropylidene-uridine in almost quantitative yield than that which had been described previously.[23]

The isopropylidene derivative XVI served as a chemical intermediate for the synthesis of the remaining anhydronucleoside, namely, 2,2'-anhydro-1-(β-D-lyxofuranosyl)-uracil (XIX). Mesylation of XVI (see Fig. 4) yielded the 2'-O-mesyl derivative XVII which was de-acetonated with acid to 1-(2'-O-mesyl-β-D-xylofuranosyl)-uracil (XVIII), obtained as a glass. Treatment of an aqueous solution of this glass slowly with one equivalent of dilute alkali afforded a 30% yield of 2,2'-anhydro-1-(β-D-lyxofuranosyl)-uracil (XIX) in crystalline form plus another component. The crystalline material XIX analyzed for an unsubstituted anhydronucleoside but differed in melting point and rotation from the known[5] 2,2'-anhydro-1-(β-D-arabinofuranosyl)-uracil (II, R', R'' = H, Fig. 1). The "twinned maxima" absorption spectrum found for the 2,2'-anhydro-arabino,[5] the 2,3'-anhydro-lyxo[8] and the 2,3'-anhydro-xylo nucleosides of uracil is also evidenced by XIX. All four anhydronucleosides exhibit a shoulder in their ultraviolet absorption spectrum at approximately 270 mμ (see Table I). Treatment of XIX with warm, dilute alkali afforded *exclusively* the expected 1-β-D-lyxofuranosyluracil (XX) with chromatographic and spectral properties identical with those reported previously.[8,15]

The second component isolated from the treatment of XVIII with dilute alkali (*vide supra*) exhibited spectral properties similar to those observed with 1-β-D-aldopentosyluracils. Unlike XVIII, this second component migrated cathodically in paper ionophoresis (borate buffer, pH 6). This component is presumably the 2',3'-epoxide of 1-β-D-lyxofuranosyluracil.[24]

The formation of this second component was avoided when XVIII was refluxed in water for one hour. A paper ionophoretic study demonstrated that two products were formed, the anhydronucleoside XIX and 1-β-D-lyxofuranosyluracil (XX, see Fig. 4). One mole of methylsulfonic acid was liberated in the process. Prolonged boiling of the reaction in water (3 hours) yielded only one product

(22) The assignment of the positions of attachment of the isopropylidene residue in XVI is based upon analogy with the synthesis of the 3',5'-O-isopropylidene derivative of 1-β-D-xylofuranosylthymine.[11]

(23) P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **106**, 113 (1934)

(24) The synthesis and properties of nucleoside epoxides of this type is the subject of another paper in this series. See J. F. Codington, R. Fecher and J. J. Fox, Abstr. 139th Meeting Am. Chem. Soc., St. Louis, Mo., 1960, p. 13-D.

TABLE I

PHYSICAL CONSTANTS OF 1-β-D-ALDOPENTOFURANOSYLURACIL DERIVATIVES

	Rotational data			Spectral data[b]			
	$[\alpha]^t{}_D$	$t,{}^\circ C.$	c, Solvent	λ_{max}, mμ	ϵ_{max}	λ_{min}, mμ	ϵ_{min}
2,2'-Anhydro-arabino[5] (II)	−20°	27	(0.4, H$_2$O)	223, 250 (270(s))[a]	7820, 7750	235	6020
2,2'-Anhydro-lyxo (XIX)	−52	22	(.15, H$_2$O)	222, 249.5, 270(s)	8600, 8270	234.5	6650
2,3'-Anhydro-lyxo[8] (IV)[c]	−13	25	(.4, H$_2$O–acetone, 1:1)	223, 249, 270(s)	8400, 8200	234	6700
2,3'-Anhydro-xylo (VI)	−19	23	(.4, H$_2$O)	229, 245, 267.5(s)	8890, 8120	239.5	8070
2,3'-Anhydro-2',5'-di-O-benzoyl-xylo (XXII)	−43	28	(.3, DMF)	232.5, 285(s)	36850		
2,3'-Anhydro-2',5'-di-O-trityl-xylo (XIII)	− 8.5	22	(.5, CHCl$_3$)	250(s), 270(s)	8330		
2',5'-Di-O-trityl-ribo (VIII)	+91.5	26	(.35, acetone)	260	9560	245	6320
2',5'-Di-O-trityl-3'-O-mesyl-ribo (IX)	+35	26	(.3, DMF)	259	10190	244.5	7000
3'-O-Mesyl-ribo (X)	−16	22	(.9, EtOH)	259.5	8810	229	1890
2',5'-Di-O-trityl-xylo (XIV)	+46	22	(.3, acetone)	260	11740	245	7530
3',5'-O-Isopropylidene-xylo (XVI)	+18	26	(.2,50%),EtOH)	263	10800	231	2170
2'-O-Mesyl-3',5'-O-isopropylidene-xylo (XVII)	+16	22	(.3, H$_2$O)	261	10500	229	2160

[a] (s) = shoulder. [b] Spectral measurements in water for II, XIX, IV, VI; in absolute ethanol for VIII, IX, X, XIII, XIV, XVIII; and in 50% ethanol for XVI and XXII. [c] IV (R = methylsulfonyl).

(XX), indicating that the anhydronucleoside XIX was an intermediate in the formation of lyxofuranosyluracil under these conditions. A similar mechanism has been elucidated previously[8] in the conversion of 3'-O-mesyl derivatives of 1-β-D-arabinofuranosyluracils (III, see Fig. 1) to 1-β-D-lyxofuranosyluracils via 2,3'-anhydronucleosides IV under the same reaction conditions.

3'-O-Mesyluridine (X) served, unexpectedly, as a chemical intermediate in the synthesis of a 2,2'-anhydro nucleoside. Benzoylation of X (see Fig. 5) yielded a sirupy di-O-benzoate (XXI) which, when treated with sodium benzoate in DMF (one hour at 110°), afforded a 10% yield[25] of a product (XXII) with physical properties (ultraviolet absorption spectrum and optical rotation) closely akin to those reported[15] for 2,2'-anhydro-1 - (3',5' - di - O - benzoyl - β - D - arabinosyl)-uracil (XXIV). The melting points of XXII and XXIV were similar (270–272°) except that the product XXII "pre-melted" at 252° with resolidification to colorless needles. These needles, upon recrystallization, melted at 270–272° without exhibiting the lower melting point behavior.

These data suggested that the product of the DMF reaction (XXII) was 2,3'-anhydro-1-(2',5'-di-O-benzoyl-β-D-xylosyl)-uracil which upon heating at 252° was converted to XXIV. Proof that such a rearrangement had occurred in the melting point apparatus was established in the following manner: First, an alternate synthesis of XXII was effected by benzoylation of 2,3'-anhydro-1-(β-D-xylofuranosyl)-uracil (VI) with benzoyl chloride in pyridine. The product obtained was identical (including melting point behavior and infrared spectrum) with that obtained by the treatment of the di-O-benzoate of 3'-O-mesyluridine (XXI) with sodium benzoate in DMF. With the structure of XXII thus established, the infrared spectrum of this anhydro nucleoside was compared before and after heating at 252°. Significant differences were observed in the two spectra (see

(25) The mother liquors of this reaction contain a benzoylated nucleoside derivative whose structure has not been determined.

Fig. 6). Moreover, the infrared spectrum of the heated material was identical with that given by an authentic sample of XXIV prepared by an independent route.[15] Thus it is established that, upon heating, XXII is rearranged to XXIV.
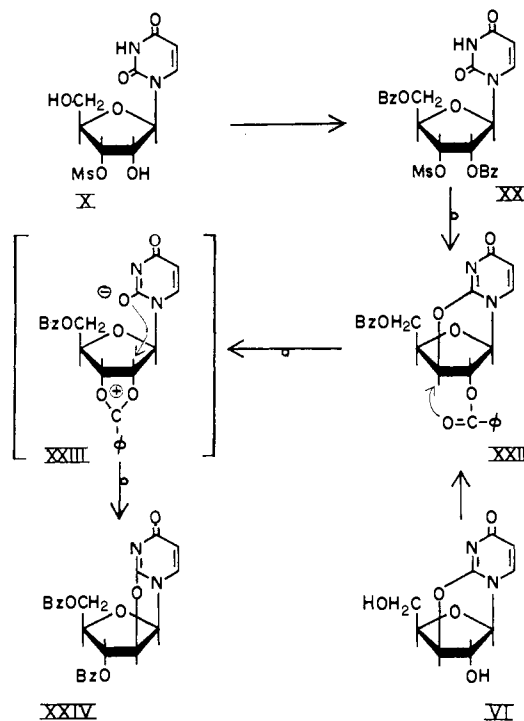

Fig. 5.

A plausible mechanism for this novel intramolecular conversion of XXII to XXIV would be, first, the formation of an orthoester ion (XXIII) by attack from the neighboring benzoate (inversion of C3') with rupture of the 2,3'-anhydro linkage. The orthoester is then attacked at C2' by the 2-carbonyl (second inversion) with the formation of XXIV.
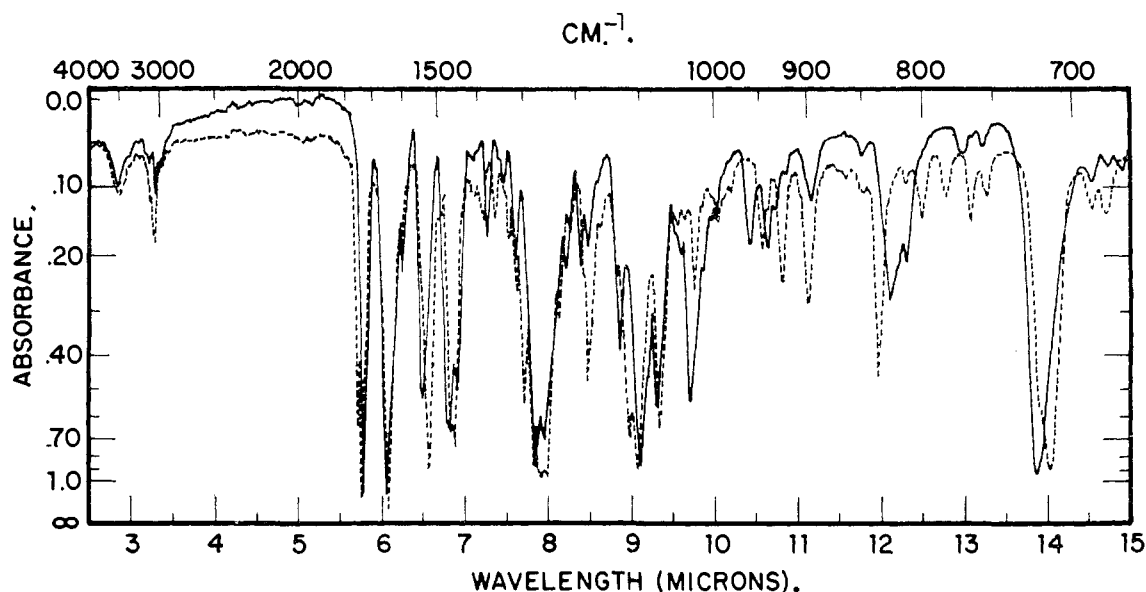
CM.⁻¹.

Fig. 6.—Infrared spectra (KBr disk): ——, 2,2'-anhydro-1-(3',5'-di-O-benzoyl-β-D-arabinosyl)-uracil (XXIV); ----------, 2,3'-anhydro-1-(2',5'-di-O-benzoyl-β-D-xylosyl)-uracil (XXII).

General Considerations.—The tritylation of uridine to the 2',5'- and the 3',5'-di-O-trityl[17] derivatives (of which the former is isolated easily in crystalline form) makes available, separately, blocked uridines in which the 2' or 3' are unsubstituted. Such uridines may be useful as intermediates for the synthesis of cytidines bearing O-methyl groups at C2' or at C3'. Nucleosides of this type (presumably the 2'-O-methyl derivatives) have been reported as minor constituents of certain nucleic acids.[26]

Acknowledgments.—The authors wish to thank the Cancer Chemotherapy National Service Center for some of the uridine used in this investigation. The authors are deeply indebted to Dr. George Bosworth Brown of this Institute for helpful suggestions and continued interest.

Experimental[27]

2',5'-Di-O-trityluridine (VIII).—Uridine (15 g.) in 150 ml. of anhydrous pyridine was treated with three equivalents of triphenylmethyl chloride and allowed to remain at room temperature overnight. The red solution was heated at 110° for 4 hours after which it was cooled, poured into water and stirred. After decantation, the gummy solid was again treated with water, stirred and the water decanted. This process was repeated several times after which the residue was dissolved in acetone and concentrated to dryness. The sirup was treated with 500 ml. of hot water, stirred and the water decanted. This process was repeated twice. The residue was dissolved in methylene chloride and dried over sodium sulfate. After filtration from the salt, the solution was concentrated to a sirup and treated with the least amount of hot benzene to effect solution. Ether was added to a point of faint opalescence. After cooling (and scratching) a reddish solid precipitated. The precipitate was collected and again dissolved in a minimal amount of hot benzene, treated with charcoal and filtered. Ether was added to incipient turbidity and cooled. Fine, colorless needles (14 g.) were obtained in two crops, m.p., sinters at ~210°, melts at 224-225°, [α]D +91° (c 0.5 g.

(26) J. D. Smith and D. B. Dunn, Biochim. et Biophys. Acta, 31, 573 (1959); B. B. Biswas and J. Myers, Nature, 186, 238 (1960).

(27) All melting points are corrected. Analyses by Spang Microanalytical Laboratory, Ann Arbor, Mich. Ultraviolet absorption spectra were run with the Cary, model 11, recording spectrophotometer. Infrared data were obtained on the Infracord, model 137.

in acetone). Levene and Tipson[16] report m.p. 223-224° and a rotation of +91.4° in acetone.

1-(3'-O-Mesyl-2',5'-di-O-trityl-β-D-ribosyl)-uracil (IX). —Methanesulfonyl chloride (0.9 ml.) was added dropwise to a cooled solution of di-O-trityluridine (7.1 g.) in 80 ml. of anhydrous pyridine and the reaction stored at 5° for 16 hours. A crystalline solid product (platelets) formed during this time, which may be worked up separately. However, it was found more practical to avoid this separate work-up. The reaction mixture was treated with 2 ml. of ethanol and let stand for 2 more hours. After concentration in vacuo to a red sirup, the residue was treated with 200 ml. of ethanol whereupon a heavy white precipitate formed. After filtration and trituration of the precipitate in ethanol, a solid was obtained (7.2 g.) which was washed repeatedly with ethanol followed by ether and dried. The product was recrystallized from ethanol and after standing at room temperature afforded pure material, m.p. 225-226°.

Anal. Calcd. for $C_{48}H_{42}O_8N_2S$: C, 71.44; H, 5.25; N, 3.47; S, 3.97. Found: C, 71.08; H, 5.26; N, 3.43, 3.28; S, 4.27.

3'-O-Mesyluridine (X).—The above derivative (IX, 0.6 g.) was suspended in 50 ml. of ethanol and saturated with hydrogen chloride with cooling. A yellow solution resulted which was refluxed for 0.5 hour and concentrated to a sirup under vacuum. The sirup was azeotroped several times with benzene and triturated several times with fresh portions of boiling ether. A glass separated which was dissolved in hot absolute ethanol. The volume of ethanol was reduced to 4-5 ml. and, upon heating the alcoholic solution, crystallization occurred. After cooling and filtration, the precipitate was washed with cold ethanol followed by ether; yield 0.2 g. Recrystallization was accomplished by dissolving the substance in ethanol and concentration of the solution to approximately 5 ml. After cooling, pure material in the form of needle clusters was obtained (160 mg.), m.p. 181-182°.

Anal. Calcd. for $C_{10}H_{14}O_8N_2S$: C, 37.26; H, 4.38; N, 8.69; S, 9.95. Found: C, 37.65; H, 4.36; N, 8.77; S, 10.12.

1-(3'-O-Mesyl-2',5'-di-O-p-tosyl-β-D-ribosyl)-uracil (XI). —3'-O-Mesyluridine (0.9 g.) in 15 ml. of anhydrous pyridine was treated with 1.4 g. of p-toluenesulfonyl chloride and the reaction kept at room temperature for 16 hours. The solution was cooled and treated with 0.5 ml. of water. After 1 hour, the reaction was poured into a well-stirred ice-water mixture. A tan, granular solid was obtained which was filtered. This precipitate was hygroscopic. It was dissolved in ethanol and concentrated in vacuo to a glass; 1.6 g. It was not purified further but used directly in the next step.

2,2'-Anhydro-1-(5'-O-benzoyl-3'-O-mesyl-β-D-arabinosyl)-uracil (XII).—A solution of 2 g. of sodium benzoate and 15 g. of acetamide at 110° was poured into a flask containing 1.0 g. of the above glass XI and the yellow solution maintained at 110–115° for 0.5 hour. The hot solution was poured into 400 ml. of cold water with stirring. A white solid (colorless needles) precipitated. After filtration, the solid was dissolved in preheated 75% ethanol, treated with charcoal, and filtered. Upon cooling, long colorless needles were obtained; 350 mg. (56% based upon the glass, XI), m.p. 233–235° dec. A mixed melting point with authentic material (obtained by treatment of tri-O-mesityluridine with sodium benzoate in acetamide)[15] melted at 233–235° dec. Spectral properties and optical rotation also agreed with those previously reported.[15]

2,3'-Anhydro-1-(2',5'-di-O-trityl-β-D-xylosyl)-uracil (XIII).—2',5'-Di-O-trityl-3'-O-mesyluridine (IX, 1.0 g.) was added to a solution of 2.0 g. of sodium benzoate in 40 ml. of DMF and heated at 130–140° (internal temperature) for 18 hours. The cooled mixture was poured into 1 l. of water and stirred for 2 hours. The precipitate was separated and washed well with water. After filtration, the precipitate was triturated with 100 ml. of ethanol whereupon a white, granular precipitate slowly formed. After 2 hours of stirring, the precipitate was filtered from the amber-colored solvent; yield 0.54 g. (62%). The product was recrystallized from a large volume of ethanol; m.p. 237° (to a yellow oil).

Anal. Calcd. for $C_{47}H_{38}O_5N_2$: C, 79.41; H, 5.39; N, 3.94. Found: C, 79.21; H, 5.28; N, 3.96.

1-(2',5'-Di-O-trityl-β-D-xylosyl)-uracil (XIV). Method A.—Ethanol (90 ml.) and 12 ml. of 1 N sodium hydroxide were added to 2.1 g. of XIII and the suspension was refluxed for 45 minutes. The clear solution was neutralized with acetic acid to pH 5–6 whereupon precipitation of a granular solid began. Most of the ethanol was removed *in vacuo*, and the suspension was cooled to complete precipitation. Filtration gave a quantitative yield of XIV. Recrystallization from ethanol gave pure material, m.p. 151.5–153.5°.

Anal. Calcd. for $C_{47}H_{40}O_6N_2·\frac{1}{2}$ C_2H_5OH: C, 76.73; H, 5.70; N, 3.73. Found: C, 76.20; H, 5.77; N, 3.88.

Method B.—Sodium hydroxide (1.0 N, 2.0 ml.) was added to 710 mg. of IX in 50 ml. of 80% ethanol and the mixture refluxed for 100 hours. Titration of the reaction solution to neutrality showed that exactly one equivalent of base per mole of IX had been consumed during the period of reflux. Crystallization occurred after the neutralization; 0.6 g. Recrystallization from ethanol afforded pure material with identical properties as described in method A.

2,3'-Anhydro-1-(β-D-xylofuranosyl)-uracil (VI).—The di-O-tritylanhydronucleoside (XIII, 1.1 g.) was placed in 50 ml. of ethanol, saturated with hydrogen chloride at 0°, and warmed for 10 minutes below 70°. The solution was concentrated *in vacuo* to a sirup and repeatedly evaporated with benzene. Absolute ethanol (10 ml.) was added to the sirup and the cooled ethanolic solution was brought to about pH 5 with ammonia whereupon crystallization took place. The prisms weighed 0.32 g., m.p. 201–204°. One crystallization from 95% ethanol gave pure material, m.p. 225–227°.

Anal. Calcd. for $C_9H_{10}O_5N_2$: C, 47.79; H, 4.46; N, 12.39. Found: C, 47.62; H, 4.38; N, 12.41.

1-β-D-Xylofuranosyluracil (XV) from XIV.—The di-O-trityl-xylonucleoside (XIV, 2.1 g.) in 30 ml. of 95% ethanol and 2 drops of concentrated hydrochloric acid was refluxed for 0.5 hour. The solution was concentrated to a sirup, treated with water and extracted with chloroform. The aqueous portion was concentrated to a sirup *in vacuo* and evaporated repeatedly with ethanol until crystallization occurred. Two crops gave a 95% yield of crude XV. Recrystallization from a minimal quantity of hot ethanol gave pure material, m.p. 148–149°. An authentic sample[15] melted at 150–150.5°, mixed m.p. 150–151°, α_D^{25}, +29° (c 0.4, H₂O) (previously reported[15] α_D^{25} +29°). Spectral properties in neutral and alkaline solution also agreed with those reported.[15]

1-(3',5'-O-Isopropylidene-β-D-xylofuranoyl)-uracil (XVI).—Crude xylofuranosyluracil was used in the formation of the isopropylidene derivative. Crude XV which was obtained from detritylation of XIV (2.1 g.) with acid, was treated with about 40 ml. of acetone. (Enough residual

acid was present in crude XV to catalyze the reaction to XVI[28]). Upon stirring, colorless prisms formed. After cooling to complete crystallization, 0.7 g. was collected on a filter, m.p. 258–264°. Another 0.1 g. was obtained from the mother liquor giving a quantitative yield. The prisms were recrystallized from a minimum quantity of hot 90% ethanol to give pure material, m.p. 264–266°.

Anal. Calcd. for $C_{12}H_{16}O_6N_2$: C, 50.69; H, 5.67; N, 9.86. Found: C, 50.77; H, 5.59; N, 9.77.

2',3'-O-Isopropylideneuridine.—Acetone (50 ml.) saturated with hydrogen chloride at 0° was added to 1 g. of uridine suspended in 100 ml. of acetone. The reaction mixture was stirred for 2–5 minutes. As the uridine dissolved, heavy needle clusters precipitated. The mixture was continually stirred while it was cooled to effect complete precipitation. (The product, though soluble in acetone, has a much lower solubility in acetone saturated with hydrogen chloride.) Evaporation *in vacuo*, bath temperature not above 40°, removed a major portion of the hydrogen chloride. Acetone (50 ml.) was added to replace that removed by distillation and the solution treated with 40 g. of anhydrous sodium carbonate. The reaction mixture was stirred until neutral (1.5 hours). The salts were filtered and washed well with acetone. The filtrate was concentrated under reduced pressure and azeotroped with benzene several times whereupon needle clusters formed (1.3 g.). Recrystallization from methanol gave pure material, m.p. 162–163°. A mixed melting point with a sample prepared according to Levene and Tipson[23] gave no depression.

1-(3',5'-O-Isopropylidene-β-D-xylosyl)-uracil (XVI) from VI. Method A (with Alkali).—The anhydronucleoside (VI, 22 mg.) in 3 ml. of 0.1 N sodium hydroxide was heated on a steam-bath for 0.5 hour. The reaction was cooled and treated with Dowex 50 (H⁺) batchwise to remove sodium ions. The filtrate was concentrated to dryness *in vacuo* and treated with 5 ml. of acetone and 1 drop of concentrated hydrochloric acid. Upon cooling and scratching, crystallization occurred; 24 mg., m.p. 256–257°. One recrystallization gave pure material (*vide supra*), m.p. 264–266°.

Method B (with Acid).—The anhydronucleoside (VI, 0.2 g.) in 20 ml. water was treated with two drops of concentrated hydrochloric acid and refluxed for 3.5 hours. The course of the reaction was followed chromatographically (paper electrophoresis in 0.1 M borate[18] at 700 volts for 3.5 hours) by withdrawal of aliquots at 30-minute intervals. Whereas at zero time only one ultraviolet-absorbing spot (VI, 3.8 cm. from origin) was in evidence, after 3.5 hours this spot was absent and three new spots appeared. The major spot (18 cm.) was 1-β-D-xylofuranosyluracil (XV). The second spot (15 cm.) was characterized as uracil both chromatographically and spectrophotometrically. The third (11 cm.), as yet uncharacterized, migrated similarly to 1-β-D-arabinofuranosyluracil. After elution this spot exhibited a 1-β-D-aldopentofuranosyluracil spectrum.

Concentration of the reaction solution to dryness *in vacuo* and treatment of the slightly acidic residue with acetone afforded a 50% yield of XVI which, after one recrystallization from 90% alcohol, melted at 264–266°.

1-(3',5'-O-Isopropylidene-2'-O-mesyl-β-D-xylosyl)-uracil (XVII).—Methanesulfonyl chloride (0.12 ml.) was added dropwise to a cooled suspension of XVI (0.0011 mole) in 8 ml. of anhydrous pyridine. The reaction mixture was agitated at room temperature (22°) for 14 hours. Ethanol (0.5 ml.) was added and let stand for 0.5 hour and finally was concentrated to a thick sirup, which was rendered crystalline from ethanol-benzene (1:1). The shiny platelets were collected on a filter; m.p. 140–141° (effervescence and resolidification and finally melting with decomposition at 200°). An additional crop from the mother liquor gave a total yield of 370 mg. of crude product. Recrystallization from the same solvent system did not alter the melting point. The analysis was consistent with the presence of one molecule of benzene. Unsolvated product was obtained after two recrystallizations from ethanol; m.p. 204–205°.

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(28) Of note is the ease with which this acetone derivative is formed. Anhydrous conditions usually advocated for such reactions need not be followed. The acetone used in this and other acetonations described in this paper was not anhydrous, nor were any precautions taken to exclude moisture from the atmosphere.

Anal. Calcd. for $C_{13}H_{18}O_8N_2S$: C, 43.09; H, 5.01; N, 7.73; S, 8.85. Found: C, 43.30; H, 5.26; N, 7.51; S, 8.54.

1-(2′-O-Mesyl-β-D-xylofuranosyl)-uracil (XVIII).—The mesyl-isopropylidene derivative (XVII, 51 mg.) was added to 50 ml. of 90% ethanol containing 5 drops of concentrated hydrochloric acid. The clear solution was refluxed for 15 minutes, then concentrated to a light acidic sirup to which benzene was added repeatedly and evaporated to obtain a glass. 2′-O-Mesyl-xylosyluracil could not be crystallized. It was demonstrated by paper electrophoresis (pH 6, borate buffer)[18] that XVII was absent and that only XVIII was present as one ultraviolet absorbing spot. The yields were shown to be quantitative by the amount of sodium hydroxide consumed in a subsequent reaction; XVIII was converted *exclusively* to 1-β-D-lyxofuranosyluracil.

2,2′-Anhydro-1-(β-D-lyxofuranosyl)-uracil (XIX).—A glass containing 0.0009 mole of XVIII obtained from the deacetonation of XVII was placed in 12 ml. of water. Sodium hydroxide (0.02 N) was added dropwise to the stirred solution until the consumption of alkali (35 ml.) ceased (phenolphthalein indicator). The neutral solution was concentrated to dryness and 40 ml. of ethanol was added. The insoluble sodium mesylate was filtered and discarded. The filtrate was again evaporated to about 10 ml. when crystallization of prisms occurred. An additional crop was obtained from the mother liquor giving a total yield of 35%. Recrystallization of XIX from 95% ethanol afforded a pure sample, m.p. 252.5–253° dec.

Anal. Calcd. for $C_9H_{10}O_5N_2$: C, 47.79; H, 4.46; N, 12.39. Found: C, 47.98; H, 4.46; N, 12.70.

Paper electrophoresis (0.1 M sodium borate[18]) revealed in the mother liquor another component which migrated similarly to the 2′,3′-epoxide of 1-β-D-lyxosyluracil,[24] and which, upon heating for 10 minutes in dilute alkali, was unaltered.

1-β-D-Lyxofuranosyluracil (XX).—An aqueous solution (10 ml.) of the 2′-O-mesyl-xylosyluracil (XVIII, 0.00014 mole) was refluxed for 3 hours. The course of the reaction was followed chromatographically and the end-point was determined when only one ultraviolet-absorbing spot, migrating identically with authentic 1-β-D-lyxofuranosyluracil,[8] remained. Exactly one molecular equivalent of methylsulfonic acid was liberated, as shown by titration with 0.01 N sodium hydroxide (methyl red). The neutral solution was taken to dryness *in vacuo*, ethanol added and warmed. Insoluble sodium mesylate was filtered and the filtrate concentrated to about 2 ml., whereupon crystallization occurred. The crude material (26 mg.) melted at 193–195°. Recrystallization from absolute ethanol afforded pure compound, m.p. 200–202°. A mixture of XX and an authentic sample[8] did not depress the melting point.

1-β-D-Lyxofuranosyluracil was also prepared from the anhydronucleoside intermediate XIX. Treatment of XIX

with warm, dilute alkali for 10 minutes followed by paper electrophoresis of the reaction solution revealed only one spot (borate buffer, pH 6)[18] corresponding to that for XX.

2,3′-Anhydro-1-(2′,5′-di-O-benzoyl-β-D-xylosyl)-uracil (XXII). **Method A.**—Benzoyl chloride (0.48 ml.) was added slowly to a stirred solution of 3′-O-mesyluridine (X, 0.64 g.) in 30 ml. of dry pyridine. The reaction mixture was kept at 50–55° for 20 hours, after which it was poured into a stirred ice–water mixture. The product was extracted with chloroform, washed with 2 N sulfuric acid, then with saturated bicarbonate solution and finally with water. 1-(2′,5′-Di-O-benzoyl-3′-O-mesyl-β-D-ribosyl)-uracil (XXI) was not obtained in crystalline form. A dried glass (1.1 g.) of XXI was treated with 1.5 g. of sodium benzoate in 20 ml. of DMF for 1 hour at 110° (internal temperature). The mixture was poured into approximately 500 ml. of water, stirred and cooled overnight. The solids were collected on a Celite pad and washed thoroughly with water. The residue was dissolved in chloroform, dried over sodium sulfate and concentrated to a sirup *in vacuo*. Two crystallizations from ethanol afford a 10% yield of XXII, m.p. 251–252° with resolidification to colorless needles which melted at 270–272° dec. Further recrystallizations did not alter the melting point. The ultraviolet spectral data for XXII are given in Table I (ratio of 230/260 mμ = 5.40). The infrared spectrum (KBr disk) of XXII is shown in Fig. 6.

Anal. Calcd. for $C_{23}H_{18}O_7N_2$: C, 63.65; H, 4.17. Found: C, 63.57; H, 4.29.

Method B.—2,3′-Anhydro-1-(β-D-xylofuranosyl)-uracil (0.1 g.) was suspended in 4 ml. of dry pyridine and treated with 0.15 ml. of benzoyl chloride. The stirred mixture was allowed to remain at 40° overnight. Fine needle crystals separated and were collected on a filter (80 mg.) and washed well with ethanol. Recrystallization from ethanol gave pure material, with identical melting point, ultraviolet and infrared spectral properties as the sample obtained by method A.

2,2′-Anhydro-1-(3′,5′-di-O-benzoyl-β-D-arabinosyl)-uracil (XXIV).—A sample of XXII was placed in a tube and immersed in a bath. The bath temperature was raised cautiously to 252° whereupon the solid melted and resolidified into needles. The bath temperature was maintained at 252° for approximately 3 minutes. The product was recrystallized from absolute ethanol to give pure XXIV, m.p. 270–272° (without pre-melting). The pure material gave no melting-point depression when admixed with an authentic sample of XXIV.[15] The ultraviolet absorption spectrum also agreed with that previously listed[15] (found 230/260 mμ = 4.52, reported 4.59). The infrared spectrum of XXIV (see Fig. 6) is significantly different from that exhibited by XXII (KBr disk). The infrared spectrum of XXIV was similar to that obtained with an authentic sample of XXIV prepared previously by another route.[15]

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA]

Synthesis of a Heptapeptide Sequence Derived from Bovine Insulin[1]

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The synthesis of glycyl-phenylalanyl-phenylalanyl-tyrosyl-threonyl-prolyl-lysine (all L), a heptapeptide sequence from bovine insulin, is described. The synthetic material appeared identical with the natural heptapeptide released by the action of trypsin on bovine insulin. The result represents a synthetic confirmation of this part of the insulin structure. A unique feature of the synthesis involved the use of the p-nitrobenzyloxycarbonyl group to cover the ε-amino function of the lysine during the preferential removal of a carbobenzoxy group from the α-amino function of lysine-containing peptides. Experiences in the preparation of p-nitrobenzyl esters of peptides and their use in peptide synthesis are described.

Since the proposal of the complete amino acid sequence of bovine insulin by Sanger and co-work-

ers in 1955,[3] a number of synthetic studies on various parts of the amino acid sequence have appeared.[4] However, to date, none of these synthetic

(3) A. P. Ryle, F. Sanger, L. F. Smith and R. Kitai, *Biochem. J.*, 60, 541 (1955).

(4) D. Chillemi, L. Scarso and E. Scoffone, *Gazzetta chim. ital.*, 87, 1356 (1957); *C. A.*, 52, 14540 (1958); J. A. Maclaren, W. E. Savige and J. M. Swan, *Australian J. Chem.*, 11, 345 (1958); G. F. Holland