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Pyrimidine Nucleosides. VII. Reactions of 2′,3′,5′-Trimesyloxyuridine[1]

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The displacement by nucleophilic attack of the three methylsulfonyloxy groups of tri-*O*-mesyluridine (I, prepared by treatment of uridine with methylsulfonyl chloride in pyridine) has been achieved with the synthesis of several new analogs of uridine. The reaction of I with sodium benzoate in refluxing N,N-dimethylformamide gave 2,2′-anhydro-(3′,5′-di-*O*-benzoyl-β-D-arabinosyl)-uracil (VI) and 1-(tri-*O*-benzoyl-β-D-xylofuranosyl)-uracil (VII) in crystalline form. The formation of VI appears to involve an anchimeric assistance by the 2,2′-anhydro oxygen in the replacement of the 3′-mesyloxy group. The reaction mixture, after removal of VI and VII, gave upon alkaline hydrolysis all four possible 1-β-D-aldopentofuranosyluracils. The mechanisms involved in these nucleoside interconversions are discussed.

As part of a program of synthesis of pyrimidine nucleosides,[2] it was of interest to investigate the behavior of a completely sulfonylated derivative of uridine toward nucleophilic reagents. Such an approach appeared to offer good possibilities for the synthesis of analogs of the naturally occuring pyrimidine nucleosides particularly with regard to variations in the sugar moiety. Neither the 2′,3′,5′-tritosyloxy- nor trimesyloxy-uridine were known. It is to be noted, however, that Levene and Tipson[3] obtained 5′-chloro-2′,3′-di-*O*-tosyluridine upon treating uridine with excess *p*-toluylsulfonyl chloride in pyridine. Since we obtained this same 5′-chloro derivative upon tosylating uridine in pyridine at 0° it was decided to concentrate upon the use of methylsulfonyl chloride. Indeed, the reaction of methylsulfonyl chloride (MsCl) and uridine at 0° in pyridine readily gave a nearly quantitative yield of crystalline 2′,3′,5′-tri-*O*-mesyluridine (I), as illustrated in Fig. 1.

Many examples of facile replacement of primary sulfonyloxy groups with various radicals are known[9] and no particular difficulty in the direct replacement of the 5′-mesyloxy function of I with a group such as iodo was anticipated.[10] In fact, it required only 20 minutes heating of I with sodium iodide in 2,5-hexanedione at 100° to give 5′-deoxy-5′-iodo-di-*O*-mesyluridine (II) in fair yield.

Primary sulfonyloxy groups are generally much more reactive to attack by iodide than secondary sulfonyloxy groups.[9,10] A notable exception to this generalization is found in the work of Levene and Tipson[11a] and of Michelson and Todd[11b] who succeeded in replacing the 3′-tosyloxy group of thymidine derivatives with halogen under relatively mild conditions.

Replacement of a 2′-sulfonyloxy group of pyrimidine nucleosides has been accomplished by intramolecular (*i.e.*, 2,2′-anhydro bond formation) nucleophilic attack. Examples of 2,2′-anhydro nucleosides are well known.[12,13-16] The 2,2′-oxygen bridge is readily formed under alkaline conditions as a result of a rearward nucleophilic attack upon C2′ by the 2-carbonyl oxygen, resulting in a displacement of the 2′-sulfonyloxy group.

Trimesyloxyuridine (I) readily underwent anhydro bond formation in 50% ethanol upon the addition of one equivalent of sodium hydroxide to form the 3′,5′-dimesyloxy-2,2′-anhydro derivative III in excellent yield. Under analogous reaction conditions, II gave a similarly good yield of 2,2′-anhydro-1-(5′-deoxy-5′-iodo-3′-*O*-methylsulfonyl-β-D-arabinosyl)-uracil (IV). The conversion of III to IV took place upon heating with sodium iodide in 2,5-hexanedione.

It is conceivable that in the reaction of I or II with base, either 2,2′- or 2,3′- or 2,5′-anhydronucleosides were formed. The evidence is conclusive, however, for a 2,2′-anhydro structure for compounds III and IV. Both III and IV show the two maxima at 225 and 247 mμ characteristic of 2,2′-anhydro nucleosides[13] rather than 2,5′-anhydro structures[17] (see Table I). In addition, a 2,5′-anhydro structure for IV would seem highly unwarranted since it would indicate a more facile replacement of the secondary 2′- or 3′-mesyloxy group than of the primary 5′-mesyloxy function in the reaction of I with sodium iodide. A 2,3′-anhydro structure for III and IV would be highly improbable in view of the experience of Brown, Parihar, Todd and Varadarajan,[18] who found 3′-*O*-tosyluridine extremely resistant to nucleophilic attack.[19] Conclusive evidence for the 2,2′-

(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) For previous publications from this Laboratory pertinent to the present work see papers I,[3] II[4] and IV[5] in this series and two recent reviews.[6,7]

(3) J. J. Fox, N. Yung, J. Davoll and G. B. Brown, THIS JOURNAL, **78**, 2117 (1956).

(4) J. J. Fox and N. Yung, *Federation Proc.*, **15**, 254 (1956); J. J. Fox, N. Yung and A. Bendich, THIS JOURNAL, **79**, 2775 (1957).

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

(6) J. J. Fox, *Rec. Chem. Prog.*, **19**, 173 (1958).

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

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

(9) For a review of the reactions of sulfonyloxy groups in carbohydrates see R. S. Tipson, *Advances in Carbohydrate Chem.*, **8**, 107 (1953).

(10) J. W. H. Oldham and J. K. Rutherford, THIS JOURNAL, **54**, 366 (1932).

(11) (a) P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **109**, 623 (1935); (b) A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, 816 (1955).

(12) Nomenclature conforms to that employed in reference 7.

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

(14) D. M. Brown, D. B. Parihar, C. B. Reese and A. Todd, *ibid.*, 3035 (1958).

(15) D. M. Brown, D. B. Parihar and A. Todd, *ibid.*, 4242 (1958).

(16) G. Shaw and R. N. Warrener, *Proc. Chem. Soc.*, 81 (1958); *J. Chem. Soc.*, 50 (1959).

(17) D. M. Brown, A. R. Todd and S. Varadarajan, *ibid.*, 868 (1957).

(18) D. M. Brown, D. B. Parihar, A. Todd and S. Varadarajan, *ibid.*, 3028 (1958).

(19) The fact that the 3′-tosyloxy group of thymidine derivatives is susceptible to both intermolecular and intramolecular nucleophilic at-

TABLE I

PHYSICAL CONSTANTS OF 1-β-D-ALDOPENTOFURANOSYLURACIL DERIVATIVES

Compound	Spectrophotometric[a] Maxima mμ	Maxima AM	Minima mμ	Minima AM	Ratio 230/260	Polarimetric[b] [α]D	T, °C.
Tri-*O*-Ms-ribo (I)	254	8970	232	5480	0.69	+35°	24
2,2'-Anhydro-3',5'-di-*O*-Ms-arabino (III)	225	8760	235	7740	1.41	−54	24
	247	8470					
2,2'-Anhydro-5'-deoxy-5'-iodo-3'-*O*-Ms arabino-($\frac{1}{2}H_2O$) (IV)	226	8730	236	7930	1.41	−45	24
	247	8500					
2,2'-Anhydro-5'-*O*-Bz-3'-*O*-Ms-arabino (V)	231	19800			3.40	−56	25
2,2'-Anhydro-3',5'-di-*O*-Bz-arabino (VI)	232.5	34000			4.59	−45	25
Tri-*O*-Bz-xylo (VII)	234	41200[c]			3.08	+82[c]	24
3',5'-Di-*O*-Bz-arabino (VIII)	233	29000	255	12600	2.14	+18	23
	262	13000					
Xylo (X)	262.5	9580	230	1860	0.20	+29	23
Tri-*O*-Bz-Bz-xylo (XI)	235				1.87		
2,2'-Anhydro-5'-deoxy-3'-*O*-Ms-arabino (XV)	225	9250	236	7980	1.46		
	247	8520					
2,2'-Anhydro-3'-*O*-Bz-5'-deoxy-arabino (XVI)	235	25000			3.37	−48	24
Tri-*O*-Bz-ribo (XXVI)	232	40700			2.81	−59	24
Tri-*O*-Bz-Bz-ribo	232.5	45500			1.87		

[a] All determinations carried out in ethanol–water (1:1). [b] For solvent and concentration data see Experimental section. [c] This value is corrected for presence of 8% volatile matter.

anhydro structure in compounds III and IV is established later.

Benzoylation Reactions.—Reist, Goodman and Baker[22] replaced the secondary tosyloxy group of methyl 6-deoxy-2,3-*O*-isopropylidene-5-*O*-tosyl-β-D-alloside with benzoate using sodium benzoate in

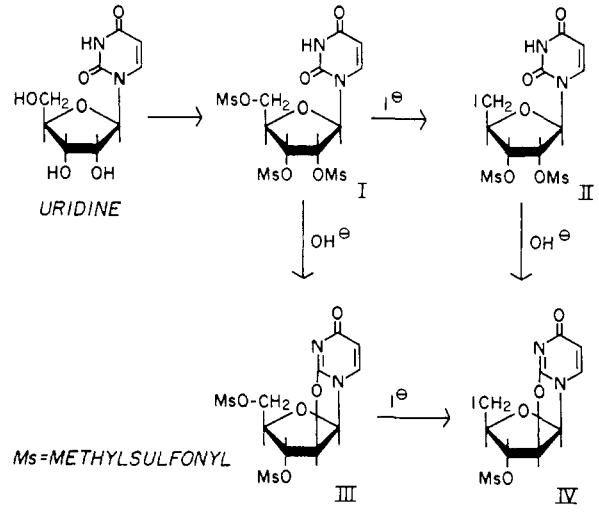

Fig. 1.

refluxing N,N-dimethylformamide (DMF). The benzoate ion entered with inversion to yield a tallose derivative. Their work suggested the possible use of these reagents to displace the mesyloxy groups of III.

In a preliminary experiment it was found that the reaction of IV with sodium benzoate in DMF at 80–90° gave 2,2'-anhydro-1-(5'-*O*-benzoyl-3'-*O*-mesyl-β-D-arabinosyl)-uracil (V) (see Fig. 2).

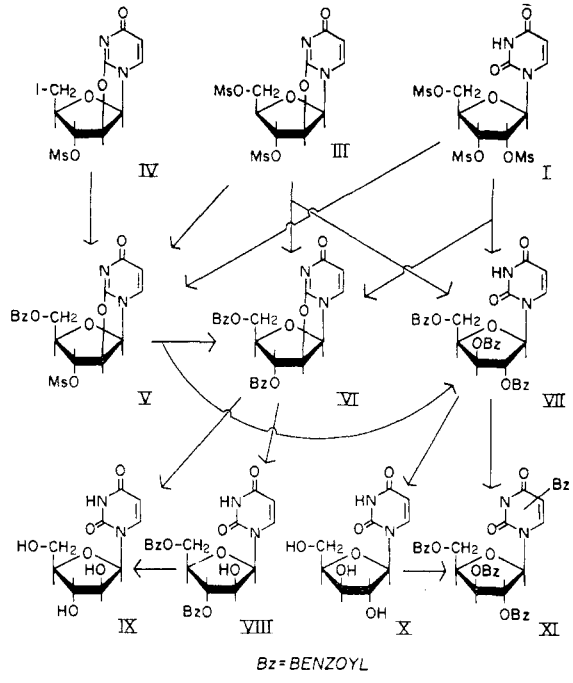

Fig. 2.

When III was heated with the same reagents a good yield of V, 84%, was obtained. It was subsequently found that V could be obtained in 89% yield when compound I was heated for only a short time at 100–110° with sodium benzoate, thus accomplishing both intermolecular and intramolecular nucleophilic displacement in a single step. N,N-Dimethylformamide may be used as a solvent in this reaction, although acetamide was found to be more satisfactory.

tack, whereas the 3'-tosyloxy group adjacent to a 2'-hydroxyl function in uridine derivatives is inert under the strenuous conditions employed by Brown and co-workers,[18] has not yet been explained. Aside from steric factors, some importance might be attributed to the fact that the 2'-OH group of uridine is more acidic than the 3'-hydroxyl function.[5,20,21] Incidentally, it would be interesting to observe whether the tosyloxy group of 3'-*O*-tosyluridine could be replaced by benzoate ion when refluxed with sodium benzoate in DMF.

(20) J. J. Fox, L. F. Cavalieri and N. Chang, THIS JOURNAL, 75, 4315 (1953).

(21) R. Kuhn and H. Sobotka, *Z. physik. Chem.*, 109, 65 (1924).

(22) E. G. Reist, L. Goodman and B. R. Baker, THIS JOURNAL, 80, 5775 (1958).

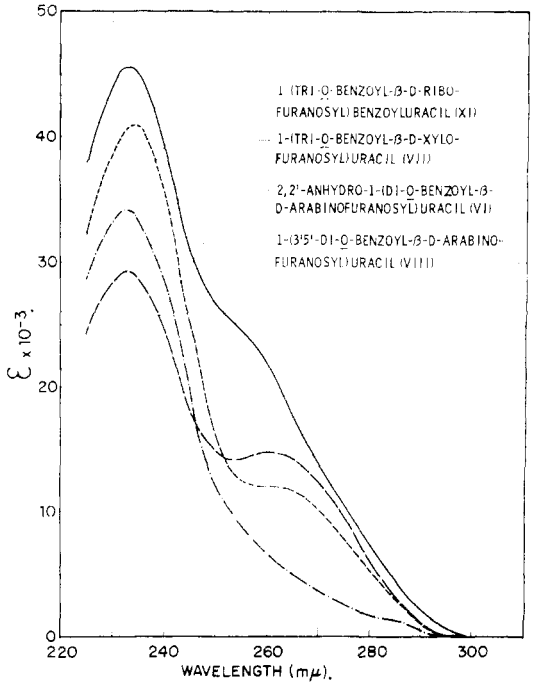

Fig. 3.—Ultraviolet absorption spectra of benzoylated nucleosides in ethanol–water (1:1).

An attempt was made to replace the 3′-mesyloxy group of V with benzoate in DMF; V proved extremely stable to further attack at temperatures below 140°. At the reflux temperature of DMF, however (*i.e.*, about 152°), reaction occurred as indicated by the appearance of color. After a 2- to 4-hour reaction period, two colorless crystalline products, VI and VII, were obtained from V without difficulty. Only 10 to 15% of the theoretical amount of VI and 7 to 9% of VII were obtained; VI and VII were obtained under the same general conditions from either I or III (see Fig. 2).

The ultraviolet absorption spectrum of VI (see Fig. 3), exhibited no maximum at 262mμ, characteristic of uridine, but showed a marked *O*-benzoyl absorption at 232.5 mμ; VI gave a ratio, 230/260, in dilute ethanol (1:1) of 4.59, far greater than that found for tri-*O*-benzoyluridine, 2.81 (see Table I). The negative optical rotation of VI (−45°) is characteristic of the 2,2′-anhydro derivatives found in Table I. Elemental analysis was consistent with a di-*O*-benzoyl-anhydro structure for VI.

When VI was warmed with aqueous hydrochloric acid in ethanol a maximum appeared at about 260 mμ, indicating rupture of the anhydro bond. When the reaction was carried out in dimethylformamide, it was possible to isolate in good yield a colorless crystalline material, VIII. Its ultraviolet absorption spectrum is shown in Fig. 3. Removal of the two benzoyl blocking groups with base gave a crystalline material, IX. Comparison with an authentic sample, kindly supplied by Dr. D. M. Brown of Cambridge University, clearly showed IX to be spongouridine, 1-β-D-arabinofuranosyluracil.[13]

It is thus established that the benzoyl group in position 3′ of VI and VIII has the *down* or arabino configuration. Therefore, in the reaction V → VI benzoate ion had replaced the 3′-mesyloxy group with no *net* inversion. These results were surprising in view of the fact that Reist, *et al.*[22] had observed inversion by a smooth SN2 displacement of a secondary tosyloxy group with benzoate under the same general reaction conditions. It should be noted here that the hydrolysis of VI may give rise to nucleosides other than IX (*vide infra*).

The arabino configuration for compound VIII, obtained in high yield from VI upon acid treatment, establishes a 2,2′-anhydro structure for VI. Since it is highly improbable that the reaction of III or V to yield VI would have resulted in a shift in the position of the anhydro linkage, a 2,2′-anhydro structure for compounds III, IV and V is also established (see Fig. 2).

It may be postulated that conversion of V to VI (without net inversion of C3′) proceeded *via* a 3′,5′-orthoester ion.[23] This plausible postulate would involve a nucleophilic attack on C3′ (XIII) (see Fig. 4) by the carbonyl oxygen of the 5′-*O*-benzoyl with the formation of a 3′,5′-orthoester ion (XIV). Attack by benzoate ion on C3′ would yield VI.

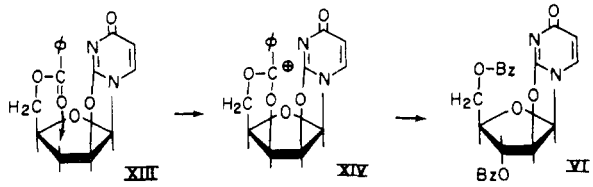

Fig. 4.

As a test of this hypothesis 2,2′-anhydro-1-(5′-deoxy-3′-*O*-mesyl-β-D-arabinosyl)-uracil (XV) (see Fig. 5) was treated with sodium benzoate under the conditions employed in the preparation of VI. Obviously, no such orthoester ion could form from XV, and the isolation of a 3′-*O*-benzoyl-anhydro-5′-deoxy-nucleoside of the arabino configuration from the reaction mixture would constitute strong evidence against the hypothesis (XV had been prepared in high yield by reduction of IV using palladium-on-charcoal).

Following a reaction period of four hours, a product, XVI, was isolated in 20% yield. This material showed a typical benzoyl absorption at 235 mμ. A negative rotation (−48°) characteristic of 2,2′-anhydronucleosides (see Table I) was observed. When XVI was warmed with aqueous hydrochloric acid a maximum appeared at about 260 mμ, indicating cleavage of the anhydro bond. Elemental analyses were consistent with a 5′-deoxy-3′-*O*-benzoyl-2,2′-anhydro-nucleoside structure for XVI.

After treatment of XVI with aqueous acid to cleave the anhydro bond, followed by alkaline treatment to remove the benzoyl group, only one product, XVII, as colorless needles, was isolated from the mixture; XVII proved to be identical

(23) The authors would like to thank Dr. B. R. Baker, Dr. C. D. Anderson and their associates for the suggestion of a 3′,5′-orthoester ion intermediate in this reaction.

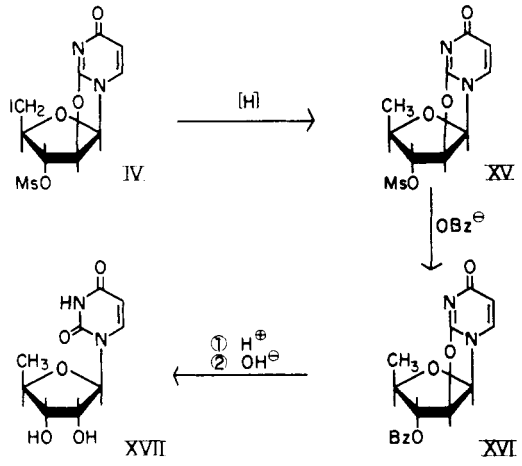

Fig. 5.

with a sample of 5′-deoxyuridine prepared by Wempen, *et al.*,[24] in an unequivocal manner.[25]

Since it is inconceivable that a 3′-*O*-benzoyl-2,2′-anhydro-lyxosyl derivative (3′-*O*-benzoyl in the *up* or lyxo position) could have given rise to XVII, it is established that compound XVI has, in fact, the same configuration as VI at C2′ and C3′. Thus, in the conversion of V to VI as well as XV to XVI, benzoate had replaced the 3′-mesyloxy group with no *net* inversion. It is therefore demonstrated that a mechanism involving a 3′,5′-orthobenzoate ion intermediate need not be invoked to explain the formation of the *down* *O*-benzoyl group in VI.

A plausible mechanism for the formation of VI as well as XVI involves anchimeric assistance[26-28] from the anhydro oxygen (see Fig. 6). The oxygen exerts an influence similar to that of a nucleophile upon C3′ as shown in structure XIX, thus assisting in the elimination of the 3′-mesyloxy group. This would result in the formation of a transitory cyclic oxonium ion intermediate, XX, of the type postulated by Winstein and co-workers.[28] Attack at C3′ of the intermediate by benzoate ion would result in the formation of VI. That C3′ attack would be favored over an attack at C2′ is consistent with previous observations regarding the opening of 2′,3′-epoxide derivatives of certain aldopentofuranosides and nucleosides.[15,29-31] This mechanism would provide the stereochemical control necessary to afford a product representing no net inversion, although two inversions would, in fact, be involved in the formation of VI.

Compound VII (see Fig. 2) showed strong absorption at 234 mμ, indicating the presence of *O*-benzoyl groups, and a shoulder at about 260 mμ

(24) I. Wempen, I. Doerr, L. Kaplan and J. J. Fox, THIS JOURNAL, **82**, 1624 (1960).

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

(26) S. Winstein, C. R. Lindegren, H. Marshall and L. L. Ingraham, THIS JOURNAL, **75**, 147 (1953).

(27) S. Winstein, C. R. Lindegren and L. L. Ingraham, *ibid.*, **75**, 155 (1953).

(28) S. Winstein, W. Allred, R. Heck and R. Glick, *Tetrahedron*, **3**, 1 (1958).

(29) S. Mukherjee and A. R. Todd, *J. Chem. Soc.*, 969 (1947).

(30) J. Davoll, B. Lythgoe and S. Trippett, *ibid.*, 2230 (1951).

(31) C. D. Anderson, L. Goodman and B. R. Baker, THIS JOURNAL, **80**, 5247 (1958).

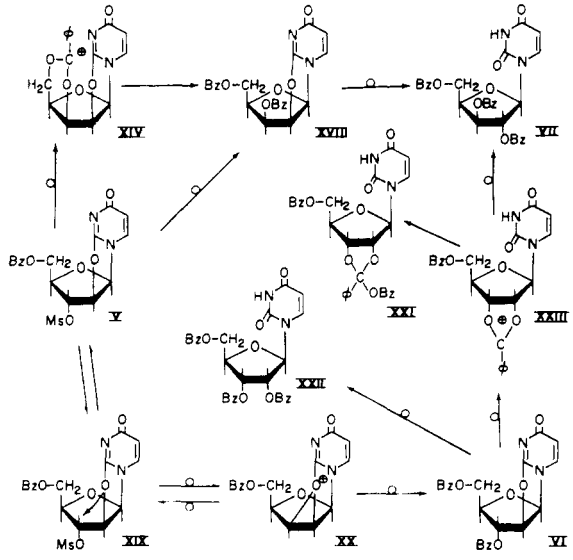

Fig. 6.

where uridine exhibits a maximum (see Fig. 3). No spectral change was noted when VII was warmed with ethanol–aqueous hydrochloric acid, indicating the absence of a 2,2′-anhydro bond. Removal of the benzoyl blocking groups with base afforded a colorless, crystalline product, X (see Fig. 2). The ultraviolet absorption spectrum of X was similar to that of uridine, but X differed in rotation (+29°) and melting point (158–158.5°) from the naturally-occurring nucleosides uridine and spongouridine. It consumed metaperiodate slowly which is characteristic of nucleosides containing vicinal *trans*-hydroxy groups.[3,5] The rotation of the solution of the dialdehyde obtained from this oxidation was +16°, a value consistent with that obtained from the other 1-β-D-aldopentofuranosyluracils. These data warrant the assignment of the 1-β-D-xylofuranosyl structure to X. A comparison of the polarimetric data for the four 1-β-D-aldopentofuranosyluracil isomers in this series and their thymine homologs is given in Table II.

TABLE II

COMPARISON OF MOLECULAR ROTATIONAL DIFFERENCES BETWEEN THE FOUR 1-β-D-ALDOPENTOFURANOSYLURACIL ISOMERS AND THEIR THYMINE HOMOLOGS

	Uracil derivatives			Thymine derivatives		
	$[\alpha]_D$	$[M]_D$	Difference	$[\alpha]_D$	$[M]_D$	Difference
Ribo	+10°[a]	+2,440		−10°[3]	−2,580	
			−28,300			−26,600
Arabino	+126°[5]	+30,700		+93°[4]	+24,000	
			+9,000			+8,500
Lyxo	+89°[31]	+21,700		+60°[3]	+15,500	
			+14,600			+16,000
Xylo	+29	+7,080		−2°[3]	−520	

[a] D. T. Elmore, *J. Chem. Soc.*, 2084 (1950).

A comparison of the molecular rotational differences of VII and tri-*O*-benzoyluridine with that of the 2′,3′,5′-tri-*O*-benzoyl-derivatives of 1-β-D-xylofuranosylthymine[3] and 1-β-D-ribofuranosylthymine[3] (see Table III) demonstrates that the precursor of X, namely, VII, is also of the xylo configuration.

TABLE III

COMPARISON OF MOLECULAR ROTATIONAL DIFFERENCES BETWEEN TRI-*O*-BENZOYL DERIVATIVES OF TWO 1-β-D-ALDOPENTOFURANOSYLURACIL ISOMERS AND THEIR THYMINE HOMOLOGS

	Uracil derivatives			Thymine derivatives		
	$[\alpha]_D$	$[M]_D$	Difference	$[\alpha]_D$	$[M]_D$	Difference
Ribo	−59°	−32,800		−83°[32]	−47,300	
			−78,400			−80,400
Xylo	+82	+45,600		+58[33]	+33,100	

Compound VII melted low and over a range, 112–118°, with the evolution of volatile material representing 8% of its weight.[32] The resulting amorphous solid, after removal of the volatile material, gave an elemental analysis corresponding to a tri-*O*-benzoyl-pentofuranosyluracil. The A_M of VII was found to be 37,800, 7% less than that found for tri-*O*-benzoyluridine. The presence of the volatile material in VII does not appreciably change the spectrum, and the ratio 230/260 of VII (3.08) and of the heated material (3.03) are essentially the same. Reaction of both X and VI (after removal of the volatile material) with excess benzoyl chloride in pyridine produced the same tetrabenzoyl nucleoside, XI (see Fig. 2), the ultraviolet absorption spectrum of which is almost identical with that of tetrabenzoyluridine[33] (ratio 230/260 = 1.87; see Fig. 4). It is concluded, therefore, that VII is 1-(tri-*O*-benzoyl-β-D-xylofuranosyl)-uracil containing 7–8% volatile material. The identity of the volatile substance has not yet been determined. The infrared spectrum of VII indicates that it contains no water of crystallization.[34]

The replacement of the 3′-mesyloxy group of V in an SN2 reaction to yield 2,2′-anhydro-1-(3′,5′-di-*O*-benzoyl-β-D-lyxofuranosyl)-uracil (XVIII) (see Fig. 6) followed by attack by benzoate ion at C2′ to cleave the anhydro bond,[15] is suggested as an explanation for the formation of tri-*O*-benzoyl-β-D-xylofuranosyluracil (VII). The formation of a di-*O*-benzoyl-anhydro derivative of the lyxo configuration XVIII by an SN2 reaction at C3′ would be consistent with the results of Reist, *et al.*[22] A discussion of evidence supporting the formation of XVIII in the reaction follows a description of material remaining in the reaction mixture after the removal of VI and VII.

The possibility of VI serving as a major precursor of VI through a 2′,3′-orthoester ion intermediate XXIII, of the type described by Winstein and collaborators,[35–37] appears unlikely. After refluxing VI with sodium benzoate in DMF it was impossible to isolate VII although, after a similar reaction time using V as a reactant, VII was readily obtained.

After the removal of VI and VII from the reaction of V with sodium benzoate in boiling DMF, the mother liquor was examined. A chloroform-soluble fraction representing 35% of the weight of starting material V was isolated as an amorphous solid XII, which resisted all attempts at crystallization. Fraction XII exhibited an ultraviolet absorption spectrum (ratio 230/260, 2.9) almost identical to that of tri-*O*-benzoyl-uridine (ratio 2.81) (see Table I) and gave a rotation (−30°) which, it may be noted, is not far removed from that of either tri-*O*-benzoyluridine (−59°) or a 2,2′-anhydro derivative (VI, −45°). Elemental analyses of XII, however, gave values in agreement with a di-*O*-benzoylated nucleoside. As will be discussed in detail in a later section, the alkaline hydrolysis of XII gave a mixture of all four 1-β-D-aldopentofuranosyluracil isomers in approximately equal quantity. From these data it is clear that XII is a mixture of difficultly separable benzoylated nucleosides and undoubtedly contains compounds of the lyxo and ribo configurations in addition to some material having the arabino, VI, and the xylo, VII, structures.

Although no 3′,5′-di-*O*-benzoyl-2,2′-anhydro derivative with the 3′-benzoyloxy group in the *up* or lyxo position has been isolated, its presence is indicated. Alkaline hydrolysis of XII gave a substantial amount of 1-β-D-lyxosyluracil. In the 5′-deoxy series, the reaction mixture, after hydrolysis, contained 1-(5′-deoxy-β-D-lyxofuranosyl)-uracil. Chromatographic evidence supports the view that a large part of the material of lyxo configuration obtained from XII is not present as 5′-*O*-benzoyl-lyxofuranosyluracil.[38] Furthermore, acid treatment of XII resulted in a marked decrease in the 230/260 mμ ratio, indicative of the presence of a large amount of 2,2′-anhydro derivatives.

It is conceivable that XVIII may be formed by yet another pathway in addition to attack by benzoate at C3′ by an SN2 mechanism, as discussed above (see Fig. 6). The possible formation of a 3′,5′-orthobenzoate ion, XIV, from V has been discussed previously (see Fig. 4). Compound XVI would be particularly vulnerable to attack at the primary carbon, C5′, which would give XVIII. Attack at the secondary carbon, C3′, to give VI seems much less likely. As discussed above, the fact that an analog of VI is formed in the 5′-deoxy series, XVI, under similar reaction conditions, mitigates strongly against XIV as a precursor to VI in the reaction.

Although no benzoylated nucleoside having the ribo configuration has been isolated from the mixture which resulted after refluxing V with sodium benzoate in DMF, the presence of such material(s) has been demonstrated. A fraction from XII showing very little water solubility but high chloroform solubility gave upon alkaline hydrolysis a high proportion of uridine. It is conceivable that the benzoylated uridine is present in two different forms, XXI and XXII, both arising from

(32) 1-(Tri-*O*-benzoyl-β-D-xylofuranosyl)-thymine melted sharply, 197.5–198.5°,[3] without gas evolution.

(33) J. J. Fox, D. Van Praag, I. Wempen, I. Doerr, L. Cheong, J. E. Knoll, M. Eidinoff, A. Bendich and G. B. Brown, THIS JOURNAL, **81**, 178 (1959).

(34) The authors wish to express their deep appreciation to Dr. David Fleischer and to Mrs. Thomas F. Gallagher for infrared spectral determinations.

(35) S. Winstein and R. E. Buckles, THIS JOURNAL, **65**, 613 (1943).

(36) S. Winstein, C. Hanson and E. Grunwald, *ibid.*, **70**, 812 (1948).

(37) S. Winstein and R. M. Roberts, *ibid.*, **75**, 2297 (1953).

(38) Although the presence of 1-(5′-*O*-benzoyl-β-D-lyxofuranosyl)-uracil in this reaction mixture has not been demonstrated, its synthesis in good yield from V has been achieved and will be reported in a forthcoming publication.

TABLE IV

HYDROLYSIS OF 1-β-D-ALDOPENTO- (AND 5′-DEOXYALDOPENTO)-FURANOSYLURACILS

Benzoylated nucleoside and 5′-deoxynucleoside	Hydrolytic agent	Products configuration
2,2′-Anhydro-3′,5′-di-*O*-Bz-arabino (VI)	HCl (DMF–water)	Arabino (VIII)[a]
3′,5′-Di-*O*-Bz-arabino (VIII)	NaOH (ethanol–water)	Arabino[a]
VI	HCl (DMF-water) followed by NaOH (water)	Arabino, xylo, ribo[b]
2,2′-Anhydro-5′-deoxy-3′-*O*-Bz-arabino (XVI)	HCl (water) followed by NaOH (water)	Ribo; arabino and/or xylo[b]
VI	NaOH (water or ethanol–water)	Arabino, xylo[b]
XVI	NaOH (water or ethanol–water)	Arabino and/or xylo[b]
Tri-*O*-Bz-xylo (VII)	NaOH (ethanol–water)	Xylo[a]
Residue (XII)	NaOH (ethanol–water)	Arabino, xylo, ribo, lyxo[b]
Residue (5′-deoxy)	NaOH (water)	Ribo, lyxo; arabino and/or xylo[b]

[a] Product isolated; mother liquor not examined by paper electrophoresis. [b] Product evaluated by paper electrophoresis.

VI through an orthoester ion intermediate XXIII[35–37] (Fig. 6). This postulate is supported by the fact that refluxing a solution of VI and sodium benzoate in DMF resulted in considerable cleavage of the 2,2′-anhydro structure after two hours. The formation of the benzoxy orthobenzoate XXI from XXIII would not be surprising, since compounds of this type have been reported by several laboratories.[35,39,40] Alkaline hydrolysis of XXI would be expected to yield uridine. Although the presence of tri-*O*-benzoyluridine XXII has not been demonstrated, it could conceivably have formed as a result of benzoate ion attack at C2′ of VI to open the oxazolidine ring.[15]

Hydrolysis Experiments.—As described above (see Fig. 2), a high yield of spongouridine (IX) was obtained when the di-O-benzoyl-anhydro derivative VI was warmed with aqueous hydrochloric acid in DMF followed by treatment of the crystalline intermediate VIII with base. When ethanol was used in place of dimethylformamide in the acid reaction followed by the same base treatment, however, no spongouridine could be isolated from the reaction mixture. Furthermore, in the cleavage of the 5′-deoxy-3′-benzoyloxy-anhydro derivative XVI with aqueous hydrochloric acid, followed by base treatment, a fair yield of 5′-deoxyuridine, (XVII) was obtained (see Fig. 5). Table IV contains a summary of the hydrolytic reactions of the benzoylated nucleosides.

In an effort to evaluate these results, the method of Gordon, Intrieri and Brown[41] (paper electrophoresis in borate buffer, *p*H 6) was employed. These investigators had obtained a good separation of the four 1-β-D-aldopentofuranosylthymine isomers synthesized in this Laboratory.[3–5] An equally good separation of the uracil analogs was obtained as shown in Fig. 7. A comparison of the anodic migrations in the different series is shown in Table V.

It was found that VIII, 1-(3′,5′-di-*O*-benzoyl-β-D-arabinosyl)-uracil (see Figs. 2 and 8), when treated with sodium hydroxide in dilute ethanol gave rise to only one compound, as evaluated by paper electrophoresis; namely, the arabino nucleoside IX. Therefore, only acyl–oxygen fission is involved in this step. When the di-*O*-benzoyl-anhydro derivative VI was refluxed under the same conditions, namely, with sodium hydroxide in dilute ethanol (and the reaction mixture separated by paper electrophoresis), two products were observed, arabinosyluracil (IX) and xylosyluracil (X). Elution of the spots with water and determination of the relative optical densities at 262 mμ for the two solutions showed both arabino and xylo isomers to be present in appreciable amounts.

(39) H. W. Post and E. R. Erickson, *J. Org. Chem.*, **2**, 260 (1937).
(40) E. Pascu, *Advances in Carbohydrate Chem.*, **1**, 81 (1945).
(41) M. P. Gordon, O. M. Intrieri and G. B. Brown, THIS JOURNAL, **80**, 5161 (1958).

TABLE V

IONOPHORETIC SEPARATION OF NUCLEOSIDES. ANODIC MIGRATIONS (IN CM.) OF THE 1-β-D-ALDOPENTOFURANOSYLURACILS COMPARED WITH THEIR THYMINE HOMOLOGS

	Uracil derivatives[a]	Thymine derivatives[b]
Arabino	− 2.7	− 5.0
Xylo	+ 6.0	+ 2.9
Ribo	+ 9.9	+ 7.3
Lyxo	+14.3	+12.5

[a] Run at 700 volts and 17–19 milliamp. for 240 minutes in borate buffer at *p*H 6.0–6.05. [b] Reported by Gordon, Intrieri and Brown[29]; run at 600–700 volts and 50 milliamp. for 180 minutes in borate buffer at *p*H 6.0.

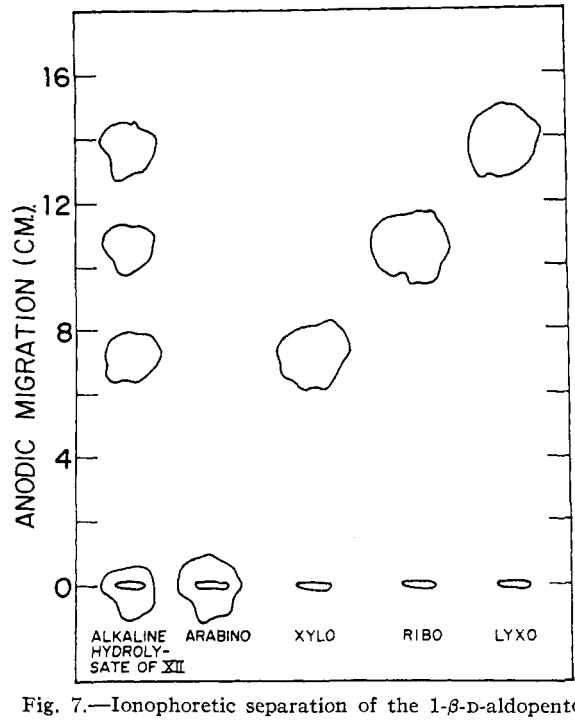

Fig. 7.—Ionophoretic separation of the 1-β-D-aldopentofuranosyluracils in borate buffer at *p*H 6.0 (900 volts, 10–13 milliamp., 285 minutes).

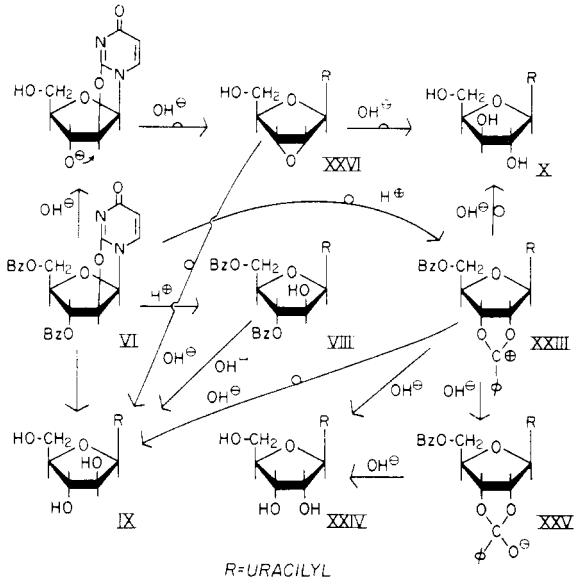

Fig. 8.

In view of the results of D. Brown, *et al.*,[18] it seems plausible to suggest that the formation of IX and X in this reaction proceeded through 2′,3′-anhydroribosyluracil (XXVI) (see Fig. 8). Opening of the *down* epoxide with base would be expected to give mainly X from attack by hydroxyl ion at C3′ and a small amount of IX from attack at C2′.[29-31] The major portion of IX formed in this reaction was probably derived from 2,2′-anhydro bond cleavage (C2-attack) without inversion, followed by removal of the benzoyl groups.

The base hydrolysis of the 5′-deoxy analog of VI, namely, XVI, was carried out under the same conditions as the hydrolysis of VI (see Table IV). When the product was subjected to paper ionophoretic separation and paper chromatography in two different solvent systems, only one spot was obtained. Although the material did not migrate in the electric field but remained at the origin, as does spongouridine, the presence of 5′-deoxyxylosyluracil cannot be eliminated. Since a 3′,5′-borate complex with the xylosyl isomer is not possible, as in the pentofuranosyl series, anodic migration would not be expected.[41] It seems probable that both isomers are formed in this reaction, as in the case in the alkaline hydrolysis of VI.

When VI was subjected to acid hydrolysis in DMF followed by treatment with sodium hydroxide in dilute ethanol (without isolation of the intermediate monohydroxy derivative VIII) two products, in addition to spongouridine, were shown to be present in substantial amounts by the technique of Gordon, *et al.*[41] These were the xylosyl and ribosyl (uridine) nucleosides.[42] The presence of lyxosyluracil was not detected. The formation of the ribosyl isomer in this reaction is consistent with the results obtained in the 5′-deoxy series where 5′-deoxyuridine was isolated in fair yield after a similar reaction. It appears reasonable to suggest that uridine is formed from XXIII under alkaline condition; XXIII could have formed from VI under acidic conditions. Base hydrolysis of the orthoester anion, intermediate XXV, which could have resulted from hydroxyl ion attack on XXIII or XXI (see Fig. 6), would have yielded uridine (XXIV). Attack by hydroxyl ion at C2′ of XXIII (see Fig. 8) followed by acyl–oxygen fission in base may represent another pathway (in addition to cleavage of the 2,2′-anhydro bond through attack at C2, followed by removal of the benzoyl groups) by which IX is formed. Attack at C3′ of XXIII would lead to the formation of X. The amorphous solid remaining after isolation of crystalline products VI and VII was subjected to hydrolysis by sodium hydroxide, and the products separated by paper electrophoresis. In the 1-β-D-aldopentofuranosyluracil series the amorphous residue XII gave the pattern shown in Fig. 7.

The presence of all four possible 1-β-D-aldopentofuranosyluracil isomers is clearly indicated. Thus, in addition to the arabino and xylo nucleosides, which were obtained by base hydrolysis of VI, and xylosyluracil, obtained from VII by base treatment, the hydrolytic product from XII contained also the ribosyl- and lyxosyluracils.[38] The relative amounts of the four isomers, ribo, arabino, xylo and lyxo, were determined spectrophotometrically after elution of the four spots from the paper. These values indicated that all four isomers were present in about the same amounts. It is thus demonstrated that in a single procedure (treatment of V with sodium benzoate in boiling DMF, followed by alkaline hydrolysis), all four possible 1-β-D-aldopentofuranosyluracil isomers are formed.

Since the alkaline hydrolysis of VI affords only the arabino and xylo isomers, and of VII only xylosyluracil, it may be postulated that the mixture XII contains in addition to these materials at least two additional benzoylated nucleosides. 1-β-D-lyxofuranosyluracil would almost certainly have resulted from the hydrolysis of compounds with lyxo configuration, such as XVIII (see Fig. 6). With regard to the origin of uridine, under alkaline conditions, it does not seem plausible that this material could have arisen from compounds other than ribosyluracil derivatives, such as XXI or tri-*O*-benzoyluridine (XXII).

Conclusion.—From the foregoing results, it is clear that tri-*O*-mesyluridine is a versatile intermediate adaptable to the synthesis of 1-β-D-aldopentofuranosyl isomers of uridine. While many of the reactions described warrant further elucidation, it is hoped to extend these studies in this Laboratory to the synthesis of new nucleosides of potential biological interest.

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

Experimental

2′,3′,5′-Tri-O-methylsulfonyluridine (I).—A solution of 12.2 g. (0.05 mole) of uridine in 100 ml. of dry pyridine at

(42) The formation of uridine and spongouridine was reported by Brown, *et al.*,[15] in the acid treatment of 2,2′-anhydro-1-(3,,5,-di-*O*-acetyl β D arabinosyl)-uracil. These authors, however, did not report the formation of xylosyluracil.

−10 to 0° was treated while stirring with 18.9 g. (0.17 mole) of methylsulfonyl chloride. The reaction mixture was allowed to stand for several hours at 0°, then poured into three liters of ice-water and stirred for 1 hour. The pale yellow solid was collected on the filter and washed with ethanol and ether. After drying in air, the material, m.p. 166–169° (uncor.), weighed 23.2 g. (98%). Upon one crystallization from 50% ethanol an 82–85% yield of the material, m.p. 181–182 dec.,[43] was recovered. Repeated crystallizations from 50% ethanol gave colorless needles, m.p. 184–186° dec., $\alpha^{24}D$ + 35° (dioxane, *c* 0.8).[44]

Anal. Calcd. for $C_{12}H_{18}N_2O_{12}S_3$: C, 30.1; H, 3.8; N, 5.9; S, 20.1. Found: C, 30.3; H, 4.0; N, 5.6; S, 20.3.

5′-Deoxy-5′-iodo-2′,3′-di-O-methylsulfonyluridine (II).—Under anhydrous conditions, 5.25 g. (0.035 mole) of dry sodium iodide was dissolved in 45 ml. of freshly distilled 2,5-hexanedione. After the addition of 3.99 g. (0.0083 mole) of I the solution was heated on a steam-bath for 20 minutes. The reaction mixture was poured into 800–900 ml. of a well-stirred ice–water mixture. The material was collected on the filter, then washed in turn with cold water, ethanol and ether. The crude material weighed 6.35 g. Upon cooling an ethanolic solution, a pale-yellow amorphous solid (1.85 g., 52%, m.p. 99–110°) was obtained. Despite repeated efforts, the product was not obtained in crystalline form. This material was used in the preparation of IV, described below.

2,2′-Anhydro-1-(5′-deoxy-5′-iodo-3′-O-methylsulfonyl-D-arabinosyl)-uracil (IV). Method I.—A hot solution of 2.61 g. (0.0051 mole) of II in 200 ml. of dilute ethanol (1:1) was allowed to cool to 40°. While stirring, 4.0 ml. of sodium hydroxide (1.13 *N*) was added. After cooling overnight, the material, 1.50 g. (75%), m.p. 160–165°, was collected on the filter. Crystallization from methanol gave colorless prisms, m.p. 176–177° (dec., uncor.), $\alpha^{24}D$ −44° (acetone–water, 1:1).

Crystallization from dilute ethanol (1:1) produced colorless needles which failed to melt sharply, m.p. 160–165° dec. Elemental analysis indicated that water of crystallization was present.

Anal. Calcd. for $C_{10}H_{11}N_2O_6SI \cdot {}^1/_2\ H_2O$: I, 30.0; S, 7.6. Found: I, 30.3; S, 7.4.

Method II.—Under anhydrous conditions 6.5 g. (0.017 mole) of III and 5.5 g. (0.037 mole) of dry sodium iodide in 110 ml. of freshly distilled 2,5-hexanedione was heated on a steam-bath for 2.2 hours. After cooling, the insoluble sodium mesylate was removed by filtration, and the volume reduced to about 10 ml. *in vacuo*. After the addition of 100 ml. of ice-water, the product was collected at the filter and washed consecutively with cold water, ethanol and finally ether. The crude material, m.p. 160–167°, weighed 6.8 g. (94%). Crystallization from methanol yielded 5.4 g., m.p. 178–179.5° dec. A mixture of this material, m.p. 176–178° (dec., uncor.), and of IV prepared by method I, m.p. 176–177° (dec., uncor.), melted at 176–177° (dec., uncor.).

2,2′-Anhydro-1-(3′,5′-di-O-methylsulfonyl-β-D-arabinosyl)-uracil (III).—A hot saturated solution of 16.0 g. (0.033 mole) of I, m.p. 180–181°, in 1600 ml. of 50% ethanol was cooled quickly to avoid crystallization. As the temperature reached 45–50°, 34.5 ml. of sodium hydroxide (1.0 *N*) was added quickly with stirring. After 5 minutes, the solution was cooled. Colorless to pale yellow needles, m.p., 179–185° dec., were collected. The material weighed 12.2 g. (95% yield). From crude uncrystallized samples of I yields of about 80% were obtained. The material was crystallized by dissolving quickly in ethanol–water (1:1) and cooling. Melting points varied somewhat with the rate of heating. Melting points as high as 195° were obtained for III; $\alpha^{24}D$ −54° (acetone–water, *c* 0.7).

Anal. Calcd. for $C_{11}H_{14}N_2O_9S_2$: C, 34.6; H, 3.7; N, 7.3; S, 16.8. Found: C, 34.9; H, 3.9; N, 7.0; S, 16.8.

2,2′-Anhydro-1-(5′-O-benzoyl-3′-O-methylsulfonyl-β-D-arabinosyl)-uracil(V). Method I.—A hot solution of 1.12 g. (0.0078 mole) of sodium benzoate in 75 ml. of N,N-dimethyl-formamide was cooled to about 100°. After the addition of 1.40 g. (0.0037 mole) of III the solution was heated on the steam-bath for two hours. The clear colorless solution was poured into 600 ml. of water. After cooling for one hour 1.26 g. (84% yield) of colorless needles, m.p. 220–222° (dec., uncor.), were collected. Crystallization from ethanol–water (1:1) yielded 1.19 g. of material, m.p. 224–224.5° (dec., uncor.).

Method II.—To a solution of 4.0 g. (0.028 mole) of sodium benzoate in 30 g. of acetamide at 100° was added 2.0 g. (0.0042 mole) of I. The solution was heated at an internal temperature of 110–115° for 12 minutes. (A small sample was removed and added to water. The colorless crystals melted at 222–226° and had a ratio in ethanol–water (1:1), 230/260 mμ, of 3.35.) The reaction mixture was heated at 110–115° for a total of 35 minutes, then added to 300 ml. of water. A yield of 1.52 g. (89%) of colorless needles, m.p. 218–220° (uncor.), ratio 230/260 mμ 3.40 (ethanol–water (1:1)). An analytically pure sample obtained by crystallization from water–ethanol (1:1) melted at 226–227° (dec., uncor.), $\alpha^{25}D$ −56° (DMF, *c* 0.4).

Anal. Calcd. for $C_{17}H_{16}N_2O_8S$: C, 50.0; H, 4.0; N, 6.9; S, 7.8. Found: C, 50.2; H, 4.0; N, 6.7; S, 7.8.

2,2′-Anhydro-1-(3′,5′-di-O-benzoyl-β-D-arabinosyl)-uracil (VI).—To a hot solution of 15.0 g. (0.104 mole) of dry sodium benzoate in 1200 ml. of N,N-dimethylformamide was added 5.64 g. (0.0138 mole) of V and the solution refluxed for two hours. The reddish-brown solution was allowed to stand several hours at room temperature, then filtered. The weight of colorless solid, consisting of sodium mesylate and unreacted sodium benzoate, was 13.8 g.

The filtrate was taken to dryness *in vacuo*, then triturated with 200 ml. of ether. The insoluble material was collected on the filter, triturated well with water and filtered. Both the ether and water extracts were discarded.

The brown solid was triturated with 150 ml. of chloroform and filtered. Recovery of VII from the chloroform solution is described later. The chloroform-insoluble material was taken up in hot ethanol and treated with charcoal. Upon cooling the ethanolic solution, colorless needles, m.p. 258–262° (dec., uncor.), were obtained. Additional material was obtained from the mother liquors and from the chloroform-soluble fraction upon re-extraction of the residue with a small amount of chloroform or chloroform containing ether. Yields of material melting within a 250–262° (dec., uncor.) range varied from 0.6 to 0.9 g. (10 to 15%). Crystallization from 95% ethanol gave colorless needles, m.p. 260–262° (dec., uncor.), $\alpha^{26}D$ −45° [CH_3OH–$CHCl_3$ (2:1), *c* 0.3].

Anal. Calcd. for $C_{23}H_{18}N_2O_7$: C, 63.6; H, 4.2; N, 6.5. Found: C, 63.4; H, 4.4; N, 6.8.

1-(2′,3′,5′-Tri-O-benzoyl-β-D-xylosyl)-uracil (VII).—The chloroform extract, described above in the preparation of VI, was evaporated to dryness *in vacuo*. The residue was dissolved in hot ethanol and treated with charcoal. Colorless needles separated readily from the cooled solution. The yield of material, m.p. 110–116° with effervescence, was 0.54 to 0.69 g. (7–8%). Recrystallization from ethanol gave colorless needles, m.p. 112–118° (eff.). When heated at 135° *in vacuo* the material lost 8.0% of its weight, leaving an amorphous solid residue, $\alpha^{24}D$ + 75° (+82°, if corrected for 8% volatile material; $CHCl_3$, *c* 0.8).

Anal. Calcd. for $C_{30}H_{24}N_2O_9$: C, 64.7; H, 4.4; N, 5.0. Found: C, 65.0; H, 4.6; N, 5.3.

Isolation of Amorphous Material XII.—The ethanolic mother liquors, after the removal of VI and VII (see above), were taken to dryness *in vacuo*, leaving an orange-yellow amorphous solid, 2.0 g. A portion of this material was triturated well with water and decanted. The residue was dissolved in benzene and the solution dried. The addition of petrolem ether (boiling range 40–60°) caused precipitation of a solid which was filtered. Trituration of the precipitate with carbon tetrachloride, followed by filtration, gave a yellow amorphous solid, m.p. 84–110°, $\alpha^{24}D$ −30° ($CHCl_3$, *c* 0.3); light absorption properties: A_M 35,900 (233 mμ, EtOH–H_2O (1:1)), ratio 230/260 mμ 2.93 (EtOH–H_2O (1:1)). Yields were about 35% of the weight of V used in the reaction.

Anal. Found: C, 61.3; H, 4.3; N, 6.0.

1-(3′,5′-Di-O-benzoyl-2′-hydroxy-β-D-arabinosyl)-uracil (VIII).—To a solution of 0.30 g. (0.00069 mole) of VI in 30 ml. of N,N-dimethylformamide was added 10 ml. of 2 *N* hydrochloric acid. The solution was warmed to 80° for 5 minutes, then allowed to cool. After 15 hours the neutral solution was taken to dryness *in vacuo*, and the residue crystallized from 80% ethanol. A yield of 0.20 g. of colorless

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

(44) Spectrophotometric data are presented in Table I.

needles, m.p. 181–184°, was obtained as a first crop and 0.12 g., m.p. 170–176°, as a second crop (total yield 0.32 g. (102%)). In a second run a yield of 81% of VII was obtained. Recrystallization yielded colorless needles, m.p. 186–187°, $\alpha^{23}D$ + 18° (EtOH, *c* 0.5).

Anal. Calcd. for $C_{23}H_{20}N_2O_8$: C, 61.1; H, 4.5; N, 6.2. Found: C, 61.0; H, 4.3; N, 6.5.

1-β-D-Xylofuranosyluracil (X).—Compound VII (0.78 g., 0.0013 mole, based upon a molecular weight of 604) was dissolved in 40 ml. of warm ethanol. After the addition of 10 ml. of sodium hydroxide (1.1 *N*), the solution was refluxed for 10 minutes, then taken to dryness *in vacuo*. The residue was dissolved in water, then triturated with Dowex 50(H^+ form). After removal of the resin the solution was taken to dryness *in vacuo*. The residue was dissolved in ethanol, and the ethanol distilled *in vacuo*. This process was repeated several times to remove all water. The residue was triturated several times with dry ether to remove benzoic acid. The colorless prisms which formed were collected on the filter. The yield of material, m.p. 145–151°, was 0.30 g. (95%). Crystallization from ethanol gave colorless prisms, m.p. 158–158.5°, $\alpha^{23}D$ + 29° (H_2O, *c* 0.4). Three hours after the addition of sodium metaperiodate the solution had reached a constant rotation, $\alpha^{24}D$ + 16° (H_2O, *c* 0.4), a value similar to that obtained for the other aldopentofuranosyluracil isomers.[38,45,46]

Anal. Calcd. for $C_9H_{12}N_2O_6$: C, 44.3; H, 5.0; N, 11.5. Found: C, 44.4; H, 5.1; N, 11.1.

Compound X was also prepared from VII by the use of anhydrous ethanolic ammonia in a sealed tube at 100° for 12 hours. Yields averaged 92%.

1-(Tri-O-benzoyl-β-D-xylofuranosyl)-benzoyluracil (XI).—A sample of 0.12 g. (0.00022 mole) of VII was heated at 140° for 10 minutes *in vacuo* until all volatile material was removed. To the dry amorphous solid was added 5 ml. of pyridine and 0.09 g. (0.00064 mole) of benzoyl chloride. The reaction mixture was allowed to stand 15 hours at room temperature, then warmed at 50–55° for 2 hours. Upon isolation of the product it appeared that the reaction had not proceeded to completion. Therefore, the dried product was again taken up in pyridine and treated with 0.10 g. (0.00071 mole) of benzoyl chloride. The mixture was heated at 60–70° for 2.5 hours then poured into ice-water with stirring. A colorless solid separated upon the addition of hydrochloric acid (12 *N*) to *p*H 6. This was collected at the filter, boiled with ethanol, then taken to dryness *in vacuo*. After triturating well with petroleum ether the material was crystallized from ethanol. Colorless needles, m.p. 183–184°, were obtained; ratio 230/260 mμ 1.87 (EtOH–H_2O (1:1)).

An identical product (based upon m.p., mixture m.p. and ultraviolet spectral properties) was obtained when 1-β-D-xylofuranosyluracil (X) was treated with excess benzoyl chloride in pyridine.

Anal. Calcd. for $C_{37}H_{28}N_2O_{10}$: C, 67.3; H, 4.3; N, 4.2. Found: C, 66.7; H, 4.4; N, 4.5.

1-β-D-Arabinofuranosyluracil ("Spongouridine") (IX).—A solution of 0.18 g. (0.00040 mole) of VIII in 30 ml. of anhydrous ethanolic ammonia was heated in a sealed tube for 12 hours at 100°. The resulting solution was taken to dryness *in vacuo*. Ethanol was added and distilled off several times to remove all ammonia. Colorless prisms, 0.072 g., 74% yield, separated from a small amount of ethanol. The material gave a typical 1-β-D-aldopentofuranosyluracil spectrum in the ultraviolet. A mixture of this material, m.p. 213–216° (uncor.), and an authentic sample kindly supplied by Dr. D. M. Brown, m.p. 208.5–215° (uncor.), melted at 210–215°.

A similar sample prepared by the reaction of base with VI gave $\alpha^{24}D$ + 126° (Bergmann and Burke[45] report + 126° and Brown, Todd and Varadarajan,[13] +131°). The addition of sodium metaperiodate brought the rotation to $\alpha^{25}D$ + 16°, a value similar to that obtained by Bergmann and Burke[45] who reported +15°.

2,2'-Anhydro-1-(5'-deoxy-3'-O-methylsulfonyl-β-D-arabinosyl)-uracil (XV).—Compound IV (2.60 g., 0.0059 mole) was dissolved in 200 ml. of warm 80% ethanol. After cooling the solution to room temperature, 2.6 g. (0.032 mole) of sodium acetate and 1.0 g. of palladium–charcoal (5%) were added. The mixture was allowed to absorb one equivalent of hydrogen at atmospheric pressure and room temperature and was then filtered through Celite. The volume was reduced to 10 ml. *in vacuo*. The addition of 30 ml. of water, followed by cooling, caused the product to separate. The material, 1.50 g., was crystallized from water to yield 1.27 g., m.p. 171–173° (uncor.). Yields averaged 70–80%. Crystallization from water gave colorless elongated prisms, m.p. 182–184.5° dec.

Anal. Calcd. for $C_{10}H_{12}N_2O_6S$: C, 41.7; H, 4.2; N, 9.7; S, 11.1. Found: C, 41.8; H, 4.5; N, 10.2; S, 11.7.

2,2'-Anhydro-1-(3'-O-benzoyl-5'-deoxy-β-D-arabinosyl)-uracil (XVI).—A solution of 1.0 g. (0.0035 mole) of XV and 2.04 g. (0.014 mole) of anhydrous sodium benzoate in 200 ml. of N,N-dimethylformamide was refluxed for 4 hours. After cooling, the insoluble salts were removed by filtration, and the solution reduced to dryness *in vacuo*. The residue was extracted with three 50-ml. portions of chloroform, and the combined chloroform solution extracted with water. After drying over sodium sulfate, the chloroform was removed *in vacuo*, and the residue dissolved in hot ethyl acetate (15 ml.). Cooling produced colorless needles, 0.22 g. (20%), m.p. 204–206° (uncor.).

Anal. Calcd. for $C_{16}H_{14}N_2O_5$: C, 61.1; H, 4.5; N, 8.9. Found: C, 60.0; H, 4.8; N, 8.8.

5'-Deoxyuridine (XVII).—To a solution of 0.23 g. (0.00073 mole) of XVI in 30 ml. of water was added 2 drops of hydrochloric acid (2 *N*). The solution was refluxed, and the course of the reaction followed spectrophotometrically. An initial ratio 230/260 mμ of 3.37 gave place to a ratio of 1.31 after 35 minutes, which showed no further change upon continued heating. The aqueous solution was extracted well with chloroform, and the chloroform solution then extracted with water. After drying over sodium sulfate the chloroform solution was reduced to dryness, leaving a residue of 0.21 g. A pure sample of the 3'-*O*-benzoxy-5'-deoxy-2'-hydroxy nucleoside was not isolated.

The above solid was dissolved in 25 ml. of methanol containing 1 ml. of sodium methylate (1 *N*). The solution was refluxed 1.5 hours. After the addition of an equal volume of water the solution was passed through Dowex 50 (H^+) then treated with charcoal to remove all color. The material was dried by the repeated addition, followed by the distillation, of ethanol. Crystallization from a small amount of ethanol gave colorless needles, m.p. 183.5–185°.

A mixture of a sample prepared in this manner, and an authentic sample of 5'-deoxyuridine,[24] m.p. 183.5–184.5°, showed no depression.

The sample gave a rotation ($\alpha^{28}D$ + 10.3°), which compares favorably with that of 5'-deoxyuridine, $\alpha^{26}D$ + 10.9°. The material consumed 1.04 equivalents of metaperiodate within 3 minutes, further confirming the presence of *cis*-vicinal-hydroxyl groups.

Electrophoretic Experiments.—All studies were made using an E. C. electrophoresis apparatus.[47] Whatman 3MM paper was employed. Borate buffer, *p*H 6.0–6.05, was prepared by dissolving 225 g. of boric acid in 4 liters of water with warming, followed by the addition of sodium hydroxide (10 *N*) until the proper *p*H was attained.

After completion of a run the paper was dried at 100–120°, and the spots viewed in ultraviolet light. When necessary, the spots were cut from the paper and triturated 1–2 hours in a measured amount of water. The paper was removed by filtration and the optical density of the filtrate determined at the maximum, 262 mμ, and the minimum, 232 mμ, making allowance for the absorption of the borate.

1. **Reaction of VI with Base.**—A sample of 0.03 g. of VI was refluxed for 30 minutes in 4 ml. of solution containing ethanol and sodium hydroxide (1.1 *N*) in the ratio of 3:2. The clear colorless product was taken to dryness *in vacuo*, and the residue dissolved in 5 ml. of water. The aqueous solution was triturated two times with Dowex-50 (H^+ form). The filtrate, *p*H 4.5–5.0, was taken to dryness *in vacuo*, and the residue taken up in 1 ml. of water. After impregnation, the paper was subjected to 700 volts and 14–17 milliamp. for 120 minutes. The dried paper was viewed in ultraviolet light. Two spots were observed with anodic migrations as follows: 0.0 cm. (1-β-D-arabinosyluracil, −0.1 cm.), 4.2 cm. (1-β-D-xylosyluracil, + 4.1 cm.).

(45) W. Bergmann and D. C. Burke, *J. Org. Chem.*, **20**, 1501 (1955).

(46) J. Davoll, B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 833 (1946).

(47) Manufactured by E. C. Apparatus Co., Swarthmore, Penna.

The same two products were formed when the hydrolysis was carried out in aqueous sodium hydroxide.

2. **Reaction of XVI with Base.**—A solution of 0.05 g. of XVI in 3 ml. of sodium hydroxide (0.22 *N*) was refluxed until completion of the reaction. Sodium ions were removed as described above, and the product subjected to ionophoretic separation in borate buffer. After four hours at 600 volts and 17–20 milliamp. only one spot was observed under ultraviolet light, anodic migration, −1.5 cm.

When XVI was refluxed in 3 ml. of ethanol and 2 ml. of sodium hydroxide (1.1 *N*) for 30 minutes, and the product evaluated as above (700 volts, 13 milliamp. for four hours) a single spot with anodic migration, −1.5 cm., was observed.

3. **Reaction of VIII with Base.**—A sample of 0.01 g. of VIII was refluxed with ethanolic aqueous sodium hydroxide as described under experiment 1 above, and an aqueous solution of the product, free of sodium ions, was subjected to separation by paper electrophoresis, 700 volts, 14–16 milliamp. for 180 minutes, as described above. Only one spot, anodic migration −0.7 cm. (1-β-D-arabinosyluracil, −0.7 cm.), was observed.

4. **Reaction of VI with Acid (HCl–DMF–Water), Followed by Reaction with Base.**—A sample of 0.025 g. of VI in 5 ml. of DMF and 1.0 ml. of hydrochloric acid (2 *N*) was heated 15 minutes at 80–90°. The solution was taken to dryness *in vacuo* and triturated with water. The ultraviolet absorption spectrum (230/260, 2.83) of the residue indicated complete cleavage of the anhydro bond. The residue was treated with ethanol–water–sodium hydroxide as described above. Ionophoretic separation of the product after removal of sodium ions (700 volts, 17–19 milliamp. for 240 minutes) gave the following anodic migrations: −2.8 cm. (1-β-D-arabinosyluracil, −2.6 cm.), + 5.9 cm. (1-β-D-xylosyluracil, + 6.0 cm.), + 9.8 cm. (1-β-D-ribosyluracil, + 10.0 cm.). No spot was found at the migration spot of 1-β-D-lyxosyluracil, + 14.3 cm.

A similar experiment carried out upon the residue after refluxing VI with sodium benzoate in DMF for 4.5 hours demonstrated the presence of the same three isomers; 1-β-D-lyxofuranosyluracil was again absent.

5. **Reaction of XII with Base.**—A sample of the amorphous residue obtained after the removal of VI and VII from the reaction mixture of V and sodium benzoate in refluxing DMF (representing about 35% by weight of the starting material V) was subjected to alkaline hydrolysis in ethanol–water–NaOH as described under (1) above. The solution was freed of sodium ions and subjected to ionophoretic separation in borate buffer, *p*H 6–6.05, as described previously. Spots having the following anodic migrations after 192 minutes at 700 volts and 18–21 milliamp. were observed: −2.3 cm. (1-β-D-arabinosyluracil, −2.2 cm.), + 4.7 cm. (1-β-D-xylosyluracil, + 4.6 cm.), + 8.2 cm. (1-β-D-ribosyluracil, + 8.2 cm)., + 12.1 cm. (1-β-D-lyxosyluracil, + 12.2 cm.).

The spots were cut from the paper and eluted with water. The relative intensities of the resulting solutions, measured at 262 mμ were: arabinosyl 1.0, xylosyl 1.2, ribosyl 0.9 and lyxosyl 1.1.

The results of a similar experiment carried out under only slightly different conditions (900 volts, 10–13 milliamp., 285 minutes) are shown in Fig. 6.

6. **Hydrolysis of the "5′-Deoxy" Residue.**—A sample of 0.20 g. of the residue remaining after isolation of XVI from the reaction of XV with sodium benzoate in refluxing DMF was refluxed with two drops of hydrochloric acid (2 *N*) in 10 ml. of water until cleavage of the anhydro bond was complete, as determined spectrophotometrically. To the mixture was added 1 ml. of sodium hydroxide (1.13 *N*) until removal of the benzoyl groups was complete.

Paper electrophoresis of the product (600 volts, 17–20 milliamp., 4 hours) gave the following spots: −1.4 cm., + 9.1 cm. (1-(5′-deoxy-β-D-ribofuranosyl)-uracil, + 9.5 cm.), + 12.1 cm. (1-(5′-deoxy-β-D-lyxofuranosyl)-uracil, + 12.1 cm.).

Polarimetric Determinations.—Techniques and equipment previously described[2] were used for all optical rotations. Calculations of the rotation of the dialdehydes of 1-β-D-xylofuranosyluracil and 1-β-D-arabinofuranosyluracil was based upon the concentrations of the nucleosides. Readings were taken until constancy was reached.

Ultraviolet Spectrophotometric Data.—The curves shown in Fig. 3 were made using the Cary recording spectrophotometer, model 11. All molar extinction coefficients were calculated from optical density values obtained using the Beckman model DU spectrophotometer.

[CONTRIBUTION FROM THE FRUIT AND VEGETABLE CHEMISTRY LABORATORY, WESTERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION, AGRICUTURAL RESEARCH SERVICE, U. S. DEPARTMENT OF AGRICULTURE]

Flavonoid Compounds of Citrus. III. Isolation and Structure of Eriodictyol Glycoside

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Lemon peel contains an appreciable quantity of a glycoside of eriodictyol which can be isolated by chromatography on silicic acid. The structure of this glycoside ("eriocitrin") is shown to be eriodictyol 7-β-rutinoside. A convenient procedure is described for preparing eriodictyol by hydrolyzing the crude mixed glycosides with hemicellulase.

Some years ago Bruckner and Szent-Györgyi[1,2] discussed the composition of "citrin," a mixture of flavonoid glycosides which had been isolated earlier from lemon peel.[3] Citrin was said to contain the flavanones hesperidin and "eriodictin" (considered to be a glycoside of eriodictyol), although no supporting experimental evidence was given. Hesperidin had long been recognized as a major constituent of lemons and oranges, but neither eriodictyol nor its glycosides had been previously reported to occur in citrus. Since citrin, or, at least, certain of its components, was thought to have an effect on capillary permeability, and was even regarded for a time as a vitamin concerned with the proper functioning of the capillaries,[3] it was of importance to establish whether eriodictyol was actually present. Several years after the appearance of the papers cited above, Mager[4] described the isolation from citrin of crystalline eriodictyol rhamnoside and its hydrolysis to eriodictyol and rhamnose, while later Higby[5] also mentioned the isolation of eriodictyol from a non-crystalline glycoside obtained from lemon peel. In both instances, however, the experimental data were fragmentary and the occurrence of this flavonoid has never been regarded as firmly established. Recently, there has been renewed interest in the question since the discovery by Masri and DeEds[6] that eriodictyol and related compounds having *o*-dihydroxyl groups

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