Tetrahedron. Vol 24, pp. 6987 to 6993. Pergamon Press 1968. Printed in Great Britain

ACETYLATION OF NUCLEOSIDES AND ACETYL MIGRATION

G. A. R. JOHNSTON

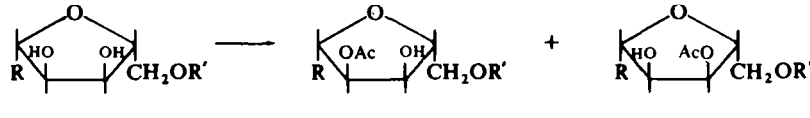
Department of Physiology, Australian National University, Canberra

(*Received in UK* 8 *July* 1968; *accepted for publication* 17 *July* 1968)

Abstract—Acetylation of the 2'-OH group predominates over acetylation of the 3'-OH group in derivatives of adenosine, uridine, 3β-D-xylosyluracil and 3β-D-lyxosyluracil. In each case subsequent equilibration of the products of acetylation results in a net acetyl migration away from the glycosidic link.

THE incorporation of amino acids into protein occurs by acylation of the 2',3'-*cis* diol system of the terminal adenosine residues of transfer ribonucleic acids (tRNA) by enzyme-bound aminoacyladenylates to give aminoacyl-tRNA, followed by amide-ester exchange with peptidyl-tRNA on the ribosomes under the direction of messenger ribonucleic acids. Subsequent to acylation, acyl migration may take place leading to problems regarding the orientation of aminoacyl-tRNA and peptidyl-tRNA as 2' and/or 3' esters. Extensive studies on acylated ribonucleoside derivatives have yielded much valuable information regarding acyl migration (cf.[1]) and the present work was initiated to investigate the *in vivo* acylation of tRNA using the acetylation of ribonucleosides as a convenient model system. The acetylation of some nucleosides with sugar moieties other than ribose was also investigated, since acetylated derivatives of such nucleosides are likely to have important pharmacological properties.[2] Aspects of this have been published in preliminary form.[3,4]

Partial acetylation of 5'-O-acetyluridine (I, R = uracil, R' = acetyl) with acetic anhydride in pyridine solution gave a mixture of mono-, di- and tri-O-acetylated uridine derivatives. The di-O-acetylated fraction was isolated by chromatography on silicic acid and was shown to be an approximately 2:1 mixture of 2', 5'- and 3',5'-di-O-acetyluridine (II and III, R = uracil, R' = acetyl) by PMR chemical shift data[5] and by a chemical procedure. The latter involved mesylation in pyridine solution at 0° and subsequent treatment with half saturated ethanolic ammonium. Under these conditions 2',5'-di-O-acetyluridine (II, R = uracil, R' = acetyl) afforded 3'-O-mesyluridine, whereas the 3',5' isomer (III, R = uracil, R' = acetyl) was converted into $O_{(2)}$,2'-cyclouridine; these products were readily separated by paper chromatography and their relative proportions estimated by UV absorption measurements. The PMR and chemical assay procedures gave similar results on individual mixtures, the percentage of 2' ester varying between 60 and 70% in repetition acetylation experiments.

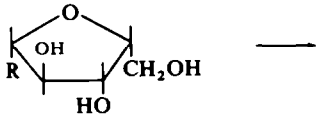


I II III

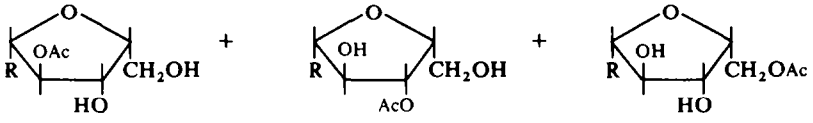
Partial acetylation of 5′-O-trityladenosine (I, R = adenine, R′ = trityl) by the above procedure gave a similar mixture of 2′ and 3′ acetates (II and III, R = adenine, R′ = trityl), assayed chemically by pyranylation and phosphorylation.[6] Partial acetylation of 5′-O-acetyladenosine (I, R = adenine, R′ = acetyl) also gave an approximately 2:1 mixture of 2′ and 3′ acetates assayed by PMR.

These approximately 2:1 mixtures of 2′ and 3′ acetates obtained by partial acetylation of 5′-O-acetyluridine, 5′-O-trityladenosine and 5′-O-acetyladenosine, when refluxed in dry pyridine for 1 hr, yielded approximately 1:3 mixtures of 2′ and 3′ acetates which represent the equilibrium proportions of these esters. Crystallization of the 2:1 or 1:3 mixture of di-O-acetyluridines from absolute ethanol required several days at 0° and yielded pure 3′,5′ ester (III, R = uracil, R′ = acetyl) after several weeks in better than 70% yield. Similar results were obtained with adenosine derivatives. Refluxing the pure 3′ esters in pyridine gave the 1:3 equilibrium mixture of 2′ and 3′ esters.

The preferential acetylation of the 2′-OH group over the 3′-OH group was not restricted to ribonucleoside derivatives. Monoacetylation of 3β-D-xylosyluracil (IV, R = uracil) gave a mixture of the three possible isomeric esters with the 2′ ester (V, R = uracil) predominating, since the mixture reacted with 0·23 equivalents of periodate during 18 hr and afforded an isopropylidene derivative in approximately 55% yield. The pure 2′ and 3′ esters were inert to periodate and the pure 2′ ester, alone of the isomeric esters, yielded an isopropylidene derivative. Refluxing the mixture of esters or any of the pure esters in pyridine solution gave pure crystalline

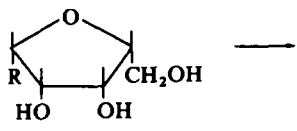


IV

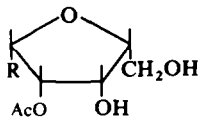


V VI VII

5′-O-acetyl-3β-D-xylosyluracil (VII, R = uracil) in good yield—this ester reacted with 0·92 equivalents of periodate during 18 hr. Authentic 2′-O-acetyl-3β-D-xylosyluracil (V, R = uracil) was prepared from 3′,5′-isopropylidene-3β-D-xylosyluracil,[7] by acetylation and subsequent acid hydrolysis and its structure was confirmed by reforming the isopropylidene derivative in high yield. Authentic 3′-O-acetyl-3β-D-xylosyluracil (VI, R = uracil) was prepared by acetylation of 2′,5′-di-O-trityl-3β-D-xylosyluracil,[7] followed by detritylation in aqueous acetic acid. The pure isomeric esters (V, VI and VII, R = uracil) were hydrolysed by base to 3β-D-xylosyluracil (IV, R = uracil), which was chromatographically and electrophoretically pure.

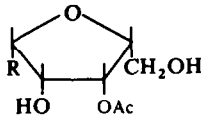


VIII



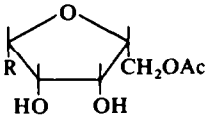
IX

+



X

+



XI

Monoacetylation of 3β-D-lyxosyluracil (VIII, R = uracil) also gave a mixture of esters with the 2′ ester (IX, R = uracil) predominating. The product reacted with 0·4 equivalents of periodate and gave a mixture of isopropylidene derivatives in better than 85% yield. Pure 2′ and 3′ esters (IX and X, R = uracil) could not be prepared due to the ease of acetyl migration, but, of the three possible isomeric esters, it is likely that the 2′ and 5′ esters would form isopropylidene derivatives and that only the 5′ ester would react with periodate. Authentic 5′ ester (XI, R = uracil) was prepared from 5′-O-acetyl-$O_{(2)}$,2′-cyclouridine[8] by mesylation followed by refluxing in aqueous solution.[9] Refluxing the mixture of esters in pyridine solution gave almost pure 5′ ester.

It is apparent that acetylation of the 2′-OH group predominated over acetylation of the 3′-OH group in derivatives of adenosine, uridine, 3β-D-xylosyluracil and 3β-D-lyxosyluracil and that subsequent equilibration of the acetylated products resulted in a net acetyl migration away from the glycosidic link. Thus the 2′-OH group appears to be effectively more nucleophilic than the 3′-OH group regardless of the stereochemistry of these OH groups. This could be due to the influence of the purine or pyrimidine base mediated via intramolecular hydrogen bonding to the 2′-OH group, similar bonding to the 3′-OH group being much less favourable;[10,11] inductive effects of the base are also likely to influence the 2′-OH group more than the 3′, since acyl groups attached to glycosides generally tend to migrate away from the glycosidic link.[12] These observations are supported by evidence in the literature pertaining to the sulphonylation,[13,14] tritylation,[15,16,17] and methylation[18,19] of ribonucleosides affording predominantly 2′-substituted products.

The 2′ and 3′ esters of 3β-D-xylosyluracil were considerably less mobile than the corresponding lyxo- and ribo-nucleoside derivatives. This may be an important factor contributing to the biological properties of the 2′,3′-*trans* nucleoside derivatives (e.g. xylosyl and arabinosyl derivatives of adenine and uracil) whose 2′ and 3′ esters, lacking the neighbouring *cis* OH group present in ribonucleoside derivatives, would be expected to be less mobile and also more stable to external nucleophilic attack than corresponding ribonucleoside esters.

There is some evidence that aminoacyl-tRNA is a relatively pure 3′-O-aminoacyladenosine derivative and contains much less of the 2′ isomer than anticipated from studies of simple nucleosides.[20] If this is indeed correct (cf.[1,6]) then, either the acylation of tRNA by enzyme-bound aminoacyladenylates leads to predominantly

the 3′ ester, or almost total net migration of 2′ esters to the 3′ position occurs after acylation. Both these possibilities differ from the behaviour of simple nucleoside derivatives *in vitro* towards acylation and acyl migration, and this might be rationalized in terms of binding of the terminal 2′-hydroxyl group to a specific receptor site during the acylation or at a subsequent stage. Alternatively the 2′/3′ ester equilibrium in aminoacyl-tRNA might be more in favour of the 3′ ester than is observed with simple nucleosides, as a result of intramolecular hydrogen bonding of the terminal 2′-OH group to the terminal adenine residue or to an internucleotide bond. Intramolecular hydrogen bonding between 2′-OH groups and the bases and/or internucleotide bonds is known to contribute to the secondary structure of polyribonucleotides[11,21] and if it extends to the terminal 2′-OH group it could result in this group becoming more nucleophilic than the corresponding group in simple nucleosides.

EXPERIMENTAL

UV absorption measurements were made on a Shimadzu QR50 or a Unicam SP500 spectrophotometer. PMR spectra were measured on a Varian A60 instrument, operating at 60 Mc/s with TMS or t-BuOH as internal reference. Periodate reactions were carried out according to the directions of Burgmann and Burke.[22] The silicic acid used for chromatography was Mallinckrodt, 100 mesh. All evaporations were carried out at reduced press below 20°.

Partial acetylation of 5′-O-*acetyluridine.* Acetic anhydride (1·0 ml) was added to a soln of 5′-O-acetyluridine (3·0 g) in dry pyridine (25 ml). After 30 min at 20°, the mixture was evaporated to dryness, the residue dissolved in $CHCl_3$, and chromatographed on a column of silicic acid (300 g) prepared in $CHCl_3$. Elution with $CHCl_3$ (10 × 30 ml) gave 2′,3′,5′-tri-O-acetyluridine (1·25 g), with 5% EtOH–$CHCl_3$ (15 × 30 ml) gave a thick oil (1·05 g) which analysed for a di-acetylated uridine. (Found: C, 47·5; H, 4·6. Calc. for $C_{13}H_{16}O_8N_2$: C, 47·5; H, 4·9%), and with 10% EtOH–$CHCl_3$ (10 × 50 ml) gave unchanged 5′-O-acetyluridine (1·2 g). The di-acetylated fraction was shown to be an approximately 2:1 mixture of 2′,5′- and 3′,5′-di-O-acetyluridine by the following chemical procedure: the oil (4 mg) was dissolved in dry pyridine (1 ml) and treated at 0° with mesyl chloride (0·01 ml). After 30 min, the mixture was taken to dryness at 0°, and treated with half-saturated ethanolic ammonia (1 ml) for 4 hr at room temp. The mixture was evaporated to dryness, dissolved in MeOH (0·1 ml) and spotted (approx 0·02 ml) on Whatman No. 1 paper. Descending chromatography in n-BuOH:AcOH:water (5:2:3) gave two UV absorbing spots corresponding to 3′-O-mesyluridine (R_f 0·65) and $O_{(2')}$,2′-cyclouridine (R_f 0·51). The spots were excised and eluted with water (5·0 ml) overnight and the relative proportions estimated by comparison of optical densities at the UV absorption maxima after correction for the appropriate blanks. 3′-O-Mesyluridine showed λ_{max} 260 mμ, ε_{max} 8,500 and $O_{(2')}$,2′-cyclouridine had λ_{max} 250 mμ, ε_{max} 7,800. The di-acetylated fraction from the partial acetylation of 5′-O-acetyluridine gave results in repetition experiments varying from 60 to 70% of 3′-O-mesyluridine. The results were individually reproducible and the reaction time for the mesylation could be extended to 4 hr without affecting the results; however, mesylation at 25° for 4 hr led to decreased yields of 3′-O-mesyluridine.

The di-acetylated fraction was also assayed by PMR studies in dimethylcyanamide soln containing 10% ^2H_2O acidified with AcOH. Measurement of the relative heights of the H(1′) resonances gave a reliable estimate of the relative proportion of 2′,5′ and 3′,5′-di-O-acetyluridine, which showed chemical shifts at 4·01 and 4·12 respectively.[5]

The di-acetylated fraction (125 mg) crystallized from abs EtOH after several days at 0° and after 3 weeks filtration gave pure 3′,5′-di-O-acetyluridine (80 mg), m.p. and mixed m.p. 135–138°. (Found: C, 47·4; H, 5·0%).

Equilibrium mixture of 2′,5′- *and* 3′,5′-*di*-O-*acetyluridine.* Refluxing the above approximately 2:1 mixture 2′,5′- and 3′,5′-di-O-acetyluridine (25 mg) in dry pyridine (10 ml) for 1 hr and evaporation of the solvent gave a thick oil which assayed, by PMR and mesylation, as an approximately 1:3 mixture of esters. The percentage of 2′,5′-di-O-acetyluridine in the product varied from 23 to 28%. This mixture crystallized from abs EtOH after 2 days at 0° and after 3 weeks pure 3′,5′-di-O-acetyluridine (21 mg), m.p. 135–138°, was collected.

Refluxing pure 3',5'-di-O-acetyluridine (18 mg) in dry pyridine (8 ml) for 1 hr afforded the 1:3 equilibrium mixture of 2',5'- and 3',5'-di-O-acetyluridine. Refluxing this mixture for prolonged periods in dry pyridine failed to alter the relative proportions of the isomeric esters but did lead to some de-acetylation.

Partial acetylation of 5'-O-*trityladenosine.* 5'-O-Trityladenosine (500 mg) was dissolved in dry pyridine (10 ml) and a soln of Ac_2O (0·1 ml) in dry pyridine (1 ml) added. The mixture was evaporated to dryness after 30 min at 20°.

A small portion of the oily residue (approx 20 mg) was dissolved in a cooled standard pyranylation soln[6] (1 ml), prepared by mixing dimethylformamide (1 ml, freshly distilled), trifluoroacetic acid (0·1 ml), and 2,3-dihydropyran (1 ml, freshly distilled from KOH). After 5 min at room temp the mixture was taken to dryness at 0°, made alkaline by the addition of conc ammonia (1 ml) and heated for 30 min at 50°. The ammonia was removed under reduced press, the residue dissolved in $CHCl_3$ (5 ml) and chromatographed on a column of silicic acid (2 g) prepared in $CHCl_3$. Gradient elution progressing from $CHCl_3$ to 10% MeOH–$CHCl_3$ afforded two fractions. The fraction eluted with approximately 9% MeOH–$CHCl_3$ was periodate-positive and corresponded to the di-esterified product of the acetylation. The fraction eluted with approximately 2% MeOH–$CHCl_3$ was periodate-negative and gave, on phosphorylation with β-cyanoethyl phosphate and dicyclohexylcarbodiimide followed by acid hydrolysis,[6] a mixture of adenosine and adenosine 2'(3') monophosphates. This mixture was separated by paper chromatography, the spots excised and eluted, and the ratio of 2' and 3' phosphates estimated by UV absorption as approximately 2:1. This ratio corresponds to the 2':3' ester ratio in the mono-esterified product of the acetylation.

The main portion of the acetylation product was refluxed in pyridine (20 ml) for 1 hr. Evaporation gave an oil which assayed for a 1:3 mixture of 2' and 3' acetates in addition to some di-acetylated product and some starting material. This oil was dissolved in 80% aqueous AcOH (20 ml) and heated at 50° for 30 min. The mixture was taken to dryness, dissolved in 10% MeOH–$CHCl_3$ (5 ml) and chromatographed on a column of silicic acid (50 g) prepared in $CHCl_3$. Elution with $CHCl_3$ gave triphenylcarbinol (210 mg). Elution with 5% MeOH–$CHCl_3$ (5 × 50 ml) gave 2',3'-di-O-acetyladenosine (130 mg). Elution with 10% MeOH–$CHCl_3$ (25 × 50 ml) gave a mixture of 2'- and 3'-O-acetyladenosine as an oil (102 mg) which assayed for a 1:3 mixture. Elution with 25% MeOH–$CHCl_3$ (20 × 50 ml) gave adenosine (40 mg). The mixture of 2'- and 3'-O-acetyladenosine slowly crystallized from abs EtOH at 0° to give after several weeks pure 3'-O-acetyladenosine (75 mg), m.p. 172–174°. (Found: C, 46·8; H, 4·7. Calc. for $C_{12}H_{15}O_5N_5$: C, 46·7; H, 4·9%).

Partial acetylation of 5'-O-*acetyladenosine.* Partial acetylation of 5'-O-acetyladenosine under the above conditions gave a mixture of acetylated products, from which the di-acetyladenosines were separated by column chromatography on silicic acid. Measurement of the H(1') resonances at 3·80 and 3·98 in ^2H_2O solution acidified with AcOH indicated that the relative proportions of 2',5'- and 3',5'-di-O-acetyladenosine were approximately 2:1. Refluxing this mixture in dry pyridine afforded a 1:3 mixture of 2',5'- and 3',5'-di-O-acetyladenosine.

2'-O-*Acetyl*-3β-D-*xylosyluracil.* Ac_2O (0·2 ml) was added to a soln of 3',5'-O-isopropylidene-3β-D-xylosyluracil[7] (225 mg) in dry pyridine (10 ml). After 4 hr at room temp the mixture was taken to dryness, the residue dissolved in glacial AcOH (1 ml) and treated with a sat HBr in glacial AcOH (1 ml) for 15 min at 20°. Evaporation gave an oil which crystallized on trituration with abs EtOH. Several recrystallizations from dry MeOH gave 2'-O-*acetyl*-3β-D-*xylosyluracil* (164 mg), m.p. 137–138°. (Found: C, 46·3; H, 4·8; N, 10·2. $C_{11}H_{14}O_7N_2$ requires: C, 46·2; H, 4·9; N, 9·8%). This material was inert to aqueous sodium periodate during 24 hr.

2'-O-Acetyl-3β-D-xylosyluracil (20 mg) was dissolved with stirring at 0° in dry acetone (10 ml) containing traces of HCl. After 1 hr the mixture was evaporated to dryness and treated with half-saturated ethanolic ammonia (10 ml) for 4 hr at room temp. Evaporation to dryness and crystallization from 95% EtOH gave 3',5'-O-isopropylidene-3β-D-xylosyluracil (18 mg), m.p. and mixed m.p. 260–262°.

3'-O-*Acetyl*-3β-D-*xylosyluracil.* 2',5'-Di-O-trityl-3β-D-xylosyluracil[7] (700 mg) was dissolved in a dry pyridine (15 ml) and treated with Ac_2O (0·2 ml) for 4 hr at room temp. The product was dissolved in 80% aqueous AcOH (20 ml) and heated at 60° for 30 min. The mixture was taken to dryness and the residue extracted with dry ether (2 × 25 ml). The ether-insoluble residue was recrystallized several times from abs EtOH, to give 3'-O-*acetyl*-3β-D-*xylosyluracil* (180 mg), m.p. 158–159° depressed on admixture with the 2' ester. (Found: C, 46·4; H, 5·0; N, 9·7. $C_{11}H_{14}O_7N_2$ requires: C, 46·2; H, 4·9; N, 9·8%). This material was inert to aqueous sodium periodate during 24 hr and did not form an isopropylidene derivative when treated at 0° with a sat soln of HCl in dry acetone.

5'-O-*Acetyl*-3β-D-*xylosyluracil.* 2'-O-Acetyl-3β-D-xylosyluracil (25 mg) or the 3' ester was refluxed for

1 hr in pyridine. The pyridine was removed under reduced press and the resulting oil crystallized by trituration with EtOH. Several recrystallizations from dry MeOH gave 5'-O-*acetyl*-3β-D-*xylosyluracil* (11 mg), m.p. 163–165° depressed on admixture with either starting material. (Found: C, 45·9; H, 4·8; N, 9·7. $C_{11}H_{14}O_7N_2$ requires: C, 46·2; H, 4·9; N, 9·8%).

5'-O-Acetyl-3β-D-xylosyluracil reacted with 0·92 equivs of periodate during 18 hr at room temp. Treatment at 0° with a sat soln of HCl in acetone failed to yield an isopropylidene derivative.

Partial acetylation of 3β-D-*xylosyluracil.* A soln of 3β-D-xylosyluracil[7] (250 mg) in dry pyridine (5 ml) was treated with Ac_2O (0·05 ml) for 1 hr at 25°. The mixture was evaporated to dryness, dissolved in 10% MeOH–$CHCl_3$, and chromatographed on a column of silicic acid (25 g) prepared in $CHCl_3$. Elution with 5% MeOH–$CHCl_3$ (10 × 50 ml) gave an oil (170 mg) which could not be further purified. Elution with 25% MeOH–$CHCl_3$ (15 × 50 ml) gave starting material (95 mg).

The above oil (25 mg) reacted with 0·23 equivs of periodate during 18 hr at room temp. Treatment of the oil (100 mg) at 0° for 1 hr with acetone (20 ml) containing traces of HCl, followed by treatment of the product with half-saturated ethanolic ammonia (20 ml) for 4 hr at room temp gave 3',5'-O-isopropylidene-3β-D-xylosyluracil (54 mg), crystallizing from 95% EtOH with m.p. and mixed m.p. 259–262°.

Refluxing the above oil (50 mg) in dry pyridine (20 ml) afforded 5'-O-acetyl-3β-D-xylosyluracil (38 mg), after two recrystallizations from dry MeOH, m.p. and mixed m.p. 163–165°.

5'-O-*Acetyl*-3β-D-*lyxosyluracil.* 5'-O-Acetyl-$O_{(1)}$,2'-cyclouridine[8] (135 mg) in dry pyridine (5 ml) was treated at 0° with mesyl chloride (0·1 ml). After 18 hr at 5°, EtOH (5 ml) was added and the mixture allowed to stand a further 2 hr. The red oil obtained on evaporation was triturated with MeOH and the resulting white solid was refluxed with water (100 ml) for 6 hr. The mixture was then neutralized with dilute ammonia and evaporated to dryness. The residue was extracted with EtOH (2 × 10 ml) and the extract concentrated at 0° to give a solid, which was collected and recrystallized from dry MeOH to give 5'-O-*acetyl*-3β-D-*lyxosyluracil* (95 mg), m.p. 173–175°. (Found: C, 46·2; H, 4·8; N, 10·1. $C_{11}H_{14}O_7N_2$ requires: C, 46·2; H, 4·9; N, 9·8%). This ester reacted with 0·95 equivts of periodate during 18 hr.

5'-O-*Acetyl*-2',3'-O-*isopropylidene*-3β-D-*lyxosyluracil.* 5'-O-Acetyl-3β-D-lyxosyluracil (25 mg) was treated at 0° with acetone containing HCl. After 1 hr the mixture was evaporated to dryness and the residue crystallized from MeOH. Two recrystallizations from MeOH gave 5'-O-*acetyl*-2',3'-O-*isopropylidene*-3β-D-*lyxosyluracil* (19 mg), m.p. 181–183°. (Found: C, 51·2; H, 5·8; N, 8·7. $C_{14}H_{18}O_7N_2$ requires: C, 51·5; H, 5·6; N, 8·6%).

Partial acetylation of 3β-D-*lyxosyluracil.* A soln of 3β-D-lyxosyluracil (250 mg) in dry pyridine (5 ml) was treated with Ac_2O (0·05 ml) for 1 hr at 25°. The mixture was evaporated to dryness, dissolved in 10% MeOH–$CHCl_3$ and chromatographed on a column of silicic acid (25 g) prepared in 5% MeOH–$CHCl_3$. Elution with 5% MeOH–$CHCl_3$ (5 × 50 ml) gave only traces of material, while elution with 10% MeOH–$CHCl_3$ gave an oil (185 mg) which could not be purified further and elution with 25% MeOH–$CHCl_3$ (10 × 50 ml) gave starting material (90 mg). The oil (50 mg) was refluxed for 1 hr in pyridine (20 ml) yielding, after two recrystallizations from dry MeOH, 5'-O-acetyl-3β-D-lyxosyluracil (42 mg) m.p. and mixed m.p. 173–175°.

The above oil (25 mg) reacted with 0·4 equivs of periodate during 18 hr at room temp. Treatment of the oil (100 mg) at 0° with acetone (20 ml) containing HCl gave material after 1 hr crystallizing from EtOH as an amorphous solid (90 mg), m.p. 110–125°, which was inert to periodate and could not be purified further.

Acknowledgements—The author wishes to thank Drs. D. M. Brown, G. M. Blackburn, and V. M. Clark for many helpful discussions. Part of this work was done during the tenure of an Overseas Studentship awarded by the Royal Commissioners for the Exhibition of 1851 at the University Chemical Laboratory, Cambridge.

REFERENCES

[1] B. E. Griffin, M. Jarman, C. B. Reese, J. E. Sulston and D. R. Trentham, *Biochemistry* **5**, 3638 (1966).
[2] A. P. Martinez, W. W. Lee and L. Goodman, *J. Med. Chem.* **9**, 268 (1966).
[3] G. A. R. Johnston, *Tetrahedron Letters* 2769 (1967).
[4] G. A. R. Johnston, *Biochim. Biophys. Acta* **145**, 831 (1967).
[5] H. P. M. Fromageot, B. E. Griffin, C. B. Reese, J. Sulston and D. R. Trentham, *Tetrahedron* **22**, 705 (1966).
[6] R. Wolfenden, D. H. Rammler and F. Lipmann, *Biochemistry* **3**, 329 (1964).
[7] N. C. Yung and J. J. Fox, *J. Am. Chem. Soc.* **83**, 3060 (1961).

[8] D. M. Brown, D. B. Parihar and Sir Alexander Todd, *J. Chem. Soc.* 4242 (1958).
[9] R. Fecher, J. F. Codington and J. J. Fox, *J. Am. Chem. Soc.* **83**, 1889 (1961).
[10] H. Witzel, *Prog. Nucleic Acid Res.* **2**, 221 (1963).
[11] P. O. P. Ts'o, S. A. Rapaport and F. J., *Biochemistry* **5**, 4153 (1966).
[12] J. M. Sugihara, *Adv. Carbohydrate Chem.* **8**, 1 (1953).
[13] D. M. Brown, D. B. Parihar, Sir Alexander Todd and S. Varadarajan, *J. Chem. Soc.* 3028 (1958).
[14] Sir Alexander Todd and T. L. V. Ulbricht, *Ibid.* 3275 (1960).
[15] J. Zemlicka, *Coll. Czech. Chem. Comm.* **29**, 1734 (1964).
[16] C. B. Reese and D. R. Trentham, *Tetrahedron Letters* 2459 (1965).
[17] G. A. R. Johnston, *Aust. J. Chem.* **21**, 513 (1968).
[18] W. Szer and D. Shugar, *Biokhimiya* **26**, 840 (1961).
[19] A. D. Broom and R. K. Robins, *J. Am. Chem. Soc.* **87**, 1145 (1965).
[20] H. G. Zachau and H. Feldmann, *Prog. Nucleic Acid Res.* **4**, 217 (1965).
[21] J. Brahms and C. Sadron, *Nature, Lond.* **212**, 283 (1966).
[22] W. Burgmann and D. C. Burke, *J. Org. Chem.* **20**, 1501 (1955).