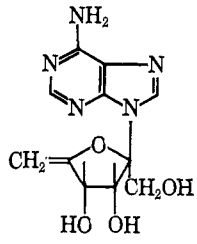


I

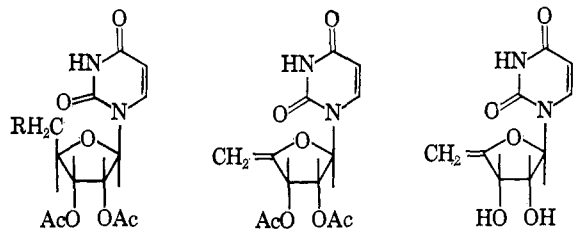
been known for many years.[6,7] Very recently Hough and Otter[8] have extended this reaction to the synthesis of protected 5-deoxy 4,5-unsaturated furanose derivatives.

Iodination of 2′,3′-di-O-acetyluridine[9] (IIa, 6.40 g, 19.5 mmoles) with methyltriphenoxyphosphonium iodide (13 g) in dimethylformamide (100 ml) for 2 hr at room temperature gave 7.2 g (84%) of 2′,3′-di-O-acetyl-5′-deoxy-5′-iodouridine (IIb), mp 163–164°, by direct crystallization of the extracted reaction mixture from chloroform–hexane,[10] λ_{max}^{MeOH} 258 mμ (ε 10,400). *Anal.* Calcd for $C_{13}H_{15}N_2O_7$: C, 35.64; H, 3.45; N, 6.39. Found: C, 35.89; H, 3.49; N, 6.29.

A solution of IIb (876 mg, 2 mmoles) in anhydrous pyridine (20 ml) was shaken in the dark at room temperature for 4 days with powdered silver fluoride (600 mg, 4.8 mmoles). The mixture was then filtered and the filtrate thoroughly shaken with water (50 ml) and ethyl acetate (50 ml). Evaporation of the ethyl acetate phase left 800 mg of a light-brown syrup that was purified by preparative thin layer chromatography on silicic acid using ethyl acetate as eluant, giving 525 mg (84%) of chromatographically homogeneous 1-(2,3-di-O-acetyl-5-deoxy-β-D-erythropent-4-enofuranosyl)uracil (III) as a dry froth which has not yet been obtained crystalline; λ_{max}^{MeOH} 258 mμ (ε 9250). *Anal.* Calcd for $C_{13}H_{14}N_2O_6$: C, 50.32; H, 4.55; N, 9.03. Found: C, 50.43; H, 4.86; N, 8.75. The structure was convincingly confirmed by the nmr spectrum in deuteriochloroform which showed the 5′-methylene group as a pair of doublets (J = 2.5 cps) centered at 4.45 and 4.69 ppm. Both the 4′ and 5′ protons of the starting material (IIb), which were present as a multiplet centered at 4.15 ppm and a doublet (J = 5 cps) centered at 3.52 ppm, respectively, were completely absent. Most of the other protons in III appeared at positions similar to those in IIb except that C_6–H was shifted upfield by 0.29 ppm and appeared as a doublet (J = 8 cps) at 7.32 ppm.

Treating III (450 mg) in a mixture of methanol (4.5 ml) and concentrated ammonium hydroxide (4.5 ml) for 1 hr at room temperature gave a single product upon examination by thin layer chromatography or paper chromatography. Evaporation of the solvent gave a crystalline residue (87%) of 1-(5-deoxy-β-D-erythropent-4-enofuranosyl)uracil (IV) which could be recrystallized from acetone and had mp 169–170°; λ_{max}^{MeOH} 261 mμ (ε 9600). *Anal.* Calcd for $C_9H_{10}N_2O_5$: C, 47.79; H, 4.96; N, 12.39. Found: C, 47.89; H, 4.62; N, 12.47. The nmr spectrum in deuteriodimethyl sulfoxide was less clear than that of III since the 5′-methylene protons and the C_2′ and C_3′ protons overlapped and appeared as a complex four-proton multiplet between 4.15 and 4.50 ppm. All other protons in IV were, however, clearly resolved.

Further work on the synthesis of other 4′,5′-unsaturated nucleosides is in progress and will be reported later.



IIa, R = OH
b, R = I

III

IV

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(7) For a comprehensive review on unsaturated sugars see R. J. Ferrier, *Advan. Carbohydrate Chem.*, **20**, 67 (1965).
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(9) A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, 3459 (1956).
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A Method for the Determination of Nucleotide Sequences near the Terminals of Ribonucleic Acids of Large Molecular Weight

Sir:

We wish to report a method by which the terminal fragments produced by specific cleavage of ribonucleic acids can be oxidized by periodate, selectively absorbed on aminoethylcellulose, and subsequently recovered in a pure condition. The approach is especially suited to the study of the sequences of large ribonucleic acids, and some preliminary results are reported for the nucleic acid of the bacteriophage f2.

The absorption of periodate-oxidized ribonucleic acid to aminoethylcellulose has been used by Zubay[1] to purify sRNA specific for a particular amino acid. In this procedure the terminal *cis*-glycol groups of the bulk of the sRNA are not protected by the selected amino acid, and they are converted to dialdehydes by the periodate oxidation. When passed through an aminoethylcellulose column these ribonucleic acid chains are retained, presumably by chemical condensation of the aldehyde groups with the amino groups on the cellulose, while the unoxidized amino acyl sRNA passes through the column in an enriched condition. More recently, using similar columns, Habermann, *et al.*,[2] have also studied the absorption of periodate-oxidized oligonucleotides and their subsequent recovery with hydrochloric acid as the eluting agent.

In the present work the model compounds, uridylyl-(3′–5′)-adenosine and guanylyl-(3′–5′)-adenosine have been used to study the absorption and recovery of periodate-oxidized fragments on aminoethylcellulose, and, on the basis of these studies, a procedure for the isolation of such fragments from ribonucleic acids has been developed. In this approach the ribonuclease

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(2) V. Habermann, E. Maidlová, and R. Černy, *Collection Czech. Chem. Commun.*, **31**, 139 (1966).

digest of the nucleic acid is treated with periodate and then passed through a column of the cellulose at 4° in the presence of 4 *M* sodium chloride. Under these conditions the unoxidized internal fragments (containing phosphate groups at their 3′ ends) are eluted while the oxidized terminal fragment is retained. Recovery is effected by the subsequent displacement of the chloride ions by bicarbonate ions followed by elution of the column at 45° with a solution of a primary amine (in the form of its volatile bicarbonate). The conditions used for the recovery are similar to the reaction conditions used by Neu and Heppel[3] for the removal of the terminal base from a periodate-oxidized oligonucleotide by a β-elimination reaction, and thus the recovered terminal fragment is expected to be found lacking its terminal nucleoside.

Bacteriophage f2 RNA (150 mg, 84 mμmoles) was dissolved in water (5 ml), and ribonuclease T_1 (1.5 mg) was added. The pH of the reaction was kept at 7.5 by the controlled addition of 0.1 *M* NaOH. After 24 hr at 25°, 0.1 *M* sodium periodate (2.5 ml) was added. The mixture was allowed to stand for 0.5 hr at 25° and the excess periodate was then destroyed by the addition of sucrose (83 mg). After 40 min at 25° the mixture was cooled to 0°, sodium chloride (1.75 g) was added, and the solution was adjusted to pH 8.5. Aminoethylcellulose (Cellex AE, 0.4 mequiv/g) was washed with 0.5 *M* *n*-propylamine bicarbonate at 45° and then with 4 *M* NaCl (pH 8.5). The cellulose was then packed at 4° into a water-jacketed column (1 × 84 cm) and washed with the NaCl solution. The oxidized enzymic digest of the RNA was applied to the column and eluted at 4° with the 4 *M* NaCl (pH 8.5) at 8–10 ml/hr. After all the internal oligonucleotides were eluted, sufficient 0.5 *M* $KHCO_3$ (pH 8.5) was passed through at 100 ml/hr, 4°, to displace all chloride ions from the column. The potassium bicarbonate was removed by elution with water at the same temperature and flow rate. The temperature of the column was then raised to 45°, and elution of the terminal fragment was carried out with 0.5 *M* *n*-propylamine bicarbonate (pH 7.5) at 5–6 ml/hr, 45°. In model experiments where *ca.* 3 ODU (260 mμ) of uridylyl-(3′–5′)-adenosine or guanylyl-(3′–5′)-adenosine was used in place of the RNA, uridine 3′-phosphate and guanosine 3′-phosphate were obtained in 60% yield by paper chromatography of the respective propylamine bicarbonate eluates.

The entire propylamine bicarbonate eluate (200 ml) was evaporated to dryness *in vacuo*, and the volatile salt was completely removed by repeated solution of the product in water and evaporation to dryness. The product was applied to a DEAE-Sephadex A-25 column (0.6 × 20 cm). Elution was effected with 600 ml of 0.05 *M* Tris acetate buffer–7 *M* urea (pH meter reading: 8.0) containing a linear gradient of sodium acetate (0.15–0.95 *M*) at a flow rate of 2.5 ml/hr. The terminal fragment appeared as a peak (4.6 ODU at 260 mμ, 60%) at a position which corresponded to the position of elution of the octanucleotides. The position of elution of the latter was determined by the chromatography of a ribonuclease T_1 digest of f2 RNA on an identical column under the same conditions. For analysis, the peak fractions were combined, dialyzed against water, and concentrated to 1.5 ml. The product was treated with alkaline phosphatase (0.1 mg) for 20 hr at 37°. The reaction mixture was adjusted to 0.3 *M* potassium hydroxide by addition of 1 *M* KOH and was kept at 37° for 20 hr. Control experiments have shown that alkaline phosphatase does not survive this treatment nor does it have enzyme activity during the alkaline hydrolysis. Thus, there was no problem arising from the dephosphorylation of nucleotides formed by the alkaline hydrolysis. Analysis of the hydrolysate by a modification of a method previously described[4] showed cytidine as the only nucleoside present. Another preparation of the terminal fragment was directly treated with 0.3 *M* potassium hydroxide for 20 hr at 37°. The mixture was neutralized with perchloric acid, and the nucleotides present were determined by a modification of the method described by Cohn and Volkin;[5] found: Up, 93 mμmoles; Ap, 96 mμmoles; Cp, 189 mμmoles; ratio Up:Ap:Cp = 2.00:2.06:4.06.

It thus appears that the terminal fragment isolated from f2 RNA has a terminal cytidine and has the composition: (3Cp,2Up,2Ap)-Cp. Other experiments in this laboratory have shown conclusively that the terminal base of f2 RNA is adenosine[4] and that the penultimate base is cytidine. The treatment of f2 RNA with the uridine blocking agent, 1-cyclohexyl-3-(2-morpholinyl-4-ethyl)carbodiimide metho-*p*-toluenesulfonate, and then pancreatic ribonuclease under previously described conditions[6] yields the same amount of adenosine end group as is obtained without prior treatment with the blocking agent. Also, treating the f2 RNA with periodate under published conditions[3,7] followed by removing the terminal phosphate so produced gave a molecule which, on treating with alkali, yielded cytidine as its terminal nucleoside. These results are consistent with the isolation, after periodate oxidation, of a fragment containing a terminal cytidine as described above. Thus, together with the fact that the above fragment was produced by an enzyme that is specific for the cleavage of guanosine 3′-phosphoryl bonds, the present experiments indicate that the terminal nucleotide sequence of the f2 RNA is -Gp-(3Cp,2Up,2Ap)-CpA.

Acknowledgment. This work was supported by the National Institutes of Health.

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Biogentic-Type Synthesis of an Acylphloroglucinol[1]

Sir:

Birch has postulated that polyketo acids (or esters) are formed as intermediates in the biosynthesis of phenolic compounds from acetate.[2] He concluded

(1) Supported by grants from the National Institute of General Medical Sciences (5-RO1-GM12848-02) and from the Research Corporation.

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