183. *Polynucleotides. Part IV.*[1] *Synthesis of Oligonucleotide Analogues Substituted in the Sugar Portion.*

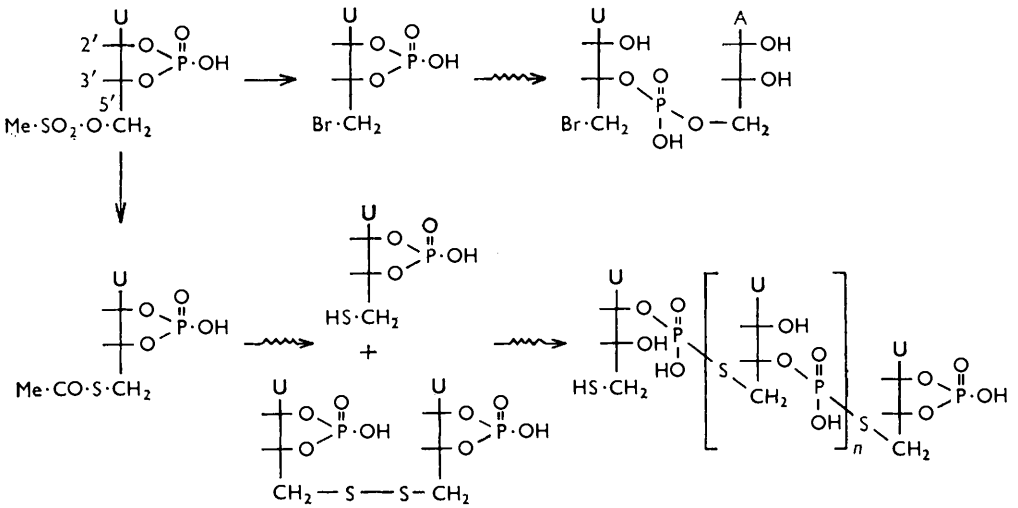
By A. M. Michelson.

Under suitable conditions, treatment of uridine-2′,3′ cyclic phosphate with methanesulphonyl chloride (or ethanesulphonyl chloride) gives 5′-*O*-methanesulphonyluridine-2′,3′ cyclic phosphate (or the 5′-*O*-ethanesulphonyl derivative) which with lithium bromide is converted into 5′-bromo-5′-deoxyuridine-2′(3)′ cyclic phosphate. Further treatment with 2′,3′-di-*O*-acetyladenosine and diphenyl phosphorochloridate followed by removal of protecting groups from the product gives a mixture of 5′-(5′-bromo-5′-deoxyuridyl-3′-yl)adenosine and the 2′,5′-linked isomer. Treatment of 5′-*O*-alkanesulphonyluridine-2′,3′ cyclic phosphate with sodium thioacetate and hydrolysis of the product with alkali give 5′-thiouridine-2′(3′) phosphate which on polymerisation yields a mixture of oligo-5′-thiouridylic acids.

The reaction of alkanesulphonyl chlorides with nucleoside-2′,3′ cyclic phosphates in pyridine gives polynucleotide material.[1] In dioxan, however, in the presence of a hindered tertiary base, the sole product is the 5′-*O*-alkanesulphonylnucleoside-2′,3′ cyclic phosphate. Replacement of sulphonyloxy-groups in nucleoside derivatives by treatment with various salts has been described[3] and it has now been found that the same reactions can occur

[1] Part III, Letters and Michelson, *J.*, 1962, 71.
[2] Michelson, *J.*, 1959, 1371.
[3] Michelson and Todd, *J.*, 1955, 816; Baddiley and Jamieson, *J.*, 1955, 1085.

with the analogous nucleotide derivatives. When uridine-2',3' cyclic phosphate was treated with methanesulphonyl chloride (or ethanesulphonyl chloride) in dioxan the 5'-*O*-alkanesulphonyl derivative was obtained quantitatively, as shown by paper chromatography and by isolation of the calcium salts of 5'-*O*-alkanesulphonyluridine-2'(3') phosphate obtained by acidic hydrolysis of the cyclic phosphate. 5'-*O*-Methanesulphonyluridine-2',3' cyclic phosphate was readily converted into 5'-bromo-5'-deoxyuridine-2',3' cyclic phosphate by treatment with lithium bromide. The ethanesulphonyl derivative, though less reactive, was also converted into this compound. Enzymic hydrolysis of the cyclic phosphate with pancreatic ribonuclease gave 5'-bromo-5'-deoxyuridine-3' phosphate, isolated as the calcium salt. Further reaction of 5'-bromo-5'-deoxyuridine-2',3' cyclic phosphate with 2',3'-di-*O*-acetyladenosine and diphenyl phosphorochloridate,[4] followed by removal of protecting groups from the product, gave a mixture of 5'-(5'-bromo-5'-deoxyuridyl-3'-yl)adenosine and the 2',5'-linked isomer; the former dinucleoside phosphate was



degraded to adenosine and 5'-bromo-5'-deoxyuridine-3' phosphate by the action of pancreatic ribonuclease. Since such dinucleoside phosphates would presumably act as incorporated primers for polynucleotide phosphorylase, they are of possible interest for *X*-ray examination of biosynthetic polymers and their helical complexes, as the heavy bromine atom would occur at one end of the polynucleotide chain.

In a similar manner, 5'-*O*-methanesulphonyluridine-2',3' cyclic phosphate, on treatment with sodium thioacetate, was converted into the 5'-acetylthio-5'-deoxy-derivative. This was not isolated as such, but was hydrolysed with alkali to 5'-thiouridine-2'(3') phosphate and isolated as the calcium salt. Under the conditions used a considerable amount of the disulphide form was also obtained, but this was readily reduced to the thiol derivative by treatment with 2-mercaptoethanol. Polymerisation of 5'-thiouridine-2'(3') phosphate (after conversion into the 2',3'-cyclic phosphate by the action of ethyl chloroformate) by treatment with diphenyl phosphorochloridate and base [2] then gave poly-5'-thiouridylic acid. Unlike poly-N^1-methyluridylic acid, this was degraded by pancreatic ribonuclease to nucleoside-3' phosphate and resistant oligonucleotides containing 2',5'-internucleotide linkages only. Enzymic hydrolysis of poly-5'-thiouridylic acid with crude rattlesnake (*Crotalus atrox*) venom gave 5'-thiouridine (identified by chromatographic comparison with an authentic specimen) as a result of the combined action of the 5'-monoesterase and diesterase that are present in the venom.

[4] Michelson, *J.*, 1959, 3655.

Experimental

5′-O-*Methanesulphonyluridine-2′,3′ Cyclic Phosphate.*—Tri-n-butylamine (2·5 c.c.) and ethyl chloroformate (0·7 c.c.) were added to a solution of uridine-2′(3′) phosphate (3·5 mmoles) in water (9 c.c.) and shaken, then kept at room temperature for 5 min. Solvent was removed under reduced pressure and the residue dried by repeated concentration of an ethanolic solution. Dry ether (100 c.c.) was added to the residue with shaking and the mixture kept at 0° for 30 min. The precipitated tri-n-butylammonium uridine-2′,3′ cyclic phosphate was dissolved in dioxan (30 c.c.) with the addition of tri-n-butylamine if necessary, and the solution concentrated to half its volume under reduced pressure, then cooled to 0°. Methanesulphonyl chloride (0·5 c.c.) and tri-n-butylamine (2·5 c.c.) were added, and the mixture was shaken and then left at room temperature overnight. Solvent was removed under reduced pressure and dry ether (50 c.c.) added to precipitate *tri-n-butylammonium* 5′-O-*methanesulphonyluridine-2′,3′ cyclic phosphate* as a gum that was washed with dry ether, then dissolved in dioxan (10 c.c.). The compound was not purified further; paper chromatography and electrophoresis in several solvents showed complete homogeneity; hydrolysis with pancreatic ribonuclease gave a single product with the properties expected of 5′-*O*-methanesulphonyluridine-3′ phosphate, and hydrolysis of an aliquot part with alkali as previously described [2] or under acidic conditions (pH 2·5 at room temperature for 4 hr.) gave 5′-*O*-methanesulphonyluridine-2′(3′) phosphate. This was isolated as the calcium salt in the usual way in 85% overall yield (Found, in material dried at 120°/10^{-3} mm. for 24 hr.: P, 7·1. Calc. for $C_{10}H_{13}CaN_2O_{11}PS$: P, 7·0%).

5′-O-*Ethanesulphonyluridine-2′,3′ Cyclic Phosphate.*—This was prepared as described for the methanesulphonyl derivative but with ethanesulphonyl chloride (0·65 c.c.). Hydrolysis of the cyclic phosphate gave calcium 5′-O-*ethanesulphonyluridine-2′(3′) phosphate* in 90% overall yield (Found, in material dried at 120°/10^{-3} mm. for 24 hr.: N, 5·9; P, 6·6. $C_{11}H_{15}CaN_2O_{11}PS$ requires N, 6·2; P, 6·8%).

5′-*Bromo-5′-deoxyuridine-2′(3′) Phosphate.*—Anhydrous lithium bromide (0·45 g., 5 mmoles) in dimethylformamide (6 c.c.) was added to a solution of tri-n-butylammonium 5′-*O*-methanesulphonyluridine-2′,3′ cyclic phosphate (1 mmole) in dioxan (4 c.c.) and kept at 100° for 2 hr. with the exclusion of moisture. Solvent was removed under reduced pressure, the residue dissolved in water (5 c.c.), and the solution adjusted to pH 2 with 2N-hydrochloric acid and then kept at room temperature for 4 hr. The solution was neutralised with lithium hydroxide and then the calcium salt was isolated in the usual way (0·405 g., 95%). A similar yield was obtained from 5′-*O*-ethanesulphonyluridine-2′,3′ cyclic phosphate. The two preparations (0·79 g.) were combined and dissolved in water (10 c.c.), and two volumes of ethanol added to precipitate the *calcium 5′-bromo-5′-deoxyuridine-2′(3′) phosphate* (0·69 g.) (Found, in material dried at 100°/10^{-3} mm. for 24 hr.: N, 6·3; P, 7·2. $C_9H_{10}BrCaN_2O_8$ requires N, 6·6; P, 7·3%).

5′-[5′-*Bromo-5′-deoxyuridyl-2′*(*and* 3′)*yl*]*adenosine.*—An aqueous solution of calcium 5′-bromo-5′-deoxyuridine-2′(3′) phosphate (1 mmole) was passed through a column of IR-120 (H^+ form), and the free acid converted into the cyclic phosphate in the usual way.[4] To a solution of the anhydrous tri-n-butylammonium 5′-bromo-5′-deoxyuridine-2′,3′ cyclic phosphate in dioxan (3 c.c.) were added 2′,3′-di-*O*-acetyladenosine (1·5 mmoles), tri-n-butylamine (0·6 c.c.), and diphenyl phosphorochloridate (0·3 c.c.), and the mixture was kept at room temperature for 12 hr. The product was deacetylated and isolated in the usual way,[4] then purified by chromatography on a column (2 × 6 cm.) of Dowex 2 × 8 (formate form). Unchanged material was eluted with 0·02N-formic acid. Elution with 0·075N-formic acid removed a mixture of 5-(5′-bromo-5′-deoxyuridyl-2′- and -3′-yl)adenosine isolated as the free acid (0·305 g.) (Found, in material dried at 100°/10^{-3} mm. for 24 hr.: N, 14·7; P, 4·5. Calc. for $C_{19}H_{23}BrN_7O_{11}P,H_2O$: N, 15·0; P, 4·7%).

5′-*Thiouridine-2′(3′) Phosphate.*—Tri-n-butylammonium 5′-*O*-methanesulphonyluridine-2′,3′ cyclic phosphate (1·5 mmoles) in dioxan (6 c.c.) and thioacetic acid (0·5 c.c.) were added to a solution of sodium thioacetate (4·5 mmoles) in dimethylformamide (9 c.c.) and left at room temperature overnight, then at 65° for 20 min. The dark brown solution was evaporated to dryness under reduced pressure and the residue dissolved in water (10 c.c.) and extracted with ether, the extracts being discarded. The aqueous solution was adjusted to pH 11 with lithium hydroxide, again extracted with ether, and then kept at pH 11 at room temperature for 48 hr. Hydrochloric acid was added to pH 7·5, then the pale yellow solution was evaporated to dryness under reduced pressure. The residue was dissolved in water (5 c.c.) and filtered,

and ethanolic calcium chloride and ethanol (15 c.c.) were added, to give a crude calcium salt which was collected, then redissolved in a small volume of water. Undissolved calcium diuridine-2′(3′) phosphate-5′ disulphide (0·175 g.) (Found, in material dried at 135°/10^{-3} mm. for 24 hr.: N, 7·1; P, 8·0. Calc. for $C_{18}H_{20}Ca_2N_4O_{16}P_2S_2$: N, 7·4; P, 8·2%) was collected and mercaptoethanol (0·2 c.c.) was added to the clear filtrate. The solution was kept at room temperature overnight, then ethanol (2 vol.) was added and the precipitated *calcium 5′-thiouridine-2′(3′) phosphate* collected by centrifugation, washed once with ethanol, then twice with ether, and dried (0·325 g.) (Found, in twice precipitated material, dried at 130°/10^{-3} mm. for 24 hr.: N, 7·6; P, 8·4. $C_9H_{11}CaN_2O_8PS$ requires N, 7·4; P, 8·2%). The thiol was also obtained by reduction of the disulphide with mercaptoethanol in aqueous solution at room temperature.

R_F of nucleotides.

	Nucleoside-2′,3′ cyclic phosphate		Nucleoside-2′,3′ phosphate	
Parent nucleoside	A	B	A	B
Uridine	0·44	0·48	0·23	0·45
5′-*O*-Methanesulphonyluridine	0·51	0·54	0·29	0·55
5′-*O*-Ethanesulphonyluridine	0·57	0·59	0·33	0·58
5′-Bromo-5′-deoxyuridine	0·57	0·58	0·29	0·58
5′-Acetylthio-5′-deoxyuridine	0·63	0·58	—	—
5′-Thiouridine (A)	—	—	0·19	0·49
Disulphide from (A)	—	—	0·03	0·28

{5′-[5′-Bromo-5′-deoxyuridyl-(2′ + 3′)-yl]adenosine: A, 0·38; B ,0·40.}

Poly-5′-thiouridylic Acid.—Calcium 5′-thiouridine-2′(3′) phosphate (0·33 mmole) was converted into the tri-n-butylammonium salt in the usual way, and then into the cyclic phosphate by treatment with tri-n-butylamine (0·25 c.c.) and ethyl chloroformate (0·07 c.c.) in water (1 c.c.). Diphenyl phosphorochloridate (0·1 c.c.) and tri-n-butylamine (0·2 c.c.) were added to a solution of the anhydrous nucleotide in dioxan (1 c.c.), and the product was isolated as the calcium salt in the usual way (0·085 g.). The product was purified by dialysis in Visking tubing against water. The dialysis residue showed the usual chromatographic properties characteristic of polymeric material. Conditions for degradation with alkali, pancreatic ribonuclease, and rattlesnake (*Crotalus atrox*) venum were as described earlier.[2] 5′-Mercapto-5′-deoxyuridine for comparison was prepared as described by Baddiley and Jamieson [3] and by Bannister and Kagen [5] for the synthesis of 5′-deoxy-2′,3′-*O*-isopropylidene-5′-mercaptouridine, but with 5′-*O*-toluene-*p*-sulphonyluridine [6] instead of 2′,3′-*O*-isopropylidene-5′-*O*-toluene-*p*-sulphonyluridine or 5′-deoxy-5′-iodo-2′,3′-isopropylideneuridine.

Paper Chromatography.—Ascending chromatograms on Whatman No. 1 paper were used with solvent systems: A, ethanol–M-ammonium acetate (5 : 2); B, t-pentyl alcohol–formic acid–water (3 : 2 : 1). Results are tabulated. Compounds containing thiol or disulphide groups were detected on paper chromatograms (after location of ultraviolet-absorbing spots by means of a Chromatolite lamp) with the nitroprusside or sodium cyanide–nitroprusside dips described by Toennies and Kolb.[7]

ARTHUR GUINNESS SON & CO. (DUBLIN) LTD.,
DUBLIN, EIRE.

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[5] Bannister and Kagen, *J. Amer. Chem. Soc.*, 1960, **82**, 3363.
[6] Brown, Todd, and Varadarajan, *J.*, 1957, 868.
[7] Toennies and Kolb, *Analyt. Chem.*, 1951, **23**, 823.