H-Pyr-Leu-Tyr-Gln-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH——The above protected tridecapeptide ester (400 mg, 0.17 mmol) was treated with MSA (3 ml) in the presence of anisole–thioanisole–*o*-cresol (1 : 1 : 1, v/v, 0.5 ml) in an ice-bath for 10 min and at room temperature for 60 min, then dry ether was added. The residue was again treated with MSA and precipitated with ether as described above. The deprotected product was dissolved in a small amount of H_2O and the solution was treated with Amberlite CG-4B (acetate form, approximately 2 g) for 30 min. The pH of the filtrate was adjusted to 9 with 10% NH_4OH/in an ice-bath and then to 6 with AcOH. After lyophilization, the resulting powder was dissolved in a small amount of the upper phase of a solvent system consisting of *n*-BuOH–AcOH–H_2O (4 : 1 : 5), and the solution was applied to a column of Sephadex G-25 (fine) equilibrated with the lower phase of the above solvent system. The column was then developed with the upper phase and individual fractions (7.5 ml each) were collected. Determination of the UV absorbancy at 275 nm indicated the presence of five peaks (Fig. 2). The front peak (F-1, tube No. 50—67) was found to be due to contaminating scavengers and two other peaks (F-3, tube No. 99—118 and F-4, tube No. 122—140) were minor components. Fractions corresponding to the main peak (F-5, tube No. 149—178) were collected and the solvent was evaporated off. Lyophilization of the residue afforded a white fluffy powder; yield 178 mg (57%), $[\alpha]_D^{20}$ −94.1° (c=0.4, H_2O), Rf^3 0.57. Amino acid ratios in 4 N MSA hydrolysate: Asp 1.00, Glu 2.29, Pro 2.02, Ile 0.90, Leu 1.96, Tyr 1.95, Lys 1.05, Arg 2.00 (average recovery 92%). Amino acid ratios in aminopeptidase M (Merck) digest: Asn 1.40, Gln 0.72, Pro=0, Ile 1.00, Leu 1.00, Tyr 1.48, Lys=0, Arg 1.09 (Ile recovery was taken as the basis of calculation). *Anal.* Calcd. for $C_{78}H_{122}N_{22}O_{19}\cdot 3CH_3COOH\cdot 8H_2O$: C, 50.54; H, 7.57; N, 15.44. Found: C, 50.67; H, 7.45; N, 15.60.

The material obtained from F-4 (Tube No. 68—89); 41 mg (13%), Rf^3 0.63 (tailing spot). Amino acid ratios in 4 N MSA hydrolysate: Asp 1.00, Glu 2.43, Pro 2.18, Ile 0.91, Leu 1.97, Tyr 1.67, Lys 0.97, Arg 1.84 (average recovery 72%).

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Selective Removal of Protecting Groups for Phosphomonoesters of Nucleotides by Anodic Oxidation

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Cyclic volutammetry of phenyl derivatives of various nucleotides was carried out, using the parent nucleosides as a control. The *p*-*N*-benzylaminophenyl ester of 3′-O-acetylthymidine 5′-phosphate showed a peak at low anodic potential, and the *N*-benzylaminophenyl group could be removed by controlled potential electrolysis at this potential. *p*-*N*-Tritylaminophenyl-, *p*-*N*-acetylaminophenyl and *p*-methoxyphenyl esters of nucleotides were also subjected to controlled potential electrolysis; the oxidative removal of these phenyl groups was less effective than that of the *N*-benzylaminophenyl ester.

Keywords——*p*-*N*-benzylaminophenyl phosphate; *p*-*N*-tritylaminophenyl phosphate; *p*-*N*-acetylaminophenyl phosphate; *p*-methoxyphenyl phosphate; cyclic voltammetry; controlled potential electrolysis

We have previously reported that the *N*-trityl-*p*-aminophenyl group served as a useful protecting group for terminal phosphate residues in the synthesis of oligonucleotides.[2] This protecting group could be removed by oxidative hydrolysis with iodine from protected oligo-

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1) Location: *133-1 Yamadakami, Suita 565, Japan.*

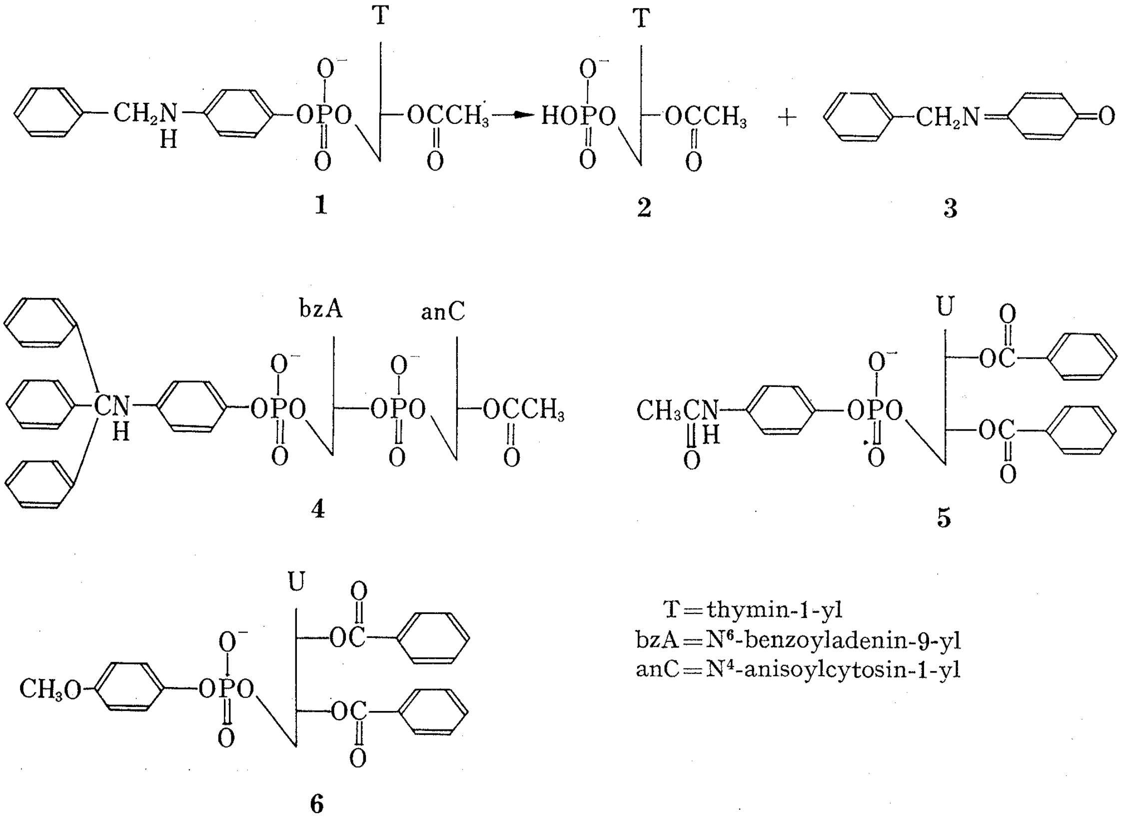
2) E. Ohtsuka, S. Morioka, and M. Ikehara, *J. Am. Chem. Soc.*, **95**, 8437 (1973).

nucleotides.[2,3] Phosphoro-*p*-hydroxyanilidate derivatives of nucleosides are also converted to the phosphate by mild oxidative hydrolysis.[4] This type of protecting group was reported to be removed by anodic oxidation.[5] In the present paper we report voltammetric peak potentials of *p*-*N*-benzylaminophenyl-, *p*-*N*-tritylaminophenyl-, *p*-*N*-acetylaminophenyl- and *p*-methoxyphenyl-esters of some nucleotides as well as those of parent nucleosides and nucleotides. Controlled potential electrolyses of these phenyl esters are also reported.

TABLE I. Results of Cyclic Voltammetry of Various Phenyl Derivatives[a]

Compound (concentration)	Background solution	First peak V *vs.* S.C.E.	Second peak V *vs.* S.C.E.
1 (2 mM)	DMF: H_2O (7 : 3) sodium perchlorate (0.1 M)	0.53	0.95
4 (3 mM)	DMF: 80% EtOH (1 : 7) triethylammonium benzoate (pH 7.5, 0.2 M)	0.70	0.90
5 (3 mM)	80% EtOH triethylammonium acetate (pH 7.5, 0.3 M)	0.66	1.20
6 (3 mM)	DMF: 95% EtOH (1 : 6) triethylammonium benzoate (pH 7.5, 0.3 M)	1.40	1.65

a) At a glassy carbon electrode: electrode area, 0.071 cm^2. Voltage sweep rate, 50 mV s^{-1}.



T = thymin-1-yl
bzA = N^6-benzoyladenin-9-yl
anC = N^4-anisoylcytosin-1-yl

Chart 1

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3) E. Ohtsuka, S. Morioka, and M. Ikehara, *Chem. Pharm. Bull.* (Tokyo), **24**, 560 (1976).

4) E. Ohtsuka, S. Morioka, and M. Ikehara, *Tetrahedron Lett.*, **1972**, 2553.

5) C.A. Chambers and J.Q. Chambers, *J. Am. Chem. Soc.*, **88**, 2922 (1966).

Results and Discussion

Cyclic Voltammetry of Phosphorophenyl Derivatives

Cyclic voltammetry of *p*-*N*-benzylaminophenyl 5′-phosphoro-3′-O-acetylthymidine (**1**) (Chart 1), *p*-*N*-tritylaminophenyl 5′-phosphoro-*N*-benzoyldeoxyadenylyl-(3′—5′)-3′-*O*-acetyl-*N*-anisoyldeoxycytidine (**4**), *p*-*N*-acetylaminophenyl 5′-phosphoro-2′,3′-*O*-dibenzoyluridine (**5**) and *p*-methoxyphenyl 5′-phosphoro-2′,3′-*O*-dibenzoyluridine (**6**) was performed under the conditions given in Table I, which also shows the results. Cyclic voltammetry of **1** shows two anodic peaks and two cathodic peaks (Fig. 1). An additional anodic peak was observed on subsequent anodic scan. Although the exact nature of the voltammetric peaks is not clear from the present results alone, the shape of the voltammogram together with the results of controlled potential electrolysis (see below) suggest an electrode process similar to that reported for 4′-methoxybenzanilide.[6]

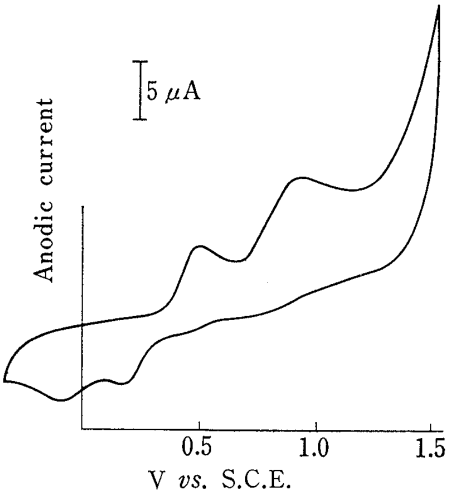

Fig. 1. Cyclic Voltammogram of 3′-O-Acetylthymidine 5′-*p*-N-Benzylaminophenyl Phosphate

For conditions, see Table I.

Cyclic Voltammetry of Protected and Non-protected Nucleosides

To avoid damage to the parent nucleosides during electrolysis of the protecting group on phosphates, oxidation potentials of nucleosides were measured by cyclic voltammetry. No peaks for cytidine, *N*-benzoyl-2′,3′-*O*-dibenzoylcytidine, uridine and 2′,3′-*O*-dibenzoyluridine were detected beyond 1.8 V in 80% ethanol containing 0.1M sodium perchlorate. However, under the same conditions, guanosine, guanosine 2′(3′)-phosphate and *N*-isobutyryl-2′,3′-*O*-dibenzoylguanosine showed anodic peaks at 1.5 V, 1.18 V and 1.0 V, respectively. Adenosine (1.4 V) and *N*,*N*,2′,3′-*O*-tetrabenzoyladenosine (1.5 V) also showed anodic peaks in 80% acetonitrile containing 0.1 M sodium perchlorate. In the case of purine nucleosides, decreases of anodic current were observed upon multiple anodic scanning. This may mean that electrode filming can occur at a glassy carbon anode.

Controlled Potential Electrolysis of Phenyl Derivatives

Compounds **1**, **4**, **5** and **6** were subjected to controlled potential electrolysis under the conditions described in Table I unless otherwise stated. The *N*-benzylaminophenyl group of **1** was removed completely by electrolysis at 0.6 V or at 1.0 V for 3 hr. The hydrolyzed product pT(Ac)[7] (**2**) was characterized by paper chromatography and paper electrophoresis, and no starting material (**1**) was detected in these systems. The dinucleotide (**4**) was unchanged after electrolysis at 0.7 V for 5.5 hr. About one-third of **4** was converted to the corresponding phosphomonoester after treatment at 0.9 V for 3.5 hr and another one-third of **4** lost the trityl group as judged from paper electrophoresis after ammonia treatment. Compound **5** lost 28% of the acetyl group on electrolysis at 1.2 V for 3.5 hr in an atmosphere of nitrogen. **6** was converted to the corresponding phosphomonoester by electrolysis at 1.7 V in a yield of 70%. It was concluded that the *p*-*N*-benzylaminophenyl group could be used as a protecting group removable by anodic oxidation. For the synthesis of oligonucleoitdes,

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6) S. Ikenoya, M. Masui, H. Ohmori, and H. Sayo, *J.C.S. Perkin II*, **1974**, 572.

7) For IUPAC-IUB Commission recommendations on biochemical nomenclature see *J. Biol. Chem.*, **245**, 5171 (1970): *Proc. Nat. Acad. Sci. U.S.*, **74**, 2222 (1977).

however, the recovery of oligonucleotides containing purine bases from a carbon electrode should be further investigated.

Experimental

Nucleosides and Nucleotides——1, 5 and 6 were synthesized by a method similar to that described for *p*-*N*-tritylaminophenyl 5′-phosphorothymidine[2] from the corresponding phosphomonoesters. 4,[2] pU(Bz)$_2$,[8] bzC(Bz)$_2$,[9] U(Bz)$_2$,[9] ibG(Bz)[10] and bzA(Bz)$_2$[9,11] were prepared as described previously.

Apparatus——Cyclic voltammetry was carried out as reported by Sayo and Masui.[12] Controlled potential electrolysis was carried out in an open beaker using a Hokutodenko HA-101 potentiostat/galvanostat with a glassy carbon plate anode, platinium wire cathode, and saturated calomel electrode was separated from the anolyte by a unglazed cylinder and the reference electrode was connected to the anolyte through an agar bridge. When a samall volume (6 ml) of solution of compound 6 was subjected to controlled potential electrolysis, the solution was circulated by means of a peristaltic pump through a funnel bearing a glassy carbon rod anode (ϕ 3 mm × 10 cm), a carbon rod cathode (ϕ 4 mm × 4 cm) and the S.C.E. connected to the anolyte through an agar bridge.

Product Analysis——Paper chromatography was performed using ethanol-1 M ammonium acetate, pH 7.5 (7: 3, v/v). Paper electrophoresis was performed using 0.05 M triethylammonium bicarbonate (pH 7.5) at 900 V/40 cm.

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8) E. Ohtsuka, A. Honda, H. Shigyo, S. Morioka, T. Sugiyama, and M. Ikehara, *Nucleic Acids Res.*, **1**, 223 (1974).
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11) M. Smith, D.H. Rammler, I.H. Goldberg, and H.G. Khorana, *J. Am. Chem. Soc.*, **84**, 430 (1962).
12) H. Sayo and M. Masui, *J.C.S. Parkin II*, **1973**, 1640.

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An Ultramicro Enzymatic Method for the Fluorimetric Determination of Uric Acid in Serum

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A highly sensitive fluorimetric method is presented for the determination of uric acid in serum, based on the uricase-catalized oxidation of the acid to hydrogen peroxide, which is measured by a fluorimetric technique using the peroxidase-*p*-hydroxyphenylacetic acid system. The method is rapidly and readily performed with only 2 μl of serum without deproteinization.

Keywords——uric acid in serum; fluorimetry; enzymatic assay; uricase-peroxidase system; *p*-hydroxyphenylacetic acid; ultramicro analysis

Many colorimetric and fluorimetric methods have been reported for the determination of uric acid in serum (or plasma). Reduction methods are based on the color development

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