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Syntheses of Polymers and Oligomers Having Adenine Derivatives

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ABSTRACT

9-(2',3'-Dihydroxyethyl)-8-bromoadenine was synthesized by the reaction of 9-(2',3'-dihydroxyethyl)-adenine with bromine. The reaction of 9-(2',3'-dihydroxyethyl)-8-bromoadenine with phosphorus oxychloride in trimethyl phosphate produced 9-(2',3'-dihydroxypropyl)-8-bromoadenine-3'-phosphate. The condensation polymerization of 9-(2',3'-dihydroxypropyl)-8-bromoadenine-3'-phosphate was conducted in refluxing dimethylformamide-water (9:1) using dicyclohexylcarbodiimide as a dehydrating agent. The oligomer obtained is soluble in water and has a molecular weight of more than 1000 according to gel-filtration measurement. This oligomer showed hypochromicity of 3% with denatured yeast RNA. The condensation polymerization of 9(2',3'-dihydroxypropyl)-8-bromoadenine-3'-phosphate was also carried out using imidazole or a triethylamine-hydrochloric acid system.

The polymers having adenine derivatives were also synthesized by the polymer reaction of substituted 9-(2'-dihydrogenphosphatoethyl)-adenines with polyvinyl alcohol.

INTRODUCTION

Polymers which contain nucleic acid bases in their main chains or in side groups present many interesting problems with respect to their physicochemical properties. Jones et al. have synthesized many vinyl monomers and polymers containing nucleosides [1]. Also, Imoto, Takemoto, and co-workers reported on the syntheses of vinyl monomers, such as 1-vinyl and 1-(2-acryloyloxyethyl) derivatives of thymine, adenine, uracil, hypoxanthine, and theophylline, and their polymers [2]. Poly-9-vinyladenine showed a hypochromicity of 13% mixed with denatured RNA [3]. Details of complex formation between poly-9-vinyladenine and polyuridylic acid was shown by Kaye [4]. Ts'o et al. reported on the syntheses of N-vinyl compounds having pyrimidines or uracil [5].

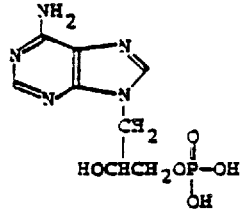
The water-insolubility of these polymers caused some difficulties for the study of their physicochemical properties in aqueous media. This problem was solved by the introduction of phosphoric ester into the main chain or into the side group of the polymer.

We have also synthesized water-soluble polymers, particularly paying attention to the new structural analogs of nucleic acid.

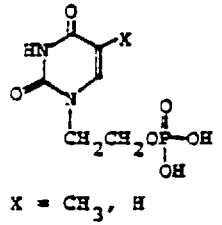
Our recent work in this field was carried out by two routes.

1. By condensation polymerization. Condensation polymerization of the monomers of the type shown in Structure A was carried out using N,N-dicyclohexylcarbodiimide (DCC) as a dehydrating agent in refluxing dimethylformamide (DMF) [6].

2. By polymer reaction. Condensation of compounds of Type B with polyvinyl alcohol was carried out under the same condition as mentioned above to give polymers containing nucleic acid bases as side groups.



(A)



X = CH_3, H

(B)

Polymers and oligomers obtained by the above two methods were very soluble in water. These polymers and oligomers also showed significant hypochromicities when mixed with yeast RNA or sperm DNA in 0.1 M Na_2HPO_4 solution.

In the present paper we describe the syntheses of polymers containing adenine derivatives through the condensation polymerization or polymer reaction, and the physicochemical properties of the polymers obtained.

EXPERIMENTAL

9-(2'-Hydroxyethyl)-8-bromoadenine (8-Br-9-AM)(I)

To a solution of 9-(2'-hydroxyethyl)-adenine (4 g, 22.5 mmole) in 10% aqueous disodium hydrogen phosphate (320 ml), p-dioxane (320 ml) and bromine (8 ml) were added. The solution was stirred for 12 hr at room temperature and kept overnight. The reaction mixture was filtered and filtrate was evaporated. Decolorizing carbon was added in a filtrate and the solution was boiled for 20 min. The charcoal was filtered and evaporated. The residue was recrystallized from ethanol to give I (2.32 g, 40%) as light-yellow prism crystal. mp 216-218°C. UV: $\lambda_{max}^{H_2O}$ 268 mμ (ϵ = 15,800).

Analysis: Calculated for $C_7H_8N_5OBr$: C, 32.57; H, 3.17; N, 27.13. Found: C, 32.24; H, 3.54; N, 26.82.

9-(2'-Dihydrogenphosphatoethyl)-8-bromoadenine-(8-Br-9-AP')(II)

Compound I (2 g, 7.4 mmoles) was suspended in trimethyl phosphate (20 ml) and cooled at 0°C. To this suspension phosphorus oxychloride (1.2 g, 7.8 mmoles) was added and stirred for 7 hr at 0°C. At the end of the reaction the suspension became clear. Water was then added, and the mixture was stirred for 1 hr. After the evaporation of water, a mixture of 5 ml ethanol and 30 ml ether was added. The white precipitate was recrystallized from ethanol to give an analytical pure product. The yield was 0.8 g (35%), mp 232°C (decomp), UV: $\lambda_{max}^{H_2O}$ 268 mμ (ϵ = 15,000).

Analysis: Calculated for $C_7H_9N_5O_4PBr$: C, 24.86; H, 2.68; N, 20.72. Found: C, 25.31; H, 2.69; N, 21.18

9-(2'-Hydroxyethyl)-6-methylmerucaptopurine (6-SCH_2-AM) (III)

Dimethylformamide solution (270 ml) containing 6-methylmerucaptopurine (6.8 g, 41.2 mmoles), ethylene carbonate (4.7 g, 42 mmoles), and a trace of sodium hydroxide was refluxed for 6 hr. The reaction mixture was cooled at room temperature, filtered, and evaporated to dryness. The residue was recrystallized from ethanol to give III (2.8 g, 32.4%) as colorless prism crystals, mp 186-187° C, UV: $\lambda_{max}^{H_2O}$ 287 mμ (ϵ = 16,200), 292 mμ (ϵ = 16,200).

Analysis: Calculated for $C_8H_{10}N_4OS$: C, 45.70; H, 4.79; N, 26.65. Found: C, 45.61; H, 4.62; N, 26.30

9-(2'-Dihydrogenphosphatoethyl)-6-methylmerucaptopurine-(6-SCH_2-AP') (IV)

Compound III (2.8 g, 13.3 mmoles) was treated with phosphorus oxychloride (2.0 g, 13.5 mmoles) in trimethyl phosphate (30 ml) as in the case of II. Water was then added to the reaction mixture, and the mixture stirred for an additional hour. After the evaporation of water, a mixture of ethanol-ether (2:8) was added. The white suspension was kept overnight. This white precipitate was recrystallized from ethanol-ether to give IV (1.16 g, 30%) as colorless prism crystals, mp 217-219°C, UV: $\lambda_{max}^{H_2O}$ 287 mμ (ϵ = 17,500), 292 mμ (ϵ = 17,500).

Analysis: Calculated for $C_8H_{11}N_4O_4PS$: C, 33.1; H, 3.83; N, 19.70. Found: C, 33.42; H, 3.91; N, 18.96.

9-(2',3'-Dihydroxypropyl)-8-bromoadenine (8-Br-9-AD) (V)

V was prepared in the same manner as described for I. 9-(2',3'-Dihydroxypropyl)-adenine (2 g, 9.62 mmoles) was treated with bromine (4 ml) in 10% aqueous disodium hydrogen phosphate and p-dioxane. V (1.25 g, 45%) was recrystallized from ethanol to give colorless prism crystals, mp 210-211° C, UV: $\lambda_{max}^{H_2O}$ 264 mμ (ϵ = 15,800).

Analysis: Calculated for $C_8H_{10}N_5O_2Br$: C, 33.35; H, 3.50; N, 24.31. Found: C, 32.35; H, 3.29; N, 23.90

9-(2',3'-Dihydroxypropyl)-8-bromoadenine-3'-phosphate (8-Br-9-AP) (VI)

VI was prepared in the same manner as described for II. V (1.2 g, 4.17 mmoles) was treated with phosphorus oxychloride (0.68 g, 4.42

mmoles) in trimethyl phosphate (15 ml). The white precipitate was recrystallized from ethanol to give VI (0.6 g, 41%) as colorless needle crystals, mp 219-221°C, UV $\lambda_{max}^{H_2O}$ 264 mμ (ϵ = 15,200).

Analysis: Calculated for $C_9H_{11}N_5O_5PBr$: C, 26.83; H, 2.81; N, 19.56. Found: C, 26.21; H, 2.69; N, 19.21.

Condensation Polymerization of VI in the Presence of DCC

After dissolving IV (0.5 g) in boiling DMF (8 ml), DCC (5.2 g) was added and refluxed for 10 hr. The reaction mixture was cooled and poured into a large amount of water and the solution was allowed to stand at room temperature overnight. After the dicyclohexylurea was completely removed, the filtrate was concentrated under reduced pressure. The residue thus obtained was dissolved in a small amount of water and the solution was poured into a 7:3 mixture (50 ml) of ether and ethanol. The precipitated oligomer was purified by reprecipitation from water/ether-ethanol (7:3) and through a Sephadex G-25 column using water as a solvent (Oligomer 1).

Condensation Polymerization of VI in the Presence of Imidazole

VI (2 mmoles) was added to a DMF (10 ml) solution of imidazole (1 mmole). The mixture was refluxed for 30 min. The reaction mixture was poured into a water-pyridine mixture. The solution was evaporated under reduced pressure. The residue was fractionated by a column packed with Sephadex G-25 using water as solvent (Oligomer 2).

Condensation Polymerization of VI in the Presence of Triethylamine-Hydrogen Chloride

The precedure was the same as that described in a previous paper [7].

Gel Filtration of the Oligomers

The procedure was described in a previous paper [7]. UV spectra of each fraction were measured at 264 mμ. With Sephadex G-25 (57 × 2.0 cm, 3.8 ml/45 min), 40-45% of Oligomer 1 was found in Tubes 13 to 35 and Trypan Red (molecular weight 1000) in Tubes 32 to 50. The gel-filtration pattern of Oligomer 2 is shown in Fig. 1.

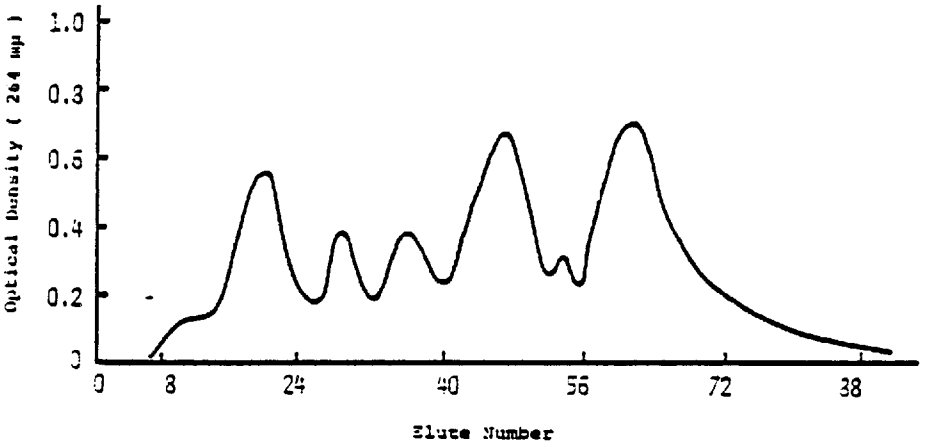


FIG. 1. Gel filtration pattern of oligo-(8-Br-9-AP) (Oligomer 2). Column: Sephadex G-25. Solvent: water. Sample volume: 0.4 ml. Elute rate: 4 ml/47 min. Column length: 57 × 2.2 cm.

Condensation of II of IV with PVA

The procedure was described in a previous paper [8].

Oligomer-Polymer Interaction

A hypochromicity was calculated according to a previous paper [6].

Synthesis of 9-(2'-Hydroxyethyl)-adenine-N-oxide

9-(2'-Hydroxyethyl)-adenine (2 g) was dissolved in acetic acid (15 ml) and 30% hydrogen peroxide (9 ml) was added to the solution. The mixture was allowed to stand at room temperature for 4 days. The reaction mixture was stirred with 10% palladium-charcoal (2 g) to decompose excess hydrogen peroxide. The solution was filtered and the solvents were evaporated to dryness. The residue was recrystallized from water to give colorless needle crystal. Yield 78%, mp 142-143°C (decomp).

Analysis: Calculated for $C_7H_9N_5O_2$ (195.18); C, 43.08; H, 4.65; N, 35.88. Found: C, 43.12; H, 4.62; N, 36.01.

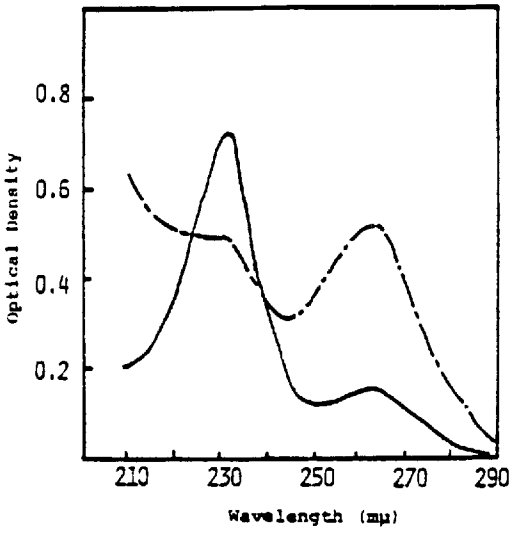


FIG. 2. UV absorption spectra of 9-(2'-hydroxyethyl)-adenine-N-oxide (—) and PVA—adenine-N-oxide (-·).

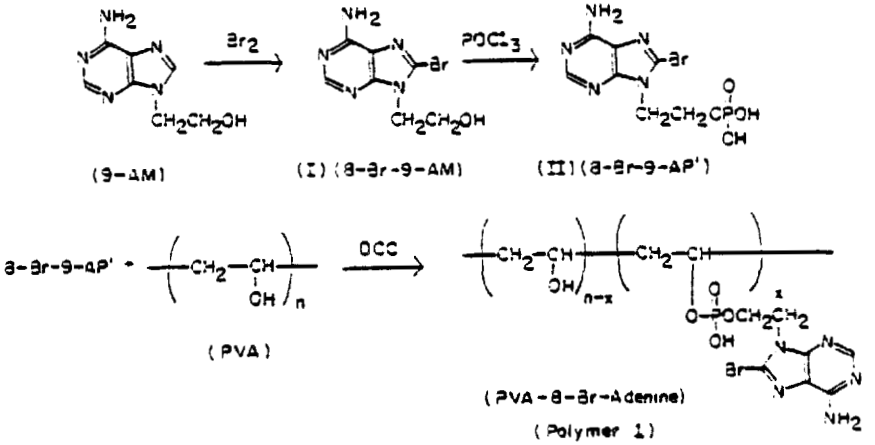
Oxidation of PVA-Adenine to PVA-Adenine-N-oxide

PVA-adenine (0.5 g) was dissolved in hot acetic acid (4 ml). After the solution was cooled, 30% hydrogen peroxide (3 ml) was added and the solution was allowed to stand at room temperature for 5 days. Excess hydrogen peroxide was decomposed by stirring with 10% palladium-charcoal (0.8 g), the solution was filtered, and the filtrate was evaporated to dryness. The residual polymer (PVA-adenine-N-oxide) was purified by reprecipitation from water-methanol. Comparing the optical density of the polymer with that of 9-(2'-hydroxyethyl)—adenine-N-oxide (Fig. 2), the content of N-oxide was determined to be about 20% based on the amount of adenine residue used (PVA-adenine).

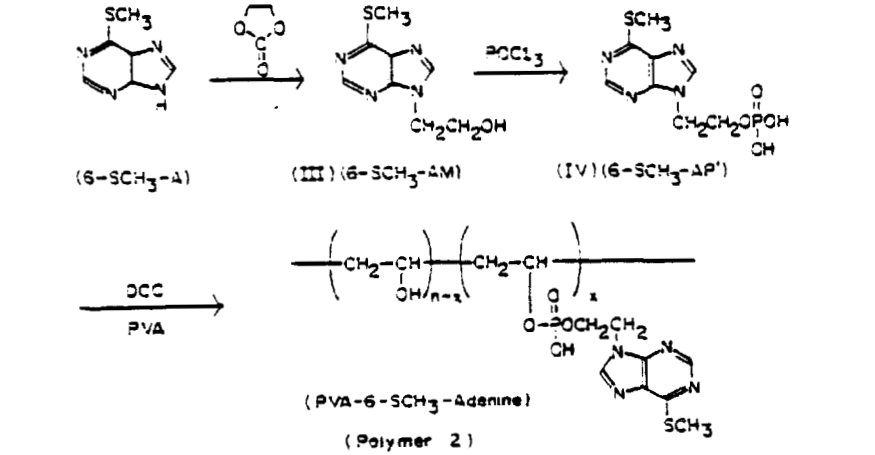
Figure 2 shows the oxidation of PVA-adenine. The content of N-oxide was determined to be about 20% based on the amount of adenine in PVA-adenine.

RESULTS AND DISCUSSION

The reaction paths are summarized in Schemes 1-6. Compound I was synthesized by the reaction of 9-(2'-hydroxyethyl)-adenine



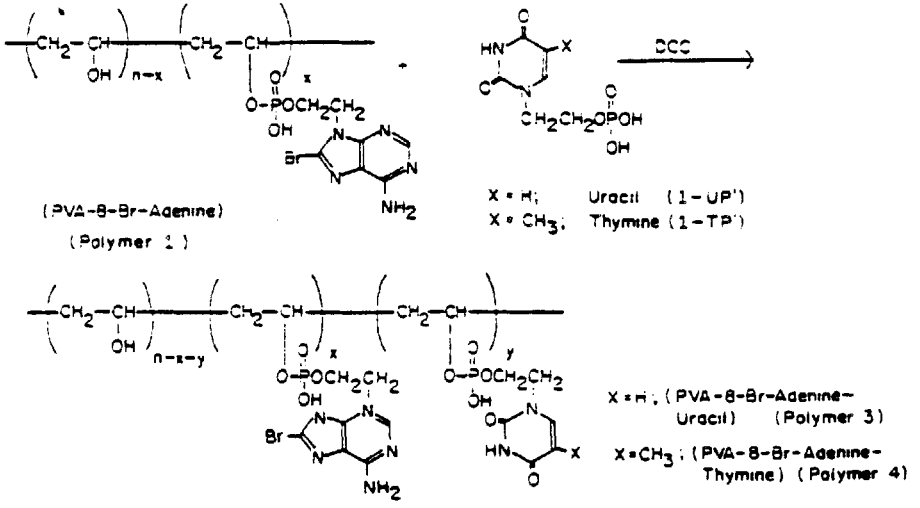
Scheme 1



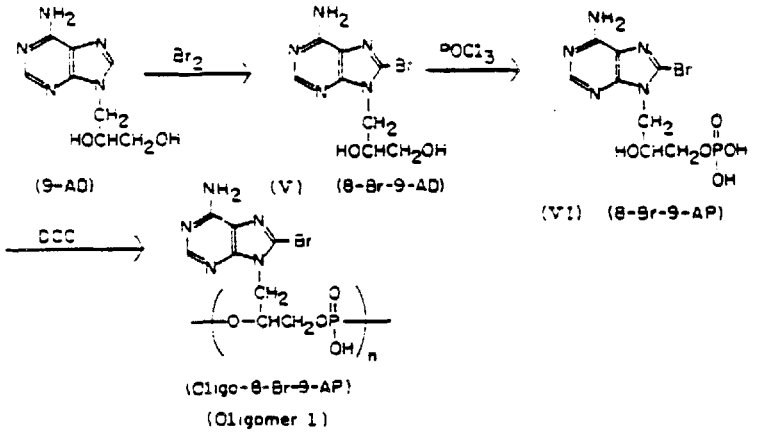
Scheme 2

with bromine in a mixture of disodium hydrogen phosphate and p-dioxane. The position of substitution of the bromine atom was confirmed by the absence of the characteristic sharp NMR absorption peak at 8.36 ppm due to 8-*H* adenine. Compound VI was also synthesized and recognized in a similar manner. III was synthesized by the system DMF/ethylene carbonate. The phosphorylation of I and III was carried out using phosphorus oxychloride in trimethyl phosphate as previously described.

Yoshikawa et al. reported the selective phosphorylation of nucleoside at position 5' using phosphorus oxychloride in trialkyl phosphate [9].



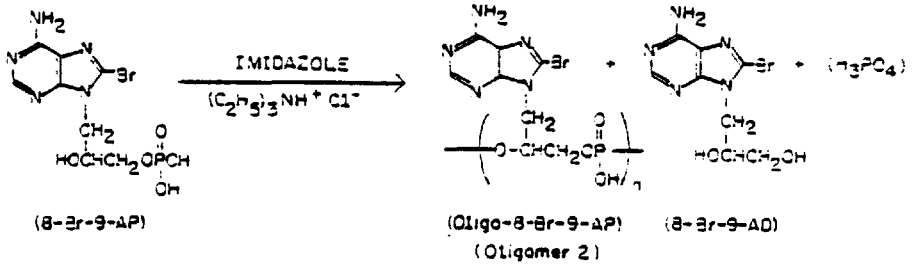
Scheme 3



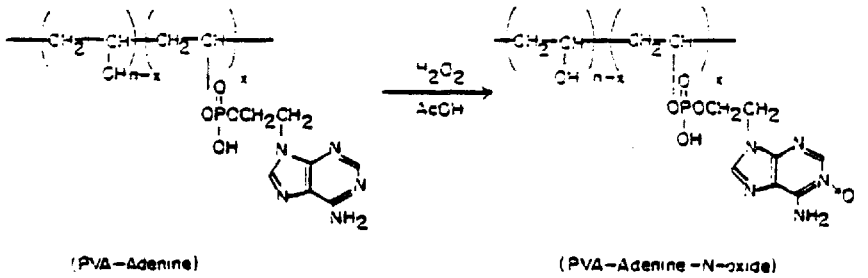
Scheme 4

The authors reported the selective phosphorylation at primary hydroxy group of nucleoside analogs, such as 9-(2',3'-dihydroxypropyl)-adenine and 7-(2',3'-dihydroxypropyl) theophylline, using phosphorus oxychloride in trimethyl phosphate [6]. All the compounds were identified by elementary analysis together with IR, NMR, and UV spectra.

PVA with adenine derivatives as pendant groups was synthesized



Scheme 5



Scheme 6

through condensation using DCC as a dehydrating agent in refluxing DMF. The polymers obtained were a white hygroscopic powder and very soluble in water, but insoluble in the usual organic solvents such as benzene, ether, and alcohols. The contents of nucleic acid bases incorporated into PVA were calculated from nitrogen content elementary analysis, and the optical density of the UV absorption corresponding to the bases.

IR spectra of polymers showed strong absorption at 1300-1000 cm^{-1} which depend on phosphoric ester bond. On the other hand, the UV spectra of polymers agreed with those of II and IV used as starting materials, respectively. The higher incorporation rate of II into PVA than expected depended on the good solubility of II in the solvent mixture. The condensation of Polymer 1 with 1-(2'-dihydrogenphosphato)-ethyl uracil and 1-(2'-dihydrogenphosphato)-ethyl thymine was also carried out using DCC as a dehydrating agent in a refluxing mixture of DMF-water (9:1). The polymers obtained were also hygroscopic white powder. The results of incorporation of pyrimidine derivatives into Polymer 1 are summarized in Table 2.

The condensation polymerization of VI was carried out using DCC, imidazole, or triethylamine-hydrochloric acid in refluxing DMF.

The oligomers obtained were separated and purified by reprecipitation with water/(ethanol-ether) [2/(2:8)] and by elution through a

TABLE 1. Condensation with PVA

Reactant	Degree of polymerization of PVA	Time (hr)	Yield (%)	Content of the reactant (%)	UV spectra $\lambda_{max}^{H_2O}$ (mμ)
II	500	24	50	32 (25)[a]	264
II	8	24	38	20 (17)	264
IV	500	20	61	38 (34)	287, 292
IV	8	20	40	24 (20)	287, 292

[a]Values in parentheses were calculated from the intensity of UV absorption.

TABLE 2. Condensation of Polymer I with 1-(2'-Dihydrogenphosphato)-ether Uracil or 1-(2'-Dihydrogenphosphato)-ethyl Thymine

Nucleic acid base	Time (hr)	Yield (%)	Content of the nucleic acid bases (%)	UV spectra $\lambda_{max}^{H_2O}$ (mμ)
Thymine	24	59	40 43	270
Uracil	24	65	45 42	265

TABLE 3. Results of Condensation Polymerization of VI

Dehydrating agent or catalyst	Time (hr)	Solvent	Yield (%)	UV spectra $\lambda_{max}^{H_2O}$ (mμ)
DCC	24	DMF	42	264
Imidazole	0.5	DMF	15	264
Imidazole	0.5	Pyridine	0	-
Triethylamine-hydrochloric acid	0.5	DMF	28	264

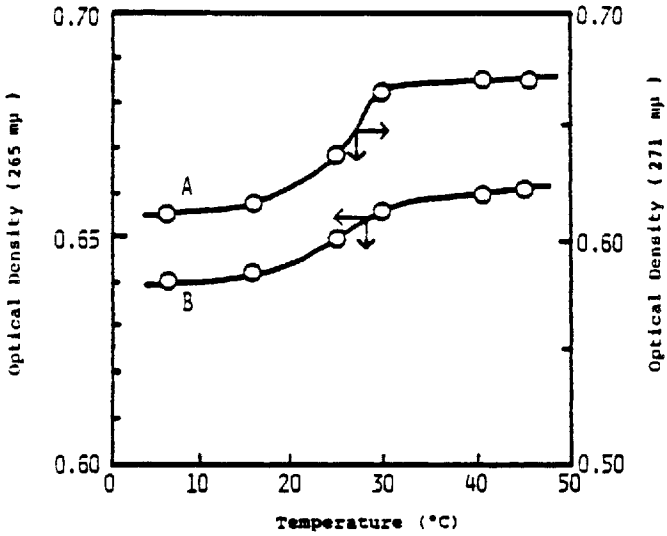


FIG. 3. Relationship between temperature and the optical density of PVA-8-Br-adenine-thymine and PVA-8-Br-adenine-uracil in 0.1 M Na_2HPO_4 solution. A: PVA-8-Br-adenine-thymine. B: PVA-8-Br-adenine-uracil.

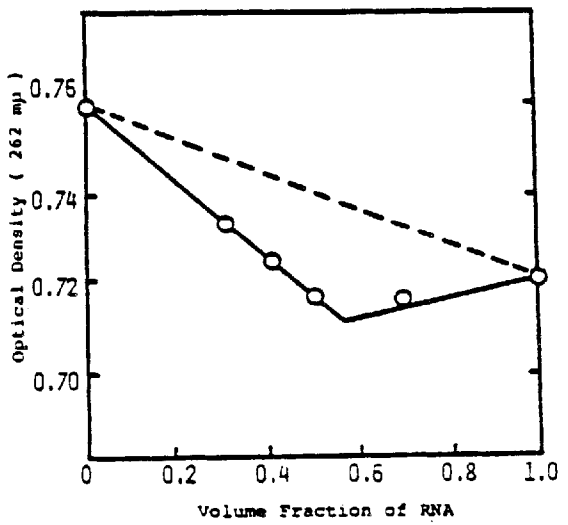


FIG. 4. Comparison of observed (full line) and Beer's law absorptions (dashed line) for the mixture of PVA-8-Br-adenine and RNA in 0.1 M Na_2HPO_4 solution.

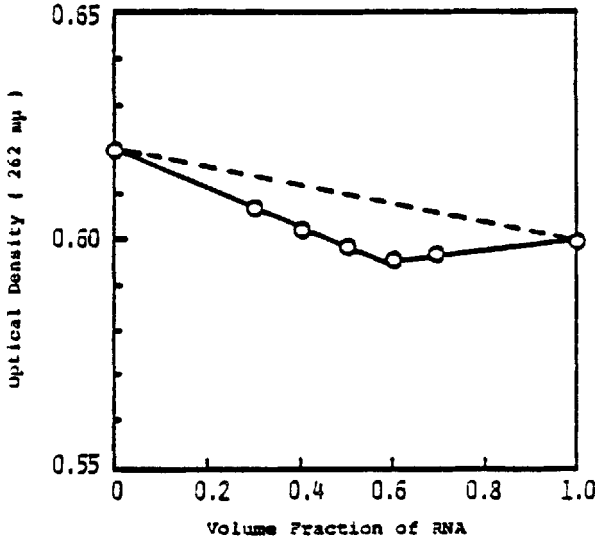


FIG. 5. Comparison of observed (full line) and Beer's law absorptions (dashed line) for the mixture of oligo-8-Br-9-AP and RNA in 0.1 M Na_2HPO_4 solution.

Sephadex G-25 column. Compound V was also separated from the reaction mixture, thus showing the dephosphorylation of VI. On the other hand, when DCC was used as a dehydrating agent, dephosphorylation was not observed. The results are shown in Table 3.

The molecular weight of oligomers obtained was determined using the Sephadex G-25 column. About 40-60% of the oligomers obtained were found to have a molecular weight of more than 1000. Figure 1 show the gel-filtration pattern of oligomer obtained in the presence of imidazole as a catalyst.

Figure 3 shows the relationship between temperature and optical density of polymer 3 (PVA-8-Br-adenine-uracil) and 4 (PVA-8-Br-adenine-thymine) in 0.1 M Na_2HPO_4 solution. This illustrates the increase of optical density and thus the decrease of hypochromicity with temperature. This result suggests the complex formation between 8-bromoadenine and thymine or uracil as side groups. The hypochromicity of the mixture of Polymer 1 or Oligomer 1 and yeast RNA in 0.1 M Na_2HPO_4 solution was about 4 and 3% measured at 0°C, respectively. Figures 4 and 5 show the comparison of observed and Beer's law absorption of the mixed system.

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