

NOTES

Polymeric Phospholipid Analogues XIII.[†] Synthesis and Properties of Vinyl Polymers Containing Phosphatidyl Choline Groups

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During the past ten years, a considerable amount of effort has been directed toward the synthesis and properties of polymers containing phosphatidylethanolamine,^{1,2} and choline³⁻⁵ analogues in the side chains and phosphatidylcholine analogues in the main chains.⁶ In the 14th paper of this series,³ we reported the monomer synthesis of 2-(methacryloyloxy)ethyl 2-(trimethylammonio)ethyl phosphate (IV), following the method of Thoung and Chabrier.⁷ On the other hand, it appeared to be of interest to synthesize the same monomer by another route, that is, by a modification of Bear *et al.*⁸ The present note is mainly concerned with the synthesis and polymerization of IV and the properties of the resultant polymer.

EXPERIMENTAL

Reagents

Trimethylamine hydrochloride and silver carbonate were commercially obtained and used without further purification. 2-Hydroxyethyl methacrylate was commercially obtained and distilled under reduced pressure in an atmosphere of nitrogen before use. AIBN was purified by two recrystallizations

from a ethanol/water (volume ratio 1:1) mixture. Trimethylamine was prepared by reaction of trimethylamine hydrochloride with 40% sodium hydroxide by the procedure of Adams and Brown.¹⁰ 2-Bromoethyl dichlorophosphate, bp 84°C (20 mmHg), was prepared by reaction of 2-bromoethanol with phosphorus oxychloride according to the method of Renshaw and Hopkins.⁷ 2-(Methacryloyloxy)ethyl 2-bromoethyl hydrogen phosphate (I), mp 52°C, was obtained by our method described previously.³ Chloroform and carbon tetrachloride were dried by distillation from diphosphorus pentoxide. Diethyl ether was distilled lithium aluminium hydride to ensure dryness. Amberlite IR-120 (B) of cation exchange resin was acidified with aqueous hydrochloric acid and washed with pure water several times before use. All other reagents were commercially supplied and purified by usual methods.

2-(Methacryloyloxy)ethyl 2-(trimethylammonio)ethyl Phosphate (IV)

Into a 300 cm³ glass pressure bottle (Top Model E 1435, Type A) were placed 13.0 g (0.041 mol) of I and 100 ml of dry methanol, 18.3 g (0.310 mol) of trimethylamine, and 80 ml

[†] For Part XII of this series, see ref 9.

of dry methanol were rapidly added to the solution. The pressure bottle was closed and then shaken in a thermostat maintained at 40°C for 3 h. After the bottle was opened, the solvent was evaporated *in vacuo* in a stream of nitrogen to give ammonium salt (II). To the product II, 70 ml of methanol were added and then 15.2 g (0.055 mol) of silver carbonate and 2 g of water were added to the solution. After the mixture was vigorously shaken for 6 min, it was allowed to stand overnight. Then, it was filtered with a tight filter paper. The filtrate was evaporated *in vacuo* with a stream of nitrogen for 2 h to give 10.0 g (65.5%) of the corresponding ammonium salt (III) as a colorless viscous liquid: ¹H NMR (D₂O): δ 1.92 (3H, s, -CH₃), 2.87 (9H, s, -CH₃), 3.16 (9H, s, -CH₃), 3.60–3.96 (2H, m, -CH₂-), 4.10–4.60 (6H, m, -CH₂-), 5.70 (1H, m, -CH=), 6.20 ppm (1H, s, -CH=).

30.0 g of product III, 38 ml of methanol, 5 ml of water, and a small Teflon covered magnetic stirrer bar were placed in a 200 ml beaker. To the stirred solution, 35 ml of cation exchange resin (Amberlite IR-120 (B)) were added. After the mixture was stirred for 2 h at 20°C, it was filtered. The filtrate was extracted with 50 ml of chloroform. The chloroform layer was filtered with a filter paper and evaporated *in vacuo* in a stream of nitrogen to give 28.8 g (97.3%) of IV as a colorless viscous liquid. The ¹H NMR (D₂O) and IR (neat) spectra of the product obtained here were, respectively, identical with those of the material obtained previously.³

Anal. Calcd for C₁₁H₂₂NO₆P·2H₂O: C, 39.88%; H, 7.85%; N, 4.23%. Found: C, 39.47%; H, 7.53%; N, 3.86%.

Polymerization of IV

The polymerization of IV was carried out in methanol, using AIBN as initiator. The solution of 5.07 g (16.0 mmol) of IV, 51 mg (0.31 mmol) of initiator, and 15 ml of methanol were mixed in a glass tube. The tube was flushed three times with nitrogen, sealed *in*

vacuo and shaken at 60°C for 9 h. After the polymerization, the content of the tube was poured into an excess of dry diethyl ether to isolate the polymer. The resulting polymer was purified by reprecipitation from methanol solution with diethyl ether to give a pure polymer as a white powder melting at 50°C. Yield: 4.83 g (95.2%). Its IR spectrum (KBr) showed the disappearance of the absorption band at 1630 cm⁻¹ due to the vinyl double bond in the monomer, but otherwise, the spectrum was unchanged.

Hydrolysis of Poly(IV)

1.5 g of poly(IV) were dissolved in 20 ml of concd. sulfuric acid with constant stirring at room temperature. The reaction mixture became homogenous after several hours. By pouring the resulting solution into an excess of ice water, the polymer was precipitated and then purified by repeated precipitation from methanol solution with ethyl acetate. Yield, 0.12 g (30%). Identified as poly(methacrylic acid) by comparison of the IR spectrum with that of authentic poly(methacrylic acid).

Intrinsic viscosity of the poly(methacrylic acid) sample was measured in 2 N sodium nitrate at 25°C, using a Ubbelohde viscometer. From the intrinsic viscosity, the number-average molecular weight of the polymer was calculated as 26800 ($P_n=315$), using the following relationship of Arnold and Caplan.¹¹

$$[\eta] = 4.49 \cdot 10^{-4} M^{0.65}$$

Viscosity Measurement

The measurements were carried out using a Ubbelohde type viscometer.

pH Measurements

The measurements were carried out by a Hitachi-Horiba Model F-8L pH meter.

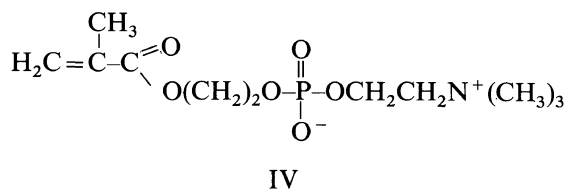
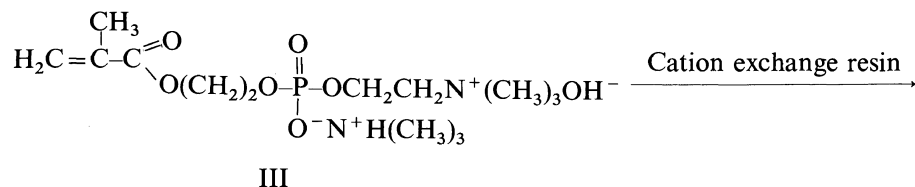
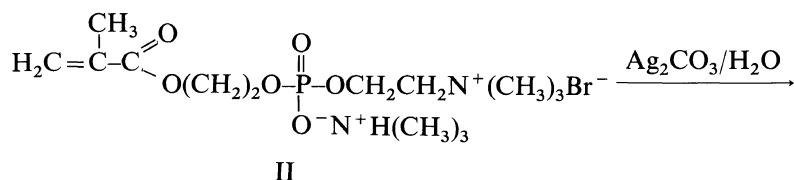
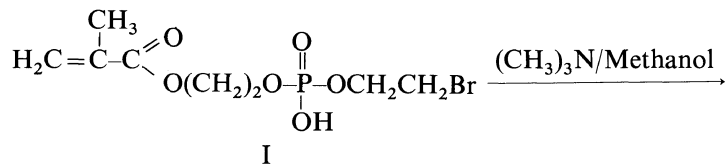
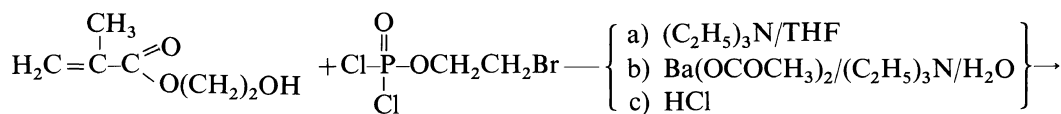
Spectroscopic Measurements

The ¹H NMR and IR measurements were carried out on 60 MHz Hitachi-Perkin Elmer

Model R-20 NMR spectrometer and Jasco Model A202 spectrometer.

RESULTS AND DISCUSSION

The monomer IV was synthesized according to the following scheme:



The starting material for the synthesis was 2-bromoethyl dichlorophosphate obtained by the reaction of 2-bromoethanol with phosphorus oxychloride in carbon tetrachloride, according to the procedure described by Renshaw and Hopkins.⁷ The reaction of 2-bromoethyl dichlorophosphate with 2-hydroxyethyl methacrylate in THF in the presence of triethylamine at $-20-0^\circ\text{C}$ for 2 h, followed by successive treatment with a water solution of barium acetate and triethylamine

and then treatment with aqueous hydrochloric acid afforded 2-methacryloyloxyethyl 2-bromoethyl hydrogen phosphate (I) in 56% yield. The reaction of I with trimethylamine in methanol in a pressure bottle at 40°C for 15 h with shaking gave di(trimethylammonio)salt (II) as a viscous liquid. By the displacement of bromide ion of II by hydroxonium ion, it was converted to the corresponding di(trimethylammonio)salt (III) by the action of silver carbonate in water at

room temperature for 15 h; its identification was made using ^1H NMR (see EXPERIMENTAL). Subsequent treatment of III with a cation exchange resin (Amberlite IR-120 (B)) in water afforded the desired 2-(methacryloyloxy)ethyl 2-(trimethylammonio)ethyl phosphate (IV), which was obtained as a viscous liquid in the present synthesis because the product contained a small amount of water in spite of drying *in vacuo*, while the product obtained by the method which was described previously,³ was a white crystalline solid; mp 15°C . The ^1H NMR (D_2O) and IR (neat) spectra of the product obtained here were, respectively, identical with those of the material obtained previously.³ It was hygroscopic and its water solution, acidic.

IV was polymerized in methanol at 60°C for 8 h with 2,2'-azoisobutyronitrile (AIBN) to give in 60% yield a white polymer whose IR spectrum showed the disappearance of the absorption band at 1630 cm^{-1} due to the vinyl double bond in the monomer. In the ^1H NMR spectrum of the polymer, the absorption peaks at $\delta = 5.80$ and 6.17 ppm due to the terminal vinyl protons disappeared, whereas a broad absorption peak at $\delta = 0.50\text{--}0.40\text{ ppm}$ due to the methyl and methylene protons of the polymer appeared. The poly(IV) was soluble in water, methanol, and ethanol, but almost insoluble in acetone as well as diethyl ether. It was hydroscopic and its water solution, acidic.

In order to determine the molecular weight of the polymer, it was converted to the corresponding poly(methacrylic acid) by treating it with conc. sulfuric acid at room temperature for 2 days and pouring the resulting solution into an excess of ice water. This procedure resulted in complete hydrolysis, which was confirmed by the IR spectrum of the polymer formed. From the measurement of the intrinsic viscosity of the polymer in 2 N sodium nitrate solution, the number-average degree of polymerization (P_n) of the polymer was evaluated as 315 by using the equation of

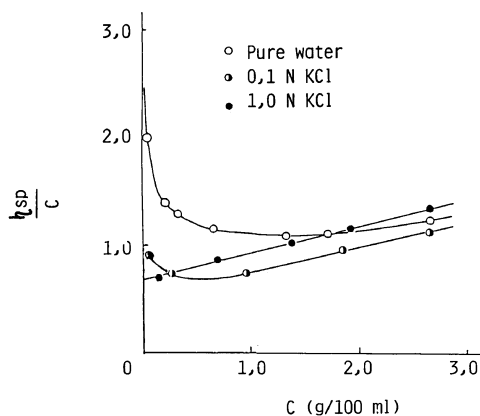


Figure 1. Reduced viscosity of poly(IV) in aqueous solutions.

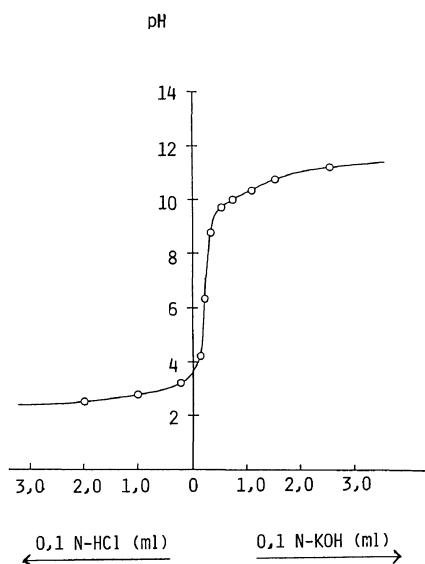


Figure 2. Titration curve of poly(IV).

Arnold and Caplan.¹¹

In order to check whether poly(IV) is a polyelectrolyte, we examined the viscosity behaviour of a water solution of poly(IV). The viscosity of the polymer was measured at 30°C in water with and without the addition of potassium chloride. Figure 1 shows the plots of the reduced viscosity, η_{sp}/C , vs. C for poly(IV) ($P_n = 315$) in aqueous solution, where C is expressed in grams per 100 ml.

In pure water, η_{sp}/C has a tendency to in-

crease rapidly upon dilution, whereas the addition the potassium chloride eliminates it. The general trend of these plots agrees with that reported by a number of investigators for various polyelectrolytes.^{12,13}

As mentioned above, the water solution of poly(IV) was acidic. In order to check this point, the titration curve was obtained by addition of 0.1 N hydrochloric acid or 0.1 N potassium hydroxide to 50 ml of 0.053 g/100 ml of pure water solution of poly(IV). The curve is shown in Figure 2.

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