73-87.
19. D. T. Cromer, *Acta. Crystallogr.*, 18, 17 (1965).
20. Reference 18, pp. 149-150.
21. D. S. Kim, S. H. Song, and Y. Kim, *Bull. Korean Chem. Soc.*, 9, 303 (1988).
22. Y. Kim and K. Seff, *J. Phys. Chem.*, 82, 1307 (1978).
23. "Handbook of Chemistry and Physics" 67th Ed. pF-159, 1986/1987.
24. Y. Kim, S. H. Song, D. S. Kim, and D. K. Park, *J. Korean Chem. Soc.*, 33, 18 (1989).
25. C. Ronay and K. Seff, *J. Phys. Chem.*, 9, 1965 (1985).

Synthesis and Biological Activities of the Alternating Copolymers Containing Cyclic Ether Rings along with Carboxyl or Hydroxyl Groups on Their Backbones

Man Jung Han*, Shin Duk Kang, and Won Young Lee†

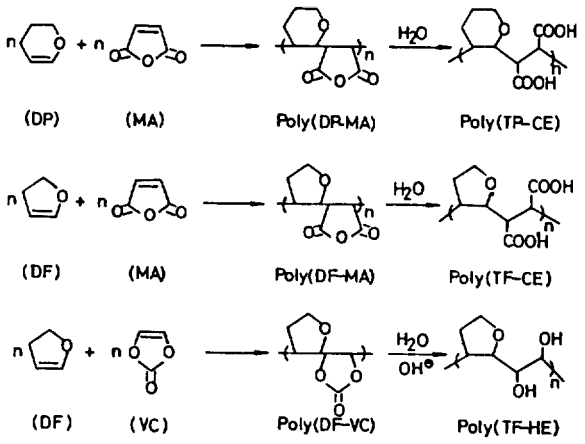
Department of Applied Chemistry, Ajou University, Suwon 440 - 749
†Department of Microbiology, School of Medicine, Yonsei University, Seoul 120 - 749. *Received January 15, 1989*

The alternating copolymers of dihydropyran(DP)-maleic anhydride(MA), dihydrofuran(DF)-MA and DF-vinylene carbonate(VC) were prepared by free radical copolymerization of DP or DF with MA or VC. The reactivity ratios for poly(DF-VC) were found to be less than unity (0.05, 0.04) and its alternating sequences were obtained by feeding an equimolar amount of the comonomers at the onset of copolymerizations. The copolymers were hydrolyzed to give poly(TP-CE), poly(TF-CE) and poly(TF-HE), whose cytotoxicities against normal and tumor cells (3LL, B16) were measured *in vitro*.

Introduction

Several synthetic polymers are known to exhibit a broad range of biological activities against tumor, virus and fungi.[1-5] It was well documented that polymers with high density of carboxylic acid functionalities as hydrophilic groups along the polymer chain have exhibited high antitumor activities.[6] Recently we have demonstrated that the tetrahydropyran(THP) and/or tetrahydrofuran rings as hydrophobic groups on the polymer chain also play a significant role in the antitumor activity of the polymer.[7-9] It is therefore of interest whether the copolymers shown in Scheme 1, which are containing cyclic ether rings as hydrophobic groups together with carboxyl or hydroxyl as hydrophilic groups along the polymer chain, would exhibit the relevant biological activities.

In this paper we report the synthesis and biological activities of poly[(tetrahydropyran-2,3-diyl)(1,2-dicarboxyethylene)] [poly(TP-CE)], poly[(tetrahydrofuran-2,3-diyl)(1,2-dicarboxyethylene)] [poly(TF-CE)] and poly[(tetrahydrofuran-2,3-diyl)(1,2-dihydroxyethylene)] [poly(TF-HE)]. The outline of syntheses for the alternating copolymers obtained by free radical copolymerizations are illustrated in Scheme 1.



Scheme 1

Experimental

Purification of chemicals; 3,4-dihydro-2H-pyran (Bp: 86 °C), 2,3-dihydrofuran (Bp: 55 °C) and vinylene carbonate (Bp_{15}: 47 °C) were distilled before use. AIBN was recrystalized from methanol. Maleic anhydride (MA) was sublimed under vacuum. Acetone was refluxed over P_2O_5 and distilled under N_2 before use.

Copolymerization. Calculated amount of monomers, solvent and initiator (AIBN) were charged in the copolymerization tube, which were immersed in a Dewar flask containing dry ice and acetone. Following conventional freeze-thaw treatment under N_2, the tubes were sealed and placed in a temperature controlled bath for a fixed period of time. The solutions of poly(DP-MA) and poly(DF-MA) in acetone, and poly(DF-VC) in chloroform were precipitated in ether several times and dried in vacuo over P_2O_5 at 50 °C in a drying pistol.

Hydrolysis. poly(tetrahydropyran-2,3-diyl)(1,2-dicarboxyethylene)] [poly(TP-CE)] and poly[(tetrahydrofuran-2,3-diyl)(1,2-dicarboxyethylene)] [poly(TF-CE)]. Aqueous solutions (25 m*l*) of poly(DP-MA) (1g) or poly(DF-MA) (1g)

Table 1. Copolymerization Data and Molecular Weight of the Copolymer

Copolymer	Copolymerization condition						Copolymer	
	[DP] or [DF]	[MA] or [VC] (mol/ℓ)	[AIBN] ×10	time [hr]	temp. [°C]	solvent	yield [%]	Mn[a]
poly(DP-MA)	3.64	3.64	0.25	20	70	Acetone	65	2150
poly(DF-MA)	5	5	1	8	25	Acetone	86	3100
poly(DF-VC)	7.19	7.19	2.82	12	100	none	40	$[\eta] = 0.16^b$
DIVEMA	0.35	0.64	0.18	20	70	Acetone	80	5500

[a]Measured in acetone or THF by VPO. [b]Intrinsic viscosity in DMF. The solubility of poly(DF-VC) in the solvents for VPO is too low to measure its Mn.

were stirred for 1 hr under N_2 gas. After evaporation of the solvent, the residues were taken in methanol, then precipitated in ether, filtered and dried in vacuo over P_2O_5 at 50 °C in a drying pistol [yield; poly(TP-CE): 90%, poly (TF-CE): 87%].

Poly[tetrahydrofuran-2,3-diyl](1,2-dihydroxyethylene)] [poly(TF-HE)]. To 25 m*l* aq. solution containing poly (DF-VC) (1g) was added 40 m*l* aq. 0.1N-NaOH and refluxed for 4 hrs under N_2 gas. After acidification of the solution with dilute HCl to pH = 4 and evaporation of the solvent, the residue was taken in DMF and precipitated in ether. Poly (TF-HE) was filtered and dried in vacuo over P_2O_5 at 50 °C in a drying pistol. The characteristic peak for the carbonyl group at 1800 cm^{-1} in IR spectrum disappeared completely. (yield: 73%).

Measurements. ^1H nmr spectra were recorded on a Varian T-60 spectrometer. Chemical shifts were reported as δ units relative to Me_4Si as the internal standard. IR spectra were obtained with a Perkin-Elmer Model 283B spectrophotometer. Measurement of molecular weights was carried out in acetone at 45 °C with the aid of vapor pressure osmometer (Knauer Co.).

Results and Discussion

The copolymers poly(TP-CE) and poly(TF-CE) were obtained simply by hydrolyses of poly[(2,3-dihydro-2H-pyran)-alt-(maleic anhydride)] (poly(DP-MA)][10] and poly[(2,3-dihydrofuran)-alt-(maleic anhydride)] [poly(DF-MA)],[11] which were synthesized by radical copolymerizations of each comonomer pair and the copolymerization data were summarized in Table 1. The hydrolysis of the copolymers was accomplished by stirring the copolymer in water at room temp. for 1 hr.

Vinylene carbonate(VC) is known, in the presence of radical initiator, to homopolymerize[12] and to copolymerize with vinyl ether to form an alternating copolymer.[13] The copolymerization of VC with cycloalkenyl ether has not been reported.

The equimolar amount of 2,3-dihydrofuran (DF) (M_1) and VC (M_2) was copolymerized in bulk in the presence of AIBN as initiator (2 mole % of total monomers) at 100 °C for 12 hrs. The copolymer, poly[(2,3-dihydrofuran)-co-(vinylene carbonate)] [poly(DF-VC)] was isolated by precipitating the polymerization mixture in ether (yield: 40%). Poly(DF-VC) is soluble in polar solvent, such as DMSO, DMF and slightly soluble in acetone, chloroform, THF, benzene and insoluble in *n*-pentane, CCl_4, isopropanol, ethanol, methanol and H_2O. The structure of poly(DF-VC) was confirmed by ir, *e.g.* characteristic peaks for carbonyl group at 1800, for the ether group of THF ring at 1050-1100 and for the ether group of ethylene carbonate at 1170-1200 cm^{-1}

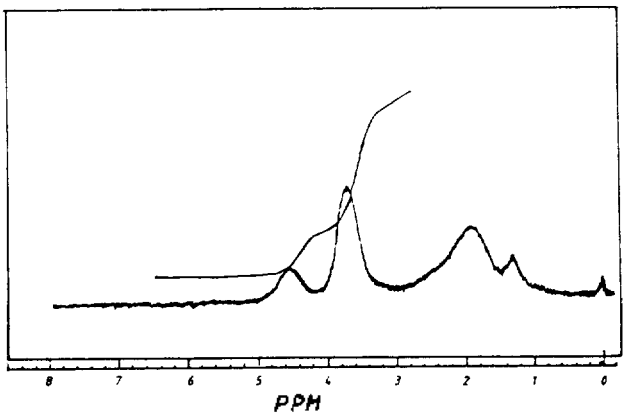


Figure 1. ^1H NMR spectrum of poly[(2,3-dihydropyran)-co-(maleic anhydride)] in DMSO-d_6.

The nmr spectrum of poly(DF-VC) in DMSO-d_6 is shown in Figure 1. The assignments were made for the peak at $\delta = 4.7$ as protons of ethylene carbonate, at 3.8 as 2,5-protons and at 2.0 as 3,4-protons of THF rings in the copolymer, according to the δ-values of protons in the relevant organic compounds at $\delta = 4.55$[14], 3.77 and 1.87[15], respectively, although slight downfield shifts have occurred by the formation of polymer. The composition of the copolymer can be determined from the integration values at $\delta = 4.7$ and 3.8, which were originated from ethylene carbonate and 2,5-protons of THF rings in the copolymer, respectively.

Determination of the reactivity ratios were performed by changing mole fraction of the comonomer pairs and measuring mole fractions of the comonomer components in the copolymer before a 10% conversion proceeds. By plotting the Fineman-Ross equation[16], the reactivity ratios, r_1 and r_2 are found to be 0.14 and 0.05 from the slope and intercept, respectively.

As the reactivity ratios are smaller than unity, an alternating copolymer is obtainable by controlling the feeding concentrations of the comonomers and the conversion of the copolymerization. In Figure 2 are plotted the feeding mole fractions of the comonomers as a function of mole fractions of monomers incorporated into the copolymers obtained by

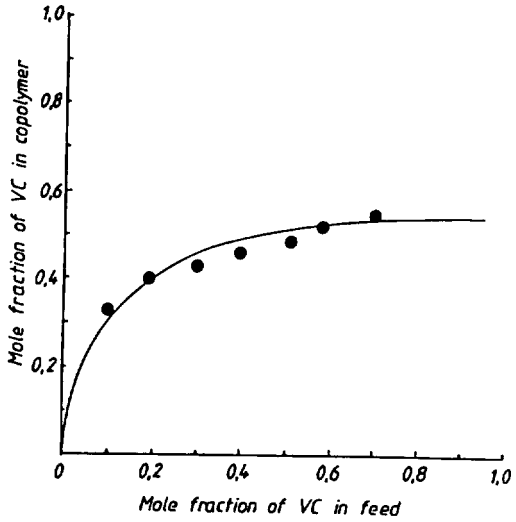


Figure 2. Vinylene Carbonate (VC) mole fractions incorporated into the copolymers as a function of VC feeding mole fractions in the copolymerization of VC with 2,3-dihydrofuran in bulk at 100 °C for 12 hours.

Table 2. Biological Activities of the Copolymers Compared with DIVEMA

Copolymer	ID_{50}[a] (μg/ml)		
	3LL[b]	B16[c]	MEF[d]
poly(TP-CE)	1289	1518	80
poly(TF-CE)	863	610	105
poly(TF-HE)	908	663	1042
DIVEMA	2504	1511	765

[a]ID_{50} was defined as the concentration which reduced absorbance by 50% of control untreated well in the MTT assay. All results represent the average of 8 wells. [b]Lewis lung carcinoma originated from C57BL/6 mouse. [c]Malignant melanoma originated from C57BL/6 mouse. [d]Secondary mouse embryo fibroblast.

bulk copolymerization of 30–40% conversion at 100 °C for 12 hrs. It is derivable from Figure 2 that an alternating copolymer can be obtained by feeding an equimolar amount of the comonomers at the onset of the copolymerization under the same condition as those given above.

Poly(DF-VC) were hydrolyzed by refluxing the polymer in aq. 0.1N NaOH. This reaction was monitored by IR spectra where the characteristic peak for the carbonyl group at 1800 cm^{-1} completely disappeared at the end of the reaction. After acidification of the solution to PH = 4 with dil. HCl and evaporation of the solvent, poly(TF-HE) was obtained by precipitating the DMF solution of the residue in ether.

For the purpose of bioactivity comparison, an alternating copolymers of divinyl ether-maleic anhydride (1:2) (DIVEMA)[17] having weight-average molecular weight (Mw) of 5,500 with polydispersity of 1.3 was synthesized for the authentic sample in this study, since DIVEMA of Mw range of 1,000–10,000 has been shown to exhibit high antitumor and antiviral activities while at the same time low toxicity.[1]

Biological activities of the copolymers were measured by MTT method[9] and ID_{50}-values are given in Table 2. ID_{50} values of poly(TP-CE) and poly(TF-CE) for normal mouse embryo fibroblast are 80 and 105, respectively, which are relatively low compared with those of other polymers. There are, however, no striking differences in ID_{50} values for neoplastic cells between DIVEMA and the polymers synthesized. The anticancer effects of DIVEMA *in vivo* have been speculated to be mediated via a macrophage system[18-21] which can not be reflected by simple direct cytotoxicity *in vitro*, as shown by this experiment. Studies on the anticancer effect of the copolymers synthesized in this study *in vivo* are currently in progress.

Acknowledgement. This work was supported by a grant from Korea Research Foundation.

References

1. R. M. Ottenbrite, K. Kuus, and A. M. Kaplan, Polymers in Medicine, E. Chiellini and P. Giusti, Eds.: Plenum Press, New York, p.3 (1983).
2. H. G. Batz, *Advances Polym. Sci.*, **23**, 25 (1977).
3. R. M. Ottenbrite, W. Regelson, A. Kaplan, R. Carchman, P. Moarahan, and A. E. Muson, Polymeric Drugs, L. G. Donaruma and O. Vogl, Eds. Academic Press, New York, p.263 (1978).
4. T. C. Merigan and W. Regelson, *N. Engl. J. Med.*, **277**, 1283 (1967).
5. W. Broun, W. Regelson, Y. Yajima, and M. Ishizuka, *Proc. Soc. Exp. Biol. Med.*, **133**, 171 (1970).
5. W. Broun, W. Regelson, Y. Yajima, and M. Ishizuka, *Proc. Soc. Exp. Biol. Med.*, **133**, 171 (1970).
6. R. J. Fiel, H. E. Mark, and H. I. Levine, Anionic Polymeric Drugs, R. M. Ottenbrite and O. Vogl Eds., John Wiley & Sons Inc., New York, p.21 and 143 (1980).
7. M. J. Han, K. B. Choi, J. P. Chae, B. S. Hahn, and W. Y. Lee, Bioactive and Compatible Polymers in press
8. M. J. Han, D. H. Lee, Y. Y. Lee, and B. S. Hahn, *Bull. Korea Chem. Soc.*, **10**, 212 (1989).
9. M. J. Han, K. H. Kim, T. J. Cho, and K. B. Choi, *J. Polymer Sci. Chem. Ed.* in press
10. R. D. Kimbrough Jr., W. P. Dickson, and J. M. Wilkenson III, *J. Polymer. Sci. Polymer Letter* **2**, 85 (1964).
11. G. B. Butler, J. T. Badgett, and M. Sharabash, *J. Macromol. Sci. Chem.*, **A4**, 51 (1790).
12. N. D. Field and J. R. Schaefgen, *J. Polymer Sci.*, **58**, 533 (1962).
13. R. C. Schulz and R. Wolf, *Kolloid-Zeitschrift und Zeitschrift fuer Polymere*, **220**, 148 (1967).
14. Asahi Research Center Ed. Handbook of proton-NMR Spectra and Data Vol. 1, p.65, p.185 and Vol. 4, p.65, Academic Press, Tokyo (1985).
15. *Ibid* p.185.
16. Fineman, and S. D. Ross, *J. Polymer. Sci.*, **2**, 101 (1947).
17. D. S. Breslow, E. I. Edward, and N. R. Newburg, *Nature*, **246**, 16 (1973).
18. P. S. Morahan and A. M. Kaplan, *Int. J. Cancer*, **17**, 82 (1976).
19. F. H. Dean, M. L. Padarathsingth, and L. Keys, *Cancer Treat. Rep.*, **62**, 1807 (1978).
20. N. A. Pavidis, R. M. Schulz, M. A. Chirigos, and Luetzer., *J. Cancer Treat. Rep.*, **62**, 1817 (1978).
21. A. M. Kaplan, J. M. Collins, P. S. Morahan, and M. J. Snodgress, *Cancer Treat. Resp.*, **62**, 1823 (1978).