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Synthesis and Biological Activity of Poly [(tri-O-acetyl-D-glucal)-*alt*-(maleic anhydride)] Derivatives

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Poly[(tri-O-acetyl-D-glucal)-*alt*-(maleic anhydride)] was synthesized by free radical copolymerizations of the relevant comonomers. The alternating sequence of the copolymer was confirmed by ^{1}H-NMR, elemental analysis, and titration of anhydride groups incorporated into the copolymer. Hydrolysis of the copolymer under different conditions resulted in poly[(2-acetoxymethyl-3,4-diacetoxytetrahydropyran-5,6-diyl) (1,2-dicarboxyethylene)] and poly[(2-hydroxymethyl-3,4-dihydroxytetrahydropyran-5,6-diyl) (1,2-dicarboxyethylene)]. The cytotoxicities of these polymers measured against normal and tumor cells (3LL, B16) *in vitro* were found to be higher than that of DIVEMA, a prototype polymer having a high antitumor activity.

Introduction

It is well known that polymers with a high density of carboxylic acid functionality along the polymer chain can exhibit antitumor, antiviral and/or antifungal activities[1]. The tetrahydropyran (THP) rings as hydrophobic groups on the polymer chain may also play a significant role in the biological activity of the polymers[2]. We have synthesized several polymers containing THP rings and carboxyl groups on their backbone, which exhibit antitumor activity *in vitro* and *in vivo* comparable or superior to that of DIVEMA[3]. Among these polymers, poly[(2-acetoxytetrahydropyran-5,6-diyl) (1,2-dicarboxyethylene)] (1) and poly[(2-hydroxytetrahydropyran-5,6-diyl) (1,2-dicarboxyethylene)] (2) have been found to exhibit very high antitumor activities *in vitro* and *in vivo*[3,6].
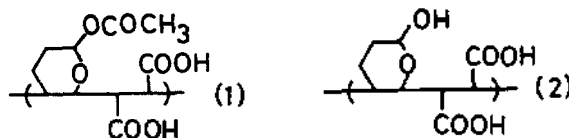
Table 1. Bulk Copolymerization Data of tri-O-acetyl-D-glucal (TAG) and Maleic Anhydride (MA) at 95°C with 1 mole-% of AIBN

TAG: MA (mole ratio)	Polym. Time (hs)	Yield (%)	MA content (mole-%)	Mn^a
1:2	20	17	–	–
1:2	48	29	52	2800
1:1	48	27	49	4200

^{a}Number-average molecular weights were measured by vapour pressure osmometry.
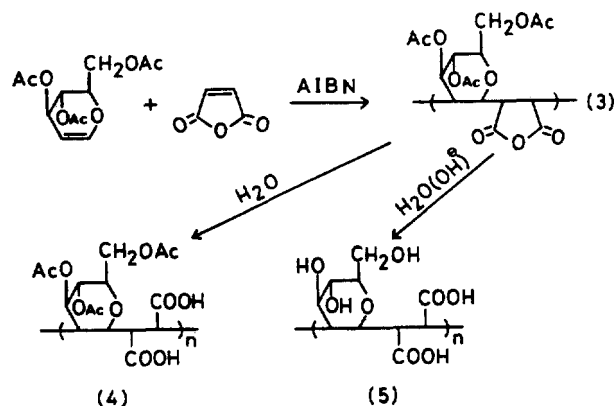
Scheme 1.

Since the polymers have acetoxyl or hydroxyl group substituents on the THP rings, it is of interest to find whether the antitumor activity is increased when the acetoxyl or hydroxyl groups are enriched on the THP rings for the same polymer skeleton. Poly[(tri-O-acetyl-D-glucal)-alt-(maleic anhydride)] (3) can be obtained by copolymerization of the relevant comonomers. Hydrolysis of the copolymer under different conditions results in poly[(2-acetoxymethyl-3,4-di-acetoxytetrahydropyran-5,6-diyl) (1,2-dicarboxyethylene)] (4) and poly [2-hydroxymethyl-3,4-dihydroxytetrahydropyran-5,6-diyl) (1,2-dicarboxyethylene)] (5), which contain either three acetoxyl or three hydroxyl groups on the THP rings of the copolymers, respectively, as shown in Scheme 1.

In this paper we report the synthesis and characterization of polymers (3), (4) and (5), and their biological activities *in vitro*.

Experimental

Material. Tri-O-acetyl-D-glucal (TAG) (mp.=51°C) and AIBN were crystallized from ether-n-heptane (1:1, v/v) and methanol, respectively. MA was sublimed under vacuum. Acetone was refluxed over P_2O_5 and distilled under N_2 before use. Other materials were commercially available reagent chemicals.

Copolymerization. The calculated amounts of monomers and initiator were charged without solvent into the polymerization tubes (Table 1). These were immersed into a Dewar flask containing dry ice and acetone. Following conventional freeze-thaw treatments under N_2, the tubes were sealed and placed in an oil bath 95°C for a fixed period of time. The polymerization mass was dissolved in acetone and

precipitated in ether-petroleum ether (l:l, v/v) several times and dried *in vacuo* over P_2O_5 at 50°C in a drying pistol. Elemental analysis; Calcd. for $C_{16}H_{18}O_{10}$: C, 51.89; H, 4.86. Found: C, 51.95; H, 4.62.

Hydrolyses Reactions. Polymer (4); Polymer (3) was hydrolyzed by stirring in water-THF (1:1, v/v) at room temp. for 4 h. Evaporation of the solvent under reduced pressure gave (4) quantitatively.

Polymer (5); Polymer (3) was refluxed in 1 N aq. NaOH for 3 h. After acidification wtih HCl to pH=2.5, the water was evaporated. The residue was dissolved in DMF and NaCl was removed by filtration. The product in the filtrate was precipitated in ethyl acetate and dried (yield: 85%).

Biological Activity. 50% inhibitory doses (ID_{50}) of the polymers against normal (secondary mouse embryo fibroblast) and tumor cells (3LL and B16) were measured by MTT assay as described earlier[5].

Titration. Analysis of anhydride groups in (3) was performed by dissolving the polymer in DMF and titrating with a solution of sodium methoxide (0.1 N) in DMF-methanol with the aid of a potentiometer[4].

Measurements. Number-average molecular weights were measured in acetone at 45°C with the aid of vapour pressure osmometer (Knauer Co.). ^{1}H-NMR spectra were recorded on a Varian T-60 spectrometer. Chemical shift were recorded as δ units relative to Me_4Si as the internal standard. IR-spectra were obtained with a Perkin-Elmer Model 283B spectrophotometer. Elemental analysis was performed by an elemental analyzer (Perkin Elmer Model 240C) at KRICT.

Results and discussion

It is known that the dihydropyran (DHP) derivatives can be copolymerized with maleic anhydride (MA) in the presence of radical initiators to yield alternating copolymers[3]. The solution copolymerization of tri-O-acetyl-D-glucal (TAG) with MA, however, failed due to the steric hindrance of the three acetyl groups on the glucal, whereas the copolymer was obtained by a bulk copolymerization with AIBN as an initiator at 95°C. The copolymerization data and molecular weight of the polymers are given in Table 1. Polymer (3) was found to be soluble in polar solvents, such as acetone, THF, DMF, DMSO and ethyl acetate, and insoluble in nonpolar solvents, such as ether, petroleum ether, carbon tetrachloride, n-hexane and toluene. The polymer was a very hygroscopic white powder.

The ^{1}H-NMR of polymer (3) reveals a typical polymer spectrum with broad peaks at 0.9 ppm for C_5 proton of THP, at 4.4-5.8 and 3.0-4.3 for all the other protons on polymer backbone, and a peak at 1.95 ppm representing acetyl protons. The integral value of backbone protons are found to be equal to that of acetyl protons. This indicates a ratio of 1:1 TAG to MA composition which corroborate the C, H elemental analysis data.

To confirm the alternating structure of the copolymers, the anhydride groups incorporated in the copolymer backbone were titrated with sodium methoxide[4] and MA contents in the polymers are found to be 49-52 mole-% as given in Table 1. TAG and MA are not homopolymerizable under the condition used. Hence it is reasonable that the copoly-

Table 2. Biological Activity of the Copolymers

Copolymer	ID_{50} ($\mu g/ml$)[a]		
	3LL[b]	B16[c]	MEF[d]
(1)	45.8	39.6	16.9
(2)	822	610	–
(4)	276	1141	227
(5)	1047	1700	804
DIVEMA[e]	2504	1511	765

[a] ID_{50} was defined as the concentration which reduced absorbance by 50% of control untreated wells 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] Mouse embryo fibroblast from C57BL/6 mouse. [e] An alternating copolymer of divinyl ether and maleic anhydride (1:2).

mers obtained have an alternating sequence between TAG and MA. The number-average molecular weight (Mn) of the copolymers were found to be low (Table 1). This is attributable to the chain transfer reaction which generally occurs in the radical polymerization of dihydropyran derivatives[3].

The hydrolyses of (3) were accomplished under different conditions as shown in Scheme 1. These reactions were monitored by IR and NMR spectra where peaks at 1825 cm^{-1} for cyclic anhydride and at 1.95 ppm for acetyl protons disappeared while a peak at 1730 cm^{-1} for carboxyl group emerged. The polymers (4) and (5) are soluble in DMF, DMSO, methanol and water, and insoluble in acetone, THF, ethyl acetate and other nonpolar solvents.

The biological activity of these copolymers were measured by MTT method[5] and ID_{50}-values against tumor cells (B16, 3LL) and normal cells are given in Table 2. The cytotoxicities of the copolymers *in vitro* are found to be low in comparison with those of the polymer (1) containing one acetoxyl group on THP ring[6], but higher than that of DIVEMA[7], an alternating copolymer of divinyl ether and maleic anhydride (1:2), which is known to exhibit a high antitumor activity. Studies on their anticancer effect *in vivo* are currently in progress.

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Dual Capillary Column System for the Qualitative Gas Chromatography: 1. Comparison Between Split and Splitless Injection Modes

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A dual capillary column system is described for the simultaneous measurement of retention index (RI) and area ratio (AR) values of each peak on two capillary columns of different polarity, DB-5 & DB-1701. Both capillary columns were connected to a common splitless injector *via* a deactivated fused-silica capillary tubing of 1 m length and a 'Y' splitter, the dead volume effect of which was found to be negligible. RI and AR were measured with high reproducibility($\leq$0.05% RSD) and with high accuracy ($<$10% RE), respectively. When applied to the test samples of the organic acid mixture, each acid was positively identified by the combined computer RI library search-AR comparison.

Introduction

With the advent of high resolution fused silica capillary columns and modern high performance gas chromatographs, gas chromatography (GC) which is primarily a separation technique, is now implemented into routine laboratory quali-

tative analysis of samples such as essential oils, organic acids, pollutants, and drugs[1-12]. Temperature programmed retention index (RI) system is most conveniently used as criteria for the identification of GC peaks without resorting to gas chromatography-mass spectrometry (GC-MS).

Confidence in the peak identification is greatly enhanced