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NOTE

고분자 항균제의 합성 및 특성

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Synthesis and Biocidal Activities of Polymeric Bactericides

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요 약 : 항박테리아성 단량체인 2,4,4′-트리클로로-2′-아크릴로일옥시디페닐 에테르(AcDP)를 트리에틸 아민 존재하의 건조 THF용매에서 2,4,4′-트리클로로-2′히드록시디페닐 에테르(DP)와 아크릴로일 클로리드로부터 합성하였다. 합성된 AcDP는 IR 및 ^{1}H-NMR 스펙트라로 확인하였다. AcDP의 단독중합체는 70℃의 톨루엔 용매하에서 개시제인 BPO를 사용하여 중합하였다. AcDP와 메틸메타크릴레이트(MMA)의 공중합체를 70℃에서 합성하였다. Poly(AcDP) 및 poly(AcDP-*co*-MMA)는 IR과 ^{1}H-NMR 스펙트라로 확인하였다. Poly(AcDP) 및 poly(AcDP-*co*-MMA)의 중량 평균 분자량은 각각 4100 및 9200이었다. AcDP, poly(AcDP) 및 poly(AcDP-*co*-MMA)의 항박테리아성은 agar dish법으로 확인하였다. DP뿐만 아니라 AcDP 및 그 중합체의 *Staphylococcus aureus*에 대한 항박테리아성은 PMMA 및 poly(ethylene-*co*-vinyl acetate)와 같은 기준 물질에 비하여 매우 우수하였다. *Staphylococcus aureus*에 대한 항박테리아성의 크기는 DP>AcDP>poly(AcDP)>poly(AcDP-*co*-MMA) 순서이었다.

Abstract : The bactericidal monomer, 2,4,4′-trichloro-2′-acryloyloxydiphenyl ether(AcDP) was synthesized from 2,4,4′-trichloro-2′-hydroxydiphenyl ether(DP) and acryloyl chloride in the presence of triethylamine in dry THF. The synthesized AcDP was identified by IR and ^{1}H-NMR spectra. The homopolymer of AcDP was obtained using BPO as an initiator in toluene at 70℃. Copolymer of AcDP and methyl methacrylate(MMA) was synthesized with an initiator at 70℃. Poly(AcDP) and poly(AcDP-*co*-MMA) were identified by IR and ^{1}H-NMR spectra. The weight average molecular weights of poly(AcDP) and poly(AcDP-*co*-MMA) were 4100 and 9200, respectively. The bactericidal activities of AcDP, poly(AcDP), and poly(AcDP-*co*-MMA) were studied using agar dish test. The bactericidal activities of AcDP and its polymers as well as DP against *Staphylococcus aureus* were vey excellent compared to those of control polymers such as PMMA and poly(ethylene-*co*-vinyl acetate). The bactericidal activities were decreased in the order DP>AcDP>poly(AcDP)>poly(AcDP-*co*-MMA) against *Staphylococcus aureus*.

INTRODUCTION

Polymeric biocides have elicited considerable interest in recent years because of their long-lasting biocidal activities. Polymeric bactericides can significantly reduce losses associated with volatilization, photolytic decomposition, dissolution, and transport. Moreover, increased efficiency, selectivity, and handing safety are additional benefits which may be realized.

Pittman[1] synthesized copolymers of pentachlorophenyl acrylate with ethyl acrylate or vinyl acetate as polymeric biocides and found that the copolymers exhibited excellent bactericidal activities against *Pseudomonas* sp. Some of the halogen-o-hydroxydiphenyl ether derivatives[2] have been used for protection of organic materials, such as synthetic resins, paper treatment liquors, printing thickners, lacquers, paints, and cosmetic articles, because of their remarkable biocidal activities.

In this work, we synthesized 2,4,4′-trichloro-2′-acryloyloxydiphenyl ether(AcDP) by reacting acryloyl chloride(Ac) with 2,4,4′-trichloro-2′-hydroxydiphenyl ether(DP). DP was selected for its bactericidal activity against both *Pseudomonas aeruginosa* and *Staphylococcus aureus*, existing in fiber, paper, latex, rubber, machine oil, leather, plastic, coatings, cosmetic articles, and packaging materials.[3~5] Apostolatos et al.[6] reported that the mixture of 2,4,4′-trichloro-2′-hydroxydiphenyl ether and 3,5,4′-tribromosalicylanilide were effective against both Gram-positive and Gram-negative bacteria. Finzi et al.[7] studied bactericidal power of DP against hospital-isolated bacteria. Carol et al.[8] reported antimicrobial effect of a mixture of DP with soap and acyl isethionate salts having a cleaning composition. They found that the antimicrobial activity of DP-containing soap was greater than that of soap itself.

AcDP was polymerized by a free radical initiator. Copolymer of AcDP with methyl methacrylate (MMA) was also synthesized. The copolymer composition was analyzed quantitatively by UV spectroscopy. The bactericidal activities of DP, AcDP, poly(AcDP), and poly(AcDP-*co*-MMA) were investigated against *Staphylococcus aureus*.

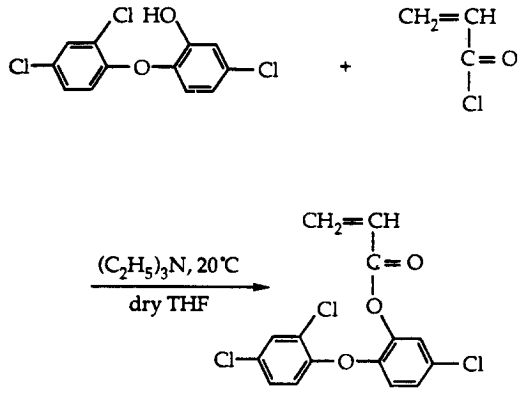
EXPERIMENTAL

Materials. 2,4,4′-Trichloro-2′-hydroxydiphenyl ether(DP ; Ciba-Geigy) was recrystallized from n-hexane. Acryloyl chloride(Ac ; Aldrich) was used without further purification. Triethylamine (Junsei) was refluxed with acetic anhydride and with KOH, and finally distilled. Methyl methacrylate(MMA ; Junsei) was washed twice with 5% aq. NaOH and three times with water, then dried with Na_2SO_4 and distilled under nitrogen at reduced pressure. Benzoyl peroxide(BPO ; Junsei) was dissolved in $CHCl_3$ and precipitated by adding an equal volume of MeOH. Toluene(Junsei), THF (J. T. Baker), and other chemicals were purified by the standard procedures. Poly(ethylene-*co*-vinyl acetate)(EVA) having 40% of vinyl acetate(Inherent viscosity ; 0.70 dlg^{-1}, Melt index ; 57) was used as received from Aldrich. Beef extract(Difco), bacto-pepton(Difco), agar(Difco), and the bacteria, *Staphylococcus aureus* ATCC 6538P, were kindly supplied from PUSAN URETHANE Co., Korea.

Instruments. IR spectra were taken on a Nicolet 710 FT-IR spectrophotometer using KBr pellet. ^{1}H-NMR spectra were recorded on a Jeol JSM-PMX 60SI spectrophotometer. UV spectra were taken on a Shimadzu 2100 spectrophotometer. The purity of a synthesized monomer was identified with a Waters(detector ; 481, pump ; 510, data module ; 745 B) liquid chromatograph(HPLC). Average molecular weight was determined by gel permeation chromatography(GPC ; Waters, 150-C). Thermal properties were recorded on a du Pont 910 differential scanning calorimeter(DSC).

Synthesis of 2,4,4′-Trichloro-2′-Acryloyloxydiphenyl Ether(AcDP). AcDP was prepared by the reaction of 2,4,4′-trichloro-2′-hydroxydiphenyl ether(DP) and acryloyl chloride(Ac) in the presence of triethylamine as follows ;

A mixture of 300 ml of THF. 23.5 g(8.12×



10^{-2} mol) of DP, and 10.6 ml of triethylamine was put into 1 L of three-necked round bottom flask equipped with a thermometer, a condenser, a dropping funnel, and a magnetic stirring bar. The flask was then maintained at 20℃, while 6.68 g (7.38×10^{-2} mol) of Ac dissolved in 50 ml of dry THF was added by dropping funnel for 1.5 h. The reaction mixture was stirred at 20℃ for 6 h. After the flask was allowed to room temperature, the THF solution was filtered and the filtrate was condensed by vacuum rotoevaporation. The remaining viscous liquid was poured into *n*-hexane and washed three times with 5% $NaHCO_3$, then four times with water. The n-hexane layer was dried over 24 h with anhydrous Na_2SO_4. After removing filtrate from the n-hexane solution by vacuum rotoevaporation, the remaining slightly yellow liquid was recrystallized twice from dry MeOH to yield 15.81 g(62.3%) AcDP as a transparent crystal, mp 49.2~50.5℃. The purity of synthesized AcDP was 99.3% by HPLC.

Synthesis of Poly(2,4,4′-trichloro-2′-acryloyloxydiphenyl ether) [poly(AcDP)]. a solution of 0.334 g (9.72×10^{-4} mol) AcDP and 4.95×10^{-6} mol of BPO in 10 ml dry toluene was introduced into a glass ampoule equipped with a magnetic stirring bar and a septa cap. The solution was deoxygenated by purging with purified N_2 gas. The ampoule was sealed and placed in a regulated thermostat bath at 70℃ for 12 h. The resulting polymer was precipitated into MeOH. The precipitate was collected by filtration and dried under vacuum to constant weight.

Synthesis of Poly(methyl methacrylate) (PMMA). A solution of 0.28 g(2.08×10^{-2} mol) MMA and 5.70×10^{-5} mol of BPO in 10 ml dry toluene was introduced into a glass ampoule equipped with a magnetic stirring bar and a septa cap. The solution was deoxygenated by purging with purified N_2 gas and was sealed. Polymerization was carried out at 70℃ for 48 h and the resulting polymer was precipitated into MeOH. The precipitate was collected by filtration and dried at room temperature under vacuum to constant weight.

Synthesis of Poly(2,4,4′-trichloro-2′-acryloyloxydiphenyl ether-*co*-methyl methacrylate)[poly (AcDP-*co*-MMA)]. Copolymerization of AcDP with MMA was carried out with BPO in toluene at 70℃. A solution of 5.41×10^{-3} mol AcDP, 5.61×10^{-4} mol MMA, and 3.01×10^{-5} mol BPO in 20 ml dry toluene was introduced into a glass ampoule tube equipped with a magnetic stirring bar and a septa cap. The solution was deoxygenated by purging with purified N_2 gas. The tube was sealed and placed in a regulated thermostat bath at 70℃ for 48 h. The polymer solution obtained was precipitated in excess MeOH. The precipitate was collected by filtration and dried under vacuum to constant weight.

Characterization of Polymers. The copolymer composition was determined quantitatively by UV spectroscopy according to the literature.[9] For the analysis of poly(AcDP-*co*-MMA), a definite amount of copolymer was dissolved in chloroform of spectroscopic grade. The solution was placed in a 1.0 cm quartz cell and quantitative analysis was performed on UV spectrum.

Average molecular weights of poly(AcDP), poly (AcDP-*co*-MMA), and PMMA were determined by GPC using non-aqueous Microstyragel column and monodisperse polystyrene as a standard at 40℃. The concentrations of polymers were 0.1% or less.

The glass transition temperature(Tg) was determined using a DSC on sample sizes averaging 10 mg under nitrogen at a heating rate of 10℃/min.

Accelerated Bacteria Growth Test. DP, AcDP, poly(AcDP), poly(AcDP-*co*-MMA), and PMMA were blended individually with poly(ethylene-*co*-vinyl acetate)(EVA ; VA content, 40%) at various concentrations(0.1~1.0 wt.%) and dissolved in THF(5% solution). Then, test sample films of 0.1~0.13 mm thickness were prepared by casting the solutions on Petri dish(diameter ; 95 mm). Control films of pure EVA were also prepared by casting from its THF solution. The Petri dishes containing test samples were dried over 24 h at room temperature and dried under vacuum at 30℃ to constant weight. The test specimens were prepared by cutting the test sample to yield circle-shaped films of 16 mm diameter. 20 ml of the nutrient agar(0.5% beef extract, 1.0% bacto-pepton, 0.5% NaCl, and 1.5% agar in distilled water) inoculated with *Staphylococcus aureus* was poured into Petri dishes. The test specimens were carefully pressed onto the centers of the plates of the nutrient agar inoculated with *Staphylococcus aureus* to ensure good contact between the specimen and the agar surface, and the plates were incubated at 30℃ for 24 h. After 24 h incubation, the growth of bacteria on the surface of the test specimens were observed by naked eyes.

RESULTS AND DISCUSSION

Identification of 2,4,4′-trichloro-2′-acryloyloxydiphenyl ether. Synthesized AcDP was identified from its IR, ^{1}H-NMR, and UV spectra. The IR spectrum(Fig. 1-a) exhibited characteristic absorption bands at 1633, 980, and 920 cm^{-1}(vinyl) and 1750 cm^{-1}(C=O). ^{1}H-NMR spectrum(Fig. 2-a) of AcDP(solvent ; Acetone-d_6) exhibited several peaks at 5.70~6.13(m, =CH), 6.13~6.70(m, $=CH_2$), 6.70~8.00 ppm(m, $C_{12}H_6$). From UV spectrum, the wavelength and molar absortivity at maximum absorption were 246.6 nm and 6550, respectively.

Characterization of Homopolymers. Poly(AcDP) was identified from its IR spectrum(Fig. 1-b) indicating absorption at 2930 cm^{-1}, characteristics of

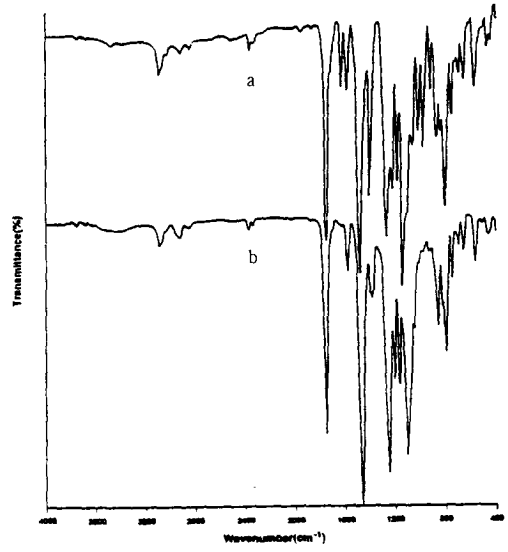


Fig. 1. IR spectra of (a) 2,4,4′-trichloro-2′-acryloyloxydiphenyl ether and (b) poly(2,4,4′-trichloro-2′-acryloyloxydiphenyl ether).(Solid phase, KBr).

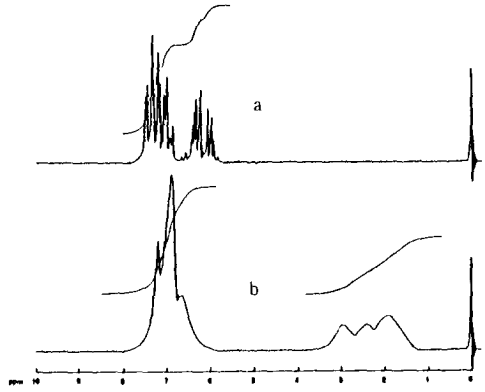


Fig. 2. ^{1}H-NMR spectra of (a) 2,4,4′-trichloro-2′-acryloyloxydiphenl ether in acetone-d_6 and (b) poly(2,4,4′-trichloro-2′-acryloyloxydiphenyl ether) in $CDCl_3$.

the vinyl polymer backbone, with disappearance of vinyl absorptions of monomeric AcDP at 1633, 980, and 920 cm^{-1}. ^{1}H-NMR spectrum of poly(AcDP) (solvent ; $CDCl_3$)(Fig. 2-b) exhibited several peaks at 1.07~2.67(-CH_2-), 2.67~3.43(-CH-), and 6.03~8.03 ppm($C_{12}H_6$). The glass transition temperature of poly(AcDP) was 73.4℃. The number and weight average molecular weights of poly

(AcDP) were 2500 and 4100, respectively.

The number and weight average molecular weights of PMMA were 10600 and 19100, respectively.

Characterization of Copolymer. IR spectrum of poly(AcDP-*co*-MMA) indicated absorptions at 3095 cm^{-1}(phenyl ring of AcDP), 1765 cm^{-1}(C=O, AcDP), and 1729 cm^{-1}(C=O, MMA) with disappearance of vinyl absorptions at 1633(AcDP) and 1637 cm^{-1}(MMA). Poly(AcDP-*co*-MMA)(solvent ; $CDCl_3$) was also identified by the several peaks on its ^{1}H-NMR spectrum ; 0.53~1.37(α-CH_3), 1.37~2.77(-CH_2-), 2.77~2.90(-CH-), 3.03~3.93 (-O-CH_3), 6.33~7.57 ppm($C_{12}H_6$).

The number and weight average molecular weights of poly(AcDP-*co*-MMA) were 3100 and 9200, respectively.

The copolymer composition was determined by quantitative UV analyses, where 276.6 nm is selected as the characteristic wavelength for analyses of poly(AcDP-*co*-MMA), because PMMA scarcely absorbs the light of wavelength.

The details of quantitative UV analysis are found in the literatures[9,10] but a brief explanation can be described as follows ; The UV spectrum of copolymer of AcDP and MMA was used to find absorbance of π-π* transiton of phenyl ring in AcDP. The straight-line calibration curve was obtained from the absorbances of the solution mixtures of poly(AcDP)/PMMA with given weight fractions (concentrations of each homopolymers ; 12 mg/100 ml chloroform). From the calibration curve, the following equation was derived ;

$$\varepsilon = 6.79X + 0.11(1-X)$$

where ε is the specific extinction coefficient of the copolymer and X is the weight fraction of AcDP unit in the copolymer.

The content of AcDP of the copolymer obtained in this work was determined to be about 72 mol %.

Accelerated Growth Studies of Bactericidal Activity. The biocidal properties of AcDP and its polymers were studied in agar dish tests. Films cast on Petri dish were individually inoculated with *Staphylococcus aureus*. The samples inoculated with a single organism were used for the agar dish tests at 30℃. Visual rating of bacterial growth was then set up as follows : 5, no growth on film, zone of inhibition present ; 4, no growth on film, growth occurs on agar up to the edge of film(no zone of inhibition) ; 3.5, very sparse growth detected in places on film ; 3, sparse growth on film ; 2, moderate growth on film ; 1, heavy growth on film.

Table 1. Results of Agar Dish Accelerated Growth Test on DP, AcDP, Poly(AcDP), Poly(AcDP-*co*-MMA), and Control Polymers

Sample	Concentration of Bactericidal agent (wt %)	*Staphylococcus aureus*[c] (ATCC 6538P)
EVA[a]	none	1
PMMA[b]	none	1
DP	0.1	3.5
	0.5	4
	1.0	5
AcDP	0.1	3.5
	0.5	4
	1.0	4
Poly(AcDP)	0.1	3
	0.5	3.5
	1.0	4
Poly(AcDP-*co*-MMA)	0.1	2
	0.5	3.5
	1.0	4

[a] poly(ethylene-*co*-vinyl acetate) without bactericide. (vinyl acetate content, 40%).
[b] poly(methyl methacrylate) without bactericide.
[c] Scale of growth : 5, no growth on film, zone of inhibition present ; 4, no growth on film, growth occurs on agar up to the edge of film(no zone of inhibition) ; 3.5, very sparse growth detected in places on film ; 3, sparse growth on film ; 2, moderate growth on film ; 1, heavy growth on film.

In Table 1, it is seen that the inhibition area in creases with rising concentration regardless of the kinds of those monomers and polymers. The specimens of AcDP, poly(AcDP), and poly(AcDP-*co*-MMA) prepared by blending with EVA, exhibited excellent bactericidal activites without growth on the film containing 1 wt% bactericidal agent, but exhibited sparse or moderate growth of *Staphylo-*

coccus aureus on the film containing 0.1 wt% bactericidal agent. The bactericidal activities against *Staphylococcus aureus* were decreased in the order DP>AcDP>poly(AcDP-*co*-MMA). This is probably attributed to the easiness of leach or migration of DP or AcDP from the sample films compared to the polymer-anchored DP such as poly(AcDP) and poly(AcDP-*co*-MMA). This result is in agreement with the study of Pittman[1] stating that the blended pentachlorophenol can leach or migrate from sample films, whereas polymer-anchored pentachlorophenol cannot. Therefore, bactericidal activities of polymers are plausibly due to the bactericidal action of polymer itself. The difference in the bactericidal activities of specimens containing DP, AcDP, poly(AcDP), and poly(AcDP-*co*-MMA) may be attributed to the difference in the content of DP moiety in AcDP and its polymers, even though the same concentrations of bactericidal agents were blended with EVA. It is also noted that the bactericidal activity of poly(AcDP-*co*-MMA) was not better than that of poly(AcDP), probably due to the effect of PMMA having no bactericidal activity.

Staphylococcus aureus showed, however, abundant growth on the control polymers such as EVA and PMMA, meaning that this bacteria is excellent organism to evaluate the biocidal effects of chemically anchored biocides and blended biocides.

The results of the agar dish accelerated bacteria growth test are summarized in Table 1.

CONCLUSIONS

In this work, the bactericidal monomer, 2,4,4′-trichloro-2′-acryloyloxydiphenyl ether(AcDP), was synthesized. The yield and purity were 62.3 and 99.3%, respectively. Poly(2,4,4′-trichloro-2′-acryloyloxydiphenyl ether)[(poly(AcDP)] was synthesized by a free radical initiator at 70℃. Its number and weight average molecular weights were 2500 and 4100, respectively. Poly(2,4,4′-trichloro-2′-acryloyloxydiphenyl ether-*co*-methyl methacrylate) [(poly(AcDP-*co*-MMA)] was also synthesized, whose copolymer composition was analyzed by UV spectroscopy. The bactericidal activities of 2,4,4′-trichloro-2′-acryloyloxydiphenyl ether, poly(2,4,4′-trichloro-2′-acryloyloxydiphenyl ether), and poly (2,4,4′-trichloro-2′-acryloyloxydiphenyl ether-*co*-methyl methacrylate) against *Staphylococcus aureus* were excellent compared to those of poly(methyl methacrylate) and poly(ethylene-*co*-vinyl acetate). It was found that the bactericidal activities against *Staphylococcus aureus* were decreased in the order DP>AcDP>poly(AcDP)>poly(AcDP-*co*-MMA).

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