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생물활성을 갖는 1-메타크릴로일옥시메틸-5-플루오로우라실 및 그 중합체의 합성과 가용매반응

이 능 주*·오 상 훈·하 창 식·이 진 국·조 원 제

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Syntheses and Solvolysis of Biological Active 1-(Methacryloyloxymethyl)-5-fluorouracil and Its Polymers

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요 약

생물활성을 가질 것으로 예상되는 1-(methacryloyloxymethyl)-5-fluorouracil(MAOMFU) 을 2, 4-bis(trimethylsilyloxy)-5-fluoropyrimidine 으로 부터 합성하고, MAOMF 와 메틸메타크릴레이트(MMA) 를 cyclohexanone 용매로 사용하여 60 °C 에서 라디칼 공중합을 하였다. 공중합체 내의 단량체조성은 공중합체의 UV 스펙트럼으로 부터 정량분석하여 구하였다. Kelen-Tüdös 법에 의해 구한 각각의 단량체 반응성비의 값은 r_1(MAOMFU)＝0.72, r_2(MMA)＝1.24 이었다. 얻어진 단량체 반응성비의 값들로부터 MAOMFU 와 MMA 의 공중합에서 MAOMFU 의 입체장애 효과가 영향을 미치는 것을 알 수 있다. MAOMFU 와 poly(MAOMFU) 의 가용매분해속도상수는 각각 6.42×10^{-5} sec^{-1} 와 7.40×10^{-6} sec^{-1} 이었다. 얻어진 가용매분해속도상수로부터 poly(MAOMFU) 에서 5-fluorouracil 의 가용매분해속도는 MAOMFU 보다 약 5배 느리다는 것을 알 수 있었다.

Abstract : The biological active monomer, 1-(methacryloyloxymethyl)-5-fluorouracil(MAOMFU) was synthesized from 2, 4-bis(trimethylsilyloxy)-5-fluoropyrimidine. Poly(MAOMFU) poly(1-methacryloyloxymethyl-5-fluorouracil-co-methyl methacrylate), and poly(MAOMFU-co-MMA) were also obtained by radical polymerization at 60 °C. The monomer reactivity ratios, r_1 and r_2 were determined by Kelen-Tüdös method ; r_1(MAOMFU)＝0.72, and r_2(MMA)＝1.24. These reactivity values imply that the copolymerization was mainly affected by the steric hindrance of MAOMFU. It was found from kinetic measurements that the rate constants of solvolysis are given as 6.42×10^{-5} sec^{-1} and 7.40×10^{-6} sec^{-6}, respectively, for MAOMFU and poly(MAOMFU).

1. INTRODUCTION

Many attempts have been made to develope controlled release drug delivery systems that contain a drug or chemotherapheutic unit as part of a polymer backbone and as a pendant group or as a terminal group on the polymer chain[1~4].

The copolymer of divinyl ether-maleic anhydride has been attracted considerable interests because of its interesting biological activity. The pyran copolymer is active against several viruses ans diseases, including leukaemia, sarcoma, and vesiculas stomatitis[5].

The inhibitory properties of 5-fluorouracil(5-FU) for tumor growth and its use in cancer chemotherapy have also resulted in extensive work on related compounds. It was observed that 5-FU has strong side effect such as gastrointestinal toxicity and delivery problems[6, 7]. Several investigations have been made to reduce the latter effects; Butler et al[8] synthesized 1-(2-carbomethoxyacryloyl)-5-fluorouracil (CMAFU) and the copolymers of CMAFU with vinyl monomers such as styrene, 2-chloroethyl vinyl ether, and vinyl ether. They concluded that the CMAFU hydrolyzes rapidly in water but the copolymers more slowly. Gebelen and Morgan[9] synthesized 5-fluoro-N-(N-allylcarbamoyl)uracil, 5-fluoro-N-(N-vinyl carbamoyl) uracil, and their polymers. Akashi[10, 11] reported on the syntheses of N-methacryloyloxyethyl-5-fluorouracil, 1-N-acryloyl-5-fluorouracil, 1-N-methacryloyl-5-fluorouracil, and their polymers and copolymers. They also studied on the in vivo antitumor activity of the polymers in Ehrlich's Ascites tumor cells and found that the antitumor activity of polymers is greater than an equivalent amount of 5-FU alone, due to the fact that sustained release of 5-FU from the polymer chain is effective and the polyanion formed after hydrolysis may be effective against the tumor by an immune mechanism similar to pyran and other polyanions.

Even though many researches have been reported on the biological activity of polymer drugs, the reactivity of monomers in those bioactive copolymers is of equally importance. However, no work has been published on the monomer reactivity in an open literature. Studies to overcome the delivery problems by 5-FU are also essential in the field of polymeric drugs. Thus, we aimed at obtaining preliminary data for monomer reactivity and solvolysis kinetics of a new monomer, 1-(methacryloyloxymethyl)-5-fluorouracil(MAOMFU) and its polymers as potential polymeric drugs.

In this connection, we synthesized the biological active nomomer, 1-(methacryloyloxymethyl)-5-fluorouracil(MAOMFU) and its homopolymer and copolymer with methyl methacrylate by a free radical initiator. The nomomer reactivity ratios, r_1 and r_2 were determined by the Kelen-Tüdös method[12]. The kinetics of solvolysis of MAOMFU and poly(MAOMFU) was also studied in ethanol-water(50/50 by vol. %) mixed solvent.

2. EXPERIMENTAL

2. 1. Materials

5-Fluorouracil, purchased from Aldrich Chem. Co., was used as received. Methacrylic anhydride, methyl methacrylate, and 2, 2'-azobisisobutyronitrile(AIBN) were purified by the standard methods. Solvents like acetonitrile, n-hexane, benzene, tetrahydrofuran (THF), and cyclohexanone were used after purification.

2. 2. Instruments

IR spectra were taken on a Perkin-Elmer 1330 spectrophotometer using KBr pellet. Elemental analyses were taken with Elemental Analyzer (Perkin-Elmer 240 C). UV spectra were taken on a Shimadzu 200 A spectrophotometer. ^{1}H-NMR spectra were recorded on a Varian A-60 spectrophotometer.

2. 3. Synthesis of 1-(methacryloyloxymethyl)-5-fluorouracil(MAOMFU)

2. 3. 1. 1-Propionyloxymethyl-5-fluorouracil (POMFU)

POMFU was synthesized from 2, 4-bis(trimethylsilyloxy)-5-fluoro-pyrimidine(BTMSF) and chloromethyl propionate according to the work of Ozaki et al[13] with slight modification. Details of preparation of BTMSF and chloromethyl propionate are described in the literatures[14, 15]. A solution of 9.56 g(35 mmol) BTMSF and 4.90 g(40 mmol) chloromethyl propionate dissolved in 130 ml of dry acetonitrile was added in 300 ml three necked round bottomed flask equipped with thermometer, reflux condenser, and a magnetic stirring bar. The reaction mixture was refluxed for 12 days and then cooled to room temperature. After acetonitrile was distilled off under reduced pressure, the mixture was dissolved in 150 ml methanol and the precipitate was filtered off. The filtrate was distilled off and the product obtained was recrystallized from benzene and then dried until a constant weight under vacuum. (Yield ; 5.67 g, 76 % : m.p. 104～105 °C, lit. 105～106 °C[13]).

Analysis : Calculated for $C_8H_9N_2O_4F$ (216.17) : C, 44.45 ; H, 4.20 ; N, 12.96 %. Found : C, 44.18 ; H, 4.09 ; N, 12.85 %. IR(KBr, cm^{-1}) : 1720, 1630 and 1150. ^{1}H-NMR(DMSO-d_6) : δ 8.2(1H, d, 6H of pyrimidine ring), 5.7(2H, s, OCH_2), 2.4(2H, q, $C=OCH_2$) and 1.1 ppm(3 H, t, $-CH_3$).

2. 3. 2. 1-Hydroxymethyl-5-fluorouracil (HMFU)

A solution of POMFU(5.40 g, 25 mmol) and 6N HCl (30 mℓ) dissolved in 150 mℓ of methanol was added in 300 mℓ three necked round bottomed flask equipped with thermometer and reflux condenser. After the reaction mixture was stirred at 70 °C for 3 hrs, the solvent was distilled off under reduced pressure to provide a crystalline product. Recrystallization from ethanol gave 2.80 g(70 %) of HMFU. m.p. 160～161 °C. IR(KBr, cm^{-1}) : 3500, 1720 and 1025. NMR(DMSO-d_6) : 8.1(d, 6H of pyrimidine ring), 4.7(N-CH_2), and 3.5(-OH).

2. 3. 3. 1-(Methacryloyloxymethyl)-5-fluorouracil(MAOMFU)

Methacrylic anhydride(3.08 g, 0.02 mole), HMFU(1.60 g, 0.01 mole) and hydroquinone(0.01 g) were added in 200 mℓ three necked round bottomed flask equipped with thermometer, reflux condenser, and a magnetic stirring bar and stirred for 3 hrs at 90 °C to give a clear solution. The reaction mixture was cooled to room temperature to precipitate the product. The product was filtered, washed with diethyl ether several times, and recrystallized from ethanol (Yield : 52.2 %, m.p. : 144～145 °C).

2. 4. Syntheses of Polymers

2. 4. 1. Poly(1-(methacryloyloxymethyl)-5-fluorouracil) [poly(MAOMFU)]

A solution of 1.14 g(5 mmol) MAOMFU and 0.05 g AIBN in 25 mℓ dry cyclohexanone was introduced into a dry polymerization tube. The tube was sealed after degassed twice by purging with purified N_2 gas and placed in a regulated thermostat at 60± 0.05 °C for 24 hrs. The polymer solution obtained was precipitated in excess n-hexane several times. The precipitate was collected by filtration and dried until a constant weight under vacuum.

2. 4. 2. Poly(methyl methacrylate)(PMMA)

Poly(methyl methacrylate) was prepared by the polymerization of methyl methacrylate. The procedure was the same as that of poly(MAOMFU).

2. 4. 3 Poly(1-(methacryloyloxymethyl)-5-fluorouracil-co-methyl methacrylate) [Poly(MAOMFU-co-MMA)]

The synthesis of the copolymer was basically the same as that of poly(MAOMFU). For the analysis of copolymer compositions, a series of copolymerizations in which the feed ratio was varied of MAOMFU(M_1) to MMA(M_2) in cyclohexanone, ranged from 0.25 to 2.00, were carried out. Copolymerization was adjusted to make conversion less than 10 %. Taking one example as a typical copolymerization of $M_1/M_2=1$, a solution 1.14 g(5 mmol) MAOMFU, 0.50 g(5 mmol) MMA, and 0.082 g AIBN in 25 mℓ dry cyclohexanone was

introduced into a dry polymerization tube. The tube was sealed after degassed twice by purging with purified N_2 gas and placed in a regulated thermostat at 60± 0.05 °C for specified periods. The polymer solution obtained was precipitated in excess n-hexane several times. The precipitate was collected by filtration and dried until a constant weight under vacuum.

2. 5. Analysis of Copolymer Compositions

The copolymer composition was determined by using UV spectrophotometer. The specific absorptivities of polymers were measured at 267.3 nm in THF.

2. 5. 1. Measurement of intrinsic viscosity

The intrinsic viscosity(μ) of polymers, as a measure of molecular weight, was measured in N, N'-dimethylformamide at 30± 0.01 °C with Cannon-Fenske viscometer.

2. 5. 2. Kinetic measurements

The solvolysis of MAOMFU and poly(MAOMFU) was studied in ethanol-water(50/50 by vol. %) mixed solvent at 37± 0.01 °C. MAOMFU or poly(MAOMFU) (1.5×10^{-4} M) and HCl(2.4×10^{-2} M) were dissolved in the mixed solvent. The reactions were generally followed by direct UV spectrophotometry by recording the absorbance changes accompanying the solvolysis at 271 nm, where the absorption of MAOMFU and poly(MAOMFU) before and after solvolysis differed maximally. At this wavelength, it was observed that the product after solvolysis resulted in an absorbance increase wheras degradation of MAOMFU showed a decrease in absorbance. The product after solvolysis will be discussed in the results section(see also Ref 17). Pseudo-first order rate constant was calculated by Guggenheim equation[16].

3. RESULTS AND DISCUSSION

3. 1. Characterization

The monomer MAOMFU was characterized by IR and ^{1}H-NMR spectrophotometers and elemental analyzer. The IR spectrum of MAOMFU shows characteristic absorption bands at 3040(=CH), 2810(C−H), 1745(C=O), 1660(C=C) and 815 cm^{-1} (N−H), as shown in Fig. 1. Fig. 2 illustrates ^{1}H-NMR spectrum of MAOMFU. The monomer was identified with characteristic peaks at 1.9(CH_3), 4.8(N−CH_2), 6.1, 5.7 (=CH_2) and 8.5 ppm(6H of pyrimidine ring). From elemental analysis, the following data was obtained : Calculated for $C_9H_9N_2O_4F$(228.18) : C, 47.37 ; H, 3.98 ; N, 12.28 %. Found : C, 47.45 ; H, 3.76 ; N, 12.19 %.

Fig. 3 shows an IR spectrum of poly(MAOMFU-co-MMA). The copolymer was characterized with several characteristic peaks at 3430, 1720 and 815cm^{-1}.

The intrinsic viscosities of poly(MAOMFU) and

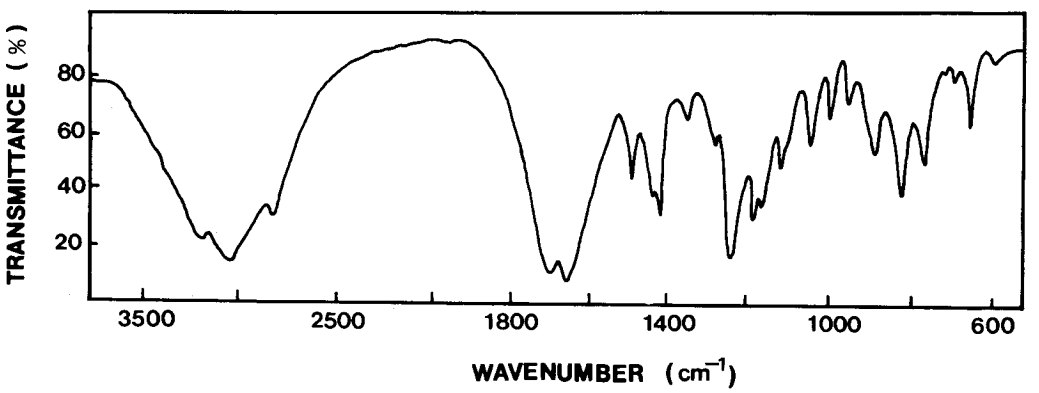


Fig. 1. IR spectrum of MAOMFU

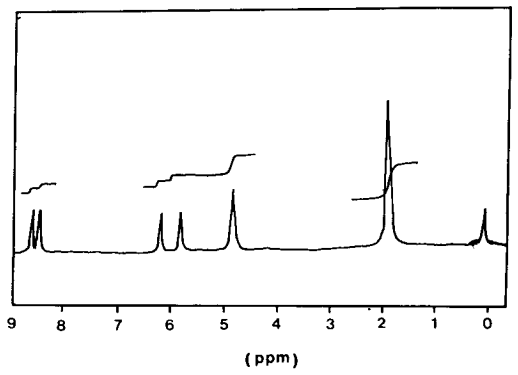


Fig. 2. NMR spectrum of MAOMFU

poly(MAOMFU-co-MMA) are determined as 0.09 and 0.08, respectively. It may be assumed from these values that these polymers are suitable for further biological tests, even though any attempts to test their biological activities were not made.

3. 2. Reactivity

The copolymer composition in poly(MAOMFU-co-MMA) was analyzed by using UV spectroscopy. The UV spectra of poly(MAOMFU) and PMMA in THF was measured at 267.3 nm, where 267.3 nm is selected as the characteristic wavelength for analysis because

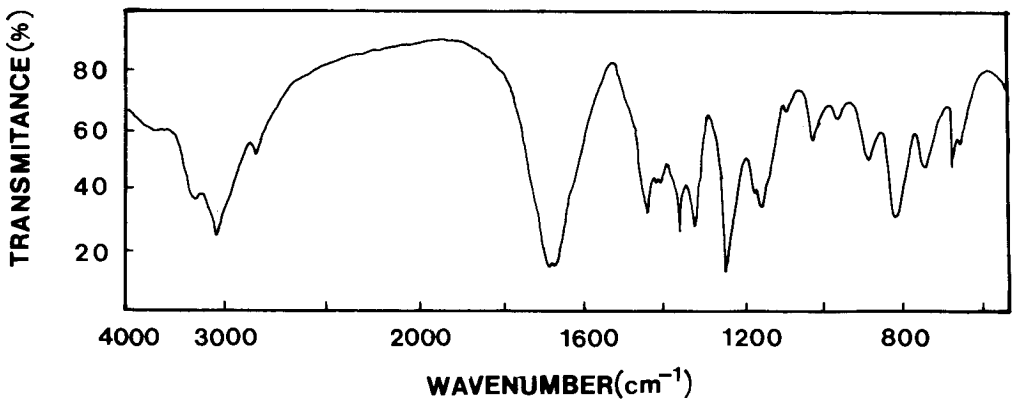


Fig. 3. IR spectrum of poly(MAOMFU-co-MMA)

PMMA scarcely absorbs the light of the wavelength. The following equation was derived from the relationship between the specific absorptivity of copolymer(k) and the weight fraction(x) of monomer unit in copolymer ;

$$x = 0.401\,k - 0.010$$

The copolymer compositions were listed in Table 1.

Table 1. Kelen-Tüös Parameters for Determination of Monoer Reactivity Ratios for the Copolymerization of MAOMFU (M_1) and MMA (M_2). $\alpha = 0.88$

EXP. NO.	$X=\frac{M_1}{M_2}$	$Y=\frac{m_1}{m_2}$	X^2	$Y-1$	$F=\frac{X^2}{Y}$	$G=\frac{X(Y-1)}{Y}$	$\alpha+F$	$\eta=\frac{G}{\alpha+F}$	$\zeta=\frac{F}{\alpha+F}$
1	0.25	0.20	0.06	−0.80	0.30	−1.00	1.18	−0.85	0.25
2	0.50	0.38	0.25	−0.62	0.66	−0.82	1.54	−0.53	0.43
3	1.00	0.80	1.00	−0.30	1.25	−0.25	2.13	−0.12	0.59
4	1.50	1.10	2.25	0.10	2.05	0.14	2.93	0.05	0.70
5	2.00	1.53	4.00	0.53	2.61	0.69	3.49	0.20	0.75

The reactivity ratio of each monomer was estimated by the Kelen-Tüdös method. In Table 1, several parameters for the Kelel-Tüdös plot were listed. From the Kelen-Tüdös plot, shown in Fig. 4, r_1 and r_2 values were estimated as 0.72(MAOMFU) and 1.24(MMA), respectively. These data show that the reaction of MAOMFU radical and MMA monomer occurs more readily than that of MAOMFU radical and MAOMFU monomer. The result may be described to the steric hindrance of MAOMFU.

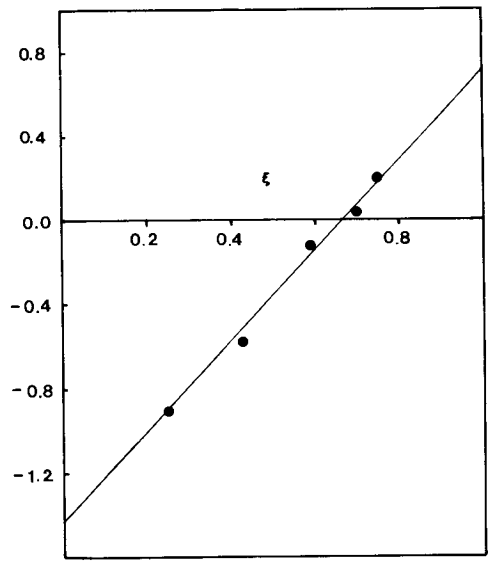


Fig. 4. Kelen-Tüdös plot for the copolymerization of MAOMFU and MMA r_1(MAOMFU)=0.72, r_2 (MMA)=1.24

3. 3. Solvolysis

In order to obtain fundamental data for drug release of MAOMFU and its polymers. synthesized in this work, we measured kinetics of their solvolysis by UV spectrophotometric method. Pseudo-first order rate constants were calculated from the slopes of linear plots of $\ln(A^{\infty}-A_t)$ against time where A^{∞} and A_t are the absorbance readings at infinity and time, t, respectively[16].

Fig. 5 shows a typical time-conversion curve and first-order plot in the solvolysis of MAOMFU in ethanol-water mixture. The rate constant k was determined as $6.42\times10^{-5}\,sec^{-1}$. By the same method, the rate constant for poly(MAOMFU) was determined as $7.4\times10^{-6}\,sec^{-1}$. It can be seen that solvolysis of poly (MAOMFU) was slower by a factor of 8 than that of MAOMFU. It should be noted that the result may be beneficial to develope controlled release drug systems.

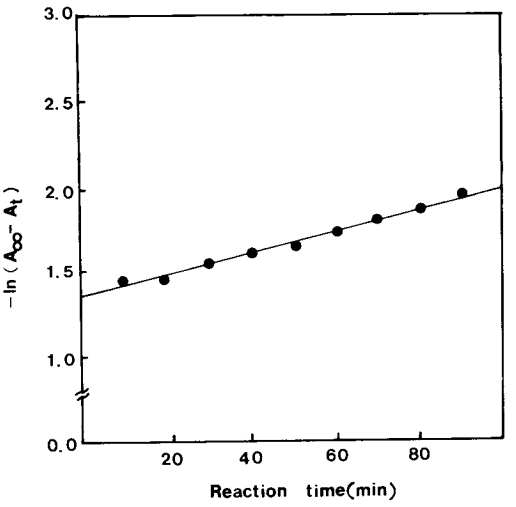
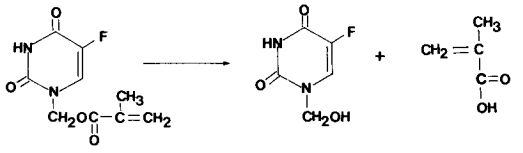


Fig. 5. Plots of $\ln(A^{\infty}-A_t)$ against time in the solvolysis of MAOMFU, where A^{∞} and A_t are described in the text.

It may be assumed that the solvolysis of the MAOMFU most likely takes place as depicted in Scheme 1. Cleavage of the ester group results in the formation of 1-hydroxymethyl-5-fluorouracil. Similar scheme has been reported for hydrolyses of other N-hydroxymethyl derivatives by Buur et al[17].



Scheme 1

It is expected that the monomer and polymers synthesized for this work may have biological activities

to some extent and further studies on their biological activities should be made. The biological test of the synthesized materials are now undertaking and will be reported in the near future.

4. CONCLUSIONS

The biological active monomer, 1-(methacryloyloxymethyl)-5-fluorouracil(MAOMFU) was synthesized from 2, 4-bis (trimethylsilyloxy)-5-fluoropyrimidine (BTMSF). Poly(MAOMFU) and poly(MAOMFU-co-MMA) were obtained by radical polymerization with AIBN in cyclohexanone at 60 °C. The monomer reactivity ratios of the copolymer were determined by the Kelen-Tüdös method ; r_1(MAOMFU)=0.72 ; r_2 (MMA)=1.24. These reactivity values imply that the copolymerization was mainly affected by the steric hindrance of MAOMFU. It was found from kinetic measurements that the rate constants of solvolysis are given as 6.42×10^{-5} sec^{-1} and 7.4×10^{-6} sec^{-1} for MAOMFU and poly(MAOMFU), respectively.

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