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K. Shiraishi[a]; K. Sugiyama[a]
[a] Department of Industrial Chemistry Faculty of Engineering, Kinki University, Hiroshima, Japan

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POLYMERIC DERIVATIVES OF CHLORAMPHENICOL AND THEIR ANTIBACTERIAL PROPERTIES

K. SHIRAISHI and K. SUGIYAMA*

Department of Industrial Chemistry
Faculty of Engineering
Kinki University
Hiro, Kure, Hiroshima 737-01, Japan

ABSTRACT

The polymerizable derivatives of chloramphenicol were prepared and free-radical copolymerized with acrylamide, methacrylic acid, and 2-(dimethylamino)ethyl methacrylate in order to obtain polymers with pharmacological activity. The monomeric and polymeric derivatives were subjected to antibacterial activity tests against *Bacillus polymyxa*.

INTRODUCTION

As the retention of drugs in the body can be increased by use of macromolecular controls, much interest has been paid recently to the synthesis of pharmacologically active macromolecular compounds that are capable of controlled slow release of a drug by enzymatic degradation or hydrolysis [1-3].

To this end, the chemotherapeutic agents have been fixed either by associating the drug by ionic interaction with charged compounds [4, 5] or by linking it to macromolecules through a covalent bond [3, 6-13]. In the

*To whom correspondence should be addressed.

latter case the active ingredients are fixed either directly or by means of spacer groups to carrier macromolecules.

Chloramphenicol has been known as a strong inhibitor of protein biosynthesis, which probably acts at or near the peptidyltransferase center of the ribosome of *Escherichia coli,* for instance [14, 15]. Chloramphenicol has been chemically modified to give some available derivatives, such as monobromoamphenicol, monoiodoamphenicol [5], and analogues of chloramphenicol in which the dichloromethyl group is replaced by alkyl, vinyl, allenyl, and monoalkyl [16], as well as palmitate, stearate, and succinate of chloramphenicol. The previous papers have shown chemical modification of chemotherapeutic agents such as anesthetics, amantadine, and others to macromolecular prodrugs [13, 17, 18]. In the present work, chloramphenicol, an antiviral agent, was modified to provide polymerizable derivatives for making macromolecular prodrugs with possibly prolonged activity. Antibacterial activities of the monomeric and polymeric derivatives of chloramphenicol were examined by using *Bacillus polymyxa.*

EXPERIMENTAL

Materials

Chloramphenicol was a commercial product of the highest purity available (Aldrich). Methacrylyl chloride and acrylyl chloride were prepared by the reaction of methacrylic acid and acrylic acid with benzoyl chloride [19].

Preparation of Chloramphenicol Methacrylate [D(-)-*threo*-2-Dichloroacetoamide-3-*p*-nitrophenyl-3-hydroxy Propyl Methacrylate], 1a

To 25 g (0.077 mol) chloramphenicol, 9.4 g (0.093 mol) triethylamine, and 5 mg 1,4-benzoquinone in ~300 mL freshly distilled ethyl acetate, 9.6 g (0.092 mol) methacrylyl chloride was added with stirring at 0°C for 3 h. After stirring for an additional 10 h at room temperature, the reaction mixture was evaporated to dryness, and ~300 mL chloroform was added to the residue. The chloroform layer was washed with water and evaporated to dryness. The product obtained was recrystallized from ethanol to give a white solid. mp = 129.5-130°C, yield 86%.

^{1}H-NMR (DMSO-d_6) δ ppm: 1.9 (s, 3H, $-CH_3$), 4.13-4.72 (m, 3H, $-CHCH_2O-$), 5.28 (br, 1H, $-NHCO-$), 5.80 (m, 1H, H–CH=), 6.25 (m, 1H, H–CH=), 6.38-6.56 (d, J = 5 Hz, 1H, PhCH(OH)–), 6.69 (s, 1H, $-CHCl_2$), 7.76-8.63 (q, 4H, phenyl).

$C_{15}H_{16}N_2O_6Cl_2$ (371.209). Calculated: C, 46.05; H, 4.12; N, 7.16. Found: C, 46.2; H, 4.3; N, 7.1.

Preparation of Chloramphenicol Acrylate [D-(-)-*thero*-2-dichloroacetoamide-3-*p*-nitorophenyl-3-hydroxy Propyl Acrylate], 1b

By the procedure given for the preparation of **1a**, the reaction of chloramphenicol and acrylyl chloride in dry ethyl acetate gave **1b** as a white solid. mp = 105-105.5°C, yield 85%.

^{1}H-NMR (DMSO-d_6) δ (ppm): 4.10-4.63(m,3H, $-\underline{C}\underline{H}\underline{C}\underline{H}_2O-$), 5.13 (br, 1H, $-N\underline{H}CO-$), 5.90 (m, 1H, $\underline{H}-CH=$), 6.05 (m, 1H, $CH_2=C\underline{H}-$), 6.14 (m, 1H, $\underline{H}-CH=$), 6.25-6.52 (d, J = Hz, 1H, $PhC\underline{H}(OH)-$) 6.72 (s, 1H, $-C\underline{H}Cl_2$), 7.46-8.43 (q, 4H, phenyl).

$C_{14}H_{14}N_2O_6Cl_2$ (377.182). Calculated: C, 44.58; H, 3.74; N, 7.42. Found: C, 44.5; H, 3.7; N, 7.4.

Preparation of Chloramphenicol Chloroacetylate

By the procedure given for the preparation of **1a**, reaction of 25 g (0.077 mol) chloramphenicol and 8.8 g (0.078 mol) chloroacetyl chloride in dry acetone gave chloroacetylate as a white solid. mp = 109.5-110°C, yield 89%.

^{1}H-NMR (DMSO-d_6) δ (ppm): 4.13-4.72 (m, 3H, $-\underline{C}\underline{H}\underline{C}\underline{H}_2O-$), 4.43 (s, 2H, $-C\underline{H}_2Cl$), 5.06-5.33 (br, 1H, $-N\underline{H}CO-$), 6.30-6.53 (d, J = 4.8 Hz, 1H, $PhC\underline{H}(OH)-$), 6.63 (s, 1H, $-C\underline{H}Cl_2$), 7.66-8.56 (q, 4H, phenyl).

$C_{13}H_{13}O_6N_2Cl_3$ (399.616). Calculated: C, 39.07; H, 3.29; N, 7.01. Found: C, 38.8; H, 3.2; N, 7.2.

Preparation of 1-Chloramphenicolmethyl-(2-methacryloyloxyethyl)-dimethylammonium Chloride, 2

Into a 100-mL Erlenmeyer flask were placed 2 g (5.00 mmol) chloramphenicol chloroacetylate and 50 mL dry dioxane. 2-(Dimethylamino)ethyl methacrylate [0.8 g (5.09 mmol)] in 30 mL of dry dioxane was added to the solution. After stirring for 50 h at 50°C, the mixture was evaporated to dryness, and the viscous residue was poured into dry diethyl ether to precipitate a white solid. The pure **2** was obtained by recrystallization from DMSO/diethyl ether. mp = 205°C (dec.), yield 89.7%.

^{1}H-NMR (DMSO-d_6) δ (ppm): 1.97 (s, 3H, $-C\underline{H}_3$), 3.47-3.53 (s, 6H,

$-N^+(C\underline{H}_3)_2-$), 3.23-3.76 (m, 2H, $-NC\underline{H}_2CO-$), 3.82-4.35 (m, 4H, $-C\underline{H}_2C\underline{H}_2N^+(CH_3)_2$), 4.40-4.90 (m, 3H, $-C\underline{H}C\underline{H}_2O-$) 5.09-5.43 (br, 1H, $-N\underline{H}-$), 5.73-5.97 (m, 1H, $\underline{H}-CH=$), 6.12-6.37 (m, 1H, $\underline{H}-CH=$), 6.57-6.73 (m, 1H, $-C\underline{H}(OH)-$), 6.78 (s, 1H, $-C\underline{H}Cl_2$), 7.70-8.57 (q, 4H, phenyl).

Polymerization

Dimethylformamide (DMF) (10 mL) solution containing the required amounts of monomeric derivatives, comonomer, and azobisisobutyronitrile (AIBN) (0.01 g) in a glass tube was degassed by the freeze-thaw technique using a Dry-Ice/methanol bath and sealed *in vacuo*. The sealed tube was shaken for the desired time at 60°C. After polymerization for 3.5-6 h, the contents of the tube were poured into a large amount of diethyl ether (or petroleum ether) to precipitate the polymer. The intrinsic viscosities [η] of the polymer in DMF were determined at 30°C with an Ubbelohde viscometer after reprecipitation from DMF/diethyl ether. The composition ratios of the copolymers were calculated from the nitrogen content as obtained by elemental analyses.

Antibacterial Test

Assay of antibacterical activity of polymeric prodrug was carried out against *B. polymyxa*. *B. polymyxa* was grown under anaerobic conditions in a modified Winogradsky's medium with constant shaking for 24 h at 30°C [20]. The antibacterial activity was evaluated from the degree of inhibition of growth of *B. polymyxa*. The growth inhibitory factor (I) is given by

$$I(\%) = (A - B)/(A - C) \times 100, \quad (1)$$

where, A, B, and C represent the turbidity of control, the turbidity of the culture containing a known amount of polymeric prodrug after cultivation for 24 h, and the turbidity just after inoculating into the fresh medium. The turbidity was determined by spectrophotometry, measuring the absorbance due to *B. polymyxa* at 660 nm.

Measurements

^{1}H-NMR spectra were obtained with a Jeol JNH-MH-100. Chemical shifts in NMR are given in the δ peak with TMS as an internal standard. The turbidity was determined with a Shimadzu UV 200S spectrophotometer.

RESULTS AND DISCUSSION

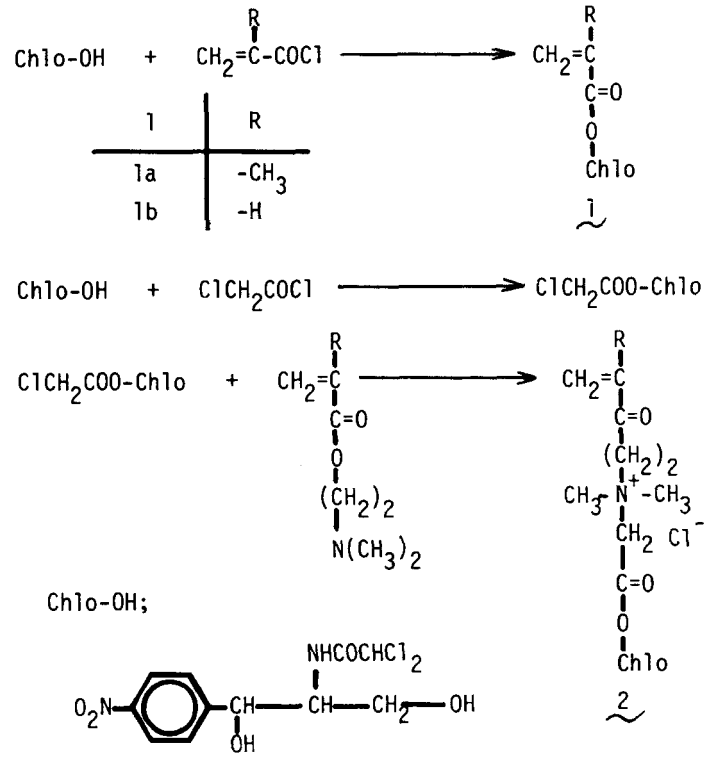
Preparation of Monomeric and Polymeric Derivatives

Polymerizable derivatives of chloramphenicol, **1a** and **1b**, were prepared by direct acylation of the hydroxy group of the drug with methacrylyl chloride or acrylyl chloride. Unsaturated derivatives containing the drug ionically fixed through ammonium salt as a spacer group, **2**, were also synthesized in order to study the effect of spacer groups on the antibacterial activity of the prodrug [5, 21] (Scheme 1).

1a and **1b** were free-radical copolymerized with acrylamide (AAm), methacrylic acid (MAA), and 2-(dimethylamino)ethyl methacrylate (DMA) to obtain the polymeric drugs, while **2** was homopolymerized radically to give poly-**2**. All of these macromolecular prodrugs were soluble in polar aprotic solvents such as DMF, dimethylsulfoxide, and hexamethylenephosphoramide, but insoluble in benzene, ethanol, and diethyl ether. Results of the copolymerization of monomeric derivatives with comonomers and characterization data of the copolymers are shown in Table 1. The molar fraction of chloramphenicol units in the copolymers was estimated to be 0.138-0.316 on the basis of elemental analyses of the copolymers. Though the molecular weight of the copolymers is known precisely, the degree of polymerization is presumed to be small as indicated by their low intrinsic viscosities.

Antibacterial Activity Against *B. polymyxa*

The monomeric and polymeric prodrugs were submitted to examination of antibacterial activities against *B. polymyxa*. The results are summarized in Table 2. The minimum inhibitory concentration (MIC) of chloramphenicol is 64 μg/mL, and all the prodrugs obtained here exhibited larger MIC values than the parent drug. The monomeric derivatives **1a** and **1b**, in which chloramphenicol is fixed directly, were found to show lower antibacterial activity against *B. polymyxa* than **2**, in which the drug is bound by means



SCHEME 1. Preparation of monomeric prodrugs.

of spacer groups to the polymerizable group. On the other hand, the homopolymer of **2** was observed to restore the activity almost to the level of the parent drug.

As the content of the active ingredients in the copolymers was relatively low, the polymeric prodrugs seemed to exhibit very little antibacterial activity. If the concentration of the active component in the copolymers is considered, the activity of polymeric prodrugs is, however, approximately comparable to that of parent drug. For instance, poly(**1a**/AAm) completely inhibits the growth of *B. polymyxa* just above 256 μg/mL, when the molar ratio of **1a** in poly(**1a**/AAm) was 0.264. Accordingly, the concentration of the active component is calculated to be 64 μg/mL, and this value corresponds to the MIC of the parent drug.

TABLE 1. Results of the Copolymerization[a] of Monomeric Derivatives **1** with Comonomer

Monomeric derivative	Comonomer	Yield, g	Elemental analysis			Mole fraction of drug units in copolymer	$[\eta]$,[b] dL/g	Abbreviation
			C, %	H, %	N, %			
1a	AAm	2.62	47.6	11.4	5.1	0.264	0.23	poly(**1a**/AAm)
1a	MAA	2.83	49.2	4.8	5.0	0.316	1.70	poly(**1a**/MAA)
1a	DMA	1.77	54.2	8.2	7.1	0.254	0.32	poly(**1a**/DMA)
1b	AAm	2.82	48.1	13.8	5.6	0.149	1.30	poly(**1b**/AAm)
1b	MAA	2.89	50.8	3.3	5.6	0.154	1.29	poly(**1b**/MAA)
1b	DMA	2.33	56.5	8.5	7.9	0.138	0.27	poly(**1b**/DMA)

[a]Polymerization conditions: **1**, 1 g; comonomer, 2 g; and AIBN, 0.01 g, in DMF at 60°C for 3.5-6 h.
[b]In DMF at 30°C.

TABLE 2. Antibacterial Activity of Monomeric and Polymeric Derivatives Against *B. polymyxa*[a, b]

Concentration (μg/mL)	Growth inhibitory factor, *I*, %										
	Chlo-OH[c]	**1a**	**1b**	**2**	poly(**2**)	**1a**/AAm	**1a**/MAA	**1a**/DMA	**1b**/AAm	**1b**/MAA	**1b**/DMA
1024	100	100	100	100	100	100	100	100	100	100	100
512	100	100	100	100	100	100	56.6	100	100	56.6	100
256	100	94.6	85.9	100	100	100	46.4	100	89.1	46.4	93.8
128	100	60.0	68.4	88.3	98.0	72	32.8	50	65.4	32.8	58.1
64	94.3	50.4	42.1	56.4	59.2	34.2	31.3	28.4	32.0	31.3	11.6
32	52.6	39.5	39.5	26.3	20.0	34.2	15.6	28.7	28.7	14.5	15.6
16	23.7	32.2	21.0	15.0	20.0	10.6	15.6	21.2	10.0	15.6	7.8
8	16.0	32.2	18.4	7.5	16.0	10.6	15.6	12.7	7.8	15.6	3.8
4	16.0	17.6	10.5	3.7	16.0	10.6	12.2	12.7	7.6	13.1	3.8
2	10.5	17.6	10.5	3.7	0	5.2	12.2	12.2	12.7	7.3	3.8
1	10.5	11.6	10.5	3.7	0	–	8.8	–	–	8.8	0

[a]At 30°C for 24 h, homopolymers, polyAAm, polyMAA, and polyDMA show ~40% of growth inhibitory factor *I* at 1024 μg/mL.

[b]The **1a** and **1b** copolymers are polymeric prodrugs.

[c]Chloramphenicol.

Antibacterial Test Against Other Bacteria

The antibacterial activity of the monomeric and polymeric derivatives of chloramphenicol was also examined against the following 25 kinds of bacteria [22]:

1. *Staphylococcus aureus* FDA 209P JC-1
2. *Staphylococcus aureus Teramima*
3. *Staphylococcus aureus* MS 353
4. *Streptococcus pyogenes Cook*
5. *Escherichia coli* NIHJ JC-2
6. *Escherichia coli* K12 C600
7. *Klebsiella pneumoniae* PCI-602
8. *Salmonella typhimurium* IID 971
9. *Salmonella typhi* 901
10. *Salmonella paratyphi* 1015
11. *Salmonella schottmuelleri* 8006
12. *Salmonella enteritidis* G 14
13. *Serratia marcescens* IAM 1184
14. *Bacillus subtilis* ATCC 6633
15. *Pseudomonas aeruginosa* IFO 3445
16. *Pseudomonas aeruginosa* NCTC 10490
17. *Pseudomonas aeruginosa* PAO 1
18. *Proteus morganii* IFO 3848
19. *Proteus mirabilis* IFO 3849
20. *Proteus vulgaris* OX-19
21. *Proteus vulgaris* HX-19
22. *Proteus rettgari* IFO 3850
23. *Enterobacter aerogenes* ATCC 13048
24. *Enterobacter cloacae* 963
25. *Micrococcus luteus* ATCC 9341

Both the monomeric and the polymeric derivatives exhibited no antibacterial activity against the bacteria 5, 6, 8, 9, 15-17, and 22-24. In addition, poly(**1a**/MAA), poly(**1a**/AAm), and poly(**1a**/DMA) showed very little activity against the 25 kinds of bacteria. Results regarded as significant activity are shown in Table 3.

TABLE 3. Antibacterial Tests of Monomeric and Polymeric Derivatives Against Miscellaneous Bacteria

	MIC, μg/mL					
Bacteria	Chlo-OH[a]	**1a**	**1b**	**1b**/MAA	**1b**/AAm	**1b**/DMA
1	3.13	>100	12.5	>100	50	100
2	3.13	>100	12.5	>100	100	100
3	3.13	>100	12.5	>100	100	100
4	1.56	100	6.25	50	25	25
7	0.78	100	3.13	25	12.5	25
10	0.78	50	3.13	25	12.5	12.5
11	0.78	100	3.13	25	25	50
12	3.13	>100	25	>100	100	100
14	1.56	>100	12.5	100	25	50
18	0.78	100	3.13	100	12.5	12.5
20	0.39	50	3.13	25	12.5	12.5
21	0.78	100	3.13	25	12.5	50
25	1.56	>100	12.5	>100	100	50

[a]Chloramphenicol.

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