Active Ester of Carboxymethyl Cellulose

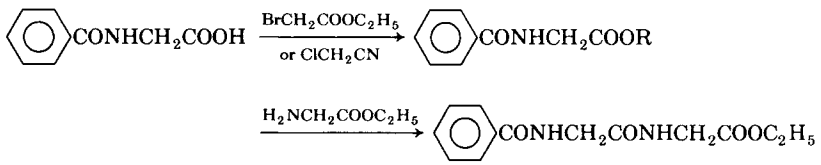
INTRODUCTION

Azid derivatives of carboxymethyl cellulose (CMC) have been widely used as the support of a variety of immobilized enzymes.[1,2] The azide derivative was prepared by reaction sequence shown in Scheme I.

$$\text{Cell—OCH}_2\text{COOH(CMC)} \xrightarrow[\text{HCl}]{\text{CH}_3\text{OH}} \text{Cell—OCH}_2\text{COOCH}_3 \xrightarrow{\text{H}_2\text{NNH}_2}$$

$$\text{Cell—OCH}_2\text{CONHNH}_2 \xrightarrow[\text{HCl}]{\text{NaNO}_2} \text{Cell—OCH}_2\text{CON}_3 \xrightarrow[6]{\text{H}_2\text{N—Enz}} \text{Cell—OCH}_2\text{CONH—Enz}$$

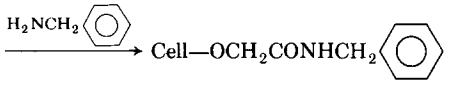
Schwyzer et al.[3] were found that ethyl glycollate and glycolonitrile esters of *N*-acylamino acids, e.g., hippuric acid, easily reacted with other amino acid esters as shown in Scheme II:



R: $CH_2COOC_2H_5$ or CH_2CN

This communication reports on the synthesis of an active ester of CMC with 2.75 of degree of substitution using the method developed by Schwyzer et al.[3] as shown in Scheme III:

$$\text{CMC} \xrightarrow[\text{Et}_3\text{N}]{\text{BrCH}_2\text{COOC}_2\text{H}_5} \text{Cell—OCH}_2\text{COOCH}_2\text{COOC}_2\text{H}_5$$



Characterization of the active ester and the reaction product with benzylamine was carried out by IR spectra recorded on films and elemental analyses. The active ester would be used as supports of immobilized enzymes and polymeric drugs.

EXPERIMENTAL

Material

Sodium salt of carboxymethyl cellulose with a high degree of substitution (2.75) was a gift from Daicel Chemical Industries, Ltd. Japan.

Procedure: Reaction of CMC with Ethyl Bromoacetate in Dimethylacetamide

A mixture of 5.0 g of finely powdered sodium salt of CMC and 20 mL of water was magnetically stirred, and 4 mL of concentrated HCl was added dropwise to the highly viscous solution. CMC gel obtained was almost dissolved by addition of 100 mL of methanol and another 50 mL of methanol was added. To the CMC solution in methanol, 10 mL of triethylamine (TEA), 10 mL of ethyl bromoacetate (EBA), 0.2 g of benzyltriethylammonium chloride (BTEAC), and 200 mL of

Journal of Applied Polymer Science, Vol. 32, 5657–5659 (1986)
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CCC 0021-8995/86/065657-03$04.00

dimethylacetamide (DMAc) were added, and the reaction mixture was refluxed for 12 h. If gelation was observed upon addition of a few drops of TEA, the reflux was continued. The reaction mixture was dialyzed against pure water and the active ester of CMC precipitated was collected on a glass funnel. The ester was soluble in DMAc, dimethylformamide (DMF), dimethylsulfoxide (DMSO), pyridine (Py), tetrahydrofuran (THF), and dioxane.

ELEMENTAL ANAL. Calcd. for $C_6H_7O_2(OH)_{0.25}(OCH_2COOH)_{0.66}(OCH_2COOCH_2COOC_2H_5)_{2.09}$: C, 47.10%; H, 5.83%. Found: C, 47.54%; H, 5.85%; N, 0%.

CMC was reacted with bromoacetonitrile in the place of EBA in a similar manner as above. The glycolonitrile ester of CMC obtained was soluble in DMAc, DMF, DMSO, and Py.

ELEMENTAL ANAL. Calcd. for $C_6H_7O_2(OH)_{0.25}(OCH_2COOH)_{1.59}(OCH_2COOCH_2CN)_{1.16}$: C, 45.22%; H, 4.54%; N, 4.43%. Found: C, 46.00%; H, 5.13%; N, 4.14%.

Reaction of Ethyl Glycollate Ester of CMC with benzylamine in DMAc

In 30 mL of DMAc was dissolved 0.3 g of the ethyl glycollate ester of CMC, and 3 mL of benzylamine was added. The solution was magnetically stirred at 30°C for 12 h. The reaction mixture was dialyzed against pure water, and the benzylamide of CMC precipitated was collected on a glass funnel.

ELEMENTAL ANAL.: Calcd. for $C_6H_7O_2(OH)_{0.25}(OCH_2COOH)_{0.66}(OCH_2CONHCH_2C_6H_5)_{1.79}(OCH_2COOCH_2COOC_2H_5)_{0.30}$: C, 59.76%; H, 5.89%; N, 4.94%. Found: C, 59.47%; H, 6.12%; N, 4.94%.

RESULTS AND DISCUSSION

The IR spectrum of ethyl glycollate ester of CMC obtained by treatment of CMC with ethyl bromoacetate was shown in Figure 1. A very strong band assigned to two ester groups of the active ester of CMC was observed at 1760–1740 cm^{-1}. Absorption band at 3600–3400 cm^{-1} assigned to OH stretching vibration of the hydroxyl group on anhydroglucose unit and the carboxyl group in carboxymethyl residue was very weak. By calculating from the result of elemental analysis the degree of esterification was 76.0% (2.09/2.75 × 100). In the case of glycolonitrile ester of CMC the degree of esterification was 42.2%.

The IR spectrum of benzylamide of CMC prepared by the reaction of ethyl glycollate ester of CMC with benzylamine was also shown in Figure 1. A very strong amide I band at 1650 cm^{-1} assigned to carbonyl group of benzylamide of CMC clearly appeared and the intensity of the band

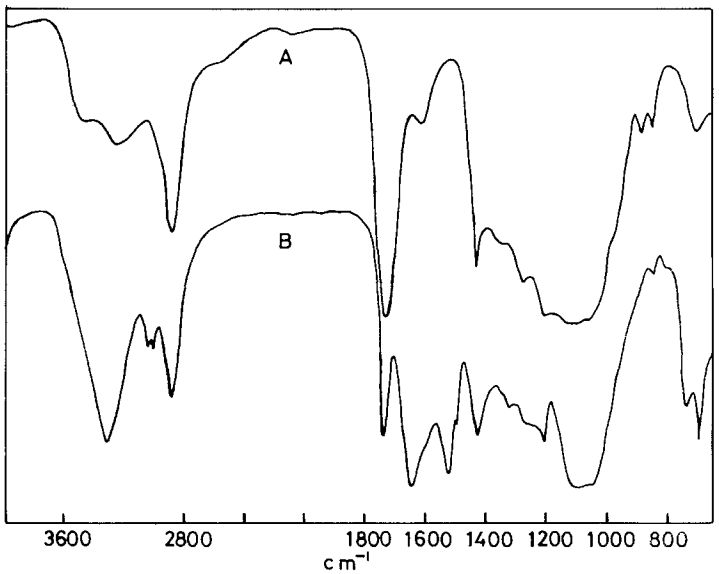

Fig. 1. IR spectra of ethyl glycollate ester (A) and benzylamide (B) of CMC.

at 1760–1740 cm^{-1} assigned to the ester groups remarkably decreased compared with that of ethyl glycollate ester of CMC. The degree of amidation calculated from the elemental analysis was 85.6% (1.79/2.09 × 100), and it was recognized that the reactivity of ethyl glycollate ester of CMC with aliphatic amine at 30°C in DMAc was fairly high. Studies on the reaction of ethyl glycollate ester of CMC with amino acids, drugs with aliphatic amino groups such as penicillins and aminosugar antibiotics, and enzyme are in progress.

In the course of this study described above, I noticed that Klemm et al. reported on the synthesis and characterization of enzymatically cleavable ethoxycarbonyl ethoxymethyl ester of CMC prepared by phase transfer reaction of sodium salt of CMC with ethyl α-bromo-α-ethoxyacetate.[4]

The author is grateful to Mr. Takeo Oomiya for kindly providing him with sodium salt of CMC with a high degree of substitution.

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Received September 18, 1985
Accepted January 16, 1986