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Carboxymethylalginic Acid Ethyl Ester and Its Reactions with *N*-Nucleophiles

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Abstract—Esters of carboxymethylalginic acid have been prepared; their acylation activity in the reactions with aromatic amines and aromatic carboxylic acids hydrazides have been studied.

Keywords: ester, polymer-analogous reaction, acylation

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Modification of drugs with polysaccharides allows for development of the existing formulations and preparation of new ones, possessing low toxicity, prolonged action, and the required lipophilic-hydrophilic balance [1]. In particular, alginic acid exhibiting a wide range of biological activity can be used for the modification [2].

Alkyl esters of carboxymethylated polysaccharides are convenient acylating agents for modification of enzymes [3], antibiotics [4], amino acids [5], and other compounds containing aliphatic or aromatic amino groups; on top of that, they can be used to prepare hydrazides of polysaccharide carboxylic acids [6].

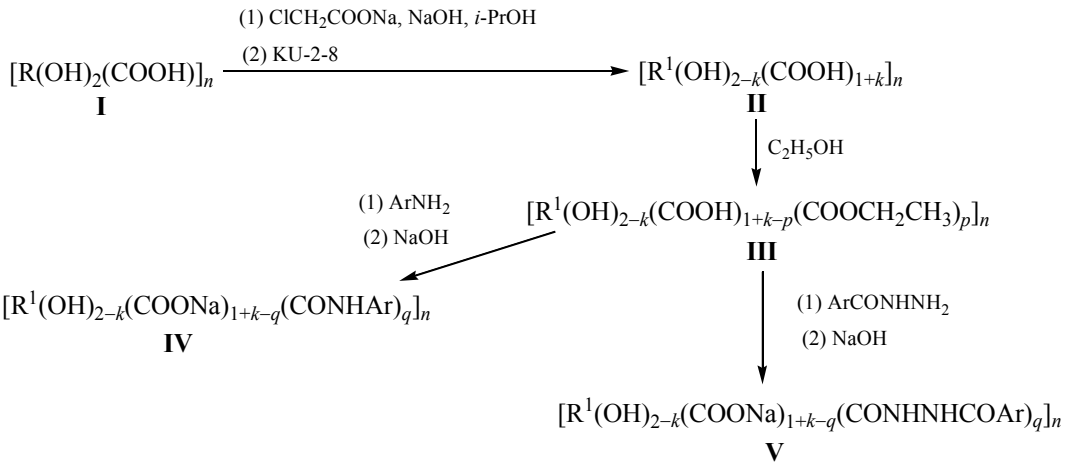
Esters of carboxymethylated polysaccharides can be prepared via a number of methods, the esterification with low-molecular alcohols in the absence of any external catalyst being the most convenient. The latter method has been recognized for the low contribution from the side reactions allowing for conversion of up to 50% of the carboxylic groups into the ester ones [7]. Unfortunately, certain polysaccharides do not form esters under these conditions (i.e., alginic acid), or the ester yield in very low (i.e., in the case of sulfated alginic acid with 0.4 sulfate groups per the monosaccharide unit the ester yield is 9%) [7, 8]. Only carboxymethyl derivatives of alginic acid readily interact with the alcohols, likely due to the higher reactivity of the carboxymethyl groups as compared to the uronic acid ones [9]. It is noteworthy that the alkylating of alginic acid with chloroacetic acid increases the polymer solubility in aqueous media; hence, carboxymethylalginic acid and its derivatives can be used for drugs modification.

The esterification of carboxymethylalginic acid and the corresponding products properties have been scarcely studied so far. In order to fill in the gap, in this work we synthesized ethyl carboxymethylalginate and investigated its acylating activity in the reactions with nucleophiles. The considered transformations of alginic acid **I** are given in the scheme below (R and R^1 representing the fragments remaining intact in the subsequent transformations; the initial uronic groups and the carboxylic groups introduced at the carboxymethylation stage are both designated as COOH).

Carboxymethylalginic acid **II** with the carboxymethylation degree D_{cm} of 0.65 to 1.62 (D_{cm} standing for the number of carboxymethyl groups per the polysaccharide monomer unit, $D_{cm} = k$ in the scheme) was transformed into the corresponding ethyl ester **III** via boiling in ethanol under the autocatalysis conditions. The so prepared esters **III** were yellow amorphous powders soluble in aqueous alkali and insoluble in water, aqueous acids, and common organic solvents including alcohols and acetone (Scheme 1).

The presence of ester groups in the products was confirmed by IR spectra. In particular, the products were treated with sodium ethylate in order to neutralize the carboxylic groups preventing identification of esters. The spectra of the so treated specimens contained the bands assigned to $\nu_{C=O}$ stretching vibrations of ester groups (1738 cm^{-1}) and of the carboxylate ones (around 1609 cm^{-1}).

Scheme 1.



Quantitative analysis of the prepared samples was performed applying the hydroxamic reaction consisting in the formation of colored complexes between hydroxamic acids and iron(III) compounds [10]. Processing of the results afforded the content of ester groups per monomer unit of polysaccharide **III** D_e ($D_e = p$ in the scheme) and the fraction of the ester groups with respect to the total amount of carboxylic groups in the acid **II** $\omega_e = p/(1 + k)$.

The equilibrium degree of esterification (D_e = 0.6) in the course of modification of carboxymethylalginic acid **II** with D_{cm} = 1.62 in the boiling ethanol was attained after 6 h; reducing the reaction duration allowed a fine tuning of the ester groups content in the product. The limiting conversion during esterification of other samples of acid **II** was attained within 6–9 h as well yielding the product with other D_e values; for instance, acid **II** with D_{cm} = 0.8 gave D_e of 0.3. The presence of tiny amounts of salt in the carboxymethylalginic sample (due to the incomplete dialysis) accelerated the esterification reaction and increased its conversion to D_e of 0.6–0.8.

Table 1. Effect of carboxymethylation degree of alginic acid on the properties of product **III** of the partial (3 h) esterification with ethanol

D_{cm} of acid **II**, mol/mol	D_e of ester **III**, mol/mol	ω_e, %
0.65	0.16	9.7
1.08	0.35	16.8
1.12	0.48	22.6
1.22	0.55	24.8
1.29	0.45	19.7
1.40	0.45	18,8
1.51	0.40	16.0
1.54	0.33	13.0

It has been earlier demonstrated that the optimal conditions of synthesis of physiologically active polymers based on carboxymethyldextran correspond to the presence of 0.3–0.4 ester groups per the monosaccharide unit [3], therefore the duration of the treatment in ethanol was reduced to 3 h in further experiments aiming to elucidate the effect of the degree of carboxymethylation of acid **II** on the properties of the product **III**.

Results of quantitative analysis of the esters **III** are given in Table 1. The esterification conversion in the excess of ethanol during 3 h was a nonmonotonic function of the content of carboxymethyl groups in the acid **II**, being the highest (D_e = 0.55 and ω_e = 25%) at D_{cm} = 1.22. The initial increase in the esterification conversion at $0.65 < D_{cm} < 1.22$ was due to the higher reactivity of carboxymethyl groups in the reaction with ethanol as compared to that of the uronic acids carboxylic groups; the reaction was additionally accelerated due to the higher concentration of protons acting as catalyst. The decreasing esterification degree with further increasing content of the carboxymethyl groups could be explained by the fact that introduction of the second CH_2COOH group at the monosaccharide fragment facilitated the formation of intra- and intermolecular hydrogen bonds thus hindering the contact with ethanol molecule.

Acylating activity of esters **III** was estimated by performing their reactions with aromatic amines and

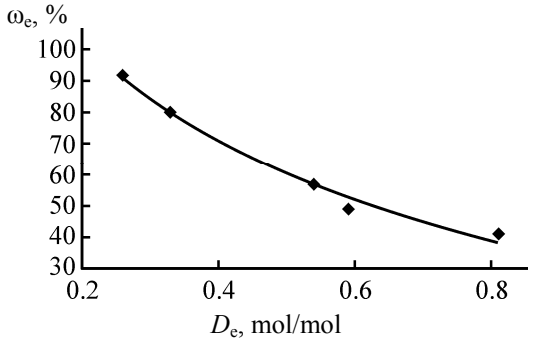


Fig. 1. Effect of the content of ester groups in the polysaccharide (D_e) on the conversion of their amidation in the reaction with *p*-ethoxyaniline (80°C, 10 h, dioxane). Degree of carboxymethylation of the corresponding acid **II** D_{cm} = 1.54.

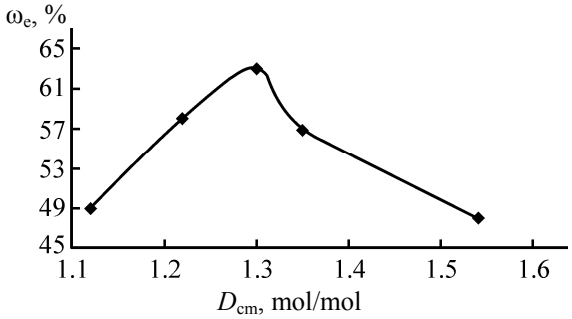


Fig. 2. Conversion of amidation of ester **III** in the reaction with *p*-ethoxyaniline (80°C, 10 h, dioxane) as a function of the content of carboxymethyl groups in acid **II**. Degree of esterification of the corresponding ester **III** D_e = 0.21–0.26.

carboxylic acids hydrazides. The acylation products **IV** and **V** were characterized by the number of the amide (or hydrazide, respectively) groups D_s per a monosaccharide unit ($D_s = q$ in the modification scheme), whereas the reaction conversion $\omega_S = q/p$ was defined as a fraction of the reacted ester groups.

The acylation products **IV** and **V** were white to cream-colored amorphous powders, soluble in neutral and alkaline aqueous media and insoluble in common organic solvents, including ethanol and acetone.

IR spectra of the prepared amides treated with acid contained the absorption bands at 1630–1640 cm^{-1} ($\nu_{C=O}$, amide **I**), 1550–1560 cm^{-1} (δ_{N-H}, amide **II**), 1736 cm^{-1} ($\nu_{C=O}$, carboxyl), and 730–770 cm^{-1} (δ_{C-H}, monosubstituted benzene ring) or 810–840 cm^{-1} (δ_{C-H}, 1,4-disubstituted benzene ring). The electron absorption spectra of the products contained the bands typical of aromatic compounds; the spectra of the products differed from those of the corresponding starting amines but were identical to those of the corresponding acetanilides, thus allowing for determination of the products yield and purity.

As expected, the yield of the acylation reaction was affected by the amine pK_a: the transformation of ester **III** into the amide was more efficient in the case of the more basic (i.e., nucleophilic) amines (Fig. 1 and Table 2). In particular, introduction of electron-donor substituents (OC_2H_5 or CH_3) in the *p*-position of the benzene ring accelerated the amine acylation, whereas the electron-acceptor substituents (Cl or $COOC_2H_5$) in the same position slowed down the acylation. In all cases the acylation conversion in ethanol medium was higher than that in dioxane (the difference being up to 40%). The mentioned observations coincided with the earlier reported data for carboxymethylated derivatives of dextran, chitin, and other polysaccharides [11, 12].

Hydrazides, stronger nucleophiles as compared to amines, were more reactive towards ester **III**. The most reactive substrate of the studied set was salicylic acid hydrazide. It contained the electron-donor hydroxyphenyl substituent and was a weaker base than the aromatic amines. Under the experimental conditions that hydrazide could be acylated by the ester groups as well as by the free carboxylic moieties. Therefore the calculated acylation degree of that substrate exceeded 100%. Hydrazides of the pyridinecarboxylic acids were weaker nucleophiles than salicylic acid hydrazide due to the electron-acceptor properties of the pyridine fragment. On top of that, the free carboxylic groups did not induce acylation of those hydrazides but rather formed the pyridinium salts. In general, the hydrazides of pyridinecarboxylic acids were acylated slower than salicylic acid hydrazides but faster than the aromatic amines (Table 2).

We further studied the effect of the ester groups amount in compound **III** (D_{cm} of the corresponding acid **II** being of 1.54) on the amidation reaction using *p*-ethoxyaniline as a model substrate. When the content of the ester groups D_e grew from 0.26 to 0.82, the substitution degree in the reaction product D_s increased from 0.24 to 0.32 but the fraction of the amide groups in the product reduced from 92% to 40% (Fig. 1).

At approximately constant content of the ester groups in compound **III** (D_e = 0.21–0.26), the degree of conversion of the ester groups in the same model reaction as a function of degree of carboxymethylation

Table 2. Conversion in the acylation of aromatic amines and carboxylic acids hydrazides with ethyl carboxymethylalginate **III** (10 h, 80°C, dioxane)

Nucleophile	Amine pK_a	D_e, mol/mol[a]	ω_e, %[a]
Amines:			
p-ethoxyaniline[b]	5.3	0.40 (0.60)	62 (93)
n-metylaniline[c]	5.1	0.35 (0.52)	56 (80)
aniline[b]	4.6	0.23 (0.34)	36 (53)
p-chloroaniline[b]	3.9	0.10 (0.14)	15 (22)
ethyl *p*-aminobenzoate[b]	2.4	0.03 (0.10)	4 (15)
Hydrazides of carboxylic acids[d]:			
salicylic	–	0.80	123
nicotinic	–	0.53	82
isonicotinic	–	0.40	62
picolinic	–	0.30	46

[a] The data in parentheses correspond to the boiling ethanol medium. Acylating agents (D_{cm} of acid **II** and D_e of ester **III**): [b] D_{cm} = 1.54, D_e = 0.65; [c] D_{cm} = 1.62, D_e = 0.63; [d] D_{cm} = 1.23, D_e = 0.65.

of the acid **II** revealed a maximum at D_{cm} = 1.29 (Fig. 2).

To conclude, we demonstrated that refluxing carboxymethylalginic acid in ethanol resulted in partial esterification of the polysaccharide carboxylic groups. The formed ester groups could be involved in the nucleophilic substitution reactions with aromatic amines and hydrazides of aromatic carboxylic acids. The degree of esterification of the carboxymethylated alginic acid and the degree of amidation of the formed ester in the mentioned reactions were nonmonotonic functions of the content of carboxymethyl groups in the polymer.

EXPERIMENTAL

The alginic acid (Acros Organics) sample has been characterized earlier [13]. Gel permeation chromatography studies (sephadex G-200 as a carrier) have revealed the presence of four major fractions, the highest *M* being below 200000. Alginic acid was purified by re-precipitation with ethanol from aqueous alkaline solution prior to the experiments. Content of the carboxylic groups after the purification was 0.97 per the monosaccharide unit.

Alginic acid was alkylated with chloroacetic acid; the product was purified via dialysis and converted into the acid form via ion-exchange chromatography [9]. Yield of the alkylation product was up to 70%, the carboxymethylation degree D_{cm} was 0.65–1.62. Ethyl esters **III** were prepared via refluxing in the excess of ethanol; the degree of esterification was ω_e = 13–32%.

Acylation of *N*-nucleophiles with the prepared products **III** was performed in ethanol or dioxane medium containing 5% of water; the reaction duration was 10 h, the nucleophiles were taken in five-fold excess with respect to the ester groups content. After the interaction aqueous sodium hydroxide solution (6 mol/L) was added to the mixture; the precipitate was centrifuged off, washed with ethanol, re-precipitated from 0.1 mol/L aqueous NaOH with ethanol, centrifuged off, washed with ethanol and with acetone, and finally dried in a vacuum at 60°C.

Purity of products **IV** and **V** and the conversion in the acylation reaction were determined from the UV spectroscopy data using the corresponding acetanilides and acetyl hydrazides as references.

IR spectra (KBr pellets) were registered using a FSM-1201 Fourier spectrometer.

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