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Carboxymethylalginic Acid Azides and Their Reactions with Nucleophiles

A. I. Cheremushkin and A. A. Iozep

St. Petersburg State Chemical-Pharmaceutical Academy, ul. Professora Popova 14, St. Petersburg, 197376 Russia
e-mail: andrei.cheremushkin@pharminnotech.com

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Abstract—The interaction of carboxymethylalginic acid hydrazides with sodium nitrite in acidic medium yielded polymers containing azide groups. Stability of aqueous solutions of the prepared products has been studied as a function of pH and temperature. Acylation ability of the products in the reactions with aromatic amines and hydrazides of aromatic carboxylic acids has been investigated.

Keywords: hydrazide, azide, polymer-analogous reaction, acylation

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Modern methods of synthesis of polymer-based drugs often include preparation polyacids azide followed by their coupling with *N*-nucleophiles to incorporate the pharmacophore into the macromolecule. This method allows modification of polyacids with thermally labile compounds (for instance, enzymes) under mild conditions. Carboxymethyl and carboxyethyl derivatives of neutral (cellulose) and acidic (alginic acid) polysaccharides are often used as the polyacid component of the reaction. Alginic acid has been widely used in food and cosmetic industry as well as in medicine [1]. Pristine alginic acid is not water-soluble; this seriously limits its study and development of applications. Therefore, carboxymethylalginic acid and its water-soluble derivatives (for example, esters, substituted amides, and *N*-acyl- or *N*-benzylidenehydrazides [2, 3]) are of particular interest. Some of the alginic acid derivatives have shown high biological activity [4]. However, to the best of our knowledge the reports on azides of carboxymethylalginic acid likely to be biologically active have been lacking so far. In order to fill in the gap in this work we prepared azides of carboxymethylalginic acid and studied their stability in aqueous solution as well as acylation ability in the reactions with low-molecular aromatic amines and carboxylic acids hydrazides.

Chemical modification of alginic acid was performed according to the following scheme, where R and R^1 stand for the fragments of macromolecules not modified in the subsequent transformations.

In particular, alginic acid **I** was alkylated in 2-propanol via addition of sodium chloroacetate and aqueous alkali. The degree of carboxylation (C_{CM} expressed as number of CH_2COOH groups per repeating unit) at that stage was 0.98 to 1.25, the product yield being of up to 70%. Carboxymethylalginic acid **II** was isolated using a KU 2-8 cationite and was further transformed into its ethyl ester **III** via esterification under autocatalysis conditions [2] (conversion of carboxylic groups into ester ones was 13 to 27% depending on the reaction duration). Ester groups were not found in the product of alginic acid reaction with ethanol under similar conditions, coinciding with the results reported in [2, 5]. However, carboxymethylation of polymer **I** could possibly change the macromolecules conformation; hence, the participation of initial carboxylic groups of alginic acid in the esterification could not be excluded.

The interaction of polymer **III** with excess of hydrazine hydrate at room temperature during 24 h led to the quantitative transformation of the esters into the corresponding hydrazides [6]. Noteworthily, attempts to prepare the carboxymethylalginic acid hydrazide **IV** directly from polymer **II** via heating with hydrazine hydrate led to undesired crosslinking of the polymer molecules due to the formation of the symmetric diacylhydrazide (Scheme 1).

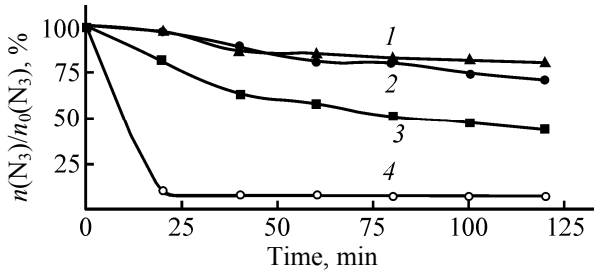


Fig. 1. Kinetics of azide groups hydrolysis in aqueous solutions of carboxymethylalginic acid azide, (C_{CM} 0.98, C_{AZ} 0.6) at (*1*, *3*, *4*) 0 and (*2*) 20°C; pH (*1*, *2*) 2, (*3*) 6, and (*4*) 10.

Carboxymethylalginic acid azides **V** were prepared via dissolution of the hydrazides **IV** in 1 mol/L aqueous sodium nitrite and addition of hydrochloric (or trifluoroacetic) acid to pH 2 (0°C, 20 min).

The so obtained azides of carboxymethylalginic acid were white amorphous powders soluble in neutral and alkaline aqueous media and insoluble in alcohols, acetone, and other common organic solvents.

The prepared azides were further used for acylation of the nucleophiles.

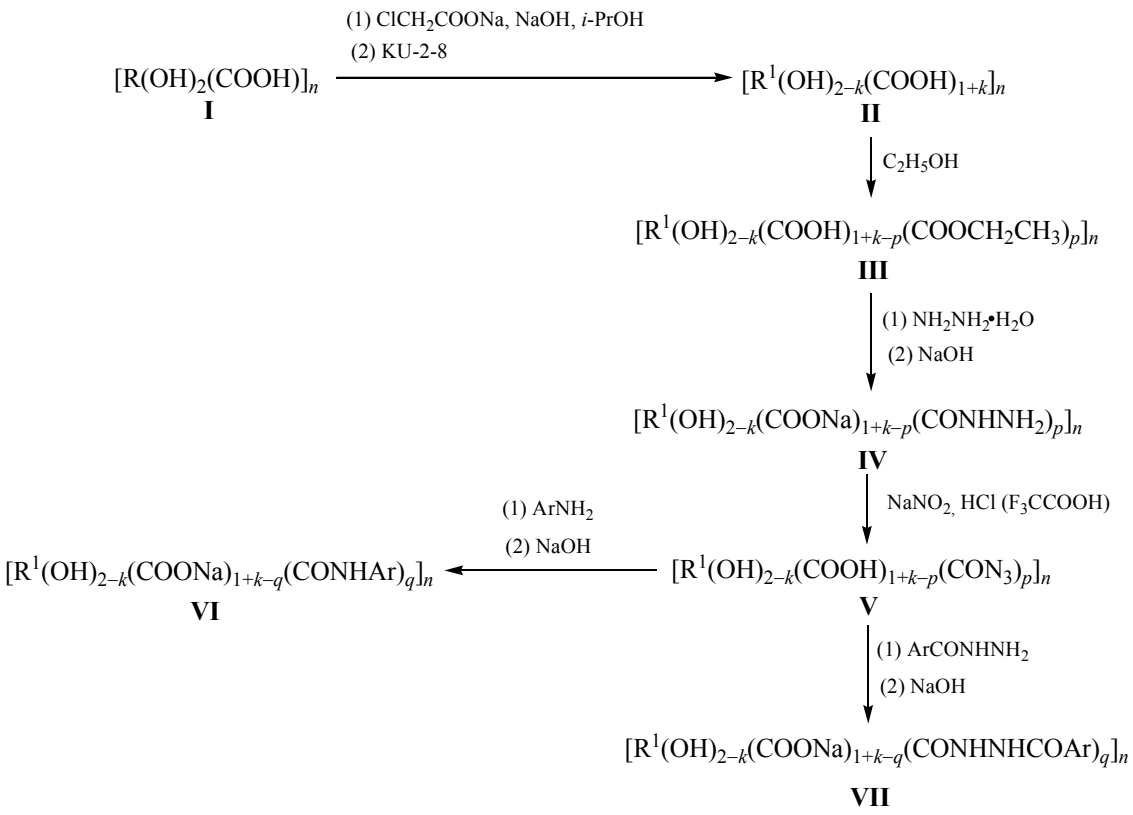
Incorporation of azide fragment into the polymer was confirmed by IR spectra of products **V**: azide group stretching vibrations band appeared at 2167 cm^{-1} along with a band characteristic of carboxylic groups (1737 cm^{-1}).

Since the carboxylic acids azides are unstable in aqueous solutions [7, 8], it was of interest to study the hydrolytic stability of the prepared carboxymethylalginic acid azides as a function of pH and temperature. The collected results on kinetics of decrease in the amount of the azide groups are shown in Fig. 1.

From the data in Fig. 1 it is seen that at 0°C and pH 2 the carboxymethylalginic acid azide was moderately stable: 80% of the initial azide groups were found in the specimen 2 h after the dissolution (curve *1*). The increase in the solution pH led to deterioration of the azide stability (curves *3* and *4*), and at pH 10 the equilibrium residual amount of azide groups (8%) was attained within 20 min after the polymer dissolution. Heating from 0°C to 20°C only slightly accelerated the azide groups hydrolysis (cf. curves *1* and *2*).

Rate of hydrolysis of the prepared carboxymethylalginic acid azides was lower than that of alginic acid

Scheme 1.



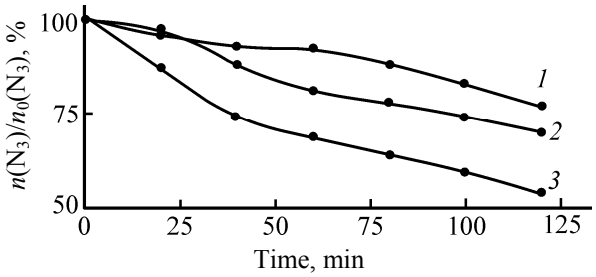


Fig. 2. Kinetics of azide groups hydrolysis in aqueous solutions of azides of (*1*) sulfated alginic acid (C_{AZ} 0.5), (*2*) carboxymethylalginic acid (C_{CM} 1.2, C_{AZ} 0.3), and (*3*) alginic acid (C_{AZ} 0.345); pH 2, 20°C.

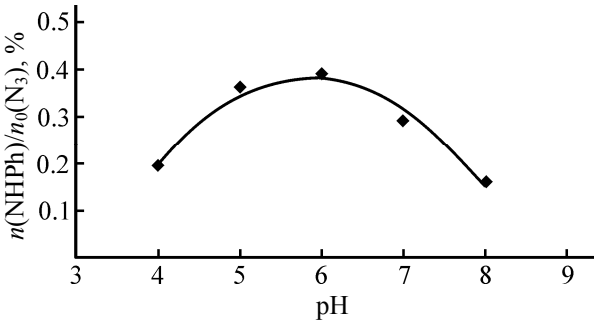


Fig. 3. Conversion of azide groups into amide ones in the reaction of carboxymethylalginic acid azide (C_{CM} 1.25, C_{AZ} 0.3) with aniline as function of pH (20°C, 24 h).

azide [8] but higher than in the case of sulfated alginic acid azide [7] (Fig. 2, data for pH 2 and 20°C).

Acylation activity of carboxymethylalginic acid azides was estimated from the yield of their reactions with aromatic amines. The yield of the corresponding amides in the case of polyacids azides is known to depend both on the amine nucleophilicity and the medium acidity [7, 8]. We determined the yields of the acylation reaction at various pH; typical experimental results are shown in Fig. 3 (acylation of aniline with carboxymethylalginic acid). The highest degree of conversion of the polymer azide groups (39%) was attained at pH 6, the result being in agreement with the outcome of similar experiments with alginic acid azide [8] and sulfated alginic acid azide [7].

In general, weak bases showed higher reaction yields in the acidic medium, whereas the yield of amidation products of strong bases was increased in neutral and alkaline solutions. The optimal pH value of amidation of amines bearing *para*-positioned electron-donor (CH_3, OC_2H_5) or electron-withdrawing (NO_2,Cl) substituents was chosen basing on the published data [5, 8].

The acylation products were white with cream-shade amorphous powders, readily soluble in neutral and alkaline aqueous media and insoluble in the studied organic solvents, including ethanol and acetone.

IR spectra of the prepared amides (after acidic treatment) contained the absorption bands at 1630–1640 ($\nu_{C=O}$, amide I), 1550–1560 (δ_{N-H}, amide II), 730–770 (δ_{C-H}, monosubstituted benzene ring), 810–840 (δ_{C-H},

Table 1. Yields in amidation reaction (20°C, 24 h) between carboxymethylalginic acid azide (C_{AZ} 0.3) and various *N*-nucleophiles

Nucleophile	pH_{opt}	λ_{max} (aqueous 0.1 mol/L NaOH), nm		ω_{max}, %
		substrate	product	
4-Ethoxyaniline	7	233	244	54
4-Methylaniline[a]	6	232	241	48
3-Methylaniline[a]	6	230	241	44
2-Methylaniline[a]	6	–[b]	–[b]	40
Aniline	6	229	238	38
4-Chloroaniline	4	240	247	27
4-Aminobenzoic acid	4	259	255	14
3-Aminobenzoic acid	4	294	269	11
2-Aminobenzoic acid	4	324	288	7
4-Nitroaniline	2	363	319	8
Isonicotinic acid hydrazide	2	296	303	67
Nicotinic acid hydrazide	2	276	286	58
Picolinic acid hydrazide	2	274	300	43
Salicylic acid hydrazide	2	266	281	77

[a] Amidation degree was determined by Nessler method. [b] ("–") no absorption.

Table 2. Yields in amidation reaction (20°C, optimal pH) between selected *N*-nucleophiles and azides of carboxymethylalginic acid (C_{AZ} 0.3), alginic acid (C_{AZ} 0.345), and sulfated alginic acid (C_{AZ} 0.5)

Nucleophile	ω_{max}, %		
	carboxymethylalginic acid	alginic acid [6]	sulfated alginic acid [7]
4-Ethoxyaniline	54[a]	36[b]	27[a]
4-Methylaniline[c]	48[a]	34[b]	26[a]
Aniline	38[a]	38[b]	39[a]
4-Chloroaniline	27[a]	21[b]	16[a]
Isonicotinic acid hydrazide	67[a]	0	64[b]

[a] Reaction time 2 h. [b] Reaction time 24 h. [c] Amidation degree was determined by Nessler method.

disubstituted benzene ring), and 1736 ($\nu_{C=O}$, carboxyl) cm^{-1}. UV spectra of the products contained the bands typical of aromatic fragments (Table 1). The spectral parameters of the substrates and the products were somewhat different, whereas the spectra of carboxymethylalginic acid amides and the corresponding acetanilides were identical, thus allowing determination of the reaction yields and the products purity from UV spectroscopy data.

Similarly to the case of alginic acid azides [8], the degree of conversion of the azide groups into the amide ones in the reaction with aromatic amines at optimal medium pH (ω_{max}, Table 1) was determined by the nature and position of the substituents in the benzene ring. In general, the electron-donor substituents increased the product yield, whereas the electron-acceptor groups decreased ω_{max}, in agreement with variation of the amines nucleophilicity. For example, the amidation degree showed a linear dependence on the σ_p constant of the substituent (the Hammett's equation): $\omega_{max} = -0.5\sigma_p + 0.402$ ($n = 5$, $R^2 = 0.98$).

Carboxylic acids hydrazides were stronger nucleophiles as compared with aromatic amines; furthermore, the degree of amidation in the reactions with hydrazides was independent on the medium pH [6]. In view of that, acylation of carboxylic acids hydrazides with carboxymethylalginic acid azide was studied at pH 2, under conditions of the highest stability of the azide.

As compared with azides of alginic acid [8] and of sulfated alginic acid [7], azides of carboxymethylalginic acid were more reactive towards acylation (Table 2). This can be assigned to the presence of carboxymethyl groups in the studied polysaccharides, stronger acylation agents than uronic acids.

EXPERIMENTAL

We used commercial alginic acid (Acros organics) with molecular mass of four major fraction up to 200000; the carboxylic groups content in the purified sample was 97% of the nominal value.

The substitution degree C_{AZ} (number of azide groups per monosaccharide unit) was determined by means of spectrophotometry after treatment with hydroxylamine and iron(III) chloride [9].

Degree of conversion of acylation giving *N*-arylamides (**VI**) and *N*-acylhydrazides (**VII**) of carboxymethylalginic acid was determined from UV spectroscopy data using the corresponding acetanilides and acetylhydrazides for calibration.

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

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