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ORGANIC SYNTHESIS AND INDUSTRIAL ORGANIC CHEMISTRY

Alginic Acid Azide and Its Reaction with Aromatic Amines

S. I. Anson, E. V. Novikova, and A. A. Iozen

St. Petersburg State Chemical Pharmacutical Academy, St. Petersburg, Russia

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Abstract—Alginic acid azide is synthesized and its stability in aqueous solutions in dependence on keeping duration and solution pH is studied. Reaction of the azide with aromatic amines is explored.

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Among the currently used methods of designing physiologically active polymers the "azide" method is of wide application. It consists in transformation of polysaccharide carboxylic acids into respective azides followed by using them for acylation of amines that allows fixation of biologically active compounds (e.g., antibiotics, ferments) of low stability toward heating on the polysaccharide matrix. As the polymeric carboxylic acids can be used carboxymethyl and carboxyethyl derivatives of neutral polysaccharides and natural polyacids such as alginic acid that is of wide application in food and cosmetics industry as well as in medicine [1]. Unfortunately chemical properties of alginic acid are studied poorly that retards its wide application to the synthesis of physiologically active polymers.

The purpose of this work is synthesis of alginic acid azide and study of its reactivity.

EXPERIMENTAL

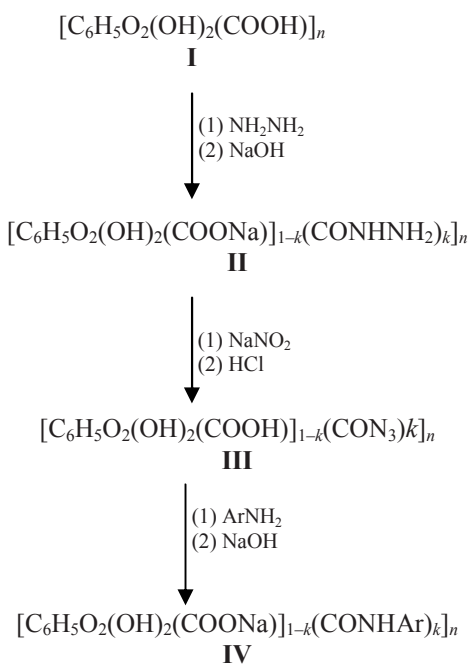
Alginic acid was chemically modified along the Scheme 1.

Alginic acid hydrazides **II** were prepared by heating the H^+ form of polysaccharide **I** in hydrazine hydrate medium for 6–10 h [2]. The alginic acid azides **III** were prepared by dissolving the hydrazides in 1 N aqueous solution of sodium nitrite followed by adding hydrochloric acid to pH 2 and keeping the reaction mixture at 0°C from 5 to 120 min. The reaction product was used for acylation of amines commonly without its isolation from the solution. For the registration of IR spectra the azide was precipitated from the solution by adding ethanol, washing with ethanol and drying in a vacuum without heating.

The samples of alginic acid azides separated from the solution are white with pink nuance amorphous powders soluble in alkaline water solutions, insoluble in most organic solvents.

The presence of azide groups in the modified polysaccharide was confirmed by IR spectroscopy, by

Scheme 1.



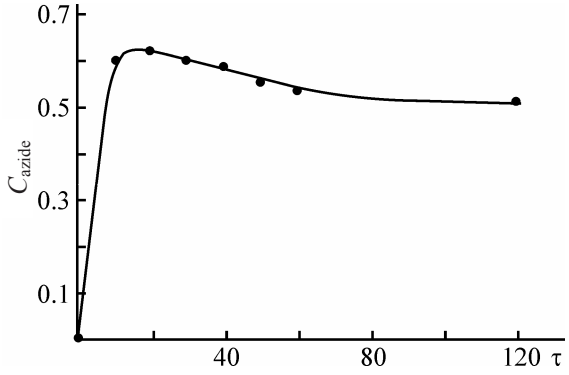


Fig. 1. Effect of duration τ (min) of the reaction of alginic acid hydrazide with nitric acid on the number of azide groups C_{azide} in the polymer.

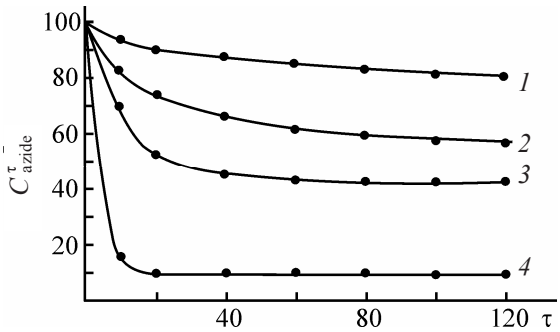


Fig. 2. Plot of azide group content in the samples of alginic acid C^{τ}_{azide} (% of initial C_{azide}) on the keeping duration τ (min) of their solutions at 20°C and pH 10: (*1*), 6 (*2*), 2 (*3*), and (*4*) at pH 2 and 0°C.

appearance of absorption band $\nu(N_3) = 2130$ cm^{-1} [3]; therewith disappeared the absorption bands characteristic of hydrazide function.

The number of azide groups in the polymer was determined by spectrophotometry using hydroxamic reaction after adding hydroxylamine in alkali and iron(III) chloride in hydrochloric acid to the solution of azide [4].

The alginic acid samples were characterized by the degree of substitution C_{azide}, that is, by the number of azide groups per a monosaccharide fragment. At the study of reaction of alginic acid hydrazides with nitrous acid we found that a 20 min period is sufficient for the reaction completing (Fig. 1). Elongation of keeping alginic acid azide in solution leads to certain decrease in the number of azide groups in the polymer that obviously is due to its damaging.

In this connection, we studied stability of aqueous solutions of alginic acid azides at the varied values of pH, reaction duration, and temperature. We found that the azide number in the polysaccharide samples capable of acylation depends considerably on the medium pH and keeping duration. The increase in pH from 2 to 6 leads to decrease in the azide groups content from 83% to 71%, and at pH 10 remain only 15% of azide groups. Increase in temperature from 0 to 20°C also decreases stability of alginic acid in aqueous solution. For example, at pH 2 at 20°C after 20-min keeping, the polymer contains 74% of azide groups and after 2 h only 54% of the initial amount, while at the same pH and 0°C the 2-h keeping leads to damage of only 20% of azide groups (Fig. 2).

At the comparison of properties of alginic acid azides and azides of carboxyethyl- and carboxymethyldextran [5, 6] we found (Fig. 3) that the alginic acid and carboxymethyldextran (CMD) azides are less stable than the related derivatives of carboxyethyldextran. Therewith, the azide groups in CMD suffered damaging faster than those in alginic acid azide. For example, after 120 min the CND samples contained 40% of azide fragments, the samples of alginic acid up to 60%.

The decrease in the number of azide groups in the aqueous solutions of alginic acid azides can be a result of either their hydrolysis affording respective carboxylic acids or rearrangement with formation of isocyanates and the products of the isocyanate hydrolysis. For elucidation of the reaction pathway were taken the samples of alginic acid azide with degree of substitution 0.259, 0.325, and 0.70. An aqueous solution of a sample was kept at pH 10 at 20°C achieving negative reaction for the azide groups. Each polysaccharide sample formed was isolated as descrybed above and studied by the methods of iodometric and conductometric titration. From the obtained data were determined the degrees of substitution with hydrazide groups $C_{hydrazide}$ (the number of hydrazide groups per one monosaccharide fragment of the polymer) and with carboxyl groups $C_{carboxyl}$ (the number of carboxyl groups per one monosaccharide fragment of the polymer). We found that the polysaccharide sample obtained contained both hydrazine and carpoxyl groups (Table 1). Moreover, with increase in $C_{hydrazide}$ the fraction of hydrazide groups that did not entered to the reaction with nitrous acid

Table 1. Results of hydrolysis of alginic acid azides

$C_{hydrazide}$ of initial hydrazides	Characteristics of samples after keeping azide in water at pH 10, 20°C, for 24 h		
	C_r	content of residual hydrazide groups, %	$C_{carboxylic}$
0.259	0.008	3.4	1.00
0.325	0.019	5.8	0.98
0.700	0.096	13.7	0.90

grew. Probably some of them are difficultly available for the reagent or are bound.

In the IR spectra of the alginic acid samples are observed absorption bands at 1618 cm^{-1} characteristic of carboxylate anion [3]. This also confirms the assumption about the hydrolysis of azide groups to carboxyl ones.

To reveale the reactivity of alginic acid azides we studied their reaction with aromatic amines. The acylation was carried out under conditions that had been determined earlier at the acylation of aromatic amines with carboxyalkylpolysaccharide azides [6].

To the obtained solution of alginic acid azide was added carbamide for binding nitrous acid excess, and 5-fold excess of respective amine, and the medium pH was brought to an optimal value [6]. The mixture was kept at 15–20°C from 1 to 24 h. The reaction product was precipitated by adding ethanol and reprecipitated from 0.1 N solution of NaOH for decomposition of the amine salt; the salt absence was confirmed by TLC. The *N*-arylamides obtained were dried in a vacuum (20–25 mm Hg) at 61°C.

The products are formed in the Na^+ form; they are yellowish-brown amorphous powder compounds well soluble in water, insoluble in ethanol, acetone, diethyl ether and most other organic solvents.

In the IR spectra of the sodium salts of alginic acid *N*-arylamides there is a strong band of absorption of carboxylate anion (1618 cm^{-1}) that retards detection and identification of amide groups. In the IR spectra of the amides after treatment with acid absorption bands occur at 1540–1550, 1660–1680, and 1490–1610 cm^{-1} characteristic of δ(N–H), "amide-II," ν(C=O), "amide-I" and benzene ring ν(C=C) respectively that are not observed in the spectra of the parent polysaccharides, and remains the absorption band of carboxyl group at 1737 cm^{-1} [3].

Table 2. Results of reactions of alginic acid azide (prepared from hydrazide with $C_{hydrazide}$ = 0.345) with aromatic amines at 20°C for 24 h

Aromatic amine	λ_{max} of solutions in 0.1 N NaOH		C_{amide}	Hammett σ-constant of substituent
	amine	alginic acid hydrazide		
4-Methoxy-aniline	231	245	0.129	–0.268
4-Ethoxyaniline	233	244	0.123	–0.240
4-Methylaniline	232	241	0.117	–0.170
Aniline	229	238	0.130	0
4-Bromoaniline	240	247	0.072	0.232
4-Ethoxy-carbonylaniline	283	265	0.046	0.450

At the analysis of UV spectra of solutions in 0.1 N NaOH of the synthesized alginic acid *N*-arylamides and respective substituted acetanilides we found that their maxima coincide (Table 2). The alginic acid azides do not absorb in this region, and the parent amines absorb with another values of absorption maxima. Hence, the method of UV spectroscopy can be applied for both analysis of the synthesized compounds and checking their purity. For construction of the calibrating plots were used the respective acetanilides.

The alginic acid *N*-arylamides were characterized by the degree of substitution C_{amide}, the number of

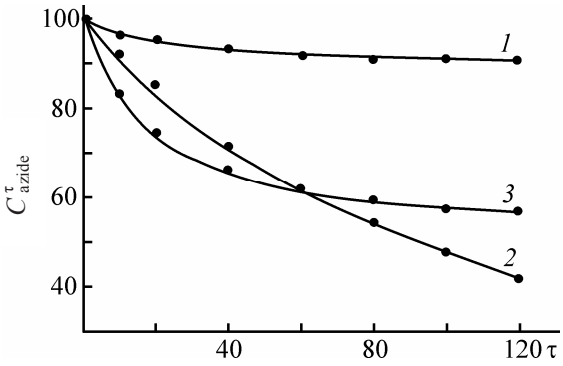


Fig. 3. Stability of azides of (*1*) carboxyethyldextran, (*2*) carboxymethyldextran, and (*3*) alginic acid in aqueous solutions at 20°C and pH 2. C^{τ}_{azide} is azide group content (% of initial C_{azide}), τ is keeping duration (min).

amide groups per one monosaccharide fragment that was calculated from the data of UV spectrophotometry.

In correspondence with our assumptions, the results of reactions of amines with alginic acid azides depend on the amine nucleophilicity. Like the case of carboxyalkylpolysaccharides [6], electron-donor substituents of the benzene ring of amine accelerate this reaction while electron-withdrawing ones retard it, therewith the plot of C_{amide} on σ (Hammett constant) of substituents is linear:

$$Y = -0.1131x + 0.0974, \quad R^2 = 0.999.$$

CONCLUSIONS

(1) In the reaction of alginic acid hydrazides with sodium nitrite in acid medium azides are formed that are the most stable at 0°C and pH 2. At elevated temperature and pH value of the azides in alginic acid azide solution are hydrolyzed to carboxylic acids.

(2) Alginic acid azides can be used for acylation of aromatic amines, therewith electron-donor substituents of the benzene ring of amine accelerate this reaction while electron-withdrawing ones decelerate it.

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