ISSN 1070-4272, Russian Journal of Applied Chemistry, 2009, Vol. 82, No. 10, pp. 1867−1870. © Pleiades Publishing, Ltd., 2009.
Original Russian Text © V.G. Dudarev, A.A. Iozep, 2009, published in Zhurnal Prikladnoi Khimii, 2009, Vol. 82, No. 10, pp. 1715−1718.

ORGANIC SYNTHESIS AND INDUSTRIAL ORGANIC CHEMISTRY

Synthesis of Hydrazide, Azide and Substututed Amides of Carboxymethylchitin

V. G. Dudarev and A. A. Iozep

St. Petersburg State Chemical-Pharmaceutical Academy, St. Petersburg, Russia

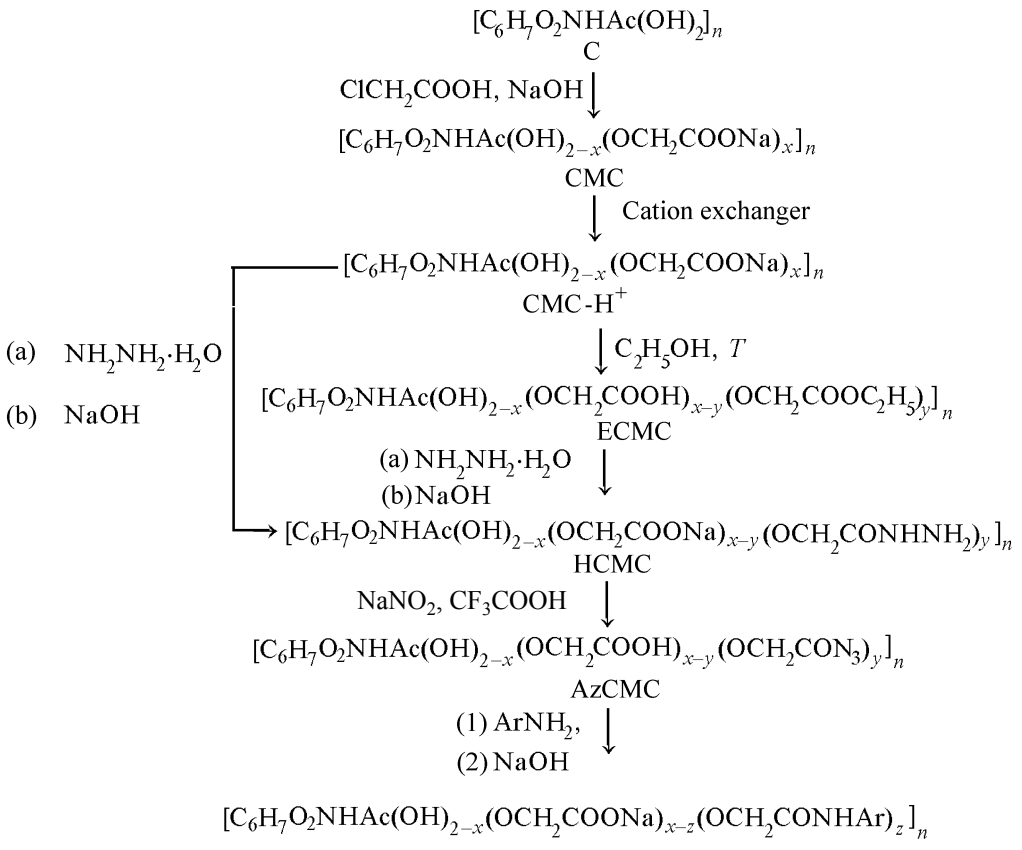
Received April 29, 2009

Abstract—Synthesis of hydrazide, azide and substututed amides of carboxymethylchitin is reported. Stability of azides in aqueous solution and their acylating ability in the reactions with aromatic amines are studied.

DOI: 10.1134/S1070427209100231

Chitin and its derivatives possess important properties and are used as auxiliary and biologically active compounds in medicine, pharmacy and cosmetics [1, 2]. Insolubility in water restricts essentially chitin application area; therefore modification of polymer with hydrophilic groups is actual problem. Carboxymethylation of chitin is a most studied process allowing synthesis of water-soluble polysaccharide derivatives [1]. Carboxyl groups

Scheme.



Ar: *p*-$CH_3OC_6H_5$, C_6H_5, *p*-BrC_6H_5, *p*-$H_5C_2OOCC_6H_5$.

Table 1. Quantitative characteristics of carboxymethylchitin hydrazides[a]

C_{cm} of the CMC sample	C_{acm} of ethyl ester	C_{hcm} of hydrazide of CMC found by	
		hydroxame reaction	iodometric titration
0.77	0.4	0.40	0.48
0.95	0.32	0.30	0.35
1.02	0.34	0.29	0.30
1.04	0.32	0.32	0.35

[a] C_{cm}, C_{acm} are the numbers of the carboxymethyl and alkoxycarbonylmethyl groups attributable to one monosaccharide fragment of CMC.

introduced by this way into polymer can be used for the further chemical modification of chitin. However their reactivity is poorly examined.

The purpose of this work is synthesis of hydrazide and azide of carboxymethylchitin and also studying an azide stability and reactivity.

A chemical modification of chitin was carried out by the scheme.

Carboxymethylchitin (CMC), its H^+ form and ethyl ester were prepared according to an earlier reported technique [3]. Hydrazide of CMC was obtained by the heating of carboxymethylchitin polyacid with hydrazine hydrate excess at 100°C for 4 h or by work up of ethyl ester of CMC with hydrazine hydrate excess at room temperature for 24 h. The product was precipitated by ethanol, reprecipitated from 0.2 N solution of sodium hydroxide by ethanol, and dried in a vacuum. Yield was 90%.

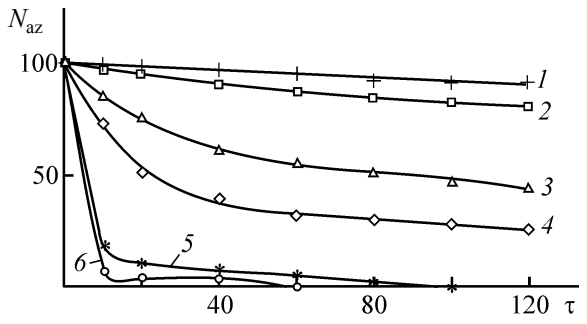


Fig. 1. Dependence of the content of the azide groups N_{az} in CMC samples (% from the number of the azide groups in polysaccharide at the time of formation) vs. the keeping time τ (min) of their solutions at 0°C. pH values: (*1*) 2, (*2*) 4, (*3*) 6, (*4*) 8, (*5*) 10, (*6*) 12.

Samples of CMC hydrazide obtained by the heating of polyacid with the hydrazide excess were insoluble in water, though, according to iodometric titration data, a transformation extent of the carboxylate groups into hydrizide was about 30%. Earlier similar result was observed at the heating of carboxymethyl derivatives of some other polysaccharides. It was explained by a diacylhydrazines formation [4]. In this connection hereafter CMC hydrazide was synthesized from its ethyl ester.

CMC hydrazide is a light-brown powder soluble in water. By comparing the IR spectra of CMC salt and hydrazide proved that an intensity of absorption bands "amide **I** and **II**" was more in the hydrazide spectra. They are more visible on a $\nu(COO^-)$ band background.

Hydrazide samples were characterized by hydazinocarbonylmethylation extent C_{hcm} (a number of the hydrazide groups accounting for a monosaccharide fragment of chitin), which was determined by the iodometric titration [4, 5] and by means of spectrophotometry method based on a hydroxame reaction [4, 5] (Table 1).

To obtain carboxymethylchitin azide (CMCA) an aqueous solution of HCMC was treated with solutions of trifluoroacetic acid and sodium nitrite for 30 min at 0°C. The product was precipitated with acetone and dried under a vacuum at 18–25°C. CMC azide represents a light-brown amorphous powder, soluble in water and insoluble in acetone. The IR spectrum of azide contains absorption band $\nu(N_3)$ at 2160 cm^{-1}.

To determine conditions for the reactions of azide with amine, we examined stability of aqueous solutions of CMCA depending on time, temperature and pH (Fig. 1). Concentration of the azide groups in the solutions was determined using the hydroxame method [4].

It was found that azides are fairly stable in strong-acidic aqueous solution at 0°C. As pH rises, azide stability decreases sharply. Thus, after 2 h from the formation time at pH 2 the number of the azide groups in the samples constituted about 90% from the initial value, at pH 4, 80%, pH 6, 45%, at pH 8, 26%. In the strong alkaline medium CMC azides decayed almost completely for 5 min. An increase in the solution temperature also reduces the azide stability. For example, after 2 h of the reaction at 17–22°C and pH 2 approximately 58% of the azide groups were remained; at pH 4, 22%; pH 6, 13%; pH 8, 7%.

These data allow comparing the azide stability of carboxymethylchitin, carboxymethyl and

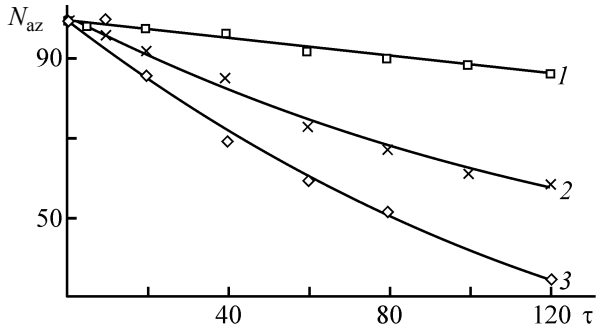


Fig. 2. The stability of carboxyethyldextran azides (*1*) [6], carboxymethylchitin (*2*) and carboxymetyldextran (*3*) [6] in the aqueous solutions at 20°C and pH 2; (N_{az}) content of the azide groups (% from the number of the azide groups in polysaccharide in the time of formation), (τ) time (min).

carboxyethyldextran in the aqueous solutions at pH 2 and 20°C (Fig. 2). We have found that CMC azide is more stable than carboxymethyldextran azide but less stable than carboxyethyldextran. Thus, after 2 h from the formation the number of the azide groups in carboxyethyldextran, carboxymethylchitin, and carboxymethyldextran were 86, 58 and 34% respectively.

For acylation of amine we used aqueous solutions of CMC azide resulted in its synthesis from HCMC. To a solution of azide was added 3–15 mol of amine per 1 mol of the azide group; pH value was adjusted to 1–10 with the aid of an acid or alkali. Ethanol was added for an increase in the amines solubility (30% from the reaction mixture volume), and the reaction was carried out at 0°C for 0.25–24. After keeping reaction mixture the product was precipitated with ethanol, reprecipitated from 0.1 N aqueous solution of sodium hydroxide in order to remove ion-bounded amine and dried under a vacuum.

CMC amides are white or light-brown colored amorphous powders, which are soluble in water, but insoluble in alcohol and acetone.

The IR spectra of CMC amides worked up with acid contain absorption bands characteristic for the substituted benzene ring in a range of 700–860, 1500 and 1600 cm^{-1}, and also bands "amide **I**" (1660 cm^{-1}), "amide **II**" (1550 cm^{-1}) and bands of carboxyl group (1730 cm^{-1}).

In the UV spectra of aqueous solutions of the synthesized amides there are absorption maxima characteristic for aromatic compounds. The values λ_{max} are coincided in the spectra of *N*-arylamides of CMC and corresponding acetanilides, but differ from λ_{max} values in the spectra of amines. Therefore UV spectrometry is convenient technique for a quantitative analysis and an estimation of purity of synthesized compounds. We used corresponding acetanilides for calibration plotting.

CMC amides were characterized by transforming extent of the azide groups into amide C_{am} (%). Along with we took into account that non-reacted azide groups were hydrolyzed to carboxylic acids in the course of reaction and isolation [4].

On studying of acylating ability of CMC azides in the reactions with aromatic amines, we suggested that the amine excess and medium acidity mostly effected on a completeness of transformation of the azide groups into the amide. A temperature can not be increased above 20°C. The reaction of CMC azide with aniline as well as with carboxymethyldextran [4] was almost completed for 30 min.

Indeed in the reaction of CMC azide with aniline at pH 6 upon the amine excess increased to 15 mol the transformation extent C_{am} rised to 75% (Fig. 3). The transformation extent of CMC azide into amide also depended on properties of a substituent in *p*-position and on pH in the reaction with aromatic amines (Table 2,

Table 2. Results of acylation of aromatic amine with CMC azide at 5 mol of amine per 1 mol of the azide group. The temperature 0°C, the reaction time 2 h

Amine	Optimum pH	C_{am}, %
p-Anisidine	5–7	70
Aniline	5–7	64
p-Bromaniline	3–5	60
Anesthesin	1	33

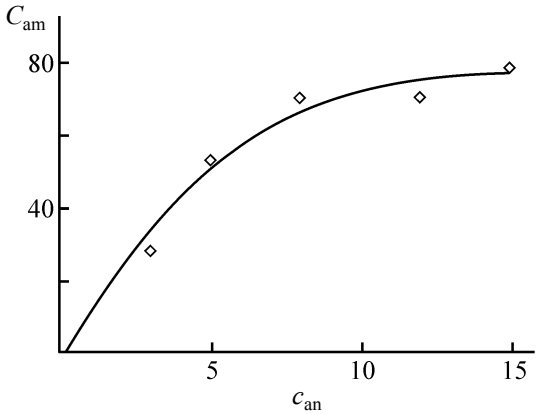


Fig. 3. Dependence of the transformation extent C_{am} (%) of CMC azide from the amine excess in the reaction with aniline. pH 6, the temperature 0°C, the reaction time 2 h, (c_{an}) aniline quantity (mol).

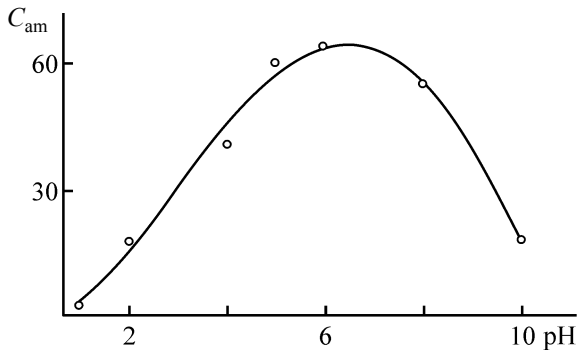


Fig. 4. Dependence of C_{am} (%) of CMC azide on pH in the reaction with aniline at 5 mol of amine per 1 mol of azide group. The temperature 0°C, the reaction time 2 h.

Fig. 4). In this case an electron-donating substituent increased C_{am}, on the contrary an electron-withdrawing substituent decreased. The stronger are substituent properties, the maximum values C_{am} are attained in the more acidic medium (Table 2).

In view of the enhanced stability of CMC azide in acidic media, the transformation extent of the azide groups into the amide may be increased by an increase in the reaction time. Thus, at pH 2, temperature 0°C and reaction time 24 h completion of the reaction of CMC azide with anesthesin was 60 % that was almost 2 times more than for 2 h (33%). Better results in the case of *p*-anisidine were obtained at pH 5–7 for 2 h (C_{am} 70%). An increase in the reaction time did not effect on the result. Presumable it is explained by the simultaneous hydrolysis of azide to carboxylic acid.

CONCLUSION

(1) Reaction of ethyl ester of carboxymethylchitin with the excess of hydrazine hydrate at room temperature forms hydrazide with 90% yield .

(2) Carboxymethylchitin hydrazide, reacting with sodium nitrite in acidic medium, transforms into azide. The latter is more stable at 0°C and pH 2.

(3) An aqueous solution of CMC azide can be used for acylation of aromatic amines at the adjustable pH values.

REFERENCES

1. Pestov, A.V. and Yatluk, Yu.G., *Karboksialkilirovannye proizvodnye khitina i khitozana* (Carboxyalkylated Derivatives of Chitin and Chitosan), Ekaterinburg: UrO RAN, 2007, 102 p.
2. Vikhoreva, G.A., Gorbacheva, I.N., and Gal'braikh, L.S., *Khim. Volokna,* 1999, no. 4, p. 25.
3. Dudarev, V.G. and Iozep, A.A., Abstracts of Papers, *Trudy nauchno-practicheskoi konferentsii "Farmatsiya iz veka v vek"* (Proceedings of Scientific-Practical Conference "Pharmacy Through The Ages", St. Petersburg, 2008, pp. 71–74.
4. Ponomarenko, M.N., *Cand. Sci. (Chem.) Dissertation,* St. Petersburg, 1998, 20 p.
5. Iozep, A.A., Kupriyanova, L.N., Ponomarenko, M.N., Ivin, B.A., and Passet, B.V., *Zh. Prikl. Khim.,* 1996, vol. 69, no. 9, p. 1537.
6. Krasnikova, A.V., *Cand. Sci. (Chem). Dissertation,* St. Petersburg, 2004, 23 p.