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PREPARATION OF ALKYL ESTERS OF BENZIMIDAZOL-2-YLCARBAMIC ACID

S. A. Khasanov, Ch. Sh. Kadyrov, and M. Akbarova

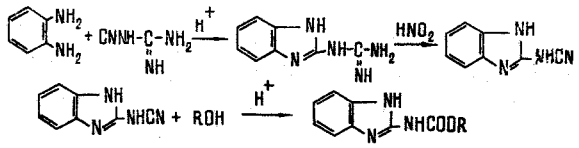
UDC 547.781+547.49+493

Alkyl esters of benzimidazol-2-ylcarbamic acid have been synthesized by the saponification of cyanamidobenzimidazole with various alcohols in the presence of concentrated hydrochloric acid.

2-Methoxycarbonylaminobenzimidazole possesses a high fungicidal activity [1-3]. A method has been described for obtaining methoxycarbonylaminobenzimidazole that is based on the reaction of chloroformic ester with cyanamide or its salts followed by the condensation of the resulting product with o-phenylenediamine. The yield of product is satisfactory.

2-Ethoxycarbonylaminobenzimidazole was synthesized in 1934 [4]. A defect of known methods for its preparation is the use of highly toxic substances or the formation of such substances during the reaction. The main methods of preparation have been described in a review [3].

We have developed a simpler method of obtaining alkyl esters of benzimidazol-2-ylcarbamic acid, by the scheme given below.



The method consists in condensing o-phenylenediamine with dicyandiamide to obtain guanidinobenzimidazole, which is converted after diazotization into 2-cyanamidobenzimidazole [5]. The latter readily undergoes alcoholysis with the formation of an alkyl benzimidazolylcarbamate.

We have used various alcohols in these ractions: the compounds CH_3OH to $C_8H_{17}OH$ of normal and iso structures, the alkoxyalcohols $HOCH_2CH_2OCH_3$ and $HOCH_2CH_2OC_2H_5$, and the halogenated alcohols $ClCH_2CH_2OH$ and $(ClCH_2)_2CHOH$. The alcoholysis of the 2-cyanamidobenzimidazole with alcohols was first carried out at a ratio of the reagents 2-cyanamidobenzimidazole: alcohol:hydrochloric acid of 1:1:1 (4 h, 60°C). Under these conditions the reaction did not take place at all and the initial 2-cyanamidobenzimidazole was recovered unchanged. The performance of the reaction under milder conditions led to the formation of 2-methoxycarbonylaminobenzimidazole. At a ratio of the reagents 2-cyanamidobenzimidazole:methanol:hydrochloric acid of 1:10:2 (3 h, 60°C), the yield of product was 62%. Methyl benzimidazol-2-ylcarbamate

Institute of The Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 704-706, September-October, 1979. Original article submitted May 25, 1979.

0009-3130/79/1505-0619$07.50

obtained by saponifying 2-cyanamidobenzimidazole with methanol gave no depression of the melting point with a known sample.

The activity of the nitrile group is increased in the presence of catalysts [6, 7].

In the presence of strong acids, alcohols giving carbonium ions play the role of electrophilic reagents in reaction with nitriles. It was of interest to consider the alcoholysis of 2-cyanamidobenzimidazole with other alcohols in order to study the influence of the structure of the alcohol on the yields of reaction products and to obtain a number of compounds possessing fungicidal activity.

At a ratio of the reagents nitrile:alcohol:hydrochloric acid of 1:5:2 (3 h, 80-100°C), depending on the structure of the alcohol, it was possible to isolate reaction products. Raising the reaction temperature (100°C) increased the yields of final product to 75%.

In the reactions with alcohols having an iso structure, a common feature was observed: at the same ratios of the reagents and the same conditions for performing the reaction the alcoholysis of nitriles took place more readily than with alcohols having the normal structure, and the yields of products were considerably higher. This is explained by the greater electron-donating capacity of the alkyl groups in branched alcohols than in normal alcohols, and also the greater basicity of the alcohols having the iso structure (secondary, tertiary) in comparison with alcohols of the normal structure.

Thus, alcohols can be arranged in the activity sequence tertiary > secondary > primary.

The saponification of 2-cyanamidobenzimidazole with halogen-containing alcohols gave low yields of products. This is connected with the decrease in the basicity of the alcohols due to the electronegative influence of the chlorine. Because of the negative inductive effect of the chlorine, dichloropropyl benzimidazol-2-ylcarbamate was obtained in lower yield (38%) than the corresponding unsubstituted ester. However, because this alcohol has a secondary structure, the yields were higher than in the case of ethylene chlorohydrin.

We studied the alcoholysis of 2-cyanamidobenzimidazole with alkoxyalcohols — methylcellosolves and ethylcellosolves. When the condensation was performed at a ratio of the reactants 2-cyanamidobenzimidazole:alkoxyalcohol:hydrochloric acid of 1:10:2 (3 h, 90-95°C) it was possible to isolate 2-alkoxyalkyl benzimidazolylcarbamates.

The structures of all the compounds obtained were shown by saponification with 10% caustic soda solution to 2-aminobenzimidazole on boiling for three hours.

The IR spectra lacked the absorption band of a nitrile group (2240 cm^{-1}) while the band of a carbonyl group appeared at 1690 cm^{-1} and a band corresponding to an NH group at 3290-3410 cm^{-1}.

The mass spectra of the alkyl benzimidazol-2-yl carbamates contained the peaks of the molecular ions the fragmentation of which then proceeded predominantly with the formation of strong peaks having m/e 159, 133, and 108.

TABLE 1. Alkyl Benzimidazol-2-ylcarbamates [8]

R	mp, °C	Yield, %	Empirical formula
Methyl	334—336	70	$C_9H_9N_3O_2$
Ethyl	324—326	75	$C_{10}H_{11}N_3O_2$
Propyl	315—318	60	$C_{11}H_{13}N_3O_2$
Isopropyl	326—329	63	$C_{11}H_{13}N_3O_2$
Butyl	318—320	50	$C_{12}H_{15}N_3O_2$
Isobutyl	348—350	52	$C_{12}H_{15}N_3O_2$
t-Butyl	212	57	$C_{12}H_{15}N_3O_2$
Amyl	192 194	70	$C_{13}H_{17}N_3O_2$
Isoamyl	333—335	71	$C_{13}H_{17}N_3O_2$
Hexyl	303—305	62	$C_{14}H_{19}N_3O_2$
Hepthyl	285—287	55	$C_{15}H_{21}N_3O_2$
Octyl	330—332	50	$C_{16}H_{23}N_3O_2$
Dichloropropyl	349—350	38	$C_{11}H_{11}N_3O_2Cl_2$
β-Chloroethyl	290—295	29	$C_{10}H_{11}N_3O_2Cl$
β-Methoxyethyl	302—304	34	$C_{11}H_{13}N_3O_3$
β-Ethoxyethyl	280—293	32	$C_{12}H_{15}N_3O_3$

EXPERIMENTAL

Alkyl Benzimidazolylcarbamates. To 0.018 mole of 2-cyanamidobenzimidazole and 0.1 mole of the appropriate alcohol was added 0.036 mole of concentrated hydrochloric acid. The reaction was carried out at the boiling point of the reaction mixture for 3 h. During the boiling of the mixture, crystals of the alkyl benzimidazolylcarbamate gradually separated out. The yields, melting points, and other characteristics of the products are given in Table 1. The analyses of the compounds obtained for nitrogen agreed with the calculated figures.

SUMMARY

A method has been developed for obtaining alkyl esters of benzimidazolylcarbamic acid using alcohols of various structures. An advantage of the method is that it has been possible to avoid the employment of toxic lachrymatory reagents and to use readily available substances.

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SOME KINETIC FEATURES OF THE HYDROLYSIS OF L-ASPARAGINE WITH *E. coli* ASPARAGINASE

I. A. Milman, I. I. Geiman,
V. A. Slavinskaya, I. A. Vina,
and R. A. Zhagat

UDC 577.155

The influence of various effectors (methanol, neutral salts) on the kinetic parameters K_M and k_{cat} has been studied. The hypothesis has been expressed that chloride ions are responsible for the worsening of the binding of the substrate to the enzyme. The temperature dependence of the kinetic parameters K_M and k_{cat} for the enzymatic hydrolysis of L-asparagine has been obtained. It has been shown that the graph of log k_{cat} versus 1/T has a break at 30°C. The effective activation energies below and above the critical point are 6.5 and 3.6 kcal/mole, respectively.

The kinetics of the enzymatic hydrolysis of L-asparagine have been little studied. In an investigation of the influence of hydroxylamine on the hydrolysis of L-asparagine, Ehrman et al. [1] put forward a hypothesis of a three-stage mechanism of the reaction. O'Leary [2] also assumed that L-asparaginase operates by a mechanism similar to that for chymotrypsin and other serine proteases:

$$E + S \rightleftarrows ES \rightarrow EA \rightarrow E + P.$$

However, the possibility of the formation of an intermediate acylenzyme (EA) has not hitherto been definitively demonstrated.

We have determined the kinetic parameters of the hydrolysis of L-asparagine in the presence of various effectors capable of selectively acting on one of the possible stages of the

Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR, Riga. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 707-709, September-October, 1979. Original article submitted April 12, 1979.

0009-3130/79/1505-0621$07.50