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Mechanism of Ring Opening Reaction of 4-Benzylidene-2-methyl-5-oxazolone. Part I. Reaction with n-Butylamine

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The reaction between 4-benzylidene-2-methyl-5-oxazolone (Ox, $c = 5.25 \times 10^{-5}$ M) with n-butylamine (nBuA, c = 0.026 to 1.66 M) in acetonitrile was studied by UV-Vis spectroscopy using steady-state and stopped-flow apparatus. The amine to Ox molar ratio higher than 500 allows to apply pseudo-first order approximation, whose rate constants depend on amine concentration according to a parabolic equation: $k_{obs} = 4.63 \pm 0.02 \times C^2_{nBuA}$, indicating a complex reaction mechanism. On the other hand, singular value decomposition (SVD) analysis, as well as global analysis of the data obtained from stopped-flow measurements support a simple A → B reaction model. Nonlinear dependence of the pseudo-first order rate constant on amine concentration can be explained, assuming that the oxazolone ring opening reaction by n-butylamine in acetonitrile proceeds as parallel reactions: A + B → C and A+2B → C. Based on our experimental results and theoretical calculations of the oxazolone ring opening reaction, a mechanism of the reaction between 4-benzylidene-2-methyl-5-oxazolone and n-butylamine is proposed.

Key words: 4-benzylidene-2-methyl-5-oxazolone, n-butylamine, stopped-flow, reaction mechanism, dehydropeptides, kinetics, activation parameters

α,β-Dehydroamino acids occur frequently in nature as components of a variety of fungally and bacterially derived peptides (metabolites, polycyclic antibiotics-lantibiotics) [1,2]. Dehydroamino acids can introduce both side chain and backbone structural constraints within peptides that leads to attractive targets for conformational studies [3,4]. Oxazolones, the activated derivatives of carboxylates formed from amino acids, are applied for the peptide bond formation. They are especially widely used in synthesis of dehydropeptides. 4-Benzylidene substituted oxazolones are very suitable intermediates for synthesis of dehydrophenylalanine containing peptides (dehydropeptides) [5,6]. The ring opening reactions of oxazolones by nucleophiles were also studied by semiempirical MNDO-based state-of-the-art MO (AM1 and PM3) methods [7–10]. 4-Benzylidene-2-methyl-5-oxazolone exhibits a characteristic absorption band at 325 nm, which disappears after treatment with a nucleophile during the ring opening reaction. In this study we used the spectrophotometric methods to investigate the kinetics and mechanism of the reaction. Different reaction

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models of the ring opening reaction of 4-benzylidene-2-methyl-5-oxazolone by n-butylamine and possible mechanisms are discussed.

EXPERIMENTAL

Synthesis: The procedure of the preparation of 4-benzylidene-2-methyl-5-oxazolone was adopted from [11]. 15 g (0.183 mole) of sodium acetate and 59 ml (0.62 mole) of acetic anhydride were added to a roundbottom flask (500 ml) containing 29 g (0.25 mole) of acetylglycine and 37.5 ml (0.37 mole) of benzaldehyde. The resulting mixture was refluxed under nitrogen for 1.5 h. After that the solution obtained was cooled in a refrigerator for crystallization. The crystals formed were filtered, washed with water and dried. Recrystallization from carbon tetrachloride yielded 35 g of the oxazolone (76%); m.p. = 150°C. Elemental analysis for $C_{11}H_9NO_2$: C, 70.56; H, 4.84; N, 7.49; O,17.10. Found: C, 70.5; H, 4.85; N, 7.49; O, 17.09. IR (KBr), ν (cm^{-1}): 3070.1, 1776.2, 1655.7, 1601.9, 1492.6, 1450.8, 1425.9, 1303.6, 1263.7, 1230.5, 1167.8; ^{1}H-NMR ($CDCl_3$), δ (ppm): 2.43 (s, 3H, CH_3), 7.16 (s, 1H, -CH=C=), 7.45–8.09 (m, 5H, C_6H_5).

Methodology of kinetic measurements: The course of reaction of the ring opening of 4-benzylidene-2-methyl-5-oxazolone (Ox) with an amine (n-butylamine, nBuA) was studied by recording changes of an absorbance of the oxazolone and product in the reaction mixture. The rate of absorbance changes (for n-butylamine concentration in range 0.0068–0.026 M) were recorded for two wavelengths: λ = 325 nm (maximum of absorption of the substrate) and λ = 270 nm (maximum absorption of the product) using Specord UV/VIS Carl Zeiss Jena spectrophotometer equipped with 12-bites analogue-to-digital converter and the computer program Mult ver.3.12 (Ambex, Warsaw, Poland). For one experimental curve 4096 data points were collected. Rate constant calculations for different kinetic models we carried out using Sigma Plot ver. 5.0 program. For higher n-butylamine concentration (in the range 0.052–1.66 M) the stopped-flow technique was applied using Applied Photophysics, SX-17MV spectrophotometer and "Glinst" program supplied by manufacturer. Global analysis of the acquired data for 35 wavelengths in the range of 420 to 250 nm with 5 nm step for different reaction models was performed. The conditions of the studied reaction were: – the starting concentration of the oxazolone ($c = 5.25 \times 10^{-5}$M) was the same in all measurements, – the ring opening reaction was curried out in acetonitrile (MeCN) at temperature 298 K in all experiments except energy of activation measurements, – concentration of n-butylamine (nBuA) varied in the range of 0.026 to 1.66 M, – to estimate an activation energy of the studied reaction the measurements were performed in the temperature range 280.2–321.4 K (for amine concentration c = 0.026 M) and 277.9–333.0 K (for amine concentration c = 0.83 M), – the reaction was monitored until more than 98% of the substrate disappeared.

RESULTS AND DISCUSSION

The spectra of the oxazolone (Ox) – n-butylamine (c = 0.014 M) mixture in acetonitrile recorded at the wavelengths range 230–370 nm at different reaction times are shown in Fig. 1. Disappearance of a long-wave band with a maximum at λ = 325 nm (a band related to the substrate) and appearance of an absorption band at the wavelength range 250–285 nm, (connected with an absorption of a product) with an isosbestic point at λ = 295 nm are observed (Fig. 1). Additionally, it has to be mentioned that HPLC analysis of the reaction course discovered that the reaction's chromatogram is always a sum of the substrates and of one product only. Reaction rates (the oxazolone absorbance decays *versus* time) were analyzed assuming of a pseudo-first order kinetics, because of the 500 times higher concentration of the amine than that of the oxazolone. This assumption leads to:

$$v = k[Ox] \cdot [nBuA] = k_{obs}[Ox] \quad (1)$$

where [Ox], [nBuA] are the molar concentrations of the appropriate reactants and the pseudo-first order rate constant is given as $k_{obs} = k \cdot [nBuA]$. The integrated rate equation describing the decay of the absorbance of the Ox is:

$$A_{Ox}(t, \lambda = 325nm) = A^0_{Ox} \exp(-k' \cdot t) \quad (2)$$

The integrated rate equation describing the increase of the absorbance of the product is:

$$A_{pr}(t) = A^0_{pr}[1 - \exp(-k' \cdot t)] \quad (3)$$

where A^0_{pr} is the absorption of product at time t = ∞. Taking into account that in the range of wavelengths, where the product has a substantial absorbance (λ^{pr}_{max} = 270 nm) also absorption of Ox occurs, the above equation has to be modified:

$$A'_{pr}(t, \lambda = 270nm) = A_{pr}(t, \lambda = 270nm) + A_{Ox}(t, \lambda = 270nm) \quad (4)$$

The calculated values of the pseudo-first order rate constants for different n-butylamine concentrations are presented in Table 1. The obtained relationship between the pseudo-first order rate constant and amine concentration $k_{obs} = f(C_{nBuA})$ is illustrated in Fig. 2.

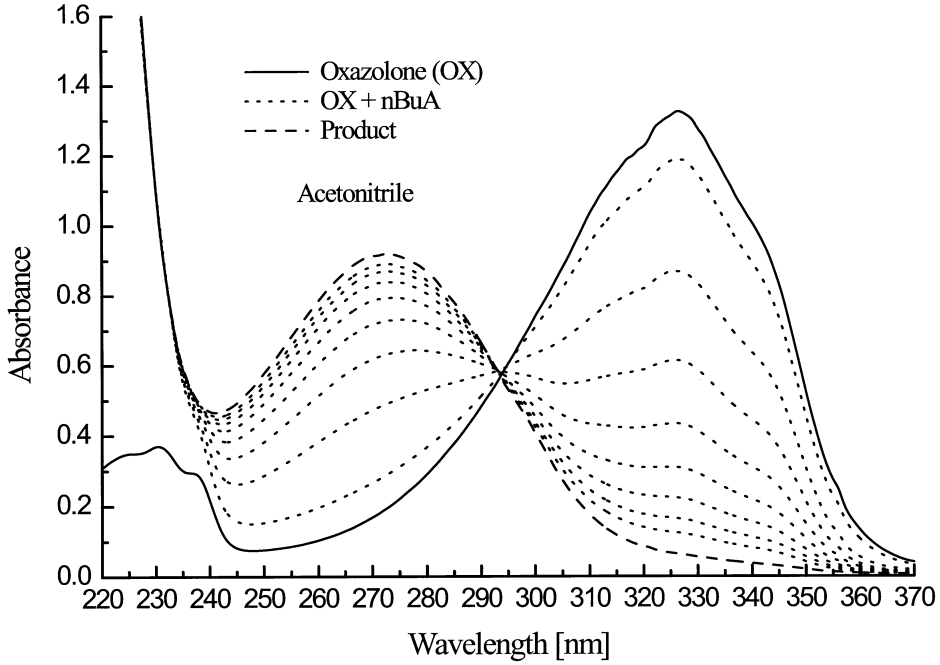

Figure 1. Reaction spectra of oxazolone and n-butylamine (c = 0.014 M) in acetonitrile.

Table 1. Rate constants for the Ox ring opening reaction obtained for different nBuA concentrations in acetonitrile at 298 K (for $C_{nBuA} \leq 0.026$ classical spectrophotometer was applied and rate constant is an average of rate constant of Ox disappearance and rate constant of product formation; for higher C_{nBuA} stopped flow technique was applied and the rate constant is a parameter obtained from a global calculation of 35 wavelengths from a range 420–250 nm; X^2 is a fitting quality parameter).

C_{nBuA} [M]	k`[1/s]	X^2
0.0068	0.00124	–
0.014	0.00378	–
0.026	0.00480	–
0.052	0.015	0.0395
0.104	0.08	0.0247
0.208	0.19	0.0257
0.42	0.69	0.0450
0.832	3.51	0.0366
1.66	13.5	0.0369

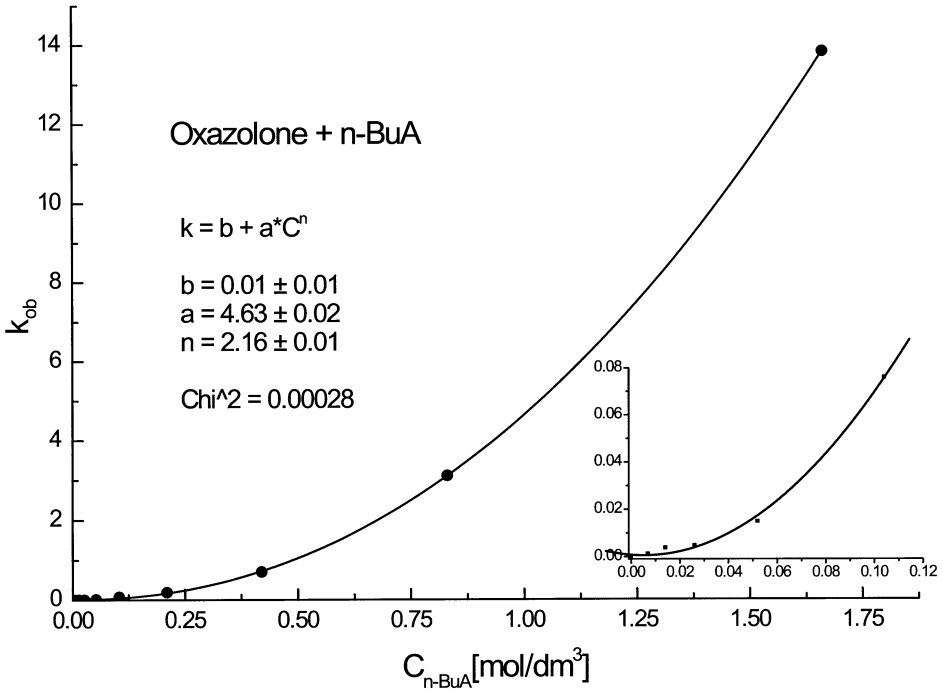

Figure 2. The dependence of rate constants on concentration of nBuA. The region of low concentration of nBuA (0 to 0.12 M) is expanded in a smaller graph inserted into lower right corner.

The dependence of k_{obs} *versus* C_{nBuA} as can be observed in Fig. 2 is nonlinear. The best fit (correlation coefficient R = 0.9985) was obtained for the following exponential function:

$$k' = a + b \cdot C_{nBuA}^{n} \tag{5}$$

with the fitted values: a = 0.01 ± 0.01, b = 4.63 ± 0.02, n = 2.16 ± 0.01. Thus, the dependence of the pseudo-first order rate constant on the amine concentration can be represented by:

$$k = b \cdot C_{nBuA}^{2} \tag{6}$$

When an isosbestic point is observed during the reaction, one can assume that it is simply a A→B type [12,13]. Our measured and calculated results (Figs. 1, 2) also suggested that this model should be considered. To find out the rate law we performed a graphical matrix rank analysis of the reaction studied [12–19] by means of difference absorption ($\Delta A_\lambda = f(\Delta A_{\lambda = 325nm})$) (Fig. 3) and absorption ($A_\lambda = f(A_{\lambda = 325nm})$, figure not shown) diagrams preparation. We found that both diagrams are linear, what indicates a simple A → B reaction mode without the presence of an intermediate product. Singular value decomposition analysis (SVD) of the Ox ring opening reaction, the methodology, which is not dependent on the reaction model chosen, also indicates that the reaction is of A → B type. During SVD analysis eight independent components have been taken into account as a standard and for each of them the obtained reactant concentration changes *versus* time. Evidently, there are only two components with concentration profiles different from zero; one is disappearing and the second is appearing during the reaction. The concentration of the other six components oscil-

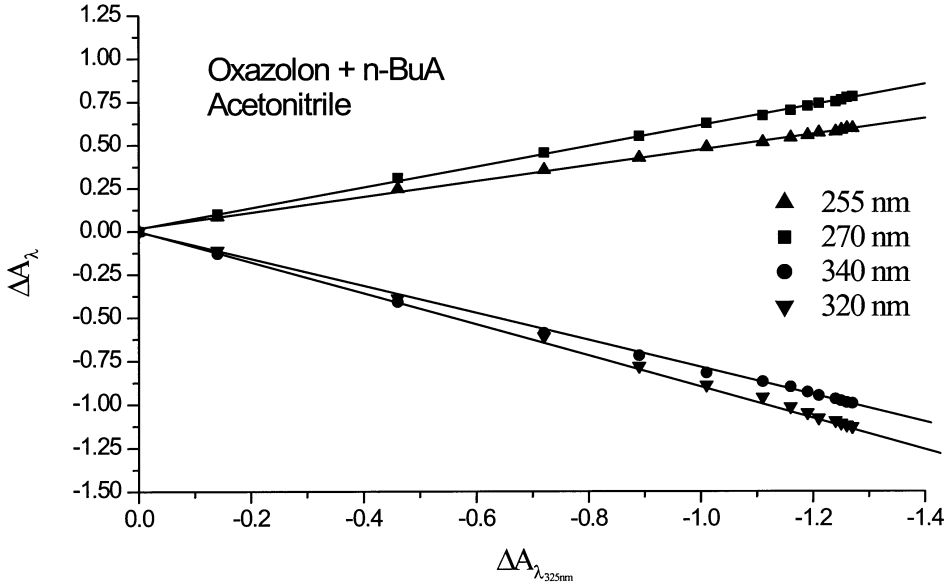

Figure 3. Difference absorbance diagram $\Delta A_\lambda = \{A_\lambda(t) - A_\lambda(t = 0)\} = f(\Delta A_{\lambda = 325nm})$, where $\Delta A_{\lambda = 325nm} = \Delta A_{\lambda = 325nm}(t) - \Delta A_{\lambda = 325nm}(t = 0)$ for the Ox + nBuA reaction in acetonitrile.

lates around zero and additionally we can treat their spectra as a baseline noise. Our previous findings about nonlinearity of the function k_{obs}= f(c_{nBuA}) suggest, however, a more complex mechanism of Ox ring opening reaction. Global analysis of the reaction was performed only for stopped flow experiments, for different concentration of nBuA (in a range of 0.052÷1.66 M). The obtained data were analyzed for three different types of the reaction: simple pseudo-first order, consecutive with all steps pseudo-first order type and consecutive reaction with a reversible first step. The obtained results indicate that for all reaction models considered the quality of the fit (X^2) of the calculated spectra and absorbance changes *versus* time to the experimental data are the same. Thus, the global analysis of the whole set of data did not give a unique answer about the rate law of the reaction studied.

During our studies of the ring opening reaction of the oxazolone with n-butylamine we performed temperature measurements in order to establish activation parameters of the process. The measurements were done for two different concentrations of nBuA; c_{nBuA}= 0.83 M (temperature range 280.2–321.4 K) and 0.026 M (temperature range 277.9–333 K).
The dependence of rate constant (k) on temperature (T) was calculated by

$$k = A \cdot \exp(-E_a/RT) \tag{7}$$

(Fig. 4). The entropy and enthalpy of activation of the reaction were calculated by [17]:

$$\log(k/T) = 10.319 + \Delta S^{\neq}/4.576 - \Delta H^{\neq}/(4.576 \times T) \tag{8}$$

The apparent activation energy (E_a) (from the linear fit of ln k *versus* 1/T) was established to be E_a=11.2 ± 1.3 kJ/mole and entropy and enthalpy of activations were $\Delta S^{\neq}$ = –258 ± 4 J/(mol· K), $\Delta H^{\neq}$ = 9.6 ± 0.1 kJ/mol for the nBuA concentration equal 0.026 M, whereas for amine concentration 0.83 M E_a = 3.4 ± 0.2 kJ/mole and $\Delta S^{\neq}$ = –232 ± 1 J/(mol·K) and $\Delta H^{\neq}$ = 1.0±0.1 kJ/mol respectively. The values of E_a are very small and even smaller than the activation energy of rotation of $-CH_3$ group for ethane (E_a = 13 kJ/mole).

The results obtained from SVD calculations, global analysis of the obtained data from stopped-flow experiments and linearity of absorbance and difference absorbance diagrams indicate that the reaction of Ox with nBuA is of simple A → B type. On the other hand, analysis of observed rate constant dependence on the amine concentration has discovered that it is exponential with exponent about 2. This nonlinear dependence, very low values of activation energy, and its dependence on the amine concentration lead unequivocally to the conclusion that the mechanism of the ring opening reaction is more complex. One of the possible explanation of the results obtained can be an assumption of the parallel reactions, as is described by:

$$-d[Ox]/dt = k_1[Ox][nBuA] + k_2[Ox][nBuA]^2 + \ldots \tag{9}$$

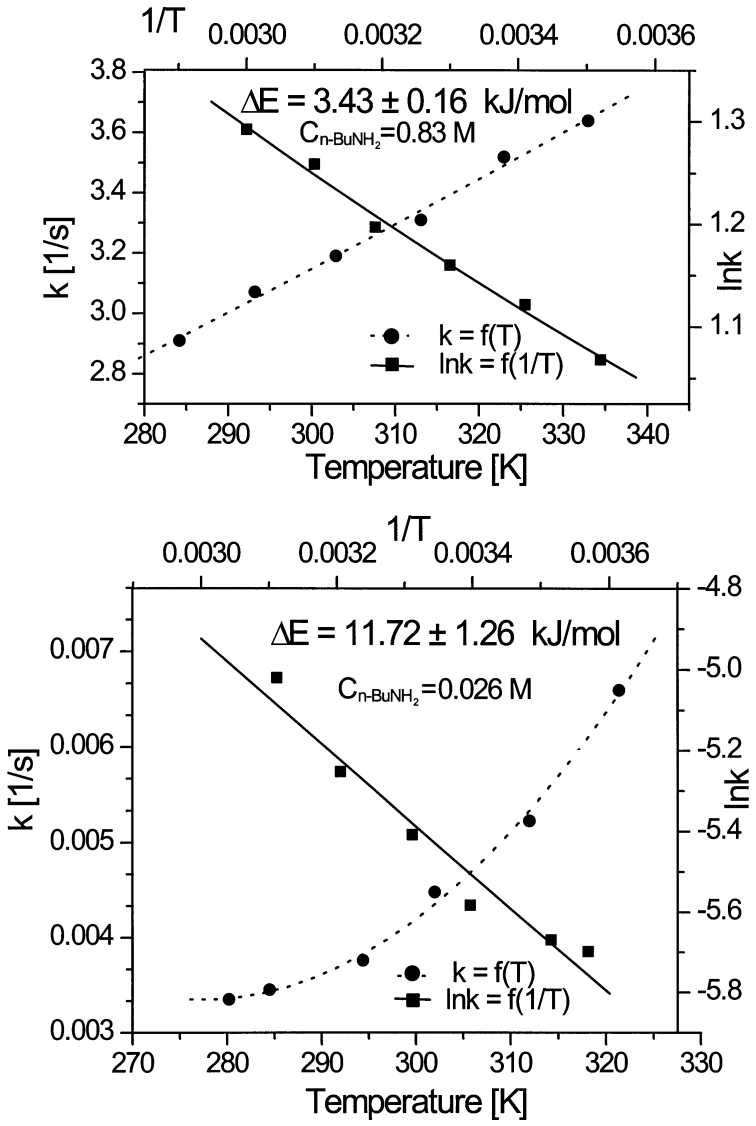

Figure 4. The dependences of the apparent rate constants on temperature – k *versus* T (left and bottom axis) and ln k *versus* 1/T (right and top axis) for n-butylamine concentrations 0.83 M and 0.026 M, upper and lower figure, respectively. The solid and dashed lines represent the best fit obtained using linear and nonlinear methods, respectively.

At a higher concentration of the amine, the second term in (9) is dominating, whereas at lower amine concentration the influence of the first-order type reaction has to be considered too. Thus, besides the ring opening reaction, in which proton transfer is involved, in a second amine molecule, the direct, intramolecular proton transfer in the intermediate product took place. This assumption was proven by the following observations: (i) the plot k_{obs}/[nBuA] *versus* [nBuA] (figure not shown) intersects the axis k_{obs}/[nBuA] above the zero point (0.19 ± 0.06) indicating that $k_1 \neq 0$; (especially when

data for higher concentration of the amine (0.832 and 1.66 mol/dm^3) are not taken into account for which the influence of the environment of reaction or higher order of reaction cannot be excluded), (ii) the apparent energy of activation depends on the amine concentration. It is distinctly lower for higher amine concentrations, for which the second-order reaction in relation to the amine is dominant (the proton transfer involving a second amine molecule is favored energetically, but disfavored entropically, compared to the direct proton transfer in the transition state), (iii) at lower amine concentrations the competition between these two reactions takes place, which results in nonlinearity of the Arrhenius`s dependence (ln k = ΔE/RT) (Fig. 4, lower panel). The direct proton transfer process is dominant at higher temperature (higher energy of activation) and the slop of the plot ln k *versus* 1/T increases with temperature increasing. At higher amine concentrations (0.83 mol/dm^3) the first term in (9) is negligible and the Arrhenius`s dependence is linear with small energy of activation.

Table 2. The rate constants and activation parameters (energy, entropy and enthalpy of activation calculated from linear dependance ln k = f(1/T)) of the reaction Ox with nBuA with assumed pseudo-first order reaction.

$C_{n\text{-}BuA}$	Temperature	k	E_a	$\Delta S^{\neq}$	$\Delta H^{\neq}$
[M]	[K]	[1/s]	[kJ/mole]	[J/(mole K)]	[kJ/mole]
0.026	280.2	0.00335	11.7 ± 1.3	–258 ± 4	9.6 ± 0.1
	284.5	0.00345			
	294.4	0.00376			
	302	0.00448			
	312	0.00523			
	321.4	0.00660			
0.83	277.9	2.51	3.4 ± 0.2	–232 ± 1	1.0 ± 0.1
	284.2	2.91			
	293.2	3.07			
	302.9	3.19			
	313.1	3.31			
	323	3.52			
	333	3.64			

The theoretical studies of the oxazolone ring opening reaction had also revealed that two molecules of amine reacted with one molecule of oxazolone. This type of reaction has a lower energy of activation than reaction when one molecule of oxazolone reacts with one amine molecule [10,20]. On the basis of the theoretical calculation and our experimental studies, the following mechanism of the reaction (for higher amine concentrations) can be proposed (Fig. 5). The lack of possibility of recording of intermediate product spectrum does not allow, in accordance with Polster and Mauser [12,17] and Vajda and Rabitz [21,22], to predict the real mechanism of the reaction studied. The theoretical [20] and our experimental data have only justified the conclusion that the parallel reactions model A + B $\rightarrow$ C and A + 2B $\rightarrow$ C is the most probable one for the explanation of the Ox ring opening reaction.

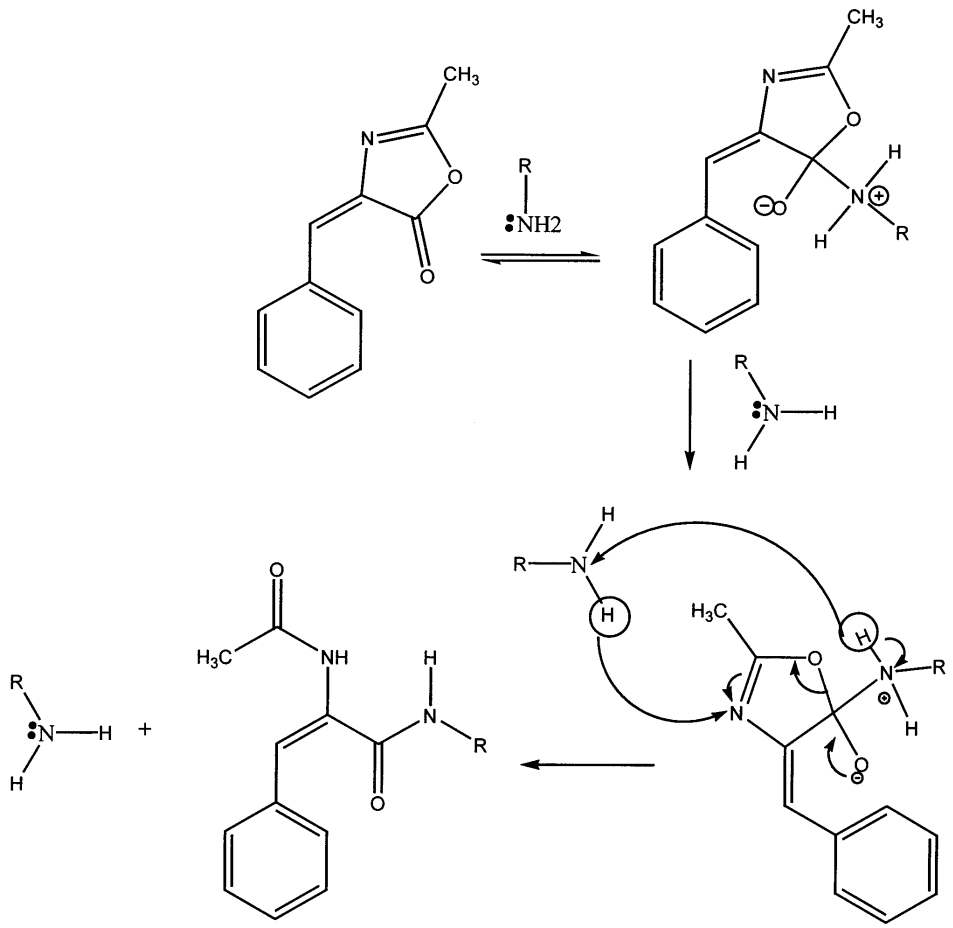

Figure 5. Proposed mechanism of the reaction of Ox with nBuA (for high amine concentration).

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