Journal of Pharmaceutical and Biomedical Analysis 50 (2009) 718–723

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

NMR analysis, protonation equilibria and decomposition kinetics of tolperisone

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ARTICLE INFO

Article history:
Received 26 March 2009
Received in revised form 28 May 2009
Accepted 29 May 2009
Available online 6 June 2009

Keywords:
Tolperisone
Decomposition kinetics
Protonation constants
Tautomerization
Tautomerization
Mannich base

ABSTRACT

The rate constants of spontaneous and hydroxide-catalyzed decomposition and the tautomer-specific protonation constants of tolperisone, a classical muscle relaxant were determined. A solution NMR method without any separation techniques was elaborated to quantitate the progress of decomposition. All the rate and equilibrium constants were determined at four different temperatures and the activation parameters were calculated. The molecular mechanism of decomposition is proposed.

1. Introduction

Tolperisone, 2-methyl-1-(4-methylphenyl)-3-piperidin-1-ylpropan-1-one hydrochloride is a centrally acting, classical muscle relaxant [1] for extrapiramidal movement disorders. Pharmacological studies on aminoketones revealed that these types of compounds cause muscle weakness with no sedation [2]. Further, clinical studies on tolperisone focused on its therapeutic value in movement disorders [3], rheumatic diseases [4], painful muscle spasms [5,6] and peripheric vasodilation [7]. Tolperisone is used as racemate, its enantiomers, however, have different pharmacologic properties: (+)-tolperisone has greater muscle relaxant activity than the (−) enantiomer, whereas (−)-tolperisone has higher broncho- and vasodilator effect [8,9]. Although it has been used for more than 40 years in therapy, its mechanism of action is still unknown. Tolperisone has a local anesthetic effect, like lidocain [10], as it blocks the voltage gated sodium channels [11,12]. A comparative assessment study shows that tolperisone is still one of the best centrally acting muscle relaxants in therapy [13].

It has no serious side effects, though anaphylactic reactions can occur [14]. Recent reviews with several pharmacological details [15,16] are available.

A definite shortcoming of tolperisone is its propensity to decompose in aqueous solution to piperidine and a vinylketone (2-methyl-1-(4-methylphenyl)prop-2-en-1-one) (Fig. 1). The decomposition is faster at higher pH [17]. Decomposition has so far been characterized at a descriptive level [18,19] with no exact kinetic parameters. Attempts have been made to stabilize tolperisione, e.g. by β-cyclodextrin [20]. The degradation product of eperisone (2-methyl-1-(4-ethylphenyl)-3-piperidin-1-ylpropan-1-one hydrochloride), the ethyl analogue of tolperisone has been fully characterized by MS, NMR, UV and IR spectroscopy [21].

Obvious reasons why its decomposition kinetics has not been quantitated were the lack of an appropriate solution analytical method to monitor the progress of decomposition, and also, the lack of input acid–base equilibrium parameters of the reactants and products.

Here we report

(a) an ^{1}H NMR method for the simultaneous determination of tolperisone and its decomposition products;
(b) the related acid–base properties at four temperatures;
(c) the kinetic constants including the activation parameters; and
(d) the proposed mechanism of decomposition.

2. Experimental methods

All experiments were performed at thermostated temperature (288, 298, 308 and 323 K), the ionic strength was held constant at 0.15 M using KCl as auxiliary electrolyte.

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doi:10.1016/j.jpba.2009.05.036

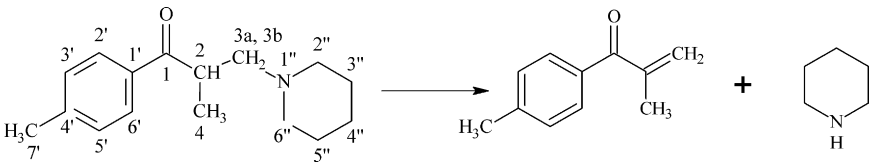

Fig. 1. Constitutional formula, numbering and decomposition of tolperisone.

Table 1
pH values of standard buffers.

	Oxalate	Phthalate	Phosphate	Borax
288 K	1.670	3.999	6.900	9.276
298 K	1.680	4.008	6.865	9.180
308 K	1.690	4.024	6.844	9.102
323 K	1.705	4.060	6.833	9.011

2.1. Chemicals

Tolperisone HCl and its vinylketone derivative were the products of Gedeon Richter Ltd., Budapest. Potassium dihydrogen phosphate, sodium hydrogenphosphate, sodium phosphate, sodium hydroxide and potassium chloride were obtained from Reanal Co. (Budapest, Hungary); potassium tetroxalate and sodium 3-trimethylsilyl-1-propanesulfonate (DSS) were obtained from Fluka (Buchs, Switzerland); borax was from Sigma; potassium hydrogen phthalate was from Merck (Darmstadt, Germany). Bidistilled water was used in all experiments.

2.2. NMR measurements

^{1}H NMR spectra of tolperisone were recorded at 599.9 MHz with Varian Unity Inova spectrometer. Double pulse field gradient spin echo pulse sequence was used to suppress the resonance of H_2O. The FID was digitized into 32,768 data points. Typically 32 transients were coadded. Chemical shifts were measured relative to internal DSS (0.0 ppm). Integration of selected peaks was also relative to the integral of the trimethylsilyl peak of DSS.

2.3. Protonation equilibria

Protonation constants were determined by NMR-pH titrations. pH was measured with Metrohm 780 pH meter equipped with a Metrohm 6.0234.110 electrode. Concentration of tolperisone was 0.001 M at all measurements. All pH data are pH meter readings based upon NBS primary standards: 0.05 M potassium tetroxalate, 0.05 M potassium hydrogen phthalate, 0.025 M KH_2PO_4 + 0.025 M Na_2HPO_4 and 0.01 M borax buffer. The buffer pH values [22] are listed in Table 1.

Tolperisone was dissolved in 0.01 M HCl and in 0.01 M NaOH solutions containing 5% (v/v) deuterium oxide. The solutions were mixed, pH was measured, and the ^{1}H NMR spectra were recorded.

2.4. Kinetic studies

The progress of decomposition was quantified by NMR experiments. Processes were followed in situ: the decomposition took place in the NMR tube, and the spectrum was recorded several times. Experiments were performed in buffer solutions containing 0.05 M KH_2PO_4 and 0.025 M $Na_2B_4O_7$ at different pH values. Tolperisone concentration was 0.001 M. Appropriate pH was set by 2 M phosphoric acid or 2 M sodium hydroxide.

The disappearance of tolperisone was followed by integration of the peak with the largest chemical shift, relative to the trimethylsilyl signal of DSS.

Nonlinear fittings were done using STATISTICA 8.0 for Windows.

3. Results and discussion

3.1. ^{1}H NMR method for the determination of tolperisone and its decomposition products

Both tolperisone and its vinylketone derivative can be determined quantitatively by ^{1}H NMR measurements, since most of their proton signals are well separated (Fig. 2).

Integrals of the aromatic protons are the best linear measures of the tolperisone and the vinylketone concentration, since the applied water suppression disturbs the shape of the proton peaks in the vicinity of the solvent signal. In tolperisone the chemical shifts of the H 2′, 6′ and H 3′, 5′ are 7.97 and 7.44 ppm, respectively. Chemical shift values of the corresponding peaks for the decomposition product are 7.69 and 7.38 ppm.

3.2. Protonation equilibria

Determination of the protonation constants of tolperisone could not be carried out with sufficient reliability by the most frequently used, standard pH-potentiometric method, since tolperisone decomposes rapidly in basic media. In addition, piperidine, one of the decomposition products is a stronger base than tolperisone. The other standard method, UV–pH titration could not be used either, because vinylketone, the other product is also UV-active in the same range of wavelength. Evaluation of the protonation constants from ^{1}H NMR–pH titration curves was based on the principle that nonexchanging NMR nuclei near the basic site sense different electronic environments upon protonation. The observed carbon-bound protons were the aliphatic methyl group and the ABX pattern of the methylene and methyne hydrogens (Fig. 3).

Since protonation processes are fast on the NMR time scale, the observed chemical shift of a nucleus can be expressed as a weighted average of chemical shifts of the protonated and unprotonated form. Weighting factors are the mole fractions.

$$\delta_{pH} = \delta_L \chi_L + \delta_{HL^+} \chi_{HL^+} = \frac{\delta_L + \delta_{HL^+} K[H^+]}{1 + K[H^+]} \quad (1)$$

where δ_{pH} is the observed chemical shift, δ_{HL^+} and δ_L are the chemical shifts of the protonated and unprotonated species, respectively.

In the process of the evaluation of the NMR–pH titrations, we used the Opium [23] software to obtain the log K values, as it calculates the protonation constants by using the data of all observed protons. log K values are listed in Table 2.

Enthalpy and entropy can be determined from the log K values at different temperatures, using the classical pair of relationships:

$$\Delta G = \Delta H - T \cdot \Delta S \quad (2)$$

$$\Delta G = -RT \cdot \ln K \quad (3)$$

Table 2
Protonation constants of tolperisone.

T (K)	log K
288	9.54 ± 0.04
298	9.37 ± 0.03
308	9.07 ± 0.04
323	8.76 ± 0.03

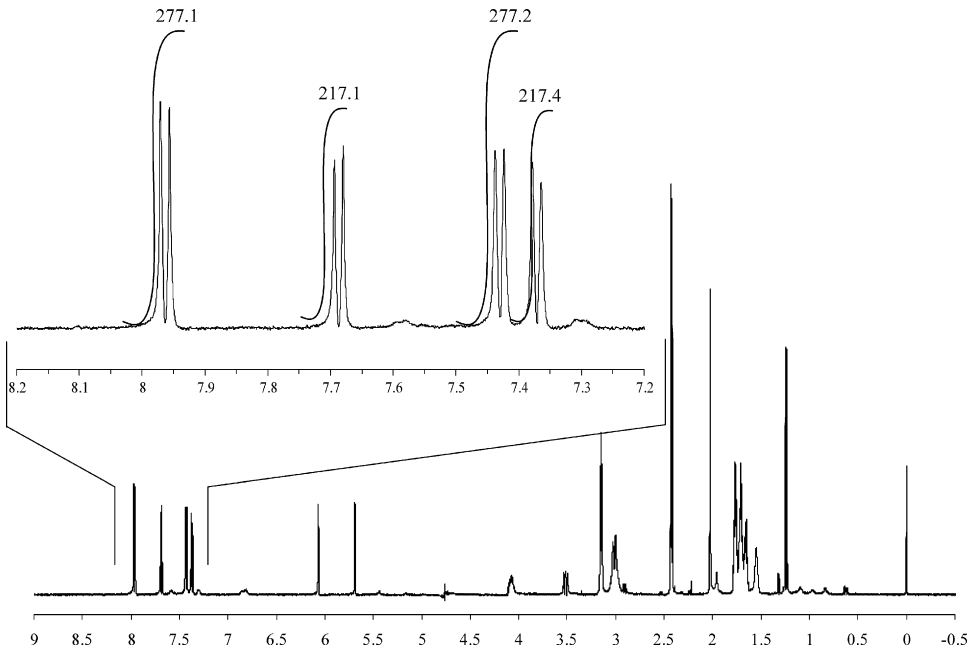

Fig. 2. ^{1}H NMR spectrum of partially decomposed tolperisone, with magnified inset with integrals from the aromatic region. Integral values are relative to internal standard DSS peak (1 0 0).

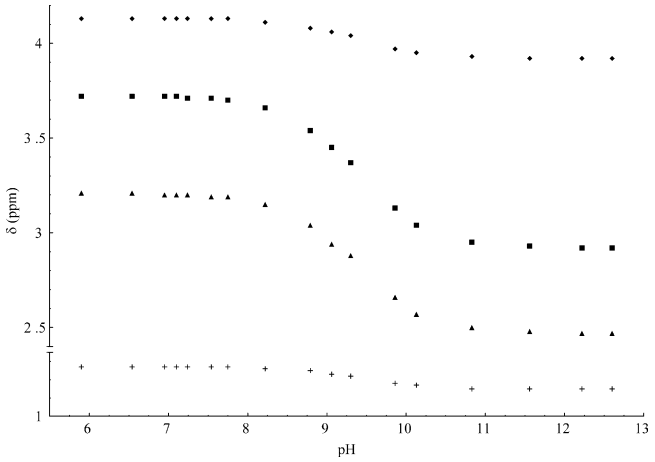

Fig. 3. Chemical shifts of the observed protons vs. pH at 298 K, where (♦), (■), (▲) and (+) stand for proton numbers 2, 3a, 3b, 4 (Fig. 1), respectively.

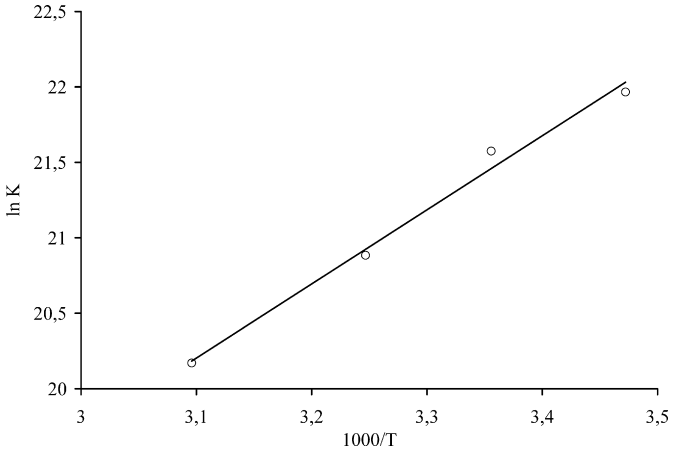

Fig. 4. ln K vs. 1/T function of tolperisone protonation.

Combining and rearranging Eqs. (2) and (3) gives Eq. (4) that can be fitted to the measured data (Fig. 4):

$$\ln K = -\frac{\Delta H}{R}\frac{1}{T} + \frac{\Delta S}{R}. \quad (4)$$

Enthalpy and entropy values were calculated from the parameters of the line (Eq. (4)): $\Delta H = -40.84 \pm 2.95$ kJ mol^{-1}, $\Delta S = 41.4 \pm 9.7$ J mol^{-1} K^{-1}, with a correlation coefficient $r = 0.995$.

3.3. Decomposition kinetics

The ^{1}H NMR method described above could be used to monitor the decomposition of tolperisone.

Disappearance of tolperisone in buffered solution showed pseudo-first-order kinetics over several half-lives (Fig. 5).

Rate constants were determined by nonlinear parameter estimation: $[T] = [T]_0 e^{-kt}$ where [T] and $[T]_0$ are the actual and the starting concentrations of tolperisone, respectively, k is the rate constant and t is the time. The observed k values are listed in Table 3.

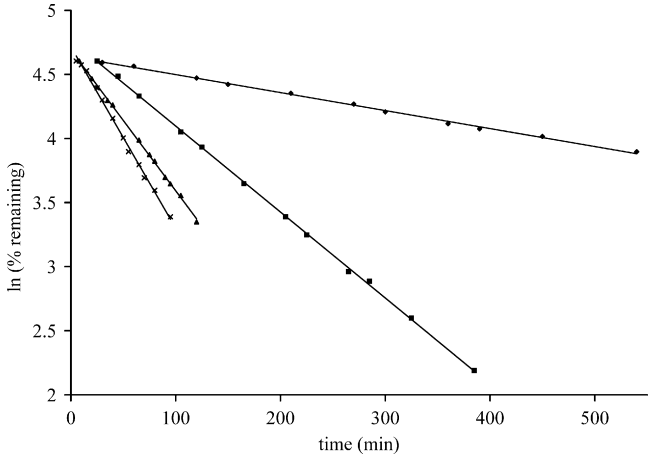

Fig. 5. Decomposition kinetics of tolperisone at 323 K at pH 6.47 (♦), 7.70 (■), 8.44 (▲) and 9.97 (×).

Table 3
Observed rate constants of tolperisone decomposition.

288 K		298 K		308 K		323 K	
pH	k_{obs} ($\times 10^{-6}$ s^{-1})	pH	k_{obs} ($\times 10^{-6}$ s^{-1})	pH	k_{obs} ($\times 10^{-5}$ s^{-1})	pH	k_{obs} ($\times 10^{-5}$ s^{-1})
6.99	0.28 ± 0.002	6.54	0.70 ± 0.01	6.50	0.31 ± 0.02	6.31	2.3 ± 0.01
7.28	0.50 ± 0.01	6.95	1.60 ± 0.02	6.97	0.90 ± 0.01	6.80	5.53 ± 0.02
7.47	0.72 ± 0.01	7.10	2.42 ± 0.02	7.13	1.23 ± 0.02	7.11	8.00 ± 0.07
7.99	1.59 ± 0.03	7.24	3.08 ± 0.04	7.24	1.48 ± 0.01	7.32	11.28 ± 0.07
8.47	3.00 ± 0.06	7.54	4.68 ± 0.06	7.45	2.05 ± 0.02	7.81	16.48 ± 0.13
8.91	4.83 ± 0.05	7.75	7.20 ± 0.07	7.72	2.92 ± 0.02	8.21	18.17 ± 0.07
9.50	9.00 ± 0.12	8.22	12.33 ± 0.16	8.22	4.25 ± 0.07	8.86	20.53 ± 0.20
10.05	11.00 ± 0.05	8.79	20.67 ± 0.67	8.72	5.78 ± 0.10	9.42	24.52 ± 0.18
11.01	12.67 ± 0.15	9.06	22.50 ± 0.50	9.40	6.81 ± 0.10	9.81	31.23 ± 0.33
12.13	25.50 ± 0.50	9.30	26.50 ± 0.15	10.47	9.28 ± 0.09	10.42	43.33 ± 0.95
		9.86	29.67 ± 0.33	11.44	17.00 ± 0.70	10.82	53.50 ± 0.87
		10.13	38.16 ± 0.33			10.97	58.00 ± 1.21
		11.56	56.33 ± 1.67			11.10	67.33 ± 1.88

Since decomposition experiments at constant pH provide observed rate constants which depend on pH, we determined the pH-independent constants. For Mannich base hydrolysis several kinetics are described in the literature [24–32]. Decomposition can be spontaneous or base-catalyzed, but only the unprotonated species decomposes. The kinetics can be described using Eq. (5)

$$-\frac{d[T]}{dt} = k_0[T_0] + k_b[OH^-][T_0] = (k_0 + k_b[OH^-])[T_0] \quad (5)$$

By integration and adequate rearrangement of Eq. (5), the observed rate constant can be expressed as follows:

$$k_{obs} = \frac{k_0 + k_b[OH^-]}{1 + K[H^+]} \quad (6)$$

In Eqs. (5) and (6) k_{obs} is the observed rate constant, k_0 and k_b are the pH-independent rate constants for the spontaneous and base catalyzed decomposition, respectively, K is the protonation constant. [T] is the total concentration of tolperisone, $[T_0]$ is the concentration of the deprotonated species.

Using Eq. (6) in a nonlinear fitting procedure resulted in the pH-independent rate constants and also the protonation constants (Fig. 6). The protonation constant values obtained in the kinetic measurements, however, appeared to be significantly different from the values obtained in the NMR–pH titrations. The rate constants and the "kinetic" protonation constants are listed in Table 4.

Since the log K values derived from the kinetic experiments are approximately 0.5 logarithm units below the corresponding values obtained by direct NMR–pH titration, we made a control experiment: the rate constant was measured at lower pH, to validate our results.

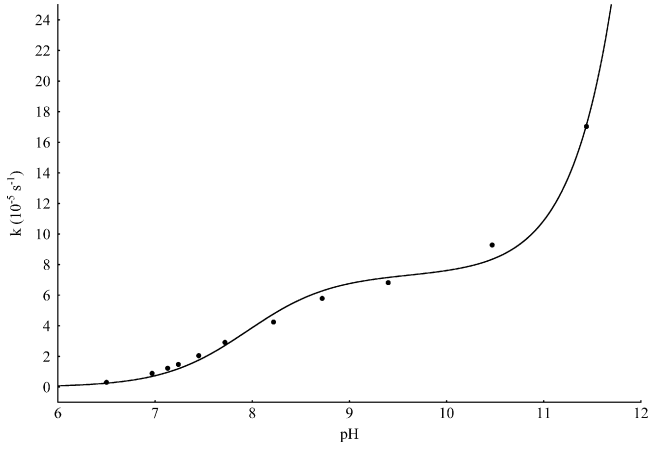

Fig. 6. The function of k_{obs} vs. pH at 308 K, where circles are experimental points, and solid line represents the calculated curve.

Table 4
Rate constants and "kinetic" protonation constants.

T (K)	k_0 (s^{-1})	k_b (s^{-1})	log K
288	$1.17 \times 10^{-5} \pm 2.8 \times 10^{-7}$	$1.33 \times 10^{-3} \pm 5 \times 10^{-5}$	8.99 ± 0.05
298	$7.13 \times 10^{-5} \pm 1.8 \times 10^{-6}$	$2.67 \times 10^{-3} \pm 1.3 \times 10^{-4}$	8.52 ± 0.13
308	$7.32 \times 10^{-5} \pm 3.3 \times 10^{-6}$	$9.82 \times 10^{-3} \pm 6 \times 10^{-4}$	7.95 ± 0.08
323	$2.69 \times 10^{-4} \pm 2.2 \times 10^{-5}$	$3.47 \times 10^{-2} \pm 3.5 \times 10^{-3}$	7.59 ± 0.18

The experiment was done at 323 K, pH 5.57. Measured rate constant was $2.52 \times 10^{-6} \pm 8.32 \times 10^{-9}$ s^{-1}. The predicted value was 2.54×10^{-6} s^{-1}. Thus, the measured value is in excellent agreement with the predicted one, verifying the other data and the measured tendency.

The apparent controversy between the two protonation constant values elucidated in two ways can be well resolved in terms of the two tautomeric forms of tolperisone. The NMR–pH titrations reflect the nitrogen-basicity of the molecule in its predominant keto form. If, however, decomposition takes place via the enolic tautomer, the "kinetic" protonation constant reflects the nitrogen-basicity of this form, even though it is the minor tautomer in solution. This is another example when the minor species is the reactive one [33,34].

The "bulk" and "kinetic" protonation constant values are in line with chemical considerations. The "bulk" values are characteristic ones of tertiary amines surrounded by distant electron-withdrawing keto and phenyl moieties.

The "kinetic" values are influenced by the hydrogen-bonded partial protonation of the piperidine nitrogen (Fig. 7), a conformation of the enolic tautomer only. This form of the molecule either predisposes decomposition, or necessitates higher hydrogen ion concentration for protonation, resulting in a lower log K value.

The activation energy of the decomposition can be calculated, using the classical Arrhenius-equation.

$$k = A \cdot e^{-E_{act}/RT} \quad (7)$$

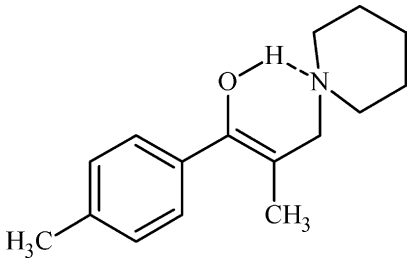

Fig. 7. Structure of the enol tautomer of tolperisone.

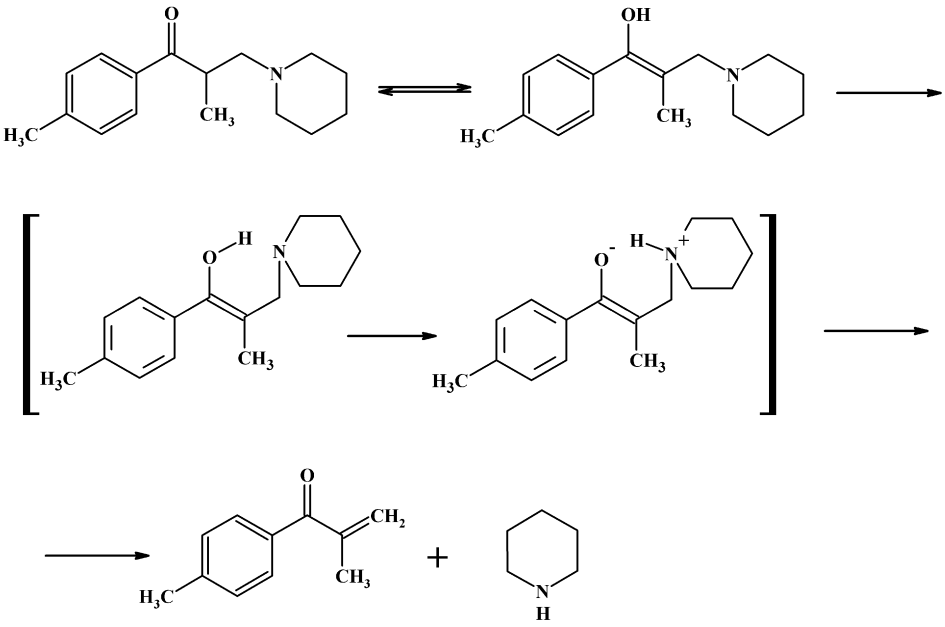

Fig. 8. Proposed mechanism for spontaneous decomposition.

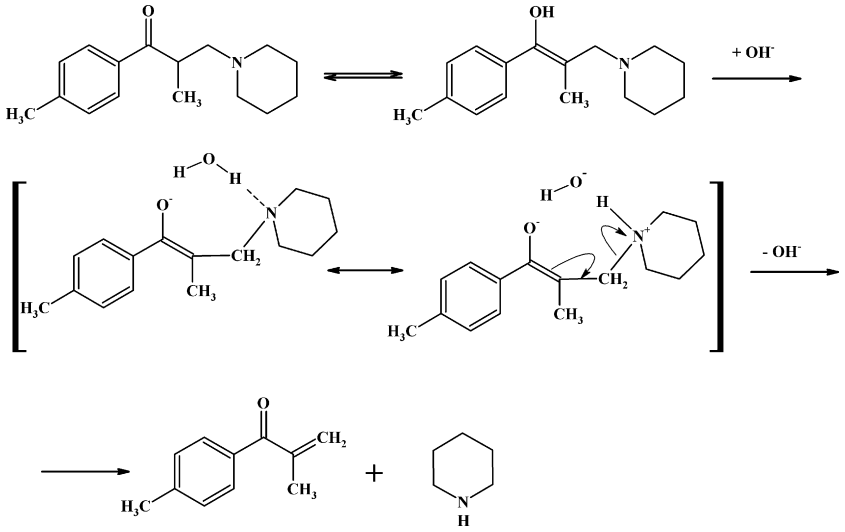

Fig. 9. Proposed mechanism for base catalyzed decomposition.

where k is the pH-independent rate constant (either the spontaneous or the catalyzed one), A is the pre-exponential factor, E_{act} is the activation energy, T is the temperature.

The activation energy was calculated for both the spontaneous and the catalyzed decomposition, the values are 68.59 ± 1.53 and $74.35 \pm 5.88\ kJ\ mol^{-1}$, respectively.

Enthalpy and entropy of protonation of the enol was calculated in the same way as those of the keto form (Eq. (4)), the results are $\Delta H = -72.96 \pm 8.02\ kJ\ mol^{-1}$, $\Delta S = -82.0 \pm 26.4\ J\ mol^{-1}\ K^{-1}$, with a correlation coefficient $r = 0.988$

ΔH values of the keto and enol forms confirm the expected tendencies. ΔS value of the keto tautomer is significantly above that of the enol form ($+41.4\ J\ mol^{-1}\ K^{-1}$ vs. $-82\ J\ mol^{-1}\ K^{-1}$). Both negative and positive entropy changes upon amino protonation are reported phenomena. Negative entropy changes were observed for amino acids [35], where the nitrogen is surrounded by groups of large electronegativity (carboxylate vs. enolic hydroxyl), whereas aliphatic amines have positive entropy of protonation [36].

Major steps in the proposed mechanism of decomposition are as follows: 1. tolperisone isomerizes into its enolic form, 2. the enol deprotonates, 3. the C–N bond breaks, 4. the enol rearranges into vinylketone (Figs. 8 and 9).

Acknowledgement

This work was supported by OTKA K73804 grant.

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