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Thermal Stability of Amino Acids

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Abstract—Thermal decomposition of L-α-amino acids $RCH_2(NH_2)COOH$ where R = Me_2CH, Me_2CHCH_2, MeEtCH, and $C_6H_5CH_2$ was studied at temperatures below the melting points of their crystals. From the effective rate constants of the first order reactions energy parameters in the Arrhenius equation were calculated. Correlations between the reaction rate constants k_R and the inductive constants σ* of substituents R and also between the rate constants of the reactions and the dipole moments of amino acids was established. Value of ρ* parameter +8.8 in the Taft equation indicates the heterolytic mechanism of transformation of the amino acids. Chromato-mass spectrometric analysis of decomposition products shows that condensation, decarboxylation, and deamination of the amino acids take place.

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Molecules of amino acids and oligopeptides in the solution and crystalline state exist in the zwitterionic form [1]. This structure is in keeping with high values of dipole moments [2], high solubility of amino acids in water, and high melting points. By means of differential thermal analysis and thermogravimetry it was established that decomposition of glycine is observed at 212–240°C, significantly lower than melting point of the pure substance [3]. Studies of the rate of thermal decomposition of glycine, alanine, and serine show that kinetic parameters may be obtained for pure substances at temperatures below the melting points of crystalline substances [4].

The aim of this work is the investigation of the effect of structure of groups R in irreplaceable L-α-amino acids on the reactivity of compounds, and also the evaluation of products of thermal decomposition of the amino acids permitting to make suggestions on the mechanism of their transformation.

We have studied the rate of thermal decomposition of valine, leucine, isoleucine, and phenylalanine in solid phase in the temperature range below the melting points of crystalline amino acids. Reaction rate was calculated from the increase in the pressure of the formed volatile products of thermal decomposition according to the procedure described in [5].

The character of the pressure increase at the decomposition of amino acids (p) with the reaction time (t) by an example of valine is presented in Fig. 1.

For the calculation of the rate constant of the first order reaction the middle part of the curve was used excluding the induction period of decomposition. The rate constant k_R was calculated according to the following equation.

$$k_R = \frac{1}{t}\ln\frac{p_\infty - p_0}{p_\infty - p_t}. \tag{1}$$

Here p_0 and p_∞ are the initial and final pressure of gaseous products in the range of intense liberation of volatile reaction products, p_t is the current pressure at the time t.

In fact, the rate of summary process was studied which included the combination of parallel and consecutive elementary reactions proceeding in the bulk of the amino acid crystal that were accompanied by liberation of volatile products at the temperature of the experiment. Kinetic parameters of thermal decomposition of the amino acids are listed in Table 1.

Rate of thermal decomposition of glycine, alanine, and serine was studied previously [4]. We have used these data for establishing the influence of the inductive effect of substituent on the rate of thermal decomposition of the amino acids with different substituents R. For comparison of reactivity of compounds using kinetic parameters the values of the reaction rate constants at 270°C were calculated.

It was found that the rate constants k_R depend on the dipole moments μ of the amino acids. The larger is the dipole moment the higher is the reaction rate (Table 2). This fact shows the heterolytic character of the reactions determining the rate of the whole process. Serine (R = $HOCH_2$) is the exception in this series. These data will be discussed further.

We have evaluated the influence of the inductive effect of substituent R on the process of thermal transformation of the amino acids in solid phase using Taft equation [6].

$$\ln k_R/k_{Me} = \rho\sigma^*. \quad (2)$$

Here k_{Me} is the rate constant of thermal decomposition of alanine carrying methyl substituent, k_R is the rate constant of decomposition of the other amino acids having electron-donor and electron-acceptor substituents, σ^* is the inductive constant of substituent R, ρ^* is the reaction constant characterizing the sensitivity of reaction center to the action of inductive effects of the group R.

Absolute value $\rho^* = +8.8$ (correlation coefficient 0.988) obtained by us serves the measure of high sensitivity of thermal decomposition to structural variations in the molecule of the amino acid (Fig. 2). This ρ^* value in Taft equation suggests a polar transition state in heterolytic mechanism of decomposition of the amino acids.

Table 1. Kinetic parameters of thermal decomposition of amino acids in the solid phase

Amino acid	T, °C	$k_R \times 10^5$, s^{-1}	E, kJ mol^{-1}	ln k_0
Valine	270	9.0	190±27	32.8
mp 315°C	295	24.4		
	301	81.4		
Leucine	269	0.204	184±25	31.3
mp 293°C	293	1.16		
	301	4.47		
Isoleucine	269	0.1	195±27	33.1
mp 285°C	291	0.79		
	301	1.39		
Phenylalanine	245	2.8	204±28	39.3
mp 315°C	284	5.1		
	275	96.2		

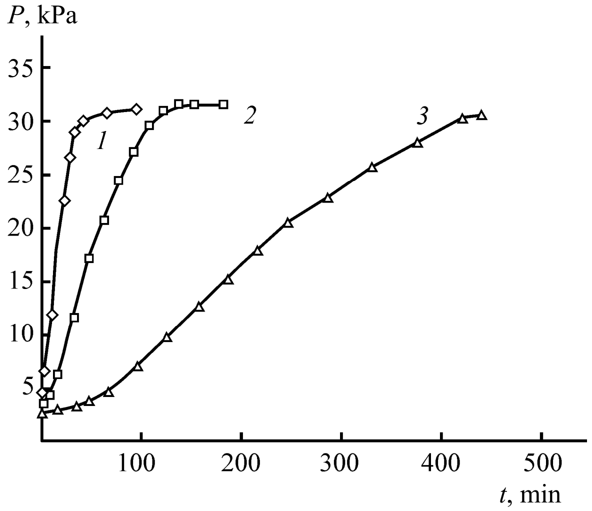


Fig. 1. Kinetic curves of the pressure of the products of valine decomposition in time at different temperatures, °C: (*1*) 301; (*2*) 285; and (*3*) 270.

It turned out that at the increase in donating inductive effect of the alkyl group R the rate constant of thermal decomposition of the amino acids decreases in the following series: H > $C_6H_5CH_2$ > CH_3 > $(CH_3)_2CHCH_2$ > $(CH_3)_2CH$ > $CH_3(C_2H_5)CH$. Serine containing the substituent $HOCH_2$- falls out of this reaction series. Hydroxy group of serine influences the process of thermal decomposition not only due to the inductive effect, but simultaneously it is the reaction center in probable condensation reactions.

The reason of thermal transformation of amino acids in solid phase far below the melting of the crystals is the location of zwitterions in the nodes of

Table 2. Comparison of rate constants of decomposition of the amino acids $RCH(NH_2)COOH$ with their dipole moments and inductive constants of substituents R[a]

R	μ, D [2]	σ* [6]	$k_R \times 10^5$, s^{-1} (270°C)	ln k_R/k_{Me}
$HOCH_2$	9.153	0.555	155270	7.62
H	10.528	0.49	2050	3.62
$C_6H_5CH_2$	10.42	0.215	317	1.76
CH_3	10.269	0.0	54.8	0.0
$(CH_3)_2CHCH_2$	10.225	–0.125	8.44	–1.87
$(CH_3)_2CH$	10.121	–0.19	9.0	–1.81
$CH_3(C_2H_5)CH$	10.079	–0.21	3.75	–2.68

[a] Rate constants of the reaction at 270°C were calculated from the kinetic parameters presented in Table 1 and in [4].

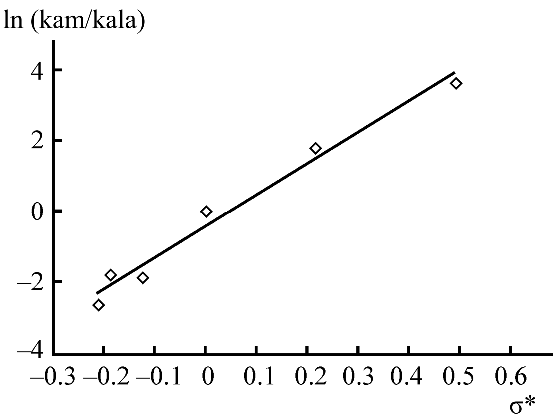
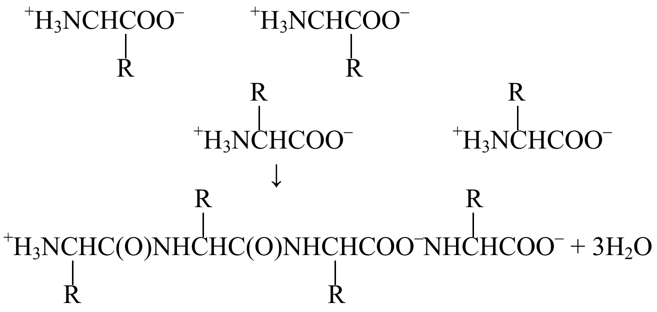


Fig. 2. Dependence of ln k_R/k_{Me} (270°C) of thermal decomposition of amino acids on the Taft inductive constants of substituents R.

the lattice. Electroneutral zwitterions carrying positive and negative charges localized on different atoms are present in the lattice nodes like in ionic crystals. The larger is the dipole moment of the zwitterion the higher is the energy of electromagnetic interaction between zwitterions, and the higher is the rate of heterolytic condensation accompanied by the formation of oligopeptides and liberation of water.

Note that the condensation of amino acids in solid phase is not a topochemial reaction. In fact the crystal of amino acid is the unique homogenic system, and the condensation proceeds in the bulk of the solid phase. The difference between the reactions in the condensed and gaseous state is that in the crystal a rigidly fixed orientation of amino acid molecules exists with two strictly oriented reaction centers of each zwitterions which is necessary for intermolecular interaction.

Depending on the relative location of zwitterions condensations leading to oligopeptides and diketopiperazines are possible. For example, the formation of oligopeptide from four zwitterions can be presented as follows.

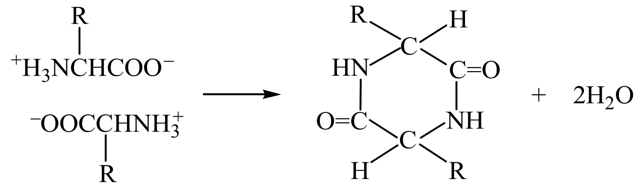


$$\begin{array}{ll} {}^{+}H_3N\underset{R}{C}HCOO^{-} & {}^{+}H_3N\underset{R}{C}HCOO^{-} \\ {}^{+}H_3N\overset{R}{C}HCOO^{-} & {}^{+}H_3N\overset{R}{C}HCOO^{-} \end{array}$$

$$\downarrow$$

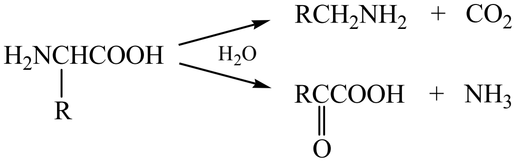
$${}^{+}H_3N\underset{R}{C}HC(O)NH\overset{R}{C}HC(O)NH\underset{R}{C}HCOO^{-}NH\overset{R}{C}HCOO^{-} + 3H_2O$$

At another location of zwitterions in crystal the formation of 2,5-piperazinediones takes place which was shown by the analysis of the reaction products.



For example, in the decomposition of valine 3,6-bis-(2-methylpropyl)-2,5-piperazinedione, 3,6-bis(2-methylpropyl)-2,5-piperazinedione in the decomposition of leucine, and 3,6-bis(1-methylpropyl)-2,5-piperazinedione in the decomposition of isoleucine where found by the chromatomass spectrometry.

Simultaneously amino acids are involved in decarboxylation and deamination.



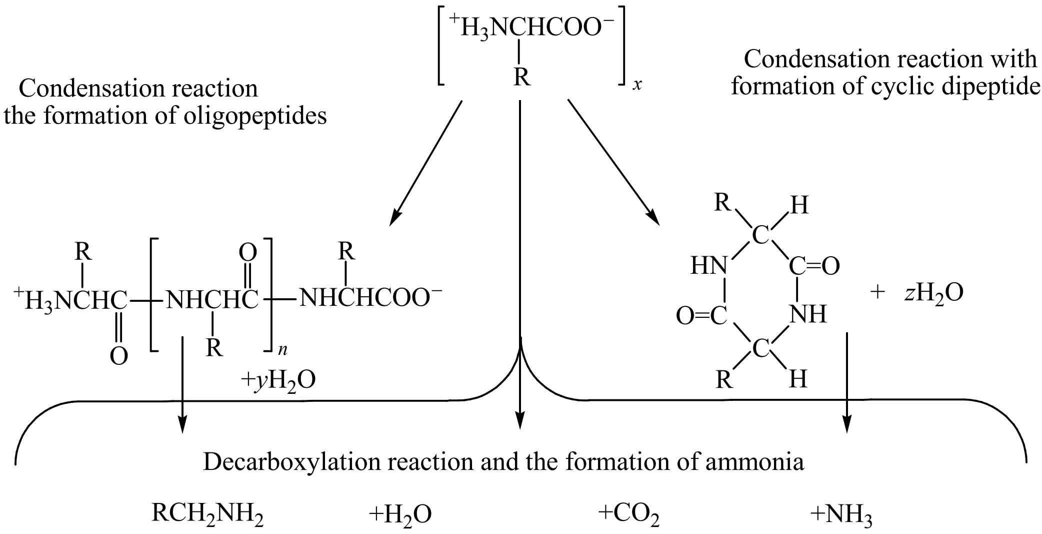
$$H_2N\underset{R}{C}HCOOH \rightarrow RCH_2NH_2 + CO_2$$

$$H_2N\underset{R}{C}HCOOH \xrightarrow{H_2O} R\underset{O}{\underset{\|}{C}}COOH + NH_3$$

As known, the decarboxylation proceeds easily at heating amino acids [7]. The reaction is accompanied by the formation of amines and CO_2. The corresponding amines were found at the decomposition of valine (isobutylamine), leucine (propylamine), and previously in the course of decomposition of glycine (methylamine) [4].

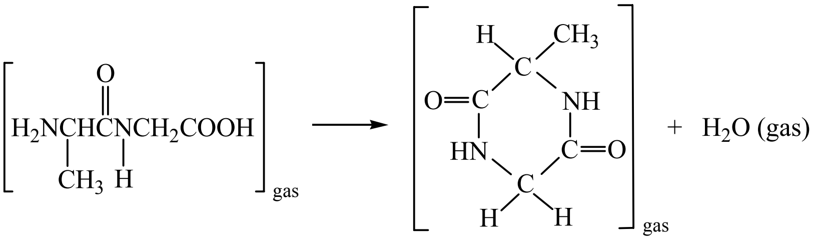
The appearance of amines and carbon dioxide corresponds to thermal decomposition of amino acids according to decarboxylation pathway, but the appearance of ammonia in the reaction of oxidative deamination takes place under the action of enzymes, and *in vitro* under the action of nitrous acid [7]. This was probably the reaction of thermal oxidative deamination in presence of water vapor formed in the course of condensation of the amino acids, and not the catalytic process. In this case the formation of ammonia must be accompanied by the appearance either of α-ketoacid R–C(O)COOH or α-hydroxyacid R–CH(OH)–COOH, but no such products were observed by means of chromatomass spectrometry.

On the basis of kinetic measurements and the analysis of the reaction products it should be concluded that the thermal decomposition of amino acids in solid phase proceeds by parallel and consecutive condensation, decarboxylation, and deamination.



In the closed reactor under the temperature of the experiment some part of amino acid enters the gas phase by sublimation. Under these conditions the amino acid molecules having no positive and negative charges localized on different atoms should be stable by energy. In this case cyclic dipeptide can form from the linear one as it was established in [8]. It was shown that during the evaporation of alanylglycine together with sublimation the formation of 2,5-piperazinedione and water vapor takes place.

It cannot be excluded that in our case the formation of cyclic dipeptides in the gas phase proceeds without the participation of zwitterions according to the reaction suggested in [8].



EXPERIMENTAL

Crystalline L-α-amino acids of "chemically pure" grade were used. The rate of decomposition of the amino acids was studied in the evacuated 30 cm^3 glass ampules having special thin membrane. In each experiment 20 mg of compound under study was used. The reaction rate was monitored by the increase in pressure of volatile decomposition products at a constant temperature. The increase in the pressure of volatile products was controlled by compensation keeping the zero position of membrane by the increase in outer pressure. Scheme of the installation used is described in [5]. After that the ampule was filled with high purity helium up to the pressure of 1.5 atm, and 1 ml of gas was taken for evaluation of composition of volatile decomposition products.

By means of a syringe the sample was placed in the injector of TraceGCUltra/DSQII chromatomass spectrometer equipped with TR 5 MS 30000×0.25 mm capillary column. The injector temperature was 250°C. Mass chromatograms were registered in the mass number range 12-200 for evaluation of light gases and 35-300 for the other volatile products. Identification of components of the mixture was carried out with the help of the NIST 2005 electronic library of mass spectra.

For the analysis of condensed phase the precipitate from the walls of ampule was dissolved in a suitable solvent. The obtained solution was analyzed by the above-described procedure.

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