# SYNTHESIS AND PHYSICO-CHEMICAL PROPERTIES OF SOME 3,5-DISUBSTITUTED 2-THIOHYDANTOINES

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Several new 3,5-disubstituted mono- and bis-2-thiohydantoines were prepared by reaction of isothiocyanates with aspartic and glutamic acids and lysine. Thiohydantoines formed were characterized by UV and IR spectra and their stability, especially in media of different pH, was examined. The rate constants of the reaction of isothiocyanates with the amino acids are reported. The rate of decyclization of 2-thiohydantoines was found to be a function of $OH^{(-)}$ ion concentration.

3,5-Disubstituted 2-thiohydantoines constitute the group of substances which are of interest both as to their physico-chemical properties (5-membered ring with two hetero atoms, the stability of which is strongly dependent on pH) and from the point of view of their biological activity. According to the current knowledge, the biological activity of thiohydantoines may be either inherent property of thiohydantoine ring or it may be assigned to the products formed by hydrolytic decomposition reaction. These are N-substituted thiocarbamoylamino derivatives, or, under appropriate conditions, also isothiocyanates. However, the assumption that thiohydantoines are precursors of isothiocyanates has been questioned, particularly in view of the fact that the derivatives studied release isothiocyanates only at pH values which are substantially higher than physiological value[1].

2-Thiohydantoines can be in general prepared by several methods[2-4]. For preparing the derivatives studied in this work (type A, derivatives *I* – *VII*, see Experimental) the method reported by Edman[4] turned out to be most convenient. This method was modified by changing reaction medium. 3,5-Disubstituted 2-thiohydantoines were all prepared by the reaction of appropriate isothiocyanates (phenyl, benzyl, *p*-nitrophenyl isothiocyanate and *p*-phenylene diisothiocyanate) with aspartic and glutamic acids and lysine. Following Edman's procedure, in the first step of the reaction, *i.e.* in addition reaction, we used a pyridine–water (1 : 1) mixture as reaction medium, but the low yields of addition products led us to seek for another reaction medium. Water–triethylamine (1 : 1) mixture was found to be most suitable. The second step of the reaction is cyclization which with all derivatives was carried out in 1M-HCl, except for *p*-phenylene isothiocyanate derivatives which were prepared with the use of glacial acetic acid.

The reaction of lysine with above isothiocyanates is an exception. Due to the presence of two amino groups of comparable basicity ($pK_a$ 8·95 and 10·53) (ref.[5]) both amino groups then react with isothiocyanates (derivative *III*, Table I.) On attempting to utilize only one amino group in the reaction (particularly during kinetic measurements) we protected ε-amino group by acetylation and prepared ε-N-acetyl-L-lysine[11].

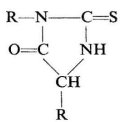
Prepared 3,5-disubstituted 2-thiohydantoines as well as corresponding bis-derivatives of type B (compounds *VIII*–*X*, see Experimental) show in UV region two absorption bands; the strong band (log $\varepsilon > 4$) at 265–270 nm is assigned to "N" conjugation in thiohydantoine ring (so called thioamide band)[6,7]. As follows from Table II, substances *VI* and *VII*, contrarily to the other derivatives, show additional absorption band at around 340 nm which can be denoted as "K" band of the conjugated system formed due to the presence of the nitro group on benzene ring.

TABLE I
3,5-Substituted 2-Thiohydantoines

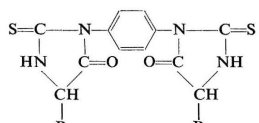
| No | Formula<br>m. w. | M. p., °C<br>yield, % | Calculated/Found<br>% N | <br>% S |
|---|---|---|---|---|
| *I* | $C_{11}H_{10}N_2O_3S$<br>250·3 | 228–229[a]<br>70 | — | — |
| *II* | $C_{12}H_{12}N_2O_3S$<br>264·3 | 165–167[a]<br>68 | — | — |
| *III* | $C_{20}H_{22}N_4OS_2$<br>398·5 | 164<br>65 | — | — |
| *IV* | $C_{12}H_{12}N_2O_3S$<br>264·3 | 152<br>60 | 10·60 10·95 | 12·13 12·05 |
| *V* | $C_{13}H_{14}N_2O_3S$<br>278·3 | 250[b]<br>58 | 10·07 10·16 | 11·52 11·76 |
| *VI* | $C_{11}H_9N_3O_5S$<br>295·3 | 94–95<br>62 | 14·23 14·24 | 10·86 10·70 |
| *VII* | $C_{12}H_{11}N_3O_5S$<br>309·3 | 139<br>65 | 13·59 13·65 | 10·37 10·23 |
| *VIII* | $C_{14}H_{14}N_4O_2S_2$<br>334·4 | 250[b]<br>68 | 16·75 16·76 | 19·18 19·00 |
| *IX* | $C_{16}H_{14}N_4O_6S_2$<br>422·4 | 300[b]<br>59 | 13·26 13·39 | 15·18 15·16 |
| *X* | $C_{18}H_{18}N_4O_6S_2$<br>450·5 | 280[b]<br>56 | 12·43 12·46 | 14·24 14·24 |

[a] Ref.[4] records m. p. 229°C (*I*) and 166°C (*II*); [b] decomposition.

The infrared spectra of studied 3,5-disubstituted 2-thiohydantoines show a number of bands, of which characteristic ones are those corresponding to the vibrations of ring C=O group (1760–1785 $cm^{-1}$) and free carbonyl group (1710–1740 $cm^{-1}$). These two different carbonyl groups could be distinguished by comparing the compounds under study with the derivatives containing amidic carbonyl only (derivatives *III* and *VIII*, Table II).



A



B

For the purpose of comparison of the reactivity of isothiocyanates with various amino acids, the rate constants of the addition of the above mentioned amino acids were determined by the method described earlier[8,9]. The conditions used (pseudomonomolecular reaction) excluded the competition reaction with $OH^-$ ions, so that the UV spectra measured after completion of the reaction were the spectra of corresponding N-substituted thiocarbamoylamino acids.

As follows from Table III, the rate constants $k$ of the addition of the amino acids (DL-aspartic and DL-glutamic acids, L-lysine) are comparable within one order of

TABLE II

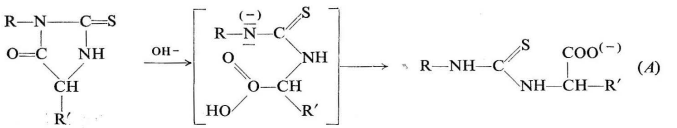
Spectral Characteristics ($\lambda_{max}$ in nm, $\tilde{\nu}$(C=O) in $cm^{-1}$) of 2-Thiohydantoines *I*–*X*

| No | $\lambda_{max,1st}$ | log $\varepsilon$ | $\lambda_{max,2nd}$ | log $\varepsilon$ | $\tilde{\nu}(C{=}O)_{ring}$ | $\tilde{\nu}(C{=}O)_{carboxyl}$ |
|---|---|---|---|---|---|---|
| *I* | 234sh | 4·07 | 270 | 4·34 | 1 780 | 1 720 |
| *II* | 235sh | 4·02 | 270 | 4·32 | 1 785 | 1 730 |
| *III* | — | — | 268 | 4·50 | 1 780 | — |
| *IV* | 234 | 4·02 | 267 | 4·27 | 1 770 | 1 740 |
| *V* | 235 | 3·94 | 267 | 4·26 | 1 760 | 1 710 |
| *VI*[a] | 225 | 4·23 | 265 | 4·30 | 1 775 | 1 740 |
| *VII*[b] | 225 | 4·22 | 265 | 4·33 | 1 760 | 1 715 |
| *VIII* | — | — | 267 | 4·30 | 1 775 | — |
| *IX* | — | — | 269 | 4·48 | 1 770 | 1 730 |
| *X* | 225 | 4·44 | 269 | 4·50 | 1 770 | 1 730 |

[a] $\lambda_{max,3rd}$ 340 nm, log $\varepsilon$ 4·05; [b] $\lambda_{max,3rd}$ 340 nm, log $\varepsilon$ 3·95.

magnitude with the values determined for the reaction of glycine by Drobnica and Augustin[8]. On reaction of L-lysine and ε-N-acetyl-L-lysine with phenyl and benzyl isothiocyanate and *p*-phenylene diisothiocyanate both amino groups of the former compound undergo transformation, which is indicated by much lower rate constant $k$ of the reaction of ε-N-acetyl derivative.

The stability of 2-thiohydantoine ring was examined at two different pH's. The rate of decyclization was found to be a function of concentration of $OH^-$ ions (Table IV). It may be assumed that $OH^-$ ion attacks electron-deficient carbon of the carbonyl group of 2-thiohydantoine ring (Equation *A*)). As outlined in the scheme, the product of the




(*A*)

decyclization is corresponding N-substituted thiocarbamoylamino acid, which was proved by comparison of the UV spectra of reaction products with the spectra of N-substituted thiocarbamoylamino derivatives.

TABLE III

Rate Constants $k'(\text{min}^{-1})$ and $k(1\ \text{mol}^{-1}\ \text{min}^{-1})$ of Addition of Amino Acids to Isothiocyanates

| Amino acid | Isothiocyanate | $k' \cdot 10^2$ | $k$ | $t_{1/2,\,\text{min}}$ |
|---|---|---|---|---|
| DL-Asp | phenyl | 2·55 ± 0·05 | 20·20 ± 0·55 | 27·1 |
| DL-Glu | phenyl | 3·15 ± 0·20 | 22·80 ± 1·4 | 22·0 |
| L-Lys | phenyl | 10·40 ± 0·40 | 40·80 ± 1·4 | 6·6 |
| Acetyl-Lys | phenyl | 2·57 ± 0·11 | 10·90 ± 0·45 | 26·9 |
| DL-Asp | benzyl | 0·315 ± 0·02 | 2·69 ± 0·15 | 220·0 |
| DL-Glu | benzyl | 0·481 ± 0·01 | 3·46 ± 0·03 | 144·0 |
| L-Lys | benzyl | 0·856 ± 0·20 | 3·37 ± 0·10 | 80·9 |
| Acetyl-Lys | benzyl | 0·419 ± 0·08 | 1·94 ± 0·04 | 165·3 |
| DL-Asp | *p*-nitrophenyl | 1·042 ± 0·05 | 20·20 ± 0·03 | 66·6 |
| DL-Glu | *p*-nitrophenyl | 6·30 ± 0·07 | 45·30 ± 0·40 | 11·0 |
| DL-Asp | *p*-phenylenedi- | 69·0 ± 1·00 | 585·0 ± 2·0 | 1·0 |
| DL-Glu | *p*-phenylenedi- | 102·0 ± 2·00 | 734·0 ± 4·0 | 0·67 |
| DL-Lys | *p*-phenylenedi- | 207·0 ± 1·50 | 815·0 ± 3·0 | 0·33 |
| Acetyl-Lys | *p*-phenylenedi- | 245·0 ± 2·00 | 1140·0 ± 5·0 | 0·28 |

TABLE IV

Rate Constants of Decyclization, $k'(min^{-1})$, at Different pH (25 ± 0·2°C)

| Derivative | $k' \cdot 10^2$ at pH | |
|---|---|---|
| | 9·77 | 10·52 |
| *I* | 0·36 ± 0·020 | 1·60 ± 0·005 |
| *II* | 0·88 ± 0·015 | 1·08 ± 0·011 |
| *III* | 0·88 ± 0·022 | 1·20 ± 0·002 |

## EXPERIMENTAL

Isothiocyanates (benzyl, phenyl, 4-nitrophenyl isothiocyanate and *p*-phenylene diisothiocyanate) used to synthesize 3,5-disubstituted 2-thiohydantoines were prepared by thiophosgene method from appropriate amines[10]. DL-aspartic and DL-glutamic acids as well as L-lysine were commercial preparations (Lachema and Koch-Light).

3,5-*Disubstituted 2-thiohydantoines.* Ten mmol of an appropriate amino acid were dissolved in 25 ml of water and 25 ml of triethylamine, pH of the solution was adjusted to 9 by 1M-NaOH, and the temperature of the solution was raised to 40°C. Then 12 mmol of corresponding isothiocyanate were added in one portion, while stirring, and the reaction mixture was allowed to stir until the reaction was completed (30—60 min). The pH of the mixture was kept during the reaction at ~9. After the reaction stopped (no change in pH value), the solvents were removed on vacuum evaporator, to yield crystalline addition product. Recrystallized products were used as standards for identification of the products of decyclization of 2-thiohydantoines (see kinetic measurements).

N-substituted thiocarbamoylamino acids so prepared were subjected to cyclization in 1M-HCl (reflux for 2—5 h). The reaction mixture was evaporated to dryness on vacuum evaporator and the residue was crystallized from 2% ethanolic HCl solution

ε-N-acetyl-L-lysine was prepared by the method reported by Leclerc and Benoiton[11], by acetylation with phenyl acetate at pH 11. The derivatives of 2-thiohydantoines with ε-N-acetyl--L-lysine were prepared in the above described way. Prepared 3,5-disubstituted 2-thiohydantoines of type A are listed in Table I, where R, R′ is for *I* ($C_6H_5$—, $CH_2COOH$); *II* ($C_6H_5$—, $(CH_2)_2COOH$); *III* ($C_6H_5$—, $(CH_2)_4$—NH—CS—NH—$C_6H_5$); *IV* ($C_6H_5CH_2$—, $CH_2COOH$); *V* ($C_6H_5CH_2$—, $(CH_2)_2COOH$); *VI* (4—$NO_2$—$C_6H_4$—, $CH_2COOH$); *VII* (4—$NO_2$—$C_6H_4$—, $(CH_2)_2COOH$), along with bis-2-thiohydantoines of type B where R is for *VIII* (—$CH_3$), *IX* (—$CH_2COOH$), *X* ($(CH_2)_2COOH$).

*The UV spectra* in the 220—360 nm region were recorded with Specord UV VIS spectrophotometer (Zeiss, Jena) using 10 mm thick cells. The IR spectra were taken with Zeiss, Model UR-20, spectrophotometer (700—3 600 $cm^{-1}$, KBr pellets: 2 mg compound/1g KBr).

*Kinetic measurements* were carried out on Specord UV VIS spectrophotometer at 25 ± 0·2°C. The buffers were adjusted on pH-meter (Radelkis, Budapest) with the accuracy of ±0·01.

Kinetics of the addition reaction was measured using the reaction mixture containing 9 ml of 0·1M borate buffer (pH 9·77), 0·5 ml of 0·05M aqueous solution of amino acid and 0·5 ml of 0·001M methanolic isothiocyanate solution. Apparent rate constants $k'$ ($min^{-1}$) were calculated

from linear dependence of log $\Delta E$ on time. Rate constants proper, $k_1$ (1 $mol^{-1}$ $min^{-1}$), were obtained by dividing $k'$ by the concentration of amino acids present in the form of the base (p$K$: DL-aspartic acid 9·60, DL-glutamic acid 9·67, L-lysine see above).

The rate of decyclization was measured by following changes in the extinction corresponding to absorption maximum of 2-thiohydantoine dissolved in 0·1M borate buffers of pH 9·77 and 10·52. Rate constants $k'$ were calculated from the expression for pseudo-first order reaction.

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