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СТРОЕНИЕ МОЛЕКУЛЫ ТРИПТОФАНА: ЭЛЕКТРОНОГРАФИЧЕСКОЕ И КВАНТОВО-ХИМИЧЕСКОЕ ИССЛЕДОВАНИЕ

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В представленной работе теоретическими (квантово-химические расчеты) и экспериментальными (газовая электронография) методами проведено исследование структуры молекулы и конформационных свойств триптофана. Расчеты выполнены в программе Gaussian 03 методом B3LYP с базисным набором cc-pVTZ. Они показали наличие шести конформеров, которые существуют в паре при температуре эксперимента (T = 495 K). Теоретически оценена чувствительность данных электронографии к конформационным изменениям в структуре молекулы триптофана. Конформеры имеют различную ориентацию как карбоксильной и аминогруппы, так и основной цепи и индольного фрагмента относительно друг друга. На основании этого их можно разделить на две группы: различимые (с разным торсионным углом C(OOH)-C(HNH$_2$)-C(H$_2$)-C(ind)) и слаборазличимые (с разными торсионными углами H-N-C-C и H-O-C-C) в рамках электронографического эксперимента. Определены молекулярные параметры конформеров триптофана. Установлено, что в конформерах этой аминокислоты присутствует внутримолекулярная водородная связь H$_2$N···HO. Анализ электронографических данных проведен на основе предположения, что насыщенный пар над триптофаном при температуре эксперимента T = 495 K представляет собой смесь как минимум двух конформеров с наименьшими энергиями. Показано, что наилучшее соотношение этих конформеров между собой I : II является 50 : 50, соответственно. Проведено сравнение геометрических параметров молекул аминокислот (глицин, аланин, триптофан), полученных из данных газовой электронографии в этой работе и ранее. Установлено влияние внутримолекулярной водородной связи на структурные параметры основной цепи молекул указанных аминокислот.

Ключевые слова: триптофан, аминокислота, газовая электронография, квантово-химические расчеты, конформер, структура молекулы

MOLECULAR STRUCTURE OF TRYPTOPHAN: GAS-PHASE ELECTRON DIFFRACTION AND QUANTUM-CHEMICAL STUDIES

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The molecular structure and conformational properties of tryptophan have been investigated by gas-phase electron diffraction and theoretical methods. Quantum chemical calculations realized by program Gaussian 03 (B3LYP/cc-pVTZ) have been predicted the existence of six conformers at the temperature of experiment (T = 495 K). The ability of gas-phase electron diffraction

method to distinguish the structure of conformers was estimated theoretically. Conformers have different orientations of carboxylic and amine group, backbone and indole fragment to each other. These conformers can be divided on two groups: distinguishable parameters (with different torsion angle C(OOH)-C(HNH_2)-C(H_2)-C(ind)) and weekly distinguishable ones (with different torsion angles H-N-C-C and H-O-C-C) by gas-phase electron diffraction. The molecular parameters of the conformers were determined. The conformers have intramolecular hydrogen bonding of the $H_2N\cdots HO$. The analysis of the gas-phase electron diffraction data have been carried out assuming the saturated vapor of tryptophan at T = 495 K consists of mixture at least of two conformers with lowest energy. It was shown that optimal ratio between conformers I : II was 50 : 50, respectively. The geometrical parameters of amino acids molecules (glycine, alanine, tryptophan) obtained by gas-phase electron diffraction were compared. The influence of the intramolecular hydrogen bond was established onto the structural parameters of the backbone of the above amino acids molecules.

Key words: tryptophan, amino acid, gas-phase electron diffraction, quantum chemical calculations, conformer, molecular structure

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INTRODUCTION

Amino acids are the building blocks of peptides and proteins having fundamental chemical, biochemical and biological significance. They take part in many biochemical processes (neuroregulation, enzyme catalysis, synthesis of bioactive substances) [1]. In the last years, the synthetic peptides with amino acid residues have gained high interest as new drugs to overcome bacterial resistance to classical antibiotics [2]. Peptides have been used in bionanotechnology (biosensor, molecular electronics) [3]. Amino acid molecules have a unique structure. Zwitterionic forms of amino acids are stabilized in the crystalline state and in solution whereas the neutral structures are found in the gas phase [4]. Therefore, the structure of amino acids are still the objects of careful study in both of these media.

In the gaseous phase the aromatic amino acids, tryptophan (Trp), phenylalanine (Phe), tyrosine (Tyr), have been studied by UV spectroscopy due to presence of UV chromophores in their side chains [5], matrix-isolation spectroscopy with quantum chemical calculations [6,7], UV and IR ion-dip spectroscopy combined with quantum chemical calculations [8], microwave spectroscopy [9] and other methods.

Amino acid molecules have low symmetry and many rotational degrees of freedom. The conformational behavior of amino acids in the gas phase is considerably complicated, particularly for the aromatic amino acids with high rotational freedom or plausible intramolecular hydrogen bonds (H-bonds). For this reason a large number of different low-energy conformers are expected on the surface of potential energy. Therefore, it is difficult to carry out a structural study of the amino acid molecule in the gas phase by experimental methods. Nevertheless, the molecular structures of some amino acids and bioactive compounds have been experimentally investigated by the gas-phase electron diffraction (GED) [4, 10-14]. The structures and preferred conformations of aromatic amino acids in gas phase are still indefinite. The molecular structures of the most stable conformers of neutral glycine (Gly), alanine (Ala), and proline (Pro) have been determined by the joint analysis of electron diffraction data and rotational constants, in conjunction with the results of an *ab initio* calculation [4, 13, 14]. Study of the Trp molecule was carried out by ultrafast electron diffraction in work [15]. The structure of Trp conformer without intramolecular hydrogen bond was obtained by experiment and quantum chemical calculations. However, the conformer obtained was no minimum on the potential energy surface in the quantum chemical studies of gaseous molecule of Trp.

The aim of our study was to determine the geometrical parameters and conformational composition of gaseous Trp using electron diffraction and quantum chemistry methods. The theoretical radial distribution functions are compared for different types of conformers in electron diffraction. It should be noted that the presented study of the Trp led to the identification of highly accurate molecular structures, which demonstrated the reliability of the GED method associated with high-level quantum-chemical calculations for determining the structure of the amino acid. The main goal of this work is experimental and theoretical investigations of the structure

and properties of the aromatic amino acid, Trp, and their conformational dependency by synchronous GED and mass spectrometry (GED/MS) along with high level quantum chemical calculations. We propose conformational classification for the neutral form of Trp with a density functional theory using the Gaussian 03 program system [16]. We investigate the differences in structure and charge distribution between the subgroups of Trp.

EXPERIMENTAL PART

Chemicals

Commercially available L-tryptophan [CASRN 73-22-3] with purity of 99%, was obtained from Aldrich Chemical Co., and was used without further purification. Before use, the sample was carefully dried under vacuum during 48 h at the temperature 323 K and then stored over P_2O_5 in desiccators, no further purification being followed.

Computational details

In this work, quantum chemical calculations of geometry and electronic properties of Trp's conformers were carried out by Gaussian 03 [16] at the B3LYP/cc-pVTZ level of theory. The charge distribution was analyzed using the natural bond orbital (NBO) analysis [17] using the Gaussian 03 package [16].

GED/MS experiment

Electron diffraction patterns were recorded simultaneously with the mass spectra using special techniques. The electron diffraction experiment was carried out using the combined GED/MS apparatus [18], consisting of the EMR-100 gas-phase electron diffraction and the APDM-1 monopole mass spectrometric units. The conditions of the GED/MS experiment are shown in Table 1.

The temperature of stainless steel effusion cell was measured by a W/Re-5/20 thermocouple, which was standardized using the melting points of Sn and Al. Polycrystalline ZnO was used to establish the wavelength of the fast electrons recorded before and after the GED experiments on Trp.

Table 1

Experimental conditions for GED/MS study of Trp

***Таблица 1.* Экспериментальные условия синхронного электронографического и масс-спектрометрического исследования Trp**

Nozzle-to-plate distance, mm	338	598
Electron beam current, μA	1.50	0.70
Ionizing voltage, V	50	50
Accelerating voltage, kV	94.0	90.8
Temperature of effusion cell, °C	222	222
Residual gas pressure diffraction chamber/MS-block, torr	$2\cdot10^{-6}$/ $6\cdot10^{-7}$	$3\cdot10^{-6}$/ $5\cdot10^{-7}$
Scattering angles s_{min} - s_{max}, Å^{-1}	3.7-31.0	1.2-17.2

The Trp sample was evaporated at T = 495±5 K from a stainless steel effusion cell. Microphotometric measurements were carried out by means of automatic techniques [19]. The scattered electrons were collected on the Kodak Electron Image films SO-163 of 9×12 cm at two camera distances, 598 and 338 mm. The electron diffraction patterns were scanned by a modified [19] computer-controlled microdensitometer Carl Zeiss Jena MD-100. For each film, a rectangular area of 10 × 130 mm was scanned at a diagonal direction (33 equidistant lines with a step of 0.1 mm along each line). The molecular intensities were obtained in the ranges 2.6-28.5 Å^{-1} (short camera) and 1.2-15.0 Å^{-1} (long camera).

The mass spectra of the effusing molecular beam were recorded simultaneously with recording diffraction patterns. These data indicate that the monomeric molecules of Trp are presented in the vapor. No volatile impurities were detected at a level of 1%, at least. Mass-spectrum is the same as a described in [20].

RESULTS AND DISCUSSION

Quantum chemical calculations

Several theoretical studies have been carried out on the conformational analyses of neutral tryptophan [21, 22]. In case of structural analyses of electron diffraction experiment, the data of geometry of molecule, force field, vibrational amplitudes, and vibrational corrections of intermolecular distances were necessary. We perform the conformational analysis of the Trp molecule at the B3LYP/cc-pVTZ level of electronic structure theory using the Gaussian 03 program [16]. The calculations predict 40 stable conformations of neutral tryptophan, energies of which lie within 10 kJ/mol. No imaginary frequencies were observed for any of the conformers examined, confirming that the optimized structures were minima on the potential energy surface. All conformers can be divided into 6 groups in relation to different orientations of carboxylic and amine group, backbone and indole fragment to each other. Relative energies and structures of conformer groups are given in Supplementary (Table S1 and Fig. S1 http://journals.isuct.ru/ctj/article/view/1985/1128). Structures of six most stable conformers with the lowest-energy are shown in Fig. 1. Each of them has an intramolecular hydrogen bond.

Because intramolecular hydrogen bonds play an important role in the molecular structures and conformational preferences of amino acids, for the six low-energetic conformers of neutral Trp, NBO-analysis at the B3LYP/cc-pVTZ level of theory was performed. The most stable Trp conformers exhibit an intramolecular hydrogen bond between the hydrogen atom of the hydroxyl group and the lone pair of electrons on the nitrogen atom of the amino group (N···H-O) (Fig. 1).

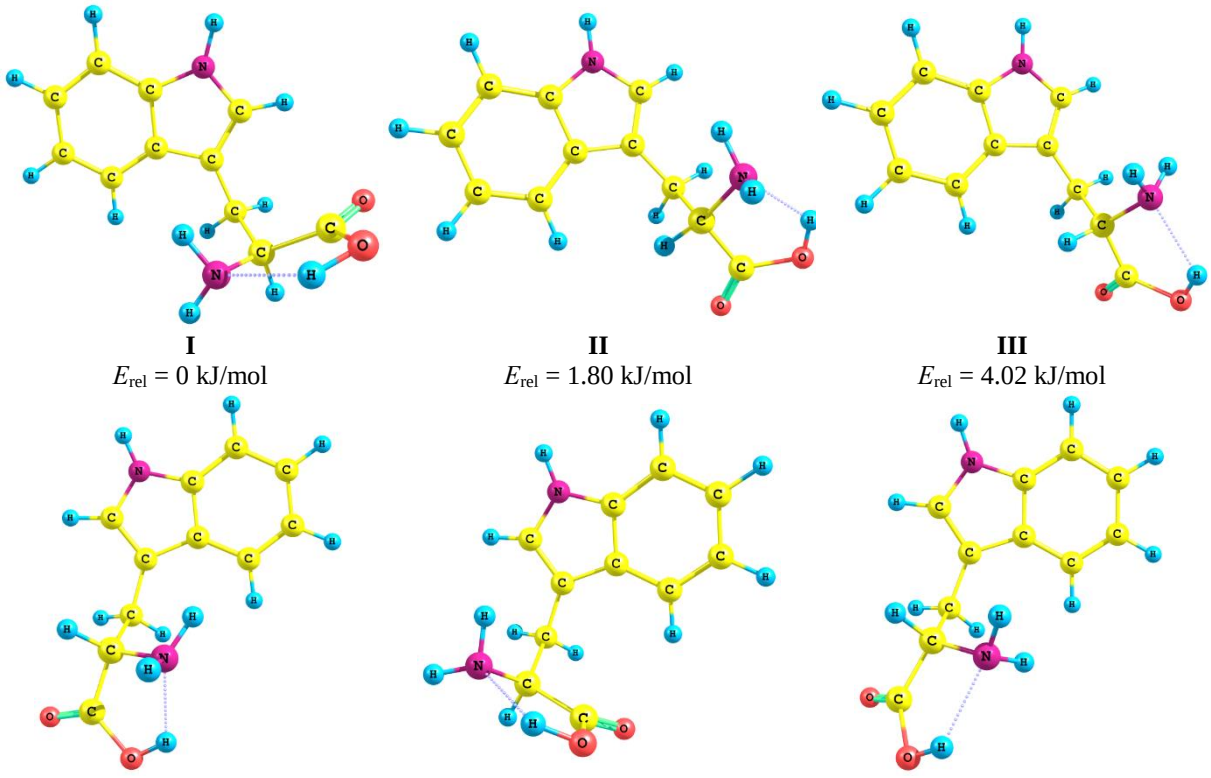

Fig. 1. Low-energetic conformers of Trp (E_{rel} - relative energy)
Рис. 1. Конформеры Trp с наименьшей энергией (E_{rel} – относительная энергия)

As in some other amino acids [23], the hydrogen bond between the carboxyl hydrogen and the nitrogen in the amino group is the strongest. The average energies of the interaction, E(LP(N) → σ*(O-H)), between atomic orbital of N with a lone electron pair (LP(N)) as a donor and an "antibonding" orbital of the O-H bond (σ*(O-H)) as acceptor, and transfer charge values (q_{tr}) are shown in table 2. The amount of transfer charge (q_{tr}) is higher than 0.01e [24].

Table 2

Energies of orbital interactions *LP*(N)-σ*(O-H) and amounts of charge transfer (q_{tr}) for main conformers of Trp based on NBO theory (at B3LYP/cc-PVTZ level of electronic structure theory)

***Таблица 2.* Энергии взаимодействия орбиталей *LP*(N) с σ*(O-H) и величины перенесенного заряда (q_{tr}) для основных конформеров Trp**

Conformer	E(LP(N)→σ*(O-H)), kJ/mol	q_{tr}
I	63.89	0.033
II	57.20	0.029
III	61.92	0.031
IV	56.57	0.029
V	60.25	0.031
VI	66.36	0.035

As it is known, the relative energy values of these conformers reflect their concentrations in the gas phase at T_{exp}. The six of the 40 Trp conformers obtained have a significant concentration in a saturated vapor (72% at T = 495 K). The concentrations of the other conformers are negligible. These minor species could not be detected in the GED study. Thus, the main contributions into the electron scattering intensities are made by the six conformers during the electron diffraction experiment.

Sensitivity of GED method to distinguish the structure of conformers

Among 6 selected conformers of Trp the conformers II and III are indistinguishable for GED because of them differs only by hydrogen orientation in amino group. The same reason explains the closeness of GED data of conformers IV and VI. Conformers I and V have the same structure of backbone and differ by indole ring orientation. Radial distribution functions *f(r)* for the Trp conformers with different orientation of indole ring and backbone were compared (Fig. 2). Electron diffraction data were found to be more sensitive to the rotation of the backbone than the indole ring.

Tryptophan conformers may be distinguishable, since the discrepancy factor R_f between theoretical functions *f(r)* for the conformers exceeds the level determined by the experimental noise of photo plate. Thus, the pair of conformers I and V, II and IV, III and

VI may be integrated. Taking into account the indistinguishing by GED conformers II and III, IV and VI, the six conformers can be reduced to I and II.

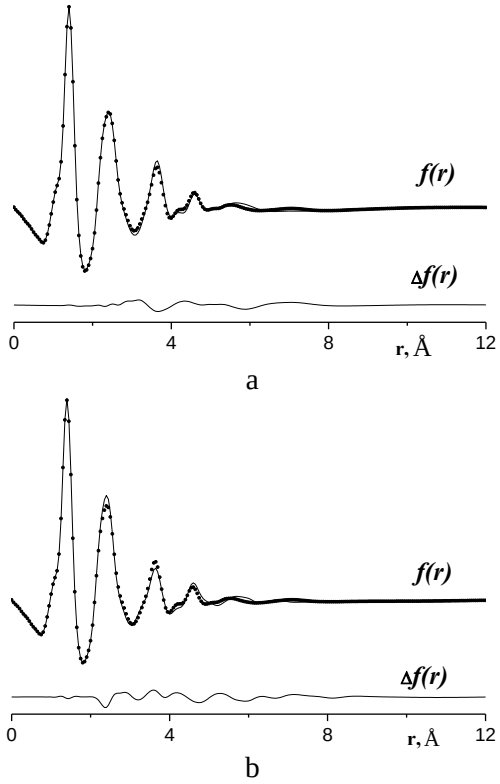

Fig. 2. Radial distribution functions of Trp. *f(r)* – theoretical radial distributions, Δ*f(r)* – difference curve a) for I and V conformers: different orientation of indole ring R_f = 5.3%; b) for I and II conformers: rotation of backbone R_f = 7.7%

Рис. 2. Функции радиального распределения Trp. *f(r)* – теоретические радиальные распределения, Δ*f(r)* – разностная кривая а) для I и V конформеров: различная ориентация индольного кольца R_f = 5,3%; b) для I и II конформеров: вращение основной цепи R_f = 7,7%

Structure analysis

Structural analysis was performed under the assumption that there are 2 conformers in the Trp vapour — I and II with different backbone locations relative to C_α-C_β. A conventional least squares analysis of sM(s) was carried out using a modified version of KCED program [25]. The starting values for bond distances and angles were taken from the B3LYP/cc-pVTZ calculations for I and II. Vibrational corrections, $\Delta r = r_{h1} - r_a$, and the starting values for vibrational amplitudes were derived from the B3LYP/cc-pVTZ force field, using the curvilinear approach of Sipachev (program SHRINK) [25]. According to Fig. 2, the indole ring has a weak influence on radial distribution functions. The two conformers, I and II, were only used for structural analysis.

The molecular structures of each conformer were constructed with 75 independent structural parameters: 28 bond distances, 25 bond angles and 22 torsion angles. In our investigation an attempt to reduce the complexity of model was done by means of decreasing the number of parameters. The following assumptions were used: (i) the 4 bond distances (N1-H16, C2-C3, C10-C3, C12-O13) were chosen independently variable (the difference between the nonequivalent distances of the same type was hold as obtained in quantum chemical calculations); (ii) the 9 bond angles (N3-C2-C1, C4-N1-C2, C6-C5-C4, C10-C3-C2, C11-C10-C3, O13-C12-C11, O14-C12-C11, N15-C11-C10, N15-C11-C12) independently modified (the differences between all angles fixed were constrained to calculated quantum chemical values); (iii) the 3 torsion angles (C1-C10-C3-C2, C12-C11-C10-C3, O13-C12-C11-C10) were chosen independently variable. With these restrictions, the number of parameters reduces to 16. The independent r_h parameters were used for the geometrical constructions. Vibrational amplitudes were refined in groups with fixed differences. The experimental molecular intensities and the radial distributing curves are shown in Fig. 3 and Fig. 4, respectively. The molecular intensities *sM*(*s*) were obtained in the ranges 3.7–31.0 $Å^{-1}$ and 1.2–17.2 $Å^{-1}$ for the short and long camera distance, respectively (Fig. 3). The molecular intensity was calculated by the formula

$$sM(s) = s \cdot [I(s) - G(s)]/G(s).$$

The results of the least squares analysis of experimental data showed that at least, two conformers of tryptophan are present as the most stable conformers

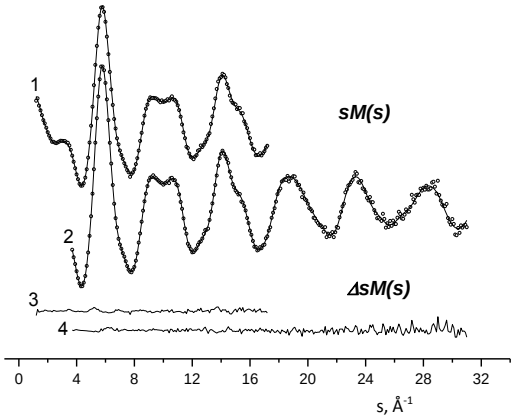

Fig. 3. Experimental (dots) and theoretical (solid line) molecular intensities *sM(s)* curves at long (1) and short (2) nozzle-to-plate distances and the difference curves Δ*sM(s)* at long (3) and short (4) nozzle-to-plate distances for Trp

Рис. 3. Экспериментальная (точки) и теоретическая (сплошная линия) функции молекулярной составляющей интенсивности рассеяния *sM(s)* для длинного (1) и короткого (2) расстояния сопло-фотопластинка и разностные кривые Δ*sM(s)* для длинного (3) и короткого (4) расстояния сопло-фотопластинка для молекулы Trp

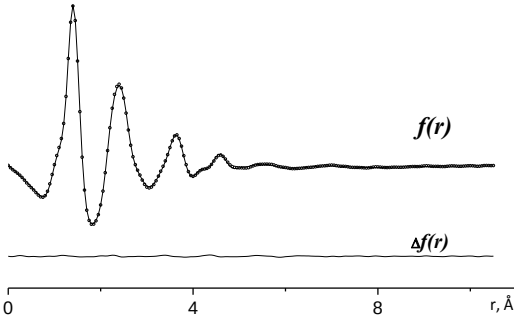

Fig. 4. Experimental (dots) and theoretical (line) radial distribution curves *f(r)* for Trp and the difference curves Δ*f(r)* for various ratio of Trp conformers

Рис. 4. Экспериментальная (точки) и теоретическая (линия) кривые радиального распределения *f(r)* для Trp и разностная кривая Δ*f(r)* для различного соотношения конформеров Trp

in the gas phase in ratio as I:II = 50:50. Under the experimental condition of T = 495 K, about 50% of the gas exists in the I and V conformers form and 50% of tryptophan in the gas is probably a mixture of II, III, IV, and VI conformers. It should be noted that the molecular constants of these conformers have no significant differences (Table 3). Final results of the least squares analysis of GED data are given in Table 3. The best fit of the experimental scattering intensities (R_f = 4.02%) was obtained for the conformer ratio 50:50. The experimental structural parameters are found to be in good agreement with the results of theoretical calculations.

Vibrational amplitudes and correction terms, $\Delta r = r_{h1}-r_a$, were derived from the theoretical force field taking into account nonlinear kinematic effects at the level of the first order perturbation theory with the use of the program SHRINK [25]. Some of the most important amplitudes and correction terms are listed in Table 4.

Table 3

Experimental (conformer I) and optimized geometric parameters (conformers I and II) of Trp

Таблица 3. **Экспериментальные (конформер I) и теоретические структурные параметры (конформеры I и II) молекулы Trp**

Conformer	I	I	II
Parameters: r, Å; ∠, º	GED, r_{h1}	B3LYP/ cc-pVTZ, r_e	B3LYP/ cc-pVTZ, r_e
N1-H16	1.014(4) [a] p_1	1.003	1.003
C2-H17	1.087(4)(p_1)	1.077	1.078
C10-H22	1.099(4)(p_1)	1.089	1.093
O14-H25	0.994(4)(p_1)	0.984	0.982
C2-C3	1.374(3) p_2	1.370	1.370
N1-C2	1.380(3)(p_2)	1.376	1.378
C10-C3	1.498(5) p_3	1.500	1.495
C12-O13	1.195(6) p_4	1.203	1.202
N3-C2-C1	110.1 (10) [b] p_5	110.0	110.1
C4-N1-C2	110.6(10) p_6	109.4	109.2
C6-C5-C4	118.7(2) p_7	118.6	118.8
C10-C3-C2	125.9(20) p_8	126.4	126.6
C11-C10-C3	112.5(13) p_9	113.9	113.6
O13-C12-C11	123.9(25) p_{10}	123.3	122.9
O14-C12-C11	110.0 (25) p_{11}	113.7	113.9
N15-C11-C10	118.2(50) p_{12}	115.9	110.7
N15-C11-C12	111.3 (18) p_{13}	109.3	109.9
C1-C10-C3-C2	82(6) [b] p_{14}	84.5	108.2
C12-C11-C10-C3	-71 (5) p_{15}	-69.9	176.4
O13-C12-C11-C10	-35 (12) p_{16}	-37.7	-73.1
mol.%	50	50	50
R_f, %	4.017	---	---

Notes: a -uncertainty in r_{h1}– distances were calculated as $\sigma=(\sigma_{sc}^2+(2.5\sigma_{LS})^2)^{1/2}$ (σ_{sc} =0.002r, σ_{LS} –standard deviation in LS-analysis); b uncertainty for angles is $3\sigma_{LS}$

Примечания: a -погрешность в r_{h1}– расстояниях вычислялась как $\sigma=(\sigma_{sc}^2+(2{,}5\sigma_{LS})^2)^{1/2}$ (σ_{sc} =0,002r, σ_{LS} –стандартное отклонение в МНК-анализе); b погрешность в величинах углов принята равной $3\sigma_{LS}$

Table 4

Bond distances, experimental and calculated vibration amplitudes and vibrational corrections (without non-bonded distances involving hydrogen atoms) [a]

Таблица 4. **Межъядерные расстояния, экспериментальные и теоретические амплитуды колебаний и колебательные поправки (за исключением термов для несвязанных атомов, включающих атомы водорода) [a]**

Interaction [c]	r_a [b]	l_{exp} [b]	l_{calc}	$\Delta r = r_{h1}-r_a$	Refinement group
1	2	3	4	5	6
O14- H25	0.9935	0.074(2)	0.072	0.0007	1
N 1- H16	1.0116	0.071	0.069	0.0020	1
C 2- H17	1.0854	0.076	0.074	0.0015	1
C 8- H20	1.0903	0.077	0.075	0.0014	1
C 6- H18	1.0915	0.077	0.075	0.0014	1
C10- H22	1.0984	0.078	0.076	0.0011	1
O14- C12	1.3282	0.046(1)	0.046	0.0005	2
C 3- C 2	1.3730	0.044	0.044	0.0012	2
C 4- N 1	1.3819	0.046	0.046	-0.0037	2
C 2- N 1	1.3843	0.046	0.046	-0.0039	2
C 6- C 7	1.3864	0.045	0.045	0.0009	2
C 9- C 8	1.3876	0.045	0.045	0.0006	2

1	2	3	4	5	6
C 4- C 9	1.3974	0.046	0.046	-0.0000	2
C 5- C 4	1.4212	0.047	0.047	0.0019	2
C 3- C 5	1.4390	0.049	0.049	0.0051	2
C11- N15	1.4743	0.052	0.052	-0.0001	2
C10- C 3	1.4973	0.051	0.051	0.0002	2
C11- C10	1.5394	0.055	0.056	0.0005	2
C11- C12	1.5411	0.056	0.056	-0.0004	2
O14…O13	2.2496	0.057(3)	0.055	0.0008	3
C 2…C 4	2.2620	0.054	0.053	0.0054	3
C 5…N 1	2.2283	0.055	0.053	-0.0038	3
C11…H25	2.1611	0.145	0.144	0.0052	3
C 3…C 4	2.3307	0.056	0.055	0.0067	3
C 5…C 7	2.4074	0.058	0.057	0.0032	3
O13…C11	2.4144	0.069	0.068	0.0052	3
C 4…C 6	2.4278	0.060	0.059	0.0062	3
C12…N15	2.4852	0.072	0.070	0.0044	3
O14…N15	2.5531	0.108	0.107	-0.0071	3
C 4…C 7	2.7602	0.065(4)	0.066	0.0072	4
C 6…C 9	2.8329	0.065	0.066	0.0067	4
O13…C10	2.8552	0.126	0.127	-0.0009	4
C 3…N15	3.0789	0.156	0.157	0.0125	4
C10…C 6	3.3218	0.114(7)	0.119	0.0136	5
O13…C 2	3.2611	0.352	0.357	0.0077	5
C 5…N15	3.5843	0.256(6)	0.263	0.0189	6
C 2…C 9	3.6329	0.056	0.063	0.0117	6
C 3…C 9	3.6569	0.055	0.062	0.0121	6
C10…N 1	3.7005	0.060	0.067	0.0051	6
C 8…N 1	3.7228	0.059	0.066	0.0076	6
C 6…N15	3.8193	0.415	0.423	0.0430	6
C10…C 4	3.7852	0.062	0.069	0.0153	6
O14…C 3	3.8775	0.265	0.272	0.0456	6
C11…C 6	4.0970	0.248(5)	0.249	0.0357	7
C 7…N 1	4.1090	0.068	0.069	0.0079	7
C12…N 1	4.4686	0.279(4)	0.275	0.0203	8
C 4…N15	4.6176	0.281	0.278	0.0285	8
C 2…C 8	4.5780	0.070	0.066	0.0166	8
O13…N 1	4.5343	0.388	0.385	0.0274	8
N15…N 1	4.7524	0.300	0.296	0.0368	8
O13…C 5	4.7228	0.273	0.270	0.0575	8
C12…C 4	5.0018	0.252(10)	0.259	0.0445	9
O14…N 1	4.8924	0.467	0.474	0.0704	9
C12…C 6	5.1567	0.292(14)	0.296	0.0719	10
O13…C 4	5.3020	0.338	0.342	0.0573	10
C 9…N15	5.6223	0.330	0.334	0.0332	10
O13…C 9	6.6539	0.371(32)	0.362	0.0775	11
O14…C 7	6.7211	0.551	0.542	0.1267	11

Notes: [a] -all values in Å; [b] - total error limit in interatomic distances was estimated by formula $\sigma=(\sigma_{sc}^2+(2.5\sigma_{LS})^2)^{1/2}$ (σ_{sc} =0.002*r*, σ_{LS} –standard deviation in LS-analysis); uncertainty for amplitudes are $3\sigma_{LS}$, [c] Numbering of atoms see at Fig. S2 http://journals.isuct.ru/ctj/article/view/1985/1128.

Примечания: [a] - все величины в Å; [b] - погрешность в межатомных расстояниях оценивалась по формуле $\sigma=(\sigma_{sc}^2+(2{,}5\sigma_{LS})^2)^{1/2}$ (σ_{sc} =0,002*r*, σ_{LS} –стандартное отклонение в МНК-анализе); погрешность для амплитуд составила $3\sigma_{LS}$, [c] Нумерация атомов дана на рис. S2 http://journals.isuct.ru/ctj/article/view/1128

Despite the great complexity of the structure of the amino acid molecule due to the need to take into account many of its conformers during the study, the experimental data obtained are in good agreement with the theoretical calculations.

Since amino acid molecules include the backbone and the various side chains, a certain structural features of the aliphatic and aromatic molecules might be expected. For three amino acids molecules, such as Gly and Ala with aliphatic side fragment and Trp with indole ring in side chain, the backbone deviates from the plane differently depending on the side chain: Gly is a flat structure, Ala is a strong distortion, Trp is the average between them.

The most stable forms of Trp molecule have the O=C-O-H dihedral angle in the *trans* orientation. As it was shown before [6, 8, 26-29], the stabilization of conformers of this type is characteristic of aromatic amino acids, contrary to the aliphatic family, where the conformers with the carboxylic group in the *cis* orientation and the bifurcated N-H…O=C bond are of lower energy [4, 14, 30]. In other words, the side chains influence the intramolecular H-bond formation. For the aliphatic (Gly [4], Ala [14]) and the hydroxyl-containing (Ser, Thr) [31] amino acids the conformers with hydrogen-bond between amino and carboxylic groups do not have the lowest energies, whereas the presence of aromatic fragment (Tyr, Phe [6] and Trp) in molecule structure is promoted to stabilize the conformers with one H-bond.

The intramolecular hydrogen bonds affect onto the structural parameters of the backbone of the above amino acids molecules. As can be seen from the data Table 5, in case of Trp molecule, ∠(C_m-C-N) increase and ∠(C-C-N) decrease, but r(C-N) longer and r(C-O) shorter on the contrary to analogical parameters of Gly and Ala molecules.

Table 5

Some geometrical parameters of amino acids by GED data
***Таблица 5.* Некоторые геометрические параметры аминокислот по данным газовой электронографии**

r, Å; ∠, º	Gly [4]	Ala [14]	Trp
C-C_m	---	1.536(11)	1.540(5)
C-N	1.467(5)	1.453(2)	1,474(3)
C-C	1.526(3)	1.527(11)	1.541(5)
C=O	1.205(1)	1.197(1)	1.195(6)
C-O	1.355(2)	1.341(2)	1.329(6)
C-C-C_m	---	111.9(2)	109.6(13)
C-C-N	112.1(5)	112.9(3)	111.3 (18)
C_m-C-N	---	110.0(2)	118.2(50)
C-C=O	125.1(5)	125.7(3)	123.9(25)
C-C-O	111.6(5)	110.3(2)	110.0(25)

CONCLUSIONS

The first study on structure determination of tryptophan molecule was performed employing the gas electron diffraction method. Geometrical parameters and conformational composition of neutral tryptophan were determined based on joint analysis of GED data and quantum chemical force constants. The DFT method with the B3LYP/cc-pVTZ level was used to optimize geometries and to obtain corrections to the total energy and Gibbs free energies to verify that all structures had real minima. A total of 40 minima have been located on the conformational space of Trp, within the 0-10 kJ/mol energy range. The conformational distribution of tryptophan at the temperature of sublimation of the compound (495 K) was calculated, and six conformers were predicted to have abundances higher than 6%. The composition of saturated vapor of tryptophan mainly contains conformers I and II. The assignment of the experimental data was based on the calculated molecular structures of these 2 conformers.

The GED refined structure of tryptophan was found to be close to the predicted one from quantum chemical calculation. The well agreement of observed and calculated molecular intensity curves was shown.

An NBO analysis was used to get quantitative evaluations of interaction energies of atomic pairs involved in intramolecular hydrogen bond. The results obtained support the presence of intramolecular hydrogen bond in all six low-energetic conformers of neutral Trp. The observed conformers for Trp are stabilized by an O–H···N interaction between the carboxylic and amino groups. Comparison of GED data of the aliphatic amino acids (Gly, Ala) with the aromatic amino acid (Trp) allowed to indicate the influence of intramolecular hydrogen bond on the structural parameters of the backbone of the molecules.

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