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ORIGINAL PAPER

DFT studies on the intrinsic conformational properties of non-ionic pyrrolysine in gas phase

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Abstract B3LYP/6-31G(d,p) level of theory is used to carry out a detailed gas phase conformational analysis of non-ionized (neutral) pyrrolysine molecule about its nine internal back-bone torsional angles. A total of 13 minima are detected from potential energy surface exploration corresponding to the nine internal back-bone torsional angles. These minima are then subjected to full geometry optimization and vibrational frequency calculations at B3LYP/6-31++G(d,p) level. Characteristic intramolecular hydrogen bonds present in each conformer, their relative energies, theoretically predicted vibrational spectra, rotational constants and dipole moments are systematically reported. Single point calculations are carried out at B3LYP/6-311++G(d,p) and MP2/6-31++G(d,p) levels. Six types of intramolecular H-bonds, viz. O…H–O, N…H-O, O…H–N, N…H–N, O…H–C and N…H–C, are found to exist in the pyrrolysine conformers; all of which contribute to the stability of the conformers. The vibrational frequencies are found to shift invariably toward the lower side of frequency scale corresponding to the presence of intramolecular H-bond interactions in the conformers.

Keywords DFT conformational analysis · Intrinsic conformational properties · Pyrrolysine conformers · Vibrational frequencies

Introduction

Pyrrolysine (Pyl), a lysine homologue with chemical identity N^6-[(4*R*,5*R*)]-4-methyl-1-*pyrro*line-5-carbonyl]-L-*lysine*, is the 22nd genetically encoded natural amino acid [1–6]. This rarely occurring amino acid is an important constituent in the active site of methylamine methyltransferases involved in methylamine metabolism in methanogenic archaea. In this group of archaea, pyrrolysine is inserted into protein in response to a canonical stop codon (UAG) which in other organisms functions as a terminator signal during the translation process of protein biosynthesis [7]. Since its discovery in 2002, there have been prolific studies to understand the biosynthetic pathway which is still unclear [8], on metal-binding affinity/selectivity of pyrrolysine [9] and on the scope of synthesizing pyrrolysine analogues [10, 11]. However, till date no research group has investigated the intrinsic conformational properties and energies of this amino acid which to a large extent determine the dynamic properties and functional specificity of pyrrolysine containing proteins and polypeptides.

Amino acids exist in non-ionic forms in the gas phase while as zwitterions in solvent and solid phases [12, 13]. Computational studies on conformational behavior of neutral amino acids in gas phase [14–20] have been performed with a view toward understanding various chemical and biochemical processes taking place in the macromolecular context of real life systems. Experimental studies on the neutral gaseous amino acids are limited due to the low thermal stability and low vapor pressure of amino acids [21, 22]. Computational techniques are indispensable in elucidating atomic level structural information about biologically active molecules owing to certain limitations of experimental techniques as pointed out in literatures [23–25]. The low-energy conformers and their related properties derived from such computations have a meaningful relationship to their presence in the macromolecular context.

Here, we provide a detailed gas phase quantum chemical conformational analysis of non-ionized pyrrolysine molecule about the nine internal back-bone torsional angles. Attempts are made to obtain full knowledge about the relative stabilities of 13 different conformers of pyrrolysine

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on the energy surface, and to provide theoretical results such as rotational constants, vibrational frequencies and dipole moments of all the 13 conformers that may be helpful for future experimentalists. Figure 1 schematically represents the nine internal rotatable bonds of the pyrrolysine molecule. The atom numbering and the torsion angle definitions are given in accordance with the schemes used earlier in various literatures [11, 26]. To facilitate a clear representation of the intramolecular hydrogen bond (H-bond) interactions present in the pyrrolysine conformers some of the hydrogen atoms are named as H_a or H_b. This gas phase DFT conformational analysis of isolated pyrrolysine molecule (in its unbound state), apart from external factors like pH effects, counter ions, proteins, water etc., would provide the opportunity to know its intrinsic conformational properties which in turn may help us to understand the dynamics and functional specificity of proteins, in discovering its biosynthetic pathway, to synthesize a new generation of pyrrolysine analogues and in understanding the nature of the genetic code or amino acid code which seems to be still evolving without deleterious changes in the amino acid sequences of proteins [27].

Computational methods

To carry the conformational search about the nine internal back-bone torsional angles, the molecular geometry of the neutral gaseous pyrrolysine molecule is subjected to full geometry optimization and vibrational frequency calculations using the B3LYP/6-31G(d,p) level of theory [28, 29]. The accuracy of DFT B3LYP/6-31G(d,p) level in carrying out conformational analysis has already been justified in literature [30]. Absence of imaginary frequency value in the vibrational frequency calculations proves that the optimized geometry is a true minimum. To determine other possible minimum/minima on the conformational potential energy surfaces (PESs) corresponding to the nine rotatable internal back-bone torsional angles of the pyrrolysine molecule, a particular torsional angle is changed progressively from 0° by a 20° step in the same direction making a full 0 to 360° rotation. For each conformation, the changed torsional angle is held fixed while the remaining variables are fully optimized. The 13 stable molecular geometries detected from potential energy surface exploration corresponding to the nine internal back-bone torsional angles are then subjected to full geometry optimization and vibrational frequency calculations at B3LYP/6-31++G (d,p) level releasing the constraints on the torsional angles. The rotational aspects around the C_5-C_6 and C_6-N_7 bonds of pyrrolysine are also examined by computing the exact maxima at B3LYP/6-31++G(d,p) level. All 13 fully optimized geometries are characterized as precise minima by the absence of imaginary frequency values in the vibrational frequency calculations while presence of one imaginary frequency value proves that the maxima are true transition states. Zero point energy (ZPE) corrections were applied to the total energies of all stable conformers and transition states using a correction factor 0.97 [21]. Single point calculations are carried out at B3LYP/6-311++G(d,p) and MP2/6-31++G(d,p) levels. The vibrational frequencies calculated using 6-31++G(d,p) basis set are also scaled using a correction factor 0.96 [31, 32]. Use of diffuse functions is important to take into account the relative diffuseness of lone pair of electrons present in the molecule under investigation [33] whereas polarization functions are useful in conformational studies where stereoelectronic effects play an important role [34]. All the calculations are performed by using the Gaussian 03 [35] and Gaussian 09 [36] programs.

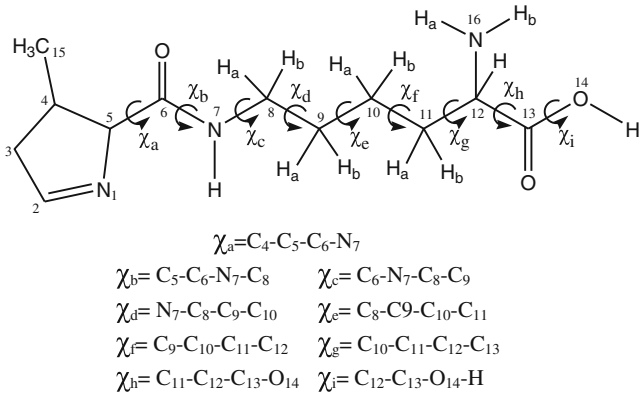

Fig. 1 Schematic representation of the nine rotatable internal back-bone torsional angles in pyrrolysine molecule

Results and discussion

Figure 2 presents the potential energy surfaces corresponding to the nine internal back-bone torsional angles, namely χ_a, χ_b, χ_c, χ_d, χ_e, χ_f, χ_g, χ_h and χ_i. As listed in Table 1, this DFT conformational analysis at B3LYP/6-31G(d,p) level reveals that a total of 1296 different conformers of the pyrrolysine molecule would result if we include all the possible combinations of rotations about the nine internal back-bone torsional angles, i.e., (i) twofold rotamers for χ_b around C_6–N_7 bond (−8.20° and 177.28°), (ii) twofold rotamers for χ_c around N_7–C_8 bond (99.09° and −103.24°), (iii) threefold rotamers for χ_d around C_8–C_9 bond (66.42°, 179.53° and −63.44°), (iv) threefold rotamers for χ_e around the C_9–C_{10} bond (68.85°, 178.25° and −66.75°), (v) threefold rotamers for χ_f around C_{10}–C_{11} bond (64.11°, −177.31° and −63.04°), (vi) threefold rotamers for χ_g around C_{11}–C_{12} bond (72.3°, 170.14° and −65.21°), (vii) twofold rotamers for χ_h around C_{12}–C_{13} bond (163.32° and −76.9°) and (viii) twofold rotamers for χ_i since the carboxyl group can possess syn- or anti-periplanar conformations

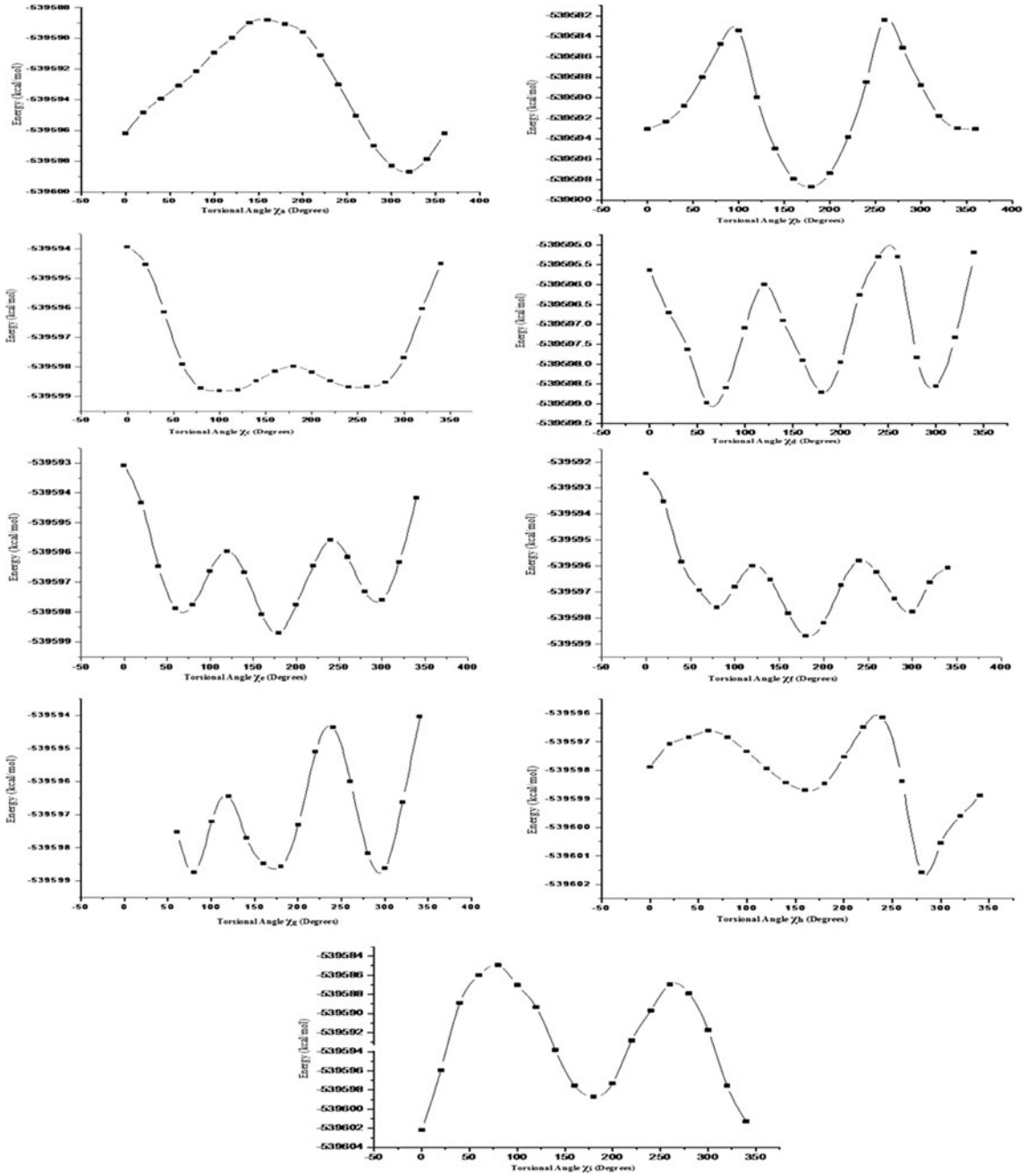

Fig. 2 The potential energy surfaces (PESs) corresponding to the nine internal back-bone torsional angles

corresponding to 0° and 180° torsions around the C_{13}–O_{14} bond (−1.99° and 178.70°).

Due to the large size of the pyrrolysine molecule it is computationally expensive to subject all 1296 conformers to full geometry optimization, and therefore only the 13 minima detected from the potential energy surface exploration are subjected to full geometry optimization as well as vibrational frequency calculations at B3LYP/6-31++G (d,p) level of theory. The optimized structures of the pyrrolysine conformers are depicted in Figs. 3 and 4 while their theoretical IR spectra are reported in the supplementary information. Table 2 lists the values of internal back-bone torsional angles of all 13 conformers and Table 3 presents their relative energies, rotational constants and dipole moments (the total energies of the conformers and transition states are given in the supplementary information). Table 4 lists some important intramolecular H-bonds that play crucial roles in stabilizing the various conformers of the pyrrolysine molecule. Table 5 lists some of the characteristic frequency and intensity values (given in brackets) of the 13 conformers calculated at the B3LYP/6-31++G (d,p) level.

Table 1 Data[a] generated from DFT conformational analysis at B3LYP/6-31G(d,p) level of theory about the nine internal backbone torsional angles of pyrrolysine

Torsional angle	Definition	No. of fold	Value of torsional angles[b]
χ_a	C_4-C_5-C_6-N_7	1 fold	134.07
χ_b	C_5-C_6-N_7-C_8	2 fold	−8.20 and 177.28
χ_c	C_6-N_7-C_8-C_9	2 fold	99.09 and −103.24
χ_d	N_7-C_8-C_9-C_{10}	3 fold	66.42, 179.53 and −63.44
χ_e	C_8-C_9-C_{10}-C_{11}	3 fold	68.85, 178.25 and −66.75
χ_f	C_9-C_{10}-C_{11}-C_{12}	3 fold	64.11, −177.31 and −63.04
χ_g	C_{10}-C_{11}-C_{12}-C_{13}	3 fold	72.30, 170.14 and −65.21
χ_h	C_{11}-C_{12}-C_{13}-O_{14}	2 fold	163.32 and −76.90
χ_i	C_{12}-C_{13}-O_{14}-H	2 fold	−1.99 and 178.70

[a]Angles in degrees; [b]Torsional angle values are listed from the fully optimized conformers at B3LYP/6-31++G(d,p)

The rotation χ_a about the C_5–C_6 bond does not result any rotamer, possibly because an electrostatic repulsion arise between the nitrogen atom of the imine group and oxygen atom of the C_6=O group as they approach a *cis* orientation to each other. The energy difference of 11.6 kcal mol^{-1} between the TSχ_a and its corresponding minimum, i.e., Pyl-1 also suggest that the above stated *cis* orientation is thermodynamically unfavorable.

Conformations and relative energies

Intramolecular H-bonds, the strongest non-covalent interactions, play an important role in stabilizing the different conformations of an amino acid molecule. The number of intramolecular H-bonds and the strength of these interactions are the two important factors responsible for bringing differences in relative energies among the various conformers of an amino acid. The strength of these H-bonds depends on two factors, (a) shorter is the distance A–H…B than the sum of their van der waals radii and (b) closer the angle A–H…B to 180° [16], where A–H is H-bond donor and B is H-bond acceptor. In the case of pyrrolysine molecule the carboxyl group, amino group, N-atom of the imine group, C_6=O and N_7–H bonds of the amide linkage and all the CH_2 groups participate actively in intramolecular H-bond formation stabilizing the various conformers. The H-bond

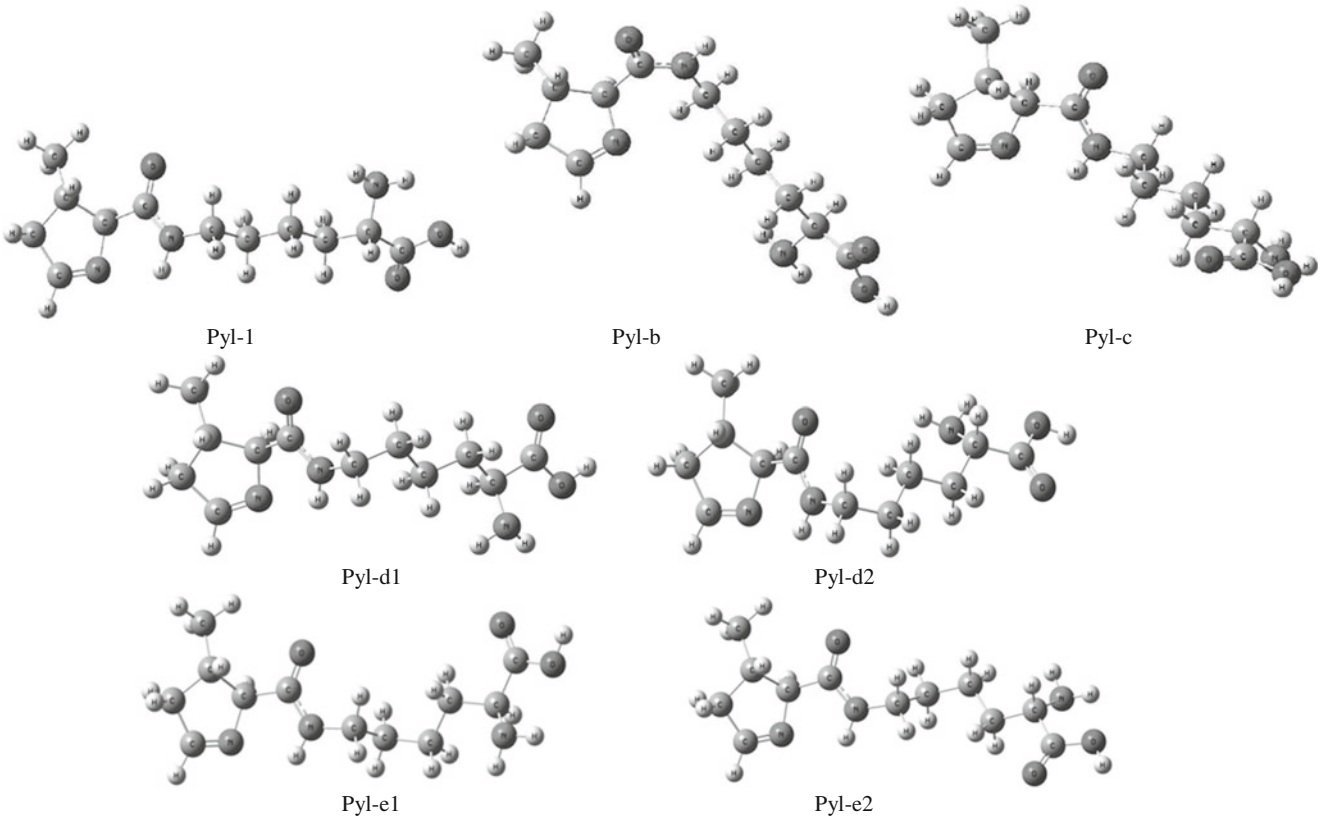

Fig. 3 The optimized structure of conformers Pyl-1, Pyl-b, Pyl-c, Pyl-d1, Pyl-d2, Pyl-e1 and Pyl-e2

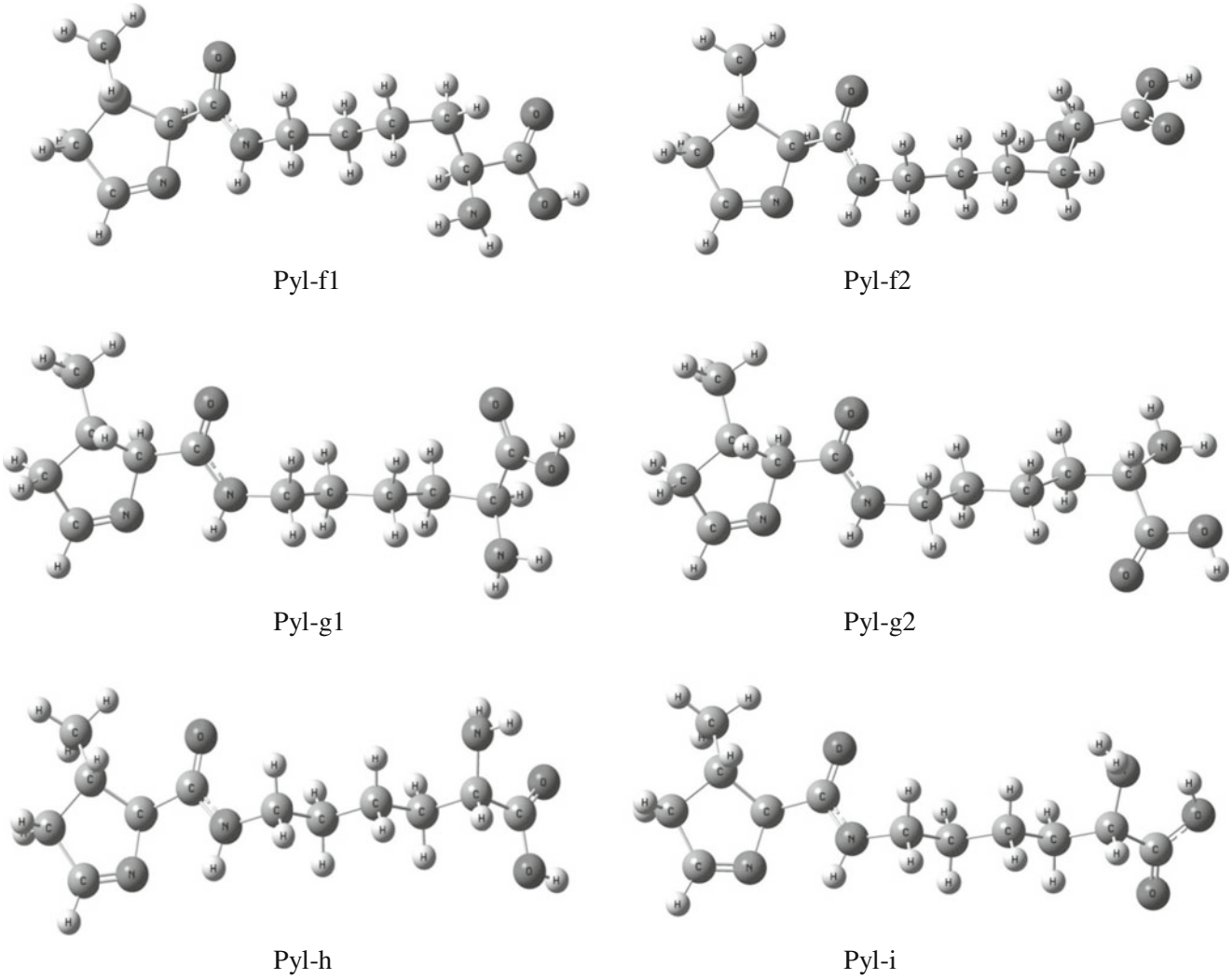

Fig. 4 The optimized structure of conformers Pyl-f1, Pyl-f2, Pyl-g1, Pyl-g2, Pyl-h and Pyl-i

Table 2 Dihedral angles (in degrees) about the nine internal back-bone torsional angles of pyrrolysine conformers after full geometry optimization at B3LYP/6-31++G(d,p)

Conformers	χ_a	χ_b	χ_c	χ_d	χ_e	χ_f	χ_g	χ_h	χ_i
Pyl-1	134.07	177.28	−103.24	179.53	178.25	−177.31	170.14	163.32	178.70
Pyl-b	−175.71	−8.20	−102.37	−178.94	178.32	−176.15	170.60	163.61	178.32
Pyl-c	136.33	179.17	99.09	−179.81	−178.55	−175.55	172.83	165.10	178.55
Pyl-d1	134.91	178.85	−89.97	−63.44	−178.84	−175.92	172.52	164.09	178.42
Pyl-d2	135.18	179.61	−105.62	66.42	−178.39	−174.51	173.48	164.22	178.39
Pyl-e1	134.12	177.66	−105.63	−174.91	−66.75	−173.35	170.64	161.05	178.04
Pyl-e2	134.33	178.21	−88.63	175.21	68.85	−178.21	171.89	163.51	178.31
Pyl-f1	134.48	177.54	−100.67	179.99	−175.08	−63.04	175.06	163.52	178.46
Pyl-f2	133.51	178.28	−87.32	178.47	172.69	64.11	149.54	165.19	178.28
Pyl-g1	134.30	178.27	−103.85	−177.80	179.80	−175.20	−65.21	152.20	176.93
Pyl-g2	133.37	177.65	−91.05	179.63	−179.43	178.85	72.30	179.74	−178.47
Pyl-h	133.98	177.92	−95.19	−178.53	−179.90	178.55	170.69	−76.90	177.85
Pyl-i	134.48	177.94	−90.10	177.88	179.85	178.38	167.62	138.21	−1.99

Table 3 The relative energies[a] (kcal mol^{-1}), theoretical rotational data (GHZ) and dipole moments (D) of the conformers

Conformers	Relative energies			Rotational constants			Dipole
	ΔE_1	ΔE_2	ΔE_3	A	B	C	moments
Pyl-i	0.00	0.00	0.00	1.272	0.101	0.098	6.802
Pyl-h	0.01	0.34	−0.29	1.234	0.101	0.098	4.005
Pyl-c	2.27	2.65	1.86	1.001	0.106	0.104	1.428
Pyl-d2	2.34	2.60	0.69	0.819	0.137	0.128	3.057
Pyl-1	2.37	2.67	2.09	1.329	0.099	0.095	3.427
Pyl-g2	2.38	2.77	1.48	1.313	0.104	0.100	1.333
Pyl-d1	2.75	2.97	1.53	0.817	0.124	0.121	5.205
Pyl-g1	2.84	3.09	2.01	1.208	0.109	0.103	3.895
Pyl-e2	3.24	3.45	2.37	1.224	0.107	0.102	1.011
Pyl-f1	3.48	3.67	2.45	0.985	0.113	0.110	5.198
Pyl-e1	3.65	3.91	2.90	1.078	0.115	0.107	3.944
Pyl-f2	4.32	4.59	3.36	1.042	0.118	0.113	4.014
Pyl-b	8.06	8.67	7.06	0.932	0.123	0.116	4.591

[a]Relative energies: ΔE_1=at B3LYP/6-31++G(d,p), ΔE_2=at B3LYP/6-311++G(d,p) and ΔE_3= at MP2/6-31++G(d,p) level of theory

combinations in pyrrolysine conformers are complex and various types of intramolecular H-bonds may coexist in one conformer. A thorough analysis reveals that six types of intramolecular H-bonds, namely O…H–O, N…H–O, O…H–N, N…H–N, O…H–C and N…H–C, are present in the conformers of pyrrolysine (Table 4). All these H-bonds play key roles in determining the energetics of the conformers. The relative energies of the 13 conformers shown in Table 3 are determined relative to the energy of Pyl-i which is predicted as the most stable conformer at B3LYP/6-31++G(d,p) level of theory. Pyl-b is the least stable in the relative stability order with an energy difference of 8.06 kcal mol^{-1} compared to Pyl-i. The single point calculations at B3LYP/6-311++G(d,p) and MP2/6-31++G(d,p) levels reveal that the stability order of the conformers depends upon the level of theory used. The change in the stability order is very limited at B3LYP/6-311++G(d,p); only the Pyl-d2 conformer is predicted to be more stable than Pyl-c by an energy difference of only 0.05 kcal mol^{-1}. Whereas the stability order predicted at MP2/6-31++G(d,p) level shows significant deviations from the one calculated at B3LYP/6-31++G(d,p) level. However, the range of relative energies calculated at the three level of theories are very similar; 8.06 kcal mol^{-1} at B3LYP/6-31++G(d,p), 7.06 kcal mol^{-1} at MP2/6-31++G(d,p) while 8.67 kcal mol^{-1} when B3LYP/6-311++G(d,p) is used (listed in Table 3). It has been pointed out that full geometry optimization of gaseous tryptophan conformers at B3LYP/6-311G(d,p) and MP2/6-311++G(d,p) levels

Table 4 H-bond distances[a] (Å) of the intramolecular H-bond interactions detected in pyrrolysine conformers in gas phase

H-bonds	Pyl-1	Pyl-b	Pyl-c	Pyl-d1	Pyl-d2	Pyl-e1	Pyl-e2	Pyl-f1	Pyl-f2	Pyl-g1	Pyl-g2	Pyl-h	Pyl-i
N_1…H-N_7	2.18	*abs*	2.18	2.19	2.18	2.19	2.19	2.19	2.19	2.18	2.19	2.18	2.19
O_6…H_a-C_8	2.43	*abs*	*abs*	2.52	2.45	2.42	2.52	2.44	2.53	2.44	2.50	2.48	2.50
O_6…H_b-C_8	*abs*	*abs*	2.45	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*
O_6…H-N_7	*abs*	2.39	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*
N_1…H_a-C_8	*abs*	2.55	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*
N_7…H_a-C_9	2.74	2.73	2.73	*abs*	*abs*	2.69	2.77	2.73	2.75	2.72	2.75	*abs*	*abs*
N_7…H_b-C_9	2.72	2.73	2.74	*abs*	2.74	2.75	2.68	2.73	2.70	2.74	2.73	2.74	2.71
N_{16}…H_b-C_{11}	2.68	2.68	2.70	2.69	2.71	2.68	2.69	2.70	2.55	*abs*	2.58	2.69	*abs*
O_{13}…H_a-C_{11}	2.54	2.55	2.58	2.57	2.58	2.55	2.54	2.58	2.38	*abs*	2.71	*abs*	2.53
O_{13}…H_a-C_{10}	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	2.59	*abs*	*abs*
O_{14}…H_a-N_{16}	2.32	2.32	2.31	2.31	2.31	2.30	2.31	2.31	2.32	2.31	2.45	*abs*	*abs*
O_{13}…H_a-N_{16}	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	2.61	*abs*
O_{13}…H-O_{14}	2.29	2.29	2.29	2.29	2.29	2.29	2.29	2.29	2.29	2.29	2.29	2.29	*abs*
N_{16}…H-O_{14}	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	*abs*	1.88

[a] Only the (B…H) distances are listed where B is H-bond acceptor; *abs* absent

Table 5 Frequencies[a] (cm^{-1}) and IR intensities (km mol^{-1}) of various vibrational modes[b] obtained from the theoretical vibrational spectra of pyrrolysine conformers in gas phase. Intensity values are given in brackets

Vibrational modes	Pyl-1	Pyl-b	Pyl-c	Pyl-d1	Pyl-d2	Pyl-e1	Pyl-e2	Pyl-f1	Pyl-f2	Pyl-g1	Pyl-g2	Pyl-h	Pyl-i
$\nu(O_{14}\text{-}H)$	3,605(73)	3,605(74)	3,604(73)	3,605(73)	3,606(72)	3,605(73)	3,605(73)	3,606(74)	3,607(74)	3,602(72)	3,602(69)	3,595(59)	3,300(288)
$\nu_{as}(N_{16}\text{-}H)$	3,468(7)	3,467(6)	3,466(6)	3,465(6)	3,464(8)	3,467(6)	3,467(7)	3,468(6)	3,472(11)	3,465(5)	3,456(3)	3,442(4)	3,470(12)
$\nu(N_7\text{-}H)$	3,445(74)	3,446(19)	3,447(74)	3,456(68)	3,440(76)	3,445(74)	3,452(75)	3,447(71)	3,452(80)	3,446(71)	3,451(76)	3,449(75)	3,451(80)
$\nu_s(N_{16}\text{-}H)$	3,384(1)	3,383(1)	3,383(1)	3,382(1)	3,382(0.9)	3,383(1)	3,384(1)	3,384(2)	3,389(0.4)	3,377(1)	3,369(0.8)	3,361(1)	3,378(0.3)
$\nu_{as}(C_9\text{-}H)$	2,952(30)	2,969(12)	2,952(28)	2,958(39)	2,930(30)	2,949(36)	2,958(18)	2,951(45)	2,952(41)	2,957(5)	2,942(2)	2,945(33)	2,954(23)
$\nu_s(C_9\text{-}H)$	2,903(14)	2,900(17)	2,905(18)	2,908(23)	2,895(8)	2,904(22)	2,907(16)	2,902(38)	2,904(31)	2,893(14)	2,908(42)	2,898(16)	2,908(20)
$\nu(C_{13}{=}O)$	1,747(271)	1,747(275)	1,748(270)	1,748(276)	1,747(280)	1,749(270)	1,746(267)	1,748(284)	1,745(289)	1,744(264)	1,751(275)	1,736(324)	1,764(323)
$\nu(C_6{=}O)$	1,666(266)	1,666(439)	1,664(266)	1,666(275)	1,663(242)	1,667(243)	1,663(250)	1,667(259)	1,663(237)	1,666(260)	1,663(248)	1,665(250)	1,663(244)
$\nu(C_2{=}N_1)$	1,632(49)	1,630(59)	1,632(45)	1,632(49)	1,632(47)	1,632(47)	1,632(49)	1,633(49)	1,632(49)	1,633(49)	1,633(49)	1,632(49)	1,632(49)
$Sis(N_{16}\text{-}H)$	1,590(56)	1,592(57)	1,590(57)	1,589(55)	1,591(52)	1,590(61)	1,590(60)	1,588(50)	1,592(39)	1,588(52)	1,588(42)	1,568(33)	1,594(33)
$\nu(C_6\text{-}N_7)$	1,501(366)	1,439(26)	1,500(355)	1,495(358)	1,501(310)	1,501(348)	1,497(363)	1,500(359)	1,497(342)	1,502(360)	1,497(355)	1,500(360)	1,497(353)
$\nu(C_{12}\text{-}H)$	2,788(49)	2,788(49)	2,794(45)	2,795(45)	2,794(46)	2,790(48)	2,793(45)	2,811(39)	2,797(39)	2,802(52)	2,822(41)	2,936(9)	2,924(20)
$\gamma(C_{10}\text{-}H)$	1,282(6)	1,279(0.6)	1,279(1)	1,282(2)	1,284(2)	1,261(14)	1,260(3)	1,268(21)	1,268(24)	1,276(1)	1,280(2)	1,266(12)	1,280(0.4)
$\nu(C_{10}\text{-}H)$	2,882(24)	2,877(26)	2,884(20)	2,883(22)	2,903(29)	2,886(31)	2,888(23)	1,938(6)	2,892(17)	2,893(14)	2,893(14)	2,891(13)	2,908(20)
$\nu(C_5\text{-}C_4)$	771(3)	772(8)	772(1)	769(3)	768(4)	768(9)	768(9)	769(6)	769(22)	770(13)	769(15)	768(12)	768(13)
$\gamma(N_{16}\text{-}H)$	251(46)	263(62)	258(36)	248(58)	266(55)	257(54)	258(43)	252(26)	248(52)	245(50)	265(12)	208(41)	287(16)

[a] Frequencies are scaled using a correction factor 0.96; [b] Vibrational modes: ν stretching; γ rocking; *Sis* scissoring; *s* symmetric; *as* asymmetric

do not produce any noticeable structural changes, only the conformer energies change by small amounts [18]. Therefore, we expect that the conformations of the 13 conformers predicted at B3LYP/6-31++G(d,p) level of theory will not change even if higher level of quantum mechanical theories are used; only the conformer energies may change little. The data on the energetics of the conformers presented in Table 3 suggest that though many of the conformers differ from one another by small energy differences but their conformations are very different (see the discussion on the vibrational spectra of the conformers given in a succeeding section of this paper); and therefore they may have different functional aspects in bio-chemical processes.

It is interesting to note that in Pyl-b the N_7–H and C_6=O bonds of the amide linkage orient themselves *cis* to each other (χ_b value being −8.20°) whereas in the other 12 conformer the N_7–H and C_6=O bonds are found to possess *trans* orientations (χ_b value range 177.28 to 179.61°). The calculated rotational barrier of the *trans*→*cis* isomerization (that is the energy difference between the *trans* minimum and TSχ_b; Pyl-1 is the *trans* minimum here) around the C_6-N_7 bond is 27.69 kcal mol^{-1}. Now as a result of this *cis* orientation of N_7–H and C_6=O bonds the value of χ_a changes to −175.71° from the usual range of 133.37 to 136.33° observed in the other conformers of pyrrolysine. This indicates that orientation of the five-membered methylpyrroline ring of pyrrolysine may be influenced by rotation about C_6-N_7 bond. Similar situations are also observed in three more cases- (a) change in χ_i value to −1.99° in Pyl-i from the usual range of 176.93 to −178.47° changes the χ_h value to 138.21°; (b) changes in χ_g , -65.21° in Pyl-g1 and 72.3° in Pyl-g2, change the χ_h values to 152.2° and 179.74° respectively; and (c) in Pyl-f2 when χ_f is changed to 64.11° the χ_g value changes to 149.54°. The other internal torsional rotations are more or less independent of each other, i.e., rotation around one dihedral angle does not alter the conformation of the other part of the molecule.

Vibrational spectra

Study of gas phase vibrational spectra is important to understand the existence and nature of various types of intramolecular H-bonds in the conformers of pyrrolysine molecule. Each conformer of the gaseous pyrrolysine molecule has a total of 111 normal modes of vibration. It is evident from Table 5 that some vibrational modes, viz. $\nu(C_2{=}N_1)$, $\nu(C_5$-$C_4)$ stretch etc., basically remain unaltered along with the change of the conformation while some are very sensitive to even small changes in the configurations of the conformers and consequently leave noticeable signatures in the IR spectra.

The frequency change reflects the internal information about each conformer and the relative strength of different intramolecular H-bond interactions. For example, in Pyl-i the $\nu(O_{14}$-H) stretching value occurs at 3300 cm^{-1} with a high intensity signal of 288 km mol^{-1} while for the other 12 conformers its stretch frequencies range from 3595 to 3607 cm^{-1} (the intensities range from 59 to 74 km mol^{-1}). This is because the Pyl-i conformer possesses a strong and unique N_{16}…H-O_{14} H-bond (with N_{16}…H distance equal to 1.88 Å) due to the anti-periplanar conformation of the carboxyl group (the value of χ_i is −1.99°). This H-bond is absent in the other 12 conformers where the carboxyl group possesses syn-periplanar conformation (the values of χ_i range from 176.93 to −178.47°). Similarly, Pyl-h conformer is unique from the other 12 conformers where the amino group lies *cis* to the carboxylic C=O group and there exists a unique O_{13}…H_a-N_{16} bond (O_{13}…H_a distance equal to 1.61 Å) which is absent in other conformers because of the *trans* orientation of the C=O group with the NH_2 group. As a result $\nu(C_{13}{=}O)$ stretch frequency appears at 1736 cm^{-1} for Pyl-h while for others it ranges from 1744 to 1764 cm^{-1}. Note that it appears at 1764 cm^{-1} in Pyl-i since in this conformer the carboxylic C=O group does not participate in H-bond formation. It can also be invoked that because of the presence of the O_{13}…H_a-N_{16} bond in Pyl-h the scissoring mode Sis(N_{16}-H) appears at 1568 cm^{-1} (for others it occurs above 1588 cm^{-1}); the asymmetric stretch frequency ν_{as} (N_{16}-H) appears at 3442 cm^{-1} (for others above 3456 cm^{-1}); the symmetric stretching $\nu_s(N_{16}$-H) appears at 3361 cm^{-1} (for others it ranges from 3369 to 3389 cm^{-1}) and the rocking bending mode $\gamma(N_{16}$-H) appears at 208 cm^{-1} (for others above 245 cm^{-1}). In Pyl-b, because of the *cis* orientation of the N_7–H and C_6=O bonds of the amide linkage, there exist a strong O_6…H-N_7 interaction with O_6…H distance equal to 2.39 Å. Consequently this conformer possesses many distinct structural features and some of the normal modes of vibration, for example the intensity of $\nu(N_7$-H) and $\nu(C_6{=}O)$, band position of $\nu(C_6$-$N_7)$ etc., differ appreciably from the other conformers. The $\nu(C_{12}$-H) mode for Pyl-1, Pyl-b, Pyl-c, Pyl-d1, Pyl-d2, Pyl-e1, Pyl-e2 and Pyl-f2 is observed below 2795 cm^{-1} whereas for others it appears above 2811 cm^{-1}. Thus, it is expected that the data listed in Table 5 would greatly aid future experimentalists in detecting the gas phase pyrrolysine conformers even though they differ by small energy differences from one another, for example, conformers Pyl-i and Pyl-h can be distinguished by simply referring to their $\nu(O_{14}$-H) and $\nu(C_{13}{=}O)$ stretching frequencies, Pyl-i and Pyl-b by their $\nu(C_6$-$N_7)$ band positions etc. The results of this DFT study also point to the fact that vibrational frequencies are lowered corresponding to the presence of the intramolecular H-bond interactions.

Rotational constants and dipole moments

Table 3 presents the rotational constants and dipole moments of the gas phase pyrrolysine conformers calculated at the B3LYP/6-31++G(d,p) level. The accuracy of DFT method in predicting the rotational constants of conformers of some aliphatic amino acids has been discussed in the literature [22,

37]. In the absence of any experimental data on rotational constants and dipole moments, the theoretical data presented in Table 3 may be useful in predicting gas phase pyrrolysine conformers with microwave spectroscopy (MW). We except that the pyrrolysine conformers that have large total dipole moments, for example 6.802 D for Pyl-i, would be easily detected by MW spectroscopy [14].

Conclusions

The conformational study at B3LYP/6-31G(d,p) level reveals that a total of 1296 different conformers of pyrrolysine would result if all the possible combinations of rotations about the nine internal back-bone torsional angles are considered. The rotation χ_a about the C_5–C_6 bond does not result any rotamer while rotation χ_b about C_6–N_7 may affect orientation of the methylpyrroline ring of pyrrolysine. The relative energies, rotational constants, dipole moments and vibrational frequencies of the 13 conformers calculated at B3LYP/6-31++G(d,p) level are listed which may be helpful for the future experimentalists. The amino group, carboxyl group, N-atom of the imine group, C_6=O and N_7–H groups of the amide linkage and all the CH_2 groups of pyrrolysine participate actively in intramolecular H-bonding stabilizing the various conformers. Six types of intramolecular H-bonds, viz. O…H–O, N…H-O, O…H–N, N…H–N, O…H–C and N…H–C, are found to exist in the pyrrolysine conformers and all of them contribute to the stability of the conformers. The calculated relative energy range of the conformers at B3LYP/6-31++G(d,p) level is 8.06 kcal mol^{-1} whereas the same obtained by single point calculations at B3LYP/6-311++G(d,p) and MP2/6-31++G(d,p) levels are 8.67 and 7.06 kcal mol^{-1} respectively. Though the pyrrolysine conformers differ from one another by only small energy differences, their conformations are very different. The vibrational frequencies are found to shift invariably toward the lower side of the frequency scale corresponding to the presence of intramolecular H-bond ineractions in the conformers.

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