J. Phys. Chem. A **1998,** *102,* 4623−4629

Conformational Behavior of α-Alanine. Matrix-Isolation Infrared and Theoretical DFT and ab Initio Study

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Received: October 28, 1997; In Final Form: February 20, 1998

Two conformers of the nonionized α-alanine and its isotopomer *N,N,O-*d_3-alanine have been observed in low-temperature Ar matrixes. Their infrared spectra have been analyzed and assigned using DFT/B3LYP/aug-cc-pVDZ and MP2/aug-cc-pVDZ geometry and frequency theoretical calculations. Two different intramolecular H-bonds, bifurcated $NH_2{\cdots}O{=}C$ and $N{\cdots}H{-}O$, were found in the observed α-alanine conformers, **I** and **IIa**. We found that the DFT/B3LYP/aug-cc-pVDZ method yields vibrational frequencies of the α-alanine conformers in excellent agreement with the experimental data.

1. Introduction

Amino acids are known to exist as zwitterions ($NH_3^+{-}CH(R){-}COO^-$, R = CH_3 for α-alanine) in the solid state and in solutions[1] and in the nonionized form ($NH_2{-}CH(R){-}COOH$) in the gas phase.[2,3] Study of the conformational behavior of the nonionized amino acids, which is considered in the present investigation, is important for understanding the dynamics of the peptides and protein backbone.

The experimental investigation of the nonionized alanine is difficult due to its low thermal stability. Alanine, like other amino acids, usually decomposes before melting. Only a very few experimental studies have been carried out for the gaseous alanine. The electron diffraction method,[3] microwave spectroscopy,[4] and low-resolution photoelectron spectroscopy[5,6] have been used in these studies, as it was shown that alanine exists in the gas phase only in the nonionized form. The rotational constants and the dipole moments of two alanine conformers—**I** and **IIa** (see Figure 1)—were determined in the microwave study of Godfrey et al.[4] For the simplest amino acid—glycine—the microwave investigations also demonstrated the presence in the gas phase of only two conformers.[7] But the IR studies allowed identification of an additional minor glycine conformer that has not been previously observed.[8,9] This additional conformer could not be identified in a microwave study due to its very low dipole moment. In the case of alanine, the dipole moment of the third low-energy conformer is also very small, similar to the third glycine conformer, and only with the IR spectroscopy method can one determine its presence or absence in the gas phase or in an inert gas matrix.

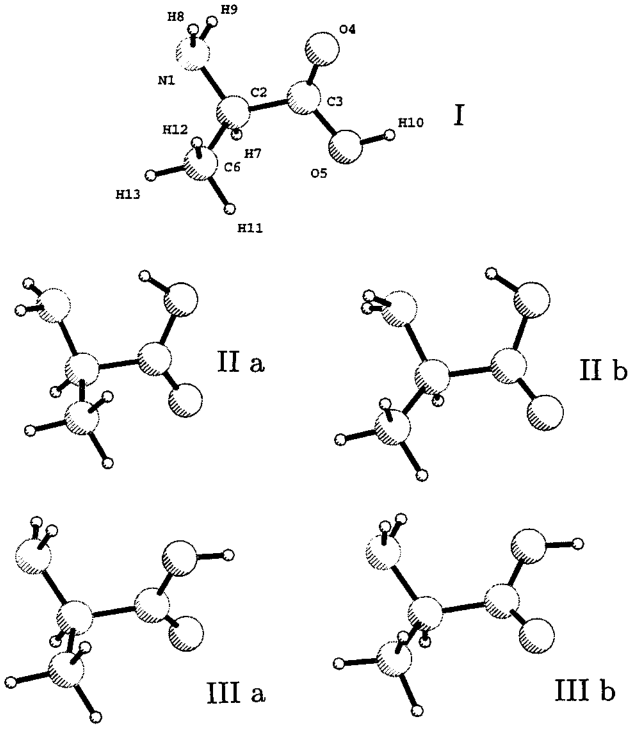

Figure 1. Lowest-energy alanine conformers. (The geometries of the comformers are available from the corresponding author: e-mail ludwik@u.arizona.edu.)

At the same time alanine was the subject of numerous theoretical calculations.[4,10−16] In the most detailed theoretical study, Császár[10] applied correlated calculations with larger basis sets and found as many as 13 minimum-energy alanine conformers. In this study the five lowest-energy alanine conformers depicted in Figure 1 were found to have relative energies smaller than 6 kJ mol^{-1}. Thus all of them should be taken into account in an experimental study of the gaseous alanine.

Recently we applied the low-temperature matrix isolation IR spectroscopy to study the conformational behavior of the simplest amino acid—glycine—and we identified three low-energy conformers.[8,9] The identification was possible due to high sensitivity of some of the IR vibrational modes and their frequencies to the intramolecular H-bonding, which occurs in the glycine conformers. The purpose of the study[8] was also to examine the performance of the density functional theory (DFT) and the second-order many-body perturbation theory (MBPT

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S1089-5639(97)03479-8 CCC: $15.00 © 1998 American Chemical Society
Published on Web 05/15/1998

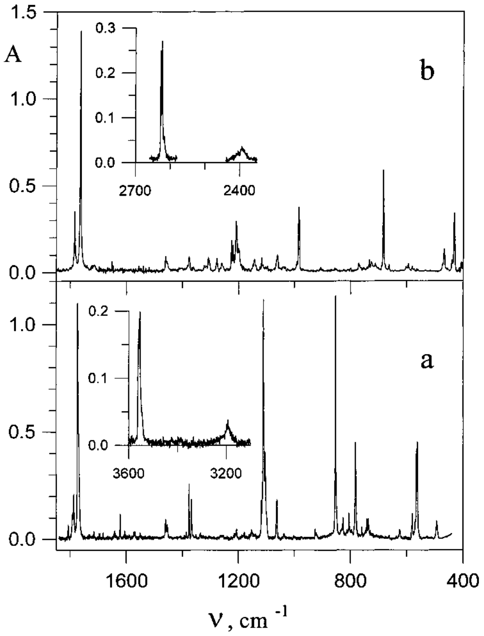

Figure 2. Matrix IR spectra of alanine-d_0 (a) and alanine-d_3 (b). The spectra are recorded for samples deposited at 15 K. The matrix ratio is 1:750.

= MP2) with different basis sets in predicting the structural and IR spectral characteristics of the amino acid conformers. It was shown that the DFT and MP2 levels of theory with the aug-cc-pVDZ basis set produce almost identical structural parameters of the glycine conformers and that the DFT calculated vibrational frequencies and intensities for this system are in the excellent agreement with the experimental data.

In the present work the conformational composition of the gaseous alanine is investigated by the IR spectroscopy method combined with the low-temperature matrix-isolation technique. Again, the DFT and MP2 methods with the aug-cc-pVDZ basis set are used to assist the analysis of the experimental spectra and to clarify the conformational behavior of the α-alanine.

2. Experimental Details

The fill-up helium cryostat used in our matrix-isolation IR experiments is described elsewhere.[17] The IR spectra were registered with the updated SPECORD IR 75 spectrometer in the range 4000−400 cm^{-1}. The resolution was 3 cm^{-1} in the range 4000−2500 cm^{-1} and 1 cm^{-1} in the range 2500−400 cm^{-1}. The spectrometer was sealed and blown through with dry nitrogen during the experiment to exclude any influence of atmospheric H_2O and CO_2.

The measurements were carried out for α-alanine and for N,N,O-d_3-α-alanine denoted here as alanine-d_0 and alanine-d_3, respectively. The matrix samples were prepared by simultaneous deposition of the substance and the matrix gas onto a cooled CsI substrate. The matrix gas was 99.99% Ar. The substrate temperature was 15 K during the matrix preparation. In the experiment, commercially available alanine-d_0 and alanine-d_3 were evaporated from the Knudsen cell at 156 °C. This temperature was found to be high enough to yield samples with sufficient concentration of the compounds, yet sufficiently low to prevent decomposition of the compounds. A low-temperature quartz microbalance was used to monitor and adjust the gaseous flows of the studied compound and the matrix gas at the level of 1:750 for the compound-to-matrix-gas ratio. This was a necessary step to avoid the formation of autoassociates in the samples.

The IR spectra of alanine-d_0 and alanine-d_3 recorded immediately after sample deposition are presented in Figure 2. They do not contain any bands of the alanine decomposition products. Only a few weak bands corresponding to trace amounts of CO_2 and H_2O are visible in the spectra, which are most probably due to small quantities of these gases absorbed on the surface of the solid alanine used in the experiments.

TABLE 1: Energies (au), Relative Stabilities (Δ*E*) (kJ mol^{-1}), Zero-Point Vibrational Energies (ZPE[a]) (au), Total Energies Including ZPE (au), and Relative Stabilities Including ZPE (kJ mol^{-1}) of the Alanine Conformers. Values Taken from the Full Optimization and Frequency Calculations of the Conformers at the B3LYP/aug-cc-pVDZ Level and from the Single-Point Calculations at the MP2/aug-cc-pVDZ//B3LYP/aug-cc-pVDZ Level

	I	IIa	IIb	IIIa
B3LYP/aug-cc-pVDZ				
energy	−323.801 175 8	−323.801 058 6	−323.800 906 0	−323.799 401 2
Δ*E*	0.00	0.31	0.71	4.66
ZPE	0.104 571 4	0.105 080 5	0.105 021 9	0.104 641 3
total energy(B3LYP + ZPE)	−323.696 604 4	−323.695 978 1	−323.695 884 1	−323.694 759 9
Δ*E*(B3LYP + ZPE)	0.00	1.64	1.89	4.84
MP2/aug-cc-pVDZ//B3LYP/aug-cc-pVDZ				
energy	−322.927 954 1	−322.927 894 2	−322.927 339 2	−322.926 187 5
Δ*E*	0.00	0.16	1.61	4.64
total energy(MP2 + ZPE[b])	−322.823 382 7	−322.822 813 7	−322.822 317 3	−322.821 546 2
Δ*E*(MP2 + ZPE[b])	0.00	1.49	2.80	4.82

[a] Zero-point vibrational energies were scaled applying the scaling factor of 0.96 for the OH, NH, and CH stretching vibrations and 0.99 for all other vibrations. [b] ZPE from the B3LYP/aug-cc-pVDZ calculation was used here.

TABLE 2: Observed and Calculated (at the B3LYP/aug-cc-pVDZ level) Rotational Constants (MHz) and Dipole Moments (Debye) of the Alanine Conformers

	I				IIa				IIb	IIIa
	obs[a]	calc			obs[a]	calc			calc	calc
A_c	5066	5042	5055[b]	5085[c]	4973	4948	4952[b]	5004[c]	4844	5022
B_c	3101	3005	3038[b]	3054[c]	3228	3196	3218[b]	3203[c]	3403	2822
C_c	2264	2274	2263[b]	2306[c]	2308	2294	2284[b]	2351[c]	2147	2445
μ	1.8	1.33			5.13	5.42			5.46	1.65

[a] The rotational constants and dipole moments of the alanine conformers **I** and **IIa** are taken from ref 4. [b] Values calculated at the B3LYP/6-311++G** level are taken from ref 10. [c] Values calculated at the MP2/6-311++G** level are taken from ref 10.

TABLE 3: Observed and Calculated (at the DFT/B3LYP/aug-cc-pVDZ level) IR Frequencies (cm^{-1}) and Intensities of Alanine-d_0

observed^a			calculated				
			alanine **I**		alanine **IIa**		
ν	A^b	$I_{\mathrm{obs}}{}^c$	ν	$I_{\mathrm{calc}}{}^d$	ν	$I_{\mathrm{calc}}{}^d$	PED^e
3560	0.165	2.298	3583	55.8			OH str [100]
3555	0.143						
3546	0.043						
			3418	3.7	3446	9.7	$\mathrm{MN_2}$ str [97]
			3345	1.3	3367	0.9	$\mathrm{NH_2}$ str [97]
3193	0.036	1.112			3320	254.5	OH str [98]
3001	0.038	0.386	3012	12.6	3015	6.8	$\mathrm{C_mH}$ str [100]
2982	0.051	0.646	2991	22.2	2980	23.2	$\mathrm{C_mH}$ str [98]
2943	0.032	0.677	2941	11.3	2925	16.6	$\mathrm{C_\alpha H}$ str [96]
2914	0.016	0.324	2918	13.8	2914	16.6	$\mathrm{C_mH}$ str [98]
2885	0.015	0.155					
1807	0.066	0.134					
1792	0.123	1.201			1808	327.5	C=O str [84]
1787	0.214	6.399	1780	289.0			C=O str [85]
1773	1.103						
1771	0.531						
1770	0.497						
1769	0.466						
1715	0.040	0.115					C=O str of the alanine dimers
1642	0.034	0.113	1637	22.6	1625	34.0	HNH bend [90]
1460	0.089	0.645	1459	7.9	1461	10.5	$\mathrm{HC_mH}$ bend [86]
1457	0.036		1455	6.5	1458	5.1	$\mathrm{HC_mH}$ bend [85]
1454	0.065						
1386	0.031	0.090	1389	8.8			$\mathrm{C_\alpha H}$ bend [37], $\mathrm{C_mH}$ bend [10]
1376	0.252	0.453			1397	353.5	OH bend [67]
1368	0.185	0.454	1367	11.3	1376	8.2	$\mathrm{C_mH}$ bend [81]
1366	0.067						
1336	0.022	0.086	1337	18.7	1331	21.6	$\mathrm{C_\alpha H}$ bend [25], OH bend [18]
			1271	0.7	1306	14.7	$\mathrm{C_\alpha H}$ bend [38], OH bend [32]
1211	0.023	0.149	1254	1.7	1247	3.9	$\mathrm{NH_2}$ bend [32], CN str [11]
1209	0.027						
1207	0.041						
1206	0.044						
1180	0.021	0.057			1181	16.2	C−O str [32], CC str [12], OH bend [11]
1178	0.027						
1153	0.038	0.202	1154	20.5			CN str [29], CC str [13]
1117	0.174	6.948	1117	271.9	1114	11.8	C−O str [48], OH bend [20], CN str [14]
1110	1.107						
1105	0.404						
1103	0.398						
1099	0.144						
1064	0.170	0.755	1067	29.3			$\mathrm{C_mH}$ bend [28], $\mathrm{C_\alpha H}$ bend [13]
1061	0.181						
1037	0.022	0.080			1039	31.4	$\mathrm{C_mH}$ bend [35], $\mathrm{C_\alpha H}$ bend [24], CN str [11], C−O str [10]
			1002	2.0	999	0.7	$\mathrm{NH_2}$ str [41], CC str [14]
925	0.043	0.203			928	36.2	CC str [19], CN str [14], $\mathrm{C_\alpha H}$ bend [12]
920	0.026		915	0.8			CC str [29], $\mathrm{C_\alpha H}$ bend [23], CN str[22]
852	1.113	3.211	878	130.3			$\mathrm{NH_2}$ bend [74]
830	0.031	0.172			866	71.2	OH tor [61]
826	0.079	0.176		835		73.5	OH tor [30], $\mathrm{NH_2}$ bend [12]
812	0.022	0.066					
805	0.102	0.280		791	8.3		CC str [32], C−O str [16], C=O bend [10]
796	0.021	0.089					
786	0.044	1.700	33.2				CC str [30], NCC=O tor [15]
782	0.435						
781	0.370						
779	0.198						
759	0.039	0.070					
741	0.078	0.296	745	26.9			NCC=O tor [37], NCC−O tor [18], CC str [14]
736	0.080	0.312			729	14.3	NCC−O tor [29], NCC=O tor [22], CCC bend [12]
625	0.045	0.211	626	10.9	626	5.6	C=O bend [50], C−O str [14], NCC bend [13]
582	0.063	0.435					
580	0.124						
570	0.092	2.819	594	88.7			OH for [84]
565	0.424						
562	0.462						

TABLE 3 (Continued)

			calculated				
observed[a]			alanine **I**		alanine **IIa**		
ν	A^b	$I_{obs}{}^c$	ν	$I_{calc}{}^d$	ν	$I_{calc}{}^d$	PED[e]
493	0.084	0.524	490	18.0	522	2.2	CC−O bend [48], O═CCC for [13], NCC═O for [12]
			372	13.4	384	0.9	NCC bend [25], O═CCC tor [25], CC−O bend [15]
					327	11.9	CC−O bend [38], NCC bend [30]
			296	8.5	268	28.3	CC═O bend [21], CCC bend [18], NCC bend [13]
			235	1.7	246	2.0	CCC bend [43], NCC bend [21], CC−O bend [14]
			217	1.8	225	1.3	$C_\alpha C_m$ tor [83]
			209	37.8			NH_2 tor [84]
			49	1.9	64	1.1	O═CCC for [55], NCC−O for [45]

[a] Ar matrix deposited at 15 K. Matrix ratio 1:750. [b] *A*, experimental relative peak intensities. [c] I_{obs}, experimental relative integral intensities measured for the single bands or for the groups of the merged bands. [d] I_{calc}, calculated intensities in km mol^{-1}. [e] Potential energy distributions are given in square brackets. Only contributions ≥ 10 are listed. Abbreviations: str, stretching; bend, bending; tor, torsion; m, methyl.

3. Theoretical Methods

In our previous study we demonstrated that the DFT method is capable of producing high-accuracy structural parameters and vibrational frequencies and intensities of the glycine conformers.[9] The IR spectral characteristics of the glycine conformers calculated at the DFT level were found to be in better agreement with the experimental IR spectra than the results calculated at the MP2 level of theory. At the same time both methods produce very similar structural parameters and relative energies of low-energy conformers of glycine. For these reasons, in the present study of alanine we have calculated the relative stabilities of the alanine conformers at both the MP2 and DFT levels of theory and the IR frequencies and intensities at the DFT level. The DFT calculations were carried out with the three-parameter density functional, usually abbreviated as B3LYP, which includes Becke's gradient exchange correction,[18] the Lee, Yang, Parr correlation functional,[19] and the Vosko, Wilk, Nusair correlation functional.[20]

The geometry optimization and the harmonic frequency calculations were carried out for the five lowest-energy alanine conformers (Figure 1) at the DFT/B3LYP level of theory with Dunning's correlation-consistent double-zeta basis set[21−23] augmented with s and p diffuse functions on hydrogens and s, p, and d diffuse functions on heavy atoms (the aug-cc-pVDZ basis set). In our studies of the conformational behavior of glycine,[9] we demonstrated an excellent performance of the DFT/B3LYP method with the aug-cc-pVDZ basis set in reproducing the experimental rotational constants and the IR frequencies. It seems that augmentation of the cc-pVDZ basis set with diffuse functions (i.e., an additional sp-shell for hydrogens and an additional spd-shell for the heavy atoms) is particularly important for generating accurate predictions of IR frequencies of amino acid conformers with inramolecular H-bonds. For the minimum-energy alanine conformers, MP2/aug-cc-pVDZ single-point calculations were also performed at the geometries obtained at the DFT/B3LYP/aug-cc-pVDZ level. All calculations in this work were done on IBM RS6000 workstations using the Gaussian94[24] quantum-mechanical program.

4. Results and Discussion

4.1. Relative Energies and Structure of the Alanine Conformers. Since the detailed energy analysis of all alanine conformers was already presented in ref 10, here we discuss only those features of the conformational structure of alanine that are important in the analysis of the experimental IR spectra. The purpose of the relative energy calculations was to determine the most thermodynamically stable alanine conformers that may be observed in low-temperature matrixes.

The calculated relative energies of the lowest-energy alanine conformers are presented in Table 1. As it is seen, both the DFT and MP2 methods predict conformer **I** to be the most stable form, although the energy difference between this conformer and conformer **IIa** is very small, only 0.31 kJ mol^{-1} (without accounting for the zero-point energy (ZPE)). Worth mentioning is that some previous calculations[10] predicted conformer **IIa** to be the global minimum, also with a very small energy difference between conformers **I** and **IIa** (less than 1 kJ mol^{-1}). Accounting for ZPE leads to an increase of the relative energies of the alanine conformers with the N···H−O H-bond—**IIa** and **IIb**—with respect to conformer **I**, making the latter the definite global-minimum structure on the alanine potential energy surface. The ZPE contributions obtained for the alanine conformers, as well as analogous results for glycine,[9] demonstrate the likely importance of accounting for ZPE in accurate prediction of the relative energies of conformers similar in structure to conformer **II** for other amino acids. The low relative energies of the conformers **IIa** and **IIb** with respect to conformer **I** allow us to conclude that at least these conformers may be observed in the matrix. The relative energy of conformer **IIIa**, being 4.84 (4.82) kJ mol^{-1} at the DFT/B3LYP (MP2) level, is much higher then for conformers **IIa** and **IIb** (Table 1); however, it is still low enough for this conformer to possibly be present in the matrix. In the case of glycine, the relative energy of conformer **III** was 6.84 (7.14) kJ mol^{-1} at the DFT/aug-cc-pVDZ (MP2/aug-cc-pVDZ) level,[9] i.e., even higher than for alanine, but still detectable amounts of this conformer were found in the Ar matrixes.

Not only the relative energies of the conformers but also the height of the energy barriers separating them can also influence their relative concentrations in the matrix. The conformer interconversion can occur easier and faster if the barrier is low, and this may effectively eliminate the presence of even those conformers with lower relative energies with respect to the most stable one. For glycine the interconversion *conformer* **III** ⟹ *conformer* **I** was observed in the Ar matrixes at a temperature higher than 13 K. There are also reasons to suspect very low energy barriers between some alanine conformers. In the microwave study of alanine, Godfrey et al.[4] observed only two alanine conformers. As it is seen from Table 2, the rotational constants obtained by Godfrey et al. correspond exactly to conformers **I** and **IIa**. The absence of conformer **IIb**, whose dipole moment is as large as that of **IIa** in the microwave spectrum, probably results from a low-energy barrier between **IIa** and **IIb** and fast interconversion **IIb** to the lower-energy conformer, **IIa**. Conformer **IIIb** was predicted to be the true minimum at the MP2(full)/6-311++G** level,[10] but calculations

TABLE 4: Observed and Calculated (at the DFT/B3LYP/aug-cc-pVDZ level) IR Frequencies (cm^{-1}) and Intensities of the Alanine-d_3

			calculated				
observed[a]			alanine **I**		alanine **IIa**		
ν	A[b]	I_{calc}[d]	ν	I_{calc}[d]	ν	I_{calc}[d]	PED[e]
3003	0.036	0.293	3012	12.5	3015	7.2	C_mH str [100]
2982	0.050	0.914	2991	22.3	2980	23.3	C_mH str [98]
2944	0.036	1.515	2941	10.9	2925	17.1	$C_\alpha H$ str [96]
2931	0.041						
2867	0.024	0.713	2918	13.9	2914	16.8	C_mH str [97]
2850	0.024						
2627	0.240	1.551	2606	36.3			OD str [100]
2624	0.262						
2617	0.051						
			2518	3.1	2541	6.8	ND_2 str [100]
			2419	1.9	2436	1.8	ND_2 str [100]
2405	0.017	0.690			2416	134.6	OD str [93]
2394	0.030						
1785	0.469	1.814			1799	331.7	$C{=}O$ str [87]
1764	1.258	6.334	1772	271.2			$C{=}O$ str [89]
1763	1.296						
1762	1.140						
1741	0.028	0.201					$C{=}O$ str in the alanine dimers
1723	0.028	0.423					$C{=}O$ str in the alanine dimers
1714	0.030						
1463	0.054	0.701	1460	5.6	1460	6.8	HC_mH bend [89]
1461	0.112						
1459	0.070		1455	8.6	1459	5.2	HC_mH bend [93]
1455	0.051						
1382	0.038	0.640	1378	11.5	1377	11.2	C_mH bend [76]
1377	0.106						
1375	0.081						
1361	0.021	0.142	1359	3.4			$C_\alpha H$ bend [53], C_mH bend [12]
1323	0.031	0.173			1319	17.7	$C_\alpha H$ bend [84]
1316	0.034	0.216					
1308	0.094	0.599	1304	17.8	1303	53.2	$C_\alpha H$ bend [62]
1305	0.08						
1303	0.053						
1281	0.041	0.554			1281	190.9	$C{-}O$ str [37], $C_\alpha H$ bend [27], $CC{=}O$ bend [13] $C_\alpha C$ str [11], OD bend [10]
1277	0.075						
1265	0.041	0.611					
1260	0.068						
1254	0.050						
1225	0.223	1.400	1221	26.4			ND_2 bend [64], CN str [19]
1220	0.186						
1209	0.335	3.203	1207	131.7			$C{-}O$ str [32], $CC{=}O$ bend [16], $C_\alpha H$ bend [16]
1198	0.136						
1189	0.057	0.227			1212	42.0	ND_2 bend [75], CN str [16]
1187	0.042						
1182	0.036	0.153					
1148	0.047	0.748	1143	26.0			ND_2 bend [36], $C_\alpha H$ bend [23]
1142	0.097						
1130	0.018	0.105			1137	11.8	$C_\alpha C_m$ str [20], C_mH bend [18], ND_2 bend [14]
1122	0.026	0.096					
1115	0.096	0.45	1112	22.8			$C_\alpha C_m$ str [25], CN str [25], ND_2 bend [11]
1111	0.041						
1103	0.040	0.102			1101	13.7	C_mH bend [23], CN str [17], $C_\alpha C_m$ str [16]
1096	0.035	0.131					
1064	0.072	0.74	1060	26.1			C_mH bend [25], $C_\alpha H$ bend [10]
1061	0.109						
1041	0.035	0.264			1037	44.7	C_mH bend [16], $C_\alpha C_m$ str [13], CN str [13], OD bend [11], $C_\alpha H$ bend [11]
1036	0.029						
1032	0.031						
990	0.072	0.217			989	13.9	OD bend [59], C_mH bend [24]
984	0.455	1.761	993	78.5			OD bend [49], $C{-}O$ str [24]
982	0.413						
905	0.021	0.047	900	2.9	905	2.9	C_mH bend [25], CN str [24], $C_\alpha C_m$ str [17]
857	0.017	0.048	852	1.4	820	1.5	ND_2 bend [52]
799	0.016	0.021			803	18.7	$C_\alpha C_m$ str [17], $C_\alpha C$ str [14], $C{-}O$ str [11]
769	0.035	0.215	760	9.6			ND_2 bend [35], $C_\alpha C$ str [12]
733	0.063	0.155	739	21.0			$NCC{=}O$ tor [36], $C_\alpha C_m$ str [17]
725	0.055	0.210			729	5.9	$NCC{-}O$ tor [30], $NCC{=}O$ tor [22], CCC bend [10]
712	0.041	0.099					
689	0.039	0.062					

TABLE 4 (Continued)

observed[a]			calculated				
			alanine **I**		alanine **IIa**		
ν	A^b	$I_{calc}{}^d$	ν	$I_{calc}{}^d$	ν	$I_{calc}{}^d$	PED[e]
683	0.732	2.086	689	73.0			$C_\alpha C$ str [27], ND_2 bend [23], OD bend [13]
678	0.043	0.093					
663	0.049	0.104			672	32.5	ND_2 bend [63], NCC bend [10]
603	0.029	0.181			620	40.0	OD tor [96]
602	0.026						
596	0.055						
581	0.037	0.063			584	20.7	CC−O bend [35], NCC bend [20]
573	0.019	0.042	573	1.5			CC═O bend [53], C−O str [11]
					510	2.3	CC═O bend [55], $C_\alpha C$ str [22]
473	0.046	0.171					
467	0.135	0.648	468	31.5			CC−O bend [39], OD tor [14], NCC═O tor [13]
440	0.069	0.276					
431	0.415	1.576	447	48.7			OD tor [77]
			347	11.8	361	0.5	O═CCC tor [25], NCC bend [20], CC−O bend [17]
			280	7.1	303	10.2	CCC bend [22], CC═O bend [18], NCC bend [13]
			224	2.2	255	11.8	CCC bend [37], NCC bend [26]
			216	0.5	225	1.1	$C_\alpha C_m$ tor [90]
			155	21.4	194	7.8	CN tor [93]
			47	2.0	61	1.2	$C_\alpha C$ tor [100]

[a] Ar matrix deposited at 15 K. Matrix ratio 1:750. [b] *A*, experimental relative peak intensities. [c] I_{obs}, experimental relative integral intensities measured for the single bands or for the groups of the merged bands. [d] I_{calc}, calculated intensities in km mol^{-1}. [e] Potential energy distributions are given in square brackets. Only contributions ≥ 10 are listed. Abbreviations: str, stretching; bend, bending; tor, torsion; m, methyl.

at the DFT/B3LYP/6-311++G** level,[10] as well as our calculations at the DFT/B3LYP/aug-cc-pVDZ level, failed to predict a local energy minimum corresponding to this conformer. Only the minimum corresponding to conformer **IIIa** was found in the DFT/B3LYP calculations. The DFT/B3LYP relative energies of the glycine conformers were in good agreement with the MP2 results.[9,25] Thus, the disagreement between the methods in predicting the existence of the alanine conformer **IIIb** is likely due to a very low interconversion barrier leading to conformer **IIIa**.

In conclusion, the relative energy calculations of the alanine conformers performed in this work predict the presence of up to five conformers in the matrix. However, due to a possible fast interconversion of **IIb** and **IIIb** into their lower-energy counterparts, the actual number of conformers in the matrix can be smaller.

4.2. IR Spectral Characterization of the Alanine Conformers.

The infrared spectra of the alanine-d_0 and alanine-d_3 are depicted in Figure 2. The observed frequencies and intensities are compared with the spectra calculated at the DFT/B3LYP/aug-ccpVDZ level for alanine-d_0 and alanine-d_3 in Tables 3 and 4, respectively. The calculated harmonic frequencies were scaled with two scaling factors: 0.96 for the X−H (X = C, N, O) stretching vibrations and 0.99 for all other vibrations. These scaling factors yielded the best agreement between the observed and calculated DFT/B3LYP/aug-cc-pVDZ frequencies for the glycine conformers.[9]

The analysis of the high-frequency region (4000−2000 cm^{-1}) allows for easy identification of conformers **I** and **IIa** of alanine in the Ar matrix. The intensive bands at 3560 cm^{-1} in the spectrum of alanine-d_0 and at 2627 cm^{-1} in the spectrum ofalanine-d_3 are assigned to the OH (OD) stretching vibration of the main alanine conformer **I**. The corresponding frequencies of glycine conformer **I** are almost identical, 3560 and 2631 cm^{-1}. There are additional bands at 3193 and 2405 cm^{-1} in the spectra of alanine-d_0 and alanine-d_3, respectively. Based on the calculated frequencies, these bands are assigned to the OH (OD) stretching vibrations of conformer **IIa**. The bands are downshifted by 367 and 222 cm^{-1} compared with the corresponding bands of conformer **I** due to the intramolecular N···H−O H-bond interaction in conformer **IIa**. As it is seen from Tables 3 and 4, the calculated frequencies are in good agreement with the observed ones except for the frequency of the OH stretching vibration of the conformer **IIa**. In this case the difference is 123 cm^{-1}. We suspect that it is due to a significant unharmonicity of this vibration. For glycine[9] we also found a larger discrepancy between the observed and calculated frequency values for the OH stretching vibration of conformer **II**. We investigated this problem further by calculating the frequencies of this mode for glycine conformers **I** and **II** by solving the vibrational problem in the potential determined over several points surrounding the minimum.[26] The calculations yielded vibrational frequencies in very good agreement with the observed frequencies.

The above presented analysis of the high-frequency region of the alanine IR spectra allowed identification of bands attributed to conformers **I** and **IIa**. The presence of the conformer **IIIa** cannot be definitely determined based on the analysis of this region because the predicted frequency differences between the conformers **I** and **IIIa** for high-frequency vibrations are only a few wavenumbers.

In the region of the C═O stretching vibrations (1800−1750 cm^{-1}) in the spectra of alanine-d_0 and alanine-d_3, the most intensive doublet at 1773 and 1763 cm^{-1} is assigned to the C═O vibration of the main conformer **I**. According to the calculations, the corresponding bands of the conformer **IIa** are upper-shifted and the bands of the conformer **IIIa** are down-shifted with respect to the bands of the main conformer. In the observed spectra, only the upper-shifted bands at 1792 (alanine-d_0) and 1784 (alanine-d_3) are present. Again it proves the presence of the conformer **IIa** in the matrix and demonstrates the absence of conformer **IIIa**.

Using the calculated frequencies and intensities of conformers **I** and **IIa**, we assigned nearly all bands that appear in the experimental spectra for alanine-d_0 and alanine-d_3. The results of this assignment are summarized in Tables 3 and 4. As it is seen, there are no manifestations of the presence in the matrix of any additional alanine conformers. A few unassigned bands in the spectra are most probably overtones or combined bands.

We do not see any spectral manifestation of the presence of conformer **IIIa**.

Assuming that only two alanine conformers, **I** and **IIa**, are responsible for the experimentally observed spectra and using the integrals of the experimental intensities and the calculated intensities of the OH stretching vibrations of the two conformers (Tables 3 and 4), we evaluated the relative conformational composition of alanine deposited in the matrix. There is approximately 90% of conformer **I** and 10% conformer **IIa** in the deposited alanine. It is noticeable that the conformational compositions derived from the spectra of alanine-d_0 and alanine-d_3 are almost identical.

5. Conclusions

In this study we determined the presence of two conformers in gas-phase α-alanine frozen in the low-temperature Ar matrix. In both conformers there is an intramolecular hydrogen-bond interaction which leaves a noticeable signature in the IR spectrum. In the first conformer, the spectrum indicates the presence of a $NH_2\cdots O{=}C$ H-bond and in the second conformer the presence of a $N\cdots H{-}O$ H-bond. We evaluated that 90% of the matrix-deposited alanine exists as the lowest-energy conformer **I**. The assignment of the experimental spectra was assisted by the frequency and intensity calculations performed at the DFT/B3LYP level of theory with the aug-cc pVDZ basis set, which appeared to be of very high quality.

We found that some alanine conformers with predicted low relative energies are not present in the matrix. We attribute this absence to low-energy barriers between these conformers and the ones observed in the matrix. Undoubtedly, in structural investigations of the nonionized amino acids, not only the relative energies of the conformers but also the interconversion barriers between them have to be taken into account in determining the possible conformers.

Acknowledgment. This work was supported in part by a COBASE grant allowing the visit of S.G.S. to the University of Arizona.

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