Experimental and Computational Thermochemical Study of N -Benzylalanines

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Experimental and Computational Thermochemical Study of *N*-Benzylalanines

Rafael Notario,* Maria Victoria Roux, and Concepción Foces-Foces

Instituto de Química Física "Rocasolano", CSIC, Serrano 119, 28006 Madrid, Spain

Manuel A. V. Ribeiro da Silva,* Maria das Dores M. C. Ribeiro da Silva, and Ana Filipa L. O. M. Santos

Centro de Investigação em Química, Department of Chemistry and Biochemistry, Faculty of Science, University of Porto, Rua do Campo Alegre 687, P-4169-007 Porto, Portugal

Ramón Guzmán-Mejía and Eusebio Juaristi*

Departamento de Química, Centro de Investigación y de Estudios Avanzados del IPN, Apartado Postal 14-740, 07000 México D.F., México

S *Supporting Information*

ABSTRACT:
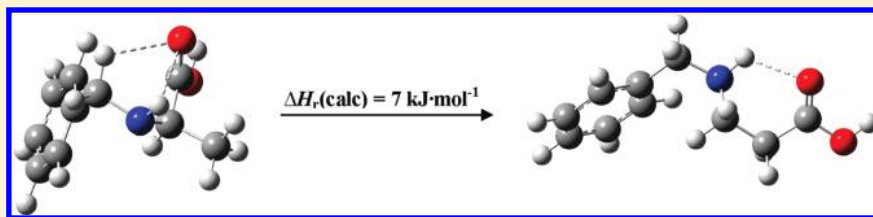
$\Delta H_r(calc) = 7 \text{ kJ} \cdot \text{mol}^{-1}$

Calorimetric measurements are expected to provide useful data regarding the relative stability of α- versus β-amino acid isomers, which, in turn, may help us to understand why nature chose α- instead of β-amino acids for the formation of the biomolecules that are essential constituents of life on earth. The present study is a combination of the experimental determination of the enthalpy of formation of *N*-benzyl-β-alanine, and high-level ab initio calculations of its molecular structure. The experimentally determined standard molar enthalpy of formation of *N*-benzyl-β-alanine in gaseous phase at $T = 298.15$ K is $-(298.8 \pm 4.8) \text{ kJ} \cdot \text{mol}^{-1}$, whereas its G3(MP2)//B3LYP-calculated enthalpy of formation is $-303.7 \text{ kJ} \cdot \text{mol}^{-1}$. This value is in very good agreement with the experimental one. Although the combustion experiments of *N*-benzyl-α-alanine were unsuccessful, its calculated enthalpy of formation is $-310.7 \text{ kJ} \cdot \text{mol}^{-1}$; thus, comparison with the corresponding experimental enthalpy of formation of *N*-benzyl-β-alanine, $-(298.8 \pm 4.8) \text{ kJ/mol}$, is in line with the concept that the more branched amino acid (α-alanine) is intrinsically more stable than the linear β-amino acid, β-alanine.

1. INTRODUCTION

Calorimetric measurements are expected to provide useful data regarding the relative stability of α- versus β-amino acid isomers, which, in turn, may help us to understand why nature chose α- instead of β-amino acids for the formation of the biomolecules that are essential constituents of life on earth. We have studied the thermochemical properties of α-alanine (DL) as representative of α-amino acids and β-alanine as representative of β-amino acids.[1] We concluded that the more branched amino acid (α-alanine) is intrinsically more stable than the linear β-amino acid (β-alanine) and that the isomerization enthalpy derived from the experimental enthalpies of formation is $5.1 \pm 3.5 \text{ kJ} \cdot \text{mol}^{-1}$.

We want to continue in the same line of study, studying another two isomeric species representative of α- and β-amino

acids, *N*-benzyl-α-alanine [*N*-(phenylmethyl)-α-alanine] and *N*-benzyl-β-alanine [*N*-(phenylmethyl)-β-alanine], respectively, whose schematic formulas are presented in Figure 1. To our knowledge, there are no previous thermochemical experimental or computational studies on these species reported in the literature. Thus, the approach selected in the present study is a combination of experimental determination of the enthalpies of formation and high-level ab initio calculations. As indicated in the Experimental Procedures, it has been impossible to obtain the enthalpy of combustion of *N*-benzyl-α-alanine, and for this species only theoretical calculations have been carried out.

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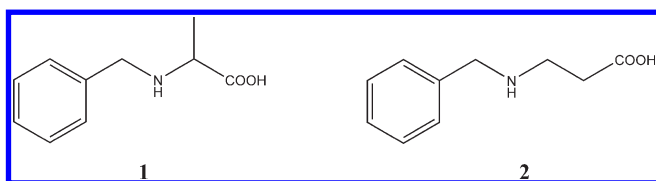dx.doi.org/10.1021/jp200891v | *J. Phys. Chem. B* 2011, 115, 9401–9409

Figure 1. Schematic formulas of N-benzyl-α-alanine (**1**) and N-benzyl-β-alanine (**2**).

2. EXPERIMENTAL PROCEDURES

2.1. Compounds and Purity Control. *N-Benzyl-α-alanine* (DL) *[CAS 40297-69-6].* In a 300 cm^3 hydrogenation flask were placed 3.0 g (33.7 mmol) of α-alanine (DL) in 150 cm^3 of MeOH/ H$_2$0 (60:40), and to this were added 3.42 cm^3 (33.7 mmol) of benzaldehyde and 0.30 g of 10% Pd/C catalyst. The resulting mixture was pressurized to 200 psi with hydrogen and shaken for 24 h at 40 °C before the removal of the catalyst by filtration over Celite. The solvent was removed at reduced pressure in a rotary evaporator, and the crude product was purified by flash column chromatography (gradient EtOAc to EtOAc-MeOH, 70:30). The expected product (3.0 g, 50% yield) was obtained as a white solid, mp 255−256 °C (lit.[2] mp 253 °C). ^{1}H NMR (D$_2$O, 270 MHz δ): 1.46 (d, 3H, J = 7.2 Hz), 3.66 (q, 1H, J = 7.2 Hz), 4.17 (AB, 2H, J = 12.8 Hz), 7.44 (s, 5H). ^{13}C NMR (D$_2$O, 67.5 MHz δ): 15.4, 49.9, 57.5, 129.4, 129.7, 130.0, 131.0, 174.9.

N-Benzyl-β-alanine [CAS 5426-62-0]. In a 500 cm^3 hydrogenation flask were placed 10.0 g (112.2 mmol) of β-alanine in 150 cm^3 MeOH/H$_2$0 (80:20), and to this were added 11.4 cm^3 (133.7 mmol) of benzaldehyde and 1.0 g of 10% Pd/C catalyst. The resulting mixture was pressurized to 60 psi with hydrogen and shaken for 24 h before the removal of the catalyst by filtration over Celite. The solvent was removed at reduced pressure in a rotary evaporator, and the crude product was purified by flash column chromatography (gradient EtOAc to EtOAc−MeOH, 70:30). The expected product (13.0 g, 65% yield) was obtained as a white solid, mp 193−194 °C (lit.[3] mp 196 °C). ^{1}H NMR (D$_2$O, 270 MHz δ): 2.43 (t, 2H, J = 20.0 Hz), 3.08 (t, 2H, J = 18.9 Hz), 4.10 (s, 2H), 7.37 (s, 5H). ^{13}C NMR (D$_2$O, 67.5 MHz δ): 32.5, 43.8, 50.8, 129.4, 129.7, 129.8, 130.9, 178.0.

The purity of the samples was checked by DSC and C, H, and N microanalysis. Calcd for C$_{12}$H$_{13}$NO$_2$: $10^2 \, w(C)$ = 0.6702, $10^2 \, w(H)$ = 0.0731, $10^2 \, w(N)$ = 0.0782. Found: $10^2 \, w(C)$ = 0.6537, $10^2 \, w(H)$ = 0.0723, $10^2 \, w(N)$ = 0.0761, and $10^2 \, w(C)$ = 0.6644, $10^2 \, w(H)$ = 0.0710, $10^2 \, w(N)$ = 0.0791 for N-benzyl-α-alanine (DL) and N-benzyl-β-alanine, respectively.

Differential Scanning Calorimetry. A differential scanning calorimeter (Pyris 1 from Perkin-Elmer) equipped with an intracooler unit was used to control the purity and to study the existence of possible phase transitions in the sample. The apparatus was previously calibrated in temperature and energy with reference materials. Temperature and power scales were calibrated[4] at heating rates of 0.04 and 0.17 K·s^{-1}. The temperature scales were calibrated by measuring the melting temperature of the recommended high-purity reference materials, hexafluorobenzene, tin, and indium.[5] The power scales were calibrated with high-purity indium (mass fraction > 0.99999) as the reference material.[5] The standards used for DSC calibration were hexafluorobenzene 99.9% purity, from Aldrich; benzoic acid, from NIST (standard reference sample 39j); and high-purity indium (mass fraction > 0.99999), tin, and synthetic sapphire, from

Perkin-Elmer. Details of the measuring procedure and calibration have been given.[4]

Thermograms of samples hermetically sealed in aluminum pans were recorded in a nitrogen atmosphere. All of the pans with the samples were weighed on a Mettler AT21 microbalance with a detection limit of 1×10^{-6} g, before and after the experiments to confirm that no product had volatilized.

For determination of purity, a heating rate of 0.04 K·s^{-1} was used, and five to eight samples weighing 1−2 mg were recorded. A fresh sample was used for each run. Determination of purities, using the fractional fusion technique,[6] indicated that the mole fraction of impurities in N-benzyl-β-alanine was less than 0.015. It was not possible to determine the purity of N-benzyl-α-alanine for decomposition of the compound in the fusion process. No solid−solid phase transitions were observed in the compounds over the temperature interval from T = 273.15 K to the corresponding fusion temperatures.

2.2. Combustion Calorimetry. The combustion experiments of N-benzyl-β-alanine were performed in a stainless steel static bomb calorimeter equipped with a twin valve combustion bomb type 1108 (Parr Instrument Company) and with an internal volume of 0.342 dm^3. All of the apparatus and techniques have been previously described in the literature.[7,8]

Benzoic acid NIST Thermochemical Standard 39j, with a certified massic energy of combustion under bomb conditions of −(26434 ± 3) J·g^{-1},[9] was used for calibration of the bomb. The calibration experiments were performed following the procedure described by Coops et al.[10] The value of the energy equivalent of the calorimeter was found to be ε(calor) = 15995.3 ± 2.0 J·K^{-1}, as a mean of six calibration experiments, for an average mass of water added to the calorimeter of 3119.6 g; the quoted uncertainty refers to the standard deviation of the mean.

In all combustion experiments, the N-benzyl-β-alanine was burned in pellet form and 1.00 cm^3 of deionized water was introduced into the bomb, which was purged twice to remove air, before being charged with 3.04 MPa of oxygen. The calorimeter temperatures were measured with a precision of ±1 × 10^{-4} K, at time intervals of 10 s, with a quartz crystal thermometer (Hewlett-Packard HP 2804 A), interfaced to a PC programmed to acquire data, control the calorimeter temperatures, and compute the adiabatic temperature change, through the LAB-TERMO program.[11] The ignition of the samples was made at T = 298.150 ± 0.001 K, and the electrical energy for the ignition was determined from the change in potential difference across a capacitor when discharged through the platinum ignition wire of diameter 0.05 mm. The empirical formula and the massic energy of combustion of the cotton thread used as fuse in all of the experiments are, respectively, CH$_{1.686}$O$_{0.843}$ and −16240 J·g^{-1}; the massic energy of combustion is a value that has been previously confirmed in our laboratory. For the n-hexadecane (mass fraction > 0.999, Aldrich), stored under nitrogen, used as an auxiliary combustion in the experiments of the N-benzyl-β-alanine, with the purpose of avoiding carbon soot residue formation, $\Delta_c u^\circ$ = −(47132.7 ± 2.6) J·g^{-1}. The corrections for nitric acid formation were based on −59.7 kJ·mol^{-1} for the molar energy of formation of 0.1 mol·dm^{-3} HNO$_3$(aq) from N$_2$(g), O$_2$(g), and H$_2$O(l).[12] After the combustion experiments, the total mass of carbon dioxide produced was recovered, and the quantity of compound used in each experiment was based on it, after allowance for that formed from the combustion of the cotton thread fuse and of the n-hexadecane. The average ratio of the mass of carbon dioxide recovered to that calculated from the
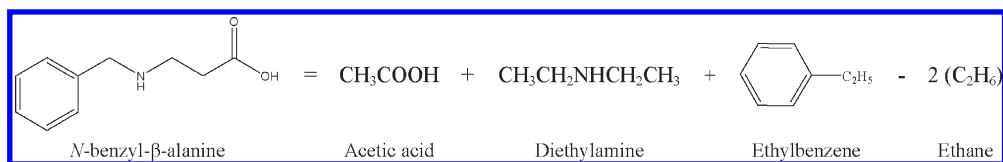
Figure 2. Group additivity scheme for $\Delta_{298.15K}^{T}H_m^\circ(g)$ estimation for N-benzyl-β-alanine.

mass of samples of N-benzyl-β-alanine used in each experiment was 0.9993 ± 0.0023, where the uncertainty is twice the standard deviation of the mean.

An estimated pressure coefficient of massic energy, $(\partial u/\partial p)_T = -0.2\,\mathrm{J\cdot g^{-1}\cdot MPa^{-1}}$, at $T = 298.15$ K, a common value for most organic solids, was used for N-benzyl-β-alanine.[13] For this amino acid, $\Delta_c u^\circ$ was calculated following the procedure given by Hubbard et al.[14]

The relative atomic masses used in the calculation of all molar quantities used in this work were those recommended by the IUPAC Commission in 2009.[15] The specific density of N-benzyl-β-alanine was assumed to be the same of β-alanine, $\rho = 1.437$ $\mathrm{g\cdot cm^{-3}}$.[16]

As it turned out, the combustion experiments of N-benzyl-α-alanine were unsuccessful because of the very large dispersion of the results and the great quantities of carbon soot residue formed in each experiment, even in the presence of n-hexadecane combustion auxiliary.

2.3. High Temperature Calvet Microcalorimetry. The standard molar enthalpy of sublimation of N-benzyl-β-alanine was measured in a high temperature Calvet microcalorimeter (Setaram, model HT 1000), employing the drop-microcalorimetric technique for vacuum sublimation described by Skinner et al.[17] The details of the apparatus and the technique have been previously described.[18]

The microcalorimeter was calibrated by making use of the reported standard molar enthalpy of sublimation of 1,3,5-triphenylbenzene, $\Delta_{cr}^g H_m^\circ(298.15\ \mathrm{K}) = 149.180 \pm 1.600\ \mathrm{kJ\cdot mol^{-1}}$.[5] The calibration constant, k, of the calorimeter was obtained as the average of six independent experiments for the predefined temperature, where the uncertainty presented is the standard deviation of the mean: $k(T = 468\ \mathrm{K}) = 1.0188 \pm 0.0012$.

In all of the experiments, the samples of about $3-5$ mg of the crystalline amino acid were introduced in thin glass capillary tubes sealed at one end. The sample tubes and blank reference capillary tubes were simultaneously dropped into the hot reaction vessel, held at a suitable predefined temperature, T, and then removed from the hot zone by vacuum sublimation.

The thermal corrections due to the differences in the mass of both capillary tubes and the different sensibilities of the two measuring cells were obtained by making separate blank correction experiments, dropping empty tubes of nearly equal mass into each of the twin cells.[18]

The standard molar enthalpy of sublimation of N-benzyl-β-alanine, at $T = 298.15$ K, was calculated from the same thermodynamic parameter, at the temperature, T, corrected to $T = 298.15$ K, using $\Delta_{298.15K}^{T}H_m^\circ(g)$ estimated by a group additivity scheme based on values of Stull et al.,[19] presented in Figure 2.

2.4. Computational Details. Standard ab initio molecular orbital calculations[20] were performed with the *Gaussian 03* series of programs.[21] Energies were obtained using the Gaussian-3 theory at the G3(MP2)//B3LYP[22] level.

G3(MP2)//B3LYP is a variation of G3(MP2)[23] theory that uses the B3LYP density functional method[24] for geometries and

zero-point energies. The B3LYP density functional method used is a linear combination of Hartree–Fock exchange, Becke[25] exchange, and Lee, Yang, and Parr (LYP)[26] correlation. Two modifications have been made to derive G3(MP2)//B3LYP. First, the geometries are obtained at the B3LYP/6-31G(d) level instead of MP2(FU)/6-31G(d). Second, the zero-point energies are obtained at the B3LYP/6-31G(d) level and scaled by 0.96 instead of HF/6-31G(d) scaled by 0.8929. All of the other steps remain the same with the exception of the values of the higher-level correction parameters.

As a result of the lack of symmetry and the internal rotational degrees of freedom by the free rotation of the NHR, COOH, and OH groups with respect to the carbon skeleton, N-benzyl-α-alanine and N-benzyl-β-alanine exhibit a large number of low-energy conformers. In this study we have optimized the molecular structures of 9 and 13 of the lowest-energy conformers of N-benzyl-α-alanine and N-benzyl-β-alanine, respectively, taking into account only those conformers that contribute significantly to the populated states.

We have also reoptimized the geometries of the lowest-energy conformers of N-benzyl-α-alanine and N-benzyl-β-alanine at the MP2(full)/6-31G(3df,2p) level to obtain more reliable molecular structures.

The charge distribution has been analyzed using a population partition technique, the natural bond orbital (NBO) analysis of Reed and Weinhold.[27−29] The NBO analysis has been performed using the NBO program[30] implemented in the *Gaussian 03* package.[21]

3. RESULTS AND DISCUSSION

3.1. Experimental Results. The detailed results of the combustion experiments of N-benzyl-β-alanine are presented in Table 1, where $\Delta m(H_2O)$ is the deviation of the mass of water added to the calorimeter from 3119.6 g, ΔU_Σ is the correction to the standard state, and the other symbols have been previously defined.[14,31] The mean value of massic energy of combustion, $\langle \Delta_c u^\circ \rangle$, and its standard deviation are also presented in Table 1.

The internal energy for the isothermal bomb process, ΔU(IBP), was calculated according to eq 1,

$$\Delta U(\mathrm{IBP}) = -\{\varepsilon(\mathrm{calor}) + c_p(H_2O,l)\cdot\Delta m(H_2O) + \varepsilon_f\}\Delta T_{ad} + \Delta U(\mathrm{ign}) \tag{1}$$

in which ΔT_{ad} is the calorimeter temperature change corrected for the heat exchange and the work of stirring. The values of $\Delta_c u^\circ$, presented in Table 1, are related to the idealized combustion reaction, represented by eq 2.

$$\mathrm{C_{10}H_{13}NO_2(cr) + 12.25O_2(g) \rightarrow 10CO_2(g) + 6.5H_2O(l) + 0.5N_2(g)} \tag{2}$$
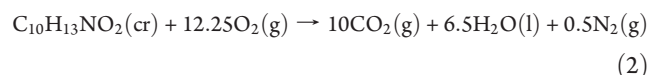
The derived standard molar value for the energy, $\Delta_c U_m^\circ(cr)$, and enthalpy, $\Delta_c H_m^\circ(cr)$, of the combustion reaction (see eq 2) as well as the standard molar enthalpy of formation in the crystalline phase, $\Delta_f H_m^\circ(cr)$, at $T = 298.15$ K, are given in Table 2.

Table 1. Experimental Standard ($p° = 0.1$ MPa) Massic Energy of Combustion of N-Benzyl-β-alanine, at $T = 298.15$ K

	1	2	3	4	5
$m(CO_2, \text{total})$,[a] g	1.49697			1.43855	1.26462
$m(\text{cmpd})$,[b] g	0.46628	0.46473	0.46924	0.42727	0.34573
$m(\text{fuse})$,[c] g	0.00293	0.00285	0.00265	0.00233	0.00210
$m(n\text{-hex})$,[d] g	0.11166	0.11162	0.11145	0.12398	0.13256
ΔT_{ad},[e] K	1.19761	1.19484	1.20260	1.16173	1.03501
ε_f,[f] J·K^{-1}	16.06	16.08	16.1	16.05	15.85
$\Delta m(H_2O)$,[g] g	0	−0.1	0	−0.2	0
$-\Delta U(\text{IBP})$,[h] J	19174.43	19129.76	19254.41	18598.80	16570.83
$\Delta U(\text{fuse})$,[i] J	47.58	46.28	43.04	37.84	34.1
$\Delta U(n\text{-hex})$,[j] J	5262.76	5260.87	5252.85	5843.68	6248.11
$\Delta U(HNO_3)$,[k] J	25.86	23.47	22.88	21.69	20.85
$\Delta U(\text{ign})$,[l] J	0.93	0.78	0.9	1.09	0.87
ΔU_Σ,[m] J	9.48	9.45	9.54	8.9	7.46
$-\Delta_c u°$,[n] J·g^{-1}	29657.61	29672.48	29677.99	29692.44	29677.23

$$-\langle\Delta_c u°\rangle = (29675.6 \pm 5.6)\ \text{J·g}^{-1}$$

[a] $m(CO_2, \text{total})$ is the mass of CO_2 recovered in each combustion. [b] $m(\text{cmpd})$ is the mass of compound burnt in each experiment. [c] $m(\text{fuse})$ is the mass of the fuse (cotton) used in each experiment. [d] $m(n\text{-hex})$ is the mass of n-hexadecane used as auxiliary of combustion. [e] ΔT_{ad} is the corrected temperature rise. [f] ε_f is the energy equivalent of the contents in the final state. [g] $\Delta m(H_2O)$ is the deviation of mass of water added to the calorimeter from 3119.6 g. [h] $\Delta U(\text{IBP})$ is the energy change for the isothermal combustion reaction under actual bomb conditions. ΔU-(IBP) includes $\Delta U(\text{ignition})$. [i] $\Delta U(\text{fuse})$ is the energy of combustion of the fuse (cotton). [j] $\Delta U(n\text{-hex})$ is the energy of combustion of n-hexadecane used as auxiliary of combustion. [k] $\Delta U(HNO_3)$ is the energy correction for the nitric acid formation. [l] $\Delta U(\text{ign})$ is the electric energy for the ignition. [m] ΔU_Σ is the standard state correction. [n] $\Delta_c u°$ is the standard massic energy of combustion.

The uncertainties of the standard molar energy and the enthalpy of combustion are twice the overall standard deviation of the mean, and they include the uncertainties in calibration and in the value of the combustion auxiliary used, n-hexadecane.[32,33]

To derive $\Delta_f H_m°(\text{cr})$ from $\Delta_c H_m°$, the standard molar enthalpies of formation of $H_2O(l)$ and $CO_2(g)$, at $T = 298.15$ K, $-(285.830 \pm 0.042)$ kJ·mol^{-1} and $-(393.51 \pm 0.13)$ kJ·mol^{-1},[34] respectively, were used.

The value of the standard molar enthalpy of sublimation of N-benzyl-β-alanine, at the experimental temperature T, obtained by Calvet microcalorimetry, together with the corrective enthalpic term, $\Delta_{298.15K}^T H_m°(g)$, estimated by a group additivity scheme based on the values of Stull et al.,[19] and the standard molar enthalpy of sublimation, at $T = 298.15$ K, are given in Table 3. The uncertainty associated with the value of $\Delta_{cr}^g H_m°(T = 298.15$ K) is twice the overall standard deviation of the mean and includes the uncertainties in calibration.

The standard molar enthalpy of formation in the crystalline phase, $\Delta_f H_m°(\text{cr})$, together with the standard molar enthalpy of sublimation, $\Delta_{cr}^g H_m°$, yields the standard molar enthalpy of formation in the gaseous phase, $\Delta_f H_m°(g)$, of the N-benzyl-β-alanine; all values are summarized in Table 4.

3.2. Molecular and Electronic Structures. To our knowledge, no experimental crystal or gas-phase structure determinations of N-benzyl-α-alanine and N-benzyl-β-alanine have been reported in the literature.

Table 2. Derived Standard ($p° = 0.1$ MPa) Molar Energy of Combustion, $\Delta_c U_m°$, Standard Molar Enthalpy of Combustion, $\Delta_c H_m°$, and Standard Molar Enthalpy of Formation, $\Delta_f H_m°$, for the Crystalline Compound, at $T = 298.15$ K

cmpd	$-\Delta_c U_m°(\text{cr})$, kJ·mol^{-1}	$-\Delta_c H_m°(\text{cr})$, kJ·mol^{-1}	$-\Delta_f H_m°(\text{cr})$, kJ·mol^{-1}
N-benzyl-β-alanine	5318.3 ± 2.8	5322.6 ± 2.8	470.4 ± 3.1

Amino acids exist as zwitterions in the crystalline state[35] as well as in aqueous solution, stabilized by electrostatic, polarization, and hydrogen-bonding interactions with their environment. In the gas phase, where the intermolecular interactions have no effect, amino acids are intrinsically flexible systems, existing as their nonionized forms. Like other amino acids, N-benzyl-α-alanine and N-benzyl-β-alanine exhibit significant internal rotational degrees of freedom due to free rotation of the NHR, COOH, and OH groups with respect to the carbon skeleton, and they exhibit a high number of low-energy conformers. Intramolecular hydrogen bonding becomes important in conformations in the gas phase. The energy barriers that separate different conformers are typically rather small for many conformations, so that thermal energy at room temperature enables the molecule to freely change from one conformation to another. Therefore, it is not generally feasible to experimentally isolate a specific conformer at room temperature.[36]

To our knowledge, no theoretical calculations have been carried out on these species. In this work, we have initially identified a set of conformers of N-benzyl-α-alanine and N-benzyl-β-alanine at low levels of theory, and then the most stable ones were reoptimized at higher levels of theory. Finally, the energies for the set of lowest-energy conformers within a $\sim$10 kJ·mol^{-1} range (9 and 13 conformers for N-benzyl-α-alanine and N-benzyl-β-alanine, respectively) were obtained at the G3(MP2)//B3LYP level. The structures of the most stable conformers of both N-benzyl-α-alanine and N-benzyl-β-alanine obtained in this work, optimized at the B3LYP/6-31G(d) level, are shown in Figures 3 and 4, respectively. Their Cartesian coordinates are collected in the Supporting Information.

The lowest-energy conformer for each of the benzylalanines has been reoptimized at the MP2(Full)/6-31G(3df,2p) level to obtain a more reliable molecular structure, and those structures are shown in Figures 5 and 6.

The most stable conformers of N-benzyl-α-alanine (see Figure 5) and N-benzyl-β-alanine (see Figure 6) are stabilized by N−H···O hydrogen bonds (2.78 and 2.29 Å, respectively). The hydrogen bond is established between the oxygen atom of the carbonyl group and the hydrogen of the NH group. In the case of N-benzyl-α-alanine, another hydrogen bond (2.46 Å) exists between the oxygen atom of the carbonyl group and one of the hydrogen atoms of the CH_2 group.

The optimized geometrical parameters for the most stable conformers of both benzylalanines have been collected in Tables S1 and S2 of the Supporting Information.
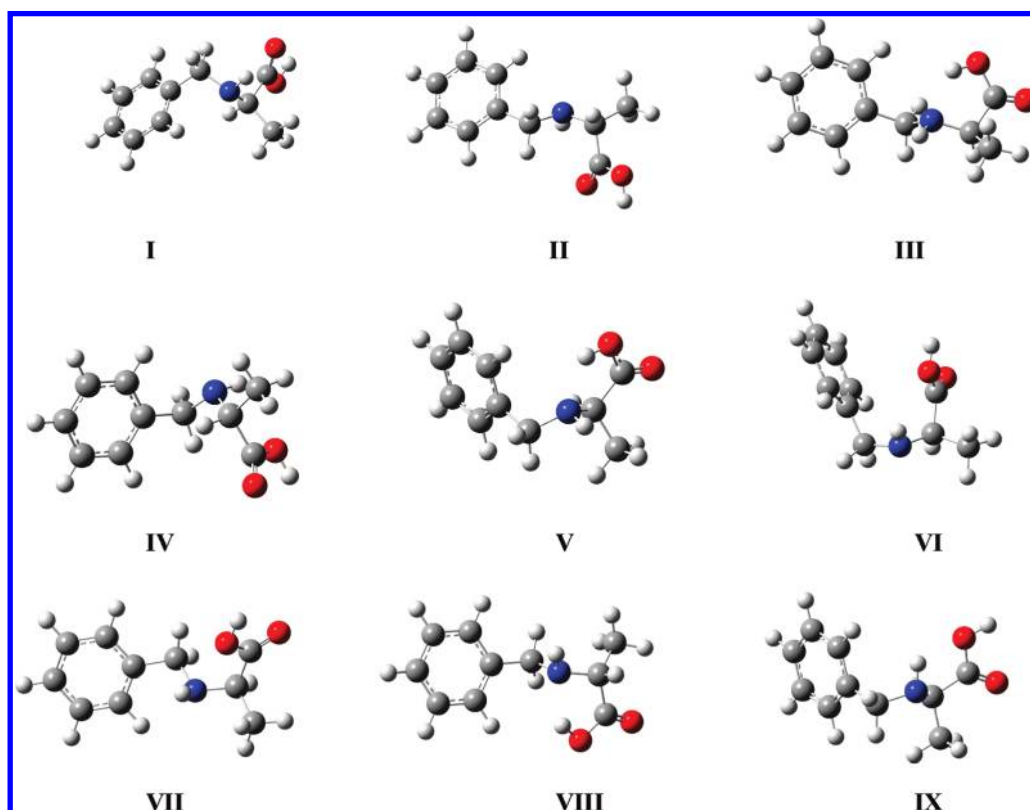
A population analysis using the natural bond orbital (NBO) analysis to obtain the natural atomic charges (the nuclear minus summed populations of the natural atomic orbitals on the atoms) that characterize the ground electronic state of the compounds studied has also been carried out. The calculated NBO charges

Table 3. Standard ($p° = 0.1$ MPa) Molar Enthalpy of Sublimation, $\Delta_{cr}^{g} H_{m}^{\circ}$, for the N-Benzyl-$\beta$-alanine, at $T = 298.15$ K, Determined by Calvet Microcalorimetry

cmpd	no. of exp	T, K	$-\Delta_{cr,298.15K}^{g,T} H_m$, kJ·mol^{-1}	$\Delta_{298.15K}^{T} H_m^{\circ}(g)$, kJ·mol^{-1}	$\Delta_{cr}^{g} H_m^{\circ}(T = 298.15$ K), kJ·mol^{-1}
N-benzyl-β-alanine	5	467.7	215.0 ± 0.3	43.41	171.6 ± 3.7

Table 4. Standard ($p° = 0.1$ MPa) Molar Enthalpy of Formation, in Crystalline and Gaseous Phases, and Standard Molar Enthalpy of Sublimation, at $T = 298.15$ K

cmpd	$-\Delta_f H_m^{\circ}(cr)$, kJ·mol^{-1}	$\Delta_{cr}^{g} H_m^{\circ}$, kJ·mol^{-1}	$-\Delta_f H_m^{\circ}(g)$, kJ·mol^{-1}
N-benzyl-β-alanine	470.4 ± 3.1	171.6 ± 3.7	298.8 ± 4.8

Figure 3. B3LYP/6-31G(d)-optimized structures of the nine lowest-energy conformers of N-benzyl-α-alanine.

located at all the atoms for the most stable structures of N-benzyl-α-alanine and N-benzyl-β-alanine are reported in Tables S1 and S2 of the Supporting Information. In N-benzyl-α-alanine, all of the heavy atoms are negatively charged except for the C atom of the carboxylic group, which has a charge of 0.970. Partial negative charges are located at the N atom (-0.729), the two O atoms (-0.726, O of the carbonyl group, and -0.784, O of the OH group), the C atoms of the CH (-0.146), CH$_2$ (-0.216), and CH$_3$ (-0.633) groups, and the C atoms of the ring. In N-benzyl-β-alanine, the behavior is very similar, with similar charges on the heavy atoms.

3.3. Theoretical Determination of the Enthalpies of Formation. G3(MP2)//B3LYP-calculated energies at 0 K, enthalpies at 298 K, and entropies, for the nine lowest-energy conformers of N-benzyl-α-alanine, and the thirteen lowest-energy

conformers of N-benzyl-β-alanine, are given in Tables 5 and 6. All of these structures are minima on the potential energy surface.

The standard procedure to obtain enthalpies of formation in Gaussian-n theories is through atomization reactions.[37,38] The G3(MP2)//B3LYP calculated enthalpies of formation of the studied conformers of N-benzyl-α-alanine and N-benzyl-β-alanine, studied using atomization reactions, are shown in Tables 5 and 6, respectively. Anantharaman and Melius[39] have developed a bond additivity correction (BAC) procedure for the G3-(MP2)//B3LYP method, which is applicable to compounds containing atoms from the first three rows of the periodic table, including H, B, C, N, O, F, Al, Si, P, S, and Cl. The BAC procedure applies atomic, molecular, and pairwise bond corrections to theoretical enthalpies of formation of molecules. The
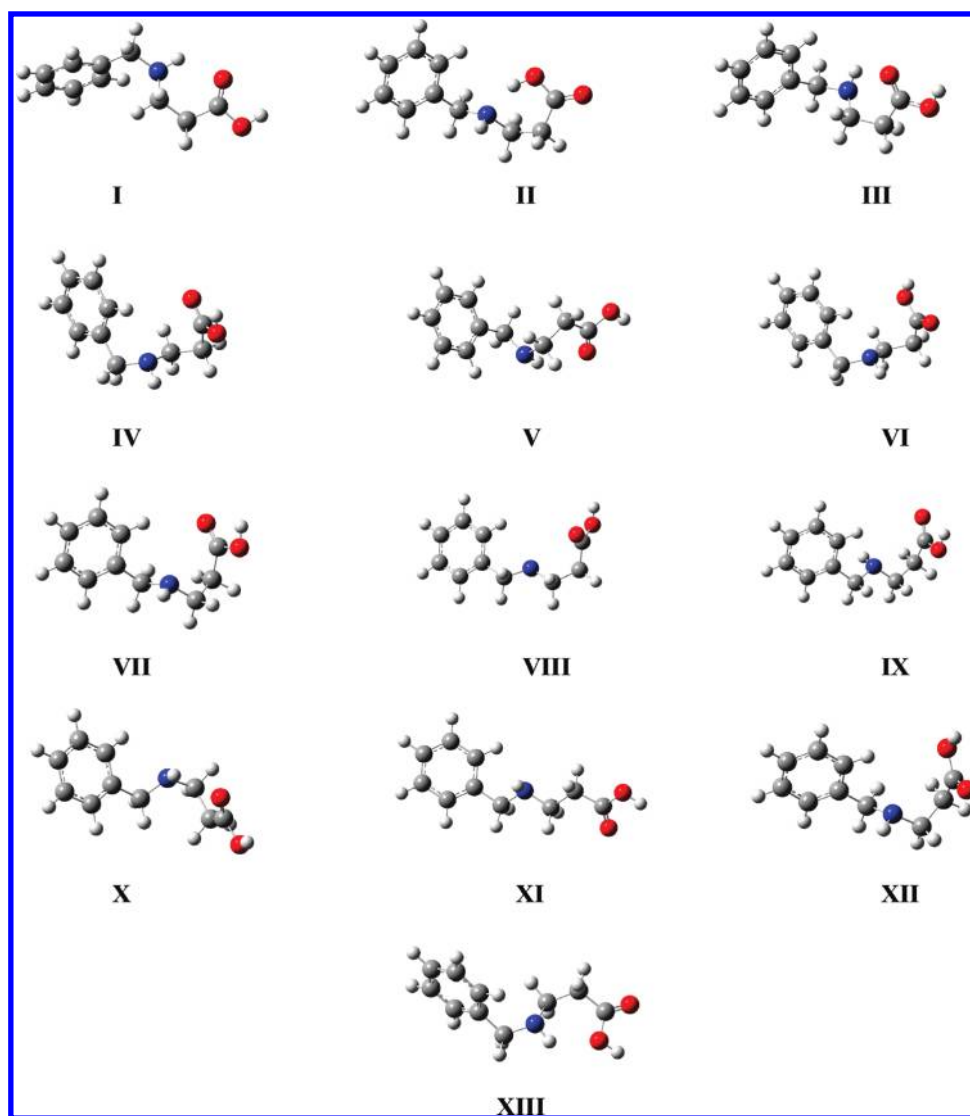
Figure 4. B3LYP/6-31G(d)-optimized structures of the thirteen lowest-energy conformers of *N*-benzyl-β-alanine.
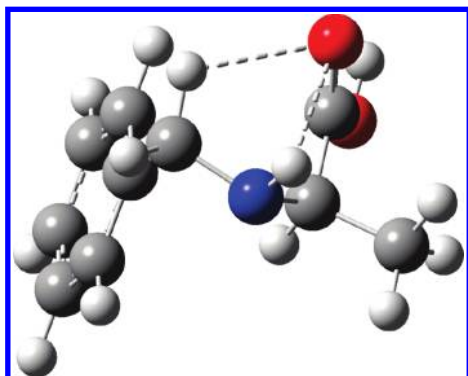

Figure 5. MP2(Full)/6-31G(3df,2p)-optimized structure of the lowest-energy conformer (conformer I) of *N*-benzyl-α-alanine.

Figure 6. MP2(Full)/6-31G(3df,2p)-optimized structure of the lowest-energy conformer (conformer I) of *N*-benzyl-β-alanine.

procedure requires parameters for each atom type but not for each bond type. The authors have applied the method to an extended test suite involving 273 compounds, neutral and ions, and the a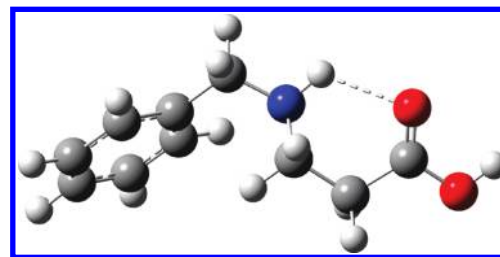verage error was only 4.4 kJ·mol^{-1}. We have carried out the BAC correction following the steps indicated in ref 39, and the values obtained are collected in Tables 5 and 6.

To obtain the conformational composition of the studied compounds in the gas phase, at $T = 298$ K, we need the $\Delta_f G_m^\circ$ values. They can be calculated with eq 3,

$$\Delta_f G_m^\circ(i) = \Delta_f H_m^\circ(i) - T[S^\circ(i) - \sum S^\circ(\text{el})] \qquad (3)$$

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Table 5. G3(MP2)//B3LYP Results for *N*-Benzyl-α-alanine Conformers

conformer	$E_0{}^a$	$H_{298}{}^a$	$\Delta\Delta H^b$	S^c	$\Delta_f H°_{298K}{}^b$	$(\Delta_f H°_{298K})_{corrected}{}^b$	$\Delta_f G°_{298K}{}^b$	χ
I	−593.200174	−593.186418	0.0	476.79	−324.70	−312.93	−95.02	0.509
II	−593.199065	−593.185226	3.1	477.39	−321.57	−309.80	−92.07	0.155
III	−593.198748	−593.185147	3.3	475.30	−321.37	−309.60	−91.24	0.111
IV	−593.198440	−593.184640	4.7	479.98	−320.03	−308.26	−91.30	0.114
V	−593.197755	−593.184282	5.6	468.86	−319.09	−307.32	−87.04	0.020
VI	−593.197694	−593.183896	6.6	471.46	−318.08	−306.31	−86.81	0.019
VII	−593.197743	−593.183875	6.7	479.79	−318.03	−306.26	−89.24	0.050
VIII	−593.196532	−593.183024	8.9	470.20	−315.79	−304.02	−84.14	0.006
IX	−593.196165	−593.182403	10.5	483.32	−314.16	−302.39	−86.43	0.016

^{a} In hartrees. ^{b} In kJ·mol^{-1}. ^{c} In J·mol^{-1}K^{-1}.

Table 6. G3(MP2)//B3LYP Results for *N*-Benzyl-β-alanine Conformers

conformer	$E_0{}^a$	$H_{298}{}^a$	$\Delta\Delta H^b$	S^c	$\Delta_f H°_{298K}{}^b$	$(\Delta_f H°_{298K})_{corrected}{}^b$	$\Delta_f G°_{298K}{}^b$	χ
I	−593.197695	−593.184179	0.0	475.84	−318.82	−307.41	−89.21	0.347
II	−593.196790	−593.183526	1.7	470.02	−317.11	−305.70	−85.77	0.087
III	−593.196985	−593.183467	1.9	474.34	−316.95	−305.54	−86.90	0.136
IV	−593.195465	−593.181949	5.9	475.94	−312.97	−301.56	−83.39	0.033
V	−593.195440	−593.181936	5.9	479.28	−312.94	−301.53	−84.36	0.049
VI	−593.195375	−593.181811	6.2	480.08	−312.61	−301.20	−84.27	0.047
VII	−593.194983	−593.181393	7.3	478.98	−311.51	−300.10	−82.84	0.027
VIII	−593.194886	−593.181188	7.9	487.12	−310.97	−299.56	−84.73	0.057
IX	−593.194774	−593.181109	8.1	481.62	−310.76	−299.35	−82.88	0.027
X	−593.194738	−593.181109	8.1	482.65	−310.76	−299.35	−83.19	0.030
XI	−593.194774	−593.180893	8.6	488.50	−310.20	−298.79	−84.37	0.049
XII	−593.194374	−593.180694	9.1	495.57	−309.67	−298.26	−85.95	0.093
XIII	−593.194264	−593.180686	9.2	481.95	−309.65	−298.24	−81.87	0.018

^{a} In hartrees. ^{b} In kJ·mol^{-1}. ^{c} In J·mol^{-1}K^{-1}.

where the sum of the entropy of the elements is calculated as eq 4

$$\sum S°(el) = 10S°(C,s) + \frac{13}{2}S°(H_2,g) + \frac{1}{2}S°(N_2,g) + S°(O_2,g) \tag{4}$$

For the elements, using the entropy values, at 298 K, taken from ref 40, $\Delta_f G_m°$ values have been obtained for all the conformers and collected in Tables 5 and 6.

Using eq 5,

$$x_i = \frac{e^{-\left[\frac{\Delta_f G_m°(i)}{RT}\right]}}{\sum\limits_{i=1}^{N} e^{-\left[\frac{\Delta_f G_m°(i)}{RT}\right]}} \tag{5}$$

we have obtained compositions in the gas phase at T = 298 K for *N*-benzyl-α-alanine and *N*-benzyl-β-alanine, as they are shown in Tables 5 and 6. As it can be observed, the most stable conformer (conformer I) of *N*-benzyl-α-alanine accounts for 50.9% of the composition in the gas phase. For *N*-benzyl-β-alanine, conformer

I accounts for 34.7% of the gas phase composition, calculated at the G3(MP2)//B3LYP level.

And, using eq 6,

$$\Delta_f H_m°(X) = \sum\limits_{i=1}^{N} x_i \Delta_f H_m°(i) \tag{6}$$

the final value for the enthalpy of formation of *N*-benzyl-α-alanine is calculated as −310.7 kJ·mol^{-1}, and the value of −303.7 kJ·mol^{-1} is obtained for *N*-benzyl-β-alanine. This value is in very good agreement with the experimental value measured in this work, −(298.8 ± 4.8) kJ·mol^{-1}. $\Delta_f G_m°$ values of −92.9 and −86.5 kJ·mol^{-1} for *N*-benzyl-α-alanine and *N*-benzyl-β-alanine, respectively, have been obtained.

In Figure 7, we compare the isomerization enthalpies of benzylalanines and alanines. Although the combustion experiments of *N*-benzyl-α-alanine were unsuccessful, and although it is not possible to obtain an experimental enthalpy of isomerization for *N*-benzyl-α-alanine to *N*-benzyl-β-alanine, the calculated value, 7.0 kJ·mol^{-1}, is comparable to the values of the enthalpy of isomerization for α-alanine to β-alanine obtained in our previous work,[1] 4.8 ± 3.5 kJ·mol^{-1} (experimental) and 5.7 kJ·mol^{-1} (calculated), and is in line with the concept that the more branched amino acid (α-alanine) is intrinsically more stable than the linear amino acid, β-alanine.[1] That is the reason for which nature selects α- instead of β-amino acids for the
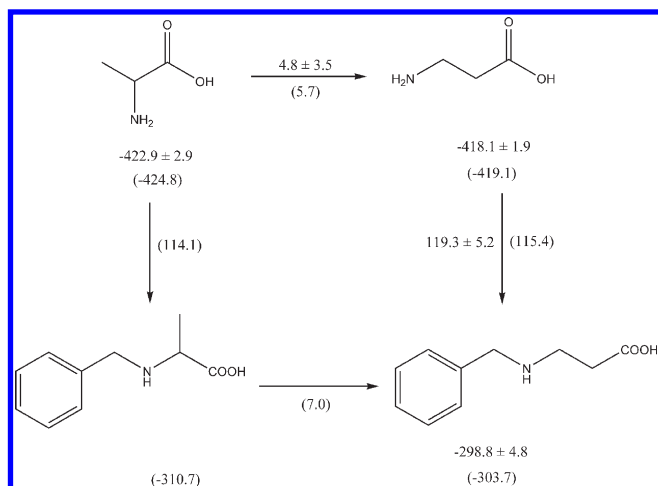
Figure 7. Experimental and calculated (in parentheses) enthalpies of formation of alanines and benzylalanines. All values in kJ·mol^{-1}.

formation of the biomolecules that are essential constituents of life on earth.

■ ASSOCIATED CONTENT

S **Supporting Information.** Optimized geometrical parameters and calculated NBO charges for the most stable conformers of N-benzyl-α-alanine and N-benzyl-β-alanine (Tables S1 and S2). Cartesian coordinates for all the lowest-energy conformers of N-benzyl-α-alanine and N-benzyl-β-alanine. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*E-mail: rnotario@iqfr.csic.es (R.N.); risilva@fc.up.pt (M.A.V.R.S.); juaristi@relaq.mx (E.J.).

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