Spectral characterization of a non-centrosymmetric organic compound: D-(–)-alanine

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HIGHLIGHTS

- The crystal growth of D-(–)-alanine (1), a non-centrosymmetric solid is reported.
- The structure was optimized computationally at the B3LYP/6-311G** level and the vibrational frequencies were calculated.
- The CD spectral measurements unambiguously confirmed that the compound (1) is D-(–)-alanine.
- Second harmonic generation (SHG) conversion efficiency is about 23% of that of the standard KDP crystals.
- The compound may have possible applications in non-linear optics.

ABSTRACT

The crystal growth of D-(–)-alanine (1), a non-centrosymmetric solid is reported. It was characterized by NMR, infrared, Raman, UV–Vis–NIR and CD spectra. Experimental vibrational frequencies are compared with theoretically calculated values. Second harmonic generation (SHG) and first hyperpolarizability measurements are reported.

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Article info

Article history:
Received 14 December 2012
Received in revised form 7 March 2013
Accepted 4 April 2013
Available online 17 April 2013

Keywords:
Crystal growth
IR
Raman
CD
UV–Vis–NIR
Introduction

α-Alanine (also known as 2-aminoopropanoic acid) is the smallest naturally occurring chiral α-amino acid and exists in two forms namely α-, L-alanine and D-alanine. Both these forms are chiral and can be distinguished by their optical rotation. A 1:1 mixture of the D- and L- forms is known as DL-alanine which is optically inactive. The compound β-alanine (3-aminoopropanoic acid) is a positional isomer of α-alanine and is an achiral molecule. Among these, D-alanine appears to have been less explored compared to L- or DL- forms. D-Alanine occurs in bacterial cell walls and in some peptide antibiotics. Recently, L-alanine [1] and DL-alanine [2] crystals have been grown by slow evaporation method and their NLO properties were reported. But, the crystal growth in reasonable sizes and the nonlinear optical properties of D-alanine (1) are yet to be reported. Low frequency vibrations which contain information on weak interactions are of importance in enzyme reactions. The vibrational spectra of the single crystal of L-alanine has been reported by Adamowicz and Fishman [3]. The temperature dependence of the far infrared spectra of L-alanine and its different possible conformations were reported by Bandekar et al. and Csaszar, respectively [4,5]. Stepanian et al. [6] have studied the IR spectra using matrix isolation technique. Few other reports also exist in the literature [7,8]. Kumar et al. [9] had reported the vibrational studies of (1) theoretically and experimentally with a resolution of 5 cm⁻¹. But, high resolution spectra are needed for accurate assignments and are important in the better understanding of the compound’s biological activity. In the present work, a single crystal of (1) of dimensions: 8 × 5 × 3 mm³ was grown and characterized by NMR and Circular dichroism (CD) spectra. The single crystal XRD was also recorded and the cell sizes checked with the literature values. The vibrational spectra (IR and Raman) were recorded with a resolution of 2 cm⁻¹ and the vibrational frequencies were theoretically calculated by optimizing the structure at the B3LYP/6-311G** level of theory using Gaussian 03 in an IBM-MERCURY [11]. It is rhombic with fourteen developed faces. Of these, the largest faces are (020), (010), (101), (011), (0–11), (110), (1–10), (–1–10) and (–110) and the smallest faces are (–10–1), (–101), (10–1) and (101).

Crystal growth

D-alanine is a white crystalline powder, the molecular formula being C₃H₅NO₂ and has a molecular weight of 89.09. Commercially available D-alanine (M/s. Loba Chemie Pvt. Ltd., purity: 99%) was further purified by recrystallising it once. Optically transparent crystals having well-defined morphology were selected as seeds and the remaining crystals were powdered and used as raw material for bulk crystal growth. The solvent (distilled water) was taken for the supersaturation, the solution temperature was raised by 2°C in a beaker and the purified material added gradually to obtain a supersaturated solution. The supersaturated solution was filtered and the solute was added till it dissolved completely. It was stirred for 30 min to obtain a homogeneous solution. The supersaturated solution was filtered and the selected seed crystal was placed carefully at the bottom of the beaker and kept covered for controlled evaporation to take place. A small amount (few mg) of sodium azide was added to the growth solution to prevent the attacks by fungi and microorganisms [10]. After about 15 days, a colorless transparent single crystal of (1) of good quality with approximate size of 8 × 5 × 3 mm³ was obtained. The photograph and the morphology of the grown crystal are shown in Fig. 1. The morphology of the crystal was obtained using the single crystal X-ray data, making use of the program MERCURY [11]. It is rhombic with fourteen developed faces. Of these, the largest faces are (020), (0–20), (01–1), (0–1–1), (011), (0–11), (110), (1–10), (–1–10) and (–110) and the smallest faces are (–10–1), (–101), (10–1) and (101).

Characterization

The unit cell parameters of the single crystal were determined using a Nonius CAD-4/MACH3 diffractometer using Mo Kα radiation of wavelength 0.71073 Å. The cell parameters were obtained from the least-squares refinement of the setting angles of 25 reflections. ¹H NMR spectra of (1) was recorded using D₂O as the solvent (Bruker AMX 400 spectrometer with tetramethylsilane as the internal standard). The infrared spectra of (1) was recorded in the frequency region of 400–4000 cm⁻¹ using a Bruker Alpha-p FTIR spectrometer, at a resolution of 2 cm⁻¹. Raman spectral measurements were made using a Bruker instrument (model RFS 100/ S). Nd:YAG laser beam of wavelength 1064 nm and power output 25 mW was used to record the spectrum over the range 400–4000 cm⁻¹, the resolution being 2 cm⁻¹. CD spectra were recorded in the wavelength range of 150–400 nm at room temperature, using a JASCO J-180 spectropolarimeter. The aqueous solution (3 mM) of the sample was kept in a quartz cuvette of path length 0.1 cm. The transmittance of (1) for a crystal of thickness:2 mm...
was measured using a Perkin–Elmer Lambda-35 spectrophotometer in the wavelength range of 200–1100 nm (with a slit of width 2 nm) with the scan speed of 240 nm/min. The second harmonic generation behavior of the powdered materials was tested using the Kurtz and Perry method [12]. A high-intensity Nd:YAG laser beam (\(\lambda = 1064\) nm) with a pulse duration of 10 ns was passed through the powdered sample for the above measurement.

Results and discussion

Single crystal X-ray diffraction

The cell parameters of (1) determined from the single crystal X-ray diffraction experiments (Table S1) are in agreement with those reported by Sullivan et al. [13]. It is known that the compound (1) crystallizes in the orthorhombic system with the space group \(P2_12_12_1\) [13]. The density of the single crystals of (1) was determined as 1.37(2) g/cm\(^3\) using the floatation method. The melting point was found out as 290(2)°C. The crystallographic, density data and a brief discussion on the reported structural aspects of \(\alpha\)-alanine [13], \(l\)-alanine [14] and \(dL\)-alanine [15] are given in Supplementary Material and Table S1 for the purpose of comparison.

Spectroscopic studies

The \(^1\)H NMR spectrum (Fig. S1) of (1) in D\(_2\)O exhibits characteristic signals assignable for the –CH and –CH\(_3\) protons (\(\delta\) 3.65 (q, 1H, CH), 1.35 (d, 3H CH\(_3\))) and reveals the spectral purity of the sample. Experimental geometries of \(\alpha\)-alanine reported in the work of Sullivan et al. [13] have been used as the starting point for the DFT optimization of the isolated molecules with the Gaussian 03 program [16], adopting the B3LYP/6-311G** hybrid-type functional theory. Harmonic frequencies and intensities were calculated with the optimized structure (Fig. 2). The mode of vibration of each bond was viewed by using the software Chemcraft(1.6) [17] and the assignments were carried out accurately. The recorded infrared spectra and the Raman are shown in Figs. 3 and 4, respectively. The experimental and calculated frequencies and the assignments are given in Table 1. The peaks occurring at 2591, 2113 and 1232 cm\(^{-1}\) in the IR spectra are due to the overtones. The IR spectra of \(\alpha\)-alanine and \(dL\)-alanine (Fig. S2) are nearly identical which can

![Fig. 2. Optimized structure of (1) at the B3LYP/6-311G** level of theory using Gaussian 03.](image)

![Fig. 3. Infrared spectrum of (1).](image)

<table>
<thead>
<tr>
<th>Infrared (cm(^{-1}))</th>
<th>Raman (cm(^{-1}))</th>
<th>Calculated value (cm(^{-1}))</th>
<th>Assignments</th>
</tr>
</thead>
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<tr>
<td>3070(^b)</td>
<td>3078(^m)</td>
<td>3089</td>
<td>H5–C3–H6 asymmetric stretch</td>
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<td>2591(^m)</td>
<td>2607(^m)</td>
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<tr>
<td>2113(^m)</td>
<td>2114(^vw)</td>
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<td>–</td>
<td>1602(^s)</td>
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<td>1588(^s)</td>
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<tr>
<td>538(^s)</td>
<td>535(^m)</td>
<td>506</td>
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</table>

\(m,\) Medium; \(s,\) strong; \(v,\) very; \(w,\) weak; \(b,\) broad.
be attributed to the similarity of their crystal structures (Supplementary material).

The CD spectrum of (1) unambiguously confirms its chiral purity and thus enables its characterization as D-(-)-alanine (Fig. 5). From the negative Cotton effect observed, it can be concluded that the compound (1) is D-(-)-alanine. In contrast, a sample of the L-isomer namely L-(+)-alanine (obtained from M/s. Loba Chemie Pvt. Ltd., purity: 99%) showed a positive Cotton effect.

The UV–Vis–NIR transmission spectrum of (1) (Fig. S3) shows that the compound (1) has no absorption in the region between 230 nm and 1100 nm. Such a transparent behavior is a desirable characteristic of non-centrosymmetric materials for possible applications.

Second harmonic generation (SHG) and Hyper Rayleigh Scattering (HRS) studies

The second harmonic signal generated in the powder sample was confirmed from the emission of green radiation (\( \lambda = 532 \) nm) from the sample. The intensity of the scattered green light was measured using a photomultiplier tube. The SHG efficiency was found to be about 0.23 times that of KDP. The efficiency of the frequency conversion will vary with the particle size and the orientation of the crystallites in the capillary tube [18]. Hence, higher efficiencies may be expected with single crystals, by optimizing the phase matching [19].

First hyperpolarizability (\( \beta \)) of the molecules was measured by second harmonic light scattering (SHLS) technique. A beam from a Q-switched Nd:YAG laser of wavelength 1064 nm was focused on the sample by a convex lens (focal length 20 cm). Vertical (90°) geometry was used to collect the scattered second harmonic photons \( I_{2\omega} \) using a photomultiplier tube after filtering out the fundamental wave. The first hyperpolarizability, \( \beta \), of a molecule dissolved in a solvent is related to the second harmonic scattered light intensity \( I_{2\omega} \) by the relation:

\[
I_{2\omega} = G(N_{\text{solvent}}\beta_{\text{solvent}}^2 + N_{\text{solute}}\beta_{\text{solute}}^2)I_x^2
\]

where \( N_{\text{solvent}} \) and \( N_{\text{solute}} \) are the number densities of the solvent and solute molecules respectively, \( G \), an instrument factor and \( I_x \) the intensity of the incident beam. The intensity of the second harmonic light was plotted against the incident laser light intensity to confirm the occurrence of second order process (Fig. 6). The slope of the plot (\( \log I_{2\omega} \) vs. \( \log I_x \)) being close to 2 confirms the occurrence of second order process, since \( I_{2\omega} \propto I_x^2 \). The first hyperpolarizability of the sample was determined by external solvent method [20]. For this, \( I_{2\omega} \) of the sample and the reference (p-nitroaniline, PNA) were
collected for different concentrations by dilution method. Water was used as the solvent for both the reference and the sample. $I_{2\text{opt}}/I_{\text{ref}}$ values were plotted against concentration (Fig. 7). From the slope of this plot, the beta value was calculated as $43.1 \times 10^{-30}$ esu, by making use of the following formula:

$$\beta_{\text{sample}} = \sqrt{\frac{\text{slop of sample}}{\text{slop of reference}}} \times \beta_{\text{reference}}$$

Conclusions

$\alpha$-$(–)$-alanine (1) was grown as a single crystal at room temperature ($30 \degree C$) in bulk size by submerged seed solution method. The structure was optimized computationally at the B3LYP/6–311$G^* \ast$ level and the vibrational frequencies were calculated. The experimentally obtained infrared and Raman frequencies are in agreement with the theoretical frequencies. The accurate assignments of the modes of vibration of the functional groups were made with the help of Chemcraft (1.6). The CD spectral measurements unambiguously confirmed that the compound (1) is $\alpha$-$(–)$-alanine. Optical studies showed that the crystal is transparent in the UV–Vis–NIR region. The SHG efficiency of the sample was investigated using Kurtz and Perry method and the first hyperpolarizability was determined by hyper Rayleigh scattering.

Acknowledgments

The authors thank Prof. B.R. Srinivasan, Department of Chemistry, Goa University, India for useful discussions. The authors also thank Prof. P.K. Das, Department of Inorganic and Physical Chemistry, Indian Institute of Science, Bangalore, India for providing some of the experimental facilities. SN thanks the Council of Scientific and Industrial Research, New Delhi for the financial support under the Emeritus Scientist Scheme.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2013.04.025.

References