Published in final edited form as:

J Mol Struct. 2015 August 15; 1094: 203–209. doi:10.1016/j.molstruc.2015.03.056.

Vibrational analysis of α -cyano-4-hydroxycinnamic acid

Elmer-Rico E. Mojica^a, Jayson Vedad^b, and Ruel Z.B. Desamero^{b,*}

^aDepartment of Chemistry and Physical Sciences, Pace University, One Pace Plaza, New York, NY 10029, United States

^bDepartment of Chemistry, York College, Institute for Macromolecular Assemblies and the Graduate Center of the City University of New York, Jamaica, NY 11451, United States

Abstract

In the present study, a comparative Raman vibrational analysis of alpha-cyano-4-hydroxycinnamic acid (4CHCA) and its derivative, alpha-cyano-3-hydroxycinnamic acid (3CHCA), was performed. The Raman spectra of the 4CHCA and 3CHCA in solid form were obtained and analyzed to determine differences between the two structurally similar derivatives. For comparison, the CHCA derivatives cyanocinnamic acid (CCA) and coumaric acid (CA) were also studied. The plausible vibrational assignments were made and matched with those obtained theoretically using density functional theory (DFT) based method employing a 6–31 g basis set. The computational wavenumbers obtained were in good agreement with the observed experimental results. This was the first reported Raman study of CCA, 3CHCA and 4CHCA.

Keywords

Raman; Cyanocinnamic acid; Alpha-cyano-4-hydroxycinnamic acid; Alpha-cyano-3-hydroxycinnamic acid; Density functional theory

Introduction

Alpha-cyano-4-hydroxycinnamic acid (4CHCA) is a member of the phenylpropanoid group, a diverse family of organic compounds synthesized from phenylalanine. This compound, which belongs to a group of hydroxycinnamic acid derivatives like sinapic/sinapinic, ferulic and caffeic acids, has been extensively used for protein and peptide analysis as a matrix for ultraviolet matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) [1,2]. Other commercially available cinnamic acids have been utilized in the analysis of proteins [1], oligodeoxyribonucleotides [3], glycopeptides [4], carbohydrates [4], and phosphopeptides [4]. Efforts to develop compounds with similar structures as sinapic acid and 4CHCA have been successful. The synthesis and applications of the α -cyanocinnamic acid derivatives such as 4-chloro- α -cyanocinnamic acid (Cl-CCA) [5], (*E*)-2-cyano-3-(naphthalen-2-yl)acrylic acid (NpCCA) [6] and (*2E*)-3-(anthracen-9-yl)-2-cyanoprop-2enoic acid

*Corresponding author. Tel.: +1 718 262 2657; fax: +1 718 262 2652. rdesamero@york.cuny.edu (R.Z.B. Desamero).

Author contributions: The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

(AnCCA) [6] have now been reported. Research studies indicate that 4CHCA is still important for peptide and protein analysis [7–9], and employing hybridization of matrix [10] or graphite supported preparation [11] makes it a more suitable matrix.

Besides its use as a matrix in MALDI-MS, 4CHCA is of biological importance. It is a classical inhibitor of pyruvate transport activity in both animal [12,13] and plant mitochondria [14] as well as of monocarboxylate transporter (MCT) activity [15]. It was also reported to partially inhibit (60% inhibition) the oxidation of branch chain α -keto acid [16]. Another study showed that 4CHCA can inhibit both the monophenolase activity and diphenolase activity of mushroom tyrosinase [17]. Lastly, 4CHCA was found to have a dose-dependent inhibitory effect on lung branching and viability [18].

Despite the reported biological activities of CHCA and the prevalent use of the compound CHCA as a MALDI matrix, there has been limited literature with regards to the vibrational analysis of the CHCA and its derivatives; most studies have been on *p*-coumaric acid, trans-3-hydroxycinnamic acid and ferulic acid [19–23]. The only vibrational study done on the 4CHCA was the one reported by Dembereldorj and Joo [24], wherein they investigated the vibration modes of solid 4CHCA using attenuated total reflectance (ATR) infrared spectroscopy as well as using temperature-dependent diffuse reflectance (DRIFT) where the compound was anchored on titanium oxide (TiO₂) surfaces. Here we present the first reported vibrational study 4CHCA and its derivative (α -cyano-3-hydroxycinnamic acid, cyanocinnamic acid and *p*-coumaric acid, Fig. 1). In addition, density functional theory (DFT) based computer simulations were carried out to correlate and assign observed Raman modes.

Experimental

Reagents

Samples of cyanohydroxycinnamic acids, 4CHCA (Sigma–Aldrich Co., St. Louis, MO), 3CHCA (Acros Organics, NJ), CCA (Sigma–Aldrich Co., St. Louis, MO) and CA (Sigma–Aldrich Co., St. Louis, MO) were used without further purification.

Raman measurements

All Raman spectra were obtained on a Jasco NRS-3100 confocal dispersive Raman spectrometer (Easton, MD). Raman scattering was induced by a 12 mW 488 nm laser and collected on a thermoelectrically cooled CCD detector. Reproducibility in the band position measurements was better than $\pm 1 \text{ cm}^{-1}$ based on sample to sample variations. The powdered samples of 4CHCA, 3CHCA, CCA and CA were prepared onto separate quartz slide for Raman measurements. All Raman spectra were obtained at room temperature.

Computational details

To help analyze the Raman spectra, computational studies were performed to aid in the peak assignments and model plausible conformational structures. After geometry optimization, vibrational frequencies were then determined. All calculations were executed on Gaussian 09 W software and carried out using DFT approximation implementing the Becke's three-

parameter exchange function in combination with the Lee, Yang, and Parr correlation function (or B3LYP) [25–27]. As a compromise between accuracy and applicability to large molecules, the 6–31 G basis set was used [27,28]. Smaller basis sets are enough in DFT based calculations because the basic functions do not have to describe correlating orbitals. GaussView05 [29] was used to prepare the input file and analyze the results of the calculations [27,30].

Results and discussion

Molecular geometry

The optimized molecular structures of both CHCA derivatives which have C1 point group symmetry are shown in Fig. 1, together with CCA and CA. The global minimum energies obtained by the DFT structure optimization by B3LYP/631G basis set level calculation for the 3CHCA is –417593.78 kcal/mol, while 4CHCA yields –417594.63 kcal/mol. Thus the optimized structure of 4CHCA is more stable than that of 3CHCA by ~0.85 kcal/mol. Greater stability of 4CHCA results from predominant resonance structures due to the hydroxyl group in the para position [31]. The hydroxyl group is an electron donating group (EDG) that stabilizes the acrylic group with the α -cyano moiety. Global minimum energies for CCA and CA are –370401.417 and –359726.23 kcal/mol, respectively.

One can observe the differences in the C–C bond lengths in the aromatic ring of the CHCA derivatives studied (Table 1). For 4CHCA, as well as CCA and CA, the C—C bond distances within the ring have the following trend, [C3—C4 and C4—C5] > [C1—C2 and C1 — C6] > [C2—C3 and C5—C6], each differing by around 0.1 Å. The longer bonds (C3 —C4, C4—C5, C1 —C2, and C1 — C6) are positioned closer to the substituents attached to the ring; the bigger the substituent is, the longer the ring C—C bonds. Ring C—C bonds also tend to be longer when EDG groups are attached due to a decrease in the p-character of the σ -bonded hybrid orbital, which consequently increases the p-character of the remaining hybrid orbitals pointing away from the substituent. Moreover, resonance effects on the ring caused by the —OH substituent cause complex angular distortions within the ring. This arises from the combination of effects from the decreased C—O bond length from resonance effects and the increase in the endocyclic C6—C1 —C2 angle due to the electron donating nature of the —OH substituent. These effects normally result in the lengthening of the C1—C2/C1—C6 and C3—C4/C4—C5 bond distances, as observed in 4CHCA [32]. Small C3—C4—C5 bond angles for CCA, CA, 3CHCA and 4CHCA result from the conjugation of the acrylic substituent with the ring which consequently yields C4—C7 bonds that are shorter than the non-conjugated C—C bonds (Table 1). The shortening of the bond increases the s-character of the bonding orbital of the σ -bond connecting C4 with C7. Consequently, this increases the p-character of the other remaining bonding orbitals, which can then account for the longer C3—C4 and C4—C5 bond lengths in CA, CCA, 3CHCA, and 4CHCA [33]. Also, conjugation of the exocyclic C=C bond with the ring π -electrons causes an increase in both π -electron density above and below the molecular plane of the C4—C7 bond as well as increase of electron density in the molecular orbitals. These increases in electron density consequently exert repulsion against the electrons on the C3—C4 and C4—C5 bonds, thus restricting the C3—C4—C5 bond angle [31]. For 3CHCA, the —OH substituent at the *meta*

position dictates a more complex pattern of bond lengths. It is expected, though, that a combination of the effects of both the acrylic and —OH substituents will be observed. Consistent with this, longer C1—C6 bond lengths were observed as the —OH substituent is bonded with C6. The C5—C6 bond appears shorter as a result of the ring's effort to conserve symmetry and planarity. The longer C4—C7 bond distance can be due to the electron withdrawing nature of carboxylic and cyano groups, wherein the group exerts larger attraction on valence electron cloud of the ring. Leading to a expanded delocalization of electrons toward the substituent, and in turn a decrease in force constant and increase in bond length [34]. Based on computer modelling, electrons are delocalized from the ring into the bigger substituent in the CHCA derivatives.

A close inspection of the other bond angles (Table 1) reveals, that for the derivatives studied, the C8—C9—O10 bond angles range from 113.1° to 118.149°, while the C9—C8—C12 bond angles were ~114°. The higher bond order of C8—C7 and C9—O10 leads to a higher electron density about these bonds which results to a decrease of bond angles opposite these bonds. Consequently, the C8—C9—O11 bond angles is the larger than 120°. For CCA, 3CHCA and 4CHCA, the C9—C8—C12 bond angle is much lower at only around 114.5° compared to that of CA. Steric effects in connection with the phenyl ring on E configuration to the —CN group may have resulted in the smaller C9—C8—C12 bond angle.

Vibrational analysis

The experimental Raman spectra of both derivatives in various spectral regions are shown in Figs. 2 and 3. The Raman spectral analysis of the CHCA derivatives was based on the characteristic vibrations of cyano, hydroxyl, carboxyl and phenyl ring modes. The assignments of the vibrational modes for all the samples used (Table 2) are based on DFT calculations and the literature data on coumaric acid derivatives [19,20,22,23,35]. All the peaks obtained for CA match those reported in previous studies [19,35].

Spectral region: 2900–3700 cm⁻¹

The *OH stretch mode* at *ca.* 3300–3600 cm⁻¹ is a vibrational mode important in studying the CHCA derivatives. Based on chemical structure (Fig. 1), CCA differs from the rest of the derivatives in that it contains a lone hydroxyl group on the carboxylic moiety while the others have an additional hydroxyl group attached to the aromatic ring. An —OH group usually gives rise to 3 vibrational modes (stretching, in-plane bending and out of plane bending). Bands caused by O—H stretching are of medium to strong intensity in the infrared spectrum and generally yield weak Raman modes. The O—H stretch modes are sensitive to hydrogen bonding. IR studies showed that intermolecular hydrogen bond broadens the O—H stretching and lowers it to 3200–3550 cm⁻¹ [36,37]. For solids, liquids and concentrated solutions, a weak broad Raman peak attributed to —OH stretching is normally observed [38]. With the exception of the sharp band at 3306 cm⁻¹ observed from a sample of 4CHCA, all observed O-H stretch bands are very weak and broad (Fig. 2, Panel A). DFT based computer simulation studies reveal that for an isolated monomer in the gas phase these derivatives (CA, 3CHCA and 4CHCA) exhibit two distinct OH stretch modes at 3528 and 3501 cm⁻¹, that can be attributed to stretching motion of the —OH attached to the ring and

the carboxyl group, respectively. Noticeably, this could not account for the number of Raman modes observed for 3CHCA and 4CHCA (Fig. 2, Panel A). We posit that perhaps many of the observed modes, including the sharp intense mode observed only for 4CHCA, could be due to intermolecular or intramolecular interactions that exist in a solid sample of these compounds. A simple approximation would be to execute theoretical calculations on a complex of these compounds, such as a dimer. Simulation results reveal that a dimer could yield a sharp vibrational mode *ca.* 3250 cm⁻¹ due to the interaction of a —OH group of one 4CHCA to a —COOH group of another 4CHCA. This could perhaps present clues as to why only 4CHCA forms an effective matrix for MALDI. The nature of the —OH group is indeed sensitive to H-bonding. It has been reported that the OH mode from a *n*-OOH-bonded donor appears at 3250 cm⁻¹ while the OH mode from a *n*-OH-bonded donor is at 3550 cm⁻¹ [39,40]. For CA, the peaks observed experimentally at 3206 cm⁻¹ can be assigned to an —OH stretch mode that results from an H-bonded COOH group. This was also evidenced by the DFT calculation simulating dimeric interactions that places the corresponding stretch mode at 3147 cm⁻¹. In a study using cinnamic acid derivatives, the —OH modes from the carboxylic and the hydroxyl ring were not detected experimentally for 3,4,5-trihydroxycinnamic acid. Those observed for caffeic acid (3321, 3341 and 3368 cm⁻¹) and ethyl 3,4,5-trihydroxycinnamate (3321, 3341 and 3370 cm⁻¹) displayed a significant shift relative to the calculated values [20]. A weak peak at 3339 cm⁻¹ and 3378 cm⁻¹ for *trans-p*-coumaric acid and syringic acid, respectively, was attributed to —OH stretching [19].

Another vibrational mode that appears in the spectral region 2900–3700 cm⁻¹ is the *CH stretch mode*. Aromatic compounds show the presence of the C—H stretching vibrations around 3000–3150 cm⁻¹ (Fig. 2, Panel B). It is usually exhibited as a multiplicity of weak to moderate bands relative to the aliphatic C—H stretch [41]. In this region, the CH stretch modes are not affected appreciably by the nature of the substituent or by molecular interactions. Observed Raman peaks (Fig. 2, Panel B) match those predicted using DFT based calculations for an isolated molecule in the gas phase. The CH stretch modes observed at 3021, 3042, 3080, 3126, and 3156 cm⁻¹ for 4CHCA are very similar to the predicted values: 3073, 3094, 3121 and 3146 cm⁻¹. Similarly, the observed Raman peaks, 3054, 3075 and 3131 cm⁻¹, for 3CHCA and those predicted, 3087, 3097, 3104 and 3127 cm⁻¹, are within the recorded spectral range. CCA samples exhibited peaks at 3063, 3092, 3145 and 3186 cm⁻¹ are again close to calculated values, 3065, 3078, 3090 and 3134 cm⁻¹. Lastly, the observed Raman peaks, 3024, 3052, 3070 and 3196 cm⁻¹, for CA samples matched predicted values, 3023, 3071, 3081, and 3123 cm⁻¹, as well as values reported in an earlier study [19]. Unlike the OH stretch modes, CH stretch bands do not vary as much between samples and are easily predicted using a gas phase single molecule approximation (Table 2).

Spectral region: 2200–2300 cm⁻¹

Nitrogen compounds containing triple bond like cyano or nitrile provide a unique spectrum with a single, intense peak in the region 2280–2200 cm⁻¹, which is the characteristic wavenumber of the C≡N *stretch vibrational mode*. The bands are not affected appreciably by the nature of constituents in this region. Literature has reported the C≡N stretching band in several compounds as 2229 cm⁻¹ for 2-hydroxy-3cyano-4-methylpyridine [42], 2231 cm⁻¹ for 5-amino-4-cyano-3-(methylthio)-1H-pyrazole-1-carbothioamide [43] and 2221

cm^{-1} for 3-cyano-4-methyl-coumarin [44]. Our results indicate a sharp peak at 2244 cm^{-1} for the 3CHCA sample and 2239 cm^{-1} for the 4CHCA sample (Fig. 2, Panel C), which is in close agreement with theoretically calculated value of 2210 cm^{-1} and 2209 cm^{-1} , respectively.

To confirm the assignment of the $\text{C}\equiv\text{N}$ mode, the Raman spectra of CCA, CA, 3CHCA and 4CHCA (Fig. 2, Panel C) were compared. The absence of a Raman mode in the spectra CA ascertains that the peak at $\text{ca. } 2200 \text{ cm}^{-1}$ can only be due to the $\text{C}\equiv\text{N}$ group, which is absent in CA. The $\text{C}\equiv\text{N}$ mode for CCA is found at 2227 cm^{-1} , which is slightly lower than those observed for 4CHCA (2239 cm^{-1}) and 3CHCA (2244 cm^{-1}). The main structural difference between CCA and the two CHCA derivatives is the presence of an extra OH group on the ring. The presence of the hydroxyl group changes the environment around the $\text{C}\equiv\text{N}$ bond, making it more polar. Polar environments have been observed to upshift $\text{C}\equiv\text{N}$ modes. For instance, the cyano mode of 4-cyanophenylalanine in water, which appears at 2237.9 cm^{-1} , shifts to 2229.5 cm^{-1} when placed in the less polar tetrahydrofuran [45].

Spectral region: 1600–1800 cm^{-1}

Predictably the CHCA derivatives should exhibit $\text{C}=\text{O}$ *stretch* Raman band indicative of its carboxyl moiety. The $\text{C}=\text{O}$ stretch mode is the strongest band in the infrared spectrum but has diminished intensity in the Raman spectrum. However, no $\text{C}=\text{O}$ stretch mode was observed from the Raman spectra of the CHCA derivatives studied. This observation, while unusual, has been previously reported for a sample of *p*-cyanobenzoic acid [34] and 4-butyl benzoic acid [46]. There was no peak at $\text{ca. } 1700 \text{ cm}^{-1}$ where the $\text{C}=\text{O}$ stretch is expected to be. Theoretical calculations indicated a peak at 1660 cm^{-1} for 3CHCA and 4CHCA as well as 1686 cm^{-1} and 1689 cm^{-1} for CCA and CA, respectively. Moreover, the calculated Raman intensity of the $\text{C}=\text{O}$ stretch mode was determined to be $\text{CA} > 4\text{CHCA} > 3\text{CHCA} = \text{CCA}$. The results obtained for CA matches those that was previously reported [35].

Spectral region: 1000–1600 cm^{-1}

Fig. 3 displays several vibrational modes observed from the Raman spectra of the different CHCA derivatives in the spectral region $1000\text{--}1600 \text{ cm}^{-1}$. Among which are the aromatic *ring stretch modes*, *in-plane* —OH *bending modes* and *aliphatic/aromatic* —CH *bending modes*. The most distinct of these modes, observed at $\text{ca. } 1600 \text{ cm}^{-1}$, can be assigned to the ring stretching vibrational modes which are very prominent in the spectrum of benzene and its derivatives, as the double bond is in conjugation with the ring [47]. Benzene has two doubly degenerate vibrations $\text{e}_{2\text{g}}$ (1596 cm^{-1}) and $\text{e}_{1\text{u}}$ (1485 cm^{-1}) [47]. The ring C—C stretch modes are expected in the range $1200\text{--}1650 \text{ cm}^{-1}$ depending on the degree of ring substitution. For instance, there are five normal modes, **8a**, **8b**, **19a**, **19b** and **14**, allowed for the asymmetrically disubstituted phenyl ring corresponding to the tangential C—C stretch [48]. The ring mode, **8a**, manifests a strong Raman signal consistent with the measured Raman spectra of the CHCA derivatives (Fig. 3). DFT based calculations reveal peaks (4CHCA = 1585 cm^{-1} ; 3CHCA = 1590 cm^{-1} ; CCA = 1597 cm^{-1} ; CA = 1607 cm^{-1}) that closely matches experimental data (4CHCA = 1595 cm^{-1} ; 3CHCA = 1599 cm^{-1} ; CCA = 1592 cm^{-1} ; CA = 1606 cm^{-1}). The Raman spectra of 4CHCA and CCA reveal the companion mode, **8b**, at 1566 and 1573 cm^{-1} , respectively. Calculation results reinforced

peak assignments with the **8b** placed at 1561 cm^{-1} and 1583 cm^{-1} for 4CHCA and CCA, respectively. In general, **8b** is relatively weak compared to the **8a** mode but surprisingly the opposite is true for the 4CHCA sample, its **8b** mode is more prominent than the **8a** mode. No **8b** peak was revealed, experimentally or theoretically, for 3CHCA and CA. Previous work on CA identified Raman peak detected at 1591 cm^{-1} as the **8b** vibrational mode [35]. The rest of the ring vibrational modes, **19b** as well as **19a**, can be assigned to peaks in the region $1300\text{--}1500\text{ cm}^{-1}$ and **14** was located in the region $1200\text{--}1300\text{ cm}^{-1}$. It is worth noting that computer simulations reveal varying degree of coupling between the C—C ring stretching modes and the neighboring —AH and C—H groups.

The O—H in-plane bending modes for phenolic group generally lie within the spectral range $1150\text{--}1250\text{ cm}^{-1}$. Unlike the stretching and out-of-plane bending frequencies (see below), the in-plane bending frequency is not as sensitive to hydrogen bonding [49]. Peaks found at 1187 , 1233 and 1266 cm^{-1} for 3CHCA and 1177 , 1229 and 1285 cm^{-1} for 4CHCA are assigned to O-H in-plane bending mode (Fig. 3). For CCA, these peaks are observed at 1162 , 1192 and 1274 cm^{-1} while for CA, these peaks are found at 1170 , 1258 and 1281 cm^{-1} . As mentioned above these vibrational modes couples with other adjacent functional groups. The theoretically computed values agree with recorded data. The O—H in-plane bending mode for carboxylic group usually appears as a strong band in the region $1260\text{--}1440\text{ cm}^{-1}$ [36,37]. These were observed in 4CHCA (1330 cm^{-1}), CCA (1324 cm^{-1}) and CA (1305 cm^{-1}) but not in 3CHCA. The interactions with the nearby hydroxyl group on the aromatic ring may explain why the in-plane O—H for the carboxyl group was not observed for a sample of 3CHCA.

The aromatic C—H in-plane bending vibrations that are observed in the $950\text{--}1350\text{ cm}^{-1}$ region are usually weak. In-plane C—H bending vibrations appear at 1187 and 1266 cm^{-1} for 3CHCA; 1177 , 1229 and 1330 cm^{-1} for 4CHCA; 1162 , 1192 , 1298 and 1324 cm^{-1} for CCA; and 1170 , 1258 and 1305 cm^{-1} for CA. DFT based calculations predicted peaks that are shifted to a higher wavenumber revealing the following values: 1197 and 1276 cm^{-1} for 3CHCA; 1191 , 1232 and 1346 cm^{-1} for 4CHCA; 1179 , 1192 , 1318 and 1346 cm^{-1} for CCA and 1184 , 1260 , and 1308 cm^{-1} for CA.

Spectral region: below 1000 cm^{-1}

The out-of-plane modes such as *aromatic ring deformation*, *OH bending* and *CH bending* modes are typically observed in the spectral region below 1000 cm^{-1} . However, the aromatic rings deformation modes only give rise to strong IR bands and are generally undetectable in the Raman spectra since the corresponding in-plane modes are more Raman active [50]. The peaks observed are the weakest for the CA sample (Fig. 3).

Medium to strong intensity C—H out-of-plane bending modes arises in the $950\text{--}600\text{ cm}^{-1}$ region [51]. The frequencies of the C—H out-of-plane bending modes mainly depend on the number of adjacent hydrogen atoms on the ring and are not very much affected by the nature of substituents. At lower frequencies, the assignments become more complex since the bands coupled and are not sensitive to substitution. The C—H out-of-plane bending mode was observed at 642 , 780 , 833 , 875 and 975 cm^{-1} for 4CHCA; 605 and 761 cm^{-1} for 3CHCA; 615 , 781 and 860 cm^{-1} for CCA; and 642 , 678 , 743 , 798 , 835 , 863 and 976 cm^{-1}

for CA. Some of these C—H out-of-plane bending modes are associated with the ring breathing modes (**1,12, 6b**), which is consistent with literature values [36,37,52].

Lastly, the O—H out-of-plane bending vibration gives rise to a broad band in the region 600–700 cm⁻¹. The peak at 605 cm⁻¹ on 3CHCA and 642 cm⁻¹ on 4CHCA can be attributed to the O—H out-of-plane bending mode. These are consistent with the calculated values of 629 cm⁻¹ and 637 cm⁻¹ for 3CHCA and 4CHCA, respectively. CCA on the other hand was found to exhibit this mode experimentally at 605 cm⁻¹, while CA has two peaks (642 and 678 cm⁻¹) pertaining to this mode. Theoretical values of 615 and 646 as well as 724 cm⁻¹ were obtained for the CCA and CA, respectively.

Summary and conclusion

In summary, there are certain peaks in the CHCA derivatives studied that can be observed and analyzed to illustrate their unique properties. Our data indicate that most likely 4CHCA, and perhaps the other derivatives, forms a self-associated complex. Computation based on a single isolated molecule cannot account for all observed peak shifts, particularly the mode at *ca.* 3300 cm⁻¹ mode. Discrepancies in the calculated and experimental peaks perhaps indicate that these derivatives do not exist as single isolated non-interacting molecules.

Acknowledgments

This work was supported in part by the Institute of General Medicine of the National Institutes of Health, Grant No. 5SC3GM89624-2. We are grateful to Ms. Jessica Desamero for careful reading of this manuscript.

References

1. Beavis RC, Chait BT. Rapid Commun Mass Spectrom. 1989; 3:432–435. [PubMed: 2520223]
2. Beavis RC, Chaudhary T, Chait BT. Org Mass Spectrom. 1992; 27:156–158.
3. Schneider K, Chait BT. Rapid Commun Mass Spectrom. 1993; 28:1353.
4. Fukuyama Y, Funakoshi N, Takeyama K, Hioki Y, Nishikaze T, Kaneshiro K, Kawabata SI, Iwamoto S, Tanaka K. Anal Chem. 2014; 86:1937–1942. [PubMed: 24498852]
5. Jaskolla TW, Lehmann WD, Karas M. Proc Natl Acad Sci U S A. 2008; 105:12200–12205. [PubMed: 18723668]
6. Porta T, Grivet C, Knochenmuss R, Varesio E, Hopfgartner G. J Mass Spectrom. 2011; 46:144–152. [PubMed: 21259393]
7. Hioki Y, Kuyama H, Hamana C, Takeyama K, Tanaka K. J Mass Spectrom. 2013; 48:1217–1223. [PubMed: 24259210]
8. Rechthaler J, Pittenauer E, Schaub TM, Allmaier G. J Am Soc Mass Spectrom. 2013; 24:701–710. [PubMed: 23595260]
9. Vergeiner S, Schafferer L, Haas H, Mueller T. J Am Soc Mass Spectrom. 2014; 25:1498–1501. [PubMed: 24894842]
10. Fleith C, Cantel S, Subra G, Mehdi A, Ciccione J, Martinez J, Enjalbal C. Analyst. 2014; 139:3748–3754. [PubMed: 24910856]
11. Gorka J, Bahr U, Karas M. J Am Soc Mass Spectrom. 2012; 23:1949–1954. [PubMed: 22993043]
12. Halestrap AP. Biochem J. 1975; 148:85–96. [PubMed: 1156402]
13. Halestrap AP. Biochem J. 1976; 156:181–183. [PubMed: 942399]
14. Day DA, Hanson JB. Plant Physiol. 1977; 59:630–635. [PubMed: 16659908]
15. Halestrap AP. IUBMB Life. 2012; 64:1–9. [PubMed: 22131303]

16. Patel TB, Waymack PP, Olson MS. Arch Biochem Biophys. 1980; 201:629–635. [PubMed: 7396523]
17. Qiu L, Chen QH, Zhuang JX, Zhong X, Zhou JJ, Guo YJ, Chen QX. Food Chem. 2009; 112:609–613.
18. Granja S, Morais-Santos F, Miranda-Goncalves V, Viana-Ferreira M, Nogueira R, Nogueira-Silva C, Correia-Pinto J, Baltazar F. Cell Physiol Biochem. 2013; 32:1845–1856. [PubMed: 24356196]
19. Clavijo RE, Ross DJ, Aroca RF. J Raman Spectrosc. 2009; 40:1984–1988.
20. Fiuza SM, Van Besien E, Milhazes N, Borges F, Marques MPM. J Mol Struct. 2004; 693:103–118.
21. Ravikumar N, Gaddamanugu G, Solomon KA. J Mol Struct. 2013; 1033:272–279.
22. Sardo M, Ruano C, Castro JL, Lopez-Tocon I, Ribeiro-Claro PJA, Otero JC. Chem Phys Lett. 2008; 467:101–104.
23. Sebastian S, Sundaraganesan N, Manoharan S. Spectrochim Acta A. 2009; 74:312–323.
24. Dembereldorj U, Joo SW. Bull Korean Chem Soc. 2010; 31:116–119.
25. Frisch MT, GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, G S, Barone V, Mennucci B, Petersson GA, Nakatsuji H, C M, Li X, Hratchian HP, Izmaylov AF, Bloino J, Zheng G, S J, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, I M, Nakajima T, Honda Y, Kitao O, Nakai H, Vreven T, M J Jr, Peralta JE, Ogliaro F, Bearpark M, Heyd JJ, B E, Kudin KN, Staroverov VN, Kobayashi R, Normand J, R K, Rendell A, Burant JC, Iyengar SS, Tomasi J, C M, Rega N, Millam JM, Klene M, Knox JE, Cross JB, Bakken V, A C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, A A, Cammi R, Pomelli C, Ochterski JW, Martin RL, M K, Zakrzewski VG, Voth GA, Salvador P, Dannenberg JJ, D S, Daniels AD, Farkas O, Foresman JB, Ortiz JV, Cioslowski J, F DJ. 2009
26. Lee C, Yang W, Parr RG. Phys Rev B. 1988; 41:785–789.
27. Rauhut G, Pulay P. J Phys Chem. 1995; 99:3093–3100.
28. Stephens PJ, Devlin FJ, Chabalowski CF, Frisch MJ. J Phys Chem. 1994; 98:11623–11627.
29. Dennington, R., II; Keith, T.; Millam, J. GaussView, Version 5. Semichem Inc; Shawnee Mission, KS: 2009.
30. Pulay P, Fogarasi G, Pongor G, Boggs JE, Vargha A. J Am Chem Soc. 1983; 105:7037–7047.
31. Arjunan V, Thillai Govindaraja S, Sakiladevi S, Kalaivani M, Mohan S. Spectrochim Acta A. 2011; 81:196–209.
32. Campanelli AR, Domenicano A, Ramondo F. J Phys Chem A. 2003; 107:6429–6440.
33. Domenicano A, Vaciago A. Acta Crystallogr B. 1975; 31:221–234.
34. Arjunan V, Rani T, Varalakshmy L, Mohan S, Tedlamelekot F. Spectrochim Acta A. 2011; 78:1449–1454.
35. Cunha VRR, Constantino VRL, Ando RA. Vib Spectrosc. 2012; 58:139–145.
36. Colthup, NB.; Daly, LH.; Wiberley, SE. Introduction to Infrared and Raman Spectroscopy. Academic Press; New York: 1990.
37. Socrates, G. Infrared and Raman of Characteristic Frequencies. 3rd. John Wiley & Sons Ltd.; Chichester: 2001.
38. Silverstein, RM.; Bassler, GC.; Morrill, TC. Spectrometric Identification of Organic Compounds. 5th. John Wiley & Sons Inc; NY: 1981.
39. Liu D, Ma G, Levering LM, Allen H. J Phys Chem B. 2004; 108:2252–2260.
40. Walker DS, Richmond GL. J Phys Chem C. 2007; 111:8321–8330.
41. Coates, J. Interpretation of infrared spectra: a practical approach. In: Meyers, RA., editor. Encyclopedia of Analytical Chemistry. John Wiley & Sons Ltd.; Chichester: 2000. p. 10815–10837.
42. Kucharska E, Sasiadek W, Janczak J, Ban-Oganowska H, Lorenc J, Weglinski Z, Talik Z, Hermanowicz K, Hanuza J. Vib Spectrosc. 2010; 53:189–198.
43. Mohamed TA, Hassan AM, Soliman UA, Zoghaib WM, Husband J, Hassan SM. Spectrochim Acta A. 2011; 79:1722–1730.
44. Sri NU, Chaitanya K, Prasad MVS, Veeraiah V, Veeraiah A. Spectrochim Acta A. 2012; 97:728–736.

45. Weeks CL, Polishchuk A, Getahun Z, DeGrado WF, Spiro TG. *J Raman Spectrosc.* 2008; 39:1606–1613. [PubMed: 20648227]
46. Karabacak M, Cinar Z, Kurt M, Sudha S, Sundaraganesan N. *Spectrochim Acta A.* 2012; 85:179–189.
47. Varsanyi, G. Academic Press; New York: 1969.
48. Sajan D, Sockalingum GD, Manfait M, Hubert Joe I, Jayakumar VS. *J Raman Spectrosc.* 2008; 39:1772–1783.
49. Sajan D, Joe IH, Jayakumar VS, Zaleski J. *J Mol Struct.* 2006; 785:43–53.
50. Machado NFL, Batista de Carvalho LAE, Otero JC, Marques MPM. *Vib Spectrosc.* 2013; 68:257–265.
51. Mortimer FS, Boldgett RB, Daniels F. *J Am Chem Soc.* 1947; 69:822–826.
52. Varsanyi G, Hilger A. *Assignments of Vibrational Spectra of Seven Hundred Benzene Derivatives.* 1974; 1–2

Highlights

- Raman spectra of α -cyanohydroxycinnamic acid (CHCA) derivatives were compared.
- CHCA Raman spectra were also compared to cyanocinnamic acid and coumaric acid.
- Assignments of modes were confirmed by DFT-based theoretical calculations.
- Data suggests formation of self-associated complex particularly for 4CHCA.

Fig. 1.
Optimized structure of α -cyano-4-hydroxycinnamic acid (A) and α -cyano-3-hydroxycinnamic acid (B), cyanocinnamic acid (C) and coumaric acid (D).

Fig. 2. Raman spectra of 4CHCA, 3CHCA, CCA and CA for the regions: (A) 3250–3500 cm^{-1} , (B) 2900–3250 cm^{-1} and (C) 2100–2400 cm^{-1} .

Fig. 3.
Raman spectra of 4CHCA, 3CHCA, CCA and CA in the spectral range 600–1700 cm^{-1} .

Table 1

Bond lengths and angles in the aromatic ring and acrylic group of the CHCA derivatives calculated using DFT based B3LYP/6-31 g simulation.

	CCA	CA	4CHCA	3CHCA
<i>Bond lengths (Å)</i>				
C1—C2	1.4004	1.4001	1.40254	1.39503
C2—C3	1.39355	1.39079	1.3903	1.39627
C3—C4	1.41615	1.41347	1.41675	1.41474
C4—C5	1.41501	1.41138	1.41984	1.41439
C5—C6	1.39469	1.39204	1.38765	1.39412
C6—C1	1.40155	1.40353	1.40361	1.40334
C2—H	1.08471	1.08307	1.0863	1.08443
C3—H	1.08647	1.08616	1.0858	1.08511
C5—H	1.08144	1.08432	1.08303	1.08326
C6—H	1.08469	1.08658	1.08133	1.08254
C4—C7	1.45736	1.4615	1.44948	1.45548
C7—H	1.08882	1.09106	1.08622	1.08576
C7—C8	1.36641	1.35209	1.37007	1.36764
C8—C9	1.49989	1.47367	1.47685	1.4795
C9—O10	1.22592	1.23202	1.23639	1.23577
C9—O11	1.38884	1.3942	1.38557	1.3829
O1—AH	0.97826	0.97792	0.98036	0.98045
C8—C12	1.42607		1.42661	1.42725
C12—N13	1.1746		1.1748	1.17465
C1—C8—H	1.085	1.08434		
C1—O/C6—O		1.38542	1.38108	1.3866
OH		0.9766	0.97687	0.97695
<i>Angles (°)</i>				
C1—C2—C3	119.791	119.417	119.287	120.379
C2—C3—C4	121.179	121.606	121.697	120.64
C3—C4—C5	118.273	117.797	117.664	118.678
C4—C5—C6	120.31	121.102	120.938	119.944
C5—C6—C1	120.61	119.83	120.068	120.991
C6—C1—C2	119.836	120.248	120.345	119.367
C3—C4—C7	116.757	119.046	116.813	116.545
C5—C4—C7	124.968	123.157	125.523	124.777
C4—C7—C8	132	127.552	131.739	131.649
C7—C8—C12	124.488		124.651	124.639
C7—C8—C9	120.835	125.215	120.656	120.755
C8—C9—O10	116.645	118.149	113.177	113.1
C8—C9—O11	123.63	123.102	125.262	125.043
O10—C9—O11	119.712	118.748	121.561	121.856

	CCA	CA	4CHCA	3CHCA
C9—C8—C12	114.523		114.693	114.606
C8—C12—N13	179.234		179.374	178.897
(C6—C1/C5—C6)—O	1122.85	116.739	122.627	
(C2—C1/C1—C6)—O	116.901	122.916	116.382

	R ₁	R ₂	R ₃
4CHCA	-OH	-H	-CN
3CHCA	-H	-OH	-CN
CCA	-H	-H	-CN
CA	-OH	-H	-H

Table 2

Plausible assignments of the Raman modes observed for each CHCA derivatives.

4CHCA	3CHCA	CCA	CA	Plausible assignments ^a
3306			3230	νOH (near the COOH of the other CHCA)
			3206	νOH (near the COOH of the other CHCA)
	3179	3186		$\nu_s\text{CH}$ ring
3156		3145		νCH ring symmetrical
3126				νCH ring symmetrical
3080	3075	3092	3070	νCH ring asymmetrical
3042		3063	3052	νCH C in C=C
			3024	νCH
2239	2244	2227		νCN
			1636	$\phi + \delta\text{CH}$ (both) + $\nu\text{CC} + \delta\text{OH} + \nu\text{CO}$
1595	1599	1592	1606	$\phi 8a + \delta\text{CH}$ (both) + $\nu\text{CC} + \delta\text{OH} + \nu\text{CO}$
1566		1573		$\phi 8b + \delta\text{CH}$ (ring) + $\nu\text{CC} + \delta\text{OH}$
1519		1536	1519	$\phi 19a + \delta\text{CH}$ (ring) + νCC
1455	1460	1449	1447	$\phi 19b + \delta\text{CH}$ (both) + $\nu\text{CC} + \delta\text{OH}$ (ring)
1382		1377		$\phi + \delta\text{CH} + \nu\text{CC} + \delta\text{OH}$
1330		1324		$\phi 14 + \delta\text{OH}$ (COOH) + $\delta\text{CH} + \delta\text{CC} + \delta\text{CO}$
		1298	1305	$\phi 14 + \delta\text{OH}$ (COOH) + δCH
1285		1274	1281	$\phi + \nu\text{CO}$ (ring) + δOH
	1266			$\phi + \delta\text{CH} + \delta\text{OH}$
			1258	$\phi + \delta\text{OH} + \delta\text{CH}$
1229	1233			$\phi + \nu\text{CC} + \delta\text{CH} + \delta\text{OH}$
		1214	1212	$\phi + \nu\text{CC}$
	1187	1192		$\delta\text{CH} + \delta\text{OH}$
1177		1162	1170	$\phi 9a + \delta\text{CH} + \delta\text{OH}$
	1003	1000		$\phi 12$
975			976	γCH (both)
875		860	863	$\phi 1 + \gamma\text{CH}$ (both)
833			835	$\phi + \omega\text{CH} + \gamma\text{OH}$
780		781	798	$\gamma\text{OH} + \gamma\text{CH}$
	761		743	$\phi 4 + \gamma\text{OH} + \gamma\text{CH}$
			678	$\phi + \gamma\text{OH} + \gamma\text{CH}$
642			642	$\phi 6b + \gamma\text{OH} + \gamma\text{CH}$
	605	615		$\phi 6b + \gamma\text{OH} + \gamma\text{CH}$

^a Assignments was based on literature comparisons and DFT based computer simulations. ν = stretching; ϕ = ring; δ = in-plane bending; γ = out-of-plane.