Structural studies of two capsaicinoids: dihydrocapsaicin and nonivamide. $^{13}$C and $^{15}$N MAS NMR supported by genetic algorithm and GIAO DFT calculations

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Capsaicinoids are alkaloid type capsaicin analogs with prospective pharmacological activity. However their solid state conformations have not been studied yet. As part of the study, cross polarization (CP) magic angle spinning (MAS) solid state $^{13}$C and $^{15}$N NMR spectra of dihydrocapsaicin (DHCAP) and nonivamide (NVA) were recorded. Solid state chemical shifts differ from their solution counterparts; remarkable differences occur for carbons C20, C60 and C70 linked to C10 in DHCAP and with methylene carbons C4–C8 in NVA. The doubling of some resonances in the spectra of solid NVA indicates that there are two molecules in the crystallographic asymmetric unit. DFT GIAO calculations of shielding constants were performed for several geometric isomers, including molecules with different orientations of aliphatic chain with respect to aromatic ring. Low-energy conformers were found by genetic algorithm methodology. Comparison of experimental $^{13}$C chemical shifts with theoretical (GIAO DFT) shielding parameters was helpful in predicting the most reliable geometry in the solid state. Cross polarization time constants $T_{CP}$ and relaxation times in the rotating frame $T_{1r}$ were obtained from variable-contact cross-polarization experiments. $T_{1r}$ are longer in the order: NVA < CAP < DHCAP.

Introduction

Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is a pungent alkaloid found in chili pepper fruit. Over the last decades, it has been described as a multipotent bioactive compound.1 According to these studies, many new capsaicin analogs with expected biological activity were sought. Nonivamide (NVA) and dihydrocapsaicin (DHCAP) (Fig. 1) are two derivatives of capsaicin (CAP)2,3 which can be found as a component of analgesic drugs or pepper spray;4 they are also used in pain-killer cream and patches.

Pharmacological properties of potential drug substances, crucial for modern pharmaceutical sciences, are widely studied using spectroscopic and theoretical methods. The biological activity of a compound defined by the affinity of a small-molecule ligand towards the macromolecular receptor can be explored using in silico methods, like the molecular docking approach. The docking process involves prediction of multiple structural conformations in a binding pocket as a basic step. However, solid state conformation analysis is also very important, because the majority of dosage forms are solid dosage forms and solid state conformation is connected with pharmacokinetic properties of an API (Active Pharmaceutical Ingredient). Therefore, low energy conformations of capsaicinoids, as well as their solid-state structure are of interest and should be studied in detail.

Crystallographic data for nonivamide and dihydrocapsaicin do not exist. Since all our attempts to obtain single crystals of capsaicinoids suitable for XRD measurement have been unsuccessful, solid-state NMR is proposed as a method for structure determination for these substances. NMR spectroscopy supported by theoretical calculations was used as the main method of structural studies. This approach was previously applied by us in the study on capsaicin structure5 and the results were compared with PXRD data. To the best of our knowledge, this work is the first to focus on the solid-state structure of nonivamide and dihydrocapsaicin. Our results may be a step towards determination of the conformation – bioavailability relationship, because neither the conformations nor the crystal structures of these capsaicinoids have been described before.

Methods

Materials

NVA (nonivamide) and DHCAP (dihydrocapsaicin) were purchased from Sigma-Aldrich (Aldrich no. V9130 and M1022 respectively).
The $^{13}$C and $^{15}$N magic angle spinning (MAS) NMR spectra of solid samples were recorded on a Bruker DRX-400 Advance spectrometer using a Bruker PH MAS VTN 400WB BL4 probe-head. Samples packed in a 4 mm ZrO$_2$ rotor were spun at 10 kHz ($^{13}$C) and 5 kHz ($^{15}$N). Contact time of 2.5 ms, repetition time of 10 s and spectral width of 20 kHz were used for accumulation of 200 scans for standard $^{13}$C MAS experiments. For the acquisition of $^{15}$N MAS spectra these parameters were: 5 ms, 10 s and 12 000 scans, respectively. $^{13}$C chemical shifts were calibrated indirectly through the glycine CO signal recorded at 176.0 ppm, relative to TMS. $^{15}$N chemical shifts were calibrated through the glycine NH signal $\delta$ $^{15}$N 10.0 ppm and referenced to nitromethane $\delta$ $^{15}$N = 380.2 ppm [liquid NH$_3$$\delta$ $^{15}$N = 0]. Solid-state NMR cross-polarization (CP) $^1$H–$^{13}$C experiments were performed with contact times in the range from 25 $\mu$s to 18 ms.

### Genetic algorithm

A conformational search was carried for each molecule using genetic algorithm (GA) with multimodal optimization. Special software (ALJAR05) was developed for the calculations and used as described earlier. Each conformation was first optimized using molecular mechanics (MMFF94 force field) to classify the conformers found by the genetic algorithm methodology.

### GIAO DFT calculations

Further calculations of NMR shielding constants were performed using the DFT method with the B3LYP functional and the 6-311G(d,p) basis set (Gaussian 09 software). Values of chemical shifts for each conformation were obtained in two ways: (i) by calculating TMS carbon atoms shielding constants and using the equation $\delta_{\text{iso}} = \sigma_{\text{TMS}} - \sigma_{\text{iso}}$ and (ii) by converting shielding constants with scaling factors. The model conformers were

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**Fig. 1** Structure of (a) dihydrocapsaicin (DHCAP) and (b) nonivamide (NVA) with numbering of carbon atoms.

**Fig. 2** $^{13}$C CPMAS NMR spectra of (a) DHCAP and (b) NVA.
verified by comparing calculated shielding constants with experimental MAS NMR chemical shifts. For $^{15}$N NMR, the reference molecule nitromethane was also optimized and its isotropic NMR shielding constants were calculated.

Results and discussion

$^{13}$C CPMAS NMR

$^1$H and $^{13}$C NMR spectra of NVA and DHCAP (in CDCl$_3$ solutions) were measured and assigned. The chemical shifts were in agreement with those reported previously by Gómez-Calvario et al.$^{17}$ $^{13}$C CPMAS NMR spectra were recorded for both solids and are illustrated in Fig. 2.

Resonances in the solid-state spectra were assigned by comparison with liquid-state chemical shifts and the results are collected in Table 1.

The $^{13}$C signals in the solid-state spectrum of DHCAP were assigned first on the basis of liquid-state chemical shifts and the results are collected in Table 1. The assignments were further confirmed by the calculation of shielding constants. The signals of carbons C1 and C7 bound to nitrogen might be slightly broader due to the interaction with quadrupolar 14 N nuclei but the effect was not observed in the spectra recorded with spinning speed of 10 kHz. Significant differences between chemical shifts in solution and solid state ($\Delta = \delta_{\text{sol}} - \delta_{\text{ss}} > 1.5$ ppm) are observed for both compounds, but for different carbon atoms.

For DHCAP, the differences appear for aromatic carbons C1', C2', C6' and also for C7'and C8'. Similar differences were noticed earlier for capsaicin.$^5$ All three neighbors of C1' exhibit an increase of shielding (3.4–3.8 ppm) in the solid state, suggesting the presence of steric hindrance. Molecular modeling shows that the rotation about C1'–C7' bond is crucial and results in intramolecular interactions of C7'–H with either C2'–H or C6'–H. Significant values of $\Delta$ may be due to the locking of this fragment into a particular conformation, for example one of the C7'–H located closer to C6'–H and OCH$_3$ group near aliphatic chain (Fig. 1).

Considering the NVA molecule, the most remarkable differences in chemical shifts (ca. −4.5 ppm) are observed for methylene carbons C4, C5 and C6 of the chain. The signals of four aromatic carbons (1', 2', 3', 6') and OCH$_3$ (C8') appear as doublets. The presence of split resonances indicates that two different molecules coexist in an asymmetric part of a unit cell of solid NVA. An attempt to assign the resonances to particular conformer was made. It is possible that one of them is similar to DHCAP, with OCH$_3$ group near aliphatic chain as suggested by comparable values of $\Delta$. But for the second conformer these differences are smaller suggesting that this molecule has aliphatic chain oriented far from aromatic ring and therefore may be less hindered.

On the basis of $^{13}$C CPMAS NMR and theoretical B3LYP/6-31G** calculations we could characterize the structure of conformers in solid phase. The chemical shifts for some conformers with different orientations of aliphatic chain, hydroxy and methoxy groups have been calculated by GIAO DFT. In solids, the rotation of hydroxy and methoxy groups is usually frozen, revealing specific environments with different chemical shifts, best seen for aromatic carbons ortho to the substituent. Better correlations between the measured and the calculated $\delta_{\text{ss}}$ values confirmed the existence of conformers with “anticlockwise” orientation of OH and OCH$_3$ groups, e.g. with OCH$_3$ pointing to C2' and OH towards OCH$_3$ oxygen (Fig. 1).

Besides conformational effects, $^{13}$C CPMAS chemical shifts reflect intermolecular interactions in solids. The formation of H-bonds results in changes of the chemical shift (deshielding) of carbonyl carbons. In CDCl$_3$ only weak interactions with the solvent occur, whereas in the solid state the C=O···HN and/or

| C atom no. | $\delta_{\text{sol}}$ | $\delta_{\text{ss}}$ | $\Delta$ |
|------------|---------------------|---------------------|---------|
| 1'         | 130.82              | 128.59              | 2.23    |
| 2'         | 111.19              | 107.77              | 3.42    |
| 3'         | 147.22              | 147.19              |         |
| 4'         | 145.62              | 145.44              |         |
| 5'         | 114.88              | 114.82              |         |
| 6'         | 121.23              | 117.39              | 3.84    |
| 7'         | 43.99               | 40.4                | 3.63    |
| 8'         | 56.39               | 53.6                | 2.76    |
| 1          | 173.38              | 176.3               | −2.9    |
| 2          | 37.32               | 37.3                |         |
| 3          | 26.30               | 28.7                | −2.3    |
| 4          | 29.85               | 31.5                | −1.6    |
| 5          | 30.11               | 31.6                | −1.5    |
| 6          | 27.72               | 28.7                |         |
| 7          | 39.43               | 40.4                |         |
| 8          | 28.41               | 28.7                |         |
| 9          | 23.10               | 24.0                |         |
| 10         | 23.10               | 23.0                |         |

| C atom no. | $\delta_{\text{sol}}$ | $\delta_{\text{ss}}$ | $\Delta$ |
|------------|---------------------|---------------------|---------|
| 1'         | 129.94              | 130.48/131.14       |         |
| 2'         | 110.74              | 111.66/112.92       | −/−2.18 |
| 3'         | 146.70              | 145.41              |         |
| 4'         | 145.19              | 143.46/145.41       | 1.73/−   |
| 5'         | 114.37              | 115.48              |         |
| 6'         | 120.86              | 121.85/122.65       | −/−1.79 |
| 7'         | 43.76               | 43.9                |         |
| 8'         | 55.98               | 54.8/56.6           |         |
| 1          | 173.53              | 171.9               | 1.5     |
| 2          | 36.55               | 36.08               |         |
| 3          | 25.88               | 26.58               |         |
| 4          | 29.29               | 33.75               | −4.46   |
| 5          | 29.14               | 33.75               | −4.61   |
| 6          | 29.14               | 33.75               | −4.61   |
| 7          | 22.63               | 24.5                | −1.95   |
| 8          | 31.79               | 33.75               | −1.96   |
| 9          | 14.08               | 15.46               |         |
| 10         | —                   | —                   |         |
C=O…HO bonds should affect the $\delta_{\text{as}}$ value. In DHCAP, chemical shift of C1=O for solution is 173.38 ppm, and for solid state 176.28 ppm. The deshielding ($\Delta = 2.9$ ppm) confirms the formation of intermolecular hydrogen bonds. For NVA, the difference of chemical shift between solution and solid state is $+1.5$ ppm and an increase of shielding suggest absence of hydrogen bond-type interaction involving C=O group.

$^{15}$N MAS NMR

$^{13}$C CPMAS spectra of solid NVA and DHCAP indicate that DHCAP has only one molecule in an asymmetric part of a unit cell, whereas NVA may have two. $^{15}$N MAS NMR spectra provide confirmation of these findings. In $^{15}$N MAS NMR spectrum of DHCAP a narrow singlet appears at $-259.3$ ppm, whereas in the spectrum of NVA a doublet is visible at $-257.2/-258.2$ ppm (Fig. 3).

The value of $^{15}$N chemical shift for both compounds is typical for aliphatic and aromatic amides.\textsuperscript{10} In both solids, hydrogen bonds may be formed by the OH…C or NH…O=C interaction. The NH…O=C interaction should be reflected in $^{15}$N MAS chemical shift. If DHCAP is stabilized by NH…O=C H-bonds, and NVA is not, their $^{15}$N chemical shifts would be significantly different. The relationship between $^{15}$N MAS chemical shift and hydrogen bond-type interactions via NH group has been previously described and the effect of H-bond formation is estimated as ca. 8 ppm.\textsuperscript{11} The $^{15}$N signals of DHCAP and NVA have almost the same chemical shift, values differ only by 1–2 ppm suggesting similar type of intermolecular interactions. Thus, one can assume that molecules of NVA and DHCAP are not linked by NH…O=C bonds. The deshielding of C=O in $^{13}$C MAS spectrum of DHCAP can be explained rather by OH…O=C interactions.

GIAO DFT calculations were performed for an isolated molecule of DHCAP and NVA and the values of $^{15}$N NMR chemical shift ($-256.0$ and $-254.6$ ppm, respectively) are in agreement with experimental data.

Cross-polarization dynamics

It seemed worth to check if DHCAP and NVA exhibit distinct cross-polarization dynamics. Therefore, a series of $^{13}$C CPMAS spectra with various contact times were recorded. According to the classic I-S model\textsuperscript{12} the signal intensity in CP spectra is a function of contact time $t$:

$$R(t) = A(1 - T_{\text{CP}}/T_{1p}^{\text{H}}) - 1[\exp(-t/T_{1p}^{\text{H}}) - \exp(-t/T_{\text{CP}})]$$  \hspace{1cm} (1)

where $A$ is the intensity amplitude. The progress of cross-polarization is characterized by the time constant $T_{\text{CP}}$ and the decrease in intensity for longer contact times $t$ is due to the effects of $^1$H spin–spin relaxation in the rotating frame, $T_{1p}^{\text{H}}$. Both parameters are specific for functional groups and differ for protonated and quaternary carbons.\textsuperscript{11} The values of $T_{\text{CP}}$ and $T_{1p}^{\text{H}}$ for dihydrocapsaicin, capsaicin and nonivamide are given in Table 2. Selected spectra of DHCAP and NVA recorded with varying contact times, are illustrated in Fig. 4a and b.

$T_{\text{CP}}$ is governed by dipolar coupling\textsuperscript{12} and is long for carbons without adjacent hydrogens. Quaternary carbon atoms of DHCAP and NVA have average $T_{\text{CP}}$ values of 0.87 and 0.7 ms respectively. $T_{\text{CP}}$ for CH groups is shorter (0.42–0.51 ms). Assuming that the average value of 0.42 ms is typical for CH carbons (Table 2), then 0.20 ms should be expected for CH$_3$ and even lower value for CH$_2$. The values for methyl carbons of 0.32–1.62 ms are longer than those expected without intramolecular dynamics, evidencing that methyl groups are fairly mobile. $T_{\text{CP}}$ increases with increasing mobility of the structural fragment bearing this carbon, and the fast rotation is typical for methyl groups.\textsuperscript{14} In the $^{13}$C CPMAS spectra of DHCAP separate signals of two methyl groups appeared, therefore we were able to characterize particular carbons. The values of $T_{\text{CP}}$ are 1.58 and 1.65 ms for C9 and C10, respectively, indicating that their interaction with intra- or intermolecular does not differ.

Relaxation times $T_{1p}^{\text{H}}$ are influenced by the short-range spatial proximity between protons and also reflect molecular dynamics. It seemed interesting to check if the two molecules

![Fig. 3](image-url) $^{15}$N MAS NMR spectra of (a) DHCAP and (b) NVA.
present in an asymmetric part of a unit cell of solid NVA exhibit distinct cross-polarization dynamics. Inspection of the spectra in Fig. 4, in which the doublets (C2, C3, C6') of almost equal intensity are seen, as well as an analysis of \( T_{CP} \) and \( \tau_{HP} \) values indicated that the behaviour of these two conformations is quite similar.

Fig. 5 shows the plot of signal intensity vs. contact time for methyl groups of NVA and DHCAP. Two signals of OCH3 groups in NVA are characterized by long decay, similar to DHCAP. The \( \tau_{HP} \) values obtained for CH3 groups (both from the aliphatic chain and methoxy groups) are the same for DHCAP and CAP.

The plot of signal intensity vs. contact time for carbonyl carbons C1 is illustrated in Fig. 6; the shape of fitted functions is distinct: steeper for NVA and plane for DHCAP.

Unfortunately, no reliable data on cross-polarization dynamics can be provided on particular methylene carbons (except C7') because of signal overlapping.

It is difficult to predict in advance which model will be better for the quantification of a particular CP signal. Therefore, we decided to analyze CP data in accordance with the I-I fusion model. Intensity decays were described by Kolodziejski and Klinowski\(^{12}\) by the following equation:

\[
R(t) = I_0[1 - \frac{1}{2} \exp(-t/T_{df}) - \frac{1}{2} \exp(-\frac{t}{2T_{df}})\exp(-\frac{t^2}{4T_{df}^2})],
\]

where \( T_{df} \) is the proton spin-diffusion constant. \( T_{df} \) values for DHCAP and NVA are collected in Table 2.

Relaxation time in the rotating frame is frequently averaged over all protons by spin diffusion and is indicative for separation of molecules or some domains (e.g. aliphatic and aromatic) by missing intimate spin contact. \( T_{df} \) are long for quaternary carbon atoms. C4' and C3' which are the most distant from aliphatic chain carbon atoms have the longest \( T_{df} \) (0.85 and 1.42 ms for DHCAP, 0.82 and 1.42 ms for NVA, respectively). There are no significant differences in \( T_{df} \) values for aromatic ring carbon atoms. However, the CH3 groups of the aliphatic chain of DHCAP and NVA differ in proton spin-diffusion constant.

Methyl groups of DHCAP require longer spin-diffusion, which may be caused by fast rotation of isopropyl chain termination, as it was described earlier for CAP.\(^7\) Shorter \( T_{df} \) value indicates that the CH3 group of NVA may be less rotating. The average relaxation times in the rotating frame for solid capsaicinoids decrease in the order: DHCAP > CAP > NVA. Although the differences may be the result of different phenomena, including intramolecular mobility and intermolecular interactions, the

| Parameter | DHCAP Mean value ± Range of values [ms] | CAP Mean value ± Range of values [ms] | NVA Mean value ± Range of values [ms] |
|-----------|----------------------------------------|----------------------------------------|----------------------------------------|
| Quaternary carbons C1', C3', C4' | \( T_{CP} 0.87 \pm 0.24 \quad 0.65-1.13 \) | \( T_{CP} 0.95 \pm 0.22 \quad 0.71-1.23 \) | \( T_{CP} 0.71 \pm 0.08 \quad 0.64-0.80 \) |
| \( \tau_{HP} \) | 159 ± 15 \quad 143-172 | 154 ± 35 \quad 124-192 | 31 ± 8 \quad 24-40 |
| \( T_{df} \) | 0.95 ± 0.43 \quad 0.58-1.42 | Nd | Nd |
| C1==O | \( T_{CP} 0.69 \quad — \) | 0.67 \quad — | 1.14 \quad — |
| \( \tau_{HP} \) | 122 \quad — | 107 \quad — | 35 \quad — |
| \( T_{df} \) (I-I-S) | 0.61 \quad — | Nd | Nd |
| C-H aromatic (C2', C5', C6') and C8 in DHCAP | \( T_{CP} 0.51 \pm 0.09 \quad 0.45-0.65 \) | 0.42 ± 0.05 \quad 0.35-0.47 | 0.42 ± 0.08 \quad 0.33-0.49 |
| \( \tau_{HP} \) | 200 ± 58 \quad 150-250 | 149 ± 20 \quad 117-160 | 39 ± 7 \quad 35-48 |
| \( T_{df} \) (I-I-S) | 0.42 ± 0.11 \quad 0.30-0.53 | Nd | Nd |
| CH3 groups | \( T_{CP} 0.38 \pm 0.11 \quad 0.30-0.60 \) | 0.27 ± 0.12 \quad 0.2-0.47 | 0.20 ± 0.06 \quad 0.15-0.3 |
| \( \tau_{HP} \) | 148 ± 23 \quad 128-180 | 129 ± 15 \quad 115-148 | 23 ± 4 \quad 21-33 |
| \( T_{df} \) (I-I-S) | 0.35 ± 0.11 \quad 0.20-0.50 | Nd | Nd |
| OCH3 groups | \( T_{CP} 1.62 \pm 0.05 \quad 1.58-1.65 \) | 0.85 ± 0 \quad — | 0.32 \quad — |
| \( \tau_{HP} \) | 160 ± 14 \quad 150-170 | 160 ± 14 \quad 150-170 | 31 \quad — |
| \( T_{df} \) (I-I-S) | 2.00 ± 0.00 \quad 2.00 | Nd | Nd |
| \( T_{df} \) (I-I-S) | 0.68 \quad — | 0.45 \quad — | 0.50 \quad — |
| \( \tau_{HP} \) | 180 \quad — | 180 \quad — | 35 \quad — |
| \( T_{df} \) (I-I-S) | 0.30 \quad — | Nd | Nd |

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lowest values for NVA (Table 2) suggest that solid NVA is more tightly packed and the chain has less space for dynamics. 

\( T_{1\rho} \) relaxation times are considered to be a probe of crystallinity degrees. For crystalline samples, \( T_{1\rho} \) takes longer values than for amorphous samples.\(^{13}\) It may suggest that NVA has a more disordered network compared to DHCAP and CAP. However, line broadening characteristic for amorphous solids is not observed neither in DHCAP nor NVA \(^{13}\)C CP MAS spectra.

Cross-polarisation dynamic parameters were analyzed for the common pharmaceutical excipients.\(^{13}\) Branched polymers of cellulose (methylcellulose, ethylcellulose, hydroxypropylmethylcellulose) have mean \( T_{1\rho} \) value of 20 ms, which is similar to NVA. For some crystalline compounds, \( T_{1\rho} \) values
were significantly higher, for example olanzapine form II ($T_{1p} = \infty$). The molecular dynamics of solid β-carotene were studied by means of $^{13}$C CPMAS NMR. The $T_{CP}$ average value of 0.79 ms was found for methine carbons and 0.65–0.78 ms for methylene carbons, which indicated that these groups must be in a flexible molecular fragment. The relaxation times $T_{1p}$ were very long (the CP curves ended in a plateau).

**Genetic algorithm search**

Low-energy conformations for NVA and DHCAP should be found for further molecular modelling, and simple DFT optimization is not efficient enough. For flexible compounds like NVA and DHCAP, systematical conformational searching (grid search) is extremely time consuming and is not recommended. A relatively new methodology of conformational analysis...
capable of locating minimum energy structures on conformational potential energy surfaces are evolutionary algorithms (GA) based on the concepts of biological evolution. A ‘population’ of possible solutions to the problem is first created with each solution being scored using a ‘fitness function’ that indicates how good they are. The population evolves over time and allows you to receive better solutions. Of the various types of evolutionary algorithm, the genetic algorithm (GA) is the most well-known. The idea of GA is based on the genetic principles of heredity transferred to the field of quantum chemistry. The best adapted, low-energy conformations are selected and promoted to the next generations.

In our research for DHCAP and NVA 11 torsion angles were defined (Fig. 7) and listed in Table 5. The GA calculations with MMFF94 force field were carried out using two different dielectric constants (ε = 1.0, ε = 4.0). The higher ε value mimics the more polar surrounding of the molecule. The purpose was to verify how electrostatic interactions affect the optimal arrangements of the aliphatic chain. A set of low-energy conformers was obtained differing by ca. 1 kcal mol⁻¹ (genetic algorithm procedures yielded ca 30 low-energy conformers for each compound). Then shielding constants calculation was carried out using DFT methods for each conformation. The calculated NMR shielding constants were converted into chemical shifts, allowing easier comparison with experimental data. The next step was to verify the agreement between experimental solid-state and theoretical NMR data (mainly in aliphatic chain region). Selected results are collected in Tables 3 and 4.

For each dielectric constant the best conformation was chosen (based on R²-tail values). Energies and dihedral angles of selected conformers are listed in Table 5.

Shielding constants are usually recalculated to chemical shifts by comparison to theoretical shielding constant of TMS (δiso = σTMS − σiso). However, there is a number of publications describing the converting of shielding constants with scaling.

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**Table 3** Chemical shifts calculated by GIAO DFT for selected DHCAP conformers from GA (R²-tail shows correlation to experimental ¹³C CPMAS data of aliphatic chain carbons, E1, E4 – dielectric constants, 01, 02… – conformer number)

|     | E1-01 | E1-03 | E1-04 | E1-06 | E1-08 | E4-01 | E4-03 | E4-05 | E4-07 | E4-08 |
|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| C1  | 141.00| 141.30| 139.14| 141.05| 140.99| 140.38| 141.28| 138.10| 140.73|
| C2  | 116.36| 116.14| 113.71| 116.24| 115.31| 116.76| 117.99| 115.89| 114.13| 115.86|
| C3  | 154.14| 154.43| 154.27| 154.17| 154.69| 154.49| 154.20| 154.25| 152.45| 154.14|
| C4  | 155.12| 153.41| 155.19| 155.27| 155.25| 154.99| 153.91| 154.71| 154.56| 155.81|
| C5  | 118.71| 118.49| 119.79| 119.57| 119.65| 119.40| 119.15| 118.97| 121.57| 119.18|
| C6  | 126.56| 126.38| 128.08| 126.62| 126.56| 126.75| 126.73| 126.38| 128.23| 126.32|
| C7  | 47.81 | 47.82 | 46.29 | 47.87 | 47.54 | 47.79 | 48.89 | 47.63 | 48.47 | 47.44 |
| C8  | 57.37 | 57.07 | 56.80 | 56.92 | 56.39 | 57.54 | 57.07 | 57.28 | 57.03 | 56.95 |
| C1  | 178.39| 178.72| 176.74| 179.03| 178.31| 178.35| 179.22| 178.90| 177.29| 178.91|
| C2  | 42.16 | 43.16 | 40.68 | 36.32 | 38.94 | 41.99 | 41.46 | 43.02 | 43.00 | 39.77 |
| C3  | 34.10 | 33.31 | 30.74 | 25.75 | 24.80 | 34.29 | 28.80 | 30.48 | 32.87 | 26.14 |
| C4  | 34.26 | 34.95 | 31.90 | 31.16 | 29.05 | 32.42 | 38.73 | 35.93 | 34.17 | 29.59 |
| C5  | 37.70 | 36.82 | 31.19 | 27.95 | 30.45 | 36.32 | 35.51 | 36.08 | 36.34 | 29.55 |
| C6  | 34.50 | 33.98 | 30.87 | 28.98 | 28.10 | 33.48 | 36.24 | 32.79 | 32.90 | 28.04 |
| C7  | 44.88 | 43.10 | 39.72 | 43.15 | 41.59 | 42.48 | 44.50 | 45.62 | 45.02 | 41.67 |
| C8  | 36.04 | 36.59 | 29.77 | 31.45 | 36.65 | 36.82 | 36.02 | 36.45 | 35.62 | 36.34 |
| C9  | 22.14 | 26.44 | 20.89 | 20.79 | 22.17 | 22.69 | 26.42 | 26.34 | 22.51 | 22.02 |
| C10 | 26.98 | 22.58 | 26.71 | 26.50 | 26.99 | 26.31 | 22.28 | 22.30 | 28.45 | 26.87 |

R²-tail 0.907 0.878 0.924 0.841 0.723 0.857 0.822 0.911 0.934 0.758

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Fig. 7 Torsion angles of (a) DHCAP and (b) NVA selected for GA calculations.
The dihedral angles (°) and energy values (kcal mol⁻¹) calculated with MMFF94 force field at two different dielectric constants (ε = 1.0, ε = 4.0) obtained for GA by the low-energy conformers of DHCAP and NVA

| Torsion angle | DHCAP-E1 | DHCAP-E4 | NVA-E1 | NVA-E4 |
|---------------|----------|----------|--------|--------|
| α (°)         | −0.5     | 1.5      | −0.9   | 1.8    |
| β (°)         | −0.4     | 0.4      | −0.4   | 0.5    |
| γ (°)         | −69.6    | 118.2    | −75.2  | 112.7  |
| θ (°)         | 125.2    | 146.7    | 93     | 155.9  |
| i (°)         | 146.3    | 97.5     | 137.7  | −133.5 |
| λ (°)         | −67.8    | 66.7     | −67.6  | −174.8 |
| μ (°)         | −177.5   | 176      | −66.8  | −177   |
| ν (°)         | 178      | 66.5     | −179.6 | 66.3   |
| ξ (°)         | 64.5     | 176      | 179.9  | −176.4 |
| τ (°)         | 60.7     | 175.3    | −179.7 | −180   |
| ΔE (kcal mol⁻¹) | 1.28    | 2.43     | 0.08   | 1.1    |
| R² | 0.997 | 0.997 | 0.999 | 0.999 |
| R²-tail | 0.924 | 0.934 | 0.914 | 0.947 |
| MAE (TMS) | 4.08 | 5.34 | 4.53 | 4.87 |
| MAE (SF) | 2.62 | 2.00 | 1.86 | 1.45 |
towards the methoxyl group (E1), which may cause steric hindrance, whereas in the second – in the opposite direction (E4). The hindrance should influence the CP kinetic parameters. The CP parameters of DHCAP for both CH₃ and OCH₃ group are longer than CP parameters of CAP. It may suggest increased mobility of these groups, which is possible in the absence of the steric hindrance (E4). Summarized GA and CPMAS NMR data recommend that the most probable conformation of DHCAP is DHCAP E4. It is bent as capsaicin conformation, but in the opposite direction.

Both GA conformers of NVA, which have the best fitting parameters to experimental NMR data, have different geometries of aliphatic chain. The NVA-E1 has a bent and the NVA-E4 extended aliphatic chain. Split resonances (doublets) in ¹³C and ¹⁵N MAS NMR spectra suggest that solid NVA sample contains two different molecules in the crystallographic unit. Taking into account the conformers obtained by GA, we suppose that the molecules coexisting in the solid may differ by the orientation of aliphatic chain. However, to confirm such assumption crystallographic data are required.

Conclusions

The solid-state structure of DHCAP and NVA were investigated. Since there is no crystallographic data and single crystals suitable for XRD were not obtained, ¹³C and ¹⁵N MAS NMR supported by genetic algorithm and GIAO DFT calculations were used for solid state structural studies. To the best of our knowledge this work is the first that focuses on solid state of DHCAP and NVA. DHCAP shows numerous similarities in NMR and GA data to those obtained for CAP. A bent aliphatic chain is probable in solid DHCAP, however the chain is not close to the methoxyl group. The molecular network of capsaicinoids is not stabilized by NH····O═C intermolecular hydrogen bonds, more probable is OH····O═C interaction in DHCAP and CAP but not in NVA. Solid state of NVA is characterized by the presence of two conformers, with bent and extended aliphatic chain.

Conflicts of interest

There are no conflicts to declare.

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