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Combined infrared spectroscopic and computational study on simpler capsaicin derivatives and their anion intermediates in the scavenging of free radicals

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ABSTRACT

Two capsaicin analogues – N-(4-hydroxy-3-methoxybenzyl)acetamide and N-(4-hydroxy-3-methoxybenzyl) benzamide, were studied by DFT methods in order to estimate their ability to act as antioxidants. A comparative study on the stability of benzylic, phenoxyl and amide radicals has outlined the most reactive site for hydrogen atom abstraction and proton transfer. The enthalpies related to the formation of those species were modeled in gas phase, benzene, DMSO and water in order to determine the most probable mechanism of antioxidant action in polar and nonpolar medium. The formation of phenoxyl anion, energetically favoured in polar medium, was investigated by infrared spectroscopy methods based on the conversion of the benzamide derivative.

1. Introduction

Capsaicin (8-methyl-N-vanillyl-trans-6-nonenamide, **1** in Scheme 1) has been extensively studied as the principle pungent component in the red hot pepper varieties [1] and is a frequently consumed food ingredient. Its structure comprises a vanillylalanine fragment (hydrophilic tail) and a nonenoic acid residue (hydrophobic tail) linked by amide bond (Scheme 1).

Many health-related beneficial effects of capsaicin have been discovered [2–7] which promoted the clinical application of capsaicin to treat diabetic neuropathy, chronic musculoskeletal pain, postherpetic neuralgia, bladder hyper-reactivity, post-operative nausea, vomiting and sore throat, pruritis associated with renal failure and to prevent non-steroidal anti-inflammatory drug induced gastritis [6,7]. Due to the crucial role of oxidative stress in the development of several diseases such as cancer, atherosclerosis, cardiovascular and neurological disorders, the antioxidant properties of capsaicin have also attracted much attention [3,4,8–18]. It has been shown that capsaicin can inhibit lipid peroxidation [8–11], reduce oxidative stress in rat brain homogenate [12] and murine hepatic mitochondrial membranes [13]. Its radical scavenging activity includes hydroxyl, azidyl, peroxyl and glutathiyl radicals [14] as well as stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) and galvinoxyl radicals [18]. On the other hand, the exact mechanism of antioxidant action of capsaicin in different environments has been subject of continuous discussion: it was suggested that in nonpolar medium capsaicin is donating a hydrogen atom either from its C7-benzylic group [8,11,15,16] or phenolic group [9,15,17], while in polar environment radical adduct formation [15,18] and/or sequential proton loss electron transfer are thought to prevail [19]. In this context, further characterization of the anionic intermediates of capsaicin might help to understand fully the reactivity of capsaicin toward free radicals.

Moreover, syntheses of capsaicin derivatives that preserve its beneficial health effects, but reduce its strong pungency, is a very useful approach to develop new therapeutically useful compounds [20–23].

In this relation a combined spectroscopic and computational study was conducted on two simpler capsaicin analogues – N-(4-hydroxy-3-methoxybenzyl)acetamide and N-(4-hydroxy-3-methoxybenzyl)benzamide (**2** and **3** in Scheme 1) containing the same deprotonation sites as capsaicin i.e. vanillylamide fragment. Our goal is to follow in more details the formation of anionic products related to the radical scavenging mechanisms and to explore the potential of the smaller-molecule derivatives to be used as antioxidant replacements of capsaicin. Density functional theory (DFT) computations will be used to predict the radical scavenging ability of the compounds through direct hydrogen atom transfer (HAT), single electron transfer (SET) and sequential proton loss electron transfer (SPLET) mechanism, as well as structural and electronic molecular characteristics related to antioxidant action. The infrared (IR) spectroscopic measurements will be applied to examine experimentally the formation of anion products of N-(4-hydroxy-3-methoxybenzyl)benzamide.

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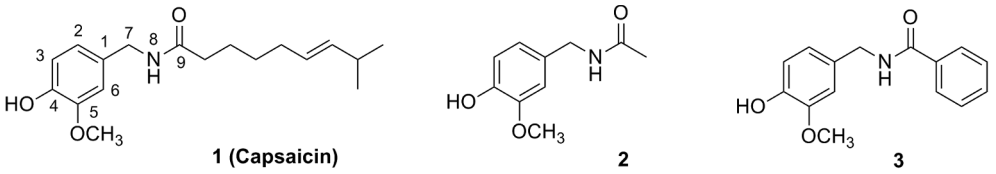


Scheme 1. Capsaicin and model compounds under study.

2. Materials and methods

4-hydroxy-3-methoxybenzylamide hydrochloride (99% purity, Sigma–Aldrich Co), benzyl chloride (99% purity, Sigma–Aldrich Co), chloroform (p.a., Sigma–Aldrich Co) were used as synthetic reagents. CD_3ONa was prepared prior the experiments by reacting spectral grade CD_3OD (99% at. enrichment, Deutero GmBh) with Na and drying the solid product under vacuum. Spectral grade deuterated dimethylsulfoxide (DMSO-d_6) and chloroform ($CDCl_3$) were purchased from Deutero GmBh.

Melting points (mp) were determined using an Büchi B-540 apparatus and were uncorrected. The FTIR measurements were carried out on a Bruker Tensor 27 FT spectrometer. The solid state spectra were measured in attenuated total reflectance (ATR) mode. The spectra in DMSO-d_6 solution were measured in transmittance mode by a 0.129 mm CaF_2 sample cell. All spectra were recorded by accumulating 64 scans at 2 cm^{-1} resolution. ^1H NMR spectra were recorded in $CDCl_3$ solution on a Bruker Avance II+ 600 MHz NMR instrument. The spectra were referred to the solvent signal. Standard Bruker pulse sequences and software were used to record and process the spectra. The reactions were monitored by thin layer chromatography, which was performed on Merck pre-coated plates (silica gel. 60 F254, 0.25 mm) and was visualized by fluorescence quenching under UV light (254 nm).

2.1. Synthesis of N-(4-hydroxy-3-methoxybenzyl)benzamide (3)

The synthesis of compound **3** was carried out by interfacial reaction between vanillylamine hydrochloride and acyl chloride in biphase $H_2O/CHCl_3$ system according to the synthetic procedure provided in [20]. The chemical structure and purity of the compound were confirmed by comparison with the previously reported data [20].

0.150 g (0.79×10^{-3} mol) 4-hydroxy-3-methoxybenzylamine hydrochloride were dissolved in 1.6 ml water and 0.218 g (2.6×10^{-3} mol) $NaHCO_3$ were added to the solution. After stirring the mixture for 30 min at 20 °C, 2.26 ml of chloroform were added. The mixture was stirred for another 15 min and then 0.1 ml $CHCl_3$ solution of benzoylchloride (0.79×10^{-3} mol in 0.62 ml $CHCl_3$) was added dropwise. The mixture was stirred for 30 min, heated to 40 °C, and the organic layer was separated. The water layer was extracted by chloroform (3×0.6 ml). The combined organic layers were washed with 2% HCl solution and dried with anhydrous $NaSO_4$. The solvent was removed under vacuum and the crude product was recrystallized in absolute ethanol.

Yield: 87%; mp 140–142 °C; Rf = 0.676 (C_6H_6:CH_3OH = 3:1); IR (ATR-FTIR): $\nu(cm^{-1})$ 3213 (ν_{N-H}), 3071 ($\nu_{(C-H)Ar}$), 2960, 2917, 2834 ($\nu_{(C-H)Alk}$), 1685 ($\nu_{C=O}$), 1523 ($\delta_{(N-H)}$), 1282, 1272 ($\nu_{(C-O)}$), 1257 ($\nu_{(C-N)}$); ^1H NMR (600 MHz, $CDCl_3$, δ): 7.76 (d, 2H, Ar), 7.50 (t, 1H, Ar), 7.40–7.44 (m, 2H, Ar), 6.90–6.82 (m, 3H, Ar), 6.30 (br s, 1H, OH), 5.60 (s, 1H, NH), 4.55 (d, 2H, CH_2), 3.88 (s, 3H, OCH_3); Analysis: Calc. for $C_{15}H_{15}NO_3$: C, 70.02; H, 5.88; N, 5.44; O, 18.66; Found: C, 70.00; H, 5.85; N, 5.42.

2.2. Conversion of N-(4-hydroxy-3-methoxybenzyl)benzamide into anion 4

DMSO-d_6 solution of N-(4-hydroxy-3-methoxybenzyl)benzamide (0.12 $mol.l^{-1}$) was mixed with excess of dry CD_3ONa and shacked for a couple of minutes. The remains of solid CD_3ONa were filtered out from the reaction mixture and the solution was transferred to the IR spectroscopic cell to record the spectra.

2.3. Computational methods

The computational study of molecular geometry, reaction enthalpies and vibrational spectra was conducted at B3LYP/6-11++G(d,p) level of theory [24–26] implementing the Gaussian 09 suite of programs [24]. The solvent effects were included by Integral Equation Formalism Polarizable Continuum Model (IEF-PCM) [27]. Analytic vibrational frequency computations at the optimized structures were done to confirm the optimized structures as minima on the potential energy hypersurface.

Dissociation enthalpy (BDE), ionization potential (IP), proton dissociation enthalpy (PDE), proton affinity (PA), and electron transfer enthalpy (ETE) of the most stable conformers were calculated at 298 K according the equations provided in the literature [28]:

$$BDE = H(ArO\cdot) + H(H) - H(ArOH)$$

$$IP = H(ArOH^+) + H(e^-) - H(ArOH)$$

$$PDE = H(ArO\cdot) + H(H^+) - H(ArOH^+)$$

$$PA = H(ArO^-) + H(H^+) - H(ArOH)$$

$$ETE = H(ArO\cdot) + H(e^-) - H(ArO^-)$$

The enthalpy of hydrogen atom, H(H), in the respective solvents were obtained using the same functional and basis set. Solvation enthalpies of proton, $H(H^+)$, and electron, $H(e^-)$, were applied in accordance with previously estimated values [29].

3. Results and discussion

3.1. Computational study on the structural properties of capsaicin and its analogues related to their radical scavenging activity

The most stable geometry of the neutral compounds and their benzylic and phynoxyl radical, radical-cationic and anionic species was determined by energy analysis. Having in mind the reports on radical scavenging activity exerted by N—H compounds [29–33] and their easy deprotonation [34–37], N-centered radicals as well as azanions (deprotonated at the amide N-atoms) were included in our study. Several possible conformers resulting from the rotation around the single bonds in the studied species were fully optimized at IEFPCM-B3LYP/6-311++G** level of theory in gas phase, benzene, water and DMSO.

The optimization showed that the geometry of vanillylamide moiety would not be substantially affected by the replacement of the octanyl fragment of capsaicin with methyl group or phenyl ring.

The total energies (E_{tot}) and relative energies (ΔE) with respect to the most stable forms of the benzylic C7-centered radicals, phenoxyl and amide radicals of the three compounds are summarized in Table 1. The relative energies of the studied radicals in gas phase, benzene and polar solvents pointed out that the formation of benzylic radical is thermodynamically favored not only for capsaicin **1**, but also for the two model compounds **2** and **3** in any kind of medium.

However, as the calculated relative energies of the benzylic and phenoxyl radicals are within the range 7–16 $kJ.mol^{-1}$, the simultaneous formation of both radicals is also plausible. The experimental studies so far have led to the conclusion that capsaicin reacts via C7-benzylic radical in nonpolar and neutral medium (pH 7) [8,11,16],

Table 1
Total energies Etot (in Hartrees) and relative energies in respect to the most stable form ΔE (in kJ.mol^{-1}) of neutral molecules, radicals, radical-cations and anions of capsaicin, compounds **2** and **3** in gas phase and various solvents.

	Compound **1**		Compound **2**		Compound **3**	
	E_{tot}	ΔE	E_{tot}	ΔE	E_{tot}	ΔE
Gas						
Molecule	−982.8825786		−669.521168		−861.3022542	
Radical C7$^{\cdot}$	−982.2451089		−668.8823741		−860.665	
Radical O$^{\cdot}$	−982.2415125	9.44	−668.8775194	12.74	−860.658807	16.26
Radical N$^{\cdot}$	−982.2040034	107.90	−668.8422505	105.32	−860.627	99.75
Radical cation C$^{\cdot+}$	−982.6137706		−669.2491953		−861.0328119	
Anion O^{-}	−982.3272719		−668.9646589		−860.7466685	
Anion N^{-}	−982.306263	55.15	−668.9427641	57.47	−860.7350195	30.58
Anion C^{-}	−982.275846	134.99	−668.9114060	139.79	−860.7045365	110.60
Benzene						
Molecule	−982.8892		−669.527		−861.308	
Radical C7$^{\cdot}$	−982.252		−668.8859		−860.672	
Radical O$^{\cdot}$	−982.2482523	9.84	−668.883	7.61	−860.66580	16.28
Radical N$^{\cdot}$	−982.209	112.88	−668.8471	101.85	−860.632	105.00
Radical cation C$^{\cdot+}$	−982.650		−669.2893		−861.071	
Anion O^{-}	−982.3722084		−669.0102		−860.792	
Anion N^{-}	−982.351	55.67	−668.9893	54.86	−860.778	36.75
Anion C^{-}	−982.318	142.30	−668.956	143.31	−860.74537	
DMSO						
Molecule	−982.8983496		−669.5353		−861.3168	
Radical C7$^{\cdot}$	−982.261		−668.899		−860.681	
Radical O$^{\cdot}$	−982.2574854	9.23	−668.894	13.12	−860.67546	14.54
Radical N$^{\cdot}$	−982.217	115.50	−668.854	118.12	−860.632	128.63
Radical cation C$^{\cdot+}$	−982.6867		−669.3212		−861.104	
Anion O^{-}	−982.4143856		−669.0526		−860.8331266	
Anion N^{-}	−982.394965	50.98	−669.031	56.70	−860.81723	41.74
Anion C^{-}	−982.358787	145.95	−668.997	146.37	−860.792	107.96
Water						
Molecule	−982.8986		−669.535562		−861.317	
Radical C7$^{\cdot}$	−982.261		−668.8982		−860.681	
Radical O$^{\cdot}$	−982.25776	8.51	−668.8945	9.71	−860.675755	13.77
Radical N$^{\cdot}$	−982.217	115.50	−668.8539	116.29	−860.6383014	112.08
Radical cation C$^{\cdot+}$	−982.688		−669.3220		−861.105	
Anion O^{-}	−982.415		−669.0537		−860.834	
Anion N^{-}	−982.396	49.88	−669.0322	56.44	−860.818	42.00
Anion C^{-}	−982.360	144.37	−668.9968	149.36	−860.785	128.62

whereas both C7-benzylic radical and C4-phenoxyl radical are formed in alkaline medium (pH > 7) [9,15,17].

Spin density on the C-atom in the benzyl radicals of **2** and **3** is only slightly increased in comparison to capsaicin which indicates an effective odd electron delocalization over the species and good stability of both radicals (Fig. 1).

In contrast to the radical intermediates, the oxyanions (deprotonated at the phenoxyl O-atoms) of the three compounds are much more stable than the respective azanions and carbanions (Table 1). For capsaicin and the acetamide analogue **2**, deprotonation is expected to occur exclusively from the hydroxyl group. For the benzamide derivative **3** the possibility for resonance stabilization by conjugation of the azanion with the phenyl ring results in a smaller energy difference between the oxyanion and the azanion. However, deprotonation of the hydroxyl groups is the most favorable in this case too.

The formation of phenoxyl anion by capsaicin has been recently discussed in connection to the accelerated rate of capsaicin reaction with DPPH and galvanoxyl radicals in water containing medium [19]. Taking into account the pH dependence of the rate constant, the authors proposed that capsaicin deactivates the free radicals by SPLET from the phenolic hydrogen [19]. Further, the authors showed that 2-methoxy-4-methyl phenol reacted with DPPH and galvanoxyl radical in a similar way to capsaicin which confirmed the essential role of the vanillylamide moiety for the radical scavenging of capsaicin [19].

Based on the structural characteristics presented above, it could be expected that the ability of **2** and **3** to donate a hydrogen atom or a

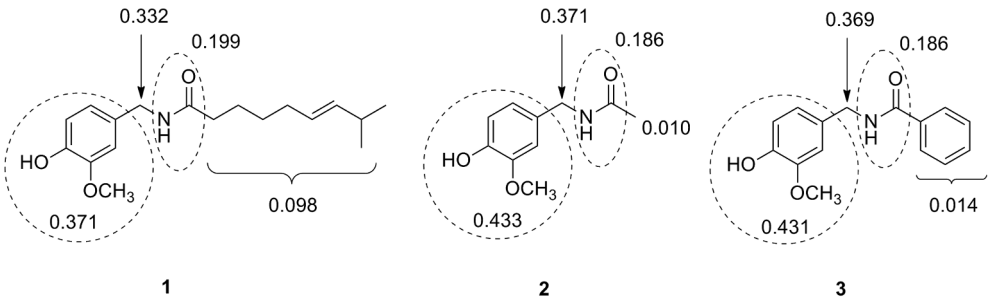


Fig. 1. Distribution of the spin densities over molecular fragments of capsaicin **1** and its analogues **2** and **3**.

Table 2
Calculated bond dissociation enthalpy, ionization potential, proton dissociation enthalpy, proton affinity, and electron transfer enthalpy values of capsaicin **1** and its analogues **2** and **3** in kJ.mol^{-1}.[1]

	HAT mechanism		SET mechanism		SPLET mechanism	
Species	BDE (C7-H)	BDE (O—H)	IP	PDE	PA (O—H)	ETE
Gas phase						
1	362	371	709	983	1464	228
2	365	377	717	982	1467	232
3	361	377	710	989	1465	234
Benzene (2.28)[a]						
1	367	376	623	168	469	321
2	378	381	621	174	470	326
3	365	381	620	176	468	327
DMSO (47.24)[a]						
1	365	375	475	18	162	331
2	364	376	481	12	158	335
3	362	376	477	17	161	333
Water (80.10)[a]						
1	356	366	452	112	253	312
2	357	366	459	94	249	304
3	354	367	455	111	252	314

[a] Relative dielectric permittivity [41] and references therein.

proton would be comparable to that of capsaicin. To ascertain these assumptions, the bond dissociation enthalpies, proton affinities and other antioxidant reaction enthalpies of **2** and **3** were calculated in gas phase and liquid media (Table 2). For this purpose, IEFPCM-B3LYP/6-311 + + G** level of theory was used as it provides sufficiently accurate estimation of the reaction enthalpies at lower computational cost [28,38,39]. The chosen level of theory will allow us to compare the reactivity of the capsaicin derivatives with other previously studied vanilloid compounds such as vanillin and syringaldehyde [40]. Calculated bond dissociation enthalpies (BDE), ionization potentials (IP), proton dissociation enthalpies (PDE), proton affinities (PA), and electron transfer enthalpies (ETE) of **1–3** are collected in Table 2.

The data summarized in Table 2 outlined HAT from the C7—H bond as the main mechanism of radical scavenging for the three compounds in nonpolar medium. In order to estimate the reactivity of compounds **2** and **3** toward different free radicals, the BDEs of (Z)-4-hydroperoxyhex-2-ene, (Z)-4-hydroxyhex-2-ene, methyl hydroperoxide, methanol, hydrogen peroxide, and water were calculated in benzene at the same level of theory. The obtained BDE values are as follows: 339 kJ.mol^{-1} ((Z)-4-hydroperoxyhex-2-enyl radical), 344 kJ.mol^{-1} ($CH_3OO^{\cdot}$), 353 kJ.mol^{-1} ($HOO^{\cdot}$), 424 kJ.mol^{-1} ((Z)-4-hydroxyhex-2-enyl radical), 418 kJ.mol^{-1} ($CH_3O^{\cdot}$), and 489 kJ.mol^{-1} ($HO^{\cdot}$). Comparing these values with the C—H and O—H BDE reported in Table 2, it could be concluded that the capsaicin derivatives might efficiently scavenge lipid alkoxyl, methoxyl and hydroxyl radicals via HAT from two sites, namely the benzyl and hydroxyl group.

It should be noted that despite the fact the O—H BDE values of **1–3** are higher than the O—H BDE value of vanillin (361 kJ.mol^{-1} in benzene) [40], a similar antioxidant capacity might be expected for them as a results of hydrogen atom donation from the benzylic C—H bonds characterized by C—H BDEs close to that of vanillin O—H group. A good advantage of the capsaicin derivatives is their ability to react simultaneously with two free radicals.

In support to the experimental evidences [19], the DFT calculated O—H proton affinities of capsaicin and its derivatives indicate the SPLET mechanism as considerably favored over HAT in polar medium. Based on the higher values of the ionization potentials of **1–3**, SET mechanism is not expected to occur neither in nonpolar nor in polar medium.

The importance of the anionic intermediates for the reactivity toward free radicals prompted us to conduct an experimental study on the conversion of vanillylaminde compounds into anion products. For this purpose, N-(4-hydroxy-3-methoxybenzyl)benzamide (**3**) was synthesized and the changes accompanying the ionization were followed by IR spectroscopy.

3.2. Synthesis of N-(4-hydroxy-3-methoxybenzyl)benzamide (3) and conversion into anion (4)

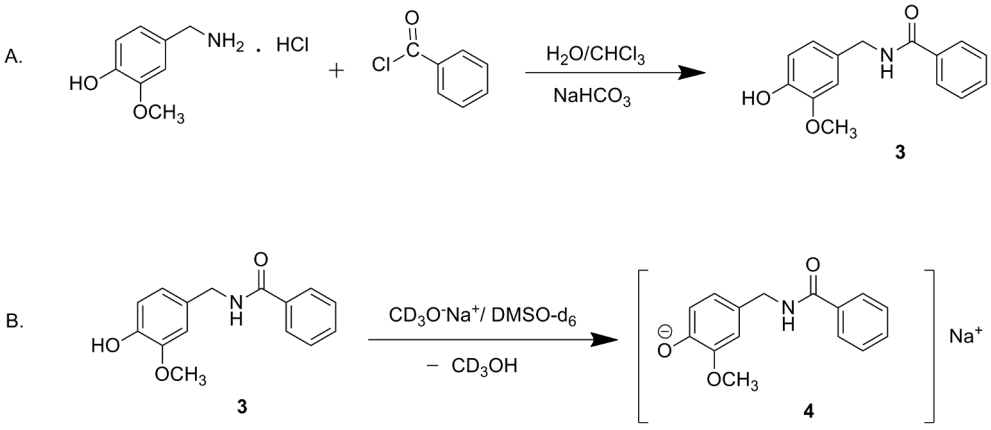
The target compound was synthesized by a fast and efficient method relying on the condensation of acyl chloride and vanillylamine in biphase $H_2O/CHCl_3$ system [20] (Scheme 2A).

Compared to other synthetic routes for preparation of capsaicin derivatives [21–23], this method offers a convenient way to enhance the reagents solubility, reduce the products of the side reactions, i.e. esterification of the vanillyl hydroxyl group or hydrolysis of the acyl chloride and increase the reaction yields [20]. Conversion of **3** into anion product was achieved in DMSO-d_6 solution by treatment with freshly prepared CD_3ONa (Scheme 2B).

3.3. Infrared spectral and structural changes accompanying the conversion of 3 into anion 4

The IR spectrum of **3** in DMSO-d_6 before treatment with CD_3ONa i.e. the neutral parent compound is displayed in Fig. 2 in dashed line. The experimental frequencies for **3** are listed together with the theoretical vibrational characteristics in Table S1 in Supplementary material. The agreement between the experimental and calculated values is very good – the mean absolute deviation between observed and calculated frequencies is 6.4 cm^{-1}.

In DMSO-d_6 it is difficult to determine exact position of the O—H



Scheme 2. Synthesis of N-(4-hydroxy-3-methoxybenzyl)benzamide **3** (A) and conversion into anion **4** (B).

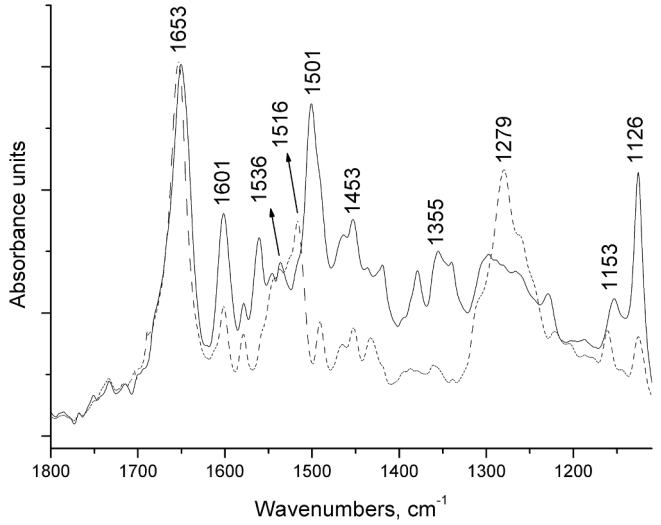


Fig. 2. Infrared spectra of the neutral parent compound **3** and its anion product **4** in deuterated dymethylsulfoxide: before the treatment with deuterated sodium methoxide (dashed line) and after the treatment (solid line).

stretching band because of the appearance of a multiplet due the formation of hydrogen bonds mainly with solvent. Therefore, it was not included in Table S1.

The DFT predicted ν(C═O) frequency is close to that measured at 1653 cm^{-1} in DMSO-d_6. In a qualitative agreement between theory and experiment, the ν(C═O) band (Amide-I) is the strongest one in the IR spectrum (Table S1).

The IR frequency of δ(HNC) of **3** in DMSO-d_6, denoted as Amide-II vibration, is also accurately estimated. It is expected to appear at 1521 cm^{-1} as a very intense band and accordingly a strong band was detected experimentally at 1516 cm^{-1}.

The second strongest band in the spectrum - measured at 1279 cm^{-1} (predicted at 1266 cm^{-1}) is assigned to ν(Ph−OCH_3) coupled with ν(Ph−OH). According to the calculation the stretching ν(N−C) coupled with ν(Ph−H), denoted as Amide-III, was predicted as an intensive band at 1259 cm^{-1}. In the experimental spectrum it appears around 1260 cm^{-1} overlapped with the stronger band at 1279 cm^{-1}. A second vibration of mixed ν(Ph−OCH_3)/ν(Ph−OH) character was predicted at 1232 cm^{-1} with predominant participation of the ν(Ph−OH) mode. The corresponding experimental band is found at 1221 cm^{-1} in DMSO-d_6. The band position is close to the values reported for phenol [42] and syringaldehyde [40].

The IR spectrum in DMSO-d_6 after the treatment of **3** with CD_3ONa is shown in Fig. 2 in solid line. Notably, the conversion into anion did not shifted the carbonyl stretching band which indicates that deprotonation has occurred from the phenol group, and did not affected the amide group (Scheme 2B). Hence, in order to assign the experimental IR bands to the corresponding vibrational modes the observed

Table 3
Theoretical and experimental bond lengths (in Å) and bond angles (in °) of compound **3** and its oxyanion **4**.

	Molecule			Anion	
	Experimental[a]	Theoretical	Δ[b]	Theoretical	∇[c]
Bond lengths					
R(C^1,C^2)	1.382	1.392	−0.010	1.403	−0.011
R(C^2,C^3)	1.379	1.398	−0.019	1.388	0.010
R(C^3,C^4)	1.383	1.385	−0.002	1.439	−0.054
R(C^4,C^5)	1.388	1.409	−0.021	1.455	−0.046
R(C^5,C^6)	1.377	1.387	−0.010	1.384	0.004
R(C^1,C^7)	1.508	1.515	−0.007	1.500	0.015
R(C^7,N^8)	1.467	1.466	0.001	1.484	−0.018
R(N^8,C^9)	1.324	1.363	−0.039	1.349	0.014
R(C^9,C^{10})	1.484	1.506	−0.022	1.515	−0.009
R(C^{10},C^{11})	1.378	1.400	−0.022	1.399	0.001
R(C^{11},C^{12})	1.381	1.393	−0.012	1.392	0.002
R(C^{12},C^{13})	1.376	1.394	−0.018	1.395	−0.002
R(C^{13},C^{14})	1.375	1.395	−0.020	1.394	0.001
R(C^{14},C^{15})	1.386	1.399	−0.013	1.394	0.005
R(C4,O16)	1.369	1.363	0.006	1.269	0.094
R(C^5,O^{17})	1.381	1.372	0.0130	1.391	−0.023
R(O^{17},C^{18})	1.422	1.425	−0.003	1.425	0.000
R(C^9,O^{19})	1.249	1.227	0.022	1.231	−0.005
Bond angles					
A(C^1,C^2,C^3)	120.78	120.91	−0.13	121.61	−0.70
A(C^2,C^3,C^4)	120.44	119.93	0.51	123.36	−3.43
A(C^3,C^4,C^5)	119.02	119.56	−0.54	113.35	6.21
A(C^4,C^5,C^6)	119.99	120.40	−0.41	122.40	−2.00
A(C^5,C^6,C^1)	121.20	120.06	1.14	122.24	−2.18
A(C^1,C^7,N^8)	112.81	113.78	−0.97	111.52	2.26
A(C^7,N^8,C^9)	122.89	122.78	0.11	124.20	−1.42
A(N^8,C^9,C^{10})	119.86	116.65	3.21	116.30	0.35
A(C^9,C^{10},C^{11})	123.85	123.34	0.51	123.59	−0.25
A(C^{10},C^{11},C^{12})	120.58	120.96	−0.38	120.66	0.30
A(C^{11},C^{12},C^{13})	119.92	120.26	−0.34	120.13	0.13
A(C^{12},C^{13},C^{14})	120.16	119.45	0.71	119.63	−0.18
A(C^{13},C^{14},C^{15})	119.68	120.26	−0.58	120.18	0.08
A(C^3,C^4,O^{16})	123.92	120.27	3.65	123.43	−3.16
A(C^4,C^5,O^{17})	115.08	113.82	1.26	120.60	−6.78
A(C^5,O^{17},C^{18})	117.73	118.43	−0.7	115.33	3.10
A(N^8,C^9,O^{19})	120.99	122.40	−1.41	123.34	−0.94

[a] See Ref.[45]; [b] Algebraic deviations (Å, degrees) between experimental and theoretical values; [c] Algebraic deviations (Å, degrees) between theoretical values of the anion and molecule; For atom numbering see Fig. 3.

numerical values were compared with those calculated for the oxyanion **4** (Table S2). The influence of the Na^+ counterion on the oxyanion frequencies might be neglected as it is known that in DMSO solvent the ions exist as free species and there are no anion/counter ion interactions [43]. As above a good agreement between the experimental and scaled theoretical frequencies, presented in Table S2 in Supplementary material, was found – mean deviation is 9.3 cm^{-1}. In contrast to other studied oxyanions of amide compounds where the oxyanion center is directly conjugated with the amide group [44], the spectral changes

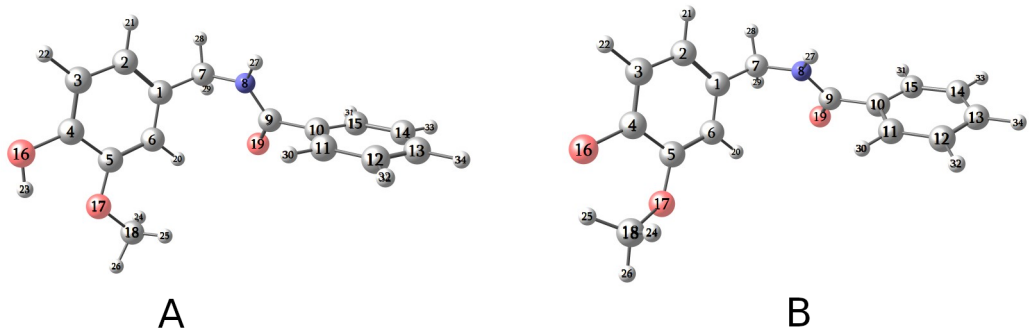


Fig. 3. Optimized structure of the most stable conformers of molecule **3** (A) and its oxyanion **4** (B).

accompanying the conversion of **3** into oxyanion are less pronounced and affect mainly the hydroxyphenyl moiety.

In full agreement between theory and experiment, conversion of the molecule into oxyanion has only a weak effect on the ν(C=O) frequency: predicted decrease 9 cm^{-1}, measured 3 cm^{-1} (Tables S1 and S2). The conversion causes also a decrease in the δ(HNC) (Amide-II) frequency: predicted 13 cm^{-1}, measured 15 cm^{-1} (Tables S1 and S2).

Removing the proton from the hydroxyl group of the oxyanion leads to a shift of the ν(Ph–O) coordinate to higher frequency (predicted at 1493 cm^{-1}), obviously due to the significant shortening of the Ph–O bond. This frequency is very close to that of the intense Amide II vibration. In the experimental spectrum the band is observed as a shoulder at 1490 cm^{-1} (Fig. 2).

As a result of the ionization, the aromatic skeletal bands of the phenolate ring ν(Ph−H) in the region 1600–1500 cm^{-1} and below 1200 cm^{-1} become more intensive than these in the spectrum of the neutral molecule.

The excellent agreement between the experimental and B3LYP/6-311++G** calculated vibrational frequencies obtained in our study is a sign that a reliable description of the structural parameters of the neutral molecule and oxyanion of **3** was achieved at the chosen level of theory. The optimized geometry parameters of the compound **3**, i.e. bond lengths and bond angles, computed at the B3LYP/6-311++G** level were also compared with those determined by X-ray crystallography [45] in Table 3. As it could be seen there, a good correspondence is found between the theoretical and experimental bond lengths and bond angles. The m.d. are 0.014 Å for bond lengths and 0.96° for bond angles.

The strongest structural variations concern the C−O bond and C−C bonds in the vanillyl fragment. As evidenced by the IR spectral studies, the conversion into oxyanion has led to lengthening of the C4−O bond, while the bond lengths of C3−C4 and C4−C5 have deceased. The bond lengths in the amide fragment were not significantly altered.

4. Conclusions

The steric structure of the molecules, radicals and anion intermediates of two simpler capsaicin analogues – N-(4-hydroxy-3-methoxybenzyl)acetamide and N-(4-hydroxy-3-methoxybenzyl)benzamide were studied by DFT computations and compared with those of capsaicin. Based on the relative energies of the studied species it was found that the formation of benzylic radical is thermodynamically favored not only for capsaicin, but also for the two model compounds **2** and **3** in any kind of medium, while the oxyanions of the three compounds are much more stable than the respective azanions and carbanions.

The DFT calculated enthalpies for HAT, SET, and SPLET mechanisms showed that the two capsaicin derivatives will react with free radicals by radical scavenging mechanisms similar to that of capsaicin itself thus supporting the essential role of the vanillyl amide fragment for the antioxidant properties of capsaicin. Moreover, this result suggested that these two smaller derivatives avoiding the capsaicin pungency have good potential to be used as antioxidant agents. Based on the calculated reaction enthalpies, in nonpolar medium the most favorable mechanism would be HAT from the benzylic groups, while in polar medium SPLET mechanism would prevail. In order to characterize in more details the anion intermediate involved in the SPLET mechanism, N-(4-hydroxy-3-methoxybenzyl)benzamide was converted into anion in DMSO-d_6 solution. The spectroscopic measurement demonstrated that deprotonation of the hydroxyl group has occurred in these conditions and a phenoxyanion was formed in accordance with the ionization mechanism suggested from the kinetic studies of capsaicin in polar medium. The resulting structural and spectral changes were described by means of IR spectroscopy and DFT computations.

CRediT authorship contribution statement

D. Yancheva: Conceptualization, Writing - original draft, Writing - review & editing. **S. Stoyanov:** Investigation. **K. Anichina:** Investigation. **S. Nikolova:** Investigation. **E. Velcheva:** Investigation, Formal analysis, Writing - original draft. **S. Stamboliyska:** Investigation, Formal analysis, Visualization, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemphys.2020.110763.

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