Published in final edited form as:

Int J Mass Spectrom. 2019 April ; 438: 107–114. doi:10.1016/j.ijms.2019.01.002.

Protonation of Curcumin Triggers Sequential Double Cyclization in the Gas-Phase: An Electrospray Mass Spectrometry and DFT Study

June Cyriac¹, Justin Paulose¹, M. George¹, R. Srinivas², Daryl Giblin³, and Michael L. Gross³

⁽¹⁾Department of Chemistry, Sacred Heart College, Thevara, Kochi, Kerala, India

⁽²⁾National center for Mass Spectrometry, IICT, Hyderabad, India

⁽³⁾Department of Chemistry, Washington University in St. Louis, St. Louis, MO 63130

Abstract

ESI-protonated natural curcumin (1) undergoes gas-phase cyclization and dissociates via competitive expulsions of 2-methoxy phenol and C₄H₄O₂ (diketene or an isomer). Evidence from mechanistic mass spectrometry and from Density Functional Theory (DFT) reveals that a two-step sequential cyclization occurs for the protonated molecule prior to the unusual loss of the elements of 2-methoxy phenol. Furthermore, the presence of the methoxy group at position-3 is essential for the second cyclization. The transformation of curcumin upon protonation in the gas phase may be predictive of its solution chemistry and explain how curcumin plays a protective role in biology.

Graphical abstract

Introduction

Curcumin is a yellow, polyphenolic pigment isolated from the rhizomes of the spice turmeric (*Curcuma longa*). Curcumin exhibits a variety of biological activities including those of an antioxidant, anticancer compound, and a mediator in A β amyloid formation with important

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

implications in Alzheimer's disease [1–5]. The mechanism of antioxidant activity of this 1,3-diketone (**1**), containing two phenolic OH groups and two double bonds, has been the subject of many experimental [6–9] and theoretical investigations [10, 11]. To determine the relationship between the structure and antioxidant activity of curcumin, investigators have focused on the role of the *phenolic*-OH group, the *enol*-OH group, and double bond [12]. The instability of curcumin under biological pH conditions [13] has led to the hypothesis that its degradation products [14] and metabolites [15, 16] may be the means whereby curcumin exhibits its biological activity. The bio-transformations of curcumin, although not fully understood, may produce metabolites including dihydrocurcumin and tetrahydrocurcumin that are biologically active [17]. Additional transformation products of autoxidation and double cyclization of curcumin are known and likely to contribute to its pharmacological activities [18].

The consumption of turmeric seems to reduce the risk of some cancers and provide salutary biological effects that correlate with curcumin content [19]. Of the three analogs present in *Curcuma longa*, curcumin (**1**) is the most biologically active component compared to the demethoxy- (**2**) and bisdemethoxy analogs [20, 21]. It is essential to understand the possible acid-catalyzed rearrangements of curcumin given that the digestive juices of stomach are at low pH. Moreover, the capability of curcumin to serve as an antioxidant is high [2] at low pH, suggesting a role for protonation of the molecule in its biological chemistry. Determining the outcome of protonation is an opportunity for electrospray ionization and mass spectrometry, which can bring a deeper understanding of this biologically important food substance and supplement.

Electrospray ionization (ESI) generates protonated/deprotonated molecules and introduces them to the gas-phase. The unimolecular transformations of both $[M + H]^+$ and $[M - H]^-$ ions and the mechanism of these reactions have been studied by various experimental approaches, including tandem mass spectrometry and DFT calculations. In fact, acid-catalyzed transformations of organic molecules in solution can be predicted on the basis of mass spectrometric fragmentations of protonated molecules [22, 23]. The use of mass spectrometer for studying various chemical reactions has led to the suggestion that it is “complete chemical laboratory” [24]. Related studies carried out in our laboratory are of 2-methoxychalcone [25], *N*-(2-nitrophenyl)alanine [26] and bis-(2-methoxybenzal)acetone [27], and these investigations demonstrate that unimolecular cyclization reactions upon ESI protonation have counterparts in solution and suggest that ESI MS in combination with DFT is an effective approach for predicting acid-catalyzed cyclizations of organic molecules in solution.

We report here an investigation of the possible rearrangements of protonated curcumin (**1**), mono-methoxycurcumin (**2**), and the synthetic analogs **3** and **4** by ESI mass spectrometry and DFT calculations. Our hypothesis is that any rearrangement occurring in the mass spectrometer also occurs in solution and provides insight on the biological activity of curcumin. LC/MS [28] and NMR [29] already show that natural curcumin containing a 1,3-diketone with conjugated double bonds, exists as a stable enol. The enol form of curcumin, upon protonation, may undergo cyclization analogous to a Nazarov reaction [30]. The collisionally-activated decomposition (CAD) product-ion mass spectrum of ESI-produced

curcumin was reported previously, but without any supporting theoretical calculations [31], and the proposed mechanisms involved forbidden and other high-energy processes. There are no reports regarding a solution rearrangement of curcumin under acidic conditions although the oxidative cyclizations of curcumin [18] and mono-methoxycurcumin [14] in buffer solutions at pH 7.4 were noted.

Experimental

Synthesis:

Natural curcumin (**1**) and mono-methoxy curcumin (**2**) were extracted from turmeric powder using acetone and purified by column chromatography on silica gel using (1:1) mixture of ethyl acetate and hexane as the eluent. Compounds **3** and **4** were synthesized from acetylacetone and the appropriate benzaldehyde by employing literature procedures [32]. The structures of the compounds were confirmed by ¹HNMR spectrum and accurate-mass MS data.

Mass Spectrometry:

Formation of the positive-ion precursors, via protonation, was achieved by ESI from 1:1 acetonitrile/water by direct infusion (5 μ L/min). Low resolving-power MS and CA (MS/MS and MS³) were with a Thermo LCQ Deca Ion-Trap (San Jose, CA) as survey experiments. Analogous high resolving-power experiments were performed with a Thermo LTQ-Orbitrap mass spectrometer operated in the positive-ion mode (RP for product ions was 30000 for both MS/MS and MS³). To track fragmentation, acidic H atoms were replaced with D, in a 1:1 D₂O/acetonitrile mixture and introduced by direct infusion, ionized by ESI, and analyzed.

Theoretical Calculations:

Theoretical calculations characterized potential-energy surface (PES) associated with fragmentation: Conformer space for precursors and intermediates were initially explored by Monte-Carlo/MMFF and then by the PM3 semi-empirical [33] algorithm. Scans for structures of associated transition states were explored by using the PM3 semi-empirical algorithm (Spartan for Linux: Wavefunction, Inc.), and if necessary, augmented by DFT: B3LYP/6-31G(d,p). Minima and transition states were re-optimized by DFT (Gaussian 98/03/09 suites) to B3LYP/6-31G(d,p) and confirmed by vibrational-frequency analysis. Transition-state connections were determined by examination, projections along normal reaction coordinates, or path calculations as necessary. Single-point energies were calculated at M06/6-311+G(2d,p)//B3LYP/6-31G(d,p) and scaled thermal-energy corrections applied. Results are reported in kJ/mol as enthalpies relative to selected precursor ion. DFT was selected because it requires less computational overhead than ab initio methods and yet incorporates correlation and performs adequately [34]. Optimized geometries are given in the Supplementary Material.

Results and discussion

Mass Spectrometry:

The CAD spectrum of the $[M + H]^+$ ion of curcumin **1**, m/z 369, shows major fragment ions of m/z 299, 285, 259 and 245, the latter being most abundant, arising from eliminations of 70, 84, 110 and 124 u (Figure 1), respectively. The measured accurate masses of the product ions (Table 1) revealed that the ions of m/z 299, 285, and 245 are formed by the eliminations of neutral species corresponding to $C_3H_2O_2$ (possibly $CH_2CO + CO$, 70 u), $C_4H_4O_2$ (possibly $2 \times CH_2CO$, 84 u), $C_6H_6O_2$, and $C_7H_8O_2$ (the elements of 2-methoxy phenol, 124 u), respectively (Scheme 1). Losses of a single ketene or CO are virtually absent, implying that losses of 84 and 70 u either occur by expelling a single moiety or as concerted losses without significant barrier to loss of the second unit. The ion of m/z 259 is likely to be formed by elimination of the elements of resorcinol (*m*-hydroxy-phenol) or its mono-keto isomer. We have found by theoretical DFT calculations that the loss of 110 u requires more proton migrations than the other losses and that the diketo-isomer loss is not feasible due to isomerization of necessary intermediates. An additional major product ion of m/z 175, observed in the lower mass region of the mass spectrum, could arise by the loss of 124 u from the ion of m/z 299 or loss of 70 u from the ion of m/z 245. The CAD mass spectra of the collision-generated fragment ions of m/z 299 and 245 (Fig 2a and 2b) show that a fragment ion of m/z 175 (MS/MS/MS experiments) is produced from these precursors.

We propose the formation of the m/z 245 product ion involves significant rearrangement involving two cyclization reactions followed by the elimination of the elements of 2-methoxy phenol. The decomposition of the ion of m/z 245 yields major ions of m/z 175 (via loss of 70 u) and m/z 213 (loss of methanol) (Fig 2c) and less abundant fragments of m/z 227, 217, 203, and 161 resulting from losses of water, CO, C_2H_2O , and 84 u. The product ion of m/z 299 upon CAD yields two fragment ions (i) that of m/z 175 by loss of 2-methoxy phenol and (ii) that of m/z 267 by loss of methanol (Fig. 2a). The product ion of m/z 285, upon CAD, fragments to produce the m/z 253 ion (loss of methanol), the m/z 225 ion (not identified), and the m/z 161 ion (loss of 2-methoxy phenol) (Fig 2b). The product ion m/z 175 loses upon CAD the methyl radical (in violation of the even-electron ion rule) and methanol to form ions of m/z 160 and 143, respectively (Fig. 2d).

There are three exchangeable H-atoms in curcumin (i.e., two phenolic OH groups and the enol-OH). ESI of curcumin in 1:1 mixture of acetonitrile D_2O affords the ion of m/z 373, $[M - 3H + 4D]^+$ ion. The CAD mass spectrum of the m/z 373 ion (Fig 1b) shows that H/D scrambling is involved in the eliminations of 2-methoxy phenol and C_2H_2O or equivalent but not for the elimination of $C_2H_2O + CO$ or equivalent (the m/z 299 ion is shifted cleanly to m/z 303). The fragment ion of m/z 245 splits into m/z 246 (minor), 247 and 248, indicating that the elimination of one D is compulsory (likely one of the phenolic-D as 2-methoxy phenol-OD). Similarly, the fragment ion of m/z 285 splits into m/z 287, 288 and 289, indicating that a maximum of 2D may be eliminated and suggesting that the phenolic OD's are retained in the process. Moreover, the ion of m/z 259 shifts to ions of m/z 261 and 262, retaining two and three D atoms, respectively.

Collisional activation of protonated mono-methoxy curcumin, **2**, m/z 339, yields fragment ions of m/z 269, 255, 229 and 245 (100%) by the eliminations 70, 84, 94 (phenol), and 110 u (resorcinol or isomer). Unlike the dissociation of protonated curcumin (**1**), the elimination of 2-methoxy phenol does not occur as for the dimethoxy counterpart (Figure 3) (as depicted in Scheme 3). Hence, the elimination of a phenol depends significantly on the substituents on the phenyl rings, specifically, the phenyl ring that bears both OH and OCH₃ is activated relative to the hydroxyl-phenyl ring for the process of double cyclization and proton migration; hence, the moiety activated for loss would be the phenol rather than the 2-methoxy-phenol. CA of the deuterium labeled analog of **2** shows H/D mixing in the fragmentation, leading to the formation of the ion of m/z 245 similar to **1**. The minor fragment ion from the elimination of the elements of resorcinol or isomer, however, is produced as seen in the CAD mass spectra of both **1** and **2**, indicating that it arises in that part of an intermediate that arises from the diketone section of curcumin that cyclizes (Scheme 2).

In addition, we explored by ESI CAD the fragmentation of synthetic curcumin analogs **3** and **4**, containing only single OCH₃ groups on the phenyl rings. The CAD mass spectra of the [M + H]⁺ ions of **3** and **4** (Fig. 4 and 5) show that both compounds eliminate 84 u (m/z 253) as a major process, whereas loss of the elements of anisole (108 u to m/z 229) is minor. Eliminations of 70 u (m/z 267) are very minor from either **3** or **4**. The di-vinyl ketone structure may perhaps undergo cyclization by another related rearrangement process [31]. Furthermore, the substitution pattern of the two substituents (one OH and one OCH₃) on the phenyl rings, as in compounds **1** and **2**, is necessary for the cyclizations that lead to the observed fragmentation. To elucidate the formation mechanisms of the m/z 299, 285 and 245 fragment ions, we turned to molecular modeling using DFT methods.

DFT calculations:

We chose the *enol* form of curcumin (**1**) for the theoretical calculations because curcumin exists in the *enol* form as a neutral molecule. Protonation can occur on the second carbonyl (**A0**) or on the bridging CH between the carbonyls (**Z**); the latter process is lower by 11 kJ/mol, but both forms would remain competitive. Because no H/D exchange or scrambling accompanies the loss of 70 u, which certainly involves the two carbonyl groups and the bridge, protonation of the bridging CH must not occur for this elimination. The lowest energy accessible structure of protonated curcumin is the *enol* form of [M + H]⁺, **A0**, and is obtained by protonation of the vacant carbonyl, Scheme 2. The rearrangement and consequent fragmentations of curcumin occur from the accessible *enol* form of the [M + H]⁺ ion. The 1,5-H shift from an oxygen atom to the alpha-carbon atom of the carbonyl group, **A1**, followed by cyclization yields an intermediate, **CD1**, which is a protonated cyclic 1,3-diketone, produced in the first cyclization. That intermediate can dissociate by two pathways.

In the first, the diketone ring opens followed by the elimination of a diketene or isomer, **K2**, to yield the m/z 285 fragment, **H1**, through decomposition of an initial ion-dipole complex, **IDC1** (Scheme 2). The sequential elimination of two ketenes or the elimination of bound C₄H₄O₂ (diketene) requires significantly more energy as does the elimination of 70 u

(**KCO**). In the second route, the benzylic carbon in intermediate, **CD1**, carrying the positive charge, attacks the *para*-position of the methoxy group of the adjacent benzene ring, leading to formation of a new five-membered ring, intermediate **F1**, Scheme 3, the second cyclization. The aromatic proton undergoes a 1,2-shift (**F2**) followed by a 1,5-shift (**F3**) and another 1,2-shift yielding intermediate, **F4**, from which the elimination of 2-methoxy phenol (**Q1**) takes place through an ion-dipole complex, **IDC3**, to generate of the m/z 245 fragment, **P1**. According to the mechanism for m/z 245 ion formation, the deuterium labeled analog of the protonated molecule most likely eliminates 2-methoxy phenol-**d**, producing m/z 248 as the major fragment ion, but mobility of the aromatic proton leads to H/D scrambling. That proposed second step of cyclization is an intramolecular aromatic substitution and requires the ring system being attacked to be electron rich, which is established by the presence of the OCH_3 and the OH groups. In the aromatic proton migration, the presence of both OCH_3 and OH facilitate such a transfer sequence. Note that the $[\text{M} + \text{H}]^+$ ion of the unsymmetrical molecule mono-methoxy curcumin (**2**) eliminates only phenol but not 2-methoxy phenol. In this case, the phenyl ring bearing both groups is tied up in the generation of the analog of **F4**, which bears only the incipient phenol for elimination. An alternative route for the formation of the fragment ion of m/z 285 could be via diketene or equivalent elimination from the intermediate (**F1**), but we were unable to discover an energetically competitive pathway.

Another mechanism, suggested by a reviewer, involves the attack of the protonated carbonyl (**Z**) on the electron-rich ring to make a feasible six-membered ring. The new cyclic molecule would fragment by the loss of the $\text{C}_7\text{H}_8\text{O}_2$ to give directly the ion of m/z 245. The difficulty with this mechanism is the formation of a vinyl carbocation, necessitating the lower energy fragmentation shown in Scheme 3.

Conclusions

The most abundant product ion (of m/z 245) in the CAD mass spectrum of protonated curcumin occurs by the elimination of 2-methoxy phenol. Intrigued by this interesting rearrangement and fragmentation, we used mass spectrometry and DFT calculations to show that the elimination of 2-methoxy phenol occurs following two consecutive cyclizations of protonated curcumin. The isomerization/cyclization of protonated curcumin, a strong antioxidant, may be biologically relevant and account for the putative health effects of curcumin. If so, the relevance of gas-phase measurements will again be underscored.

We are pleased to dedicate this article on gas-phase ion chemistry to the 75th birthday volume for Helmut Schwarz, arguably the leading and most productive figure in gas-phase ion chemistry. We are reminded of collaborations in the 1980s and 90s with Professor Schwarz where some of us contributed to early exciting studies of metal-ion reactions with organic molecules [35], endohedral complexes of buckyballs [36], and structure determination of cyclic peptides [37].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors JC, JP and MG thank Principal, S.H. College, and Thevara, Cochin for providing infrastructure. RS thank Director ICT, Hyderabad, for providing the mass spectral facility. Research at WU was supported by the National Centers for Research Resources of the NIH, Grant P41GM103422. In addition, this work made use of the Washington University Computational Chemistry Facility, supported by NSF grant CHE-0443501.

References

- [1]. Wang J, Zhao C, Zhao A, Li M, Ren J, Qu X, New Insights in Amyloid Beta Interactions with Human Telomerase. *Journal of the American Chemical Society*, 137(3) (2015) 1213–1219. 10.1021/ja511030s [PubMed: 25564872]
- [2]. Malik P, Mukherjee TK, Structure-Function Elucidation of Antioxidative and Prooxidative Activities of the Polyphenolic Compound Curcumin. *Chinese J. Biol.*, 2014 (2014) 1–8. 10.1155/2014/396708
- [3]. Heger M, van Golen RF, Broekgaarden M, Michel MC, The Molecular Basis for the Pharmacokinetics and Pharmacodynamics of Curcumin and Its Metabolites in Relation to Cancer. *Pharmacol. Rev.*, 66(1) (2013) 222–307. 10.1124/pr.110.004044 [PubMed: 24368738]
- [4]. Esatbeyoglu T, Huebbe P, Ernst IMA, Chin D, Wagner AE, Rimbach G, Curcumin-From Molecule to Biological Function. *Angew. Chem. Intl. Ed.*, 51(22) (2012) 5308–5332. 10.1002/anie.201107724
- [5]. Chen SY, Chen Y, Li YP, Chen SH, Tan JH, Ou TM, Gu LQ, Huang ZS, Design, synthesis, and biological evaluation of curcumin analogues as multifunctional agents for the treatment of Alzheimer's disease. *Bioorg. Med. Chem.*, 19(18) (2011) 5596–5604. 10.1016/j.bmc.2011.07.033 [PubMed: 21840724]
- [6]. Zhang X, Tian Y, Li Z, Tian X, Sun H, Liu H, Anna M, Ran C, Design and Synthesis of Curcumin Analogues for in Vivo Fluorescence Imaging and Inhibiting Copper-Induced Cross-Linking of Amyloid Beta Species in Alzheimer's Disease. *J. Am. Chem. Soc.*, 135(44) (2013) 16397–16409. 10.1021/ja405239v [PubMed: 24116384]
- [7]. Bhullar KS, Jha A, Youssef D, Rupasinghe HPV, Curcumin and Its Carbocyclic Analogs: Structure-Activity in Relation to Antioxidant and Selected Biological Properties. *Molecules*, 18(5) (2013) 5389–5404. 10.3390/molecules18055389 [PubMed: 23666006]
- [8]. Begum AN, Jones MR, Lim GP, Morihara T, Kim P, Heath DD, Rock CL, Pruitt MA, Yang F, Hudspeth B, Hu S, Faull KF, Teter B, Cole GM, Frautschy SA, Curcumin Structure-Function, Bioavailability, and Efficacy in Models of Neuroinflammation and Alzheimer's Disease. *J. Pharmacol. Exp. Ther.*, 326(1) (2008) 196–208. 10.1124/jpet.108.137455 [PubMed: 18417733]
- [9]. Li Q, Chen J, Luo S, Xu J, Huang Q, Liu T. Synthesis and assessment of the antioxidant and antitumor properties of asymmetric curcumin analogues. *Eur. J. Med. Chem.*, 93 (2015) 461–469. 10.1016/j.ejmech.2015.02.005 [PubMed: 25728027]
- [10]. Sun YM, Zhang HY, Chen DZ, Liu CB. Theoretical elucidation on the antioxidant mechanism of curcumin: a DFT study. *Org. Lett.*, 4(17) (2002) 2909–2911. [PubMed: 12182586]
- [11]. Worachartcheewan A, Nantasenamat C, Isarankura-Na-Ayudhya C, Prachayasittikul S, Prachayasittikul V. Predicting the free radical scavenging activity of curcumin derivatives. *Chemometr. Intell. Lab. Syst.*, 109(2) (2011) 207–216. 10.1016/j.chemolab.2011.09.010
- [12]. Bayomi SM, El-Kashef HA, El-Ashmawy MB, Nasr MNA, El-Sherbeny MA, Badria FA, Abou-Zeid LA, Ghaly MA, Abdel-Aziz NI. Synthesis and biological evaluation of new curcumin derivatives as antioxidant and antitumor agents. *Med. Chem. Res.*, 22(3) (2013) 1147–1162. 10.1007/s00044-012-0116-9
- [13]. Metzler M, Pfeiffer E, Schulz SI, Dempe JS, Curcumin uptake and metabolism. *BioFactors*, 39(1) (2013) 14–20. 10.1002/biof.1042 [PubMed: 22996406]
- [14]. Gordon ON, Luis PB, Ashley RE, Osheroff N, Schneider C. Oxidative Transformation of Demethoxy- and Bisdemethoxycurcumin: Products, Mechanism of Formation, and Poisoning of Human Topoisomerase II α . *Chem. Res. Toxicol* 28(5) (2015) 989–996. 10.1021/acs.chemrestox.5b00009 [PubMed: 25806475]

- [15]. Hassaninasab A, Hashimoto Y, Tomita-Yokotani K, Kobayashi M. Discovery of the curcumin metabolic pathway involving a unique enzyme in an intestinal microorganism. *Proc Natl Acad Sci U S A*, 108(16) (2011) 6615–6620. 10.1073/pnas.1016217108 [PubMed: 21467222]
- [16]. Anand P, Thomas SG, Kunnumakkara AB, Sundaram C, Harikumar KB, Sung B, Tharakan ST, Misra K, Priyadarsini IK, Rajasekharan KN, Aggarwal BB. Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. *Biochem. Pharmacol* 76(11) (2008) 1590–1611. 10.1016/j.bcp.2008.08.008 [PubMed: 18775680]
- [17]. Pan MH, Huang TM, Lin JK. Biotransformation of curcumin through reduction and glucuronidation in mice. *Drug Metab. Dispos*, 27(4) (1999) 486–494. <https://www.ncbi.nlm.nih.gov/pubmed/10101144> [PubMed: 10101144]
- [18]. Gordon ON, Luis PB, Sintim HO, Schneider C. Unraveling Curcumin Degradation: autoxidation proceeds through spiroepoxide and vinyl ether intermediates en route to the main bicyclopentadione. *J. Biol. Chem*, 290(8) (2015) 4817–4828. 10.1074/jbc.M114.618785 [PubMed: 25564617]
- [19]. Maheshwari RK, Singh AK, Gaddipati J, Srimal RC. Multiple biological activities of curcumin: A short review. *Life Sciences*, 75(18) (2006) 2081–2087. <https://doi.org/10.1016/j.lfs.2005.12.007>
- [20]. Rao TS, Basu N, Siddiqui HH. Anti-inflammatory activity of curcumin analogues. *The Indian J. Med. Res*, 75 (1982), 574–578. <https://www.ncbi.nlm.nih.gov/pubmed/7118227> [PubMed: 7118227]
- [21]. Somparn P, Phisalaphong C, Nakornchai S, Unchern S, Morales NP. Comparative antioxidant activities of curcumin and its demethoxy and hydrogenated derivatives. *Biol. Pharm. Bull*, 30(1) (2007) 74–78. <https://www.ncbi.nlm.nih.gov/pubmed/17202663> [PubMed: 17202663]
- [22]. Crotti S, Stella L, Munari I, Massaccesi F, Cotarca L, Forcato M, Traldi P. Claisen rearrangement induced by low-energy collision of ESI-generated, protonated benzyloxy indoles. *J. Mass Spectrom*, 42(12) (2007) 1562–1568. 10.1002/jms.1334 [PubMed: 18085549]
- [23]. Wang HY, Zhang X, Guo YL, Tang QH, Lu L. Using tandem mass spectrometry to predict chemical transformations of 2-pyrimidinyl-N-arylbenzyl amine derivatives in solution. *J. Am. Soc. Mass Spectrom*, 17(2) (2006) 253–263. 10.1016/j.jasms.2005.11.001 [PubMed: 16413200]
- [24]. O'Hair RAJ, The 3D quadrupole ion trap mass spectrometer as a complete chemical laboratory for fundamental gas-phase studies of metal mediated chemistry *Chem. Comm* 2006, 1469 <http://pubs.rsc.org/en/content/articlelanding/2006/cc/b516348j> [PubMed: 16575433]
- [25]. George M, Sebastian VS, Reddy PN, Srinivas R, Giblin D, Gross ML. Gas-phase Nazarov cyclization of protonated 2-methoxy and 2-hydroxy chalcone: An example of intramolecular proton-transport catalysis *J. Am. Soc. Mass Spectrom* 20(5) 2009, 805–818. 10.1016/j.jasms.2008.12.017 [PubMed: 19230703]
- [26]. George M, Ramesh V, Srinivas R, Giblin D, Gross ML. Deprotonated N-(2,4-dinitrophenyl)amino acids undergo cyclization in solution and the gas phase. *Int. J. Mass Spectrom*, 306(2-3) (2011) 232–240. 10.1016/j.ijms.2011.01.007 [PubMed: 21966244]
- [27]. Cyriac J, Paulose J, George M, Ramesh M, Srinivas R, Giblin D, Gross ML. The Role of Methoxy Group in the Nazarov Cyclization of 1,5-bis-(2-Methoxyphenyl)-1,4-Pentadien-3-one in the Gas Phase and Condensed Phase. *J. Am. Soc. Mass Spectrom*, 25(3) (2014) 398–409. 10.1007/s13361-013-0785-8 [PubMed: 24415061]
- [28]. Kawano SI, Inohana Y, Hashi Y, Lin JM. Analysis of keto-enol tautomers of curcumin by liquid chromatography/mass spectrometry *Chin. Chem. Lett* 24(8) (2013), 685–87. 10.1016/j.ccl.2013.05.006
- [29]. Yanagisawa D, Shirai N, Amatsubo T, Taguchi H, Hirao K, Urushitani M, Morikawa S, Inubushi T, Kato M, Kato F, Morino K, Kimura H, Nakano I, Yoshida C, Okada T, Sano M, Wada Y, Wada KN, Yamamoto A, Tooyama I. Relationship between the tautomeric structures of curcumin derivatives and their A β -binding activities in the context of therapies for Alzheimer's disease. *Biomaterials* 31(14) (2010) 4179–85. 10.1016/j.biomaterials.2010.01.142 [PubMed: 20181392]
- [30]. Spencer WT III, Vaidya T, Frontier AJ. Beyond the Divinyl Ketone: Innovations in the Generation and Nazarov Cyclization of Pentadienyl Cation Intermediates: Generation and Nazarov Cyclization of Pentadienyl Cations. *Eur. J. Org. Chem*, 2013(18) (2013) 3621–3633. 10.1002/ejoc.201300134

- [31]. Jiang H, Somogyi Á, Jacobsen NE, Timmermann BN, Gang DR. Analysis of curcuminoids by positive and negative electrospray ionization and tandem mass spectrometry. *Rapid Comm.Mass Spectrom*, 20(6) (2006) 1001–1012. 10.1002/rcm.2401
- [32]. Rao EV, Sudheer P. Revisiting Curcumin Chemistry Part I: A New Strategy for the Synthesis of Curcuminoids. *Indian J. Pharm. Sci*, 73(3) (2011) 262–270. 10.4103/0250-474X.93508 [PubMed: 22457548]
- [33]. Stewart JJP. Optimization of parameters for semiempirical methods I. *Method. J. Comput. Chem*, 10(2) (1989) 209–220. 10.1002/jcc.540100208
- [34]. Zhao Y, Truhlar DG. Density Functionals with Broad Applicability in Chemistry. *Acc. Chem. Res*, 41(2) (2008), 157–167. [PubMed: 18186612]
- [35]. Schulze C, Schwarz H, Peake DA, and Gross ML, On the Mechanism of Fe^+ -Induced Hydrogen Migrations in Gaseous Octyne/Iron(I) Complexes *J. Am. Chem. Soc*, 109, (1987) 2368–2374.
- [36]. Weiske T, Schwarz H, Giblin DE, and Gross ML. High-energy collision of Kr@C_{60}^+ with Helium. Evidence for the Formation of HeKr@C_{60}^+ , *Chemical Physics Letters*, 227, (1994) 87–90.
- [37]. Eckart K, Schwarz H, Tomer KB, and Gross ML, Tandem Mass Spectrometry Methodology for the Sequence Determination of Cyclic Peptides, *J. Am. Chem. Soc*, 107, (1985) 6765–6769.

Highlights

- Rearrangement of protonated curcumin characterized by mass spectrometry and DFT
- Protonated curcumin undergoes two consecutive cyclization reactions in the gas phase.
- The precursor upon protonation undergoes competitive eliminations of 2-methoxy phenol and $C_4H_4O_2$, presumably diketene
- This gas-phase rearrangement may be predictive of solution chemistry and be predictive for biological function.

Fig 1.

a. CAD mass spectrum of curcumin, $[M + H]^+$, m/z 369, produced by ESI.

b. CAD mass spectrum of curcumin, $[M - 3H + 4D]^+$, m/z 373, produced by ESI.

Figure 2.
CAD mass spectra (MS/MS/MS) of collision generated fragment ions of m/z 299 (a), 285 (b), 245 (c) and 175 (d) from protonated curcumin of m/z 369.

Figure 3.
CAD mass spectrum of protonated mono-methoxycurcumin (**2**), $[M + H]^+$ of m/z 339, produced by ESI.

Figure 4.
CAD mass spectrum of the $[M + H]^+$ ion of compound **3**, of m/z 337

Figure 5.
CAD mass spectrum of $[M + H]^+$ ion of compound **4** of m/z 337, produced by ESI.

Compounds under investigation

Scheme 1.

Fragment ions from protonated curcumin (1) and dimethoxy-curcumin (2).

Scheme 2.
Proposed mechanism for elimination of 84 u ($C_4H_4O_2$) and the first cyclization of protonated curcumin.

Scheme 3.

Proposed mechanism for the elimination of 2-methoxy phenol and second cyclization of protonated curcumin.

Table 1.

Measured accurate masses and elemental compositions of precursor $[M + H]^+$ and fragment ions of curcumin (**1**) and mono-methoxy-curcumin (**2**).

Compd	Precursor ion (<i>m/z</i>)		Fragment ions (<i>m/z</i>)		
	$[M + H]^+$	$[M + H - 70]^+$	$[M + H - 84]^+$	$[M + H - C_6H_6O_2]^+$	$[M + H - \text{methoxyphenol/phenol}]^+$ (100%)
Curcumin (1)	C ₂₁ H ₂₁ O ₆ 369.1326 (calc. 369.1333)	C ₁₈ H ₁₉ O ₄ 299.1274 (calc. 299.1278)	C ₁₇ H ₁₇ O ₄ 285.118 (calc. 285.1121)	C ₁₅ H ₁₅ O ₄ 259.0961 (calc. 259.0965)	C ₁₄ H ₁₃ O ₄ 245.0807 (calc. 245.0808)
Mono-methoxy curcumin (2)	C ₂₀ H ₁₉ O ₅ 339.1222 (calc. 339.1227)	C ₁₇ H ₁₇ O ₃ 269.1168 (calc. 269.1172)	C ₁₆ H ₁₅ O ₃ 255.1012 (calc. 255.1016)	C ₁₄ H ₁₃ O ₃ 229.085 (calc. 229.0859)	C ₁₄ H ₁₃ O ₄ 245.0805 (calc. 245.0808)

Compounds under investigation