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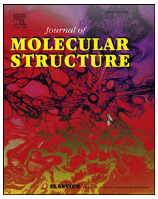

Spectroscopic and structural investigation on intermediates species structurally associated to the tricyclic bisguanidine compound and to the toxic agent, saxitoxin

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ABSTRACT

In the present work, we have studied the structural, topological and spectroscopic properties of five cyclic and of open chain species derived from tricyclic bisguanidine compound in gas and aqueous phases combining the DFT calculations with the experimental infrared and ^{1}H NMR, ^{13}C NMR and UV−visible spectra. These species are members of the saxitoxin family and they were recently synthesized by *Tsuchiya* et al. (Chemistry. A European Journal, 21 (2015) 7835–7840). Here, the self consistent reaction force (SCRF) calculations were employed in aqueous medium to study the solvation energies by using the polarized continuum (PCM) and solvation (SM) models. All the calculations were performed with the 6-31G* and 6-311++G** basis sets. The atomic charges, electrostatic potentials, bond order, stabilization energy, topological properties suggest the structural connection between the cyclic cationic and saxitoxin species while the electrophilicity and nucleophilicity indexes could be one of the explanations for the Na_v blocking activities of these species. The complete vibrational assignments for all the species are reported. The predicted spectra present a reasonable concordance with the corresponding experimental ones.

1. Introduction

Saxitoxin (STX) and its analogues forming the family of paralytic shellfish toxins (PSTs) because they inhibit sodium influx through cell membranes and, for these reasons, they are known from several decades as the most potent and selective voltage-gated sodium channel (Na_v) blocker [1–22]. At the moment, approximately 57 PST derivatives were reported in the literature, as mentioned by Wiese et al. [18]. The structure of STX present three rings, two of them of five members and the remains of six members and, moreover, STX has two NH_2 groups which form the two guanidine moieties, as determinate by Schantz et al. [4]. Recently, Tsuchiya et al. [23] have reported the synthesis and identification of two genetically predicted intermediates of STX which supporting the route proposed by Kellman et al. [24] and, also, those authors have performed the direct conversion of one of those intermediate species into a tricyclic bisguanidine compound structurally associated to STX by means of oxidative intramolecular guanidine transfer to 2-aminoimidazole catalyzed by Pd/C under basis conditions in air [25]. But so far, the biosynthetic reaction for the formation of the tricyclic ring of STX remains unknown because there are other intermediates which were not identified. The knowledge of the intermediates species and the reaction mechanisms involved in the route to obtain STX is useful and necessary taking into account the high biological activity of STX and the consequences of their toxins on the health and the environmental. Hence, the detections of all the related species to STX should be performed using specialized laboratory personnel and expensive equipment, as mentioned by different authors [12,13,17]. For instance, the chemiluminescence-based microarray immunoassay was reported as a technical fast, cheap and reliable for simultaneous detection of proteotoxins and small toxins [12,17]. The identifications of these species by using the vibrational spectroscopy will be a great advantage taking into account the toxicities of these species and

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the versatility of this spectroscopic technique. On the other hand, studies related to the effects of varying toxin structure and charge have demonstrate that there is no effect of toxin charge on the voltage dependence of toxin binding rates, and that a voltage-dependent conformational equilibrium of the protein could explain the effects of voltage on toxin binding [15]. In this context and taking into account the difficulties in handling these toxics substances the theoretical calculations are indispensable to study these species types from different points of view. Thus, the structural, topological, electronic and vibrational studies of STX and their related species are of interest to know the relation that exist between the different chemical groups and rings present in these species in function of the high activity and potency of their toxins. In this work, we have studied those mentioned properties for two cyclic and three of open chain species related to STX which were compared with the corresponding to STX in order to find correlations among their properties and to elucidate the connection between STX and their intermediates. Hence, the two cyclic species studied in this work are the tricyclic bisguanidine cationic (with two positive charges) reported by Tsuchiya et al. [25] and their corresponding neutral species while of the other three species of open chain, one is that neutral species identified by Tsuchiya et al. [23] and the other ones proposed in this work are two cationic species with one and two positive charges, respectively. These studies were performed combining the DFT calculations with the experimental infrared, UV–visible and ^{1}H NMR and ^{13}C NMR spectra. All the studied species were optimized in gas and aqueous phases using the hybrid B3LYP/6-31G* [26,27] while the effect of solvents were considered by using the self consistent reaction force (SCRF) calculations [28,29]. Thus, the atomic charges, molecular electrostatic potentials, bond orders, stabilization energies and topological properties were calculated for all the species taking into account the presence of different donor and acceptors groups of H bonds in those six structures including STX such as, NH_2 and NH groups and rings [2]. In addition, the reactivities and behaviors of these species were also studied by using the corresponding frontier orbitals and practical descriptors reported in the literature [30–34]. Here, the assignments of all the bands observed in the infrared spectra of all the species were performed from the corresponding force fields obtained using the Pulay's Scaled Quantum Mechanics Force Field (SQMFF) methodology [35] and the Molvib program [36] while their Raman spectra were predicted at the B3LYP/6-31G* level of theory. Reasonable concordances were found among the theoretical and experimental infrared, UV–visible and ^{1}H NMR and ^{13}C NMR spectra of all the species. Here, the high polarities of all the species could support the ability of these species to traverse biological membranes quickly while the correlations among the different properties reveal their probable connections.

2. Experimental section

The intermediates species of open chain and the cyclic compound were obtained according to the methodology described in Refs. [23] and [25], respectively. Both species were characterized by using FT-IR, ^{1}H NMR, ^{13}C NMR and UV–visible spectra. The attenuated total reflectance spectra were obtained using films in Zn–Se and were measured using a JASCO FT/IR-4600 spectrometer (JASCO, Tokyo, Japan). NMR spectra were recorded using an Agilent 600 MHz NMR spectrometer (Agilent Technologies, Santa Clara, CA, USA), 600 MHz (^{1}H NMR) and 151 MHz (^{13}C NMR); CD_3OD and $(CD_3)_2SO$ were used as the solvents and internal standards. The signals of CHD_2OD (3.30 ppm) and $(CHD_2)_2SO$ (2.50 ppm) and $^{13}CD_3OD$ (49.0 ppm) and $(^{13}CD_3)_2SO$ (40.0 ppm) were used as references in ^{1}H and ^{13}C NMR, respectively.

3. Computational details

Initially, STX and the three neutral and cationic species of open chains named OC_N, OC_I and OC_{II}, respectively and, the two neutral and cationic cyclic species named respectively C_N and C_C were modeled with the *GaussView* program [37] and, then these forms were optimized in gas phase and in aqueous solution phases by using the hybrid B3LYP method [26,27] and the 6-31G* and 6-311++G** basis sets with the Gaussian 09 program [38]. In this study, the properties were calculated for those most stable species determined from the potential energy curves using their optimized parameters. The cyclic and of open chains structures together with the labeling atoms can be seen in Figs. 1 and 2 while the most stable structure of STX is presented in Fig. S1 together with a detailed structure indicating the three different rings in accordance with the experimental structure reported for p-bromobenzenesulfonate saxitoxin by Schantz et al. [4]. The SCRF method together with the polarized continuum (PCM) model [28,29] were used to calculate the properties in solution taking into account the solvent effects at the same level of theory. The solvation energies of all the species were calculated using the universal solvation model (SM) [39] while the NBO program [40,41] was used to compute the atomic natural population atomic (NPA) charges, bond orders and stabilization energies for all those species at the same level of theory. The atomic Merz-Kollman (MK) charges [42] were also calculated in order to compare with the corresponding NPA while the intramolecular interactions were studied using the Bader's atoms in molecules theory [43] by means of the topological properties, hence, these parameters were calculated for all the forms with the AIM2000 program [44]. On the other hand, the prediction of the reactivities and behaviors of all those species in the different media were carried out by the frontier orbitals calculations and attractive descriptors reported [30–34,45]. Here, the volume variations that experiment the different species in solution were calculated with the Moldraw program [46] taking like reference the values in gas phase. The internal coordinates were built like those reported for molecules with similar groups [47–50] and the corresponding force fields were calculated employing the SQMFF methodology using the same level of theory [35]. After that, the Molvib program [36] was used to transform the results in Cartesian coordinates to internal coordinates. The complete vibrational assignments for all the species were performed considering the potential energy distribution components (PED) $\geq$ 10%. The chemical shifts of all the species were calculated from the predicted ^{1}H NMR and ^{13}C NMR spectra by using the Gauge-Independent Atomic Orbital (GIAO) method [51] while the ultraviolet–visible (UV–Vis) spectra were predicted using the same level of theory in aqueous solution using time dependent density functional theory (TD-DFT) calculations, as implemented in the Gaussian 09 program [38]. The calculated properties for all the studied species were compared and discussed.

4. Results and discussion

4.1. Geometry optimization

The calculated geometrical parameters for all the species in gas phase including saxitoxin are compared with that experimental structure determinate for p-bromobenzenesulfonate saxitoxin by Schantz et al. [4] by X-ray diffraction. The results expressed by means of the root-mean-square deviation (RMSD) values can be seen in Table 1. Obviously, the better correlation in the bond lengths (0.023 Å) and angles (1.5°) are observed for saxitoxin, as it is expected. Note that the C_C cyclic species whose structure is of cationic nature, as saxitoxin, also present a good correlation in the bond length (0.028 Å) and angle (5.1°) while all the open chains species