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Molecular structures of two tetrodotoxin analogs containing a monooxa-hydrocarbon cage: A computational study

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ABSTRACT

Using quantum chemical calculations we investigate the molecular structures of two tetrodotoxin (TTX) analogs recently isolated from the Japanese toxic newt *Cynops ensicauda popei*. These novel analogs are characterized by a monooxa-hydrocarbon cage with a direct C5–C10 bond that replaces one of the ether bridges in the canonical dioxa-adamantane cage of TTX. The computed change in the ^{13}C NMR chemical shifts is in good agreement with the change in the corresponding experimental values that results from the above chemical modification. This confirms the chemical structure assigned to the TTX analogs. A topological analysis of the theoretical electronic charge density indicates that the removal of the oxygen bridge in TTX increases the magnitude of the charge density at the cage critical point. A database search indicates that the monooxa-hydrocarbon cage is also present in other natural products such as cinnzeylanine and platensimycin whose molecular structures have been characterized by single-crystal X-ray diffraction analyses.

1. Introduction

Tetrodotoxin (TTX, **1**) is a potent natural toxin that is produced by symbiotic bacteria such as, for example, *Vibrio alginolyticus* [1], *Raoultella terrigena* [2] and others which are stored in the internal organs of pufferfish (called *Fugu* in Japan), newts, and toads [3–5]. When TTX-poisoned food is ingested, numbness of the tongue and limbs arise whereas in severe cases of TTX poisoning [6] the respiratory muscles become paralyzed, a threatening condition for life. As far as the mode of action of the toxin is concerned, TTX has the ability to bind the voltage-gated sodium channels thereby preventing the passage of ions through the cell membrane [7]. Crystallographic studies carried out in the middle 1960s [8] and in the early 1970s [9] indicated that the molecular structure of TTX is made of a dioxa-adamantane cage fused to a guanidinium group, as shown in Fig. 1 (guanidinium is the protonated form of the guanidine group). Six hydroxyl groups stick out from the molecular scaffold of TTX thereby conferring to the toxin a good solubility in water.

To this day, several deoxy-TTX derivatives such as 6-deoxy-TTX, 11-deoxy-TTX, and 6,11-dideoxy-TTX have been isolated and spectroscopically characterized [10]. These derivatives are all structurally similar to TTX given that the removal of the hydroxyl groups bonded to C6 and C11 does not alter the geometry of the dioxa-adamantane cage. The removal of one water molecule from the condensation of the hydroxyl groups bonded to C4 and C9 yields 4,9-anhydro-TTX (**2**) which possesses three oxo bridges, two pertaining to the dioxa-damantane cage and another one external to it, as shown in Fig. 1. This derivative has been crystallographically characterized in the diacetylated form by Tamura et al. [11].

Recently, Kudo et al. [12] isolated from the Japanese toxic newts (*Cynops ensicauda popei*) two novel TTX analogs (**3** and **4**) which are characterized by a direct C5–C10 bond. This modification corresponds to a different molecular scaffold characterized by two ether bridges, one pertaining to the cage and the other one external to it, as shown in Fig. 1. The authors characterized the structures of these new TTX analogs on the basis of extended NMR spectroscopic experiments including COSY, TOCSY, HSQC, and HMBC techniques (see Ref. [13] for an overview of these techniques). As done in the past for both **1** and **2**, a crystallographic determination of **3** and **4** would be important to corroborate the above structural assignment and to quantitatively characterize the structural parameters of these compounds, particularly the length of the C5–C10 bond. This, however, would require the isolation of a large amount of the two compounds so as to crystallize them and obtain single crystals suitable for the X-ray diffraction analyses. We were therefore intrigued to investigate in advance of a crystallographic

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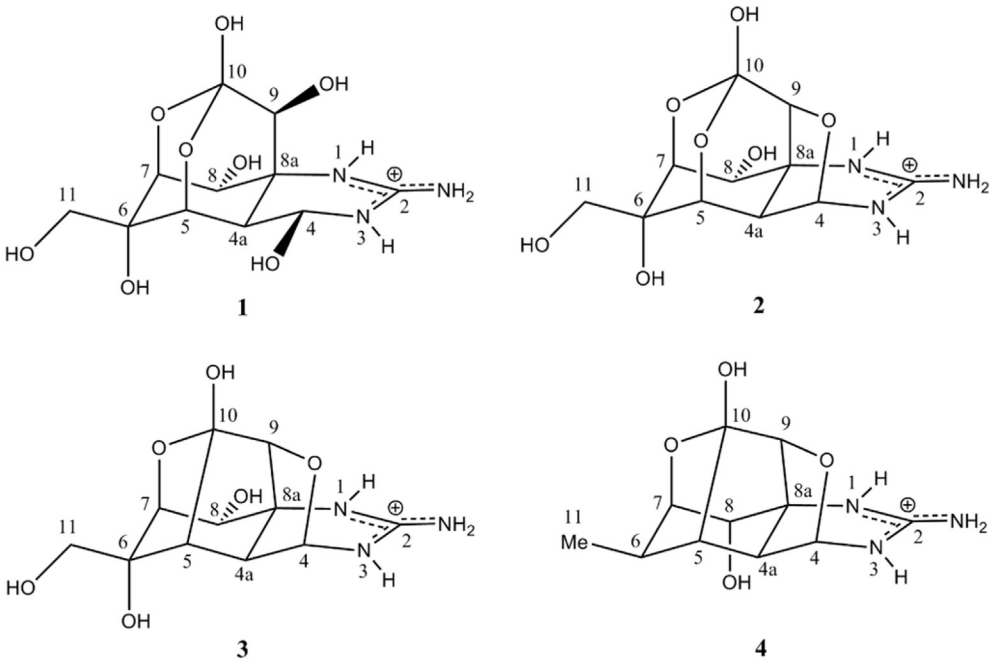

Fig. 1. Chemical structures of tetrodotoxin (**1**), 4,9-anhydro-tetrodotoxin (**2**), 4,9-anhydro-5-deoxy-tetrodotoxin (**3**), and 4,9-anhydro-8-epi-5,6,11-trideoxy-tetrodotoxin (**4**). The guanidine group of all these molecules is protonated. Atoms are numbered following reference [12].

determination the 3D molecular structures of **3** and **4** with the aid of computational quantum chemistry. This represents the main goal of the present study.

The field of computational quantum chemistry based on electronic structure calculations [14,15] has been the subject of enormous advances in the last several years and, as a result, it is now possible to corroborate structural assignments of complex natural products by comparing the computed NMR chemical shifts (as well as other spectroscopic properties) against the experimental data [16,17]. As an example, this author with the help of density functional theory (DFT) calculations has recently investigated the molecular structure and conformations of caramboxin, a natural neurotoxin that is present inside the star fruit *Averroha carambola* [18]. The availability of experimental NMR data from the original isolation study [19] along with computational methods for the accurate computation of NMR chemical shifts, optical rotation, and other spectroscopic properties [20,21] allow one to fully characterize the molecular structure of a given natural compound. In some cases, i.e. when the computed and experimental chemical shifts do not match each other within few ppm, structural revision can be suggested [22]. Furthermore, the application of methods for the analysis of the topology of the computed electron density, such as Bader's quantum theory of atoms in molecules (QTAIM) [23,24], shed light on the strength and nature of the covalent bonds along with discovering the presence of specific intramolecular (non-covalent) interactions in a molecule. For instance, from our analysis of the topology of the electron density of caramboxin [18] it emerged that besides the canonical O–H···O/N H-bonds also the weaker C–H···O/N H-bonds do contribute in the stabilization of the low-energy conformers of this neurotoxin.

2. Computational methods

All the quantum chemical DFT calculations were performed with the Gaussian 09 software package [25]. The B3LYP hybrid functional of Becke [26] was employed here in combination with the 6-31G(d,p) basis set [27] for both geometry optimizations and vibrational frequency calculations (the latter providing zero-point energy, ZPE, corrections). Molecular geometries were also optimized with the second-order Møller-Plesset perturbation theory (MP2) method [28] in combination with the above basis set.

Nuclear magnetic shieldings were computed using gauge-including atomic orbitals (GIAOs) [29] in combination with the MPW1PW91 hybrid functional of Adamo and Barone [30] and the 6-311+G(2d,p) basis set [31]. The choice of the MPW1PW91 functional is based on the good performances obtained in the simulation of NMR spectra of natural products [16]. Solvation (hydration) effects were modeled with the polarization continuum model (PCM) of Tomasi and coworkers [32].

The topology of the electronic charge density of the molecules investigated herein was analyzed with the AIMAll software package of Keith [33] which implements Bader's QTAIM. Molecular crystal structures were searched through the Cambridge Structural Database (CSD) [34], Version 5.36 (2015 release), maintained and distributed by the Cambridge Crystallographic Data Centre (CCDC). The visualization of the molecular geometries was performed with the GaussView graphical interface [35].

3. Results and discussion

3.1. Molecular structures

Two low-energy conformers were identified for both **1** and **2**, depending upon the orientation of the terminal CH_2–OH group, and their DFT-optimized geometries are shown in Fig. 2. As far as TTX (**1**) is concerned, the conformer **1a** with the torsion angle τ_1(O–C6–C11–O) of +51.3° is more stable than **1b** where this torsion angle is −50.8° (the ZPE-corrected energy difference between them is 0.19 kcal/mol). Both conformers are present in the molecular crystal (CSD id: TETXHB) of tetrodotoxin hydrobromide where the torsion angle τ_1 assumes the values of +65.03° and −56.20° [9]. The conformers **1a** and **1b** are stabilized by an intramolecular H-bond of 2.042 Å and 2.038 Å, respectively, involving the hydroxyl groups bonded to C6 and C11. There is the