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Thymidine 3′,5′-Diphosphoric Acid Derived Cations and Radicals: Ab Initio Study

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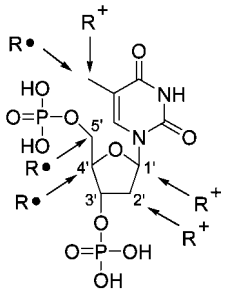
ABSTRACT



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The relative stabilities of thymidine-3′,5′-diphosphoric acid (1) derived isomeric cations and radicals were calculated and key geometric parameters were thoroughly analyzed. The most probable sites of a hydride-ion (1′, 2′, 5-Me) and H-atom (4′, 5′, 5-Me) abstraction were identified, thus allowing prediction of the regioselectivity of potential damage to the deoxyribose ring and thymine moiety caused by carcinogenic agents of electrophilic and radical nature.

Interaction of DNA with electrophilic and radical species can trigger a cascade of structural changes that affects its functionality, as well as conformational and configurational characteristics. A body of experimental evidence accumulated so far deals with a gross effect of such alterations, i.e., DNA strand cleavage.[1] Intimate mechanistic details remain mostly beyond comprehension due to the complexity of the subject, the abundance of potential reactive sites, and the scarcity of valid molecular probes and assays. While a number of electrophilic agents have been long proven to be carcinogenic to humans,[2] the mechanisms of cation(oid)−DNA interaction have been established only in a few cases involving DNA bases.[3] Oxidative damage to DNA caused by oxygen- and carbon-centered radicals is well documented;[4a−c] model studies including selective generation of the radical centers at alternative sites of a deoxyribose ring and DNA bases have shed light upon the mechanism of secondary structural changes including an ultimate DNA strand cleavage.[4d−f] Despite recent advances, an understanding of the "organic

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chemistry of DNA" is still deficient, resulting in a low degree of predictability of product distribution in electrophilic and radical reactions, an insufficient understanding of configurational and conformational changes attendant with generation of cationic and radical centers, as well as the nature of secondary transformations leading to irreversible structural alterations. Our interest in this area was initiated by the discovery of an unusual THF-mediated radical dimerization of cobalt-complexed propargyl alcohols[5a] mimicking, on a molecular level, DNA damage inflicted by electrophilic carcinogenic agents.[5b] In this Letter we report results on ab initio calculations of isomeric cations and radicals (Titan[6]) derived from thymidine-3′,5′-diphosphoric acid (**1**). Semiempirical calculations were carried out at the AM1 level[7a] with the optimized geometries being further employed in ab initio calculations by the Hartree−Fock (HF) method for cations and by the high-spin open-shell SCF Hartree−Fock (ROHF) method for radicals using the 3-21G* basis set.[7b] Single-point calculations were then performed by the Density Functional Theory (DFT) with the hybrid density functional B3LYP for cations and by ROB3LYP for radicals using the 6-31G* basis set in their optimized 3-21G* geometries.[7c−e,8]

Along with a major characteristic—relative stability of isomeric cations and radicals—we were also interested in a hybridization mode of the cationic and radical centers, as well as a spatial arrangement around them. Quantitative data on carbocations (**2**) and radicals (**3**) are summarized in Figure 1. Cation 1′ is found to be the most stable one, supposedly,

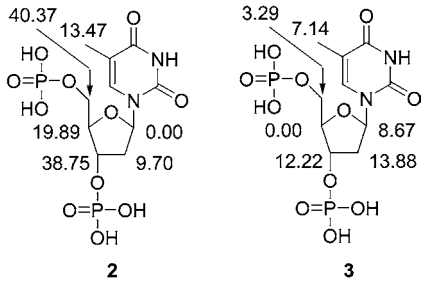

Figure 1. Relative stabilities (kcal/mol; B3LYP/6-31G*/HF/3-21G*) of isomeric cations (**2**) and radicals (**3**).

due to the *"double-stabilization" by* α*-heteroatoms*[9] manifested, first, by a shortened O−C1′ bond (1.29 Å) relative to the O−C4′ counterpart (1.50 Å; ca. 1.46 Å in **1**) and, second, by the difference in C1′−N bond lengths in cation 1′ (1.31 Å) and its neutral precursor **1** (1.45 Å). While a cationic center is ideally flat (0.002 Å out of plane defined by O/N/C2′), the angles around it vary in a wide range of 112.0−131.2°. Unexpectedly, the secondary cation 2′ exhibited stability (9.70 kcal/mol) even greater than α-oxygen stabilized cation 4′ (19.89kcal/mol): its well-emphasized pyramidal arrangement (out of plane by 0.39 Å) is caused by the stabilizing, *through-the-space* coordination between the cationic center and carbonyl group in thymine (Figure 2). The C2′−O nonbonding distance of 1.47 Å is well below

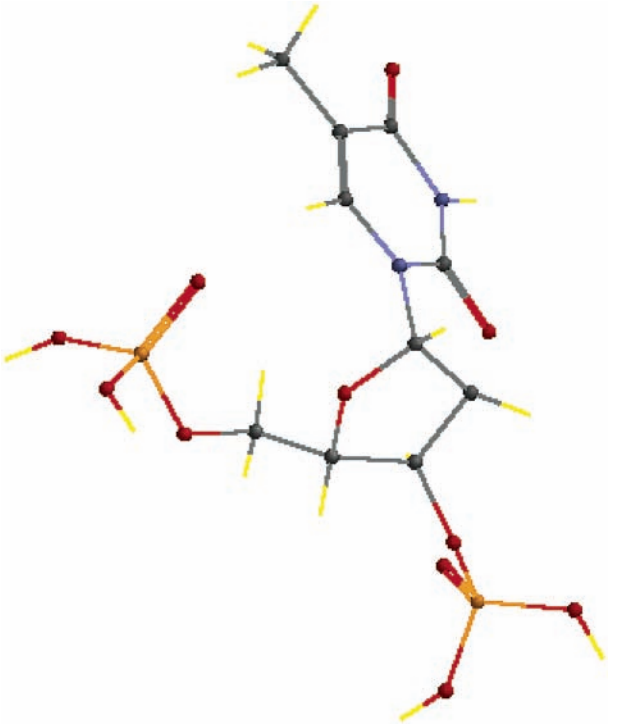

Figure 2. Cation 2′ stabilized by through-the-space interaction with a thymine moiety.

the sum of van der Waals radii (3.15−3.20 Å).[10] Bond length analysis indicates that a DNA base is represented by the most stable resonance contributor containing an amide moiety: an alternative dipolar structure with an internal charge separation, $^{+}N{=}C{-}O^{-}$, a potentially better donor for the C2′-based cationic center, failed to materialize ($N{-}C_2$ 1.377 Å vs 1.382 Å in **1**; $C_2{=}O$ 1.223 Å vs 1.226 Å in **1**). The third most stable cation is generated from the 5-Me group in the thymine moiety (13.47 kcal/mol). Its stability derives mostly from allylic delocalization; the cationic center has an ideal trigonal planar arrangement (119.8−120.2°) analogous to that of the

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(8) The dihedral angle C(4′)−C(5′)−O−P was fixed at 174.41° to avoid conformations not permissible in DNA.[13a]

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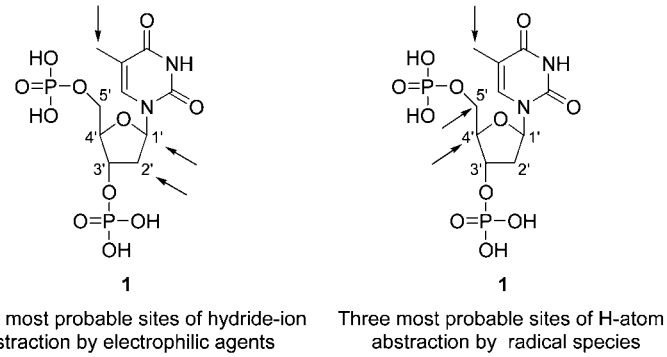

Figure 3.

least stable, C5′ cation (119.8−120.4°). Although relatively high in energy (19.89 kcal/mol), cation 4′ interacts with an α-oxygen causing significant shortening of the O−C4′ bond (1.20 Å vs 1.46 Å in **1**). It adopts a nearly flat configuration (out of plane by 0.019 Å) with a disparity in bond angles (113.6−124.0°) comparable to that of cation 1′. The two least stable species—cations 3′ and 5′—are impacted by a destabilizing effect of phosphate moieties.[11] While both are nearly flat (0.003 and 0.018 Å, respectively), an acyclic one has a nearly perfect trigonal planar arrangement (119.8−120.4°), while its cyclic counterpart exhibits a significant bond angle distortion (109.5−130.4°).

Computational data on radicals revealed a different order of relative stabilities with 4′ and 2′ radicals occupying opposite ends of the energy scale (Figure 1). In contrast to the predominantly flat, sp^2-hybridized cations—with the exception of the 2′ cation stabilized by the coordination with a thymine moiety—most of the radicals adopt pyramidal configuration. The deviation from planarity lies in the range of 0.21−0.37 Å (1′ 0.37 Å; 3′ 0.33 Å; 4′ 0.35 Å; 5′ 0.21 Å) with only 2′ and 5-Me derived radicals being nearly flat (0.03 and 0.001 Å, respectively). The generation of the radical center in the 1′ position is not favorably affected by an α-N moiety, probably due to the so-called *antagonistic effect.*[12] Bond lengths indicate a notable stabilization by an α-O atom (1.39 Å for C1′−O relative to 1.44 Å in **1**) and a lesser bond alteration for C1′−N (1.43 Å vs 1.45 Å in **1**). The contribution of the α-O atom to the stability of the most stable, 4′ radical is best substantiated by a shortened C4′−O bond (1.40 Å vs 1.46 Å in **1**). Curiously enough, despite significant difference in their thermodynamic stabilities, a total bond shortening for 1′ and 4′ radicals comes close for each (0.07 Å vs 0.06 Å). In a cation series, a bond shortening is higher in magnitude totaling 0.29 Å for the most stable, double-stabilized 1′ cation vs 0.26 Å for "monostabilized" 4′ species. It is worth mentioning that the least stable, 2′ radical, in contrast to its ionic counterpart, cation 2′, does not interact with a thymine moiety: the nonbonding distance C2′−O is equal to 2.88 Å (1.47 Å in cation 2′) and the radical center adopts a nearly flat configuration (0.03 Å). The trigonal-planar arrangement around radical centers is comparable to that in the cationic series and varies in the range of 108−126°. Calculated data on related radical species[13] revealed a different order of relative stabilities—1′ > 4′ > 5′ > 3′ > 2′—with a C1′ radical being the most stable.[13a] The energy gap between two extremes is much lower (<5.5 vs 13.88 kcal/mol, Figure 1), as well as the difference between the most implicated 1′ and 4′ radicals (<1.5 vs 8.67 kcal/mol). It is worth mentioning that replacement of the 1′-amino group with a cytosine moiety did not affect the relative energies of isomeric radicals.[13a]

The observed orders of the relative stabilities of cations (1′ > 2′ > 5-Me > 4′ > 3′ > 5′) and radicals (4′ > 5′ > 5-Me > 1′ > 3′ > 2′) are indicative of significant dissimilarities in conformational and configurational characteristics involved, and receptivity toward π-donation, both via chemical bond and through-the-space. The computation also revealed a greater energy disparity in the cation series (0−40.37 kcal/mol) relative to that in radicals (0−13.88 kcal/mol). Attendant with it is a broader energy gap between the two most stable cations, 1′ and 2,′ relative to their radical counterparts, 4′ and 5′ (9.70 vs 3.29 kcal/mol). The observed order, and disparity, of thermodynamic stabilities of cations and radicals allow, first, the prediction of the most probable sites for generation of DNA cations and radicals (Figure 3) and, second, the conclusion that DNA interaction with electrophilic carcinogenic agents—acting as hydride-ion acceptors—can be more regioselective than H-atom abstractions by "unruly" radical species, in particular, by oxygen-centered radicals. Computational data on radicals are consistent with a literature precedence. The most stable, C4′ radical is involved in DNA alterations caused by irradiation[14a] or bleomycin aided by metal ions and oxygen.[14b] Radicals C5′ are selectively generated by a H-atom abstraction by a hydroxyl radical followed by the formation of a covalent

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linkage to pyrimidine and purine bases.[15] The irradiation of DNA oligomers is also reported to produce a radical center from the 5-Me group.[4c] Although fourth by its stability, radical C1′ can be accessed by irradiation of dAMP[13a] and hydrogen abstraction by a uracilyl radical in a DNA−RNA hybrid.[16] Computational data on cations cannot be readily related to the experimental results since the latter deal mostly with structural modifications of DNA bases.[3] It is conceivable nevertheless that a direct electrophilic attack upon a ribose ring can take place; our data revealed an unexpected vulnerability of the C2′ position of a ribose ring along with the C1′ position and a 5-Me group in a thymine moiety being the most probable alternative hydride-ion sources.

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Supporting Information Available: Calculated total electronic energies (in hartrees) for thymidine 3′,5′-diphosphoric acid derived cations [HF/3-21G*; DFT (B3LYP/6-31G*//HF/3-21G*)] and radicals [ROHF/3-21G*; DFT (ROB3LYP/6-31G*//ROHF/3-21G*)]. This material is available free of charge via the Internet at http://pubs.acs.org.

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