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Investigation of the electronic structure and the structural stability of selected penicillins by density functional calculations of ^{14}N nuclear quadrupole resonance parameters

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Abstract A computational study was carried out by density functional theory (DFT) to investigate the relative stability and reactivity in three selected penicillins: penicillin-G, penicillin-V and carbenicillin. The geometry of the investigated molecules was optimized at the B3LYP/6-31G(d) level of theory. Then, the nuclear quadrupole resonance (NQR) parameters of ^{14}N and ^{2}H nuclei and natural bond orbital (NBO) analysis in these molecules were calculated on the geometrically optimized models at the B3LYP level using 6-311++G(d,p) basis sets in the gas phase. The NBO analysis shows that the occupancy of the LP(N) decreases with increasing p character of the lone pair of nitrogen. A comparison between the results obtained for these penicillins and related 6-APA structures of them indicates that the presence of a bulky side group in the acyl side chain can lead to more stability of the β-lactam ring. On the other hand, NBO analysis was applied to rationalize the ^{14}N NQR parameters in the charge distribution around nitrogen atoms. Inspection of the present results illustrates that the largest component of EFG tensor (q_{zz}), the nuclear quadrupole coupling constant, C_Q, and the NQR frequency values of nitrogens decrease with decreasing occupancy values of LP(N). We suggest that the reason for this trend can be found in increasing contribution of delocalized electrons of nitrogen in the intramolecular interactions and hence stability of these structures increases in the order: PG < PV < CA. Finally, a good relationship is found between most of the calculated ^{2}H NQR parameters and the related intramolecular hydrogen bonds.

Keywords Penicillin · Nuclear quadrupole resonance (NQR) · Natural bond orbital (NBO) analysis

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Introduction

The β-lactam antibiotics are useful and frequently prescribed antimicrobial agents that share a common structure and mechanism of action-inhibition of synthesis of bacterial peptidoglycan cell wall. The penicillins (PENs) constitute one of the most important groups of these antibiotics. The general structure of penicillins consists of a thiazolidine ring (5-membered ring), connected to a β-lactam ring (4-membered ring) to which an aminoacyl side chain is attached [1]. The study of β-lactam antibiotics covers a very wide field, dealing with its chemistry, antibacterial activity, biosynthesis, and reaction mechanisms [2]. The reactive β-lactam ring is the important structural moiety for antibacterial activity of penicillins, which requires: a ring of sufficient strain; possibilities for electron delocalization outside the lactam ring; and some conformational features [3]. On the other hand, the stereochemistry of penicillins plays an important role in explaining their biological activities. X-ray analyses have shown that the nonplanar thiazolidine ring can exist in two conformations: the axial conformation, where the C3 carbon is out of the plane formed by the other four atoms of the ring and the equatorial conformation, where the sulphur atom is out of the plane. Therefore, in-depth knowledge of the β-lactam-thiazolidine nucleus is essential for establishing a

structure–activity relationship. Previous studies demonstrate the axial conformation is more stable than the other one [4–6]. Also, the X-ray structures available in the Cambridge Structural Database (CSD) reveal that the majority of penicillins (~80 %) are in the axial conformation, which has been interpreted as an evidence for the biological relevance of this conformation. The relationship between the structure of penicillins and their biological activity has been intensively investigated for many years [7, 8]. The antibacterial activity in vitro is defined as the minimum inhibitor concentration (MIC) which is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. A lower MIC is an indication of a better antimicrobial agent [9, 10].

Nuclear quadrupole resonance (NQR) is widely used to study the geometry and electronic structure of molecules and solids [11]. It must be noted that since the NQR spectroscopy can be only studied in the solid state [12], this technique would be effective to study the electronic structure of β-lactams. Nuclei with spin angular momentum, I, greater than one half ($I > 1/2$), have electric quadrupole moment, eQ, which interacts with electric field gradient tensor, EFG. The quadrupole coupling constant, QCC, calculated from NQR frequency, is proportional to the charge on quadrupole nuclei and gives information on electron distribution in a molecule, whereas asymmetry parameter, η_Q, provides information on the direction and order of chemical bonds [13]. Generally, nuclear quadrupole resonance, NQR, spectroscopy provides a sensitive technique for electronic environment and nuclear charge distribution measurements and can be applied for better characterization of hydrogen bond properties [14, 15].

In this work, the density functional theory (DFT) was used to compare the electronic structure and reactivity of some selected penicillins: penicillin-G (PG), penicillin-V (PV) and carbenicillin (CA). Among them, penicillin-G (benzylpenicillin) has the greatest antimicrobial activity and is the only natural penicillin used clinically. The antimicrobial spectra of penicillin-G and penicillin-V (phenoxymethylpenicillin) are very similar for aerobic gram-positive microorganisms. The virtue of penicillin-V in comparison with penicillin-G is that it is more stable in an acidic medium [1], while carbenicillin is active against most gram-negative organisms and some gram-positive organisms [16]. The experimental values for the activities in vitro suggest that PG is the more active molecule, CA is the less active one, and PV is molecule of intermediate activity [9, 17]. X-ray studies on these molecules show that all three of them are in axial conformation [18–20]. Unlike most previous studies that have focused more on the structural parameters obtained from geometry optimization such as the bond orders, dipole moments, electrophilicity index, atomic charges, etc., we applied natural bond orbital (NBO) analysis and nuclear quadrupole resonance (NQR) spectroscopy to study the electronic structure and nuclear charge distribution around the nitrogen nucleus in these penicillins. Also, to obtain information about the relative stability of these structures, we have studied their energies. Since the reactivity of the β-lactam ring was linked to the lack of resonance of the amide endocyclic system caused by the pronounced pyramidal character of the β-lactam nitrogen atom [21], in the present work, we have mainly focused on the nitrogen atoms in the β-lactam ring and the side chain positions. Finally, it was seen that a comparison between ^{14}N-NQR parameters of them with the related experimental data (MIC) would be useful in the determination of the activity of penicillins.

Computational details

The quantum chemical calculations were carried out within the Gaussian 03 software package [22]. X-ray structures of PG, PV and CA molecules are already available and these structures are supplied as input [18–20]. Geometry optimizations have been performed at the DFT level with B3LYP functional [23–25] using 6-31G(d) basis set. Also, natural bond orbital (NBO) analysis and electric field gradient (EFG) calculations have been done at the B3LYP/6-311++G(d,p) level of theory on the optimized structures in gas phase. The principal components of the EFG tensor, q_{ii}, are computed in atomic unit (1 au = 9.717365 × 10^{21} V m^{-2}), with $|q_{zz}| \geq |q_{yy}| \geq |q_{xx}|$ and $q_{xx} + q_{yy} + q_{zz} = 0$. These diagonal elements relate to each other by the asymmetry parameter: $\eta_Q = |(q_{yy} - q_{xx})/q_{zz}|$, $0 \leq \eta_Q \leq 1$, that measures the deviation of EFG tensor from axial symmetry. The computed q_{zz} component of EFG tensor is used to obtain the nuclear quadrupole coupling constant (χ_{zz}) from the equation; C_Q (MHz) = $e^2 Q q_{zz}/h$ [26]. The standard values of Q reported by Pyykkö [27] are employed: Q (^{14}N) = 20.56 mb and Q (^{2}H) = 2.86 mb. For a nucleus of unit spin (such as ^{14}N and ^{2}H), we have three energy levels, so we get three nuclear quadrupole resonance frequencies: $\upsilon_+ = 3/4\chi_{zz}(1 + \eta/3)$; $\upsilon_- = 3/4\chi_{zz}(1-\eta/3)$ and $\upsilon_0 = 1/2\chi_{zz}\eta$ [28].

The occupancies and natural hybrids of lone pair orbital of nitrogen, the stabilization energies associated with LP(N) → σ* or π* delocalizations and some of intramolecular hydrogen bonds were analyzed by the natural bond orbital (NBO) method. In the second part of NBO analysis, we have focused on the common structure of penicillins (6-APA) to investigate the role of acyl side chain on the structural stability of penicillin. The

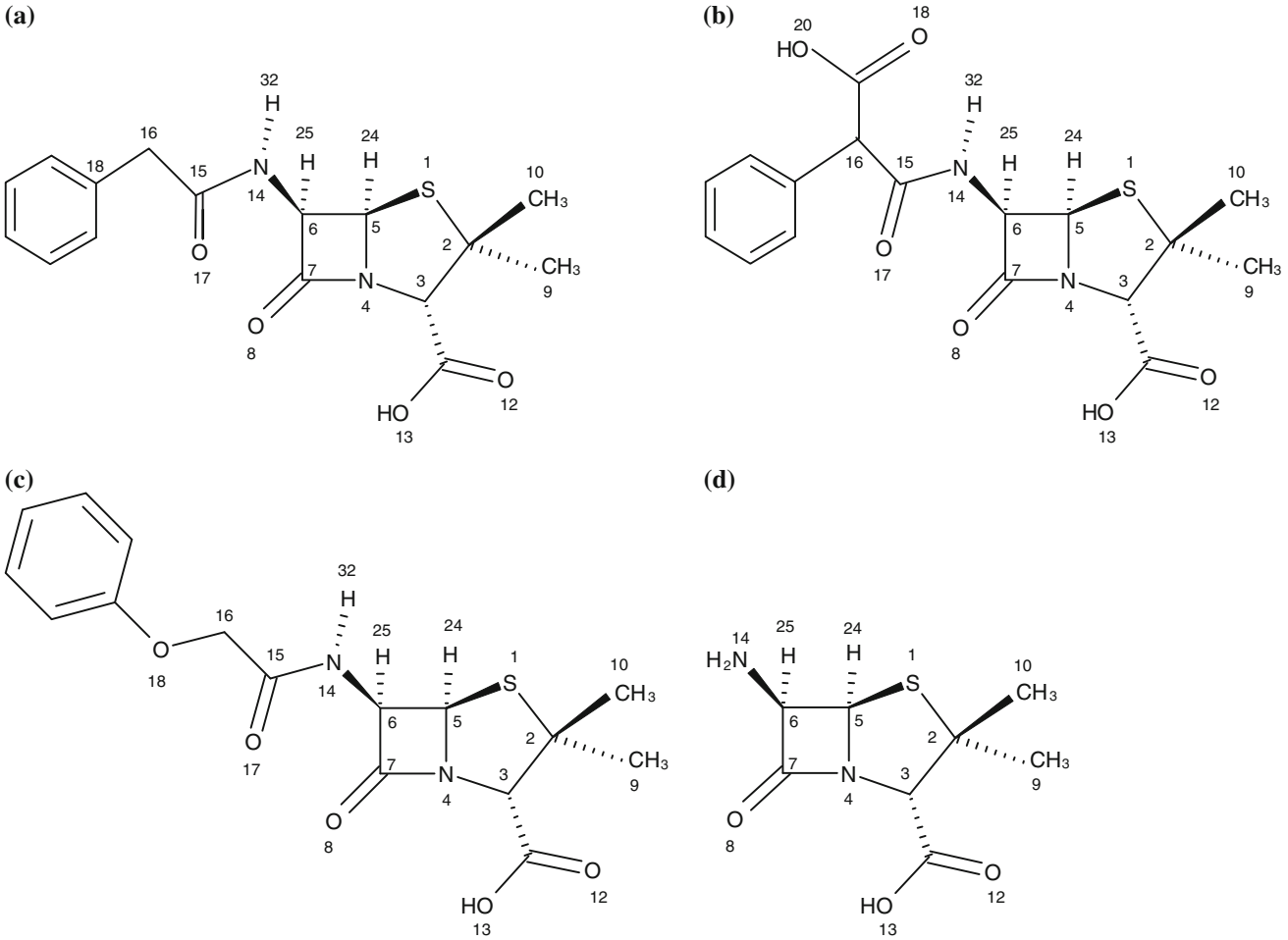

Fig. 1 The chemical structures of **a** penicillin-G (*PG*), **b** carbenicillin (*CA*), **c** penicillin-V (*PV*) and **d** 6-aminopenicillanic acid (*6-APA*)

6-aminopenicillanic acid (6-APA) is the key intermediate of semisynthetic penicillins [29] and for antibacterial activity to be achieved, an acyl group must be attached to the amino group of 6-APA [30]. The penicillin basic bicyclic structure studied in the present work is shown in Fig. 1. In the 6-APA structure of all three penicillins, the acyl group is replaced by a hydrogen atom.

Results and discussion

NBO analysis

The interactions between the filled (bonding or lone pair) Lewis-type (donor) NBOs and empty (antibonding and Rydberg) non-Lewis (acceptor) NBOs represent the deviation of the molecule from the Lewis structure and can be used as the measure of delocalization [31–34]. For each donor (i) and acceptor (j), the stabilization energy (E_2) associated with i → j delocalization can be estimated by second-order perturbation theory according to the following equation: $E_2 = q_i(F^2_{(i,j)}/(\varepsilon_i - \varepsilon_j))$, where q_i is the donor orbital occupancy, ε_i and ε_j are diagonal elements (orbital energies) and $F_{(i,j)}$ is the off-diagonal NBO Fock matrix elements [35].

The study of NBO analysis in Table 1 for each structure in gas phase revealed that the total stabilization energy for LP(N) → σ* or π* delocalization increases with increasing p character of nitrogen lone pair. In contrast, the occupancy of the LP(N) decreases with increasing p character of the lone pair of nitrogen. We observed that the occupancy values of LP(N4) and LP(N14) in CA, PV and PG molecules decrease, respectively; this trend is correlated with increase in the related total E_2 values for donor–acceptor interactions in these penicillins. Our findings indicate that the trend of the stabilization energies associated with both LP(1) N4 → π* (C7–O8) and LP(1) N14 → π* (C15–O17) delocalizations is: CA > PV > PG. This result is in agreement with the previous study which suggests that the carbonyl groups participation in the hydrolysis mechanism may contribute to increasing the stability of the β-lactam ring [36]. On the other hand,

Table 1 Calculated natural hybrids (NHOs), occupancies and resonance energies (in kcal mol^{-1}) of nitrogen lone pair using NBO analysis at the B3LYP/6-311++G**//B3LYP/6-31G* level of theory and calculated electronic energies, E_{el}, values (in Hartree) at the B3LYP/6-31G* level for PG, PV and CA molecules in the gas phase

PENs	Lewis-type NBOs			Non-Lewis NBOs	Interaction energy E_2 (kcal mol^{-1})	Total E_2	$-E_{el}$
	Type	Hybrid	Occupancy	Type			
PG	LP (1) N4	$sp^{8.70}$	1.72423	BD*(1) S1–C5	7.33	57.97	1429.14
				BD*(1) C2–C3	5.02		
				BD*(1) C3–C11	3.62		
				BD*(1) C5–H24	4.03		
				BD*(2) C7–O8	37.97		
	LP (1) N14	p	1.69910	BD*(1) C5–C6	7.35	43.15	
				BD*(1) C6–C7	8.19		
				BD*(2) C7–O8	0.54		
				BD*(1) C15–O17	13.92		
				BD*(2) C15–O17	13.15		
PV	LP (1) N4	$sp^{8.74}$	1.72344	BD*(1) S1–C5	7.36	58.07	1504.35
				BD*(1) C2–C3	5.02		
				BD*(1) C3–C11	3.62		
				BD*(1) C5–H24	4.02		
				BD*(2) C7–O8	38.05		
	LP (1) N14	p	1.68828	BD*(1) C5–C6	6.98	65.59	
				BD*(1) C6–C7	8.37		
				BD*(2) C7–O8	0.52		
				BD*(1) C15–O17	1.88		
				BD*(2) C15–O17	47.84		
CA	LP (1) N4	$sp^{8.77}$	1.72249	BD*(1) S1–C5	7.14	58.38	1617.70
				BD*(1) C2–C3	4.96		
				BD*(1) C3–C11	3.65		
				BD*(1) C5–H24	4.02		
				BD*(2) C7–O8	38.61		
	LP (1) N14	p	1.68441	BD*(1) C5–C6	8.46	77.31	
				BD*(1) C6–C7	6.64		
				BD*(2) C7–O8	–		
				BD*(1) C15–O17	0.52		
				BD*(2) C15–O17	61.69		

resistance to these antibiotics (PENs) is conferred principally via β-lactamases. Those are enzymes which hydrolyze the amide bond of the β-lactam ring and rendering the drug useless [37–40]. Thus probably, the N4–C7 bond is one of the most important bonds in determining the relative stability of penicillin. Present studies demonstrated an increasing order of stability for PG, PV and CA due to the increasing occupancy of the N4–C7 bond (1.98265, 1.98272 and 1.98287, respectively). Furthermore, the interaction energy of LP(2) O8 → σ (N4–C7) delocalization increases in the order: PG, PV and CA (29.09, 29.15 and 29.29 kcal mol^{-1}, respectively).

The stabilization energy of all intramolecular hydrogen bonds and their hydrogen bond lengths are reported in Table 2. Nangia and co-worker [7] suggested that the axial conformation in penicillins is stabilized by intramolecular N14–H32···S and C10–H30···O8 hydrogen bonds. According to our results in this table, we note that PG and CA molecules have, respectively, the shortest and longest bond lengths in both hydrogen bonds. A comparison between the basic bicyclic structure and the general structure of penicillin reveals that the sum of resonance energy for the hydrogen bonds related to β-lactam-thiazolidine nucleus increases in the order: CA, PV and PG (10.06, 10.57 and 10.66 kcal mol^{-1}, respectively), while the total E_2 value for all intramolecular hydrogen bonds in PG molecule is significantly less than that in two other molecules. Also, Abrahamsson et al. [19] in the X-ray

Table 2 Calculated resonance energies in (kcal mol^{-1}) and related distances (Å) of all hydrogen interactions for PG, PV and CA molecules using NBO analysis at the B3LYP/6-311++G**//B3LYP/6-31G* level of theory in the gas phase

PENs	Donor	Acceptor	Interaction energy E_2 (kcal mol^{-1})	Hydrogen contact distance (Å)	Total E_2
PG	LP (2) S2	BD*(1) C5–H24	3.45	2.447	10.66
		BD*(1) C9–H27	0.74	3.776	
		BD*(1) C10–H29	1.21	3.793	
		BD*(1) N14–H32	0.53	2.926	
	LP (1) N4	BD*(1) C5–H24	4.02	2.169	
	LP (1) O8	BD*(1) C10–H30	–	2.769	
	LP (2) O17	BD*(1) C6–H25	0.70	2.308	
PV	LP (2) S2	BD*(1) C5–H24	3.49	2.445	25.21
		BD*(1) C9–H27	0.74	3.777	
		BD*(1) C10–H29	1.22	3.793	
		BD*(1) N14–H32	0.51	2.932	
	LP (1) N4	BD*(1) C5–H24	4.02	2.166	
	LP (1) O8	BD*(1) C10–H30	–	2.773	
	LP (2) O17	BD*(1) C6–H25	0.59	2.333	
	LP (1) O18	BD*(1) N14–H32	1.51	2.316	
		BD*(1) C16–H33	0.81	2.085	
		BD*(1) C16–H34	0.71	2.086	
	LP (2) O18	BD*(1) C16–H33	5.68	2.085	
		BD*(1) C16–H34	5.93	2.086	
CA	LP (2) S2	BD*(1) C5–H24	3.53	2.444	16.39
		BD*(1) C9–H27	1.21	3.777	
		BD*(1) C10–H29	0.74	3.794	
		BD*(1) N14–H32	–	3.043	
	LP (1) N4	BD*(1) C5–H24	4.02	2.164	
	LP (1) O8	BD*(1) C10–H30	–	2.774	
	LP (2) O17	BD*(1) C6–H25	0.56	2.346	
	LP (1) O18	BD*(1) N14–H32	1.76	1.982	
	LP (2) O18	BD*(1) N14–H32	4.57	1.982	

crystallography study of PV explained that the only region where it seems possible for an internal hydrogen bond exists in penicillin-V is between O18 and N14–H32 in the side chain and the effect of this close contact may be sufficiently strong. From the results reported in Table 2, it is obvious that the O18···N14–H32 distance in CA molecule is shorter than that in the PV molecule (1.982 and 2.316 Å, respectively), and the corresponding estimated hydrogen bond length in the PG molecule, C18···N14–H32, is 2.410 Å. As mentioned above, the NBO analysis illustrated that the substituents (the O18 atom in PV and the carboxylic group in CA) attached to the aminoacyl side chain at the position of C16 atom play an important role in increasing the stability of both molecules through hydrogen bonds.

The next section of NBO analysis focused on the basic structure of the penicillin. Therefore, the natural hybrids (NHOs), occupancies and stabilization energies (E_2) for nitrogen lone pair calculated using NBO analysis for the 6-APA structure of PG, PV and CA molecules are listed in Table 3. By a quick look at the results reported in this table, it is obvious that the occupancy and p character values of CA and PG are close together and the occupancy of LP(N4) in these two molecules is smaller than that in the PV molecule. Also, the 6-APA structure of PV, PG and CA shows an increasing order of stability due to the increasing total stabilization energy associated with donor–acceptor interactions for lone pair electrons of N4. This trend is in parallel with their increasing electronic energy (Table 3). Furthermore, the N4–C7 bond lengths in the 6-APA structure of CA and PG are smaller than that of PV (CA = PG = 1.403 Å and PV = 1.405 Å). Also, the NBO analysis illustrated that the occupancy values of the N4–C7 bond in the 6-APA structure of CA and PG are greater than that of PV (CA = PG = 1.98271 and PV = 1.98260). Comparison of these results with those reported in Table 1

Table 3 Calculated natural hybrids (NHOs), occupancies and resonance energies (in kcal mol^{-1}) of nitrogen lone pair using NBO analysis at the B3LYP/6-311++G**//B3LYP/6-31G* level of theory and calculated electronic energies, E_{el}, values (in Hartree) at the B3LYP/6-31G* level for the 6-APA structure of PG, PV and CA molecules in the gas phase

PENs	Lewis-type NBOs			Non-lewis NBOs	Interaction energy E_2 (kcal mol^{-1})	Total E_2	$-E_{el}$
	Type	Hybrid	Occupancy	Type			
PG	LP (1) N4	$sp^{8.89}$	1.72173	BD*(1) S1–C5	7.30	58.43	1045.4257
				BD*(1) C2–C3	4.98		
				BD*(1) C3–C11	3.69		
				BD*(1) C5–H24	4.10		
				BD*(2) C7–O8	38.36		
	LP (1) N14	$sp^{4.18}$	1.92924	BD*(1) C5–C6	1.43	14.58	
				BD*(1) C6–C7	11.33		
				BD*(1) C5–H25	1.23		
				BD*(2) C7–O8	0.59		
PV	LP (1) N4	$sp^{8.78}$	1.72533	BD*(1) S1–C5	7.40	58.17	1045.4249
				BD*(1) C2–C3	5.08		
				BD*(1) C3–C11	3.71		
				BD*(1) C5–H24	4.18		
				BD*(2) C7–O8	37.80		
	LP (1) N14	$sp^{4.30}$	1.92684	BD*(1) C5–C6	11.83	15.3	
				BD*(1) C6–C7	2.73		
				BD*(1) C6–H25	0.74		
CA	LP (1) N4	$sp^{8.90}$	1.72165	BD*(1) S1–C5	7.29	58.45	1045.4258
				BD*(1) C2–C3	4.98		
				BD*(1) C3–C11	3.69		
				BD*(1) C5–H24	4.10		
				BD*(2) C7–O8	38.39		
	LP (1) N14	$sp^{4.18}$	1.92930	BD*(1) C5–C6	1.42	14.57	
				BD*(1) C6–C7	11.32		
				BD*(1) C5–H25	1.24		
				BD*(2) C7–O8	0.59		

indicate that by attaching the acyl group to the amino group of 6-APA, the β-lactam ring in CA and PV can be more stable than that in PG. So, our obtained results are in good agreement with the previous studies, suggesting that penicillins with bulky side groups can block the β-lactamases which hydrolyze the lactam ring [41–44].

Finally, for all the above reasons, we suggest that decreasing occupancy of the LP(N) and corresponding increasing interaction energy between LP(N) and non-Lewis NBO can be associated with the contribution of the electron pairs of both nitrogen atoms during the formation of intramolecular hydrogen bond and resonance interactions in the β-lactam ring and the aminoacyl side chain positions. Hence, structural stability of these structures can be increased by the delocalization of electron density of nitrogen lone pair. So, we expect that the stability order of these penicillins is as follows: CA > PV > PG, which accords with the trend of electronic energy obtained from geometry optimization (see Table 1).

^{14}N and ^{2}H NQR parameters

Tables 4 and 5 present the calculated NQR parameters (η and C_Q), principal components of the EFG tensors and the associated frequencies of ^{14}N and ^{2}H nuclei in PG, PV and CA molecules. There are only three known stable nuclei with unit spin: ^{2}H, ^{6}Li, and ^{14}N. Most of the NQR work done using integer spin nuclei is for ~100 % naturally abundant ^{14}N. Deuterium (^{2}H) and ^{6}Li have very small electric quadrupole moments, making direct observation with NQR difficult. For spin 1, there are three transition frequencies. The NQR frequency will be determined by the distribution of electric charge in the vicinity of a nucleus [45]. Homonuclear diatomic molecules (such as ^{14}N and ^{2}H) have axial symmetry.

Table 4 Calculated EFG tensor, the NQR parameters and related frequencies of ^{14}N nuclei for PG, PV and CA molecules at the B3LYP/6-311++G**//B3LYP/6-31G* level of theory in the gas phase

PENs	Nuclei	q_{xx} (10^{21} V m^{-2})	q_{yy} (10^{21} V m^{-2})	q_{zz} (10^{21} V m^{-2})	$e^2Q\ q_{zz}/h$ (MHz)	η_Q	υ_+ (MHz)	υ_- (MHz)	υ_0 (MHz)
PG	N(4)	−3.715230	−5.620514	9.335744	4.614032	0.204084	3.695937	3.225110	0.470826
	N(14)	−3.445155	−5.589467	9.034622	4.465207	0.237343	3.613853	3.083958	0.529894
PV	N(4)	−3.726619	−5.608017	9.334646	4.613489	0.201550	3.692579	3.227654	0.464924
	N(14)	−3.093620	−5.631251	8.724872	4.312118	0.290850	3.547634	2.920543	0.627090
CA	N(4)	−3.726055	−5.584589	9.310644	4.601626	0.199613	3.680857	3.221583	0.459274
	N(14)	−2.787775	−5.790305	8.578091	4.239574	0.350023	3.550668	2.808694	0.741974

Table 5 Calculated EFG tensor, the NQR parameters and related frequencies of ^{2}H nuclei for PG, PV and CA molecules at the B3LYP/6-311++G**//B3LYP/6-31G* level of theory in the gas phase

PENs	Nuclei	q_{xx} (10^{21} V m^{-2})	q_{yy} (10^{21} V m^{-2})	q_{zz} (10^{21} V m^{-2})	$e^2Q\ q_{zz}/h$ (MHz)	η_Q	υ_+ (MHz)	υ_- (MHz)	υ_0 (MHz)
PG	H24	1.392615	1.485755	−2.878370	−0.199050	0.032358	−0.150898	−0.147677	−0.003220
	H25	1.314205	1.441512	−2.755708	−0.190568	0.046197	−0.145126	−0.140725	−0.004401
	H30	1.370653	1.503606	−2.874270	−0.198766	0.046256	−0.151373	−0.146776	−0.004597
	H32	1.491353	2.277556	−3.768909	−0.260634	0.208602	−0.209068	−0.181883	−0.027184
PV	H24	1.393547	1.482889	−2.876437	−0.198916	0.031059	−0.150732	−0.147643	−0.003089
	H25	1.309152	1.449665	−2.758818	−0.190783	0.050932	−0.145516	−0.140658	−0.004858
	H30	1.370750	1.504461	−2.875203	−0.198831	0.046504	−0.151435	−0.146811	−0.004623
	H32	1.507454	2.219873	−3.727338	−0.257759	0.191133	−0.205636	−0.181003	−0.024633
CA	H24	1.391312	1.483725	−2.875037	−0.198820	0.032142	−0.150712	−0.147517	−0.003195
	H25	1.306140	1.462259	−2.768399	−0.191445	0.056393	−0.146283	−0.140885	−0.005398
	H30	1.371907	1.509689	−2.881597	−0.199273	0.047814	−0.151837	−0.147073	−0.004764
	H32	1.358186	2.120591	−3.478777	−0.240571	0.219158	−0.193609	−0.167247	−0.026361

In these molecules, $q_{xx} = q_{yy} = -q_{zz}/2$, $\upsilon_+ = \upsilon_-$, $\upsilon_0 = 0$ and also $\eta = 0$. It is concluded from Table 4 that the distribution of electric charge around the nitrogen nucleus is deviated from cylindrical symmetry. This can be attributed to the influence of electric field of neighboring nucleus on the nitrogen atoms. By convention, the z axis is along the direction of axial symmetry. Therefore, the results reported in this table reveal that the η and q_{zz} values of non-protonated nitrogen (N4) in CA, PV and PG molecules increase, respectively, while the q_{zz} value of protonated nitrogen (N14) increases with decreasing its asymmetry parameter value. Also, the asymmetry parameter measure of N4 is smaller than that of N14 in each structure. This means that the distribution of electric charge around N4 is closer to the symmetry axis. Furthermore, our findings show that q_{zz}, C_Q and υ_+ values of both nitrogens decrease with decreasing the occupancy values of LP(N). This trend corresponds with the increasing stabilization energy values of donor–acceptor interactions in the order of PG, PV and CA. Although in all three molecules, p character of the lone pair of N14 (with 100 % p character) is the same, the q_{zz} and C_Q values of N14 in CA molecule are smaller than those in two other molecules. This trend is due to the high measure of delocalization for lone pair electrons of N14 in CA molecule in compaction with those in PV and PG molecules. Also, we note that about the nitrogen of β-lactam ring (N4), the q_{zz}, C_Q and υ_+ values decrease with increasing p character of lone pairs in the order of PG < PV < CA which can be justified by a main reason: the N4 atom in CA and PV molecules has a lower degree of occupancy for lone pair electrons compared to those of PG molecule. As a main result, the ^{14}N electric field gradient (EFG) tensors have been rationalized by natural bond orbital (NBO) analysis, demonstrating that with the increasing contribution of the lone pair electrons of nitrogen in the resonance interactions, the largest component of EFG tensor (q_{zz}), the nuclear quadrupole coupling constant, C_Q, and the υ_+ frequency values of ^{14}N nuclei decrease. This result is in good agreement with the increasing order of structural stability obtained from the NBO analysis in PG, PV and CA molecules, respectively.

The calculated ^{2}H NQR parameters for some hydrogen atoms involved in hydrogen bonds are presented in Table 5. The data reported in this table indicate that the electron density distributions around H32 deviate from axial symmetry, but are nearly cylindrical for the other hydrogens (η is close to zero). Recent studies [46] have shown that intermolecular interactions, e.g., hydrogen bonding (HB) interactions, have significant influence on

the EFG tensors. Also, it has been suggested that there is a close relationship between the nuclear quadrupole coupling constant, QCC, and hydrogen bond lengths [47]. By a quick look at the present results, we found a relationship between some of the ^{2}H NQR parameters and the related hydrogen bond lengths (Table 2). Analysis of the data in Tables 2, 5 indicates that H24, H25, H30 and H32 contribute to S···C5–H24, N4···C5–H24, O17···C6–H25, O8···C10–H30 and S···N14–H32 hydrogen bonds, respectively. Comparing these three molecules using calculated ^{2}H EFG tensors illustrated that the negative values of q_{zz}, C_Q and all three transition frequencies for the H24, H25 and H30 atoms increase with the increasing related hydrogen bond lengths and also these trends can be attributed to decreasing the stabilization energy (E_2) associated with these hydrogen bonds. In contrast, with the increasing negative values of ^{2}H NQR parameters for H32 atom, the distance between the S atom and N14–H32 bond decreases, corresponding with the increase of positive values of the ^{14}N NQR parameters for N14 atom. So, our results reveal that the calculated NQR parameters of ^{2}H nuclei are sensitive to the intramolecular hydrogen bonds and there is an approximately linear relationship between some C_Q(^{2}H) values and the related hydrogen bond lengths.

Conclusions

In the present work, DFT calculations were performed to study the effects of nitrogen atom on the relative stability of some selected penicillins. The NBO analysis showed that the increasing order of total resonance between LP(N) and non-Lewis-type NBOs (σ* or π*) is in parallel with the trend of electronic energy obtained from geometry optimization. It was found that by attaching a bulkier acyl group to the amino group of 6-aminopenicillanic acid, the N4–C7 bond order of penicillin molecule decreases and the occupancy value of this bond increases. So, the volume of the substituent joined to the aminoacyl side chain plays an important role in the stability of the β-lactam ring; i.e., the high stability of carbenicillin in comparison with two other molecules can be attributed to the presence of bulky carboxylic group in acyl side chain. Study of the electronic structure of these structures using NQR calculations revealed that the electron density distribution around N4 is closer to the symmetry axis than the charge distributions around N14. Also, it was seen that there is a logical relationship between the occupancy and p character of nitrogen lone pair with the ^{14}N NQR parameters; such that the q_{zz}, C_Q and υ_+ values of nitrogen nuclei decrease with decreasing the occupancy values of LP(N). Also, our results reveal that the calculated NQR parameters of ^{2}H nuclei are sensitive to the intramolecular hydrogen bonds. Finally, the results of NQR parameter calculations with NBO analysis showed that the increasing order of stability in PG, PV and CA molecules is in relatively good agreement with the observed decreasing trend of activity (the MIC values obtained from experimental analysis) in these penicillins. Thus, in this work, we have demonstrated that the electronic and nuclear properties of nitrogen atoms in penicillins are nearly associated with their antibacterial activity.

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