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Molecular modeling of penicilloate anions: an RHF-SCF analysis

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Abstract An ab initio restricted Hartree–Fock self-consistent field (RHF-SCF) analysis of penicilloate anions was performed at the TZV level with GAMESS. Geometry optimization was initialized by the semi-empirical AM1 method followed by optimization at the 6-31++G** level. The total energy obtained was −1116.0997 a.u. for the penicilloate amine, −1115.3164 a.u. for the imine, −1115.2969 a.u. for the enamine and −1115.2017 a.u. for the amine that was deprotonated at the thiazolidine nitrogen. Formation of the free thiolate in the imine and enamine anions by deprotonation of the penicilloate amine is associated with: (1) an increase in total energy (2) an increase in the energy of the highest occupied molecular orbital (HOMO) to that of anti-bonding (3) a decrease in chemical hardness (4) an increase in the chemical potential (5) a more negative Mulliken net charge on the sulfur atom and (6) an increase in the Mulliken atomic population on the former thiazolidine sulfur atom in the HOMO. The RHF-SCF analysis presented here suggests a potential role for the thiolate sulfur of penicilloate anions, especially of the imine, as a chemically reactive soft nucleophile.

Keywords Beta-lactam antibiotics · Molecular orbital · Allergy and allergens

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Introduction

Theoretical chemistry has played an important role in elucidating the mode of action of penicillins as antibiotics. Quantum chemical analyses of penicillins have been successful in contributing to our understanding of the interactions of penicillin antibiotics with their target molecules, the penicillin-binding proteins (PBPs) of susceptible bacteria that result in inhibition of transpeptidation of bacterial cell walls. [1] Quantum chemical approaches have contributed to the determination of the most likely molecular geometry of the D-ala–D-ala dipeptide that penicillin antibiotics mimic in interacting with bacterial penicillin binding proteins. [2] Quantum chemistry is being used to study the mechanisms of inactivation of penicillin antibiotics by ß-lactamases and the mechanisms of the development of bacterial resistance to the ß-lactamases. [3]

Hydrolysis of the ß-lactam ring of penicillins produces penicilloates, which lack anti-bacterial activity [4] but which are potent allergens. [5, 6, 7] We have begun to apply quantum chemistry to the analysis of penicillin allergens [8, 9] and report here an ab initio Hartree–Fock self-consistent field (HF-SCF) analysis of penicilloate anions.

Methods

The structures of the penicilloate anions, and the numbering system used in this paper, are given in the chart shown in Fig. 1.

Each oxygen of the two carboxylate groups carries a mean net negative charge of –0.71 a.u. (Mulliken population analysis) that does not vary significantly among the penicilloate anions. The hydrogens on N4 and C6 are involved in imine/enamine formation and tautomerism. The unnumbered atoms are shown for purposes of reference.

Restricted Hartee–Fock (RHF) self-consistent field (SCF) analyses were performed with GAMESS [10] using a triple zeta valence (TZV) basis set with three polarizing *p* and *d* functions and with one polarizing *f* function. Orbital exponents were adjusted to those of the diffuse functions of anions and diffuse *s* and *sp* shells were used. The RHF-SCF computations were performed in parallel mode

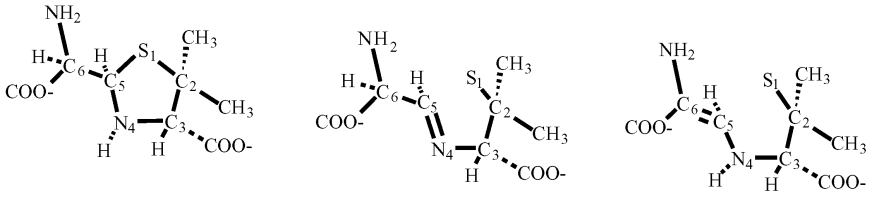

Fig. 1 The structure of penicilloate anions. Penicilloate amine (*left*), penicilloate imine (*center*) and penicilloate enamine (*right*)

on an IBM-SP computer. Molecular visualizations and bond angle determinations were performed with ViewMol3D [11] using a PC.

The geometry optimization process was begun with approximated coordinates for 6-amino-penicillanic acid (6-APA), assuming fused β-lactam and thiazolidine rings [12] and covalent bond lengths and bond angles collated by Pauling. [13] With these assumptions, the final coordinates did not vary with the details of the initial bond lengths and angles. The geometry optimization yielded coordinates for 6-APA in the "curled configuration". [12] The geometry optimization process was continued for penicilloic acid formed by hydroxylation of 6-APA at the β-lactam carbonyl, followed by ionization of the two carboxyl groups, yielding the coordinates of the penicilloate amine dianion. These coordinates, except those for the N4 proton, were also used for the N4-deprotonated penicilloate amine trianion, without further optimization.

The optimized coordinates, obtained as described in the preceding paragraph for the penicilloate amine dianion, protonated at the N4 position, were the starting coordinates for optimization of the imine and enamine trianions. Geometric optimization of the N4-deprotonated penicilloate amine trianion yielded the atomic coordinates of the penicilloate imine trianion tautomer. Geometric optimization of the C6-deprotonated penicilloate amine trianion yielded the atomic coordinates of the penicilloate enamine trianion tautomer.

Geometry optimization and Hessian analysis were performed by the semi-empirical AM1 method with PC-GAMESS [10] on a desktop PC. Final geometry optimization of the penicilloate amine, imine and enamine anions was performed with GAMESS using a 6-31++G** basis set. A Hessian analysis was performed for each type of penicilloate anion to determine whether the atomic coordinates represented equilibrium positions on the potential energy surfaces. An AM1 internal reaction coordinate (IRC) analysis was performed on the N4-deprotonated amine to determine whether the energy was at a saddle point on the potential energy surface.

Geometry optimization and RHF-SCF analyses were performed for the model disulfide, dimethyl disulfide $(CH_3S)_2$ with C_i molecular symmetry (C–S–S–C dihedral angle=180°), as described above for penicilloates, except that because $(CH_3S)_2$ is uncharged, this RHF-SCF analysis was performed with the 6-31G** and TZV basis sets without diffuse functions and shells.

Results

In preparatory studies it was determined from the Hessian analyses that the atomic coordinates for the penicilloate amine, imine and enamine anions all were at equilibrium positions on the potential energy surface and that those for the N4-deprotonated penicilloate amine trianion were not. The IRC analysis of the N4-deprotonated penicilloate amine trianion gave a negative slope in forward and backward directions on the reaction coordinate, thereby

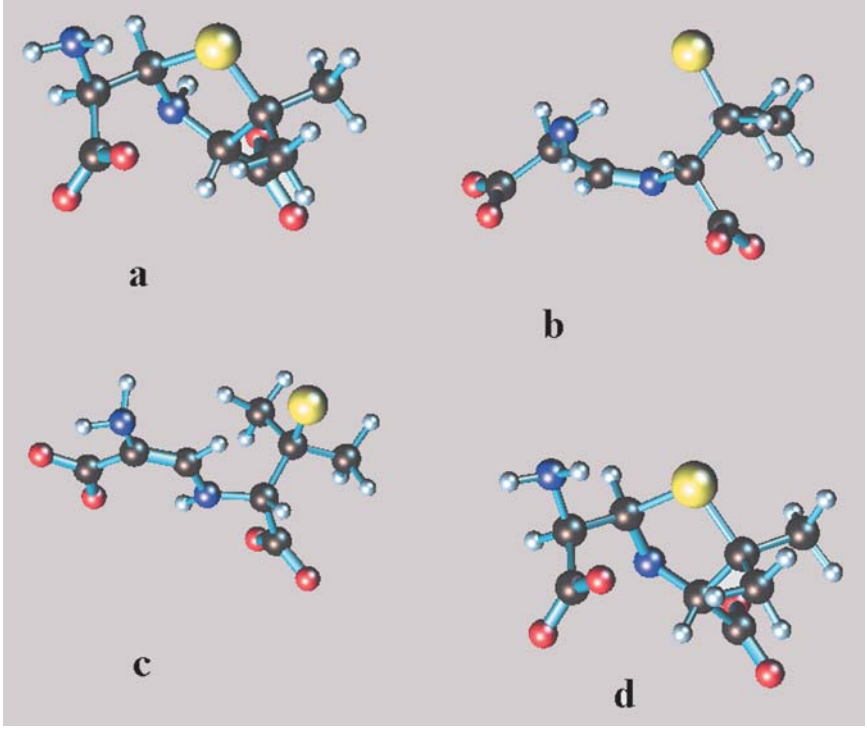

Fig. 2a–d The molecular geometry of penicilloate anions. **a** Amine. **b** Imine. **c** Enamine. **d** N4-deprotonated amine. Sulfur is in *yellow*, carbon in *black*, oxygen in *red*, nitrogen in *blue* and hydrogen in *white*. The graphics in this figure were obtained with ViewMol3D. [11]

Table 1 The molecular geometry of the thiazolidine ring of penicilloate amine dianion and various penicillins

Atoms	Parameter	Ab initio HF-SCF Penicilloate amine	Crystallography			
			Oxacillin penicilloic acid[a]	Oxacillin[b]	Benzylpenicillin sodium salt[b]	Benzylpenicillin potassium salt[b]
S1–C2	Bond length (Å)	1.85	1.88	1.89	2.06	1.78
C2–C3	Bond length (Å)	1.58	1.54	1.58	1.59	1.67
C3–N4	Bond length (Å)	1.45	1.57	1.47	1.41	1.48
N4–C5	Bond length (Å)	1.43	1.45	1.50	1.38	1.51
C5–S1	Bond length (Å)	1.85	1.88	1.83	1.81	1.81
S1–C2–C3	Bond angle (°)	104.9	100.2	104	95	107
C2–C3–N4	Bond angle (°)	110.7	107.0	107	115	114
C3–N4–C5	Bond angle (°)	113.9	110.0	117	118	117
N4–C5–S1	Bond angle (°)	106.1	104.7	104	109	107
C5–S1–C2	Bond angle (°)	94.4	96.1	97	94	94

[a] The crystallographic data for oxacillin penicilloic acid and for oxacillin are from Blanpain et al. [14]
[b] The crystallographic data for the sodium and potassium salts of benzylpenicillin are from Crowfoot-Hodgkin et al. [12]
Bond lengths are in Angstrom units (Å). Bond angles are in degrees (°)

suggesting that the coordinates of this ionic form were on a saddle point on the potential energy surface. A saddle point analysis of the penicilloate N4-deprotonated amine showed rupture of the S1–C5 bond, although the analysis was not carried to equilibrium.

The molecular geometry of the penicilloate anions is shown in Fig. 2. The penicilloate amine dianion contains 27 atoms, with the H of the N4-nitrogen of the thiazolidine ring extending behind the plane of Fig. 2a. The imine (Fig. 2b) and enamine (Fig. 2d) tautomers and the N4-deprotonated amine (Fig. 2d) each contain 26 atoms. The N4–C5 bond order is 1.049 in the amine, 1.227 in the enamine and is 2.174 in the imine. The C5–C6 bond order is 1.029 in the amine, 1.088 in the imine and 2.001 in the enamine. The S1–C5 bond length is 1.85 Å in the penicilloate amine dianion. Rupture of the covalent S1–C5 bond is associated with an increase of the S1–C5 bond length to 3.84 Å in the imine and to 3.64 Å in the enamine. The S1–C2 bond order is 1.130 in the amine, 0.951 in the imine and 0.994 in the enamine penicilloate.

The HF-SCF geometric parameters for the penicilloate amine were compared with those reported from crystallographic analysis of oxacillin, oxacillin penicilloic acid [14] and the sodium and potassium salts of benzylpenicillin. [12] The comparative values for bond lengths and bond angles are given in Table 1. The concordance between the HF-SCF parameters reported in this paper and the results from crystallography is as close as the concordance among the crystallographic results themselves.

The total energy (E_{total}) and the energies of the highest occupied molecular orbital (E_{HOMO}) and of the lowest unoccupied molecular orbital (E_{LUMO}) obtained by RHF-SCF analysis are shown in Table 2 for the various forms of penicilloate anion. The chemical hardness (η) and the chemical potential (μ) were calculated from the data in Table 2. η and μ are defined as: [15]

$$\eta = (I - A)/2 \quad \text{and} \quad \mu = -(I + A)/2 \tag{1}$$

where I is the ionization potential and A is the electron affinity.

Table 2 Total and frontier orbital energies of penicilloate anions

Penicilloate anion	E_{total}[a]	E_{HOMO}[b]	E_{LUMO}[c]
Amine	−1116.0997	−0.0860	+0.2079
N4-deprotonated amine	−1115.2017	+0.1630	+0.2905
Imine	−1115.3164	+0.0818	+0.2908
Enamine	−1115.2969	+0.0907	+0.2926
CH_3–S–S–CH_3[d]	−874.3441	−0.3081	+0.0998

[a] E_{total} is the total energy
[b] E_{HOMO} is the energy of the highest occupied molecular orbital
[c] E^{LUMO} is the energy of the lowest unoccupied molecular orbital
[d] The values for dimethyl disulfide (CH_3-S-S-CH_3) are given for purposes of comparison
Energy values are in atomic units (a.u.)

The frontier orbital energies are given by: [16]

$$E_{\text{HOMO}} = -I \text{ and } E_{\text{LUMO}} = -A \tag{2}$$

Substitution of Eq. (2) into Eq. (1) gives:

$$\eta = (E_{\text{LUMO}} - E_{\text{HOMO}})/2 \text{ and } \mu = (E_{\text{LUMO}} + E_{\text{HOMO}})/2 \tag{3}$$

Applying Eq. (3) to the data in Table 2, the values obtained for η were 0.1470 a.u. for the penicilloate amine dianion, 0.0638 a.u. for the imine trianion and 0.1010 a.u. for the enamine trianion. The values obtained for μ were 0.0610 a.u. for the penicilloate amine dianion, 0.1863 a.u. for the imine trianion and 0.1917 a.u. for the enamine trianion.

E_{total}, E_{HOMO} and E_{LUMO} values for dimethyl disulfide are given in Table 2. The RHF-SCF analysis was conducted on dimethyl disulfide in order to test the plausibility of reaction of the free thiolates of penicilloate imines and enamines with disulfides.

The total Mulliken atomic populations and the Mulliken atomic population in the HOMO of each form of penicilloate anion studied are given in Table 3.

Table 3 Total Mulliken atomic populations and the Mulliken atomic population in the highest occupied molecular orbital of each form of penicilloate anion

Penicilloate anion	Mulliken Population	Atom number[a]					
		S1	C2	C3	N4	C5	C6
Amine	Total[b]	+0.0112	–0.1052	+0.0274	–0.4264	–0.0405	+0.0660
Imine	Total[b]	–0.9751	+0.1564	+0.0032	–0.2560	+0.0965	+0.0231
Enamine	Total[b]	–0.9176	+0.0898	+0.0910	–0.2666	+0.1143	–0.1560
Amine	HOMO[c]	1.1891	0.0183	0.0001	0.0061	0.0590	0.1272
Imine	HOMO[c]	1.9534	–0.0718	0.0154	0.0124	–0.0073	–0.0158
Enamine	HOMO[c]	0.9275	0.0015	0.0280	0.2297	0.1658	0.4236

[a] Atoms are numbered according to the numbering system given in the chart in Figure 1
[b] Total denotes the net charge determined from the total Mullikem atomic population
[c] HOMO denotes the Mulliken atomic population in the highest occupied molecular orbital (HOMO)

Discussion

The molecular geometry determined for penicilloate amine dianion by HF-SCF geometry optimization at the 6-31++G** level (Table 1) agrees with that previously reported from crystallography of oxacillin, oxacillin penicilloic acid by Blanpain et al. [14] and for benzylpenicillins by Crowfoot-Hodgkin et al. [12] The sulfur in the penicilloate amine dianion is part of the five-atom heterocyclic thiazolidine ring (Fig. 2a). In contrast, the imine and enamine forms of penicilloate each contain sulfur as a free, thiolate anion (Fig. 2b and c) with an S–C bond order that is characteristic of a single bond (see Results).

The RHF-SCF analysis presented here shows that formation of the free thiolate in the imine and enamine that forms by deprotonation of penicilloate amine is associated with:

1. An increase in total energy (Table 2)
2. An increase in the energy of the HOMO to that of antibonding (Table 2)
3. A decrease in chemical hardness (see Results)
4. An increase in chemical potential (see Results)
5. A more negative Mulliken net charge on the sulfur atom (Table 3)
6. An increase in the Mulliken atomic population on the sulfur atom, in the HOMO (Table 3).

These six consequences of penicilloate imine and enamine formation from the amine suggest that the resulting free thiolate is a site with the characteristics of a potential electron donor and a soft nucleophile. [17]

An RHF-SCF analysis reported here was conducted on the model compound, dimethyl disulfide $(CH_3S)_2$, in order to test the plausibility of reaction of the free thiolates of penicilloate imines and enamines with disulfides of proteins. It was found that the E_{LUMO} of dimethyl disulfide is almost isoenergetic with those of the penicilloate imine and of the enamine (Table 2) just as expected between the HOMO of a soft nucleophile and the LUMO of the target of an orbital controlled nucleophilic attack. [17]

The optimized bond lengths and bond angles upon which the energy values given in Table 2 for dimethyl disulfide are based are essentially identical to those previously reported for gas phase dimethyl disulfide using HF/MIDI! and MIDI!6D methods. [18] The AM1/6-31++G** geometry optimization of dimethyl disulfide used here yielded a C–S–S–C dihedral angle of 180°. It has previously been shown that neither Mulliken atomic charges (HF/6-31G*) nor E_{LUMO} (EH) vary with the C–S–S–C dihedral angle of dimethyl disulfide. [19, 20] It is possible, nevertheless, that the concordance between the E_{HOMO} values for penicilloate imine and enamine anions and the E_{LUMO} of dimethyl disulfide in Table 2 either might be fortuitous, or might be caused by a cancellation of errors.

The total energies (E_{total}) of the penicilloate anions given in Table 2 rank as follows: E_{total}(amine)< E_{total}(imine)<E_{total}(enamine)<E_{total} (N4-deprotonated amine). The rank order of these energies suggests that the N4-deprotonated amine may be a transition state [21] in the molecular reorganization reaction that leads to formation of the penicilloate imine and enamine tautomers, with their free, thiolate sulfurs.

The mechanism of the hydrolysis of penicillins and cephalosporins has been the subject of previous theoretical chemical studies. Frau et al. reported an analysis of the alkaline hydrolysis of penicillins by the AM1, and other, semi-empirical methods. [22] Petrongolo et al. reported ab initio studies of the alkaline hydrolysis of penicillins performed with STO-3G and 9*s*6*p*/7*s*3*p*/3*s* basis sets. [23, 24] Hydrolysis of the β-lactam ring of penicillins and cephalosporins produces products that lack antibiotic activity and inhibitory activity against β-lactamase enzymes. To the best knowledge of the authors, these hydrolysis products, including penicilloate anions, have not been the subjects of previous quantum chemical analyses. In the ab initio study reported here, the carboxyl groups were modeled as carboxylate anions so as to represent their ionized state at physiological pH, i.e., pH 7.4. The penicilloate anions were modeled with the NH_2 group unprotonated so as to more closely resemble the electronic structure of the penicilloate anion with the penicillin side chain still attached, as in the intact penicilloate allergen. The results of the RHF-SCF analysis presented in this paper (Fig. 2, Tables 2 and 3) suggest that the free, thiolate sulfur of the penicilloate imine and enamine anions, especially of the imine, has potential as a chemically reactive soft nucleophile.

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