BIOORGANIC CHEMISTRY 19, 314–326 (1991)

Computational Studies on Isomerization of Imine from Penicillin and Electron Transfer by Carboxyiminium Derived from β-Lactams

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Received January 4, 1991

Fused-ring β-lactams readily form imine carboxylic acids upon binding to cell-wall enzymes. It has been proposed that part of the antibiotic action of β-lactams may involve electron transfer by the corresponding iminium form of these derivatives. In the case of penicillin, ring opening leads to a compound which might be expected to isomerize to two stable conjugated species. One conversion yields a conjugated carboxyimine, while the other results in loss of the imine functionality. Semiempirical calculations were carried out to determine which isomerization is favored. The heats of formation for the initial compound, the final isomers, their protonated forms, as well as the intermediates involved were calculated using MNDO. Whereas one conjugated isomer (the imine) is thermodynamically more stable, the other (an enamine) is favored kinetically. Molecular orbital energies (LUMO's) for these structures show that the thermodynamically favored isomer has greater potential to participate in electron transfer than the kinetically favored product. In addition, calculations on simple molecules bearing structural similarity to the thermodynamically preferred isomer reveal that electron transfer is facilitated if the acid portion of the carboxyiminium remains protonated and is enhanced by the presence of sulfur in the structure.

INTRODUCTION

There is increasing evidence implicating electron transfer (ET) as an important factor in the biochemistry of many endogenous and exogenous agents (*1*). The principal functionalities that participate in ET are quinones, aromatic nitro compounds, metal (Cu, Fe) complexes, flavins, and iminium ions. Of these, the iminium group has received the least amount of systematic attention. Prior reports give support to involvement of these ET classes in the mode of action of fungicides (*2*), anthelmintics (*3*), and antimycobacterials (*4*). References to other xenobiotics studied (carcinogens, anticancer agents, amebicides, antimalarials, and CNS affectors) are given elsewhere (*4*).

The present work deals in part with iminium conjugated with the carboxyl group. It has been proposed that this structural combination plays an ET role in

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0045-2068/91 $3.00

the antibiotic and nephrotoxic activities of β-lactams (*5–7*). Apparently, the charged form arises from *in vivo* protonation of the carboxyimine precursor, either inter- or intramolecularly. It may be significant that the same structural feature is present in various other agents of physiological interest, such as pyrroline or thiazoline carboxylates (*8*), lanthionine ketimine and piperidein-2-carboxylic acid (pipecolic acid) (*9*), aminoethylcysteine ketimine (*10*), and the imine intermediate in transamination (*11, 12*). Since the dihydro precursor is readily oxidized to the imine (*8*), indirect ET involvement can also be visualized for the reduced forms found in azetidine-2-carboxylic acid (*13*), domoic acid (*14*), acromelic acid (*15*), kainic acid (*16*), and bulgecinine (*17*).

An alternative ET pathway exists for the imine carboxylic acids. Since they chelate readily with metal ions present in the biological milieu, the Cu and Fe complexes might also conceivably play a role (*8*).

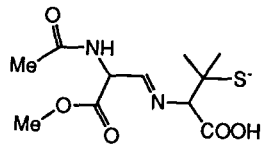
According to the theoretical framework, the imine generated by β-lactam ring opening is converted to an ET functionality by protonation. Various sources of acidic hydrogen are available at the binding site (*6*). One is from the carboxyl group of the antibiotic, and another is from the enzyme serine hydroxyl after attack on the β-lactam. Evidence has been presented for the existence of positive charges associated with protein near the carboxyl group of the bound drug. It is reasonable to adopt the proposition that imine basicity in the living system may not be identical to that for an *in vitro* solvent system. Also, calculations indicate that competition of imine and ammonia for proton (iminium–ammonium equilibrium) is significantly influenced not only by inherent basicity, but also by geometrical considerations as would pertain at the active site in a biological system (*18*). In an arrangement in which the lone pairs of the two bases point toward one another, the proton prefers the Schiff base. The presence of protons in the binding cavity is also relevant to the subsequent discussion on acid-catalyzed rearrangement of imine from penicillin.

Since the conjugated iminocarboxy group appears to provide a unifying theme for fused-ring β-lactam action, it is reasonable to hypothesize formation from penicillin (*5–7*). The requisite material, apparently not yet reported, could conceivably be derived from isomerization of the imine formed by β-lactam ring opening.

In order to determine whether carboxyimine can be produced from the initial imine, theoretical calculations were performed for various conceivable mechanisms of the isomerization. Since a competing pathway exists for conversion of the imine, both pathways must be compared to discern the one more likely to occur. If the desired compound can reasonably be expected to form, then certain structural characteristics may enhance its capacity to act as an electron transfer agent. Hence, additional calculations were performed to ascertain the optimal molecular features for ET by the carboxyiminium group.

COMPUTATIONAL ANALYSIS

The *E*-isomer of **1** was built using SYBYL 5.3 (*19*). This structure was modified to give both the *E*- and *Z*-isomers of **2** and **3** since all mechanisms yield intermedi-



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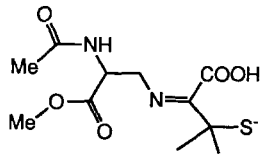
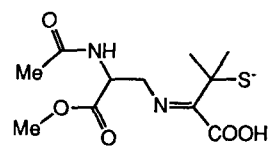
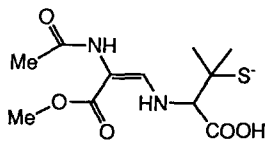


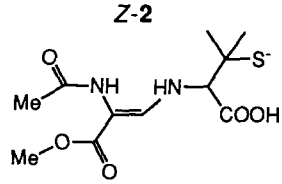
E-2



Z-2



E-3

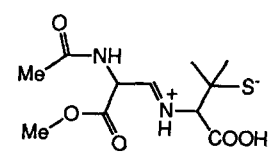


Z-3

ates which exhibit free rotation about the bond that will subsequently become the double bond in the product. The structure representing the molecular mechanics global energy minimum was determined for each compound with the SEARCH option under SYBYL.

The heat of formation for each structure was determined using MNDO (*20, 21*) (MOPAC version 5.0, available through SYBYL), rotating in 60° increments about the S–C–C–N bond, starting from a torsional value of 0°. The overall heat of formation for each compound was taken to be the Boltzmann-weighted average of the heats of formation for the individual conformations. Taking the LUMO energies for these conformations into account, the overall LUMO energy was calculated as a weighted average also, using the corresponding percentages determined from the Boltzmann distribution as weighting factors.

The heat of formation and the LUMO energy for the protonated forms of the five structures (addition of H^+ to the N atom in each case) were determined in a similar manner (compounds **4–6**). Compound **1** was further modified to give the



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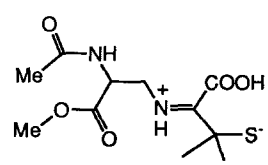

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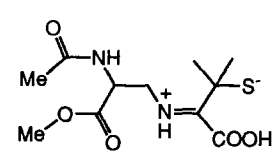
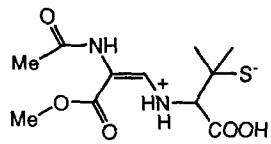
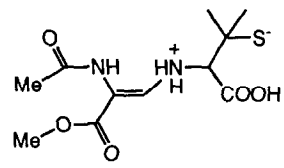


Z-5

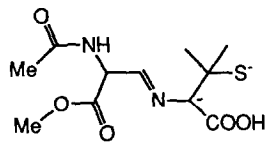


E-6



Z-6

intermediate formed in each of the mechanisms for both pathways (a total of eight intermediates, compounds **7–14**). The heat of formation of each was calculated as



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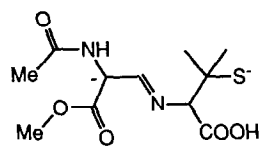

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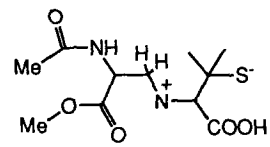

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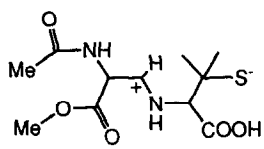

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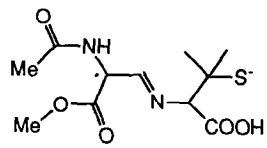

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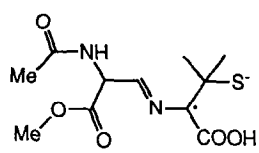

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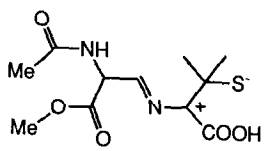

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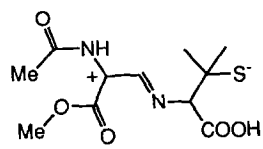

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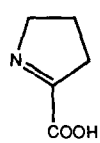
described above. The UHF method was employed for the radical species **11** and **12**. Compounds **15–26** were built, and their heats of formation and LUMO energies were determined as related previously. The UHF method was used on radicals **19, 20, 25,** and **26.**



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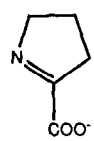

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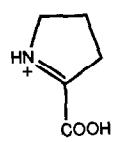

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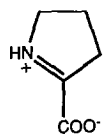

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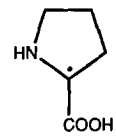

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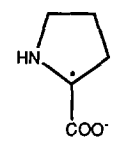

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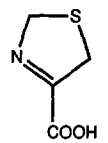

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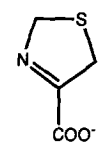

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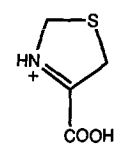

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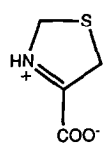

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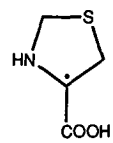

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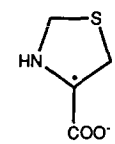

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RESULTS AND DISCUSSION

The heats of formation and energy of the LUMO's for compounds **1–6** are listed in Tables 1 and 2. The heats of formation for compounds **7–14** are reported in Table 3. The heats of formation and LUMO energies (where applicable) for structures **15–26** are shown in Table 4.

TABLE 1

Heats of Formation and LUMO Energies for Initial and Final Isomerization Compounds

Structure	ΔH_f (kcal/mol)[a]	LUMO energy (eV)	ΔH_f of low energy conformation (kcal/mol)	LUMO energy of low energy conformation (eV)
1	−206.62	3.34206	−206.73	3.36218
E-**2**	−220.65	3.37703	−220.65	3.37703
Z-**2**	−216.59	3.37119	−216.62	3.34118
E-**3**	−212.60	3.05208	−212.60	3.05208
Z-**3**	−215.05	2.98140	−215.05	2.98140

[a] The heat of formation of the low energy conformation constitutes a major portion of the Boltzmann-averaged value for each structure in Tables 1–4. Other conformations contribute less significantly.

TABLE 2

Heats of Formation and LUMO Energies for Protonated Compounds

Structure	ΔH_f (kcal/mol)[a]	LUMO energy (eV)	ΔH_f of low energy conformation (kcal/mol)	LUMO energy of low energy conformation (eV)
4	−193.70	0.04294	−193.91	−0.00725
E-**5**	−199.00	−0.15727	−199.21	−0.07900
Z-**5**	−197.12	−0.57531	−197.12	−0.57531
E-**6**	−192.46	0.07773	−192.56	0.08022
Z-**6**	−195.66	0.07796	−195.88	0.09828

The attack by enzyme serine nucleophile on penicillin (**27**) leads to ring-opening. The resultant structure can theoretically isomerize to yield two conjugated species by way of a 1,3-H shift (Scheme 1). Although compound **3** has been isolated (*22*), there is apparently no report on the formation of **2.** For simplicity, sulfur in the product is depicted in anionic form, whereas there may be reversible binding to the cavity wall (*5*).

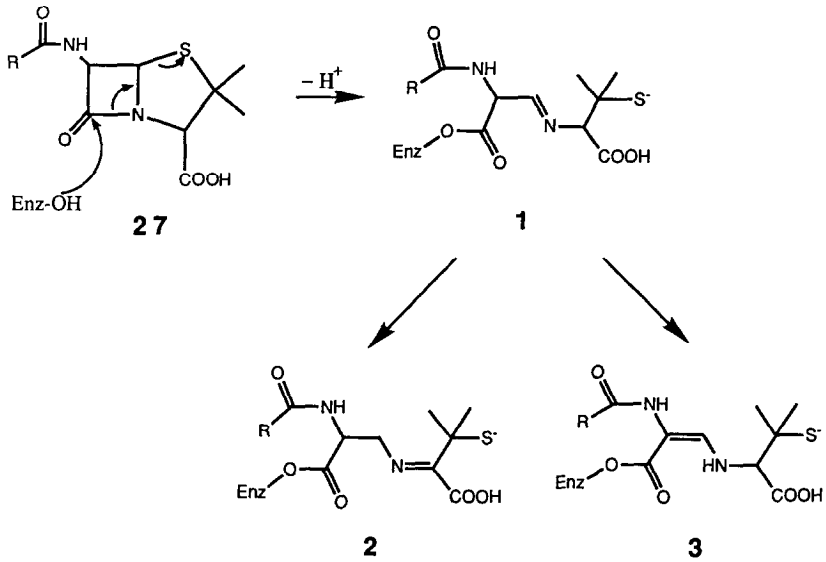
Several reasonable mechanisms may be proposed for the rearrangement. The base-catalyzed one involves H^+ abstraction from **1,** rearrangement, and then proton addition (Scheme 2). This pathway conforms to the generally accepted scheme for transamination (*12*). Davis and Page (*22*) state that at high concentrations of hydroxide, the substrate exists predominantly as tautomer **3.**

For the acid-catalyzed case, protonation of **1** occurs at the C–N double bond, followed by H^+ elimination to give the conjugated structures **2** or **3** (Scheme 3). Enamine **3** is readily accommodated by this approach. However, conversion of **1** to **2** is less straightforward. If protonation–deprotonation were a concerted process, little charge build-up would occur on N of **9,** particularly since proton attack

TABLE 3

Heats of Formation for Intermediates

Structure	ΔH_f (kcal/mol)	ΔH_f of low energy conformer (kcal/mol)
7	−158.43	−158.54
8	−179.74	−179.85
9	−185.23	−185.45
10	−193.70	−193.91
11	−197.10	−197.28
12	−198.60	−198.75
13	−159.88	−160.04
14	−168.19	−168.19



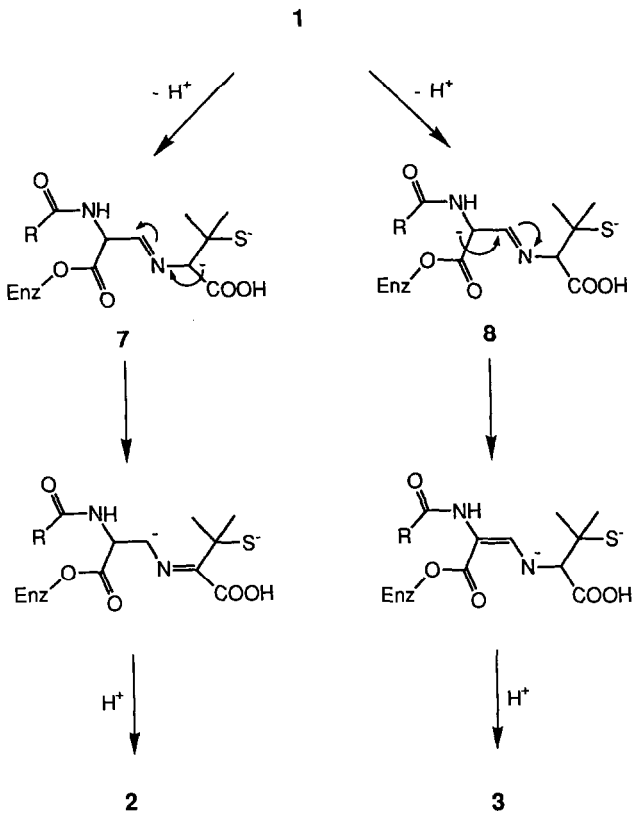
SCHEME 1. Ring-opening of penicillin and isomerization to conjugated products.

would involve the imine pi cloud and not participation of the imine carbon or the nitrogen lone pair. The amount of positive character associated with the imine carbon is not large. However, there is precedent for generation of electron-deficient nitrogen. For example, for the carbocation **28** bearing a cyano substituent,

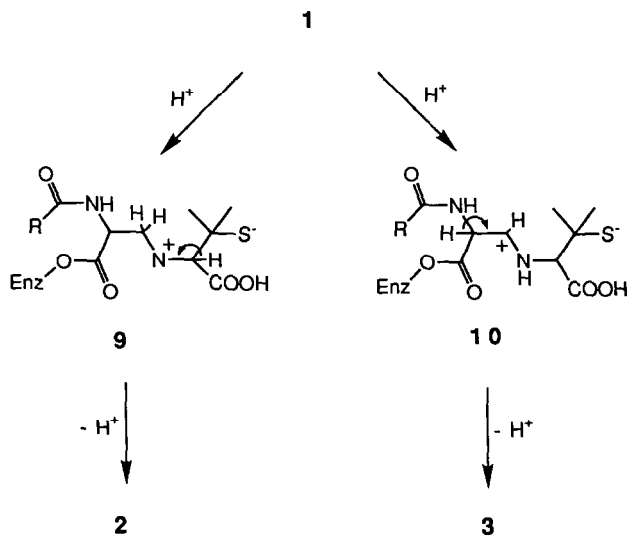
TABLE 4

Heats of Formation and LUMO Energies for Conjugated Carboxyimines and Derivatives

Structure	ΔH_f (kcal/mol)[a]	LUMO energy (eV)	ΔH_f of low energy conformation (kcal/mol)	LUMO energy of low energy conformation (eV)
15	−81.00	0.10065	−81.16	0.10079
16	−101.15	4.94002	−101.21	4.94210
17	86.32	−6.51567	86.16	−6.49701
18	−37.46	−1.31152	−37.53	−1.30968
19	−88.59	—	−88.87	—
20	−99.46	—	−99.74	—
21	−75.18	−0.43937	−75.32	−0.43771
22	−99.24	4.47282	−99.29	4.47674
23	104.09	−6.94132	103.92	−6.92981
24	−26.06	−1.69767	−26.13	−1.8520
25	−80.54	—	−80.88	—
26	−97.28	—	−97.55	—



SCHEME 2. Base-catalyzed mechanism for isomerization.



SCHEME 3. Acid-catalyzed mechanism for isomerization.

28 29

SCHEME 4. Electron-deficient nitrogen in carbocation with cyano substituent.

the nitrogen absorbs a significant amount of the positive charge (*23*) (Scheme 4). Resonance form **29** can be compared to the vinyl carbocation. Since vinyl carbocations are less stable than their saturated counterparts (*24*), **9** would be expected to assume positive character more readily than **29.**

For the sake of completeness, less likely alternative routes were also considered. For example, a hydrogen radical or hydride ion may replace the proton in the removal–addition scheme. In order to determine which conjugated form is preferred, each mechanism is considered for each isomerization pathway, and the heats of formation were calculated for the initial compound, for the final products, and for the intermediates. The heats were compared to aid in predicting the relative ease of reaction.

As already discussed, the final conjugated species, or the corresponding protonated form may act as an electron-transfer agent as part of the antibiotic action. Hence, the heats of formation for compounds **4, 5,** and **6** were determined, and the energies of the LUMO for **4, 5,** and **6,** were compared to ascertain which can accept an electron most readily.

Each of the final conjugated, nonprotonated species (*E*-**2**, *Z*-**2**, *E*-**3**, *Z*-**3**) has a lower heat of formation than the initial imine carboxylic acid **1** from which it was derived. Thus, they are thermodynamically more stable than the original structure, and isomerization is favorable for energetic reasons. Table 5 shows the heats of reaction for each isomerization. The most stable of the conjugated isomers is *E*-**2** ($\Delta H_f = -220.65$ kcal/mol), followed by *Z*-**2** ($\Delta H_f = -216.59$ kcal/mol). The most exothermic and thus the most favorable of these reactions are those leading to either isomer of compound **2,** which is the thermodynamically preferred isomer.

However, as stated previously, **3** is observed rather than **2.** Since these two are products of competing reactions from **1,** and since **2** is thermodynamically preferred, it may be argued that **3** is kinetically favored (i.e., the pathway leading to **3**

TABLE 5

Heats of Isomerization

Isomerization	Heat of reaction (kcal/mol)
1 → *E*-**2**	−14.03
1 → *Z*-**2**	−9.97
1 → *E*-**3**	−5.98
1 → *Z*-**3**	−8.43

TABLE 6

Relative Stability of Intermediates

Mechanism	Intermediate A	Intermediate B	Difference in ΔH_f of intermediates (B − A) (kcal/mol)
Base-catalyzed	**7**	**8**	−21.31
Acid-catalyzed	**9**	**10**	−8.47
H-radical transfer	**11**	**12**	−1.50
Hydride transfer	**13**	**14**	−8.31

from **1** is energetically more favorable than that for the transformation to **2**). In addition, comparison of the intermediates shows that for every proposed mechanism for the isomerizations, the intermediates in the reaction yielding **3** are more stable than those for the reaction leading to **2.** The stability of the radical intermediates compared to the other intermediates is an example of the captodative effect, in which electron-donating and electron-withdrawing groups on the same radical center lead to enhanced stabilization above that expected from the sum of the individual stabilizing influences (*8*). Table 6 shows the stability of the intermediates in reactions leading to **3** relative to the intermediates leading to **2.** According to Hammond's postulate, the geometry of the transition state, and thus the stability of the transition state, for an endothermic reaction more closely resembles the products (*25*). Inasmuch as the intermediates along the reaction path to **3** have lower heats of formation than those along the path for **2,** the transition states for these competing endothermic reactions may also be expected to reflect this difference in stability (i.e., the transition states leading to **3**'s intermediates in the various proposed mechanisms are less energetic than those leading to **2** and the activation energy required to form the intermediates will be lower in the case of **3**). Therefore, the significant increase in the stability of the intermediates in the reaction scheme for compound **3** suggests that these states will be preferentially populated relative to the intermediates giving compound **2.** It is reasonable to assume that if more of these species exist, then the final product coming from them will be preferred. Therefore, compound **3** is the favored structure in this case. Depending on the conditions, then, either isomer may be formed. Thermodynamic conditions favor the formation of compound **2** while kinetic conditions will lead to the formation of compound **3.**

Consideration should be given to the ability of each structure to accept an electron, as these compounds have been proposed to act as electron transfer agents. All of the nonprotonated species show poor tendencies to accept an electron, as evidenced by the high positive values for the LUMO energies. However, upon protonation, each of the compounds displays a marked drop in LUMO energy. This decrease is accompanied by an increase in the heat of formation (Table 7) which is relatively the same for the conjugated species **2** and **3.** However, there is a significantly larger decrease in the LUMO values for the isomers of

TABLE 7

Effect of Protonation on Initial and Final Compounds

Structure	Change in ΔH_f upon protonation (kcal/mol)	Change in ΔH_f relative to **1** (kcal/mol)	Change in energy of LUMO (eV)	Change in energy of LUMO (kcal)
1	+12.92	0	−3.29912	−76.04
E-**2**	+21.65	+8.73	−3.53430	−81.46
Z-**2**	+19.47	+6.55	−3.94650	−90.97
E-**3**	+20.14	+7.22	−2.97435	−68.56
Z-**3**	+19.39	+6.47	−2.90344	−66.92

compound **2** compared to compound **3.** Further inspection reveals that of all the protonated structures, only the iminium forms of compound **2** exhibit a negative LUMO energy. Since energy will be released when these ions accept an electron, this process can occur spontaneously.

It is unclear why compound **2,** which has definite potential to form, has yet to be observed. Further investigation is required to determine the appropriate conditions. However, if **2** can be produced, certain structural features favor its action as an ET agent. These traits are shown by comparison of heats of formation and LUMO energies for compounds **15–26.** For simplicity, only one resonance form of each structure is illustrated. The carboxylate form is more stable than the carboxylic acid. However, removing the proton greatly reduces the ability to take up an electron (Table 8). Protonation at the N atom greatly enhances the ability to accept an electron. Indeed, addition of a proton makes electron uptake (which would require an input of energy for the unprotonated imine) a spontaneous process (negative LUMO values). A similar situation energetically was found from theoretical calculations on MPP^+ (1-methyl-4-phenylpyridinium) (*26*). Table 9 shows the decrease in LUMO energy associated with protonation. Introduction of sulfur into the compounds leads to a slight decrease in stability but a significant increase in the ability to accept an electron (Table 10).

The ET process postulated for the carboxyimine **2** involves protonation at the imine N followed by electron uptake. The heat of reaction for the comparable conversion from imine to iminium to free radical for each of the compounds **15–18** is listed in Table 11. The positive heat of reaction for the examples involving the carboxylate form indicate an unfavorable reaction, whereas the cases involving the carboxylic acid show a negative heat of reaction. These values duplicate the trend noted earlier. ET by carboxyimine is more likely to occur if the acid remains intact.

Of the two cases in which ET is likely, the example not containing S is favored by a more negative heat of reaction by 2.23 kcal/mol. However, in relation to the concept of iminium ion as an ET agent, the S-containing compound favors electron-uptake by 9.81 kcal/mol (Table 10). As a result, it seems that the S atom enhances the ability of the imine to act as an ET agent. Thus, these data serve to rationalize the presence of sulfur in many of the β-lactam antibiotics.

TABLE 8

Effect of Deprotonation of Carboxyimine on LUMO Energy

Carboxylic acid A	Carboxylate B	Difference in LUMO (B − A) (eV)	Difference in LUMO (kcal/mol)
15	**16**	+4.83937	+111.55
17	**18**	+5.20415	+119.96
21	**22**	+4.91219	+113.22
23	**24**	+5.24365	+120.87

TABLE 9

Effect of Deprotonation of Carboxyimine on LUMO Energy

Imine A	Iminium B	Difference in LUMO (B − A) (eV)	Difference in LUMO (kcal/mol)
15	**17**	−6.61632	−152.50
16	**18**	−6.25154	−144.10
21	**23**	−6.50195	−149.87
22	**24**	−6.17049	−142.23

TABLE 10

Effect of S in Carboxyimine Ring on LUMO Energy

No S atom in ring A	S atom in ring B	Difference in LUMO (B − A) (eV)	Difference in LUMO (kcal/mol)
15	**21**	−0.54002	−12.45
16	**22**	−0.46720	−10.77
17	**23**	−0.42565	−9.81
18	**24**	−0.38615	−8.90

TABLE 11

Heat of Electron-Uptake by Carboxyimines

Reaction	Heat of reaction (kcal/mol)
15 → 17 → 19	−7.59
16 → 18 → 20	1.69
21 → 23 → 25	−5.36
22 → 24 → 26	1.96

It is significant that the trends outlined here correlate well with experimental data on the reduction potentials of these compounds (*5–7*). The structures with the lowest calculated LUMO values have the greatest reduction potentials.

Possible electron donors in the catalytic ET scheme are protein functionalities, such as tryptophan, tyrosine, cysteine, and histidine and perhaps methionine. The oxidation potentials for these residues are well within the realm of those achievable by biological oxidations (*27*). Electron transfer is known to take place over appreciable distances (*28*).

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