Computational Study of Pharmacophores: β-Lactams

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The thermochemistry of bicyclic lactams (penams, penems, cephams, cephems), which are key pharmacophores in β-lactam antibiotics, has been investigated by high-level ab initio methods. Particular attention has been paid to estimating magnitudes of amide resonance (ARE) and ring strain (RSE) in the four-member lactam ring because these quantities are difficult to measure and distinguish experimentally. The ring strain destabilization effect is greater than the stabilization arising from amide resonance. However, in cephemes the amide resonance stabilization slightly exceeds destabilization due to the β-lactam ring strain.

Introduction

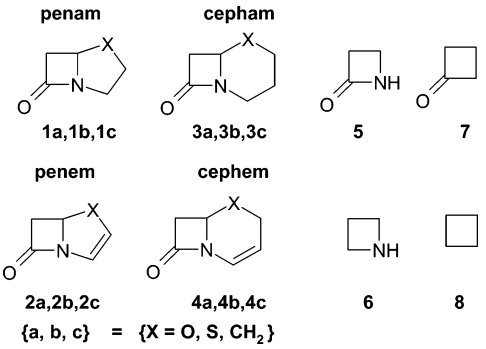
β-Lactam antibiotics (penicillins and cephalosporins) are still the most widely used pharmaceuticals in the treatment of infectious diseases.[1,2] The important pharmacophores (i.e., structural features responsible for molecule's biological activity) in these antibiotics are bicyclic molecules: cephams/cephems and penams/penems (Scheme 1). These pharmacophores contain the β-lactam ring.

Lactam antibiotics interfere with the synthesis of peptidoglycan, which is the main component of the bacterial cell membrane. The antibiotic molecules acylate irreversibly the serine active site of the enzyme transpeptidase, and as a consequence, the cross-linking of peptidoglycan strands cannot take place. This weakens the bacterial cell wall, the cell succumbs to high osmotic pressure, and bursts.

Because of their medical importance, the thermochemical properties and molecular and electronic structures of these antibiotics have been studied theoretically and experimentally for a long time.[3,4] Among the investigated properties were solvation effects on reaction barrier heights, structures of transition states, and reaction intermediates.[5,6] The C═O bond length was shown to correlate with stability toward hydrolysis.[5] On the other hand, the lactam ring strain was found not to influence the rate of hydrolysis because the rate-limiting reaction steps comprise transition states with unbroken lactam rings. The lactam ring cleavage takes place during the final reaction step. The kinetic unimportance of ring strain was established in the cases of base hydrolysis of penicillanic acid[6] and of 2-azetidinone (**5**).[7]

The 2-Azetidinone (β-lactam) ring is present in all penicillin and cephalosphorin antibiotics, so its properties are of special interest. The calorimetric and computational studies suggested that the ring strain energies (RSE) of **5** and penicillin G are very similar, in the range of 116−119 kJ/mol.[8,9] It must be noted of course that the RSE was not determined directly by experiment but inferred indirectly from the combination experimental results.[8]

SCHEME 1



The ring strain may be expected to lead to thermodynamic destabilization of β-lactams, but the β-lactam ring also contains an amide group whose resonance can be considered as a stabilizing influence. No report to date has determined amide resonance energy (ARE) and RSE separately. Since ARE and RSE cannot be readily deduced from experimental measurements, one has to resort to calculations. We present high-level calculations in which amide resonance and ring strain of cepham and penam derivatives were determined separately. Penam and cepham molecules represent more realistic models for β-lactam antibiotics than does 2-azetidinone. Details of the molecular structure and energetics of β-lactam antibiotics are important in view of the emerging bacterial resistance to these pharmaceuticals.[10]

Computational Methods

The quantum chemical calculations were performed with the Gaussian 03 program[11] using the multistep G3/B3LYP method.[12] The method includes full geometry optimization at the B3LYP/6-31G* level followed by the sophisticated post-HF steps which provide total electronic energy within chemical accuracy (<8 kJ/mol). To reduce errors associated with total energies, we used isodesmic reaction schemes for calculating individual contributions to the thermodynamic stabilities of penams and cephams (Scheme 2).

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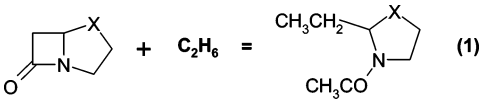
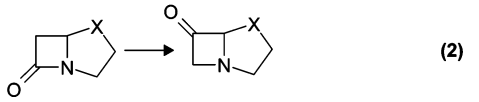
SCHEME 2



isodesmic reaction scheme for RSE



isomerisation reaction scheme for ARE

TABLE 1: Amide Resonance Energy (ARE/kJ mol^{-1}), Ring Strain (RSE/kJ mol^{-1}), and Degree of Pyramidalization of Lactam Nitrogen ($\sum\alpha_i$/deg)$^{a-c}$

compound	ARE	RSE	RSE − ARE	r_{CN}	$\sum\alpha_i$
1a	81.7	110.5	28.8	1.42	323.5
1b	75.3	97.5	22.2	1.40	334.7
1c	72.6	110.5	37.9	1.40	326.1
2a	72.5	129.1	56.6	1.43	321.5
2b	67.3	125.4	58.1	1.42	334.7
2c	57.9	129.4	71.5	1.42	326.0
3a	109.1	70.8	−38.3	1.39	348.6
3b	102.4	70.7	−31.7	1.38	355.5
3c	105.4	60.0	−45.4	1.38	353.6
4a	93.9	86.8	−7.1	1.40	348.5
4b	86.1	71.8	−14.3	1.40	354.6
4c	85.7	82.1	−3.6	1.39	352.6
5	90.4	102.4	12.0		
		(109.2)			
		110.9			
6		(105.4)			
7		108.1			
		(109.6)			
		112.5			
8		(113.8)			

[a] RSE values in parentheses are from ref 16 obtained via the CBS-Q method. [b] $\sum\alpha_i$ is the sum of CNC bond angles centered on the lactam nitrogen.

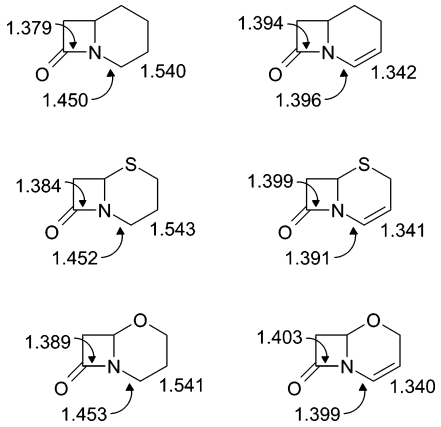
The ARE and RSE are not the sole factors governing relative thermodynamic stabilities of cephams and penams, and it was therefore important to estimate ARE and RSE independently by eqs 1 and 2. Furthermore, the resonance effect of the amide group is not due to π-electron transfer from nitrogen to oxygen but rather from nitrogen to carbon. This was pointed out by Wiberg, who used a group-transfer reaction scheme to estimate the interaction between the carbonyl group and neighboring groups.[13] Enthalpies of reactions 1 and 2 lead directly to the corresponding thermodynamic values for ARE and RSE (Table 1).

Results and Discussion

The results of our study are summarized in Table 1. The calculated geometries of penams/penems (**1** and **2**) and cephams/cephems (**3** and **4**) agree well with the available experimental geometries obtained in X-ray diffraction studies.[14,15] In particular, the calculations and X-ray data concur that the β-lactam ring is essentially planar. The six-membered ring in cephams adopts the boat conformation. It is also clear from X-ray data that the lactam ring and the larger fused ring are not coplanar. This would suggest that lactam nitrogen assumes a pyramidal configuration. However, amide resonance was also evident from the shortening of the C−N bond in the amide moiety.[14]

The RSE for **1b** calculated in this work at the G3/B3LYP level (97.5 kJ/mol) is in fair agreement with the value calculated for one of its derivatives (87.4 kJ/mol) at the MP2/6-31+G* level and reported previously.[6] This agreement supports the soundness of our isodesmic approach scheme. As expected, the amide resonance has a stabilizing and ring strain a destabilizing effect, and we estimated their magnitudes separately. The net effect is quantified by defining the energy difference: RSE − ARE. We also deduced the degree of pyramidalization of the lactam nitrogen and expressed it via the sum of CNC bond angles ($\sum\alpha_i$). The larger the $\sum\alpha_i$ value becomes, the smaller the pyramidalization of the nitrogen atom and the larger the N2p character of its lone pair. The amide resonance favors smaller pyramidalization because of a change in hybridization of the nitrogen valence orbitals from sp^3 to sp^2. The extent of nitrogen atom pyramidalization, amide resonance, and ring strain are factors which influence the final geometry and thermodynamic stability of β-lactams.

SCHEME 3



Lactam nitrogen is more pyramidal in penam/penem derivatives than in cepham/cephem analogues as $\sum\alpha_i$ values in Table 1 clearly show. This is so because the fused six-membered rings in cephams/cephems are larger and hence conformationally more flexible than the five-membered rings in penams/penems. However, this additional flexibility does not by itself favor greater or smaller pyramidalization. The final geometry and stability depends on a net balance between energy stabilization acquired by decreasing pyramidalization and concomitant increase of π-resonance energy on one hand and an increase in ring strain on the other. The increase in RSE with decreasing pyramidalization is due to the fact that in the four-membered lactam ring, small ring bond angles (close to 90°) are preferred (angular strain).

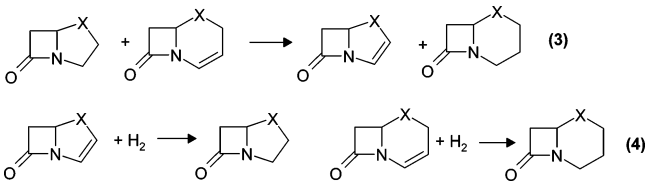
Results in Table 1 show that in penams/penems (**1** and **2**) RSE > ARE; hence, RSE is the dominant factor affecting stability. In cephams/cephems (**3** and **4**) the relative magnitudes are reversed, i.e., RSE < ARE. This reduction in RSE can be expected due to the presence of less strained six-membered rings in **3** and **4**. In cephems (**4**) RSE and ARE have comparable magnitudes with ARE being slightly larger than RSE. This can be rationalized by the enhanced π-electron delocalization between the amide group and the C═C bond in the six-membered ring. This delocalization is reflected in the variations of amide C−N and the neighboring C−C bond lengths. The variations in the calculated bond lengths are shown in Scheme 3. The π-electron delocalization makes the six-membered rings in cephems (**4**) slightly more rigid (more strained) than in cephams (**3**). This accounts for the slight rise in RSE vs ARE on going from **3** to **4**. The same effect does not appear in penems due to the greater nitrogen pyramidalization of lactam nitrogen (Table 1) and the higher rigidity of the five-membered ring

TABLE 2: Reaction Enthalpies (kJ mol^{-1}) for Hydrogenation and Exchange Reactions of β-Lactams and Their Analogues

compound	hydrogenation	exchange
1a + **4a**		9.5
1b + **4b**		15.0
1c + **4c**		16.1
2a	−127.8	
2b	−125.9	
2c	−125.1	
4a	−118.2	
4b	−110.9	
4c	−109.0	

(compared to the six-membered ring). The two rings in penems are far from coplanar.[14]

We also determined the reaction energies for exchange (eq 3) and hydrogenation (eq 4) reactions as indicated below (Table 2).

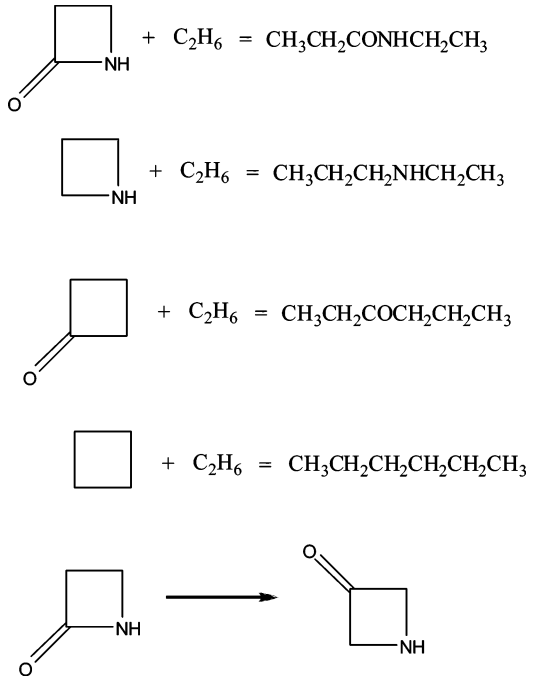


The positive enthalpies (endothermicity) for exchange reactions indicate that there is indeed a resonance between ring C═C bond and the bridgehead nitrogen in **4**. Why? In **2** there is no N−C═C resonance stabilization due to the strongly pyramidal geometry of lactam nitrogen. However, small X−C═C resonance stabilization can exist in **2** as shown for isomeric dihydrofurans.[18] In **4** there is no X−C═C resonance stabilization, so the positive enthalpy of the exchange reaction can be explained by the presence of N−C═C resonance stabilization which seems to be greater than the X−C═C in **2**. The hydrogenation enthalpies for **4c** and **2c** are comparable to the hydrogenation enthalpies of cyclohexene (−118.0 ± 6.0 kJ/mol) and cyclopentene (−111.6 ± 0.3 kJ/mol).[19]

The hydrogenation enthalpies for penems (Table 2) are smaller than −111.6 kJ/mol, which could be indicative of larger strain in five-membered rings of cephems compared to the ring strain in cyclopentene. This may be rationalized by additional rigidity imposed on the cyclopentene ring upon fusion with the lactam ring.

Changing the heteroatom X does not lead to any pronounced, discernible trends in RSE or ARE values. It was suggested earlier that ring strain does not have a significant role in the reactivity of β-lactam antibiotics. The suggestion was based on previous studies,[6] which concluded that the transition state (in the rate-determining step) retains the β-lactam ring structure. We propose that this is due to the fact that ARE and RSE largely "compensate" for each other so that the net thermodynamic destabilization due to ring strain is relatively small even in the reactant molecule. (The "compensation" is with respect to the noninteracting, isolated amino and carbonyl groups as the reference molecules.) In the transition state bonds of the lactam ring can be expected to be weakened, thus further reducing any potential thermodynamic effects of the ring strain. Comparison of RSE values for penams and cephams with **5**−**8** (Table 1) reveals that cephams have considerably lower strain energies than their four-membered ring analogues[16] while penams have RSEs which are comparable to **5**−**8**. Fusion of the β-lactam ring with the six-membered ring leads to a reduction of β-lactam strain, while fusion with a five-membered ring has no effect.

SCHEME 4



The RSE and ARE for **5**−**8** were calculated from the isodesmic reactions in Scheme 4.

The difficulty in establishing the structure−activity relationships within the family of β-lactam antibiotics was recognized some time ago.[17] This arises from different sensitivities of target enzymes toward antibiotic action, the inhibitory action of β-lactamases, and varying diffusion rates of β-lactam antibiotics through bacterial membrane.[17] In the same study the experimental rates of acylation of bacterial enzymes by penicillins and cephalosporins have been reported. The rate of lactam ring cleaving acylation is faster in penam/penem-based penicillins than in cepham/cephem-based cephalosporins. It is tempting to rationalize the difference in acylation rates with higher RSE in the penams/penem pharmacophore, which is present in penicillins. However, for reasons already noted, this rationalization is false.

Summary

We discussed the interplay of amide resonance and ring strain in β-lactam pharmacophores. The results suggest that the destabilizing effect of ring strain on the four-membered β-lactam ring is to a large extent compensated by the stabilizing effect of amide resonance. Overall ring strain is greatly reduced by amide resonant stabilization, and the old argument about penicillin derivatives being active due to strain in the four-membered ring is wrong not only from the point of view of the reaction mechanism pertaining to lactam hydrolysis,[4,5] but also on purely thermochemical grounds. We also proposed the reason why the transition state in the rate-determining step of β-lactam hydrolysis retains the ring structure.

Supporting Information Available: Coordinates of optimized geometries and total energies for penams/penems and cephams/sephems. This material is available free of charge via the Internet at http://pubs.acs.org.

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