Theoretical Studies on the Methanolysis of a Cephalosporin; Mimicking Acylation of the Active Site Serine of D-Ala-D-Ala Transpeptidases

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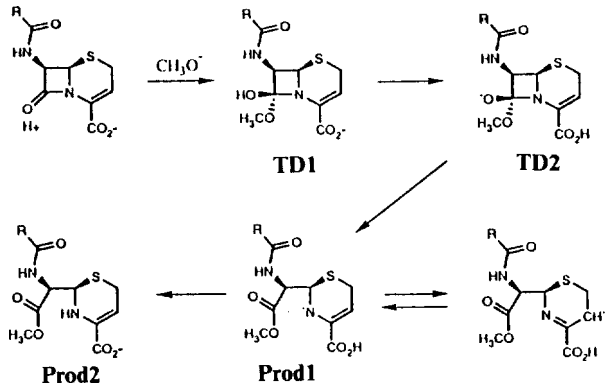
Methanolysis of a β-lactam ring of a cephalosporin was simulated with AM1 semiempirical quantum mechanical calculation. The tetrahedral intermediate **TD1** from an O-protonated cephalosporin and a methanol transfers the proton intramolecularly to the C-4 carboxylate to generate an oxyanion, *i.e.*, second tetrahedral intermediate **TD2**, which undergoes the amide bond cleavage without further protonation on the N-5. For this cleavage a low-energy barrier **TS2** was located. According to the energy diagram, tetrahedral intermediates easily undergo ring cleavage even without the protonation on the amide nitrogen.

Introdution

The active site of glycopeptide D-Ala-D-Ala transpeptidases of bacteria is now known to have a serine unit and a generally positively charged area,[1] which is similar to those of the serine proteases.[2] It is widely believed that the addition of the hydroxy group of the serine to the amide of a β-lactam ring initiates the acting mode of β-lactam antibiotics and finally the serine at the active site is acylated with β-lactam antibiotics, which reduce or terminate the activities of the D-Ala-D-Ala transpeptidase.[2] As a result, the formation of cross linkages in the peptidoglycans of bacteria can not occur and bacteria will finally burst out because of the weak cell wall.[2] The major difference between the D-Ala-D-Ala peptidase and the classical serine protease family is that the former has not a well-known Asp-His-Ser triad, which is an essential active site of the serine protease family to activate the serine hydroxy group.[3] Almost all known penicillin-binding-proteins do not have even the histidines around the active sites.[1c,4]

The chemical processes involoved in the acting mode of β-lactam antibiotics are very similar to the hydrolysis of an amide which is well studied experimentally and theoretically.[5] In solution phase, the protonated carbonyl of an amide is attacked by a hydroxide to form a tetrahedral intermediate which is further cleaved to a carboxylate and an amine. Existence of this tetrahedral intermediate is very controversial,[6] but in solution these intermediates have been detected experimentally.[7]

Antibiotic activities of β-lactam antibiotics, such as penicillins and cephalosphorins, have been thought to be originated from strain of the four-membered ring and reduced amide resonance.[2b,8] However, it is now generally accepted that the biological or chemical reactivity of β-lactam antibiotics is not determined solely by the strain or the reduced amide resonance.[9] The studies on the serine protease reveal that the active site serine is more reactive to the substrates or the substrate-like than other serines in the snzymes.[2a] Therefore, the amide bond in the β-lactam ring or the hydroxyl group or the serine should be activated to form a tetrahedral intermediate either by protonation of the amide bond (hydrogen bonding donor) or by deprotonation of the



Scheme 1

serine hydroxyl group (hydrogen bonding acceptor). The candidates for the protonating agents are His and Lys, and those for the deprotonating are His and Glu, etc. Another major feature of the antibiotic, the carboxylate at C-3 in penicillins and at C-4 in cephalosporins has been studied actively and has been postulated to participate in transpeptidase inhibition or β-lactamase catalysis by acting as an counterpart of an electrostatic anchor, such as Lys.[10]

However, it is of great difficulty to monitor the whole actual mode. Here, we report the results of the semiempirical calculation on the hypothetical methanolysis of a cephalosporin by using AMPAC program.[11] We describe the protonated cephalosporins, formation of tetrahedral intermediates with methanol, and the breakdown of the intermediates.

Calculation methods.

Semiempirical molecular orbital calculation has been performed with the AMPAC pakage and AM1 parameter has been used throughout this calculation.[11] The cephalosporin (**1**) studied here has an acetylamino group at C-7 but no leaving substituent at C-3. The NH proton of the C-7 acetylamino group is located above the β-lactam ring. The attacking direction of the methoxide anion could be either *cis* or *trans* to the C_6-S_1 bond, but in this simulation study only the *trans* direction was studied considering the transpeptidase enzyme reaction (Scheme 1). Every atoms was set free for the full

Table 1. Heat of Formation of the Protonated Cephalosporins (in kcal/mol)

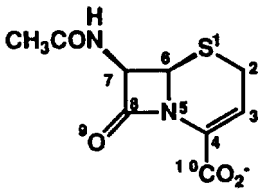
	H_f	Length					Torsional angle	
		O_9-C_8	C_8-N_5	N_5-C_6	O_9-C_{10}	C_3-C_4	C_8-N_5-C_6-C_4	C_6-S_1-C_2-C_3
Cepha (2)	−41.14	1.274	1.380	1.476	3.274	1.352	146.4	−43.2
Cepha (3)	−41.69	1.198	1.553	1.508	3.052	1.340	119.2	−42.2
Cepha (4)	−96.99	1.219	1.438	1.470	3.310	1.358	138.6	−39.8

Table 2. Geometric Informations of Reactants, Intermediates, Products, and Transition States (bond lengths in angstrom, angles in degree)

	Length					Torsional angle	
	O_9-C_8	C_8-N_5	N_5-C_6	O_9-C_{10}	C_3-C_4	C_8-N_5-C_6-C_4	C_6-S_1-C_2-C_3
Cephalosporin (1)	1.219	1.422	1.468	3.416	1.354	143.4	−35.8
TD1	1.390	1.504	1.467	3.313	1.355	133.2	−39.0
TD2	1.288	1.584	1.454	3.322	1.364	137.4	−38.1
TS1	1.339	1.535	1.465	3.021	1.359	129.8	−43.7
TS2	1.265	1.811	1.436	3.470	1.368	136.5	−35.1
Prod (1)	1.231	3.027[a]	1.402	5.246[a]	1.377	—	−29.0
Prod (2)	1.230	3.028[a]	1.421	5.151[a]	1.354	−164.4[b]	−28.3

[a] completely cleaved bond. [b] ω(H-N_5-C_6-C_4).

optimization, and the "PRESICE" key word was used. Vibrational frequencies of all intermediates and products were checked to see all positive frequencies and all transition structures were confirmed by observing only one negative frequency.



Results and Discussion

Protonated Cephalosporins. A calculated cephalosporin **1** has a similar structure of a β-lactam ring and a dihydrothiazine ring to those determined by crystallographic methods.[12] The C-4 carbon was deviated from the plane of N_5-C_6-C_8 by 36.4°. The chain of O_9-C_8-N_5-C_4-C_3 was composed by the bonds of 1.219, 1.421, 1.397, and 1.354 Å, respectively. A normal peptide bond has an amide (O=C-N-C) of 1.24, 1.32, and 1.46 Å.[2a] It is clear that cephalosporin **1** has a less conjugated amide bond. Especially the C-N bond length of **1** is similar to a C-N single bond (1.49 Å) because of the β-lactam ring strain. The enamine conjugation in the C_3-C_4-N_5 bond is believed to shorten the C_4-N_5 bond, which should decrease the electron density at N-5. This also should decrease the amide resonance on the β-lactam ring.

Three protonated cephalosporins were considered. The first one is an O-protonated cephalosporin (**2**) which has two conformation depending on the direction of the proton. The second one is an N-ptotonated (**3**) and the last one is a protonated carboxylic cephalosporin (**4**). Although the last one is most stable, we excluded this form frm further studies because it seems to be equivalent to the unprotonated **1** in the amide methanolysis. The calculated heat of formation of an O-protonated, an N-protonated, and a carboxylic acid form are −41.14, −41.69, and −96.99 kcal/mol, respectively. The detailed parameters are written on Table 1.

Methanolysis of the O-Protonated Cephalosporin. A proposed scheme for the methanolysis of the O-Protonated cephalosporin **2** was drawn in the Scheme 1. The total charge density around the ring carbonyl carbon of the O-protonated cephalosporin, **2**, was calculated to be more positive compared to the unprotonated 1 by +0.632. Not surprisingly, the proton on the carbonyl oxygen was located toward the C-4 carboxylate because of electrostatic attraction, which has been maintained throughout the methanolysis. The geometrical differences between the unprotonated and the protonated are as follows; on protonation, the carbonyl bondlength is elongated by 0.055 Å, but the C_8-N_5 bond is shortened by 0.042 Å.

Tetrahedral intermediate, **TD1**, is expected to be formed from **2** and a methoxide anion. Inside the peptidase enzymes, formation of **TD1** would be a lot more complicated; the hydroxy group of the active serine will attack the ring amide bond with assistance from the surroundings, which should lower the activation energy for the **TD1** formation. The C_8-N_5 bond in **TD1** is 1.504 Å, which is not an amide bond any more, and the O_9-C_8 bond is an almost normal hydroxyl bond (1.390 Å). The proton on the O-9 of **TD1** is directing toward the C-4 carboxylate, and in this conformation one of the lone electron pairs of the O-9 is in the antiperiplanar position of the C_8-N_5 bond.

The second tetrahedral intermediate, **TD2**, which has an O-9 anion and a protonated C-4 carboxylic group, were located. The shorter O_9-C_8 bond (1.288 Å) compared to that of the **TD1** indicates that the electron on the O-9 was moving to the N-5 through the O_9-C_8-N_5 bond, as a consequence,

Table 3 Heat of Formation and Reaction of Reactants, Intermediates, Products, and Reactions (in kcal/mol)

Intermediates and reactions	H_f or H_{rxn}	ΔE_{act}
CH_3OH	−57.03	
CH_3O^-	−38.50	
Cephalosporin (1)	−127.28	
TD1	−200.60	
TD2	−198.71	
Prod(1)	−217.32[a]	
Prod(2)	−217.18[b]	
TS1	−191.80	
TS2	−196.73	
$CH_3OH+1\rightarrow$**TD1**	−16.29	
TD1→**TD2**	+1.89	+8.80
TD2→**Prod(1)**	−18.61	+1.98
Prod(1)→**Prod(2)**	+0.24	

[a] $\omega(N_5\text{-}C_6\text{-}C_7\text{-}C_8) = -70.9°$. [b] $\omega(N_5\text{-}C_6\text{-}C_7\text{-}C_8) = -72.2°$.

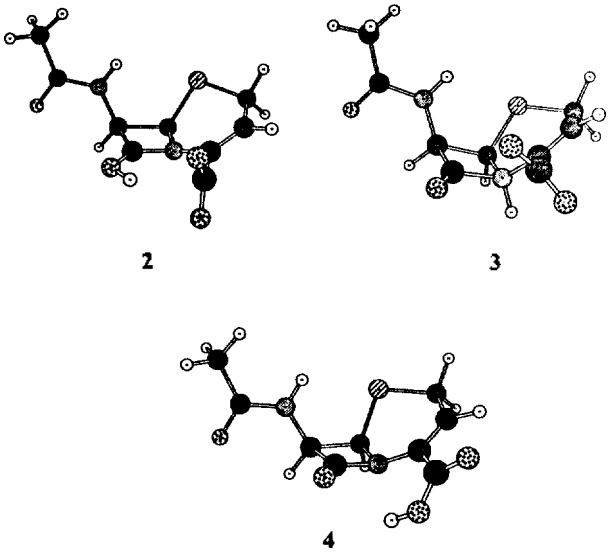


Figure 1. Various protonated cephalosporins.

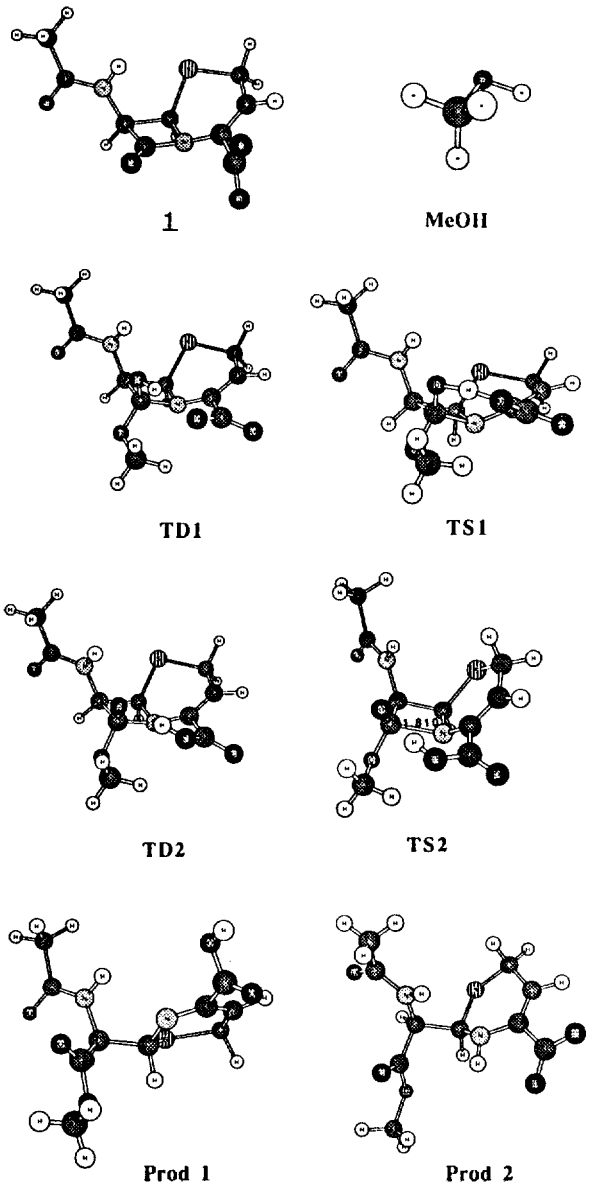


Figure 2. Various reactants, intermediates, and transition structures.

the C_8-N_5 bond was elongated to 1.584 Å.

The approaching directon of the methanol (a serine equivalent) in both **TD1** and **TD2** was fixed only to the β-side (*trans* to the sulfur) as shown in Figure 1, which is well known from crystallographic studies.[1c] However, in solution phase, the methanol should approach to either the α or β side.[2b] The position of the methoxy group obeys the stereoelectronic effect; *i.e.*, either one of the nonbonding electron pairs is located to the antiperiplanar position of the C_8-N_5 bond and therefore the methyl has the staggered position to the bond.

TD2 could be formed from **TD1** by many ways; *i.e.*, protonation and deprotonation by external catalysts. However, **TD1** and **TD2** are similar in energies (**TD1** has a slightly lower energy compared to **TD2** by 1.9 kcal/mol) and the C-4 carboxylate group can participate in the proton transfer from **TD1** to **TD2**. In that sense, we located the transition state, **TS1**, between two tetrahedral intermediates. It has a proton partially attached between the oxygens of an oxide and a carboxylate, and is located above **TD1** by 8.8 kcal/mol. The corresponding C_8-N_5 and C_8-O_9 bonds are 1.535 and 1.339 Å, respectively, and their values are somewhat inbetween those of **TD1** and **TD2**. Our calculation results show that the C-4 carboxylic group in **TS1** behaves as an internal catalyst. Recent experimental work also shows that a protonated tetrahedral intermediate (**TD1**) should convert an unprotonated tetrahedral intermeidate (**TD2**) to undergo methanolysis.[13]

In the amide bond cleavage in the intermediate **TD2** to yield a product (**Prod1**), a transition state (**TS2**) was located and this process was calculated to have a barrier of 1.98 kcal/mol. The angles of $<C_8\text{-}C_7\text{-}C_6$ and $<C_7\text{-}C_6\text{-}N_5$ are 93.3° and 95.9°, respectively, and the β-lactam ring strain has been relieved by 5°. The torsional angle of $\omega(C_6\text{-}S_1\text{-}C_2\text{-}C_3)$ was calculated to be −35.1° in this transition state. The bond length of C_8-N_5 is 1.811 Å, which is longer than those of **2**, **TD1**, and **TD2** by 0.2-0.3 Å. Otherwise, **TS2** and **TD2** are very similar in geometry; for examples, the torsional

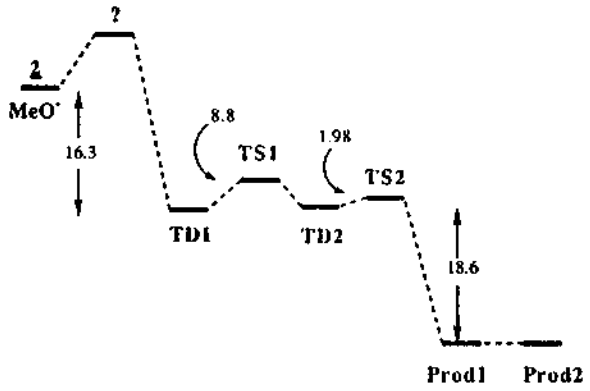


Figure 3. Energy diagram for the methanolysis of the O-protonated cephalosporin (units in kcal/mol, not scaled).

angles of $\omega(C_4\text{-}N_5\text{-}C_6\text{-}C_7)$ and $\omega(S_1\text{-}C_6\text{-}C_7\text{-}C_8)$ are $-140.0°$ and $-120.9°$ for **TS2**, and $-141.5°$ and $-119.4°$ for **TD2**. The similarity in geometry of **TS2** with **TD2**, not with **Prod1**, is also expected from the low energy barrier for the conversion to **Prod1**. It should be emphasized that the amide bond cleavage of **TD2** can occur without protonation on N-5, which is the major difference from the ordinary amide hydrolysis.[5e] Almost all known cephalosporins have leaving groups on C-3 ($C_3\text{-}CH_2\text{-}X$) and they may not need protonation on N-5 to repel the leaving group (X) during the methanolysis with a serine residue in DD-prptidases.

Prod1 has a planar ester bond around C-8. The dihydrothiazine ring was rotated away from the methoxy group on C-8 to have $C_8\text{-}C_7\text{-}C_6\text{-}C_5$ torsional angle of $-70.9°$, which is well correlated with crystallgraphic data.[1c] Charge densities on N-5 were changed from -0.206 in **1** to -0.308 in **TS2**, and finally to -0.438 in **Prod1**. Those on C-3 also were changed from -0.304 in **1** to -0.344 in **TS2**, and to -0.489 in **Prod1**. The negative charge created by the amide bond cleavage is observed to be stabilized by being distributed over the C-3 carbon, which is one of the major differences between the cephalosporin methanolysis and the normal amide methanolysis.[9a] There are some previous studies on the leaving group effect at the C-3 position.[14] From our charge density study on **Prod1** also shows that when there is a leaving group at the C-3 position, the leaving group will be easily detached from the C-3 position.

Prod2 is expected to be the final product in the methanolysis and has a $\omega(C_8\text{-}C_7\text{-}C_6\text{-}N_5)$ torsional angle of $-72.2°$. Even though there is another conformer which has slightly higher energy (by 3.6 kcal/mol) than **Prod2** and has the torsional angle of 42.8°, it was not considered because only **Prod2** fits the cavity suggested by the crystallogrphic work.[1c]

In conclusion, the proton on the β-lactam amide oxygen can be removed by the C-4 carboxylate group to give the cleavage-ready tetrahedral intermediate **TD2**, which has an oxyanion. The amide cleavage has been calculated to have a low activation energy, and the negative charge on the N_5 of **Prod1** stabilized well by the $C_3=C_4$ double bond.

The whole processes involved in the deactivation of the transpeptidase by β-lactam is not clear yet and it will take more time to figure out the exact mechanism. Because even modern X-ray crystallogrphy and kinetic studies can not provide the exact pictures of acting modes of drugs, it is important to consider the experimental results in conjuntion with theoretical data

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Theoretical Studies on the Gas-Phase Wittig-Oxy-Cope Rearrangement of Deprotonated Diallyl Ether[1]

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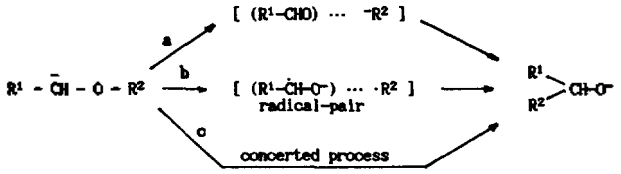
The Wittig-oxy-Cope rearrangements of deprotonated diallyl ether, I, $CH_2=\bar{C}H$-CH-O-CH_2-CH=CH_2, have been investigated theoretically by the AM1 method. A two step mechanism forming a Wittig product ion, II, (CH_2=CH) (CH_2=CH-CH_2) CHO^-, through a radical-pair intermediate was found to provide the most favored reaction pathway in the Wittig rearrangement. The subsequent oxy-Cope rearrangement from species II also involves a two step mechanism through a biradicaloid intermediate. The intramolecular proton transfer in I (to form CH_2=CH-CH_2-O-$\bar{C}$H-CH=CH_2) is a higher activation energy barrier process compared to the Wittig and oxy-Cope rearrangements and is considered to be insignificant. These results are in good agreement with the condensed-phase as well as gas-phase experimental results.

Introduction

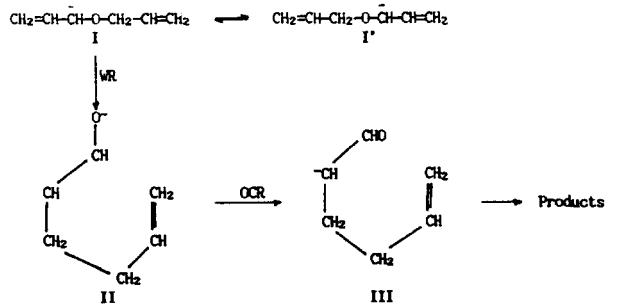
The Wittig rearrangement (WR)[2] has been extensively studied as one of the better known carbanion rearrangement in the condensed phase. Two-step mechanisms (reaction pathways *a* and *b*) involving either of the intermediate shown in Scheme 1 are proposed for the rearrangement.[3] However the possibility of a concerted one-step process (reaction pathways *c*) can not be entirely ruled out.[4] Various experimental results seem to support the two-step mechanism involving a ridical pair, pathway *b*, rather than that involving an ionic intermediate, pathway *a*.[5] Some of the condensed-phase experimental results in favor of the radical-pair mechanism are: (i) the migratory aptitude of substituents R^2 is in the order of free-radical stabilities,[6] allyl≅benzyl>methyl>ethyl>phenyl, (ii) partial racemization of R^2 is observed,[7] (iii) when ketyl radicals and R radicals from different precursors were brought together, similar products resulted.[8] Despite these experimental evidence in support of the radical-pair intermediate pathway, *b*, the radical mechanism is unable to account for all the reaction products and a concerted mechanism, *c*, is suggested as a possible alternative.[4] It is also known that when R^2 is an allyl group, the [2,3] sigmatropic rearrangement can take place.[9]

The Wittig rearrangement can also occur in the gas-phase.[10] Eichinger *et al.*,[10a] have shown in their gas-phase studies on the Wittig rearrangement of deprotonated diallyl ether that (i) the proton-transfer reaction I to I′ (Scheme 2) is insignificant, (ii) I undergoes facile reaction leading to 1,2- and 1,4-rearrangement products, (iii) the Wittig ions rearrange further by an oxy-Cope mechanism (OCR)[11] (Scheme 2).

In this work, we explore MO theoretically the most possible reaction pathway for the Wittig rearrangement of deprotonated diallyl ether (Scheme 1) and the mechanism of the subsequent oxy-Cope rearrangement of the Wittig product ion, II (Scheme 2).



Scheme 1



Scheme 2

Computation

The AM1 procedure[12] implemented in AMPAC package[13] was used throughout in this work. The AM1 method accommodates some electron correlation effect through its parametrization[14] and it requires 2-3 orders of magnitude lesser computing time than even those using the relatively low level ab initio (3-21G) method.[12,14] It has been reported that AM1 gives good results for reactions of anionic species by giving

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