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AM1 STUDY ON TETRAHEDRAL INTERMEDIATES OF THE AMIDES OF β-LACTAM ANTIBIOTICS AND METHANOL

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Abstract: Tetrahedral intermediates of β-lactam antibiotics were calculated with semiempirical SCF-MO calculation (AM1). Intrinsic strain of the β-lactam rings and steric hindrance of the thioazine rings of β-lactam antibiotics are expected to elongate the N-C(O) bonds, and lower the transition barriers for the next cleavage step.

β-Lactam antibiotics have been widely used as important anti-infective drugs since Fleming observed the antibacterial properties of *Penicillium notatum* in 1928. The antibiotics inhibit bacterial D-Ala-D-Ala-transpeptidases to prevent the formation of the bacterial peptidoglycan which consists of the bacterial cell walls.[1] The chemical reactions for the inhibition of the transpeptidases which have an active serine residue, are the addition of the serine gamma hydroxy group to the β-lactam carbonyl carbon to form tetrahedral intermediates, then the breakage of the tetrahedral (TD) intermediates to acylate the serine with the antibiotics.[2] The antibiotic-acylated enzymes are observed to be stable toward hydrolysis, which prevent further function of the enzyme.[3]

We have studied the methanolysis of β-lactam antibiotics with the AM1 semiempirical theoretical method to estimate their intrinsic reactivities in the enzyme active sites.[4] Here we report the semiempirical MO study of the breakage of the TD intermidiates from several β-lactam antibiotics, and compare these results with that of the ordinary amide bond methanolysis to elucidate the acting-mode of the β-lactam antibiotics.[3,5]

Procedure

All semiempirical SCF-MO calculations were done with the AMPAC package, and AM1 parameters were used throughout.[6] The model antibiotics were i) a cephalosporin **1** which has an acylated amine at the C-7 as shown in Fig 1, ii) a carbapenem **2** with an (R)-1-hydroxy-ethyl group on the C-6 position which is *trans* to the C-1, and iii) a penicillin **3** with an acylamido group on the C-6 position. The methoxy groups of the tetrahedral structures from these antibiotics and methanol have been fixed to *trans* to the S-1 (or C-1), which

seems reasonable from the X-ray crystallographic study of the D-Ala-D-Ala transpeptidase systems.[2] The ring-cleaved products could have several conformations, but again the leaving nitrogen ring moieties have been maintained to the away-direction from the approaching methanol to follow the results of the enzyme crystallographic study. To study the effect of thiazine ring to the reactivity of the β-lactam amide, the methanol adduct of N-methyl-β-lactam, **4**, was also calculated. We selected N-vinyl acetamide (**5**) as a linear amide model and a methyl acetate and a vinylamine anion as products from **5**, where the vinyl group is expected to stabilize the developing anion on the nitrogen in the cleavage process. Every intermediates has been confirmed by checking the F-matrix elements, and all transition structures (TS) have only one negative elements. The numbering of each atoms is followed as shown in **4**.

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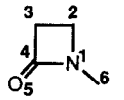

Table 1. Heat of Formation and Geometric Parameters of Various Calculated Structures of β-Lactams. (energy in kcal/mol, length in angstom and angle in degree. For numberings, see the text).

β-Lactams	H_f	C_4=O_5	N_1-C_4	θ(N_1-C_2-C_3)	θ(C_2-C_3-C_4)	ω(C_6-N_1-C_2-C_3)
Cephalosporin **1**	-96.99	1.219	1.438	89.3	85.1	-142.7
TD1	-198.71	1.288	1.584	92.1	88.7	-142.0
TS1	-196.73	1.265	1.811	95.8	93.3	-139.9
Ester1	-217.32	1.231	3.027	113.0	107.5	-152.2
Carbapenem **2**	-114.59	1.219	1.461	89.6	86.9	-122.4
TD2	-205.55	1.290	1.587	92.3	89.4	-118.4
TS2	-202.17	1.264	1.875	97.3	95.3	-117.0
Ester2	-224.13	1.234	2.747	113.4	109.8	-123.3
Penicillin **3**	-122.03	1.218	1.448	89.7	85.0	-136.7
TD3	-215.17	1.286	1.580	92.6	87.7	-126.6
TS3	-210.73	1.258	1.905	98.3	94.2	-122.9
Ester3	-228.83	1.233	3.203	120.6	106.8	-91.8
N-Me-β-lactam **4**	-5.40	1.225	1.414	88.3	86.2	-151.8
TD4	-64.80	1.282	1.522	90.1	88.9	179.2
TS4	-56.55	1.253	1.900	96.6	97.2	178.9
Ester4	-73.53	1.236	2.979	112.4	112.0	178.9
N-Vinyl Acetamide **5**	-27.52	1.245	1.391			
TD5	-88.94	1.298	1.511			
TS5	-72.78	1.252	2.062			

Results and Discussion

Structures: The calculated β-lactam structures show general agreement with the X-ray crystallographic structures.[7] The carbonyl bonds are 1.22 Å and the N-C(=O) bonds are 1.43 Å. Compared with an acyclic amide bond (1.25Å and 1.39Å, respectively), the β-lactam rings have less amide bond character, i.e., the formers are shorter and the latters are longer, which is believed to be caused by the 4-membered ring strain. Each β-lactams has one extra thiazine ring fused to the β-lactam ring. The angle between two rings decreases in the order of the cephalosporin **1**, the penicillin **3**, and the carbapenem **2**. (The torsional angles, $\omega(C_6\text{-}N_1\text{-}C_2\text{-}C_3)$, are -143°, -137°, and -123°, respectively) Considering that ordinary amide bonds have planar structures, the C-6's of the carbapenem **2**, the penicillin **3**, and the cephalosporin **1** are deviated from the β-lactam ring planarity by 77°, 54°, and 40°, respectively. These twisted amide bonds should be more reactive toward the nucleophilic substitution than the planar amide, even though this is not the only cause for the high reactivity.[8] The N-methyl of the β-lactam **4** is calculated to be out of plane by 30°. Many monocyclic β-lactams on Cambridge Structures Database have planar N-substituents, therefore the AM1 method overestimates the steric hindrance, but only slightly, because only 0.2 kcal/mol of energy was necessasry to maintain the N-methyl of **4** on the plane.

The tetrahedral intermediates have oxyanions which are known to be important in the process of the tetrahedral intermediate breakage.[9] In our models, they are stabilized by carboxylic protons and amido protons. As shown in the Table 1, the oxyanionic **TD**'s of the β-lactam antibiotics have longer N-C(O) bonds compared to those of N-methyl-β-lactam **TD4** by 0.06Å and N-vinyl acetamide **TD5** by 0.08Å.

The N-methyl group of the N-methyl-β-lactam **TD4** is located in-between the oxyanion oxygen and the methoxy group (see Fig 1). In the **TD1**, **TD2**, and **TD3**, the oxyanion is in the eclipsed position to the N-methylene of the thiazine ring. Becasuse of this steric hindrance by the extra thiazine rings, we believe the bonds of N-C(O) are elongated by 0.06 Å. When compared with that of the N-vinyl actamide which is shorter by 0.08 Å than those of the antibiotic **TD**'s, the elongation by the β-lactam ring and the second thiazine ring are expected to be 0.02Å and 0.06Å, respectively. Considering these geometric parameters, we can expect that the antibiotic **TD** intermediates have more product characters.

The carbonyl bond lengthes of transition structures of **TS1**, **TS2**, and **TS3** are about 1.260Å, which is equivalent to about 50% conversion to the product ester carbonyls from the tetrahedral intermediates. The bond lengthes of N-C(O) are about 1.900 Å for the β-lactam **TS**'s including **TS4**. Unlike the tetrahedral intermediate case, at this distance the steric hindrance between the oxyanion and the neighboring methylene seem to be deminished considerably. The carbonyl bond of N-vinyl acetamide **TS5** is 1.25Å, which is about 70% conversion to the ester product. The N-C(O) bond of **TS5** is 2.062Å, which indicates that this transition state is more product-like than those of β-lactams.

Energetics: Although transition states for the formation of **TD**'s were not calculated, these transiton states in enzymes are known to be catalyzed by acid-base and/or hydrogen bonds. Because β-lactam rings are

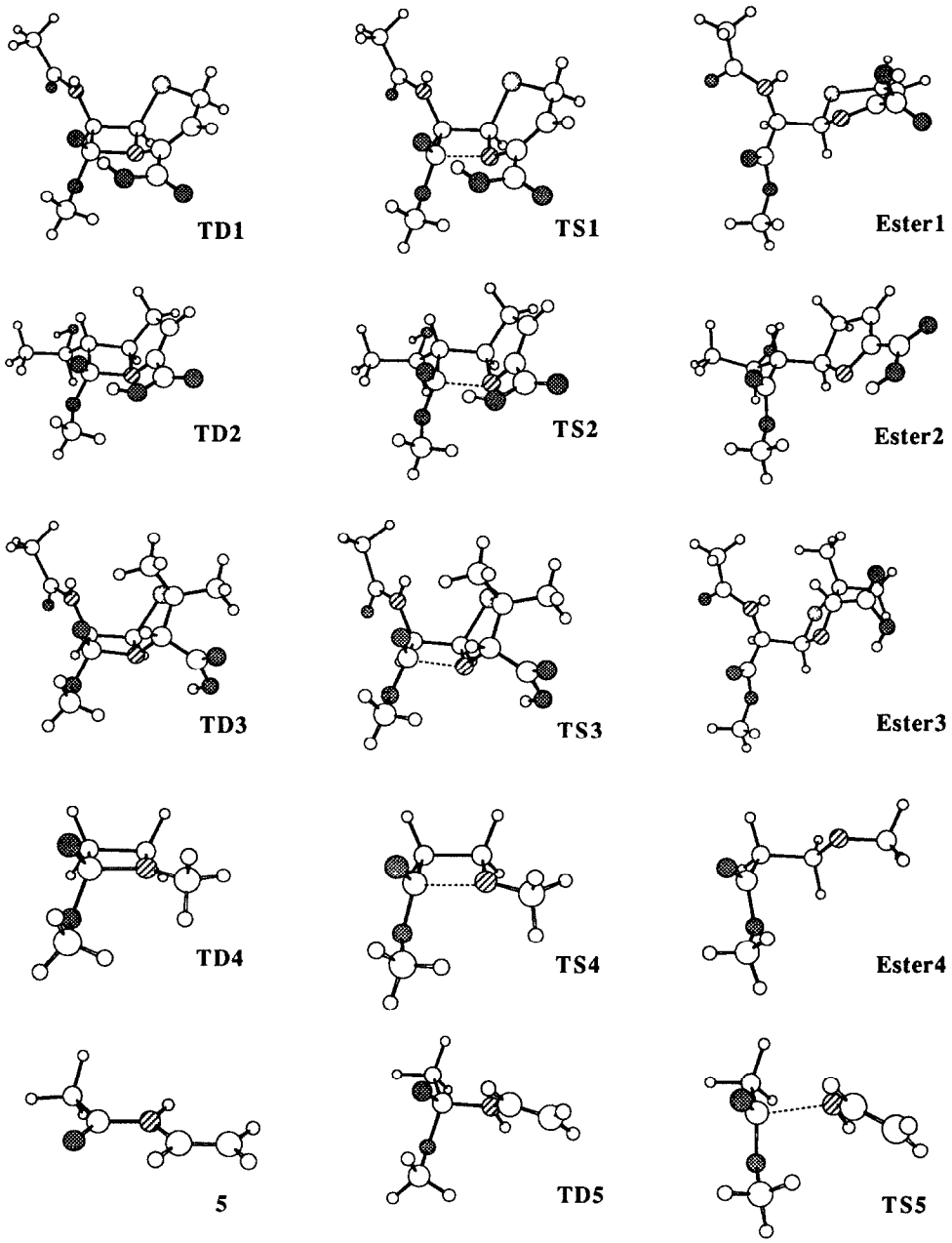

Figure 1. Optimized structures of tetrahedral intermediates (**TD**), transition states (**TS**) for the C-N(O) bond-cleavage, and products (**Ester**). (oxygens are shadowed, nitrogens are lined, and sulfurs are dotted)

strained and the amide bonds of the β-lactam antibiotics are further twisted by the second rings, it is expected that formation of **TD**'s from the amides and a methanol is exothermic and have not high barrier.[8] It is believed that formation of antibiotic **TD**'s is highly exothermic because of the twisted amide and the stabilization of the anions of **TD**'s by hydrogen bonds with the amido NH and the carboxylic protons.[10]

Formation of the ester products from the **TD**'s is also exothermic. In the case of β -lactam antibiotics, the β-lactam ring strain and the steric hindrance caused by the the thiazine rings are relieved by the formation of the esters. Not surprisingly, the transition state energies of **TS1**, **TS2**, and **TS3** are within 4.4 kcal/mol. The energy of N-methyl-β-lactam **TS4** is calculated to be 8.3 kcal/mol. When we compare these **TS**'s energies, we are expecting that the thiazine ring lower the **TS** energy by 4-6 kcal/mol. An *ab initio* calculation on the hydrolysis of 3-cephem[11] show that a fused thiazine ring reduces the **TS** energy by 8.1 kcal/mol. When we consider the energy of the N-vinylacetamide **TS5** (16.2 kcal/mol), the β-lactam ring contributes about 8 kcal/mol in lowering the **TS**'s energies. In the β-lactam antibiotics, the calculated low barriers even without the protonation on the leaving N-4 atom can rationalizes that many β-lactam antibiotics have leaving groups at the conjugated position of the anionic nitrogen. Experimentally, the rate of enhancement of 30-500-fold shown by β-lactams has been observed, and the bicyclic antibiotics are ca. 100-fold more reactive than similar β-lactams.[12]

In conclusion, from the calculated geometries of the antibiotic tetrahedral intermediates, the β-lactam ring strain and the steric hindrance between the eclipsed oxyanion and the C-6 group are believed to lower the conversion barrier considerably. In this calculation, the low activation barriers predict that the cleavage process of the tetrahderal intermediates of β-lactam rings may not be a rate-limiting step in the methanolysis of the β-lactam antibiotics even without the N-protonation.

Table 2. Calculated Heat of Reactions and Activation Energy for the Formation and Breakage of Tetrahedral Intermediates. (in kcal/mol)

	β-Lactam + MeO^- —> TD[a]	ΔH (TD —> ester)	ΔH (TS)
Cephalosporin **1**	-66.22	-18.61	+1.98
Carbapenem **2**	-52.46	-18.58	+3.38
Penicillin **3**	-54.64	-13.66	+4.44
N-methyl-β-lactam **4**	-20.90	-8.73	+8.25
N-vinyl acetamide **5**	-22.92	+19.70[b]	+16.16

[a] Heat of formation of methoxy anion was calculated to be -38.50 kcal/mol.

[b] Heats of formation of methyl acetate and vinyl amine anion are -96.41 and +27.17 kcal/mol, respectively. Actual ester product will be a complex of methyl acetate and vinylamine anion and have a lower energy.

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References

1. For reviews, see (a) Boyd, D. B. in *Chemistry and Biology of β-Lactam Antibiotics*; Ed. by Morin, R. B. and Gorman, M.; Vol 1, Academic Press, New York, NY, 1982. (b) Kelly, J. A.; Knox, J. R.; Moews, P. C., Moring, J., Zhao, H. C., in *Antibiotic Inhibition of Bacterial Cell Surface Assembly and Function* Ed Actor, A., Daneo-Moore, L., Higgins, M. L., Salton, M. R. J., Shockman, G. D.; American Society for Microbiology, Washington, D.C., 1988; Chapter 24.
2. (a) J. A.Kelly, J. R. Knox, P. C. Moews, G. J. Hite, J. B. Bartolone, H. Zhao, B. Joris, J. M. Frere, J. M. Ghuysen, *J. Biol., Chem.* **1985**, *260*, 6449 (b) Herzberg, O., Moult, J. *Science* **1987**, *236*, 694. (c) Oefner, C.; D'Arey, A.; Daly, J. J.; Gubernator, K.; Charnas, R. L.; Heinze, I.; Hubschwerlen, C.; Winkler, F. K. *Nature (London)* **1990**, *343*, 284. (d) Knox, J. R., Moews, P. C. *J. Mol. Biol.* **1991**, *220*, 435.
3. (a) Kelly, J. A.; Dideberg, O.; Charlier, P.; Wery, J. P.; Libert, M.; Moews, P. C.; Knox, J. R.; Duez, C.; Fraipont, C.; Joris, B.; Dusart, J.; Frere, J. M.; Ghuysen, J. M. *Science* **1986**, *231*, 1429. (b) Escobar, W. A.; Tan, A. K.; Fink, A. L. *Biochemistry* **1991**, *39*, 10783.
4. Nahm, K. *Bull. Korean Chem. Soc.* **1991**, *12*, 674.
5. (a) Page, M. I.; Webster, P.; Bogdan, S.; Tremerie, B.; Ghosez, L. *J. Chem. Soc., Chem. Commun.* **1986**, 1039. (b) Page, M. I. *Adv. Phys, Org. Chem.* **1987**, 165.
6. (a) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902. (b) AMPAC, Version 2.1, QCPE 506, Indiana Univcersity, Indiana, U. S. A.
7. (a) Stert , R. M.; Dahl, L. F. *J. Am. Chem. Soc.* **1970**, *92*, 5489. (b) Boyd, D. B. *J. Org. Chem.* **1985**, *50*, 886.
8. (a) Wiberg, K. B.; Laidig, K. E. *J. Am. Chem. Soc.* **1987**, *109*, 5935. (b) Laidig, K. E.; Bader, R. F. W. *J. Am. Chem. Soc.* **1991**, *113*, 6312. (c) Bennet, A. J.; Wang, Q-P.; Slebocka-Tilk, H.; Somayaji, V.; Brown, R. S. *J. Am. Chem. Soc.* **1990**, *112*, 6383.
9. (a) Guthrie, J. P. *J. Am. Chem. Soc.* **1974**, *96*, 3608. (b) Proctor, P.; Gensmantel, N. P.; Page, M. I. *J. Chem. Soc., Perkin Trans.* II **1982**, 1185. (c) Rao, S. N.; More O'Ferrall, R. A. *J. Am. Chem. Soc.* **1990**, *112*, 2729.
10. Nakagawa, S.; Umeyama, H. *J. Mol. Biol.* **1984**, *179*, 103.
11. Fixed-geometry calculation with a primitive GTO basis set; Petrongolo, C.; Pescatori, E.; Ranghino, G.; Scordamaglia, R. *Chem. Physics* **1980**, *45*, 291.
12. Page, M. I. *Acct. Chem. Res.* **1984**, *17*, 144.