STRUCTURES OF THE ALKALINE HYDROLYSIS PRODUCTS OF PENEM ANTIBIOTIC, SUN5555

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β-Lactam compounds break down under alkaline conditions to give ring opened products which in the case of cephalosporin derivative[1] undergo further degradation. This hydrolytic ring opening of the β-lactam structure is usually observed *in vivo*, resulting in biologically inactive metabolites[2,3].

The penem derivatives are also unstable in alkaline media to afford biologically inactive compounds[4,5]. Upon administration of the penem derivative, SUN 5555 (**1**), sodium [5*R*,6*S*,8*R*,2′*R*]-2-(2′-tetrahydrofuryl)-6-hydroxyethylpenem-3-carboxylate, to rat orally, two major metabolites (M-1 and M-2) were detected in urine by HPLC analysis.

Hydrolysis of SUN5555 with 1.2 equiv NaOH in water at room temperature for 3 hours afforded two products (B-1 and B-2) in almost quantitative yield. Examination of these products using HPLC revealed that they are the same as the metabolites M-1 and M-2, respectively. Separation of the mixture of B-1 and B-2 using preparative LC on Kaseisorb LC-ODS-100-5 gave each pure compound in a ratio of 4:1.

Compounds B-1 and B-2 showed very similar UV (λ_{max} 300 nm) and IR (1610 and 1400 cm^{-1}) spectra and FAB-MS (*m/z* 348, $(M+H)^+$). The ^1H and ^{13}C NMR (see Table 1) also indicated a close resemblance of the structure, suggesting that they are diastereomeric compounds. Since ^1H NMR of B-1 showed the presence of a vinylic proton signal at 6.15 ppm and the absence of the characteristic methine proton at C-2′ and methylene protons for the tetrahydrofuran ring observed in SUN5555, the hydrolysis should be accompanied by the opening of the tetrahydrofuran ring to give an exo-olefinic alkyl alcohol (4′-hydroxybutylidene group) at C-2 position. The minor product B-2 showed very similar chemical shifts for the exo-olefin side chain at C-2 in its ^1H and ^{13}C NMR. In addition, the compound (**2**) which is a diastereomer of SUN5555 (**1**) having *S* configuration at C-2′ position was also converted to the same mixture of B-1 and B-2. This suggests no influence of the stereochemistry at C-2′ on the formation of the *E* or *Z* exo-olefinic bond during the opening of the tetrahydrofuran ring. Thus, it will be reasonable to assume the *Z* configuration for this exo-olefinic bond as observed in the clavulanic acid derivatives which should be thermodynamically more stable than the *E* olefin[6]. On the other hand, the characteristic difference of the chemical shifts and the coupling constants between C-5-H, C-6-H and C-8-H of B-1 an B-2 suggested that B-1 and B-2 are the epimers at C-5 or C-6 (see Table 1).

The CD spectra of B-1 and B-2 which showed the maximum positive Cotton effect (θ 8,460) at 304 nm for B-1 and the opposite effect (θ 12,000) at 306 nm for B-2 clearly indicated the difference of the chirality at the C-5 position as the consequence of the opposite dihedral angles of the conjugated Schiff base chromophore (N-4, C-3, C-2, C-2′). Molecular mechanics calculation using MM2 program in

Table 1. ^1H and ^{13}C NMR data of the hydrolysis products of SUN5555.

Proton	^1H NMR[a]	
	B-1	B-2
5-H	6.02 d[b] (J=9.9)	6.00 d (J=7.4)
6-H	2.65 dd (J=7.2, 9.9)	2.64 t (J=7.4)
8-H	4.25 dq (J=6.4, 7.2)	4.13 dq (J=6.4, 7.4)
9-H_3	1.27 d (J=6.4)	1.27 d (J=6.4)
11-H	6.15 t (J=7.4)	6.10 t (J=7.4)
12-H_2	2.22 q (J=7.4)	2.22 q (J=7.4)
13-H_2	1.75 tt (J=6.9, 7.4)	1.75 tt (J=6.9, 7.4)
14-H_2	3.62 t (J=6.9)	3.63 t (J=6.9)

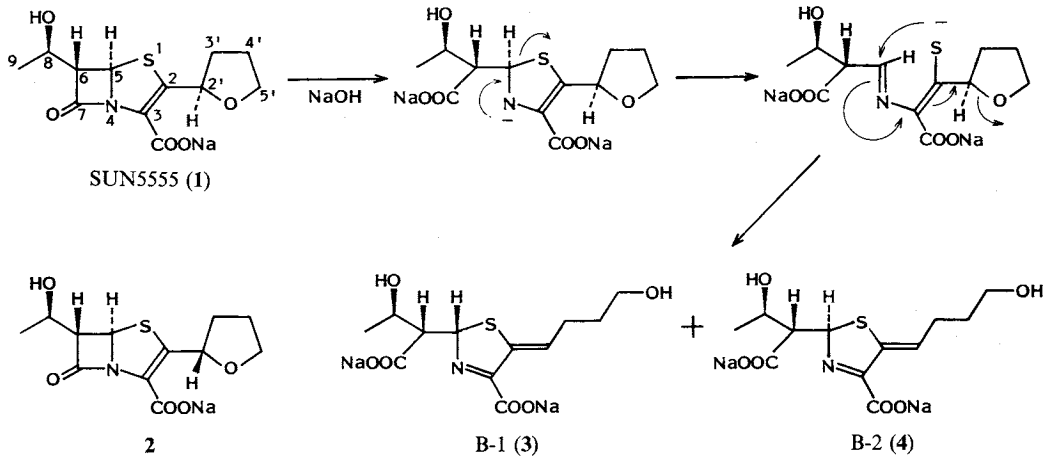
Carbon	^{13}C NMR[c]	
	B-1	B-2
C-2	141.7	142.9
C-3	171.7	171.5
C-5	84.7	83.8
C-6	66.9	66.1
C-7	180.7	181.3
C-8	71.1	70.7
C-9	22.3	23.8
C-10	173.1	173.5
C-11	129.8	128.7
C-12	32.0	31.8
C-13	32.7	32.8
C-14	64.0	64.0

[a] Measured at 270 MHz in D_2O; chemical shifts in ppm from 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TSP), J in Hz.

[b] Multiplicity.

[c] Measured at 68 MHz in D_2O; chemical shifts in ppm from TSP.

Scheme 1. Mechanism of the formation of two epimers B-1 and B-2.



MACROMODEL[7] gave the preferable conformation for each diastereomer and by analogy with CD spectra of the conjugated oxime compounds[8] suggested the *S* configuration for the C-5 position of B-1 and the *R* for B-2 judging from the dihedral angle, $+175°$ and $-175°$, respectively, of the conjugated Schiff base chromophore. Thus, it can be concluded that the epimerization occurred at C-5 to give two isomeric products B-1 (**3**) and B-2 (**4**).

This result may suggest two possible mechanisms for the epimerization reaction, one of which is a deprotonation-protonation reaction to epimerize the configuration at C-5 of B-1 and B-2. The other possible mechanism is shown in the scheme which indicates the cleavage of S1-C5 bond prior to the opening of the tetrahydrofuran ring. Since deuterium was not incorporated in the C-5 position by the hydrolysis condition using deuterium oxide as solvent and the compounds B-1 and B-2 were not interconvertible in basic media (1.2 equiv $NaOH-H_2O$), the epimerization by deprotonation-protonation reaction can be excluded and the other mechanism will be more plausible. Another example of epimerization at the corresponding position has been reported for a penicillin derivative[9] where the mechanism of the epimerization would be similar to that for penem derivatives.

As described here, hydrolysis of SUN5555 (**1**) in basic media gives the ring opening of the β-lactam, followed by cleavage of the tetrahydrofuran ring together with epimerization at C-5 position to afford the products B-1 and B-2 as shown in the scheme.

It is suggested that metabolites M-1 and M-2 could also be obtained by the same pathway *in vivo* in the kidney where dehydropeptidase-I[3] hydrolyzes the β-lactam ring of the penem.

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References

1) EGGERS, S. H.; V. V. KANE & G. LOWE: Studies related to cephalosporin C. Part III. A synthetical route to 6H-1,3-thiazines and the synthesis of a new fragmentation product of a cephalosporanic acid derivative. J. Chem. Soc. 1965: 1262~1270, 1965
2) PFAENDLER, H. R.; J. GOSTELI, R. B. WOODWARD & G. RIHS: Structure, reactivity, and biological activity of strained bicyclic β-lactams. J. Am. Chem. Soc. 103: 4526~4531, 1981
3) CASSINELLI, G.; R. CORIGLI, P. OREZZI, G. VENTRELLA, A. BEDESCHI, E. PERRONE, D. BORGHI & G. FRANCESCHI: Structure determination of the primary renal metabolite of the penem FCE 22101. J. Antibiotics 41: 984~987, 1988
4) ISHIGURO, M.; H. IWATA, T. NAKATSUKA, R. TANAKA, Y. MAEDA, T. NISHIHARA, T. NOGUCHI & T. NISHINO: Studies on penem antibiotics. I. Synthesis and *in vitro* activity of novel 2-chiral substituted penems. J. Antibiotics 41: 1685~1693, 1988
5) NISHINO, T.; Y. MAEDA, E. OHTSU, S. KOIZUKA, T. NISHIHARA, H. ADACHI, K. OKAMOTO & M. ISHIGURO: Studies on penem antibiotics. II. *In vitro* activity of SUN5555, a new oral penem. J. Antibiotics 42: 977~988, 1989
6) CHERRY, P. C.; G. I. GREGORY, C. E. NEWALL, P. WARD & N. S. WATSON: Reactions of sulphur nucleophiles with activated derivatives of clavulanic acid. J. Chem. Soc. Chem. Commun. 1978: 467~468,

1978
7) STILL W. C. (*Ed.*): MACROMODEL (version 1.1), the Columbia Chemistry Molecular Modeling System. Columbia University, 1986
8) CRABBÉ, P. (*Ed.*): ORD and CD of organic functional groups. *In* ORD and CD in Chemistry and Biochemistry, an Introduction. pp. 58～59, Academic Press, 1972
9) HAGINAKA, J. & J. WAKAI: Epimerization of benzylpenicilloate in alkaline aqueous solutions. Chem. Pharm. Bull. 33: 2605～2608, 1985