SYNTHESIS AND β-LACTAMASE INHIBITORY ACTIVITY OF 7α-HYDROXYETHYL CEPHEM DERIVATIVES

Sir:

Recent advances in the chemistry of β-lactam antibiotics have created novel nuclei, such as carbapenems. One of the structural peculiarity of carbapenems is to have 6α-hydroxyethyl side chain as well as the highly strained ring system. On the other hand, the most of the cephalosporin antibiotics has the amide side chain at the 7β-position, and so the biological property of the corresponding cephalosporin having hydroxyethyl side chain at the 7α-position has become of interest in recent years.

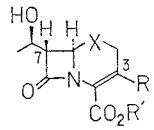
Accordingly, the Merck group investigated homothienamycin **1**[1] as a new carbacephem and reported that its antibacterial activity was quite low level. We thought that the poor activity was mainly due to the low reactivity of β-lactam ring because there was no electron-withdrawing group (EWG) at the both of the 3 and 7 positions.

In cephalosporin chemistry, it was said that the EWG at the 3 position played an important role for reactivity of β-lactam ring[2]. Therefore, we attempted to introduce strong EWG's at the 3 position of 7α-hydroxyethyl cephem derivatives in order to find new biologically active cephem derivatives as shown in **2**.

Silver salt **7** was obtained from 3-chloro-1,2-propanediol *via* 4 steps (Scheme 1). Azetidinone **8**[3] was treated with **7** and sodium iodide in acetonitrile to give **9**. Phosphorane **10** was obtained by the condensation of allyl glycoxylate with **9**, conversion to the chloride with $SOCl_2$ and subsequent reaction with PPh_3.

Cephem **12** was obtained by an intramolecular Wittig reaction[4] of **10** in the presence of hydroquinone[5] followed by desilylation and subsequent oxidation with Collins reagent. Deprotection of **12** was effected with hydrochloric acid to give **13**† (Scheme 2). Oxime **14**, derived from **13** by the treatment with hydroxyamine hydrochloride, was dehydrated to give the cyano derivative **15**. Deprotection of **15** was accomplished with palladium(0) - catalyzed exchange[6] to form **16**††, sodium 7α-[(1*R*)-1-hydroxyethyl]-3-cyano-3-cephem-4-carboxylate. Similarly, **18**†††, sodium 7α-[(1*R*)-1-hydroxyethyl]-3-[(*Z*)-2-cyano-

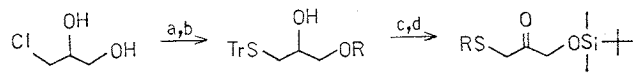
Fig. 1. Structures of **1** and **2**.



1 X=CH_2 R=$-S\diagup\!\!\diagdown NH_2$ R′=H

2 X=S R=EWG R′=Na

Scheme 1.



3 **4** R=H (yield 80%) **6** R=Tr (yield 46%)

5 R=$-OSi(t\text{-}Bu)Me_2$ (yield 66%) **7** R=Ag (yield 98%)

a) TrSH, *tert*-BuOK - THF, b) *tert*-$Bu(CH_3)_2SiCl$, imidazole - DMF, c) $(CF_3CO)_2O$, DMSO - CH_2Cl_2, d) $AgNO_3$, pyridine, THF - MeOH.

† IR and ^1H NMR data of **13**: IR (CH_2Cl_2) cm^{-1} 3600, 1790, 1735, 1670, 1600, 1380, 1345, 1235; ^1H NMR (90 MHz, $CDCl_3$) δ 1.31 (3H, d, *J*=7 Hz), 2.48 (1H, br s), 3.28 and 3.97 (2H, ABq, *J*=17 Hz), 3.38 (1H, dd, *J*=3 and 5 Hz), 4.35 (1H, m), 4.72~4.90 (3H, m), 5.70~6.20 (1H, m), 9.65 (1H, s).

†† IR and ^1H NMR data of **16**: IR (Nujol) cm^{-1} 3500~3250, 2200, 1760, 1620, 1590, 1450, 1330; ^1H NMR (90 MHz, D_2O) δ 1.28 (3H, d, *J*=7 Hz), 3.48 and 3.78 (2H, ABq, *J*=17 Hz), 3.57 (1H, dd, *J*=3 and 5 Hz), 4.33 (1H, m), 4.87 (1H, d, *J*=3 Hz).

††† IR and ^1H NMR data of **18**: IR (Nujol) cm^{-1} 2210, 1750, 1610, 1340; ^1H NMR (90 MHz, D_2O) δ 1.31 (3H, d, *J*=7 Hz), 3.52 (1H, dd, *J*=3 and 5 Hz), 3.83 and 4.10 (2H, ABq, *J*=17 Hz), 4.32 (1H, m), 4.86 (1H, d, *J*=3 Hz), 5.35 (1H, d, *J*=12 Hz), 7.00 (1H, d, *J*=12 Hz).

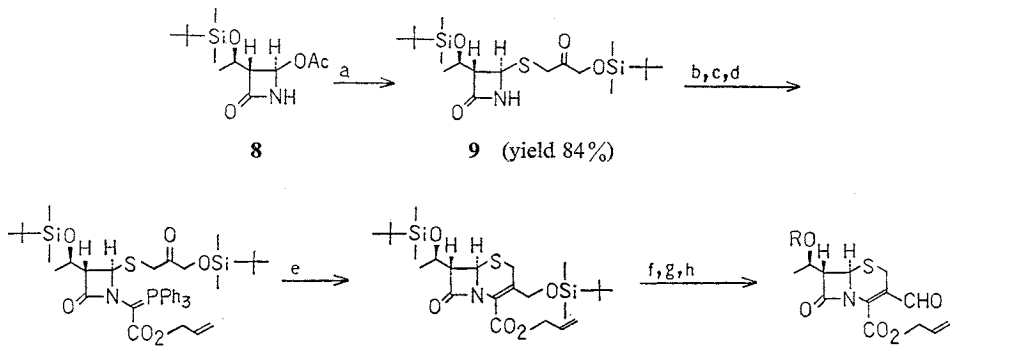
1-vinyl]-3-cephem-4-carboxylate, and **20**†, sodium 7α-[(1*R*)-1-hydroxyethyl]-3-[(*E*)-3-(3-pyridyl)-3-oxo-1-propen-1-yl]-3-cephem-4-carboxylate were obtained as shown in Scheme 4.

The MICs of **16**, **18** and **20** against *Staphylococcus aureus* and *Escherichia coli* are shown in Table 1. The cephems of this series showed only poor activity. Fig. 2 shows the binding affinities of **20**, imipenem and cefazolin (CEZ) for penicillin-binding proteins in *E. coli*. Interestingly, the affinity pattern of **20** is similar to that of imipenem. This fact suggested that the hydroxyethyl moiety determined the affinity pattern regardless of the ring systems, and that the relative weakness of the affinities of **20** resulted in poor MIC values.

Interestingly, it was found that **16**, **18** and **20** had potent β-lactamase inhibitory activity. As can be seen from Table 2, the degree of activity seems to be in order of the electron-withdrawing effect of the side chain at the 3 position. In particular, **16** exhibits superior inhibitory activity against cephalosporinase to that of sulbactam and clavulanic acid. Furthermore, **16** displayed synergistic activity with ceftizoxime (CZX). The MIC data of 1 : 1 combination of CZX plus **16** against several representative β-lactamase producing bacteria is shown in Table 3.

In summary, 7α-hydroxyethyl cephems, which have EWG at the 3 position, have poor antibacterial activity, however exhibit potent β-lactamase inhibitory activity and synergistic activity in

Scheme 2.



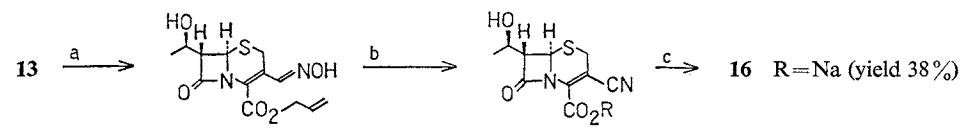
8 **9** (yield 84%)

10 (yield 61%) **11** (yield 91%) **12** R= $-Si-$ (yield 53%)

13 R=H (yield 86%)

a) **7**, NaI - CH_3CN, b) CH_2=$CHCH_2OOCCHO \cdot H_2O$ - toluene, reflux 3 hours, c) $SOCl_2$, 2,6-lutidine - THF, d) PPh_3, 2,6-lutidine, KI - DMF, e) hydroquinone - xylene, reflux 13 hours, f) $BF_3 \cdot Et_2O$ - CH_3CN, g) Collins reagent - CH_2Cl_2, h) 2 N HCl - THF.

Scheme 3.

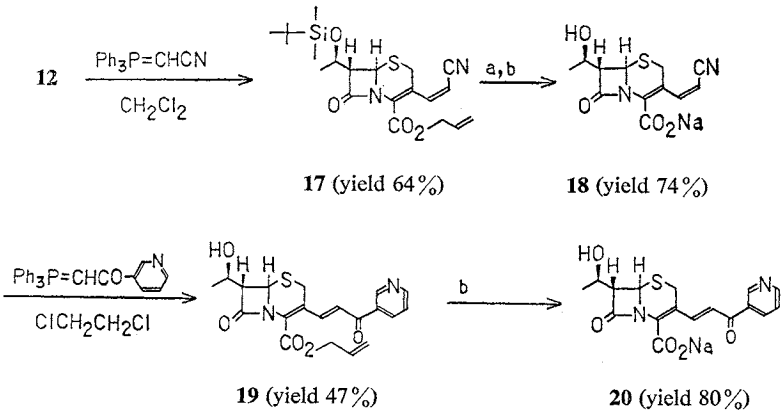


14 (yield 46%) **15** R= ~ (yield 48%)

a) $NH_2OH \cdot HCl$ - $(CH_3)_2CHOH$, b) $SOCl_2$ - $CHCl_3$, reflux 1.5 hours, c) PPh_3, sodium 2-ethyl hexanoate, $Pd(PPh_3)_4$ - EtOAc.

† IR and ^1H NMR data of **20**: IR (Nujol) cm^{-1} 1770, 1625, 1595, 1390, 1365, 1320, 1240~1210; ^1H NMR (90 MHz, D_2O) δ 1.32 (3H, d, J=7 Hz), 3.55 (1H, dd, J=3 and 6 Hz), 3.73 (2H, s), 4.33 (1H, m), 4.92 (1H, d, J=3 Hz), 6.98 (1H, d, J=15 Hz), 7.54 (1H, dd, J=5 and 8 Hz), 7.66 (1H, d, J=15 Hz), 8.23 (1H, dt, J=2 and 5 Hz), 8.98 (1H, d, J=2 Hz).

Scheme 4.



a) 2 N HCl - THF, b) PPh_3, sodium 2-ethyl hexanoate, $Pd(PPh_3)_4$ - EtOAc.

Table 1. MICs[a] of **16**, **18** and **20**.

Organism	MIC (μg/ml)		
	16	**18**	**20**
Staphylococcus aureus 209P JC-1	50	100	25
Escherichia coli NIHJ JC-2	100	>100	100

[a] Mueller-Hinton agar 10^{-2}: Stamp method; 37°C, 20 hours.

Table 2. β-Lactamase inhibitory activity[a].

β-Lactamase	ID_{50} (μg/ml)				
	16	**18**	**20**	Sulbactam	Clavulanic acid
TEM PCase (*Escherichia coli* 18)	17	>500	30	1.2	1.0
Ia CSase (*Enterobacter cloacae* 91)	<0.03	<0.78	33	42	12
Ib CSase (*E. coli* HB101/pCF3)	<0.5	14	450	14	7.8
Ic CSase (*Proteus vulgaris* 9)	0.9	<7.8	<0.5	<0.5	0.6

[a] Serial dilutions of a β-lactamase inhibitor were incubated with enzyme solution for 10 minutes at 37°C. Residual β-lactamase activity was determined spectrophotometrically using the chromogenic substrate nitrocefin at 482 nm. ID_{50} was calculated as the concentration inhibiting 50% of activity.

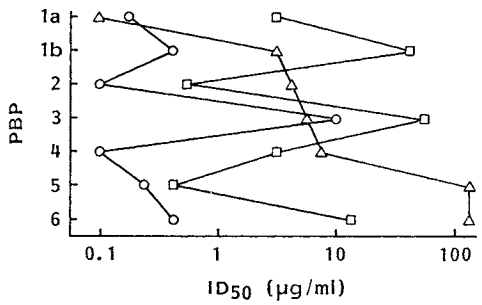
Table 3. MIC data[a] of 1 : 1 combination of **16** with ceftizoxime (CZX).

Organism	MIC (μg/ml)		
	16 - CZX (1 : 1)	**16**	CZX
Morganella morganii 181	25	>100	100
Citrobacter freundii 3007	3.13	100	6.25
C. freundii 3014	12.5	50	25
Enterobacter cloacae 3011	12.5	100	100
E. cloacae 3022	1.56	100	25
Pseudomonas aeruginosa FP1457	12.5	>100	25

[a] Mueller-Hinton agar 10^{-2}: Stamp method; 37°C, 20 hours.

Fig. 2. Binding affinities[a] of **20**, imipenem and cefazolin (CEZ) for penicillin-binding proteins (PBP) in *Escherichia coli*.

□ **20**, ○ imipenem, △ CEZ.



[a] Concentration required to inhibit binding of [^{14}C]benzylpenicillin to each protein by 50%.

combination with CZX. There are few reports of the cephalosporins which have β-lactamase inhibitory activity[7]. Further detailed descriptions and synthesis of 1-oxacephem derivatives are underway.

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