Communications to the Editor

Conscripting β-Lactamase for Use in Drug Delivery. Synthesis and Biological Activity of a Cephalosporin C_{10}-Ester of an Antibiotic Dipeptide

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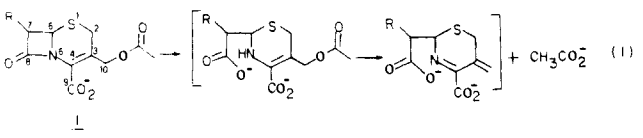
β-Lactamase-promoted fragmentation of the penicillins and cephalosporins is the principal microbial activity which confers resistance to the β-lactam antibiotics, a problem that has been addressed traditionally by strategies designed chiefly to circumvent it. Considerable attention, for example, has been given to the development of new β-lactams with improved lactamase stability; these include the penems,[1] the cephamycins,[2] thienamycin,[3] and the monobactams.[4] An alternate approach centers on the possibility of coadministration of a β-lactam and a lactamase inhibitor,[5] such as clavulanic acid,[6] a carbapenem,[7] a pencillinate sulfone,[8] or a 6-halopenem.[9]

Our efforts in this area are based on the suspicion that the β-lactamases, rather than having to be circumvented, might actually be coopted. This idea arises from an appreciation of the fact that lactamase-catalyzed hydrolysis of Δ^3-cephalosporins is followed by release of a good leaving group from the cephem.[10] Acetate, for example, is formed when cephalothin (**1**) is hydrolyzed (eq 1). This feature of the β-lactamase reaction suggested to



(1)

us that a cephalosporin might be used to "carry" a second bactericidal compound, provided that the latter could be linked—as an ester, carbamate, or ether—to the C_{10}-position of a Δ^3-cephem. Reaction with a β-lactam might release the toxophore in vivo. We report here our initial findings in support of this concept.

The cephem **7** was chosen as our first synthetic target. The key preparative reaction (Scheme I) was coupling of the allylic iodide **3** with the dipeptide **4**, which proceeds readily under mild conditions.[12] Deprotection of **5**, ironically, was the major challenge in synthesis. For example, replacement of Pt with Pd or Rh in **5** → **6** gave, together with the desired removal of the C_9-nitrobenzyl group, the undesired reductive cleavage of the newly formed C_{10}-ester. Similarly, methods used routinely for removal of the BOC group from simple peptides, when applied to **6**, gave cleavage of either the β-lactam or the C_{10}-ester. Thus we devised the sequence **6** → **7**, which involves (1) silylation of the C_9-carboxylate of **6**, (2) treatment with Me_3SiI to effect substitution of the *N*-BOC group with a silyl carbamate, and (3) methanolysis. Compound **8** was prepared by replacement of **4** with *N*-BOC-L-Ala-L-Ala in reaction with **3**.[13]

Both **7** and **8** are excellent substrates for the purified TEM β-lactamase, and they gave identical kinetic constants (K_M = 0.3 mM, V_{max} = 340 μmol min^{-1} mg^{-1}). These cephems are actually better substrates for the TEM enzyme than is cephalothin, **1** (K_M = 0.2 mM, V_{max} = 80 μmol min^{-1} mg^{-1}). Analysis of the enzymatic reactions by high-field ^1H NMR showed that β-Cl-L-Ala-β-Cl-L-Ala was released from **7**, and L-Ala-L-Ala from **8**.[14]

Table I reports the data for in vitro microbial susceptibility to **7** and **8**.[15] Six of eight organisms screened are sensitive to **7**. Cephem **8** has no activity against any strain (MIC > 200 μg/mL), a finding which implies that the cephem nucleus makes little, if any, contribution to the antibiotic properties of **7**.

Susceptibility to **7** generally reflects the sensitivity of an organism to the antibiotic dipeptide. For example, *Ent. aerogenes* is insensitive to haloalanyl peptides and is not affected by **7**. But *Ent. cloacae* is sensitive both to β-Cl-L-Ala-β-Cl-L-Ala (MIC = 12.5 μg/mL) and to **7** (MIC = 14.1 μg/mL). This relationship is most obvious when comparing the data for *E. coli* strain JSR-O and *E. coli* JSR-O Cl-Pep^R; the latter was selected for resistance to β-Cl-L-Ala-β-Cl-L-Ala. While the parental strain is sensitive to **7** (MIC = 14.1 μg/mL), the peptide resistant mutant is not (MIC > 200 μg/mL).

The susceptibilities to **7** of *E. coli* JSR-O (pBR322) and *S. aureus* Pen^R are of special interest. *S. aureus* Pen^R is a lactamase producer, and *E. coli* (pBR322) carries the TEM β-lactamase. The sensitivity of these organisms is consistent with the expectation that the activity of **7** may arise from lactamase-dependent release of the haloalanyl peptide.

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(1) Doyle, F. P.; Naylor, J. H. C. *Adv. Drug Res.* **1964**, *1*, 1.
(2) Nagarajan, R.; Bocek, L.; Gorman, M.; Hamill, M.; Higgens, R. L.; Hoehn, C. E.; Stark, M.; Whitney, J. G. *J. Am. Chem. Soc.* **1971**, *93*, 2308. Kahan, J. S.; Kahan, F. M.; Golgelman, R.; Currie, S.; Jackson, M.; Stapley, E. O.; Miller, F. M.; Hendlin, A. K.; Mochales, S.; Hernandez, S.; Woodruff, H. B.; Zirnbaum, J. *J. Antibiot.* **1974**, *32*, 1.
(3) Komatsu, Y.; Nishikawa, F. *Antimicrob. Agents Chemother.* **1980**, *17*, 316.
(4) Sykes, R.; Bonner, D.; Bush, K.; Georgopapadakou, N. *Antimicrob. Agents Chemother.* **1982**, *21*, 85.
(5) Combination drugs called Augmentin and Timentin (sodium clavulanate with amoxicillin or ticarcillin, respectively) have come into clinical use recently.
(6) Brown, A. G.; Butterworth, D.; Coli, M.; Hanscomb, G.; Hood, J. D.; Reading, C. *J. Antibiot.* **1976**, *29*, 668.
(7) Brown, A. G.; Corbett, F.; Eglington, A. J.; Horvath, T. T. *J. Chem. Soc., Chem. Commun.* **1977**, 523. Maeda, K.; Takahashi, S.; Sezaki, M.; Sinuma, K.; Naganawa, H.; Kondo, S.; Ohno, M.; Umezawa, H. *J. Antibiot.* **1977**, *30*, 770. Okamura, K.; Sakamoto, K.; Ishikura, T. *J. Antibiot.* **1980**, *33*, 293.
(8) English, A. R.; Retsema, J. A.; Girard, A. E.; Lynch, J. E.; Barth, W. E. *Antimicrob. Agents Chemother.* **1978**, *14*, 414. Labia, R.; Lelievre, V.; Puduzzi, J. *Biochim. Biophys. Acta* **1980**, *611*, 351.
(9) Pratt, R. F.; Loosemore, M. J. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 4145. Knott-Hunziker, V.; Orlek, B. S.; Sommers, P. A.; Waley, S. G. *Biochem. J.* **1979**, *117*, 365. Knott-Hunziker, V.; Waley, S. G.; Orlek, B. S.; Sommers, P. G. *FEBS Lett.* **1979**, *99*, 59. Cartwright, S. J.; Coulson, A. F. W. *Nature (London)* **1979**, *278*, 360.
(10) Sabath, L. D.; Jago, M.; Abraham, E. P. *Biochem. J.* **1965**, *96*, 739. Boyd, D. B.; Hermann, R. B.; Presti, D. E.; March, M. M. *J. Med. Chem.* **1975**, *18*, 408. Boyd, D. B.; Lunn, W. H. W. *J. Med. Chem.* **1979**, *22*, 778. Faraci, W. S.; Pratt, R. F. *J. Am. Chem. Soc.* **1984**, *106*, 1489. Page, M. I.; Procter, P. *J. Am. Chem. Soc.* **1984**, *106*, 3829. Page, M. I. *Acc. Chem. Res.* **1984**, *17*, 144.
(11) We had previously prepared the haloalanyl peptide β-Cl-L-Ala-β-Cl-L-Ala and found it to be a broad-spectrum antibacterial agent: Cheung, K. S.; Wasserman, S. A.; Dudek, E.; Lerner, S. A.; Johnston, M. *J. Med. Chem.* **1983**, *26*, 1733.

(12) Product **5** was obtained in 62% yield after 3 h at room temperature. We have not yet attempted the optimization of this coupling.
(13) All of the intermediates of Scheme I, and the products **7** and **8**, are crystalline and have been characterized by elemental analysis, IR, NMR, and FAB mass spectrometry. Complete synthetic details together with characterization of products and intermediates will be published subsequently.
(14) We shall provide a detailed account of these findings elsewhere.
(15) Microbiologic testing was carried out as described by Cheung et al.[11]

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Scheme I

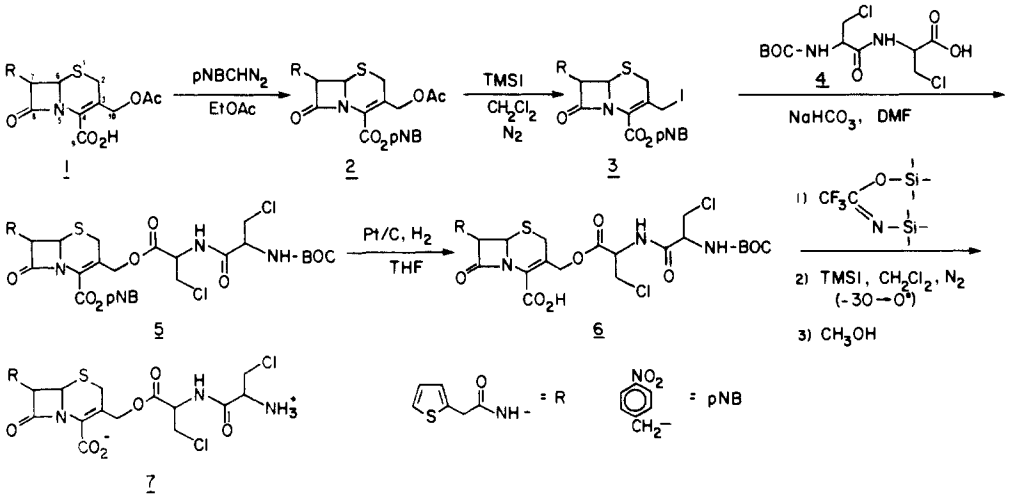

Table I. Susceptibility of Selected Microorganisms to Cephalosporin Dipeptidyl Esters

bacterial species	MIC, μg/mL[a] 7	8
Enterobacter aerogenes	100	>200
Enterobacter cloacae	14.1	>200
Escherichia coli JSR-O	14.1	>200
Escherichia coli JSR-O (Cl-Pep^R)[b]	>200	>200
Escherichia coli JSR-O (pBR322)[c]	7.05	>200
Escherichia coli (Ceph^R)[d]	7.05	>200
Staphylococcus aureus (Pen^R)[e]	0.85	>200
Corynebacterium JK	1.70	>200

[a] MIC = minimum inhibitory concentration. [b] This is an *E. coli* JSR-O strain selected for resistance to the dipeptide β-Cl-L-Ala-β-Cl-L-Ala (MIC >100 μg/mL).[11] [c] Contains the plasmid gene encoding for the TEM β-lactamase. [d] Resistant to cephalothin, I (MIC >200 μg/mL). [e] Resistant to penicillin G (MIC >200 μg/mL).

If the action of a periplasmic lactamase releases β-Cl-L-Ala-β-Cl-L-Ala in vivo,[16] as it does in vitro, this is likely to be only the first in a series of events that render **7** an antibiotic. Subsequent transformations are likely to include: (1) transport of the dipeptide across the inner plasmalemma, (2) hydrolysis of the peptide by a cytoplasmic peptidase, and (3) attendant inactivation by β-Cl-L-Ala[17] of alanine racemase, an enzyme essential for cell wall biosynthesis. Consistent with these expectations, exposure to β-Cl-L-Ala-β-Cl-L-Ala gives inactivation of alanine racemase in *E. coli* JSR-O; the loss of enzyme activity appears to require cleavage of the dipeptide by an aminopeptidase. We have also found that **7** leads to inactivation of the racemase in vitro, in a sequence that involves processing first of **7** by TEM β-lactamase and subsequent aminopeptidase hydrolysis of the liberated dipeptide.[18] We will provide later a more detailed account of the mechanism of action of this novel cephalosporin peptide ester.

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Registry No. 1, 153-61-7; **2**, 41625-53-0; **3**, 100206-63-1; **4**, 100206-64-2; **5**, 100206-65-3; **6**, 100206-66-4; **7**, 100206-67-5; **8**, 100228-77-1; *N*-BOC-L-Ala-L-Ala, 27317-69-7; $pNBCHN_2$, 100206-68-6; β-lactamase, 9073-60-3.

(16) The mechanism of action of **7** in Gram-positive species may not be the same as that outlined for Gram-negative organisms. For example, the lactamases in Gram-positive bacteria are mostly extracellular.

(17) β-Chloroalanines are only weakly antibacterial, probably because they are poorly transported. Incorporation of the amino acid into a transportable peptide substantially increases its antibiotic potency (see ref 11).

(18) Cheung, K. S.; Mobashery, S.; Johnston, M., unpublished experiments.

Reactivity of $Cr(CO)_4$ in the Gas Phase

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Coordinatively unsaturated transition metals can affect the activation of alkanes[2] and the hydrogenation of olefins.[3] Pulsed lasers have been employed to generate such species and in studying the kinetics of their subsequent reactions.[4-6] Time-resolved infrared laser absorption methods provide a means to observe coordinatively unsaturated metal complexes, to characterize their vibrational spectroscopy and to directly determine their reaction kinetics.[4,5] Efforts toward understanding the chemistry of coordinatively unsaturated transition metals have focused primarily on the reactions of metal atoms[7] or monounsaturated metal complexes.[8] We report here new data on kinetics of association of a bisunsaturated complex, $Cr(CO)_4$, with a variety of ligands.

(1) Fellow of the Alfred P. Sloan Foundation (1985-1987).
(2) Janowicz, A. H.; Bergman, R. G. *J. Am. Chem. Soc.* **1982**, *104*, 352.
(3) Miller, M. E.; Grant, E. R. *J. Am. Chem. Soc.* **1984**, *106*, 4635.
(4) Fletcher, T. R.; Rosenfeld, R. N. *J. Am. Chem. Soc.* **1985**, *107*, 2203.
(5) Ouderkirk, A.; Wermer, P.; Schultz, N. L.; Weitz, E. *J. Am. Chem. Soc.* **1983**, *105*, 3354. Ouderkirk, A. J.; Weitz, E. *J. Chem. Phys.* **1983**, *79*, 1089.
(6) Welch, J. A.; Peters, K. S.; Vaida, V. *J. Am. Chem. Soc.* **1982**, *104*, 1089. Simon, J. D.; Peters, K. S. *Chem. Phys. Lett.* **1983**, *98*, 53.
(7) For example, see: (a) Jacobsen, D. B.; Freiser, B. S. *J. Am. Chem. Soc.* **1983**, *105*, 7487. (b) Houriet, R.; Halle, L. F.; Beauchamp, J. L. *Organometallics* **1983**, *2*, 1818.
(8) Seder, T. A.; Church, S. P.; Ouderkirk, A. J.; Weitz, E. *J. Am. Chem. Soc.* **1985**, *107*, 1432.

0002-7863/86/1508-1686$01.50/0