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Registry No. (toluene)$Cr(CO)_3$, 12083-24-8; (*p*-xylene)$Cr(CO)_3$, 12129-27-0; (benzene)$Cr(CO)_3$, 12082-08-5; (mesitylene)$Cr(CO)_3$, 12129-67-8; *m*-xylene, 108-38-3; ethylbenzene, 100-41-4; iodine, 7553-56-2.

Supplementary Material Available: FTIR spectra of (benzene)tricarbonylchromium(0) complexes before and after iodine addition (4 pages). Ordering information is given on any current masthead page.

Elimination of a Good Leaving Group from the 3′-Position of a Cephalosporin Need Not Be Concerted with β-Lactam Ring Opening: TEM-2 β-Lactamase-Catalyzed Hydrolysis of Pyridine-2-azo-4′-(*N*′,*N*′-dimethylaniline) Cephalosporin (PADAC) and of Cephaloridine

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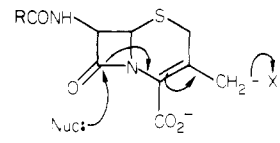
The antibacterial effectiveness of cephalosporin antibiotics, **1**, is strongly influenced by substituents at the 3-position.[1,2] These substituents are capable not only of affecting the binding of the cephalosporins to bacterial β-lactam binding enzymes but also of exerting electronic effects on the reactivity of the β-lactam carbonyl group, which can be correlated to antibiotic activity.[3,4] Furthermore, substituents at the 3′-position may have additional influence through their leaving group ability since it is known[5-8] that nucleophilic β-lactam ring cleavage of cephalosporins that have a good leaving group in the 3′-position, as many clinically important cephalosporins in fact do, is accompanied by elimination of the leaving group, as shown in Scheme I for hydrolysis in neutral aqueous solution.

Both experiment[9-11] and theory[4,12,13] have been interpreted in terms of β-lactam ring opening concerted with departure of the leaving group X (**4**); i.e., no intermediate corresponding to **2** has



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until now been observed in cases where X is a good leaving group such as acetoxy or pyridinium. Our experiments described below, however, indicate that the departure of even a good leaving group need not in general be concerted with β-lactam ring opening, although, in the presence of certain enzymes, it may be.

Reaction of the TEM-2 β-lactamase[14] (3–12 μM) with the chromogenic cephalosporin PADAC (pyridine-2-azo-4′-(*N*′,*N*′-dimethylaniline) cephalosporin, **1a**) (13 μM) was followed spectrophotometrically by the stopped-flow method.[15] Hydrolysis of this substrate is accompanied by a color change from purple (λ_{max} 570 nm) to yellow (λ_{max} 468 nm)[17] which arises through discharge of the *N*,*N*-dimethylaniline-4-azo-2′-pyridine leaving group. Observation at 468 or 570 nm showed that the reaction is two phased with an induction period before the color change. Figure 1 shows the absorbance changes at 498 nm, where the two-step nature of the reaction was clearly evident, as a function of enzyme concentration. These curves and those obtained at 468 and 570 nm could be qualitatively and quantitatively fitted by the reaction scheme of eq 1, where E represents the β-lactamase

$$E + C\text{–}P \underset{K_m}{\overset{\text{fast}}{\rightleftharpoons}} E\cdot C\text{–}P \xrightarrow{k_{cat}} E + C'\text{–}P \xrightarrow{k_3} C' + Y \quad (1)$$

and C–P the substrate, which is composed of the cephalosporin nucleus C and the purple leaving group P, which becomes yellow, Y, after cleavage from C in the third step of the reaction.

The two phases of reaction can thus be identified as the enzyme-catalyzed formation of a purple product, C′–P, followed by its transformation, in a step not enzyme catalyzed, to the final yellow product. We identify the intermediate C′–P as the hitherto hypothetical intermediate **2**, where the β-lactam ring has been opened but the leaving group is still present. Curve fitting,[18] assuming K_m = 48 μM and k_{cat} = 192 s^{-1}, which are the values obtained under steady-state, i.e., (C–P) >> (E), conditions, gives k_3 = 11 s^{-1}. Note that the final and slowest step above cannot be decay of an enzyme–substrate complex since it is slower than enzymic turnover at the enzyme concentrations used.

A two-phase reaction that could be quantitatively fitted by equation 1 was also seen on reaction of TEM-2 β-lactamase (3–14 μM) with cephaloridine (**1b**) (20 μM). In this case the reaction was followed at 260 nm, and the kinetic parameters were K_m = 800 μM, k_{cat} = 1100 s^{-1}, and k_3 = 0.44 s^{-1}. The smaller value of k_3 is expected on the basis of the higher pK_a of the conjugate acid of the leaving group.[19] Further evidence for the nature of the two phases of reaction was also obtained in this case by measurement of the rate of proton release.[20] Two phases of proton release of equal amplitude were observed whose time dependence was the same as those observed directly. One would anticipate that one proton would be released during each step of Scheme

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(14) Obtained from the PHLS Centre for Applied Microbiology and Research, Porton Down, England, and used without further purification.
(15) All reactions were carried out in 0.1 M phosphate buffer at pH 7.5 and at 20 °C. The stopped-flow apparatus has been previously described.[16]
(16) Anderson, E. G.; Pratt, R. F. *J. Biol. Chem.* **1981**, *256*, 11401–11404. Anderson, E. G.; Pratt, R. F. *Ibid.* **1983**, *258*, 13120–13126.
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(18) A previously described[16] simplex optimization method was used.
(19) Spectrophotometric titration of *N*,*N*-dimethylaniline-4-azo-2′-pyridine in a 50 mM each formate/acetate/phosphate buffer yielded a pK_a of 4.3; the pK_a of pyridine is 5.14 (Jencks, W. P.; Regenstein, J. In Handbook of Biochemistry and Molecular Biology", 3rd ed.; Fasman, G. D., Ed.; CRC Press: Cleveland, 1976; p 331).
(20) The experiment here consisted of mixing degassed solutions at pH 7.5 of the enzyme in 100 μM phosphate buffer with the substrate in 100 μM phosphate buffer also containing 50 μM bromthymol blue in the stopped-flow instrument; the reactions were monitored at 620 nm.

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Scheme I

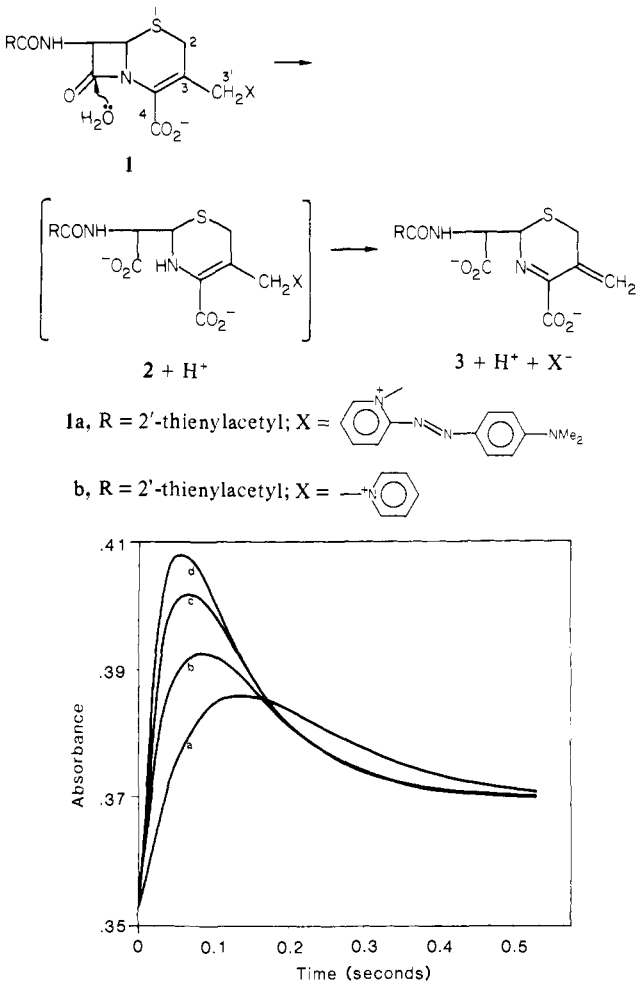

Figure 1. Absorption changes at 498 nm on mixing 13 μM PADAC with (a) 3.0, (b) 6.0, (c) 9.0, and (d) 12.0 μM TEM-2 β-lactamase.

I. It is well-known that two protons are released on hydrolysis of cephaloridine.[5]

The above evidence shows that elimination of pyridinium leaving groups from the 3'-position of these cephalosporins is not concerted with β-lactam ring opening when the latter is catalyzed by the TEM-2 β-lactamase. This may also be true with other leaving groups and when the β-lactam ring is opened by nonenzymic nucleophiles.[21] Other enzymes, either β-lactamases or bacterial cell wall transpeptidases, may influence the situation differently. For example, we have found that the reaction of PADAC (12 μM) with the β-lactamase II of *Bacillus cereus*[22] (60 μM) occurs in a single enzyme-catalyzed step yielding Y and at a rate much faster than the rate of breakdown of C'-P to C' and Y under the same conditions. In this case the enzyme must be catalyzing departure of the leaving group in what could be either a concerted or a nonconcerted reaction on the enzyme surface. The application of these findings to further studies of the active sites of the β-lactam-specific enzymes is being pursued.

Acknowledgment. We are grateful to Hoechst-Roussel Pharmaceuticals Inc. and to Eli Lilly and Co. for generous gifts of PADAC and cephaloridine, respectively. This work was supported by Wesleyan University and the National Institute of Health.

(21) Only one phase of reaction was observed in reactions of PADAC or cephaloridine with hydroxide ion (up to 0.5 M). Either the elimination step is concerted with ring opening or ring opening is rate determining in a two-step mechanism.

(22) Obtained as a mixture with β-lactamase I from the PHLS Centre for Applied Microbiology, Porton Down, England, and purified by the method of Davies et al. (Davies, R. B.; Abraham, E. P.; Melling, J. *Biochem. J.* **1974**, *143*, 115-127).

Asymmetric Synthesis of Amino Acids by Pyridoxamine Enzyme Analogues Utilizing General Base-Acid Catalysis

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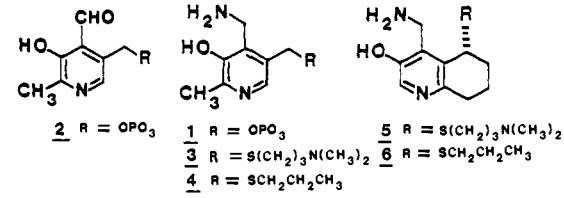
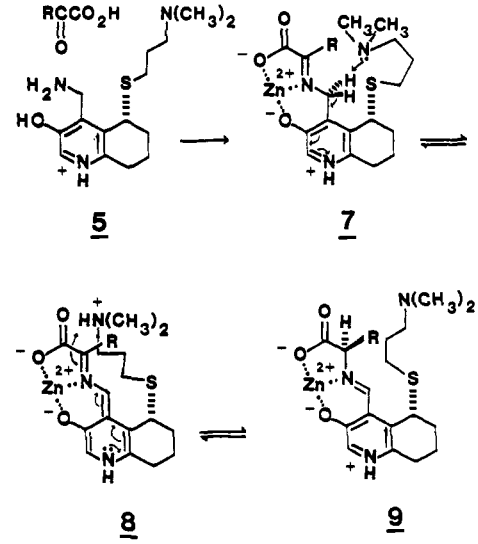
Pyridoxamine (**1**) and pyridoxal (**2**) are the coenzymes for numerous transformations in amino acid metabolism[1] including transaminations that interconvert α-keto acids and amino acids. In transaminations a single enzyme catalytic group, possibly a lysine amino group,[2] acts as a general base to remove the *pro-S* hydrogen from the 4'-methylene of a ketimine intermediate (analogous to **7**). It then moves up the same face of the intermediate and, acting as an acid, reprotonates it in the α-position on the *si* face, yielding the chiral aldimine (analogous to **9**).[1,3] The chiral amino acid is then released. We recently demonstrated[4]



that this base-acid sequence could be duplicated in a transaminase analogue by pyridoxamine derivatives carrying basic side arms, such as **3**. However, only modest stereoselectivity was observed[4] when the side arm was chiral; some stereoselectivity has also been observed in transaminations by pyridoxamine-cyclodextrin derivatives[5,6] or other chiral pyridoxamines.[7] We not wish to describe a closely biomimetic system (**5**) in which a rigidly mounted



side arm is constrained to perform proton transfers on one face of the transamination intermediate, as in the enzyme. Extraordinary stereoselectivity results from this constraint.

As Scheme I indicates, the synthesis of **5** proceeded through

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