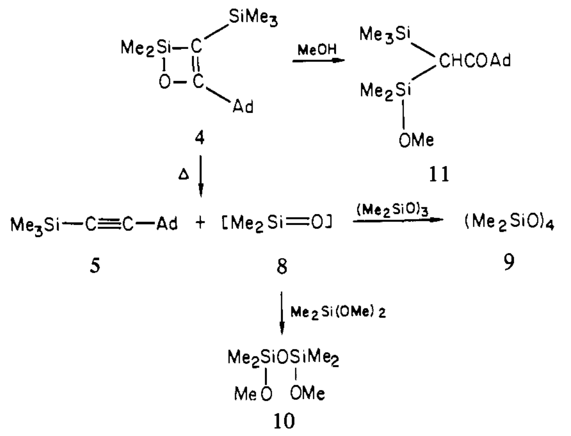
Scheme II



silaoxetene **4** is formed quantitatively. The molecular ion (M^+, 306) is observed on electron impact ionization with intensity 23, the highest mass ion being 291 ($M^+ - Me$, 100), with relatively strong ion peaks at 232 (9, $AdC{\equiv}CSiMe_3^{+\cdot}$) and 217 (53, $AdC{\equiv}CSiMe_2^{+\cdot}$).[5] The IR spectrum (in C_6H_6) had a characteristic C=C stretching vibration at 1530 cm^{-1}, which immediately disappeared on addition of methanol.[6]

The photolysate was readily converted to **5** (69% yield) by heating at 120 °C for 1 h in benzene. Neither hexamethylcyclotrisiloxane nor octamethylcyclotetrasiloxane expected from dimethylsilanone (**8**) was found in the thermal decomposition of **4**.[7] However, the heating of the benzene solution of **4** at 120 °C in the presence of hexamethylcyclotrisiloxane resulted in octamethylcyclotetrasiloxane (**9**) as the apparent product of the silanone **8** in 47% yield as well as **5** (62%). Likewise, dimethyldimethoxysilane trapped **8** to give *sym*-tetramethyldimethoxydisiloxane (**10**) in 26% yield (Scheme II).[8]

The silaoxetene **4** is a thermally very labile molecule, and the half life is $t_{1/2}$ = ca. 24 min. (first-order rate constant $k = 4.8 \times 10^{-4}\ s^{-1}$) at 90 °C in benzene-$d_6$.[9] Attempts to isolate **4** as a pure form were thwarted by its high instability, but is does survive in solution.

Most convincingly, when a benzene solution of **4** was treated with methanol at room temperature, a mildly exothermic reaction occurred to result in the formation of β-silyl ketone **11** in 86% yield (Scheme II).[10] The high reactivity of the silaoxetene may be ascribed to the strain of a four-membered ring and to the weakened Si-O bond.

It is quite interesting to note that the intramolecular reaction of the silene **3** with the adamantylcarbonyl exceedingly prevails over the intermolecular one. Thus, photolysis of **2** in the presence of carbonyl compounds (benzophenone and acetone) or 2,3-dimethylbutadiene led to the silaoxetene **4**, but no intermolecular reaction products from the silene **3**.

Acknowledgment. We are grateful to the Shinetsu Chemical Co. for partial support of this work.

Registry No. 2, 87594-05-6; **4**, 88703-87-1; **5**, 82094-48-2; **6**, 88703-88-2; **9**, 556-67-2; **10**, 18187-24-1; **11**, 88729-58-2; $(Me_2SiO)_3$, 541-05-9; $Me_2Si(OMe)_2$, 1112-39-6; MeOH, 67-56-1.

---

(5) Fragmentation pattern of the silaoxetene **4** is similar to that of the silyl diazo ketone **2**, but the relative intensities are quite different each other. Mass spectrum of **2**, *m/e* 306 ($M^+ - N_2$, 8), 291 (36), 260 (10), 233 (11), and 217 (100).

(6) Carbonyl absorption (1640 cm^{-1}) attributed to the compound **11** immediately appeared on addition of methanol.

(7) It is common not to observe hexamethylcyclotrisiloxane and octamethylcyclotetrasiloxane in solution reactions. (a) Barton, T. J.; Wulff, W. D. *J. Am. Chem. Soc.* **1979**, *101*, 2735. (b) Hussmann, G.; Wulff, W. D.; Barton, T. J. *J. Am. Chem. Soc.* **1983**, *105*, 1263. However, pyrolysis of **2** at 400 °C with a nitrogen stream produced hexamethylcyclotrisiloxane (21%) and octamethylcyclotetrasiloxane (20%) along with **5** (73%).

(8) Hexamethylcyclotrisiloxane and dimethyldimethoxysilane are well-known to trap dimethylsilanone. For a general review of π-bonded silicon see: Gusel'nikov, L. E.; Nametkin, N. S. *Chem. Rev.* **1979**, *79*, 529.

(9) The rate was measured by monitoring the disappearance of the ^1H NMR for the methyl protons of the dimethylsiloxy group.

(10) Compound **11** was isolated by preparative high-pressure liquid chromatography. ^1H NMR (CCl_4, δ) 0.09 (s, 9 H, $SiMe_3$), 0.15 (s, 6 H, $SiMe_2$), 1.53-2.22 (m, 15 H, adamantyl CH), 2.68 (s, 1 H, CHCO), 3.40 (s, 3 H, OMe); IR (neat) 1640 cm^{-1} (C=O); mass spectrum *m/e* 338 (M^+). Anal. Calcd for $C_{18}H_{34}O_2Si_2$: C, 63.84, H, 10.12. Found: C, 64.12, H, 10.09.

Iodine-Catalyzed Arene Exchange of (Arene)tricarbonylchromium(0) Complexes

J. J. Harrison

Gulf Research & Development Company
Pittsburgh, Pennsylvania 15230

Received October 28, 1983

Arene exchange reactions of (arene)tricarbonylchromium(0) complexes, although known almost since the discovery of such complexes,[1-4] are in general difficult processes normally requiring elevated temperatures and the use of electron-rich arenes, electron-donating solvents, etc.[5-12] One consequence of this has been that the use of (arene)tricarbonylchromium(0) complexes in organic synthesis has usually required stoichiometric quantities of chromium.[13-15] We have found that iodine catalyzes the room-temperature arene exchange reaction of (arene)tricarbonylchromium(0) complexes is noncoordinating solvents.

Guided by the recent reports by Kochi[16] and Brown,[17] which teach the importance for ligand exchange reactions of metal-centered radicals with 17 electrons in the valence orbital of the metal, we reasoned that arene exchange of (arene)tricarbonylchromium(0) complexes might become more facile after oxidation of the metal (Scheme I).

Scheme I

$$\underset{\mathbf{1}}{[(\text{arene})Cr(CO)_3]} \rightarrow \underset{\mathbf{2}}{[(\text{arene})Cr(CO)_3]^{+\cdot}} \quad (1)$$

$$[(\text{arene})Cr(CO)_3]^{+\cdot} + \text{arene}' \rightleftharpoons [(\text{arene}')Cr(CO)_3]^{+\cdot} + \text{arene} \quad (2)$$

$$[(\text{arene}')Cr(CO)_3]^{+\cdot} + [(\text{arene})Cr(CO)_3] \rightleftharpoons [(\text{arene}')Cr(CO)_3] + [(\text{arene})Cr(CO)_3]^{+\cdot} \quad (3)$$

To test this possibility we reacted (arene)tricarbonylchromium(0) complexes dissolved in aromatic solvents with a substoichiometric quantity of iodine as oxidizing agent.[18] Arene

---

(1) Natta, G.; Ercoli, F., Calderazzo, F.; Santambrogio, E., Chim. e Ind. (Milan) 1958, 40, 1003.

(2) Ercoli, R.; Calderazzo, F.; Abberola, A. *Chim. Ind.* (*Milan*) **1959**, *41*, 975.

(3) Nicholls, B.; Whiting, M. C. *J. Chem. Soc.* **1959**, 551.

(4) Manuel, T. A.; Stone, F. G. A. *Chem. Ind.* (*London*) **1960**, 231.

(5) Nesmeyanov, A. N.; Vol'kenau, N. A.; Shilovtseva, L. S.; Petrakova, V. A. *J. Organomet. Chem.* **1983**, *61*, 329.

(6) Mahaffy, C. A. L.; Pauson, P. L. *J. Chem. Res., Synop.* **1979**, 126-127; *J. Chem. Res., Miniprint* **1979**, 1752-1775, and references cited therein.

(7) Mahaffy, C. A. L.; Pauson, P. L. *J. Chem. Res., Synop.* **1979**, 128; *J. Chem. Res., Miniprint* **1979**, 1776-1794.

(8) Meyer, A.; Jaouen, G. *J. Organomet. Res.* **1975**, *97*, C21.

(9) Goasmat, F.; Dabard, R.; Patin, H. *Tetrahedron Lett.* **1975**, 2359-2362.

(10) Muetterties, E. L., Bleeke, J. R.; Sievert, A. C. *J. Organomet. Chem.* **1979**, *178*, 197-216.

(11) Barbieri, G.; Benassi, A. Synth. React. Inorg. Met.-Org. Chem. **1974**, *4*, 545.

(12) Semmelhack, M. F.; Seufert, W.; Keller, L. *J. Organomet. Chem.* **1982**, *226*, 183-190.

(13) Semmelhack, M. F. *J. Organomet. Chem. Libr.* **1976**, *1*, 361.

(14) Semmelhack, M. F. *Org. Synth.: Today Tomorrow, Proc. iUPAC Symp. Org. Synth., 3rd* **1980**, 64.

(15) Jaouen, G. *Org. Chem.* (*N.Y.*) **1978**, *39*, 65.

(16) (a) Hershberger, J. W.; Klingler, R. J.; Kochi, J. K. *J. Am. Chem. Soc.* **1982**, *104* 3034-3043. (b) Hershberger, J. W.; Amatore, C.; Kochi, J. K. *J. Organomet. Chem.* **1983**, *250*, 345-371.

(17) McCullen, S. B.; Walker, H. W.; Brown, T. L. *J. Am. Chem. Soc.* **1982**, *104* 4007-4008.

(18) Excess quantities of iodine has been shown to completely oxidize (arene)tricarbonylchromium(0) complexes to free arene, CO, and CrI_3.[19-22]

(19) Semmelhack, M. F.; Hall, H. T. *J. Am. Chem. Soc.* **1974**, *96*, 7091.

Table I. Equilibrium Composition and Equilibrium Constant for Iodine Exchange Reaction[a]

arene	arene′	(arene′)-$Cr(CO)_3$,[b] %	(arene)-$Cr(CO)_3$,[b] %	K_{eq}[c]
toluene	*p*-xylene	70	30	2.3 ± 0.3
benzene	toluene	79	21	3.8 ± 0.5
benzene	mesitylene	99.6	0.4	>250
benzene	*p*-xylene	93	7	14 ± 4

[a] 0.1 mmol of (arene)$Cr(CO)_3$ was reacted at 22-24 °C with an excess amount (5 mL) of 1:1 arene/arene′ under argon. Iodine (1 mg) was added successively until a constant composition was obtained. Usually 2-3 mg were required. The equilibrium composition was the same approached from either direction within limits of determination (±2%). [b] Determined by HPLC.[24] Samples were dissolved in methylene chloride prior to injection. Uncertainty in measurement is ±2%. [c] Defined by eq 4; see text. Error limits relate to ±2% uncertainty in percent composition from HPLC.

exchange occurred at room temperature in good yield! A typical procedure is as follows: To 0.23 ± 0.01 g (toluene)tricarbonylchromium(0) (1 mmol) and 10 mL of *p*-xylene (distilled from sodium/benzophenone) under argon were added while stirring 2 mg of iodine (0.008 mmol). After 30 min, 2 mg of iodine (0.008 mmol) were again added and the reaction mixture was stirred at room temperature overnight. Then the reaction was diluted with ether and filtered through silica gel. The solvent was removed in vacuo to give 0.25 ± 0.01 g of a 85:15 mixture of (*p*-xylene)tricarbonylchromium(0):(toluene)tricarbonylchromium(0) (H^1 NMR, HPLC[24] comparison with authentic sample). A control experiment without iodine showed no exchange. Although the equilibrium composition has not been reached, this experiment shows that as little as 4 mg of iodine (0.016 mmol) catalyzes the production of 0.85 mmol of (*p*-xylene)tricarbonylchromium(0). This corresponds to a catalytic efficiency of greater than 50:1. Equilibrium compositions can be obtained using more iodine (see Table I).

When cosolvents such as THF, methanol, *p*-dioxane, acetonitrile, acetone, propylene carbonate, Me_2SO, and trifluoroacetic acid were used, the arene exchange reaction proceeded poorly if at all. In addition, methylene chloride and chloroform reacted to quench the reaction with formation of a gas and green precipitate.[23] Exchange was observed with cyclohexane as solvent.

The iodine-catalyzed exchange reaction has been observed with benzene, toluene, *p*-xylene, *m*-xylene, mesitylene, and ethylbenzene. Little, if any, exchange was observed with chlorobenzene and anisole.

The kinetics of the exchange reaction were briefly examined (Figure 1) by using a solution of (benzene)tricarbonylchromium(0) (0.1 mmol) in 5 mL of benzene/mesitylene (1/1). After the addition of 0.03 equiv of iodine, the decrease in concentration of (benzene)tricarbonylchromium(0) and increase in concentration of (mesitylene)tricarbonylchromium(0) were followed by HPLC.[24] After 120 min, exchange appeared to stop. This was shown to be due to deactivation of the active catalyst since further addition of 0.05 equiv of iodine restored activity and exchange proceeded to equilibrium.

$$\text{(arene)}Cr(CO)_3 + \text{(arene}') \rightleftharpoons \text{(arene}')Cr(CO)_3 + \text{arene} \quad (4)$$

$$K_{eq} = \frac{[\text{(arene}')Cr(CO)_3][\text{arene}]}{[\text{(arene)}Cr(CO)_3][\text{arene}']} \simeq \frac{[\text{(arene}')Cr(CO)_3]}{[\text{(arene)}Cr(CO)_3]} \quad (5)$$

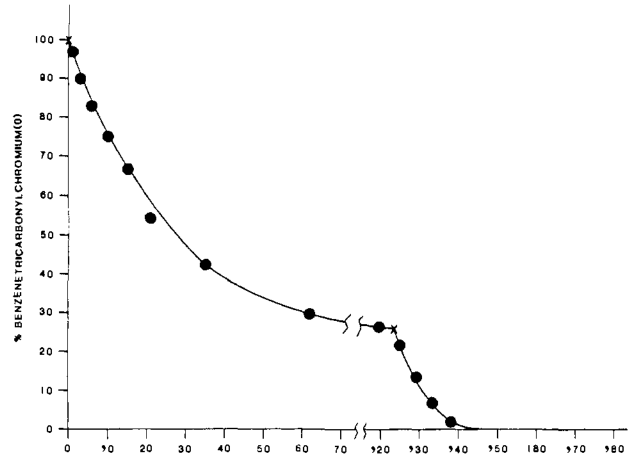


Figure 1. Rate of disappearance of (benzene)tricarbonylchromium(0) in 1:1 benzene/mesitylene at 23 °C.

If the reaction is carried out in approximately equimolar mixtures of arenes, and the concentrations of arene and arene′ are much greater than the concentration of (arene)$Cr(CO)_3$, the equilibrium constant K_{eq} can be approximated by the ratio of (arene′)$Cr(CO)_3$/(arene)$Cr(CO)_3$. This ratio was measured from both directions for a variety of arenes. These are shown in Table I.

The ESR spectrum of the benzene/methylene system reported above was examined immediately after the addition of iodine. Since the radical cation **2** in Scheme I is formally a d_5 species we had hoped to see evidence for it in ESR. No signal was observed over the range 1330-5330 G.[25]

Our attempts to generate **2** by electrochemical oxidation[26-28] have been hampered by the lack of a suitable solvent that will permit both the flow of current and the exchange reaction.

Other oxidizing agents such as bromine, methyl-*p*-benzoquinone, DDQ, 2,6-dimethylbenzoquinone, manganese(III) acetylacetonate, and copper(I) iodide gave little, if any, exchange. Chromium(III) iodide and chromium(II) iodide did catalyze the exchange reaction at a reduced rate.

In light of the above, we have considered a mechanism involving an iodine bridged or an inner-sphere process involving a chromium carbonyl iodide species as the active catalyst in the exchange reaction. The FTIR spectrum[29] of a solution of (benzene)tricarbonylchromium(0) in benzene (0.1 M) was examined in the region 1700-2100 cm^{-1} as increasing amounts of iodine in benzene (0.1 M) were added. A detectable concentration of a carbonyl-containing species other than (benzene)tricarbonylchromium(0) was not observed in this experiment (see supplementary material).

The mechanistic details of the exchange reaction, therefore, remains an open question. However, the mechanism appears to differ significantly from published mechanisms that require a donor solvent.[6,10,30-32]

In conclusion, we have demonstrated for the first time a room-temperature iodine-catalyzed arene exchange reaction of (arene)tricarbonylchromium(0) complexes with arenes in noncoordinating solvent. We are currently interested in pursuing an understanding of the scope, mechanism, and synthetic application of the reaction.

---

(20) Semmelhack, M. F.; Hall, H. T.; Yoshifuji, M.; Clark, G. *J. Am. Chem. Soc.* **1975**, *97*, 1247.

(21) Semmelhack, M. F.; Thebtaranonth, Y.; Keller, L. *J. Am. Chem. Soc.* **1977**, *99*, 959.

(22) Hoff, C. D. *J. Organomet. Chem.* **1983**, *246*, C53-C56.

(23) Presumably carbon monoxide and Cr^{+3} salts, respectively.

(24) A Varian 5000 series HPLC was used; solvent, hexane, methylene chloride; column, micropak CN-10, alkyl nitrile (Varian Co.); flow, 1 mL/min, UV detector. Relative response factors: toluene $Cr(CO)_3$, 1.0; *p*-ylene $Cr(CO)_3$, 0.96; benzene $Cr(CO)_3$, 0.92; mesitylene $Cr(CO)_3$, 0.70.

(25) We thank D. W. Grandy for running this experiment.

(26) Milligan, S. N.; Tucker, I.; Rieke, R. D. *Inorg. Chem.* **1983**, *22*, 987-989.

(27) Degrand, C.; Radecki-Sudre, A.; Besancon, J. *Organometallics* **1982**, *1*, 1311-1315.

(28) We are grateful to Dr. J. Solash and Dr. J. E. Lester for assistance in running electrochemical experiments.

(29) We thank Dr. T. V. Harris for running this experiment. See supplementary material.

(30) Zimmerman, C. L.; Sandra, S. L.; Roth, S. A.; Willeford, B. R. *J. Chem. Res., Synop.* **1980**, 108; *J. Chem. Res. Miniprint* **1980**, 1289-1297.

(31) Muetterties, E. L.; Blecke, J. R.; Wucherer, E. J.; Albright, T. A. *Chem. Rev.* **1982**, *82*, 499-525.

(32) Albright, T. A.; Hofmann, P. Hoffmann, R.; Lillya, C. P.; Dobosh, P. A. *J. Am. Chem. Soc.* **1983**, *105*, 3396-3411.

Acknowledgment. We acknowledge the technical expertise of T. Tabacchi and D. Malecki. We also thank Dr. M. F. Semmelhack, Dr. D. C. Hrncir, Dr. T. V. Harris, and Dr. W. R. Pretzer for helpful discussion.

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

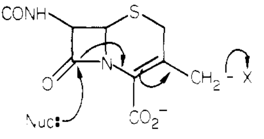
W. Stephen Faraci and R. F. Pratt*

Department of Chemistry, Wesleyan University
Middletown, Connecticut 06457

Received October 19, 1983

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 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.



4

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

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

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

---

(1) Gorman, M.; Ryan, C. W. In "Cephalosporins and Penicillins"; Flynn, E. H., Ed.; Academic Press: New York, 1972; pp 544–569.

(2) Sassiver, M. L.; Lewis, A. In "Structure-Activity Relationships among the Semisynthetic Antibiotics"; Perlman, D., Ed.; Academic Press: New York, 1977; pp 87–160.

(3) Boyd, D. B.; Herron, D. K.; Lunn, W. H. W.; Spitzer, W. A. *J. Am. Chem. Soc.* **1980**, *102*, 1812–1814.

(4) Boyd, D. B. In "Chemistry and Biology of β-Lactam Antibiotics, Vol. 1"; Morin, R. B., Gorman, M., Eds.; Academic Press: New York, 1982; pp 437–545.

(5) Sabath, L. D.; Jago, M.; Abraham, E. P. *Biochem. J.* **1965**, *96*, 739–751.

(6) Newton, G. G. F.; Abraham, E. P.; Kuwabara, S. *Antimicrob. Agents Chemother.* **1968**, 449–455.

(7) Hamilton-Miller, J. M. T.; Newton, G. G. F.; Abraham, E. P. *Biochem. J.* **1970**, *116*, 371–384.

(8) Hamilton-Miller, J. M. T.; Richards, E.; Abraham, E. P. *Biochem. J.* **1970**, *116*, 385–395.

(9) O'Callaghan, C. H.; Kirby, S. M.; Morris, A.; Waller, R. E.; Duncombe, R. E. *J. Bacteriol.* **1972**, *110*, 988–991.

(10) Waller, R. E. *Analyst* **1973**, *98*, 535–541.

(11) Bundgaard, H. *Arch. Pharm. Chemi, Sci. Ed.* **1975**, *3*, 94–123.

(12) Boyd, D. B.; Hermann, R. B.; Presti, D. E.; Marsh, M. M. *J. Med. Chem.* **1975**, *18*, 408–417.

(13) Boyd, D. B.; Lunn, W. H. W. *J. Med. Chem.* **1979**, *22*, 778–784.

(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.

(17) Schindler, P.; Huber, G. In "Enzyme Inhibitors"; Brodbeck, U., Ed.; Verlag Chemie: Weinheim, 1980; pp 169–176. Jones, R. N.; Wilson, H. W.; Novick, W. J. Jr. *J. Clin. Microbiol.* **1982**, *15*, 677–683.

(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.