

Figure 1. Electronic absorption spectra of **9–11** recorded in CH_2Cl_2 , $T = 293 \text{ K}$, $d = 1 \text{ cm}$; $c = 1.4 \times 10^{-5} \text{ M}$ for the complete spectra of **9** (---), **10** (—), and **11** (---); $c = 1.7 \times 10^{-4} \text{ M}$ for the long wavelength absorptions of **10** (---).

octadehydro[24]annulene **13**¹⁴ are very unstable in the solid state, the crystalline compounds **9**, **11**, and, especially, **10** are kinetically quite stable and can be kept for weeks at room temperature and ambient atmosphere without noticeable decomposition.

The electronic absorption spectra of **9–11** (Figure 1) provide valuable information on the geometries of the cyclobutenoannulenes. The spectrum of **9** closely resembles the spectra of **12**¹² and a new hexadehydro[18]annulene for which the planar annulene perimeter was shown by X-ray analysis.^{3,15} The strong absorptions at $\lambda_{\text{max}}(\text{nm}) = 314$ (ϵ 41 000), 332 (75 600), 356 (142 200) in the spectrum of **10** show bathochromic shifts as compared to **9**. In addition, weaker absorptions (ϵ between 3500 and 6500) are visible at $\lambda = 400\text{--}550 \text{ nm}$ which account for the orange-red color of **10**. From the shape similarity of the absorptions of **9** and **10** in the region between $\lambda = 300$ and 400 nm , we conclude that **10** also possesses a planar, conformationally rather rigid annulene perimeter.¹⁶ The spectrum of **10** differs strikingly from the one described for **13**.¹⁴ The longest wavelength absorption of **13** is reported at $\lambda_{\text{max}} = 352 \text{ nm}$ ($\epsilon = 45\,100$), and all bands have extinction coefficients below $\epsilon = 50\,000$. On the basis of its electronic absorption spectrum and the absence of paratropicity in the ¹H NMR spectrum, a nonplanar, cyclooctatetraene-like structure was postulated for **13**. The spectrum of pentamer **11** resembles in its shape the one reported for the tetramer **13**. We take the low extinction coefficients, the considerably reduced vibrational structure, and the lack of long wavelength transitions as strong evidence for a nonplanar, conformationally flexible chromophore in **11**.

Although the ethylene ketal protons in **9–11** are at a remote distance of the π -systems, their ¹H NMR resonances provide additional evidence for planar annulene perimeters in **9** and **10**. The comparison of the centers of the AA'BB' multiplets for the ethylene ketal protons in the spectra of **8** (δ 4.04), **9** (δ 4.27), **10** (δ 3.95), and **11** (δ 4.07) indicates that **9** has a diatropic and **10** a paratropic character.

Preliminary computational studies (AM1)¹⁷ show that the peculiar stereochemistry of the 1,2-diethynyl-1-cyclobutene unit

defines the unique properties of the cyclobutenoannulenes.¹⁸ The monomer *cis*-3-hexene-1,5-diyne with a C(2)–C(3)–C(4) angle of 125.4° is preferentially incorporated into the planar trimer **12**. The accommodation into a planar tetramer **13** generates angle strain, and a cyclooctatetraene-like conformation is preferred. At 136.3°, the C(1')–C(1)–C(2) angle in **8** is considerably larger. The incorporation of **8** into the planar trimer **9** generates angle strain. On the other hand, **8** can be accommodated in a nearly strain-free way into the planar tetramer **10**, which is of lower energy than a nonplanar, cyclooctatetraene-like conformer.

The use of the cyclobutenoannulenes **9–11** for the generation of ketones **1–3** and the corresponding cyclo[*n*]carbons is now under way.

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Conformational Changes in the Inactivation of β -Lactamase by Penicillin Sulfones

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Mechanism-based inhibitors of enzyme are a source of potential new drugs. We wish to point out that even though there may be a good *chemical* rationale for inhibition in such cases, physical (conformational) processes may play a critical role, especially if the inhibitor or enzyme are conformationally mobile. For example, the inactivation of β -lactamase by penicillin sulfones with large hydrophobic side chains (Type A substrates¹) is accompanied by significant change in the protein conformation, as determined by circular dichroism (CD), whereas no such change is observed with penicillanic acid sulfone, which lacks the C6 side chain. We believe it is the conformational change which is responsible for the observed irreversible inhibition at high pH.

The sulfones of several penicillins have been shown to be inhibitors^{3–12} of β -lactamases; the inhibition appears to be a form of suicide inactivation in which the lability of the C–S bond results in the formation of a transient imine acyl-enzyme intermediate (**1a**) which tautomerizes to the more stable enamine form (**1b**),⁸ a β -aminoacrylate of reduced hydrolytic sensitivity. The inhibitory reaction has been studied in the most detail with penicillanic acid sulfone (**2**), especially by Knowles and co-workers^{5–8} using the TEM β -lactamase. They conclude that the acyl-enzyme can undergo three fates: hydrolysis leading to turnover, conversion to the enamine leading to transient inhibition, and transimination by a suitably positioned lysine residue in the active site leading

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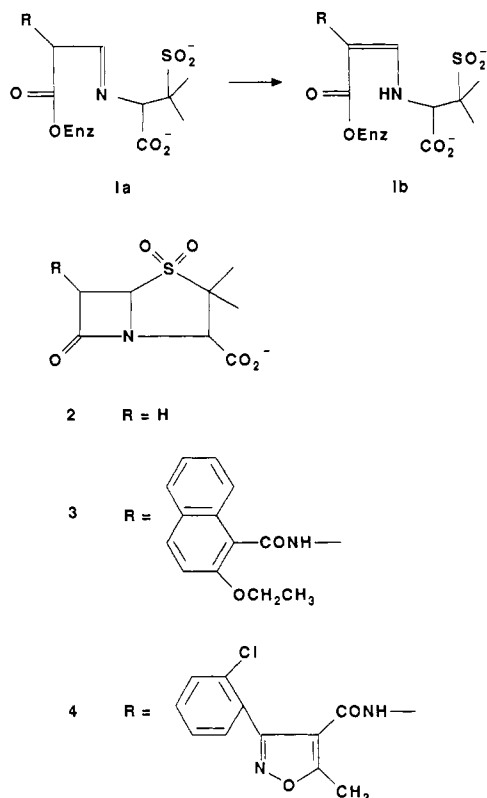
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to irreversible inactivation. No evidence for a conformational change was reported, and the proposed mechanism provides a reasonable chemical rationale for the inactivation.



In contrast, Dmitrienko et al.¹¹ have reported that inactivation of *B. cereus* β -lactamase I by 6- β -(trifluoromethanesulfonyl)-amidopenicillanic acid sulfone is not accompanied by transimination and leads to a species which differs from the native enzyme in terms of its thermostability and ORD spectrum, suggesting a non-native conformation in the inactivated enzyme.

β -Lactamase I from *Bacillus cereus*¹³ (strain 569/H/9) and the sulfones of nafcillin¹⁴ (3), cloxacillin¹⁴ (4), and penicillanic acid¹⁶ were used. Sulfones 3 and 4 are derived from Type A substrates.^{1,17}

β -Lactamase (5 μ M) was inactivated at pH 9.0 (30 °C or 37 °C) with 3 or 4, with [S]/[E] = 20:1 and 100:1. Inactivation was monitored by removing aliquots and diluting them into a benzylpenicillin assay. Essentially complete inactivation occurs in 2 h under these conditions. The inactivated enzyme is stable for long periods (days at 4 °C) at alkaline pH but can be completely reactivated on acidifying the solution to pH < 7. The far-UV circular dichroism spectra of β -lactamase inactivated by 3 and the native enzyme are shown in Figure 1A. Inactivation leads to spectral changes consistent with the loss of α -helix (8% by deconvolution¹⁸); reactivation leads to the reversal of these changes. Similar changes in the far-UV CD are observed for inactivation and reactivation with 4. Under the experimental conditions the contribution of 3 or 4 or their products to the far-UV circular dichroism spectrum is negligible. We therefore conclude that inactivation by the sulfones of Type A substrates leads to a change in the secondary structure of the enzyme.

The kinetics of the inactivation reaction, as measured by loss of activity toward benzylpenicillin, were compared with those for

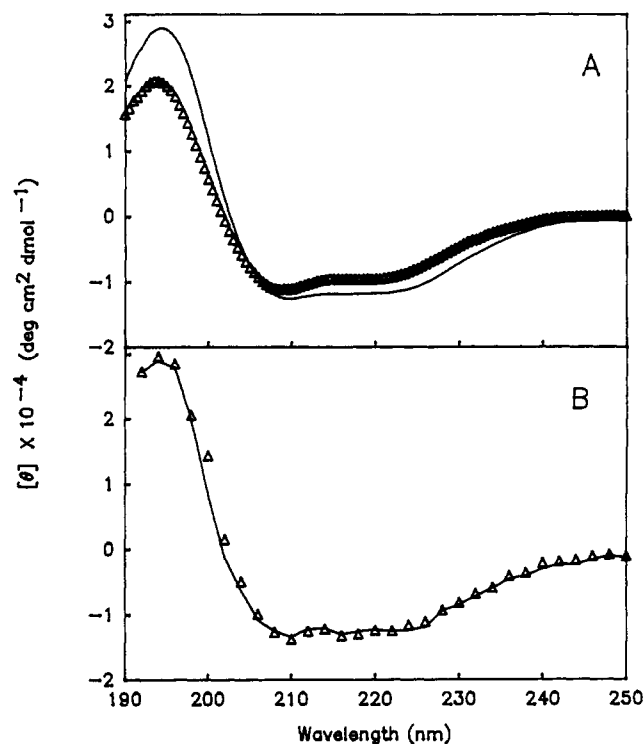


Figure 1. The far-UV circular dichroism spectra of native and sulfone-inactivated β -lactamase. A. Native (solid line) and nafcillin sulfone-inactivated enzyme (Δ). Conditions were pH 9.0, 4 °C, [E] = 5.7 μ M, [S] = 120 μ M. B. Native (solid line) and penicillanic acid sulfone-inactivated enzyme (Δ). Conditions were pH 8.9, 4 °C, [E] = 5.7 μ M. The inactivation was carried out at 30 °C with [penicillanic acid sulfone] = 8.6 mM.

the change in ellipticity at 196 nm in an experiment with 3 using 5.7 μ M enzyme (the rates are proportional to the enzyme concentration), [S]/[E] = 20:1, 36.8 °C. The observed first-order rate constants were identical within experimental error, namely 6.9×10^{-4} and 7.3×10^{-4} s⁻¹, respectively. Thus under these experimental conditions the conformational change and the inactivation process occur simultaneously.

In contrast to the case of penam sulfones derived from Type A substrates which give *stable* inactivated enzyme at high pH, the penicillanic acid sulfone-inactivated enzyme was *only transiently* inactivated at high pH (even at [S]/[E] of several 1000). β -Lactamase (5.6 μ M) was inactivated with 2 at pH 8.9 with [S]/[E] = 3000:1.¹⁹ Lowering the temperature to 4 °C reduced the rate of reactivation to 3.3×10^{-4} s⁻¹. The large concentration of sulfone necessitated its removal prior to collecting the circular dichroism data.²⁰ Spectra were collected at pH 8.9 (at 4 °C) at frequent time periods during the reactivation process, which was monitored simultaneously. As shown in Figure 1B the spectrum of the inactivated enzyme was identical with that of the native enzyme. Therefore, for penicillanic acid sulfone-inactivated enzyme there was no significant change in the secondary structure due to inactivation.

These observations illustrate that there are differences in the mechanism of inactivation of penam sulfones due to the nature of the side chain on C6. In addition it appears that the combi-

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(20) This was accomplished by using two passages through a centrifugal mini ion-exchange column.²¹ The eluant was pH 8.9 pyrophosphate buffer, either 0.05 or 0.3 M in sodium sulfate; the column packing was DEAE-Sephadex. The first column used 5 mL of packing, the second 2 mL. The separation was done at 1 °C. Due to the significant rate of reactivation under these conditions some 20–25% of the inactivated enzyme had reactivated by the time the first spectrum was collected.

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nation of the flexibility introduced into the inhibitor by conversion from a fused bicyclic ring system to an acyclic one in the enamine form of the acyl-enzyme as well as the inherent mobility of the enzyme¹⁷ leads to a significant conformational change on formation of the enamine acyl-enzyme. On the basis of the behavior of enzyme inactivated by **2**, which lacks a side chain on C6 and shows neither the change in secondary structure nor stability at alkaline pH, we believe it is the conformational change, rather than the slow hydrolysis of the β -aminoacrylate, which leads to the irreversible inactivation at alkaline pH by the sulfones of Type A substrates. This conformational change results in displacement of the catalytic groups with respect to the acyl-enzyme bond such that catalysis of deacylation is prevented.

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Methylchlorocarbene: Measurement of Rate Constants for 1,2-Hydrogen Migration and Addition to Alkenes

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There have been no reports so far of the intermolecular capture of dialkylcarbenes, probably because of the ease of intramolecular 1,2-H shift. Moss and Mamantov¹ demonstrated that the presence of the chlorine atom stabilizes methylchlorocarbene (**2**) generated from photolysis of 3-chloro-3-methyldiazirine (**1**) enabling the intermolecular addition to alkenes to compete with the intramolecular 1,2-H shift of the carbene to form vinyl chloride. Absolute rate constants for the addition of a variety of substrates to a number of carbenes generated from arylhalodiazirines have been measured² by laser flash photolysis (LFP). For alkylchlorocarbenes, similar measurements have not been reported until recently,³ mainly because the alkylchlorocarbenes do not absorb in a region where they can be monitored. However, the "pyridine probe technique"³ renders the methylchlorocarbene visible. We now report the rate constant for 1,2-H migration in **2**, as well as the rate constants for the reaction of **2** with various olefins. These results indicate an usually slow H migration and the ambiphilic character of this carbene.

3-Chloro-3-methyldiazirine was prepared by Graham's method⁴ and the LFP setup has been described previously.⁵ LFP of **1** (≈ 5 mM in isoctane at 25 °C) in the presence of pyridine produces a transient absorption ($\lambda_{\max} = 360$ nm) that is not observed in the absence of pyridine and is attributed to the pyridinium ylide, **3**. The rate constants for 1,2-H migration, k_i , and for ylide formation, k_y , are determined by measuring, at 360 nm, the rate of growth of the ylide absorption, $k_{\text{growth}} = k_i + k_y[\text{pyridine}]$, as a function of [pyridine]. Least-squares analysis of ten measurements for [pyridine] ranging from 0.2 to 6 mM gives

$$k_y = 8.86 \pm 0.10 \times 10^9 \text{ M}^{-1} \text{ s}^{-1} \text{ and}$$

$$k_i = 3.04 \pm 0.05 \times 10^6 \text{ s}^{-1}$$

When a constant amount of pyridine (0.45 mM) is used, the addition of alkene substrates such as tetramethylethylene (TME)

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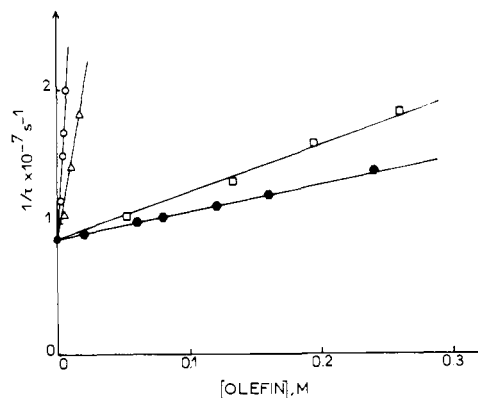


Figure 1. Plot of the pseudo-first-order rate constants for the growth of the ylide ($1/\tau$) vs [olefin], at 25 °C in isoctane with [pyridine] = 0.45 mM: TME (○); HEX (●); ACN (□); CIACN (△).

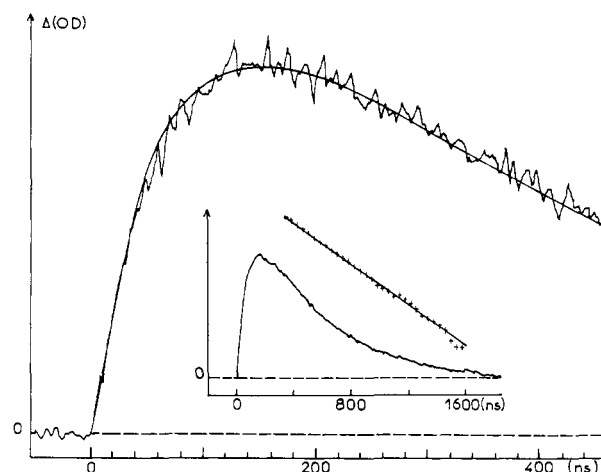


Figure 2. Growth and decay of the ylide absorption with [pyridine] = 0.45 mM and [CIACN] = 12.4 mM at 50 ns/div sweep rate. The solid line is the theoretical curve calculated with eq 1 with $\tau_1 = 70$ ns and $\tau_2 = 400$ ns. Inset: same conditions, at 200 ns/div sweep rate, for determining τ_2 .

and 1-hexene (HEX) decreases the yield of ylide formation because the cycloaddition of **2** to the alkene competes with ylide formation and increases the rate of growth of **3** which becomes $k_{\text{growth}} = k_i + k_y[\text{pyridine}] + k_c[\text{olefin}]$. Excellent linearity was obtained for k_{growth} vs [TME] and [HEX]. The slopes of the plots shown in Figure 1 give the following values for the rate constant, k_c , for addition of **2** to TME and HEX respectively

$$1.32 \pm 0.06 \times 10^9 \text{ and } 2.10 \pm 0.05 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$$

With α -chloroacrylonitrile (CIACN) and acrylonitrile (ACN), the determination of k_c is not simple because the ylide **3** is also quenched by the nitriles, most probably to give the corresponding indolizines as it has been demonstrated in the case of phenylchlorocarbene.⁶ Then, the relevant mechanistic scheme is

