

Table I. Decomposition of 6-Nitrobenzoxazole-3-carboxylate in Water in the Presence of Poly(vinylbenzo-18-crown-6) (concentration of carboxylate $2 \times 10^{-4} M$; $T = 25^\circ C$)

| Ratio of crown to carboxylate | Ratio of salt to carboxylate | $10^5 k, \text{sec}^{-1}$ |
|-------------------------------|------------------------------|---------------------------|
| 0 | — | 0.4 |
| 2 | — | 11 |
| 2 | 10:1 NaCl | 16 |
| 2 | 10:1 KCl | 45 |
| 5 | — | 45 |
| 20 | — | 111 |
| 50 | — | 198 |

zene, acetone, and dimethyl sulfoxide. We observed that the decomposition of this carboxylate is significantly accelerated by addition of P18C6. The reaction is first order up to at least 80% decomposition. The pertinent rate constants are given in Table I. The carboxylate, on binding to P18C6 apparently is partially dehydrated causing the acceleration. The binding is weaker than that found for picrates, since the rate constant continues to go up even at concentrations of P18C6 as high as 0.05 M.

At a crown to carboxylate ratio of 50 the first-order rate constant is of the same order of magnitude as that found in benzene (using tetramethylguanidine as base the value in benzene was found to be $4.8 \times 10^{-3} \text{sec}^{-1}$, see ref 8). Complexable cations will further enhance the rate, as shown in Table I. Addition of KCl converts the poly(crown ether) into a polycation, resulting in a significant rate enhancement as more carboxylate is now attracted electrostatically to the polymer domain. Binding of Na^+ to P18C6 is known to be weaker than that of K^+ , hence the effect of added NaCl is less pronounced. The behavior of P18C6 in some respects resembles the micellar catalysis of this decarboxylation reaction with cationic soap⁹ although in the latter case electrostatic effects play a more important role and the effects of added electrolyte are different. Other types of reactions are presently being investigated, including the catalytic behavior of copolymers containing crown moieties.

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References and Notes

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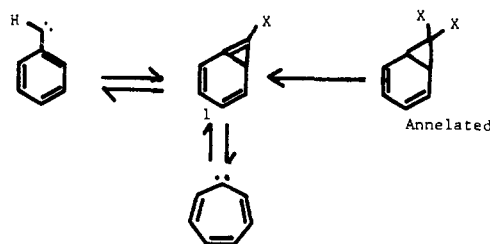
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Formation of a Bicyclo[4.1.0]heptatriene by Intramolecular Addition of a Carbene to a Carbon-Carbon Triple Bond¹

Sir:

Bicycloheptatrienes (1), tautomers of benzocyclopropenes, have been postulated as intermediates in both the interconversion of aryl and aromatic carbenes² and the base induced dehydrohalogenation of gem-dihalocyclopropanes.³ Al-

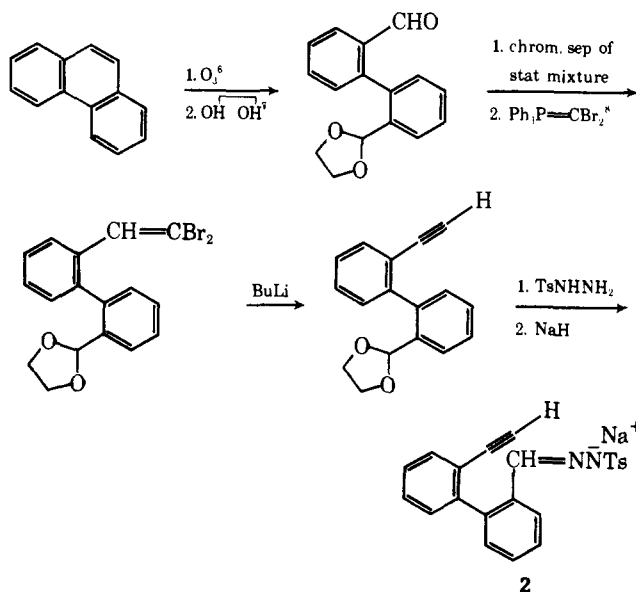


though to date no direct evidence has been obtained for the parent hydrocarbon—although it is probably an intermediate in the interconversion of phenylcarbene and cycloheptatrienylidene⁴—there has been reported rather convincing evidence for its intermediacy in both mono- and dibenzannelated systems.² At this time, we would like to report evidence for the generation of dibenzbicycloheptatriene 7 from the intramolecular addition of a carbene to a carbon-carbon triple bond.

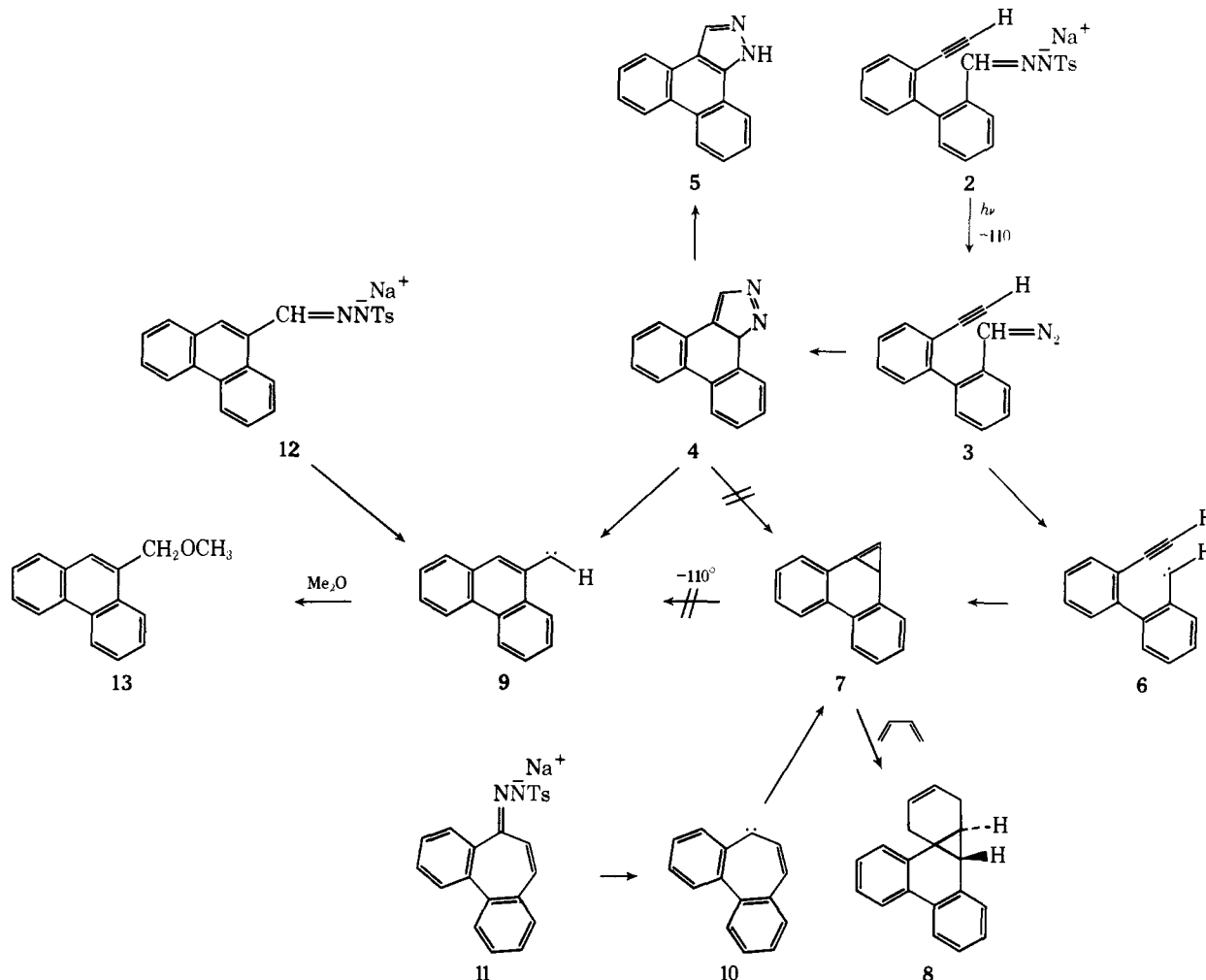
Schemes I and II describe the synthesis and chemistry of the carbene precursor 2.⁵ A study of the carbene that would result from nitrogen expulsion from the presumed diazoalkane 3 was severely complicated by the tendency of the diazoalkane to add to the triple bond to give the 1*H*-pyrazole 5. For example, pyrolysis of 2 in benzene at 125° gave 5 in essentially quantitative yield. It was found, however, that 1*H*-pyrazole formation could be modestly suppressed⁹ by lowering the temperature and, at -110° , photolysis of the tosylhydrazone salt 2 in a mixture of butadiene and dimethyl ether did, in fact, give a low but reproducible yield ($5 \pm 1\%$) of the adduct (8) expected from reaction of the bicycloheptatriene with the diene.^{11,12} Photolysis of the tropono tosylhydrazone salt¹³ 11 in the presence of butadiene gave the same adduct (30%), thus supporting the bicycloheptatriene as an intermediate in both reactions.

Photolysis of 2 at -110° also gave 11% of the ether 13¹⁴

Scheme I



Scheme II



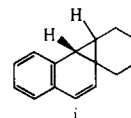
which was shown to also be the product (40% isolated) from photolysis of the tosylhydrazone salt **12** in dimethyl ether at -60° . Interestingly, at -110° in dimethyl ether, dibenzocycloheptatrienyliene **10** gave none of the phenanthrylcarbene product **13**. This apparent dichotomy is readily resolved by assuming: (1) the bicycloheptatriene **7** does not ring open to the arylcarbene at this low temperature (even at -60° the ring opening is almost completely eliminated) and (2) under the reaction conditions, *3H*-pyrazole **4** loses nitrogen to give *9H*-phenanthrylcarbene.¹⁶ Support for the latter assumption was obtained by photolyzing **2** at -110° in the presence of a base that could divert the *3H*-pyrazole to its isomeric and photostable *1H*-isomer **5**.¹⁷ In the presence of triethylamine, the yield of phenanthrylcarbene product **13** was reduced from 11% to less than 1%. Under the same conditions, however, the yield of the bicycloheptatriene adduct **8** was essentially unaffected thus excluding photolysis of *3H*-pyrazole **4** as the precursor to **7**.

In summary, it is concluded that at -110° in dimethyl ether and 1,3-butadiene, photolysis of **2** gives the diazoalkane which partitions to the *3H*-pyrazole **4** and the carbene **6**. The latter cyclizes to give the dibenzocycloheptatriene **7** which can be trapped with butadiene. The *3H*-pyrazole tautomerizes to its *1H*-isomer in competition with loss of nitrogen to give *9H*-phenanthrylcarbene. This partitioning can be diverted to the *1H*-pyrazole with triethylamine.

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- (11) The structure of **8** was assigned on the basis of its high resolution MS (calcd *m/e* 244.1251; found *m/e* 244.1247), its ir ((neat): 3068, 3028, 2925, 2855, 1600, 1445, 1295, 1260, 1005, 890, 785, and 738 cm^{-1}), and its NMR (CDCl_3): δ 8.10–7.0 (m, 8 H), 5.62 (str. d, 2 H), 2.55 (m, 5 H), and 0.7–0.4 (m, 1 H). The NMR was particularly important in assignment of structure in its similarity to previously characterized² compounds. For example, although the high-field cyclopropane proton of **8**



appeared at slightly lower field than the corresponding hydrogen in **i**, the multiplet patterns were nearly superimposable and coupling constants agreed well. In addition, the unconjugated vinyl hydrogens of both com-

pounds appeared at identical chemical shift although those of **8** were more of a structured doublet than a multiplet. Butadiene was used as the trap because other dienes (e.g., cyclopentadiene) freeze at -110° .

- (12) No adduct was observed at -60° .
 (13) Prepared by T. T. Coburn to whom the authors express grateful appreciation.
 (14) This surprising product finds ample precedent in the reaction of other carbenes with methyl ethers although the fate of the displaced CH_2 is not known.¹⁵
 (15) Cf. W. Kirmse, "Carbene Chemistry", 2nd ed, Academic Press, New York, N.Y., 1971.
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The Mechanism of Carbonic Anhydrase Studied by ^{13}C and ^{18}O Labeling of Carbon Dioxide

Sir:

The very rapid rate of hydration of CO_2 catalyzed by carbonic anhydrase (EC 4.2.1.1) has been explained by the hypothesis that there is a proton transfer step in the mechanism involving the enzyme and buffers in solution.¹⁻³ Supporting this explanation, a buffer dependence of the carbonic anhydrase-catalyzed exchange of oxygen-18 between bicarbonate and water at equilibrium and alkaline pH has been reported.⁴ We present here results which further support this hypothesis by measuring the buffer dependence of the catalysis by bovine carbonic anhydrase of two types of ^{18}O exchange involving species of CO_2 and water. Type I: the ^{18}O exchange between bicarbonate and water^{5,6} has been measured near neutral pH where the enzyme is most active. Type II: the exchange of ^{18}O between species of CO_2 in solution, measured as the exchange of ^{18}O between ^{12}C - and ^{13}C -containing molecules,^{7,8} has been measured in the same pH region.

The atom fraction of ^{18}O in carbon dioxide, α , includes all ^{12}C - and ^{13}C -containing molecules. However, we designate as $^{(12)}\alpha$ the atom fraction of ^{18}O in ^{12}C -containing CO_2 only.⁸ In type I exchange, the decay of $(\alpha - \alpha_\infty)$ is a first-order process with the following rate constant:⁴

$$\theta_1 = \theta_{\text{cat}} + \theta_{\text{uncat}} = (f_{\text{HCO}_3^-})(k_c + k_{\text{uncat}})/3$$

where k_{uncat} is the rate constant for the uncatalyzed dehydration of HCO_3^- and k_c is the first-order rate constant describing the catalyzed dehydration of HCO_3^- at equilibrium. The fraction of all CO_2 species existing as bicarbonate is $f_{\text{HCO}_3^-}$. The kinetic equations describing type II exchange have been derived.⁸ The first-order rate constant ϕ ($\phi = \phi_{\text{cat}} + \phi_{\text{uncat}}$) describes the exchange of ^{18}O between ^{12}C - and ^{13}C -containing species of CO_2 in solution. The sum $(\theta_1 + \phi)$ is obtained as the slope of a plot of $-\ln(^{(12)}\alpha - \alpha)$ vs. time.⁸

The preparation of ^{18}O - and ^{13}C -enriched bicarbonate, as well as bovine carbonic anhydrase, is identical with that described earlier.^{4,8} The isotopic enrichments were measured on a Finnigan 3000 mass spectrometer and monitored continuously using a CO_2 inlet system which allows CO_2 to pass across a membrane in contact with the reaction solution.⁸ All solutions were $1.6 \times 10^{-9} M$ bovine carbonic anhydrase (BCA), 10 mM total CO_2 species, and were maintained at an ionic strength of 0.2 with Na_2SO_4 .

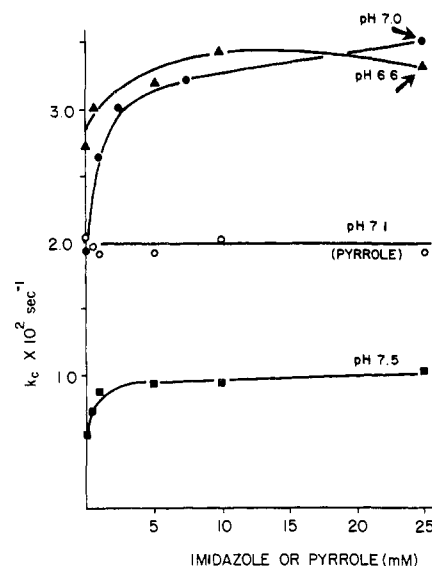


Figure 1. The first-order rate constant k_c at 25° for catalyzed dehydration of bicarbonate at equilibrium as a function of concentration of imidazole at pH 7.5 (■), pH 7.0 (●), and pH 6.6 (▲) or pyrrole (○) at pH 7.1. The concentration of bovine carbonic anhydrase was $1.6 \times 10^{-9} M$ and the total concentration of CO_2 species was 10 mM. Ionic strength was maintained at 0.2 with the noninhibitory Na_2SO_4 .

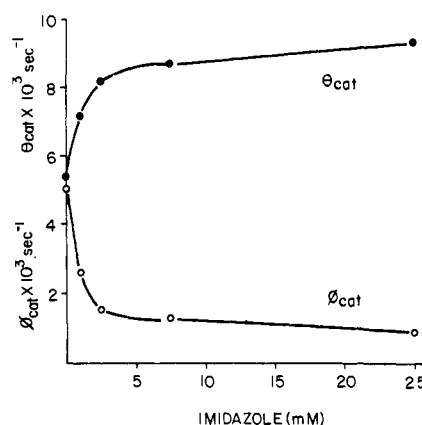


Figure 2. The first-order rate constants at 25° and pH 7.0 for the catalyzed exchange of ^{18}O between bicarbonate and water, θ_{cat} (●), and for the catalyzed exchange of ^{18}O between ^{12}C - and ^{13}C -containing CO_2 species, ϕ_{cat} (○), as a function of imidazole concentration. The concentration of bovine carbonic anhydrase was $1.6 \times 10^{-9} M$ and the total concentration of CO_2 species was 10 mM with ionic strength held constant at 0.2 using Na_2SO_4 . The ^{13}C enrichment was 44–46% in a final volume of 8 ml.

As in previous studies at alkaline pH,⁴ k_c near neutral pH is dependent on low concentrations of buffer (imidazole, as shown in Figure 1, *N*-methylmorpholine, and 2,4-lutidine), but becomes essentially invariable at higher buffer concentrations. This buffer dependence is not obtained using compounds similar to imidazole in structure but lacking a proton transfer capability, such as 1,3-dimethylimidazolium sulfate and pyrrole (shown in Figure 1). That k_c is relatively large even in the absence of added buffer may be attributed to the buffering capabilities of the substrate and enzyme themselves, and to the fact that alternating hydration–dehydration steps at equilibrium do not require proton transfer.

Figure 2 compares the effect of imidazole buffer at pH 7.0 on θ_{cat} and ϕ_{cat} . The rate constant for the uncatalyzed exchange, ϕ_{uncat} , is small ($\sim 6 \times 10^{-5} \text{ sec}^{-1}$) and independent of imidazole concentration under these conditions. A