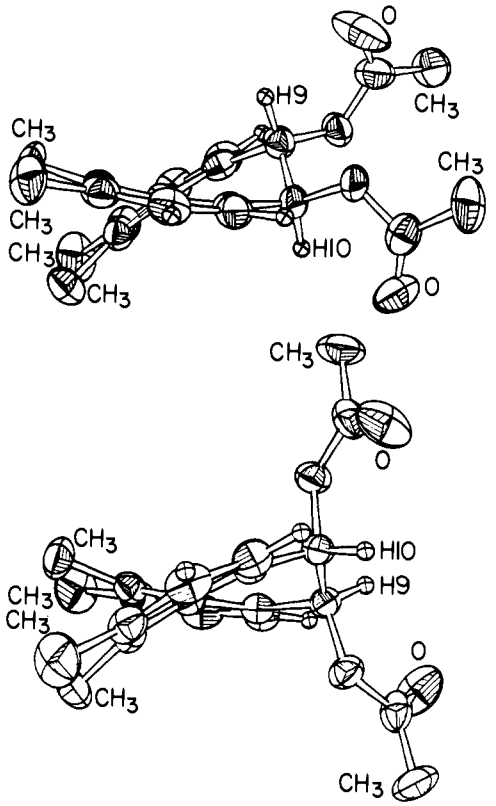

Figure 1. ORTEP representations of the diactate of **5E** (top) and **5A** (bottom) viewed down the biphenyl axis. Twist angle of the biphenyl is 42°. Final R ($\sum|F_o - F_c|\sum F_o$) values were 0.092 and 0.080 for **5E** + **6E** and **5A** + **6A**, respectively.

Table I. Kinetics of the Enzyme-Catalyzed Glucuronidation of the Six Stereoisomeric 3,4,5,6-Tetramethyl-9,10-dihydroxy-9,10-dihydrophenanthrenes[a]

substrate	$\Delta\epsilon_{dis}$,[b] M^{-1} cm^{-1}	abs config[c]	k_c, s^{-1}	k_c/K_{mapp}, M^{-1} s^{-1}
4P	+143	*R,S,P*	0.020[d]	
4M	−143	*R,S,M*	0.0058[d]	
5A	+144	*S,S,P*	<0.0005[d,e]	
6A	−143	*R,R,M*	0.0069[d]	
5E	−120	*S,S,M*	0.41 ± 0.01	980 ± 100
6E	+116	*R,R,P*	0.20 ± 0.004	910 ± 60

[a] Reactions were run as described in ref 2. [b] Circular dichroic extinction coefficients for the dissymmetry transition at 233 (**4M** and **4P**), 232 (**5A** and **6A**), and 230 nm (**5E** and **6E**) were determined in 2-propanol. [c] Designations *R* and *S* described absolute configuration of the carbinol carbons. *M* and *P* designate the helicity of the biphenyl axis. [d] Turnover numbers were estimated by using saturating 1.5–2.2 mM substrate concentrations. Reactions were too slow for accurate determination of k_c/K_{mapp} due to lability of the enzyme at 25 °C. [e] No product detected.

resolved by synthesis and resolution of the diastereomeric mono[(−)-α-methoxy-α-(trifluoromethyl)phenylacetates]. All pairs of enantiomers gave mirror image CD spectra (supplementary material, Figures 2–4) with an intense dissymmetry transition at ~232 nm characteristic of the 2,2′-bridged biphenyl chromophore. Absolute configurations of the six stereoisomers were determined from the sign of this transition (Table I), which reports the helicity of the biphenyl axis.[6,9] The conformers are quite stable at room temperature. For example the half-life for racemization of **4** is 2.0 years at 25 °C.[3]

Enzyme-catalyzed reactions of the six conformationally locked substrates were monitored by reversed-phase HPLC.[10] Three interesting observations can be made. First, only one product from each of the two *cis*-antipodes is evident by HPLC even at long reaction times, suggesting that only one of the two topochemically distinct carbinol groups of **4M** or **4P** is glucuronidated. Second, the trans diaxial stereoisomer **5A** is not a substrate for the enzyme. Finally, the most interesting feature of the enzyme-catalyzed reaction is the striking kinetic discrimination between the six stereoisomers by UDPglucuronosyltransferase (Table I). In particular, the trans diequatorial isomers **5E** and **6E** are turned over 30 to >800 times more rapidly than their corresponding conformational diastereomers with axial hydroxyl groups. It therefore seems likely that the preferred conformation of kinetically labile *trans*-dihydro diols such as **2** or **3** in a productive enzyme–substrate complex is that with diequatorial hydroxyl groups. In view of the conformer specificity of the enzyme toward the trans isomers it appears probable that only the equatorial hydroxyl group in **4M** and **4P** is recognized by the enzyme. This suggests that the two diasteromeric glucuronides formed from **1**[2] arise via reaction of the equatorial hydroxyl groups of the two conformational enantiomers **1M** and **1P** in the enzyme–substrate complex. Work is in progress to clarify this point.

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Supplementary Material Available: Description of synthesis and resolution of **4M**, **4P**, **5A**, **6A**, **5E**, and **6E**, complete NMR data for the racemic diacetates, circular dichroism spectra of all six stereoisomeric dihydro diols (Figures 2–4), and crystallographic data including atomic coordinates and temperature factors (29 pages). Ordering information is given on any current masthead page.

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(10) Retention times (min) for substrates and products: **4M** and **4P**, 42.6; **4M** glucuronide, 36.0; **4P** glucuronide, 36.5; **5A** and **6A**, 29.6; **6A** glucuronide, 17.6; **5E** and **6E**, 45.0; **5E** glucuronide, 42.2; **6E** glucuronide, 40.9, found by using a Rainin Microsorb C18 column (4.6 mm × 25 cm) eluted at 0.5 mL/min with 50% CH_3OH in 0.1 M acetic acid for 10 min then a gradient of 1%/min to 80% CH_3OH. Products were identified by their CD spectra.

Calculation of Substrate Dissociation Constants from Steady-State Isotope Effects in Enzyme-Catalyzed Reactions

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Isotopic probes of enzyme reactions provide a measure of the extent to which bond cleavage steps limit catalysis.[1-7] Further, when the magnitude of the intrinsic isotope effect on isolated bond

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Table I. Initial Rate Parameters and Isotope Effects Describing Eq 1

parameter	isotope effect	isotope effect, minus one
$k_{cat} = \frac{k_3k_5}{(k_3 + k_4)(1 + C_{vf} + C_r)}$ (2)	$^*k_{cat} = \frac{^*k_5 + C_{vf} + {}^*KC_r}{1 + C_{vf} + C_r}$	$(^*k_{cat} - 1) = \frac{(^*k_5 - 1) + (^*K - 1)C_r}{1 + C_{vf} + C_r}$ (4)
$k_{cat}/K_m = \frac{k_1k_3k_5}{k_2k_4(1 + C_f + C_r)}$ (3)	$^*(k_{cat}/K_m) = \frac{^*k_5 + C_f + {}^*KC_r}{1 + C_f + C_r}$	$[^*(k_{cat}/K_m) - 1] = \frac{(^*k_5 - 1) + (^*K - 1)C_r}{1 + C_f + C_r}$ (5)

cleavage step(s) is available, kinetic isotope effects can permit the calculation of microscopic rate and dissociation constants.[8-10] Recently, observed steady-state isotope effects on k_{cat} and k_{cat}/K_m have been shown to provide substrate dissociation constants without the requirement for knowledge of the intrinsic isotope effect, provided that the equilibrium isotope effect on the chemical step is small relative to the kinetic isotope effect.[10] In this paper, we show that this constraint is *not* a prerequisite for the calculation of K_d values, greatly expanding the scope of enzyme reactions that will permit direct calculation of K_d from steady-state data.

Consider the single-substrate, two-product enzyme reaction, characterized by two preisotopic and one postisotopic enzyme complexes, eq 1, where k_5 and k_6 are the isotopically sensitive steps.

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E{\cdot}S \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E{\cdot}S' \underset{k_6}{\overset{k_5}{\rightleftharpoons}} E{\cdot}P_1{\cdot}P_2 \xrightarrow{k_7} E{\cdot}P_1 + P_2 \xrightarrow{k_9} E + P_1 \quad (1)$$

The expressions for the steady-state parameters, k_{cat} and k_{cat}/K_m, and isotope effects on these parameters, $^*k_{cat}$ and $^*k_{cat}/K_m$, are given in Table I, where $^*k_5$ and $^*K$ are the intrinsic kinetic and equilibrium isotope effects for the conversion of E·S′ to E·P_1·P_2 and C_f, C_{vf}, and C_r are the forward and reverse commitment factors as described by Cleland.[5] As originally noted by Northrop,[11] subtraction of one from both sides of the isotope effect equations allows reduction of these expressions, leading to eq 4 and 5 in Table I. Note that this algebraic rearrangement yields equations *that contain the same term in their numerators. Hence, division of* $(^*k_{cat} - 1)$ *by* $[^*(k_{cat}/K_m) - 1]$ *leads to cancellation of both the kinetic,* $^*k_5$, *and equilibrium,* $^*K$, *effects.* Combining eq 2–5 in Table I we obtain

$$\frac{(^*k_{cat} - 1)}{[^*(k_{cat}/K_m) - 1]} = \frac{1 + C_f + C_r}{1 + C_{vf} + C_r} = K_m/K_d \quad (6)$$

where

$$K_d = \frac{k_2k_4}{k_1(k_3 + k_4)} \quad (7)$$

i.e., where K_d represents the dissociation constant of substrate from all preisotopic complexes. Where there is only one preisotopic E·S complex or where additional preisotopic complexes are present at low concentrations relative to E·S in the steady state, $K_d = k_2/k_1$.

The above expression is a general one and will apply to any enzyme reaction characterized by nonunitary values for $^*k_{cat}$ and $^*(k_{cat}/K_m)$, with the exception of reactions in which more than one isotopically sensitive step occurs.[13] As long as experimental error can be minimized, measurements of heavy atom and secondary hydrogen isotope effects can yield K_d. However, as a consequence of the small magnitude of these effects, they are frequently measured by competitive methods, which provide $^*(k_{cat}/K_m)$ but not $^*k_{cat}$. Thus, it is expected that eq 6 will see greatest application in the measurement of primary hydrogen isotope effects in enzyme-catalyzed redox and proton abstraction reactions.

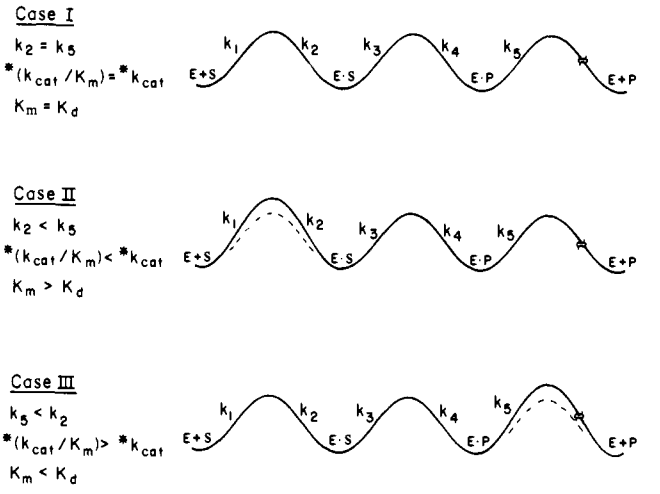

Figure 1. Free energy profiles for a single-substrate, single-product enzyme reaction, illustrating the interplay between eq 6 and the nature of rate determining step(s) in an enzyme reaction. Under initial rate conditions, the loss of product is assumed to be irreversible, indicated by a break in free energy profiles.

While it is recognized that a kinetically obtained K_m value will, in general, be different from the substrate dissociation constant, K_m values continue to be used as estimates for K_d values in the literature. The large discrepancy that can be expected between K_d and K_m can be estimated from eq 6. *Only in the special instance that* $^*k_{cat} = {}^*(k_{cat}/K_m)$ *will* $K_m = K_d$. The interplay between eq 6 and the rate determining step(s) in an enzyme reaction is illustrated in Figure 1 for a single-substrate, single-product reaction, where $C_r + C_{vf} = k_4/k_5 + k_3/k_5$ and $C_r + C_f = k_4/k_5 + k_3/k_2$. Note that C_r is common to both expressions and the relationship of K_m to K_d is determined by the partitioning ratios k_3/k_5 and k_3/k_2 or, following cancellation of k_3, k_2/k_5. Hence, with the exception of reactions in which the observed isotope effects approach the intrinsic value[14] or unity,[15] K_m/K_d reflects the relative barrier heights for the conversion of central complexes to either E + S (k_2) or E + P (k_5). For a symmetrical enzyme reaction, i.e., one in which $k_2 = k_5$, $^*k_{cat} = {}^*(k_{cat}/K_m)$ and $K_m = K_d$ (case I, Figure 1). Deviations from this symmetry can lead to a marked increase (case II, Figure 1) or reduction (case III, Figure 1) in K_m relative to K_d.

In conclusion, we also note that the availability of substrate dissociation constants from steady-state data should facilitate an analysis of the energetics of substrate binding and the role of substrate binding energy in catalysis. For reversible reactions in which K_d values can be calculated for both the forward and reverse directions, the magnitude of the equilibrium constant associated

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(13) It should be noted that exchange of the isotopically substituted atom with solvent prior to release of the first product may alter the form of eq 4 and 5; however, eq 6 remains valid.
(14) In this instance, the chemical step limits k_{cat} and k_{cat}/K_m, all commitment factors are zero and K_m equals K_d.
(15) Very small isotope effects can result from $k_4/k_5 > 1$ and hence obscure differences between k_2 and k_5.

with the isotopically sensitive step (K_{int}) can also now be evaluated readily.[16]

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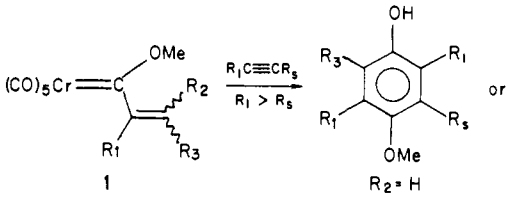
(16) Where multiple preisotopic complexes occur in a reversible reaction and contribute more significantly to the steady-state composition in one direction, the calculated value for K_{int} will deviate from a value measured directly. For example, in the mechanism described by eq 1

$$K_{int}(calcd) = K_{int}/(1 + k_4/k_3)$$



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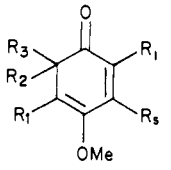

3

Two-Alkyne Annulations of Transition-Metal Carbene Complexes via in Situ Generated Vinyl Carbene Complexes[1]

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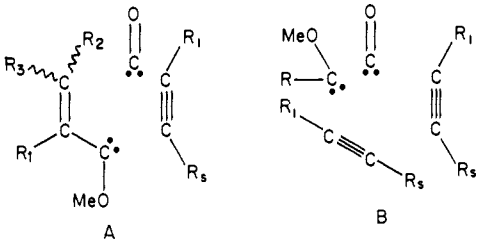
Among the many transition-metal-mediated six-membered ring syntheses,[2] the benzannulation of vinyl carbene complexes of chromium with alkynes is quite novel and still relatively unexplored.[3,4] This annulation involves the overall incorporation of an acetylene, a carbon monoxide ligand, the carbene carbon, and its vinyl substituent as indicated schematically by A. Depending



on the substitution pattern of the vinyl group in complex **1** the ultimate annulation products can be either cyclohexadienones or phenols[3c] and have recently been employed as intermediates in natural product synthesis.[3]

We wish to describe the first examples of a new type of six-membered annulation (indicated schematically by B) of transition-metal carbene complexes which involves the incorporation of 2 equiv of an acetylene, a carbon monoxide ligand, and the carbene carbon. This new annulation is based upon the premise that the in situ generated tetracarbonyl vinyl carbene complex **8** (Scheme I) will react with an acetylene in the same manner as the pentacarbonyl vinyl carbene complex **1** and give the cyclohexadienone **10**. On the basis of the proposed[4d,5] mechanism for the annulation of the pentacarbonyl vinyl carbene complexes **1**, the coordinatively unsaturated vinyl carbene complex **8** is envisioned to form from the methyl complex **4** via dissociation of a carbon monoxide ligand, followed by reaction with an alkyne to give the metallacyclobutene **7** and electrocyclic ring opening. On this same basis, the vinyl carbene complex **8** could be expected to react with an acetylene to give the annulated product **10** that is a result of the overall reaction of **4** with 2 equiv of the acetylene.[6a]

The reactions of the chromium methyl methoxy carbene complex **4**[7] with 1-pentyne and propyne proceed as expected with the incorporation of two equivalents of the alkyne. The ultimate products are, however, the phenols **15**,[8] the formation of which can be attributed to an in situ reduction of **10** via a chromium(0) species (vide infra).[6b] The reaction of complex **4** with phenylacetylene has previously been reported to give as the sole product a 12% yield of the naphthol **16** as its chromium tricarbonyl complex.[10] However, we have found that this reaction in fact gives primarily the two-alkyne annulated phenol **15c** (28%) along with the naphthol **16** as the minor product in 8% yield. The formation of these two different types of annulated products may be attributed to the fact that two different isomeric forms of intermediate **11c** (Scheme II) are possible.

The addition of the second equivalent of alkyne to the intermediate **8** is apparently disfavored for more sterically hindered acetylenes. It has been observed[11] that the reaction of complex **4** with diphenylacetylene produces the cyclobutenone **13** in 28% yield and we have been unable to find any two-alkyne annulated products from this reaction. The reaction of the corresponding

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(6) (a) We recently observed the reaction of a vinyl carbene complex that gave a low yield of a two-alkyne annulated product along with the normal "monoalkyne" annulated product.[3c] (b) An alternative mechanism for formation of the phenols of **15** (or **22**) involves insertion of a carbon monoxide into **8** to give a vinyl ketene complex[4] that subsequently reacts with the second equivalent of alkyne.

(7) For preparation of these complexes, see: Wulff, W.; Gilbertson, S. R. *J. Am. Chem. Soc.* **1985**, *107*, 503.

(8) Despite the low yield, the phenols **15a** and **15b** were the major products and small amounts of unidentified products were obtained. The substitution pattern of these phenols is that expected from the known regiochemistry of acetylene incorporation[9,3b] and is confirmed in the case of **15b** by comparisons of its spectral data with that of an authentic sample (see supplementary material). The reactions of complex **4** with propyne and 1-pentyne were run at 0.07 M in carbene complex, whereas the reaction with phenylacetylene was carried out exactly as described in ref 10 (0.53 M).

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