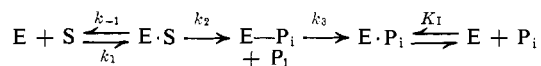Plots of $1/b$ vs. $1/S$ indeed yield excellent straight lines for a given constant value of P_i, and from these plots values of b_{lim}, K_S, and K_I can be calculated. Values of V have been measured more conveniently on the expanded slidewire of a Cary 15 recording spectrophotometer at 400 mμ and in a 5-cm path-length quartz cuvette. Normal Michaelis–Menten type kinetics were observed in all cases, and phosphate ions acted here too as purely competitive inhibitors, i.e. the rates were adequately described by $V = k_{cat}E_oS/(S + K_m(1 + P_i/K_I))$.

The data obtained in these experiments are summarized in Table I. These results are consistent with the kinetic scheme[6]

$$E + S \underset{k_1}{\overset{k_{-1}}{\rightleftarrows}} E \cdot S \xrightarrow{k_2} \underset{+\ P_1}{E-P_i} \xrightarrow{k_3} E \cdot P_i \overset{K_I}{\rightleftarrows} E + P_i$$

with the following values for the individual constants: $K_s = k_{-1}/k_1 = 8 \times 10^{-7}M$; $k_2 = 24.6 \text{ sec}^{-1}$; $k_3 = 0.8 \text{ sec}^{-1}$; and $K_I = 2.4 \times 10^{-6}M$. Since k_3 is much smaller than k_2 and since in all our stopped-flow experiments the condition $S \gg K_m$ was satisfied, the amount of P_1 released in the presteady state is a direct measurement of the concentration of active sites in the reaction mixture.[7] Using $\epsilon_{400} = 11,130$ for the 2,4-dinitrophenolate ion and a molecular weight of 86,000 for the enzyme we found that in all our measurements on the stopped-flow instrument the ratio of the molarity of the active sites vs. the molarity of the enzyme was 0.9 ± 0.1. This ratio remained the same in the absence of phosphate ions and with phosphate ion concentrations as high as 1×10^{-3} M. Also, preincubation of the enzyme at pH 6.3 for 10 min with or without phosphate ions present had no effect on the ratio.

The following conclusions are suggested by our results. (1) The E. coli alkaline phosphatase catalyzed hydrolysis of 2,4-dinitrophenyl phosphate is adeq uately described by a kinetic scheme involving the format on of an enzyme–substrate complex followed by a fast phosphorylation and a slower dephosphorylation of the enzyme, and no other steps involving changes in covalent bonds need be invoked. (2) In our experimental conditions phosphate ion acts as a competitive inhibitor, and it phosphorylates less than 10% of the active sites reacting with the substrate. (3) If one assumes that k_1 measures a diffusion-controlled process, then by analogy with the ribonuclease-catalyzed reactions[8] a lower limit of 10^9 M^{-1} sec^{-1} can be estimated for k_1. Then from the experimental value of K_S the lower limit for k_{-1} will be 800 sec^{-1}. Thus k_{-1} is much greater than k_2 and for all practical purposes K_S is a true equilibrium constant measuring the binding of the substrate to the enzyme. (4) The unusually low value of the binding constants K_S and K_I is suggestive of binding forces other than "hydrophobic," possibly of metal ion–ligand coordination complexes. (5) Finally, the titration of the active sites would indicate a 90% pure enzyme preparation, each molecule of mol wt 86,000 possessing one active site.[9]

(8) R. E. Cathou and G. G. Hammes, J. Am. Chem. Soc., 87, 4674 (1965).

(9) The specific activity of our enzyme preparation was 86 ± 5% of that measured with the most pure crystallized enzyme (M. H. Malamy and B. L. Horecker, Biochemistry, 3, 1893 (1964)). This then argues against the possibility of a 45% pure enzyme preparation with two active centers per molecule.

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Table I. Kinetic Parameters of the E. coli Alkaline Phosphatase Catalyzed Hydrolysis of 2,4-Dinitrophenyl Phosphate[a]

$P_i \times 10^5$, M	b_{lim}, sec^{-1b}	$K_S{}^{app} \times 10^7$, M^b	k_{cat}, sec^{-1c}	$K_m \times 10^8$, M^c
0	25.4	8 ± 5	0.7	1.4 ± 0.7
1.5	25.3	25 ± 4	0.8	10.6 ± 3
5	25.4	91 ± 9	0.8	28.5 ± 5

[a] pH 5.7, 0.05 M acetate buffer, 25°. The enzyme used was Worthington alkaline phosphatase BAPC 71A, activity 30 units/mg. A stock solution of the enzyme was obtained by using the procedure described by Fife.[4] The enzyme concentration was determined from the absorption of that solution at 280 mμ using the value of 0.77 AU = 1 mg of protein/ml and a molecular weight of 86,000 (M. J. Schlesinger and K. J. Barrett, J. Biol. Chem., 240, 4284 (1965)). 2,4-Dinitrophenyl phosphate monolutidinium salt was used (the authors wish to thank Dr. A. Thomson and Professor M. L Bender for a sample of this compound), mp 140° (lit. mp 142° (A. J. Kirby and A. G. Vargolis, J. Am. Chem. Soc., 88, 1824 (1966)). [b] $S_o = 6$–50×10^{-6} M; $E_o = 6$–30×10^{-7} M. [c] $S_o = 2$–7×10^{-7} M; $E_o = 1.8 \times 10^{-9}$ M.

However, at pH 5.0 a reversible partial loss of activity of the enzyme is observed in the few seconds following the presteady state, thus decreasing slowly the value of V to a constant value. Since phosphate ions and excess substrate seem to protect the enzyme from this loss of activity, this phenomenon offers a possible explanation of the abnormally high titration values observed at pH 4.7 using 2-nitrophenyl phosphate as a substrate.[4]

(6) J. A. Stewart and L. Ouellet, Can. J. Chem., 37, 751 (1959).
(7) M. L. Bender, F. J. Kézdy, and F. C. Wedler, J. Chem. Educ., 44, 84 (1967).

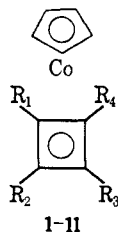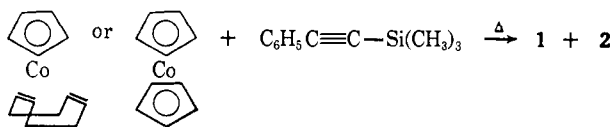## New Cyclobutadiene Complexes of Cobalt

Sir:

The recent communication by Rausch and Genetti[1] prompts us to report some initial results of our research which is also directed at the synthesis and study of π-cyclopentadienyl-π-cyclobutadienecobalt(I) and its derivatives. Until recently only one complex of this type, π-cyclopentadienyl-π-tetraphenylcyclobutadienecobalt(I),[2] had been prepared despite the fact that considerations of electronic arrangement and symmetry lead to the expectation that these compounds should parallel ferrocene and cyclobutadieneiron tricarbonyl in reactivity and thermal stability. Since some previous syntheses of π-cyclopentadienyl-π-tetraphenylcyclobutadienecobalt(I) had involved the reaction of diphenylacetylene with π-cyclopentadienyl-π-1,5-cyclooctadienecobalt(I)[2a] or cobaltocene,[2b] it seemed desirable to investigate possible extensions of these techniques.

We find that a number of arylacetylenes bearing a variety of functional groups attached to the triple bond react with π-cyclopentadienyl-π-1,5-cyclooctadienecobalt(I) or cobaltocene to produce cyclobutadiene complexes, and we have for the first time prepared derivatives of π-cyclopentadiene-π-cyclobutadienenecobalt(I)

(1) M. D. Rausch and R. A. Genetti, J. Am. Chem. Soc., 89, 5502 (1967).
(2) (a) A. Nakamura and H. Hagihara, Bull. Chem. Soc. Japan, 34, 452 (1961); (b) J. L. Boston, D. W. A. Sharpe, and G. Wilkinson, J. Chem. Soc., 3488 (1962); (c) P. M. Maitlis, A. Efraty, and M. L. Games, J. Organometal. Chem. (Amsterdam), 2, 284 (1964).

bearing unsubstituted positions on the cyclobutadiene ring.

competitive electrophilic substitution in four-, five-, and six-membered ring systems. The significance of the silyl derivatives as intermediates is evident from the fact that phenylacetylene has thus far formed no cyclobutadiene complexes with either the cyclooctadiene complex or cobaltocene but rather undergoes some trimerization.

The structures of the new cyclobutadiene complexes reported here are supported by elemental analyses, molecular weights (mass spectra), and nmr, ultraviolet and infrared spectra. The products may be isolated using column and thin layer chromatography. Most of them may be sublimed under reduced pressure at elevated temperatures. Although the stability limits of these compounds have not been fully defined, they are stable to air and water and seem to have good thermal stability. In the nmr spectra of these compounds in CCl_4 the position of absorption of the cyclopentadienyl protons varies from τ 5.02 (9) to 5.52 (4). The cyclobutadiene protons appear at τ 5.18 in 10 and at τ 5.70 in 11. These spectra support the structures assigned to 10 and 11 since in 10 two flanking aryl groups would be expected to exert a greater deshielding effect on the cyclobutadiene protons than would a single flanking aryl group which would be the case in 11. The mass spectra of these compounds always contain a peak at m/e 124 $(C_5H_5Co^+)$. No peaks are found which can be ascribed to initial cleavage of C_5H_5. The other major peaks in the spectra may be rationalized in terms of degradation of the cyclobutadiene ring or substituent groups.

The reactions of the cyclooctadiene complex and cobaltocene with acetylenes are not completely general. Diiodoacetylene, for example, does not undergo complex formation. Neither does 3-phenylpropynamide. The results of the reactions reported here and a number of others which we have examined suggest that formation of cyclobutadiene complexes using these routes requires acetylenes bearing an aryl group conjugated with the triple bond. Even then cyclobutadiene complex formation may not occur if trimerization, more extensive polymerization, or other processes occur readily.

Our preliminary, independent investigation of substitution reactions of π-cyclopentadienyl-π-tetraphenylcyclobutadienecobalt(I) confirms the observations of Rausch and Genetti[1] that Friedel–Crafts acetylation occurs only in low yield and that metalation may be effected with n-butyllithium.[3a]

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(Phenylethynyl)trimethylsilane and the cyclooctadiene complex in refluxing xylene gave a mixture of two isomers, 1 and 2, in 59% yield. The ratio 1:2 was approximately 2:1. (Phenylethynyl)trimethylsilane and cobaltocene yielded the same mixture of isomers in refluxing diethylbenzene in 38% yield. (Phenylethynyl)triphenyltin also reacts with the cyclooctadiene complex to produce 3 in 53% yield. The structure assigned seems reasonable on steric grounds. No cyclobutadiene complexes could be detected following the reaction of cobaltocene and the tin-substituted acetylene. Both the cyclooctadiene complex and cobaltocene reacted with phenylmethylacetylene, but for reasons which are not clear at present only a single cyclobutadiene complex, 4, was isolated.

Arylacetylenes bearing electron-withdrawing substituents also yield cyclobutadiene complexes. Phenylacetylacetylene reacts with either the cyclooctadiene complex or cobaltocene to give the expected mixture of isomers 5 and 6. Phenylpropargylaldehyde led only to a rather insoluble product which did not contain cyclobutadiene complexes and is believed to be polymeric. However, this difficulty may be circumvented by reaction of the cyclooctadiene complex with the diethyl acetal of phenylpropargylaldehyde. After hydrolysis of the reaction products, the corresponding isomeric aldehydes 7 and 8 may be isolated. Phenyltrifluoromethylacetylene[3] reacts with either the cyclooctadiene complex or cobaltocene to produce 9. Chromatography gave evidence of a second complex, but too little was present to permit identification.

Compounds 1 and 3 react with HCl to give the same product, 10. Compound 2 reacts with HCl to give the expected isomer, 11. Compounds 10 and 11 are the first examples of the parent complex system which possess unsubstituted positions on the cyclobutadiene ring and present interesting possibilities for the study of

(3) Phenyltrifluoromethylacetylene was prepared by sunlamp irradiation of CF_3I and phenylacetylene followed by dehydrohalogenatic of the product by dropping it onto powdered KOH at 220°.

Two New Chromium–Carbonyl–Carbene Complexes

Sir:

When gaseous ammonia is passed through a benzene solution containing methylmethoxycarbenechromium