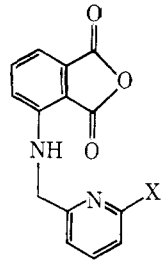
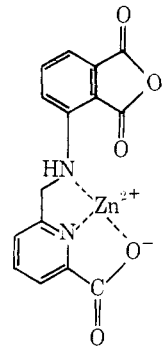
The rate was extrapolated to the enzymatically important pH of 7.50 since it was too fast to measure in this region by simple conventional techniques.

The rate enhancement of this cleavage due to coordinated metal ion is of a magnitude similar to that for other known metal ion accelerations, but with a relatively reactive anhydride function the rate constant at pH 7.50 is 3.0 ± 0.5 sec^{-1}. This falls in the region of values of k_{cat} mentioned for the enzyme, and it does indicate that such a zinc-catalyzed cleavage of the anhydride is a reasonable step to invoke in the course of the enzymatic reaction. The additional acceleration required to make this step fast compared with the hydrolysis of the fastest substrates for the enzyme could presumably be supplied if some of the freedom still present in our model III were restricted in the enzyme–substrate complex.

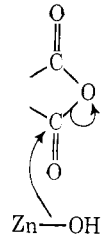
The data in Figure 1 contain an indication of the mechanism of this catalysis. The pH dependence indicates that Zn^{2+} catalyzes the attack of hydroxide on the anhydride but does not detectably catalyze the attack of neutral water. Such an effect would not be expected if zinc were functioning as a Lewis acid to facilitate attack on the coordinated anhydride by an external nucleophile; in a more reactive coordinated anhydride the preference for a better nucleophile should be decreased, not increased. However, if the mechanism of this attack involves coordination of hydroxide to the Zn^{2+}, followed by nucleophilic attack by such a coordinated hydroxide on an uncoordinated anhydride carbonyl (IV), then the effect would be understandable. This mechanism is the one expected for catalyzed cleavage of an anhydride, in which the catalytic problem to be solved is that of supplying a sufficient concentration of nucleophile, not of stabilizing the leaving group.



I, X = CO_2H
II, X = H



III



IV

Studies of the opening of anhydride I with hydroxylamine also support this mechanism. In the absence of Zn^{2+}, hydroxylamine is an effective nucleophile toward I, but its attack is *not catalyzed* by Zn^{2+}. Instead the data fit eq 1

$$-\frac{d[I]}{dt} = k_1[I \cdot Zn^{2+}][OH^-] + k_2[I_{total}][NH_2OH] \quad (1)$$

under a variety of conditions. When [I] is 5×10^{-5} M and $[NH_2OH_{total}]$ is 5×10^{-4} M at pH 5.50 in the absence of Zn^{2+}, for example, the rate for the disappearance of I due to hydroxylamine attack completely dominates that due to spontaneous hydrolysis ($\sim 10^2$ faster). When enough Zn^{2+} is added to completely saturate I with the metal under these conditions, an increase in the overall rate by 30% is observed. This rate increase is exactly that expected from the addition of the now important first term in eq 1.[9] The balance between these two terms will be determined by pH and the effectiveness of Zn^{2+} catalysis. Thus, in the enzyme at pH 7.50, with decreased conformational mobility and good proximity of Zn^{2+}, we would expect that the catalyzed attack by coordinated OH^- would be much faster than the uncatalyzed attack by hydroxylamine.

The results from our model systems indicate that the failure to trap an anhydride intermediate in the enzymatic reaction[5] is expected because of the mechanism by which Zn^{2+} would catalyze its cleavage, and this would explain[10] the observed preference of carboxypeptidase A for water (OH^-) over any other lytic agent. The two-step mechanism thus remains one of the most attractive explanations of all the data on the reactions catalyzed by carboxypeptidase A. In this mechanism the Zn^{2+} catalyzes the first step, anhydride formation, by acting as a Lewis acid. It catalyzes the second step by the delivery of a specific nucleophile to the anhydride intermediate.

Acknowledgments. Helpful discussions with Mr. David Wernick are gratefully acknowledged. This work was supported by the National Institutes of Health.

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(1) For reviews, see (a) E. T. Kaiser and B. L. Kaiser, *Accounts Chem. Res.*, **5**, 219 (1972); (b) J. A. Hartuck and W. N. Lipscomb, "The Enzymes," Vol. III, 3rd ed, P. D. Boyer, Ed., 1971, p 1; (c) W. N Lipscomb, *Tetrahedron*, **30**, 1725 (1974).
(2) Of the three identified catalytic groups, the tyrosine hydroxyl appears to be required for peptidase activity but not for esterase activity.[1] Since the glutamate residue has been incorporated into the anhydride (acyl–enzyme) intermediate, only Zn^{2+} is still available for catalysis.
(3) L. M. Ginodman, N. I. Mal'tsev, and V. N. Orekhovich, *Biochemistry (USSR)*, **31**, 931 (1966).
(4) D. Wernick, in unpublished calculations, has shown that the ^{18}O exchange rate is as fast as expected for the enzyme-catalyzed endothermic resynthesis of an anhydride. The calculation involves the known equilibrium constant for anhydride hydrolysis and an enzyme-catalyzed rate of hydrolysis fast enough to fit the enzyme time scale for substrate hydrolyses.
(5) D. Wernick, unpublished work. Mr. Wernick has also shown that carboxypeptidase A prefers $H_2O(OH^-)$ over $CH_3OH(CH_3O^-)$ by at least 10^2. Related observations have apparently been made in other laboratories.
(6) (a) G. Tomalin, B. L. Kaiser, and E. T. Kaiser, *J. Amer. Chem. Soc.*, **92**, 6046 (1970); (b) P. L. Hall, B. L. Kaiser, and E. T. Kaiser, *ibid.*, **91**, 485 (1969); (c) E. T. Kaiser and F. W. Carson, *ibid.*, **86**, 2922 (1966).
(7) The compounds I, mp 229–232°, and II, mp 148–150°, were prepared by sequences involving reductive condensation of aminophthalic acid with the appropriate pyridinecarboxaldehyde. Both exhibited the correct spectral and mass spectral properties.
(8) (a) J. W. Thanassi and T. C. Bruice, *J. Amer. Chem. Soc.*, **88**, 747 (1966); (b) C. A. Bunton, N. A. Fuller, S. G. Perry, and V. J. Shiner, *J. Chem. Soc.*, 2918 (1963).
(9) Conversion of I to its Zn^{2+} complex III neither increases nor decreases its reaction rate with NH_2OH (a 10% change would be detectable). The flexibility in III permits the Zn^{2+} to lie away from the anhydride, and electronic effects are apparently small.
(10) Most other nucleophiles could not be delivered by Zn^{2+}. Even CH_3O^- would be selected against, for reasons discussed by R. Breslow, R. Fairweather, and J. Keana, *J. Amer. Chem. Soc.*, **89**, 2135 (1967). An additional likely possibility is that in mechanism IV the hydroxide is not transferred away from zinc, but instead undergoes a further deprotonation after it attacks the carbonyl group. This would generate a carboxylate ion still coordinated to zinc. Such a further deprotonation is not possible for methoxide.
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Trans Addition of Halogens to Tetrakis(triphenylphosphine)platinum(0)

Sir:

The oxidative additions of bromine and iodine to $Pt(PPh_3)_4$ have been reported both explicitly and implicitly to give invariably *cis*-$PtBr_2(PPh_3)_2$ and *cis*-$PtI_2(PPh_3)_2$,

respectively.[1-5] Although, the reaction of $Pt(PPh_3)_4$ with Cl_2 does not appear to have been reported, the general concensus among chemists is that *cis*-$PtCl_2(PPh_3)_2$ would result. This is evidenced by the fact that, hitherto, synthetic methods for the synthesis of *trans*-$PtX_2(PPh_3)_2$ (X = Cl, Br, or I) involve either photochemical[6] or thermal[7] isomerization of the cis isomers, or, from the reaction of *trans*-$PtHCl(PPh_3)_2$ with $HgCl_2$[8] instead of the obvious, direct reaction of $Pt(PPh_3)_4$ with the halogens. We now report that, under suitable conditions, the direct oxidative addition of the halogens to $Pt(PPh_3)_4$ leads exclusively to *trans*-$PtX_2(PPh_3)_2$ (X = Cl, Br, or I) and that, under any conditions reported, the first step in these oxidative additions is a trans addition of X_2 to $Pt(PPh_3)_4$.

The fact that previous investigators have reported the isolation of cis isomers is a consequence of isomerization, in the presence of triphenylphosphine, of the initially formed trans isomers to the cis isomers. This trans–cis isomerization has previously been observed to occur rapidly for $PtCl_2(PPh_3)_2$ in chloroform.[8] We have observed that the rate of trans–cis isomerization in benzene for $PtX_2(PPh_3)_2$ (X = Cl, Br, or I) follows the order Cl > Br ≫ I. Thus, in order to demonstrate that the addition of halogens to $Pt(PPh_3)_4$ is a trans addition by isolating trans isomers, the experimental conditions must be such that no free triphenylphosphine is present after the formation of *trans*-$PtX_2(PPh_3)_2$. In this manner, the phosphine-catalyzed trans–cis isomerization reaction can be prevented.

One method available to prevent isomerization of the trans products is to "tie up" the free triphenylphosphine present in the reaction mixture by using more than the stoichiometric amount of halogen required for the oxidative-addition reaction. The excess halogen reacts with PPh_3 to form $[PPh_3X]X$ (and/or other products depending on the amount of X_2 used). Previous investigators, who obtained cis isomers, were reluctant to use an excess of the halogen, presumably for fear of further oxidation of the initially formed $PtX_2(PPh_3)_2$ to $PtX_4(PPh_3)_2$. However, we have observed that the first oxidative addition of X_2 to $Pt(PPh_3)_4$ proceeds faster than the second oxidative addition of X_2 to $PtX_2(PPh_3)_2$; thus, by limiting the reaction time to 3 min or less, the formation of Pt(IV) complexes can be avoided. For I_2 and Br_2, the reaction with $Pt(PPh_3)_4$ for 3 min using 4:1 mole ratio of halogen to Pt(0) complex was found to be sufficient to yield exclusively *trans*-$PtI_2(PPh_3)_2$ and *trans*-$PtBr_2(PPh_3)_2$, respectively. The reactions were carried out by mixing an ethereal solution of the halogen with a benzene solution of $Pt(PPh_3)_4$. The conditions were modified for the reaction of chlorine with $Pt(PPh_3)_4$. Since *trans*-$PtCl_2(PPh_3)_2$ is more rapidly isomerized in the presence of PPh_3 than *trans*-$PtBr_2(PPh_3)_2$ or *trans*-$PtI_2(PPh_3)_2$, it was found necessary to add the $Pt(PPh_3)_4$ solution in a fast dropwise fashion to an excess of a stirred benzene solution of Cl_2 so that at any given time during the addition no free phosphine can be present in the solution. The reaction time was also limited to 1 min to prevent oxidation to $PtCl_4(PPh_3)_2$ because Cl_2 is more reactive with both $Pt(PPh_3)_4$ and $PtCl_2(PPh_3)_2$ than are either Br_2 or I_2. In each instance only the trans isomer was obtained demonstrating that the addition of the halogens to $Pt(PPh_3)_4$ is a trans addition reaction.

The isomers were identified by their elemental compositions, their solubilities in benzene (from which they were recrystallized), their decomposition points, and their infrared spectra in which the 500–600-cm^{-1} region is of particular use. Mastin[7] has reported that there is a very strong absorption at 550 ± 5 cm^{-1} in all *cis*-$PtXY(PPh_3)_2$ and *cis*-$PtX_2(PPh_3)_2$ complexes, but he reports this absorption to be very weak in the trans isomers. We confirm his observation for the cis dihalogen complexes, but we find this absorption to be entirely absent in our spectra of the trans complexes prepared both by the oxidative-addition reactions reported herein and by independent methods.[9]

The reaction of I_2 with $Pt(PPh_3)_4$ was also repeated using the stoichiometric amount of reactants (1:1) for the formation of $PtI_2(PPh_3)_2$. The reaction time was also extended to 15 min. It was found that *trans*-$PtI_2(PPh_3)_2$ was formed in spite of the presence of free triphenylphosphine being present under these conditions. This demonstrates that *trans*-$PtI_2(PPh_3)_2$ isomerizes rather slowly in the presence of PPh_3 and suggests that earlier investigators had obtained the *trans*-$PtI_2(PPh_3)_2$ instead of the reported *cis*-$PtI_2(PPh_3)_2$.[3,4] An error, if made, might have resulted from an analogy to the reaction of Cl_2 and Br_2 with $Pt(PPh_3)_4$ where cis isomers are indeed formed under the conditions of a 1:1 mole ratio of the reactants.

With *trans*-$PtI_2(PPh_3)_2$, cis–trans isomerization appears to occur more readily than trans–cis isomerization. Mastin has reported[7] that the thermal isomerization of the cis isomer occurs in a refluxing chloroform solution containing 2% ethanol. We have found that the isomerization also proceeds in refluxing solution of benzene and even in the solid state at 200°. Thus, *trans*-$PtI_2(PPh_3)_2$ appeárs to be relatively more thermodynamically stable with respect to *cis*-$PtI_2(PPh_3)_2$ than are *trans*-$PtBr_2(PPh_3)_2$ and *trans*-$PtCl_2(PPh_3)_2$ with respect to their cis isomers.

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Atropisomeric Streptovaricins[1,2]

Sir:

The streptovaricins (and other ansamycin antibiotics)[3] derive an intrinsic helicity from their ansa rings. In connection with extensive studies of the biological activities of the streptovaricins and their derivatives,[2] we have examined the effect of this helicity on the activities displayed. Heating streptovaricin C (**1**) in refluxing toluene overnight (Figure 1) gave a mixture of **1** and atropisostreptovaricin C[4] (**2**, $C_{40}H_{51}NO_{14}$,[5b,c] properties in Table I), in the approximate ratio of 17:1, which were separated by chromatography over silicic acid. Heating a sample of **2** gave a similar mixture of **1** and **2**. Most spectral properties (uv, ir, pmr, mass spectral) of **1** and **2** are identical or nearly so, but the nature of the isomerism of **1** and **2** is established by their rotations of similar magnitude but opposite sign (Table I), by the nearly mirror image relationship of their CD curves,[6] and by the conversion of both **1** and **2** to streptoval C [**3**, $C_{40}H_{49}NO_{14}$,[5] mp 140–143°, $[\alpha]^{24}D$ −92.3° (*c* 0.013, $CHCl_3$)] on treatment with sodium metaperiodate.[7] Significantly, while most cmr chemical shifts for **1** and **2** are nearly identical,[8a] those for C-15 and C-16, which lie *above* the acetate group in **1** but *below* the acetate group in **2**, differ considerably (C-15 at 153.9 ppm from TMS for **1**, 149.3