$AlEt_2(OEt)$ were inactive; $AlEt_2Cl$ was weakly active.

It had been reported[7] that the major product of the reaction of benzene and $AlCl_3$ (0.18 M) at reflux temperature is phenylcyclohexane, and we have confirmed this result. At higher $AlCl_3$ concentration, increasing amounts of alkylbenzenes (mainly toluene and ethylbenzene) are observed, suggesting the possibility that phenylcyclohexane as a primary product undergoes cracking reactions to the observed volatile products. However, at higher temperature the reaction is complicated by condensations, secondary cracking, and catalyst deactivation, most likely by π complexation to higher benzenoids[13] (Figure 2). Such complexation evidently prevents further turnover of benzene and its reaction products. Addition of more $AlCl_3$ leads to a new spurt in benzene turnover. The hydrogen necessary for alkylbenzene production is envisaged to arise via bi- and polyphenyl formation as well as Scholl-type condensation reactions.[3,7,9,10,12] Further evidence for the intermediacy of phenylcyclohexane is derived from the observation of very similar product formation to that depicted in Figure 1 on its reaction with $AlCl_3$.

The major alkylbenzene products are toluene and ethylbenzene. This is consistent with the fact that $AlCl_3$ catalyzes the cleavage of diphenylalkanes to alkylbenzenes[14] and the higher members of the latter are fragmented to the above products.[10] The finding that no xylenes and polysubstituted benzenes are detected is most likely due to the low turnover of the reaction. Thus, such products do appear when toluene and other alkylbenzenes react with $AlCl_3$.

In order to shed further light on the mechanism of the benzene cleavage–hydrogenation process, several additional labeling experiments were run. Reaction of C_6D_6 gave completely labeled products. Not unexpectedly, a 1:1 mixture of C_6H_6 and C_6D_6 gave complete scrambling. Therefore an equimolar mixture of C_6H_6 and $^{13}C_6H_6$ (90% enriched) was exposed to $AlCl_3$ [N_2 (1 atm), 160 °C, 48 h]. Surprisingly, ^{13}C–^{12}C exchange (ca. 5%) is observed in recovered "unreacted" benzene and additional scrambling in all other volatile products[15] as analyzed by GC/MS. Despite this perturbation, the mass spectral peak patterns indicate substantially intact incorporation of alkyl chains derived from the original benzene ring. Thus, the connectivity of the initial carbon arrays is extensively preserved in the alkylbenzenes (including annulated and cycloalkylbenzenes) formed. We do not presently understand the mechanism by which label exchange occurs but suspect it to be independent of the alkylation process.

It might perhaps be emphasized that reactions such as those described in this communication must play a significant role in any process that attempts to liquefy coal in the presence of Lewis acids.[14,16] In any event it appears that the report on the "Fischer–Tropsch alkylation" of benzene should be approached with caution.

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(15) Toluene *m/e* (% rel intensity): 91 (86.6), 92 (100 normalized), 93 (39.7), 94 (12.4), 95 (16.1), 96 (34), 97 (56.8), 98 (40), 99 (8.7). Ethylbenzene: 105 (28.0), 106 (100), 107 (60.7), 108 (51.2), 109 (10.5), 110 (15.1), 111 (37.2), 112 (50), 113 (28.5), 114 (13.4). *n*-Propylbenzene: 120 (100), 121 (56.1), 122 (46.6), 123 (39.7), 124 (4.67), 125 (24.0), 126 (50.8), 127 (31.5), 128 (26.5), 129 (14.0). *n*-Butylbenzene: 134 (100), 135 (70.9), 136 (56.0), 137 (46.3), 138 (35.9), 139 (26.8), 140 (51.1), 141 (38.0), 142 (28.8), 143 (19.2), 144 (10.1). Attempts to fit these patterns to calculated intensities based on a statistical analysis of the label distribution expected based on the extent of scrambling observed in the recovered benzene gave variable data, always indicating additional but not random scrambling. Similar results were observed for the higher oligophenyls, aromatic systems, and cycloalkylbenzenes.

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Carboxypeptidase A Catalysis of an α,β-Elimination Reaction

Nashaat T. Nashed and E. T. Kaiser*

Department of Chemistry, The University of Chicago Chicago, Illinois 60637

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We wish to report our finding that carboxypeptidase A (CPA) catalyzes the α,β elimination of a suitably designed ketonic substrate containing in addition to the activated α-methylene group a good leaving group β to the ketone function. In early studies[1,2] CPA was demonstrated to catalyze hydrogen–deuterium exchange with retention of configuration at the activated methylene group of (−)-2-benzyl-3-(*p*-methoxybenzoyl)propionic acid, (−)-**1**, a ketonic analogue of ester and peptide substrates such as *N*-acyl-L-phenylalanines or *O*-acyl-L-β-phenyllactates, respectively. Stereochemical analysis[2] showed that it was the pro-*R* hydrogen of the 3-methylene group of (−)-**1** which undergoes hydrogen–deuterium exchange at the active site of CPA_γ, and this observation was consistent with the hypothesis that (−)-**1** binds to CPA in a mode similar to that which has been deduced for hydrolytic substrates from X-ray crystallographic studies on peptide–enzyme complexes.[3] Subsequently, an X-ray structure determination[4,5] was reported for the complex of CPA with (−)-**1**, and the arrangement of (−)-**1** at the enzyme's active site was found to be in accord with the binding picture proposed in the stereochemical studies. The γ-carboxylate moiety of Glu-270 is the functional group in the enzyme responsible for the abstraction of the pro-*R* proton from the 3 position of (−)-**1**. With the demonstration in hand that CPA catalyzes stereospecific enolization at the methylene group α to the ketone function in (−)-**1**, a logical step was to ask whether introduction of a good leaving group β to the ketone function would provide a compound susceptible to CPA-catalyzed α,β elimination. To test this possibility, we have synthesized 3-benzoyl-2-[(*p*-nitrophenyl)thio]propionic acid (**2**) and have examined the interaction of carboxypeptidase A with this substrate.

Compound (±)-**2** was obtained by the addition at room temperature of *p*-nitrothiophenol to 3-benzylpropenoic acid (**3**) in acetic acid, employing a catalytic amount of sulfuric acid. Anal. Calcd for $C_{16}H_{13}NO_5S$: C, 57.99; H, 3.96; N, 4.23; S, 9.68. Found: C, 58.08; H, 4.07; N, 4.17; S, 9.50. The ^1H NMR, IR, and mass spectra were consistent with the assigned structure.

Resolution of (±)-**2** was accomplished by repeated recrystallization from acetonitrile of the salt formed between the ethylene ketal of the 3-benzylpropionic acid,[6] (**4**) and enantiomerically pure α-methylbenzylamine. The enantiomers, (−)-**4**, mp 143–145 °C, α^{25}_D −31.5° (*c* 2, acetone), and (+)-**4**, mp 144–145 °C, α^{25}_D +33.1° (*c* 2, acetone), were obtained by using (−)- and (+)-α-methylbenzylamine, respectively. Treatment of (−)-**4** and (+)-**4** with 10% HCl in acetic acid at room temperature for 2 h gave (−)-**2**, mp 101–102 °C, α^{25}_D −108.1° (*c* 1, acetone) and (+)-**2**, mp 103–105 °C, α^{25}_D +111.8° (*c* 2, acetone), respectively.

Under conditions usually optimal for the hydrolytic reactions of CPA[7] (0.05 M 4-morpholinopropanesulfonic acid, pH 7.5, 25.0 °C, 3% (v/v) acetonitrile), the enzyme was found to catalyze the

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(6) Due to the lability of **2** in the presence of a base, it was necessary to transform **2** to **4** to achieve enantiomeric resolution, employing α-methylbenzylamine as the resolving agent. Compound **2** was esterified at room temperature in methanol containing a catalytic amount of sulfuric acid to give the methyl ester. Then the methyl ester was transformed to the ethylene ketal by heating under reflux for 24 h in benzene containing ethylene glycol and *p*-toluenesulfonic acid. The resultant ethylene ketal–methyl ester derivative of **2** was saponified with 2% potassium hydroxide in 1:1 (v/v) ethanol–water at room temperature, yielding **4**.

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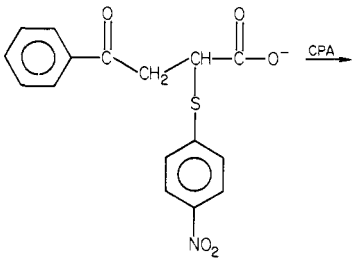
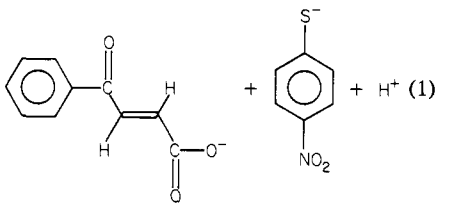
elimination of *p*-nitrothiophenol from (+)-**2**, a reaction which was followed spectrophotometrically at 412 nm (see eq 1 below).



2



3

When the enzyme concentration was much greater than the substrate concentration, pseudo-first-order kinetics were observed for the liberation of 1 equiv of *p*-nitrothiophenol from (+)-**2** under anaerobic conditions. The dependence of the observed first-order rate constant on the concentration of the enzyme yielded the kinetic parameter k_{cat}/K_m which had a value of 4.97 ± 0.34 M^{-1} s^{-1} under the conditions mentioned above. The CPA-catalyzed elimination of *p*-nitrothiophenol from (+)-**2** was completely inhibited upon the addition of 2.37 mM of *dl*-benzylsuccinic acid. Since *dl*-benzylsuccinic acid is a potent inhibitor of the hydrolytic action of CPA (K_i = 1.1 μM),[8] the inhibition observed for the elimination reaction provides strong evidence that the latter process occurred at the active site. On the other hand, CPA does not catalyze the elimination of *p*-nitrothiophenol from (−)-**2** even though (−)-**2** binds to the enzyme active site. Compound (−)-**2** is a competitive inhibitor for the CPA-catalyzed hydrolysis of *O*-(*trans*-*p*-chlorocinnamoyl)-L-β-phenyllactate with a K_i value of 640 ± 40 μM at pH 7.5, 0.5 M NaCl, 0.05 M Tris–HCl buffer, 3% acetonitrile and 25.0 °C. From kinetic experiments under substrate in excess conditions using (+)-**2**, the kinetic parameters K_m = 420 ± 10 μM and k_{cat} = (1.4 ± 0.1)10^{-3} s^{-1} were obtained at pH 7.5, 0.5 M NaCl, 0.05 M Tris–HCl buffer, 6.3% acetonitrile at 25.0 °C. Thus it appears that both enantiomers of **2** bound to the active site of the enzyme approximately equally, but only (+)-**2** is a substrate, consistent with the enzymic nature of the elimination reaction. Because *p*-nitrothiophenol readily adds to the unsaturated acid **3** in organic solvents, the product analyses which we performed on the elimination reactions of **2** were carried out in the presence of 1 mM potassium ferricyanide which oxidizes the *p*-nitrothiophenol produced in the α,β-elimination reaction to bis(*p*-nitrophenyl)disulfide. Control experiments showed that the presence of potassium ferricyanide up to a concentration of 1.5 mM in the reaction mixture does not affect the rate of the enzymic or nonenzymic elimination reactions carried out at pH 7.5. The disulfide formed by the oxidation of *p*-nitrothiophenol precipitates from the reaction mixture under these conditions and was removed by filtration. Then, the filtrate was acidified to pH 2 by using cold HCl and extracted immediately with ether three times. Subsequently, the organic layer was dried over $MgSO_4$, and the solvent was evaporated by using a rotary evaporator in the cold. A 270-MHz ^1H NMR spectrum showed that the residue contained only carboxylic acid **3** as the product obtained in either the enzymic or nonenzymic elimination reactions of (+)-**2** or (±)-**2**. The cis isomer of **3** can be distinguished from *trans*-**3** by NMR spectroscopy.[9]

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Although the absolute configuration of (+)-**2** is not known yet, it would be reasonable from the above data to assume that (+)-**2** binds to the active site of the enzyme in a fashion similar to that observed for (−)-**1**. In that event, the *p*-nitrothiophenyl group would be placed in a hydrophobic pocket, the carboxylate anion of the substrate with its negative charge interacting with the positively charged side chain of Arg-145, the carbonyl oxygen of **2** coordinated to the reactive site zinc ion, and a hydrogen of the α-methylene group within striking distance from the γ-carboxylate group of Glu-270.[10] According to this proposal, either the γ-carboxylate group of Glu-270 or a water molecule assisted by this residue acting as a general base catalyst would be the species removing the hydrogen from the α-methylene position of (+)-**2**.

In summary, in this communication we have demonstrated that CPA can catalyze an α,β-elimination reaction with an appropriately designed ketone substrate containing a labile group β to the ketone function. The k_{cat}/K_m value for the catalytic action of CPA on (+)-**2** is comparable to the second-order rate constant for the hydroxide ion catalyzed elimination of *p*-nitrothiophenol from **2** (k_{OH^-} = 4.7 ± 0.1 M^{-1} s^{-1} at 30.0 °C) and at least 10^5 greater than the rate constant observed with acetate ion (k_{OAc^-} = (2.6 ± 0.2)10^{-5} M^{-1} s^{-1} in 33% ethanol (v/v), 30.0 °C). The possibility that elimination reactions can be employed as the basis for the design of "suicide" substrates for the action of the hydrolytic enzyme CPA is under active investigation in our laboratory.

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(10) One question which must be raised with regard to this suggestion is whether the *p*-nitro substituent of the *p*-nitrothiophenyl moiety can fit in the hydrophobic pocket of CPA. We are probing this point by examining the reactivity of a variety of substrates related to **2** in which there is a considerable range in the size of the para substituent attached to the thiophenyl ring.

Direct Observation of Alkyl/Nitrosyl Migratory Insertion in an Organotransition Metal Complex

Wayne P. Weiner, Mary Ann White, and Robert G. Bergman*

Department of Chemistry, University of California, Berkeley, Berkeley, California, 94720
and Division of Chemistry and Chemical Engineering California Institute of Technology Pasadena, California 91125

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Migratory insertion of CO into metal carbon bonds (eq 1) is one of the most ubiquitous and well-studied reactions in organotransition metal chemistry. In addition to its fundamental importance, CO migratory insertion is a critical step in many important carbon–carbon bond-forming processes involving homogeneous transition-metal catalysts.[1] In contrast, migratory insertion of NO into metal–carbon bonds (eq 2) is much less

$$R\text{-}M(CO) + L \rightarrow R\text{-}CO\text{-}M(L) \quad (1)$$

$$R\text{-}M(NO) + L \rightarrow (RNO)M(L) \quad (2)$$

* To whom correspondence should be addressed at the University of California, Berkeley.

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