The isomerization of allyl alcohols can also be readily carried out at 25 °C using such complexes as catalysts, the stable and easily prepared $MoO_2(OBu^t)_2$ (**5**)[5] serving conveniently as the catalyst precursor. For instance, using a 2.8×10^{-2} M solution of **5** in CD_2Cl_2, 50 equiv of $Me_2C{=}CHCH_2OH$ is converted in 2.5 h into the equilibrium mixture of this alcohol with $CH_2{=}CHCMe_2OH$ (ca. 37:63), at an initial rate of 35 turnovers/h. We have shown by ^1H NMR that in the presence of excess $Me_2C{=}CHCH_2OH$ the initial replacement of OBu^t ligands and the displacement of rearranged ligand $CH_2{=}CHCMe_2O$ by $Me_2C{=}CHCH_2O$ is rapid (≪ 5 min). Further, the isomerization rate is zero order in allyl alcohol under these conditions. These observations show that the allyl rearrangement step of the type found in complexes **1a–3a** is turnover limiting in the catalytic process.

(10) If we assume the equations (M = monomer), the rate of inversion will

$$\text{inversion} \xleftarrow[k_1]{} M \overset{K}{\rightleftharpoons} \tfrac{1}{2}M_2 \xrightarrow[k'_1]{} \text{inversion}$$

be expressed by $v = k_1[M] + k'_1[M_2]$. If K is small, this tends toward $v = k_1[M] + k'_1K^2[M]^2 = k_1[M] + k_2[M]^2$. Allyl migration in this dimer (built via bridging oxo or allyloxo ligands) may also take place on a single Mo center, as described above. The Lewis acidity of the second Mo center would thereby lower the transition state by stabilizing the developing negative charge on the first Mo, thereby increasing the rate. We also observe that addition of excess $MoO_2(OBu^t)_2$ (**5**)[5] to CD_3CN solutions of **1a**, which will increase the overall concentration of dimeric species, increases the initial rearrangement rate.

$^{11}C/^{14}C$ Kinetic Isotope Effects in Enzyme Mechanism Studies. $^{11}C/^{14}C$ Kinetic Isotope Effect of the Tyrosine Phenol-Lyase Catalyzed α,β-Elimination of L-Tyrosine

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Received October 8, 1991

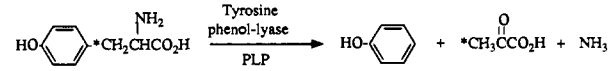
In this paper a method for determination of $^{11}C/^{14}C$ kinetic isotope effects (KIEs) in enzyme-catalyzed reactions is presented.

The KIE was determined for the elimination reaction of L-(β-*C)tyrosine (*C = ^{11}C or ^{14}C), catalyzed by tyrosine phenol-lyase (EC 4.1.99.2) from *Citrobacter freundii*; see Scheme I. This reaction was chosen as a model to develop methodology for determination of $^{11}C/^{14}C$ KIEs in enzyme-catalyzed reactions.

Isotope-effect studies[1] are crucial in enzyme mechanism investigations in that they yield information on the kinetic mechanisms, rate-limiting steps, and chemical mechanisms. In favorable cases, transition-state structures for single steps may also be inferred from KIE data.[2] In carbon KIE studies of enzyme reactions, the isotopes ^{12}C, ^{13}C, and ^{14}C have so far been utilized. The isotope ^{11}C is a short-lived (half-life 20.34 min) accelerator-produced positron-emitting radionuclide. ^{11}C is frequently used in positron emission tomography (PET) studies[3] in biomedical research and clinical diagnosis. This has accelerated the development of rapid labeling synthesis, so that today a large range of ^{11}C-labeled molecules can be synthesized.[4]

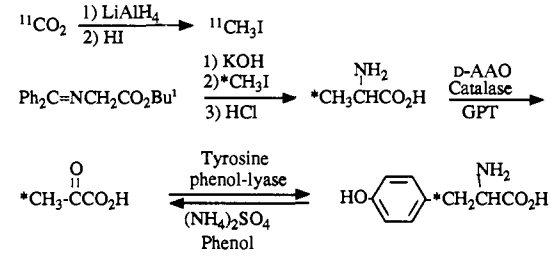
The $^{11}C/^{14}C$ method[5a] is based on internal competition between two radioactive isotopically labeled substrates. The rate constants

Scheme I[a]



[a] An asterisk (*) denotes 11 or 14; PLP = pyridoxal 5-phosphate.

Scheme II. Multienzymatic Synthesis of β-Labeled Tyrosine[a]



[a] An asterisk (*) denotes 11 or 14; D-AAO = D-amino acid oxidase; GPT = glutamic-pyruvic transaminase.

for the two isotopic (^{11}C and ^{14}C) reactions are calculated from the measurements by liquid scintillation counting of substrate and product fractions, isolated from the reaction mixture via a technique of quenching and HPLC separation.

Three different methods have been used earlier to determine KIEs for enzyme reactions:[2a] the direct comparison method, the equilibrium perturbation method, and the internal competition method. The highest accuracy has been achieved by the last method, using an isotope ratio mass spectrometer.

The present method may become an alternative and complement to that based on mass spectrometry and has certain advantages;[5] e.g., the largest practical mass range of carbon isotopes is utilized, resulting in relatively large isotope effects. The kinetic techniques involved in the $^{11}C/^{14}C$ method are quite easy to use. HPLC is a versatile technique which can be applied to many different systems. Moreover, no cumbersome workup or degradation of the samples is required. Neither are the analyses sensitive to unlabeled impurities, as long as these do not cause scintillation quenching. The $^{11}C/^{14}C$ method has recently been demonstrated to be useful in the determination of carbon[5] and deuterium[5b] isotope effects for some organic reactions. In the present study, the $^{11}C/^{14}C$ method described earlier[5a] was used with some modifications.

The synthesis of L-[β-^{11}C]tyrosine was performed using a multienzymatic reaction route;[6] see Scheme II. The radiochemical yield was 30% (decay corrected), and the total synthesis time, including purification, was usually 50 min. The same reaction route was used for the synthesis of L-(β-^{14}C)tyrosine, except that in the ^{14}C-labeling synthesis (^{14}C)methyl iodide was used as starting material.

The KIE experiments were performed under first-order reaction conditions. The conditions were chosen so that the reverse reaction was negligible. In the KIE experiments, the ^{11}C- and ^{14}C-labeled tyrosines were mixed with a standard solution of unlabeled tyrosine. The kinetic experiments were performed in the following way: equal volumes (400–1000 μL) of thermostated enzyme and substrate solutions[8] were mixed and replaced in the thermostat. The reaction mixture was agitated during the entire experiment. At time intervals, small volumes (50–100 μL) of the reaction mixture were withdrawn and immediately added to vials containing trifluoroacetic acid, cooled in an ice bath. During the first 10 min of the reaction, 10–15 samples were withdrawn from the

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(8) The tyrosine phenol-lyase stock solution, purified from *C. freundii*, had a protein concentration of 56.7 mg mL^{-1} and a specific activity of 1.2 (μmol of tyrosine) min^{-1} (mg of protein)$^{-1}$. The enzyme concentration was a 200-fold dilution of the stock solution containing 0.2 mM PLP, and the L-tyrosine concentration was 0.6 mM. The experiment was performed in phosphate buffer at pH 6.8 and 18 °C.

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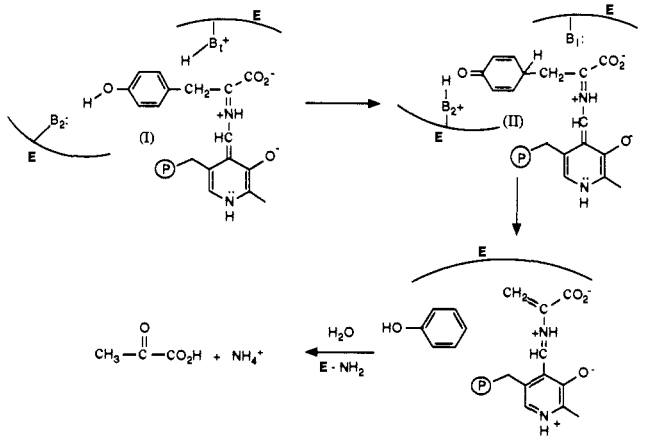

Figure 1. Part of the mechanism for the β-tyrosine phenol-lyase-catalyzed elimination reaction of tyrosine. P is a phosphate group and E the enzyme.

reaction mixture. The vials were then centrifuged at 5000 rpm for a few minutes; 5–10 μL of the quenched reaction mixtures were injected on an HPLC column,[9] and the separated labeled substrate and product were collected in liquid scintillation bottles. The radioactivity of the fractions was measured immediately, and after total decay of the ^{11}C, the ^{14}C-radioactivity was measured with a liquid scintillation counter. The ratio $k_{11}/k_{14} = \ln(1 - f_{11})/\ln(1 - f_{14})$, where f is the fraction of reaction, was calculated for each reaction point.

The results from three KIE experiments, performed in phosphate buffer pH 6.8 at 18 °C,[10] were 1.068 ± 0.017 (n = 10), 1.083 ± 0.013 (n = 11), and 1.051 ± 0.012 (n = 14), where n is the number of reaction points and the standard deviation is reported. The mean $^{11}C/^{14}C$ KIE value is 1.067 ± 0.009.

Several contributions to the elucidation of the mechanism of tyrosine phenol-lyase action have recently been reported.[11] For reviews, see, e.g., Snell and DiMari[12] and Miles.[13] The chemical mechanism involves the formation of an aldimine between L-tyrosine and pyridoxal 5-phosphate (PLP). The substrate α-proton is abstracted by an enzyme-bound base (see Figure 1; B_1, pK_a = 7.6[11c]) with the formation of a quinonoid structure (I). Another base (B_2, pK_a = 8.0[12c]) then abstracts the hydroxyl proton, and the first base (B_1) returns a proton to the aromatic C-4 position with the formation of a cyclohexadienone moiety (II). The activated carbon–carbon bond now breaks with simultaneous electron-push from the PLP, and electron-pull when the hydroxyl proton is returned by the base B_2. Phenol is released, and after transamination and hydrolysis, pyruvic acid and ammonia are released from the enzyme. In a study of the pH dependence of kinetic parameters and the primary deuterium KIE of tyrosine phenol-lyase from *C. freundii*, it was concluded that the α-proton abstraction was a partially rate-limiting step.[11c]

For the nonenzymatic malonic acid decarboxylation, a reaction in which carbon–carbon bond breaking (as in the present case) is accompanied by formation of a double bond to the isotopic carbon atom,[14] k_{12}/k_{14} for acid labeled in the 2-position was determined to be 1.076 by Ropp and Raaen.[15] Using the relation $\ln(k_{11}/k_{14})/\ln(k_{12}/k_{14}) \cong 1.6$[5b] between the different carbon isotope effects, the $^{12}C/^{14}C$ KIE corresponding to our value may be estimated to be 1.04. Our results, in combination with earlier conclusions,[11c] therefore suggest that the C–C bond breaking is at least partially rate limiting.

Acknowledgment. We thank Dr. Petter Malmborg for his help with the radionuclide production and Prof. Y. Watanabe for supplying the tyrosine phenol-lyase. This project is supported financially by the Swedish Natural Science Research Council.

(9) A RP C-18 column was used, and the mobile phase was 0.01 M acetic acid, pH 3.5: methanol (75:25, v/v) isocratic flow 2.0 mL min^{-1}.

(10) The optimum is pH 8, but because of a slow sampling technique, the rate of the reaction was decreased by lowering the pH and temperature.

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One-Pot Synthesis of Aromatic Methyl Esters by Electrochemical Oxidation of Aldehydes Mediated by Biscoenzyme Catalysis

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Received October 24, 1991

The direct oxidation of aldehydes to esters under mild conditions is a useful transformation in organic synthesis.[1] Here we describe an efficient, one-pot synthesis of aromatic methyl esters by electrochemical oxidation of aldehydes, mediated by two coenzyme catalysts: the thiazolium ions **1a/b** and flavin **MeFl**. The use of the macrocyclic catalyst **1b** in electroorganic synthesis elegantly combines the principles of electrocatalysis[2] with molecular recognition (Chart I).[3]

Thiazolium ions are known to catalyze the oxidation of aldehydes to esters.[4] The thiazolium ylide **2**[5] reacts to give the "active aldehyde" **3** (Scheme I).[6] This reactive intermediate can condense with another aldehyde to give an acyloin or can be oxidized to give the 2-acylthiazolium ion **4**. This ion reacts readily in alcohol[7] to give an ester (Scheme I). Stoichiometric amounts of oxidizing agents like nitrobenzene,[8] potassium ferricyanide,[9] and flavins[10] cause solubility problems and complicate product isolation. Also, the thiazolium catalyst is destroyed oxidatively in basic solution by ferricyanide,[9,11] iodine,[12] and air.[11,13] Attempts to regenerate catalytic amounts of **MeFl** with air resulted in the oxidation of **1a/b** by air (Table II, entry e).[13]

Our investigation by cyclic voltammetry shows that the anodic peak potential of **1a/b** in a 0.05 M solution of NEt_4Br in MeOH is ~0.2 V (vs Ag/AgCl at 0.02V s^{-1}), while that of **MeFl** is ca. −0.47 V ($E_{1/2}$ ca. −0.52 V at 0.02V s^{-1}). Under argon atmosphere,

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