and the mixture was stirred under nitrogen for 1 h. A solution of alkyl halide (4.5 mmol) in tetrahydrofuran (5 mL) was added to the mixture and stirred for 48 h. During workup the mixture was acidified (pH 2) with 2 N hydrochloric acid and the tetrahydrofuran was removed on the rotorvapor. The residue was transferred on to the membrane filter and washed with 50% methanol water mixture (2 × 200 mL) and water. The washings were tested for halide ions with silver nitrate solution. The coal samples were dried under vacuum (60 °C).

Alkylation Using Tosylates. A solution of the tosylate (4.5 mmol per gram of coal) in tetrahydrofuran was added to a suspension of coal in tetrahydrofuran containing an appropriate base (2.6 mmol per gram of coal). The reaction was carried out as already described except that the product was first washed with ether (50 mL per gram of coal) to remove the alkylation agent. When 2-phenylethyl tosylate and 2-[4-propylphenyl]ethyl tosylate were used, it was necessary to wash the product with 4 portions of ether to remove the alkylating agent. The coal residue was then washed with 50% methanol water mixtures and the washings were tested with barium chloride solution and sodium tetraphenylboron to test for the removal of sulfonates and tetrabutylammonium compounds. Anal. of the coal alkylated with methyl tosylate: C, 68.50; H, 5.25; S, 2.80; ash, 10.8.

C-Alkylation of Coal. The carbanions were prepared from O-methylated coal (3 g) with sodium amide (41 mmol) in liquid ammonia (150 mL) for 3 h. The alkyl halide (127 mmol) was added and the reaction mixture was stirred for an additional 6 h. The ammonia was allowed to evaporate under a slow stream of nitrogen and the residue was taken up in a mixture of tetrahydrofuran (50 mL) and water (50 mL) and neutralized using 6 N hydrochloric acid. The residue after evaporation of tetrahydrofuran was filtered and washed with 50% methanol water mixture (3–5 L) until the washings were free of halide ions. The coal sample was dried under vacuum (65 °C) and then transferred under nitrogen into a flask containing freshly distilled collidine (35 mL per gram of coal) and lithium iodide (2.1 g per gram of coal). The mixture was refluxed under nitrogen for 48 h and then collidine was distilled under vacuum. The residue was acidified (pH 4), filtered through a membrane filter, and washed with 50% methanol water until washings were free of any halide ion. Anal. of C-methylated coal: C, 69.46; H, 5.29; ash, 8.82.

Enzyme-Catalyzed Synthesis of L-Acetylcarnitine and Citric Acid Using Acetyl Coenzyme A Recycling

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Natural products chemists have developed extensive methodology to construct complex organic compounds. A great deal of effort has been directed at stereoselective bond formation to produce optically pure materials. Enzymes are involved in virtually every reaction occurring in the biosynthesis of natural products; however, they have been used only recently for large scale synthesis of organic compounds. Enzymes offer many advantages for synthesis; they are selective, are usually stereospecific, and operate at ambient temperature and pressure.[1] The most widely used enzymes are the isomerases and hydrolases.[2] There

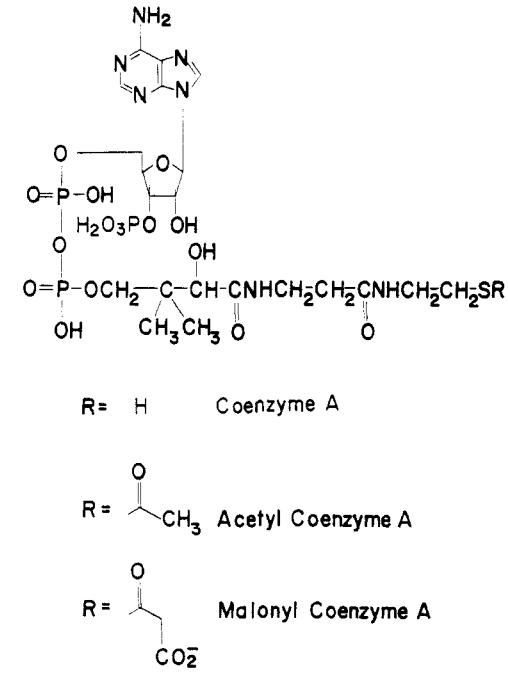

Figure 1.

is great interest in using enzymes to form carbon–carbon bonds, because complex molecules can be prepared from simpler starting materials. Very few examples of enzymatic carbon–carbon bond-forming reactions have been reported.

A large percentage of enzymes require cofactors such as XTP (X = A, C, G), NAD(P)(H), and coenzyme A (CoA). The cost of these cofactors precludes their use in large scale synthesis if stoichiometric quantities of cofactor are required. In order to make synthesis with cofactor-requiring enzymes economically feasible, the cofactors have to be regenerated and recycled in situ. Successful efforts have been made to recycle ATP[3] and NAD(P)(H),[4] but little effort has been made to recycle CoA to furnish isolable quantities of product. CoA and its derivatives (Figure 1) are the most expensive of the above cofactors (CoA - $400 000/kg, acetyl-CoA - $1 400 000/kg, based on Sigma catalog prices) and are useful for activating substrates toward carbon–carbon and carbon–oxygen bond formation. Acetyl-CoA is a common cellular currency for acetyl transfers and is involved in the citric acid cycle, fatty acid metabolism, glucose metabolism, and malic acid production. CoA and its derivatives are therefore indispensable for many reactions that involve stereospecific bond formation and complex molecule construction. Synthesis of organic compounds using CoA dependent enzymes will become practical only if these CoA thio esters can be recycled. We report here our successful efforts in recycling CoA and acetyl-CoA in synthesis.

As a demonstration of acetyl-CoA recycling, we chose to examine the enzyme-catalyzed aldol condensation of

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(2) Selected examples include: (a) Abbot, B. J. *Adv. Biochem. Eng.* **1979**, *12*, 41. (b) Chibata, I.; Tosa, T.; Sato, T. *Methods Enzymol.* **1976**, *44*, 739. (c) Strandberg, G. W.; Smiley, K. L. *Appl. Microbiol.* **1971**, *21*, 588. (d) Cambou, B.; Klibanov, A. M. *J. Am. Chem. Soc.* **1984**, *106*, 2687. (e) Laumen, K.; Reimerdes, E. H.; Schneider, M. *Tetrahedron Lett.* **1985**, *26*, 407.

(3) (a) Pollak, A., Baughn, R. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1977**, *99*, 2366. (b) Rios-Mercadillo, V. M.; Whitesides, G. M. *J. Am. Chem. Soc* **1979**, *101*, 5828.

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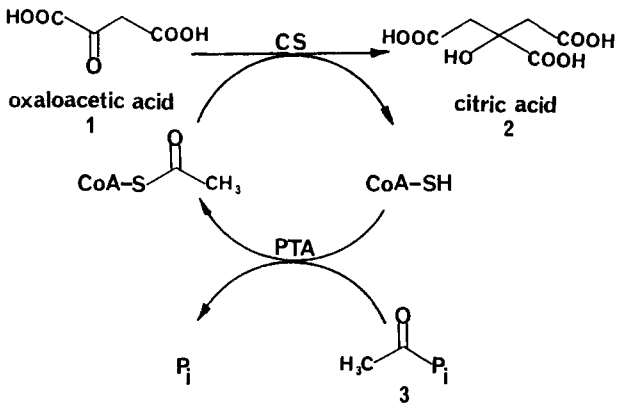
Scheme I[a]



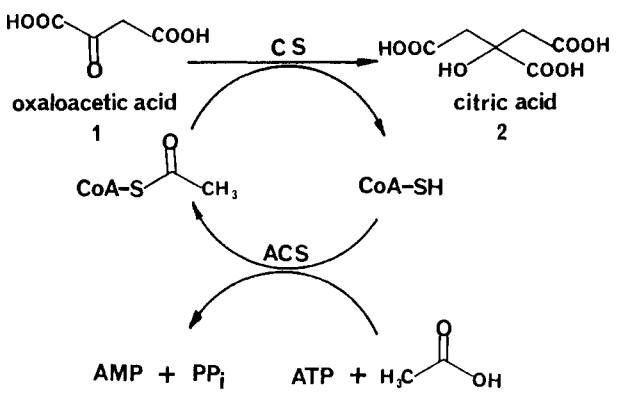
[a] PTA, phosphotransacetylase (E.C. 2.3.1.8); CS, citrate synthase (E.C. 4.1.3.7).

acetyl-CoA with oxaloacetic acid (1) to form citric acid (2). This reaction is catalyzed by the enzyme citrate synthase. Our choice of reaction was based on the ready availability of citrate synthase and the high equilbrium constant for the formation of citric acid.

Two methods of acetyl-CoA regeneration have been employed as shown in Schemes I and II. In Scheme I phosphotransacetylase (PTA, E.C. 2.3.1.8) catalyzes the acetylation of CoA with acetyl phosphate to furnish the acetyl-CoA used in the aldol condensation. The byproduct of citric acid formation is CoA which is then reacetylated by PTA. A similar recycling scheme has been used on an assay scale to quantitate CoA.[5] Acetyl phosphate (3) and oxaloacetic acid are unstable to hydrolysis and decomposition in solution under the reaction conditions and were therefore added in aliquots during the reaction period.[6] The progress of the reaction was monitored by following the formation of citric acid with proton NMR using methanol as an internal standard. A small scale reaction furnished an acetyl CoA recycling of 11 800. The recycling number is calculated from the molar ratios of citric acid produced to CoA used. In a larger scale reaction, 860 mg of citric acid was synthesized with a recycling number of 560. The high ionic strength of the reaction mixture and the use of catalytic amounts of CoA affect the enzyme activity such that the enzymes do not operate at their maximum velocity. Hence a considerable amount of enzyme activity is required to obtain the desired amount of product in a reasonable time period. For practical purposes, it is desirable to use immobilized enzymes so that the enzyme activity can be recovered after the reaction. PTA and citrate synthase can be immobilized either separately or together on a PAN[7] [poly(acrylamide-co-*N*-(acryloxy)succinimide)] gel. We observed similar amounts of product formation in both cases. At the end of the reaction the enzyme activity is recovered by centrifuging and washing the gel. The enzyme retains 80% of its activity after 3 days in the reaction mixture at 40 °C and 15 days of storage at 0–4 °C.

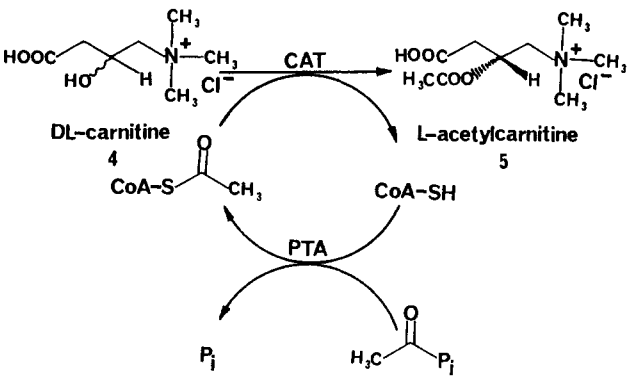
The second method of acetyl-CoA recycling is shown in Scheme II and uses the enzyme acetyl-CoA synthetase (E.C. 6.2.1.1). When this enzyme is used, acetic acid and ATP replace the easily hydrolyzed acetyl phosphate. This

Scheme II[a]



[a] ACS, acetyl-CoA synthetase (E.C. 6.2.1.1); CS, citrate synthase (E.C. 4.1.3.7).

Scheme III[a]



[a] PTA, phosphotransacetylase (E.C. 2.3.1.8); CAT, carnitine acetyltransferase (E.C. 2.3.1.7).

alternative reaction has furnished a recycling number of 1000.

This successful demonstration of acetyl-CoA recycling on a multimillimole scale prompted us to examine a practical use for the method. L-Carnitine (4) is used clinically in the treatment of lipid storage myopathy.[8] Present methods of preparing L-carnitine are expensive and involve chemical synthesis and enantiomer resolution.[9] Alternative methods have been reported recently, most notably a combined chemical–microbiological synthesis by Sih and co-workers.[10] Carnitine acetyltransferase (E.C. 2.3.1.7) catalyzes the acetylation of carnitine with acetyl-CoA and is specific for the L isomer.[11] Using acetyl CoA recycling, we have prepared pure L-acetylcarnitine (5) from DL-carnitine. Since L-acetylcarnitine can be easily hydrolyzed to L-carnitine, this route offers a simple and practical enzymatic method for resolving DL-carnitine. Scheme III shows the reactions used for the synthesis of millimolar quantities of L-acetylcarnitine. In this reaction acetyl-CoA recycling numbers ranged from 400 to 2500. The equilibrium constant of the reaction leading to the formation of acetylcarnitine is 1.67,[12] so that only 60% of the L-carnitine is converted to L-acetylcarnitine. L-Acetylcarnitine is separated from carnitine by using alu-

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(6) The neutralized solutions of acetyl phosphate and oxaloacetate were kept frozen between additions to the reaction mixture.
(7) Pollak, A.; Blumenfeld, H.; Wax, M.; Baughn, R. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1980**, *102*, 6324.
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(9) Fraenkel, G.; Friedman, S. *Vitam. Horm. (N.Y.)* **1957**, *16*, 73.
(10) Zhou, B. N.; Gopalan, A. S.; Van Middlesworth, F.; Shieh, W. R.; Sih, C. J. *J. Am. Chem. Soc.* **1983**, *105*, 5925.
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(12) Fritz, I. B.; Schultz, S. K.; Srere, P. A. *J. Biol. Chem.* **1963**, *238*, 2509.

minum oxide chromatography. Optical purity was determined by proton NMR using the chiral shift reagent tris[3-((trifluoromethyl)hydroxymethylene)-*d*-camphorato]europium (III)[13] and showed no detectable amounts of the other enantiomer.

In summary, we have shown that CoA and acetyl-CoA can be used to generate millimole quantities of product using enzyme-catalyzed carbon–carbon bond-forming and esterification reactions. While recycle numbers of 11 800 have been achieved, the high cost of CoA requires further optimization of this system except for very high valued products (>$100/mol). Further studies are in progress to apply this method to the production of fatty acids and polyketide-derived natural products.

Experimental Section

Proton NMR were recorded on a 300-MHz Bruker instrument. A coaxial NMR tube (Wilmad) was used to record the spectra. The inner 5 mm tube was filled with the reaction mixture in water and the outer 10 mm tube with D_2O as the locking solvent. A Waters HPLC instrument with reverse phase C-18 column was used to monitor the reaction and assay the enzymes.

Oxaloacetic acid, DL-carnitine, and tris[3-((trifluoromethyl)-hydroxymethylene)-*d*-camphorato]europium(III) were purchased from Aldrich. Acetyl phosphate, phosphotransacetylase, citrate synthase, and carnitine acetyltransferase were purchased from Sigma. PAN was prepared by a reported procedure.[7]

Acetyl-CoA Recycling: Synthesis of Citric Acid (Scheme I). A typical small scale reaction was carried out as follows: Acetyl phosphate K^+, Li^+ salt (55 mg, 0.3 mmol) and oxaloacetic acid (40 mg, 0.3 mmol) [each dissolved separately in 0.5 mL of 0.5 M Tris buffer of pH 7.5 and neutralized with 2 M Tris base to pH 7.8] were added in small aliquots (0.1 mL) to the reaction mixture consisting of CoA (1.24×10^{-5} mmol), dithiothreitol (6×10^{-4} mmol), ammonium sulfate (1×10^{-2} mmol), and the enzymes phosphotransacetylase (E.C. 2.3.1.8) (500 U) and citrate synthase (E.C. 4.1.3.7) (200 U) in Tris buffer pH 7.5 at 40 °C. (Final volume of the reaction was 1.5 mL). The reaction was carried out under N_2 at 40 °C. The presence of CoA was followed by HPLC,[14] and the progress of the reaction was monitored by the formation of citrate using ^1H NMR. After 7 days, 0.147 mmol of citrate was formed as determined from ^1H NMR using methanol as an internal standard. This corresponds to a recycling number of 11 800 for acetyl-CoA. The reaction was repeated on a larger scale by using acetyl phosphate (0.9 g, 5.0 mmol), oxaloacetate (0.66 g, 5.0 mmol), CoA (7.3×10^{-3} mmol), 1500 U of phosphotransacetylase, and 500 U of citrate synthase in a total volume of 40 mL of 0.5 M Tris buffer pH 7.8. After 7 days citric acid (0.86 g, 4 mmol) was formed corresponding to a recycling of 560 for acetyl-CoA. Citric acid was purified from the reaction mixture as follows: The reaction mixture was acidified to pH 1 and lyophilized to a white powder which was extracted with ethyl acetate (4 × 20 mL). Ethyl acetate was removed under reduced pressure to give an oil containing 80% citric acid as determined by ^1H NMR. The remaining material consisted entirely of Tris base.

Acetyl-CoA Recycling Using Immobilized Enzymes. The above procedure was repeated using PAN immobilized[7] phosphotransacetylase (50 U) and citrate synthase (50 U). Acetyl-CoA was recycled 400 times to furnish 80 mg of citric acid after 3 days of reaction. At the end of the reaction, the enzyme activity was recovered by centrifuging the gel and washing with 0.5 M Tris buffer pH 7.4. The enzymes were assayed using HPLC[14] and immobilized PTA retained 80% of its activity after 3 days in the reaction mixture at 4 °C and 15 days storage at 0–4 °C.

Acetyl-CoA Recycling: Synthesis of L-Acetylcarnitine (Scheme III). DL-Carnitine (1 g, 5 mmol) and acetyl phosphate (0.5 g, 2.7 mmol) [each dissolved separately in 1 mL of 0.5M Tris buffer of pH 7.5 and neutralized with 2 M Tris base of pH 7.8 at 25 °C] were added to CoA (3.3×10^{-3} mmol), dithiothreitol (0.03 mmol), manganese sulfate (0.01 mmol), and the enzymes phosphotransacetylase (200 U) and carnitine acetyltransferase (2.3.1.7, 160 U) in 0.5 M Tris buffer in a round-bottomed reaction flask (total volume 3.3 mL). The reaction mixture was purged with argon and stirred at 40 °C. After 12 h, the reaction reached equilibrium with 60% conversion of L-carnitine to L-acetylcarnitine as determined from the 300-MHz proton NMR. The trimethylammonium protons of carnitine appears at 3.0 ppm and those of acetylcarnitine at 2.96 ppm. The ratio of these peaks for the conversion corresponds to a recycling number of 420 for acetyl coenzyme A. A similar small scale reaction with lesser amounts of coenzyme A and kept for longer periods has furnished a recycling number of 2500 for acetyl coenzyme A. L-Acetylcarnitine was purified from DL-carnitine by aluminum oxide preparative chromatography developed with 14:5:1 methylene chloride/methanol/ammonium hydroxide. Its optical purity was confirmed by proton NMR using the chiral shift reagent, tris-[3-((trifluoromethyl)hydroxymethylene)-*d*-camphorato]europium(III). When an equimolar quantity of the chiral shift reagent was added to DL-acetylcarnitine, resolution of the two enantiomers was observed on the proton NMR. The acetyl group appears as a doublet at 2.06 ppm and the trimethylammonium group as a doublet at 3.4 ppm. Addition of the chiral shift reagent to the acetylcarnitine purified from the above reaction showed only one isomer in the proton NMR.

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Registry No. 1, 328-42-7; 2, 77-92-9; 4, 406-76-8; 5, 3040-38-8; PTA, 9029-91-8; CoA, 85-61-0; CoA-SAc, 72-89-9; E.C.6.2.1.1, 9012-31-1; E.C.4.1.3.7, 9027-96-7; E.C.2.3.1.7, 9029-90-7.

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(14) HPLC was done on a Versapack C-18, 10-μm column using potassium phosphate (0.22 M), 12% methanol buffer, pH 4 as th eluant.

Carbon–Carbon Bond Fragmentation through Oxidative Electrolysis of Carboxylic Acids and Its Application to the Synthesis of Malyngolide

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The pioneering work of Corey[1] established a paradigm for oxidative electrolysis of γ-hydroxy acid **1** which proceeds through initial carbonium ion formation followed by fragmentation to form an oxygen stabilized cation **2**. Loss of a proton, then gives keto olefin **3** (Scheme I).

This paradigm and the expectation that ketals should exert a stronger carbocation stabilizing effect led us to examine the oxidative electrolysis of γ-ketal carboxylic acid **4** to olefinic ester **5** (Scheme II).

We now wish to report the success of this design which represents the first case in which a ketal stabilized carbocation participates in an electrolytically induced carbon–carbon bond fragmentation.

The desired substrate **4** was readily prepared from 3-methoxybenzoic acid (**6**). Reductive alkylation[2] with 1-bromononane gave acid **7**, which upon distillation cyclized to lactone **8** in 82.8% overall yield. Hydrogenation, methanolysis, and ketalization secured ester **9** in 98.0% yield. Basic hydrolysis gave acid **4** in 98.8% (Scheme III).

With acid **4** in hand we examined the electrolysis under a variety of conditions. Experimentally, all electrolysis reactions were run at 50 applied V DC at 0.5 ampere be-

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