tively dilute solution, have characteristic frequencies of the order of 0.2 kHz.[24] Accordingly, whole molecule reorientation can be eliminated as a source for this greater than 100 000-dalton polymer at its very viscous coacervate concentration where there is less than 70% water. This leaves the rocking motion of the peptide moieties as the source of the 25-MHz relaxation. Direct nuclear magnetic relaxation studies on peptide carbonyls of elastic fiber[25] containing the polypentapeptide and of the polypentapeptide coacervate itself[26,27] provide estimates in the 10-ns range for the carbonyl carbon correlation time. The relaxation, therefore, is readily assigned to a peptide libration mode in the polypentapeptide of elastin.

The peptide librational mode builds in intensity (see Figure 2A) over the same temperature range that has been identified as the interval wherein an inverse temperature transition occurs which leads to an increase in order of the polypentapeptide with increases in temperatre.[28] This, of course, is confirmed by the present data. Rather than a broad distribution of correlation times characteristic of a distribution of conformations, a single correlation time is found with the relaxation being well fit by the Debye equation or by a single Cole–Cole term in which $\alpha = 0$. In fact the drop in the background curve with increasing temperature, followed as ϵ(1 GHz) vs. temperature (see Figure 2B), taken together with the simultaneous building of intensity of the relaxation near 25 MHz, becomes a new demonstration of an inverse temperature transition. Below 20 °C, the perspective is one of pentamers that exhibit rocking motions with a range of different correlation times derived from a dispersity of conformational states. Above 30 °C, the pentamers become arranged in a regular manner to give a single characteristic correlation time. The single correlation time represents a common dynamic conformational state for the pentamers. Therefore the polypentapeptide is describable as a regular, nonrandom array of pentamers at 40 °C.

To our knowledge this is the first demonstration of a peptide librational mode and its relevance to the entropic component of the elastomeric force of cross-linked polypentapeptide will treated in a future report.

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Stereochemical Control of Yeast Reductions. 5. Characterization of the Oxidoreductases Involved in the Reduction of β-Keto Esters[1]

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Recently, we demonstrated the feasibility of altering the stereochemical course of reduction of 4-chloroacetoacetic esters by bakers' yeast via modification of the size of the ester substituent.[2] In this paper, we examine the underlying factors governing this striking stereochemical observation. We now report the isolation of three dominant competing β-keto oxidoreductases and show a distinct correlation of the ester substituent with the specificity constant,[3,4] k_{cat}/K. Moreover, ethyl (*R*)-4-chloro-3-hydroxybutanoate (**5**) of high optical purity (ee = 0.90) may now be prepared by using a mutant of *S. cerevisiae* lacking one of the competing enzymes of opposite stereochemical preference.

Table I. Kinetic Constants of β-Keto Reductases of Bakers' Yeast

$ClCH_2C(=O)$-$CH_2C(=O)OR$	fatty acid synthetase K, mM	fatty acid synthetase k_{cat}, s^{-1}	D enzyme K, mM	D enzyme k_{cat}, s^{-1}	L enzyme K, mM	L enzyme k_{cat}, s^{-1}
R = C_2H_5	1.82	303	1.00	0.21	1.00	6.60
R = C_4H_9	1.33	202	0.10	0.11	0.094	7.13
R = C_6H_{13}	1.82	252	0.20	0.23	0.028	6.87
R = C_8H_{17}	1.60	69	0.29	0.47	0.01	6.12

We have purified three enzymes to homogeneity from the cytosolic fraction of bakers' yeast (Red Star) capable of actively reducing 4-chloroacetoacetic esters to yield corresponding carbinolic products of high optical purity[5] (>0.97 ee). All of them utilize NADPH preferentially as the coenzyme. One of these enzymes (MW 2 400 000) possesses physical and chemical properties reminiscent of fatty acid synthetase[6] and reduces β-keto esters to yield carbinols of D configuration.[7] Although the natural substrates for the other two enzymes have not yet been defined they may be readily resolved on a hydroxyapatite column. The faster moving protein (D enzyme) has an MW of 38 000 and reduces β-keto esters to yield D carbinolic products, whereas L enzyme (MW 74 000) affords carbinols of L configuration. Because mammalian L-3-(hydroxyacyl)-CoA dehydrogenase[8] (EC 1:1:35) in the presence of NADH reduced 4-chloroacetoacetic esters to (*R*)-4-chloro-3-hydroxybutanoates of high enantiomeric excess (>0.97), we carefully searched for this reductase activity in bakers' yeast. While mitochondrial fractions of bakers' yeast actively reduced acetoacetyl-CoA, only a trace of reductase activity was detectable using either **1** or **3** as substrates. This observation indicates that L-3-(hydroxyacyl)-CoA dehydrogenases of different species have marked differences in substrate specificities.[9]

To gain an insight into the influence of the ester substituent on the enantioselective reduction of γ-chloro-β-keto esters by intact bakers' yeast, the k_{cat} (turnover number) and K (Michaelis constant) for the three enzymes on various 4-chloroacetoacetic esters were measured (Table I).

A moderate decrease in k_{cat}/K[4] is noted for fatty acid synthetase as the ester grouping is enlarged (Figure 1). The more pronounced drop in k_{cat}/K for the octyl ester is attributable to a decrease in the value of k_{cat} (Table I). More dramatic is the increase in the value of k_{cat}/K as the ester grouping is changed from ethyl to octyl for the L enzyme (Figure 1). Interestingly, this increase is due to a decrease in K (Table I). These complementary relationships between ester substituents and specificity constants for the two competing enzymes are compatible with the observed stereochemical results of resting bakers' yeasts. That is, **1** is reduced predominantly to **2** whereas **3** is reduced to **4**.

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(7) Because all three enzymes reduce β-keto esters and 4-chloro-β-keto esters, the L and D designation is preferred.
(8) Sigma H4626, type III from porcine heart.
(9) Yeast alcohol dehydrogenase in the presence of NADH is unable to reduce ethyl 4-chloroacetoacetate, whereas horse liver alcohol dehydrogenase readily reduced this substrate.

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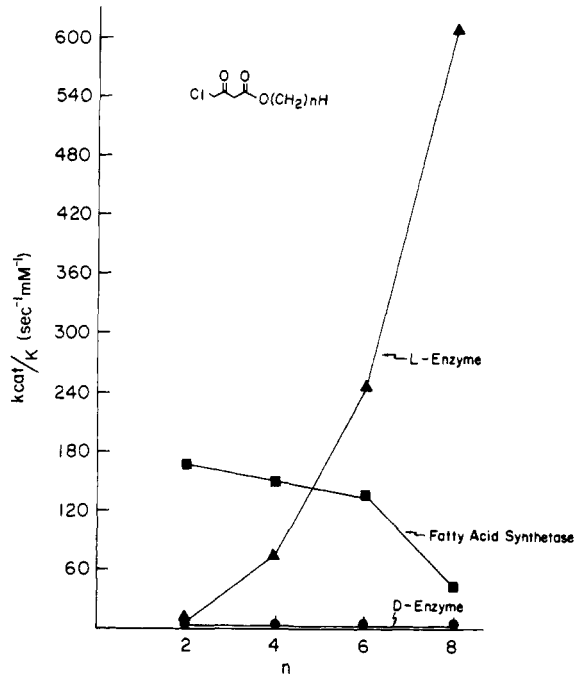
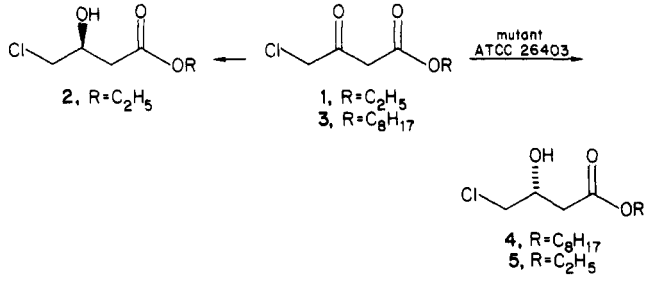


Figure 1. Relationship of specificity constants and ester substituents.

Because mutants of *S. cerevisiae* devoid of various components of the fatty acid synthetase complex had been prepared,[10] we envisaged that one of these mutants may be suitably used for the synthesis of **5**, a requisite intermediate for L-carnitine synthesis.[2] To test this supposition, **1** was exposed to two mutants of *S. cerevisiae*, ATCC 26403 (β-keto reductase negative) and ATCC 26404 (pantotheine free). As expected, the latter mutant behaved like the parent and afforded **2** (ee = 0.57) in 50% yield. In contrast, mutant ATCC 26403 gave indeed the desired **5** in 55%



yield but the optical purity of the product was low[11] (ee = 0.16). Nevertheless, these results confirm the notion that the β-keto reductase component of the fatty acid synthetase complex is indeed a major competing enzyme engaged in the reduction of β-keto esters in vivo. Moreover, the successful prediction of the stereochemical outcome encouraged us to make further refinements. By taking maximal advantage of the differences in the k_{cat}/K[12] of the L and D reductases, **1** was continuously fed to a growing culture of mutant ATCC 26403 at a slow rate.[13] Under these conditions, the optical purity of the desired **5** obtained after 72 h (53% yield) was enhanced markedly (ee = 0.90).

Acknowledgment. This investigation was supported in part by grants from the University of Wisconsin Graduate School and the 3M Foundation.

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(12) Most enzymes are not saturated with substrate [S] under physiological conditions (the ratio of $[S]/K$ is in the range of 0.01-1.0). Hence the relative rates of enantioselective reduction by two competing enzymes in intact cells depends on the V/K (first-order rate constant) ratio of the two enzymes, ee = $(V_L/K_L) - (V_D/K_D)/(V_L/K_L) + (V_D/K_D)$; see: Chen, C. S.; Zhou, B. N.; Girdaukas, G.; Shieh, W. R.; VanMiddleswroth, F.; Sih, C. J. *Biorg. Chem.* **1984**, *12*, 98.

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Synthesis and Structural Characterization of $Co(NO)_2[PhP(OCH_2CH_2)_2NH]Cl$: A Novel Carbon Dioxide Carrier

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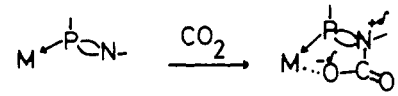
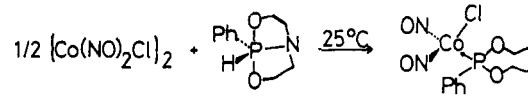
In the last decade, interest in the coordination chemistry of CO_2 has continued to grow.[1] Several CO_2-transition metal complexes, definitively characterized, point out different bonding modes for CO_2.[2]

This paper describes a novel mode of CO_2 coordination which involves, as the primary bonding site, a nucleophilic center located on a ligand of a metal complex. The oxophilic character of the metal center contributes to the stabilization of the adduct (eq 1).



(1)

The reaction of $[Co(NO)_2Cl]_2$[3] with the bicyclic phosphorane[4] **1** affords the monomeric complex **2**[5] (eq 2). In this complex,



(2)

1 **2**

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