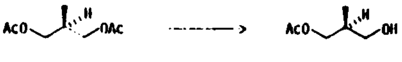
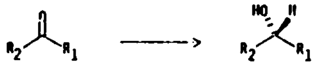
BIOCHEMICAL ASYMMETRIC CATALYSIS

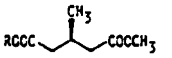
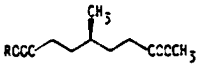
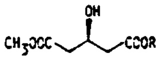
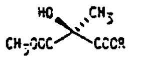
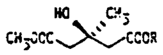
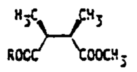
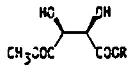
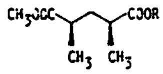
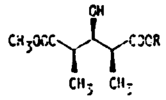
1) Enantiotopic group differentiation (Esterases)



2) Enantioface discrimination (Oxido-reductases)



PLE-CATALYZED HYDROLYSIS OF DIESTERS

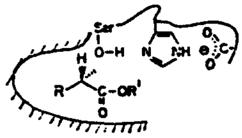
Substrate (R = CH_3) Product (R = H)	% e.e.
	90
	10
	12 (100)
	46
	99 (≈ 100)
	18
	48
	60
	98

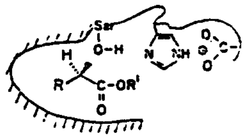
1) There are only a limited number of commercial enzymes suitable for synthetic applications.

2) Most enzymes lack high stereochemical specificity towards many substrates.

3) Enzymes of opposite stereochemical preference are often present in commercial preparations and microorganisms.

$$\begin{tikzcd}
 & Enz\cdot S \arrow[r, shift left, "k_2"] \arrow[dl, shift left, "k_{-1}"] & Enz\cdot S^* \arrow[l, shift left, "k_{-2}\cdot ROH"] \arrow[r, shift left, "k_3\cdot H_2O"] & Enz\cdot P \arrow[l, shift left, "k_{-3}"] \arrow[r, shift left, "k_4"] & Enz+P \arrow[l, shift left, "k_{-4}"] \\
Enz \arrow[ur, shift left, "k_1S"] \arrow[dr, shift left, "k_1'S'"] & & & & \\
 & Enz\cdot S' \arrow[r, shift left, "k_2'"] \arrow[ul, shift left, "k_{-1}'"] & Enz\cdot S'^* \arrow[l, shift left, "k_{-2}'\cdot ROH"] \arrow[r, shift left, "k_3'\cdot H_2O"] & Enz\cdot P' \arrow[l, shift left, "k_{-3}'"] \arrow[r, shift left, "k_4'"] & Enz+P' \arrow[l, shift left, "k_{-4}'"]
\end{tikzcd}$$





Maximal Enantioselection

$$\begin{tikzcd}
 & Enz\cdot S \arrow[r, shift left, "k_2"] \arrow[dl, shift left, "k_{-1}"] & Enz\cdot S^* \arrow[l, shift left, "k_{-2}"] \arrow[r, shift left, "k_3"] & Enz\cdot P \arrow[l, shift left, "k_{-3}"] \arrow[r, shift left, "k_4"] & Enz + P \arrow[l, shift left, "k_{-4}"] \\
Enz \arrow[ur, shift left, "k_1S"] \arrow[dr, shift left, "k_1'S'"] & & & & \\
 & Enz\cdot S' \arrow[r, shift left, "k_2'"] \arrow[ul, shift left, "k_{-1}'"] & Enz\cdot S'^* \arrow[l, shift left, "k_{-2}'"] \arrow[r, shift left, "k_3'"] & Enz\cdot P' \arrow[l, shift left, "k_{-3}'"] \arrow[r, shift left, "k_4'"] & Enz + P' \arrow[l, shift left, "k_{-4}'"]
\end{tikzcd}$$

$$E' = \frac{[P]}{[P']} = \frac{k_2\cdot[Enz\cdot S]}{k_2'\cdot[Enz\cdot S']} = \frac{k_2}{k_2'}\cdot K_{eq}; \qquad K_{eq} = \frac{k_1/k_{-1}}{k_1'/k_{-1}'}$$

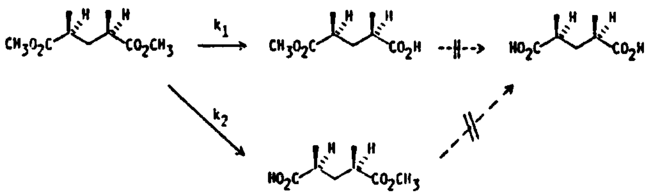
Partial Enantioselection

$$\begin{tikzcd}
 & Enz\cdot S \arrow[r, shift left, "k_2"] & Enz\cdot S^* \arrow[l, shift left, "k_{-2}"] \arrow[r, shift left, "k_3"] & Enz\cdot P \arrow[l, shift left, "k_{-3}"] \arrow[r, shift left, "k_4"] & Enz + P \arrow[l, shift left, "k_{-4}"] \\
Enz \arrow[ur, "k_1S"] \arrow[dr, "k_1'S'"'] & & & & \\
 & Enz\cdot S' \arrow[r, shift left, "k_2'"] & Enz\cdot S'^* \arrow[l, shift left, "k_{-2}'"] \arrow[r, shift left, "k_3'"] & Enz\cdot P' \arrow[l, shift left, "k_{-3}'"] \arrow[r, shift left, "k_4'"] & Enz + P' \arrow[l, shift left, "k_{-4}'"]
\end{tikzcd}$$

$$E' = \frac{[P]}{[P']} = \frac{k_1}{k_1'}$$

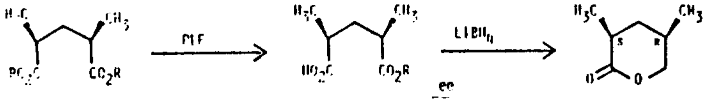
Enantioselective properties of enzymes may be changed by

1) pH
2) Temperature
3) Substrate modification
4) Protein modification



$$\text{Relative rate: } \alpha = \frac{k_1}{k_2}$$

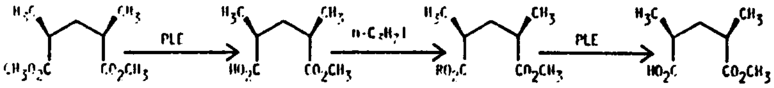
$$\text{Optical purity: } \beta = \frac{(\alpha - 1)}{(\alpha + 1)}$$



1 R = CH_3
2 R = C_2H_5
3 R = n-C_3H_7

ee
4 R = CH_3 0.60
5 R = C_2H_5 0.63
6 R = n-C_3H_7 0.83

7

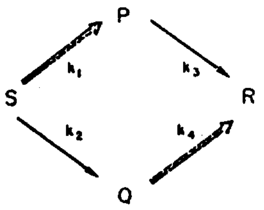


ee = 0.64

R = n-C_3H_7

ee = 0.85

[70% conversion, 57% yield]

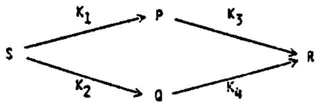


$k_1 > k_2$

$k_4 > k_3$

$$k_1 + k_2 = \frac{k_{cat(s)}}{K_s}\ ; \quad k_3 = \frac{k_{cat(p)}}{K_p}\ ; \quad k_4 = \frac{k_{cat(Q)}}{K_Q}$$

k_{cat} = turnover number
K = Michaelis constant



$$P = \frac{\alpha S_0}{(\alpha + 1)(1 - E_1)}\left[\left(\frac{S}{S_0}\right)^{E_1} - \left(\frac{S}{S_0}\right)\right]$$

$$Q = \frac{S_0}{(\alpha + 1)(1 - E_2)}\left[\left(\frac{S}{S_0}\right)^{E_2} - \left(\frac{S}{S_0}\right)\right]$$

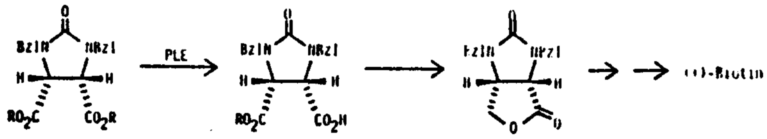
$$R = S_0 - S - P - Q$$

$$E_1 = \frac{\ln\left\{\frac{P_2}{P_1}\left[\left(\frac{S_1}{S_0}\right)^{E_1} - \left(\frac{S_1}{S_0}\right)\right] + \left(\frac{S_2}{S_0}\right)\right\}}{\ln\left(\frac{S_2}{S_0}\right)}$$

$$E_2 = \frac{\ln\left\{\frac{P_2}{P_1}\left[\left(\frac{S_1}{S_0}\right)^{E_2} - \left(\frac{S_1}{S_0}\right)\right] + \left(\frac{S_2}{S_0}\right)\right\}}{\ln\left(\frac{S_2}{S_0}\right)}$$

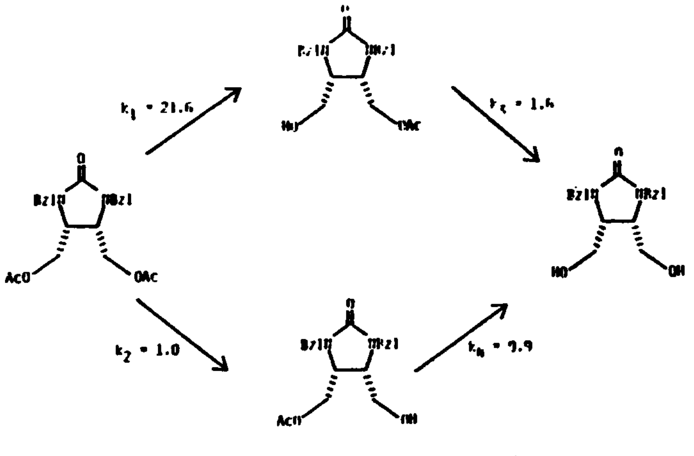
$$\alpha = \frac{P_1(1 - E_1)}{S_0\left[\left(\frac{S_1}{S_0}\right)^{E_1} - \left(\frac{S_1}{S_0}\right)\right] - P_1(1 - E_1)}$$

$$\alpha = \frac{K_1}{K_2} \qquad E_1 = \frac{K_3}{K_1 + K_2} \qquad E_2 = \frac{K_4}{K_1 + K_2}$$

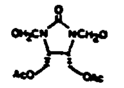


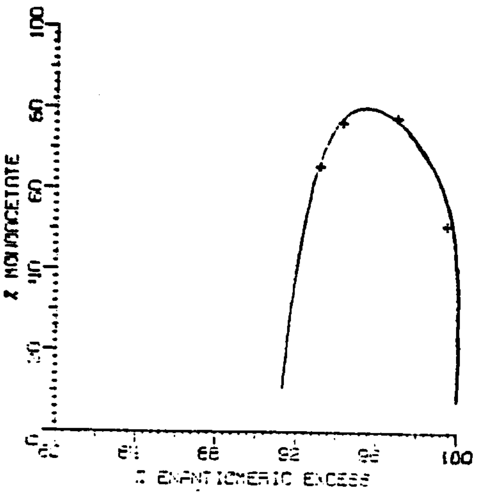
R = CH_3, ee = 0.39, 71%
R = n-C_3H_7, ee = 0.75, 85%

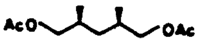
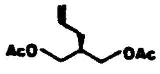
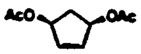
S. Iriuchijima et al., Agri. Biol. Chem. 46, 1907 (1982).

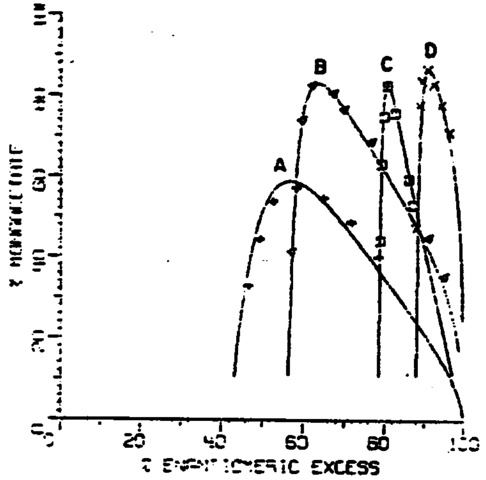


$\alpha = \frac{k_1}{k_2} = 21.6$ $E_1 = \frac{k_3}{k_1 + k_2} = 0.07$ $E_2 = \frac{k_4}{k_1 + k_2} = 0.44$

			α	E_1	E_2
Pig liver esterase		pro-R	21.6	0.07	0.44

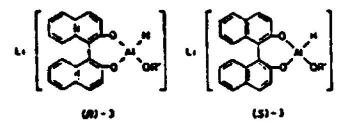


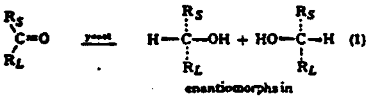
Pig liver esterase		pro-R	2.47	0.216	0.601
Pig pancreatic lipase		pro-R	3.60	0.041	0.152
Pig liver esterase		pro-R	8.44	0.058	0.120
Pig pancreatic lipase		pro-S	15.6	0.036	0.179



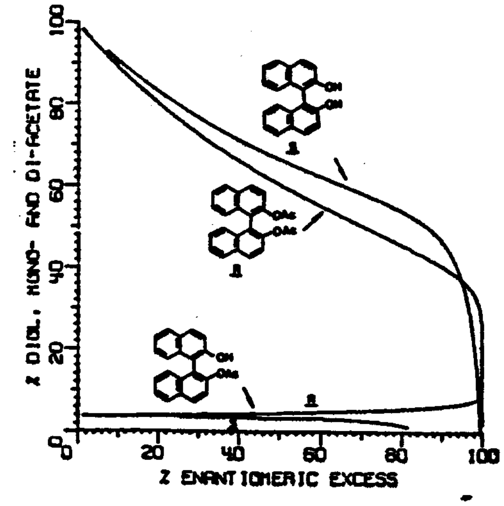
$$A \xrightarrow[\text{(fast)}]{k_1} P \xrightarrow[\text{(fast)}]{k_2} R$$

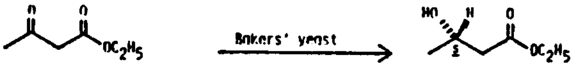
$$B \xrightarrow[\text{(slow)}]{k_3} Q \xrightarrow[\text{(slow)}]{k_4} S$$



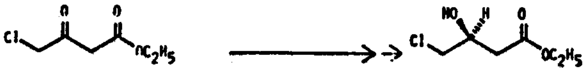


enantiomorphs in
unequal amounts

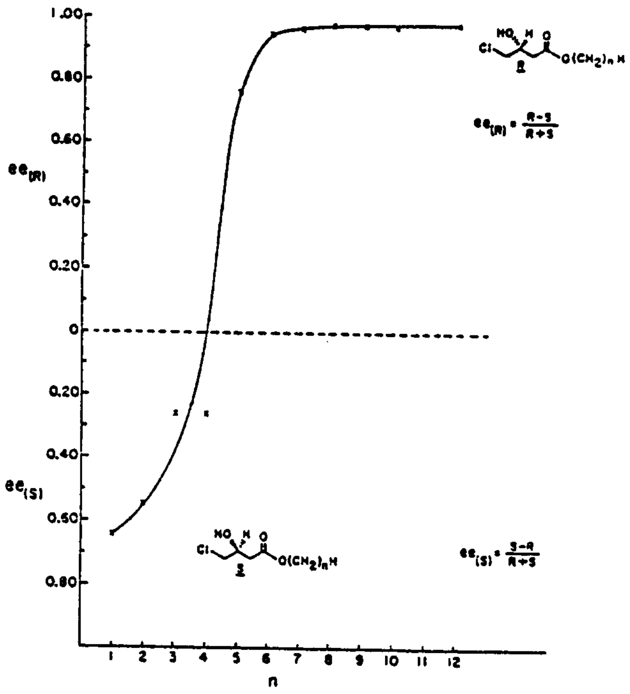


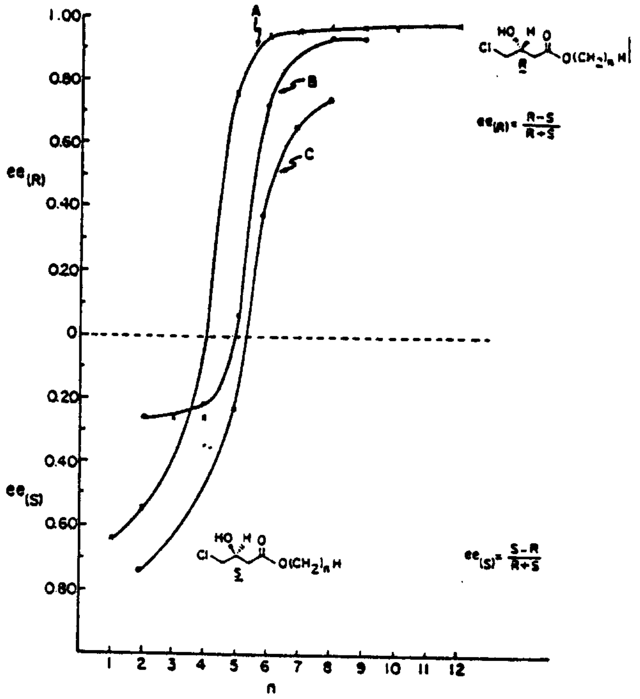


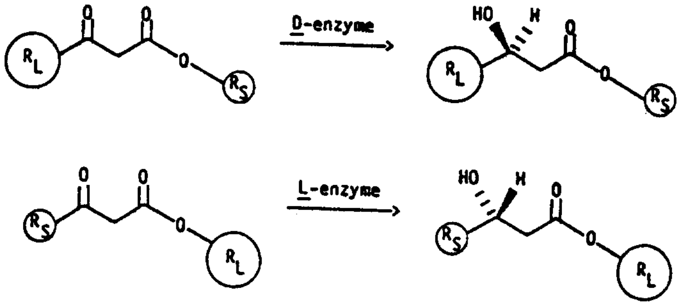
ee: 0.70-0.97

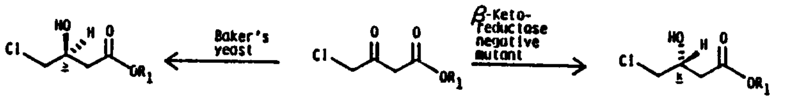


ee: 0.55









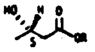
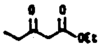
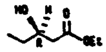
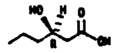
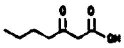
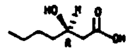
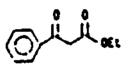
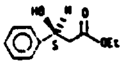
2 $R_1 = C_2H_5$
(ee = 0.55)

1 $R_1 = C_2H_5$
3 $R_1 = C_8H_{17}$

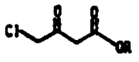
4 $R_1 = C_2H_5$
(ee = 0.90)

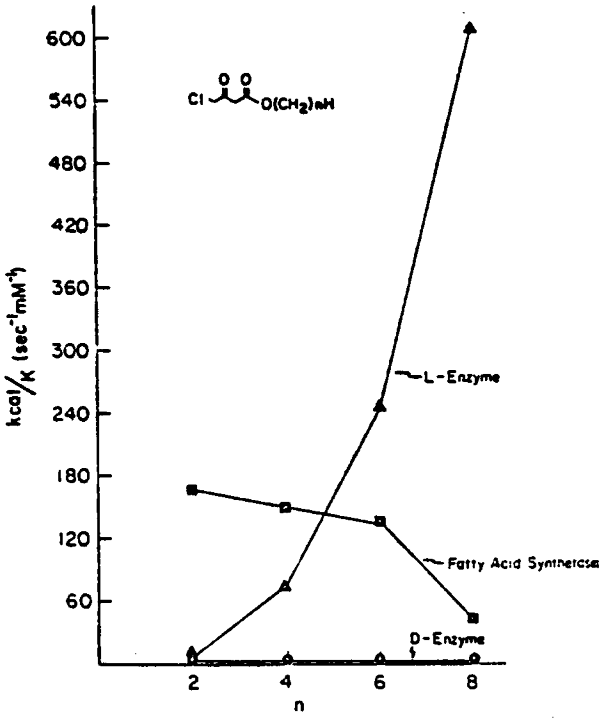
5 $R_1 = C_8H_{17}$
(ee = 0.97)

TABLE 2. Enantioselective reduction of β-keto-carbonyl derivatives by bakers' yeast.

Substrate	Product	R/S ratio
 (R = H or Et)		>2/98
		70/30
		Predominantly R
		Predominantly R
		>99/1
		Predominantly S

Properties of β-keto reductases from bakers' yeast

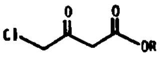
	Fatty acid synthetase complex	L-Enzyme	D-Enzyme
Intracellular localization	Cytosol	Cytosol	Cytosol
Coenzyme	NADPH	NADPH	NADPH
M.W.	2,400,000	74,000	38,000
	S	R	S



$$S_0 \longrightarrow \infty \qquad ee = \frac{V_R - V_S}{V_R + V_S}$$

$$S_0 \longrightarrow 0 \qquad ee = \frac{\frac{V_R}{K_R} - \frac{V_S}{K_S}}{\frac{V_R}{K_R} + \frac{V_S}{K_S}}$$

Kinetic constants of β-keto reductases of bakers' yeast.

	Fatty Acid Synthetase			D-Enzyme			L-Enzyme		
	K (mM)	k_{cat} (sec^{-1})	k_{cat}/K	K (mM)	k_{cat} (sec^{-1})	k_{cat}/K	K (mM)	k_{cat} (sec^{-1})	k_{cat}/K
$R = C_2H_5$	1.82	303	166	1.00	0.21	0.21	1.00	6.60	6.60
$R = C_4H_9$	1.33	202	152	0.10	0.11	1.10	0.094	7.13	76
$R = C_6H_{13}$	1.82	252	138	0.20	0.23	1.15	0.028	6.87	245
$R = C_8H_{17}$	1.60	69	43	0.29	0.47	1.62	0.01	6.12	612

TWO ENZYMES ACTING ON ONE SUBSTRATE YIELDING TWO ENANTIOMERIC PRODUCTS

$$\begin{tikzcd} & P \\ S \arrow[ur, "Enz_a"] \arrow[dr, "Enz_b"'] & \\ & Q \end{tikzcd}$$

$$\bar{v}_a = \frac{dp}{dt} = \frac{V_a[S]}{K_a + [S]} = \frac{V_a[S_0 - P - Q]}{K_a + [S_0 - P - Q]} \tag{1}$$

$$\bar{v}_b = \frac{dq}{dt} = \frac{V_b[S]}{K_b + [S]} = \frac{V_b[S_0 - P - Q]}{K_b + [S_0 - P - Q]} \tag{2}$$

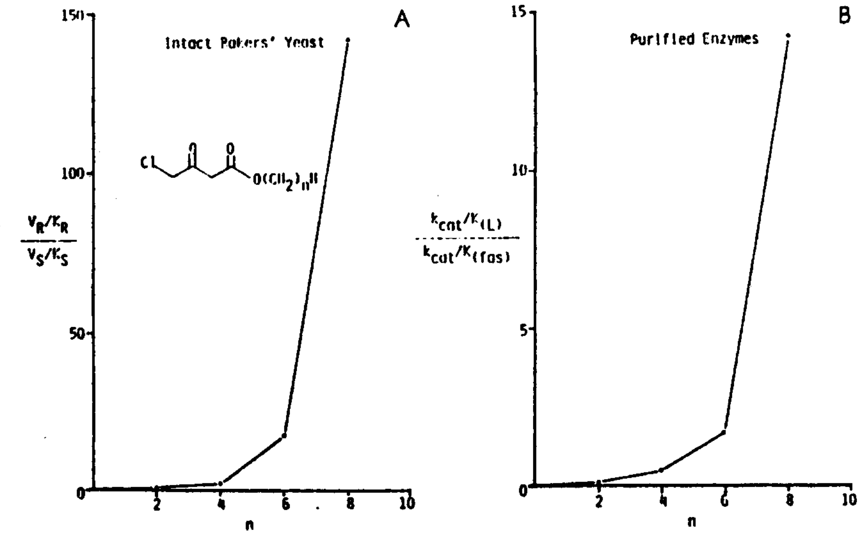
$$P + Q + \frac{y(K_a - K_b)}{(1 + y)} \ln \frac{(1 + y)S_0 + (yK_b + K_a)}{(1 + y)(S_0 - P - Q) + (yK_b + K_a)} = (1 + y)Q \tag{3}$$

$$P = \frac{1 + ee}{2} S_0C \qquad C = \frac{P + Q}{S_0} \qquad x = \frac{yK_b + K_a}{(1 + y)}$$

$$Q = \frac{1 - ee}{2} S_0C \qquad y = \frac{V_a}{V_b} \qquad (K_a - x) = \frac{y(K_a - K_b)}{(1 + y)}$$

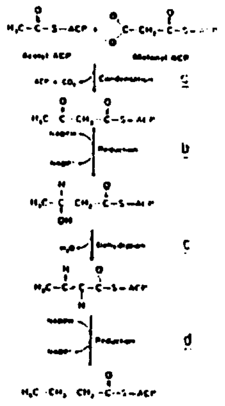
$$\left[\frac{1 - ee}{2}(1 + y) - 1\right] S_0C = (K_a - x) \ln \frac{S_0 + x}{S_0(1 - C)x} \tag{4}$$

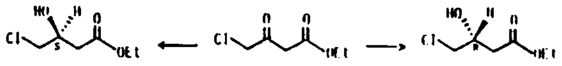
Unknowns: y, K_a, and x



Component enzyme activities in mutant fatty-acid-synthetase complexes of fas-complementation groups

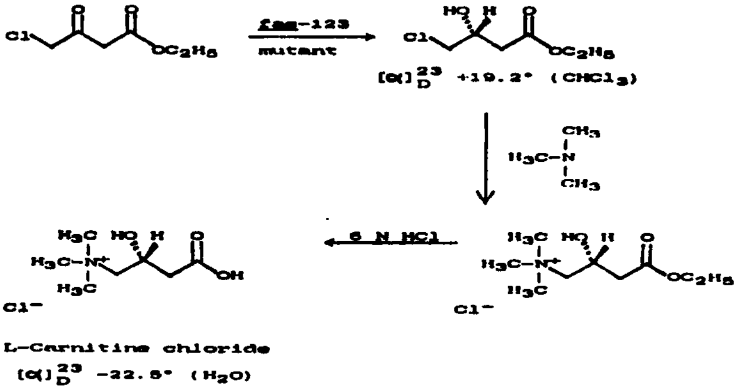
Complementation group	Strain	Condensing enzyme (a)	First reductase (b)	Dehydratase (c)	Second reductase (d)
		m-units/mg			
	Wild-type	0.20	3800	2.6	6300
IV	*fas*-182	-	-	-	-
VII	*fas*-158	-	720	2.0	3800
VIII	*fas*-123	0.03	-	4.0	8000





Strain	ee
Wild-type	0.55
fas-158	0.57

Strain	ee
fas-182	0.87
fas-123	0.90



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