Studies on the Stereochemical Control of Fermenting Baker's Yeast Mediated Reductions: Some 3- and 4-Oxo Esters

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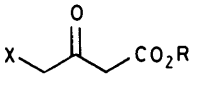
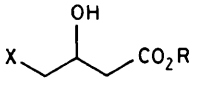
Ethyl 4-benzyloxy-3-oxobutanoate (**1c**) is reduced by fermenting baker's yeast with stereochemical control which is dependent on the yeast:substrate ratio and the presence or absence of ethanol. Contrary to earlier reports, ethyl levulinate (**3a**) can be stereospecifically reduced to (+)-(*S*)-ethyl 4-hydroxypentanoate (**4a**) (15% yield, >95% optical purity) as shown by the conversion of (**4a**) into (−)-(*S*)-5-methyl-γ-valerolactone (**5b**). Also, ethyl 4-oxo-4-phenylbutanoate (**3b**) is stereospecifically reduced to the previously unreported (−)-(*S*)-5-phenyl-γ-butyrolactone (**5c**). The stereochemistry of compound (**5c**) has been established by chemical correlation with (−)-(*S*)-ethyl 3-hydroxy-3-phenylpropanoate (**4c**), which in turn was prepared by the baker's yeast reduction of ethyl 3-oxo-3-phenylpropanoate (**3e**).

The use of fermenting baker's yeast for the enantioselective reduction of oxo esters to the corresponding hydroxy esters is well documented. Following a first report[1] on reduction of ethyl acetoacetate, Ridley more recently demonstrated in two papers that various oxo esters can be successfully reduced to optically active 3-hydroxy esters, whereas the reduction of 2-oxo esters is severely limited by extensive racemization.[2,3] In his preliminary report,[2] Ridley also indicated that ethyl levulinate, the simplest 4-oxo ester, is not reduced by fermenting yeast. However, it had also been reported that *Saccharomyces cerevisiae* efficiently reduces 4- and 5-oxo-acids to the corresponding lactones, presumably of high optical purity.[4] Since then, a great number of 4-hetero-substituted 3-oxobutanoates have been shown to be substrates for the bioreducing baker's yeast.[5] The general feature for the bioreduction of substituted carbonyl compounds is currently well explained by the so-called Prelog's rule.[6] When an exception to this rule is found, it is generally assumed that an enzyme system other than an alcohol dehydrogenase is the biochemical system used by the yeast for 'anomalous' biohydrogenations. Also, several enzymes operating together can sometimes alter the stereochemical course or reduce the enantiomeric excess of the reduction product.[7,8]



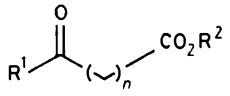
(1)

a: X = Cl; R = Et
b: X = Cl; R = Octyl
c: X = $PhCH_2O$; R = Et



(2)

a: X = Cl; R = Octyl
b: X = $PhCH_2O$; R = Et
c: X = OH; R = Et



(3)

a: R^1 = Me; R^2 = Et; *n* = 2
b: R^1 = Ph; R^2 = Et; *n* = 2
c: R^1 = Ph; R^2 = H; *n* = 2
d: R^1 = Alkyl; R^2 = H; *n* = 2 or 3
e: R^1 = Ph; R^2 = Et; *n* = 1

Reduction of Ethyl 4-Benzyloxy-3-oxobutanoate (**1c**).—We carefully investigated the reduction of ethyl 4-benzyloxy-3-oxobutanoate (**1c**), since the corresponding hydroxy ester (**2b**) was potentially an excellent chiral building block.[5,†] Ethyl 4-benzyloxy-3-oxobutanoate (**1c**) was prepared from the corresponding 4-chloro ester (**1a**) essentially according to a described procedure[9] in 40—50% yield after purification. Several procedures exist for the incubation of the ester (**1c**) with fermenting yeast, such as the classical method described by Levene[10] or by varying the amounts of yeast, adding fermenting yeast to the incubating mixture well before the reduction is complete, using a solvent such as ethanol, and adding the substrate, either dropwise or all at once. We found dramatic differences in the optical purity of the corresponding hydroxy ester (**2b**) which were related to the incubation conditions. For instance, opposite optical rotations were found when the ester (**1c**) was incubated according to Levene[10] (low yeast:substrate ratio, 0.75 g $mmol^{-1}$; substrate added in one portion; no EtOH) or according to Nakamura *et al.*[11] (high yeast:substrate ratio, 38 g $mmol^{-1}$; slow addition of an ethanolic solution of the substrate). Results of further investigations on the effect of ethanol and variations of the yeast:substrate ratio are collected in the Table. From the results it is evident that for the specific substrate (**1c**), the best optical purity of the product (**2b**) $[\alpha]_D$ +8° (e.e. 71%), is achieved by the Nakamura procedure[11] (Method H in the Table). The stereochemistry of C-3 of the hydroxy ester (**2b**) obtained as above was established as *R* by hydrogenolysis of the benzyl group (Pd black–room temperature; 93%) and converting the optically active ethyl 3,4-dihydroxybutanoate (**2c**) to the corresponding 3-hydroxy-γ-butyrolactone (**5a**) $[\alpha]_D$ +66° (lit.,[5] $[\alpha]_D$ +94°, 70% o.p.). This result is in agreement with the stereochemical outcome of other 4-substituted-3-oxo esters.[5] In the case of high yeast:substrate ratio, the solubility of the bioreduction product allows a good recovery from the large amount of yeast used (100 g l^{-1}) and from the low concentrations of substrate (2.5 mmol). Yields (70%) compare favourably with the experimental protocol of Seebach and Eberle,[5] whereas the incubation procedure according to Levene[10] (Method A in the Table), leads to lower yields (25%) and lower optical purity. However, in this last example, the

† Reduction of this substrate was also observed by Professor Seebach[5] when our work was drawing to a conclusion. However, the ester (**1c**) was less efficiently reduced than the corresponding 4-t-butoxy analogue and the stereochemistry the hydroxy ester (**2b**) was apparently not ascertained.

Table. Bioreduction of ethyl 4-benzyloxy-3-oxobutanoate (**1c**) under various incubation procedures

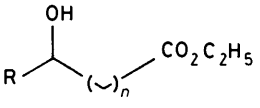
Method[a]	A	B	C	D	E	F	G	H
Ethanol addition	–	+	–	+	–	+	–	+
Time of addition (h)	–[b]	1	1.5	1.5	10	3	6	6
Incubation time (days)	6	6	6	6	4	4	1	1
Yeast/substrate (g mmol^{-1})	0.75	0.75	1.5	1.5	6	6	38	38
Configuration	*S*	*S*	*S*	*S*	*R*	*R*	*R*	*R*
Enantiomeric excess (%)	48	45	13	42	56	20	54	71
$[\alpha]_D$ (°)[c]	−5.4	−5.1	−1.5	−4.7	+6.3	+2.3	+6.2	+8.0
Yield of (**2b**) (%)	11	11	54	34	58	42	55	73

[a] A: according to ref. 9. E: Quoted from ref. 5. H: According to ref. 11. [b] All substrate added at once to fermenting yeast. [c] *c* 1.5 in $CHCl_3$; e.e can be calculated assuming a 56% value for $[\alpha]_D$ +6.3° (ref. 5).

stereochemical outcome of the bioreduction of the oxo ester (**1c**) is reversed. In fact, the (+)-(*R*)-hydroxy ester (**2b**) is produced with variable e.e. at high yeast:substrate ratio (entries E, F, G, and H), whereas at low yeast:substrate ratio (entry A, B, C, and D) product (**2b**) is produced in the 'wrong' *S*-configuration. The effect of ethanol on the normal bioreduction path is at present less clear and can be compared to a similar situation recently reported for yeasts grown in methanol.[12]

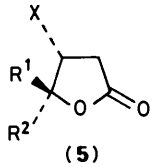
We also found that the ester (**1c**) is a substrate for the purified horse liver alcohol dehydrogenase (HLADH) (14% activity compared to 100% activity observed for acetaldehyde) and D-3-hydroxybutyrate dehydrogenase (HBDH) from *Pseudomonas lemoignei* [6% activity compared to 100% activity for (**1b**)] which is able to reduce stereospecifically the 4-chloro-3-oxobutanoates (**1a**) and (**1b**).[13]

Reduction of Ethyl Levulinate (**3a**).—We went on to examine the yeast-mediated reduction of 4-oxo esters, which had been reported not to occur by Ridley.[2] This result seemed to contrast with the fact that substrates possessing a range of structural features are acceptable to baker's yeast for bioreductive processes. We therefore treated ethyl levulinate (**3a**) with fermenting yeast, and it was indeed reduced to only a modest extent under a variety of incubation conditions, the most acceptable yields being achieved under Levene's conditions[10,*] (15—10% of pure, isolated product). It should be noticed that in all cases, the starting material (**3a**) was still present in considerable amounts (70—85%) and that the reduction product was invariably ethyl 4-hydroxypentanoate (**4a**), no lactone (**5b**) being detected in the incubation mixture. Low yields of the hydroxy ester (**4a**) were partially the result of solubility problems and some experimental expedient was necessary to achieve acceptable yields of the reduction product. The 4-hydroxy ester (**4a**) thus obtained exhibited a positive optical rotation $[\alpha]_D$ +12° and was cyclized to the corresponding γ-lactone (**5b**) to assess the stereochemical outcome of the bioreduction. The purified lactone (**5b**) had an optical rotation $[\alpha]_D$ −32° and was therefore assigned the *S*-configuration (lit.,[14] +30.1° for the *R*-isomer). Thus, the bioreduction of the 4-oxo ester (**3a**) proceeds according to the Prelog's rule on the reduction of substituted carbonyl groups, Me being 'S (small)' and $CH_2CH_2CO_2CH_2Me$ 'L (large)' groups.[6] Low yields obtained from the bioreduction of the ester (**3a**) seem to suggest that this 4-oxo ester is not reduced by the 3-oxo ester-reducing enzyme, which may be able to reduce only enolizable oxo esters. Also, other non-specific oxidoreductases which are present in fermenting yeast do not seem to be able to carry out the efficient



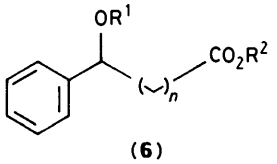
(**4**)

a; R = Me; *n* = 2
b; R = Ph; *n* = 2
c; R = Ph; *n* = 1



(**5**)

a; R^1 = R^2 = H; X = OH
b; R^1 = Me; R^2 = X = H
c; R^1 = X = H; R^2 = Ph



(**6**)

a; R^1 = $SiBu^tMe_2$; R^2 = Et; *n* = 1
b; R^1 = $SiBu^tMe_2$; R^2 = H; *n* = 1
c; R^1 = $SiBu^tMe_2$; R^2 = Me; *n* = 2

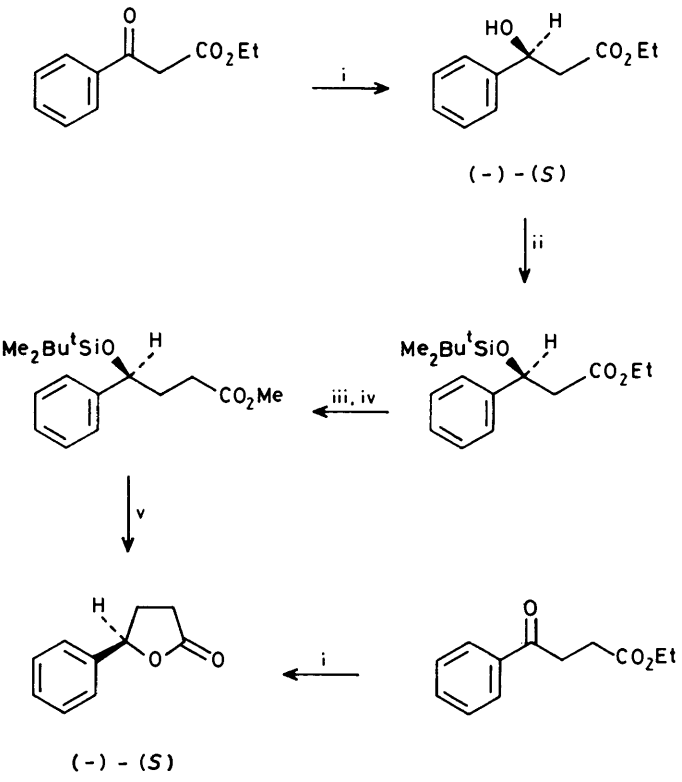
bioreduction of the 4-oxo ester (**3a**). Although not completely clear from an enzymatic point of view, the simplicity of the preparation of the optically active (−)-(S)-lactone (**5b**) can be favourably compared to the overall yields obtained from the completely chemical synthesis from glutamic acid as reported by Mori.[14]

Reduction of Ethyl 4-Oxo-4-phenylbutanoate (**3b**).—The bioreduction of ethyl 4-oxo-4-phenylbutanoate (**3b**) [prepared from commercially available benzoylpropanoic acid (**3c**)] was also studied. The bioreduction of the ester (**3b**) could be of synthetic significance, since, according to the literature, no chiral syntheses of the corresponding hydroxy ester (**4b**) or its synthetic equivalent, the lactone (**5c**), exist at present. Muys *et al.*[4] reported on the baker's yeast-mediated reduction of γ- and δ-oxo acids with a common aliphatic framework [compounds of formula (**3d**)]. For these oxo acids, the same stereochemical outcome should be observed, since the lactones formed all exhibited positive optical rotations.† Incubation of the ester (**3b**) with baker's yeast under Levene's conditions[10] led to no reduction of the substrate, whereas the addition of an ethanolic solution of (**3b**) by Nakamura's procedure[11] led to bioreduction. After 7 days, the starting material was completely

* Also, incubation conditions according to Nakamura[11] afforded the hydroxy ester (**4a**) and starting material (**3a**), but the crude products recovery was much lower (15%).

† It has recently been discovered that positive optical rotations for these lactones correspond to the *R*-configuration, T. Sugai and K. Mori, *Agric. Biol. Chem.*, 1984, **48**, 2497; M. Utaka, H. Watabu, and A. Takeda, *Chem. Lett.*, 1985, 1475.

consumed and after column chromatography, the products isolated were the lactone (**5c**) (31%) and the hydroxy ester (**4b**) (2%). The lactone (**5c**) obtained so far exhibited a negative optical rotation ($[\alpha]_D$ −32°) and, although this value was of opposite sign with respect to the lactones obtained from aliphatic keto acids, no conclusions could be drawn as regards its chirality. On the other hand, when the 4-keto acid (**3c**) was incubated with fermenting yeast, the lactone (**5c**) was formed in much better isolated yields (84%), with the same optical rotation as the previous experiment on the ester (**3b**) ($[\alpha]_D$ −32°). The optical purity of the lactone (**5c**) could be established by the method described by Jones[15] and found to be >95% by ^{1}H n.m.r. spectroscopy. When the bioreduction was carried out on the ester (**3b**) and the acid (**3c**) excluding ethanol at high yeast:substrate ratio, the optical purity of the lactone (**5c**) was nearly identical to the lactone obtained by Nakamura's procedure.[11] Some problems arise in the interpretations of these results, since nothing is known with certainty about the yeast's enzymatic systems which are capable of reducing variously substituted keto esters, except the work of Sih *et al.*[16] It is conceivable that under Nakamura's conditions, for instance, the ester (**3b**) is hydrolysed by some non-specific esterase to the corresponding acid (or salt), which is the true substrate for the bioreduction. The enzyme could carry out the reduction of the oxo acid (**3c**) to the corresponding hydroxy acid [and thus the lactone (**5c**)]. This is different to the oxidoreductase, which reduces 3-oxo esters. However the possibility cannot be excluded that a reductase different from the others and able to reduce all previous substrates, is acting on the ester (**3b**), reducing it to the hydroxy ester (**4b**), which is spontaneously cyclized to the lactone (**5c**). This facile cyclization is a well known outcome of the chemical reduction of 4-oxo esters, since $NaBH_4$ reduction of the ester (**3c**) affords the lactone (**5c**),[17] presumably as a mixture with the hydroxy ester (**4b**). The same behaviour has also been reported in the case of catalytic hydrogenation of (**3c**).[18] In any event, we considered that since no methods are currently available for the preparation of optically active (**5c**), it was worthwhile investigating the lactone configuration. Although optically active styrene oxides are commercially available, synthesis described from racemic aromatic epoxides and ethyl malonate[19] were not satisfactory in our hands and we were discouraged from using drastic conditions for some of the critical steps for the preparation of chirally pure (**5c**). We decided to start with the optically active 3-hydroxy ester (**4c**), which could in turn be prepared in the *S*-configuration ($[\alpha]_D$ −25.8°) by baker's yeast reduction of ethyl 3-oxo-3-phenylpropanoate (**3e**), according to Ridley.[3] Repetition of this incubation procedure was, in our hands, not as satisfactory as described [20% yield for the hydroxy ester (**4c**)]. However, the addition of an ethanolic solution of the ester (**3e**) at high yeast:substrate ratio afforded, after 7 days, compound (**4c**) in 70% isolated yields ($[\alpha]_D$ −40°). For correlation of the lactone (**5c**) with the hydroxy ester (**4c**), it was protected as the dimethyl-t-butylsilyl ether (**6a**)[20] (60%), which was hydrolysed to the corresponding silylated acid (**6b**). Homologation of (**6b**) under classical Arndt–Eistert conditions[21] proceeded in good yield to give the protected 4-hydroxy ester (**6c**) (70% yield). Cleavage of the silyl protecting group could constitute a problem for the integrity of the benzylic chiral centre and therefore we considered two different methods, studying this reaction on easily prepared, optically active (**6a**). Cleavage with tetrabutylammonium fluoride adsorbed on silica gel[22] afforded the hydroxy ester (**4c**) (65%) with no appreciable racemization. Repetition of the same reaction on the 4-keto ester (**6c**) proceeded less satisfactorily, and a desilylation–cyclization of the ester (**6c**) to the lactone (**5c**) was devised. Treatment of the silylated ester (**6c**) with trifluoroacetic acid (70% yield) proceeded with substantial retention of optical purity. In fact, starting from optically pure (**4c**) ($[\alpha]_D$ −39°), the chiral lactone (**5c**) was obtained ($[\alpha]_D$ − 31°), thus establishing an *S*-configuration for the lactone (**5c**) obtained from either the 4-oxo ester (**3b**) or 4-oxo acid (**3c**) by baker's yeast fermentation (see the Scheme).



Scheme. *Reagents:* i, Baker's yeast; ii, Bu^{t}SiMe$_2$Cl–imidazole; iii, OH^-, H^+; iv, CH_2N_2–Ag_2O; v, CF_3CO_2H

Experimental

Unless otherwise stated, materials were obtained from commercial suppliers and were used without further purification. Baker's yeast was from ERIDANIA (Italy). All m.p.s are uncorrected. I.r. spectra were recorded on a 1420 Perkin-Elmer spectrometer for solutions in chloroform or for Nujol mulls. ^{1}H N.m.r. spectra were recorded on a Varian 360 L spectrometer for solutions in $CDCl_3$, using $SiMe_4$ as an internal or external standard. Mass spectra were recorded on a LKB 2091 Gas chromatograph–mass spectrometer. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter. Gas chromatographic analyses were performed on a Carlo Erba Fractovap 2101. Distillation for analytical purposes was performed on a glass tube oven Buchi GKR-50. T.l.c. analyses were carried out on silica gel Merck 60 F_{254} plates. Flash column chromatography[23] was performed on silica gel Merck 60 (230—400 mesh).

(+)-(R)-*Ethyl* 4-*Benzyloxy*-3-*hydroxybutanoate* (**2b**).—(*Method H*, *Table*). A solution of ethyl 4-benzyloxy-3-oxobutanoate (**1c**) (0.5 g, 2.1 mmol) in ethanol (40 ml) was added over 6 h to fermenting baker's yeast [yeast (80 g) and sucrose (80 g) in tap water (800 ml)]. The mixture was shaken at 30 °C for 1 day, then filtered through a Celite pad and extracted with diethyl ether (5 × 100 ml). The extracts were dried and evaporated to dryness to leave a residue (0.8 g) which was purified by column chromatography. Elution with light petroleum (b.p. 40—70 °C)–ethyl acetate (8:2) afforded title ester (**2b**) (0.4 g,

73%) as a viscous oil, b.p. 242 °C (11 mmHg) (Found: C, 65.1; H, 7.8. $C_{13}H_{18}O_4$ requires C, 65.5; H, 7.6%); $[\alpha]_D$ +8.0° (*c*, 1.5 in $CHCl_3$); δ 1.24 (3 H, t, *J* 7 Hz, $MeCH_2$), 2.54 (2 H, d, *J* 6 Hz, OCH_2CHOH), 3.0 (1 H, br s, OH), 3.50 (2 H, d, *J* 6 Hz, CH_2CO), 4.19 (2 H, q, *J* 7 Hz, $MeCH_2$), 4.1—4.5 (1 H, m, CHOH), 4.69 (2 H, s, CH_2Ph), and 7.32 (5 H, s, Ph); *m/z* 238 (M^+), 202, 193, 164, 132, 131, and 117.

Methods B, D, and F. Experimental procedures were as described for Method H, but with varying addition and incubation times, and the yeast:substrate ratio as indicated in the Table. Volumes of ethanol used were always 1/20 of that of the water.

Method A. To a slurry of fermenting baker's yeast [yeast (4 g) and sucrose (4 g) in tap water (40 ml)], was added the ester (**1c**) (1.3 g, 5.5 mmol). The mixture was shaken at 30 °C for 6 days, then filtered through a Celite pad and extracted with diethyl ether (4 × 40 ml). The extracts were dried and evaporated under reduced pressure to leave a residue (0.75 g) which was purified by column chromatography. Elution with light petroleum (b.p. 40—70 °C)–ethyl acetate (8:2) afforded the title ester (**2b**) (0.14 g, 11%) as a viscous oil.

Methods C and G. Experimental procedures were as described for Method A, but with varying addition and incubation times. The yeast:substrate ratios were as indicated in the Table.

(+)-(R)-*Ethyl* 3,4-*Dihydroxybutanoate* (**2c**).—(+)-(*R*)-4-Benzyloxy-3-hydroxybutanoate (**2b**) ($[\alpha]_D$ +8°; 0.4 g, 1.7 mmol) in methanol (4 ml) was hydrogenated in the presence of palladium black (40 mg). Hydrogen was generated by addition of 4M-ethanolic $NaBH_4$ to 5M-HCl. When the reaction was complete [t.l.c., dichloromethane–acetone (9:1) as the eluant], the reaction mixture was filtered through a Celite pad, evaporated under reduced pressure, taken up in dichloromethane, dried (anhydrous Na_2SO_4), and evaporated. Silica gel column chromatography eluting with light petroleum (b.p. 40—70 °C)–ethyl acetate (1:1) yielded (+)-(*R*)-ethyl 3,4-dihydroxybutanoate (**2c**) (0.23 g, 93%), $[\alpha]_D$ +14° (*c*, 2.8 in $CHCl_3$); δ 1.25 (3 H, t, *J* 7.5 Hz, CH_2Me), 2.50 (2 H, d, *J* 6.5 Hz, CH_2CO), 3.45—3.75 (2 H, complex, $HOCH_2CHOH$), 3.70 (2 H, exchangeable s, OH), 4.20 (2 H, q, *J* 7.5 Hz, CH_2Me), and 3.95—4.45 (1 H, m, CHOH). The above diol (**2c**) was cyclized with trifluoroacetic acid,[24] to yield (+)-(*R*)-3-hydroxytetrahydrofuranone (**5c**), $[\alpha]_D$ +66° (*c*, 3 in $CHCl_3$) (lit.,[5] +94°) with spectroscopic characteristics (n.m.r. and i.r.) and boiling point in agreement with the literature values.[5]

(+)-(S)-*Ethyl* 4-*Hydroxypentanoate* (**4a**).—To a slurry of fermenting baker's yeast [yeast (15 g) and sucrose (15 g) in tap water (150 ml)] was added ethyl levulinate (**3a**) (2.84 ml, 20 mmol) and the flask was stoppered with aluminium foil. The mixture was shaken at 30 °C for 4 days, then cooled and filtered through a Celite pad, and extracted with diethyl ether (5 × 25 ml). The extracts were dried and carefully evaporated under reduced pressure at room temperature. Flash chromatography eluting with dichloromethane–acetone (92:8) yielded unchanged ethyl levulinate (**3a**) (2.0 g, 70%) with physical and spectroscopic characteristics identical to an authentic sample and (*S*)-ethyl 4-hydroxypentanoate (**4a**) (0.4 g, 15%) (Found: C, 57.3; H, 9.7. $C_7H_{14}O_3$ requires C, 57.5; H, 9.65%); $[\alpha]_D$ +12° (*c*, 2.8 in $CHCl_3$); δ 1.2—1.4 (5 H, complex, CH_2Me and $CHMe$), 1.5—2.0 (2 H, complex, $CHCH_2$), 2.1 (1 H, br s, OH), 3.48 (2 H, t, *J* 7 Hz, CH_2CO), 3.35—4.0 (1 H, m, $MeCH$), and 4.2 (2 H, q, *J* 7 Hz, CH_2Me).

(−)-(S)-5-*Methyl*-4,5-*dihydrofuran*-2(3H)-*one* (**5b**).—A solution of (+)-(*S*)-ethyl 4-hydroxypentanoate (**4a**) (0.17 g, 1.16 mmol) in dichloromethane (1 ml) was treated with trifluoroacetic acid, according to Saito *et al.*[24] The reaction was worked up and bulb-to-bulb distillation (100 °C, 11 mmHg) of the residue yielded the title compound (**5b**) (0.11 g, 95%), $[\alpha]_D$ −32° (*c*, 0.85 in CH_2Cl_2), with physical and spectroscopic characteristics identical to published values.[14]

(−)-(S)-5-*Phenyl*-4,5-*dihydrofuran*-2(3H)-*one* (**5c**).—A solution of the ester (**3b**) (0.44 g, 2.1 mmol) in ethanol (40 ml) was added over 2 h to fermenting baker's yeast [yeast (80 g) and sucrose (80 g) in tap water (800 ml)]. The mixture was shaken at 30 °C for 7 days, then cooled and filtered through a Celite pad and extracted with ethyl acetate (5 × 100 ml). The extracts were dried and evaporated to dryness to leave a residue (0.4 g) which was purified by flash chromatography. Elution with dichloromethane–acetone (99:1) gave the title compound (**5c**) (0.11 mg, 31%) which was distilled (200 °C, 2 mmHg) to yield a solid, m.p. 35—36 °C (Found: C, 73.8; H, 6.35. $C_{10}H_{10}O_2$ requires C, 74.05; H, 6.2%); $[\alpha]_D$ −32.5° (*c*, 4.3 in $CHCl_3$); δ 1.8—3.0 (4 H, m, CH_2CH_2), 5.57 (1 H, t, *J* 7 Hz, CH), and 7.45 (5 H, s, Ph); *m/z* 162 (M^+, 100%), 133, 117, 107, 105, and 91. Elution with dichloromethane–acetone (9:1) afforded ethyl 4-hydroxy-4-phenylbutanoate (**4b**) (0.008 g, 2%); δ 1.24 (3 H, t, *J* 7 Hz, CH_2CH_3), 1.85—2.65 (4 H, m, CH_2CH_2), 4.18 (2 H, q, *J* 7 Hz, CH_2Me), 4.78 (1 H, t, *J* 6 Hz, CH), and 7.4 (5 H, s, Ph).

(−)-(S)-*Ethyl* 3-*Hydroxy*-3-*phenylpropanoate* (**4c**).—A solution of the ester (**3e**) (5 g, 26 mmol) in ethanol (100 ml) was added over 2 h to fermenting baker's yeast [yeast (200 g) and sucrose (200 g) in tap water (2 l)]. The mixture was shaken at 30 °C for 7 days, then cooled and filtered through a Celite pad and extracted with ethyl acetate (5 × 100 ml). The extracts were dried and evaporated to dryness to leave a residue (3.8 g) which was purified by flash chromatography. Elution with dichloromethane–acetone (9:1) afforded the ester (**4c**) (3.5 g, 70%), b.p. 195 °C (11 mmHg) (Found: C, 68.05; H, 7.4. Calc. for $C_{11}H_{14}O_3$: C, 68.0; H, 7.3%); $[\alpha]_D$ −39.8° (*c*, 1.5 in $CHCl_3$) (lit.,[3] −25.8°); δ 1.20 (3 H, t, *J* 7 Hz, $MeCH_2$), 2.60 (2 H, d, *J* 6 Hz, CH_2CHOH), 3.40 (1 H, br s, OH), 4.12 (2 H, q, *J* 7 Hz, CH_3CH_2), 5.03 (1 H, t, *J* 6 Hz, CHOH), and 7.28 (5 H, br s, Ph); *m/z* 194 (M^+), 177, 176, 165, 149, 147, 135, and 131.

(−)-(S)-*Ethyl* 3-*Dimethyl*-t-*butylsilyloxy*-3-*phenylpropanoate* (**6a**).—Imidazole (1.02 g, 15.0 mmol) was dissolved in dichloromethane distilled over calcium hydride (8.75 ml) under a nitrogen atmosphere. A solution of chlorodimethyl-t-buthylsilane (1.13 g, 7.5 mmol) in anhydrous dichloromethane (2.5 ml) was added and the resulting mixture was stirred for 10 min. A solution of (*S*)-ethyl 3-hydroxy-3-phenylpropanoate (**4c**) (1 g, 5.15 mmol) in anhydrous dichloromethane (2.5 ml) was then added dropwise. The mixture was stirred for 18 h at room temperature, after which time it was poured in a separatory funnel containing dichloromethane (60 ml) and water (12.5 ml). The organic phase was washed with brine (15 ml), and the combined aqueous washings were extracted with dichloromethane (30 ml). The combined organic fractions were dried and evaporated to dryness. Chromatography of the residue on grade III neutral alumina eluting with light petroleum (b.p. 40—70 °C)–ethyl acetate (99:1) yielded the title compound (**6a**) (0.95 g, 60%), b.p. 100 °C (11 mmHg) (Found: C, 66.3; H, 9.1. $C_{17}H_{28}O_3Si$ requires C, 66.2; H, 9.15%); $[\alpha]_D$ −50.4° (*c*, 2 in $CHCl_3$); $\nu_{max.}$ (liquid film) 1 735 (CO); δ −0.28 (3 H, s, SiMe), 0.06 (3 H, s, SiMe), 0.86 (9 H, s, CMe_3), 1.12 (3 H, t, *J* 7 Hz, $MeCH_2$), 2.46—2.73 (2 H, m, CH_2CO), 4.06 (2 H, q, *J* 7 Hz, $MeCH_2$), 4.97—5.27 (1 H, m, CHOSi), and 7.27 (5 H, s, Ph); *m/z* 293 (M^+ − Me) and 251 (M^+ − CMe_3, 100%).

(−)-(S)-3-(*Dimethyl*-t-*butylsilyloxy*)-3-*phenylpropanoic Acid* (**6b**).—To a solution of the ester (**6a**) (1.6 g, 5.1 mmol) in methanol (24 ml) was added ground NaOH (0.2 g, 5.1 mmol).

The mixture was stirred under a nitrogen atmosphere for 24 h. The solution was then poured into cold water (200 ml) made slightly acidic with hydrochloric acid, and was extracted with dichloromethane (4 × 50 ml). The extract was dried and evaporated to dryness, to leave a residue (1.3 g) which was purified by silica gel chromatography. Elution with dichloromethane–acetone (95:5) yielded the acid (**6b**) (1.07 g, 75%), m.p. 30—31 °C (Found: C, 64.0; H, 8.9. $C_{15}H_{24}O_3Si$ requires C, 64.2; H, 8.6%); $[\alpha]_D$ −55.0° (*c*, 2 in $CHCl_3$); $\nu_{max.}$(CCl_4) 3.500—2 850 (OH), 1 700sh and 1 740; δ −0.28 (3 H, s, SiMe), 0.06 (3 H, s, SiMe), 0.86 (9 H, s, CMe_3), 2.46—2.73 (2 H, m, CH_2CO), 4.97—5.27 (1 H, m, CHOSi), 7.27 (5 H, s, Ph), and 12.1 (1 H, br s, CO_2H).

(−)-(S)-*Methyl* 4-(*Dimethyl-t-butylsilyloxy*)-4-*phenylbutanoate* (**6c**).—The acid (**6b**) (0.34 g, 1.27 mmol) was dissolved in a mixture of dimethylformamide in dichloromethane distilled from calcium hydride (1 drop DMF in 5 ml CH_2Cl_2; 1.6 ml) under an argon atmosphere. The solution was μl, cooled to 0 °C, and freshly distilled oxalyl chloride (140 μl, 1.6 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for 1.5 h and then 0.5 h at room temperature. The solution was evaporated under reduced pressure under a stream of argon. Benzene (3 × 0.1 ml) was sequentially added and evaporated, to yield the acid chloride as a clear oil. This oil was dissolved in dry diethyl ether (4 ml) and the solution was added dropwise at 0 °C to an ethereal solution of diazomethane (0.3M; 14 ml). After 4 h at room temperature the solution was evaporated under a stream of nitrogen. The residue was dissolved in anhydrous methanol and the solution was warmed first to 40 °C and then to reflux. A suspension of freshly prepared silver oxide (0.58 g, 2.5 mmol) in methanol was added portionwise during 1 h. The mixture was refluxed for a further 3 h, filtered through a pad of Celite and evaporated to dryness. The residue (0.35 g) was purified by chromatography on neutral alumina (grade III), eluting with light petroleum (b.p. 40—70 °C)–ethyl acetate (99:1) to yield the title ester (**6c**) (0.27 g, 70%), b.p. 150 °C (11 mmHg) (Found: C, 65.9; H, 9.0. $C_{17}H_{28}O_3Si$ requires C, 66.2; H, 9.15%); $[\alpha]_D$ −45.9° (*c*, 2 in $CHCl_3$); $\nu_{max.}$ (liquid film) 1 735 (CO); δ −0.28 (3 H, s, SiMe), −0.097 (3 H, s, SiMe), 0.95 (9 H, s, CMe_3), 1.75—2.6 (4 H, m, CH_2CH_2), 3.68 (3 H, s, OMe), 4.82 (1 H, t, *J* 9 Hz, CHOSi), and 7.30 (5 H, s, Ph); *m/z* 293 (M^+ − Me) and 251 (M^+ − CMe_3, 100%). Relative proportions of the ester (**6c**) and the methyl ester of the starting phenylpropanoic acid were established by gas chromatography–mass spectrometry (SE 30 1%; 2 m; 170 °C; He 0.8) focussing peaks at 251 (M^+ − CMe_3) for the phenylbutanoate, (98.6%) and 237 (M^+ − CMe_3) for the phenylpropanoate, (1.4%).

Compound (**5c**).—A solution of the methyl ester (**6c**) (0.2 g, 0.65 mmol) in anhydrous dichloromethane (0.75 ml) was treated with 3 drops of trifluoroacetic acid. The mixture was left for 7—8 days at room temperature, during which time the solvent evaporated through a calcium chloride drying tube. The residue was dissolved in dichloromethane (15 ml), washed with aqueous sodium hydrogen carbonate and brine, dried, and evaporated to dryness to afford a residue which was purified by flash chromatography. Elution with light petroleum (b.p. 40—70 °C)–ethyl acetate (75:25) yielded title lactone (**5c**) (0.08 mg, 70%), $[\alpha]_D$ −31° (*c*, 4.3 in $CHCl_3$) with physical and spectroscopic characteristics identical to the lactone obtained by the baker's yeast-mediated reduction of ethyl 4-oxo-4-phenylbutanoate.

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