Biotransformation of αβ-unsaturated carbonyl compounds: sulfides, sulfoxides, sulfones, nitriles and esters by yeast species: carbonyl group and carbon–carbon double bond reduction

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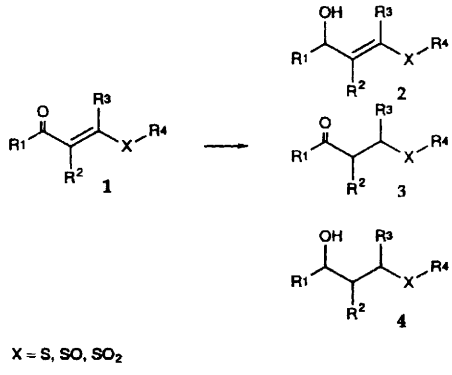
The reduction of αβ-unsaturated ketones with γ-sulfide, sulfoxide, sulfone, nitrile and ester functions has been investigated. Both C=O and C=C reduction was observed. In the sulfur series, C=O bond reduction was always observed, but significant C=C bond reduction was observed only with the sulfide. The unsaturated nitriles gave the corresponding alcohol as the major bioreduction product, with smaller but significant amounts of fully reduced product. A similar result was obtained with the ester substrate. Relative and absolute configurations of bioreduction products were determined. A comparison was made between reductions catalysed by bakers' yeast (*Saccharomyces cerevisiae*) and by other yeasts (*Zygosaccharomyces rouxii*, *Pichia capsulata*, *P. farinosa*, *Candida chalmersi* and *C. diddensiae*). The tendency of *Z. rouxii* to give products enantiomeric with those obtained using *S. cerevisiae* was noted. The relationship between substrate structure and the stereochemistry of C=C double bond reduction is discussed.

The search for efficient routes to enantiomerically pure compounds has led many workers to investigate biocatalytic reductions of unsaturated compounds. Because the enzymes responsible for these reductions operate *via* redox co-factors, the problem of co-factor recycling has to be addressed. Although many co-factor recycling systems have been developed for use *in vitro*, for convenience many studies have been carried out using whole-cell microbial systems in which co-factor recycling is effected through the normal metabolic activity of the microorganism. Often, the trade-off for this convenience is sub-optimal enantiomeric purity in chiral reduction products. This is attributable to the biocatalytic activity of different enzymes catalysing reactions that follow different stereochemical courses. Nevertheless, microbial species have been widely used for reductions, principally of carbonyl groups, and continue to be used as an alternative to methods using non-enzymatic catalysts. Because of its familiarity, safety and ready availability, baker's yeast (*Saccharomyces cerevisiae*) has been used more often than all other species combined.[1,2]

Bioreduction of carbonyl compounds has been shown to provide a versatile route to an astonishing variety of optically active compounds of synthetic usefulness.[1,2] A useful extension of the biocatalytic capabilities of baker's yeast has been the reduction of carbon–carbon double bonds. Without exception, such reductions require conjugation of the double bond, usually with a carbonyl group (which may be generated *in situ* by reversible oxidation of a hydroxyl function), but occasionally with other electronegative groups such as the nitro group.[3,4] Such reductions are attractive as routes to chiral compounds since, in principle, they can lead to the introduction of one, two or three new chiral centres into a non-chiral structure (Scheme 1). Bioreduction of species such as the ketone **1** could give products such as **2** or **3** formed by reduction of the carbonyl group or the carbon–carbon double bond, respectively, or products such as **4** in which both functions are reduced. The last possibility was attractive as a goal as enzymatic stereocontrol might lead to the formation of products in which two or more chiral centres have been introduced diastereoselectively and enantioselectively. The products would have functionality that would make them valuable building blocks in chiral syntheses. We were interested to study reductions of systems not

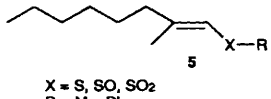


Scheme 1

previously investigated. In this paper is described a survey of reductions of αβ-unsaturated ketones with γ-sulfide, sulfoxide, sulfonyl, cyano and ethoxycarbonyl substituents.

Sulfur compounds, and in particular sulfoxides[5] and sulfones,[6] have been used extensively in organic synthesis. It was therefore of interest to study biotransformation routes to examples of such systems. A few baker's yeast reductions of sulfur compounds are to be found in the literature.[7–13]

It is clearly important to match the redox characteristics of the substrate to be reduced to those of the enzymatic reducing system. A substrate with a reduction potential outside the range of available biocatalysts will not undergo reduction. However, once this fundamental criterion has been satisfied, the remaining questions concern the selectivity and specificity of the enzyme. Preliminary investigations with six compounds of structure **5** (data not reported) showed that apart from a slight

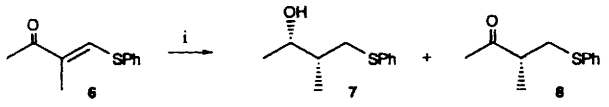


X = S, SO, SO2
R = Me, Ph

reduction (2–3%) of the sulfoxide to the corresponding sulfide, none of the compounds was transformed; starting material was recovered in >70% yield in each case. Experiments with

substrates containing a conjugated carbonyl group gave more positive results and are reported here.

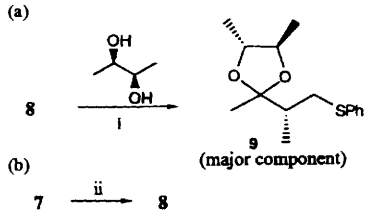
The simplest type of system containing the functionalities proposed above was chosen for investigation. This is shown as the keto sulfide **6** (Scheme 2). Initial experiments were carried



Scheme 2 *Reagent:* i, *S. cerevisiae*

out with baker's yeast (*Saccharomyces cerevisiae*). The yeast used in most experiments was a commercial supermarket yeast. This is a hybrid yeast that is extremely stable and contaminant free. (Samples grown in a chemostat revealed the presence of no other microorganisms). The commercial product is grown to the point of nitrogen depletion (stationary phase). (Personal communication, Dr J. B. van der Plaat, Gist-Brocades, Delft, The Netherlands) We have previously used this strain for biotransformation studies and it has been deposited as NCYC1765.[14] In these studies, a comparison was made between the commercially obtained yeast and the deposited strain NCYC 1765. There was no detectable difference in the results obtained and therefore, for convenience, the commercially available material was used in these studies.

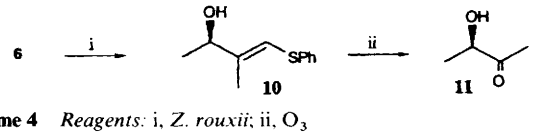
Reduction of the sulfide **6** with this strain gave the fully saturated compound **7** (18%) and the keto sulfide **8** (20%, isolated as a 34:64 mixture with substrate **6**). The fully reduced compound **7** was of 90% de (by ^{1}H NMR). Both diastereoisomers were found to be enantiomerically pure (> 96% ee) by ^{1}H NMR spectral studies of the acetyl derivatives using the chiral solvating agent (*S*)-1-(9-anthryl)-2,2,2-trifluoroethanol (referred to below as 'the chiral solvating agent'). The absolute configuration of the major diastereoisomer was determined as 2*S*,3*R* by X-ray crystallography of the (*R*)-1-methylbenzylamine salt of the hydrogen phthalate (Fig. 1). The minor product **8** proved difficult to separate from unreduced substrate. Chiral analysis proved difficult and so it was converted into the cyclic ketal **9** using (2*R*,3*R*)-butane-2,3-diol [Scheme 3(a)]. ^{1}H NMR analysis of the mixture indicated



Scheme 3 *Reagents:* i, H^+; ii, CrO_3·$(C_5H_5N)_2$

an enantiomeric excess of 72%. Its absolute configuration was determined by oxidation of the fully reduced product **7** to the corresponding keto sulfide with the same sign of optical rotation as that of the reduction product **8** [Scheme 3(b)].

In other studies,[15,16] we have found that the yeast *Zygosaccharomyces rouxii* gave products enantiomeric with those obtained using *S. cerevisiae*. When the keto sulfide **6** was reduced using this species, the sole product, after 3 days' incubation, was the allylic alcohol **10** (Scheme 4) which was obtained in 10% yield and 72% ee. The absolute configuration



Scheme 4 *Reagents:* i, *Z. rouxii*; ii, O_3

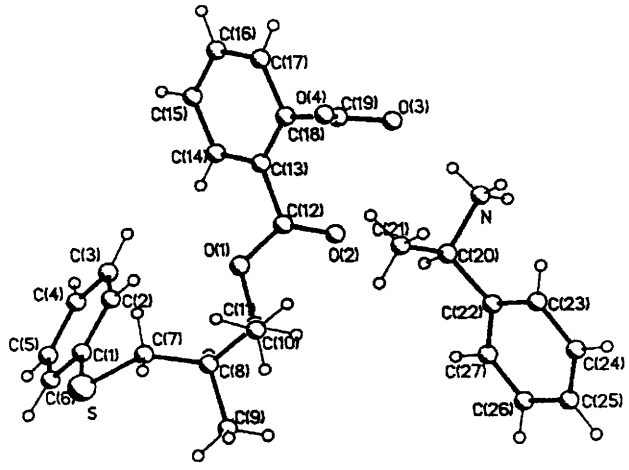
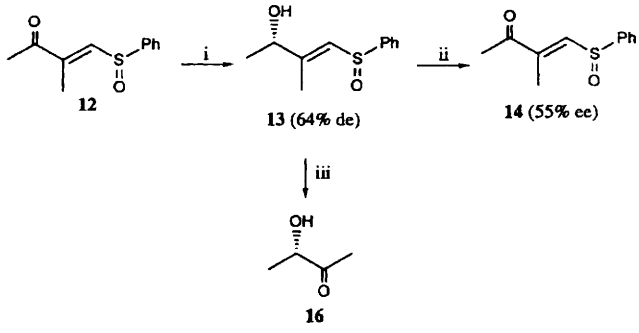


Fig. 1 X-Ray crystal structure of hydrogen (*R*)-1-methylbenzylammonium (2*S*,3*R*)-3-methyl-4-phenylsulfanylbutan-2-yl phthalate

was determined by ozonolysis to (*R*)-acetoin [(*R*)-3-hydroxybutan-2-one] **11**[17] and comparison with authentic material by chiral GLC using cyclodextrin-based columns. Although 10% of starting material was also recovered, the remainder of the product was unaccounted for. Longer incubation times (4–5 days) brought about complete disappearance of the starting material but also a lowering of the yield of the reduction product **10** to 3–4%.

The sulfoxide **12** corresponding to the sulfide **6** was next investigated. The major product isolated (19%) was the alcohol **13** (Scheme 5) together with small amounts of the



Scheme 5 *Reagents:* i, *S. cerevisiae*; ii, $CrO_3(C_5H_5N)_2$; iii, O_3

corresponding sulfide and sulfone. The ^{1}H NMR spectrum of the hydroxy sulfoxide **13** indicated a diastereoisomeric excess of 64%. Both diastereoisomers were found to be enantiomerically pure by ^{1}H NMR analysis using the chiral solvating agent. To determine whether the diastereoisomerism arose from configurational differences at the carbinol centre or the sulfoxide centre, the alcohol **13** was oxidised (Scheme 5) to the keto sulfoxide **14**, constitutionally identical with the substrate **12**. The scalemic product **14** was shown by ^{1}H NMR analysis using the chiral solvating agent to have an enantiomeric excess of 55%, from which it could be concluded that the bioproduct **13** was configurationally homogeneous at the carbinol centre. The reduction was therefore stereoselective with respect to reduction of the carbonyl group and there was partial discrimination between the two sulfoxide enantiomers. There remained the question of the absolute and relative configurations of the diastereoisomeric products (as **13**). Attempts to form a well-defined crystalline salt with 1-methylbenzylamine were unsuccessful. To determine the relative configuration, a synthetic mixture of racemic diastereoisomers corresponding to the sulfoxide **13** was converted into the hydrogen phthalates.

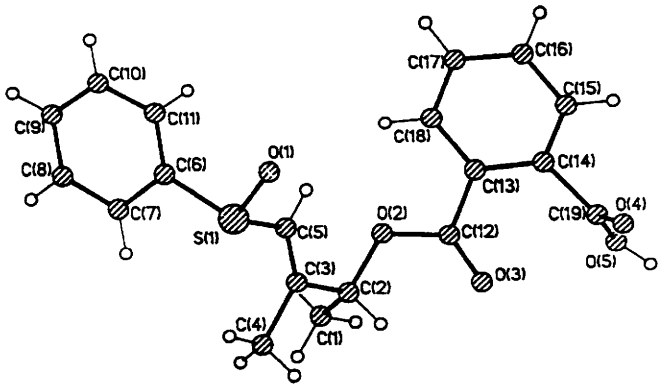
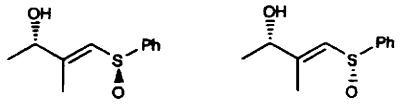


Fig. 2 X-Ray crystal structure of hydrogen (2*S*,5*R*,*E*)-3-methyl-4-phenylsulfinylbut-3-en-2-yl phthalate

Fractional crystallisation from acetone furnished a higher melting diastereoisomer, crystals of which proved to be unsuitable for X-ray structure determination. Fractional crystallisation of the isomer remaining in the mother liquor gave the lower melting diastereoisomer which proved (by ^{1}H NMR) to be identical (as the racemate) with the major diastereoisomer of the hydrogen phthalate of the bioreduction product **13**. Crystals of this compound proved to be suitable for X-ray analysis (Fig. 2) by which it was shown to have the relative configuration 2*R*,5*R* or 2*S*,5*S* (**15**). The absolute



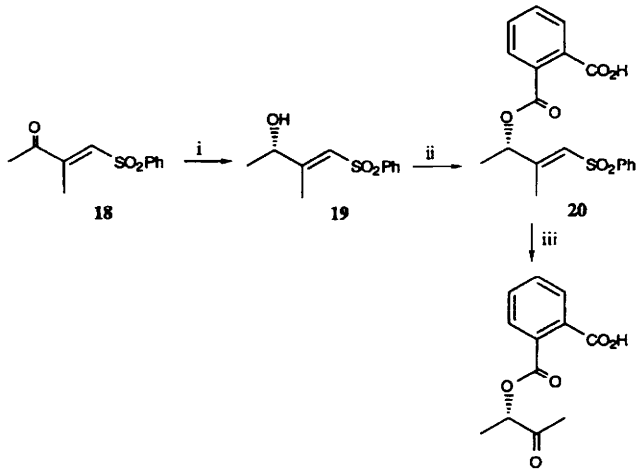
configuration was shown to be 2*S*,5*S* by ozonolysis of the bioreduction product **13** to give (*S*)-acetoin (**16**, Scheme 5). The configuration (2*S*,5*R*) **17** could therefore be assigned to the minor bioreduction product.

When the sulfoxide **12** was reduced using *Z. rouxii*, the sulfoxide **13** was again isolated as the major product (25%), together with unchanged starting material (40%) and minor amounts of sulfide and sulfone corresponding to the starting material. The major diastereoisomer (72% de) proved to be the same as that produced in the reduction by *S. cerevisiae*. ^{1}H NMR analysis as before showed that both diastereoisomers were enantiomerically pure (> 96% ee). Since the mixture was strongly dextrorotatory (as was the product **13** of the *S. cerevisiae* reduction), it could be concluded that the major and minor diastereoisomers again had relative and absolute configurations as shown for structures **15** and **17**, respectively.

To complete the series, reduction of the sulfone **18** was studied. Using *S. cerevisiae*, the only product formed, in excellent yield (70–75%) after 26 h was the corresponding alcohol **19** (Scheme 6), with no recovery of starting material. Chiral analysis by ^{1}H NMR using the chiral solvating agent showed that this product was enantiomerically pure (> 96% ee). The absolute configuration was determined by conversion into the hydrogen phthalate **20** and ozonolysis to give the hydrogen phthalate of (*S*)-acetoin (Scheme 6). Confirmation was obtained by direct ozonolysis of sulfone **19** to (*S*)-acetoin.

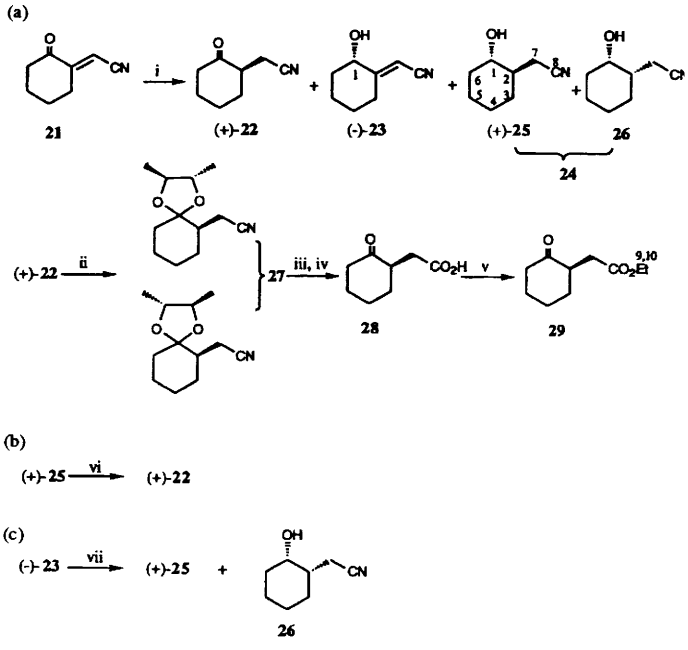
Reduction of the sulfone **18** by *Z. rouxii* gave hydroxy sulfone **19** in high yield (80–85%) but only in modest enantiomeric purity (60% ee).

Next studied were the γ-cyano-αβ-unsaturated ketones **21** and **33**. Such compounds had not previously been subjected to microbial reduction. Thus, (*E*)-(2-oxocyclohexylidene)ethanenitrile **21** was incubated with *Saccharomyces cerevisiae* at 30 °C to give a mixture of reduction products **22**, **23** and **24** in a



Scheme 6 *Reagents:* i, *S. cerevisiae*; ii, phthalic anhydride; iii, O_3

combined yield of 61% [Scheme 7(a)]. Separation of the



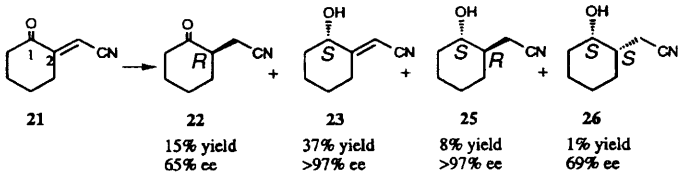
Scheme 7 *Reagents:* i, baker's yeast; ii, H^+/(±)-butane-2,3-diol; iii, OH^-/MeOH–H_2O; iv, $HClO_4$; v, DBU, EtI; vi, CrO_3·2C_5H_5N; vii, Pt–C/H_2

products by flash chromatography yielded (2-oxocyclohexyl)ethanenitrile **22** (15%), (*E*)-(2-hydroxycyclohexylidene)ethanenitrile **23** (28%) and a mixture of the nitrile **23** and (2-hydroxycyclohexyl)ethanenitrile **24** (1:1, 18%). The fully saturated product **24** could not be fully separated from the alcohol **23**. Accordingly, the mixture was treated with osmium tetraoxide and *N*-methylmorpholine *N*-oxide to hydroxylate the double bond of the nitrile **23** giving a mixture from which the fully saturated compound **24** could be isolated by chromatography.

The saturated ketone (+)-**22** was found to have an enantiomeric excess (ee) of 65% by chiral GLC. To determine its absolute configuration it was converted *via* the spiro ketal **27** and the keto acid **28** into (+)-ethyl (2-oxohexyl)ethanoate **29** of known (*R*) absolute configuration [Scheme 7(a)].[18] The fully saturated compound **24** was found by chiral GLC to have a diastereoisomeric excess of 72%. The major enantiomer was enantiomerically pure; the minor enantiomer had an ee of 69%. The major diastereoisomer **25** of the diastereoisomeric mixture

24 was separated from the minor diastereoisomer **26** by OsO_4 oxidation and flash chromatography. The absolute configuration of the major diastereoisomer **25** was determined by oxidation (Collins) to (+)-**22** from which the *R*-configuration at C-2 could be assigned [Scheme 7(b)]. This assignment was confirmed by chiral GLC in comparison with the biotransformation product **22**. The relative configuration of the cyano alcohol **25** was assigned by analysis of the ^{1}H NMR signal at δ 3.3 attributable to 1-H (the carbinol methine proton). Energy minima for structure **25** and its C-2 epimer **26** were computed using PCMODEL. Coupling constants for both chair forms corresponding to structure **26** were calculated. Coupling constants for the four spin system of protons attached to C-1, C-2 and C-6 were extracted using the in-built Karplus routine. The results are shown in Table 1. By inspection of the ^{1}H NMR spectrum, the couplings to 1-H appeared to consist of one of small magnitude (~4 Hz) and two larger couplings of about 10 Hz. These data were more consistent with structure **25** than with either conformer of structure **26** (Table 1). Further confirmation of the structure of compound **25** was obtained by irradiation of the signal attributable to 2-H in the ^{1}H NMR spectrum, which removed one large coupling to leave a doublet of doublets with *J* 10 and 4.4 Hz. The absolute configuration of compound **25** could thus be assigned as shown (1*S*,2*R*).

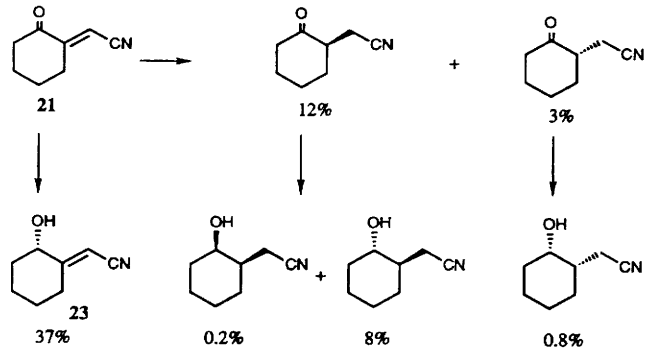
The unsaturated hydroxy nitrile (−)-**23** was found by chiral GLC to be enantiomerically pure (>97% ee). Its absolute configuration was determined by comparing the chiral GLC behaviour of its reduction product (as **24**) with that of the major diastereoisomer (+)-**25** of the fully saturated product and with that of a synthetic mixture of both racemic diastereoisomers corresponding to saturated alcohol **24**. The latter mixture gave two sets of peaks designated 1,2 and 3,4 (in order of increasing retention time) and corresponding to the two diastereoisomeric pairs of enantiomers. By comparison with the GLC behaviour of (+)-**25** of established 1*S*,2*R* absolute configuration, it could be shown that peak 2 corresponded to this enantiomer. When product (−)-**23** was hydrogenated [Scheme 7(c)], two products were obtained. One of these co-chromatographed with (+)-**25**, from which result the 1*S*-configuration could be assigned to the reduction product (−)-**23**. The other product, **26**, was therefore the *1S*,*2S*-isomer and co-eluted with peak 4 of the synthetic mixture. The order of elution of the stereoisomers of the fully saturated product (as **24**) were: peak 1, (1*R*,2*S*), peak 2 (1*S*,2*R*), peak 3 (1*R*,2*R*), peak 4 (1*S*,2*S*). The composition of the product mixture was therefore as summarised in Scheme 8. The absolute configuration of the minor diastereoisomer, **26**, of the fully saturated product **24** was found to be 1*S*,2*S* as it co-eluted with peak 4 of the synthetic mixture.



Scheme 8

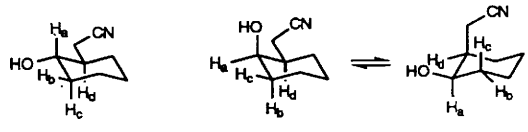
Reduction of the unsaturated keto nitrile **21** by a variety of yeast species (*Zygosaccharomyces rouxii*, *Candida chalmersi*, *Candida diddensii*, *Pichia farinosa* and *Pichia capsulata*) was studied. The results are shown in Table 2. It was noteworthy that all of these species except *Candida chalmersi* gave the saturated ketone **30** of predominantly the *R* configuration and all gave the unsaturated alcohol **31** of predominantly the *S* configuration.

Reduction of the unsaturated ketonitrile **21** by *S. cerevisiae* can be interpreted as shown in Scheme 9. In this scheme it is



Scheme 9

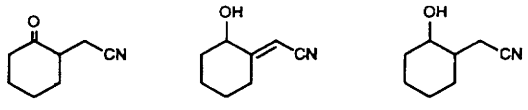
Table 1 Calculated energies and coupling constants for the hydroxy nitriles **25** and **26**



25 **26**

Energy (kJ mol^{-1})	26.0	37.5	40.0
J_{ab}/Hz	4.6	3.9	5.0
J_{ac}/Hz	11.2	2.3	11.0
J_{ad}/Hz	10.2	1.5	5.5

Table 2 Reduction of the keto nitrile **21** by yeasts[a]



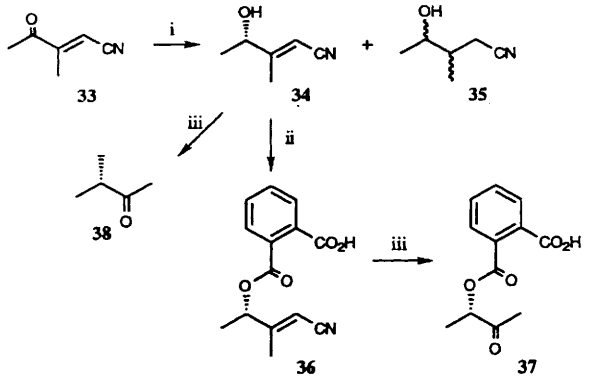
30 **31** **32**

Species	**30** ee% (config.)	**31** ee% (config.)	**32** de%, ee% (config.)	Ratio **30**:**31**:**32**
Z. rouxii	50 (*R*)	—	99, 50 (1*S*, 2*S*)	82:18 (**30**:**32**)
C. chalmersi	19 (*S*)	91 (*S*)	50, >99 (1*R*, 2*S*)[b] 27 (1*S*, 2*S*)	20:70:10
C. diddensiae	8 (*R*)	60 (*S*)	—	34:66 (**30**:**31**)
P. farinosa	87 (*R*)	13 (*S*)	14, 70 (1*R*, 2*S*)[c] 28 (1*R*, 2*R*)	28:30:42
P. capsulata	43 (*R*)	44 (*S*)	*d*	69:23:8

[a] Overall yields were in the range 60–62%. [b] Minor isomer. [c] Major isomer. [d] < 10% saturated nitrile **32** formed.

assumed that reduction of the carbon–carbon double bond precedes reduction of the carbonyl group. This assumption is supported by the relatively high yield of the unsaturated alcohol **23**, suggesting that once formed, it is not subject to further transformation. Presentation of the results as in Scheme 9 emphasises the marked stereochemical bias towards reduction to give an *R*-configured side-chain and *re*-face reduction of the carbonyl group to give the *S*-configured alcohol. The major preference therefore is for hydrogen delivery to opposite faces of the substrate **21** at C-1 and C-2.

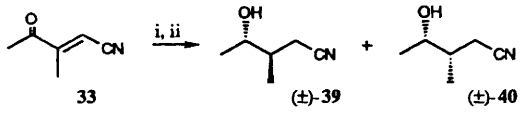
Reduction of (*E*)-3-methyl-4-oxopent-2-enenitrile **33** by *Saccharomyces cerevisiae* gave the unsaturated nitrile **34** and a diastereoisomeric mixture of fully saturated nitriles **35** (Scheme 10) in 70–75% overall yield. The major product **34** was



Scheme 10 *Reagents:* i, *S. cerevisiae*; ii, phthalic anhydride; iii, O_3

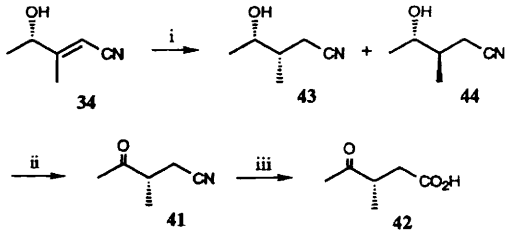
separated by flash chromatography and was found to be enantiomerically pure by chiral GLC. For determination of its absolute configuration it was converted into the hydrogen phthalate **36** and ozonised to the dextrorotatory hydrogen phthalate **37** of acetoin, of known (*S*) absolute configuration.[17] As a further check on the configurational assignment, the nitrile **34** was ozonised directly to (*S*)-acetoin **38**, its identity with which was confirmed by chiral GLC by comparison with a sample of known absolute configuration.

The saturated diastereoisomer mixture **35** could not be separated completely from the unsaturated nitrile **34** by chromatography. Accordingly the mixture was treated with osmium tetraoxide–*N*-methylmorpholine *N*-oxide to convert the unsaturated nitrile **34** into the corresponding diol from which the diastereoisomers **35** were readily separated. The major diastereoisomer was isolated and purified by flash chromatography. For chiral analysis, a mixture of diastereoisomers was prepared from the keto nitrile **33** (Scheme 11). One



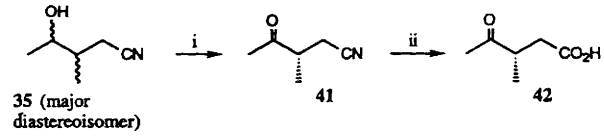
Scheme 11 *Reagents:* i, H_2/Pd–C; ii, $NaBH_4$

diastereoisomer was resolved by chiral GLC but not the other. The three GLC peaks observed, in order of elution, are referred to below as peaks 1 and 1′ (attributable to the enantiomers of one diastereoisomer) and 2 (unresolved second diastereoisomer). The major component (37% de) of the biotransformation product **35** corresponded to peak 2, and the minor product to peak 1′. When biotransformation product **34** (Scheme 10) was hydrogenated and the product was examined by chiral GLC only peaks 1′ and 2 were observed. The major component corresponded to peak 2. The absolute configuration of this major product was determined by oxidation of the mixture and non-stereoselective hydrolysis of the product nitrile **41** under mild conditions (to avoid racemisation) by an immobilised *Rhodococcus* nitrilase SP361 (Novo)[19] (Scheme 12). The



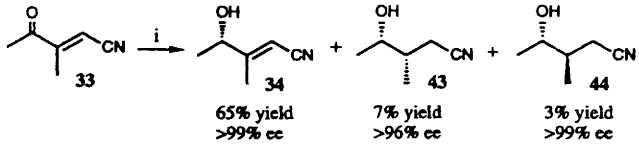
Scheme 12 *Reagents:* i, H/Pd–C; ii, Collins' reagent; iii, *Rhodococcus* nitrilase

product was the dextrorotatory 3-methyllaevulinic acid **42** of known (*S*) absolute configuration.[20] Accordingly, peak 2 in the chiral GLC analysis of the product from the hydrogenation of the hydroxy nitrile **34** could be assigned to the (3*S*,4*S*)-isomer **43** and peak 1′ to the (3*R*,4*S*)-isomer **44**. Peak 1 could accordingly be assigned to the (3*S*,4*R*)-isomer of **44**. These results established that peak 2 in the chiral GLC trace of the saturated bioreduction product **35** (Scheme 10) corresponded either to the (3*S*,4*S*)- or (3*R*,4*R*)-enantiomer, or a mixture of the two. Collins oxidation of product **35** (purified by flash chromatography) followed by hydrolysis using the *Rhodococcus* nitrilase (Scheme 13) again gave (*S*)-3-methyllaevulinic acid,



Scheme 13 *Reagents:* i, Collins' reagent; ii, *Rhodococcus* nitrilase

confirming the (3*S*,4*S*)-configuration **43** for the major component of the bioreduction product **35**. Since chiral analysis by GLC of the racemate corresponding to the major component of saturated product **35** was not possible, an NMR method was devised based on the chiral solvating agent. By this means an enantiomeric excess of >96% for this biotransformation product was established. The biotransformation mixture **35** and the mixture from hydrogenation of the unsaturated hydroxy nitrile **34** had similar diastereoisomeric compositions [both contained an excess of the (3*S*,4*S*)-isomer, as **43**, Scheme 12]. The overall results of the biotransformation of the unsaturated keto nitrile **33** are summarised in Scheme 14.



Scheme 14 *Reagent:* i, baker's yeast

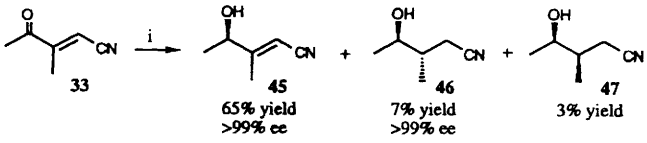
Bioreduction by *Saccharomyces cerevisiae* clearly provides an efficient procedure for the production of the enantiomerically pure (*S*)-hydroxy nitrile **34**. However, it was desirable to develop a method for entry into the enantiomeric series and for this purpose a number of other yeasts were investigated. Reduction using *Zygosaccharomyces rouxii* provided a solution. The keto nitrile **33** again proved to be an excellent substrate and was reduced with overall yields of 70–75%. The isomer composition was investigated using the chiral GLC system developed for the chiral analysis of the reductions by *S. cerevisiae* described above. The overall results are illustrated in

Table 3 Reduction of 3-methyl-4-oxopent-2-enenitrile **33** by yeasts[a]

Species	4-Hydroxy-3-methylpent-2-enenitrile %ee (config.)	%Yield	4-Hydroxy-3-methylpentanenitrile Major isomer %ee (config.)	Minor isomer %ee (config.)	%de
P. capsulata	>99 (*S*)	70	—	—	58
P. farinosa	>99 (*R*)	60	99 (3*S*,4*R*)	—	23
C. chalmersi	93 (*S*)	70	—	59 (2*S*,3*R*)	60

[a] Yields were in the range 70–75%.

Scheme 15. The major product, enantiomerically pure, was the



Scheme 15 *Reagent:* i, *Z. rouxii*

enantiomer **45** of the corresponding product **34** (Scheme 14) from the reduction by *S. cerevisiae*. The major isomer **46** of the mixture of saturated hydroxy nitriles corresponded to the diastereoisomer that was resolvable by chiral GLC and was shown by this method of analysis to be enantiomerically pure and to have the 3*S*,4*R*-configuration shown. Confirmation of the absolute configuration was obtained by oxidation (Collins) to (3*S*)-3-methyl-4-oxopentanenitrile **41**, the same enantiomer as had been obtained from the major stereoisomer **43** of bioreduction product **35** (Scheme 12) from the *S. cerevisiae* reduction. The enantiomeric excess of the minor component **47** (assumed configuration 3*R*,4*R*) was not determined. Results obtained using other yeasts are given in Table 3.

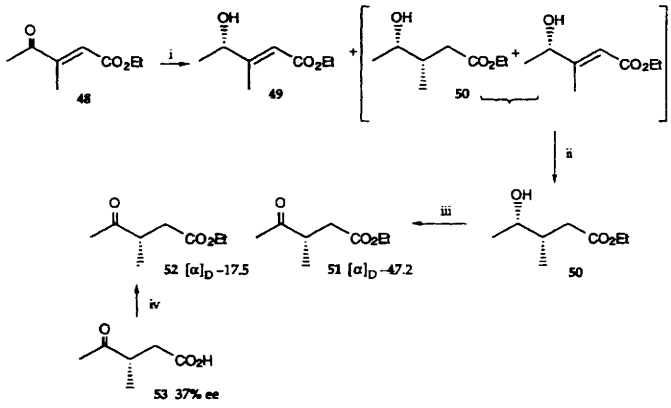
These studies showed that all of the yeasts investigated were able to reduce the carbon–carbon double bond of the unsaturated nitriles to some degree. The results indicate that reduction of the carbonyl group was generally more stereospecific than reduction of the carbon–carbon double bond.

Finally, the reduction of the ester **48** by *Saccharomyces cerevisiae* was studied. From the ester **48**, two products were formed, an unsaturated hydroxy ester **49** and a fully saturated product **50**. Overall yields were 50–55% with the unsaturated alcohol **49** forming the bulk (80%) of the product. The unsaturated hydroxy ester **49** was isolated and purified by flash chromatography. A pure sample of the saturated ester **50** could not be obtained by this means, but only as a mixture with ester **49**. Accordingly, this mixture was hydroxylated (osmium tetraaoxide–*N*-methylmorpholine *N*-oxide). The ester **50** was then readily separated from the water-soluble dihydroxy ester formed from contaminating unsaturated hydroxy ester **49**.

By chiral GLC on an α-cyclodextrin-based column, and by comparison with racemic material, the unsaturated hydroxy ester **49** was shown to be enantiomerically pure (>98% ee). The absolute configuration of the ester was established by conversion into the hydrogen phthalate and ozonolysis to the dextrorotatory hydrogen phthalate of acetoin of known (*S*) absolute configuration. Confirmation was obtained by direct ozonolysis of the ester **49** to (*S*)-(+)-acetoin, the configuration of which was confirmed by chiral GLC using either α- or γ-cyclodextrin-based columns.

The purified saturated ester **50** was found to have a de of >96% by ^{1}H NMR spectroscopy. Its enantiomeric purity was investigated by chiral GLC. The synthetic, racemic material, was obtained as a mixture of diastereoisomers. One of these was resolved by chiral GLC but not the other. The GLC peak corresponding to the unresolved diastereoisomer is referred to below as peak 1, and the peaks corresponding to the enantiomers of the resolved diastereoisomer as peaks 2 and 3. The bioreduction product **50** corresponded to the diastereoisomer that could be resolved; it was found to be enantiomerically pure (>98% ee).

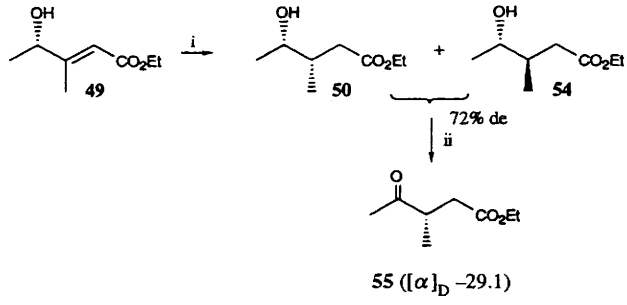
The absolute configuration of the saturated ester was determined in two ways. First (Scheme 16) it was oxidised



Scheme 16 *Reagents:* i, *S. cerevisiae*; ii, OsO_4–*N*-methylmorpholine *N*-oxide/flash chromatography; iii, Collins' reagent; iv, EtI, DBU

(Collins) to laevorotatory ethyl 3-methyllaevulinate **51**. (*S*)-3-Methyllaevulinic acid **53** of 37% ee was available from the parallel studies of the bioreduction of unsaturated keto nitriles. This was converted into the corresponding ethyl ester (**52**, Scheme 16) which was also found to be the laevorotatory enantiomer, thus establishing the *S*-configuration for the bioreduction product **50**. The ester **52**, which was derived from the acid **53** of 37% ee, had $[\alpha]_D$ −17.5, entirely consistent with the value obtained for the product **51** ($[\alpha]_D$ −47.2), the latter being derived from the enantiomerically pure saturated ester **50**.

To establish the configuration at C-4 in the saturated ester **50**, the unsaturated bioreduction product **49** was hydrogenated (Scheme 17). The product obtained (72% de) was examined by



Scheme 17 *Reagents:* i, H_2/Pd–C; ii, Collins' reagent

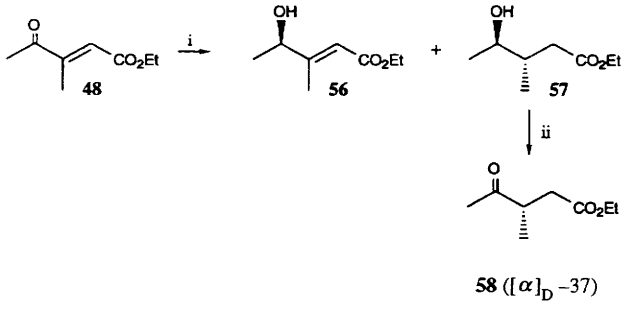
chiral GLC. One peak corresponded to the bioreduction product **50** and not its enantiomer. The other peak could

Table 4 Reduction of ethyl 3-methyl-4-oxopent-2-enoate **48** by yeasts

Yeast species	Ethyl 4-hydroxy-3-methylpent-2-enoate (as **49**) %ee (config.)	Ethyl 4-hydroxy-3-methylpentanoate (as **50**) Major diastereoisomer %ee (config.)	Minor diastereoisomer %ee (config.)	%de
S. cerevisiae	>99 (*S*)	>99 (3*S*,4*S*)	—	>96
Z. rouxii	>94 (*R*)	>78 (3*S*,4*R*)	>98 (2*R*,3*R*)	>95
C. chalmersi	91 (*S*)	>99 (3*S*,4*S*)	—	43
C. diddensiae	77 (*S*)	>99 (3*S*,4*S*)	—	17
P. capsulata	92 (*S*)	>99 (3*S*,4*S*)	—	72
P. farinosa	93 (*R*)	—	>99 (2*R*,3*R*)	74

therefore be attributed to enantiomer **54** of the unresolved diastereoisomer. For confirmation, the mixture of esters (**50** + **54**) was oxidised (Collins) to laevorotatory ethyl (*S*)-3-methyllaevulinate **55** (Scheme 17). The optical rotation of this material ($[\alpha]_D$ −29.1) when compared with that of the ester derived from enantiomerically pure material (**51**, Scheme 16, $[\alpha]_D$ −47.2) indicated an ee of 62%, in good agreement with the de of 72% of the precursor mixture **50** + **54** (Scheme 17) determined by ^{1}H NMR. The configuration 3*S*,4*S* for the saturated ester **50** was thus established. At the same time, the chiral GLC peak 1 could be assigned to the 3*SR*,4*RS* diastereoisomer and peaks 2 and 3 to the 3*R*,4*R*- and 3*S*,4*S*-isomers, respectively.

We had already observed in other series (above) that bioreduction with *Zygosaccharomyces rouxii*, gave products enantiomeric with those obtained using *S. cerevisiae*. When the unsaturated keto ester **48** was reduced by *Z. rouxii*, the unsaturated hydroxy ester **56** was produced together with the saturated ester **57** in 50–55% combined yield (Scheme 18). The



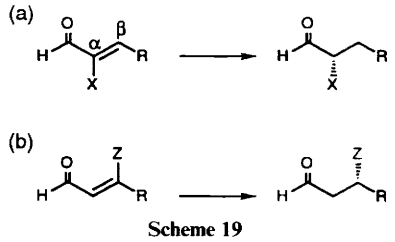
Scheme 18 *Reagents:* i, *Z. rouxii*; ii, Collins' reagent

unsaturated hydroxy ester **56** (the major product, 40–45% yield) was isolated and purified by flash chromatography, but the ester **57** could only be obtained as a mixture with the unsaturated ester **56**. The ester **56** was laevorotatory and was thus assigned the *R*-configuration. However, as the optical rotation was low ($[\alpha]_D$ −3.6), confirmation of this assignment was obtained by conversion into (*R*)-acetoin and the hydrogen phthalate of (*R*)-acetoin, as for the product **49** of the *S. cerevisiae* reduction. By chiral GLC., the ester **56** was shown to be of 94% ee.

The fully reduced ester **57** was shown by chiral GLC to have de >95%. However, the major diastereoisomer corresponded to peak 1 (see above), the diastereoisomer that was not resolved. Oxidation gave laevorotatory ethyl 3-methyllaevulinate **58** and accordingly the ester **57** could be assigned the 3*S*-configuration (compare **50** ⟶ **51**, Scheme 16). The configuration 3*S*,4*R* could thus be assigned to the saturated hydroxy ester **57**. The enantiomeric purity of this compound could be estimated as >78% ee by comparison of the optical rotation ($[\alpha]_D$ −37) with that of the enantiomerically pure product **51** ($[\alpha]_D$ −47.2) obtained *via* the bioreduction with *S. cerevisiae*. The saturated hydroxy ester **57** proved to be unstable on chromatography (possibly owing to lactone formation). Its 3,5-dinitrobenzoate was prepared and fully characterised. However, its intended use for X-ray crystallographic determination of the relative configuration of the ester **57** was frustrated by its lack of crystallinity.

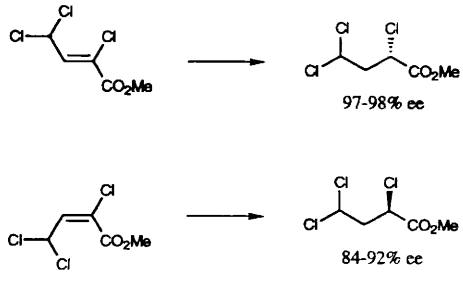
The results obtained in this study are summarised in Table 4, which also gives results obtained using other yeast strains.

For biotransformations such as those described above, it is important to analyse them with respect to consistency, even if the mechanistic basis for such consistency might be obscure. With respect to the examples of carbon–carbon double bond reduction already described in the literature, Servi [1] has pointed out that the observed product stereochemistry for reduction of α- and of β-substituted αβ-unsaturated aldehydes is remarkably consistent. It can be described as in Scheme 19(a), (b) respec-



Scheme 19

tively. α-Substituted compounds are reduced as shown in Scheme 19(a) [21–37] and β-substituted compounds are reduced as shown in Scheme 19(b). [30,32,34] The correlation extends to other classes of αβ-unsaturated compounds. The effect of introducing a bulky *cis*-β-substituent is graphically illustrated by the example shown in Scheme 20. [40] Compounds in which the



Scheme 20

cis-β-arrangement is imposed by a cyclic structure provide particularly clear examples of the second [Scheme 19(b)] mode of reduction. [8,38,39]

There are a few apparent exceptions. Thus, a substrate with ethoxycarbonyl and an incipient (dimethyl acetal) aldehyde groups in a *cis* arrangement apparently reacted in the mode of Scheme 19(a). [41] However, here, the presumed ethyl (*Z*)-3-formylbut-2-enoate may have undergone *Z*–*E* isomerisation

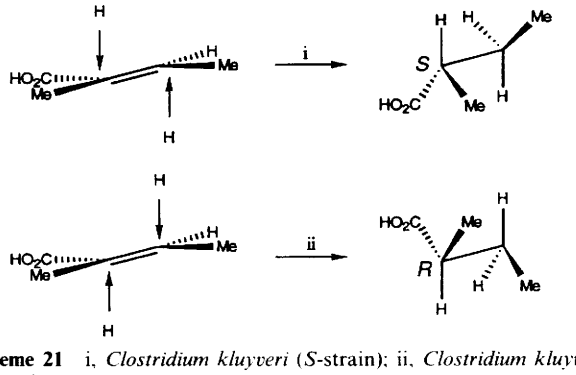
before reduction. (2*Z*)-3-Methylpenta-2,4-dien-1-ol was reduced with low selectivity (the product had 25% ee) by the mode of Scheme 19(a).[42] However, here the diene system in the presumed dienal intermediate might have undergone partial isomerisation to the *E*-isomer before reduction. In another apparent exception, the substrate was submitted to reduction as an *E*,*Z*-mixture leading to uncertainty as to the configuration of the substrate actually giving rise to the observed product.[43]

Thus, with a few such apparent exceptions, the stereochemical pattern of reduction noted by Servi [1] is followed with impressive consistency.

However, a problem arises in interpreting these results when account is taken of the investigations of Fuganti *et al.* on the stereochemistry of C=C bond reduction by baker's yeast. Using cinnamyl alcohol[44] or the lactone of 5-hydroxydec-2-enoic acid[45] as substrates, deuterium labelling experiments clearly showed that in both cases, *trans* addition of hydrogen took place. With respect to the substrate of Scheme 19a, this implies hydrogen addition to C-α from the front and therefore hydrogen addition to C-β from the rear. However, if the substrate of Scheme 19b is reduced with the same conformation with respect to the catalytic groups on the enzyme, the configuration at C-β in the product clearly shows that hydrogen addition takes place from the front and therefore from the rear at C-α. Thus, if *trans* addition is assumed, hydrogen addition to the two substrate types must take place from opposite sides of the molecule at C-α and C-β. Two possibilities must be considered: first, that two different enzymes are responsible for reduction of the two types, second that the two substrate types adopt different conformations at the active site of the enzyme.

A closely comparable situation was revealed by Veschambre and co-workers during their studies of the stereochemical course of reduction of αβ-unsaturated aldehydes and ketones by *Beauveria sulfurescens*.[46–48] It was found that bulky β-substituents (*cis* to the carbonyl function) caused the stereochemistry of addition to be reversed in reductions that were also shown to proceed by overall *trans* addition of hydrogen.[49]

trans Addition–elimination has been demonstrated for a variety of enzymes, ranging from dihydroorotate dehydrogenase[50] to acyl CoA dehydrogenases,[51–53] succinate dehydrogenase,[54] and clostridial enoate reductases.[55–63] The acyl CoA reductases are *re*-face stereospecific (α-centre addition). However, the situation is more complex with the enoate reductases. In *Clostridium kluyveri* ('S-strain') an enoate reductase was identified that was *re*-face stereospecific in the reduction of (*E*)-crotonate and tiglate, giving 2*S*-products.[55–58] However, a further strain (the '*R*-strain') was identified that gave (*R*)-2-methylbutanoate by *si*-face addition at the α-centre.[59,60] The same strain gave products with corresponding stereochemistry from tiglate and (*E*)-2-methylcinnamate as illustrated for tiglate reduction in Scheme 21.[62,63] Given that



Scheme 21 i, *Clostridium kluyveri* (*S*-strain); ii, *Clostridium kluyveri* (*R*-strain)

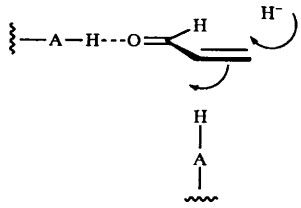


Fig. 3 Postulated arrangement of catalytic groups at the active site of a C=C dehydrogenase. The arrows indicate the overall stereochemistry of hydrogen addition, without mechanistic implications.

microbial reductases thus exist that are either *re*- or *si*-face specific, it seems probable that the correlation noted by Servi [1] is attributable also to the activities of different enzymes and that the switch in substrate specificity is highly sensitive to the presence and steric volume of substituents at the α- and β-centres. This conclusion is reinforced by a consideration of the probable nature and disposition of the catalytic centres involved in reductions. Three centres are probably involved: a hydrogen bond donor to the carbonyl oxygen atom, the hydride ion donor to the β-centre and a proton donor placed so as to give overall *trans* addition (Fig. 3). Regardless of the degree of concertedness of the reduction, the arrangement of the three catalytic centres will be complementary specifically to one face of an αβ-unsaturated carbonyl system. If binding in the optimum manner were to be inhibited by steric interactions between, say, a bulky β-substituent and the enzyme, this might be relieved by rotation about the carbonyl carbon-C-α bond so as to present the opposite face of the αβ-double bond to the catalytic groups (as suggested by Veschambre for reductions by *Beauveria sulfurescens*) or by binding of the entire αβ-unsaturated carbonyl system on its opposite face. In either case, it seems unlikely that the positioning of all three key centres (Fig. 3) would be sufficiently close to the optimum arrangement to allow the catalytic mechanism to operate even if the substrate were to bind in such a way as to give the minimum root mean square deviation from the optimum positions. The different stereochemical courses observed in reduction of the two classes of substrate are therefore more probably attributed to the operation of different enzymes.

It is noteworthy that in all of the reductions using bakers' yeast in the present work, the methyl ketone system was reduced to give the *S*-configured secondary alcohol with remarkable consistency and high enantioselectivity. Similar results have been obtained by other workers, using bakers' yeast from widely differing sources.[64–66] It has been observed that in bakers' yeast, at least four different dehydrogenases may participate in the reduction of keto esters.[67–69] It thus appears probable that the rigid (relative to the fully saturated system) αβ-unsaturated structure limits the enzymes capable of reducing the carbonyl group to one only of these dehydrogenases (or another, so far undescribed), with manifestation of minor or negligible activity with respect to the other enzymes.

Experimental

^{1}H NMR spectra were determined at either 250 MHz or 400 MHz using either Bruker AC 250 or WH 400 spectrometers respectively. ^{13}C NMR spectra were determined at either 62.89 or 100.62 MHz using the same instruments. Mass spectra were determined with a Kratos MS 80 mass spectrometer. IR spectra were obtained on a Perkin–Elmer FT-IR 1720X spectrometer. Chiral analysis by NMR was carried out using the chiral solvating agent (*S*)-1-(9-anthryl)-2,2,2-trifluoroethanol in [$^2H_6$] benzene using 5 equiv. of solvating agent. Optical rotations were determined using an Optical Activity AA-1000 polarimeter and with a 2 dm cell. Optical rotations are given in

units of 10^{-1} deg cm^2 g^{-1}. GLC was carried out using a Shimadzu GC-14A gas chromatograph with a C-R5A Chromatopac integrator, with H_2 as carrier gas and FID detection. For normal analysis, an FSCC 25A02/BP20025 column (25 m, 0.22 mm i.d., SGE, Richmond, Ringwood, Australia) was used with H_2 linear velocity 46 cm s^{-1}, injector temperature 250 °C, detector temperature 250 °C. For chiral analysis either a Lipodex A (perpentyl α-cyclodextrin in OV 1701, 25 m) and/or a Lipodex D2 (6-methyl-2,3-di-*O*-pentyl γ-cyclodextrin in OV 1701 25 m) column was used. Light petroleum had bp 40–60 °C.

The yeast strains used were obtained as follows. *Zygosaccharomyces rouxii* NCYC 564 and *Saccharomyces cerevisiae* NCYC 1765 were obtained from the National Collection of Yeast Cultures, Norwich, UK. *S. cerevisiae* was also obtained in bulk from Sainsbury's Supermarket, Cannon Park, Coventry, UK. (This yeast is deposited as NCYC 1765.) *Pichia capsulata* CBS 837 and *Pichia farinosa* CBS 2006 were obtained from the Centraal Bureau voor Schimmelcultures, Baarn, the Netherlands. *Candida chalmersi* NRRL Y 311 was obtained from the Northern Utilisation Research Laboratory and Development Division, Peoria, Illinois, USA. *Candida diddensiae* ATCC 20213 was obtained from the American Type Culture Collection. Rockville, Maryland, USA. Collins' reagent is dipyridine chromium(VI) oxide; although the name sodium borohydride has been used throughout, the compound described is more correctly named according to the IUPAC rules of nomenclature as sodium boranuide.

Growth conditions

For the production of biomass, the following growth solution was used: (g dm^{-3}) KH_2PO_4 (2.72), $(NH_4)_2SO_4$ (1.8), $MgSO_4 \cdot 7H_2O$ (1.8), $FeCl_3$ (0.001), D-glucose (15) and yeast extract (5). Trace element solution (1 cm^3) was added containing (g dm^{-3}) $CuSO_4 \cdot 2H_2O$ (0.02), $MnSO_4 \cdot 4H_2O$ (0.1), $ZnSO_4 \cdot 7H_2O$ (0.1), $CaCl_2 \cdot 2H_2O$ (2.232). The pH of the medium was adjusted to pH 6.3. The medium was autoclaved at 120 °C for 20 min. The solution of D-glucose was autoclaved separately and was added to the medium before inoculation. Flasks (500 cm^3) filled with either 150 cm^3 or 200 cm^3 of medium were inoculated and cultivated under anaerobic conditions (*i.e.* under CO_2) on a rotary shaker at 200 rpm at 28 °C for 24 h. Growth was followed by measuring absorbance at 610 nm. Cell dry mass was determined by centrifugation of 100 cm^3 of the culture and drying the separated cells to constant weight at 105 °C. The yeast cultures were centrifuged, the cells were washed with sterile 5% aqueous sucrose and again centrifuged. The cell pellet was transferred to a 250 cm^3 flask containing aqueous sucrose (5%; 60 cm^3). The substrate was added, the flask was closed with a fermentation cock and the mixture was cultivated anaerobically. For 200 mg substrate an amount of wet cells corresponding to 4–5 g dry weight was used.

(*E*)-3-Methyl-4-phenylsulfanylbut-3-en-2-one 6

To a suspension of sodium hydride (80% dispersion in oil; 870 mg, 30 mmol) in diethyl ether (20 cm^3) under N_2 at 0 °C was added diethyl phenylsulfanylmethylphosphonate (7.93 g, 30 mmol) in diethyl ether (20 cm^3) dropwise over 10 min. 3,3-Dimethoxybutan-2-one (4.9 g, 30 mmol) was added over 10 min. The mixture was stirred at 0 °C for 1.5 h and then at room temperature for 8 h. The mixture was diluted with water (30 cm^3) and hydrochloric acid (4 mol dm^{-3}; 20 cm^3) after which it was stirred for 1 h and extracted with ethyl acetate (4 × 50 cm^3). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure and the residue was purified by flash chromatography [ethyl acetate–light petroleum (1:19)] to give the title compound **6** as a pale yellow liquid (3.92 g, 67%) (Found: M^+, 192.0609. $C_{11}H_{12}OS$ requires 192.0609); ν_{max}(neat)/cm^{-1} 1659 (C=O) and 1580 (C=C); δ_H(250 MHz; $CDCl_3$) 1.93 (3 H, s, CH_3C=), 2.30 (3 H, s, CH_3CO) and 7.50 (6 H, m, ArH and CH=); δ_C(60 MHz; $CDCl_3$) 12.87 (CH_3C=), 24.97 (CH_3CO), 128.08 (C-3), 129.38 (C-4′), 130.73 (C-2′, 6′), 133.64 (C-3′, 5′), 133.72 (C-1′), 143.15 (C-4) and 194.66 (CO); *m*/*z* (EI) 192 (56%), 177 (43), 149 (7), 134 (41), 125 (5), 115 (40), 77 (24), 71 (10), 65 (29), 51 (23) and 43 (100).

(2*S*,3*R*)-3-Methyl-4-sulfanylbutan-2-ol 7

A 250 cm^3 flask fitted with a fermentation cock (bubbler) and a magnetic stirrer was charged with sucrose (5 g) and baker's yeast (5 g), followed by distilled water (120 cm^3). After the contents had been stirred at 30 °C for 15 min, a solution of the butenone **6** (200 mg) in ethanol (2 cm^3) was added and stirring of the mixture was continued. A fresh charge of baker's yeast (2 g) and sucrose (1 g) was added every 24 h. After 8 days the mixture was filtered (Celite) and the filtrate was extracted with ethyl acetate (5 × 40 cm^3); the filter pad was washed with ethyl acetate (2 × 30 cm^3). The combined extracts and washings were dried ($MgSO_4$) and evaporated under reduced pressure at < 40 °C and the residue was purified by flash chromatography [light petroleum–ethyl acetate (9:1)] to give a mixture of starting material **6** and (*R*)-3-methylsulfanylbutan-2-one **8** (110 mg, ratio **6**:**8** = 64:36), and the title compound **7** (36 mg, 18%) as an oil (Found: M^+, 196.0922. $C_{11}H_{16}OS$ requires *M*, 196.0922); $[\alpha]_D^{20}$ −13.6 [*c* 0.3 in MeOH (for material of 90% de)] ν_{max}(neat)/cm^{-1} 3500 (OH); δ_H(400 MHz; $CDCl_3$) 1.02 (3 H, d, *J* 6.9, CH_3CH), 1.18 [3 H, d, *J* 6.4, $CH_3CH(OH)$], 1.77 (1 H, m, MeC*H*), 2.79 (1 H, dd, *J* 7.6, 12.8, C*H*HS), 3.13 (1 H, dd, *J* 6.3, 12.8, CH*H*S), 3.99 [1 H, m, C*H*(OH)] and 7.14–7.35 (5 H, ArH); δ_C(100 MHz; $CDCl_3$) 13.61 (CH_3CH), 20.06 [$CH_3CH(OH)$], 37.08 (CH_2S), 39.06 (CH_3C), 69.50 [C(OH)], 125.69 (C-4′), 128.80 (C-2′, 3′, 5′, 6′) and 136.79 (C-1′); *m*/*z* (EI) 196 (62%), 151 (13), 123 (45), 110 (100), 91 (7), 86 (60), 77 (19), 71 (85), 58 (15), 51 (20) and 45 (45).

(±)-3-Methyl-4-sulfanylbutan-2-ol (as 7)

To a stirred solution of the butenone **6** (600 mg) in tetrahydrofuran–methanol (9:1; 10 cm^3) at −20 °C was added sodium borohydride (50 mg) in three portions over 20 min. The mixture was stirred for 45 min and then poured into ice-cold water (40 cm^3) and extracted with diethyl ether (5 × 40 cm^3). The combined extracts were washed with hydrochloric acid (1 mol dm^{-3}; 2 × 10 cm^3) and water (2 × 25 cm^3), dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by flash chromatography with light petroleum–ethyl acetate (19:1) as eluent to give (±)-(*E*)-3-methyl-4-phenylsulfanylbut-3-en-2-ol (as **10**) (550 mg, 91%). (Spectroscopic and NMR data were as for the optically active compound **10**, below.) This compound (450 mg) in ethyl acetate (20 cm^3) was hydrogenated over 10% Pd–C at 45 psi at room temperature for 24 h. The product was purified by flash chromatography [ethyl acetate–light petroleum, 91:9)] to give (±)-3-methyl-4-phenylsulfanylbutan-2-ol (as **7**) as a mixture of diastereoisomers (110 mg, 21%). The spectroscopic data of one diastereoisomer were as described for the bioreduction product **7**, above. The other diastereoisomer had the following spectroscopic properties: δ_H(400 MHz; $CDCl_3$) 1.03 (3 H, d, *J* 6.7, CH_3CH), 1.19 [3 H, d, *J* 6.2, $CH_3CH(OH)$], 1.77 (1 H, m, MeC*H*), 2.78 (1 H, dd, *J* 8.2, 12.6, C*H*HS), 3.19 (1 H, dd, *J* 4.6, 12.6, CH*H*S), 3.97 [1 H, m, C*H*(OH)] and 7.16–7.35 (5 H, m, ArH); δ_C(100 MHz; $CDCl_3$) 15.25 (CH_3CH), 20.21 [$CH_3C(OH)$], 37.06 (CH_3CH), 39.98 (CH_2S), 70.91 [C(OH)], 125.64 (C-4′), 128.74 (C-2′, -3′, -5′, -6′) and 136.79 (C-1′).

Acetylation of the hydroxy sulfide 7

To the hydroxy sulfide **7** (13 mg) in pyridine (0.3 cm^3) was added 4-dimethylaminopyridine (3 mg) followed by acetic

anhydride (0.2 cm^3). The mixture was stirred for 8 h at room temperature, evaporated under reduced pressure and diluted with water (5 cm^3). The mixture was extracted with diethyl ether (5 × 5 cm^3) and the combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The product was purified by chromatography over silica [ethyl acetate–light petroleum (1:19)] to give (1*S*,3*R*)-1,3-dimethyl-3-phenylsulfanylpropyl acetate (15 mg, 90%). The acetate of the corresponding racemic compound was prepared in the same way. Data are reported for the derivative of the bioreduction product (major isomer), which was used for chiral analysis in [$^2H_6$]benzene using the chiral solvating agent (Found: M^+, 238.1027. $C_{13}H_{18}SO_2$ requires *M*, 238.1025): ν_{max}(neat)/cm^{-1} 1735 (CO); δ_H(400 MHz; [$^2H_6$]benzene) 0.94 (3 H, d, *J* 6.9, CH_3CHCH$_2$), 0.97 [3 H, d, *J* 6.5, CH_3CH(OAc)], 1.67 (3 H, s, OCOCH_3), 1.77 (1 H, m, MeC*H*CH$_2$), 2.50 (1 H, dd, *J* 8.8, 13.0, C*H*HS), 2.99 (1 H, dd, *J* 5.0, 13.0, CH*H*S), 5.09 [1 H, dq, *J* 6.5, 3.9, MeC*H*(OAc)], 6.96 (1 H, apparent t, *J* 7.3, 4-H′), 7.05 (2 H, m, 3′-, 5′-H) and 7.31 (2 H, d, *J* 8.29, 2′-, 6′-H); *m/z* (EI) 238 (44%), 178 (24), 163 (18), 123 (38) and 55 (7).

Hydrogen phthalate of the hydroxy sulfide 7

To the hydroxy sulfide **7** (30 mg) in pyridine (0.5 cm^3) was added phthalic anhydride (40 mg). The mixture was stirred for 48 h at 30 °C after which it was diluted with water (40 cm^3), acidified (2 mol dm^{-3} HCl) to pH 3, and extracted with ethyl acetate (5 × 15 cm^3). The combined extracts were washed with water (2 × 15 cm^3), dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by flash chromatography [ethyl acetate–light petroleum (30:70)] to give the hydrogen (2*S*,3*R*)-3-methyl-4-phenylsulfanylbutan-2-yl phthalate as a gum (40 mg, 80%) [Found: $(M + H)^+$, 345.1160. $C_{19}H_{21}SO_4$ requires $(M + H)$, 345.1150]; $[\alpha]_D^{20}$ +48.7 (*c* 1.4 in MeOH); ν_{max}($CHCl_3$)/cm^{-1} 3503br (CO_2H) and 1718 (ester CO); δ_H(400 MHz; $CDCl_3$) 1.20 (3 H, d, *J* 6.9, CH_3CH), 1.35 [3 H, d, *J* 6.4, CH_3C(OPhth)], 1.99 (1 H, m, MeC*H*), 2.76 (1 H, dd, *J* 8.6, 13.0, C*H*HS), 3.14 (1 H, dd, *J* 8.2, 13.0, CH*H*S), 5.33 [1 H, dq, *J* 3.7, 6.4, C*H*(OH)], 7.23 (5 H, m, ArH), 7.60 (3 H, m, ArH) and 7.80 (1 H, m, ArH); *m/z* [(FAB (NBA)] 344 (15%), 179 (39), 154 (100), 77 (59) and 52 (28).

(*R*)-1-Methylbenzylammonium (2*S*,3*R*)-3-methyl-4-phenylsulfanylbutan-2-yl phthalate

To hydrogen (2*S*,3*R*)-3-methyl-4-phenylsulfanylbutan-2-yl phthalate (25 mg) in acetone (3 cm^3) was added (*R*)-1-methylbenzylamine (12 mg). After the mixture had been stored at room temperature for 1 h it was evaporated under reduced pressure and the residue was dissolved in ethyl acetate–light petroleum–cyclohexane (2:5:3; 10 cm^3) the solution was left for 48 h to afford a crystalline product which was recrystallised from the same solvent mixture to give the title compound mp 95–96 °C. A single crystal was submitted for X-ray structure determination, the details of which are as follows.

Crystal data. $C_{27}H_{31}NO_4S$, *M* 465.59, *T* = 293(2) K, λ = 0.710 73 Å. Orthorhombic *a* = 6.111(7) Å; *b* = 15.85(2), *c* = 25.96(4), *V* = 2514(5) $Å^3$; space group *P*2(1)2(1)2(1), *Z* = 4; D_x = 1.230 mg m^{-3}, μ = 0.161 mm^{-1}, *F*(000) = 992. Crystal size 0.7 × 0.13 × 0.07 mm; θ range for data collection 2.03–22.55°; index ranges $0 \leqslant h \leqslant 6$, $0 \leqslant k \leqslant 17$, $0 \leqslant l \leqslant 28$; reflections collected 1947; independent reflections 1947 [*R*(int) = 0.00]; absorption correction none; refinement method full-matrix least squares on F^2; data/restraints/parameters 1944/60/298; goodness-of-fit on F^2 1.007; *R*(*F*) [$I > 2\sigma(I)$] = 0.0696, $wR(F^2)$ = 0.1910; absolute structure parameter −0.2(5); largest diff. peak and hole 0.196 and −0.231 e $Å^{-3}$.

(*R*)-3-Methyl-4-phenylsulfanylbutan-2-one 8

The title compound **8** was obtained as an inseparable mixture with recovered starting material compound **6** (see above). Accordingly, it was analysed as the mixture. The spectroscopic data were identical with those of the same compound prepared by oxidation of compound **7** (see below). The optical rotation was determined on the mixture: $[\alpha]_D^{20}$ +35.4 (*c* 1.4 in MeOH).

Compound 8 by oxidation of bioreduction product 7

The hydroxy sulfide **7** (20 mg) in dichloromethane (15 cm^3) was treated with Collins' reagent (120 mg) at 15 °C for 4 h after which the reaction mixture was diluted with light petroleum (20 cm^3) and filtered, and the residue was washed with dichloromethane–light petroleum (1:1; 10 cm^3). The filtrate was evaporated under reduced pressure and the product was purified by flash chromatography [ethyl acetate–light petroleum (1:19)] to give the title compound **8** (10 mg, 50%) and recovered starting material (8 mg) (Found: M^+, 194.0761. $C_{11}H_{14}OS$ requires *M*, 194.0765); $[\alpha]_D^{20}$ +57.9 (*c* 0.19 in MeOH); ν_{max}(neat)/cm $^{-1}$ 1714 (CO); δ_H(400 MHz; $CDCl_3$) 1.20 (3 H, d, *J* 6.9, CH_3CH), 2.15 (3 H, s, CH_3CO), 2.75 (1 H, m, MeC*H*), 2.85 (1 H, dd, *J* 6.9, 13.1, C*H*HS), 3.24 (1 H, dd, *J* 6.8, 13.1, CH*H*S) and 7.19–7.31 (5 H, m, ArH); δ_C(100 MHz; $CDCl_3$) 16.31 (*C*H_3CH), 28.7 (*C*H_3CO), 36.12 (CH$_3$*C*H), 46.37 (CH_2S), 126.24 (C-4′), 128.8 (C-2′, -6′), 129.55 (C-3′, -5′), 135.64 (C-1′) and 210.53 (CO); *m/z* (EI) 194 (52%), 151 (17), 123 (48), 110 (83), 85 (49), 77 (16), 65 (18), 51 (15) and 43 (100).

(±)-3-Methyl-4-phenylsulfanylbutan-2-one (as 8)

To a solution of oxalyl chloride (0.7 cm^3) in dichloromethane (10 cm^3) at −70 °C was added dimethyl sulfoxide (1 cm^3) slowly *via* a syringe. The mixture was stirred for 5 min after which 3-methyl-4-sulfanylbutan-2-ol (as a mixture of racemic diastereoisomers, 40 mg) in dichloromethane (7 cm^3) was added to it during 10 min. The mixture was stirred at −70 °C for 30 min after which triethylamine (2 cm^3) was added dropwise to it; the mixture was then stirred for a further 1 h. After dilution with water (20 cm^3) the mixture was stirred for 2 h as it was allowed to warm to room temperature. The organic layer was separated and the aqueous phase was extracted with dichloromethane (2 × 25 cm^3). The combined extracts were dried ($MgSO_4$) and evaporated to give the title compound (30 mg, 75%). Spectroscopic data were as described for the product obtained from oxidation of the bioreduction product **7**, above.

(*R*,*E*)-3-Methyl-4-phenysulfanylbut-3-en-2-ol 10

Cells of *Zygosaccharomyces rouxii* (5 g equiv. dry weight) were transferred to a 250 cm^3 flask which was then charged with distilled water (110 cm^3) and sucrose (5 g). The mixture was stirred for 20 min at 30 °C under anaerobic conditions as described above for the reductions using *S. cerevisiae* after which a solution of the butenone **6** (200 mg) in ethanol (2 cm^3) was added to it. The mixture was stirred for 2 days, treated with Celite (5 g) and filtered. The filtrate was extracted with ethyl acetate (4 × 30 cm^3) and the filter pad was washed with ethyl acetate (2 × 30 cm^3). The combined organic phases were dried ($MgSO_4$) and evaporated under reduced pressure and the product was purified by flash chromatography [light petroleum–ethyl acetate (19:1)] to give the title compound **10** (20 mg, 10%) as a colourless gum (Found: M^+, 194.0765. $C_{11}H_{14}OS$ requires *M*, 194.0765); $[\alpha]_D^{20}$ −10 (*c* 0.2 in MeOH); ν_{max}(neat)/cm^{-1} 3356 (OH) and 1631 (C=C); δ_H(400 MHz; $CDCl_3$) 1.32 [3 H, d, *J* 6.4, CH_3C(OH)], 1.84 (3 H, s, CH_3C=), 4.32 [1 H, q, *J* 6.4, C*H*(OH)], 6.27 (1 H, s, CH=) and 7.17–7.38 (m, 5 H, ArH); δ_C(100 MHz; $CDCl_3$) 13.70 (*C*H_3C=), 21.74 [*C*H_3C(OH)], 72.33 [C(OH)], 118.17 (C=*C*S), 126.19 (C-

4′), 128.82 (C-2′, -6′ or C-3′, -5′), 128.93 (C-3′, -5′ or C-2′, -6′), 129.91 (CH_3C=) and 142.12 (C-1′); *m/z* (EI) 194 (38%), 179 (20), 161 (17), 149 (13), 110 (100), 85 (44), 77 (15), 65 (24), 51 (23) and 43 (55).

Ozonolysis of the butenol 10

Compound **10** (40 mg) in methanol (3 cm^3) was ozonised at −20 °C for 3 h after which it was treated with a solution of sodium sulfite (0.26 mmol) in water (5 cm^3) and then diluted with water (15 cm^3). After passage of nitrogen through the solution to remove the excess of ozone it was extracted with diethyl ether (3 × 10 cm^3). The combined extracts were reduced to 4 cm^3 by passage through them of a stream of dry nitrogen. The residual liquid was then dried ($MgSO_4$) and analysed by chiral GLC using the Lipodex D_2 (α-cyclodextrin) column at 20 °C. The acetoin was eluted with the retention time of (*R*)-acetoin **11**, confirmed by co-injection with authentic material.

Cyclic ketal 9 of (*R*)-3-methyl-4-phenylsulfanylbutan-2-one 8 with (2*R*,3*R*)-butane-2,3-diol

To a solution of the butenone **8** (as a mixture with starting material **6**; 20 mg, 0.1 mmol) in dry benzene (2.5 cm^3) was added (2*R*,3*R*)-butane-2,3-diol (25 mg), toluene-*p*-sulfonic acid (1 mg) and $MgSO_4$ (anhydrous, 1 mg). After the mixture had been boiled under reflux for 3.5 h, TLC indicated complete disappearance of starting material. The mixture was therefore evaporated under reduced pressure and applied to a column of activated alumina (Brockmann 1, neutral, pH 6.5–7.5). The column was eluted with light petroleum–ethyl acetate (49:1) to give (2*R*,4′*R*,5′*R*)-2-methyl-2-(2′,4′,5′-trimethyldioxolan-2-yl)ethanethiol **9** (24 mg, 85%) (Found: M^+, 266.1344. $C_{15}H_{22}SO_2$ requires *M*, 266.1347); δ_H(400 MHz; $CDCl_3$) 1.10 (3 H, d, *J* 6.9, CH_3CHCH_2S), 1.22 (6 H, d, *J* ~6.0, 2 × CH_3CO), 1.30 [3 H, s, $OC(CH_3)O$], 1.92 (1 H, m, MeC*H*), 2.50 (1 H, dd, *J* 10.9, 12.9, C*H*HS), 3.40 (1 H, dd, *J* 2.7, 12.9, CH*H*S), 3.51 (1 H, m, MeC*H*CO), 3.67 (1 H, m, MeC*H*CO) and 7.10–7.47 (5 H, ArH) (major isomer); δ_H 1.11 (3 H, d, *J* 6.9, CH_3CHCH_2S), 1.226 (3 H, d, *J* 6.0, CH_3CHCO), 1.228 (3 H, d, *J* 5.9, CH_3CHCO), 1.30 [3 H, s, $OC(CH_3)O$], 1.92 (1 H, m, MeC*H*), 2.49 (1 H, dd, *J* 10.9, 12.8, C*H*HS), 3.42 (1 H, dd, *J* 2.6, 12.8, CH*H*S), 3.51 (1 H, m, MeC*H*CO), 3.67 (1 H, m, MeC*H*CO) and 7.10–7.47 (5 H, ArH) (minor isomer); *m/z* (EI) 266 (0.5%), 177 (2), 151 (2), 115 (100), 109 (9), 85 (3), 73 (12), 55 (11) and 45 (3).

Cyclic ketal (as 9) of (±)-3-methyl-4-phenylsulfanylbutan-2-one with (2*R*,3*R*)-butane-2,3-diol

The cyclic ketal (as **9**) of (±)-3-methyl-4-phenylsulfanylbutan-2-one with (2*R*,3*R*)-butane-2,3-diol was prepared as for the optically active sample above, in 81% yield. Spectroscopic data were as for the diastereoisomeric mixture prepared from *S. cerevisiae* bioreduction product **8**.

(*E*)-3-Methyl-4-phenylsulfinylbut-3-en-2-one 12

Sodium metaperiodate (10 g, 500 mmol) in water (60 cm^3), methanol (20 cm^3) and tetrahydrofuran (10 cm^3) were added to a solution of compound **6** (0.9 g, 4.65 mmol) dissolved in methanol and cooled to 0 °C. The mixture was stirred at 0 °C for 27 h after which it was filtered, diluted with water (60 cm^3) and extracted with ethyl acetate (4 × 40 cm^3). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure and the residue was purified by flash chromatography [ethyl acetate–light petroleum (60:40)] to give the title compound **12** as a thick oil (0.75 g, 77%) (Found: M^+, 208.0551. $C_{11}H_{12}SO_2$ requires *M*, 208.0558); ν_{max}(neat)/cm^{-1} 1722 (CO), 1686 (C=C) and 1044 (SO); δ_H(250 MHz; $CDCl_3$) 2.23 (3 H, s, CH_3C=), 2.32 (3 H, s, CH_3CO), 6.98 (1 H, s, CH=), 7.54 (3 H, m, ArH) and 7.65 (2 H, m, ArH); δ_C(60 MHz; $CDCl_3$) 13.90 (CH_3C=), 25.88 (CH_3CO), 124.0 (C-2′, -6′), 129.64 (C-3′, -5′), 131.44 (C-4′), 143.09 (C-1′), 143.20 (*C*=CSO), 145.59 (=CSO) and 197.43 (CO); *m/z* (EI) 208 (12%), 166 (27), 126 (16), 77 (15), 51 (12) and 43 (100).

(*S*,*E*)-3-Methyl-4-phenylsulfinylbut-3-en-2-ol 13: reduction by baker's yeast

To a stirred mixture of baker's yeast (5 g) and sucrose (5 g) in distilled water (110 cm^3) at 30 °C under anaerobic conditions was added a solution of compound **12** (200 mg, 0.96 mmol) in ethanol (2 cm^3). The mixture was stirred for 4 days after which the product was isolated as described for the reduction of compound **6** above. Flash chromatography of the crude product in ethyl acetate–light petroleum [gradient from 0% ethyl acetate to ethyl acetate–light petroleum (1:1)] gave first (*E*)-3-methyl-4-phenylsulfanylbut-3-en-2-one (6 mg) followed by 3-methyl-(*E*)-4-phenylsulfonylbut-3-en-2-one (8 mg) and then (with the 1:1 solvent mixture) starting material, compound **12** (113 mg), $[\alpha]_D^{23}$ −1.4 (*c* 1.6, MeOH) and compound **13** (39 mg, 19%) (Found: M^+, 210.0719. $C_{11}H_{14}SO_2$ requires *M*, 210.0715); $[\alpha]_D^{20}$ +111.6 (*c* 0.6 in MeOH); ν_{max}(neat)/cm^{-1} 3383 (OH), 1627 (C=C) and 1017 (SO); δ_H(400 MHz; [$^2H_6$]benzene) 1.29 [3 H, d, *J* 6.5, $CH_3C(OH)$], 2.13 (3 H, s, CH_3C=), 4.24 [1 H, q, *J* 6.5, C*H*(OH)], 6.30 (1 H, S, CH=) and 7.45–7.56 [5 H, m, ArH (major diastereoisomer)]; δ_H 1.27 [3 H, d, *J* 6.5, $CH_3C(OH)$], 2.12 (3 H, s, CH_3C=), 4.25 [1 H, q, *J* 6.5, MeC*H*(OH)], 6.39 (1 H, s, CH=) and 7.47–7.56 [5 H, m, ArH (minor diastereoisomer)]; δ_C(60 MHz; $CDCl_3$) 15.33 (CH_3C=), 21.39 [$CH_3C(OH)$], 70.68 [C(OH)], 124.03 (C-2′, -6′), 129.19 (C-3′, -5′), 129.67 (C-4′), 130.55 (CH=), 144.07 (C-1′) and 155.62 (CH_3C=) (major diastereoisomer); *m/z* [CI (NH_3)] 211 $(M + H)^+$ (100%), 193 (34), 177 (90) and 161 (20).

Hydrogen (*S*,*E*)-3-methyl-4-phenylsulfinylbut-3-en-2-yl phthalate 13

A mixture of compound **13** (103 mg, 0.5 mmol) and phthalic anhydride (86 mg, 0.6 mmol) dissolved in pyridine (0.8 cm^3) was maintained at 30 °C for 36 h after which it was diluted with water (30 cm^3). The solution was adjusted to pH 3 (dilute HCl) and extracted with ethyl acetate (5 × 30 cm^3). The combined extracts were washed with water (2 × 10 cm^3), dried ($MgSO_4$) and evaporated under reduced pressure to give the hydrogen phthalate (180 mg, 100%). The product was twice recrystallised from acetone–light petroleum to give the compound **13**, mp 148–149 °C [Found: $(M + H)^+$, 359.0950. $C_{11}H_{19}SO_5$ requires 359.0952]; $[\alpha]_D^{20}$ +148.3 (*c* 0.24 in MeOH); *m/z* [FAB (NBA)] 359 (37%), 307 (27), 193 (25), 89 (68) and 77 (100). ^1H NMR data were as for the synthetic racemic compound, below. A single crystal of this material was submitted for X-ray crystallographic analysis, the details of which are as follows.

Crystal data. $C_{19}H_{18}O_5S$, $M = 358.39$, $T = 220(2)$ K, $\lambda = 0.710\,73$ Å. Monoclinic $a = 9.634(6)$ Å, $b = 15.85(2)$, $c = 11.708(4)$ ($\beta = 111.88(4)°$), $V = 854.1(10)$ Å^3; space group $P2(1)$; $Z = 2$; $D_x = 1.393$ mg/m^3; $\mu = 0.216$ mm^{-1}; $F(000) = 376$. Crystal size 0.67 × 0.19 × 0.05 mm; θ range for data collection 2.28–25.05°; index ranges $0 \leqslant h \leqslant 11$, $0 \leqslant k \leqslant 17$, $-13 \leqslant l \leqslant 12$; reflections collected 1719; independent reflections 1623 [R(int) = 0.0208]; absorption correction none; refinement method full-matrix least squares on F^2; data/restraints/parameters 1622/1/226; goodness-of-fit on F^2 1.058; $R(F)$ [$I > 2\sigma(I)$] = 0.0442, $wR(F^2) = 0.0912$; absolute structure parameter −0.06(14); largest diff. peak and hole 0.216 and −0.222 e Å^{-3}.

(±)-(*E*)-3-Methyl-4-phenylsulfinylbut-3-en-2-ol

(±)-(*E*)-3-Methyl-4-phenylsulfinylbut-3-en-2-one (210 mg, 1 mmol) was reduced with sodium borohydride as for the reduction of (*E*)-3-methyl-4-phenylsulfanylbut-3-en-2-one, above, to give (±)-(*E*)-3-methyl-4-phenylsulfinylbut-3-en-2-ol (as a mixture of diastereoisomers) (190 mg, 90%) (Found: M^+, 210.0719. $C_{11}H_{19}SO_2$ requires *M*, 210.0715).

Hydrogen (±)-(*E*)-3-methyl-4-phenylsulfinylbut-3-en-2-yl phthalate

A solution of (±)-(*E*)-3-methyl-4-phenylsulfinylbut-3-en-2-ol (as a mixture of diastereoisomers) (*rac*-**15** + *rac*-**17**) (140 mg, 0.67 mmol) and phthalic anhydride (102 mg, 0.7 mmol) in pyridine (0.5 cm^3) was left for 48 h at 35 °C. The product was isolated as for the optically active compound, above, to give the title compound (diastereoisomeric mixture) (206 mg, 85%). A solution of the product (200 mg) in acetone (4 cm^3) was refrigerated at 0 °C for 12 h to give a crystalline product. This was filtered off and twice recrystallised from the same solvent to give the racemate of the (2*S*, sulfur-*R*) diastereoisomer of the bioreduction product **13**, mp 180–182 °C; δ_H(250 MHz; $CDCl_3$) 1.48 [3 H, d, *J* 6.6, CH_3C(OCOAr)], 2.26 (3 H, s, CH_3C=), 5.54 [1 H, q, *J* 6.6, C*H*(OCOAr)], 6.45 (1 H, s, CH=), 7.50 (5 H, ArH), 7.61 (3 H, m, ArH) and 7.80 (1 H, m, ArH).

Hydrogen (2*SR*,5*RS*,*E*)-3-methyl-4-phenylsulfinylbut-3-en-2-yl phthalate. The mother liquor was concentrated and redissolved in acetone and the solution was refrigerated at 0 °C for 24 h to give a crystalline precipitate of the above diastereoisomer. This was filtered off and the process was repeated once more. NMR analysis of the mother liquor showed that very little of this less-soluble diastereoisomer remained. The mother liquor was dissolved in the minimum amount of acetone and the solution was diluted with light petroleum until a permanent turbidity persisted; it was then stored at 0 °C for 12 h. The resulting crystalline product was filtered off to give the racemate of the hydrogen phthalate of the bioreduction product **13**, mp 126–129 °C; δ_H(250 MHz; $CDCl_3$) 1.41 [3 H, d, *J* 6.6, CH_3C(OCOAr)], 2.25 (3 H, s, CH_3C=), 5.54 [1 H, q, *J* 6.6, C*H*(OCOAr)], 6.52 (1 H, s, CH=), 7.51 (5 H, m, ArH), 7.65 (3 H, m, ArH) and 7.80 (1 H, m, ArH). A single crystal of this material was submitted for X-ray crystallographic analysis.

Oxidation of the bioreduction product 13

The bioreduction product **13** (18 mg, 0.09 mmol) in dichloromethane (5 cm^3) was stirred and treated with Collins' reagent (146 mg, 8 equiv.) at 15 °C for 4 h after which it was diluted with light petroleum (20 cm^3) and dichloromethane (10 cm^3) and filtered. The filtrate was evaporated under reduced pressure and the residue was purified by flash chromatography [light petroleum–ethyl acetate (1:1)] to give optically active (*E*)-3-methyl-4-phenylsulfinylbut-3-en-2-one **14** (6 mg, 33%), $[\alpha]_D^{20}$ +48.3 (*c* 0.3 in MeOH) and starting material (10 mg, 55%).

Ozonolysis of the bioreduction product 13

The bioreduction product **13** (55 mg) was ozonised as described above for ozonolysis of compound **10**. The optically active acetoin produced was analysed on the γ-cyclodextrin column at 20 °C. The acetoin had the same retention time as (*S*)-acetoin **16**, confirmed by co-injection with authentic material.

Reduction of compound 12 by *Z. rouxii*

Compound **12** (200 mg) was added to a mixture of *Z. rouxii cells* (4.5 g) and sucrose (5 g) with distilled water (110 cm^3). The mixture was allowed to ferment under anaerobic conditions at 30 °C for 4 days. The product was isolated and purified as for the corresponding reduction with baker's yeast, above, to give compound **13** (51 mg, 25%), $[\alpha]_D^{20}$ +122.6 (*c* 0.5 in MeOH) together with the sulfide (5 mg), the sulfone (8 mg) and starting material **12** (82 mg).

(*E*)-3-Methyl-4-phenylsulfonylbut-3-en-2-one 18

A solution of compound **6** (1.08 g) in acetic acid (6 cm^3) was treated dropwise with hydrogen peroxide (35%; 2.6 cm^3). After the mixture had been stored at 70 °C for 48 h it was cooled to room temperature, diluted with water (30 cm^3) and extracted with ethyl acetate (4 × 50 cm^3). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure and the product was isolated by flash chromatography [ethyl acetate–light petroleum (1:4)] to give the title compound **18** as an oil (0.9 g, 72%) (Found: M^+, 224.0519. $C_{11}H_{12}SO_3$ requires *M*, 224.0508); ν_{max}(neat)/cm^{-1} 1693 (CO), 1617 (C=C), 1308 (SO_2) and 1151 (SO_2); δ_H(250 MHz; $CDCl_3$) 2.25 (3 H, d, *J* 1, CH_3C=), 2.35 (3 H, s, CH_3CO), 7.01 (1 H, s, d, *J* 1, CH=), 7.64 (3 H, m, ArH) and 7.95 (2 H, m, ArH); δ_C(60 MHz; $CDCl_3$) 12.33 (*C*H_3C=), 26.26 (*C*H_3CO), 127.56 (C-2′, -6′), 129.44 (C-3′, -5′), 134.02 (C-4′), 136.74 (=CSO_2), 140.39 (C-1′), 147.74 (*C*=CSO_2) and 197.84 (CO); *m*/*z* (EI) 224 (55%), 141 (34), 105 (16), 83 (12), 77 (73), 69 (9) and 43 (100).

Reduction of compound 18 by baker's yeast

Compound **18** (200 mg) in ethanol (2 cm^3) was added to a mixture of baker's yeast (5 g) and sucrose (5 g) in distilled water (100 cm^3) and the mixture was stirred for 26 h under anaerobic conditions at 30 °C. The crude product, isolated as described for the bioreduction product **7** (see above), was purified by flash chromatography [ethyl acetate–light petroleum (1:1)] to give (*S*,*E*)-3-methyl-4-phenylsulfonylbut-3-en-2-ol **19** (148 mg, 75%) as a thick oil [Found: $(M + H)^+$, 227.0729. $C_{11}H_{15}SO_3$ requires 227.0727]; $[\alpha]_D^{20}$ −3.0 (*c* 1.02 in MeOH); ν_{max}(neat)/cm^{-1} 3482 (OH), 1634 (C=C), 1302 (SO_2) and 1144 (SO_2); δ_H(250 MHz; $CDCl_3$) 1.25 [3 H, d, *J* 6.4, CH_3C(OH)], 2.07 (3 H, s, CH_3C=), 4.19 [1 H, q, *J* 6.4, C*H*(OH)], 6.53 (1 H, s, CH=), 7.56 (3 H, m, ArH) and 7.88 (2 H, m, ArH); δ_C(60 MHz; $CDCl_3$) 14.29 (*C*H_3C=C), 21.38 [*C*H_3C(OH)], 71.36 [C(OH)], 124.2 (CSO_2), 126.97 (C-2′, -6′), 129.11 (C-3′, -5′), 133.14 (C-4′), 141.7 (C-1′) and 159.80 (*C*=CSO_2); *m*/*z* [CI (NH_3)] 244 $(M + NH_4)^+$ (100%); 227 (17), 218 (3), 209 (19), 183 (2), 142 (2) and 78 (5).

Hydrogen (*S*,*E*)-3-methyl-4-phenylsulfonylbut-3-en-2-yl phthalate

The bioreduction product **19** (250 mg, 1.1 mmol) and phthalic anhydride (180 mg, 1.3 mmol) were dissolved in pyridine (0.8 cm^3) and the mixture was stirred at 35 °C for 72 h. The product was isolated as for the corresponding derivative of compound **13** (see above), to give the hydrogen phthalate **20** (430 mg, 100%), mp 118–120 °C (MeOH–EtOAc) [Found: $(M + H)^+$, 375.0900. $C_{19}H_{19}SO_5$ requires $(M + H)$, 375.0902]; $[\alpha]_D^{20}$ +26.5 (*c* 1.6 in MeOH); ν_{max}($CHCl_3$)/cm^{-1} 1726 (CO), 1636 (C=C), 1287 (SO_2) and 1146 (SO_2); δ_H(250 MHz; $CDCl_3$) 1.35 [3 H, d, *J* 6.6, CH_3C(OCOAr)], 2.10 (3 H, s, MeC=), 5.35 [1 H, q, *J* 6.6, C*H*C(OCOAr)], 6.43 (1 H, s, CH=), 7.51 (5 H, m, ArH) and 7.82 (4 H, m, ArH); *m*/*z* [FAB (NBA)] 375 $(M + H)^+$ (18%), 307 (100), 227 (10), 209 (55), 192 (13), 176 (32) and 167 (48).

Ozonolysis of compound **19**. Compound **19** (100 mg) was ozonised as described above for bioreduction products **10** and **13**. The acetoin produced was analysed by GLC using the α- and γ-cyclodextrin columns. The retention time corresponded to (*S*)-acetoin on both columns.

Ozonolysis of the hydrogen (*S*,*E*)-3-methyl-4-phenylsulfonylbut-3-en-2-yl phthalate 20

A solution of compound **20** (240 mg, 1.05 mmol) in methanol (6 cm^3) was ozonised for 3 h. The reaction was quenched as

described for the ozonolysis of compound **10**, above. The product was extracted with ethyl acetate (4 × 20 cm^3) and the combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give the hydrogen phthalate of (*S*)-acetoin (0.157 g, 0.66 mmol). This, dissolved in acetone (4 cm^3), was treated with *N*,*N*-dicyclohexylamine (127 mg, 0.7 mmol). After the reaction mixture had been evaporated under reduced pressure, the residue was dissolved in acetone (10 cm^3) and the solution was diluted with pentane until a permanent turbidity persisted. The mixture was left at 0 °C overnight after which the crystalline precipitate was filtered off and washed with pentane–acetone (1:19, 10 cm^3). The salt (130 mg), dissolved in water (5 cm^3), was passed through a column of Dowex 50W-X8 ion exchange resin (4 g) with water (15 cm^3) as eluent. The eluate was extracted with diethyl ether (4 × 100 cm^3) and the combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give the hydrogen phthalate of (*S*)-acetoin, $[\alpha]_D^{20}$ +13.4 (*c* 0.16 in acetone) {lit.,[17] $[\alpha]_D$ +27 (*c* 1.0, acetone)}.

Reduction of compound 18 by *Z. rouxii*

Compound **18** (200 mg) was reduced by *Z. rouxii* as for the reduction of compound **12**, above. The product was isolated as for the corresponding reduction by *S. cerevisiae*, above, to give compound **19** (163 mg, 81%), $[\alpha]_D^{20}$ −2.0 (*c* 0.5 in MeOH).

2,2-Dimethoxycyclohexanone

Cyclohexane-1,2-dione (10 g, 90 mmol) was treated with trimethyl orthoformate (10 cm^3) and one drop of conc. H_2SO_4. The mixture was stirred at room temperature for 72 h after which it was diluted with water (50 cm^3) and extracted with diethyl ether (4 × 40 cm^3). The combined extracts were washed with water (2 × 20 cm^3), dried ($MgSO_4$) and concentrated under reduced pressure and the residue was purified by flash chromatography. 2,2-Dimethoxycyclohexanone [70] (7.5 g, 53%) was eluted with light petroleum–ethyl acetate (95:5); ν_{max}(neat)/cm^{-1} 1737 (CO); δ_H($CDCl_3$) 1.77 (4 H, m, $CH_2CH_2CH_2CH_2$), 1.92 [2 H, apparent t, *J* 5.9, $CH_2C(OMe)_2$], 2.49 (2 H, apparent t, *J* 6.3, CH_2CO) and 3.22 (6 H, s, OMe); δ_C($CDCl_3$) 21.58 ($CH_2CH_2CH_2CH_2$), 27.14 ($CH_2CH_2CH_2CH_2$), 35.02 [$CH_2C(OMe)_2$], 39.73 (CH_2CO), 48.92 (OMe), 100.46 [$C(OMe)_2$] and 206.89 (CO); *m*/*z* [CI (NH_3)] 176 $(M + NH_4)^+$ (81%), 159 $(M + H)^+$ (26), 144 (82), 130 (72), 127 (94), 112 (100), 101 (85) and 99 (36).

Further elution with light petroleum–EtOAc (85:15) gave 2-methoxycyclohex-2-enone (5 g, 44%); ν_{max}(neat)/cm^{-1} 1692 (CO) and 1628 (C=C); δ_H($CDCl_3$) 1.98 (2 H, m, $CH_2CH_2CH_2$), 2.49 (4 H, m, $CH_2CH_2CH_2$), 3.60 (3 H, s, OMe) and 5.90 (1 H, apparent t, *J* 4.5, CH=); δ_C($CDCl_3$) 22.74 ($CH_2CH_2CH_2$), 24.13 ($CH_2CH_2CH_2CH$=), 38.47 ($CH_2CH_2CH_2CO$), 54.46 (OMe), 116.45 (CH=), 151.05 (MeO*C*=) and 194.38 (CO); *m*/*z* (EI) 126 (M^+) (66%), 111 (23), 96 (55), 84 (81), 67 (46) and 55 (100).

(*E*)-(2-Oxocyclohexylidene)ethanenitrile 21

To sodium hydride (80% dispersion in mineral oil; 750 mg) in dimethylformamide (5 cm^3) was added over 15 min at room temperature under N_2 with stirring diethyl cyanomethylphosphonate (4.6 g). The mixture was stirred for 15 min after which 2,2-dimethoxycyclohexanone (4 g) was added to it with stirring over 15 min. The initially slightly warm solution was stirred at room temperature for 6 h after which it was diluted with water (40 cm^3), treated with conc. hydrochloric acid (20 cm^3) and stirred for a further 2 h. The mixture was extracted with dichloromethane (4 × 40 cm^3) and the combined extracts were washed with water (4 × 25 cm^3), dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography [light petroleum–ethyl acetate (80:20)] to give the title compound **21** (1.35 g, 40%) and the impure *Z*-isomer (Found: M^+, 135.0685. C_8H_9NO requires *M*, 135.0684); ν_{max}(neat)/cm^{-1} 2219 (CN), 1697 (CO) and 1604 (C=C); δ_H(250 MHz; $CDCl_3$) 1.92 (4 H, m, $CH_2CH_2CH_2CH_2$), 2.55 (2 H, apparent t, *J* 7.0, CH_2CO), 2.90 (2 H, apparent t, *J* 6.0, CH_2C=C) and 6.06 (1 H, s, CH=); δ_C(100 MHz; $CDCl_3$) 22.9, 23.18 ($CH_2CH_2CH_2CH_2$), 30.99 (CH_2C=), 40.24 (CH_2CO), 102.70 (*C*HCN), 115.60 (CN), 156.47 (CO*C*=C) and 197.86 (CO); *m*/*z* (EI) 135 (9%), 107 (100), 101 (8), 92 (11), 79 (56), 67 (69) and 52 (34).

(*Z*)-(2-Oxocyclohexylidene)ethanenitrile

The fraction containing the *Z*-isomer was re-chromatographed to give the *Z*-isomer as an unstable (with respect to *Z*–*E* isomerisation) oil (0.38 g, 12%). The configuration was established from the chemical shift of the olefinic proton (δ 5.42) relative to the (*E*)-isomer above (δ 6.06), and from NOE experiments which showed on irradiation of the vinyl proton an enhancement of the allylic methylene signals for the (*Z*)-isomer but not for the (*E*)-isomer **21**, above; ν_{max}(neat)/cm^{-1} 2222 (CN), 1699 (CO) and 1622 (C=C); δ_H(250 MHz; $CDCl_3$) 1.92 (4 H, m, $CH_2CH_2CH_2CH_2$), 2.56 (2 H, app t J_{app} 7.0, CH_2C=C), 2.72 (2 H, m, CH_2CO) and 5.42 (1 H, appt, J_{app} 1.5, CH=); δ_C($CDCl_3$) 24.10, 24.26 ($CH_2CH_2CH_2CH_2$), 34.71 (CH_2C=C), 41.58 (CH_2CO), 99.48 (*C*HCN), 115.49 (CN), 158.5 (*C*=CHCN) and 198.48 (CO); *m*/*z* (FAB) 136 (61%), 106 (20), 91 (12), 89 (17), 79 (27), 77 (35), 67 (41), 52 (31), 47 (13) and 40 (100).

(*R*)-(2-Oxocyclohexyl)ethanenitrile 22

A mixture of baker's yeast (13 g) and sucrose (13 g) in distilled water (250 cm^3) was stirred at 30 °C for 10 min after which a solution of compound **21** (600 mg) in ethanol (4 cm^3) was added to it. The mixture was stirred at 30 °C for 18 h and then filtered through a pad of Celite and extracted with ether (5 × 50 cm^3). The Celite pad was washed with diethyl ether (2 × 50 cm^3). The combined ethereal phases were dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography [ethyl acetate–light petroleum (10:90)] to give the title compound **22** (90 mg), (*E*,*S*)-(2-hydroxycyclohexylidene)ethanenitrile **23** (170 mg) and a mixture of (1*R*,2*S*)-(2-hydroxycyclohexyl)ethanenitrile **24** and **23** (108 mg, *ca.* 1:1 by ^1H NMR). The keto nitrile **22** gave the following data (Found: M^+, 137.0845. $C_8H_{11}NO$ requires *M*, 137.0841); $[\alpha]_D^{20}$ +5.8 (*c* 0.44 in MeOH); ν_{max}(neat)/cm^{-1} 2219 (CN) and 1698 (CO); δ_H($CDCl_3$) 1.44 (1 H, m, 3_{ax}-H), 1.65 (2 H, m, 4-, 5_{ax}-H), 1.92 (1 H, m, 4_{eq}-H), 2.09 (1 H, m, 5_{eq}-H), 2.35 (3 H, m, 3_{eq}-H, 6_{ax}-H, 6_{eq}-H), 2.37 (1 H, dd, *J* 9.3, 18.24, 7a-H), 2.62 (1 H, dd, *J* 5.2, 18.2, 7b-H) and 2.64 [1 H, m, 2_{eq}-H (assignments from the COSY experiment)]; δ_C($CDCl_3$) 17.68 (C-7), 24.70 (C-5), 27.40 (C-4), 33.25 (C-3), 41.45 (C-6), 46.64 (C-2), 118.50 (C-8) and 208.62 (C-1); *m*/*z* (EI) 137 (15%), 109 (10), 94 (6), 84 (24), 67 (13), 55 (100) and 41 (50).

(*R*)-(2-Oxocyclohexyl)ethanenitrile phenylhydrazone

To a solution of compound **22** (200 mg) in methanol (3 cm^3) were added phenylhydrazine (200 mg) in methanol (2 cm^3), acetic acid (one drop) and water (1 cm^3). The mixture was kept at 4 °C for 1 h after which the crystalline product was filtered off and recrystallised (methanol) to give the title compound, mp 93–95 °C (Found: M^+, 227.1415. $C_{14}H_{17}N_3$ requires *M*, 227.1422); $[\alpha]_D^{20}$ +20.7 (*c*, 0.26 in MeOH); *m*/*z* (EI) 227 (85%), 187 (100), 104 (20), 92 (20), 84 (26), 77 (68), 65 (69) and 41 (61).

Cyclic ketal 27 of (*R*)-(2-oxocyclohexyl)ethanenitrile 22 with (±)-butane-2,3-diol

A solution of (*R*)-2-oxohexylethanenitrile **22** (104 mg), (±)-

butane-2,3-diol (180 mg) and toluene-*p*-sulfonic acid (1 mg) in benzene (6 cm^3) was treated with $MgSO_4$ (1 mg) for 6 h under reflux. After this the solvent was removed under reduced pressure and the product was purified by flash chromatography [ethyl acetate–light petroleum (10:90)] to give 2-cyanomethyl-4′,5′-dimethylspiro[cyclohexane-1,2′-[1,3]dioxolane] **27** (150 mg, 90%) as a mixture of diastereoisomers; ν_{max}(neat)/cm^{-1} 2240 (CN); *m*/*z* [CI (NH_3)] 227 $(M + NH_4)^+$ (100%), 210 $(M + 1)^+$ (35), 166 (13), 155 (22), 141 (11), 127 (14), 108 (11), 94 (8), 86 (11), 79 (24) and 72 (13).

Cyclic ketal of (*R*)-(2-oxohexyl)ethanoic acid 28 with (2*R*,3*R*)-butane-2,3-diol

A solution of the mixture of ketals **27** (140 mg) in a mixture of methanol (2 cm^3) and aqueous sodium hydroxide [16% (w/v); 10 cm^3] was boiled under reflux for 13 h. The cooled solution was extracted with dichloromethane (2 × 15 cm^3), acidified to pH 2 (1 mol dm^{-3} HCl) and extracted with ethyl acetate (4 × 25 cm^3). The ethyl acetate extracts were washed with water (2 × 10 cm^3), dried ($MgSO_4$) and evaporated under reduced pressure to give the (4′*R*,5′*R*)-2-carboxymethyl-4′,5′-dimethylspiro[cyclohexane-1,2′-[1,3]dioxolane] (130 mg, 80%), $[\alpha]_D^{20}$ +3.2 (*c* 0.2 in MeOH); ν_{max}(neat)/cm^{-1} 1708 (CO); *m*/*z* (EI) 229 $(M + H)^+$ (35 %), 185 (18), 154 (71), 139 (100), 89 (28), 77 (41) and 52 (20).

Ethyl (2-oxocyclohexyl)ethanoate

To a solution of the preceding compound (56 mg) in acetone (3 cm^3) was added one drop of perchloric acid. The solution was stirred for 30 min, diluted with water (5 cm^3) and extracted with dichloromethane (5 × 5 cm^3). The combined extracts were washed with water (5 cm^3), dried ($MgSO_4$) and evaporated under reduced pressure to give the crude title compound as a liquid (60 mg). The product (40 mg) was dissolved in benzene (4 cm^3) and DBU (50 mg) was added to the solution followed by a solution of iodoethane (45 mg) in benzene (3 cm^3). After 12 h the mixture was treated with water (5 cm^3). The organic layer was separated, washed with water (2 × 5 cm^3), dried ($MgSO_4$) and evaporated under reduced pressure to give ethyl (*R*)-2-oxocyclohexyl)ethanoate **29**. This was subjected to flash chromatography [ethyl acetate–light petroleum (15:18)] to give the pure ester **29** (35 mg), $[\alpha]_D$ +3.2 (*c* 0.4 MeCN), $[\alpha]_D^{20}$ +1.7 (*c* 0.7 in MeOH) [for the *S*-isomer, lit.,[18] −7.0 (*c* 1.69 in MeOH)]; ν_{max}(neat)/cm^{-1} 1784 (ketone CO) and 1735 (ester CO); δ_H(400 MHz; $CDCl_3$) 1.23 (3 H, t, *J* 7.1, CH_3), 1.38 (1 H, m, 3_{ax}-H), 1.65 (2 H, m, 4_{ax}-, 5_{ax}-H), 1.85 (1 H, m, 4_{eq}-H), 2.10 (2 H, m, 3_{eq}-, 5_{eq}-H), 2.11 (1 H, dd, *J* 6.0, 16.4, 7-H), 2.36 (2 H, m, 6_{ax}-, 6_{eq}-H), 2.73 (1 H, dd, *J* 7.2, 16.4, 7-H), 2.84 (1 H, m, 2_{eq}-H) and 4.10 (2 H, m, $MeCH_2$); δ_C(100 MHz; $CDCl_3$) 14.03 (C-10), 25.05 (C-4), 27.64 (C-3), 33.73 (C-3), 34.30 (C-7), 41.68 (C-6), 46.96 (C-2), 60.29 (C-9), 172.47 (C-8) and 210.93 (C-1); *m*/*z* [CI (NH_3)] 202 $(M + NH_4)^+$ (49 %), 185 $(M + H)^+$ (100), 156 (12), 139 (7), 124 (12) and 110 (2).

(1*S*,2*R*)-(2-Hydroxycyclohexyl)ethanenitrile 25

The mixture of compounds **23** and **24** (108 mg) dissolved in a mixture of acetone (8.8 cm^3) and water (1.2 cm^3) was treated with *N*-methylmorpholine *N*-oxide (200 mg) and osmium tetraoxide (20 mg) and the mixture was stirred for 15 h. After this the mixture was diluted with $CHCl_3$ (20 cm^3), extracted with water (10 cm^3) and aqueous sodium hydrogen sulfite (45%; 10 cm^3), dried ($MgSO_4$) and evaporated under reduced pressure. The residue (55 mg) was purified by flash chromatography [light petroleum–ethyl acetate (85:15)] to give the title compound **25** (39 mg) as an oil. This was shown by chiral GLC (α-cyclodextrin column) to consist of a single enantiomer of >99% ee [Found: $(M + H)^+$, 140.1075. $C_8H_{14}NO$ requires 140.1071]; $[\alpha]_D^{20}$ +66.3 (*c* 0.39 in MeOH); ν_{max}(neat)/cm^{-1} 3421 (OH) and 2249 (CN); δ_H(400 MHz; $CDCl_3$) 1.14–1.35 (4 H, m, 3_{ax}-, 4_{ax}-, 5_{ax}-, 6_{ax}-H), 1.50–1.61 (1 H, m, 2_{ax}-H), 1.66–1.79 (2 H, m, 3_{eq}-, 5_{eq}-H), 1.86–1.92 (1 H, m, 4_{eq}-H), 1.94–2.01 (1 H, m, 6_{eq}-H), 2.47 (1 H, dd, *J* 7.3, 16.8, 7-H), 2.61 (1 H, dd, *J* 3.9, 16.8, 7-H) and 3.30 (1 H, ddd, *J ca.* 4.4, 4, 10, 1-H); δ_C(100 MHz; $CDCl_3$) 20.54 (C-7), 24.56, 24.99, 30.24 (C-3,4,5), 35.49 (C-2), 41.68 (C-6), 72.71 (C-1) and 118.99 (C-8); *m*/*z* (EI) 140 $(M + H)^+$ (30%), 122 (86), 110 (100), 96 (47), 83 (83), 79 (33), 68 (40) and 57 (51).

Collins oxidation of compound 25

Compound **25** (14 mg, 0.1 mmol) in dichloromethane (4 cm^3) was treated with Collins reagent (100 mg, 0.5 mmol) and the mixture stirred for 3 h at room temperature. After this it was diluted with light petroleum (15 cm^3), filtered, evaporated and passed through a small column of flash silica with ethyl acetate–light petroleum (1:9) as eluent. The eluate was evaporated under reduced pressure and the residue (as **22**) was analysed by chiral GLC using the α-cyclodextrin column. The R-isomer had the lowest retention time.

Compound **23** [Found: $(M + H)^+$, 138.0912. $C_8H_{12}NO$ requires *M*, 138.0919]; $[\alpha]_D^{20}$ −75.4 (*c* 0.44 in MeOH); ν_{max}(neat)/cm^{-1} 3431 (OH), 2221 (CN) and 1632 (C=C); δ_H(400 MHz; $CDCl_3$) 1.30–1.55 (3 H, m, 4_{ax}-, 5_{ax}-, 6_{ax}-H), 1.78–1.85 (2 H, m, 4_{eq}-, 5_{eq}-H), 1.92 (1 H, m, 3_{ax}-H), 2.11 (1 H, m, 6_{eq}-H), 2.93 (1 H, m, 3_{eq}-H), 4.12 [1 H, m, C*H*(OH)] and 5.45 (1 H, m, CH=); δ_C(100 MHz; $CDCl_3$) 23.98 (C-5), 27.08 (C-4), 31.82 (C-3), 37.00 (C-6), 72.09 (C-1), 89.98 (C-7), 117.17 (CN) and 169.56 (C-2); *m*/*z* (EI) 138 $(M + H)^+$ (100%), 120 (22), 109 (64), 95 (24), 80 (81), 67 (77) and 53 (34).

Preparation of racemic standards for compounds 22, 23 and 24

To a cold solution of compound **21** (200 mg, 1.5 mmol) in tetrahydrofuran–methanol (9:1; 10 cm^3) was added sodium borohydride (35 mg, 0.8 mmol). The mixture was stirred at −20 °C for 40 min and then diluted with water (10 cm^3) treated with hydrochloric acid (1 mol dm^{-3}; 3 cm^3) and extracted with diethyl ether (4 × 25 cm^3). The combined extracts were washed with water (2 × 20 cm^3), dried ($MgSO_4$) and evaporated under reduced pressure to give (±)-(*E*)-(2-hydroxycyclohexylidene)ethanenitrile (as **23**) (190 mg, 94%). This compound (70 mg, 0.5 mmol) in ethanol (10 cm^3) was hydrogenated over 5% Pd–C at 40 lb in^{-2} for 8 h after which the mixture was filtered and evaporated under reduced pressure. The product was purified by flash chromatography [ethyl acetate–light petroleum (15:85)] to give the racemic mixture of diastereoisomers of (2-hydroxycyclohexyl)ethanenitrile (as **24**) (58 mg, 80%). To this compound (46 mg, 0.3 mmol) in dichloromethane (6 cm^3) was added Collins reagent (400 mg, 2.5 mmol). The mixture was stirred at room temperature for 2.5 h after which it was diluted with light petroleum (20 cm^3), filtered, dried ($MgSO_4$) and evaporated under reduced pressure. The product was purified as for the optically active product, (*R*)-(2-oxocyclohexyl)ethanenitrile **22**, above, to give the racemic compound (40 mg, 86%). Spectroscopic data for all three compounds were identical with those reported above for the optically active forms **22**, **23** and **24**.

(*E*)-3-Methyl-4-oxopent-2-enenitrile 33

To a stirred suspension of sodium hydride (80% suspension in mineral oil, 0.6 g; *ca.* 20 mmol) in diethyl ether (10 cm^3) at 0 °C was added dropwise, under N_2, half of a solution of diethyl cyanomethylenephosphonate (3.4 g, 20 mmol). A solution of 3,3-dimethoxybutan-2-one (2.7 g, 20 mmol) in diethyl ether (10 cm^3) was then added to the mixture over 15 min after which addition of the phosphonate was then completed. The mixture was stirred at 0 °C for 2 h and at room temperature for 12 h after which it was treated with water (20 cm^3) and hydrochloric

acid (10 mol dm^{-3}; 10 cm^3). Stirring was continued at room temperature for 2 h after which the mixture was extracted with dichloromethane (4 × 30 cm^3). The combined organic extracts were washed with water (3 × 20 cm^3), dried ($MgSO_4$) and evaporated under reduced pressure and the residue was purified by flash chromatography with light petroleum–diethyl ether (85:15) as eluent to give the title compound **33**[70] (1.21 g, 55%) as an oil (Found: M^+, 109.0527. C_6H_7NO requires *M*, 109.0528); ν_{max}(neat)/cm^{-1} 2223 (CN), 1689 (CO) and 1615 (C=C); δ_H(250 MHz; $CDCl_3$) 2.20 (3 H, d, *J* 1.4, CH_3C=), 2.40 (3 H, s, MeCO) and 6.15 (1 H, d, *J* 1.1, CH=); δ_C(100 MHz; $CDCl_3$) 16.17 (CH_3C=), 25.97 (CH_3CO), 106.80 (CH=), 115.42 (CN), 155.42 (MeC=) and 196.52 (CO); *m*/*z* (EI) 109 (62%), 94 (43), 66 (74) and 43 (100).

(*S*,*E*)-4-Hydroxy-3-methylpent-2-enenitrile 34

To a mixture of baker's yeast (5 g) and sucrose (5 g) in distilled water (100 cm^3) was added with stirring a solution of the nitrile **33** (200 mg) in ethanol (2 cm^3). The mixture was stirred under anaerobic conditions at 30 °C for 96 h. After every 24 h period a further quantity (5 g) of sucrose was added. The mixture was cooled, filtered (Celite) and the filtrate was extracted with diethyl ether (4 × 40 cm^3). The Celite pad was washed with diethyl ether (2 × 20 cm^3) after which the combined ethereal solutions were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by flash chromatography with light petroleum–ethyl acetate (4:1) as eluent to give the title compound **34** (110 mg, 50%) followed by a mixture of the nitrile **34** and the fully saturated nitrile **35** (40 mg, 20%) as an oil (Found: $(M + H)^+$, 112.0762. $C_6H_{10}NO$ requires *M*, 112.0759); $[\alpha]_D^{25}$ +2.3 (*c* 0.32 in MeOH); ν_{max}(neat)/cm^{-1} 3422 (OH), 2223 (CN) and 1635 (C=C); δ_H(250 MHz; $CDCl_3$) 1.31 [3 H, d, *J* 6.6, CH_3C(OH)], 2.01 (3 H, d, *J* 1.1, CH_3C=), 4.28 [1 H, q, *J* 6.6, CH(OH)] and 5.52 (1 H, q, *J* 1.1, CH); δ_C(100 MHz; $CDCl_3$) 17.40 (CH_3C=), 21.71 [CH_3CH(OH)], 70.44 [CH(OH)], 93.94 (CH=), 117.05 (CN) and 167.09 (C=CCN); *m*/*z* (EI) 111 (2%), 110 (8), 96 (58), 78 (16), 68 (100) and 55 (19).

(*S*,*E*)-4-(*o*-Carboxybenzoyloxy)-3-methylpent-2-enenitrile 36

To a solution of the nitrile **34** (145 mg) in pyridine (1 cm^3) was added phthalic anhydride (192 mg). The mixture was stored at 30 °C for 72 h, after which it was cooled, diluted with water (20 cm^3), treated with hydrochloric acid (1 mol dm^{-3}) to pH 3 and extracted with ethyl acetate (4 × 25 cm^3). The combined extracts were washed with water (10 cm^3), dried ($MgSO_4$) and evaporated under reduced pressure to give the title compound **36** as an oil (330 mg, 100%) [Found: $(M + NH_4)^+$, 277.1188. $C_{14}H_{17}N_2O_4$ requires *M*, 277.1198]; $[\alpha]_D^{20}$ +27.5 (*c* 1.4 in MeOH); ν_{max}(neat)/cm^{-1} 2223 (CN), 1725 (CO) and 1641 (C=C); δ_H(250 MHz; $CDCl_3$) 1.49 (3 H, d, *J* 6.6, CH_3CHO), 2.09 (3 H, s, CH_3C=), 5.46 (1 H, s, CH=), 5.55 (1 H, q, *J* 6.6, CH_3CHO), 7.54–7.70 (3 H, m, Ar) and 7.95 (1 H, m, Ar); *m*/*z* [CI (NH_3)] 277 $(M + NH_4)^+$ (11%), 260 $(M + H)^+$ (13), 166 (32), 149 (13), 129 (100), 112 (12), 104 (46), 93 (38) and 80 (35).

(±)-(*E*)-4-Hydroxy-3-methylpent-2-enenitrile (as 34)

To a stirred solution of the nitrile **33** (330 mg) in tetrahydrofuran–ethanol (9:1; 10 cm^3) at −20 °C was added sodium borohydride (60 mg) in four portions over 45 min. The mixture was stirred for 30 min after which it was diluted with water, adjusted to pH 6 (2 mol dm^{-3} HCl) and extracted with diethyl ether (3 × 30 cm^3). The combined ethereal extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give the title compound[71] (as **34**) as an oil (330 mg, 100%) [Found: $(M + H)^+$, 112.0762. $C_6H_{10}NO$ requires *M*, 112.0759]; ν_{max}(neat)/cm^{-1} 3422 (OH), 2224 (CN) and 1636 (C=C); δ_H(250 MHz; $CDCl_3$) 1.32 [3 H, d, *J* 6.6, CH_3CH(OH)], 2.03 (3 H, s, CH_3C=), 4.30 [1 H, q, *J* 6.6, CH(OH)] and 5.54 (1 H, s, CH=); δ_C(100 MHz; $CDCl_3$) 17.47 (CH_3CH(OH), 21.56 (CH_3C=), 70.25 [CH(OH)], 93.59 (CH=), 117.16 [C(Me)=] and 167.77 (CN); *m*/*z* (EI) 111 (2%), 110 (8), 96 (57), 68 (100) and 55 (18).

(3*S*,4*S*)-4-Hydroxy-3-methylpentanenitrile 43

To a mixture of (*S*,*E*)-4-hydroxy-3-methylpent-2-enenitrile **34** and mixture of diastereoisomers of 4-hydroxy-3-methylpentanenitrile **35** from the reduction of 3-methyl-4-oxopent-2-enenitrile **33** by baker's yeast (500 mg) in acetone–water (88:12; 10 cm^3) was added *N*-methylmorpholine *N*-oxide (1 g) and osmium tetraoxide (60 mg). The mixture was stirred at room temperature for 24 h after which it was diluted with chloroform (30 cm^3), washed with water (10 cm^3), aqueous sodium hydrogen sulfite (45% w/v, 15 cm^3), and water (15 cm^3), dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by flash chromatography with light petroleum–ethyl acetate (80:20) as eluent to give the title compound (see **43**) as an oil (73 mg) [Found: $(M + H)^+$, 114.0920. $C_6H_{12}NO$ requires *M*, 114.0919]; $[\alpha]_D^{20}$ +24.3 (*c* 0.8 in MeOH); ν_{max}(neat)/cm^{-1} 3426 (OH) and 2249 (CN); δ_H(400 MHz; $CDCl_3$) 1.05 (3 H, d, *J* 6.4, CH_3CHCCN), 1.18 [3 H, d, *J* 6.4, CH_3C(OH)], 1.94 (1 H, m, CH_3CH), 2.27 (1 H, dd, *J* 7.8, 16.7, CHHCN), 2.49 (1 H, dd, *J* 6.4, 16.7, CHHCN) and 3.92 [1 H, dq, *J* 3.9, 6.4, CH(OH)]; δ_C(100 MHz; $CDCl_3$) 13.53 (CH_3CH), 19.90 [CH_3C(OH)], 20.69 (CH_2CN), 36.79 (MeCCCN), 69.03 [C(OH)] and 119.35 (CN); *m*/*z* (EI) 114 $(M + H)^+$ (100%), 96 (69), 80 (1), 73 (8), 69 (17), 54 (10) and 45 (11).

(3*R*,4*S*)-4-Hydroxy-3-methylpentanenitrile 44

The title compound, the minor diastereoisomer, gave the following NMR data: δ_H(400 MHz; $CDCl_3$) 1.07 (3 H, d, *J* 6.4, CH_3CH), 1.22 [3 H, d, *J* 6.4, CH_3C(OH)], 1.79 (1 H, m, MeCHCCN), 2.43 (1 H, dd, *J* 6.4, 16.7, CHHCN), 2.55 (1 H, dd, *J* 7.8, 16.7, CHHCN) and 3.91 [1 H, dq, *J* 6.3, 7.4, CH(OH)]; δ_C(100 MHz; $CDCl_3$) 16.06 (CH_3CCCN), 20.69 (CH_2), 21.28 [CH_3C(OH)], 37.67 (MeCHCCN), 70.27 [C(OH)] and 119.14 (CN).

(3*S*)-3-Methyl-4-oxopentanenitrile 41

A solution of the major diastereoisomer **43** of the product **35** from the reduction of the nitrile **33** by baker's yeast (90 mg) in dichloromethane (6 cm^3) was treated with Collins' reagent (900 mg). The mixture was stirred at room temperature for 24 h after which it was diluted with light petroleum (40 cm^3) and filtered. The residue was washed with dichloromethane (10 cm^3) and the combined dichloromethane solutions were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by flash chromatography with light petroleum–ethyl acetate (85:15) as eluent to give the title compound **41** (79 mg, 87%) as an oil (Found: M^+, 111.0682. C_6H_9NO requires *M*, 111.0684); $[\alpha]_D^{20}$ −25.2 (*c* 1.12 in MeOH); ν_{max}(neat)/cm^{-1} 2219 (CN) and 1715 (CO); δ_H(400 MHz; $CDCl_3$) 1.27 (3 H, d, *J* 7.3, CH_3CH), 2.17 (3 H, s, CH_3CO), 2.36 (1 H, dd, *J* 16.9, 7.7, CHHCN), 2.52 (1 H, dd, *J* 16.9, 5.8, CHHCN) and 2.80 (1 H, m, MeCH); δ_C(100 MHz; $CDCl_3$) 16.22 (CH_3CH), 19.46 (CH_3CO), 27.77 (CH_2CN), 43.22 (MeCH), 118.32 (CN) and 207.61 (CO); *m*/*z* (EI) 111 (10%), 96 (5), 68 (14), 54 (3) and 43 (100).

(*S*)-3-Methyllaevulinic acid [(*S*)-3-methyl-4-oxopentanoic acid] 42

The nitrile **41** (70 mg of the above sample) in potassium phosphate buffer (0.1 mol dm^{-3}, pH 7; 50 cm^3) was treated with immobilised *Rhodococcus* nitrilase (0.5 g). The mixture was stirred at 220 r.p.m. at 30 °C for 12 h, with the hydrolysis being monitored by TLC. When the reaction was complete, the mixture was filtered (Celite) and the Celite was washed with

water (5 cm^3). The filtrate was adjusted to pH 10 (2 mol dm^{-3} NaOH) and extracted with ethyl acetate (2 × 10 cm^3). The solution was adjusted to pH 3 (1 mol dm^{-3} HCl) and extracted with ethyl acetate (4 × 20 cm^3). The extracts were combined, dried ($MgSO_4$) and evaporated under reduced pressure to give the title compound **42** (56 mg, 80%) [Found: $(M + H)^+$, 131.0699. $C_6H_{11}O_3$ requires *M*, 131.0707]; $[\alpha]_D^{20}$ −14.1 (*c* 0.24 in MeOH) {lit.,[20] (for the *R*-isomer) $[\alpha]_D^{17}$ +43 (*c* 1.56 in H_2O)}; ν_{max}(neat)/cm^{-1} 1712 (CO); δ_H(400 MHz; $CDCl_3$) 1.10 (3 H, d, *J* 7.1, CH_3CH), 2.15 (3 H, s, CH_3CO), 2.27 (1 H, dd *J* 17.0, 5.1, $CHHCO_2H$), 2.73 (1 H, dd, *J* 17.0, 8.7, $CHHCO_2H$) and 2.80 (1 H, m, CHMe); δ_C(100 MHz; $CDCl_3$) 16.35 (CH_3CH), 28.14 (CH_3CO), 36.40 (CH_2), 42.45 (CH_3-CH), 177.71 (CO_2H) and 210.63 (MeCO); *m*/*z* (EI) 130 (21%), 113 (17), 87 (19), 73 (27), 70 (39), 61 (44), 55 (9) and 43 (100).

(±)-4-Hydroxy-3-methylpentanenitrile

(±)-(*E*)-4-Hydroxy-3-methylpentenenitrile (200 mg) in ethanol (15 cm^3) was hydrogenated at 30 p.s.i. over Pt–C (5%; 40 mg) for 24 h after which the mixture was filtered, diluted with water and extracted with diethyl ether (4 × 30 cm^3). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure and the product was purified by flash chromatography with light petroleum–ethyl acetate (4:1) as eluent to give the title compound (0.1 g, 50%). NMR and mass spectral data were as for the optically active diastereoisomer mixture **35** apart from different relative intensities of signals attributable to the different diastereoisomers [Found: $(M + H)^+$, 114.0920. $C_6H_{12}NO$ requires *M*, 114.0919].

(*R*,*E*)-4-Hydroxy-3-methylpent-2-enenitrile 45

A suspension of *Z. rouxii* cells (4.7 g) in a solution of sucrose (5 g) in distilled water (100 cm^3) was stirred for 10 min. A solution of the nitrile **33** (200 mg) in ethanol (2 cm^3) was added to the mixture which was then stirred at 30 °C for 96 h. The product was isolated as described above for the corresponding reduction using *S. cerevisiae*, to give the title compound **45** (118 mg) and a mixture of this nitrile and the diastereoisomeric mixture of (3*S*,4*R*)-4-hydroxy-3-methylpentanenitrile **46** and (3*R*,4*R*)-4-hydroxy-3-methylpentanenitrile **47** [Found: $(M + H)^+$, 112.0749. $C_6H_{10}NO$ requires *M*, 112.0759]; $[\alpha]_D^{20}$ −4.4 (*c* 1.4 in MeOH); ν_{max}(neat)/cm^{-1} 3447 (OH), 2223 (CN) and 1634 (C=C); δ_H(400 MHz; $CDCl_3$) 1.31 (3 H, d, *J* 6.4, CH_3C(OH), 2.01 (3 H, d, *J* 1.1, CH_3C=), 4.20 [1 H, q, *J* 6.4, CH(OH)] and 5.52 (1 H, q, *J* 1.1, CH=); δ_C(100 MHz; $CDCl_3$) 17.34 (CH_3C=), 20.44 [CH_3C(OH)], 70.13 [CH(OH)], 93.46 (CH=), 117.04 (CN) and 167.7 (MeC=); *m*/*z* (EI) 112 $(M + H)^+$ (24%), 96 (33), 82 (4), 78 (10), 68 (100), 54 (11) and 42 (40).

(4*R*,*E*)-4-(*o*-Carboxybenzoyloxy)-3-methylpent-2-enenitrile 45 (OH replaced by $OCOC_6H_4CO_2H$-*o*)

The derivative (470 mg), 100%) was prepared as for the enantiomer **36** from the nitrile **45** (200 mg) (Found: M^+, 259.0858. $C_{14}H_{13}NO_4$ requires *M*, 259.0856); $[\alpha]_D^{20}$ −27.2 (*c* 0.2 in MeOH); ν_{max}(neat)/cm^{-1} 2223 (CN), 1725 (CO) and 1641 (C=C); δ_H(400 MHz; $CDCl_3$) 1.47 [3 H, d, *J* 6.6, CH_3C(OH)], 2.10 (3 H, d, *J* 1.1, CH_3C=), 5.47 (1 H, q, *J* 1.1, CH=), 5.56 [1 H, q, *J* 6.6, CH(OH)], 7.56–7.68 (3 H, m, Ar) and 7.93–7.98 (1 H, m, Ar); *m*/*z* [CI (NH_3)] 277 $(M + NH_4)^+$ (30%), 260 $(M + H)^+$ (16), 223 (4), 184 (5), 166 (31), 149 (3), 129 (100), 104 (19), 93 (29) and 76 (6).

(3*S*,4*R*)-4-Hydroxy-3-methylpentanenitrile 46 and (3*R*,4*R*)-4-hydroxy-3-methylpentanenitrile 47

A mixture of the unsaturated nitrile **45** and the 4-hydroxy-3-methylpentanenitrile diastereoisomers **46** and **47** from the reduction of the nitrile **33** by *Z. rouxii* (100 mg, 0.9 mmol) was dissolved in a mixture of acetone (4.4 cm^3) and water (0.6 cm^3). *N*-Methylmorpholine *N*-oxide (150 mg, 1.2 mmol) and osmium tetraoxide (25 mg) were added to the mixture which was then stirred at room temperature for 24 h. The product was isolated as described above for the diastereoisomeric mixture **43** and **44** from the reduction by baker's yeast to give the mixture of the title compounds **46** and **47** (44 mg). The IR and NMR data were similar to those of the mixture **43** and **44** except that in the ^1H NMR spectrum the relative integrals of the two diastereoisomers were reversed [Found: $(M + H)^+$, 114.0920. $C_6H_{12}NO$ requires *M*, 114.0919]; $[\alpha]_D^{20}$ −21.05 (*c* 0.38 in MeOH).

(3*S*,4*R*)-46 and (3*R*,4*R*)-4-hydroxy-3-methylpentanenitrile 47

The mixture of hydroxy nitriles **46** and **47** (100 mg) from three batches of the product from reduction of the keto nitrile **33** by *Z. rouxii* were separated from the major product, the keto nitrile **45**, by osmium tetraoxide hydroxylation and chromatography as before. The pooled material (102 mg) was purified by flash chromatography as for the corresponding mixture **43** and **44**, above, to give the title compound **46** (44 mg) [Found: $(M + H)^+$, 114.0920. $C_6H_{12}NO$ requires *M*, 114.0919]; $[\alpha]_D^{25}$ −21.05 (*c* 0.38, MeOH). IR and ^1H and ^{13}C NMR data were as described for hydroxy nitrile **44**, above.

(−)-3-Methyl-4-oxopentanenitrile from the saturated nitrile 46

The nitrile **46** from the *Z. rouxii* reduction, above, (20 mg, 0.18 mmol) in dichloromethane (2.5 cm^3) was treated with Collins' reagent (150 mg, 7 equiv.). The mixture was stirred at room temperature for 6 h after which the product was isolated as described for the corresponding oxidation of the hydroxy nitrile **43**, above, to give (−)-3-methyl-4-oxopentanenitrile **41** (8 mg); $[\alpha]_D^{20}$ −29.8 (*c* 0.25 in MeOH).

(*R*)-3-(*o*-Carboxybenzoyloxy)butan-2-one

Ozone was passed through a solution of (4*R*,*E*)-4-(*o*-carboxybenzoyloxy)-3-methylpent-2-enenitrile **45** (OH replaced by $OCOC_6H_4CO_2H$-*o*) (460 mg) in methanol (4 cm^3) at −20 °C for 3 h. The solution was treated with aqueous sodium sulfite (1.8 mmol in 10 cm^3 water) and diluted with water (30 cm^3). The mixture was extracted with diethyl ether (5 × 30 cm^3) and the combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified *via* formation of the dicyclohexylamine salt as follows. The product was treated with a solution of dicyclohexylamine (280 mg) in acetone (4 cm^3) for 2 h after which the mixture was concentrated, redissolved in acetone (2 cm^3) and treated with light petroleum until a slight turbidity persisted. The mixture was left at 0 °C overnight after which the crystalline precipitate was filtered off, dissolved in water (3 cm^3) and the solution was passed through a column of Dowex 50W-X8 (H^+ form). The column was eluted with water and the aqueous eluate (15 cm^3) was extracted with diethyl ether (3 × 10 cm^3). The combined extracts were dried ($MgSO_4$) and evaporated to give the title compound $[\alpha]_D^{23}$ −23 (*c* 0.6, acetone) (lit.,[17] −27). The hydrogen phthalate **36** derived from the baker's yeast reduction product **34** was ozonised in the same way to give the hydrogen phthalate **37** of (*S*)-acetoin, $[\alpha]_D^{25}$ +10.5.

Ozonolysis of the nitriles 34 and 45

Ozone was passed through a solution of the nitrile **45** (110 mg, 1 mmol) in methanol (4 cm^3) at −20 °C for 3 h after which it was treated with a solution of sodium sulfite [1 mmol in water (3 cm^3)], diluted with water and extracted with diethyl ether (4 × 10 cm^3). The combined extracts were dried ($MgSO_4$) and concentrated to 500 mm^3 by passage through them of a current of dry nitrogen. The solution was analysed by chiral GLC on an α-cyclodextrin column. Ozonolysis of the enantiomeric derivative

34 was carried out in a similar manner. The (*R*)-enantiomer of acetoin was eluted first.

(*S*)-3-Methyllaevulinic acid from nitrile 34

A solution of the nitrile **34** (120 mg) in ethyl acetate (16 cm^3) was hydrogenated over palladium-on-carbon (30 mg) at 32 psi for 6 h. The mixture was filtered and evaporated under reduced pressure to give a mixture of diastereoisomeric reduction products **43** and **44**, $[\alpha]_D^{25}$ +14.4 (*c* 0.5, MeOH). Reduction of the *Z. rouxii* reduction product **45** gave a product with a similar (positive) optical rotation. The reduction product (100 mg) was oxidised using Collins' reagent (800 mg) as for the oxidation of the corresponding bioreduction product **35**, above, to give the keto nitrile **41** (80 mg), $[\alpha]_D^{25}$ −10.2 (*c* 0.2, MeOH). Hydrolysis of this compound (60 mg) using the *Rhodococcus* nitrilase, as for the corresponding hydrolysis of the keto nitrile (as **41**), above, gave (*S*)-3-methyllaevulinic acid **42** (60 mg), $[\alpha]_D^{26}$ −6.1 (*c* 1.2, MeOH).

Ethyl (*E*)-3-methyl-4-oxopent-3-enoate 48

A mixture of sodium hydride (80% dispersion in mineral oil; 0.85 g) was added to diethyl ether (10 cm^3) and the mixture was cooled under N_2. A solution of triethyl phosphonoacetate (6.42 g) in diethyl ether (15 cm^3) was then added to the mixture over 20 min after which it was stirred at 0 °C for 30 min. After this a solution of 3,3-dimethoxybutan-2-one (3.96 g) in diethyl ether (20 cm^3) was added dropwise to the mixture which was then stirred for 6 h, diluted with water (10 cm^3) and hydrochloric acid (4 mol dm^{-3}; 30 cm^3), and extracted with diethyl ether (4 × 50 cm^3). The combined extracts were washed with water (3 × 25 cm^3), dried ($MgSO_4$) and evaporated under reduced pressure. The residue was subjected to flash chromatography [ethyl acetate–light petroleum (1:9)] to give the title compound **48**[72] (2.62 g, 56%) (Found: M^+, 156.0785. $C_8H_{12}O_3$ requires *M*, 156.0786); ν_{max}(neat)/cm^{-1} 1723 (ester CO), 1686 (ketone CO) and 1640 (C=C); δ_H(250 MHz; $CDCl_3$) 1.33 (3 H, t, *J* 7.1, OCH_2CH_3), 2.21 (3 H, d, *J* 1.5, CH_3C=), 2.39 (3 H, s, CH_3CO), 4.25 (2 H, q, *J* 7.1, CH_2O) and 6.58 (1 H, q, *J* 1.5, CH=); δ_C(60 MHz; $CDCl_3$) 12.91 (CH_3CH_2O), 14.07 (CH_3C=), 26.06 (CH_3CO), 60.68 (OCH_2), 126.42 (CH=), 150.28 [$C(CH_3)$=], 166.06 (CO_2) and 199.80 (CH_3CO); *m*/*z* (EI) 157 (M + H)$^+$ (11%), 156 (6), 141 (7), 125 (5), 110 (91), 85 (31), 67 (54) and 43 (100).

Ethyl (4*S*,*E*)-4-hydroxy-3-methylpent-2-enoate 49

A mixture of *S. cerevisiae* (5 g) and sucrose (5 g) in distilled water (100 cm^3) was stirred for 20 min after which a solution of the ester **48** (200 mg) in ethanol (2 cm^3) was added and the mixture was incubated under anaerobic conditions for 96 h. Sucrose (1 g) was added to the mixture at intervals of 24 h. The mixture was filtered (Celite) and the Celite was washed with diethyl ether (30 cm^3) and the filtrate was extracted with diethyl ether (5 × 40 cm^3). The combined organic phases were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by flash chromatography [ethyl acetate–light petroleum (8:92)] to give the ethyl compound **49** (80 mg, 40%) and a mixture of **49** and (3*S*,4*S*)-4-hydroxy-3-methylpentanoate **50** (39 mg). For ester **49** (Found: M^+, 158.0944. $C_8H_{14}O_3$ requires *M*, 158.0940); $[\alpha]_D^{20}$ +3.6 (*c* 0.46 in MeOH); ν_{max}(neat)/cm^{-1} 3420 (OH), 1717 (CO) and 1654 (C=C); δ_H(250 MHz; $CDCl_3$) 1.21 (3 H, t, *J* 7.1, CH_3CH_2O), 1.23 [3 H, d, *J* 6.5, $CH_3C(OH)$], 2.04 (3 H, d, *J* 1.3, CH_3C=), 4.08 (2 H, q, *J* 7.1, OCH_2), 4.18 [1 H, q, *J* 6.5, CH(OH)] and 5.87 (1 H, q, *J* 1.3, CH=); δ_C(60 MHz; $CDCl_3$) 14.14 (CH_3C=), 14.80 (CH_3CH_2O), 21.54 [$CH_3C(OH)$], 59.68 (OCH_2), 72.15 [C(OH)], 113.80 (CH=), 161.35 (CH_3C=) and 167.00 (CO); *m*/*z* (EI) 158 (M^+) (4%), 140 (25), 115 (100), 97 (25), 87 (85), 69 (58), 57 (11) and 43 (92).

(3*S*,4*S*)-4-Hydroxy-3-methylpentanoate 50

To a mixture of the unsaturated ester **49** and saturated ester **50** (190 mg, pooled product from several reductions of the unsaturated ester **48** by *S. cerevisiae*, after removal of most of the major reduction product, ester **49**), in a mixture of acetone (4.4 cm^3) and water (0.6 cm^3) was added *N*-methylmorpholine *N*-oxide (278 mg) and osmium tetraoxide (30 mg). The mixture was stirred at room temperature for 24 h after which it was diluted with chloroform (20 cm^3) and washed with water (10 cm^3). The organic layer was separated, washed with aqueous sodium hydrogen sulfite (45%; 10 cm^3), dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by flash chromatography with ethyl acetate–light petroleum (1:9) as eluent to give the title compound **50** as an oil (76 mg) [Found: (M + H)$^+$ 161.1176. $C_8H_{17}O_3$ requires (*M* + H), 161.1178]; $[\alpha]_D^{20}$ −17.2 (*c* 1.3 in MeOH); ν_{max}(neat)/cm^{-1} 3426 (OH) and 1734 (CO); δ_H(250 MHz; $CDCl_3$) 0.86 (3 H, d, *J* 6.7, CH_3CH), 1.06 [3 H, d, *J* 7.2, $CH_3C(OH)$], 1.19 (3 H, t, *J* 7.1, CH_3CH_2O), 2.0 (1 H, m, CH_3CH), 2.09 (1 H, dd, *J* 8.0, 14.6, $CHHCO_2$), 2.43 (1 H, dd, *J* 5.5, 14.6, $CHHCO_2$), 3.71 [1 H, m, CH(OH)] and 4.06 (2 H, q, *J* 7.1); δ_C(60 MHz; $CDCl_3$) 14.03 (CH_3CH), 14.27 (CH_3CH_2O), 19.20 [$CH_3C(OH)$], 36.52 (CH_2CO_2), 37.43 (CH_3CH), 60.22 (OCH_2), 70.04 [C(OH)] and 173.72 (CO); *m*/*z* (EI) 161 (M + H)$^+$ (43%), 143 (61), 116 (100), 99 (29), 88 (34), 73 (12), 55 (16) and 43 (24).

(*S*,*E*)-4-*o*-(Carboxybenzoyloxy)-3-methylpent-2-enoate 49 (OH replaced by $OCOC_6H_4CO_2H$-*o*)

The ester **49** (218 mg) in pyridine (1 cm^3) was treated with phthalic anhydride (235 mg) and the mixture was stirred at 30 °C for 72 h after which it was diluted with water (25 cm^3) and hydrochloric acid (1 mol dm^{-3}; 5 cm^3) and extracted with ethyl acetate (5 × 25 cm^3). The combined extracts were washed with water (3 × 15 cm^3), dried ($MgSO_4$) and evaporated under reduced pressure. The product was purified by flash chromatography with ethyl acetate–light petroleum (6:4) as eluent to give the title compound **49** (400 mg, 95%) [Found: (M + NH_4)$^+$, 324.1447. $C_{16}H_{22}NO_6$ requires (M + NH_4)$^+$, 324.1440]; $[\alpha]_D^{20}$ +41.0 (*c* 1 in MeOH); ν_{max}(neat)/cm^{-1} 1715 (CO) and 1659 (C=C); δ_H(250 MHz; $CDCl_3$) 1.24 (3 H, t, *J* 7.1, CH_3CH_2O), 1.45 [3 H, d, J 6.5, $CH_3C(OH)$], 2.17 (3 H, d, *J* 1.1, CH_3C=), 4.10 (2 H, q, *J* 7.1, CH_3CH_2O), 5.50 [1 H, q, *J* 6.5, CH(OH)], 5.95 (1 H, q, *J* 1.1, CH=), 7.58–7.85 (3 H, m, ArH) and 7.89–7.93 (1 H, m, ArH).

Ozonolysis of ethyl (*S*,*E*)-4-(*o*-carboxybenzoyloxy)-3-methylpent-2-enoate 49 (OH replaced by $OCOC_6H_4CO_2H$-*o*)

The title compound (146 mg) in methanol (4 cm^3) was ozonised at −20 °C for 3 h after which the solution was treated with sodium sulfite (0.1 mol dm^{-3}; 5 cm^3), diluted with water (15 cm^3) and extracted with diethyl ether (4 × 20 cm^3). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The crude product (75 mg) was dissolved in acetone (3 cm^3) and treated with dicyclohexylamine (75 mg). After 1 h the solvent was evaporated under reduced pressure and the residue was crystallised (acetone–pentane). The crystalline product was dissolved in water (5 cm^3) and passed through a column of Dowex 50W-X8 ion exchange resin (H^+ form). The column was eluted with water (12 cm^3). The combined eluates were extracted with diethyl ether, dried and evaporated to give the hydrogen phthalate of (*S*)-acetoin; $[\alpha]_D^{20}$ +25.1 (*c* 0.8 in acetone).

Ozonolysis of ethyl (*S*,*E*)-4-hydroxy-3-methylpent-2-enoate 49

The title compound **49** (110 mg) in methanol (4 cm^3) was ozonised for 3 h after which the mixture was treated with sodium sulfite (0.1 mol dm^{-3}; 5 cm^3) and extracted with diethyl ether (3 × 10 cm^3). The extracts were concentrated to 5 cm^3 using a

current of N_2 and dried ($MgSO_4$) and the acetoin present was analysed by chiral GLC using a α-cyclodextrin column.

Ethyl (*S*)-3-methyllaevulinate 51

The ester **50** (22 mg) was dissolved in dichloromethane (4 cm^3) and Collins' reagent (180 mg) was added in four portions to the solution. After the mixture had been stirred for 8 h at room temperature, it was diluted with light petroleum, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography with ethyl acetate–light petroleum (5:95) as eluent to give the title compound **51** (16 mg, 72%) (Found: M^+, 158.0947. $C_8H_{14}O_3$ requires *M*, 158.0943); $[\alpha]_D^{20}$ −47.2 (*c* 1.1 in MeOH); ν_{max}(neat)/cm^{-1} 1736 (CO); δ_H(250 MHz; $CDCl_3$) 1.13 (3 H, d, *J* 7.3, CH_3CH), 1.25 (3 H, t, *J* 7.1, CH_3CH_2O), 2.20 (3 H, s, CH_3CO), 2.27 (1 H, dd, *J* 5.3, 16.7, CHHCO), 2.73 (1 H, dd, *J* 8.6, 16.7, CHHCO), 3.05 (1 H, m, CH_3CH) and 4.11 (2 H, q, *J* 7.1, CH_3CH_2O); δ_C(60 MHz; $CDCl_3$) 14.03 (CH_3CH), 16.38 (CH_3CH_2O), 28.27 (CH_3CO), 36.81 (CH_2CO), 42.61 (CH_3CH), 60.43 (OCH_2), 172.16 (CO_2) and 210.68 (CH_3CO); *m*/*z* (EI) 158 (11%), 143 (14), 113 (100), 101 (23), 95 (9), 88 (34), 73 (40) and 57 (28).

Ethyl (*S*)-3-methyllaevulinate 52 from (*S*)-3-methyllaevulinic acid 53

To a solution of the acid **53** (37% ee, 25 mg) in dry benzene (3 cm^3) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (30 mg) and iodoethane (40 mg). After 12 h at room temperature the mixture was filtered, diluted with ethyl acetate (15 cm^3), washed with water (2 × 10 cm^3), dried ($MgSO_4$) and evaporated under reduced pressure to give the ester **52** (26 mg, 85%). Spectroscopic data were as for the corresponding ester **51** obtained from the ester **48** *via* bioreduction using *S. cerevisiae*: $[\alpha]_D^{20}$ −17.5 (*c* 0.5 in MeOH).

Oxidation of the diastereoisomer mixture 50 + 54 obtained by hydrogenation of bioreduction product 49

The mixture of diastereoisomers **50** + **54** (30 mg) from hydrogenation of the bioreduction product **49** (see below) was oxidised using Collins' reagent as described for the oxidation of the ester **50**, above, to give ethyl (3*S*)-3-methyllaevulinate **55** (18 mg, 60%), $[\alpha]_D^{20}$ −29.1 (*c* 0.17 in MeOH) and recovered starting material (10 mg).

Ethyl (*R*,*E*)-4-hydroxy-3-methylpent-2-enoate 56

A mixture of *Zygosaccharomyces rouxii* cells (4.5 g) and sucrose in distilled water (100 cm^3) was stirred for 20 min after which a solution of the unsaturated keto ester **48** (200 mg) in ethanol (2 cm^3) was added to it. The mixture was stirred and incubated at 30 °C for 96 h after which it was treated as described above for the corresponding reduction using *S. cerevisiae*, to give the title compound **56** (70 mg, 35%) and a mixture of ester **56** and ethyl (3*S*,4*R*)-4-hydroxy-3-methylpentanoate **57** (~ 30 mg). For ester **56** [Found: $(M + H)^+$, 159.1020. $C_8H_{15}O_3$ requires $(M + H)$, 159.1017]; $[\alpha]_D^{20}$ −3.6 (*c* 0.44 in MeOH); ν_{max}(neat)/cm^{-1} 3436 (OH), 1717 (CO) and 1654 (C=C); δ_H(250 MHz; $CDCl_3$) 1.21 (3 H, t, *J* 7.1, CH_3CH_2O), 1.23 [3 H, d, *J* 6.5, $CH_3C(OH)$], 2.04 (3 H, d, *J* 1.35, CH_3C=), 4.08 (2 H, q, *J* 7.1, OCH_2), 4.18 [1 H, q, *J* 6.5, CH(OH)] and 5.87 (1 H, q, *J* 1.25, CH=); δ_C(60 MHz; $CDCl_3$) 14.15 (CH_3C=), 14.82 (CH_3CH_2O), 21.57 [CH_3C(OH)], 59.69 (OCH_2), 72.21 [C(OH)], 113.85 (CH=), 161.26 (CH_3C=) and 167.00 (CO); *m*/*z* (EI) 159 $(M + H)^+$ (2%), 140 (13), 115 (87), 101 (10), 97 (32), 87 (100), 69 (78), 55 (14) and 45 (22).

Ethyl (4*R*,*E*)-4-(*o*-Carboxybenzoyloxy)-3-methylpent-2-enoate 56 (OH replaced by $OCOC_6H_4CO_2H$-*o*)

The hydrogen phthalate (410 mg, 100%), was prepared from the ester **56** (208 mg) as for the corresponding derivative of the (4*S*)-ester **49** [Found: $(M + NH_4)^+$, 324.1447. $C_{16}H_{22}NO_6$ requires $(M + NH_4)$, 324.1440]; $[\alpha]_D^{20}$ −40.2 (*c* 0.42 in MeOH); ν_{max}(neat)/cm^{-1} 1715s (CO) and 1659 (C=C); δ_H(250 MHz; $CDCl_3$) 1.25 (3 H, t, *J* 7.1, CH_3CH_2O), 1.47 [3 H, d, *J* 6.5, $CH_3C(OH)$], 2.20 (3 H, d, *J* 1.1, CH_3CH=), 4.13 (2 H, q, *J* 7.1, CH_3CH_2O), 5.51 [1 H, q, *J* 6.5, CH(OH)], 5.94 (1 H, s, CH=), 7.58–7.85 (3 H, m, ArH) and 7.89–7.93 (1 H, m, ArH); *m*/*z* [FAB (glycine)] 307 $(M + H)^+$ (16%), 167 (33), 149 (58), 141 (100), 113 (76), 105 (11), 95 (29), 85 (16), 69 (21), 55 (17) and 44 (45).

Ozonolysis of the phthalate of 56 (OH replaced by $OCOC_6H_4CO_2H$-*o*)

The title ester was ozonised as for the corresponding derivative of the (*S*)-ester **49**, to give the hydrogen phthalate of (*R*)-acetoin; $[\alpha]_D^{20}$ −22.9 (*c* 0.54 in acetone).

Ozonolysis of ethyl (4*R*,*E*)-4-hydroxy-3-methylpent-2-enoate 56

The ester **56** (95 mg) was ozonised as for the (*S*)-ester **49**, above. The acetoin produced was analysed by chiral GLC using the α-cyclodextrin column.

Ethyl (*R*)-3-methyllaevulinate 58

To the product mixture consisting mainly of (3*S*,4*R*)-4-hydroxy-3-methylpentanoate **51** after separation of most of the unsaturated ester **50** from the reduction of the ester **48** by *Z. rouxii* (100 mg) in dichloromethane (4 cm^3) was added Collins' reagent (1.0 g). The mixture was stirred for 26 h at room temperature. The product was isolated as for the corresponding (*S*)-ester **51**, above and purified by flash chromatography with ethyl acetate–light petroleum (5:95) as eluent to give ethyl (*S*)-3-methyllaevulinate **58** (7 mg); $[\alpha]_D^{20}$ −37 (*c* 0.35 in MeOH).

3,5-Dinitrobenzoate of ethyl (3*S*,4*R*)-4-hydroxy-3-methylpentanoate 57

To a mixture (200 mg) of products from the reduction of the ester **48** using *Z. rouxii* in dichloromethane (8 cm^3) was added 4-dimethylaminopyridine (20 mg), dicyclohexylcarbodiimide (300 mg) and 3,5-dinitrobenzoic acid (360 mg). The mixture was stirred for 24 h at room temperature, diluted with pentane (20 cm^3) and dichloromethane (5 cm^3) and filtered. The residue was washed with pentane–dichloromethane (9:1 20 cm^3). The combined organic phases were evaporated under reduced pressure and the residue was dissolved in pentane–dichloromethane (1:1: 10 cm^3). The solution was stored at 4 °C for 6 h after which the solid was filtered off and the filtrate was evaporated under reduced pressure and the mixture was again dissolved in pentane–dichloromethane (1:1 10 cm^3). After being stored at 4 °C for 6 h, the mixture was filtered and the filtrate was evaporated under reduced pressure. The residue (490 mg) was dissolved in a mixture of acetone (8.8 cm^3) and water (1.2 cm^3) and to this solution were added *N*-methylmorpholine *N*-oxide (350 mg) and osmium tetraoxide (40 mg). The mixture was stirred at room temperature for 24 h after which the product was isolated as described for the ester **50**, above. The crude product was purified by flash chromatography with light petroleum–ethyl acetate (85:15) as eluent to give the title compound **57** (66 mg) [Found: $(M + NH_4)^+$, 372.1407. $C_{15}H_{22}N_3O_8$ requires $(M + NH_4)$, 172.1398]; ν_{max}(neat)/cm^{-1} 3102 (OH) and 1729 (CO); δ_H(250 MHz; C_6D_6) 0.91 (3 H, d, *J* 6.9, CH_3CH), 1.05 (3 H, t, *J* 7.1, CH_3CH_2O), 1.11 [3 H, d, *J* 6.4, $CH_3(OCO)$], 2.10 (1 H, dd, *J* 9.3, 16.6, CHHCO), 2.30 (1 H, m, CHHCO), 2.38 (1 H, m, CH_3CH), 4.03 (2 H, m, OCH_2), 5.09 [1 H, dq, *J* ~6.2, 6.2, CH(OH)], 8.62 (1 H, m, ArH) and 8.76 (2 H, m, ArH); δ_C(60 MHz; $CDCl_3$) 14.06 (CH_3CH), 15.88 (CH_3CH_2O), 16.87 [$CH_3CH(OCO)$], 34.82 (CH_3CH), 37.60 (CH_2CO), 60.44 (OCH_2), 76.47 [CH(OCO)], 122.23 (Ar-4′), 129.24 (Ar-2′, -6′), 134.06 (Ar-1′), 148.52 (Ar-3′, -5′), 161.80

(CO) and 172.06 (CO); *m/z* [CI(NH_3)] 372 $(M + NH_4)^+$ (100%), 355 $(M + H)^+$ (30), 326 (5), 295 (1), 247 (2), 195 (3), 176 (1), 160 (3), 143 (54), 132 (8), 114 (1), 97 (3), 73 (1) and 58 (3).

Preparation of mixtures of stereoisomers of ethyl (*E*)-4-hydroxy-3-methylpent-2-enoate (as 49) and ethyl 4-hydroxy-3-methylpentanoate (as 50) for GLC analysis

(a) To a stirred solution of the ester **48** (0.5 g) in tetrahydrofuran (9 cm^3) and methanol (1 cm^3) at 20 °C was added sodium borohydride (70 mg) in three portions over 30 min. The mixture was stirred for 1 h after which it was diluted with water (60 cm^3) and extracted with diethyl ether (5 × 30 cm^3) The combined organic extracts were washed with hydrochloric acid (0.1 mol dm^{-3}; 15 cm^3) and water (10 cm^3), dried ($MgSO_4$) and evaporated under reduced pressure to give ethyl (±)-4-hydroxy-3-methylpent-2-enoate (as **49**[71]) (0.5 g, 100%). Spectroscopic data (IR ^{1}H NMR and ^{13}C NMR were as for the optically active material (**49**, **56**). This material was used as a gas chromatographic standard with no further purification.

(b) A solution of the above ester (0.3 g) in ethanol (15 cm^3) was hydrogenated over 5% Pd–C at 15 psi for 3 h. The mixture was filtered and the filtrate was diluted with water (40 cm^3) and extracted with diethyl ether (4 × 30 cm^3). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give ethyl 4-hydroxy-3-methylpentanoate (as **50**, **57**) (0.3 g, 100%). Spectroscopic data (IR, ^{1}H NMR and ^{13}C NMR) were as for the optically active materials (**50**, **57**). This material was used as a gas chromatographic standard without further purification.

Hydrogenation of ethyl (*S*,*E*)-4-hydroxypent-2-enoate 49

The ester **49** (60 mg) in ethanol (10 cm^3) was hydrogenated as for the racemic compound, above, to give a mixture of ethyl (3*S*,4*S*)- and (3*R*,4*S*)-4-hydroxy-3-methylpentanoates **50** and **54** (50 mg); $[\alpha]_D^{20}$ −4.4 (*c* 0.25 in acetone).

Hydrogenation of ethyl (*R*,*E*)-4-hydroxypent-2-enoate 56

The ester **56** (70 mg) in ethanol (10 cm^3) was hydrogenated as for the racemic compound, above, to give a mixture of ethyl (3*S*,4*R*)- and (3*R*,4*R*)-4-hydroxy-3-methylpentanoates (70 mg); $[\alpha]_D^{20}$ −7 (*c* 1.1 in MeOH).

X-Ray crystallography

All measurements were made using a Siemens P3R3 four-circle diffractometer equipped with an Oxford Cryostream Cooler (version 2.4). Graphite monochromated Mo-*K*α radiation (λ = 0.710 73 Å) was used to collect the intensity data in the ω–2θ mode. Unit cell parameters and orientation matrices were obtained by least-squares refinement of the setting angles of 15 high angle reflections. The crystallographic programme was SHELXTL PLUS[73] and SHELXL-93;[74] the refinement program uses atomic scattering factors taken from International Tables for Crystallography.[75] The structures were solved by direct methods and refined using full-matrix least-squares on F^2. All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were inserted using a riding model and given isotropic thermal parameters equal to 1.2 (or 1.5 for methyl groups) times the equivalent isotropic displacement parameter of the atom to which it is attached. The weighting scheme was of the form $w^{-1} = [\sigma^2(F_o)^2 + (aP)^2 + bP]$ where $P = [\max(F_o^2, 0) + 2F_c^2]$ 3. The *R* factors are defined as $R(F) = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ and $wR(F^2) = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{\frac{1}{2}}$.

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