Biohydrogenation of Unsaturated Compounds by *Saccharomyces cerevisiae*. Part 1.† Stereochemical Aspects of the Reaction and Preparation of Useful Bifunctional Chiral Synthons

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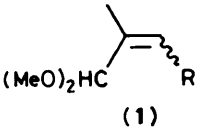
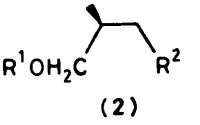
Ethyl 4,4-dimethoxy-3-methylbut-2-enoate (**1**; R = CO_2Et) has been prepared as a mixture of (*E*)- and (*Z*)-isomers, the (*E*)/(*Z*) ratio depending on the base used. Each isomeric mixture of (**1a**) and (**1b**) has been used as substrate for biohydrogenation with fermenting *Saccharomyces cerevisiae* (baker's yeast) and the (*Z*)-isomer seems to be the preferred substrate. (*E*)-Unsaturated alcohols such as (**3a**) and (**5d**) are not reduced to the corresponding saturated hydroxy derivatives by baker's yeast. The (*E*)-aldehyde (**3c**) and its acetal (**3d**) are mainly reduced to the corresponding (*E*)-alcohol (**3a**), the saturated hydroxy ester (**2a**) being formed to a minor extent, especially with (**3d**). In contrast, biohydrogenation is also successful with the (*E*)-isomers of compounds (**3e**), (**3f**), and (**3b**) (R^2 = alkyl or alkenyl). If the allylic oxygenated group to be reduced is not α-methyl substituted, reduction to the corresponding saturated alcohols readily occurs with the (*E*)-isomers as in the case of (**5f**). For this last biohydrogenation, the stereochemistry of the methyl-bearing carbon has been established by chemical correlations. The α,β-disubstituted allylic acetal (**6a**) is not biohydrogenated by the yeast, but a mixture of unsaturated hydroxy ester (**6b**) and γ-hydroxy lactone (**8**) is recovered from the incubation.

Biological systems such as purified enzymes and micro-organisms can be an invaluable tool for the synthesis of chiral molecules.[1] Of the micro-organisms so used, *Saccharomyces cerevisiae* (the common baker's yeast) has been widely employed to effect stereospecific reductions of variously substituted carbonyl compounds to the corresponding chiral secondary alcohols.[2] Recently, it has been reported by several authors that the biohydrogenation of unsaturated compounds to the corresponding saturated compounds can also be of synthetic significance.[3–6] We became interested in utilizing a biohydrogenation effected by baker's yeast for the preparation of alkylphenyl sulphones bearing a chiral centre, which could be useful chiral synthons for the construction of unusual stereoidal side chains.[7] Thus, we used the transformation of ethyl 4,4-dimethoxy-3-methylbut-2-enoate (**1**; R = CO_2Et) to the corresponding saturated hydroxy ester, namely ethyl (*S*)-(−)-4-hydroxy-3-methylbutanoate (**2a**) as described by Leuenberger *et al.*[3] Preparation of the unsaturated ester (**1**; R = CO_2Et) was easily realized by a Wittig-Horner reaction of 1,1-dimethoxyacetone and ethyl diethylphosphorylacetate using either sodium ethoxide in ethanol or BuLi in THF as base. The ^1H n.m.r. spectra of the products from these two preparations, showed that the (*E*)/(*Z*) isomer ratios were different; the latter were established from the ratio of the two methyl signals at δ 2.10 (*E*) and 1.90 (*Z*), and $CH(OCH_3)_2$ at 4.56 (*E*) and 5.95 (*Z*).[3] Incubation of the esters (**1a**) and (**1b**) with different proportions of (*E*)/(*Z*) isomers gave the saturated hydroxy ester (**2a**) {$[\alpha]_D$ = −4.7° (*c* 8 in MeOH)} and the unsaturated (*E*)-hydroxy ester (**3a**), the relative proportions of each depending on the (*E*)/(*Z*) isomer ratio in the starting material used. Thus, yields of unsaturated ester (**3a**) were 39% from (**1a**) and 37% from (**1b**) and compound (**2a**) was obtained in 15 and 25% from (**1a**) and (**1b**) respectively. These results suggested that the (*Z*)-isomer was the best substrate for the biohydrogenation



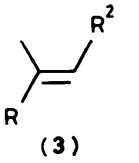
(**1**)

a; R = CO_2Et; (*E*)/(*Z*), 7:3
b; R = CO_2Et; (*E*)/(*Z*), 6:4
c; R = CH_2OH; (*E*)/(*Z*), 6:4
d; R = CH_2OCH_2Ph; (*E*)/(*Z*), 6:4



(**2**)

a; R^1 = H, R^2 = CO_2Et
b; R^1 = H, R^2 = Me
c; R^1 = H, R^2 = CH_2OCH_2Ph
d; R^1 = CH_2OEt, R^2 = CO_2Et
e; R^1 = CH_2OEt, R^2 = CH_2OH
f; R^1 = CH_2OEt, R^2 = CH_2OCH_2Ph



(**3**)

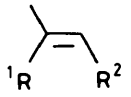
a; R^1 = CH_2OH, R^2 = CO_2Et
b; R^1 = CH_2OH, R^2 = Alkenyl
c; R^1 = CHO, R^2 = CO_2Et
d; R^1 = $CH(OMe)_2$, R^2 = CO_2Et
e; R^1 = CHO, R^2 = Me
f; R^1 = CH_2OH, R^2 = Me
g; R^1 = $CH(OMe)_2$, R^2 = CH_2OCH_2Ph
h; R^1 = CHO, R^2 = CH_2OCH_2Ph

examined by us. One path proposed was *via* the following steps: (*a*) hydrolysis of the acetal function of (**1**; R = CO_2Et); (*b*) biohydrogenation of the unsaturated aldehyde to afford the corresponding saturated aldehyde; (*c*) reduction of the saturated aldehyde to the corresponding alcohol (**2a**). Alternatively, in step (*b*) it was thought that the unsaturated aldehyde could be reduced to the corresponding unsaturated alcohol, which upon biohydrogenation would afford the saturated hydroxy ester (**2a**). The literature offers examples of both paths occurring with baker's yeast. Thus, cinnamaldehyde and cinnamyl alcohol are reduced to 3-phenylpropanol[4] and a *trans* addition of hydrogens stereospecifically takes place across the double bond. Also, recently it has been reported that an allylic alcohol such as geraniol is stereospecifically reduced by baker's yeast to (*R*)-(+)-citronellol.[5] Also (*E*)-geranial afforded the same chiral alcohol, whereas (*Z*)-neral apparently afforded the same alcohol with an *R*/*S* ratio of 6:4.[6] Recently, it has

† Part 2, see accompanying paper. Preliminary accounts on this work have been presented at the 4th European Symposium on Organic Chemistry (OC-11), Aix-en-Provence, 1985.

also been reported that (*E*)-allylic alcohols of the general formula (**3b**) can be hydrogenated by baker's yeast.[6]

With compounds (**1a**) and (**1b**), however, the presence and the position of the methyl group, along with the nature of the groups present in the substrate to be transformed could be the factors which differentiate their transformations from those previously described.[4–6] The chemical synthesis of (*Z*)-substrates such as (**4a**) or (**4b**) is more difficult than for the



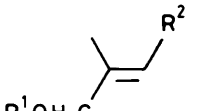
(**4**)

a; R^1 = CHO, R^2 = CO_2Et
b; R^1 = CH_2OH, R^2 = CO_2Et
c; R^1 = $CH(OMe)_2$, R^2 = CO_2Et

corresponding (*E*)-isomers, and, furthermore, the (*Z*)-compounds could be isomerized in the biological medium (see ref. 5). We therefore prepared the (*E*)-aldehyde (**3c**) by acidic hydrolysis of the acetal group of (**1a**) and incubated it with baker's yeast. If all the aldehyde was added at once to the fermenting yeast, there was no reaction and only starting material (**3c**) along with minute amounts of unsaturated hydroxy ester (**3a**) were recovered from the incubation. We reasoned that the unsaturated aldehyde might inhibit some enzymatic reaction along the biological process and in a repeat experiment, added the substrate (**3c**) slowly to the incubation mixture. With this procedure, starting material was completely transformed within 1 day, and the unsaturated (*E*)-hydroxy ester (**3a**) was isolated as the main product (37%) along with 14% of saturated hydroxy ester (**2a**). The unsaturated (*E*)-hydroxy ester (**3a**) itself, prepared by $NaBH_4$ reduction of (*E*)-aldehyde (**3c**), was incubated with yeast and not surprisingly was recovered unchanged after 5 days. Also the (*E*)-dimethyl acetal (**3d**) was prepared from the (*E*)-aldehydo ester (**3c**) and incubated with fermenting yeast (6 days) and again the (*E*)-hydroxy ester (**3a**) was isolated (40%) along with only slight amounts of (**2a**) (5%). The results obtained from incubations of (**3a**), (**3c**), and (**3d**) indicate that the preferred stereochemical prerequisites for a substrate such as the previously examined ones, still require a (*Z*)-configuration. In fact, the (*E*)-hydroxy ester (**3a**) is not biohydrogenated to (**2a**), although this result is in sharp contrast with the recently reported[6] transformations of compounds (**3b**). On the other hand, the (*E*)-aldehyde (**3c**) is rapidly reduced to the (*E*)-hydroxy ester (**3a**) and, probably *via* a concurrent double bond isomerization, biohydrogenated to the corresponding saturated aldehyde, which is rapidly reduced to the saturated hydroxy ester (**2a**) [(**3a**)/(**2a**), 3:1]. The acetal group in (**3d**) is not completely equivalent to the aldehyde moiety, since slow hydrolysis of (**3d**) probably produces the (*E*)-aldehydo ester (**3c**) which is reduced to the (*E*)-alcohol (**3a**) more rapidly than any possible isomerization; this limits the extent of biohydrogenation to (**2a**). With the (*E*)-acetal (**3d**), in fact, formation of unsaturated hydroxy ester is favoured over production of the saturated product [(**3a**)/(**2a**), 9:1].

In order to obtain further information on the steric requirements of the biohydrogenating system towards an α-methyl unsaturated aldehyde as substrate, (*E*)-2-methylbut-2-enal (**3e**) was reduced by baker's yeast to afford the corresponding saturated (*S*)-alcohol (**2b**), $[\alpha]_D$ −6.3° [only 28% yield because of product(s) solubility and difficulty with recovery; estimated optical purity 95%]. This result indicated that for (**3**; R^1 = CHO), changing R^2 from EtO_2C to Me caused a dramatic change in the stereochemical demand of the biohydrogenation process, a finding which is in agreement with the results of Gramatica *et al.* for compounds (**3b**).[6]

In extending our studies to other substrates, in the hope of preparing useful bifunctional chiral synthons, we prepared a number of methyl substituted unsaturated compounds as



(**5**)

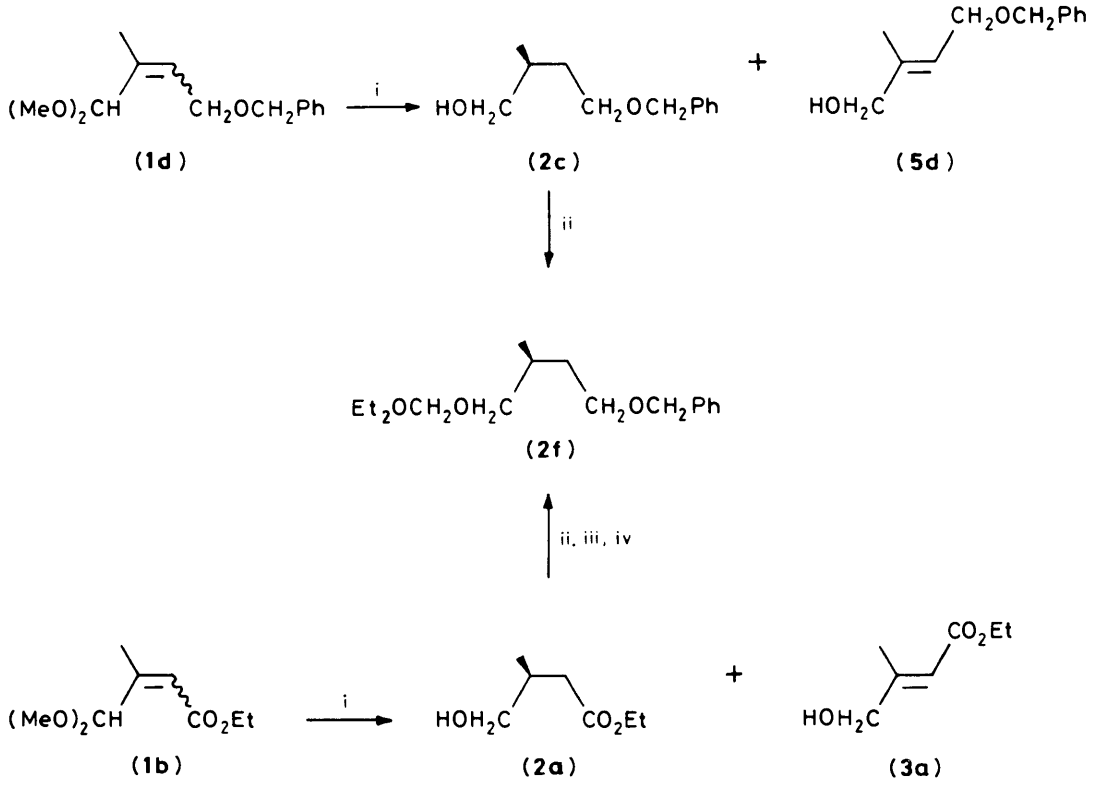
a; R^1 = Ac, R^2 = CO_2Et
b; R^1 = THP, R^2 = CO_2Et
c; R^1 = CH_2OEt, R^2 = CO_2Et
d; R^1 = H, R^2 = CH_2OCH_2Ph
e; R^1 = CH_2OEt, R^2 = CH_2OH
f; R^1 = CH_2OEt, R^2 = CHO

possible substrates for the yeast biohydrogenation. As expected, *O*-protected unsaturated (*E*)-hydroxy esters such as (**5a**—**c**) were not transformed by the yeast, although for compound (**5a**), some hydrolysis of the acetate was observed [40% of the hydroxy ester (**3a**) after 1 day].

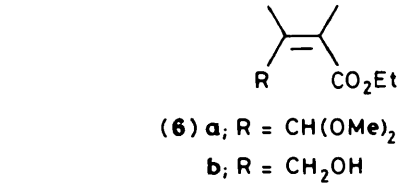
We also prepared a series of unsaturated aldehydes and alcohols and observed again that if a methyl group was α to the function to be reduced, stereoselective demands directed the course of the biohydrogenation. Thus, we prepared a mixture of (*E*)- and (*Z*)-unsaturated acetals (**1d**) starting from (*E*)/(*Z*) mixtures of the hydroxy esters (**1b**). Reduction of (**1b**) with $LiAlH_4$ under controlled conditions afforded the allylic alcohol (**1c**) as an isomeric mixture (79% yield). Benzylation of (**1c**) under basic conditions (NaH, $PhCH_2Br$ in tetrahydrofuran, 54%) afforded the mixture (**1d**) necessary for our experiments. Incubation of the isomeric mixture of acetals (**1d**) with baker's yeast afforded after 6 days a mixture of the saturated alcohol (**2c**) and the (*E*)-allylic alcohol (**5d**) (32 and 60%, respectively). Again this result indicated that the (*Z*)-acetal (**4c**) or its equivalent aldehyde was the substrate for the biohydrogenating enzyme system, whereas the (*E*)-acetal (**3g**) was only hydrolysed and reduced to the corresponding alcohol (**5d**). The configuration of the saturated alcohol (**2c**) was established as (*S*) by chemical correlation with the product obtained from the (*S*)-hydroxy ester (**2a**) (Scheme). In fact, starting from (**2a**) {$[\alpha]_D$ −4.7° (*c* 8 in MeOH)} the hydroxy group was protected as the ethoxymethyl ether[8] and compound (**2d**) was then reduced to the corresponding alcohol (**2e**), which was benzylated to afford the protected (*S*)-diol (**2f**) {$[\alpha]_D$ +0.4° (*c* 2.5 in $CHCl_3$)}. Also compound (**2f**) prepared from (**2c**), deriving from yeast biohydrogenation of (**1d**), exhibited $[\alpha]_D$ +0.4° (*c* 2.5 in $CHCl_3$). This allowed assignment of the (*S*) configuration to the saturated diol monobenzyl ether (**2c**) obtained from baker's yeast biohydrogenation and bioreduction of (**1d**). In order to achieve additional proof of the stereoselective prerequisite of the yeast enzymes, the (*E*)-monobenzyl diol (**5d**) was independently prepared from the acetal (**1d**), which was hydrolysed (HCl) to the (*E*)-unsaturated aldehyde (**3h**). $NaBH_4$ Reduction of the latter afforded exclusively (**5d**). As expected, incubation of the latter with baker's yeast led after 5 days only to recovery of starting material.

Different behaviour was observed when the (*E*)-aldehyde (**5f**) [prepared from controlled $LiAlH_4$ reduction of the (*E*)-hydroxy ester (**5c**) and chromium trioxide–pyridine oxidation[9] of the (*E*)-monoprotected diol (**5e**)] was incubated with baker's yeast. The aldehyde (**5f**) was transformed only into a monofunctionalized saturated diol (**2e**) {$[\alpha]_D$ −2.4° (*c* 2 in $CHCl_3$)}, which showed an optical rotation identical with that of the same compound prepared from (**2a**) *via* $LiAlH_4$ reduction of the protected ester (**2d**). In this case, it should be noted that the (*E*)-aldehyde deriving from the acetal (**1d**) is reduced only to the (*E*)-alcohol (**5d**), whereas the (*E*)-aldehyde (**5f**) in which the methyl group is differently placed is completely transformed into the saturated compound (**2e**).

Finally, we have also synthesized the dimethyl unsaturated acetal (**6a**), probably as a mixture of (*E*) and (*Z*) isomers, by condensation of 1,1-dimethoxyacetone and ethyl diethylphosphorylpropionate (**7**) (in turn prepared from triethyl phosphite and ethyl 2-bromopropionate[10]). When incubated with baker's yeast (10 days) compound (**6a**) afforded a mixture of the unsaturated hydroxy ester (**6b**) [probably (*E*) isomer]

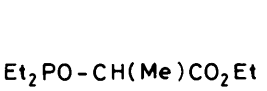


Scheme. *Reagents:* i, Baker's yeast; ii, $CH_2(OEt)_2$, *p*-TsOH; iii. $LiAlH_4$; iv, NaH, $PhCH_2Br$

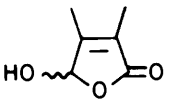


(6) a; R = $CH(OMe)_2$

b; R = CH_2OH



(7)



(8)

and the γ-hydroxy lactone (**8**) (22 and 52%, respectively). Results suggest that under biohydrogenating conditions, the acetal function of compound (**6a**) is hydrolysed to the corresponding aldehyde, which may then undergo two different reactions, depending on the type of isomer; the intermediate aldehyde can be either reduced to the corresponding hydroxy derivative (**6b**) or cyclized to the lactone (**8**) probably after hydrolysis of the ester group of (**6a**). The above results are consistent with other unsuccessful attempts of similar microbial hydrogenations of tetrasubstituted double bonds.[1]

Experimental

Distillations for analytical purposes were performed with a glass tube oven Buchi GKR-50 at reduced pressure (15 mmHg). ^1H N.m.r. spectra were recorded on a Varian 360 L spectrometer for solutions in C^2HCl_3 using Me_4Si as internal standard. Mass spectra were recorded on a LKB 2091 Gas Chromatograph-Mass spectrometer by D.I.S. unless otherwise stated. The progress of all reactions and column chromatographies was monitored by t.l.c. on Merck silica gel HF_{254} plates visualized by u.v. absorption, exposure of the plates to iodine vapour, or spraying with a 5% ethanol solution of phosphomolybdic acid. Gas chromatographic analyses were carried out on a Carlo Erba Fractovap 2001 (column 1% OV 17), carrier N_2. Optical rotations were recorded on a 141 Perkin-Elmer polarimeter.

General Conditions for Incubations with Fermenting Baker's Yeast.—Unless otherwise indicated, for incubations with baker's yeast the following amounts were used per mmol of substrate. To a solution of saccharose (0.78 g) in water (14 ml) commercial baker's yeast (Eridania, Italy) (1.58 g) was added. The mixture was kept at 30 °C (1 h) in order to start the fermentation and then the substrate (1 mmol) was added. The mixture was kept at 30 °C for the time required in each case (monitored by t.l.c. or ^1H n.m.r. when of necessity) and after filtration on a Celite pad, the solution was saturated with sodium chloride and extracted with diethyl ether (5 × 20 ml). The organic solution was dried over sodium sulphate and carefully evaporated at reduced pressure.

Ethyl 4,4-Dimethoxy-3-methylbut-2-enoate (**1a**).—A solution of triethyl phosphonoacetate (19 g, 85 mmol) in absolute ethanol (25 ml) was slowly added to a solution of sodium (2.5 g, 108 mmol) in absolute ethanol (500 ml) cooled in an ice-bath. The mixture was stirred at 0 °C for 20 min, after which a solution of 1,1-dimethoxyacetone (10 g, 85 mmol) in absolute ethanol (20 ml) was added dropwise. The mixture was then stirred at room temperature for 3 h after which it was neutralized with 1M HCl, concentrated under reduced pressure, and extracted with dichloromethane (3 × 100 ml). The extract was dried (Na_2SO_4) and evaporated under reduced pressure to afford the title compound (14.4 g, 90%) (Found: C, 57.2; H, 8.7. Calc. for $C_9H_{16}O_4$: C, 57.4; H, 8.6%). Physicochemical results were in agreement with the literature;[3] from the ^1H n.m.r. spectrum the relative proportions of (*Z*) and (*E*) isomers could

be evaluated by signals at δ 1.90 (s, *Z*-Me), 2.10 (s, *E*-Me), 4.56 (s, OCHO, *E*), and 5.95 (s, OCHO, *Z*); g.c. (temp. 90 °C): T_R 3 min for *Z* and 4 min for *E*.

Ethyl 4,4-*Dimethoxy*-3-*methylbut*-2-*enoate* (**1b**).—A solution of butyl-lithium (1.6M in hexane; 40 ml) was added under argon to a solution of ethyl diethylphosphorylacetate (14.2 g, 63.5 mmol) in anhydrous tetrahydrofuran (40 ml), cooled in an ice-bath. After the mixture had been stirred for 20 min at 0 °C, a solution of 1,1-dimethoxyacetone (7.5 g, 63.5 mmol) in tetrahydrofuran (10 ml) was added dropwise. After 6 h at 0 °C, the reaction mixture was neutralized with 1M HCl, concentrated under reduced pressure, and extracted with dichloromethane (3 × 100 ml). The combined organic extracts were dried (Na_2SO_4) and evaporated under reduced pressure to afford the title compound (10.7 g, 90%). Physicochemical results were in agreement with literature;[3] from the ^1H n.m.r. spectrum and g.l.c. the relative proportions of (*Z*)- and (*E*)-isomers (4:6) could be evaluated as for ester (**1a**).

Baker's Yeast Incubations of (**1a**) *and* (**1b**).—Ethyl 4,4-dimethoxy-3-methylbut-2-enoate (**1a**) (1 g, 5.3 mmol) was incubated (96 h) and after work-up a mixture (0.688 g) of (*S*)-ethyl 4-hydroxy-3-methylbutanoate (**2a**) and (*E*)-ethyl 4-hydroxy-3-methylbut-2-enoate (**3a**) which was purified by silica gel column with hexane–ethyl acetate (6:4) as eluant: (**2a**) (0.116 g, 15%), b.p. 155—160 °C (Found: C, 57.6; H, 9.8. Calc. for $C_7H_{14}O_3$: C, 57.5; H, 9.6%); δ 0.90 (d, CH_3CH, 3 H), 1.30 (t, CH_3CH_2O, 3 H), 2.00—2.50 (m, CH and CH_2, 3 H), 3.40 (d, CH_2OH, 2 H), and 4.30 (q, CH_3CH_2O, 2 H); $[\alpha]_D$ −4.7° (*c* 8 in MeOH); (**3a**) (0.298 g, 39%), b.p. 160—165 °C (Found: C, 58.4; H, 8.45. Calc. for $C_7H_{12}O_3$: C, 58.3; H, 8.3%); δ 1.30 (t, CH_3CH_2O, 3 H), 2.10 (s, $CH_3C{=}C$, 3 H), 4.15 (q + s, CH_3CH_2O and CH_2OH, 4 H), and 6.10 (s, HC=C, 1 H).

Similar results were obtained starting from (**1b**), prepared as above with butyl-lithium, except that yields of saturated and unsaturated hydroxy ester (**2a**) and (**3a**) were 25 and 37% respectively.

(E)-*Ethyl* 3-*formyl*-3-*methylbut*-2-*enoate* (**3c**).—A stirred mixture of ethyl 4,4-dimethoxy-3-methylbut-2-enoate (**1a**) (5 g, 26.5 mmol) and dioxane (5 ml) was treated with 1M HCl (3.3 ml) at room temperature (4 h). Two phases were formed and the oil corresponding to the aldehyde (phase superior) was separated, an additional amount of product being then recovered by extraction with hexane (3 × 10 ml). The combined organic phases were washed with aqueous $NaHCO_3$, dried (Na_2SO_4), and carefully evaporated under reduced pressure to afford the desired product (**3c**) (2 g, 53%), which was directly used for the next reaction(s) (Found: C, 59.5; H, 7.2. Calc. for $C_7H_{10}O_3$: C, 59.15; H, 7.0%); δ 1.30 (t, $CO_2CH_2CH_3$, 3 H), 2.10 (s, $C{=}CCH_3$, 3 H), 4.30 (q, $CO_2CH_2CH_3$, 2 H), 6.60 (s, C=CH, 1 H), and 9.65 (s, CHO, 1 H).

Incubation of Compound (**3c**) *with Baker's Yeast.—Method A* (*rapid addition of substrate*). The aldehyde (**3c**) (1 g) was added in one portion to a suspension of baker's yeast (4.36 g) in a solution of saccharose (4.36 g) in water (66 ml) under the usual conditions. After 72 h at 30 °C and work-up, an oil (0.4 g) was recovered corresponding to unchanged starting material. Only traces of the unsaturated hydroxy ester (**3a**) were present in the mixture (t.l.c. and g.c.).

Method B (*dropwise addition of the substrate*). The aldehyde (**3c**) (1 g) was added dropwise during 8 h to the suspension of baker's yeast in the saccharose solution prepared as above. The reaction was monitored by t.l.c. and ^1H n.m.r. analysis at various intervals of time and after addition of aldehyde (**3c**) (8 h), the incubation was continued for an additional 16 h. A mixture was obtained in which no starting material was present. The reaction mixture consisted of (*E*)-ethyl 4-hydroxy-3-methylbut-2-enoate (**3a**) and ethyl 4-hydroxy-3-methylbutanoate (**2a**) (ratio 7:3, by ^1H n.m.r.).

(E)-*Ethyl* 4-4-*Dimethoxy*-3-*methylbut*-2-*enoate* (**3d**).—Trimethyl orthoformate (0.75 g, 7.1 mmol) and ammonium chloride (0.014 g, 0.26 mmol) were added to a solution of the aldehyde (**3c**) (1 g, 7 mmol) in absolute methanol (1 ml). The mixture was stirred under reflux for 30 min after which it was washed with aqueous sodium carbonate and extracted with dichloromethane (3 × 4 ml). The organic extract was dried (Na_2SO_4) and evaporated under reduced pressure to afford (**3d**) as an oil (0.8 g, 60%), b.p. 110 °C (Found: C, 57.6; H, 8.7. Calc. for $C_9H_{16}O_4$: C, 57.4; H, 8.6%); δ 1.30 (t, CO_2Et, 3 H), 2.10 (s, $CH{=}CCH_3$, 3 H), 3.35 (s, 2 × OCH_3, 6 H), 4.30 (q, $CO_2CH_2CH_3$, 2 H), 4.60 (s, OCHO, 1 H), and 6.10 (s, CH=C, 1 H).

Incubation of Compound (**3d**).—The reaction of the (*E*)-isomer (**3d**) (1 g) with baker's yeast was carried out as for the mixture of *E*/*Z* isomers of (**1a**), except that the mixture was kept at 30 °C for 24 h: none of the corresponding saturated ester (**2a**) was formed (as established by ^1H n.m.r. analysis). Since the situation remained unchanged after an additional 5 days the reaction was stopped. After work-up small amounts of the saturated compound (**2a**) and the unsaturated hydroxy ester (**3a**) (0.5 g) were obtained. Column chromatography (silica gel) and elution with hexane–ethyl acetate (6:4) afforded the unsaturated hydroxy ester (**3a**) (0.32 g, 40%) and the saturated hydroxy ester (**2a**) (0.04 g, 5%).

(E)-*Ethyl* 4-*ethoxymethoxy*-3-*methylbut*-2-*enoate* (**5c**).—The hydroxy ester (**3a**) (2.6 g, 18 mmol) obtained from an incubated mixture of (**1a**) was stirred and treated with formaldehyde diethyl acetal (33 ml) and toluene-*p*-sulphonic acid (2.3 g, 12 mmol) at room temperature (12 h). The mixture was neutralized with aqueous $NaHCO_3$ and extracted with dichloromethane (3 × 50 ml). After work-up, an oil (3 g) was obtained which was purified by neutral aluminium oxide chromatography. Elution with hexane–ethyl acetate (9:1) afforded the desired ethoxymethyl ether (**5c**) (2 g, 72%), b.p. 200—205 °C (Found: C, 59.5; H, 9.00. $C_{10}H_{18}O_4$ requires C, 59.4; H, 8.9%); δ 1.10—1.40 (m, CO_2Et, OEt, 6 H), 2.10 (s, $CH{=}CCH_3$, 3 H), 3.65 (q, OCH_2CH_3, 2 H), 4.00—4.40 (q + s, CO_2Et, $=CCH_2O$, 4 H), 4.70 (s, OCH_2O, 2 H), 6.0 (s, CH=C, 1 H); *m*/*z* 173 (M^+ − Et), 157, 143, 128, 127, 114, 98, and 59.

(E)-4-*Ethoxymethoxy*-3-*methylbut*-2-*enol* (**5e**).—A solution of the ester (**5c**) previously prepared (1.38 g, 6.8 mmol) in THF (27 ml) was cooled to 0 °C. Lithium aluminium hydride (0.25 g, 6.8 mmol) was very slowly added to it, care being taken to keep the temperature at 0 °C. After 1 h, water (0.25 ml), aqueous NaOH (15%; 0.25 ml), and water (0.75 ml) were added. The precipitate was removed by filtration through a Celite pad and the filtrate concentrated under reduced pressure to give the unsaturated alcohol (1 g, 92%), b.p. 160—162 °C (Found: C, 60.1; H, 10.1. $C_8H_{16}O_3$ requires C, 60.0; H, 10.0%); δ 1.20 (t, OCH_2CH_3, 3 H), 1.70 (s, $CH{=}CCH_3$, 3 H), 3.65 (q, OCH_2CH_3, 2 H), 4.00 (s, OCH_2CH_3, 2 H), 4.20 (d, $OCH_2CH{=}$, 2 H), 4.70 (s, OCH_2O, 2 H), and 5.70 (t, C=CH, 1 H).

(E)-4-*Ethoxymethoxy*-3-*methylbut*-2-*enal* (**5f**).—Chromium trioxide (3.75 g, 37.5 mmol) was added to a stirred solution of pyridine (5.93 g, 75 mmol) in dichloromethane (93 ml). The solution was stirred for 15 min at room temperature and a solution of the alcohol (**5e**) (1 g, 6.25 mmol) in dichloromethane (10 ml) was added at once. After the mixture had been stirred for 20 min at room temperature the solution was

decanted from the residue which was washed with diethyl ether (80 ml). The combined organic solutions were washed with 5% aqueous sodium hydroxide, 5% aqueous hydrochloric acid, 5% aqueous sodium hydrogen carbonate and water, dried (Na_2SO_4), and evaporated under reduced pressure to leave a brown oil. This after purification by column chromatography [Al_2O_3, elution with hexane–ethyl acetate (8 : 2)] afforded the aldehyde (**5f**) (0.4 g, 40%), b.p. 160—165 °C (Found: C, 60.7; H, 8.9. $C_8H_{14}O_3$ requires C, 60.75; H, 8.85%); δ 1.20 (t, OCH_2CH_3, 3 H), 2.20 (s, CH=CCH_3, 3 H), 3.75 (q, OCH_2CH_3, 2 H), 4.20 (s, =CCH_2O, 2 H), 4.80 (s, OCH_2O, 2 H), 6.20 (d, CH=C, 1 H), and 10.15 (d, CHO, 1 H).

Incubation of the Aldehyde (**5f**) *with Baker's Yeast*.—The aldehyde (**5f**) (0.15 g, 0.95 mmol) was added dropwise to the incubation mixture and after 60 h the reaction was worked up to give a residue (82 mg), the ^1H n.m.r. spectrum of which showed the presence of the saturated alcohol with a little unsaturated alcohol. Purification by column chromatography (Al_2O_3) afforded pure 4-ethoxymethoxy-3-methylbutan-1-ol (**2e**) (54 mg, 36%), b.p. 165—168 °C (Found: C, 59.15; H, 11.0. $C_8H_{18}O_3$ requires C, 59.25; H, 11.1%); δ 0.90 (d, CH_3CH, 3 H), 1.20 (t, OCH_2CH_3, 3 H), 1.50—2.00 (m, CH_2CH_2OH, CHCH_3, 3 H), 3.50 (d, OCH_2CH, 2 H), 3.70 (t, CH_2CH_2OH, 2 H), and 4.70 (s, OCH_2O, 2 H); $[\alpha]_D$ −2.42° (*c* 2.06 in $CHCl_3$).

4,4-*Dimethoxy*-3-*methylbut*-2-*en*-1-*ol* (**1c**).—Lithium aluminium hydride (0.544 g, 14.36 mmol) was slowly added to the ester (**1a**) (2.7 g, 14.3 mmol) in tetrahydrofuran (50 ml) at 0 °C. The mixture was stirred at 0 °C for 1 h and after work-up, the unsaturated alcohol (**1c**) was recovered (1.65 g, 79%) as a mixture of (*E*)/(*Z*) isomers in the same ratio as that in the starting ester (**1a**), b.p. 140—145 °C (Found: C, 57.5; H, 9.6. $C_7H_{14}O_3$ requires C, 57.5; H, 9.6%); δ 1.65 (s, CH=CCH_3, *E*), 1.75 (s, CH=CCH_3, *Z*), 3.35 (s, OCH_3, *E*), 3.45 (s, OCH_3, *Z*), 4.25 (d, CH_2O, 2 H), 4.54 (s, OCHO, *E*), 5.00 (s, OCHO, *Z*), and 5.85 (m, CH=C, 1 H); *m*/*z* 146 (M^+), 128, 115, and 83.

4-*Benzyloxy*-2-*methylbut*-2-*en*-1-*al* 1,1-*Dimethylacetal* (**1d**).—80% Sodium hydride (0.38 g, 12.6 mmol) was added to a solution of the previously prepared alcohol (**1c**) (1.28 g, 8.76 mmol) in tetrahydrofuran (25 ml). The mixture was stirred at room temperature for 10 min after which benzyl bromide (1.5 g, 8.76 mmol) was added to it; the mixture was then stirred at room temperature for 12 h. It was then diluted with water (20 ml) and the tetrahydrofuran was evaporated off under reduced pressure; the aqueous solution was then extracted with dichloromethane (3 × 25 ml). The organic extract was dried (Na_2SO_4) and evaporated under reduced pressure to afford an oil (1.3 g) which was purified by column chromatography (Al_2O_3). Elution with hexane–ethyl acetate (95:5) afforded the pure title benzyl ether (**1d**) (0.8 g, 38%), b.p. 200—210 °C (Found: C, 71.3; H, 8.6. $C_{14}H_{20}O_3$ requires C, 71.2; H, 8.5%); δ 1.60 (s, CH=CCH_3, *E*), 1.75 (s, CH_3, *Z*), 3.30 (s, 2 × OCH_3, 6 H), 4.20 (d, CH_2O, 2 H), 4.55 (s + s, $PhCH_2O$, OCHO, 2 H + 1 H *E*), 4.95 (s, OCHO, *Z*), 5.55—6.00 (m, CH=C, 1 H), 7.40 (s, Ar, 5 H); *m*/*z* 235 (M^+ − 1), 204, 173, and 129.

Incubation of Compound (**1d**).—The above acetal (**1d**) (0.8 g, 3.38 mmol) was incubated for 6 days at 30 °C. After work-up, the products (0.6 g) were purified by column chromatography on silica gel. Elution with hexane–ethyl acetate (6:4) afforded (**2c**) (0.208 g, 32%), b.p. >245 °C (Found: C, 74.35; H, 9.4. $C_{12}H_{18}O_2$ requires C, 74.2; H, 9.3%); $[\alpha]_D$ −4.87° (*c* 3.16 in CH_2Cl_2); δ 0.90 (d, CH_3CH, 3 H), 1.20—1.90 (m, CH_2CH, CH_3CHCH_2, 3 H), 3.30—3.70 (m, CH_2OH, CH_2OCH_2Ph, 4 H), 4.50 (s, OCH_2Ph, 2 H), 7.35 (s, ArH, 5 H); *m*/*z* 194 (M^+), 167, 149, 107, and 91. The unsaturated monoprotected diol (**5d**) was also eluted with the same eluant (hexane–ethyl acetate, 6:4) (0.39 g, 60%), b.p. 240—245 °C (Found: C, 75.0; H, 8.4. $C_{12}H_{16}O_2$ requires C, 75.0; H, 8.3%); δ 1.55 (s, CH_3CH=, 3 H), 3.80—4.20 (m, CH_2OH, CH_2OCH_2Ph, 4 H), 4.40 (s, OCH_2Ph, 2 H), 5.60 (t, CH=C, 1 H), and 7.20 (s, ArH, 5 H).

Incubation of Compound (**3g**).—Compound (**1d**) after exposure to an aluminium column for 6 h provided with hexane–ethyl acetate (95:5) as eluant compound (**3g**). Incubation and work-up of the latter were as for (**1d**). Purification by column chromatography on silica gel and elution with hexane–ethyl acetate (6:4) afforded the unsaturated monoprotected diol (**5d**) (0.4 g, 58%).

(S)-*Ethyl* 4-*Ethoxymethoxy*-3-*methylbutanoate* (**2d**).—(*S*)-Ethyl 4-hydroxy-3-methylbutanoate (**2a**) (2.28 g, 15.6 mmol) was treated with toluene-*p*-sulphonic acid (2.95 g, 15.5 mmol) and formaldehyde diethyl acetal (35 g, 0.337 mol) at room temperature with stirring for 24 h. The mixture was then brought to pH 7 with saturated aqueous $NaHCO_3$ and extracted with dichloromethane (3 × 25 ml). After work-up of the extract the crude product was purified by column chromatography [neutral Al_2O_3 and hexane–ethyl acetate (9:1) as eluant] to afford the protected hydroxy ester (**2d**) (2.4 g, 75%), b.p. 160 °C (Found: C, 59.0; H, 9.9. $C_{10}H_{20}O_4$ requires C, 58.8; H, 9.8%); δ 0.90—1.50 (m, CH_3, 9 H), 2.00—2.50 (m, 3 H), 3.20—3.90 (m, OCH_2CH_3, OCH_2CH, 4 H), 4.20 (q, OCH_2CH_3, 2 H), and 4.70 (s, OCH_2O, 2 H); $[\alpha]_D$ −4.38° (*c* 1.14 in $CHCl_3$); *m*/*z* 204 (M^+), 159, and 129.

(S)-4-*Ethoxymethoxy*-3-*methylbutan*-1-*ol* (**2e**).—A solution of compound (**2d**) (1.83 g, 8.97 mmol) in anhydrous tetrahydrofuran (5 ml) was added dropwise to a suspension of lithium aluminium hydride (1 g, 26.3 mmol) in tetrahydrofuran (40 ml). The reaction was heated under reflux for 5 h after which work-up and column chromatography [Al_2O_3 with hexane–ethyl acetate (6:4) as eluant] afforded the product (**2e**) (1.4 g, 96%), b.p. 165 °C (Found: C, 59.3; H, 11.2. $C_8H_{18}O_3$ requires C, 59.25; H, 11.1%); δ 0.95 (d, CH_3CH, 3 H), 1.25 (t, CH_3CH_2, 3 H), 1.50—2.10 (m, CH_2CH, 3 H), 3.35—3.85 (m, 3 × CH_2O, 6 H), and 4.70 (s, OCH_2O, 2 H); $[\alpha]_D$ −2.62° (*c* 2.06 in $CHCl_3$); *m*/*z* 117 (M^+ − 45), 103, 85, 69, and 57.

(S)-4-*Benzyloxy*-1-*ethoxymethoxy*-2-*methylbutane* (**2f**).—80% Sodium hydride dispersion (0.114 g, 4.7 mmol) was added under nitrogen to the diol (**2e**) (0.51 g, 3.14 mmol) dissolved in anhydrous tetrahydrofuran (10 ml). The stirred mixture was kept at room temperature for 8 h and upon work-up provided the crude product (1.05 g). Purification of this by column chromatography (neutral Al_2O_3, elution with hexane–ethyl acetate, 9:1) afforded pure (**2f**) (0.35 g, 44%), b.p. 235—245 °C (Found: C, 71.5; H, 9.6. $C_{15}H_{24}O_3$ requires C, 71.4; H, 9.5%); δ 1.00 (d, CH_3CH, 3 H), 1.20 (t, CH_3CH_2, 3 H), 1.50—2.10 (m, CH_2CH, 3 H), 3.35—3.9 (m, 3 × CH_2O, 6 H), 4.55 (s, CH_2Ph, 2 H), 4.70 (s, OCH_2O, 2 H), 7.25—7.35 (s, ArH, 5 H); $[\alpha]_D$ +0.4° (*c* 2.5 in $CHCl_3$); *m*/*z* 251 (M^+ − 1), 207, 193, 167, 149, 105, and 91.

(S)-4-*Benzyloxy*-1-*ethoxymethoxy*-2-*methylbutane* (**2f**) *from* (**2c**) *obtained from Incubation of Compound* (**1d**).—Toluene-*p*-sulphonic acid (0.18 g, 0.95 mmol) was added to the ether (**2c**) (0.188 g, 0.97 mmol) dissolved in formaldehyde diethyl acetal (2.13 g, 20.5 mmol). After 24 h at room temperature, work-up of the reaction mixture afforded crude product (0.215 g) which was purified by column chromatography (neutral Al_2O_3, elution with hexane–ethyl acetate, 9:1) to give pure (**2f**) (0.125 g, 51%); this had chemicophysical properties identical with the product obtained by the previously described method; $[\alpha]_D$ +0.4° (*c* 2.5 in $CHCl_3$).

Incubation of (E)-2-*Methylbut*-2-*enal* (**3e**).—Tiglic aldehyde (**3e**) (0.5 g) was incubated for 18 h. Work-up gave the crude product (0.35 g) which was distilled to afford pure (*S*)-(−)-2-methylbutan-1-ol (**2b**) (0.15 g, 28%), b.p. 126—128 °C/760 mmHg (Found: C, 68.3; H, 13.75. Calc. for $C_5H_{12}O$: C, 68.2; H, 13.6%); δ 0.90 (d, CH_3C=, 3 H), 1.00—1.80 (m, 3 H), 3.45 (d, CH_2OH, 2 H); $[\alpha]_D$ −6.3° (*c* 10 in EtOH).

Ethyl 2-*Diethylphosphorylpropionate* (**7**).—Triethyl phosphite (4.58 g, 27.6 mmol) was added, with stirring, in two portions to ethyl 2-bromopropionate (5 g, 27.6 mmol). After the first addition (1 g) the reaction was kept for 30 min at room temperature and when the reaction temperature began to rise, the remaining triethyl phosphite was added, the ethyl 2-bromopropionate being maintained at reflux. The reaction mixture was then heated at 170 °C for 8 h. The product was recovered essentially pure (6 g, 91%) after removal of the ethyl bromide by distillation (at 170 °C for 1 h without the condenser) (Found: C, 45.45; H, 8.0. Calc. for $C_9H_{19}O_5P$: C, 45.4; H, 8.0%); δ 1.20—1.90 (m, CH_3, 12 H), 2.60—3.60 (m, CH, 1 H), and 3.90—4.40 (m, CH_2O, 6 H).

Ethyl 4,4-*Dimethoxy*-2,3-*dimethylbut*-2-*enoate* (**6a**).—1.6M Butyl-lithium in hexane (15.75 ml) was added to a solution of ethyl 2-diethylphosphorylpropionate (6 g, 25.2 mmol) in anhydrous tetrahydrofuran (20 ml) cooled in an ice-bath. The reaction was kept at 0 °C for 20 min and then 1,1-dimethoxyacetone (2.97 g, 25.2 mmol) in tetrahydrofuran (20 ml) was added dropwise at 0 °C. After 4 h at 0 °C work-up of the mixture gave a crude product (5 g) which was purified on aluminium oxide column. Elution with hexane–ethyl acetate (9:1) afforded the pure ester (**6a**) (3 g, 60%) (Found: C, 59.5; H, 9.0. $C_{10}H_{18}O_4$ requires C, 59.4; H, 8.9%); δ 1.30 (t, CH_3CH_2O, 3 H), 1.70 (s, CH_3C=C, *Z*), 1.90 (s, CH_3C=C, *E*), 3.30 (s, OCH_3, 6 H), 4.20 (q, CH_3CH_2O, 2 H), 5.00 (s, OCHO, *Z*, 1 H), and 5.25 (s, OCHO, *E*, 1 H).

Incubation of Ethyl 4,4-*Dimethoxy*-2,3-*dimethylbut*-2-*enoate* (**6a**).—The title ester (**6a**) (0.624 g, 3.08 mmol) was incubated for 10 days. The crude mixture (0.4 g) was purified on silica gel column with hexane–ethyl acetate (6:4) as eluant to afford ethyl 4-hydroxy-2,3-dimethylbut-2-enoate (**6b**) (0.107 g, 22.6%) and the γ-hydroxy-γ-lactone (**8**) (0.207 g, 52.6%); (**6b**) (Found: C, 60.9; H, 9.0. $C_8H_{14}O_3$ requires C, 60.75; H, 8.9%); δ 1.30 (t, CH_3, 3 H), 1.90 (s, CH_3C=, 3 H), 2.05 (s, CH_3, 3 H), 4.20 (q, CH_2, 2 H), and 4.25 (s, CH_2OH, 2 H); (**8**) δ 1.80 (s, CH_3C=C, 3 H), 2.00 (s, CH_3C=C, 3 H), and 5.90 (s, OCHOH, 1 H); *m/z* 127 ($M^+ - 1$), 111, and 99.

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