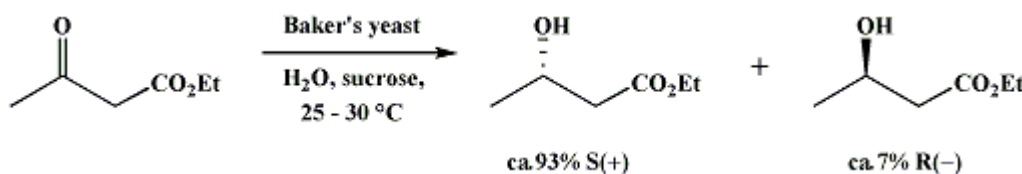


*Organic Syntheses, CV 7, 215*

## YEAST REDUCTION OF ETHYL ACETOACETATE: (S)-( + )-ETHYL 3-HYDROXYBUTANOATE

[Butanoic acid, 3-hydroxy-, ethyl ester, (S)]



Submitted by Dieter Seebach, Marius A. Sutter, Roland H. Weber, and Max F. Züger<sup>1</sup>.

Checked by Terry Rosen and Clayton H. Heathcock.

### 1. Procedure

A 4-L, three-necked, round-bottomed flask equipped with mechanical stirrer, bubble counter, and a stopper is charged with 1.6 L of tap water, 300 g of [sucrose](#) (Note 1), and 200 g of baker's yeast (Note 2), which are added with stirring in this order. The mixture is stirred for 1 hr at about 30°C, 20.0 g (0.154 mol) of [ethyl acetoacetate](#) (Note 3) is added, and the fermenting suspension (Note 4) is stirred for another 24 hr at room temperature. A warm (ca. 40°C) solution of 200 g of [sucrose](#) (Note 1) in 1 L of tap water is then added, followed 1 hr later by an additional 20.0 g (0.154 mol) of [ethyl acetoacetate](#) (Note 3). Stirring is continued for 50–60 hr at room temperature. When the reaction is complete by gas chromatographic analysis (Note 5), the mixture is worked up by first adding 80 g of Celite and filtering through a sintered-glass funnel (porosity 4, 17-cm diam). After the filtrate is washed with 200 mL of water, it is saturated with [sodium chloride](#) and extracted with five 500-mL portions of [ethyl ether](#) (Note 6). The combined [ether](#) extracts are dried over [magnesium sulfate](#), filtered, and concentrated with a rotary evaporator at 35°C bath temperature to a volume of 50–80 mL. This residue is fractionally distilled at a pressure of 12 mm through a 10-cm Vigreux column, and the fraction boiling at 71–73°C (12 mm) is collected to give 24–31 g (59–76%) of (S)-( + )-ethyl 3-hydroxybutanoate (Note 7) and (Note 8); the specific rotation  $[\alpha]_D^{25} + 37.2^\circ$  ([chloroform](#),  $c$  1.3) corresponds to an enantiomeric excess of 85% (Note 9).

The enantiomeric excess may be enhanced by several crystallizations of the 3,5-dinitrobenzoate derivative (Note 10) or else by using "starved" yeast (Note 11).

### 2. Notes

1. Commercially available sugar ([sucrose](#)) from a grocery store is used.
2. Commercially available baker's yeast can be used. The submitters used baker's yeast from E. Klipfel & Co. AG, CH-4310 Rheinfelden (Switzerland). The checkers used Fleischmann's yeast (cubes), obtained from a supermarket, or Red Star Baker's yeast (Universal Food Corporation), obtained from a bakery. The optical rotation of the final product was essentially the same for runs in which the two brands were employed.
3. [Ethyl acetoacetate](#) is freshly distilled before use (bp 65°C/12 mm).
4. One to two bubbles per second of [CO<sub>2</sub>](#) are developed.
5. A small sample (ca. 1 mL) is removed from the mixture and extracted with [ethyl ether](#). The [ether](#) solution is analyzed for remaining [ethyl acetoacetate](#) by capillary gas chromatography: 0.3-mm × 20-m glass capillary column Carbowax 20 M, oven

temperature 100°C, carrier gas: [hydrogen](#) (0.4 atm); retention time of [ethyl acetoacetate](#): 450 sec, of [\(S\)-\( + \)-ethyl 3-hydroxybutanoate](#): 610 sec. It is important that all the starting material be consumed. If small mounts of [ethyl acetoacetate](#) are detected, 100 g of [sucrose](#) is added and the mixture is stirred for a further period of 2 days. The checkers detected the presence of residual [ethyl acetoacetate](#) by TLC on 250- $\mu$ m silica gel plates with 1 : 1 [ether](#) : [hexane](#) as eluant. Plates are developed by dipping the dried plate into a solution of 10% [vanillin](#) and 5% [sulfuric acid](#) in 95% [ethanol](#) and then gently warming over a hot plate; [ethyl acetoacetate](#) appears as an intense blue spot with  $R_f$  0.45.

6. In the case of emulsions, addition of [methanol](#) may be helpful. The very fine and stable emulsion that still remains is included with the aqueous phase.

7. The spectral properties of [\(S\)-\( + \)-ethyl 3-hydroxybutanoate](#) are as follows: IR<sup>2a</sup> (film)  $\text{cm}^{-1}$ : 3440, 2980, 1730, 1375, 1300, 1180, 1030; <sup>1</sup>H NMR<sup>2b</sup> ( $\text{CCl}_4$ )  $\delta$ : 1.15 (d, 3 H,  $J = 6.5$ ,  $\text{CH}_3$ ), 1.28 (t, 3 H,  $J = 7$  Hz,  $\text{CH}_3$ ), 2.35 (d, 2 H,  $J = 6.5$ ,  $\text{CH}_2\text{CO}$ ), 3.15 (s, 1 H, OH), 4.05 (q, 2 H,  $J = 7$ ,  $\text{CH}_2\text{O}$ ), 4.15 (m, 1 H, CHOH).

8. This ester should be stored in a refrigerator as there has been some indication that it may undergo a transesterification-oligomerization upon standing at room temperature.

9. The specific rotation  $[\alpha]_D^{25}$  varies from +35.5° to +38° (82–87% enantiomeric excess). The enantiomeric purity can also be checked by formation of the ester with *(R)*-( + )-1-methoxy-1-trifluoromethylphenylacetyl (MTPA) chloride.<sup>2</sup> The <sup>19</sup>F NMR chemical shifts of the diastereomeric esters are 6.13 (*R,R*) and 6.01 (*R,S*) ppm downfield of external [trifluoroacetic acid](#).

10. The procedure of enriching the *(S)*-( + )-enantiomer to 100% enantiomeric excess by the previously described crystallization method is tedious.<sup>3</sup> It provides optically pure [ethyl \(S\)-\( + \)-3-\(3',5'-dinitrobenzoyloxy\)butanoate](#) of  $[\alpha]_D^{25} +26.3^\circ$  ([chloroform](#),  $c$  2), which after cleavage gives enantiomerically pure [\(S\)-\( + \)-ethyl 3-hydroxybutanoate](#) of  $[\alpha]_D^{25} +43.5^\circ$  ([chloroform](#),  $c$  1.0). This optically pure compound has recently become commercially available from Fluka AG, CH-9470 Buchs (Switzerland), but it is very expensive. After submission and checking of this procedure, it was shown<sup>4</sup> that the ee of the product can be increased to >95% by working under aerobic conditions and by adding the keto ester more slowly; see also (Note 11).

11. The analysis of the published procedures for reductions of  $\beta$ -keto esters by baker's yeast indicated<sup>5</sup> that aerobic conditions,<sup>4</sup> the presence of 5–15% [ethanol](#) in the medium,<sup>4,6</sup> and "aging" of the yeast<sup>4</sup> might be important for high selectivity. The optimum conditions—"starving" the yeast for at least 4 days in 5% aqueous [ethanol](#) aerobically—lead to an activation of the enzyme(s)<sup>7</sup> producing the *S*-enantiomer of [ethyl-3-hydroxybutanoate](#).

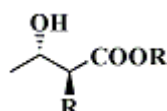
The procedure<sup>5</sup> was as follows. A suspension of 125 g of baker's yeast in 1000 mL of  $\text{H}_2\text{O}/\text{EtOH}$  (95 : 5) was shaken (120 rpm) at 30°C in a 2-L Erlenmeyer flask with indentations for 4 days. After the addition of 5 g (38 mmol) of [ethyl acetoacetate](#) the reaction was followed by GLC. When the reaction had reached completion (2–3 days), the mixture was centrifuged and the supernatant was extracted continuously with [ether](#) (4 days). The organic layer was dried over [magnesium sulfate](#), filtered, and concentrated with a rotary evaporator at 35°C bath temperature. The crude product was purified by bulb to bulb distillation to give [ethyl 3-hydroxybutanoate](#), 3.54 g (70%), as a colorless liquid with an optical purity of 94% e.e. (enantiomeric excess).

### 3. Discussion

[3-Hydroxybutanoic acid](#) in both enantiomeric forms has been obtained by resolution

of the racemic mixture.<sup>8</sup> Hydrogenation of methyl acetoacetate using a Raney nickel catalyst that had been treated with tartaric acid resulted in methyl 3-hydroxybutanoate with an enantiomeric excess of 83–88%.<sup>9</sup> Most recently it was found that enantiomerically pure (*R*-) or (*S*-)-ethyl 3-hydroxybutanoate is available by enantioselective hydrogenation with a chiral homogeneous ruthenium catalyst.<sup>10</sup> Furthermore, optically active 3-hydroxybutanoic acid has been obtained in good chemical and optical yield by condensation of chiral  $\alpha$ -sulfinyl ester enolates with aldehydes followed by desulfurization.<sup>11</sup> (*R*-)(–)-Ethyl 3-hydroxybutanoate in 100% enantiomeric excess resulted from depolymerization of poly-(*R*)-3-hydroxybutanoate, an intracellular storage product of *Alcaligenes eutrophus* H 16.<sup>12</sup> The method presented in the Seebach–Züger paper<sup>12</sup> is easy to perform. The (*S*-)(+)-ethyl 3-hydroxybutanoate obtained may be enriched to 100% enantiomeric excess by crystallization of its 3,5-dinitrobenzoate derivative, followed by alcoholysis.<sup>3</sup>

Optically active ethyl 3-hydroxybutanoate is a very useful chiral building block for natural product synthesis. Some applications are shown in Table 1. Alkylation of doubly deprotonated ethyl 3-hydroxybutanoate gives branched structures of the following type:<sup>13,14</sup>



The yeast reduction is not limited to ethyl acetoacetate. It has been applied to other  $\beta$ -keto esters,  $\alpha$ -keto esters,  $\alpha$ -keto alcohols,  $\alpha$ -keto phosphates, and some ketones (Table II). The reductions show a high degree of stereoselectivity. The absolute configuration of the product obtained by reduction of a carbonyl group containing a large group L and a small group S to the alcohol may be determined by application of Prelog's rule.<sup>15,16</sup>

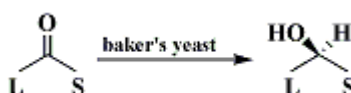
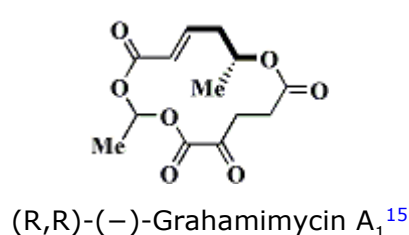
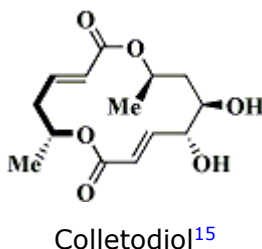
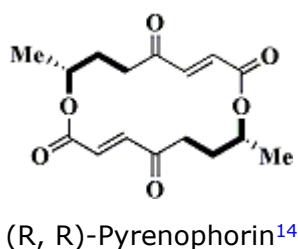
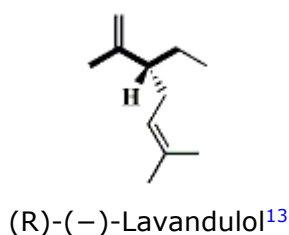
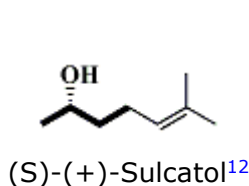
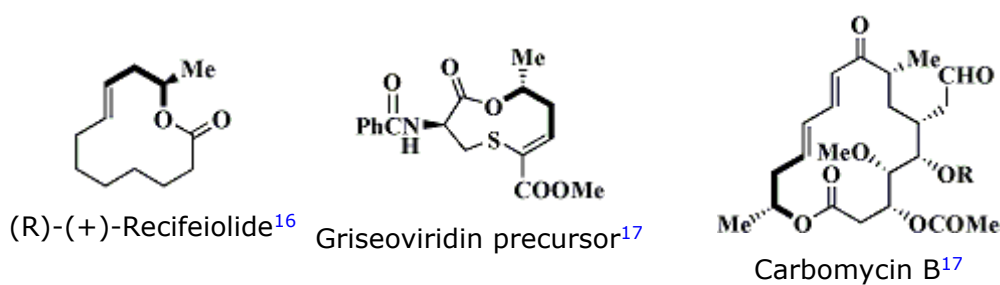


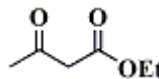
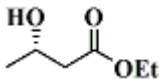
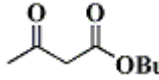
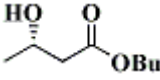
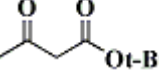
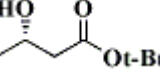
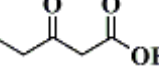
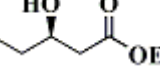
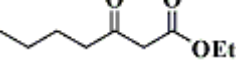
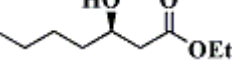
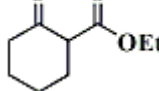
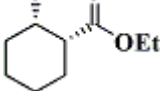
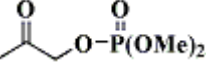
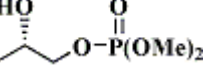
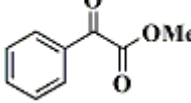
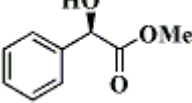
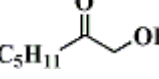
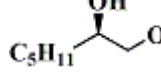
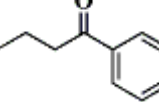
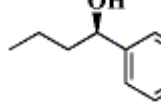
TABLE I  
NATURAL PRODUCTS FROM (*S*)- OR (*R*)-ETHYL 3-HYDROXYBUTANOATE<sup>a</sup>

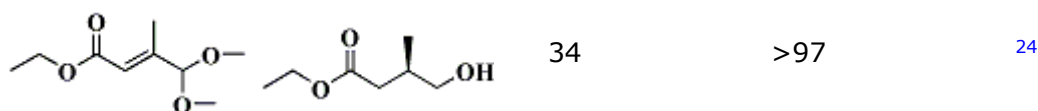




<sup>a</sup>The skeleton of [ethyl 3-hydroxybutanoate](#) is indicated by heavy lines.

TABLE II  
ENANTIOSELECTIVE PREPARATION OF ALCOHOLS FROM THE  
CORRESPONDING KETONE BY YEAST REDUCTION

Substrate	Product	Yield (%)	Enantiomeric Excess (%)	Ref.
		57-67	84-87	<a href="#">18</a> , <a href="#">19</a> , <a href="#">20</a>
		58	90	<a href="#">12</a>
		61	85	<a href="#">12</a>
		67	40	<a href="#">13</a>
			>90	<a href="#">13</a>
		65	86	<a href="#">19</a> , <a href="#">21</a>
		57	74	<a href="#">22</a>
		59	>97	<a href="#">19</a>
		56	100	<a href="#">23</a>
		45	85-87	<a href="#">16</a>



This preparation is referenced from:

- [Org. Syn. Coll. Vol. 8, 332](#)
- [Org. Syn. Coll. Vol. 8, 420](#)
- [Org. Syn. Coll. Vol. 9, 483](#)
- [Org. Syn. Coll. Vol. 9, 589](#)

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31. See the Sadtler Standard Spectra; (a) no. 17507; (b) no. 4253 M.
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## Appendix

### Compounds Referenced (Chemical Abstracts Registry Number)

(R)-( + )-1-methoxy-1-trifluoromethylphenylacetyl (MTPA) chloride  
(R-) or (S-)-ethyl 3-hydroxybutanoate  
(R-)-( - )-Ethyl 3-hydroxybutanoate  
(S)-( + )-Sulcatol  
(R)-(-)-Lavandulol  
(R,R)-(-)-Grahamimycin A<sub>1</sub>  
(R)-( + )-Recifeiolide  
Griseoviridin precursor  
Carbomycin B  
[ethanol](#), [EtOH](#) (64-17-5)  
[sulfuric acid](#) (7664-93-9)  
[methanol](#) (67-56-1)  
[ether](#), [ethyl ether](#) (60-29-7)  
[hydrogen](#) (1333-74-0)  
[chloroform](#) (67-66-3)  
[sodium chloride](#) (7647-14-5)  
[CO<sub>2</sub>](#) (124-38-9)  
[sucrose](#)  
[Raney nickel](#) (7440-02-0)  
[tartaric acid](#) (87-69-4)  
[Ethyl acetoacetate](#) (141-97-9)  
[magnesium sulfate](#) (7487-88-9)  
[vanillin](#) (121-33-5)  
[hexane](#) (110-54-3)  
[Methyl acetoacetate](#) (105-45-3)  
[trifluoroacetic acid](#) (76-05-1)  
[ruthenium](#) (7440-18-8)  
[ethyl-3-hydroxybutanoate](#), [ethyl 3-hydroxybutanoate](#) (5405-41-4)  
[3-Hydroxybutanoic acid](#) (300-85-6)  
[methyl 3-hydroxybutanoate](#)  
[Butanoic acid, 3-hydroxy-, ethyl ester, \(S\), \(S\)-\( + \)-ETHYL 3-HYDROXYBUTANOATE](#) (56816-01-4)  
[ethyl \(S\)-\( + \)-3-\(3',5'-dinitrobenzoyloxy\)butanoate](#)

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