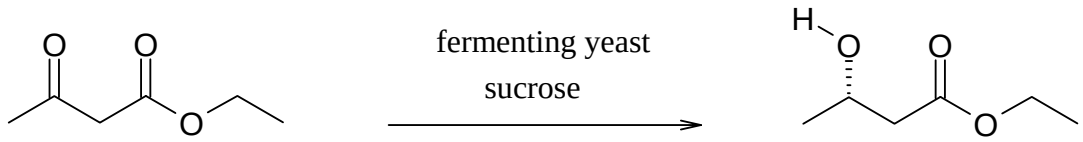
4022 Synthesis of (S)-(+)-3-hydroxybutyric acid ethyl ester



$C_6H_{10}O_3$ (130.1) → $C_{12}H_{22}O_{11}$ (342.3) → $C_6H_{12}O_3$ (132.2)

Classification

Reaction types and substance classes
stereoselektive reduction
ketone, alcohol, enzyme, natural product

Work methods
stirring with KPG stirrer, stirring with magnetic stir bar, adding dropwise with an addition funnel, extracting, evaporating with rotary evaporator, filtering, distilling under reduced pressure, column distillation, vacuum pump, heating with oil bath

for batch scale 10 mmol:
without stirring with KPG stirrer

Instruction (batch scale 10 mmol)

Equipment
250 mL three-neck flask, bubble counter, internal thermometer, heatable magnetic stirrer, magnetic stir bar, addition funnel with pressure balance, suction flask, Buechner funnel (Ø = 5.5 cm), apparatus for continuous extraction of liquids, separating funnel, rotary evaporator, 8 cm Vigreux column or packed column, distillation apparatus, vacuum pump, oil bath

Substances

yeast (baker's yeast))	10 g
D(+)-sucrose (mp 169-170 °C)	16.3 g (47.5 mmol)
acetoacetic acid ethyl ester (bp 180 °C)	1.30 g (1.26 mL, 10.0 mmol)
diethyl ether (bp 35 °C)	80 mL
Celite 545 (filter help)	5 g

Reaction
The reaction apparatus consists of a 250 mL three-neck flask with magnetic stir bar, internal thermometer and addition funnel with pressure balance equipped with a bubble counter filled with paraffin oil. A solution of 9.76 g (28.5 mmol) sucrose in 52 mL fresh tap water, heated to 30 °C, is filled in the flask and 10 g yeast are suspended in the solution under moderate stirring. When the fermentation of the yeast starts, visible at the continuous CO_2-development

(after about one hour), 0.65 g (0.63 mL, 5.0 mmol) acetoacetic acid ethyl ester are added dropwise with an addition funnel and the reaction mixture is stirred for further 24 hours at room temperature. Afterwards a solution of 6.50 g (19.0 mmol) sucrose in 33 mL tap water, heated to 40 °C, is added and stirred for one further hour. Then 0.65 g (0.63 mL, 5.0 mmol) acetoacetic acid ethyl ester are added dropwise, and the suspension is sligthly stirred for further 2 days at room temperature.

Work up

After filtration over a Buechner funnel (Ø = 5.5 cm, Blauband filterpaper), on which about 5 g Celite (filter help) are equally spread, the filtrate is extracted in an apparatus for continuous extraction of liquids with 80 mL diethyl ether for 24 hours. The aqueous phase is separated, from the organic phase the solvent is evaporated at a rotary evaporator at 40 °C. A liquid residue remains as crude product.
Crude yield: 1.01 g

The crude product is fractional distilled under reduced pressure over a short Vigreux or packed column.
Yield: 0.800 g (6.05 mmol, 61%); head temperature 73-74 °C (18 hPa), oil bath temperature 150 °C), colourless liquid; n_D^{20} = 1.4150; $[\alpha]_D^{20}$ = +13 ° (EtOH, c = 1), +38.5 ° ($CHCl_3$,c = 1)

Comments

The substrate should be added only after the fermentation of the yeast has started, which is visible through the steady CO_2-development in the bubble counter. The second addition of substrate can be made also one day later than described above. At low room temperature the fermentation can take more time than given above. A complete reaction is important, since this makes the distillation easier.

The yield can be increased, if the filtered yeast is suspended again in a small amount of water. When the yeast has sedimented, the suspension above the sediment can be filtered and the filtrate can be combined with the other filtrates and can be extracted.

Waste management

Recycling

The evaporated diethyl ether is collected and redistilled.

Waste disposal

Waste	Disposal
filter residue	solid waste, free from mercury
distillation residue	organic solvents, halogen free

Time

5 hours, inclusive 1 hour for filtration
3 days stirring and 24 hours extraction

Break

Before distillation

Degree of difficulty
Medium

Instruction (batch scale 100 mmol)

Equipment

2 L three-neck flask, bubble counter, internal thermometer, KPG-stirrer, heatable magnetic stirrer, magnetic stir bar, addition funnel with pressure balance, Buechner funnel (Ø = 9.5 cm), suction flask, apparatus for continuous extraction of liquids, separating funnel, rotary evaporator, 8 cm Vigreux column or packed column, distillation apparatus, vacuum pump, oil bath

Substances

yeast (baker's yeast)	100 g
D-(+)-sucrose (mp 169-170 °C)	163 g (475 mmol)
acetoacetic acid ethyl ester (bp 180 °C)	13.0 g (12.6 mL, 100 mmol)
diethyl ether (bp 35 °C)	400 mL
(Celite 545)	40 g

Reaction

The reaction apparatus consists of a 2 L three-neck flask with KPG stirrer, internal thermometer and addition funnel with pressure balance equipped with a bubble counter filled with paraffin oil. A solution of 97.6 g (285 mmol) sucrose in 520 mL fresh tap water, heated to 30 °C, is filled in the flask and 100 g yeast are suspended in the solution under moderate stirring. When the fermentation of the yeast starts, visible at the continuous CO_2-development (after about one hour), 6.50 g (6.30 mL, 50.0 mmol) acetoacetic acid ethyl ester are added dropwise with an addition funnel and the reaction mixture is stirred for further 24 hours at room temperature. Afterwards a solution of 65.0 g (190 mmol) sucrose in 325 mL tap water, heated to 40 °C, is added and stirred for one further hour. Then 6.50 g (6.30 mL, 50.0 mmol) acetoacetic acid ethyl ester are added dropwise, and the suspension is sligthly stirred for further 2 days at room temperature.

Work up

The reaction solution is filtered in two charges over a Buechner funnel (Ø = 9.5 cm, Blauband filterpaper), on which at each time about 20 g Celite are equally spread (it is of advantage to filter not before the yeast suspension has sedimented). The filtrate is extracted in an apparatus for continuous extraction of liquids with 400 mL diethyl ether for 24 hours. The aqueous phase is separated, from the organic phase the solvent is evaporated at a rotary evaporator at 40 °C. A liquid residue remains as crude product.
Crude yield: 10.2 g

The crude product is fractional distilled under reduced pressure over a short Vigreux or packed column.

Yield: 8.26 g (62.5 mmol, 63%); head temperature 73-74 °C (18 hPa), oil bath temperature 150 °C), colourless liquid; n_D^{20} = 1.4150; $[\alpha]_D^{20}$ = +13 ° (EtOH, c = 1), +38.8 ° ($CHCl_3$,c = 1)

Comments

The substrate should be added only after the fermentation of the yeast has started, which is visible through the steady CO_2-development in the bubble counter. The second addition of substrate can be made also one day later than described above. At low room temperature the fermentation can take more time than given above. A complete reaction is important, since this makes the distillation easier.

The yield can be increased, if the filtered yeast is suspended again in a small amount of water. When the yeast has sedimented, the suspension above the sediment can be filtered and the filtrate can be combined with the other filtrates and can be extracted.

Waste management

Recycling

The evaporated diethyl ether is collected and redistilled.

Waste disposal

Waste	Disposal
filter residue	solid waste, free from mercury
distillation residue	organic solvents, halogen free

Time

8 hours, inclusive 4 hours for filtration
3 days stirring and 24 hours extraction

Break

Before distillation

Degree of difficulty

Medium

Analytics

Reaction monitoring with TLC

TLC-conditions:	
adsorbant:	TLC-aluminium foil (silica gel 60)
eluent:	*n*-Hexan : diethyl ether = 2:1
visualizing:	After the elution solvent is evaporated, the foil is dipped in a 2% ninhydrine solution and then dried with a hot air dryer. The substances appear as red-violet marks
R_f (product)	0.14
R_f (acetoacetic acid ethyl ester)	0.52

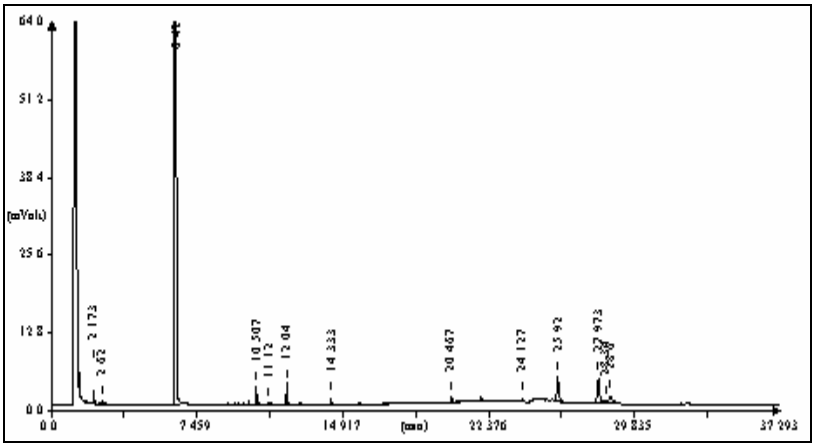
GC

GC-conditions:

column:	DB-1, 28 m, internal diameter 0.32 mm, film 0.25 µm
inlet:	on-column-injection
carrier gas:	hydrogen (40 cm/s)
oven:	50 °C (5 min), 10 °C/min at 240 °C (40 min)
detector:	FID, 270 °C

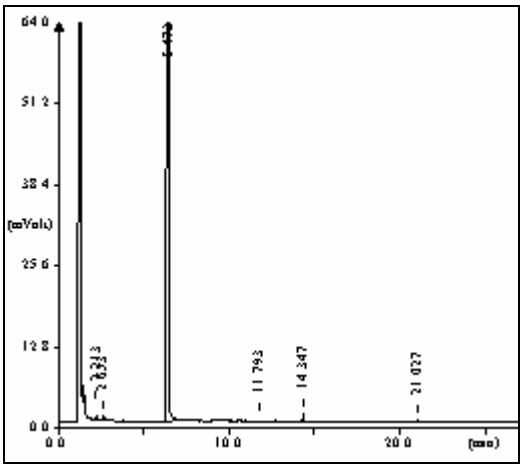
Percent concentration was calculated from peak areas.

GC of the crude product



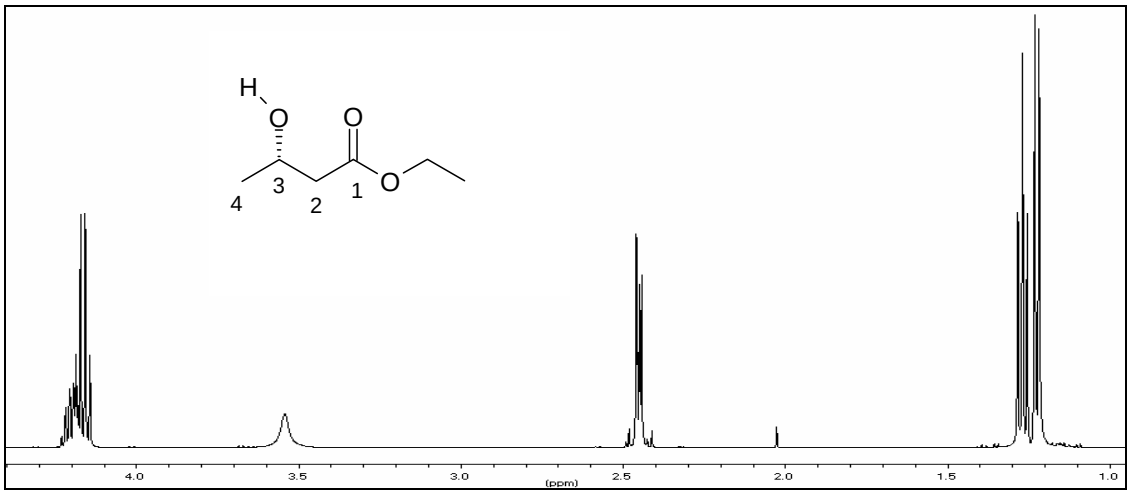
Retention time(min)	Substance	Peak area %
6.42	product	91.7
25.9	side product (unknown)	1.6
28.0	side product (unknown)	2.6

GC of the pure product



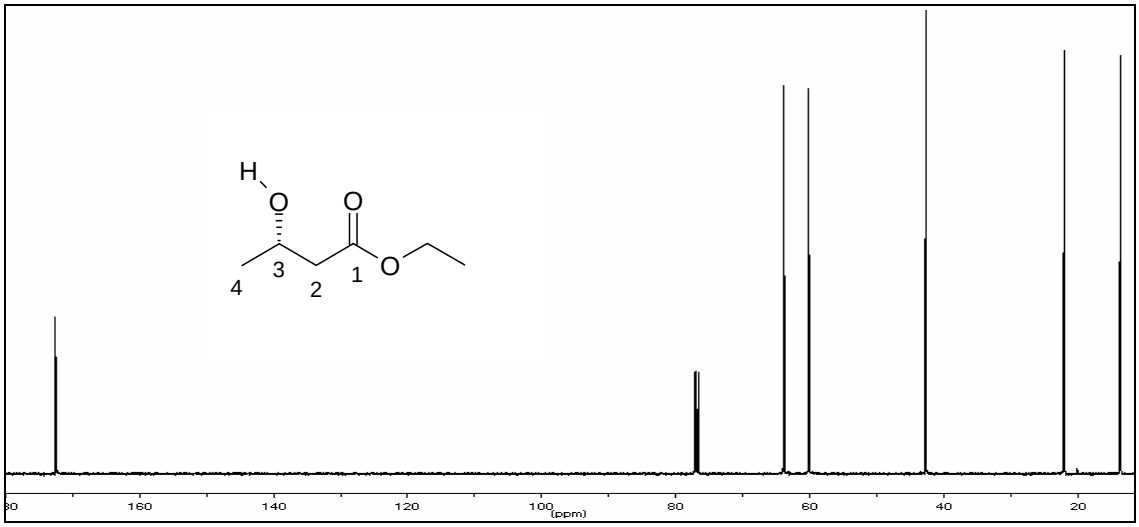
Retention time (min)	Substance	Peak area%
6.47	product	99.2
	other impurities	< 0.2

^{1}H NMR spectrum of the pure product (500 MHz, $CDCl_3$)



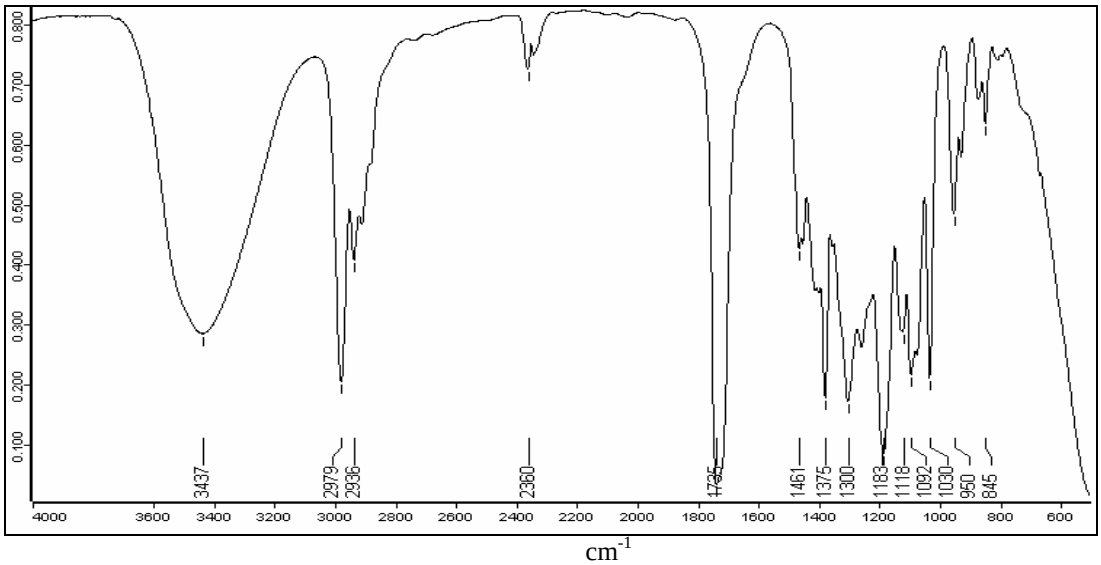
δ (ppm)	Multiplicity	Number of H	Assignment
1.23	D	3	4-H
1.27	t	3	O-$CH_2C\mathbf{H_3}$
2.46	m	2	2-H
3.55	s	1	OH
4.16	q	2	O-$C\mathbf{H_2}CH_3$
4.15 - 4.23	m	1	3-H

^{13}C NMR spectrum of the pure product (125 MHz, $CDCl_3$)



δ (ppm)	Assignment
13.8	$OCH_2\mathbf{C}H_3$
22.3	C-4
42.8	C-2
60.3	O-$\mathbf{C}H_2CH_3$
64.0	C-3
172.4	C-1
76.5-77.5	solvent

IR spectrum of the pure product (film)



(cm^{-1})	**Assignment**
3437	O-H-valence
2979, 2936	C-H-valence, alkane
1735	C=O-valence, ester