Pergamon

Tetrahedron: Asymmetry, Vol. 8, No. 7, pp. 1049–1054, 1997

Printed in Great Britain
PII: S0957-4166(97)00050-5 0957-4166/97 $17.00 + 0.00

The stereoselective preparation of β-hydroxy esters using a yeast reduction in an organic solvent

Caroline Medson, Andrew J. Smallridge * and Maurie A. Trewhella
Department of Chemistry and Biology, Victoria University of Technology, PO Box 14428, MCMC, Melbourne, 8001, Australia

Abstract: A range of (S)-β-hydroxy esters has been prepared in high yield (56–96%) and with very high enantioselectivity (>94%) using a yeast mediated reduction in light petroleum. It was found that the ester functionality had a marked effect on both the isolated yield and the quantity of yeast required to effect complete reduction. © 1997 Elsevier Science Ltd

Introduction

Homochiral β-hydroxy esters can be readily prepared *via* the stereoselective reduction of β-keto esters and have been used as chiral starting materials for the synthesis of a variety of important chemicals including β-lactams,[1] insect pheromones[2] and carotenoids.[3] One of the most widely used means of effecting stereoselective reductions of this type is through the use of the biological catalyst, yeast. A major limitation of this methodology is the difficulty in extracting the product from fermentation broth and consequently often only moderate yields of products are obtained. Recently we have shown that similar reductions are possible using a considerably simpler reaction system involving non-fermenting yeast in an organic solvent.[4,5] North,[6] and more recently Rotthaus *et al.*,[7] have reported the reduction of a variety of keto esters using our reaction protocol although considerably more yeast was employed to achieve complete reduction. We would now like to report our results concerning the reduction of β-keto esters containing differing ester groups utilising our yeast/organic solvent reaction system.

Results

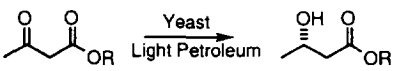
Yeast reduction of β-ketoesters

A series of β-ketoesters, prepared using standard literature procedures,[8] was subjected to yeast mediated reduction using the conditions previously reported to be optimum for the reduction of ethyl acetoacetate **1b**: 1mmol substrate, 1g yeast, 0.8ml water, 50ml light petroleum at room temperature for 24h. For the majority of the ketoesters, 1g of yeast was insufficient to effect complete reduction. Reactions utilising increasing amounts of yeast were therefore conducted to determine the minimum amount of yeast required. Removal of the yeast by filtration followed by washing with ethyl acetate and distillation gave high to near quantitative yields of the (S)-β-hydroxy esters (Table 1). For comparison purposes, results reported for the reduction of the β-ketoesters using an aqueous reaction system with fermenting yeast are also given.

As can be seen, the yield of isolated material varied considerably with the nature of the ester group. The methyl, ethyl, *tert*-butyl and benzyl esters gave good yields, 56–72%. These yields are better than those reported for reduction with fermenting yeast in an aqueous system or in organic solvents; North[6] obtained complete conversion and isolated yields of 15–39%, Rotthaus *et al.*[7] also achieved 100% conversion but did not report isolated yields. Virtually quantitative yields were obtained for the *iso*-propyl, butyl and *sec*-butyl esters. These are considerably higher than reported yields for these

* Corresponding author. Email: AndrewSmallridge@vut.edu.au

Table 1. Yeast mediated reduction of β-ketoesters[9–12]



(1) (2)

	R	Yeast (g/mmol)	Isolated Yield (%)	ee (%)	Aqueous system Yield (ee)
a	Me	1	57	98(*S*)	23 (87)[9]
b	Et	1	69[4]	99(*S*)	76 (85)[10]
c	*i*Pr	2	96	97(*S*)	N/A[b]
d	*n*Bu	3	89	>99(*S*)	58 (90)[11]
e	*t*Bu	11	68	98(*S*)	61 (85)[11]
f	*s*Bu	4	89	97(*S*)[a]	45 (14)[12]
g	Bn	5	72	94(*S*)	N/A[b]

[a] ee at the hydroxy centre, no enantiomeric excess was observed at the ester centre.
[b] not reported using an aqueous reaction system.

esters using fermenting bakers yeast and are among the highest yields reported for a yeast reduction reaction. The reason for the marked variation in the yields is not clear, however it does not appear to be due to absorption of substrate or product onto the yeast since similar isolated yields were obtained using 1g yeast/mmol **1b** and 11g yeast/mmol **1e**. If material was being absorbed onto the yeast it would be expected that the greater the amount of yeast employed the more material would be absorbed and the lower the isolated yield would be. Yeast catalysed ester hydrolysis is also unlikely to be the cause of the difference in isolated yields as no trace of liberated alcohol could be detected in the reaction mixture using gas chromatography.

In all cases the reduction proceeded with a high degree of stereoselectivity (94–99%ee) to give the (S)-β-hydroxy esters. The preferential formation of the (S)-enantiomer is consistent with reported reductions of these esters utilising both aqueous and organic reaction systems although the enantioselectivity achieved is generally higher than that obtained using the other systems.

Yeast reduction of the *sec*-butyl ester **1f** gave high enantioselectivity but showed no evidence of diastereoselectivity. Incomplete reduction (62% conversion by gas chromatography) of *sec*-butyl acetoacetate was achieved using 1g of yeast/mmol, examination of the product using chiral GC also showed no evidence of diastereoselectivity. (S)-*sec*-Butyl acetoacetate did not react with yeast faster than the racemic material and required the same amount of yeast for complete reduction. This indicates that both enantiomers of the β-ketoester were reduced by the yeast at a similar rate which is consistent with reported results for this compound using an aqueous reaction system.[12]

The amount of yeast required to effect complete consumption of starting material (as measured by gas chromatography) increased with the size of the ester group. The smaller methyl and ethyl esters are reduced with just 1g of yeast whilst the more sterically demanding *tert*-butyl group requires 11g. The requirement for increased amounts of yeast strongly suggests that the compounds with the larger ester groups react at a slower rate, possibly due to increased steric interactions with the enzyme binding site. If the requirement for larger quantities of yeast is purely rate related then it would be expected that longer reaction times should allow complete reaction using lesser amounts of yeast. Longer reaction times do not lead to increased conversion due to a significant decrease in yeast reductase activity after exposure to the reaction system for 24h. This can be demonstrated by stirring yeast in light petroleum/water for 24h prior to the addition of substrate (ethyl acetoacetate) and then allowing the reaction to proceed for a further 24h. Very little reduction (13%) is observed in this case compared to complete consumption of substrate if it is added immediately.

Conclusion

The yeast mediated reduction of β-ketoesters in light petrol is a simple procedure for the stereoselective preparation of (S)-3-hydroxy butanoates. By judicious choice of the ester group it is possible to obtain a quantitative yield of enantiomerically pure material. The yields and stereoselectivity obtained using this method are far superior to that reported for reductions using fermenting yeast in an aqueous environment.

Experimental

General

Methyl acetoacetate **1a**, benzyl acetoacetate **1g**, (S)-(+)-2-butanol and 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one were purchased from Sigma Aldrich.

Gas chromatography was performed on a Shimadzu GC-17A with FID. The column was an HP-1 (12m×0.22mm) with a thickness of 0.33mm and a non-polar, crosslinked, methylsiloxane phase. Chiral gas chromatography was performed on the trifluoroacetates of the reduced products as previously described.[4] The chiral column was a Chiraldex G-TA (30m×0.25mm) with a thickness of 0.125mm and a cyclodextrin phase. Bulb to bulb distillations were performed on a Buchi GKR-50. 300MHz ^{1}H n.m.r. were recorded on a Bruker DPX300 instrument and refer to deuteriochloroform solutions with tetramethylsilane as the internal reference (δ=0.00ppm). Optical rotation measurements were carried out on a Optical Activity Polaar 2000-AA series polarimeter.

'Mauripan-Instant Dry Yeast' (*Saccharomyces cerevisiae*) was obtained from Mauri Foods Ltd, Australia and stored at room temperature.

Light petroleum refers to the fraction boiling at 40–60°C.

Preparation of β-keto esters

iso-*Propyl acetoacetate* ***1c*** *(R=iPr)*

2,2,6-Trimethyl-4*H*-1,3-dioxin-4-one (5g, 0.035mol) was added to *iso*-propanol (20ml) with catalytic quantities of *p*-toluene sulfonic acid and refluxed for 4h. Removal of *iso*-propanol under reduced pressure gave a mixture of starting material and product. The product was isolated by forming the copper chelate followed by recrystallisation and regeneration of the desired β-keto ester using the procedure described by White *et al.*[13] Bulb to bulb distillation (100°/20mm) gave *iso*-propyl acetoacetate (0.9g, 17%). lit.[14] b.p. 69°/11mm. ^{1}H n.m.r. δ($CDCl_3$) 1.28, d, *J* 6.2Hz, $(CH_3)_2$; 2.28, s, H4; 3.43, s, H2; 5.08, sept, *J* 6.2Hz, CH. ^{13}C n.m.r. δ 21.08, $(CH_3)_2$; 29.47, C4; 49.88, C2; 68.41, CH; 166.06, C1; 200.19, C3.

tert-*Butyl acetoacetate* ***1e*** *(R=tBu)*

Using a similar procedure 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (5g, 0.035mol) and *tert*-butanol (20ml) gave the title compound after purification using the copper chelate methodology (0.8g, 14%). b.p. 120°/20mm. lit.[15] b.p. 85°/20mm. ^{1}H n.m.r. δ($CDCl_3$) 1.49, s, *t*Bu; 2.27, s, H4; 3.36, s, H2. ^{13}C n.m.r. δ 27.34, *t*Bu; 29.4, C4; 50.91, C2; 80.6, C1′; 165.94, C1; 200.33, C3.

sec-*Butyl acetoacetate* ***1f*** *(R=sBu)*

Using a similar procedure 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (5g, 0.035mol) and 1-methyl propanol (20ml) gave the title compound after purification by distillation (120°/20mm), (3.94g, 72%). ^{1}H n.m.r. δ($CDCl_3$) 0.924, t, *J* 7.5Hz, H3′; 1.25, d, *J* 6.2Hz, H4′; 1.59, m, H2′; 2.29, s, H4; 3.45, s, H2; 4.91, m, H1′. ^{13}C n.m.r. δ 8.9, C3′; 18.7, C4′; 28.2, C2′; 29.4, C4; 49.8, C2; 72.9, C1′; 166.2, C1; 200.0 C3. The n.m.r. data is identical to that previously reported.[12]

*(S)-(+)-*sec*-Butyl acetoacetate* ***1f*** *(R=*s*Bu)*

Using a similar procedure 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (0.9g, 6.3mmol) and (S)-(+)-1-methyl propanol (0.5g, 6.7mmol) gave the title compound after purification by distillation (120°/20mm) (0.4 g, 47%). $[\alpha]_D$ +19.1.

Butyl acetoacetate ***1d*** *(R=Bu)*

Ethyl acetoacetate (15g, 0.125mol) was refluxed in BuOH (50ml) and *p*-toluene sulfonic acid (100mg) for 2h. Removal of the BuOH gave a mixture of butyl acetoacetate and the enol ether. It was not possible to readily separate these compounds so the acetoacetate was used without further purification (4.8g, 27%). lit.[16] b.p. 81°/5mm. ^{1}H n.m.r. $\delta(CDCl_3)$ 0.95, t, *J* 7.3Hz, H4′; 1.41, m, H3′; 1.65, m, H2′; 2.28, s, H4; 3.45, s, H2; 4.16, t, *J* 6.5Hz, H1′. ^{13}C n.m.r. δ 12.97, C4′; 18.41, C3′; 29.43, C2′; 29.89, C4; 49.52, C2; 64.65, C1′; 166.6, C1; 199.9, C3.

General procedure for yeast reduction of β-keto esters

(S)-(+)-Methyl 3-hydroxybutyrate ***2a***

Methyl acetoacetate **1a** (1g, 8.6mmol) was added to a 500ml round-bottom flask with yeast (8.6g), water (6.9ml) and light petroleum (300ml) and stirred at room temperature for 24h. The reaction mixture was filtered and the yeast washed with ethyl acetate (100ml). The solvent was then removed under reduced pressure, and bulb to bulb distillation (b.p. 170°) gave the desired product (0.56g, 57%). lit.[17] b.p. 71–73°/17mm. ^{1}H n.m.r. $\delta(CDCl_3)$ 1.25, d, *J* 6.3Hz, H4; 2.44, dd, *J* 8.4, 16.5Hz, H2; 2.53, dd, *J* 16.5, 3.9Hz, H2; 3.73, s, CH_3; 4.22, m, H3. ^{13}C n.m.r. δ 21.81, C4; 41.93, C2; 51.07, CH_3; 63.63, C2; 172.6, C1. $[\alpha]_D$ +46.89 ($CHCl_3$, c=1), lit.[18] +33.3 ($CHCl_3$, c=1.2). Chiral gas chromatography showed a ratio of 99:1 (98%ee).

iso-*Propyl (S)-(+)-3-hydroxybutyrate* ***2c***

Using a similar procedure 1-methyl ethyl 3-acetoacetate (**1c**) (1g, 6.3mmol) was reacted with yeast (27.4g) and water (22ml). Bulb to bulb distillation (100°/20mm) gave the desired product. (0.95g, 96%). lit.[19] b.p. 78–79°/21mm. ^{1}H n.m.r. $\delta(CDCl_3)$ 1.23, d, *J* 6.3Hz, H4; 1.27, d, *J* 6.3Hz, $(CH_3)_2$; 2.40, dd, *J* 16.2, 8.4Hz, H2; 2.48, dd, *J* 16.2, 3.6, H2; 2.66, s(br), OH; 4.21, m, H3; 5.07, sept, *J* 6.3Hz, CH. ^{13}C n.m.r. δ 21.1, C4; 21.8, $(CH_3)_2$; 42.5, C2; 63.7, C3; 67.5, CH; 171.8, C1. $[\alpha]_D$ +38.89 ($CHCl_3$, c=1). Chiral gas chromatography showed a ratio of 98.4:1.6 (97%ee).

Butyl (S)-(+)-3-hydroxybutyrate ***2d***

Using a similar procedure butyl 3-acetoacetate **1d** (1g, 6.3mmol) was reacted with yeast (18.9g) and water (15.1ml). Bulb to bulb distillation (100°/1mm) followed by radial chromatography (light petroleum:ether, 90:10) gave the desired product (0.9g, 89%). lit.[15] b.p. 81°/5mm. ^{1}H n.m.r. $\delta(CDCl_3)$ 0.94, t, *J* 7.5Hz, H4′; 1.23, d, *J* 6.3Hz, H4; 1.36, m, H3′; 1.62, m, H2′; 2.41, dd, *J* 16.5, 8.4Hz, H2; 2.49, dd, *J* 16.5, 3.6; 2.95, d, *J* 3.9Hz, OH; 4.12, t, *J* 6.6Hz, H1′; 4.18, m, H3. ^{13}C n.m.r. δ 13.0, C4′; 18.5, C3′; 21.8, C4; 30.0, C2′; 42.2, C2; 63.6, C3; 63.9, C1′; 172.3, C1. $[\alpha]_D$ +36.70 ($CHCl_3$, c=1), lit.[20] $[\alpha]_D$ +35.1 (97%ee). Chiral gas chromatography showed only one enantiomer (>99%ee).

tert-*Butyl (S)-(+)-3-hydroxybutyrate* ***2e***

Using a similar procedure *tert*-butyl 3-acetoacetate **1e** (0.6g, 3.8mmol) was reacted with of yeast (42g) and water (33.4ml). Bulb to bulb distillation (100°/1mm) gave the desired product. (0.41g, 68%). lit.[12] b.p. 74–79°/11mm. ^{1}H n.m.r. $\delta(CDCl_3)$ 1.23, d, J 6.3Hz, H4; 1.49, s, *t*Bu; 2.34, dd, *J* 16.2, 8.4, H2; 2.43, dd, *J* 16.2, 3.6Hz, H2; 3.12, s(br), OH; 4.15, m, H3. ^{13}C n.m.r. δ 21.7, C4; 27.5, $(CH_3)_3$; 43.2, C2; 63.7, C3; 80.6, C1′; 171.7, C1. $[\alpha]_D$ +34.05 ($CHCl_3$, c=1), lit.[12] $[\alpha]_D$ +30.8 ($CHCl_3$, c=1). Chiral gas chromatography showed a ratio of 99:1 (98%ee).

sec-*Butyl (S)-(+)-3-hydroxybutyrate 2f*

Using a similar procedure 1-methyl propyl 3-acetoacetate **1f** (1g, 6.3mmol) was reacted with yeast (25.3g) and water (20.2ml). Bulb to bulb distillation (140°/20mm) gave the desired product. (0.9g, 89%). ^{1}H n.m.r. δ($CDCl_3$) 0.92, t, *J* 7.5Hz, H3′; 1.240, dd, *J* 6.3Hz, H4′ (RS); 1.242, d, *J* 6.3Hz, H4′ (SS); 1.245, d, *J* 6.3Hz, H4; 1.6, m, H2′; 2.409, dd, *J* 16.2, 5.4Hz, H2 (RS); 2.413, dd, *J* 16.2, 5.4Hz, H2 (SS); 2.499, dd, *J* 16.2, 3.9Hz, H2 (SS); 2.501, dd, *J* 16.2, 3.9Hz, H2 (RS); 3.027, s(br), OH; 4.2, m, H3; 4.9, sextet, *J* 6.3Hz, H1′. ^{13}C n.m.r. δ 9.0, C3′; 18.8, C4′; 21.8, C4; 28.1, C2′; 42.4, C2; 63.7, C3; 72.1, C1′; 172.0, C1. $[\alpha]_D$ +31.11, ($CHCl_3$, c=1), lit.[12] $[\alpha]_D$ +13.7, ($CHCl_3$, c=1). Chiral gas chromatography showed a ratio of 98.7:1.3 (97%ee).

(S)-sec-*Butyl (S)-(+)-3-hydroxybutyrate 2f*

Using a similar procedure (S)-*sec*-butyl 3-acetoacetate **1f** (0.158g, 1mmol) was reacted with yeast (4g) and water (3.2ml). ^{1}H n.m.r. δ($CDCl_3$) 0.92, t, *J* 7.5Hz, H3′; 1.242, d, *J* 6.3Hz, H4′; 1.245, d, *J* 6.3Hz, H4; 1.6, m, H2′; 2.413, dd, *J* 16.2, 5.4Hz, H2; 2.499, dd, *J* 16.2, 3.9Hz, H2; 3.027, s(br), OH; 4.2, m, H3; 4.9, sextet, *J* 6.3Hz, H1′. ^{13}C n.m.r. δ 9.0, C3′; 18.8, C4′; 21.8, C4; 28.1, C2′; 42.4, C2; 63.7, C3; 72.1, C1′; 172.0, C1. $[\alpha]_D$ +52.3, ($CHCl_3$, c=1). Chiral gas chromatography showed a ratio of 98:2 (96%ee).

Benzyl (S)-(+)-3-hydroxybutyrate 2g

Using a similar procedure benzyl 3-acetoacetate **1g** (1g, 5.2mmol) was reacted with yeast (26g) and water (28.8ml). Bulb to bulb distillation (100°/1mm) gave the desired product. (0.72g, 72%). ^{1}H n.m.r. δ($CDCl_3$) 1.25, d, *J* 6.3Hz, H4; 2.49, dd, *J* 16.8, 8.4Hz, H2; 2.58, dd, *J* 16.8, 3.6Hz, H2; 4.24, m, H3; 5.18, s, H1′; 7.38, s, Ph. ^{13}C n.m.r. δ 21.9, C4; 42.3, C2; 63.7, C3; 65.9, C1′; 127.6, 128.0, (*o, m*); 127.8 (*p*); 135.0, (*i*); 172.0, C1. The n.m.r. data is identical to that previously reported.[21] $[\alpha]_D$ +29.0 ($CHCl_3$, c=1). Chiral gas chromatography showed a ratio of 97:3 (94%ee).

Acknowledgements

The authors wish to thank Mauri Foods Ltd for providing the yeast samples and assistance with technical support. We would also like to acknowledge the financial support of Polychip Pharmaceuticals Pty. Ltd. and the Victorian Education Foundation.

References

1. Tsachen, D. M., Fuentes, L. M., Lynch, J. E., Laswell, W. L., Volante, R. P., Shinkai, I., *Tetrahedron Letters*, 1988, **29**, 2779.
2. Mori, K., *Tetrahedron*, 1989, **45**, 3233.
3. Kramer, A., Pfader, H., *Helv. Chim. Acta*, 1982, **65**, 293.
4. Jayasinghe. L. Y., Kodituwakku, D., Smallridge, A. J., Trewhella, M. A., *Bull. Chem. Soc. Jpn.*, 1994, **67**, 2528.
5. Bak, R. A., Mc Anda, A. F., Smallridge, A. J., Trewhella, M. A., *Aust. J. Chem.*, 1996, **49**, 1257.
6. North, M., *Tetrahedron Letters*, 1996, **37**, 1699.
7. Rotthaus, O., Kruger, D., Demuth, M., Schnaffner, K., *Tetrahedron*, 1997, **53**, 935.
8. Carroll, M. F., Bader, A. R., *J. Am. Chem. Soc.*, 1953, **75**, 5400.
9. Csuk, R., Glanzer, B. I., *Chem. Rev.*, 1991, **91**, 49; Muller, R., Lingens, F., *Angew. Chem. Int. Ed. Engl.*, 1986, **25**, 779.
10. Seebach, D., Sutter, M. A., Weber, R. H., *Org. Synth.*, 1984, **63**, 1.
11. Seebach, D., Zuger, M., *Helv. Chim. Acta.*, 1982, **65**, 495.
12. Hudlicky, T., Tsunoda, T., Gadamasetti, K. G., Murry, J. A., Keck, G. E., *J. Org. Chem.*, 1991, **56**, 3619.
13. White, W. L., Anzeveno, P. B., Johnson, F., *J. Org. Chem.*, 1992, **47**, 2379.
14. Triebs, A., Hintermeier, K., *Chem. Ber.*, 1956, **87**, 1167.

15. Lawesson, S., Gronwall, S., Sandberg, R., *Org. Synth., Coll. Vol. 5*, 1973, 155.
16. Ojima, I., Kogure, T., Kumagai, M., *J. Org. Chem.*, 1977, **42**, 1673.
17. Levene, P. A., Haller, H. L., *J. Biol. Chem.*, 1926, **69**, 165.
18. Lemieux, R. U., Giguere, J., *Can. J. Chem.*, 1951, **29**, 678.
19. Tai, A., Harada, T., Hiraki, Y., Murakami, S., *Bull. Chem. Soc. Jpn.*, 1983, **56**, 1414.
20. Spilliotis, V., Papahatjis, D., Ragoussis, N., *Tetrahedron Letters*, 1990, **31**, 1615.
21. Schmidt, G. H., Reiner, J., Suss-Fink, G., *J. Organomet. Chem.*, 1988, **355**, 379.

(*Received in UK 8 January 1997*)