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THE SYNTHESIS OF ISOTOPICALLY LABELLED N-ACETYLCYSTEAMINE THIOESTERS UTILISING A BAKER'S YEAST REDUCTION IN D_2O

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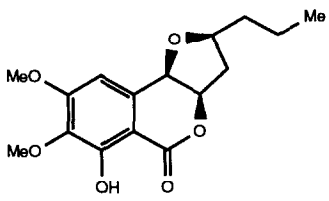
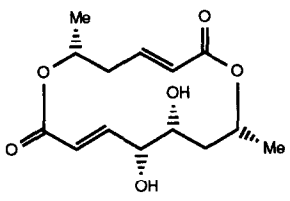
Abstract: Bakers' yeast reduction of ethyl acetoacetate and ethyl 3-oxo-hexanoate in D_2O gives the corresponding β-hydroxy esters bearing an isotopic label at the activated methylene position, in good yield and high enantiomeric excess. These compounds have been converted to their N-acetylcysteamine thioesters for use in biosynthetic studies.

Isotopically labelled N-acetylcysteamine thioesters are much used probes for studying the fatty acid and polyketide biosynthetic pathways.[1] In connection with studies on polyketide chain assembly processes in the biosynthesis of the metabolites monocerin **(1)**, colletodiol **(2)**, and compactin **(3)**, we required N-acetylcysteamine thioesters of (*S*)-3-hydroxybutyrate **(4)** and



(1)



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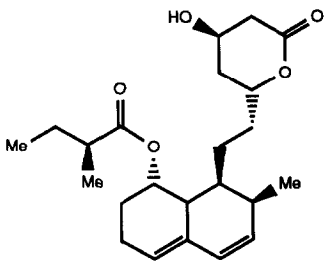
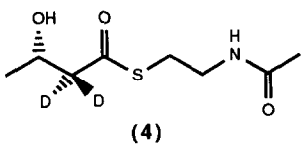


(3)

(*R*)-3-hydroxy hexanoate **(5)** in isotopically labelled form. An obvious route to the unlabelled β-hydroxy esters was *via* Bakers' yeast (*Saccharomyces cerevisiae*) reduction of the corresponding β-keto esters.[2]



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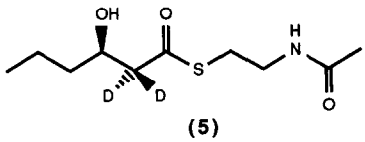
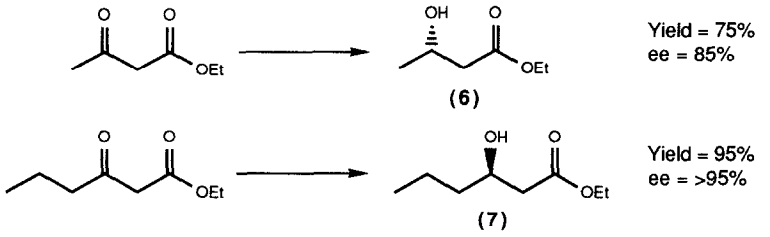


(5)

Bakers' yeast reductions of β-keto esters is well known[3] to give β-hydroxy esters in variable yields and optical purities. Work in our laboratory using *S. cerevisiae*[4,5] has shown that ethyl acetoacetate and ethyl 3-oxo-hexanoate can be reduced to (*S*)-ethyl 3-hydroxybutyrate **(6)**[2] and (*R*)-ethyl 3-hydroxyhexanoate **(7)**[6], respectively, in reproducibly good yield; furthermore, the products are formed with high enantiomeric excess[7] (**Scheme 1**). Our aim was to utilize this method to synthesize **(6)** and **(7)** incorporating an isotopic label at a defined position.

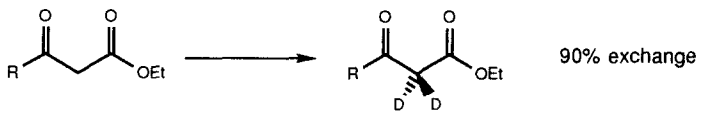
Scheme 1



S. cerevisiae, H_2O, Glucose, 48hr., 30°C, 250rpm.

Preliminary investigations suggested that introduction of ^2H label into the β-keto ester precursors is readily achieved by stirring the substrate in D_2O at room temperature, this procedure leading to 90% exchange of ^1H for ^2H at the doubly activated methylene position, as shown by ^1H nmr (**Scheme 2**). No exchange was observed at the singly activated position[8] as determined by ^1H (270MHz) and ^2H (61.37MHz) NMR. Subsequent reaction of these substrates under the previously mentioned conditions[5] gave the corresponding ethyl 3-hydroxy esters with complete loss of the isotopic label. As no ^2H label is retained in the product, it is reasonable to infer that the rate of the enzymatic reduction must be slower than that of the exchange reaction with the medium.

Scheme 2



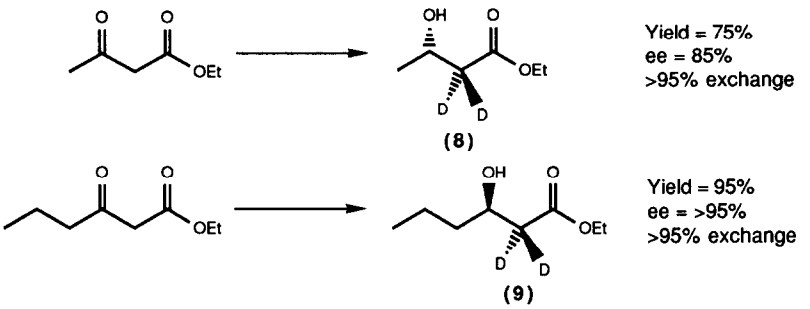
D_2O, r.t., 12hr.

Therefore, we next examined the reaction of protiated β-keto esters with *S.cerevisiae* using D_2O as solvent in the hope that both exchange with solvent and the desired reduction would be carried to completion. Although the use of Bakers' yeast as a reagent in organic synthesis has been recorded from the beginning of the century[9] no literature precedent appears to exist for performing the reaction in D_2O.

Gratifyingly, we observed that the reaction with *S.cerevisiae* of protiated keto esters in D_2O as solvent gave >95% ^2H incorporation at C-2 giving ^2H labelled ethyl 3-hydroxy esters[10] **(8)-(9)** (**Scheme 3**). The yields and optical purities were identical to those seen in the reaction using

protiated solvent. A small amount of exchange (approximately 20%) was also observed at C-3 presumably arising from exchange between NAD(P)H and solvent, leading to transfer of deuteride in the reduction reaction.

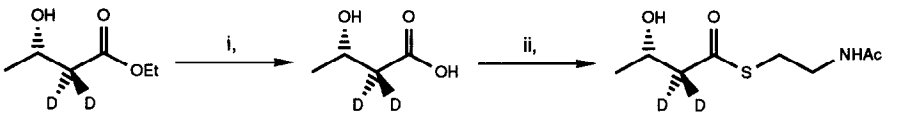
Scheme 3



S. cerevisiae, D_2O, Glucose, 48hr., 30°C, 250rpm.

The ^2H labelled compounds could be converted to the corresponding thioester intermediates by ester hydrolysis and coupling with N-acetylcysteamine[11,12], **Scheme 4**, giving the deuterium labelled compounds[13] required for biosynthetic studies. Experiments towards the synthesis of other isotopically labelled thioesters and their use in feeding studies are currently in progress.

Scheme 4



i, 1M NaOH, r.t., 24hr.
ii, DCC, DMAP, N-acetylcysteamine, CH_2Cl_2

We believe that this process allows for incorporation of ^2H label into the many substrates known to undergo Bakers' Yeast reduction and therefore represents a synthetic process of some utility.

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Sedgewick B., Cornforth J.W., French S.J., Gray R.T., Kelstrup E. and Willadson P., *Eur. J. Biochem.*, 1977, **75**, 481.

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3. For a review of the use of Baker's yeast in organic synthesis see: Servi S., *Synthesis*, 1990, **1**,1.
4. Pure dried strain commercially available from The Distiller's Company (Yeast) Limited, Head Office: Collingwood House, Sutton, Surrey, SM3 9AT, U.K.
5. Experimental procedure - Glucose (20g) was dissolved in water (100ml). Dried yeast (10g) was added to this solution and the mixture incubated at 35°C with gentle shaking for 1 hour. Substrate (1g) was added to the resulting suspension and the mixture shaken (250rpm) for 48 hours at 30°C. Celite (10g) was added and the mixture filtered. The aqueous solution was extracted with diethyl ether (4x50ml), the combined organic extracts were washed with brine (2x25ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure yielding a pale yellow oil. The products were then isolated by Kugelrohr distillation.
6. Crump D.R., *Aust. J. Chem.*, 1982, **35**, 1945.
7. The enantiomeric excess of all products was determined by ^{19}F NMR (90 MHz) and capillary G.C. (Perkin Elmer 8500, column SGE BPX70, 25QC3, using He as carrier gas) analysis of the corresponding Mosher's ester derivatives. (Dale J.A., Dull D.L. and Mosher H.S., *J. Org. Chem.*, 1969, **34**, 2543.
8. No incorporation of label into the ethyl 3-hydroxy esters is observed under these conditions.
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   Neuberg C. and Hirsch J., *Biochem. Z.*, 1921, **115**, 282.
10. **8.** ^1H NMR ($CDCl_3$, 270MHz): δ 1.21 (d, 3H, J=6Hz, $C\underline{H}_3CH$), 1.27 (t, 3H, J=7Hz, $C\underline{H}_3CH_2O$), 4.16 (q, 2H, J=7Hz, $CH_3C\underline{H}_2O$), 4.16 (q, 1H, J=6Hz, $C\underline{H}OH$), 4.57 (s, 0.5H, $O\underline{H}$).
    ^2H NMR ($CDCl_3$, 61.37MHz): δ 2.39 (s, 2D, $COC\underline{D}_2$), 3.92 (s, 0.5D, $O\underline{D}$).
    9. ^1H NMR ($CDCl_3$, 270MHz): δ 0.92 (t, 3H, J=7Hz, $C\underline{H}_3CH_2CH_2$), 1.26 (t, 3H, J=7Hz, $C\underline{H}_3CH_2O$), 1.34-1.54 (m, 4H, $CH_3C\underline{H}_2C\underline{H}_2$), 3.42 (s, 0.5H, $O\underline{H}$), 4.01 (m, 0.8H, $C\underline{H}OH$), 4.16 (q, 2H, J=7Hz, $CH_3C\underline{H}_2O$).
    ^2H NMR ($CDCl_3$, 61.37MHz): δ 2.30 (s, 1D, $COC\underline{D}_2$), 2.40 (s, 1D, $COC\underline{D}_2$), 3.40 (s, 0.5D, $O\underline{D}$), 3.95 (s, 0.2D, $C\underline{D}OH$).
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12. Steglich W. and Neises B., *Angew. Chem., Int. Ed. Engl.* 1978, **17**, 522.
13. All spectral and analytical data for the compounds were consistent with their expected structures.