Tetrahedron Letters,Vol.26,No.1,pp 101-104, 1985 0040-4039/85 $3.00 + .00
Printed in Great Britain

ON THE STERIC COURSE OF BAKER'S YEAST MEDIATED REDUCTION OF ALKYL 4-AZIDO- AND 4-BROMO-3-OXOBUTYRATE. SYNTHESIS OF (R)- AND (S)-CARNITIN

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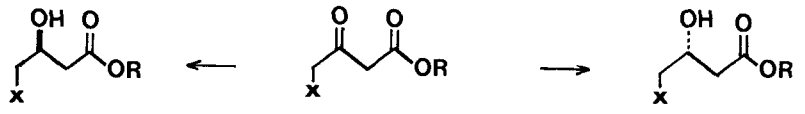
Summary. Baker's yeast reduction of ethyl 4-azido- and 4-bromo-3-oxobutyrate affords (3R) (8) and (3S) (2), respectively, in high optical purity.

Substrate specificity and reaction stereospecificity are the two major problems faced with the use of microbial transformations of non-conventional substrates as sources of chiral educts. A current example[1] is represented by the baker's yeast mediated conversion of alkyl 3-oxoalkanoates into the corresponding 3-hydroxy derivatives, where a variety of substituents at positions 2 and/or 4 of the substrates are tolerated by the enzymic system(s) involved. The absolute configuration and the optical purity of the reduced products depend strongly upon the nature of the 4-substituents and of the ester moiety and, in some instances, upon the substrate concentration. Indeed, whereas ethyl 3-oxobutyrate is reduced to the (3S) alcohol of high optical purity,[2] ethyl 3-oxo-4-chlorobutyrate affords a reduced material containing an excess of the enantiomer (1).[3] Increase of the lenght of the n-alkyl chain of the 3-oxo-4-chlorobutyrate causes inversion of the absolute configuration of the reduced products, optically pure (14) being obtained from the n-octyl ester.

These results have been explained by invoking the participation within the reduction process of two or more different enzymes acting with opposite stereochemistry. The preferred substrates for the enzymes leading to R-type products are those with a large hydrophobic substituent at position 4, whereas enzymes yielding S-type educts should prefer substrates bearing large hydrophobic ester moieties (Scheme).[1]



R-type S-type

Scheme

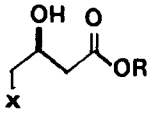
In this context, we report now on the steric course of the baker's yeast mediated reduction of alkyl 3-oxo-4-azidobutyrate showing that, irrespective of the lenght of the ester moiety, S-type reduction product are formed.

Thus, the 3-oxo-4-azido esters (3)-(7) prepared from the corresponding 3-oxo-4-bromo derivatives by azide displacement afforded, on yeast treatment, products (8)-(12) in 70-80% isolated yield. Typically, 0.4 mole of substrate in ethanol are added dropwise to 2.5 kg of commercial baker's yeast in 10 L tap water containing 1 Kg D-glucose at pH 7.5-8 and 25-30°C. The ee values[1] for compounds (8)-(12) (Table) were determined by ^{1}H-n.m.r. studies on the O-acetyl derivatives in the presence of tris [3-(heptafluoropropylhydroxymethylene)-d-camphorato] europium (III). The (3R) absolute configuration was determined as follows. Basic hydrolysis of the 3-hydroxy-4-azidoesters (8)-(12) afforded 3-hydroxy-4-azidobutyric acid which was converted directly upon hydrogenation (PtO_2, acetic acid) into (3R) 3-hydroxy-4-aminobutyric acid (13). Comparison of its optical properties with that of an authentic sample (Table) indicated the absolute configuration.

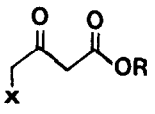
Table: ee values for products (8)-(12) and $[\alpha]_D^{20}$ of derived (13)[a,b]

	(8)	(9)	(10)	(11)	(12)
ee	0.8	0.95	0.95	~1	~1
$[\alpha]_D^{20}$	-16°[c]	-20.4°	-19.7°	-21.4°	-21°

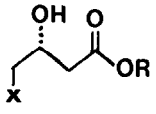
[a] lit.[4] -21°; [b] C 2, water; [c] recryst. material



(1) X = Cl; R = C_2H_5
(2) X = Br; R = C_2H_5



(3) X = N_3; R = C_2H_5
(4) X = N_3; R = CH_2Ph
(5) X = N_3; R = $(CH_2)_2Ph$
(6) X = N_3; R = $(CH_2)_6CH_3$
(7) X = N_3; R = $(CH_2)_7CH_3$



(8) X = N_3; R = C_2H_5
(9) X = N_3; R = CH_2Ph
(10) X = N_3; R = $(CH_2)_2Ph$
(11) X = N_3; R = $(CH_2)_6CH_3$
(12) X = N_3; R = $(CH_2)_7CH_3$
(13) X = NH_2; R = H
(14) X = Cl; R = $(CH_2)_7CH_3$

The significance of the results obtained in the reduction of ethyl 3-oxo-4-azidobutyrate (3) is enhanced by the fact that ethyl 3-oxo-4-bromobutyrate, on yeast treatment at pH 8, afforded

in 40-50% yield a reduction product showing $[\alpha]_D^{20}$ -11° (c 1, EtOH). The latter material was enantiomerically pure on the basis of ^{1}H-n.m.r. studies on the O-acetyl derivative in the presence of the above chiral shift reagent. The (3S) absolute configuration depicted in (2) was assigned to the above product because of its conversion, on NMe_3 treatment, into (S) carnitin with $[\alpha]_D^{20}$ +30.6° (c 2, H_2O). When the yeast reduction of ethyl 3-oxo-4-bromo butyrate was carried out at pH 6 the ee value[1] of (2) was ca. 0.7. However, the 4-bromo analogs of the esters (4)-(7) afforded, on yeast reduction, the corresponding (3R) alcohols in enantiomerically pure forms, as indicated by their conversion into (R) carnitin and ^{1}H-n.m.r. studies on the O-acetyl derivatives.

Finally, we submitted ethyl 4-bromo and 4-azido-3-oxobutyrate to the action of available strains[5] of Aspergillus niger (IPV 283 and CBS 102.12), which are known to reduce ethyl 3-oxobutyrate to the alcohols of opposite stereochemistry. From the above substrates, in the two series of experiments, we obtained ethyl (3S) 4-bromo-3-hydroxybutyrate and ethyl (3R) 4-azido-3-hydroxybutyrate, respectively. Similarly, Beauveria bassiana, Epicoccum nigrum and Geotrichum candidum (CBS 233.76 and 109.12[5]) reduced ethyl 3-oxo-4-bromobutyrate to (2) with ca. 0.75, 0.8 and 0.9 ee., respectively.

The present observations thus lend support to the above mentioned idea[1] that in the microbial transformations leading to the reduction of carbonyl compounds, using intact cells, the steric outcome is the result of the conflicting action of different enzymes operating on the same substrate with different stereochemistry, the preference of each enzyme being strongly affected by subtle substrate modifications. In the present case, it seems that ethyl 3-oxo-4-azidobutyrate, at variance with other substrates bearing at position 4 a large hydrophobic substituent and an ethyl ester moiety, is a rather 'poor' substrate for R-type enzymes. Furthermore, the recent observation that Thermoanaerobium brockii[6] reduces methyl 3-oxo-4-chlorobutyrate to the (3R) alcohol with high optical purity should stimulate screening of different microorganisms for R- and S-type reducing enzymes as an entry to the two enantiomeric forms of a synthetically useful chiral educt.

We thank dr. Rosanna Cardillo for the microbial transformations.

This work has been financially supported by Piano Finalizzato CNR Chimica Fine e Secondaria.

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(Received in UK 11 September 1984)