Tetrahedron Letters,Vol.28,No.42,pp 5033-5036,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

NEW CHIRAL BUILDING BLOCKS BY MICROBIAL ASYMMETRIC REDUCTION: A DIRECT ACCESS TO FUNCTIONALIZED 2R,3R- AND 2S,3R-2-METHYL-3-HYDROXY BUTYRATE SYNTHONS[1]

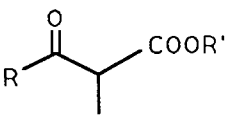
Didier BUISSON[a], Serge HENROT[b], Marc LARCHEVEQUE[b,*] and Robert AZERAD[a,*]

[a]Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques associé au CNRS, Université R. Descartes, 45 rue des Saints Pères, 75270-Paris Cedex 06, FRANCE

[b]Laboratoire de Chimie de l'Ecole Normale Supérieure, ER 12 du CNRS, 24 rue Lhomond, 75231 PARIS Cedex 05,FRANCE

Abstract: *The reduction of 4-O-benzyl-2-methyl-3-oxo-butyrate esters by several fungal strains produces predominantly (2S,3R)-anti-hydroxyesters in high optical purity. The corresponding (2R,3R)-syn isomer was obtained, with other strains, only in mixture with the anti isomer. These esters are useful precursors for chiral 2-methyl-3-hydroxy-butyrolactone synthons.*

Functionalized 2-methyl-3-hydroxy butyric acids or esters of definite stereochemistry constitute useful building blocks for the synthesis of polyhydroxylated methylated natural products biosynthetically derived from polypropionate-polyacetate pathways. The direct microbial reduction of 2-methyl-3-oxosuccinate esters **1a** by various yeast strains has been described to give generally (2R,3R)- and (2S,3R)-isomer mixtures, which are difficult to separate, in variable optical purity[2,3]. Several attempts have been made to differentiate the two ester functions and to replace the 4-carboalkoxy function by a group considered as a carboxylic group (or other functional group) equivalent. Benzylidene (**1b**)[4], furyl (**1c**)[5] and thienyl (**1d**)[6] substituted compounds have been tested as microbial reduction substrates, with moderate success : in most cases, notwithstanding the easy enolization of such esters, mixtures of diastereoisomers were obtained, indicating a low selectivity towards the enantiomers of the keto-substrate. Moreover , despite the easier separation of such isomers, the ozonolysis procedure required for the conversion to a carboxylic acid rendered this method difficult in practice[3]. One attempt to use enantioselective hydrolysis of the corresponding racemic 3-acetoxy esters by lipases has been recently described[7].



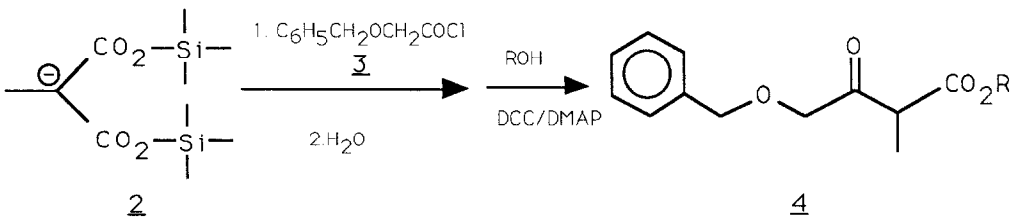
1a : R = COOMe or COOEt; R' = Me or Et

1b : R = CH=CH-C6H5 ; R' = Et

1c : R =  R' = Me

1d : R' =  R' = Me

4-O-Benzyl-2-methyl-3-oxoesters such as **4a-e** were easily obtained by condensation of the acid chloride **3** with the anion of the methylmalonic acid disilyl ester **2**[8], followed by hydrolysis and carbodiimide esterification with various alcohols (Scheme 1).



2 4

Scheme 1

4a: R= CH_3 **4d**: R= n-C_5H_{11}
4b: R= C_2H_5 **4e**: R= n-C_8H_{17}
4c: R= i-C_3H_7

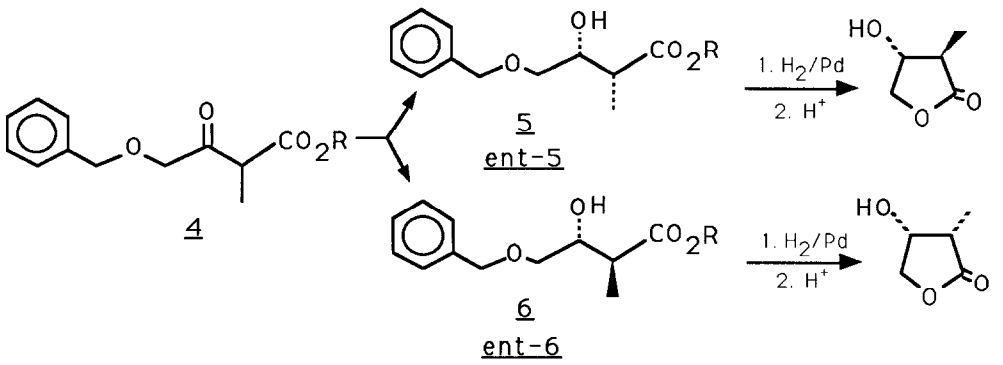
The reduction of the ethyl ester **4b** by yeasts or fungi precedently used for the diastereoselective reduction of simpler α-methyl-β-oxoesters[9] gave moderate yields of reduced product, probably because of

Table 1: Microbial reduction of 4-O-benzyl-2-methyl-3-oxobutyrate esters **4**

		Yield	syn/anti	syn		anti	
	Ester	%	ratio	config.	ee%	config.	ee%
1- Saccharomyces cerevisiae[c]	**4a**	50	10/90	2R,3R[a]	68	2S,3R[b]	6
	4b	46	54/46	2R,3R	36	2R,3S	24
	4c	64	24/76	2R,3R	96	2R,3S	50
2- Rhodotorula mucilaginosa[d,e]	**4b**	-	30/70	2R,3R	98	2S,3R	18
	4c	75	43/57	2R,3R	97	2S,3R	86
	4d	20	80/20	-	-	-	-
3- Curvularia lunata[d,f]	**4b**	20	5/95	-	-	2S,3R	96
	4c	52	31/69	2R,3R	80	2S,3R	92
	4e	no reduction					
4- Geotrichum candidum[d,e]	**4a**	66	2/98	2R,3R	60	2S,3R	96
	4b	70	2/98	-	-	2S,3R	90
	4d	50	4/96	-	-	2S,3R	82
	4e	no reduction					
5- Mucor racemosus[d,e]	**4a**	50	40/60	2R,3R	42	2R,3S	6
	4b	40	48/52	2R,3R	90	2R,3S	7
6- Rhizopus arrhizus[d,g]	**4b**	-	4/96	-	-	2S,3R	81

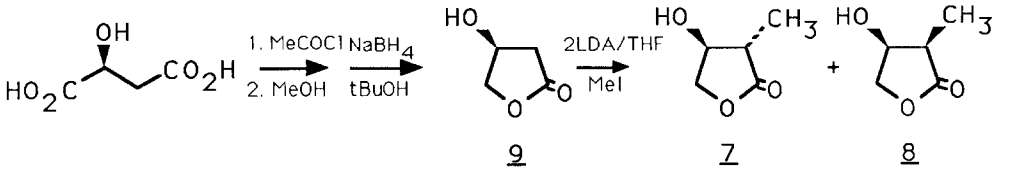
a) 2R,3R = **5**; b) 2S,3R = **6**; c) Baker's yeast (5.5 g) incubated with ester (250 mg) and saccharose (3 g) in water (100 ml) during 2-3 days at 30°C ; d) cells or mycelium freshly grown in complex culture medium (100 ml)[9], then added with ester (100 mg) and incubated at 27°C until complete reduction (2-4 days) ; e) local strain ; f)NRRL 2380 ; g) ATCC 11145.

some hydrolytic activity of the cells. Moreover the ratio and optical purity of the recovered syn and anti hydroxyesters **5b** (and/or ent-**5b**) and **6b** (and/or ent-**6b**)[10] were dependent on the microorganism (Table 1). These isomers were easily separated by column chromatography[11] and converted to trans and cis lactones by debenzylation (H_2/Pd) followed by cyclization (CF_3COOH/CH_2Cl_2) (Scheme 2).



Scheme 2

The configuration of these lactones[12] was determined by comparison with authentic samples of (2S,3S)-lactone 7 and (2R,3S)-lactone 8, either directly by GC on a chiral column [13] or after derivatization with MTPA[14] and HPLC separation (Fig.1). These lactones have been obtained in a 98:2 ratio by monoalkylation [15] of the dianion of the lactone 9 (Scheme 3) and separated by medium pressure chromatography[16] .



Scheme 3

Some selected fungal strains are highly enantioselective, producing high amounts of the single (2S,3R)-anti-isomer 6b, generally in a good optical purity (Table 1, entries 3, 4 and 6) ; this hydroxyester has been converted to the ent-8 lactone. This method is thus complementary to the known alkylation of lactones like 9 [15a] to trans lactones like 7, which have been used as intermediates in the synthesis of syn α-substituted β-hydroxyesters[15b]; the microbial method compares advantageously with the alkylation of 3-hydroxyesters, which has been currently used for the preparation of optically active anti α-substituted β-hydroxyesters[17,18]. However, none of our strains was able to produce a prevailing syn-isomer, nor substantially enriched (3S)-enantiomers.

The effect of a modification of the ester group upon the stereoselectivity of the reduction was investigated (Table 1). In some cases, specially with yeasts, some increase in the enantioselectivity of the carbonyl group reduction was observed with higher esters 4c-d. On the other hand, when the size of the alkoxy group was increased, the diastereoselectivity of the reduction was significantly shifted to the syn isomer, as precedently reported [19] ; however, with R.mucilaginosa, which produced more syn ester, but in low yields (Table 1, entry 2), it appears that this effect may be the result of selective hydrolytic activities rather than a modification of the reduction stereochemistry. Moreover, when the size of the ester group was too large, the reduction was strongly inhibited.

In conclusion, one of the isomeric hydroxyesters (anti-2S,3R-6a[21], for example with G.candidum) could be obtained in good yield and sufficient optical purity for synthetic applications [15b]. The syn-2R,3R

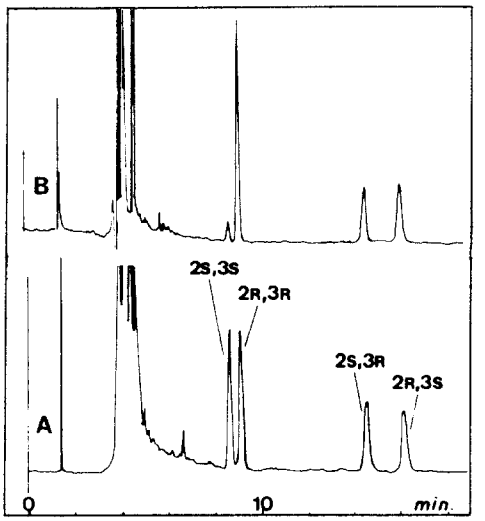


Figure 1: HPLC separation of MTPA esters of enantiomers of lactones 7 and 8, obtained by cyclisation of mixtures of hydroxyesters

(A) obtained by $NaBH_4$ reduction of ester 4b.

(B) obtained by M. racemosus reduction of ester 4b.

Zorbax 5µ silica column (25 cm x 4.6 mm) solvent : isooctane-EtOAc (85/15), 1.5 ml/min

isomer was obtained, in high optical purity, only in mixture with the anti isomer. On the other hand, the (3S) isomers could be obtained, but their enantiomeric purity is always low.

REFERENCES AND NOTES

1- This work was presented in part at the IVth European Symposium of Organic Chemistry, Aix-en-Provence (France), Sept. 1985
2- H.Akita, A.Furuichi, H.Koshiji, K.Horikoshi, T.Oishi, Chem.Pharm.Bull., 1983, **31**, 4384
3- H.Akita, H.Matsukara, T.Oishi, Chem. Pharm. Bull., 1986, **34**, 2656
4- H.Akita, H.Koshiji, A.Furuichi, K.Horikoshi, T.Oishi, Tetrahedron Lett., 1983, **24**, 2009
5- H.Akita, A.Furuichi, H.Koshiji, K.Horikoshi, T.Oishi, Tetrahedron Lett., 1982, **23**, 4051 ; Chem. Pharm. Bull., 1984, **32**, 1333
6- A.Furuichi, H.Akita, H.Koshiji, K.Horikoshi, T.Oishi, Chem. Pharm. Bull., 1984, **32**, 1619
7- H.Akita, H.Matsukara, T.Oishi, Tetrahedron Lett., 1986, **43**, 5241
8- J.W.F.K.Barnick, J.L.Vanderbaan and F.Bickelhaupt, Synthesis, 1978, 787
9- D.Buisson and R.Azerad, Tetrahedron Lett., 1986, **27**, 2631 ; D.Buisson, C.Sanner, M.Larchevêque and R.Azerad, Tetrahedron Lett., in press, 1987
10- **5b** : δ ($CHCl_3$) 7.32 (s,5H,ArH), 4.55 (s,2H,$ArCH_2$), 4.14 (q,J=7Hz,2H,$COOCH_2$), 4.05 (m,1H,CHOH), 3.51 (2d,J=4Hz and 6Hz,2H,OCH_2), 2.65 (dq, J_1=6Hz,J_2=5Hz,1H,CHMe), 1.60 (s,1H,OH), 1.21 (t,J=7Hz,3H,CH_3), 1.18 (d,J=6Hz,3H,CH_3) ; **6b**: δ ($CHCl_3$) 7.30 (s,5H,ArH), 4.55 (2d,J=11Hz,2H,Ar-CH_2), 4.14 (q,J=7Hz,2H,$COOCH_2$), 3.85 (ddd, J_1=7Hz,J_2=6Hz,J_3=4Hz,1H, CHOH),3.57 (dd,J_1=9Hz,J_2=4Hz,1H,OCH_2), 3.48 (dd,J_1=9Hz,J_2=6Hz,1H,OCH_2), 2.75 (dq,J_1=J_2= 7Hz,1H,CHMe), 1.25 (t,J=7Hz,3H,CH_3), 1.18 (d,J=7Hz,3H,CH_3)
11- Silicagel 60H (Merck) ; solvent : Cyclohexane:AcOEt- 8:2
12-These lactones have been obtained recently in mixtures (see ref.3) by acidic cyclization of the BH_3-Me_2S reduction product of diethyl 2-methyl-3-hydroxysuccinates, produced by microbial reduction of the corresponding 3-oxoester, and were similarly used for the identification of the isomeric hydroxyesters.
13- GC of the hydroxylactones for separation of the cis enantiomers or their isopropyl carbamates (see ref.20) for the separation of the trans enantiomers on a Chrompack XE60-polysiloxane S-valine-S-α-phenylethylamide silica capillary column (50 m x 0.25 mm).
14- J.A.Dale and H.S.Mosher, J.Org.Chem., 1969, **34**, 2543
15- a) H.Shieh and G.D. Prestwich, J.Org.Chem., 1981, **46**, 4321 ; b) M.Larchevêque and S.Henrot, Tetrahedron Lett., 1987, **28**, 1781
16- Modulprep (Jobin & Yvon) ; Merck 60H (230-400 mesh) silica column; solvent cyclohexane-AcOEt (30:70)
17- G.Frater, U.Müller and W.Günther, Tetrahedron, 1984, **40**, 1269
18- D.Seebach and M.Eberle, Synthesis, 1986, 37
19- K.Nakamura, T.Miyai, K.Nozaki, K.Ushio, S.Oka and A.Ohno, Tetrahedron Lett., 1986, **27**, 3155
20- W.A.König, I.Benecke and S.Sievers, J.Chromatogr., 1982, **238**, 427.
21-$[\alpha]_D^{21}$=+24,6° (c=1,3 MeOH) after distillation. ee: 96 % .

(Received in France 30 July 1987)