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On the Mode of Baker's Yeast Reduction of Enantiomeric 4-Acyl Butanolides

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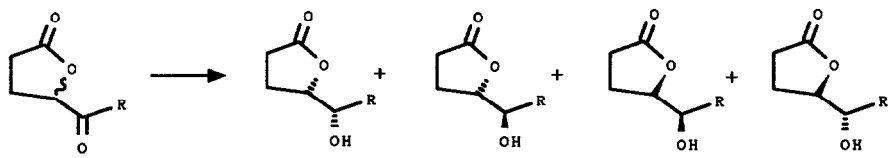
Abstract: 4-acyl butanolides (*R*)-(**1a**) and (*R*)-(**1b**), bearing *n*-alkyl chains, on yeast treatment afford exclusively the *syn* reduction products (**4a**) and (**4b**), whereas the (*S*) enantiomers yield *syn* (**2a**) and (**2b**) in ca. 6:4 ratio with the *anti* diastereoisomers (**3a**) and (**3b**), respectively. Under the same conditions, (*S*)-(**1c**) and (*R*)-(**1c**) give rise to *syn* and *anti* reduction products in ca. 1:4 and 4:1 ratios, respectively.

One of the topic of interest in the field of the applications of baker's yeast to the production of optically active carbinols through reduction of carbonyl compounds is the definition of empirical rules for the structure of the flanking substituents enabling one to predict the stereochemical outcome of the transformation.[1] For some time now, in the work related to studies on the baker's yeast mediated decarboxylative incorporation of pyruvate into aromatic and α,β-unsaturated aldehydes which gives (2*S*,3*R*) methyl diols through the intermediacy of (*R*) α-hydroxy methyl ketones we have been studying the yeast reduction of racemic α-hydroxy and α-acyloxy ketones, bearing at the two sides substituents of quite different nature and degree of functionalization.[2] Whereas in the former case the reduction proceeds with the prevalent formation of enantiomerically pure *anti* diols,[3] in the instance of the α-acyloxy substrates the product distribution appears more complicated, yielding *syn* and *anti* materials of different optical purity, close to deacylated diols.[4,5] In these circumstances we have been always dealing with racemic substrates ending up into chiral adducts conceivably formed under kinetic control and we thought it was worthwhile to compare stereochemistry and rate of yeast reduction of the two enantiomeric forms of configurationally stable, optically pure α-acyloxy ketones bearing different side chains. To this end we chose as substrates the enantiomeric 4-acyl butanolides **1**. Compounds of this type, with R= alkyl from C-2 to C-10, thus including (*R*)- and (*S*)-**1b**, recently became synthetically accessible via high

yield sequences from the two enantiomeric forms of glutamic acid. They were prepared[6] in a synthesis of (+)-exobrevicomin, which included also an extended study of the mode of reduction to the corresponding diastereoisomeric carbinols using a variety of metal hydrides under different experimental conditions. Products (1) (Scheme) appeared particularly suited for our stereochemical studies. Indeed, they are configurationally stable, the diastereoisomeric composition of the reduction products can be easily



1 2 3 4 5

a R = nC_6H_{13}

b R = $nC_{10}H_{21}$

c R = $(CH_2)_2C_6H_5$

Scheme

determined through ^1H NMR studies and *glc* analysis. In fact previous studies on the configurational assignement of secondary diols[7] led to establish that generally the *anti* isomers show lower field chemical shifts and smaller coupling constants with respect to the *syn* isomers. This rule has been successfully applied to the determination of the stereochemistry of the reduction products of some 4-acyl butanolides, and its validity confirmed in a synthesis of (+)-*exo*brevicomin.[6] Thus for carbinols **2-5** the products with *anti* configuration (4*S*,5*R* or 4*R*,5*S*) show H-5 at ca. 3.9 ppm and J(4,5) of ca. 3.5 Hz, while for the products with *syn* configuration, H-5 resonates at ca. 3.5 ppm and J(4,5) is ca. 4.5Hz. In transformation experiments using racemic precursors the enantiomeric composition of both surving ketones and of the derived carbinols can be established through *glc* and *hplc* analyses, using chiral columns.[6] Furthermore, carbinols of the type accessible by reduction of 4-acyl butanolides occur in nature with different alkyl side chains in *anti* form, but with different optical purities[8] and although the direct participation of ketonic intermediates to the biosynthesis of these acetogenins appears unlikely, it seemed worthwhile to define the mode of their production through enzymic carbonyl reduction. Accordingly, products (*R*)- and (*S*)-**1**,a-c were prepared in 60-70% yields from (*S*) and (*R*) tetrahydro-5-oxo-2-furan-carboxylic acid chloride and 1-hexyl bromide, 1-decyl bromide and phenethylbromide, respectively, according to the reported procedure.[6] Thus (*S*)-**1**, (*R*)-**1** and (*R*,*S*)-**1**, obtained mixing the first two together in 1:1 ratio, were submitted to yeast reduction in parallel experiments. The product

distribution in the transformation mixture obtained after 4 h fermenting baker's yeast incubation, isolated in 70-80% yield by solvent extraction, is reported in **Table 1** and **2**. The diastereoisomeric composition of the reduction products was determined by comparison with the reported data[6] through ^1H NMR studies and glc analysis of the crude mixture, whereas the enantiomeric purities of the products in the series with R= n-hexyl and n-decyl, respectively, was established through *glc* analysis on chiral capillary column[6], and those of products in the series R= phenethyl were determined through *hplc* analysis using a chiral column. However, in this instance, the best separations were observed when the carbinols were transformed into the benzoate esters. This was achieved separating the carbinols from the unreacted ketones by column chromatography and transforming the former into the esters upon reaction with benzoyl chloride/pyridine. Examination of the results of **Table 1**, relative to the transformations of (*S*)-**1** and (*R*)-**1**, points to the following considerations.

PRODUCTS FROM THE BAKER'S YEAST REDUCTION OF (*R*) and (*S*)-1a-c

Substrate	Products	1a-c recovered (%)	Alcohols % *syn*	% *anti*	Yields* (%)
(*S*)-1a	2a + 3a	0	60	40	100
(*S*)-1b	2b + 3b	0	56	44	100
(*S*)-1c	2c + 3c	75	20	80	25
(*R*)-1a	4a + 5a	91	100	0	9
(*R*)-1b	4b + 5b	90	100	0	10
(*R*)-1c	4c + 5c	12	79	21	88

* GC yields on the recovered material which accounts for 70 to 80 % of the starting ketones

Table 1

In first instance there is the significant change in the transformation rates within the same enantiomeric series, associated with the substitution of the *n*-alkyl side chain for the phenethyl moiety. The consequence is the complete reduction of (*S*)-**1a** and (*S*)-**1b** while 75% of (*S*)-**1c** survives, and conversely, the survival of ca. 90% of (*R*)-**1a** and (*R*)-**1b** while ca. 90% of (*R*)-**1c** is reduced. Secondly, in the reduction of the (*S*)-configurated materials **1**, the ratio between the *syn* and *anti* carbinols **2** and **3** changes from ca. 6:4 for the products with the aliphatic side chain to 1:4 for the

products bearing the phenethyl moiety, respectively. Conversely, within the *R*-configurated series 2 carbonyl reduction takes place exclusively onto the *si* face in the case of (*R*)-**1a** and (*R*)-**1b** to produce the *syn* (*R*)- configurated carbinols **4a** and **4b**, whereas in the case of (*R*)-**1c** *syn*-**4c** is produced in ca. 7:2 ratio with the *anti* diastereoisomer **5c**. Thus, the face selectivity in the yeast- mediated reduction of the carbonyl moieties present in enantiomeric 4-acylbutanolides bearing the aliphatic side chain is dramatically influenced by the absolute configuration of the attached α-lactone moiety. The same phenomenon is much less marked within the set of products with the phenethyl side chain. The trends observed in the reduction experiments of enantiomerically pure **1** and **2** are confirmed when the racemic materials (*R,S*)-**1** were submitted to the yeast reduction . As consequence of the kinetic resolution, enantiomerically pure (*S*)-**1c**, were obtained in reasonable yields as survived materials. Subsequent experiments indicated that at 24 h incubation (*R*)-**1b** yields 56% of *syn* **4b** without noticeable presence of the *anti* diastereoisomer **5b**. It thus follows a similarity between the diastereoisomeric excess obtained in the yeast and in the metal hydride reduction of the ketones bearing R= *n*-alkyl chain, with the notable exception that in the case of the baker's yeast this happens only in the case of the (*R*) enantiomer.

PRODUCTS DISTRIBUTION FROM THE BAKER'S YEAST REDUCTION OF (*R,S*)-1a-c

Substrate	% Survived ketone (ee)	*syn*-Products*(%, ee)	*anti*-Products*(%, ee)	Yields # %
(*R,S*)-1a	(*S*)-1a 0 % (*R*)-1a 42 % (100)	(2a) 4a (62, 64)	(3a) 5a (38, 100)	58
(*R,S*)-1b	(*S*)-1b 0 % (*R*)-1b 38 % (100)	(2b) 4b (63, 38)	(3b) 5b (37, 100)	62
(*R,S*)-1c	(*S*)-1c 40 % (100) (*R*)-1c 0 %	2c, (4c) (71, 90)	3c, 5c (29, 0)	40

* Prevailing enantiomer circled
\# GC yields of alcohols on the recovered material which accounts for 70 to 80% of the starting ketones

Table 2

Indeed, amongst the metal hydrides employed[6] several showed excellent *syn* selectivity, as baker's yeast does in the case of (*R*)-**1a** and (*R*)-**1b**. Conversely, the metal hydrides which gave rise in the above instances prevalently to *anti* materials

showed *de* values comparable with those here observed in the yeast reduction of (*S*)-1c to *anti* 3c and *syn* 2c. Seen together, these results, which indicate the dramatic change in the mode of yeast transformation of substrates with the same functionalities even on subtle structural modifications some of which occurring far away from the place where the transformation occurs, point to the empiricism still informing the area of applied enzymology attempting to formulate structural rules for acceptability of non conventional substrates by synthetically useful enzymes. The ongoing experiments on the mode of yeast and metal hydrides reduction of racemic and optically active 5-acylpentanolides should be helpful in the above context.

Experimental

The synthesis of ketolactones 1,a-c has been performed exactly as reported in ref.6, using the inverse addition of the Grignard reagents onto the THF solution of the two enantiomeric forms of the chloride of tetrahydro-5-oxo-2-furancarboxylic acid at -78 °C, with yields ranging from 55 to 70%. GLC chiral analysis was performed on Megadex 1, permethylated beta cyclodextrin coated with fused silica capillary column, 25 m x 0.25 mm i.d.. Analysis conditions: 75 °C, 20 °C/min to 165 °C, 165 °C for 2 min, then 1 °C/min to 190 °C, H_2 carrier gas. HPLC on chiral column: Daicel Chiracel OD, hexane/isopropanol 9:1, 218 nm, 0.8 ml/min.

Yeast reduction. In a 2 L glass jar a mixture is made up composed of 250 g of commercial baker's yeast and 200 g of D-glucose in 1 L of tap water at 32 °C. As the fermentation starts, 5 g of substrate in 5 ml of ethanol are added under stirring. After the required time, 100 g of Celite are added, the reaction mixture is filtered on a large Buchner funnel, the solid pad is washed with 200 ml of ethyl acetate and the filtrate is extracted twice with 200 ml portions of ethyl acetate. The organic phase, once dried, is evaporated, leaving a residue of ca. 4-4.5 g. This material is subjected directly to NMR and glc analysis and, when required, is chromatographed on silica gel with hexane-ethyl acetate to separate the unreacted ketone from the carbinols. GLC behaviour of (*S*)-1a, (*R*)-1a and of their reduction products on the chiral column: (*S*)-1a, (*R*)-1a, 2a, 4a, 5a, and 3a, Rt 29.37, 29.422, 42.505, 45,73 and 47.363 min, respectively. The reduction products of (*S*)-1b and (*R*)-1b were identified by comparison with authentic samples.[6] HPLC on chiral column: (*S*)-1c, Rt 36.3 min, (*R*)-1c, Rt 33.2 min. The carbinols were separated from the starting ketones by column chromatography and converted quantitatively into the corresponding benzoates by treatment at room temperature overnight of the methylene chloride solution with 1.1 mol eq of benzoyl chloride and 2 mol eq of dry pyridine in the presence of 10 mol % of 4-N,N-dimethylaminopyridine. Aqueous workup, followed by washings with sodium hydrogen carbonate and hydrochloric acid solutions afforded on evaporation of the dried organic phase the ester mixture. Benzoates of: 2c, 4c, 5c and 3c, Rt 30.21, 32.75, 38.46 and 52.72 min, respectively.

^{1}H NMR and analytical data of compounds 1-5

1a, R = nC_6H_{13}. ^{1}H NMR ($CDCl_3$) δ 4.80 (1H, m, H-4), 2.1-2.7 (6H, m, CH_2-3, CH_2-4 and CH_2-6), 1.15-1.7 (6H, m, 3 CH_2), 0.89 (3H, dist t, CH_3). Anal. Calcd for $C_{11}H_{18}O_3$: C, 66.64; H, 9.15. Found: C, 66.68; H, 9.10.

1b, R = n $C_{10}H_{21}$. ^{1}H NMR ($CDCl_3$) δ 4.82 (1H, m, H-4), 2.1-2.7 (6H, m, CH_2-2, CH_2-3 and CH_2-6), 1.15-1.7 (16H, m, 8 CH_2), 0.89 (3H, dist t, CH_3). Anal. Calcd for $C_{11}H_{18}O_3$:

C, 66.64; H, 9.15. Found: C, 66.68; H, 9.10.

1c, R = $(CH_2)_2C_6H_5$. ^{1}H NMR ($CDCl_3$) δ 4.78 (1H, m, H-4), 2.92 (4H, m, S broad, CH_2-6 and CH_2-7), 2.1-2.6 (4H, m, CH_2-2 and CH_2-3), 7.1-7.3 (5H, m, C_6H_5). Anal. Calcd for $C_{13}H_{14}O_3$: C, 71.54; H, 6.47. Found: C, 71.35; H, 6.49.

2a, 4a R = nC_6H_{13}. ^{1}H NMR ($CDCl_3$) δ 4.42 (1H, td, H-4, $J_{3,4}$ 7.0, $J_{4,5}$ 4.5 H_2), 3.50 (1H, m, H-5), 2.0-2.6 (5H, m, CH_2-2 , CH_2-3 and OH), 1.2-1.6 (10H, m, 5 CH_2), 0.90 (3H, dist t, CH_3). Anal. Calcd for $C_{11}H_{20}O_3$: C, 65.97; H, 10.07. Found: C, 70.02; H, 10.12.

3a, 5a R = nC_6H_{13}. ^{1}H NMR ($CDCl_3$) δ 4.42 (1H, td, H-4, $J_{3,4}$ 7.2, $J_{4,5}$ 3.5 H_2), 3.89 (1H, m, H-5), 2.1-2.6 (5H, m, CH_2-2 , CH_2-3 and OH), 1.2-1.6 (10H, m, 5 CH_2), 0.90 (3H, dist t, CH_3)

2b, 4b R = $nC_{10}H_{21}$. ^{1}H NMR ($CDCl_3$) δ 4.40 (1H, dt, H-4, $J_{3,4}$ 7.0, $J_{4,5}$ 4.5 H_2), 3.54 (1H, m, H-5), 2.0-2.6 (5H, m, CH_2-2 , CH_2-3 and OH), 1.2-1.6 (18H, m, 9 CH_2), 0.91 (3H, dist t, CH_3). Anal. Calcd for $C_{15}H_{28}O_3$: C, 70.27; H, 11.01. Found: C, 70.37; H, 10.82.

3b, 5b R = $nC_{10}H_{21}$. ^{1}H NMR ($CDCl_3$) δ 4.40 (1H, dt, H-4, $J_{3,4}$ 7.0, $J_{4,5}$ 3.5 H_2), 3.90 (1H, m, H-5), 2.0-2.7 (5H, m, CH_2-2 , CH_2-3 and OH), 1.2-1.6 (18H, m, 9 CH_2), 0.91 (3H, dist t, CH_3)

2c, 4c R = $(CH_2)_2C_6H_5$. ^{1}H NMR ($CDCl_3$) δ 7.15-7.45 (5H, m, C_6H_5),4 41 (1H, td, H-4, $J_{3,4}$ 7.4, $J_{4,5}$ 4.5 Hz), 3.56 (1H, dt, H-5, $J_{5,6}$ 9.0 and 4.5 Hz), 2.4-3.0 (5H, m, CH_2-2, CH_2-7 and OH), 1.7-2.3 (4H, m, CH_2-3 and CH_2-6). Anal. Calcd for $C_{13}H_{16}O_3$: C, 70.89, H, 7.32. Found: C, 70.80, H, 7.43.

3c, 5c R = $(CH_2)_2C_6H_5$. ^{1}H NMR ($CDCl_3$) δ 7.15-7.45 (5H, m, C_6H_5), 4.41 (1H, td, H-4, $J_{3,4}$ 7.4, $J_{4,5}$ 3.2 Hz), 3.94 (1H, ddd, H-5, $J_{5,6}$ 8.0 and 5.5 Hz), 2 4-3.0 (5H, m, CH_2-2, CH_2-7 and OH), 1.7-2 3 (4H, m, CH_2-3 and CH_2-6).

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