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REMOTE SUBSTITUENT EFFECTS IN MICROBIAL REDUCTIONS OF 3-KETOGLUTARATE AND 3-KETOADIPATE ESTERS

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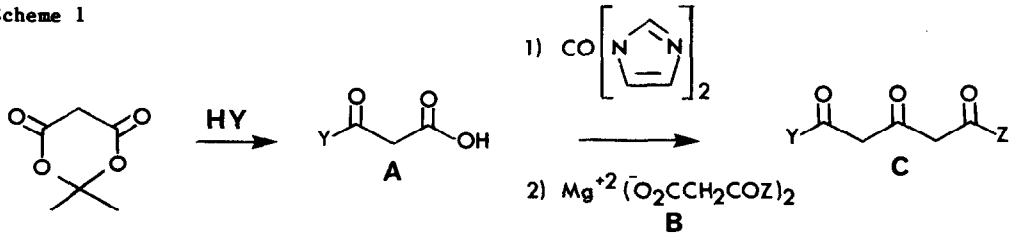
Summary: The enantioselectivity of yeast mediated reductions of prochiral 3-ketoglutarate and 3-ketoadipate esters to the corresponding 3-hydroxyesters can be influenced by simple differences in the ester groups.

Asymmetric microbial reduction of a carbonyl group by bakers' yeast is a useful preparative method to obtain optically active secondary hydroxy compounds.[1] The ability of an enzyme to make a prochiral distinction by selective reduction of one enantiotopic face of a carbonyl group requires the formation of a preferred enzyme-substrate complex. Factors which control this selectivity have been examined and the " Prelog rule " is one result of early work in this regard.[2] More recently, substituents relatively remote from the carbonyl group have been shown to influence the enantiomeric outcome of yeast reductions.[3] We have investigated yeast mediated reduction of prochiral 3-ketoglutarate and 3-ketoadipate esters and have found that simple differences in the remote ester functionality affect the enantioselectivity of these enzymatic reductions.

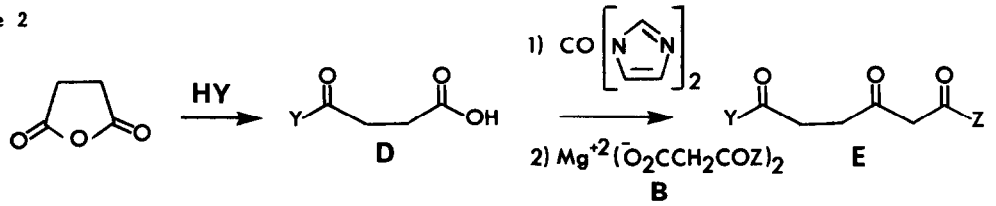
A series of prochiral 3-ketoglutarates 1-7 (see Table 1) were prepared as outlined in Scheme 1. Meldrum's acid[4] (1 equiv) was treated with an alcohol or mercaptan (**HY**, 1 equiv) and heated at reflux for 24 h to provide the malonate half-acid esters **A** in 70-85% yield.[5] Treatment of the malonate half-acid esters **A** first with carbonyldiimidazole in THF, 25°C, 1-2h, followed by the C-acylating reagent **B**[6] and stirring at 25°C for 16h gave the 3-ketoglutarates **C** (50-70% yield) which were purified by chromatography (silica gel, 10% ethyl acetate in hexane).

Scheme 1



A series of 3-ketoadipates **15-20** (see Table 1) were prepared as outlined in Scheme 2. Succinic anhydride (1 equiv) was treated with an alcohol or mercaptan (**HY**, 1 equiv or excess) and heated at reflux for 24h to provide the succinate half-acid esters **D** in 80-95% yield. C-Acylation as described previously gave the 3-ketoadipates **E** (75-90% yield) which were purified by chromatography (silica gel, 10% ethyl acetate in hexane).[7]

Scheme 2



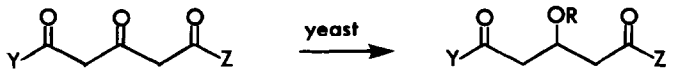
The following general procedure was used for the microbial reduction of the carbonyl substrates. To a stirred solution of 50ml of water buffered to pH 7 (18.9g Na_2HPO_4, 9.1g KH_2PO_4 in 1 l water)containing 5g of D-glucose, 0.25g yeast extract, warmed to 35°C, was added 5g of dry active bakers' yeast (Fleischmann's from Standard Brands Inc.) and the mixture was stirred at 35°C for 30 min, after which 0.5g of 3-ketoester was added. The mixture was vigorously stirred at 25-30°C for 24h and then continuously extracted with dichloromethane for 48h providing a crude product after evaporation of the solvent which consisted mainly of the 3-hydroxyester and starting material. The products were purified by chromatography (silica gel, 20-40% ethyl acetate in hexane). The enantiomeric composition of the hydroxyesters was determined by analysis and comparison of the ^{19}F (188MHz) spectra of the corresponding (+)-α-(trifluoromethyl)phenyl acetic acid (MTPA) esters[8] with those derived from the racemic ketols prepared by reduction with $NaBH_4$ (1 equiv of 1 M aqueous $NaBH_4$ solution added to a 0.1 M THF solution of the substrate at 0°C and stirred for 15 min.) In each case the diastereomeric CF_3 signals were clearly resolved and carefully integrated to provide the results shown in Table 1.[9]

The results of the yeast reductions of prochiral 3-ketoglutarates **C** reveal that, in general, the enzyme reducing system does not readily differentiate small differences in the size of the ester group (entry 1), the presence of an aromatic ring (entry 2), or a trifluoromethyl group (entry 3). It is interesting to note that a distinction between an ethyl ester versus a ethanethiol ester (entry 5) is similar to that of a tert-butyl versus a methyl ester (entry 4). The best distinction of this set of substrates was provided by a ethanethiol ester versus a tert-butyl ester (entry 7).

The symmetry of this substrate system provides a good test of the ability of the yeast reducing system to distinguish differences in substituents remote from the carbonyl group. These results provide support for developing further refinements in adjusting groups Y and Z such that optical purities approaching 100% might be obtained. Also, if Y and Z are designed as in the case for entry 7, to allow selective reaction at either ester site specifically, an **R**- or **S**-3-hydroxyglutarate synthon from the same substrate would be available as a useful precursor in natural product synthesis.

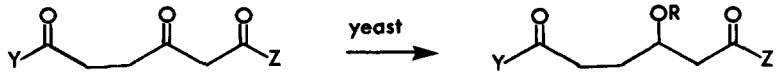
For the 3-ketoadipate substrates **E**, the dimethyl ester (entry 8) might be considered as an starting point for comparison of the effect of changes in the ester groups. In some cases (entries 9, 10, and 11) the distinction became worse. Entries 11 and 12 show that the effect of the tert-butyl ester is greater when it is beta to the carbonyl. Entry 13 provides a 3-hydroxyadipate **26** of 84% ee that can be selectively reacted at either ester group.

Table 1. Yeast Mediated Reduction of Prochiral 3-Oxoglutarate Esters and 3-Oxoadipate Esters



R= (+)-OCC(CH_3)(CF_3)C_6H_5

Entry	Substrate	Y	Z	Product	(+)MTPA diastereomeric composition	isolated yield (%)[a]
1	**1**	OCH_2CH_3	OCH_3	**8**	50:50	20
2	**2**	$OC(CH_3)_3$	$OCH_2C_6H_5$	**9**	50:50	20
3	**3**	$OC(CH_3)_3$	OCH_2CF_3	**10**	50:50	10
4	**4**	$OC(CH_3)_3$	OCH_3	**11**	62:38	36
5	**5**	OCH_2CH_3	SCH_2CH_3	**12**	65:40	10
6	**6**	OCH_3	$SC(CH_3)_3$	**13**	57:43	8
7	**7**	$OC(CH_3)_3$	SCH_2CH_3	**14**	72:28	32

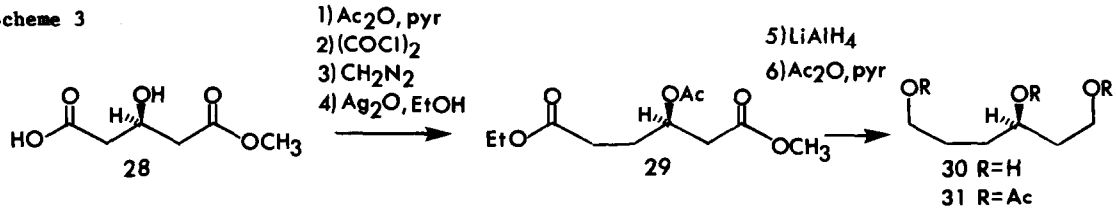


					S:R	
8	**15**	OCH_3	OCH_3	**21**	74:26	19
9	**16**	$OCH_2C_6H_5$	OCH_3	**22**	51:49	24
10	**17**	OCH_3	SCH_2CH_3	**23**	52:48	56
11	**18**	$OC(CH_3)_3$	OCH_3	**24**	65:35	26
12	**19**	OCH_3	$OC(CH_3)_3$	**25**	83:17	29
13	**20**	$OCH(CH_3)_3$	SCH_2CH_3	**26**	92:8	26

a. The isolated yield represents the amount of 3-hydroxy ester isolated after a 24 h reaction time using the general procedure described in the text. A mass balance of about 80% was obtained in each case by the sum of 3-hydroxy ester and recovered starting material.

The absolute configuration of the predominant enantiomer in the products **24** and **26** was established as [3S] by a correlation sequence outlined in Scheme 3. Chymotrypsin catalyzed hydrolysis of dimethyl 3-hydroxyglutarate provides the [3R] half-acid ester **28**,[10] which was acetylated and then homologated by a standard Arndt-Eistert reaction.[11] Reduction with $LiAlH_4$ and acetylation provided [3R]-1,3,6-hexanetriol triacetate (**31**), $[\alpha]_D^{\bar{=}}$ - 8.0°, c=2 ($CHCl_3$) as a reference compound.[12] The yeast products **24** and **26** were reduced with $LiAlH_4$ and acetylation provided the corresponding triacetates with $[\alpha]_D^{\bar{=}}$ + 4.0° and + 11.4° respectively. The ^{19}F chemical shift of the CF_3 group in the predominant (+)MTPA diastereomer for products **21-26** was consistently upfield and this observation was used as the basis for assigning the [3S] configuration to the major enantiomer of the uncorrelated 3-hydroxyadipate product mixtures.[13]

Scheme 3



These results illustrate that simple changes in the ester group of ß-ketoesters can be used to control the enantioselectivity of yeast mediated reductions.

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