Tetrahedron: Asymmetry Vol. 4, No. 3, pp. 301-302, 1993
Printed in Great Britain

0957-4166/93 $6.00+.00
Pergamon Press Ltd

STEREOSELECTIVE REDUCTION OF SUBSTITUTED BENZOPHENONES BY MICROORGANISMS I.

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(*Received in UK* 22 *January* 1993)

In Memory of Prof. Dr. Günther Snatzke: He combined the deep human relationships with his selflessness to science in a magnificent way, worthy of following.

Abstract: Debaryomyces marama enantioselectively reduces p-Cl-benzophenone to the S-alcohol with high enantiomeric excess.

Prochiral compounds with different substituents were subjected to microbial reduction with strains of yeast, fungi and bacteria to obtain products with high enantiomeric purity[1-6]. An excellent review has described the use of baker's yeast as a reagent in organic synthesis, with an emphasis on the developments of the last 15 years[7]. Aryl methyl ketones have been reduced to S-alcohols in a modest yield and approximately 90% ee[8]. Chromium tricarbonyl complexed aromatic ketones have also been treated with bakers yeast, and in the case of acetophenone, S-1-phenylethanol has been obtained in 96% yield and 100 ee. The reduction of cyclic aromatic ketones has been performed in a low yield[9]. The present study is the beginning of a wide investigation of the stereoselective reduction of benzophenone derivatives to the corresponding alcohols (Figure 1).

$$R^1C_6H_4COC_6H_4R^2 \rightarrow R^1C_6H_4CH(OH)C_6H_4R^2$$

1. $R^1 = H$; $R^2 = o-Cl$
2. $R^1 = H$; $R^2 = m-Cl$
3. $R^1 = H$; $R^2 = p-Cl$
4. $R^1 = o-Cl$; $R^2 = m-Cl$
5. $R^1 = H$; $R^2 = p-F$
6. $R^1 = H$; $R^2 = p-CH_3$

Figure 1: *Microbial reduction of benzophenones with different substituents.*

154 strains were subjected to screening to establish their reduction ability in creating a stereogenic centre. In a typical procedure, 4 mg of the substrate in 20 ml of growing strain mixture liquid, were stirred at 150 rpm, for 48 h. The reaction was stopped by extraction with ethyl acetate. The products were analyzed by HPTLC. The spots were visualized by their yellow colouring on spraying with Ce-reactive and heating at 150°C. After the screening, all promising strains were investigated in preparative quantities in a 20L fermenter with 10L volume containing 1% corn steep liquor, 1% treacle, 0.17% KH_2PO_4, 0.22% Na_2HPO_4, plus 1 ml silicone anti-foam, pH 6.0. The fermenter was sterilized for 60 mins. at 1 atu, and 120°C in situ; the nutritive medium was stirred throughout. The conditions of the fermentations were: 0.3 VVM and 300 RPM. The inoculum of the fermenter was prepared previously in 100 ml flasks containing 25 ml nutritive medium each and incubated on a mechanical shaker for 24 hrs at 220 RPM and 30°C. It represents 5.0% (vol) of the total liquid medium in the fermenter. With the yeast, the growth rate is determined every 4 hours by measuring the optical density at 546 nm (OD_{546}), while with the fungi the quantity of the biomass is estimated by centrifugation. To achieve reduction, at the end of the exponential phase of the microorganisms development, a substrate was added in concentration 0.2 g/L, dissolved in 25 ml DMFA. The entire process took place under sterile conditions. After approximately 24 hrs. of contact between the substrate and the yeast, respectively the fungus, the fermentation was terminated. The reduction rate was checked by the aid of TLC, eluent benzene, on silica gel plates 60 F_{254}, Merck. When the fermentation was over, the microbial mass was extracted several times with ethylacetate amounting to 1/5 to 1/3 of the culture broth volume. The combined extracts were concentrated at low pressure, 40°C to 0.1L. The product was left overnight at 4°C to

crystallize and was then recrystallized from chloroform at 4°C. Ten strains yielded positive TLC results. Corynespora cassicola DSM 62475, Debaryomyces marama DSM 70250, Absidia blakesleana ATCC 70148A, Syncepthalastrum racemosum ATCC 18192, Rhizopus fusiformis CBS 26630, Rhizopus arrhizus ATCC 11145, Enterobacter liquefaciens DSM 30063, Corynespora melonis CBS 12925, Rhizopus triticii CBS 12808 and Debaryomyces phaffii IFO 1362. Especially successful was the reduction in the presence of the p-substituents (p-F, p-Cl, p-CH_3) with Debaryomyces marama DSM 70250, Corynespora cassicola DSM 62475, Rhizopus arrhizus ATCC 11145, Absidia blakesleana ATCC 70148A and Syncephalastrum racemosum ATCC 18192. An attempted reduction of the ketone with Debaryomyces marama gave the isomeric (S)-alcohol as the main product with the following physical parameters: white needle crystals; m.p. 52-55°C (Koffler aparatus); $[\alpha]_D^{22}$ -11.2 (c 1.3 $CHCl_3$); NMR δ -2.25/S 1H, 5.75/S 1H; CD: $[\theta]_{272}$ – 123, $[\theta]_{266}$ 94, $[\theta]_{260}$ 207, $[\theta]_{254}$ 132, $[\theta]_{249}$ 52, $[\theta]_{247}$ 61; e.e. = 100%. Starting from 2g of **3** (Figure 1), 0.96g of the corresponding S-alcohol was obtained, 0.68g of the ketone being recovered unreacted. The enantiomeric purity was determined by chemical modification of the alcohol with (+)-α-methoxy-α-(trifluoromethyl)-phenylacetylchloride in pyridine. The resulting esters were analyzed in HPTLC. The assignment of the structure of **3** (Figure 1) was based on CD-analysis (Figure 2). The two phenyl rings are enantiotopic, therefore CD-series of opposite signs within the α-bands are to be expected for p-chlorosubstitution with the O-O line of 36.3 cm^{-1}, which is negative, belonging to the p-chlorophenyl chromofore. The other series, at somewhat shorter wavelengths, are indeed of opposite signs. This result shows also that for such a diarylcarbinol p-chlorosubstitution does not change the sign of the CD of the α-band, as we have also recently proved for other, similar systems.[10]

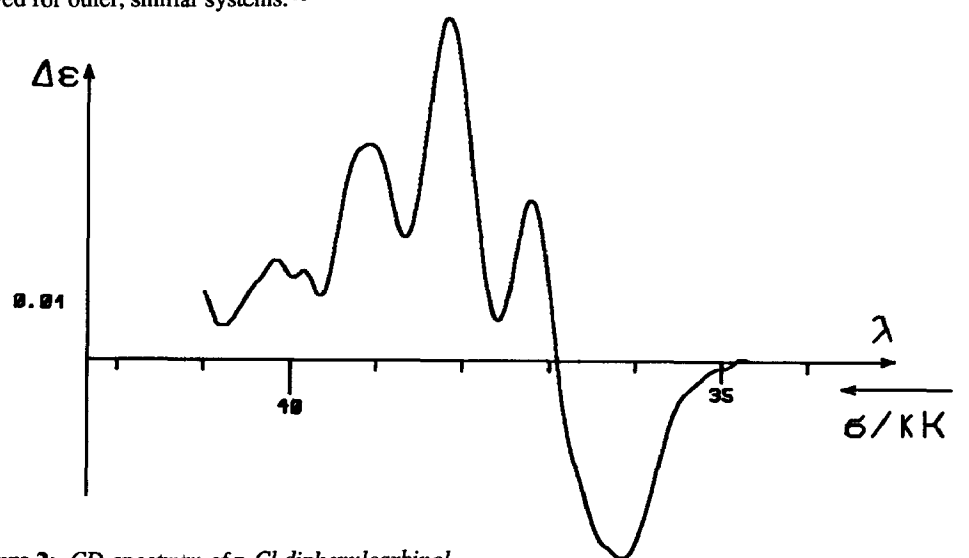

Figure 2: *CD-spectrum of p-Cl diphenylcarbinol*

Acknowledgements: The authors wish to thank Dr. J. Vlahov for the determination of the e.e.

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