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Chemo-enzymatic synthesis of the active enantiomer of the anorressant 2-benzylmorpholine

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Abstract

Baker's yeast reduction of (*Z*)-α-bromocinnamaldehyde **1** in the presence of absorbing resins allows the easy preparation of the corresponding saturated bromo-alcohol **2** in high yields and enantiomeric excess. The absolute configuration is assigned through conversion into the (*R*)-phenyl oxirane **3** and 1-phenyl-2-propanol **4** of (*S*) absolute configuration. The (*R*)-epoxide is transformed into **6**, the pharmacologically active enantiomer of the appetite suppressant 2-benzylmorpholine, to which the (*R*) configuration is assigned.

1. Introduction

The ability of microorganisms to reduce stereoselectively triply substituted C=C double bonds of α,β-unsaturated carbonyl compounds is a unique property of some yeasts and bacteria and has been exploited in the preparation of enantiomerically enriched compounds.[1–17] Recently homogeneous hydrogenation catalysts have been successfully used for the same purpose.[18] Because of the mild and environmentally friendly reaction conditions, whole cell biocatalysts are used in stereoselective reduction even on an industrial scale.[19,20] We have previously shown, however, that compounds of type **1** undergo reduction with unsatisfactory yields and selectivity, giving rise to the formation of several reduction products with incomplete enantioselectivity.[21] Recently we have applied absorbing resins in what we define as extractive biocatalysis and have shown that by controlling the substrate concentration of hydrophobic substrates, not only is high product recovery achieved, but improved enantio-chemoselectivity is obtained.[22,27] In this article we report on the baker's yeast reduction of **1**, resulting in the preparation of **2** of (*S*) absolute configuration and its transformation into benzylmorpholine **6**, a potent appetite suppressant drug. Compound **6** has previously been prepared in a racemic form and the two enantiomers

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resolved with (−)-dibenzoyltartaric acid.[23] Appetite suppressant tests on dogs showed that the biological activity completely resides in the enantiomer showing positive rotation in plane polarised light. The absolute configuration of the active enantiomer was not assigned.[23]

2. Results and discussion

Baker's yeast reduction of **1** was reported to give the bromo-alcohol **2** of undefined enantiomeric excess, to which the (*S*) absolute configuration was assigned by analogy with similar products.[24] The study of the yeast reduction of **1** in different operating conditions shows that the enantiomeric purity of the saturated bromo-alcohol obtained strongly depends on the initial substrate concentration. With an initial substrate concentration of 5 g/l the enantiomeric excess was only 62.7%, but could be raised to 91.8% when the concentration was kept as low as 0.1 g/l. A behaviour of this type can be explained in terms of competition of the same substrate between similar enzymes with different K_m.[25,26] Dilutions of this order cannot be considered for preparatively useful biotransformations. Absorbing resins are able to effectively control the substrate activity in the medium. High substrate concentration on the resin still results in a low substrate activity in the water phase and in the yeast environment, resulting in a direct control of the enantiomeric excess of the product: with a resin:substrate ratio of 1 and an initial substrate concentration of 5 g/l, compound **2** was obtained in nearly quantitative yield and 98.6% e.e.[27]

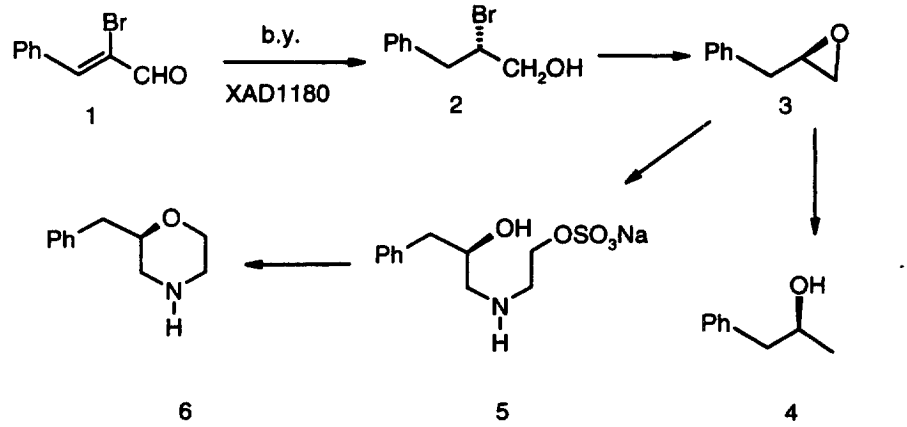

Fig. 1 summarises the enantiomeric excess of the product **2** obtained in different conditions, as a function of the initial substrate concentration. The control of the enantiomeric excess by means of absorbing resins added is evident. Already at a resin:substrate ratio of 0.1, the increase in ee of **2** is dramatic (95.6%), although a slight improvement is still observed by increasing the ratio. The improved performance makes it possible to work at practically interesting concentrations (5 g/l). A higher resin:substrate ratio improves the efficiency of product extraction. In this case, solvent extraction can be minimised. The enantiomeric excess of **2** was evaluated from chiral GC and comparison with the racemic bromohydrines obtained from allylbenzene and N-bromosuccinimide† in water.[23]

Treatment of **2** with base gave the epoxide **3** which was then converted into 1-phenyl-2-propanol **4** of (*S*) absolute configuration, as established by comparison with physical data from the literature.[28] Compound **3** of high enantiomeric excess could easily be prepared on a 10 g scale. The same epoxide

† NBS treatment of allylbenzene gives a mixture of isomeric bromoalcohols in racemic form: they are separated into four well resolved signals in GC on chiral stationary phase (see experimental).

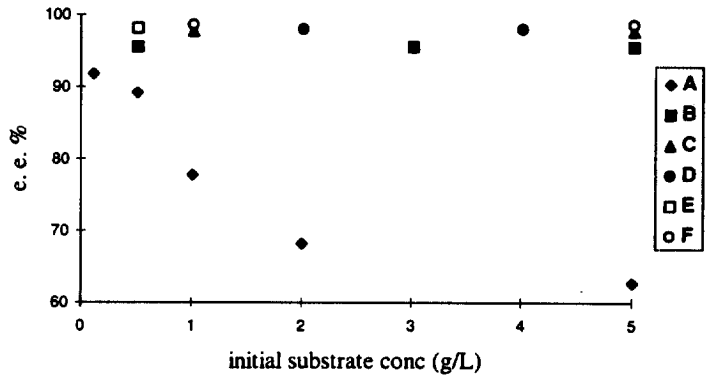

Figure 1. Enantiomeric excess of **2** as a function of initial concentration of **1**: with no resin added (A); with addition of XAD1180, resin:substrate ratio (g:g) (B) 0.1; (C) 0.5; (D) 1; (E) 2; (F) 5

can be prepared in about 25% yield in a tedious three step procedure from the corresponding amino-acid, involving nitrous acid treatment, LAH reduction and base cyclisation.[29,30] Treatment of **3** with ethanolamine sulphate gave **5** as the major product in a mixture with the nitrogen substituted compound, from which it could be purified by column chromatography after base catalysed ring closure to **6**.[23] Compound **6**, showing a positive rotation sign, was identified as the biologically active enantiomer of the appetite suppressant drug. Since the hydrochloride of compound **6** showed a specific rotation value sensibly higher than reported in the literature for the resolved enantiomer, the MTPA derivatives of **6** with (*R*)-MTPACl were prepared and analysed on an achiral HPLC column. Experiments with the racemic derivatives showed that the limit of detectability of a second enantiomer was lower then 1% and thus an enantiomeric excess of approximately 99% could be assigned to **6**.

3. Experimental

3.1. Analytical

GC chiral analyses were performed on a DANI 8610 with a FID detector, fitted with a glass capillary column, Megadex DACTBSβ-cdx (Mega, Legnano Italy), 25 m×0.25 mm i.d., film thickness 0.25 μm. HPLC analyses were recorded on a Merck Hitachi L6000 equipped with L4000 UV detector and D2500 integrator. $[\alpha]_D^{20}$ were recorded with a Propol automatic digital polarimeter. ^1H NMR were recorded on a Varian EMX 250 MHz with TMS as internal standard. All spectra are recorded in $CDCl_3$ unless otherwise indicated.

3.2. Absorption of the substrates onto the resin

The crude commercial resin XAD 1180 was washed successively with deionised water and acetone (3 ml for 1 ml of resin). The substrate was dissolved in acetone and the resin, once dried, added to the solution (i.e. 10 g of substrate, 100 ml of acetone and 10 g of dry resin). The mixture was shaken for 10 min, avoiding the use of the magnetic bar which can damage the resin, and then the acetone was evaporated at reduced pressure. The solid so obtained was poured directly into the fermentation vessel.

3.3. (S)-2-Bromo-3-phenyl-propan-1-ol 2

In a 5 l beaker containing 2 l of tap water at 30°C were added, in one portion, 100 g of D-glucose and 500 g of fresh baker's yeast (Distillerie Italiane, Eridania group). The mixture was pre-fermented for 30 min. 10 g (47 mmol) of **1** adsorbed onto 10 g of XAD1180 resin were added in one portion and the mixture was stirred at 25°C for 48 h. The reaction was filtered on a glass sintered funnel (porosity 0) and the collected resin was washed three times with tap water. The resin and the water phase were extracted with ethyl acetate. The collected organic phases gave crude **2** (9.7 g). The alcohol was purified by distillation to give pure **2** (9.1 g, 43, mmol, 91% yield). An analytical sample was obtained by bulb to bulb distillation, 110°C at 0.1 mmHg. ^{1}H NMR δ ($CDCl_3$) 2.41 (1H, OH, s), 3.20 (2H, CH_2, m), 3.77 (2H, CH_2, m), 4.31 (1H, CH, m), 7.27 (5H, Ph, m). $[\alpha]_D^{20}$ −22.6 (c 5, $CHCl_3$). Anal. calcd for $C_9H_{11}BrO$: C, 50.26; H, 5.15; Br, 37,15. Found: C, 50.25; H, 5.12; Br, 37.19. Temperature program for the GLC chiral analysis on the MEGA column: 40°C 1 min, 20°C/min, 125°C 2 min, 1°C/min, 210°C; injector 250°C, detector 250°C. Retention times: (*R*)-**2** 31.50, (*S*)-**2** 32.32. Racemic **2** was synthesised as reported in the literature,[23] by treating allyl benzene with NBS in water. The two peaks assigned to the enantiomeric couple **2** were accompanied by a second pair of peaks attributed to the isomer of α-Br addition. The e.e. determined by GLC was 98.6%. In a parallel experiment without the use of resin, the product recovery was similar but the e.e. was only 68%.

3.4. (R)-2-Benzyl-oxirane 3

Literature procedure[23] was followed for the preparation of **3**. 8 g (37 mmol) of **2** were suspended in 15 ml of water and 2 g (50 mmol) of sodium hydroxide pellets were added to the suspension. The mixture was heated at 65°C for 45 min, cooled to 25°C and diluted with water (30 ml). The water was extracted with diethyl ether so as to obtain after distillation (95°C, 15 mmHg) pure **3**, 3.6 g (27 mmol, 73% yield), $[\alpha]_D^{20}$ +25.9 (c 5, benzene), $[\alpha]_D^{20}$ +19.1 (neat), lit.[30] +19.2 (neat). ^{1}H NMR δ ($CDCl_3$) 2.55 (1H, CH_2, q), 2.79 (1H, CH_2, m), 2.87 (2H, CH_2, m), 3.15 (1H, CH, m) and 7.28 (5H, Ph, m). The e.e. determined by GLC was 98.8%. Temperature program for the GLC chiral analysis on the MEGA column: 40°C 1 min, 20°C/min, 110°C 2 min, 1°C/min, 210°C; injector 250°C, detector 250°C. Retention times: (*R*)-**3** 12.01, (*S*)-**3** 12.43 (racemic **3** was prepared in the same way from the mixture of racemic **2** obtained as described earlier).

3.5. (S)-1-Phenyl-propan-2-ol 4

3 g (22 mmol) of **3**, dissolved in 15 ml of dry diethyl ether, were added dropwise to a suspension of $LiAlH_4$ (0.23 g, 6 mmol) in 50 ml of anhydrous diethyl ether. The mixture was stirred at reflux for 2 h, after which time the reaction was quenched with ice and the organic phase separated; the aqueous layer was extracted twice with diethyl ether and the combined organic phases were dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure gave 3 g of crude material which was purified by bulb to bulb distillation (10 mm Hg, 100°C) so as to obtain 2.6 g (19 mmol, 86% yield) of pure **4**, $[\alpha]_D^{20}$ +40.7 (c 5, benzene, lit.[28] +41). Anal. calcd for $C_9H_{12}O$: C, 79.37; H, 8.88. Found: C, 79.35; H, 8.74. ^{1}H NMR δ ($CDCl_3$) 1.22 (3H, CH_3, d), 1.71 (1H, OH, s), 2.72 (2H, CH_2, m), 3.99 (1H, CH, m), 7.25 (5H, Ph, m). The e.e. determined by GLC was 98.4%. Temperature program for the GLC chiral analysis on the MEGA column: 40°C 1 min, 20°C/min, 75°C 2 min, 1°C/min, 113°C, 10°C/min, 210°C; injector 250°C, detector 250°C. Retention times: (*S*)-**4** 35.50, (*R*)-**4** 36.52 [racemic **4** was of commercial origin (Fluka)].

3.6. (+)-(R)-2-Benzylmorpholine 6

Ethanolamine-O-sulphate (17.5 g) was added to a mixture of **3** (4 g, 29.9 mmol), 16 M NaOH solution (9.5 g) and MeOH (6 ml) and the mixture stirred for 2 h at 40°C. To this mixture, solid NaOH (7.5 g) and toluene (26 ml) were added and the reaction heated at 65°C for 7 h. To the cooled mixture, 45 ml of water and 15 ml of toluene were added and the organic phase separated. The toluene was extracted with 2 N HCl and the acid extract was made alkaline to pH 11 with NaOH 4 M and extracted with toluene. The toluene was washed with brine, dried, and evaporated to an oil (4.5 g). Crude **6** was chromatographed on silica gel eluting first with ethyl acetate so as to obtain the N-substituted morpholine and then with ethyl acetate:EtOH:Et_3N (4:1:0.1) to obtain pure **6** (3.5 g, 19.8 mmol, 66% yield), $[\alpha]_D^{20}$ +1.32 (c 5, $CHCl_3$) and +28.99 (c 5, benzene), ^1H NMR δ ($CDCl_3$) 2.42 (1H, NH, s), 2.63 (2H, CH_2, m), 2.84 (4H, $2CH_2$, m), 3.64 (2H, CH_2, m) 3.88 (1H, CH, m) and 7.25 (5H, Ph, m).

3.7. (+)-(R)-2-Benzylmorpholine 6 hydrochloride

Compound **6** (3.5 g) was dissolved in diethyl ether (50 ml), and ethanol saturated with HCl (10 ml) was added. The precipitate was collected by filtration and crystallisation from ethanol–ether gave pure **6**·HCl, $[\alpha]_D^{20}$ +13.8 (c 5, 2 N HCl), (lit.[23] +8.4), m.p. 153, ^1H NMR δ (DMSO-d_6) 3.40 (9H, m), 7.22 (5H, m) and 9.45 (1H, m). Anal. calcd for $C_{11}H_{16}NOCl$: C, 61.82; H, 7.55; N, 6.55. Found: C, 61.84; H, 7.51; N, 6.49.

3.8. MTPA derivatives of 6

The hydrochloride of **6** (50 mg) was suspended in dry pyridine (0.5 ml) and (*R*)-(+)-MTPA-Cl (60 mg) was added in one portion. The mixture was stirred for 18 h at room temperature. Pyridine was evaporated at reduced pressure and the crude residue used directly for the HPLC analysis. The sample was dissolved in hexane:*i*-PrOH:ethyl acetate (8:1:1) so as to obtain a solution of 1 mg/ml of both (*R*)-**6** and racemic **6**. 100 μL of this solution were injected into the HPLC column (Lichrospher Si60 5 μm, 25×4 mm, eluent *n*-hexane:*i*-PrOH, 95:5; flow 0.6 ml/min, retention times, 8.68 for the derivative of the (*R*) enantiomer and 9.96 for that of the (*S*) enantiomer) and no traces of the (*S*) enantiomer could be detected. The presence of the second enantiomer became detectable when 2 μl of racemic mixture was added to 100 μl of the solution containing the single enantiomer.

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