ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 11 (2001) 367–371

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

www.elsevier.com/locate/molcatb

Asymmetric bioreduction of racemic 5,6,7,8-tetrahydro-8-methyl-1,3-dimethoxynaphthalen-6-one to the corresponding chiral β-tetralols

Gabriella Aina, Gianluca Nasini, Orso Vajna de Pava *

Dipartimento di Chimica, Politecnico, Centro di Studio del CNR sulle Sostanze Organiche Naturali, Via Mancinelli 7, 20121 Milan, Italy

Abstract

The synthesis of racemic 5,6,7,8-tetrahydro-8-methyl-1,3-dimethoxynaphthalen-6-one **1** was performed and the bioreduction to the corresponding β-tetralols was studied with respect to the stereochemical course and optical purity of the products; in particular the 6*S*,8*S* enantiomer corresponding to the dimethyl derivative of the natural compound feroxidin was isolated. The biomass of: *Aspergillus niger*, *Cladosporium cladosporioides*, *Candida lypolitica*, *Bacillus megatherium*, *Rhodotorula minuta*, *R. flava*, *R. rubra*, *Beauveria bassiana* and Baker's yeast were used as biocatalysts. Relative and absolute configurations of the obtained β-tetralols were established by comparison with those of the natural feroxidin.

Keywords: Bioreduction; Microbial screening; β-tetralols; Enantiomeric purity

1. Introduction

The α- and β-tetralols (1,2,3,4-tetrahydro-1-naphthols) and their derivatives are useful synthons for drugs with dopaminergic agonistic activity. Of the possible optical isomers, only one is active [1]. Feroxidin (6*S*,8*S*-5,6,7,8-tetrahydro-8-methyl-1,3,6-naphthalenetriol) is a natural tetralol isolated from *Aloe ferox* [2] as a pure enantiomer.

Due to the current interest in the synthesis of biologically active compounds in enantiomerically pure form, many microbial reductions of different hydroaromatic ketones were carried out. In particular, a 6-bromo-β-tetralone was reduced to the corresponding tetralol, precursor of an anti-arrhythmical drug, with high enantiomeric excess (ee) in gram quantities using a yeast as biocatalyst [3] Reduction of 4-substituted α- and β-tetralones was carried out with several microorganisms to give tetralols with good ee and conversion yields [4]. Rationalisation of the course of tetralones bioreduction is, at present, not available for all substrates: in some cases, structural effects due to substituents in various positions were not investigated because of the unavailability of the starting molecules. Recently, the reduction with Baker's yeast of β-tetralones to β-tetralols with variable yields was reported [5].

* Corresponding author. Tel.: +39-02-2399-3064; fax: +39-02-2399-3080.

E-mail address: vajna@dept.chem.polimi.it (O. Vajna de Pava).

1381-1177/01/$ - see front matter
PII: S1381-1177(00)00026-6

In this paper, we wish to report on the stereochemistry of reduction of 5,6,7,8-tetrahydro-8-methyl-1,3-dimethoxynaphthalen-6-one **1** to give with variable ee the corresponding tetralols and in particular, the dimethyl derivative **3a** of feroxidin, which occurs in *Aloe ferox* [5,6].

2. Results and discussion

β-tetralone **1** was synthesized by a Friedel–Crafts raction of 3,5-dimethoxyphenylacetyl chloride with propylene in the presence of $AlCl_3$ [7].The desired ketone **1** besides the open chain compounds **4** and **5** was obtained in low yields; the reaction was not optimized (Fig. 1).

From the reduction of ketone **1** with $NaBH_4$, a mixture of racemic *cis*- and *trans*-1,3-dimethoxyphenyl-6-hydroxy-8-methyltetralin **2** and **3** was obtained. Compound **3** was identified from the ^1H NMR spectrum (Table 1) as [5,6] dimethylferoxidin, while to compound **2**, the structure was assigned on the basis of the magnitude of the vicinal coupling constants exhibited by the 7-methylene protons with H-8 (7.8 and 8.2 vs. 1.9 and 5.9 Hz) because of the *cis* relationship of the OH and Me groups. HPLC chromatography using a chiral column showed that the reaction is in part diastereoselective. In fact, four peaks were detected, attributable to two couples of diastereoisomers **2a**,**b** ($t_R = 24$ and 37) and **3a**,**b** ($t_R = 32$ and 21.5) in 80:20 ratio; their absolute configuration was deduced by isolation of the pure enantiomer (6*R*,8*R*)-**3b** by preparative HPLC using

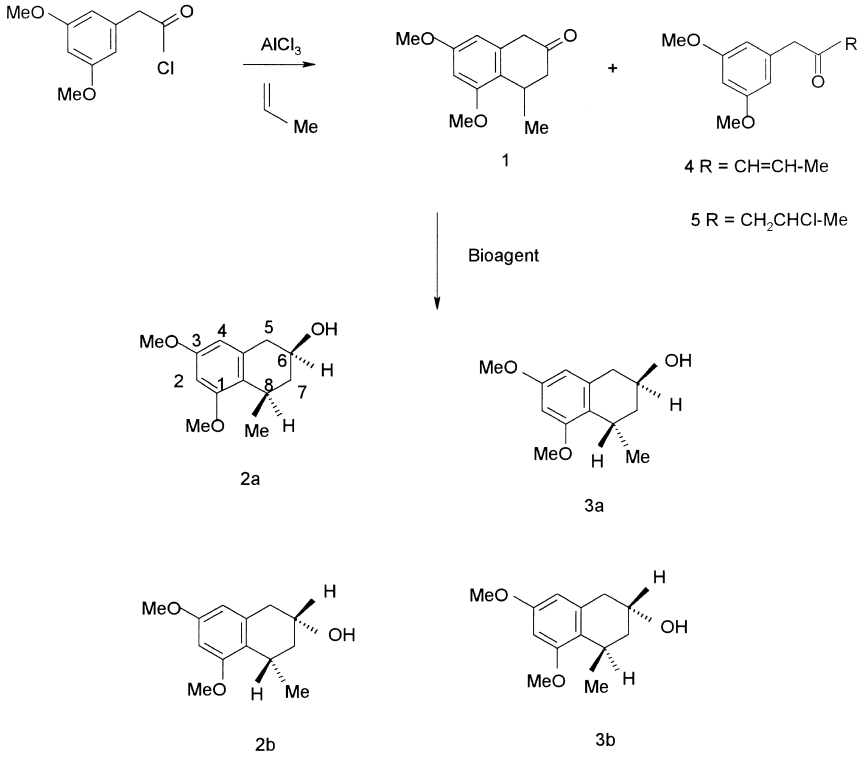

Fig. 1. Synthesis and bioreduction of tetralones.

Table 1
^{1}H NMR data of compounds **2a**, **b** in $CDCl_3$

	δ (ppm)	J (H,H) (Hz)	
H-2	6.32 (6.32)[a]	2,4	2.4 (2.4)
H-4	6.21 (6.21)	5ax,5eq	15.5 (15.5)
H-5ax	2.75 (2.63)	5ax,6	8.5 (9.2)
H-5eq	2.90 (2.81)	5eq,6	4.5 (4.5)
H-6	3.99 (3.80)	5eq,7eq	2.2 (2.3)
H-7ax	1.50 (1.38)	6,7ax	9.7 (10.3)
H-7eq	2.26 (2.24)	6,7eq	3.8 (3.8)
H-8	3.14 (3.08)	7ax,7eq	13.0 (13.0)
Me-8	1.31 (1.25)	7ax,8	7.6 (8.2)
OH	1.60 (4.90)	7eq,8	7.9 (7.8)
OMe	3.79 (3.76)	8,Me-8	6.8 (6.8)
OMe	3.77 (3.73)		

[a]Values in parentheses are chemical shifts and coupling constants in CD_3OD.

a chiral column (see Experimental Section). Compound **3b** with a retention time of 21.5 min shows an $[\alpha]_D$ value $+10.0°$ with opposite sign to natural dimethylferoxidin ($[\alpha]_D$ $-12.1°$) the absolute configuration of which is (6*S*,8*S*) [6] (Table 2). On the contrary, by reduction with *Aspergillus niger*, a mixture optically active *cis*-diastereoisomers **2b**(62%, ee 92%) and *trans*-diastereoisomers **3b**(38%, ee 40%) was obtained, the predominant *trans*-enantiomer was identified as the previously isolated (6*R*,8*R*)-**3b** $t_r = 21.5$ min, $[\alpha]_D$ $+10.0°$; the predominant *cis*-enantiomer was identified as compound (6*R*,8*S*)-**2b**, $t_R = 37$, because the enantiomeric composition of the unreacted ketone was *R*, $[\alpha]_D$ $+5°$ (ee 24%). In fact, diastereoisomers deriving from the prochiral ketone *R* (6*R*,8*R* and 6*S*,8*R*) accounts for the 29% while the products deriving from the prochiral ketone *S* (6*S*,8*S* and 6*R*,8*S*) constitute the major isomers (71%). This fact shows that the reductases of *A. niger* reduces enantiospecifically β-tetralones to 6*R* β-tetralols with low diastereoselectiviy. *Cladosporium cladosporioides* and *Candida lypolitica* are instead *trans*-diastereoselective giving rise respectively to the opposite enantiomers 6*S*,8*S* and 6*R*,8*R* with moderate ee (together with **2b**). *Rhodotorula rubra*, in accor-

Table 2
Bioreduction of racemic tetralone **1**

Bioagent	Medium[a]	Tetralol	ee (%)	Residual ketone	ee (%)	Conversion (%)
A. niger (IPV 283)	a	*cis*-(6*R*,8*S*) **2b** (62%)	92	*R*[b]	24	40
		trans-(6*R*,8*R*) **3b** (38%)	40			
C. cladosporioides (IPV F167)	d	*cis*-(6*R*,8*S*) **2b** (5%)	30	*R*	56	50
		trans-(6*S*,8*S*) **3a** (95%)	56			
Can. lypolitica (CBS 2074)	b	*cis*-(6*R*,8*S*) **2b** (14%)	29	*S*	20	35
		trans-(6*R*,8*R*) **3b** (86%)	34			
Bac. megatherium (DSM 32)	d	*cis*-(6*S*,8*R*) **2a** (58%)	68	*R*		35
		trans-(6*S*,8*S*) **3a** (42%)	60			
R. minuta (CBS 2177)	c	*cis*-(6*R*,8*S*) **2a** (22%)	18	*R*	20	34
		trans-(6*S*,8*S*) **3a** (78%)	28			
R. flava (ATCC 2503)	c	*cis*-(6*R*,8*S*) **2b** (38%)	12	*R*		22
		trans-(6*R*,8*R*) **3b** (62%)	15			
R. rubra (ATCC 10656)	c	*cis*-(6*R*,8*S*) **2a** (52%)	61	*S*	60	45
		trans-(6*R*,8*R*) **3a** (48%)	25			
B. bassiana (ATCC 7159)	a	*cis*-(6*S*,8*R*) **2a** (41%)	95	*S*	50	34
		trans-(6*R*,8*R*) **3b** (59%)	42			
Baker's yeast	e	*cis*-(6*R*,8*S*) **2b** (13%)	50	*S*	10	45
	8	*trans*-(6*R*,8*R*) **3b** (87%)	68			

IPV: Istituto di Patologia Vegetale Università degli Studi-Milano; CBS: Centraal Bureau vor Schimmelcultures-Baarn-Delft; DSM: Deutsche Sammlung von Mikroorganismen und Zellkulturen; ATCC: American Type Culture Collection; Baker's yeast: Eridania Lievito Stabilimento San Quirico Trecasali-PR.

[a]See Experimental.

[b]$[\alpha]_D + 5$ (c 0.36, methanol).

dance with data obtained for 4-methyl-1-tetralone [4], is enantiospecific but not diastereoselective; on the contrary, *Beauveria bassiana* is enantioselective giving rise to *cis* 6*S*,8*R* enantiomer with ee = 95%, but scarcely diastereoselective. Baker's yeast gives rise to the *trans*-diastereoisomer 6*R*,8*R* with an ee of 68%, this fact is in accordance with a previously reported reduction of mono and di-substituted 2-tetralones with Baker's yeast giving rise to 2-tetralols with usually moderate ee [5]. Reduction of prochiral ketones is dependent on the action of different reductases and from the stereochemistry of the substituents, in our case, probably the two faces of the molecule are very similar; therefore, ee of the reduction products are low. In conclusion, we obtained natural dimethylferoxidin with moderate ee, by bioreduction of tetralone **1** with *C. cladosporioides* and *Bacillus megatherium*; on the other hand, reductions of racemic tetralone with *A. niger* and *B. bassiana* gave rise to the 6*R*,8*S* and 6*S*,8*R* enantiomers, respectively with good yields and high ee (> 90%). Since diastereoisomeric tetralones are easily separated by column chromatography, this method allows the preparation of the four stereoisomeric tetralols.

3. Experimental

3.1. Analytical methods

HPLC analyses for compounds were performed on a LiChrograph L-6000A (Merck-Hitachi) equipped with a L-4000 Detector ($\lambda = 225$ nm) and D-2500 Integrator, using a Daicel Chiralcel OD 0.45 × 25 cm column with hexane-Pr^i-OH (95:5) as eluent (a) at a nominal flow rate of 0.5 cm^3 min^{-1} (fr), retention time (t_R). Optical rotations were obtained on a JASCO Dip-181 polarimeter and values are given in 10^{-1} deg cm^2 g^{-1}. NMR spectra were acquired on a Bruker AC 250L spectrometer operating at 250.1 MHz for ^{1}H. Chemical shifts are in ppm (δ) from $SiMe_4$ as internal standard, and J values are given in Hz. Mass spectra were obtained with a Finnigan-MAT TSQ70 spectrometer. TLC and PLC were performed with Merck HF_{254} silica gel.

3.1.1. Synthesis of 5,6,7,8-tetrahydro-8-methyl-1,3-dimethoxynaphthalen-6-one *1*

A mixture of oxalyl chloride (2 g) and 3,5-dimethoxyphenylacetic acid (0.5 g, 2.5 mmol) was stirred under nitrogen at room temperature for 2 h and then refluxed for 4 h. The excess of oxalyl chloride was removed from the cooled solution under vacuum and the reaction product was not further purified.

In a stirred suspension of aluminium chloride (0.16 g, 1.25 mmol) in dry methylene chloride (100 ml) under nitrogen, cooled to −20°C propylene was bubbled. Subsequently, under the flow of propylene, crude 3,5-dimethoxyphenylacetyl chloride, previously obtained, dissolved in methylene chloride (10 ml) was added dropwise in an hour. The mixture was poured over ice, extracted with methylene chloride and the extracts were washed with $NaHCO_3$ solution, dried over Na_2SO_4 and evaporated under reduced pressure. The crude extracts were chromatographed on silica gel using a mixture of hexane/ethyl acetate 60/40 as eluent to give tetralone **1** (110 mg), ^{1}H NMR ($CDCl_3$): δ 6.37 ad 6.23 (2H brd, $J = 2.5$ Hz, H-2 ad -4), 3.83 and 3.79 (6H, s, 2 × OMe), 3.68 (1H, m, H-8), 3.55 (2H, brs, H_2-5), 2.65 (1H, dd, $J = 15.5$ and 6.5 Hz, H-7a), 2.50 (1H, dd, $J = 15.5$ ad 2.5 Hz, H-7b), and 1.11 (3H, d, $J = 6.5$ Hz, Me-8); compound **4** (10 mg), ^{1}H NMR ($CDCl_3$): δ 6.93 (1H, dq, $J = 15.4$ and 6.2 Hz, H-2), 6.36 (2H, brs, H-2′ and 6′), 6.17 (1H, dq, $J = 15.4$ and 1.2 Hz, H-3), 3.78 (6H, s, 2 × OMe), 1.87 (3H, dd, $J = 6.2$ and 1.2 Hz, H_3-1); EIMS: m/z 254 [M^+]; compound **5** (50 mg), ^{1}H NMR ($CDCl_3$): δ 6.38 and 6.34 (3H, m, H-2′, -4′ and -6′), 4.45 (1H, m, H-2), 3.77 (6H, s, 2 × OMe), 3.72 and 3.62 (2H, d, $J = 17.6$ Hz, H_2-5), 3.0 (1H, dd, $J = 17.1$ ad 7.2 Hz, H-3a), 2.73 (1H, dd, $J = 17.1$ and 6.0 Hz, H-3b), and 1.48 (3H, d, $J = 6.1$ Hz, H_3-1); EIMS: m/z 256 [M^+].

3.1.2. Reduction of tetralone *1*

Tetralone **1** (50 mg) was treated with $NaBH_4$ (10 mg) in MeOH (5 cm^3); work-up gave a mixture 80:20 of two racemic compounds (40 mg) **2a**,**b** and **3a**,**b**, which may be separated by chiral HPLC: $t_R = 24$ (40%); 37 (40%); 32 (10%); 21.5 (10%); $[\alpha]_D$ −10.0 (c 0.05, CH_2Cl_2).

3.1.3. Microbial transformations

The strains were grown for 48 h at 28°C in shaken (180 rev^{-1}) Erlenmeyer flasks (100 ml) containing each 50 cm^3 of liquid medium: (a) MPG: malt extract (code 5397, Merck, D-61 Darmstadt) 20 g/l; peptone-M66 (code 7043, Merck, D-61 Darmstadt) 2 g/l; D-glucose (code 8346, Merck, D-61 Darmstadt) 30 g/l; (b) GYP: glucose 30 g/l; yeast extract (code 3753, Merck, D-61 Darmstadt) 30 g/l; peptone 10 g/l; (c) GYPM: glucose 30 g/l, yeast extract 20 g/l, peptone 20 g/l, malt 20 g/l; (d) YMP: yeast extract 3 g/l, malt 2 g/l, peptone 10 g/l.

Tetralone **1** (20 mg per flask) dissolved in DMSO (100 μl) was added to the grown culture and the incubation was continued for 3 days. For Baker's yeast reduction (e), a suspension of the same (1 g) in distilled water (100 cm^3) and glucose (3 g) was kept at 37 °C for 30 min, then the substrate dissolved in EtOH (0.5 ml) was gradually added to the mixture under stirring. Reduction was carried out in a phosphate buffer (6.8 g of NaH_2PO_4 in 1000 ml of aqueous solution pH = 6.5), bioconversion was stopped after 3 days because with longer times the substrate was metabolised by the micro-organisms.

Each resulting mixture was extracted with ethyl acetate, and the combined organic phases were dried and evaporated under reduced pressure; the residue was then subjected to PLC in hexane/EtOAc (2:1) to yield unchanged tetralone enantiomers and the obtained tetralols. The composition of the last was determined either by HPLC with a chiral column or by ^{1}H NMR techniques.

References

[1] J.G. Cannon, T. Lee, H.D. Goldman, J. Med. Chem. 20 (1977) 1111.
[2] G. Speranza, P. Manitto, D. Monti, F. Lianza, Tetrahedron Lett. 31 (1990) 3077.
[3] J. Reddy, D. Tschaen, Y.J. Shi, V. Pecpre, L. Katz, R. Greasham, M. Chartrain, J. Ferment. Bioeng. 81 (1996) 304.
[4] J.P. Beguè, C. Cerceau, A. Dogbeavou, A. Mathè, S. Sicsic, J. Chem. Soc., Perkin Trans. 1 (1992) 3141.
[5] P. Manitto, G. Speranza, D. Monti, G. Fontana, E. Panosetti, Tetrahedron 51 (1995) 11531.
[6] G. Speranza, P. Manitto, D. Monti, Chirality (1991) 263.
[7] J.J. Sims, L.H. Selman, M. Cadogan, Org. Synth. 51 (1971) 108.