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Microbial Reduction of Methyl-substituted Bicyclo[3.2.0]hept-3-en-6-ones : a Screening to Homochiral *endo*- and *exo*-Alcohols

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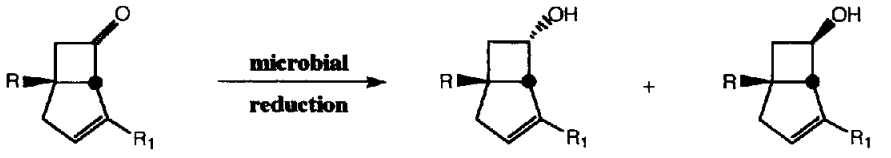
Key Words: Microbial reduction, Methyl-substituted bicyclo[3.2.0]hept-3-en-6-ones, Bicyclo[3.2.0]hept-2-en-6-one, Bicyclo[3.3.0]oct-7-en-2-one

Abstract: Various yeast and mould strains were tested in the microbial reduction of methyl-substituted bicyclo[3.2.0]hept-3-en-6-ones **1a-c**. The *endo*-alcohols **2a-c** were obtained with good yields and enantiomeric excess. Lower yields are described for the *exo*-alcohols **3a-c** which are normally enantiomerically pure. Comparisons with microbial reduction of bicyclo[3.2.0]hept-2-en-6-one **1d** and bicyclo[3.3.0]oct-7-en-2-one **1e** are also reported.

Bicyclic compounds with different functionalities in each ring are suitable for the stereocontrolled synthesis of wide variety of natural products. In this field bicyclo[3.2.0]hept-2-en-6-one and its derivatives are important starting materials for the synthesis of prostaglandins,[1] bicyclo[3.2.0]hept-3-en-6-ones are used to obtain pheromones as grandisol and lineatin,[2] and *endo*-bicyclo[3.3.0]oct-7-en-2-ol is well known as an inexpensive starting material for cyclopentanoid natural products.[3] Owing their importance many efforts have been undertaken in the preparation of enantiomerically pure bicyclo[3.2.0]hept-2-en-6-one and bicyclo[3.3.0]oct-7-en-2-one: reduction of the ketones with microrganisms[4] and enzymes[5,6] and resolution of the corresponding *endo*-alcohol with lipases,[7,8] or by chemical method[9]. Moreover, recently we reported the kinetic resolution of these *endo*-bicyclic alcohols *via* oxidation with *Bacillus stearothermophilus*.[10] Since bicyclo[3.2.0]hept-3-en-6-ones, only recently prepared by bicyclization of 3-hydroxy-6-alkenoic acids with potassium acetate in acetic anhydride,[2] are interesting building blocks in the synthesis of different natural products, their synthetic potential will be greatly enhanced if a procedure to have them enantiomerically pure would be developed. Since the mechanism of bicyclization precludes the EPC synthesis starting from the enantiomerically pure 3-hydroxy-6-alkenoic acids,[11] in the present work we describe the microbial reduction of methyl-substituted bicyclic hept-3-en-6-ones **1a-c** using a selection of yeast and mould strains[12] (Scheme).

Scheme

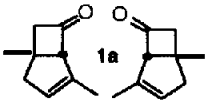
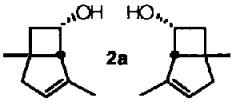
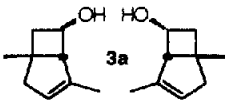
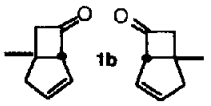
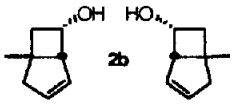
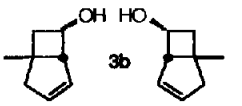


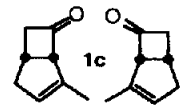
1a-c *endo*-**2a-c** *exo*-**3a-c**

a; R = R_1 = Me **b**; R = Me, R_1 = H **c**; R = H; R_1 = Me

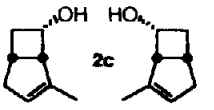
Similar approach has been used also for bicyclo[3.2.0]hept-2-en-6-one **1d** and bicyclo[3.3.0]oct-7-en-2-one **1e**. The results are summarized in the Table.

Table. Microbial reduction of bicyclic compounds **1a-e**.

yield % **1** (ee%)[a]		microrganism[b]	yield %*endo*-**2** (ee%)[a]		yield %*exo*-**3** (ee%)[a]	
		microbial reduction				
(-)-1R,5S[c]	(+)-1S,5R[c]		(-)-1R,5S,6S[c]	(+)-1S,5R,6R	(-)-1R,5S,6R	(+)-1S,5R,6S
	13(54)	*Saccharom. cerev.* RM1	46(100)			12(100)
36(0)		*Saccharom. cerev.* RM74[d]	3(100)			31(100)
	25(100)	*Yarrowia lipolytica* Y2[d]	48(100)			7(14)
	26(100)	*Yarrowia lipolytica* Y9	43(100)			6(2)
25(24)		*Fusarium*		28(38)		7(100)
	4(100)	*Thricoderma sp.*	46(98)			20(100)
	40(44)	*Penicillum roqueforti*	30(100)			3(100)
		microbial reduction				
(-)-1R,5R	(+)-1S,5S[e]		(-)-1R,5R,6S[e]	(+)-1S,5S,6R	(-)-1R,5R,6R	(+)-1S,5S,6S[e]
		Saccharom. cerev. RM1	72(80)			13(100)
	15(99)	*Saccharom.cerev.* ML31[d]	58(95)			13(97)
		Mucor spirescens	70(70)			15(100)
		Thricoderma sp.	77(36)			8(100)



microbial reduction



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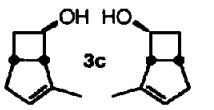
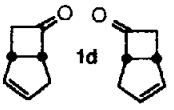
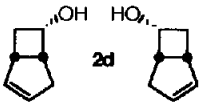


(-)-1R,5S[c]	(+)-1S,5R[c]		(-)-1R,5S,6S[c]	(+)-1S,5R,6R[c]	(-)-1R,5S,6R	(+)-1S,5R,6S
	29(50)	*Saccharom. cerev.* RM1	60(40)			
	27(25)	*Saccharom.cerev.* ML31	54(50)			9(100)
	45(22)	*Mucor spirescens*	43(20)			2(100)
	21(72)	*Thricoderma sp.*[d]	63(80)			5(100)



microbial reduction



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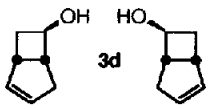
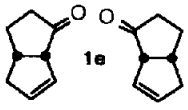
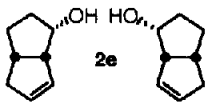


(-)-1S,5R[f]	(+)-1R,5S		(+)-1S,5R,6S[g]	(-)-1R,5S,6R[f]	(+)-1S,5R,6R	(-)-1R,5S,6S[g]
42(30)		*Saccharom. cerev.* RM1		27(70)		4(38)
29(2)		*Saccharom. cerev.* RM9	20(60)			16(94)
23(10)		*Saccharom.cerev.* ML31		52(16)		3(100)
	34(2)	*Kluveromyces lactis* CBS141	32(76)			5(8)
	34(10)	*Penicillum digitatum*	21(62)			8(100)
	35(8)	*Rhizopus nigricans*	22(66)			7(100)
	30(38)	*Thricoderma sp.*	32(72)			3(100)



microbial reduction



(-)-1S,5S[f]	(+)-1R,5R		(-)-1S,2S,5S	(+)-1R,2R,5R[f]
	30(86)	*Saccharom. cerev.* RM1	29(24)	
43(4)		*Saccharom.cerev.* ML31	26(28)	
	39(72)	*Yarrowia lipolytica* Y2	40(82)	
	18(2)	*Mucor spirescens*	46(20)	
	26(60)	*Thricoderma sp.*	73(30)	

[a] Yields and enantiomeric excesses are calculated by GLC on a chiral column using cyclohexanone as internal standard [b] Yeast and mould strains, except those labeled CBS, belong to DPVA (Dipartimento di Protezione e Valorizzazione Agroalimentare, University of Bologna, Italy) collection. [c] Ref. 14. [d] The reactions are repeated on preparative scale (see experimental). [e] The optical rotation are obtained by reduction with *Saccharom. cerev.* ML31 on preparative scale while the absolute configurations are assigned on the basis of the retention time in a homologous series. [e] Ref. 10. [f] Ref. 4.

The reactions are carried out through the incubation with a yeast or mould culture at 28°C for 48 h. The yeast or mould culture were grown in the presence of small amounts of the substrate solution in order to induce or activate the production of particular enzymes during the growth phase.

The microbial reduction of 1,4-dimethyl-bicyclo[3.2.0]hept-3-en-6-one **1a** produces the enantiomerically pure (-)-(*1R,5S,6S*)-*endo* alcohol **2a** (30-48%) and the (+)-(*1S,5R,6S*)-*exo* **3a** (20% and 31% by *Trichoderma* and *Saccharom. cerev.* RM74, respectively). Moreover, with *Yarr. lip.* Y2 and Y9 the recovered ketone (+)-**1a** is also resolved (ee 100%) and with *Fusarium* the *endo*-alcohol (+)-**2a** is prevalently produced (28%, ee 38%). The absolute configuration of (-)-**2a** is determined by single crystal X-ray diffraction of the compound obtained by esterification with (-)-(*1S,4R*)-camphanic acid chloride.[14] The absolute configuration of (+)-**3a** is assigned oxidizing the enantiomerically pure alcohol to the corresponding ketone (+)-**1a** ($[\alpha]_D$ = 745) and comparing the sign of the specific rotation with that of the ketone *1R,5S*-**1a** ($[\alpha]_D$ = -821 (c 0.96, $CHCl_3$)) obtained by oxidation of the enantiomerically pure (-)-**2a**.[14]

1-Methylbicyclo[3.2.0]hep-3-en-6-one **1b** is almost quantitatively reduced to (-)-*endo* alcohol **2b** (58-77%, ee 36-95%) and the pure (+)-*exo* alcohol **3b** (8-15%). The best results is obtained with *Saccharom. cerev.* ML31 that gives both the diastereomers enantiomerically pure and the ketone is also resolved. The absolute configurations of compounds **1b**, **2b** and **3b** have been assigned comparing the retention time of GLC on chiral column assuming that in a homologous series (**2a-c** and **3a-c**) the enantiomers with the lower retention time have the same configuration.

For the 4-methyl derivative **1c** the microbial reduction affords good yields of the (-)-*endo* **2c** (43-63%) but with lower enantiomeric excesses (20-80%) and small amounts (2-9%) of the (*1S,5R,6S*)-*exo* derivative **3c** (ee 100%). The absolute configuration of (-)-**2c** is determined, as for **2a**, by single crystal X-ray diffraction.[14] On the other hand the configuration of (+)-**3c** is assigned by chemical oxidation[15] to the corresponding ketone (+)-**1c** ($[\alpha]_D$ = 797.3) which is the same obtained from the oxidation of pure (+)-**2c**.[14] This reaction assignes also the *1S,5R* configuration to the ketone (+)-**1c**.

Similar approach has been used for the microbial reduction of bicyclo[3.2.0]hept-2-en-6-one **1d** but with poorer results: the (+)-*endo* alcohol **2d** is produced with low yields (14-32%) but with good e.e. (60-76%) together with small amounts (3-8%) of the pure (-)-*exo* alcohol **3d**. *Saccharom. cerev.* RM1 and ML31 are the only exceptions to this behaviour affording prevalently the (-)-*endo* -**2d** (27 and 52%, respectively) and in one case (for RM1 strain) with good ee (70%). On the other hand, only the (-)-*endo*-alcohol **2e** (26-70%) with not excellent ee (20-80%) is obtained by microbial reduction of bicyclo oct-7-en-2-one **1e**.

In conclusion, the microbial reduction of the selected bicycloheptenones **1a-d** and bicyclo octenone **1e** is enantioselective (always S-enantiomer) but not diastereoselective (both *endo* and *exo* diastereomers). The only exception is **1e**. However, it is worth mentioning that *Saccharomyces cerevisiae* RM1 (yeast) and *Trichoderma sp.* (mould), reducing all the substrates, can be used for the reduction of other bicyclic compounds.

Experimental

Optical rotations were measured on a Perkin Elmer Model 241 polarimeter. Gas chromatographic analyses were performed on a Carlo Erba GC 6000 Vega series 2. The bicyclo[3.2.0]hept-3-en-6-ones **1a-c**[2] are prepared according to the literature procedure. Bicyclo[3.2.0]hept-2-en-6-one **1d** is commercially available (Merck). The bicyclo[3.3.0]oct-7-en-2-one **1e** is prepared by oxidation with Jones' reagent[11] of the *endo*-alcohol **2e** obtained according the literature procedure.[13]

Enantiomer separation on Megadex 5 column (25 m X 0.25 mm) containing n-pentyl dimethyl β-cyclodextrin in OV 1701 from Mega s.n.c.: carrier gas: helium 0.8 atm; temp. 100-200°C (1.5°C/min). Retention time in min: (-)-**1a**, 7.09; (+)-**1a**, 7.39; (-)-*endo*-**2a**, 10.43; (+)-*endo*-**2a**, 10.78; (-)-*exo*-**3a**, 11.23; (+)-*exo*-**3a**, 11.59; (-)-**1b**, 5.83; (+)-**1b**, 6.14; (-)-*endo*-**2b**, 8.15; (+)-*endo*-**2b**, 8.64; (-)-*exo*-**3b**, 9.48; (+)-*exo*-**3b**, 9.82; (-)-**1c**, 9.12; (+)-**1c**, 9.44; (-)-*endo*-**2c** (as acetyl derivative), 14.10; (+)-*endo*-**2c** (as acetyl derivative), 14.51; (1R,5S,6S)-*exo*-**3c**, 14.15; (1S,5R,6S)-*exo*-**3c**, 14.53; (-)-**1d**, 5.74; (+)-**1d**, 5.97; (+)-*endo*-**2d** (as acetyl derivative), 10.99; (-)-*endo*-**2d** (as acetyl derivative), 11.73; (+)-*exo*-**3d** (as acetyl derivative), 9.29; (-)-*exo*-**3d** (as acetyl derivative), 9.40; (-)-**1e**, 10.28; (+)-**1e**, 10.54; (-)-*endo*-**2e** (as acetyl derivative), 8.53; (+)-*endo*-**2e** (as acetyl derivative), 8.75.

Screening of microbial reduction of ketones 1a-e. General Procedure. The synthetic culture medium is prepared dissolving in 1 L of water glucose (50 g), $(NH_4)_2SO_4$ (5g), KH_2PO_4 (2 g), $CaCl_2$ (0.25 g), $MgSO_4.7H_2O$ (0.25 g), inositol (25 mg), H_3BO_3 (1 mg), $ZnSO_4$ (1 mg), $MnCl_2$ (1 mg), $FeCl_2$ (0.5 mg), $CuSO_4$ (0.1 mg), tiamine (0.3 mg), biotine (0.025 mg), calcium pantothenate (0.3 mg), pyridoxine (0.3 mg) and nicotinic acid (0.3 mg). The culture medium (8 mL) is inoculated with a spore suspension (yeast or mould) and grown for 48 h in the presence of small amounts of the selected substrate (20 μL) (the solution is prepared dissolving 0.2 g of the selected substrate in 2 mL of DMSO). To a yeast or mould culture is added a further 80 μL of the substrate solution and the incubation is continued for a further 48 h at 28°C. The suspension is removed by centrifugation, the mixture is extracted with diethyl ether and dried over anhydrous Na_2SO_4. The crude reaction products are analyzed by GLC on a chiral column using cyclohexanone as internal standard. The most significative results are summarized in the Table.

Microbial reduction of the ketones 1a-c on preparative scale. General procedure. The reaction is carried out as above starting from 160 mL of culture medium, inoculated with the appropriate spore suspension, and 0.2 g of the selected ketone (see Table) dissolved in 2 mL of DMSO. The reaction mixture was extracted with diethyl ether (250 mL) with a continous liquid-liquid extractor, dried over anhydrous Na_2SO_4 and concentrated under vacuum. The products were purified by a short column (silica gel, petroleum ether/diethyl ether 80/20) to give the unreacted ketones and the mixture of the *endo*- and *exo*-alcohols (total yields 90%). GLC analyses on chiral column of the crude reaction mixture give the yields and the enantiomeric excesses (Table). The enantiomerically pure compounds were separated by flash chromatography (silica gel, n-pentane/diethyl ether 80/20).

(-)-*1R,5S,6S-endo*-1,4-Dimethylbicyclo[3.2.0]hept-3-en-6-ol (2a):[14] $[\alpha]_D$ = -104.5 (c 1.05, $CHCl_3$).

(+)-*1S,5R,6S-exo*-1,4-Dimethylbicyclo[3.2.0]hept-3-en-6-ol (3a): $[\alpha]_D$ = 2.93 (*c* 1.07, $CHCl_3$); oil; ^1H NMR (300 MHz, $CDCl_3$) δ 1.34 (s, 3 H), 1.73 (s, 3 H), 1.78 (ddd, 1 H, *J* = 1.9, 2.9 and 13.0 Hz), 2.18-2.26 (m, 2 H), 2.27 (ddd, 1 H, *J* = 1.2, 6.8, and 13.0 Hz), 2.53 (br s, 1 H), 3.1 (br s, 1 H, OH), 4.00 (ddd, 1 H, *J* = 1.9, 2.9 and 6.8 Hz), 5.28 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.30, 28.16, 40.55, 44.89, 48.08, 64.80, 71.79, 126.24, 140.40.

(-)-*1R,5R,6S-endo*-1-Methylbicyclo[3.2.0]hept-3-en-6-ol (2b): $[\alpha]_D$ = -165.3 (*c*, 3, $CHCl_3$); oil; ^1H NMR (300 MHz, $CDCl_3$) δ 1.25 (s, 3 H), 1.72 (dd, 1 H, *J* = 8, and 13 Hz), 1.8 (br s, 1 H), 2.14 (m, 3

H), 3.15 (m, 1 H), 4.45 (q, 1 H, J = 8 Hz), 5.74 (m, 1 H), 5.95 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.50, 35.89, 45.03, 48.45, 58.80, 67.33, 128.83, 135.12.

(+)-*1S,5S,6S-exo*-1-Methylbicyclo[3.2.0]hept-3-en-6-ol (3b): $[\alpha]_D$ = 82.3 (*c*, 1.2, $CHCl_3$); oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19 (s, 3 H), 1.71 (br s, 1 H), 1.80 (dt, 1 H, J = 2.5, and 13 Hz), 2.18-2.36 (m, 3 H), 3.97 (m, 1 H), 5.72 (s, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) 27.39, 39.22, 43.94, 47.75, 61.33, 70.05, 130.56, 132.00.

(-)-*1R,5S,6S-endo*-4-Methylbicyclo[3.2.0]hept-3-en-6-ol (2c)[14] : oil; $[\alpha]_D$ = -136.8 (*c* 1.065, $CHCl_3$).

(+)-*1S,5R,6S-exo*-4-Methylbicyclo[3.2.0]hept-3-en-6-ol (3c) :$[\alpha]_D$ = 12.7 (*c* 1.5, $CHCl_3$); oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.73 (s, 3 H), 2.00-2.18 (m, 4 H), 2.5-2.6 (m, 1 H), 2.92-3.06 (m, 1 H), 4.1 (ddd, 1 H, J = 1.6, 3.0, and 4.6 Hz), 5.35 (br s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.68, 32.21, 38.14, 39.62, 59.47, 73.81, 125.83, 139.49.

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