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Chemoenzymatic preparation of a biologically active naphthoquinone from *Tabebuia impetiginosa* using lipases or alcohol dehydrogenases

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ABSTRACT

Cancer chemopreventive agent (*S*)-5-hydroxy-2-(1-hydroxyethyl)naphtho[2,3-*b*]furan-4,9-dione and its counterpart (*R*)-acetate have been obtained through a lipase-catalyzed transesterification process in organic solvent. *Candida antarctica* lipase B and *Pseudomonas cepacia* lipase have demonstrated their potential as excellent biocatalysts for the production of enantiomerically pure compounds under mild reaction conditions. At the same time different commercially available alcohol dehydrogenases have been tested in the bioreduction of the corresponding naphthoquinone in an aqueous system. Biologically active (*S*)-alcohol has been isolated in enantiopure form with different conversion values depending on the biocatalyst employed and the reaction conditions.

1. Introduction

Tabebuia impetiginosa, also known as *Tabebuia avellanadae Lorentz ex Griseb*, or popularly called Pau d'arco, is a tree from the Bignoniaceae family distributed from northern of Mexico to northern of Argentina. It has been used with medicinal purposes for centuries and for example its inner bark has been employed in the treatment of pain, arthritis, inflammation of the prostate gland, fever, dysentery, boils, ulcers and a wide range of cancers [1], being nowadays commercialized as tablets, dried bark tea or tincture.

The isolation of secondary metabolites of this specie has been previously reported [2], identifying 5-hydroxy-2-(1-hydroxyethyl)naphtho[2,3-*b*]furan-4,9-dione (**1**) as an antitumor-active substance [3]. However as far as we now there are just a couple of examples describing the production of optically active (*S*)-5-hydroxy-2-(1-hydroxyethyl)naphtho[2,3-*b*]furan-4,9-dione (**1**), based on a preparative HPLC resolution [3] and alternatively a catalytic chemical approach relying on an asymmetric transfer hydrogenation process using a chiral ruthenium catalyst complex [4] (Fig. 1).

Nowadays biocatalysis is certainly considered as a useful tool for the preparation of optically active compounds. Along the last decades many strategies have been developed for the production of interesting intermediates for the fine chemical and pharmaceutical industry [5]. Lipases and oxidoreductases are by far the biocatalysts most widely employed in asymmetric biotransformations because of their versatility and simple work-up [6]. For this reason, in our ongoing project we have paid attention to the production of optically active naphthoquinones with applications in medicinal chemistry and herein we wish to report the chemoenzymatic synthesis of the cancer chemopreventive alcohol (*S*)-**1**, based on the use of biocatalyzed processes for the development of the stereoselective transformation. Lipases and alcohol dehydrogenases (ADHs), a class of oxidoreductases, have been tested in organic solvents or aqueous systems, respectively, for the preparation of 5-hydroxy-2-(1-hydroxyethyl)-naphtho[2,3-*b*]furan-4,9-dione (**1**) in enantiopure form.

2. Experimental

2.1. Materials and methods

Candida antarctica lipase type B (CAL-B, Novozyme 435, 7300 PLU/g) was a gift from Novo Nordisk Co. *Pseudomonas cepacia* lipase PSL-C I (1638 U/g) was acquired from Sigma–Aldrich. Alcohol dehydrogenases and glucose dehydrogenase were purchased

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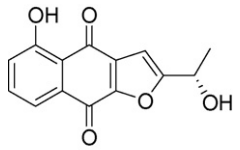


(*S*)-**1**

Fig. 1. Structure of antitumoral drug (*S*)-5-hydroxy-2-(1-hydroxyethyl) naphtho[2,3-*b*]furan-4,9-dione.

from Codexis. All other reagents were purchased from Acros Organics, Aldrich or Lancaster and used without further purification. Solvents were distilled over an adequate desiccant under nitrogen. *Flash* chromatographies were performed using silica gel 60 (230–240 mesh). Melting points were taken on samples in open capillary tubes and are uncorrected. IR spectra were recorded on using NaCl plates or KBr pellets in a Perkin-Elmer 1720-X FT. ^{1}H, ^{13}C NMR and DEPT were obtained using AC-300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz) or DPX-300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz) Bruker spectrometers. The chemical shifts are given in delta (δ) values and the coupling constants (J) in Hertz (Hz). HP1100 chromatograph mass detector was used to record mass spectra experiments (MS) through ESI$^+$ experiments and HRMS spectra data were acquired on a Shimadzu LCMS-IT-TOF (225-07100-34): electrospray quadrupole ion trap-time of flight mass spectrometer. Measurement of the optical rotation was done in a Perkin-Elmer 241 polarimeter.

High performance liquid chromatography (HPLC) analyses were carried out in a Hewlett Packard 1100 chromatograph UV detector at 210 nm using a Daicel Chiralcel OD (25 cm × 4.6 mm I.D.) varying the conditions depending on the specific substrate. For lipase-mediated acetylation reactions a Chiralcel OD column was used, employing a mixture of hexane/2-propanol (90:10) as eluent and a flow of 0.8 mL/min at 20 °C. Retention times are detailed: (a) acetate **8**: 23.2 for the (*S*)-enantiomer and 24.8 min for the (*R*)-enantiomer; (b) alcohol **1**: 29.3 for the (*R*)-enantiomer and 47.9 for the (*S*)-enantiomer. To analyze bioreduction reactions we used the same Chiralcel OD column but with an eluent composed by a mixture of hexane/2-propanol (85:15) and a 0.8 mL/min flow at 20 °C, obtaining the following retention times: (a) alcohol **1** 19.7 min for the (*R*)-enantiomer and 30.1 min for the (*S*)-enantiomer; (b) ketone **7** appeared at 41.3 min.

Gas chromatography (GC) analyses were performed on a Hewlett Packard 6890 Series II chromatograph equipped with a HP-1 column (30 m × 0.25 mm × 0.25 μm, 1.0 bar N_2) from Varian for the following of the reaction time courses: 150 °C (hold 5 min) 3 °C/min 210 °C (hold 10 min). Retention times were: (a) ketone 20.3 min; (b) alcohol 23.5 min; (c) acetate 25.8 min.

2.2. Preparation of 5-hydroxynaphthoquinone (*3*)

A solution of 1,5-dihydroxynaphthalene (1 g, 6.24 mmol) in a mixture of acetonitrile–water (2:1, v/v, 60 mL) was added dropwise PIFA (5.4 g, 12.48 mmol) in acetonitrile–water (2:1, v/v, 60 mL) under cooling 0 °C. The solution was stirred for 2 h, then the solvent was evaporated under reduced pressure and the resulting suspension was redissolved in H_2O and extracted with EtOAc (3× 200 mL). The organic layers were combined, dried over Na_2SO_4 and the solvent evaporated under reduced pressure. Reaction crude was finally purified by *flash* chromatography (50% CH_2Cl_2/hexane), yielding 5-hydroxynaphthoquinone as a yellow solid (58%). R_f (1/1 hexane/CH_2Cl_2): 0.42; Mp = 129–131 °C; IR (KBr): ν 3406, 3056, 2921, 1642, 1592, 1451, 1353, 1288, 1223, 1150, 1080, 839, 748 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 6.95 (2H, s), 7.29 (1H, dd, J_{HH} = 1.89 and 7.70 Hz), 7.61–7.67 (2H, m), 11.90 (1H, s); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ: 115.0, 119.1, 124.5, 131.8, 136.5, 138.6, 139.6, 161.5, 184.2, 190.3; MS (ESI$^+$, m/z): 175 [(M+H)$^+$, 100%], 174 (M$^+$, 18%).

2.3. Preparation of 2-(dimethylamino)-5-hydroxynaphthoquinone (*4*)

A solution of the naphthoquinone **3** (510 mg, 2.93 mmol) in toluene (30 mL) was bubbled gas of dimethylamine (10 min) under cooling −55 °C. The reaction was stirred at room temperature during 1 h until complete consumption of the starting material, and then the solvent was evaporated under reduced pressure. Reaction crude was purified by *flash* chromatography (100% CH_2Cl_2), yielding quinone **4** as a red solid (71%). R_f (CH_2Cl_2): 0.46; Mp = 144–146 °C; IR (KBr): ν 3485, 3068, 2930, 1671, 1618, 1565, 1471, 1408, 1348, 1278, 1233, 1153, 1049, 926, 860, 765 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 3.22 (6H, s), 5.66 (1H, s), 7.15 (1H, dd, J_{HH} = 2.3 and 7.2 Hz), 7.39–7.46 (2H, m), 12.93 (1H, s); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ: 42.7, 105.1, 114.6, 118.8, 124.3, 132.5, 133.7, 152.9, 160.3, 182.8, 187.9; MS (ESI$^+$, m/z): 218 [(M+H)$^+$, 100%].

2.4. Preparation of 2,5-dihydroxy-1,4-naphthalenedione (*5*)

A solution of **4** (391 mg, 1.79 mmol) in HCl 3N (2 mL) under reflux was stirred during 1 h until complete consumption of the starting material. The suspension was extracted with CH_2Cl_2 (3× 30 mL) and the solvent was evaporated under reduced pressure yielding compound **5** as an orange solid (95%). R_f (4/1 CH_2Cl_2/MeOH): 0.45; Mp = 216–219 °C (decomp.); IR (KBr): ν 3907, 3565, 3080, 2914, 2607, 1659, 1609, 1567, 1464, 1392, 1339, 1266, 1214, 1159,1086, 1049, 846, 785, 703 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 6.30 (1H, s), 7.26 (1H, s), 7.33 (1H, dd, J_{HH} = 1.2 and 8.4 Hz), 7.58 (1H, t, J_{HH} = 7.8 Hz), 7.69 (1H, dd, J_{HH} = 1.0 and 7.3 Hz), 12.32 (1H, s), ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ: 111.5, 116.5, 120.1, 126.5, 132.2, 134.7, 155.8, 161.7, 182.3, 192.3; MS (ESI$^+$, m/z): 191 [(M+H)$^+$, 100%], 190 (M$^+$, 14%).

2.5. Preparation of 2-acetyl-2,3,4,9-tetrahydronaphtho[2,3-b]furan-4,9-dione (*6*)

To a stirred solution of but-3-en-2-one (210 μL, 2.5 mmol) in pentane (2 mL) was added bromine (134 μL, 2.6 mmol) at −15 °C. After being stirred for 20 min the reaction was quenched evaporating pentane under reduced pressure. To a solution of 2,5-dihydroxy-1,4-naphthalenedione (100 mg, 0.53 mmol) in THF (5 mL) was added DBU (550 μL, 3.6 mmol) and stirred for 20 min at 0 °C, then the crude 3,4-dibromo-2-butanone was added into the solution under nitrogen atmosphere. The resulting red solution was stirred at room temperature for 2 h. DBU (75 μL, 0.49 mmol) was added again and stirring was continued for 4 h. The reaction was quenched by addition of an aqueous solution of ammonium chloride (5 mL) and the product was extracted with CH_2Cl_2 (3× 5 mL) then the solvent was evaporated under reduced pressure. Corresponding naphthoquinone **6** was isolated after flash chromatography (20% hexane/CH_2Cl_2) as a yellow solid (28%) and also naphthodihydrofuran **7** (37%). Data for compound **6** are R_f (92/8 CH_2Cl_2/EtOAc): 0.36; Mp = 141–143 °C; IR (KBr): ν 3381, 2920, 1717, 1677, 1634, 1450, 1352,1276, 1223, 1153, 1070, 1036, 874, 764 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 2.41 (3H, s), 3.40 (2H, d, J_{HH} = 9.7 Hz), 5.29 (1H, t, J_{HH} = 9.5 Hz), 7.27 (1H, dd, J_{HH} = 0.9 and 8.1 Hz), 7.57 (1H, t, J_{HH} = 7.9 Hz), 7.65 (1H, dd, J_{HH} = 1.0 and 7.3 Hz), 12.10 (1H, s); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ: 26.5, 29.5, 87.4, 114.7, 119.7, 123.5, 125.9, 131.7, 135.4, 159.9, 161.3, 176.4, 187.5, 204.2; MS (ESI$^+$, m/z): 259 [(M+H)$^+$, 100%].

Table 1
Stereoselective bioreduction of ketone **7** at 30 °C and 140 rpm during 24 h (1 U of enzyme per 5 mg of substrate).

Entry	ADH	ee_P (%)[a]	c (%)[b]	Config. alcohol **1**
1	T	>99	7.5	*S*
2	LB	>99	4	*R*
3	CP	>99	<3	*S*
4	PR2	–	<3	–
5	RS1	>99	16	*S*
6	A	>99	10	*S*

[a] Enantiomeric excesses of the optically active alcohol were determined by chiral HPLC.
[b] Conversion values were calculated by gas chromatography.

2.6. Preparation of 2-acetyl-4,9-dihydronaphtho [2,3-b]furan-4,9-dione (**7**)

A stirred solution of **6** (158 mg, 0.61 mmol) in CH_2Cl_2 (10 mL) was heated with MnO_2 (534 mg, 6.1 mmol) at reflux for 24 h. The mixture was filtered through a pad of celite and the filtrate was concentrated in vacuum. The residue was purified by silica chromatography using as eluent (4/1 CH_2Cl_2/hexane), affording ketone **7** (60%) as yellow crystals. R_f (92/8 CH_2Cl_2/EtOAc): 0.72; Mp = 229–231 °C; IR (KBr): ν 3368, 3098, 2920, 2110, 1668, 1642, 1567, 1446, 1350, 1291, 1236, 1196, 1070, 1027, 868, 834, 754 cm^{-1}; ^1H NMR ($CDCl_3$, 300.13 MHz) δ: 2.66 (3H, s), 7.34 (1H, dd, J_{HH} = 1.1 and 7.4 Hz), 7.60 (1H, s), 7.67 (1H, t, J_{HH} = 8.0 Hz), 7.82 (1H, dd, J_{HH} = 1.1 and 7.4 Hz), 12.1 (1H, s); ^{13}C NMR (DMSO, 75.5 MHz) δ: 26.7, 111.8, 118.3, 120.4, 125.9, 128.6, 129.7, 136.6, 151.9, 154.5, 161.1, 169.9, 170.6, 202.3; MS (ESI$^+$, m/z): 257 [(M+H)$^+$, 17%], 149 [(M−$C_6H_4O_2$)$^+$, 100%].

2.7. Typical procedures for the bioreduction of ketone **7** depending on the ADH used in the enzymatic process (see Tables 1 and 2)

2.7.1. ADH T
In an Eppendorf tube were added 2.5 μL (1 U) of enzyme, 5 mg of **7** (0.0194 mmol, 19.4 mM) and 700 μL of TRIS–HCl 0.1 M buffer of pH 7.0. Then 100 μL of a NADP$^+$ 10 mM solution were added as cofactor and 200 μL of 2-propanol for cofactor regeneration. Reaction was shaken at 140 rpm and 30 °C, and after 24 h the reaction was extracted with EtOAc (3× 400 μL) and dried over Na_2SO_4.

2.7.2. ADH LB
In an Eppendorf tube were added 1 μL (1 U) of enzyme, 5 mg of **7** (0.0194 mmol, 19.4 mM) and 600 μL of TRIS–HCl 0.1 M buffer of pH 7.0 and 100 μL $MgCl_2$ 10 mM. Then 100 μL of a NADP$^+$ 10 mM solution were added as cofactor and 200 μL of 2-propanol for cofactor regeneration. Reaction was shaken at 140 rpm and 30 °C, and after 24 h the reaction was extracted with (3× 400 μL) and dried over Na_2SO_4.

2.7.3. ADH CP
In an Eppendorf tube were added 3 μL (1 U) of enzyme, 5 mg of **7** (0.0194 mmol, 19.4 mM) and 700 μL of TRIS–HCl 0.1 M buffer of pH 7.0. Then 100 μL of a NADP$^+$ 10 mM solution were added as cofactor and 200 μL of 2-propanol for cofactor regeneration. Reaction was shaken at 140 rpm and 30 °C, and after 24 h the reaction was extracted with (3× 400 μL) and dried over Na_2SO_4.

2.7.4. ADH PR2
In an Eppendorf tube were added 7.7 mg (1 U) of enzyme, 5 mg of **7** (0.0194 mmol, 17.6 mM) and 800 μL of a phosphate buffer 0.1 M pH 7.5. Then 100 μL of a NADP$^+$ 10 mM solution were added as cofactor and 200 μL of 2-propanol for cofactor regeneration. Reaction was shaken at 140 rpm and 30 °C, and after 24 h the reaction was extracted with EtOAc (3× 400 μL) and dried over Na_2SO_4.

Table 2
Stereoselective bioreduction of ketone **7** at 30 °C and 140 rpm.

Entry	ADH	Enzyme (U)[a]	t (h)	ee_P (%)[b]	c (%)[c]	Config. alcohol **1**
1	LB	1	96	>99	9	*R*
2	RS1	1	96	>99	33	*S*
3	A	4	48	>99	92	*S*
4	A	4	96	>99	94	*S*

[a] Units of enzyme per 5 mg of substrate.
[b] Enantiomeric excesses of the optically active alcohol were determined by chiral HPLC.
[c] Conversion values were calculated by gas chromatography.

2.7.5. ADH RS1
In an Eppendorf tube were added 3.6 μL (1 U) of enzyme, 5 mg of **7** (0.0194 mmol, 17.6 mM), 10 mg of glucose (0.0555 mmol, 0.05 mM) and 1 mL of TRIS–HCl 0.1 M buffer of pH 7.0. Then 100 μL of a NADP$^+$ 10 mM solution were added as cofactor and 2 μL (1U) of glucose dehydrogenase for cofactor regeneration. Reaction was shaken at 140 rpm and 30 °C, and after 24 h the reaction was extracted with EtOAc (3× 400 μL) and dried over Na_2SO_4.

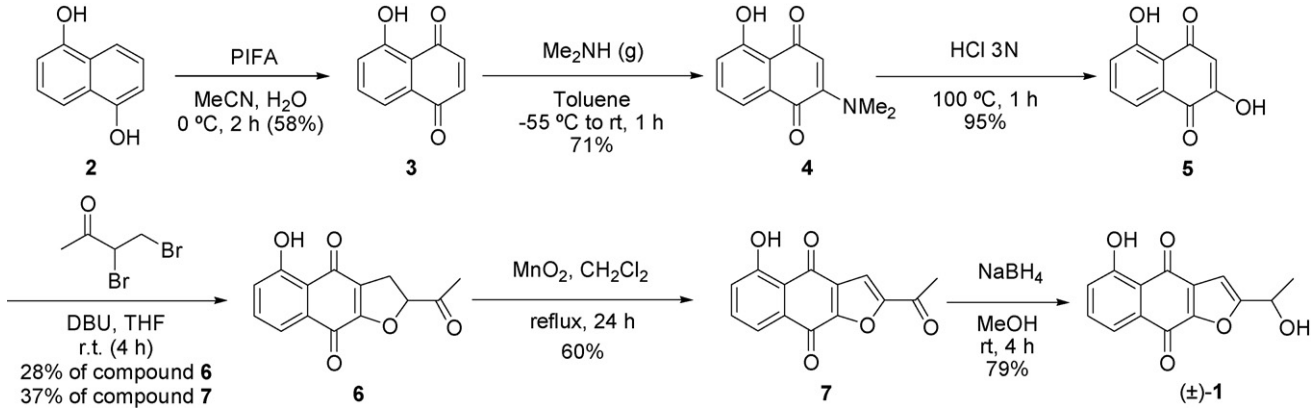
2.7.6. ADH A
In an Eppendorf tube were added 2 mg (4 U) of enzyme, 5 mg of **7** (0.0194 mmol, 16.2 mM) and 900 μL of TRIS–HCl 0.1 M buffer of pH 7.0. Then 100 μL of a NADP$^+$ 10 mM solution were added as cofactor and 200 μL of 2-propanol for cofactor regeneration. Reaction was shaken at 140 rpm and 30 °C, and after 24 h the reaction was extracted with EtOAc (3× 400 μL) and dried over Na_2SO_4.

2.8. Preparation of racemic 5-hydroxy-2-(1-hydroxyethyl)-naphtho[2,3-b]furan-4,9-dione (**1**)

To a solution of ketone **7** (134 mg, 0.52 mmol) in dry MeOH (5 mL) under nitrogen atmosphere, $NaBH_4$ (39 mg, 1 mmol) was added at 0 °C. The mixture was left to warm until room temperature and stirred for 1 h. Hydride excess was destroyed carefully adding HCl aqueous 1N (2 mL) and MeOH was then evaporated. The mixture was extracted with EtOAc (3× 2 mL). The organic layers were combined, dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The reaction crude was purified by *flash* chromatography (80% CH_2Cl_2/hexane) affording racemic alcohol **1** as a yellow solid (79%). R_f (92% CH_2Cl_2/EtOAc): 0.32; Mp = 167–169 °C; IR (KBr): ν 3387, 2914, 2847, 1736, 1636, 1530, 1444, 1371, 1303, 1214, 1153, 1064, 1024, 938, 862, 825, 753, 700 cm^{-1}; ^1H NMR ($CDCl_3$, 300.13 MHz) δ: 1.57 (3H, d, J_{HH} = 6.9 Hz), 2.94 (1H, s), 4.92 (1H, q, J_{HH} = 6.6 Hz), 5.26 (1H, s), 6.77 (1H, s), 7.20 (1H, dd, J_{HH} = 1.2 and 8.4 Hz), 7.55 (1H, t, J_{HH} = 7.8 Hz), 7.67 (1H, dd, J_{HH} = 0.9 and 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ: 21.1, 63.1, 103.1, 115.0, 119.9, 125.1, 131.1, 132.6, 136.2, 151.7, 161.9, 166.4, 172.8, 186.4; HRMS (ESI) m/z calcd for $C_{14}H_9O_5$ (M−H)$^-$: 257.0450. Found 257.0462. $[\alpha]_D^{20} = -23.4$ (c = 0.58, MeOH) for >99% ee.

2.9. Preparation of 1-(5-hydroxy-4,9-dioxo-4,9-dihydronaphtho [2,3-b]furan-2-yl) ethyl acetate (**8**)

Over a solution under nitrogen atmosphere of alcohol (±)-**1** (26 mg, 0.10 mmol) in dry CH_2Cl_2 (1.0 mL), Et_3N (20.8 μL, 0.15 mmol), DMAP (3.7 mg, 0.03 mmol) and Ac_2O (14.2 μL, 0.15 mmol) were successively added. The reaction was stirred at room temperature during 2 h, until complete consumption of the starting material was observed by TLC analysis (80%



Scheme 1. Chemical synthesis of racemic alcohol **1**.

Table 3
Lipase-catalyzed acetylation of (±)-**1** using vinyl acetate and THF at 30 °C and 250 rpm obtaining the (*S*)-alcohol **1** and the (*R*)-acetate **8**.

Entry	Lipase	*t* (h)	ee_S (%)[a]	ee_P (%)[a]	*c* (%)[b]	*E*[c]
1	CAL-B	1	92.5	>99	48	>200
2	CAL-B	2	>99(42)	>99(43)	50	>200
3	PSL-C I	1	72	>99	42	>200
4	PSL-C I	2	94.5	>99	49	>200
5	PSL-C I	3	>99(45)	>99(44)	50	>200

[a] Enantiomeric excesses were determined by chiral HPLC and isolated yields in brackets.
[b] $c = [ee_S/(ee_S + ee_P)]$.
[c] $E = \ln[(1-c)\times(1-ee_S)]/\ln[(1-c)\times(1+ee_S)]$ [12].

CH_2Cl_2/hexane). The solvent was then evaporated under reduced pressure affording a crude that was finally purified by *flash* chromatography (80% CH_2Cl_2/hexane), yielding (±)-**8** as a yellow solid (52%). R_f (80% CH_2Cl_2/hexane): 0.42; Mp 135–137 °C; IR (KBr): ν 3395, 3117, 2920, 1743, 1671, 1640, 1541, 1450, 1374, 1309, 1222, 1159, 1065, 1032, 935, 829, 755, 703 cm^{-1}; ^1H NMR ($CDCl_3$, 300.13 MHz) δ: 1.69 (3H, d, J_{HH} = 6.9 Hz), 2.13 (3H, s), 6.04 (1H, q, J_{HH} = 6.6 Hz), 6.88 (1H, s), 7.28 (1H, dd, J_{HH} = 0.6 and 8.4 Hz), 7.63 (1H, t, J_{HH} = 7.9 Hz), 7.77 (1H, dd, J_{HH} = 1.2 and 7.4 Hz), 12.1 (1H, s); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ: 18.5, 20.9, 64.8, 105.4, 115.2, 120.0, 125.3, 130.8, 132.7, 136.3, 152.3, 161.0, 162.3, 169.8, 172.6, 186.3; HRMS (ESI) *m*/*z* calcd for $C_{16}H_{13}O_6$ $(M+H)^+$: 301.0712. Found 301.0990. Calcd for $C_{16}H_{12}O_6Na$ $(M+Na)^+$: 323.0532. Found 323.0527. $[\alpha]_D^{20} = +134.4$ (*c* = 0.58, CH_2Cl_2) for >99% ee.

*2.10. Typical procedure for the enzymatic kinetic resolution of racemic alcohol **1***

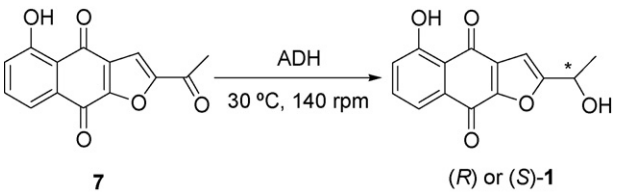
To a solution of alcohol (±)-**8** (25 mg, 0.10 mmol) in dry THF (1.0 mL) was added the corresponding enzyme (CAL-B or PSL-C I, ratio 1:1 in weight respect to the alcohol), vinyl acetate (17.2 μL, 0.20 mmol) was added and the reaction was shaken at 30 °C and 250 rpm. Aliquots were regularly analyzed by HPLC until conversion value reached around 50%, then the reaction was stopped, the enzyme filtered and the solvent was evaporated under reduce pressure. The reaction crude was finally purified by *flash* chromatography (eluent gradient 80% CH_2Cl_2/hexane and then 92% CH_2Cl_2/EtOAc) affording the corresponding optically active (*R*)-**8** and (*S*)-**1**. See Table 3.

3. Results and discussion

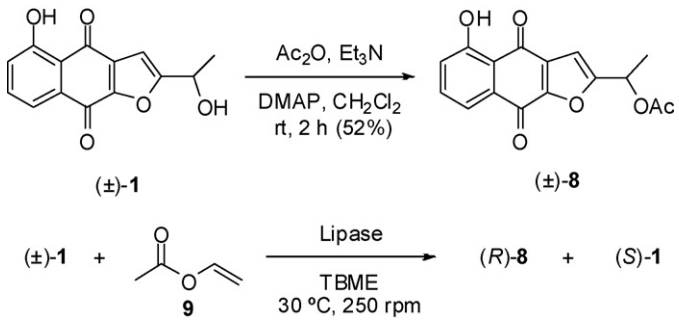
Initially we carried out the synthesis of the naphthofuran **7** starting from commercially available 1,5-dihydroxynaphthalene **2**, following in most of the cases adapting procedures already described in the literature. Thus, the chemoselective oxidation of **2** using bis(trifluoroacetoxy)iodobenzene in a system acetonitrile–water [7] led to the quinone **3** in moderate yield (Scheme 1) [8]. Dimethylamine gaseous was bubbled over a solution of 5-hydroxynaphthoquinone in toluene at −55 °C leading to **4** in 71% isolated yield [9]. Hydrolysis of **4** using an aqueous solution of HCl at 100 °C allowed the isolation of **5** in almost quantitative yield. Then the synthesis of **7** was performed in a similar manner than Hagiwara et al. described [10]. Reaction of **5** with 3,4-dibromobutan-2-one generated *in situ* from but-3-en-2-one and bromine, led in the presence of 8-diazabicyclo[5.4.0]undec-7-ene (DBU) to a mixture of furans **6** and **7**. It must be highlighted the importance to perform the reaction in short reaction times as longer periods led to decomposition products as major compounds in the reaction crude. Then the recovered compound **6** was selectively oxidized to **7** using MnO_2 and CH_2Cl_2 as solvent, obtaining the desired ketone in moderate yield, due to the fact that conversion was low, recovering a high amount of the starting material. Final chemical reduction employing sodium borohydride led to the isolation of racemic alcohol (±)-**1** in high yield.

In order to obtain enantiopure alcohol **1**, several bioreduction processes were carried out using different commercially available ADHs (Scheme 2). In all the reactions 1 unit (U) of biocatalyst was employed for 5 mg (0.02 mmol) of starting ketone **7**. Final data for the stereoselective reduction of ketone **7** when working at optimal conditions for each enzyme (see Section 2) are summarized in Table 1.

Low or moderate activities were found for the different ADHs, yielding alcohol **1** in enantiopure form in all cases except for ADH PR2, which led to the racemic alcohol (entry 4). From all the enzymes tested, ADH RS1 (entry 5) and ADH A (entry 6) showed the highest activity values, reaching 16 and 10% conversion value, respectively, after 24 h when the processes were carried out at 30 °C and 140 rpm. As expected, the (*S*)-enantiomer of alcohol **1** was achieved for the four Prelog ADHs [11] (entries 1, 3, 5 and 6) while an opposite stereopreference was observed for ADH LB, unique oxi-



Scheme 2. Bioreduction of **7** using ADH in aqueous systems.



Scheme 3. Synthesis of racemic acetate **8** and lipase-catalyzed transesterification processes of alcohol **1**.

doreductase that allowed the recovery of the alcohol (*R*)-**1** as shown in entry 2.

From this starting point, ADHs A, RS1 and LB were selected for further optimization in order to obtain higher conversion values, studying the effect of longer reaction times or more units of enzyme in the bioreduction process (Table 2). A slight increase in the conversion values was observed, yielding again in all cases the enantiopure (*R*)- or (*S*)-alcohol **1**. Thus, comparing the conversion values reached by ADH LB and ADH RS1 at 24 h (entries 2 and 5 of Table 1) with the results obtained after 4 days of reaction (entries 1 and 2 of Table 2), it can be observed that higher conversion of alcohol was obtained although far from an optimal synthetic point of view (maximum obtained 33% for ADH RS1). Oxidoreductases require redox cofactors in their mechanism action, being this issue and the corresponding cofactor recycling of crucial importance for the economy of the enzymatic process. Taking advantage of the easier and less expensive cofactor recycling system used by ADH A (isopropanol in a coupled-substrate method) *versus* ADH RS1 (glucose dehydrogenase in a coupled-enzyme method), we decided next to optimize the bioreduction of the ketone **7** using ADH A. Thus, we increased the amount of enzyme from 1 unit (entry 6 of Table 1) to 4 units and the reaction time was raised in additional 24 h (entry 3 of Table 2), leading to a 92% conversion in the enantiopure (*S*)-alcohol **1**. Unfortunately, much longer reaction times had almost no influence in the conversion value, as shown in entry 4 after 96 h.

Once that the bioreduction of ketone **7** was extensively studied and optimized in the production of enantiopure alcohol (*S*)-**1**, we decided to move forward and explore the lipase-catalyzed acetylation of the racemic alcohol **1** (Scheme 3). Lipase-mediated kinetic acylations allow the recovery of both enantiomers of the alcohol, one as the starting material and the other as the acylated product. This fact presents a remarkable importance for the pharmaceutical industry because both isomers of a racemate often display opposite therapeutic effects.

For all these reasons, first of all, the racemic acetate **8** was chemically prepared, and reliable chiral HPLC conditions were developed for the separation of alcohol (±)-**1** and acetate (±)-**8** enantiomers, in order to follow the reaction time courses (see Section 2). Enzymatic processes were carried out using two equivalents of vinyl acetate (**9**) and *Candida antarctica* lipase B (CAL-B) or *Pseudomonas cepacia* lipase (PSL-C I) as biocatalysts. All the experimental data have been summarized in Table 3. Reactions were performed at 30 °C observing that both lipases showed a complete stereopreference for the acetylation of the (*R*)-isomer although a higher reaction rate was observed for CAL-B (entries 1 and 2) rather than PSL-C I (entries 3–5). In this manner 50% conversion values were reached at 2 h for CAL-B and 3 h for PSL-C I. Acetate (*R*)-**8** and alcohol (*S*)-**1** were both isolated in enantiopure form with very high isolated yields (42–45%) after *flash* chromatography.

Assignments of the absolute configuration for the (*S*)-alcohol **1** and (*R*)-acetate **8** were tentatively done considering that the substituents size of the chiral center for alcohol **1** can be accepted in the Kazlauskas' rule model [13]. That was confirmed by comparison of the optical rotation value for the alcohol isolated after the lipase-catalyzed process $\{[\alpha]_D^{20} = -23.4\ (c = 0.58, \text{MeOH})\}$ with the one previously reported in the literature for the (S)-alcohol $\{[\alpha]_D^{20} = -22.7\ (c = 0.58, \text{MeOH})\}$ [4].

4. Conclusions

In summary, we have chemoenzymatically prepared the antitumoral agent (*S*)-5-hydroxy-2-(1-hydroxyethyl)naphtho[2,3-*b*]furan-4,9-dione based on an enzymatic process using lipases or oxidoreductases for the production of optically active compounds. The use of both class of enzymes led to the chiral alcohol **1** in enantiopure form. The optimization of the reaction conditions for the biocatalyzed reduction of ketone **7** employing different ADHs has allowed us to obtain the (*S*)-alcohol with an excellent conversion. The biocatalyzed acetylation of the synthesized racemic alcohol (±)-**1** by two well known lipases led to the recovery in high isolated yields of the (*S*)-alcohol and the corresponding (*R*)-acetate with high enantioselectivities and conversions close to 50%. This new synthetic strategy avoids the use of transition metal catalysts as source of chirality.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcatb.2009.08.006.

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