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Asymmetric bioreduction of natural xenobiotic diketones by *Brassica napus* hairy roots

Alejandro A. Orden, Fabricio R. Bisogno, Diego A. Cifuente, Oscar S. Giordano, Marcela Kurina Sanz *

INTEQUI-CONICET, Área de Química Orgánica, Facultad de Química, Bioquímica y Farmacia, UNSL, Chacabuco y Pedernera, 5700 San Luis, Argentina

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Abstract

Enantioselective bioreduction of natural prochiral diketones and some chemical derivatives were mediated by *Brassica napus* hairy root cultures. The natural bioactive diketones 1-(5-acetyl-2-hydroxyphenyl)-3-methylbut-2-en-1-one (**1**) and 6-acetyl-2,2-dimethyl-2,3-dihydro-4*H*-chromen-4-one (**2**) were both transformed into 6-(1(*S*)-hydroxyethyl)-2,2-dimethyl-2,3-dihydro-4*H*-chromen-4-one (**4**) in high enantiomeric excesses (>97%) and with good biotransformation rates (>78%). The assignment of the absolute configuration of the new stereogenic center was established by ^{1}H NMR using modified Mosher's method. Moreover, *B. napus* hairy roots showed the ability to perform glycosylation of phenolic-hydroxyl groups. The natural ketone 1-(5-acetyl-2-hydroxyphenyl)-3-methylbutan-1-one (**3**) was biotransformed into 4-acetyl-2-(3-methylbutanoil)-phenyl-*O*-β-D-glucopyranoside (**5**). Additionally, the acetate derivative 4-(acetyl-2-(3-methylbut-2-enoyl)-phenylacetate (**6**) was hydrolyzed in the culture media and transformed into **4**. The *O*-methyl derivative 1-(5-acetyl-2-metoxyphenyl)-3-methylbut-2-en-1-one (**7**) was recovered untransformed.

Keywords: Enantioselective bioreduction; Glycosylation; *Brassica napus* hairy roots; Natural prochiral ketones

1. Introduction

The natural acetophenone derivatives 1-(5-acetyl-2-hydroxyphenyl)-3-methylbut-2-en-1-one (**1**), 6-acetyl-2,2-dimethyl-2,3-dihydro-4*H*-chromen-4-one (**2**), and 1-(5-acetyl-2-hydroxyphenyl)-3-methylbutan-1-one (**3**) were isolated from *Ophryosporus axilliflorus* (Griseb.) Hieron (Asteraceae). The former was also reported in *Argeratina altissima* L. (Eupatorieae) [1], while the second one was previously isolated in *Madia sativa* Molina (Asteraceae) and *Bedfordia salicina* DC. (Asteraceae) [2]. These and other related compounds showed anti-inflammatory activity [3]. Some attempts have been made in obtaining new derivatives by chemical transformations. However, an environmentally sensitive, milder, energy saving and regio- and stereo-selective method has been required to complement traditional approaches. Plant cell cultures, as well as fungi and bacteria, are considered to be useful biocatalysts for oxidation–reduction-like reactions of alcohols and ketones [4–8]. Hairy roots formed after the infection of wounded plant tissues by the soil bacteria *Agrobacterium rhizogenes*, have been widely used for both plant science research and production of highly valuable plant metabolites [9,10]. Compared with other plant cell and tissue culture systems, they exhibit two important characteristics that give them potential applicability in fine chemistry. The *A. rhizogenes* plasmid integration to the plant genome confers genetic stability and high growth rates in culture media without the addition of phytoregulators. Moreover, they are mechanically stronger than cell cultures, which is better for scaling up processes [11,12]. Consequently, hairy root cultures represent a convenient experimental system to use plant enzymes in xenobiotic biotransformations as it was recently reported in bioreductions mediated by *Daucus carota* hairy roots [13]. *Brassica napus* hairy root cultures have also been used for phytoremediation of 2,4-dichlorophenol [14].

In this work, we report for the first time the stereo- and regio-selective reduction of natural prochiral diketones into the corresponding (*S*)-1′-hydroxy compound catalyzed by *B. napus* hairy roots in high ee and conversion rates. Furthermore, some

* Corresponding author. Tel.: +54 2652 439909; fax: +54 2652 426711.
E-mail address: mkurina@unsl.edu.ar (M. Kurina Sanz).

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chemical derivatives were prepared in order to study the biocatalytic process towards different chemical moieties, as well as possible associated reactions.

2. Experimental

2.1. General methods

The NMR spectra were recorded on Bruker AC-200 or AMX-400 instruments. For ^{1}H NMR instruments were operated at 200.13 MHz or 400 MHz and for ^{13}C NMR at 50.23 MHz or 100 MHz. Chemical shifts are expressed in ppm values using TMS as an internal standard. Optical rotations were obtained on a Perkin-Elmer 341 polarimeter. Melting points were taken on a Leitz hot plate microscope and are uncorrected. Biotransformation reactions were monitored by GC (FID) analysis in a Perkin-Elmer Clarus 500 instrument equipped with an Elite 5-column (30 m, 0.25 mm i.d. and 0.25 μm d.f.) or a β-DEX-column (60 m, 0.25 mm i.d. and 0.25 μm d.f.). CC procedures were performed on silica gel G 70–230 mesh Merck. TLC was carried out on Si gel 60 F_{254} Merck (0.2 mm-thick plates).

2.2. Substrates and standards

2.2.1. Isolation of *1*–*3*

Compounds **1**–**3** were isolated from dried aerial parts *of O. axilliflorus* (Griseb.) Hieron as it was described in [3]. Comparison of their spectral data with those reported confirmed their structures.

*1-(5-Acetyl-2-hydroxyphenyl)-3-methylbut-2-en-1-one (**1**)*: Yellow crystals, mp 65–67 °C, ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.11 (3H, s, H-5), 2.26 (3H, s, H-4), 2.60 (3H, s, *Me*CO), 6.89 (1H, s, H-2), 7.02 (1H, d, J = 8.8 Hz, H-3′), 8.03 (1H, dd, J = 2.2, 8.8 Hz, H-4′), 8.48 (1H, d, J = 2.2 Hz, H-6′), 13.36 (1H, s, –O*H*). ^{13}C NMR (200 MHz, $CDCl_3$): δ 197.1 (s, C-1″), 195.9 (s, C-1), 167.1 (s, C-2′), 161.0 (s, C-3), 136.2 (d, C-4′), 131.9 (d, C-6′), 128.0 (s, C-5′), 120.2 (s, C-1′), 119.3 (d, C-2), 118.0 (d, C-3′), 28.1 (q, C-5), 25.9 (q, C-2″), 22.0 (q, C-4).

*6-Acetyl-2,2-dimethyl-2,3-dihydro-4H-chromen-4-one (**2**)*: White crystals, mp 94–96 °C, ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.50 (6H, s, H-3′, H-4′), 2.60 (3H, s, H-2′), 3.28 (2H, s, H-3), 7.00 (1H, d, J = 8.2 Hz, H-8), 8.15 (1H, dd, J = 2.0, 8.2 Hz, H-7), 8.45 (1H, d, J = 2.0, H-5). ^{13}C NMR (200 MHz, $CDCl_3$): δ 195.3 (s, C-4), 192.1 (s, C-1′), 162.8 (s, C-9), 134.3 (d, C-7), 129.2 (s, C-6), 127.0 (d, C-5), 118.8 (s, C-10), 118.6 (d, C-8), 80.0 (s, C-2), 48.1 (t, C-3), 26.2 (q, C-3′, C-4′), 26.3 (q, C-2′).

*1-(5-Acetyl-2-hydroxyphenyl)-3-methylbutan-1-one (**3**)*: White crystals, mp 64–66 °C, ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.06 (6H, d, J = 7.7 Hz, H-4, H-5), 2.33 (1H, m, H-3), 2.60 (3H, s, H-2″), 2.98 (2H, d, J = 7.7 Hz, H-2), 7.02 (1H, d, J = 9.3 Hz, H-3′), 8.06 (1H, dd, J = 2.0, 9.3 Hz, H-4′), 8.47 (1H, d, J = 2.0 Hz, H-6′), 13.4 (1H, s, –O*H*). ^{13}C NMR (200 MHz, $CDCl_3$): δ 206.8 (s, C-1″), 195.7 (s, C-1), 166.3 (s, C-2′), 136.1 (d, C-4′), 131.2 (d, C-6′), 128.5 (s, C-5′), 119.2 (s, C-1′), 118.7 (d, C-3′), 47.0 (t, C-2), 26.3 (d, C-3), 25.3 (q, C-2″), 22.7 (q, C-4, C-5).

2.2.2. Preparation of *6* and *7*

*Compound **6***: To a solution of **1** (100 mg, 0.46 mmol) in pyridine (2 ml) Ac_2O was added and incubated in darkness at room temperature for 24 h. The reaction mixture was washed with $CuSO_4$ 10%, extracted with Et_2O and dried over anhydrous Na_2SO_4. Chromatography over Si gel (*n*-hexane:EtOAc 8:2) yielded 107 mg of **6**.

*4-(Acetyl-2-(3-methylbut-2-enoyl)-phenylacetate (**6**)*: Oil, ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.02 (3H, s, H-5), 2.21 (3H, s, H-4), 2.33 (3H, s, *CH*$_3$–COO), 2.64 (3H, s, H-2″), 6.46 (1H, s, H-2), 7.21 (1H, d, J = 9.7 Hz, H-3′), 8.09 (1H, dd, J = 2.0, 9.7 Hz, H-4′), 8.25 (1H, d, J = 2.0 Hz, H-6′). ^{13}C NMR (200 MHz, $CDCl_3$): δ 196.2 (s, C-1″), 190.1 (s, C-1), 168.8 (–CH$_3$–*C*OO), 158.3 (s, C-3), 151.9 (s, C-2′), 134.7 (s, C-5′), 133.6 (s, C-1′), 132.0 (d, C-4′), 129.9 (d, C-6′), 123.7 (d, C-3′), 123.2 (d, C-2), 24.1 (q, *C*H$_3$–COO), 23.8 (q, C-5), 21.1 (q, C-2″), 20.9 (q, C-4).

*Compound **7***: A stirred suspension of **1** (100 mg, 0.46 mmol), K_2CO_3 (222 mg, 1.61 mmol), and dimethyl sulfate (172 mg, 1.36 mmol) in acetone (5 ml) was heated to gentle reflux for 2 h and then cooled at room temperature. The undissolved material was removed by filtration and the filter cake was washed with acetone. The combine filtrate was evaporated and chromatographed on Si gel (*n*-hexane:EtOAc 8:2) to afford 87 mg of a more polar compound (**7**).

*1-(5-Acetyl-2-metoxyphenyl)-3-methylbut-2-en-1-one (**7**)*: white crystals, mp 54–56 °C, ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.98 (3H, s, H-5), 2.24 (3H, s, H-4), 2.58 (3H, s, H-2″), 3.95 (3H, s, OMe), 6.57 (1H, s, H-2), 7.00 (1H, d, J = 8.8 Hz, H-3′), 8.07 (1H, dd, J = 2.6, 8.8 Hz, H-4′), 8.13 (1H, d, J = 2.6 Hz, H-6′). ^{13}C NMR (200 MHz, $CDCl_3$): δ 191.8 (s, C-1″), 161.2 (s, C-1), 157.0 (s, C-3), 133.1 (d, C-4′), 132.3 (d, C-6′), 131.0 (s, C-2′), 129.9 (s, C-5′, C-1′), 125.1 (d, C-3′), 111.5 (d, C-2), 56.5 (q, –O*C*H$_3$), 28.0 (q, C-5), 16.5 (q, C-2″), 11.5 (q, C-4).

2.2.3. Non-enzymatic preparation of *2*

Compound **1** (250 mg) was dissolved in 200 ml of pH 9 phosphate buffer solution, stirred at room temperature for 48 h and partitioned with EtOAc yielding 235 mg of compound **2**. Comparison of its spectral data with those reported confirmed its identity.

2.2.4. Preparation of the racemic mixture of ±6-(1-hydroxyethyl)-2,2-dimethyl-2,3-dihydro-4H-chromen-4-one

To a solution of **2** (100 mg, 0.46 mmol) in MeOH (10 ml) $NaBH_4$ was added (42 mg, 1.1 mmol) and the mixture was stirred at room temperature for 2 h. After work up with water, the aqueous layer was extracted with EtOAc. The organic phase was dried over Na_2SO_4. The solvent was removed in vacuum and the residue was purified by preparative TLC, eluting with *n*-hexane–EtOAc (5:5) to afford 47 mg of (*R*, *S*)-**2**.

2.3. Hairy root cultures

B. napus hairy roots were obtained by inoculation of sterile leaf explants with *A. rhizogenes* LBA 9402 as described previously by Agostini et al. [15]. They were subcultured in Murashige and Skoog (MS) liquid medium enriched with vita-

mins [16] and incubated in an orbital shaker at 120 rpm and 22 ± 2 °C under darkness.

2.4. Biotransformation procedures

Hairy roots (four tips) were transferred to fresh MS-media (30 ml) and incubated in the above-mentioned conditions. After 12 days, substrates (3 mg/flask) dissolved in DMF (50 μl) were added to the cultures. Blank assays without substrates and without hairy roots were carried out. The results are the media of the repetition of three experiments.

2.5. Biotransformation products isolation and structural determination

After 4 days of incubation, the filtered culture media were extracted with EtOAc, the solvent dried over Na_2SO_4 and removed in vacuo.

2.5.1. Bioreduction product

The residues were subjected to preparative TLC on Si gel, using *n*-hexane–EtOAc (7:3) to afford 25 mg (41% isolated yield) of compound **4**.

*6-(1-Hydroxyethyl)-2,2-dimethyl-2,3-dihydro-4H-chromen-4-one (**4**)*: Oil, $[\alpha]_D^{25} = +36.4°$ (acetone; *c* 1.1) ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.46 (6H, s, H-3′, H-4′), 1.49 (3H, d, *J* = 7.0 Hz, H-2′), 2.71 (2H, s, H-3), 4.87 (1H, q, *J* = 7.0 Hz, H-1′), 6.93 (1H, d, *J* = 8.9 Hz, H-8), 7.56 (1H, dd, *J* = 2.0, 8.9 Hz, H-7), 7.83 (1H, d, *J* = 2.0 Hz, H-5). ^{13}C NMR (200 MHz, $CDCl_3$): δ 192.5 (s, C-4), 159.1 (s, C-9), 138.5 (s, C-6), 133.0 (d, C-7), 122.8 (d, C-5), 120.3 (s, C-10), 118.5 (d, C-8), 79.0 (s, C-2), 69.5 (d, C-1′), 48.5 (t, C-3), 27.0 (q, C-3′, C-4′), 25.2 (q, C-2′).

2.5.2. Glycosylation product

Extraction was carried out as above described. The residues were subjected to preparative TLC on Si gel, using EtOAc as solvent to give 17 mg (16% isolated yield) of compound **5**.

*4-Acetyl-2-(3-methylbutanoyl)-phenyl-O-β-*D*-glucopyranoside (**5**)*: Pale yellow crystals which decomposed at >108 °C, $[\alpha]_D^{25} = -40.8°$ (MeOH; *c* 0.56). ^{1}H NMR (200 MHz, CD_3OD): δ 0.95 (6H, d, *J* = 6.8 Hz, H-4, H-5), 2.19 (1H, m, H-3), 2.58 (3H, s, H-2″), 3.0 (2H, dd, *J* = 3.5, 6.4 Hz, H-2), 7.38 (1H, d, *J* = 8.8 Hz, H-3′), 8.12 (1H, dd, *J* = 2.0, 8.8 Hz, H-4′), 8.16 (1H, d, *J* = 2.0 Hz, H-6′); D-Glucose: δ 3.45 (1H, m, H-5), 3.51 (3H, m, H-2, H-3, H-4), 3.70 (1H, d, *J* = 12.3 Hz, H-6b), 3.92 (1H, dd, *J* = 2.5, 12.3 Hz, H-6a), 5.18 (1H, d, *J* = 8.3 Hz, H-1). ^{13}C NMR (200 MHz, CD_3OD): δ 204.0 (s, C-1″), 198.2 (s, C-1), 161.0 (s, C-2′), 134.3 (d, C-4′), 132.5 (s, C-5′), 131.0 (d, C-6′), 130.5 (s, C-1′), 116.1 (d, C-3′), 54.4 (t, C-2), 27.2 (q, C-2″), 26.5 (d, C-3), 23.0 (q, C-4, C-5); D-Glucose: δ 108.1 (d, C-1), 75.0 (d, C-2), 78.0 (d, C-3), 71.1 (d, C-4), 78.2 (d, C-5), 62.5 (t, C-6).

The glycoside was hydrolyzed with 2 N HCl in dilute MeOH–H_2O (9:1) at 100 °C for 2 h. The reaction mixture was diluted with H_2O and neutralized with Ag_2CO_3. Solids were removed by filtering through Celite and the filtrate was extracted with EtOAc. The aqueous layer was lyophilized to give the sugar residue. The analysis of the sugar as TMSi derivative was performed using a FID *T* = 350 °C, T_1 = 160 °C, T_2 = 200 °C (Δ*t* = 8 °C/min). Carrier gas N_2 20 cm/seg. Standard of D-glucose (R_t = 6.8 min) was run in the same condition and the retention time of the sugar was in agreement with it.

2.6. Conversion rates analysis

Batches of 30 ml were harvested at different incubation times. Filtered culture media was extracted with EtOAc. The organic extracts were acetylated as it was described in Section 2.2.2. Aliquots of the residues (1 μl) were analyzed by GC–FID equipped with Elite-5 column (30 m, 0.25 mm i.d. and 0.25 μm d.f.) T_1 = 100 °C, T_2 = 320 °C (Δ*t* = 25 °C/min), injector *T* = 240 °C, carrier gas N_2 35 cm/seg, FID detector *T* = 350 °C. Compound **2** (R_t = 5.49 min) and **4** (R_t = 5.82 min).

2.7. Compound **4** C-1′ absolute stereochemistry determination

2.7.1. Preparation of (R)- and (S)-MTPA esters

To a H_2CCl_2 (30 μl) solution of alcohol **4** (5 mg, 22.7 μmol) was added (*R*)-(+)-methoxy-(trifluoromethyl)-phenyl acetic acid ((*R*)-(+)-MTPA acid) (6.37 mg, 27.24 μmol), (dimethylamino)-pyridine (DMPA) (0.13 mg, 1.14 μmol) and *N*,*N*′-dicyclohexylcarbodiimide (DCC) (3.26 mg, 15.9 μmol). The solution was allowed to stand at room temperature for 24 h. The residue obtained after evaporation of the solvent was applied to preparative TLC (Merck, Kieselgel 60, F_{254}, *n*-hexane–EtOAc (8:2)) to give the (*R*)-MPTA ester (11 mg, 95%). The (*S*)-(−)-MTPA ester was obtained in the same manner.

2.7.2. Absolute stereochemistry determination

The ^{1}H NMR data of the (*R*)- and (*S*)-MTPA esters were recorded on a Bruker AMX 400 spectrometer. The assignments were based using COSY spectrum.

*(S)-(MTPA) ester of **4***: ^{1}H NMR (400 MHz, $CDCl_3$) 1.56 (3H, d, *J* = 7.0 Hz, H-2′), 2.72 (2H, s, H-3), 6.07 (1H, q, *J* = 7.0 Hz, H-1′), 6.91 (1H, d, *J* = 8.9 Hz, H-8), 7.38 (1H, dd, *J* = 2.0, 8.9 Hz, H-7), 7.86 (1H, d, *J* = 2.0 Hz, H-5).

*(R)-(MTPA) ester of **4***: ^{1}H NMR (400 MHz, $CDCl_3$) 1.62 (3H, d, *J* = 7.0 Hz, H-2′), 2.71 (2H, s, H-3), 6.04 (1H, q, *J* = 7.0 Hz, H-1′), 6.85 (1H, d, *J* = 8.9 Hz, H-8), 7.35 (1H, dd, *J* = 2.0, 8.9 Hz, H-7), 7.77 (1H, d, *J* = 2.0 Hz, H-5).

2.8. GC analysis for determination of the enantiomeric excess (ee)

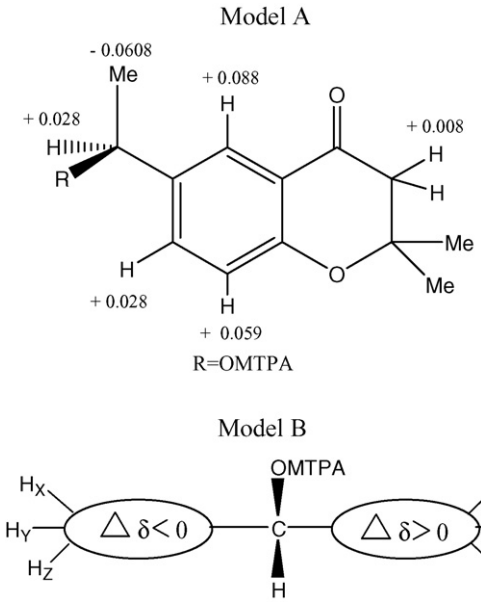
The reaction progress was monitored every 24 h by collecting 30 ml samples. These samples were extracted with EtOAc. The organic phase was derivatized as it was described in Section 2.2.2. The acetyl derivatives (1 μl) were analyzed by chiral GC on a β-DEX-column (60 m, 0.25 mm i.d. and 0.25 μm d.f.), injector *T* = 240 °C, oven *T* = 185 °C, FID *T* = 350 °C, Carrier gas N_2 27 cm/seg. The products of the biotransformation reaction were compared with a racemic mixture previously obtained

from chemical reduction (see Section 2.2.4). Retention times (*S*)-**4** = 82.87 min, (*R*)-**4** = 83.91 min.

3. Results and discussion

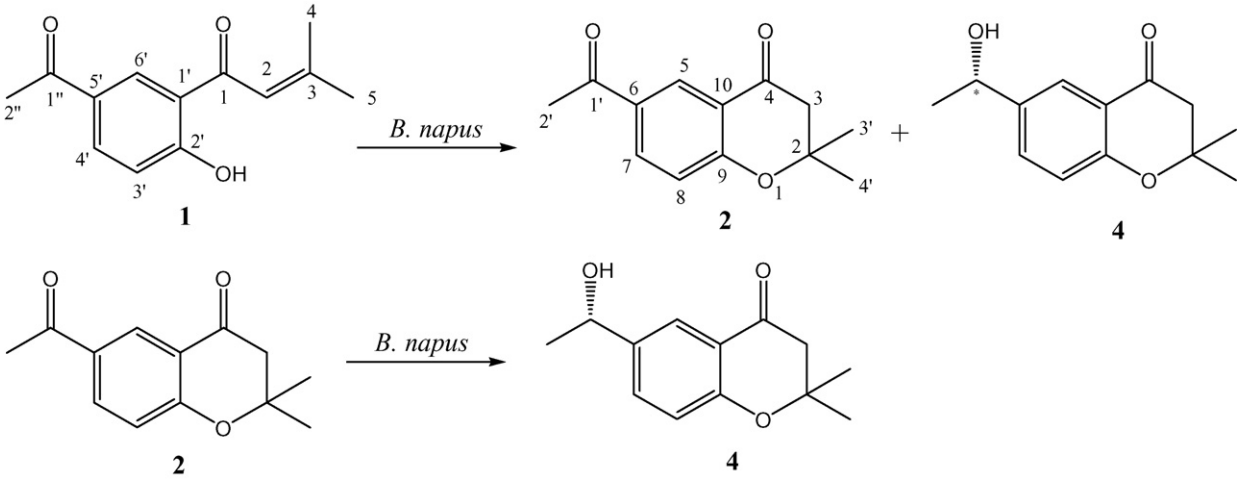
3.1. Biotransformation of *1*

The natural acetophenone derivative **1** shows two different prochiral ketone moieties attached to the phenolic ring, so it constitutes an interesting substrate for the screening of stereo- and regio-selective reductions. When it was incubated with 12-day-old hairy root cultures of *B. napus* for 4 days, two metabolites were obtained: compound **2** and the optically active compound **4**. At 96 h of incubation, substrate **1** was totally converted into bicyclic metabolites **2** and **4** with conversion rate 25% and 75%, respectively (Scheme 1). ^{1}H and ^{13}C NMR spectral data of the former agree with those reported for the natural product **2**. On the other hand, by comparison of ^{1}H NMR spectral data of the reduced metabolite **4** with the ones of the substrate **1** and compound **2**, it was evident the presence of a new signal corresponding to the new carbinolic proton at 4.87 ppm (1H, q, J = 7.0 Hz). In addition, the singlet at 2.60 ppm (3H, s) corresponding to the methyl group of the acetate moiety in both **1** and **2** spectra was here replaced by a doublet at 1.49 ppm (3H, d, J = 7.0 Hz). Moreover, in the ^{13}C NMR a new signal at 69.5 ppm due to the presence of the new carbinol chiral carbon replaces one of the oxygenated quaternary carbons at δ = 192 ppm. The signal corresponding to the olefinic proton in the ^{1}H NMR spectra of **1** at 6.89 ppm (1H, s) is now replaced by a singlet at 2.71 ppm (2H, s). These evidences prompted us to postulate structure of compound **4** as 6-(1-hydroxyethyl)-2,2-dimethyl-2,3-dihydro-4*H*-chromen-4-one. Although compound **4** was not detected in the phytochemical study of *O. axilliflorus*, its presence was previously reported in *Doranicum grandiflorum* Lam. (Asteraceae) [17] and *Trichogonia grazielae* (DC) Gardn. (Eupatorieae) [18]. Even though C-1′ absolute stereochemistry was not previously reported, the spectral data are in agreement with the ones here discussed.



Scheme 2. Models to elucidate the configuration at C-1′.

Modified Mosher's method, one of the most useful techniques among those employed to determinate the absolute configuration of organic substances [19–22], was applied to elucidate the absolute configuration of C-1′ in compound **4** since it was obtained as an oil. The (*R*)- and (*S*)-MTPA (methoxy-(trifluoromethyl)-phenyl acetic acid) esters of **4** were prepared in the presence of DCC and DMAP. ^{1}H NMR spectra of MTPA esters were recorded for calculation of anisotropic chemical differences ($\Delta\delta = \delta_S - \delta_R$) for each proton. The proton signals of the respective derivative were assigned using the COSY spectrum. The value of the chemical shift differences between the (*R*)- and the (*S*)-MTPA esters $\Delta\delta$ ($\delta_S - \delta_R$) (ppm) were calculated (model A, Scheme 2). The protons with positive $\Delta\delta$ were put on the right side and those with negative $\Delta\delta$ on the left side of model B (Scheme 2). The $\Delta\delta$ values for H-2′ were positive while negative $\Delta\delta$ values were observed for H-3, H-1′, H-8, H-7



Scheme 1. Biotransformation of **1** and **2**.

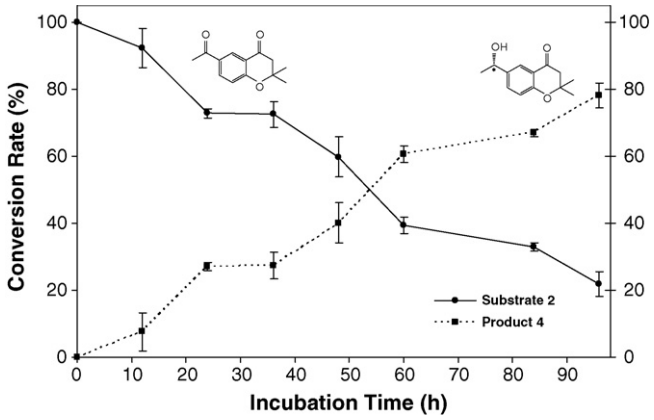


Fig. 1. Biotransformation of **2** by *B. napus* hairy roots.

and H-5. Thus, the absolute configuration at C-1′ was assigned to be *S*.

The observed enantioselectivity was in accordance with Prelog's rule, leading to (*S*)-**4** with an enantiomeric purity of 97.2% determinated by chiral GC. In parallel, using modified Mosher's method, it was possible to estimate ee values ≥97%, consistent with the GC chiral data.

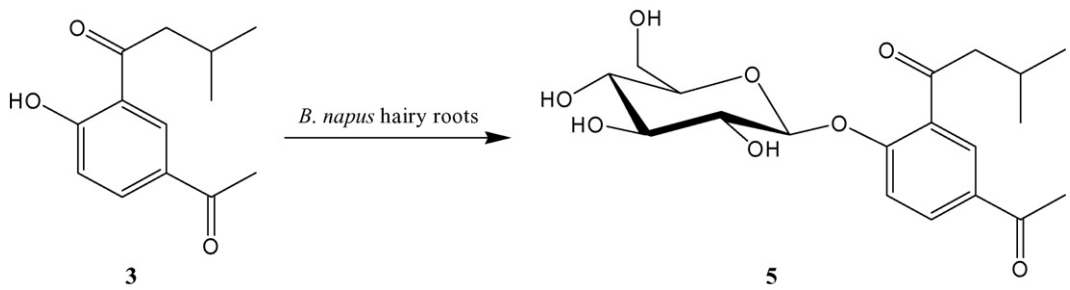
3.2. Biotransformation of *2*

On the basis of the above described biotransformation results, it is reasonable to propose that the cyclation of the substrate leading to the chromanone ring occurs previous to the reduction of the ketone moiety. It could even be attributed to an artifact rationalized through a prototropic Michael addition mechanism. In order to prove this hypothesis, the non-enzymatic cyclation of **1** in culture media without root development was studied. In these conditions, after 48 h of incubation compound **1** was transformed into **2** in a 100% yield. Moreover, compound **2** was prepared by stirring **1** in pH 9 phosphate buffer solution, isolated and inoculated to 12 days old hairy root cultures of *B. napus*. After incubation during 4 days, compound (*S*)-**4** resulted to be the only detected biotransformation product in the two procedures previously described (Scheme 1). Fig. 1 depicts the kinetic of the bioreduction of **2**. In accordance with the results observed in the biotransformation of **1**, conversion rates increased up to 78%. The value of the optical yield of (*S*)-**4** remained constant throughout the incubation time with an ee higher than 97%. These observations indicate that the bioreduction of **2** by *B. napus* hairy roots can take place stereoselectively. These results are in agreement with the ones reported for immobilized cells of *D. carota* [7].

3.3. Biotransformation of *3*

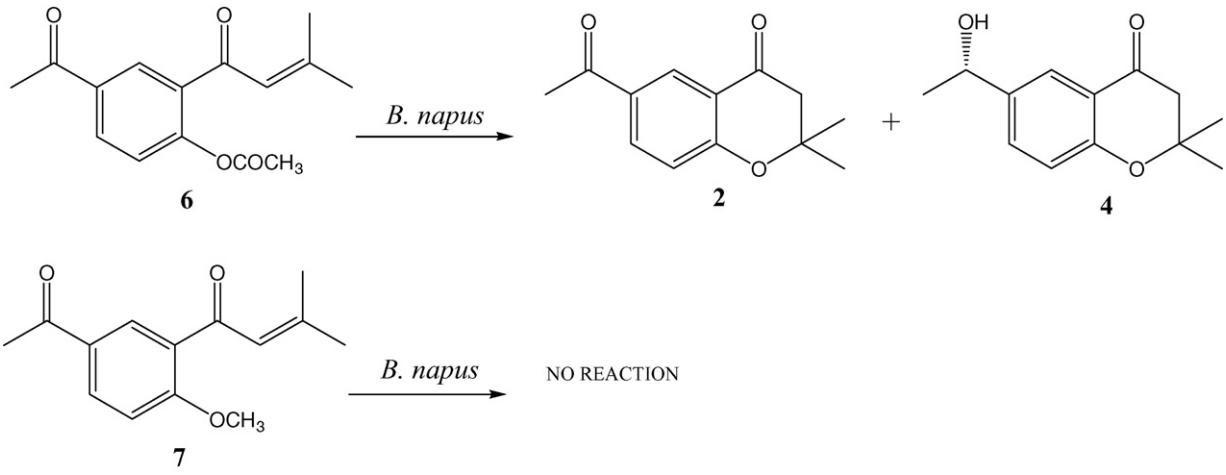
In order to study if the dehydrogenases system could also reduce hydroxyphenyl metabolites that cannot form the chroman-4-one derivative, compound **3** was purified from the plant extracts and added as substrate to *B. napus* hairy roots cultures in the above mentioned conditions. This metabolite does not possess a double bond in the side chain. Thus, it cannot undergo the intramolecular Michael's addition reaction. After 4 days of incubation, a more polar compound was isolated. Combinations of 1D and 2D NMR (COSY, DEPT and HETCOR) experiments allow us to deduce the structure of the new glycoside characterized as 4-acetyl-2-(3-methylbutanoil) phenyl-*O*-β-D-glucopyranoside (**5**) (Scheme 3). The ^{1}H NMR spectrum showed the absence of the described signals corresponding to the chromanone arrangement and the characteristic singlet at 13.4 ppm of the phenolic hydroxyl group. In addition, a typical doublet at 5.18 ppm (1H, d, $J = 8.3$ Hz) originated by an axial anomeric proton prompted us to think about the presence of a β-glycoside. The 2D COSY spectrum furnished evidence of cross-peaks between H-6a ($\delta = 3.92$, 1H, dd, $J = 12.3$, 2.5 Hz), H-6b ($\delta = 3.70$, 1H, d, $J = 12.3$ Hz), and H-5 ($\delta = 3.45$, 1H, m), and between H-2 together with H-3 and H-4 ($\delta = 3.5$, 3H, m), and the anomeric proton H-1 ($\delta = 5.18$, 1H, d, $J = 8.3$ Hz). After acid hydrolysis, the sugar residue of compound **5** (TMSi derivative) was confirmed as D-glucose by comparison with an authentic sample.

Although it has been postulated that the polymerization catalyzed by peroxidases would be the main mechanism in phytoremediation of phenol derivatives by *B. napus* hairy roots [13], the glycosylation here observed may contribute to the overall removal process. Glycosylation occurs in plant cell cultures and this ability has been widely reported for several suspension cultures such as the glycosylation of terpenes and phenolic compounds ([23,24] and references herein). Hairy roots have also been used in the glycoside-bond formation in low molecular weight alcohols [25] and the triterpene 18-β-glycyrrhetinic acid [26].



3 **5**

Scheme 3. Biotransformation of **3**.



Scheme 4. Biotransformation of **6** and **7**.

3.4. Biotransformation of *6* and *7*

With the aim of broadening the observation pointed out in the previous paragraphs, two new derivatives of **1** were prepared by blocking the phenolic hydroxyl group either forming an acetyl derivative (**6**) or an *O*-methyl compound (**7**). When the acetate ester, 4-(acetyl-2-(3-methylbut-2-enoyl)-phenylacetate (**6**), was added to the hairy root cultures, the already known chroman-4-ona type compounds **2** and (*S*)-**4** were recovered after 4 days of incubation (Scheme 4). This evidence suggests that the ester moiety was hydrolyzed under the culture conditions producing in situ compound **1**, which was converted into **2** and further biotransformed as it was above discussed.

Conversely, in the incubation of the ether derivative, 1-(5-acetyl-2-metoxyphenyl)-3-methylbut-2-en-1-one (**7**), the substrate was recovered unchanged from the culture media after 4 days. When incubation time was extended up to 10 days, no changes were observed.

4. Conclusions

At the best of our knowledge, this is the first report of the use of *B. napus* hairy roots cultures to reduce prochiral diketones with stereo- and regio-control. This system results in a good alternative to use plant enzymes because of the easy-handling of this type of cultures. The bioreduction system demonstrated interesting stereo- and regio-selection to the 5-acetyl group. The latter is a valuable characteristic to produce secondary alcohols with regiocontrol, not feasible to be obtained by chemical transformations using usual reductive reagents. In addition, the glycosylation of phenolic-hydroxyl group is another valuable reaction that may be applied on other phenolic compounds particularly in phytoremediation approaches.

In this case the *B. napus* hairy root-mediated-biotransformation represents a useful tool to obtain the new compound **5**, which was not reported to be found neither in *O. axilliflorus* nor by chemical transformation of its natural products.

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