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Microbiological reductions of chromen-4-one derivatives

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Abstract

From the microbiological reductions of 2-acetyl or 2-benzoylchromen-4-one both enantiomers of the corresponding alcohols were obtained with high enantiomeric excess. The absolute configurations were determined directly by an X-ray structural determination. The results obtained showed that for most of the microorganisms tested, an inversion of the configuration of the alcohol occurred with the change of the substituent (methyl to phenyl group) in position 2, but also with the presence of a bromine atom in position 6 of the aromatic ring, positioned quite far from the prochiral centre.

1. Introduction

The stereochemistry of amino alcohol groups is often the main factor responsible for the pharmacological activity of numerous drugs and neuromediators.[1] The role of the absolute configuration has been time and again established: for instance, (−)-adrenaline and (−)-ephedrine are far more efficient than their corresponding (+)-enantiomers.

Such a specificity has been widely used in the syntheses of aminoalcohol ligands of the β-receptors, which received great attention in the treatment of cardiovascular or respiratory diseases. It is well-known that benzopyrones are used with success in the treatment of asthma and miscellaneous allergies.[2]

Taking into account the antiallergic tropism of chromone derivatives, it seemed interesting to join this heterocycle and the aminoalcohol group in the same molecule. The synthetic scheme requires the initial obtaining of 2-hydroxyalkyl (or aryl) chromones by a regio- and, if possible, an enantioselective reduction of the prochiral exocyclic carbonyl of the chromones.

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With this main aim, we have studied the microbial reduction of compounds **1** to **4** by several strains of microorganisms (yeasts, fungi, bacteria) which have been previously shown to be able to reduce α-substituted ketones (Fig. 1).[3]

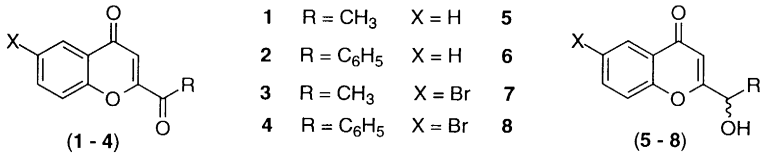
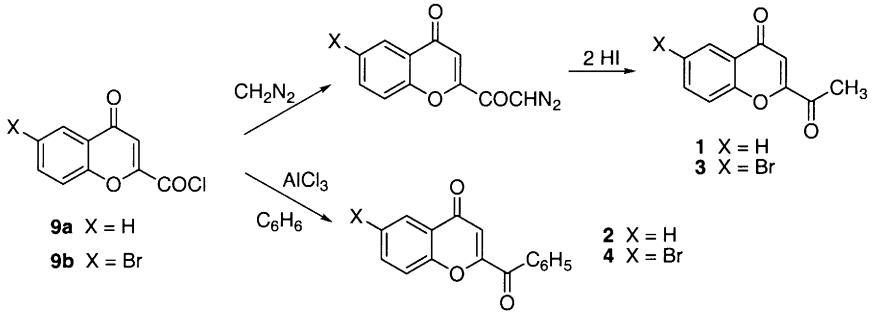


Fig. 1.

2. Results and discussion

2.1. Synthesis of the chromone derivatives *1* to *4*

Two different synthetic routes were used according to the substituent to be introduced on the chromone structure (acetyl or benzoyl group), but they were both starting from the same materials: the appropriate acyl chloride **9a** or **9b** (Scheme 1), whose synthesis has already been described.[4]



Scheme 1.

For the preparation of the acetylchromen-4-ones **1** and **3**, **9a** and **9b** were first converted into the corresponding diazoketones, which then reacted with 2 equiv. of hydriodic acid according to the Wolfrom method.[5]Compounds **1** and **3** were obtained in a 82%[4] and 30% overall yield, respectively. The presence of the bromine atom on the aromatic ring disturbed the second step whose yield was only 42% (instead of 89% without bromine).

Both benzoylchromen-4-ones **2** and **4** were obtained, with excellent yields (88% for **2** and 83% for **4**), by a Friedel–Crafts acylation using already published conditions.[6]

2.2. Microbiological reductions of *1* and *2*

The aim was to find microorganisms yielding each enantiomer of alcohol enantiomerically pure for each substrate. The strains of microorganism were selected according to our previous assays on various α-substituted ketones.[3] The yeast was used freeze-dried under non-fermenting conditions. Bioconversions with the other microorganisms were carried out using washed resting cells. The incubation time was 24 h. The results are reported in Table 1.

Chromones **1** to **4** possess three functions that could be reduced: the cyclic and/or exocyclic ketone, and/or the double bond. In fact, in our case, the reduction was highly regioselective: the sole exocyclic ketone function was reduced with all the microorganisms tested as proved by comparison with racemic

Table 1
Microbiological reductions of **1** and **2**

	Alcohol 5			Alcohol 6		
	$[\alpha]_D^{25}$	ee[a]	Yield[b]	$[\alpha]_D^{25}$	ee[a]	Yield[b]
Bakers' yeast	**- 60**	**≥ 98%**	**87%**	**+ 27**	**94%**	**60%**
Rhodotorula glutinis	**- 60**	**≥ 98%**	**83%**	+ 2	3%	40%
Zygosaccharomyces rouxii	**- 60**	**≥ 98%**	**80%**	+ 23	82%	46%
Yamadazyma farinosa (O_2)	- 58	97%	75%	+ 24	84%	48%
Yamadazyma farinosa (N_2)	+ 53	89%	50%	+ 18	62%	7%
Aspergillus niger	- 47	78%	51%	+ 0.5	0%	49%
Beauveria bassiana	- 13	8%	59%	- 22	79%	32%
Cunninghamella elegans	- 37	62%	50%	- 8[c]	30%	20%
Geotrichum candidum	- 60	≥ 98%	50%			
Lactobacillus kefiri	**+ 53**	**89%**	**58%**	**- 24**	**84%**	**33%**

[a] Determined by HPLC equipped with a Chiralcel OB column (see Experimental section).
[b] Yield after purification. [c] Presence in small quantities of other products; they were not identified.

authentic samples.[7] None of them touched the double bond, which was expected because of the presence of two substituents on one carbon of the double bond.[8]

The comparison of the results obtained with our substrates shows that the enantioselectivity of the reduction is less efficient in the case of the benzoylchromen-4-one **2**, which could be due to the fact that generally high chiral discrimination is only achieved with groups of greatly different size. However, by choosing the appropriate microorganisms, that are the same for the two classes of substrates (baker's yeast and *Lactobacillus kefiri*), both enantiomers of each alcohol were obtained with high enantiomeric excesses. It is worth noting that the signs of the specific rotation of the alcohols obtained by the reduction with the same microorganism are often opposite.

2.3. Remarks on the melting points of the optically active alcohols

During the characterization of the products formed by microbiological reductions of **1** and **2**, we noticed that the value of the melting point of the racemic mixture was different from that of the corresponding optically active compound, as often observed in asymmetric organic syntheses. The racemic mixture can have either a higher or a lower melting point than each pure enantiomer. These two possibilities are illustrated with the examples listed in Table 2. The precise values of the melting points were determined by DSC (differential scanning calorimetry).

Table 2
Melting points of each enantiomer of **5** and **6** determined by DSC

	5	**6**
Racemic	83.3°C	103.7°C
Bakers' yeast	103.2°C (R, ee ≥ 98%)	62.0°C (S, ee = 94%)
Lactobacillus kefiri	99.2°C (S, ee = 89%)	73.2 °C (R, ee = 82%)

2.4. Determination of the absolute configuration of both alcohols

None of the optically active alcohols isolated from the reduction of **1** or **2**, or their derivatives, have been described in the literature before. As each alcohol was being isolated as a solid, the easiest way to determine its absolute configuration was by X-ray diffraction. However, the determination of such a crystallographic structure requires the presence of either another stereogenic centre of known absolute configuration or an heavy atom (halogen) in the structure. Our first attempts corresponded to the derivatization of the alcohol function with (*S*)-*O*-acetyllactoyl chloride[9] or with (*S*)-1-ethylphenylisocyanate,[10] or that of the cyclic ketone function with (*S*)-α-benzylmethylamine,[11] but they were all unsuccessful as the derivatives were always obtained in an oily form. We decided to take the second option: the synthesis of compounds bearing an heavy atom. That is the reason why **3** and **4** were synthetized (analogues of **1** and **2** with a bromine atom in position 6 of the aromatic ring). Their microbiological reduction has been achieved, in order to obtain at least one pure enantiomer for each compound.

2.5. Microbiological reduction of *3* and *4*

Only the four microorganisms giving the best results for both **1** and **2** were tested: baker's yeast, *Zygosaccharomyces rouxii*, *Yamadazyma farinosa* under anaerobic conditions and *L. kefiri*. The incubation time was 48 h. The results obtained are collected in Table 3.

Table 3
Microbiological reductions of **3** and **4**

	Alcohol 7			**Alcohol 8**		
	$[\alpha]_D^{25}$	ee[a]	Yield[b]	$[\alpha]_D^{25}$	ee[a]	Yield[b]
Bakers' yeast	**- 54**	**≥ 98%**	**53%**	- 4	14%	10%
Zygosaccharomyces rouxii	**- 54**	**≥ 98%**	**58%**	+ 26	88%	12%
Yamadazyma farinosa (N_2)	**+ 54**	**≥ 98%**	**69%**	**+ 30**	**≥ 98%**	**7%**
Lactobacillus kefiri	+ 49	90%	60%	- 17	57%	3%

[a] Determined by HPLC equipped with a Chiralcel OB column (see Experimental section). [b] Yields after purification

Although the presence of the bromine atom on the aromatic ring did not affect the way of reduction for the acetyl derivative to a great extent — except in the yield which was lowered —, the increase in the steric hindrance, in the case of the benzoyl derivative, almost prevented the reduction: the yields like the enantiomeric excesses of the alcohol formed were very low. This compound was not studied further.

2.5.1. Determination of the absolute configuration of the chromone derivatives

In fact, in all cases, it was possible to assign the absolute configurations of the alcohols **5** to **7** derived from chromones, in a solid state, directly by X-Ray analysis of a single crystal. The crystallographic structures of (−)-**5**, (+)-**6** and (−)-**7**, obtained by microbiological reduction with baker's yeast, are presented in Fig. 2.

Compound (−)-**5** has an *R* absolute configuration whereas (+)-**6** and (−)-**7** are *S*. Although the configuration change between (−)-**5** and (+)-**6** could be envisaged (inversion of the sign of the specific

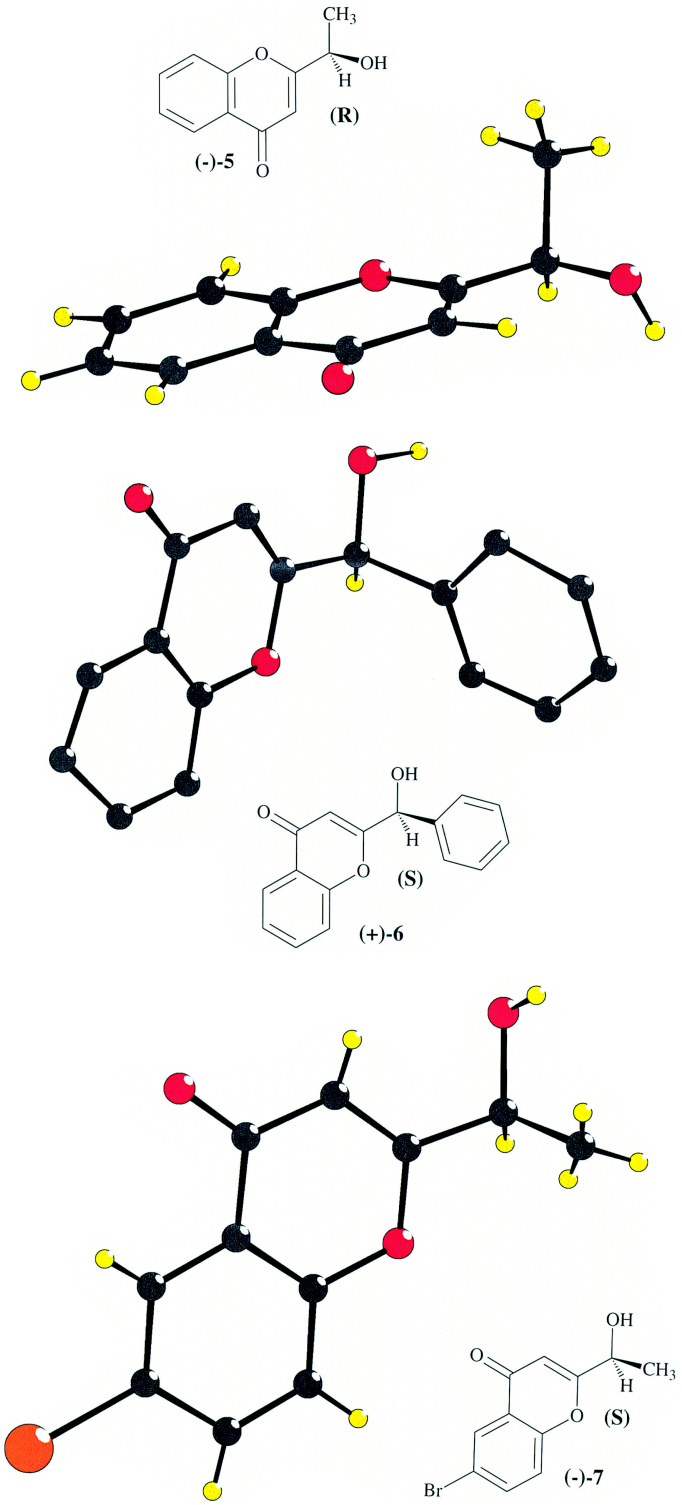


Fig. 2. Crystallographic structures of (−)-(*R*)-2-(1-hydroxyethyl)chromen-4-one (−)-**5**, (+)-(*S*)-2-(1-hydroxyphenylmethyl)-chromen-4-one (+)-**6** and (−)-(*S*)-6-bromo-2-(1-hydroxyethyl)chromen-4-one (−)-**7**

rotation for both alcohols obtained by microbiological reduction with the same microorganism), that between (−)-**5** and (−)-**7** is more surprising. That means that the sole presence of a bromine atom, whose position is quite far from the prochiral centre, implies a change in the enantioselectivity of the reduction by baker's yeast and also by almost all the microorganisms tested. The replacement of a methyl by a phenyl group has also the same effect: an inversion of the absolute configuration. No change of the steric priority between the methyl and the phenyl group being possible for this molecule, an hypothesis could be that these substrates are not reduced by the same alcohol dehydrogenase. The absolute configurations of (+)-**5**, (−)-**6** and (+)-**7** were determined by comparison of the sign of their optical rotation with that of their enantiomer of known absolute configuration: compound (+)-**5** has an *S* absolute configuration whereas (−)-**6** and (+)-**7** are *R*.

These results show once again the complexity of the microorganisms due to the great number of reductases present[12] in whole-cells and the necessity of determining the absolute configuration for each new product.

3. Conclusion

By choosing the appropriate microorganism, both enantiomers of 2-(1-hydroxyethyl)- and of 2-(1-hydroxyphenylmethyl)chromen-4-one were obtained with high enantiomeric excesses. The absolute configurations of these new products were determined by X-ray diffraction. A change in the absolute configuration was observed for the two series of alcohols **5** and **6**, when the substituent changes from a methyl to a phenyl group. The presence of a bromine atom in a position quite far from the stereogenic centre on the 2-acetylchromen-4-one also gave the same inversion of configuration of the corresponding alcohol **7**.

The optically active alcohols are now going to be tested for their anti-asthmatic properties. The different stereochemistries of the alcohols, corresponding to the reduction of the exocyclic ketone of the chromen-4-ones, can be obtained by bioconversion. These results evidenced the microorganisms able to reduce such compounds and should allow us to obtain easily the enantiomers of aminoalcohols, whose β-adrenergic activity depends essentially on their stereochemistry.

4. Experimental

4.1. General methods

4.1.1. Analysis methods

For ^{1}H (400.13 MHz) and ^{13}C (100.61 MHz) NMR spectra, the chemical shifts were relative to chloroform. HPLC experiments for the enantiomeric excess determination were performed using a Waters 600E liquid chromatograph fitted out with a CHIRALCEL OB column at room temperature and were monitored at 254 nm. The eluent was ethyl acetate/cyclohexane in various proportions depending on the product. Optical rotations were determined on a JASCO polarimeter. Microanalyses were performed by the Service Central d′Analyses du CNRS, Vernaison, France.

4.1.2. Microbiological methods

The microorganisms were all laboratory-grown except freeze-dried baker's yeast which was a commercial product (VAHINE, Monteux, France).

4.1.2.1. Culture conditions. Bioconversions are performed with resting cells of microorganisms previously grown at 27°C in one of the following media: for the yeast *Rhodotorula glutinis* NRLL Y 1091: glucose 20 g; malt extract (Difco) 10 g; yeast extract (Difco) 5 g; peptone 5 g; H_2O 1 L; preculture: 48 h and culture: 60 h. For the yeast *Z. rouxii* ATCC 13356: glucose 20 g; malt extract 6 g; yeast extract 6 g; tryptone (Difco) 10 g and H_2O 1L; preculture and culture: 48 h. For the yeast *Y. farinosa* IFO 10896: glucose 50 g; tryptone (Difco) 7 g; yeast extract 5 g; K_2HPO_4 2 g; KH_2PO_4 3 g and H_2O 1 L; preculture and culture: 48 h. For the fungus *Aspergillus niger* ATCC 9142: yeast extract 5 g; soyoptim (Roquette) 5 g; glucose 20 g; NaCl 5 g; KH_2PO_4 5 g and H_2O 1 L; preculture and culture: 24 h. For *Cunninghamella elegans* ATCC 9245: same medium as *A. niger*; preculture: 48 h and culture: 24 h. For *Beauveria bassiana* ATCC 7159: *medium 1*: corn steep 12 g; glucose 10 g and H_2O 1 L; *medium 2*: peptone 10 g; glucose 30 g; K_2HPO_4 1 g; $FeSO_4 \cdot 7\ H_2O$ 0.01 g; $MgSO_4 \cdot 7H_2O$ 0.5 g; $ZnSO_4 \cdot 7H_2O$ 0.3 g; KCl 0.5 g and H_2O 1 L; preculture in m*edium 1*: 48 h and culture in m*edium 2*: 24 h. For *Geotrichum candidum* CBS 233-76: yeast extract 10 g; peptone 10 g; glucose 50 g and H_2O 1 L; preculture: 24 h and culture 48 h. For the bacterium *L. kefiri* DSM 20587: commercially available MRS medium was used; preculture and culture: 24 h.

4.1.2.2. Bioconversion conditions. General case: microorganisms in metabolic resting phase and aerobic conditions. After culture at 27°C for the times indicated previously, the microorganisms were filtered on sintered glass or centrifuged, and then washed four times with NaCl solution (8 g L^{-1}). Mycelium (5 g) was placed in a 500 mL conical flask with 50 mL of distilled water and 50 μL of substrate. After incubation at 27°C on a rotating table set at 200 rpm, the mixture was filtered on sintered glass or centrifuged for 10 min at 8000 rpm. The liquor was then continuously extracted with ether for 24 h. The ether phase was dried on $MgSO_4$ and the solvent evaporated off under vacuum.

Yamadazyma farinosa: The bioconversion reaction was carried out under either aerobic or anaerobic conditions according to Ohta and co-workers.[13]

4.2. Synthesis of the chromen-4-one derivatives

The synthesis of **1** and **2** have already been described in the literature.[6,7]

*4.2.1. Synthesis of 2-acetyl-6-bromochromen-4-one **3***

4.2.1.1. Preparation of 6-bromo-2-diazoacetylchromen-4-one. To an ethereal solution of diazomethane, containing approximately 5 g (0.12 mol) of diazomethane, cooled at 0°C was added slowly 14.5 g (0.05 mol) of 6-bromochromen-4-one 2-carboxylchloride **5b**, previously prepared according to the literature.[4] After stirring for an additional 15 min at room temperature, the mixture was filtered on a Büchner apparatus. The solid was dried for obtention of 10.3 g of 6-bromo-2-diazoacetylchromen-4-one. Yield: 70%. R_f=0.13 (CH_2Cl_2). Mp=192°C. IR (KBr) ν (cm^{-1}): 1450, 1600 (C=C); 1640 (CO pyrone); 1660 (CO ketone); 2120 (CHN_2); 3070 (CH). ^{1}H NMR (400.13 MHz) δ: 6.23 (s, 1H, CHN_2); 7.02 (s, 1H, H_3); 7.39 (d, 1H, H_8, J=8.9 Hz); 7.80 (dd, 1H, H_7, J=8.9 Hz, J=2.5 Hz); 8.31 (d, 1H, H_5, J=2.5 Hz).

*4.2.1.2. Preparation of 2-acetyl-6-bromochromen-4-one **3**.* To a stirred aqueous solution of hydriodic acid (0.084 mol, 2 equiv., 10.7 g) heated at 50°C was added slowly, by small quantities, 12.3 g (0.042 mol) of 6-bromo-2-diazoacetylchromen-4-one. Gas bubbling (N_2) occured and the mixture became brown in colour. The mixture was stirred for an additional hour and then filtered. The brown solid was treated with sodium thiosulfate to eliminate the excess of iodine. The crude solid was then recrystallized in ethanol. White solid **3** (4.7 g) was obtained. Yield: 42%. R_f=0.74 (CH_2Cl_2). Mp=144°C (DSC). IR (KBr)

ν (cm^{-1}): 1460, 1600, 1620 (C=C); 1650 (CO pyrone); 1700 (CO ketone); 2920, 3080 (CH). ^{1}H NMR (400.13 MHz) δ: 2.65 (s, 3H, CH_3); 7.05 (s, 1H, H_3); 7.50 (d, 1H, H_8, J=9.2 Hz); 7.84 (dd, 1H, H_7, J=1.6 Hz, J=9.2 Hz); 8.86 (d, 1H, H_5, J=1.6 Hz). ^{13}C NMR (100.61 MHz) δ: 26.2 (CH_3); 111.9 (C-3); 119.3 (C-6); 120.6 (C-8); 125.8 (C-10); 128.6 (C-5); 137.9 (C-7); 154.4 (C-9); 156.7 (C-2); 177.3 (C-4); 192.2 (CO exocyclic). Anal. calcd for $C_{11}H_7BrO_3$: C 49.47; H 2.64; O 17.97. Found: C 48.83; H 2.55; O 18.45.

*4.2.2. Synthesis of 2-benzoyl-6-bromochromen-4-one **4***

To a stirred solution of 14.5 g (0.05 mol) of 6-bromochromen-4-one 2-carboxylic chloride **5b** in 200 mL of dry benzene was added slowly 15 g (0.113 mol) of $AlCl_3$ while maintaining the temperature under 10°C. The solution was stirred for 6 h, then poured into a mixture of 100 g of crushed ice and 100 mL of HCl. The organic layer was washed three times with water. After evaporation of the solvent, the crude product was recrystallized in ethanol, yielding 13.6 g of **4** (yield: 83%). R_f=0.43 (CH_2Cl_2). Mp=162°C. IR (KBr) ν (cm^{-1}): 1595, 1610 (C=C); 1653 (CO pyrone); 1675 (CO ketone); 3033, 3057, 3085 (CH). ^{1}H NMR (400.13 MHz) δ: 6.90 (s, 1H, H_3); 7.50 (d, 1H, H_8, J=8.9 Hz); 7.54–7.61 (m, 2H, $H_{3'}$ and $H_{5'}$); 7.68–7.76 (m, 1H, $H_{4'}$); 7.85 (dd, 1H, H_7, J=2.5 Hz, J=8.9 Hz); 7.93–8.00 (m, 2H, $H_{2'}$ and $H_{6'}$); 8.37 (d, 1H, H_5, J=2.5 Hz). ^{13}C NMR (100.61 MHz) δ: 115.1 (C-3); 119.7 (C-6); 120.8 (C-8); 125.8 (C-10); 128.6 (C-5); 129.0 (C-3′ and C-5′); 130.1 (C-2′ and C-6′); 134.5 (C-4′); 134.6 (C-1′); 137.9 (C-7); 154.7 (C-9); 158.1 (C-2); 177.0 (C-4); 187.7 (CO exocyclic). Anal. calcd for $C_{16}H_9BrO_3$: C 58.38; H 2.75; O 14.58. Found: C 58.59; H 2.78; O 14.65.

4.3. Microbiological reductions

In each case, the yields indicated are overall yields after work-up.

*4.3.1. Microbiological reductions of 2-acetylchromen-4-one **1***

Incubation time: 24 h. The products from the residue were separated on a silica gel column. The eluent was cyclohexane/ethyl acetate 35/65.

Baker's yeast: (*R*)-(−)-**5**. Yield: 87%. R_f=0.71 (ethyl acetate). Mp=107.7°C (DSC). IR (KBr) ν (cm^{-1}): 1635 (CO); 1655 (C=C); 3300 (OH). ^{1}H NMR (400.13 MHz) δ: 1.59 (d, 3H, CH_3, J=6.7 Hz); 3.66 (br s, 1H, exchangeable with D_2O); 4.74 (q, 1H, CHOH, J=6.6 Hz); 6.52 (s, 1H, H_3); 7.30–7.41 (m, 2H, H_5 and H_6); 7.56–7.65 (m, 1H, H_7); 8.10 (dd, 1H, H_8, J=1.6 Hz, J=7.9 Hz). ^{13}C NMR (100.61 MHz) δ: 21.4 (CH_3), 67.1 (CHOH); 107.2 (C-3); 118.0 (C-8); 123.6 (C-10); 125.2 (C-6); 125.6 (C-5); 133.8 (C-7); 156.2 (C-9); 172.2 (C-2); 179.2 (C-4). MS (EI) *m/z*: 190 ($M^{+\cdot}$); 175 ($M-CH_3)^{+\cdot}$; 145 ($M-CHOH-CH_3)^{+\cdot}$; 131; 91; 77; 63; 39. $[\alpha]_D^{25}=-60$ (c 2.85, $CHCl_3$); ee≥98%.

Lactobacillus kefiri: (*S*)-(+)-**5**. Yield: 58%. Same physical constants and NMR spectra as those described previously for (−)-**5**. $[\alpha]_D^{25}=+53$ (c 3.2, $CHCl_3$); ee=89%.

*4.3.2. Microbiological reductions of 2-benzoylchromen-4-one **2***

Incubation time: 24 h. The products from the residue were separated on a silica gel column. The eluent was cyclohexane/ethyl acetate 50/50.

Baker's yeast: (*S*)-(+)-**6**. Yield: 60%. R_f=0.76 (ethyl acetate). Mp=70.4°C (DSC). IR (KBr) ν (cm^{-1}): 1635 (CO); 1650 (C=C); 3400–2600 (OH). ^{1}H NMR (400.13 MHz) δ: 3.55 (br s, 1H, exchangeable with D_2O); 5.64 (s, 1H, CHOH); 6.66 (s, 1H, H_3); 7.32–7.43 (m, 5H); 7.47–7.52 (m, 2H); 7.60 (td, 1H, J=1.2 Hz, J=7.0 Hz); 8.14 (dd, 1H, H_5, J=1.8 Hz, J=6.5 Hz). ^{13}C NMR (100.61 MHz) δ: 73.2 (CHOH); 108.0 (C-3); 118.0 (C-8); 123.6 (C-10); 125.2 (C-6); 125.5 (C-5); 127.0 (C-3′ and C5′); 128.7 (C-4′);

128.8 (C-2′ and C-6′); 133.8 (C-7); 139.3 (C-1′); 156.2 (C-9); 170.1 (C-2); 179.2 (C-4). MS (EI) *m/z*: 252 ($M^{+\cdot}$); 223; 147; 121; 105; 77. $[\alpha]_D^{25}$=+27 (c 1.05, $CHCl_3$); ee=94%.

Lactobacillus kefiri: (*R*)-(−)-**6**. Yield: 33%. Same physical constants and NMR spectra as those described previously for (+)-**6**. $[\alpha]_D^{25}$=−24 (c 3.6, $CHCl_3$); ee=84%.

*4.3.3. Microbiological reductions of 2-acetyl-6-bromochromen-4-one **3***

Incubation time: 48 h. The products from the residue were separated on a silica gel column. The eluent was cyclohexane/ethyl acetate 50/50.

Baker's yeast: (*S*)-(−)-**7**. Yield: 53%. R_f=0.43 (cyclohexane/AcOEt 30/70). Mp=130.2°C (DSC). IR (KBr) ν (cm^{-1}): 1592 (C=C); 1650 (CO); 2850–3000 (CH); 3200 (OH). ^{1}H NMR (400.13 MHz) δ: 1.60 (d, 3H, CH_3, J=7.5 Hz); 3.90 (s, 1H, exchangeable with D_2O); 4.67–4.80 (m, 1H, CHOH); 6.51 (s, 1H, H_3); 7.34 (d, 1H, H_8, J=9.0 Hz); 7.73 (dd, 1H, H_7, J=2.5 Hz, J=9.0 Hz); 8.28 (d, 1H, H_5, J=2.5 Hz). ^{13}C NMR (100.61 MHz) δ: 21.4 (CH_3); 67.0 (CHOH); 107.1 (C-3); 118.6 (C-6); 120.0 (C-8); 124.9 (C-10); 128.2 (C-5); 136.8 (C-7); 154.9 (C-9); 172.6 (C-2); 177.7 (C-4). MS (EI) *m/z*: 268, 270 ($M^{+\cdot}$); 225, 227 ($M-CHOH-CH_3)^{+\cdot}$; 146 ($M-C_2H_5O-Br)^{+\cdot}$; 118; 77. $[\alpha]_D^{25}$=−54 (c 2.45, $CHCl_3$); ee≥98%. Anal. calcd for $C_{11}H_9BrO_3$: C 49.10; H 3.37; O 17.84. Found: C 50.09; H 3.37; O 17.79.

Yamadazyma farinosa (anaerobic conditions): (*R*)-(+)-**7**. Yield: 69%. Same physical constants and NMR spectra as those described previously for (−)-**7**. $[\alpha]_D^{25}$=+54 (c 3.18, $CHCl_3$); ee≥98%.

*4.3.4. Microbiological reductions of 2-benzoyl-6-bromochromen-4-one **4***

Incubation time: 48 h. The products from the residue were separated on a silica gel column. The eluent was cyclohexane/ethyl acetate 50/50.

Zygosaccharomyces rouxii: (+)-**8**. Yield: 7%. R_f=0.65 (cyclohexane/AcOEt 30/70). ^{1}H NMR (400.13 MHz) δ: 3.95 (br s, 1H, exchangeable with D_2O); 5.60 (s, 1H, CHOH); 6.66 (s, 1H, H_3); 7.23 (d, 1H, H_8, J=8.9 Hz); 7.33–7.40 (m, 3H, $H_{3'}$, $H_{4'}$ and $H_{5'}$); 7.44–7.51 (m, 2H, $H_{2'}$ and $H_{6'}$); 7.66 (dd, 1H, H_7, J=2.5 Hz, J=8.9 Hz); 8.20 (d, 1H, H_5, J=2.5 Hz). ^{13}C NMR (100.61 MHz) δ: 73.3 (CHOH); 108.2 (C-3); 118.7 (C-6); 120.1 (C-8); 125.1 (C-10); 127.0 (C-3′); 128.3 (C-5); 128.5 (C-4′); 129.0 (C-2′); 136.8 (C-7); 139.0 (C-1′); 155.0 (C-9); 169.9 (C-2); 177.5 (C-4). MS (EI) *m/z*: 330, 332 (M^+); 226, 228. $[\alpha]_D^{25}$=+30 (c 0.85, $CHCl_3$); ee≥98%. Anal. calcd for $C_{16}H_{11}BrO_3$: C 58.03; H 3.35; O 14.49. Found: C 57.85; H 3.39; O 13.99.

4.4. Determination of the enantiomeric excesses

The enantiomeric excess of each alcohol obtained by microbial reduction of **1** to **4** were determined by HPLC on a Chiralcel OB column. For **5**: eluent: cyclohexane/isopropanol 95/5; flow rate: 0.1 mL/min; R_t (min)=121 (*R*) and 132 (*S*). For **6**: eluent: cyclohexane/isopropanol 60/40; flow rate: 0.6 mL/min; R_t (min)=19 (*R*) and 64 (*S*). For **7**: eluent: cyclohexane/isopropanol 99/1; flow rate: 0.45 mL/min; R_t (min)=75 (*S*) and 91 (*R*). For **8**: eluent: cyclohexane/isopropanol 95/5; flow rate: 0.2 mL/min; R_t (min)=60 (−) and 78 (+).

4.5. Determination of the absolute configurations

The absolute configurations of (−)-**5**, (+)-**6** and (−)-**7** were determined by X-ray diffraction. Single crystals of **5** and **7** were easily obtained from solutions in DMF after a slow evaporation of the solvent. Concerning the phenyl derivative **6**, single crystals were very difficult to obtain. After many attempts, only few crystals were isolated after evaporation of a mixed solution of ethyl acetate and hexane (50:50). In all cases, a crystal was mounted on a STOE IPDS single phi axis diffractometer with a 2D area

detector based on imaging plate technology. The diffraction pattern was stored as a latent image on the image plate, after an exposure time depending on the crystal. A classical 80 mm crystal-to-detector distance was used to satisfy to a good reflection resolution. A recording time close to 30 h was required for the full data collection. The images were processed with the set of programs from STOE (Display, Index, Cell, Profile, Integrate, Merge).[14] Cell parameters were refined from 2000 reflections. This data set was used for structure solution attempts. Direct methods were used to localize the first atoms and the remaining from succesive Fourier difference maps. All calculations were made using the SHELTX program. The absolute configurations were obtained after refinement of the absolute structure parameter (Flack parameter) according to the SHELX97 program.

Atomic coordinates have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from CCDC, Union Road, Cambridge CB2 1EZ, UK.

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