Synthesis of (*S*)-4-hydroxy-α-lapachone and biotransformation of some 4-chromanones by *Mortierella isabellina* ATCC 42613

Herbert L. Holland, Jia Qi, and T. Samuel Manoharan

Abstract: 1-Methoxy-2-naphthol was converted by MOM ether formation, lithiation at C-3, reaction with 3,3-dimethylacryloyl chloride, cyclization, and oxidation using cerium ammonium nitrate to 4-keto-α-lapachone (2,2-dimethyl-4-keto-1-oxa-1,2,3,4-tetrahydroanthra-5,10-quinone). Reduction by biotransformation using *Mortierella isabellina* ATCC 42613 gave (*S*)-4-hydroxy-α-lapachone in high (>95%) optical purity. Reduction of several 2,2-dimethyl-4-chromanones to the corresponding (*S*)-alcohols by *M. isabellina* is also reported.

Key words: biotransformation, chemoenzymatic, 4-hydroxy-α-lapachone, *Mortierella isabellina*.

Résumé : Faisant appel à la série de réactions suivantes, formation d'éther MOM, lithiation en C-3, réaction avec du chlorure de 3,3-diméthylacryloyle, cyclisation et oxydation à l'aide du nitrate d'ammonium cérique, on a transformé le 1-méthoxynapht-2-ol en 4-céto-α-lapachone (2,2-diméthyl-4-céto-1-oxa-1,2,3,4-tétrahydroanthra-5,10-quinone). Sa réduction par biotransformation utilisant de la *Mortierella isabellina* ATCC 42613 conduit à la (*S*)-4-hydroxy-α-lapachone avec une pureté optique élevée (>95%). On rapporte aussi la réduction de plusieurs 2,2-diméthyl-4-chromanones en alcools-(*S*) correspondants par la *M. isabellina*.

Mots clés : biotransformation, chemoenzymatique, 4-hydroxy-α-lapachone, *Mortierella isabellina*.

Introduction

The biotransformation of 4-chromanone (**1**) by *Mortierella isabellina* ATCC 42613 can be used to prepare (*S*)-4-chromanol (**2**) in high yield and enantiomeric purity (Scheme 1) (1). (*S*)-4-Hydroxy-α-lapachone (**3**), which possesses the (*S*)-4-chromanol functional unit, has been isolated from the Japanese Kisasage tree *Catalpa ocata* (2, 3) and from the heartwood of the Brazilian *Zeyhera tuberculosa* (4), and has been shown to possess antineoplastic properties (4). The racemate of **3** was prepared by Gupta and Khanna in a non-regioselective synthesis (5, 6), and by Tapia's group in a route involving construction of the aromatic ring via Diels–Alder addition to a suitably substituted quinone (7), but preparation of the natural chiral material has not hitherto been reported. To develop a route to (*S*)-**3** based on chiral reduction of an appropriately substituted keto precursor we have examined the biotransformation by *M. isabellina* of a number of substituted 4-chromanones (**4**, **8**, and **13**). The syntheses of these compounds and their biotransformations by *M. isabellina* are presented, together the preparation of (*S*)-4-hydroxy-α-lapachone (**3**) by *M. isabellina*-catalyzed reduction of 2,2-dimethyl-4-keto-1-oxa-1,2,3,4-tetrahydroanthra-5,10-quinone.

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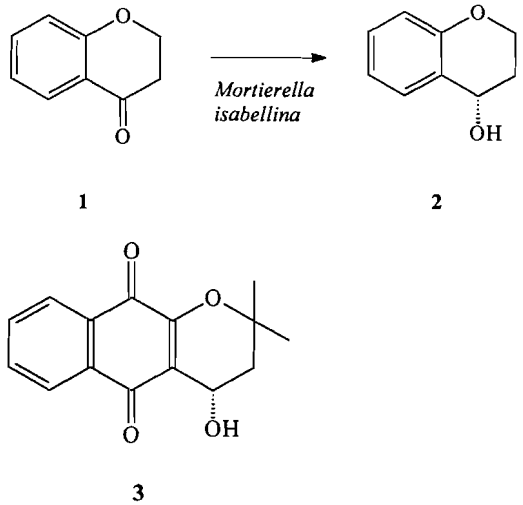
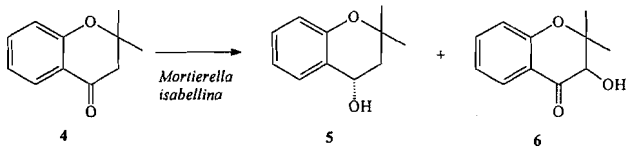
Scheme 1. Preparation of (*S*)-4-chromanol.



Results and discussion

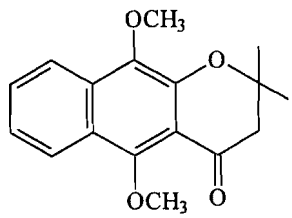
To investigate the effect of methyl substitution at C-2 on the stereochemistry of biotransformation of a chromanone, the 2,2-dimethyl-substituted substrate **4** was prepared by a modification of ApSimon's method (8) and subjected to biotransformation using *M. isabellina* (Scheme 2). The desired (*S*)-alcohol **5** was produced in good yield, but its enantiomeric excess (40%) was low. The absolute stereochemistry of this and other reduction products described below was assigned in two ways: firstly, in accord with the observation that *M. isa-*

Scheme 2. Biotransformation of 2,2-dimethyl-4-chromanone.



bellina reduces benzylic ketones according to Prelog's rule to give (*S*) benzylic carbinols (1, 9), and secondly by the observation that addition of the chiral ^{1}H nmr shift reagent tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorato] europium(III) to a non-racemic benzylic alcohol results in a ^{1}H nmr spectrum in which the carbinol hydrogen of the -C*H*(OH)- unit can be resolved into two baseline-separated signals in the 10–20 ppm region. In all cases examined using alcohols of known configuration, the signal attributable to the (*S*) enantiomer is upfield and that of the (*R*) enantiomer downfield (1, 10, 11). In addition to the production of **5**, biotransformation of **4** by *M. isabellina* also resulted in formation of a minor product, hydroxyketone **6**, whose stereochemistry was not examined.

A route to 4-hydroxy-α-lapachone via a precursor such as **7** was first considered. Prior to undertaking synthesis of the latter, we examined reduction of 2,2-dimethyl-5,8-dimethoxychroman-4-one (**8**). Chroman-4-ones with this substitution



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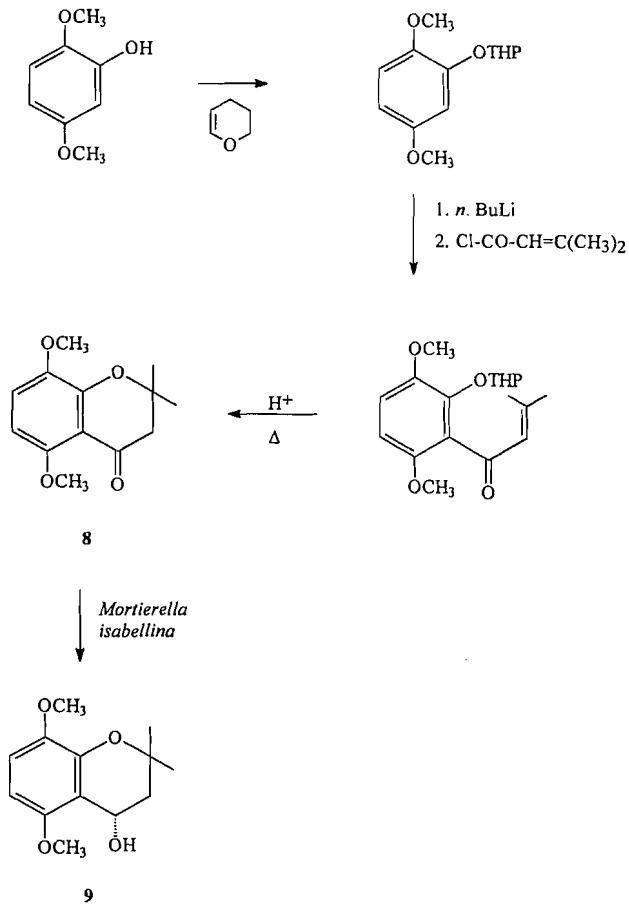
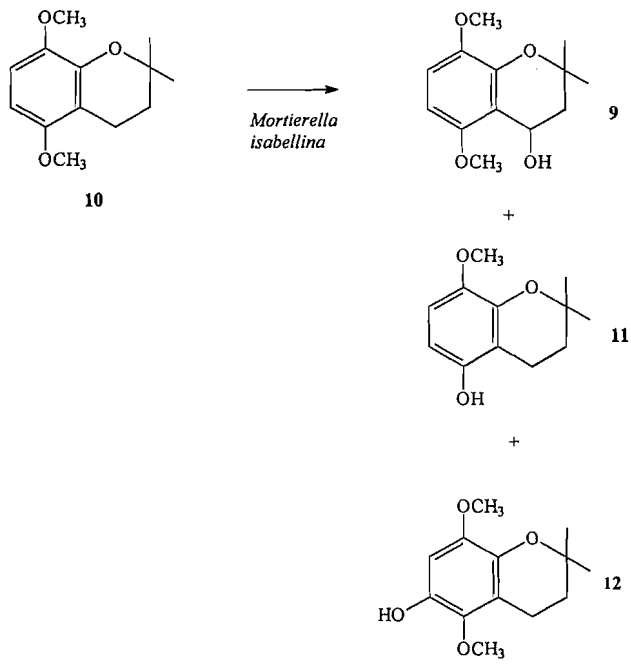
pattern have not been reported, and we developed the synthesis of **8** from 2,5-dimethoxyphenol as outlined in Scheme 3. Biotransformation of **8** by *M. Isabellina* (Scheme 3) gave the alcohol (*S*)-**9** in high yield (99%) and in an enantiomeric purity exceeding 95%. A ^{1}H nmr analysis in the presence of tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorato] europium(III) failed to reveal any (*R*) enantiomer; addition of controlled amounts of synthetic (±)-**9** to the biotransformation product followed by nmr analysis confirmed the (*S*) configuration of the latter by the gradual appearance of a C*H*OH signal downfield from that of the original sample.

Biotransformation of the chroman **10** (Scheme 4) also gave **9**, but in racemic form. Additional products were obtained and assigned structures **11** and **12** on the basis of homonuclear nOe experiments. It was clear from mass spectral and ^{1}H nmr analysis that **11** was a mono-O-demethyl derivative of the substrate, and the site of demethylation was shown to be O(5) by the absence of nOe enhancement of the benzylic methylene protons upon irradiation of the remaining O-methyl signal at δ 3.77 ppm. Similarly, **12** was clearly a phenolic derivative of substrate; nOe experiments involving irradiation of the C-5 methoxy signal at δ 3.74 ppm showed an enhancement of only the benzylic methylene protons at δ 2.74, while irradiation of the C-8 methoxy resonance resulted only in enhancement of

Scheme 3. Synthesis and biotransformation of 5,8-dimethoxy-2,2-dimethyl-4-chromanone (**8**).



Scheme 4. Biotransformation of 5,8-dimethoxy-2,2-dimethylchroman (**10**).



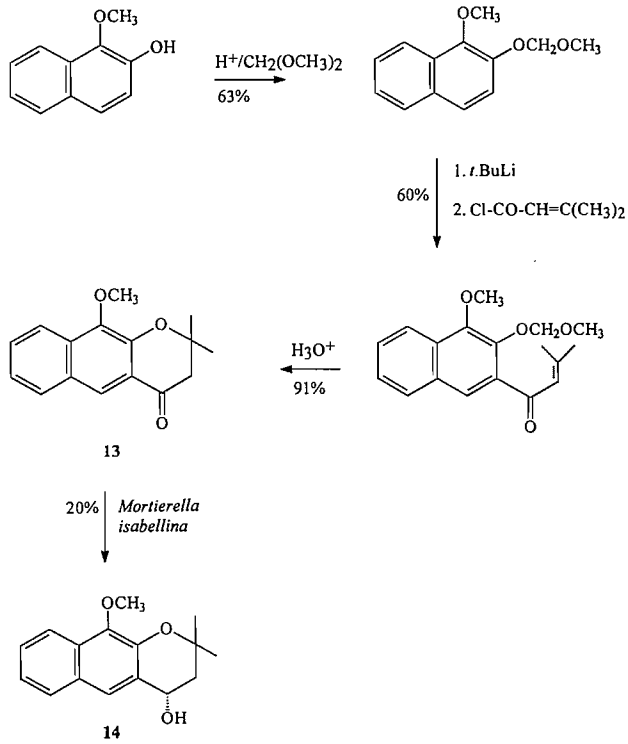
the C-7 aromatic hydrogen signal. During investigation of the synthesis and biotransformation of 6,7-benzo-5,8-dimethoxy-2,2-dimethyl-4-chromanone **7** as a route to **3**, we were unsuccessful in attempts to prepare **7** by a route analogous to that of Scheme 3. Although lithiation of a protected phenol intermediate analogous to that shown in Scheme 3 was achieved (evidenced by incorporation of deuterium label when quenched with D_2O), reaction of this species with dimethylacryolyl chloride did not afford the desired ketone, resulting only in formation of intractable product mixtures. We therefore turned our attention to the monomethoxy analogue **13**, whose synthesis from 1-methoxy-2-naphthol is shown in Scheme 5. In contrast to the THP protection of Scheme 3, we found here that protection of the phenolic group as a MOM ether facilitated purification of intermediates. It was also necessary, again in contrast to the route of Scheme 3, to use *tert*-butyllithium for lithiation of the intermediate protected phenol.

Biotransformation of **13** by *M. isabellina* gave the (*S*) alcohol **14** (Scheme 5), but the enantiomeric purity was low (32%) and phenolic side-products were obtained. However, given that the presence of a substituent *ortho* to the carbonyl group undergoing biocatalytic reduction apparently results in enhancement of the optical purity of the resulting (*S*) alcohol (cf. results from **4** vs. **8**), we examined the quinone **15** (4-ketolapachone), obtained by ceric ion oxidation of **13** (Scheme 6). Biotransformation of **15** by *M. isabellina* resulted in the formation of (+)-4-hydroxy-α-lapachone (**3**) in acceptable yield and high (>95%) enantiomeric purity. The absolute configuration of the (+) (natural) enantiomer of **3** has been assigned as (*S*) by application of the extended dibenzoate chirality rule to the CD spectrum of its benzoate ester (2). The configuration of our product is thus established. Furthermore, under conditions described above where racemic alcohols give cleanly resolved signals in chiral nmr analysis, we were unable to detect any (*R*)-**3** in our product. The reactions of Schemes 5 and 6 therefore represent the first asymmetric synthesis of the natural enantiomer of (*S*)-(+)-4-hydroxy-α-lapachone.

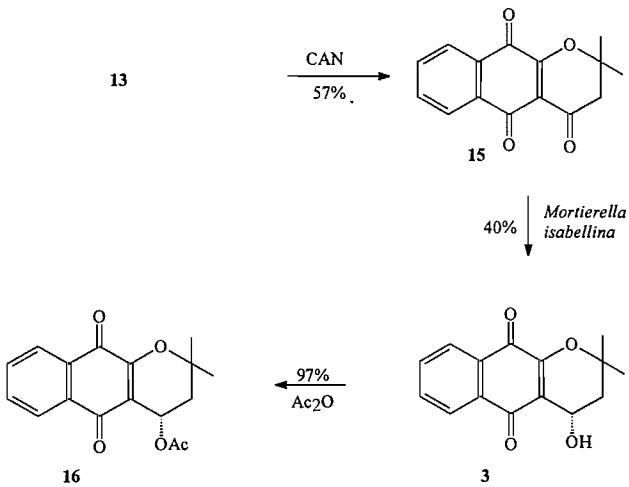
Although spectral data of our synthetic sample matched those reported in the literature for both natural (2–4) and synthetic (5–7) 4-hydroxy-α-lapachone, we observed discrepancies in physical data. This material has been variously reported as a yellow oil (natural (2, 4) and synthetic racemate (7)) and a yellow solid with mp 117–119°C (synthetic racemate (6)), whereas our material melted at 67°C. Furthermore, natural 4-hydroxy-α-lapachone has a reported rotation, $[\alpha]_D$ +27.4, whereas our sample showed $[\alpha]_D$ +39.7 under the same conditions. From this it may be inferred that the natural material is not optically pure, possessing an enantiomeric purity of 69%. To confirm this our sample of **3** was converted to the acetate **16** in order to compare the data obtained therefrom with those reported for synthetic racemic **16** and for **16** obtained by acetylation of natural 4-hydroxy-α-lapachone. Again, although our spectral data for **16** matched those reported (2), the mp and rotation data were disparate, the latter confirming the conclusion that natural 4-hydroxy-α-lapachone is not optically pure as isolated. Our sample of **16** has $[\alpha]_D$ −22.7 whereas the acetate of natural 4-hydroxy-α-lapachone has $[\alpha]_D$ −14.2, corresponding to ee = 63% (2).

We therefore suggest that our synthesis of (*S*)-(+)-4-hydroxy-α-lapachone gives enantiomerically pure material, whereas the natural product is not enantiomerically homogeneous. Whether this results from an aspect of its biosynthesis or the occurrence of partial racemization during the extraction procedure is unknown.

Scheme 5. Synthesis and biotransformation of 6,7-benzo-2,2-dimethyl-8-methoxy-4-chromanone (**13**).



Scheme 6. Preparation of (*S*)-4-hydroxy-α-lapachone (**3**) and the acetate (**16**).



Experimental

Apparatus, materials, and methods

Melting points were determined on a Kofler heating stage. Infrared spectra were recorded with an Analect 6260FX spec-

trometer. The nmr spectra were recorded at 200 MHz (routine ^1H) or 50 MHz (routine ^{13}C) with a Bruker AC200 spectrometer using $CDCl_3$ as solvent and $CHCl_3$ as internal standard. ^{13}C signals are assigned as primary (p), secondary (s), tertiary (t), or quaternary (q) from analysis of JMOD spectra. Enantiomeric ratios were examined by ^1H nmr analysis in the presence of sufficient tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorato] europium(III) to cause shifting of the C*H*OH signals under investigation to δ 10–20 ppm. Homonuclear nOe difference spectra were obtained at McMaster University using a Bruker AM500 spectrometer. Optical rotations were obtained in the stated solvent at ambient temperature with a Rudolph Autopol III polarimeter. Mass spectra were obtained with a Kratos 1S instrument operating in EI mode. Thin-layer chromatography (tlc) was performed on Merck silica gel 60F-254 and flash column chromatography used silica gel, 230–400 mesh. Microanalyses were performed by Guelph Chemical Laboratories, Guelph, Ontario.

Maintenance of microorganisms

Mortierella isabellina ATCC 42613 was obtained from the American Type Culture Collection, Rockville, Md., and was maintained on 4% malt agar slopes, grown at 27°C, and stored at 4°C.

Preparation of bicyclic substrates

*2,2-Dimethyl-4-chromanone (**4**)*

Based on a procedure of ApSimon et al. (8), a mixture of *o*-hydroxyacetophenone (13.5 g), pyrrolidine (12.2 g), dry acetone (6 g), and toluene (100 mL) was stirred under argon for 4 days. The mixture was then acidified with 5% HCl, and the organic layer was separated and washed with saturated aqueous NaCl (3 × 50 mL), dried, and evaporated. The residue was dissolved in dichloromethane and passed through a short column of Florisil using dichloromethane as eluent. The effluent was washed with 5% KOH (4 × 50 mL), dried, and evaporated. Crystallization of the residue from ethanol gave the title compound in 65% yield; mp 90°C; ^1H nmr δ: 1.46 (6H, s), 2.72 (2H, s), 6.90–7.01 (2H, m), 7.46 (1H, d of t), and 7.85 (1H, d of d) ppm; ^{13}C nmr δ: 26.6 (2C, p), 48.8 (s), 77.6 (q), 118.3 (t), 120.3 (q), 120.6 (t), 126.5 (t), 136.1 (t), 160.0 (q), and 192.4 (q) ppm.

*5,8-Dimethoxy-2,2-dimethyl-4-chromanone (**8**)*

A mixture of 2,5-dimethoxyphenol (7 g), dihydropyran (16.5 mL), and concentrated HCl (2 drops) was stirred overnight under an argon atmosphere. Dichloromethane (100 mL) was then added, and the resulting solution washed with 10% NaOH (2 × 20 mL). The organic layer was dried and evaporated, and the residue purified by Kugelrohr distillation (120–130°C/0.7 Torr; 1 Torr = 133.3 Pa) to yield 8.7 g (82%) of 2,5-dimethoxyphenol tetrahydropyranyl ether as a colourless oil; ^1H nmr δ: 1.55–1.75 (4H, m), 1.85–2.0 (6H, m), 3.55–4.05 (8H, m and two s), 5.59 (1H, t), 6.45–6.51 (1H, m), and 6.78–6.85 (2H, m) ppm; ms *m/z*(%): 238(1.4), 180(1), 166(1), 154(100), 139(63), 125(9), 111(22), 85(24). This material was dissolved in dry THF (400 mL), and the solution was placed under an argon atmosphere and cooled to 0°C. A solution of *n*-BuLi (14 mL, 2.8 M in hexane) was then added slowly and with stirring at such a rate as to maintain the reaction temperature below 5°C. On completion of addition, the cooling bath was removed and the mixture stirred at room temperature for 2 h. The system was then cooled to −65°C and 3,3-dimethylacryloyl chloride (4.2 mL) was added slowly. The system was left to warm to ambient temperature overnight, then water (100 mL) was added. The resulting mixture was stirred for 15 min and then extracted with chloroform. The organic phase was dried and evaporated to yield a viscous oil (14 g) that was directly dissolved in chloroform (30 mL). To this solution was added dilute HCl (60 mL, 6 M), and the resulting mixture was stirred at room temperature for 2 h. The solution was then extracted with chloroform (3 × 200 mL) and the extract dried and evaporated. The residue was subjected to Kugelrohr distillation at 125–135°C/0.5 Torr to give 5,8-dimethoxy-2,2-dimethyl-4-chromanone (1.27 g). Further product was obtained by chromatography of the residue from the Kugelrohr distillation using 50–60% ethyl acetate – hexane as eluent; mp 143–145°C (from ethyl acetate); ir (KBr) ν_{max}: 1694 cm^{-1}; ^1H nmr δ: 1.50 (6H, s), 2.72 (2H, s), 3.84 and 3.87 (each 3H, s), 6.39 and 7.0 (2H, ABq) ppm; ^{13}C nmr δ: 26.3 (2C, p), 50.2 (s), 56.1 (p), 56.9 (p), 79.2 (q), 101.6 (t), 111.3 (q), 118.4 (t), 143.0 (q), 151.3 (q), 153.8 (q), 191.3 (q) ppm; ms *m/z*(%): 236(64), 221(31), 206(6), 181(58), 180(100), 165(21), 151(31), 137(54), 123(45). Anal. calcd. for $C_{13}H_{16}O_4$: C 66.09, H 6.83%; found: C 65.79, H 6.65%.

*5,8-Dimethoxy-2,2-Dimethylchroman (**10**)*

Lithium aluminum hydride (1.0 g) was added to a stirred solution of 5,8-dimethoxy-2,2-dimethyl-4-chromanone (2.7 g) in dry THF (200 mL), and resulting mixture stirred and heated under reflux for 1 h. The mixture was then cooled, ethyl acetate (5 mL) added, and the reaction mixture stirred for a further 30 min at room temperature. A saturated aqueous solution of sodium sulfate (200 mL) was then added, and the mixture extracted with chloroform (3 × 150 mL). The extract was dried and evaporated and the residue chromatographed on silica gel. Elution with 30–40% ethyl acetate – hexane gave (±)-5,8-dimethoxy-2,2-dimethyl-4-chromanol (**9**, 2.3 g, 85%), obtained as a colourless solid following crystallization from ethyl acetate – hexane; mp 99–101°C; ir (KBr) ν_{max}: 3520 cm^{-1}; ^1H nmr δ: 1.41 and 1.48 (each 3H, s), 1.95–2.15 (2H, m), 3.50 (1H, s, exchanges with D_2O), 3.81 and 3.85 (each 3H, s), 5.0 (1H, t), 6.35 and 6.74 (2H, ABq) ppm; ^{13}C nmr δ: 26.8 (p), 27.2 (p), 40.2 (s), 55.5 (p), 56.7 (p), 60.8 (t), 75.1 (q), 100.1 (t), 111.2 (t), 114.0 (q), 132.1 (q), 143.7 (q), 152.3 (q) ppm; ms m/z(%): 238(66), 205(36), 183(33), 182(100), 167(18), 153(5), 139(19), 107(11). Anal. calcd. for $C_{13}H_{18}O_4$: C 65.53, H 7.61%; found: C 65.56, H 7.80%.

Concentrated HCl (12 mL) was added to a solution of the alcohol **9** (0.44 g) in chloroform (6 mL) and the resulting mixture stirred overnight at room temperature. Water (10 mL) was then added and the mixture was extracted with chloroform (3 × 25 mL). The extract was dried and evaporated, and the residue subjected to chromatography. Elution with 30–40% ethyl acetate – hexane gave 2,2-dimethyl-5,8-dimethoxychromene (0.34 g, 84%); mp 62–64°C; ^1H nmr δ: 1.45 (6H, s), 3.76 and 3.79 (each 3H, s), 5.56 and 6.63 (2H, ABq), and 6.29 and 6.68 (2H, ABq) ppm; ms *m/z*(%): 220(25), 205(100), 190(12), 175(24), 157(6), 91(5), 77(5).

To a solution of the above olefin (0.34 g) in ethyl acetate (6 mL) was added 10% palladium/carbon catalyst (50 mg). Overnight hydrogenation using a Parr apparatus at 19 psig (1

psi = 6.9 kPa) followed by filtration and evaporation of the filtrate gave 5,8-dimethoxy-2,2-dimethylchroman (**10**, 0.31 g, 91%); mp 56–58°C; ^{1}H nmr δ: 1.38 (6H, s), 1.78 (2H, t), 2.66 (2H, t), 3.78 and 3.81 (each 3H, s), 6.29 and 6.66 (2H, ABq) ppm; ^{13}C nmr δ: 17.4 (s), 26.6 (2C, p), 32.2 (s), 55.5 (p), 56.6 (p), 74.1 (q), 99.5 (t), 109.2 (t), 111.4 (q), 120.5 (q), 143.3 (q), 151.8 (q); ms *m/z*(%): 222(100), 167(84), 166(69), 151(6), 137(21), 123(15). Anal. calcd. for $C_{13}H_{18}O_3$: C 70.24, H 8.16%; found: C 70.62%, H 7.82%.

Biotransformations of bicyclic substrates

General procedures

Two slopes of *Mortierella isabellina* ATCC 42613 were used to inoculate 15 1 L Erlenmeyer flasks each containing 200 mL of an autoclaved medium composed of glucose (40 g), soybean flour (5 g), yeast extract (5 g), sodium chloride (5 g), and dibasic potassium phosphate (5 g) per L of distilled water. The flasks were allowed to stand overnight at 27°C, then were placed on a rotary shaker at 180 rpm, and growth continued for a further 72 h at 27°C. The fungus was then harvested by centrifugation (IEC chemical centrifuge) and resuspended in 15 1 L Erlenmeyer flasks each containing 200 mL of distilled water. Substrate (1 g in 30 mL of 95% ethanol) was then distributed among the flasks, which were replaced on the rotary shaker at 180 rpm, at 27°C, for a further 72 h. The fungus and aqueous medium were then separated by filtration as before, the aqueous medium extracted with dichloromethane (continuous extraction, 72 h), and the fungus discarded. Concentration of the medium extract gave the crude product, which was examined by tlc, using ether or 10% methanol – ether as solvent, and then subjected to flash chromatography. The yields and ee values quoted below refer to purified, homogeneous material, and arise from the combination of (only) homogeneous column fractions without further purification (e.g., crystallization) that could lead to changes in stereochemical enrichment values.

*2,2-Dimethyl-4-chromanone (**4**)*

Biotransformation of **4** (1 g) by the above procedure gave the following products in order of elution. (*S*)-2,2-Dimethylchroman-4-ol (**5**, 0.78 g); ir (KBr) ν_{max}: 3353 cm^{-1}; ^{1}H nmr δ: 1.26 and 1.39 (each 3H, s), 1.71–2.11 (2H, m), 2.90 (1H, br. s, exchanges D_2O), 4.75 (1H, d of t), and 6.73–7.42 (4H, m) ppm; ^{13}C nmr δ: 25.8 (p), 26.4 (p), 42.5 (s), 63.5 (t), 75.1 (q), 117.1 (t), 120.2 (t), 124.3 (q), 127.6 (t), 128.1 (t), and 153.2 (q) ppm; ms *m/z*(%): 178(60), 145(70), 123(57), 122(100), 121(71); $[\alpha]_D$ +8.4 (*c* 2.0, chloroform); ee 40% based on integration of C*H*OH and C-5H nmr signals in the presence of Eu(tfc)$_3$. 2,2-Dimethylchroman-3-ol-4-one (**6**, 42 mg); ir (KBr) ν_{max}: 1694, 3467 cm^{-1}; ^{1}H nmr δ: 1.22 (3H, s), 1.65 (3H, s), 3.78 (1H, br s, exchanges D_2O), 4.43 (1H, s), and 6.92–7.84 (4H, m) ppm; ^{13}C nmr: 17.2 (p), 26.9 (p), 77.0 (s), 83.5 (q), 118.4 (t), 188.9 (q), 121.1 (t), 126.7 (t), 136.6 (t), 159.5 (q), 194.4 (q); ms *m/z*(%): 192(68), 178(12), 163(53), 148(17), 121(100). M$^+$ calcd. for $C_{11}H_{12}O_3$: 192.0786; found: 192.0790.

*5,8-Dimethoxy-2,2-dimethyl-4-chromanone (**8**)*

Biotransformation of **8** (0.25 g) using four 1 L Erlemeyer flasks as described above gave, following extraction and chromatography with 30–50% ethyl acetate – hexane, (*S*)-5,8-dimethoxy-2,2-dimethylchroman-4-ol (**9**, 0.25 g, 99%); mp 67–69°C; nmr and mass spectral data identical with those described above for (±)-**9**; $[\alpha]_D$ +7.5 (*c* 1.5, ethanol); ee >95% based on ^{1}H nmr analysis of the C*H*OH signal in the presence of Eu(tfc)$_3$.

*5,8-Dimethoxy-2,2-dimethylchroman (**10**)*

Exposure of **10** (0.35 g) to *M. isabellina* (2 L of culture in 10 Erlenmeyer flasks) for 72 h followed by extraction as described above gave a crude extract (0.15 g) that was subjected to chromatography using 20–60% ethyl acetate – hexane as eluent and gave, in order of elution, 5,8-dimethoxy-2,2-dimethylchroman (**10**, 50 mg); 2,2-dimethyl-5-hydroxy-8-methoxychroman (**11**, 6 mg), as an oil; ^{1}H nmr δ: 1.37 (6H, s), 1.80 (2H, t, *J* = 7 Hz), 2.65 (2H, t, *J* = 7 Hz), 3.77 (3H, s), and 6.24/6.58 (2H, ABq) ppm; ^{13}C nmr δ: 17.4 (s), 26.6 (2C, p), 32.1 (s), 56.7 (p), 74.1 (q), 104.1 (t), 110.0 (q), 110.1 (t), 143.1 (q), 144.5 (q), 147.8 (q) ppm; ms *m/z*: M$^+$ 208; an oil (33 mg), which was further purified by preparative tlc (alumina) using chloroform as eluent to give 5,8-dimethoxy-2,2-dimethylchroman-4-ol (**9**, 5 mg), identified by spectral comparisons with authentic material; $[\alpha]_D$ 0; and 5,8-dimethoxy-2,2-dimethyl-6-hydroxychroman (**12**, 10 mg), as an oil; ^{1}H nmr δ: 1.35 (6H, s), 1.76 (2H, t, *J* = 7 Hz), 2.74 (2H, t *J* = 7 Hz), 3.74 and 3.77 (each 3H, s), and 6.43 (1H, s) ppm; ^{13}C nmr δ: 17.8 (s), 26.6 (2C, p), 32.2 (s), 56.2 (p), 60.8 (p), 73.6 (q), 98.2 (t), 115.5 (q), 137.0 (q), 137.4 (q), 140.8 (q), and 145.6 (q) ppm; ms *m/z*: M$^+$ 238.

Synthesis of 6,7-benzo-2,2-dimethyl-8-methoxy-4-chromanone (13)

A solution of 1-methoxy-2-naphthol (12) (6.26 g) and *p*-toluenesulfonic acid (0.1 g) in dichloromethane (80 mL) containing dimethoxymethane (25 mL) was heated under reflux (argon atmosphere) overnight in an apparatus fitted with a Soxhlet apparatus containing 40 g of activated 3 Å molecular sieves. The mixture was then cooled, triethylamine (1 mL) added, and the resulting solution washed (2 × 10% aqueous sodium hydroxide followed by saturated NaCl), dried, and evaporated. The residue was passed through a column of silica gel using 2% ethyl acetate – hexane as eluent to give 4.85 g (63%) of 1-methoxy-2-(methoxymethoxy)-naphthalene as a pale yellow oil; ^{1}H nmr δ: 3.53 (3H, s), 3.99 (3H, s), 5.27 (2H, s), 7.4–8.1 (6H, m) ppm; ^{13}C nmr δ: 58.2 (p), 61.1 (p), 96.0 (s), 116.7 (t), 121.4 (t), 124.1 (t), 124.5 (t), 126.0 (t), 127.6 (t), 129.0 (q), 130.6 (q), 144.0 (q), 145.0 (q) ppm; ms *m/z*(%): 218(100), 188(24), 173(71), 145(37), 127(33). M$^+$ calcd. for $C_{13}H_{14}O_3$: 218.0943; found: 218.0941.

A solution of *tert*-butyllithium (8.2 mL, 1.4 M in pentane) was added slowly to a stirred solution of 1-methoxy-2-(methoxymethoxy)-naphthalene (2.0 g) in dry THF (30 mL) under an argon atmosphere at −80°C. The resulting green solution was allowed to reach room temperature, stirred at that temperature for 1 h, and then recooled to −80°C. Upon slow addition of 3,3-dimethylacryolyl chloride (1.4 mL), the reaction mixture became pale yellow. The reaction was stirred overnight at room temperature, and then worked up by addition of aqueous THF and extraction with chloroform. The organic extract was washed with 10% aqueous sodium hydroxide, saturated sodium bicarbonate solution, and saturated sodium chloride, and then dried and evaporated. Chromatography (silica gel, 30% ethyl acetate – hexane) afforded 1.7 g (60%) of 3-(3′,3′-

dimethylacryloyl)-1-methoxy-2-(methoxymethoxy)-naphthalene as a pale yellow oil; ^{1}H nmr δ: 2.01 (3H, s), 2.26 (3H, s), 3.52 (3H, s), 4.04 (3H, s), 5.18 (2H, s), 6.68 (1H, t), and 7.5–8.1 (5H, m) ppm; ms *m/z*(%): 300(21), 285(9), 256(26), 200(34) relative to 83(100). M^+ calcd. for $C_{18}H_{20}O_3$: 300.1362; found: 300.1386.

The above compound (4.32 g) was dissolved in a mixture of THF (90 mL) and 6 M sulfuric acid (10 mL) and the resulting solution refluxed overnight. The solution was then cooled, neutralized by addition of 5% aqueous sodium hydroxide solution, and the resulting mixture extracted with chloroform. The extract was washed with saturated sodium bicarbonate solution, dried, and evaporated to give a residue that, following chromatography, afforded 6,7-benzo-2,2-dimethyl-8-methoxy-4-chromanone (**13**, 3.35 g, 91%); mp 94–95.5°C from ethyl acetate – hexane; ^{1}H nmr δ: 1.55 (6H, s), 2.85 (2H, s), 4.03 (3H, s), 7.36 (1H, t), 7.54 (1H, t), 7.67 (1H, d), 8.08 (1H, d), and 8.28 (1H, s) ppm; ^{13}C nmr δ: 27.0 (2C, p), 49.7 (s), 61.0 (p), 70.0 (q), 121.3 (t), 122.0 (t), 123.0 (t), 124.7 (t), 126.1 (q), 126.6 (t), 130.0 (t), 132.6 (q), 142.5 (q), 146.2 (q), and 193.0 (q) ppm; ms *m/z*(%): 256(100), 241(16), 201(49), 200(64), 185(25), 157(44). M^+ calcd. for $C_{16}H_{16}O_3$: 256.1099; found: 256.1119. Anal. calcd. for $C_{16}H_{16}O_3$: C 74.98, H 6.29%; found: C 74.61, H 6.60%.

Biotransformation of 6,7-benzo-2,2-dimethyl-8-methoxy-4-chromanone (13)

Using a 72 h growth of *M. isabellina* (600 mL of culture in three 1 L Erlenmeyer flasks), the title ketone (0.18 g) was converted to (*S*)-6,7-benzo-2,2-dimethyl-8-methoxychroman-4-ol (**14**, 35 mg), isolated as an oil by chromatography on silica gel using a 2% ether – benzene stepwise gradient elution; ^{1}H nmr δ: 1.36 (3H, s), 1.53 (3H, s), 1.92 and 2.24 (each 1H, dd), 2.4 (1H, br. s, exchanges D_2O), 3.95 (3H, s), 5.02 (1H, t), 7.36 (2H, m), 7.69 (1H, d), 7.73 (1H, s), and 8.04 (1H, d) ppm; ^{13}C nmr δ: 25.9 (p), 29.4 (p), 42.8 (s), 60.7 (p), 64.1 (t), 75.8 (q), 120.9 (t), 121.5 (t), 123.7 (t), 125.9 (t), 127.6 (t), 128.0 (q), 128.4 (q), 128.6 (q), 141.4 (q), and 142.8 (q) ppm; ms *m/z*(%): 258(46), 240(60), 225(100), 210(30), 202(81), 187(55). M^+ calcd. for $C_{16}H_{18}O_3$: 258.1256; found: 258.1270; $[\alpha]_D$ +24.8 (*c* 0.5, ethanol), ee 32% based on ^{1}H nmr analysis of the C*H*OH signal in the presence of Eu(tfc)$_3$. Also obtained from chromatography was a pale green oily material (11 mg) tentatively identified as a phenolic derivative of **14** by the following spectral data: ^{1}H nmr δ: 1.33 (3H, s), 1.51 (3H, s), 1.9 and 2.2 (each 1H, dd), 3.92 (3H, s), 4.95 (1H, t), 6.31 (1H, br. s, exchanges D_2O), 6.94 (1H, s), 7.03 and 7.88 (2H, ABq), and 7.41 (1H, s) ppm; ms *m/z*(%): 274(2), 256(10), 241(9), 226(4) relative to 83(100). Lack of material and instability of the product precluded further analysis.

Synthesis of 4-hydroxy-α-lapachone

4-Keto-α-lapachone (15)

A solution of 6,7-benzo-2,2-dimethyl-8-methoxy-4-chromanone (**13**, 0.2 g) in acetonitrile (2 mL) was cooled to 0°C and then a solution of ceric ammonium nitrate (2.18 g) in water (10 mL) was added at 0°C over a period of 5 min. The mixture was stirred for a further 30 min at 0°C, water (20 mL) was added, and the resulting solution was extracted with chloroform. The extract was dried and concentrated to a volume of 30 mL. The resulting solution was then aerated and refluxed for 24 h. After this period the cooled solution was washed (saturated aqueous sodium bicarbonate), dried, and evaporated. Chromatography of the residue gave 4-keto-α-lapachone (**15**, 0.11 g, 57%) as a yellow solid, mp 161–164°C (from benzene–hexane) (lit. (2) mp 163–165°C); ir ν_{max}: 1689, 1714 cm^{-1}; ^{1}H nmr δ: 1.62 (6H, s), 2.77 (2H, s), 7.76 (2H, m), and 8.10 (2H, m) ppm; ^{13}C nmr δ: 26.1 (2C, p), 48.7 (s), 84.4 (q), 112.9 (q), 126.3 (t), 127.0 (t), 130.8 (q), 132.0 (q), 133.3 (t), 135.4 (t), 162.4 (q), 180.0 (q), 180.6 (q), and 189.7 (q) ppm; ms *m/z*(%): 256 (73), 201 (98), 173(47), 104(100).

(S)-4-Hydroxy-α-lapachone (3)

Biotransformation of the ketone **15** (0.41 g) with a culture of *M. isabellina* in eight 1 L Erlenmeyer flasks as outlined above, followed by chromatography of the extract, gave (*S*)-4-hydroxy-α-lapachone (**3**, 0.2 g, 49%) as a yellow solid, mp 67°C (lit. (2) yellow syrup (*S*); (6) mp 117–119°C (racemate); (7) yellow oil (racemate); (4) yellow oil (*S*);); ir ν_{max}: 1645, 1683, 3505 cm^{-1}; ^{1}H nmr δ: 1.44 (3H, s), 1.55 (3H, s), 2.08 (2H, dd), 3.80 (1H, br s, exchanges D_2O), 4.97 (1H, t), 7.70 (2H, m), and 8.08 (2H, m) ppm; ^{13}C nmr δ: 26.7 (p), 27.1 (p), 39.6 (s), 60.0 (t), 79.8 (q), 120.4 (q), 126.0 (t), 126.6 (t), 131.2 (q), 131.9 (q), 133.4 (t), 134.3 (t), 154.1 (q), 180.1 (q), and 186.1 (q) ppm; ms *m/z*(%): 258(24), 243(10), 203(93), 176(69), 146(87), 105(50), 83(100). M^+ calcd. for $C_{15}H_{14}O_4$: 258.0892; found: 258.0888. $[\alpha]_D$ +39.7 (*c* 1.3, methanol) (lit. (2) $[\alpha]_D$ +27.4 (methanol)), ee >95% by ^{1}H nmr analysis of the C*H*OH signal in the presence of Eu(tfc)$_3$.

(S)-4-Acetoxy-α-lapachone (16)

Acetic anhydride (1 mL) was added to a solution of (*S*)-4-hydroxy-α-lapachone (**3**, 0.071 g) in dry pyridine (2 mL) and the mixture allowed to stand overnight at room temperature. The usual work-up afforded a yellow residue that, on chromatography, afforded (*S*)-4-acetoxy-α-lapachone (**16**, 0.08 g, 97%). Crystallization from ethanol gave a pale yellow solid, mp 145–148.5°C (lit. (6) mp 136–138°C (racemate); (7) mp 138.5–139.5°C (racemate); (4) mp 118–120°C (*S*); (2) mp 139.5–140.5°C (*S*)); ir ν_{max}: 1616, 1651, 1683, 1739, 2931, 2979 cm^{-1}; ^{1}H nmr δ: 1.51 (3H, s), 1.56 (3H, s), 2.15 (5H, s + m), 6.10 (1H, dd), 7.72 (2H, m), and 8.10 (2H, m) ppm; ^{13}C nmr δ: 21.0 (p), 25.4 (p), 28.8 (p), 38.1 (s), 60.6 (t), 78.7 (q), 116.7 (q), 126.3 (t), 131.1 (q), 132.0 (q), 133.2 (t), 134.4 (t), 155.8 (q), 170.0 (q), 179.9 (q), and 182.7 (q) ppm; ms *m/z*(%): 300(0.6), 258(100), 243(12), 225(9), 201(71). M^+ calcd. for $C_{16}H_{16}O_5$: 300.0998; found: 300.1004. $[\alpha]_D$ −22.7 (*c* 1.3, methanol) (lit. (2) $[\alpha]_D$ −14.2 (*c* = 1.03, methanol)).

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