# Enzymatic Kinetic Resolution of Flavanone and cis-4-Acetoxyflavan

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The microbial, asymmetric reduction of flavanone with fermenting bakers' yeast led to the formation of (2S,4S)-(+)-cis-4-hydroxyflavan and (2R)-(+)-flavanone. The kinetic resolution of racemic cis-4-acetoxyflavan with lipase PS yields (2R,4R)-(−)-cis-4-hydroxyflavan, and the (2S,4S)-(+)-enantiomer remains as the acetate.

Flavanones are widely distributed in plants, and are interesting compounds with respect to the existence of optically active compounds such as pinocembrin (5,7-dihydroxyflavanone),[1] matteucinol (5,7-dihydroxy-4'-methoxy-6,8-dimethylflavanone),[2] farrerol (4',5,7-trihydroxy-6,8-dimethylflavanone),[3] cryptostrobin (5,7-dihydroxy-8-methylflavanone),[4] sophoranone (4',7-dihydroxy-3',5,5'-triprenylflavanone),[5] dihydrowogonin (5,7-dihydroxy-8-methoxyflavanone),[6] strobopinin (5,7-dihydroxy-6-methylflavanone),[7] and obtusifolin.[8] Furthermore, Corey and Mitra,[9] and Bognar and co-workers[10] reported the synthesis of enantiomers of flavanone 1 by optical resolution or synthesis. On the other hand, asymmetric synthesis by biological systems has become important in organic synthesis.[11] In this paper, we report the enantioselective microbial kinetic resolution of racemic 1 with bakers' yeast and the enantioselective hydrolysis of racemic cis-4-acetoxyflavan 2 with lipase PS (*Pseudomonas* sp. lipase, Amano).

It has been shown recently that reduction of ketones, α-substituted by an electron-withdrawing group, with actively fermenting bakers' yeast (*Saccharomyces cerevisiae*) leads to the formation of the corresponding optically active alcohols in good chemical and high optical yields.[11e] The reduction of racemic flavanone 1 with bakers' yeast under standard conditions provided (2S,4S)-(+)-cis-4-hydroxyflavan [(2S,4S)-3] in 32% yield (83% e.e.) and the optically active (2R)-(+)-flavanone [(2R)-1] in 51% yield (20% e.e.) (Scheme 1). The enantiomeric purity of (2S,4S)-(+)-3 was determined by means of high-performance liquid chromatography (HPLC) of the
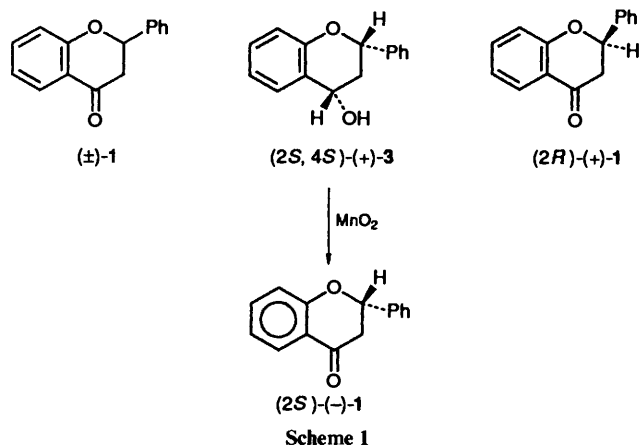
corresponding (S)-(−)-2-methoxy-2-(trifluoromethyl)(phenyl)-acetate [3,3,3-trifluoro-2-methoxy-2-phenylpropionate] (MPTA) derivative. On the other hand, the e.e.-value of (2R)-(+)-1 was determined by chemical correlation to (2R,4R)-(−)-cis-4-hydroxyflavan (2R,4R)-3 by reduction with LiAlH₄ and analysis (HPLC) of its MPTA ester.

The oxidation of (2S,4S)-(+)-3 (83% e.e.) with active manganese dioxide in chloroform led to the formation of (2S)-(−)-flavanone [(2S)-1] ([α]$_D^{20}$ −54.1)† in 95% yield (Scheme 1).

Meanwhile, Bevinakatti et al.[12] reported that a lipase-catalysed esterification is effective for primary alcohols, but secondary alcohols require the use of activated esters to achieve reaction, and more sterically hindered secondary alcohols no longer react with activated esters. Enzymatic transesterification of (±)-cis-4-hydroxyflavan 3 with lipase PS using vinyl acetate as the acyl donor proceeded to afford acetate 2 which, however, showed no optical rotation. On the other hand, the lipase PS-catalysed kinetic resolution of racemic acetate 2 was performed at room temperature in a phosphate buffer solution (pH 7.4) (Scheme 2). The reaction processes were monitored by HPLC and the reactions were terminated at, or close to, the 50%-of-hydrolysis point. The products were extracted with chloroform and purified by column chromatography. The results are summarized in Table 1.

Scheme 1

Scheme 2

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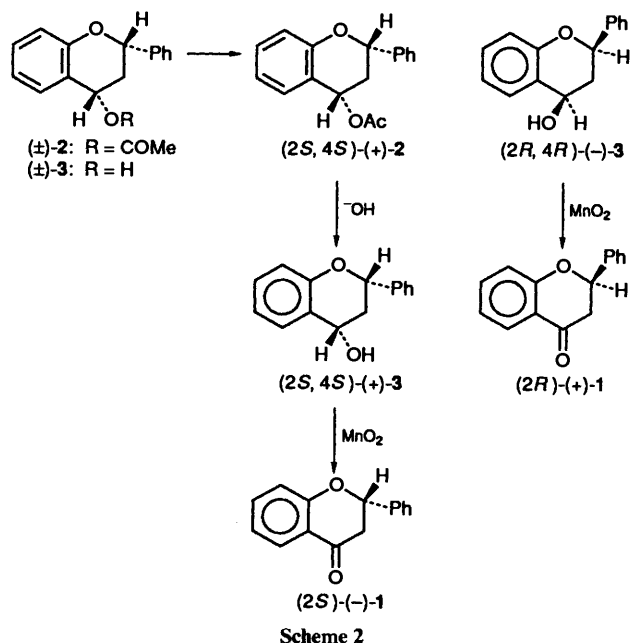
† New IUPAC recommendations for [α]$_D$-values suggest that these should now have units of 10^{-1} deg cm² g⁻¹.

Table 1 Enzymatic hydrolysis of racemic acetate **2** in the presence of lipase PS

Entry	Time/day	Conversion/%	Product (2R,4R)-(−)-3			Recovered acetate (2S,4S)-(+)-2		
			Yield/%	$[\alpha]_D^{20}$	e.e./%	Yield/%	$[\alpha]_D^{20}$	e.e./%[a]
1	2	14	14	−11.3 (CHCl$_3$)	75	80	+6.7 (CHCl$_3$)	20
2	4	26	26	−12.8 (CHCl$_3$)	83	68	+14.8 (CHCl$_3$)	41
3	6	38	38	−14.4 (CHCl$_3$)	98	55	+23.9 (CHCl$_3$)	70
4	8	47	47	−13.8 (CHCl$_3$)	93	45	+33.1 (CHCl$_3$)	95

[a] Determined on the basis of the optical purity of the alcohol obtained from the acetate by alkaline hydrolysis (see the Experimental section).

After 6 days, the lipase PS-catalysed hydrolysis afforded (2R,4R)-(−)-cis-4-hydroxyflavan [(2R,4R)-3] (38% yield, 98% e.e.) and (2S,4S)-(+)-cis-4-acetoxyflavan [(2S,4S)-2] (55% yield, 70% e.e.). Meanwhile, after 8 days, the reaction products were (2R,4R)-(−)-3 (93% e.e.) and (2S,4S)-(+)-2 (95% e.e.). The e.e.-value (98%) of the alcohol [(2R,4R)-(−)-3] was determined by HPLC analysis of the corresponding MTPA derivative, and the e.e.-value (70%) of the acetate [(2S,4S)-(+)-2] was also determined by derivatization to the corresponding alcohol [(2S,4S)-(+)-3].

By oxidation with active manganese dioxide, the alcohol (2R,4R)-(−)-3 with 98% e.e. was converted into flavanone (2R)-(+)-1 ([α]$_D^{20}$ +66.5). Furthermore, oxidation of the alcohol (2S,4S)-(+)-3 (70% e.e.) with manganese dioxide led to the formation of flavanone (2S)-(−)-1 ([α]$_D^{20}$ −44.4).

Experimental

M.p.s were taken with a Gallenkamp melting point apparatus and are uncorrected. IR and ^{1}H NMR spectra were recorded using Hitachi 260-10 and Hitachi R90H spectrometers, respectively, in deuteriochloroform with (CH$_3$)$_4$Si as internal standard in the latter case. Mass spectra were recorded on a Hitachi RMU-6M spectrometer. J values are given in Hz.

HPLC analysis of enantiomers was carried out on a silica gel column (NUCLEOSIL 50-5, 8 mm × 25 cm; hexane–ethyl acetate mixtures as eluent). Optical rotations were measured on a JASCO DIP-140 digital polarimeter. Bakers' yeast and lipase PS were purchased from Oriental Yeast Co. and Amano Pharmaceutical Co., respectively. All organic solvents used in this work were of analytical grade, and prior to use they were dried overnight over 3 Å molecular sieves. Racemic flavanone[13] **1** and cis-4-acetoxyflavan[14] **2** were prepared according to the respective literature methods. The e.e.-values were determined on the basis of comparison of the observed rotation either with literature-reported values or with the values of a chemically synthesized, optically pure, authentic sample.

Enzymatic Resolution of Racemic Flavanone **1** *with Bakers' Yeast.*—A mixture of dry yeast (60 g) and D-glucose (40 g) in water (600 cm^3) was stirred for 15 min at 30 °C. To the well stirred fermenting bakers' yeast was added, in portions, a solution of racemic **1** (600 mg, 2.68 mmol) in ethanol (10 cm^3) during 1 h, and the mixture was stirred at 30 °C with monitoring of the conversion by HPLC [hexane–ethyl acetate (10:1)]. After 21 days, the reaction was terminated by addition of acetone, the mixture was filtered, and the solids were washed with methanol. The combined filtrate and washings were evaporated under reduced pressure to ∼150 cm^3 and extracted with diethyl ether. After being dried over anhydrous magnesium sulfate, the extract was evaporated and the crude products were purified by column chromatography on silica gel [hexane–ethyl acetate (3:1)]. The first eluate, followed by recrystallization from ethanol, afforded optically active flavanone (2R)-(+)-1 as crystals (306 mg, 51% yield)

based on expected (±)-1, m.p. 74.5–75 °C; [α]$_D^{20}$ +12.4 (c 0.44, CHCl$_3$) (e.e. = 20%) (Found: C, 80.3; H, 4.5. Calc. for C$_{15}$H$_{12}$O$_2$: C, 80.33; H, 4.54%) {lit.,^{10a} m.p. 77 °C; [α]$_D^{20}$ + 67.2 (c 0.35, CHCl$_3$)}; ν_{max}(KBr)/cm^{-1} 1680 (C=O), 1600, 1580, 760, 745 and 690; δ(CDCl$_3$) 2.90 (2 H, m, 3-H$_2$), 5.36 (1 H, dd, J 5.5 and 11.5, 2-H) and 6.89–7.96 (9 H, m, ArH); m/z 224 (M$^+$).

The second eluate with hexane–ethyl acetate afforded (2S,4S)-(+)-cis-4-hydroxyflavan [(2S,4S)-3] as crystals (194 mg, 32%), m.p. 116 °C (from EtOH); [α]$_D^{20}$ +13.6 (c 0.27, CHCl$_3$) (e.e. 83%) (Found: C, 79.6; H, 6.15. Calc. for C$_{15}$H$_{14}$O$_2$: C, 79.62; H, 6.24%) {lit.,^{15} m.p. 116 °C; [α]$_D^{20}$ +16 (c 0.5, CHCl$_3$)}; ν_{max}(KBr)/cm^{-1} 3400 (OH), 1600, 1580, 760, 750 and 690; δ-(CDCl$_3$) 1.90–2.08 (2 H, m, 3-H$_2$), 4.50 (1 H, br s, OH), 5.03 (1 H, dd, 2-H), 5.25 (1 H, dd, 4-H) and 6.72–7.90 (9 H, m, ArH); m/z 226 (M$^+$).

Determination of Enantiomeric Purity.—The alcohol (2S,4S)-(+)-3 was treated with (S)-(−)-2-methoxy-2-(trifluoromethyl)(phenyl)acetyl chloride to form the corresponding MPTA ester. The MTPA derivative was analysed by HPLC on a silica gel column (8 mm × 25 cm) with hexane–ethyl acetate (4:1) as the mobile phase at a flow rate of 1.8 cm^3 min^{-1}. The retention times for the (−)-MTPA esters of the alcohol (2S,4S)-(+)-3 were: (2S,4S)-isomer, 12 min; (2R,4R)-isomer, 13 min 10 s.

The enantiomeric purity of flavanone (2R)-(+)-1 (100 mg) was determined after conversion into the alcohol (2R,4R)-(−)-3 by reduction with LiAlH$_4$ (100 mg) in dry diethyl ether (20 cm^3) and analysed by HPLC of the corresponding (−)-MTPA ester.

The Oxidation of (2S,4S)-(+)-3 *with Manganese Dioxide to give Flavanone* (2S)-(−)-1.—Active manganese dioxide (2 g) was added to a solution of the alcohol (2S,4S)-(+)-3 ([α]$_D^{20}$ +13.6) (45 mg) in chloroform (10 cm^3) at room temperature, and the mixture was stirred in the dark for 3 h. After filtration to remove manganese dioxide and evaporation to dryness under reduced pressure, the residue was recrystallized from ethanol to afford crystals of flavanone (2S)-(−)-1 (38 mg, 95%), m.p. 75–77 °C; [α]$_D^{20}$ −54.1 (c 0.13, CHCl$_3$) (Found: C, 80.2; H, 4.45. C$_{15}$H$_{12}$O$_2$ requires C, 80.33; H, 4.54%) {lit.,^{10a} m.p. 76–77 °C; [α]$_D^{20}$ −64.4 (c 0.35, CHCl$_3$)}. The spectroscopic data were identical with those of (2S)-(+)-1.

Enzymatic Resolution of Racemic cis-4-Acetoxyflavan **2** *with Lipase PS.*—The (±)-acetate **2** (160 mg, 0.59 mmol) was dissolved in the mixture of diisopropyl ether (5 cm^3) and ethanol (0.5 cm^3, nucleophile). To this solution was added 2% (w/w) aq. poly(vinyl alcohol) (2.5 cm^3), followed by 0.15 mol dm^{-3} potassium phosphate buffer (pH 7.4; 2.5 cm^3) to form an emulsion. To the vigorously stirred emulsion was added portionwise lipase PS (300 mg) and the mixture was stirred at room temperature (23 °C) while the conversion was monitored by HPLC [hexane–ethyl acetate (5:1)]. After 6 days, the reaction mixture was extracted with chloroform four times. The

combined organic phase was dried over anhydrous magnesium sulfate, and evaporated to dryness under reduced pressure, and the residue was chromatographed on a silica gel column. The first eluate with chloroform afforded the *optically active acetate* (2S,4S)-(+)-**2** (88 mg, 55%) as crystals, m.p. 94–95 °C (from EtOH); $[\alpha]_D^{20}$ +23.9 (*c* 0.96, CHCl$_3$) (Found: C, 75.9; H, 5.9. C$_{17}$H$_{16}$O$_2$ requires C, 76.10; H, 6.01%); ν_{max}(KBr)/cm^{-1} 1700 (OAc), 1600, 1580, 765, 750 and 690; δ(CDCl$_3$) 1.63–2.79 (m, 2 H, 3-H$_2$), 2.04 (s, 3 H, Ac), 5.01 (1 H, dd, 2-H), 6.13 (1 H, dd, 4-H) and 6.75–7.48 (9 H, m, ArH); *m/z* 268 (M$^+$).

The e.e.-value (70%) of (2S,4S)-(+)-**2** was determined by derivatization to the alcohol (2S,4S)-(+)-**3** and analysis (HPLC) of the corresponding MTPA ester.

The second eluate with chloroform gave the alcohol (2R,4R)-(−)-**3** (51 mg, 38%) as crystals, m.p. 115–116 °C (from EtOH); $[\alpha]_D^{20}$ −14.4 (*c* 0.5, CHCl$_3$) (Found: C, 79.55; H, 6.1. Calc. for C$_{15}$H$_{14}$O$_2$: C, 79.62; H, 6.24%) {lit.,[15] m.p. 116 °C; $[\alpha]_D^{20}$ −14.5 (*c* 0.5, CHCl$_3$)}. The spectroscopic data were identical with those of (2S,4S)-(+)-**3**. The e.e.-value (98%) was determined by HPLC of the corresponding MTPA ester.

Hydrolysis of the Acetate (2S,4S)-(+)-**2** *to the Alcohol* (2S,4S)-(+)-**3**.—The acetate (2S,4S)-(+)-**2** ($[\alpha]_D^{20}$ +23.9) (80 mg) was dissolved in methanol (10 cm^3) and the solution was refluxed with 15% aq. sodium hydroxide (1 cm^3) for 15 min. After cooling, the reaction mixture was neutralized with a few drops of hydrochloric acid and then extracted with chloroform. The extracts were washed with water, dried over anhydrous magnesium sulfate, and evaporated to dryness under reduced pressure. The residue was recrystallized from ethanol–water (2:1) to afford (60 mg) (2S,4S)-(+)-*cis*-4-hydroxyflavan **3**, m.p. 115–116 °C; $[\alpha]_D^{20}$ +11.2 (*c* 0.35, CHCl$_3$) {lit.,[15] m.p. 116 °C; $[\alpha]_D^{20}$ +16 (*c* 0.5, CHCl$_3$)}. The e.e.-value (70%) was determined by HPLC of the corresponding MTPA ester. The analytical data were identical with those of (2S,4S)-(+)-**3** which was synthesized by reduction of flavanone (±)-**1** with baker's yeast.

Synthesis of Flavanone (2R)-(+)-**1** *from the Alcohol* (2R,4R)-(−)-**3** ($[\alpha]_D^{20}$ −14.4).—The oxidation of (2R,4R)-(−)-**3** ($[\alpha]_D^{20}$ −14.4, e.e. 98%) with manganese dioxide was carried out in the same way as that described for (2S,4S)-(+)-**3** ($[\alpha]_D^{20}$ +13.6), to give the flavanone (2R)-(+)-**1** as crystals, m.p. 76–77 °C; $[\alpha]_D^{20}$ +66.5 (*c* 0.48, CHCl$_3$) {lit.,[10a] m.p. 77 °C; $[\alpha]_D^{20}$ +67.2 (*c* 0.35, CHCl$_3$)}. The spectroscopic data were identical with those of (2R)-(+)-**1** obtained by reduction of flavanone (±)-**1** with bakers' yeast.

Synthesis of Flavanone (2S)-(−)-**1** *from the Alcohol* (2S,4S)-(+)-**3** ($[\alpha]_D^{20}$ +11.2).—The oxidation of the alcohol (2S,4S)-(+)-**3** ($[\alpha]_D^{20}$ 11.2, e.e. 71%) with manganese dioxide was carried out in the same way as that described for the alcohol (2S,4S)-(+)-**3** ($[\alpha]_D^{20}$ +13.6), to give flavanone (2S)-(−)-**1**, crystals, m.p. 76 °C; $[\alpha]_D^{20}$ −44.4 (*c* 0.38, CHCl$_3$) {lit.,[10a] m.p. 76–77 °C; $[\alpha]_D^{20}$ −64.4 (*c* 0.35, CHCl$_3$)}. The spectroscopic data were identical with those of flavanone (2R)-(+)-**1**.

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