Enzymatic Resolution of Flavanone Oximes

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The optically active (*R*)- and (*S*)-flavanones were prepared by an enzymatically enantioselective hydrolysis of (±)-flavanone oxime *O*-acylates employing lipases, followed by hydrolysis with acid.

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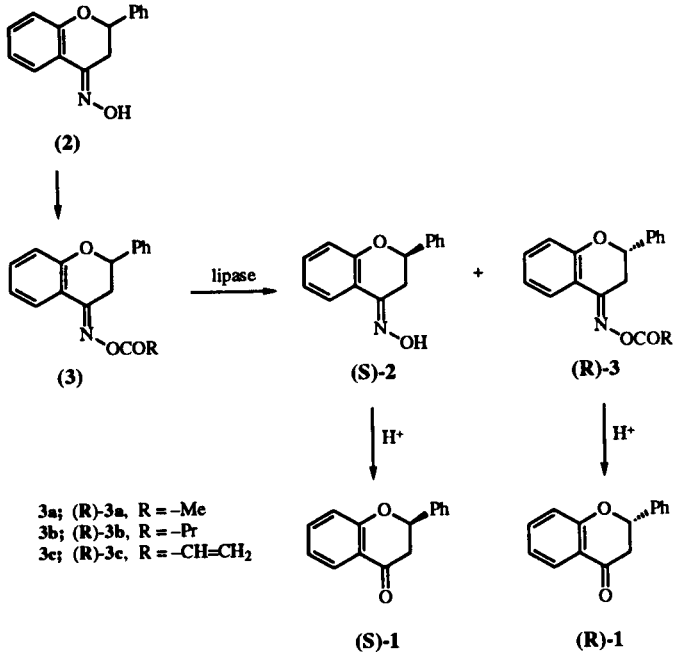
Flavanones (2,3-dihydro-2-phenyl-4*H*-benzo[*b*]pyran-4-ones) are widely distributed in plants, leaves, fruits, and flowers, and are interesting substances with respect to the existence of optically active compounds such as hesperetin, matteucinol and naringin, *etc* [1]. Corey and Mitra [2] and Bognár *et al.* [3] reported the synthesis of the enantiomers of flavanone (**1**) by optical resolution or synthesis. We also reported the enzymaticaly kinetic resolution of **1** by the enantioselective reduction using Baker's yeast [4]. On the other hand, lipases have been extensively used to catalyze stereoselective esterification, transesterification and hydrolysis in nonaqueous media [5]. Moreover, oxime esters have an intermediate reactivity as irreversible acyl transfer agents for lipase catalysis and, therefore, can be used under mild conditions in enzymatical transesterification reaction [6]. Ketone *O*-acyloximes can also be prepared by reaction of oximes with vinyl esters, using lipases as biocatalysts [7]. In this paper, we wish to report the enzymatic resolution of **1** by an enantioselective hydrolysis of (±)-flavanone oxime *O*-acylates (**3**) [4-acyloxyimino-2,3-dihydro-2-phenyl-4*H*-benzo[*b*]pyran] employing lipases, followed by hydrolysis with acid.

Scheme 1



Results and Discussion.

In an initial attempt, the enzymatic transesterification of (±)-flavanone oxime **2** using lipase from *Pseudomonas cepacia* and vinyl acetate as acylating agent in diisopropyl ether at room temperature for 9 days led to the formation of the esterified flavanone oxime *O*-acetate (±)-**3a** and unesterified oxime (±)-**2**, however, the reaction proceeded with a very low degree of enantioselectivity and afforded the products which showed no optical rotation (entry 1 in Table 1). Moreover, *Candida cylindracea* lipase exhibited very low catalytic activities in the esterification of (±)-**2**, and did not afford the enzymatically transesterification products (entry 2 in Table 1). On the other hand, the enzymatic hydrolysis of (±)-**3a** using *Porcine pancreatic* lipase proceeded smoothly with a high degree of enantioselectivity to afford (*S*)-flavanone oxime (*S*)-**2** (83% ee) and (*R*)-flavanone oxime *O*-acetate (*R*)-**3a** (81% ee) (entry 1 in Table 2). The absolute configuration of (*S*)-**2** and (*R*)-**3a** was secured by the conversion into the known (*S*)-flavanone (*S*)-**1** and (*R*)-flavanone (*R*) **1**, respectively, using the hydrolysis by 1*N* hydrochloric acid in acetone aqueous solution. The results of enzymatic hydrolysis of *O*-acylates (**3a-c**) using lipases are summarized in Table 2.

The enzymatic hydrolysis of (±)-**3a** with lipase from *Candida rugosa* (lipase MY) also yielded (*R*)-**3a** and (*S*)-**2** with lower degrees of enantioselectivity, compared with that using *porcine pancreatic* lipase (entry 2 in Table 2). On the other hand, in the biochemical reaction of (±)-**3a** with lipase from *Pseudomonas cepacia* (lipase PS), the enzymatic hydrolysis proceeded very rapidly, however, the optical purities of the products were not so high (entry 3 in Table 2). In the enzymatic hydrolysis using *Candida cylindracea* lipase, the acetate (±)-**3a** was not hydrolyzed even though the substrate was allowed to react for 10 days (entry 4 in Table 2).

The enzymatic hydrolysis of flavanone oxime *O*-butyrate (±)-**3b** using *Porcine pancreatic* lipase, lipase from *Candida rugosa* (lipase MY), lipase from *Pseudomonas cepacia* (lipase PS) or *Candida cylindracea*

Table 1
Enzymatic Transesterification of Flavanone Oxime (±)-2 using Lipases and Vinyl Acetate

Entry	Enzyme [a]	Time (day)	Esterified Oxime			Unesterified Oxime		
			Yield	Configuration	ee	Yield	Configuration	ee
1	PS	8	25%	(±)-2	0%	65%	(±)-3a	0%
2	CCL	9	0%	–	–	99%	(±)-3a	0%

[a] PS = *Pseudomonas cepacia* lipase; CCL = *Candida cylindracea* lipase.

Table 2
Enzymatic Hydrolysis of Flavanone Oxime *O*-Acylates Using Lipases

Entry	Substrate [a]	Lipase [b]	Reaction Time (day)	Hydrolyzed Oxime [c]			Unhydrolyzed Oxime Acylate [c]			E [d]
				Yield (%)	Configuration	ee (%)	Yield (%)	Configuration	ee (%)	
1	3a	PPL	9	42	(*S*)-2	83	46	(*R*)-3a	81	29
2	3a	MY	9	28	(*S*)-2	46	60	(*R*)-3a	17	5
3	3a	PS	9.5 hours	60	(*S*)-2	20	35	(*R*)-3a	28	2
4	3a	CCL	10	–	–	–	97	(±)-3a	0	–
5	3b	PPL	12	36	(*S*)-2	65	54	(*R*)-3b	44	18
6	3b	MY	10	51	(*S*)-2	24	45	(*R*)-3b	26	2
7	3b	PS	9 hours	40	(*S*)-2	26	53	(*R*)-3b	19	2
8	3b	CCL	9	14	(*S*)-2	42	76	(*R*)-3b	7	2
9	3c	PPL	9	34	(*S*)-2	77	54	(*R*)-3c	68	21
10	3c	PS	9 hours	53	(*S*)-2	17	38	(*R*)-3c	16	2

[a] 3a = flavanone oxime *O*-acetate, 3b = flavanone oxime *O*-butyrate, 3c = flavanone oxime *O*-acrylate. [b] PPL = porcine pancreatic lipase; MY = lipase from *Candida rugosa;* PS = *Pseudomonas cepacia* lipase; CCL = *Candida cylindracea* lipase. [c] The ee value of the products was determined by chiral hplc analysis with Daicel chiralcel OG column (4.6 mm x 250 mm) with hexane-propan-2-ol (10:1) as mobile phase at a flow rate of 0.4 ml; detection, uv at 220 nm. [d] $E = \ln[1 - c(1 + ee_p)]/\ln[1 - c(1 - ee_p)]$, $c = ee_p/(ee_S + ee_p)$.

lipase, proceeded to afford (*S*)-2 and (*R*)-butyrate (*R*)-3b, however, the enantioselections of the reaction, except *Candida cylindracea* lipase, were lower than those using (±)-3a as the substrate (entries 5, 6, 7, 8 in Table 2).

The enzymatic hydrolysis of the oxime *O*-acrylate (±)-3c using *Porcine pancreatic* lipase, also afforded (*S*)-2 and (*R*)-acrylate (*R*)-3c, however, the enantioselectivity of the reaction was lower, compared with those using (±)-3a, but was much higher than those using (±)-3b as the starting substrate (entry 9 in Table 2).

EXPERIMENTAL

All melting points were taken on a Gallenkemp melting points apparatus and are uncorrected. The ir spectra were recorded on a Hitachi 260-10 spectrometer. The ^1H nmr spectra were taken with a Hitachi R-90H (90 MHz) instrument in deuteriochloroform using tetramethylsilane as internal standard. Mass spectra were recorded on a JEOL JMS-AX505HA spectrometer. Optical rotations were measured with a JASCO DIP-140 digital polarimeter. The enantiomeric excess (ee) value of the products was determined by chiral high performance liquid chromatography analysis using a Daicel chiralcel OG column (4.6 mm x 25 cm) with hexane-propan-2-ol (10:1) as mobile phase at a flow rate of 0.4 ml; detection, uv at 220 nm.

Flavanone oxime (2) was prepared as described in the literature [8].

Porcine pancreatic lipase (PPL, Type II) and *Candida cylindracea* lipase (CCL, Type VII, 900 u mg^{-1}) were obtained from Sigma Chemical Co. Lipase from *Candida rugosa* (lipase MY, 6200 u g^{-1}) and lipase from *Pseudomonas cepacia* (lipase PS) were purchased from Meito Sangyo Co. and Amano Pharmaceutical Co., respectively.

Synthesis of Flavanone Oxime *O*-Acylate (3). Typical Procedure.

In a round-bottomed flask fitted with magnetic stirrer and addition funnel, acetyl chloride, butyryl chloride or acryoyl chloride (12 ml) is added dropwise to a solution of flavanone oxime (2) (10 mmoles) in pyridine (2.7 ml, 30 mmoles) at 0–5°. After stirring for 5 hours at the same temperature, the mixture was poured into 3 *N* hydrochloric acid (12 ml), and extracted (3 x 50 ml) with chloroform. The combined extracts were successively washed with saturated aqueous sodium chloride solution, saturated aqueous sodium hydrogen carbonate solution and saturated aqueous sodium chloride solution, then dried over anhydrous magnesium sulfate and concentrated to yield a white solid. The solid was recrystallized from ethanol to give colorless crystals of the desired oxime *O*-acylate; spectroscopic and analytical data of the product were as follows:

Flavanone Oxime *O*-Acetate (4-Acetoxyimino-2,3-dihydro-2-phenyl-4*H*-benzo[*b*]pyran) (3a).

This compound was obtained from 2 and acetyl chloride as colorless crystals, yield 98%, mp 174-175°, ir (potassium bromide):

1780 (ester), 1620 (-C=N), 780, 760, 710 cm^{-1} (Ar-H); ^1H nmr: δ 2.20 (s, 3H, $-CH_3$), 2.82 (dd, 1H, J = 17 Hz and 12.5 Hz, $-C_3H_{ax}$), 3.49 (dd, 1H, J = 17 Hz and 3 Hz, $-C_3H_{eq}$), 5.08 (dd, 1H, J = 12.5 Hz and 3 Hz, $-C_2H$), 6.84~7.06 (m, 2H, Ar*H*), 7.17~7.50 (m, 6H, Ar*H*), 8.04 ppm (dd, 1H, $-C_5H$); ms: m/z 281 [M^+].

Anal. Calcd. for $C_{17}H_{15}NO_3$: C, 72.58; H, 5.37; N, 4.98. Found: C, 72.46; H, 5.31; N, 4.85.

Flavanone Oxime *O*-Butyrate (4-Butyryoxyimino-2,3-dihydro-2-phenyl-4*H*-benzo[*b*]pyran) (**3b**).

This compound was obtained from **2** and butyryl chloride as colorless crystals, yield 90%, mp 123-124°; ir (potassium bromide): 1760 (ester), 1620 (-C=N), 770, 760, 700 cm^{-1} (Ar-H); ^1H nmr: δ 1.00 (t, 3H, $-CH_3$), 1.74 (sex, 2H, $-CH_2Me$), 2.45 (t, 2H, $-CH_2CO$), 2.82 (dd, 1H, J = 17 Hz and 12.5 Hz, $-C_3H_{ax}$), 3.49 (dd, 1H, J C_3H_{ax}), 3.49 (dd, 1H, J = 17 Hz and 3 Hz, $-C_3H_{eq}$), 5.08 (dd, 1H, J = 12.5 Hz and 3 Hz, $-C_2H$), 6.84~7.06 (m, 2H, Ar*H*), 7.17~7.50 (m, 6H, Ar*H*), 8.04 ppm (dd, 1H, $-C_5H$); ms: m/z 309 [M^+].

Anal. Calcd. for $C_{19}H_{19}NO_3$: C, 73.77; H, 6.19; N, 4.53. Found: C, 73.68; H, 6.23; N, 4.47.

Flavanone Oxime *O*-Acrylate (4-Acryloyloxyimino-2,3-dihydro-2-phenyl-4*H*-benzo[*b*]pyran) (**3c**).

This compound was obtained from **2** and acryloyl chloride as colorless crystals, yield 63%, mp 147~150°, ir (potassium bromide): 1750 (ester), 1620 (-C=N), 965 (-CH=CH_2), 760, 700 cm^{-1} (Ar*H*); ^1H nmr: δ 2.87 (dd, 1H, J = 17.5 Hz and 12.5 Hz, $-C_3H_{ax}$), 3.53 (dd, 1H, J = 17.5 Hz and 3 Hz, $-C_3H_{eq}$), 5.12 (dd, 1H, J = 12.5 Hz and 3 Hz, C_2H), 5.90 (dd, 1H, J = 10 Hz and 2.5 Hz, -COC=C*H*-), 6.23 (dd, 1H, J = 17 Hz and 10 Hz, -COC*H*=C-), 6.57 (dd, 1H, J = 17 Hz and 2.5 Hz, -COC=C*H*-), 6.93~7.08 (m, 2H, Ar*H*), 7.28~7.51 (m, 6H, Ar*H*), 8.15 ppm (dd, 1H, C_5H); mz: m/z 293 [M^+].

Anal. Calcd. for $C_{18}H_{15}NO_3$: C, 73.71; H, 5.15; N, 4.78. Found: C, 73.58; H, 5.03; N, 4.66.

Enzymatic Transesterification of Flavanone Oxime (**2**). Typical Procedure.

To a solution of (±)-**2** (0.90 g, 3.76 mmoles) in dry diisopropyl ether (40 ml) was successively added lipase from *Pseudomonas cepacia* (lipase PS) (1.0 g), 4 Å molecular sieves powder (1.0 g) and vinyl acetate (0.350 g, 4.07 mmoles), and the mixture was vigorously stirred at 23°. The conversion was monitored by thin layer chromatography analysis (silica gel, benzene), and quenched by filteration of the enzyme and 4 Å molecular sieve. The filtrate was evaporated to dryness under reduced pressure, and the products were purified by column chromatography on silica gel with chloroform as eluent. The first elution with chloroform yielded the (±)-*O*-acetylated oxime (±)-**3** (mp 174-175°, 20% yield) and the second elution with chloroform afforded (±)-unesterified oxime (±)-**2** (mp 164-165°, 78% yield). *Candida cylindracea* lipase catalyzed transesterification of **2** proceeded to the no formation of the esterified product. The results were summarized in Table 1.

Enzymatic Hydrolysis of Flavanone Oxime *O*-Acylate (**3**). Typical Procedure.

The enzyme (500 mg) was added to a solution of the substrate **3** (4.0 mmoles) in a mixture of tetrahydrofuran (20 ml) and *n*-butanol (0.28 ml). The mixture was stirred at 23° and the conversion was monitored by thin layer chromatography analysis (silica gel, chloroform). When the appropriate degree of conversion was accomplished, the reaction was stopped by the addition of a mixture of chloroform and acetone (4:1, 200 ml). After the enzyme was removed by filtration, the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in chloroform and was chromatographed on silica gel column. The first elution with chloroform-ethyl acetate (100:1) afforded the unhydrolyzed ester (*R*)-**3**, and the second elution with chloroform-ethyl acetate gave the hydrolyzed oxime (*S*)-**2**. The absolute configuration of the unhydrolyzed oxime ester (*R*)-**3** was determined according to the optical rotation given in the literature after conversion by hydrolysis with sodium hydroxide aqueous solution to (*R*)-flavanone oxime and followed with hydrolysis by 5% hydrochloric acid in methanol to the corresponding (*R*)-flavanone (*R*)-**1**. On the other hand, the absolute configuration was determined according to the optical rotations given in the literature after conversion by hydrolysis with hydrochloric acid to the (*S*)-flavanone (*S*)-**1**. The results are summarized in Table 2.

(*S*)-Flavanone Oxime (*S*)-**2**.

This compound (entry 1 in Table 2) was obtained from (±)-**3a** by the enzymatic reaction with *Porcine pancreatic* lipase, accompanied by (*R*)-**3a**, colorless crystals, mp 168-169°, $[\alpha]_D^{20}$ = -50.28° (c 0.35, chloroform), ee 83%; ir (potassium bromide): 3220 (-OH), 1640 (-C=N-), 770, 760, 700 cm^{-1} (Ar*H*); ^1H-nmr: δ 2.75 (dd, 1H, one proton of $-CH_2$-), 3.58 (dd, 1H, one proton of $-CH_2$-), 5.09 (dd, 1H, -O-C*H*-), 6.83-7.04 (m, 2H, $-C_6H$ + $-C_8H$), 7.16-7.53 (m, 6H, $-C_7H$ + Ph*H*), 7.80 (dd, 1H, $-C_5H$), 9.00 ppm (s, 1H, -O*H*); ms: m/z 239 (M^+).

Anal. Calcd. for $C_{15}H_{13}NO_2$: C, 75.30; H, 5.48; N, 5.85. Found: C, 75.18; H, 5.32; N, 5.76.

The hydrolysis of (*S*)-**2** (0.199 g) using 1*N* hydrochloric acid (4 ml) in acetone (8 ml) afforded (*S*)-flavanone (*S*)-**1** (colorless crystals, mp 75-76°, $[\alpha]_D^{20}$ = -54.5° (c 0.42, chloroform); lit [3] mp 76-77°, $[\alpha]_D^{20}$ = -64.4° (c 0.35, chloroform).

(*R*)-Flavanone Oxime *O*-Acetate (*R*)-**3a**.

This compound (entry 1 in Table 2) was obtained from (±)-**3a** by the enzymatic hydrolysis using *Porcine pancreatic* lipase, accompanied by (±)-**2**, colorless crystals, mp 176-178°, $[\alpha]_D^{20}$ = +28.7° (c 0.35, chloroform), ee 81%; spectroscopic and analytical data were identical with those of (±)-**3a**.

The hydrolysis of (*R*)-**3a** using hydrochloric acid in acetone afforded (*R*)-flavanone (*R*)-**1** (colorless crystals, mp 75-76°, $[\alpha]_D^{20}$ = +53.8° (c 0.31, chloroform); lit [3], mp 77°, $[\alpha]_D^{20}$ = +67.2° (c 0.35, chloroform).

(*R*)-Flavanone Oxime *O*-Butyrate (*R*)-**3b**.

This compound (entry 5 in Table 2) was obtained from (±)-**3b** by the enzymatic hydrolysis using *Porcine pancreatic* lipase, accompanied by (*S*)-**2** (ee 44%), colorless crystals, mp 126-128°, $[\alpha]_D^{20}$ = +14.9° (c 0.38, chloroform), ee 44%; spectroscopic and analytical data were identical with those of (±)-**3b**. The hydrolysis of (*R*)-**3b** using 1*N* hydrochloric acid in acetone (1:2) solution for 7 hours refluxing afforded (*R*)-**1**, $[\alpha]_D^{20}$ = +29.3° (c 0.41, chloroform).

(*R*)-Flavanone Oxime *O*-Acrylate (*R*)-**3c**.

This compound (entry 9 in Table 2) was obtained from (±)-**3c** by the enzymatic hydrolysis using *Porcine pancreatic* lipase, accompanied by (*S*)-**2** (ee 77%), mp 153-154°, $[\alpha]_D^{20}$ = +41.8°

(c 0.35, chloroform), ee 68%; spectroscopic and analytical data were identical with those of (±)-**3c**.

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