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Paradisin C: a new CYP3A4 inhibitor from grapefruit juice

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Abstract—A new furanocoumarin derivative, paradisin C, was isolated from grapefruit juice as an inhibitor of cytochrome P450 (CYP) 3A4. Its stereochemistry was elucidated by spectroscopic methods. The stereochemistries of 17,18-dihydroxybergamottin and 17-epoxybergamottin were also elucidated.

1. Introduction

Cytochrome P450 (CYP) enzymes are recognized to be responsible for drug metabolism, degradation of xenobiotics, and carcinogenesis. Concomitant oral administration of grapefruit juice affected the bioavailability of dihydropyridine-type calcium channel blockers, such as felodipine and nifedipine,[1] and similar phenomena in pharmacokinetics have been reported for various clinically important drugs. In the course of our study on CYP3A4[2] inhibitors in the diet, we have already reported the isolation of two furanocoumarin dimers, GF-I-1 (**1**) and GF-I-4 (**2**), newly named paradisins A and B, respectively, from grapefruit juice[3–6] and bisalkaloids from white pepper.[7,8] Recently, we found new CYP inhibitors in which a semisynthetic 17,18-dihydroxybergamottin caproate having a more stable and simpler structure than **1** and **2**, exhibited comparable activity.[9] This paper describes the isolation, structure determination, and CYP inhibitory activity of a new furanocoumarin dimer, paradisin C (**3**),[10] along with the elucidation of the absolute stereochemistries of 17,18-dihydroxybergamottin (**4**)[11] and 17-epoxybergamottin (**5**)[12] (Chart 1).

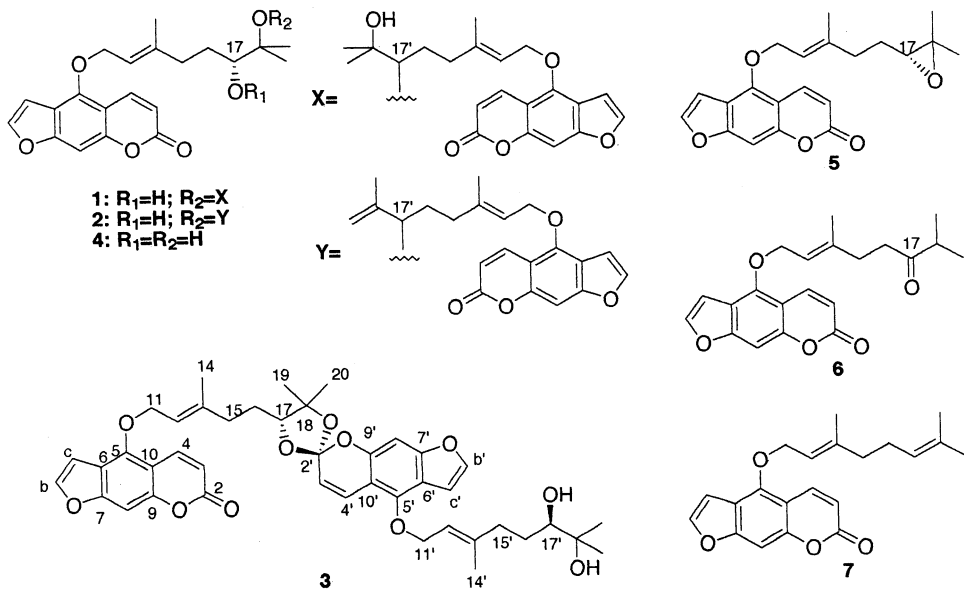

Chart 1.

Keywords: grapefruit juice; paradisin; furanocoumarin; CYP3A4 inhibition; absolute stereochemistry.
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Table 1. NMR spectral data for paradisin C (**3**) in $CDCl_3$

	δ_H	δ_C	HMBC		δ_H	δ_C	HMBC
2		161.3 s		2′		117.6 s	
3	6.26 (1H, d, 9.8)	112.6 d	C-2, C-10	3′	5.59 (1H, d, 9.8)	116.6 d	C-2′, C-10′
4	8.14 (1H, d, 9.8)	139.5 d	C-2, C-5, C-9	4′	7.22 (1H, d, 9.8)	124.7 d	C-3′, C-5′, C-9′
5		148.9 s		5′		147.8 s	
6		114.1 s		6′		113.3 s	
7		158.1 s		7′		156.5 s	
8	7.16 (1H, s)	94.3 d	C-7, C-9, C-10	8′	6.81 (1H, s)	94.5 d	C-6′, C-7′, C-9′, C-10′
9		152.6 s		9′		150.5 s	
10		107.5 s		10′		108.0 s	
11	4.94 (2H, d, 7.8)	69.6 t	C-5, C-12, C-13	11′	4.80 (2H, d, 7.8)	69.5 t	C-5′, C-12′, C-13′
12	5.58 (1H, t, 7.8)	119.6 d	C-14, C-15	12′	5.54 (1H, t, 7.8)	120.2 d	C-14′, C-15′
13		141.9 s		13′		141.9 s	
14	1.71 (3H, s)	16.7 q	C-12, C-13, C-15	14′	1.61 (3H, s)	16.3 q	C-12′, C-13′, C-15′
15	2.32 (1H, m)	36.4 t	C-12, C-13	15′	2.26 (1H, m)	36.4 t	C-12′, C-13′
	2.21 (1H, m)				2.16 (1H, m)		
16	1.72 (1H, m)	27.2 t	C-13, C-15	16′	1.57 (1H, m)	29.1 t	
	1.57 (1H, m)				1.40 (1H, m)		
17	4.24 (1H, dd, 3.4, 9.8)	82.6 d		17′	3.15 (1H, dd, 2.6, 10.5)	77.6 d	
18		82.7 s		18′		73.0 s	
19	1.47 (3H, s)	22.6 q	C-17, C-18, C-20	19′	1.13 (3H, s)	23.0 q	C-17′, C-18′, C-20′
20	1.20 (3H, s)	26.4 q	C-17, C-18, C-19	20′	1.15 (3H, s)	26.5 q	C-17′, C-18′, C-19′
b	7.59 (1H, d, 2.4)	144.9 d	C-6, C-7, C-c	b′	7.44 (1H, d, 2.4)	143.3 d	C-6′, C-7′, C-c′
c	6.94 (1H, d, 2.4)	105.0 d	C-6, C-7, C-b	c′	6.80 (1H, d, 2.4)	104.7 d	C-6′, C-7′, C-b′

2. Results and discussion

2.1. Isolation and structure elucidation of paradisin C (3)

Grapefruit juice (12 L) was extracted with hexane/EtOAc (1:1). The concentrated extract (1.2 g) was purified by silica gel (hexane/EtOAc) and ODS chromatography to afford a new furanocoumarin derivative paradisin C (**3**, 2.0 mg, 0.17% from the extract) as well as known paradisins A (**1**) and B (**2**) and 17,18-dihydroxybergamottin (**4**).

The FAB mass spectrum of paradisin C (**3**) showed a pseudomolecular ion peak at *m/z* 727 $[M+H]^+$ and the formula of $C_{42}H_{46}O_{11}$ was established by HRFABMS. The ^1H NMR spectrum in $CDCl_3$ showed six singlet methyl (δ 1.13, 1.15, 1.20, 1.47, 1.61, and 1.71), two oxymethylene (δ 4.80 (d, J=7.8 Hz) and 4.94 (d, J=7.8 Hz)), two oxymethine (δ 3.15 (dd, J=2.6, 10.5 Hz) and 4.24 (dd, J=3.4, 9.8 Hz)), 12 olefinic signals (δ 5.54 (t, J=7.8 Hz), 5.58 (t, J=7.8 Hz), 5.59 (d, J=9.8 Hz), 6.26 (d, J=9.8 Hz), 6.80 (d, J=2.4 Hz), 6.81 (s), 6.94 (d, J=2.4 Hz), 7.16 (s), 7.22 (d, J=9.8 Hz), 7.44 (d, J=2.4 Hz), 7.59 (d, J=2.4 Hz), and 8.14 (d, J=9.8 Hz)) (Table 1), which indicated the presence of two *O*-prenylfurocoumarin moieties. The ^{13}C NMR spectrum of **3** (Table 1) was almost superimposable on those of **1** and **2** except for the presence of a quaternary carbon at δ 117.6 (C-2′) instead of a carbonyl carbon. Interpretation of the HMBC spectrum confirmed that **3** was composed of two *O*-prenylfurocoumarin moieties (Fig. 1). HMBC correlation, δ 5.59 (H-3′)/δ 117.6 (C-2′) (Fig. 1), and NOE correlation between H-3′ and H_3-20 (Fig. 2) suggested the presence of an orthoester structure in **3**. The acid hydrolysis of **3** (10.0 mg) afforded 17,18-dihydroxybergamottin (**4**, 4.0 mg), 17-ketobergamottin (**6**, 1.0 mg),[12] and **3** (2.0 mg) which supported the gross structure of **3**. During the hydrolysis of **3**, partial dehydration of **4** may be occurred to afford **6**. The compound **4** was led to 17-*O*-*R*-(+)-MTPA ester (**4r**), whose NMR data were identical to those of the corresponding ester with 17*R*-dihydroxybergamottin, indicating 17*R*,17′*R*-configuration of **3**. Thereby, together with the result from the NOESY correlation (Fig. 2), 2′*S*-configuration of **3** was established.

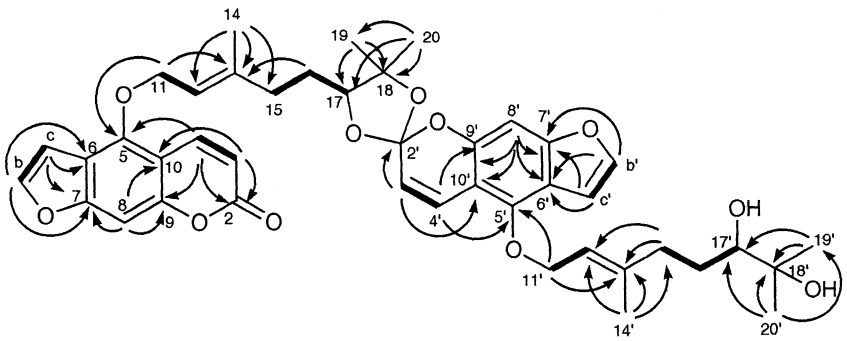


Figure 1. COSY (bold lines) and HMBC (arrows) correlations observed for paradisin C (**3**).

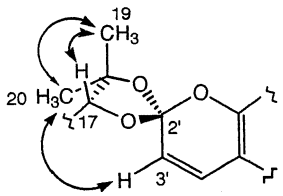


Figure 2. NOE correlations observed for paradisin C (**3**).

2.2. Absolute stereochemistry of 17,18-dihydroxybergamottin (4) and 17-epoxybergamottin (5)

The structures of 17,18-dihydroxybergamottin (**4**)[11] and 17-epoxybergamottin (**5**)[12] were reported while the absolute stereochemistry remained undetermined. Here, we establish the absolute configurations of the chiral centers of the compounds.

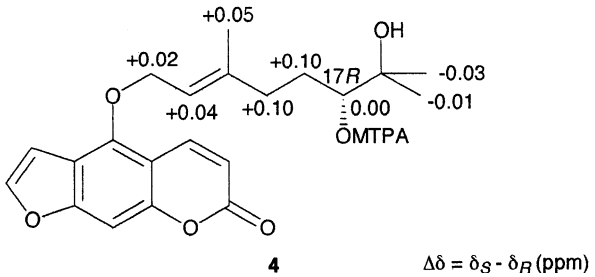


Figure 3. Application of modified Mosher's method to 17,18-dihydroxybergamottin (**4**).

17,18-Dihydroxybergamottin (**4**) was isolated from both grapefruit juice and its peel oil, while 17-epoxybergamottin (**5**) was only from the peel oil. The configuration at C-17 of **4** was determined by the Mosher method (Fig. 3).[13] The chemical shift differences for 17-(*R*)- and 17-(*S*)-MTPA esters of **4** indicated 17*R* configuration. The absolute stereochemistry of 17-epoxybergamottin (**5**) was determined by comparison of the optical rotation of synthetic 17*R*-epoxybergamottin (17*R*-**5**). Bergamottin (**7**) was converted to 17*S*,18-dihydroxybergamottin (17*S*-**4**) and then to 17*R*-epoxybergamottin (17*R*-**5**) following the mesylation (Scheme 1). Optical rotation of the synthetic 17*R*-**5** ($[\alpha]_D$=+4.6°) was consistent with that of the natural **5** ($[\alpha]_D$=+5.3°). Thus, 17-configuration of **5** from the grapefruit peel oil was established as *R*.

2.3. CYP3A4 inhibitory activity of paradisin C (3)

CYP activity was based on nifedipine oxidation. Paradisin C (**3**) showed moderate CYP3A4 inhibitory activity with IC_{50} values of 1.0 μM compared to paradisins A (**1**) and B (**2**) (IC_{50}, 0.07 and 0.07 μM).

By means of the inhibitory effect of drug metabolizing enzyme CYP3A4, administration of grapefruit juice could reduce the drug dose and lead to cost-savings for patients. Elucidation of the interaction between CYP3A4 and the inhibitors may be an interesting subject in the pharmacokinetics of clinically used drugs.

3. Experimental

3.1. General

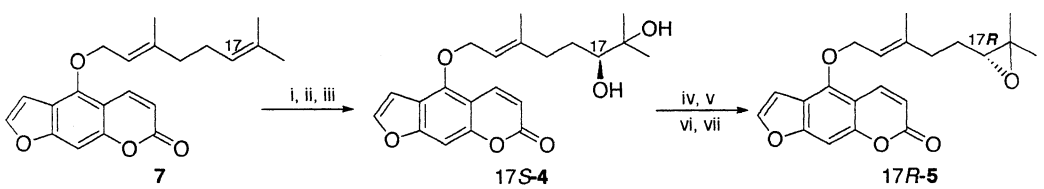
Optical rotations were determined with a HORIBA SEPA-300 high sensitive polarimeter. UV spectrum was measured on a SHIMADZU UV-1600 UV-visible spectrophotometer. IR spectrum was recorded on a SHIMADZU IR-460 infrared spectrophotometer. NMR spectra were recorded on a JEOL GSX-500 NMR spectrometer in $CDCl_3$. All chemical shifts were reported with respect to TMS. Mass spectra were measured on a JEOL SX-102 mass spectrometer.

3.2. Extraction and isolation

Grapefruit juice (12 L) was extracted with hexane/EtOAc (1:1). The concentrated extract (1.2 g) was purified by silica gel (hexane/EtOAc) and ODS (82% MeOH/H_2O) chromatography to afford known furanocoumarin derivatives, paradisins A (**1**, 5.0 mg, 0.42% from the extract) and B (**2**, 15.0 mg, 1.3%), bergamottin (**7**, 25.2 mg, 2.1%), and a crude fraction (15.0 mg). The crude fraction was purified by ODS HPLC (95% MeOH/H_2O) to afford a new furanocoumarin derivative, paradisin C (**3**, 2.0 mg, 0.17%). 17,18-Dihydroxybergamottin (**4**) and 17-epoxybergamottin (**5**, 99.8 mg, 1.0%) isolated from grapefruit oil[14] were used in this experiment.

3.2.1. Paradisin C (3). A colorless solid. $[\alpha]_D^{25}$=−17° (*c* 0.12, $CHCl_3$). UV (MeOH) λ_{max} (log ε) 225 (4.3), 245 (5.7), 267 (3.2), 311 nm (2.0). IR ($CHCl_3$) ν_{max} 3581, 1723, 1627, 1608, 1582, 1140, 1080 cm^{-1}. ^1H and ^{13}C NMR ($CDCl_3$) see Table 1. FABMS (positive) *m/z* 727 $[M+H]^+$; HRFABMS (positive) *m/z* 727.3115 ($C_{42}H_{47}O_{11}$, Δ −0.4 mmu).

3.2.2. Acid hydrolysis of paradisin C (3). A solution of **3** (10.0 mg) in dimethoxyethane (210 μL) was added to 0.01N HCl (150 μL), and the mixture was kept at room temperature for 4 h. The solvent was evaporated, and the residue was purified by ODS HPLC (70% MeOH/H_2O) to afford 17,18-dihydroxybergamottin (**4**, 4.0 mg), 17-ketobergamottin (**6**, 1.0 mg), and **3** (2.0 mg).



Scheme 1. (i) 3.0 equiv. $K_3Fe(CN)_6$, 3.0 equiv. K_2CO_3, 1.0 equiv. $CH_3SO_2NH_2$, 0.025 equiv. $(DHQ)_2$-PHAL, H_2O, 0°C; (ii) 0.005 equiv. OsO_4, *t*-BuOH, H_2O, 7°C, 21 h; (iii) 12 equiv. Na_2SO_3, 7°C, 30 min; (iv) 1.2 equiv. MsCl, pyridine, rt, 3 h; (v) 1.2 equiv. MsCl, rt, 4 h; (vi) 2.0 equiv. DBU, CH_2Cl_2, rt, 1 h; (vii) 2.0 equiv. DBU, rt, 2 h.

4: ^{1}H NMR ($CDCl_3$) δ 1.16 (3H, s, H_3-19), 1.20 (3H, s, H_3-20), 1.45 (1H, m, H-16), 1.59 (1H, m, H-16), 1.70 (3H, s, H_3-14), 2.15 (1H, m, H-15), 2.36 (1H, m, H-15), 3.31 (1H, d, J=8.0 Hz, H-17), 4.94 (2H, d, J=6.8 Hz, H_2-11), 5.59 (1H, t, J=6.8 Hz, H-12), 6.26 (1H, d, J=9.8 Hz, H-3), 6.94 (1H, d, J=1.5 Hz, H-c), 7.14 (1H, s, H-8), 7.59 (1H, d, J=1.5 Hz, H-b), 8.14 (1H, d, J=9.8 Hz, H-4). EI-MS m/z 372 $[M]^+$.

6: ^{1}H NMR ($CDCl_3$) δ 1.10 (6H, d, J=6.8 Hz, H_3-19 and H_3-20), 1.70 (3H, s, H_3-14), 2.34 (2H, t, J=7.3 Hz, H_2-15), 2.58 (2H, t, J=7.3 Hz, H_2-16), 2.60 (1H, septet, J=6.8 Hz, H-18), 4.93 (2H, d, J=6.8 Hz, H_2-11), 5.53 (1H, t, J=6.8 Hz, H-12), 6.28 (1H, d, J=9.8 Hz, H-3), 6.94 (1H, d, J=2.4 Hz, H-c), 7.17 (1H, s, H-8), 7.60 (1H, d, J=2.4 Hz, H-b), 8.15 (1H, d, J=9.8 Hz, H-4). FABMS (positive) m/z 355 $[M+H]^+$.

A solution of **4** (4.1 mg) derived from **3** in THF (1.0 mL) was treated with (*S*)-MTPA chloride (6.0 μL) in the presence of DMAP (5.0 mg) at room temperature for 23 h. After evaporation, the residue was purified by silica gel chromatography (hexane/EtOAc, 3:1) and ODS HPLC (82% MeOH/H_2O) to afford (*R*)-MTPA ester (**4r**, 3.1 mg).

4r: ^{1}H NMR ($CDCl_3$) δ 1.15 (3H, s, H_3-19), 1.20 (3H, s, H_3-20), 1.61 (3H, s, H_3-14), 1.62 (1H, m, H-16), 1.75 (1H, m, H-16), 1.97 (2H, t, J=7.3 Hz, H_2-15), 4.92 (2H, d, J=6.8 Hz, H_2-11), 4.97 (1H, dd, J=10.3, 2.4 Hz, H-17), 5.47 (1H, t, J=6.8 Hz, H-12), 6.28 (1H, d, J=9.8 Hz, H-3), 6.94 (1H, d, J=2.4 Hz, H-c), 7.17 (1H, s, H-8), 7.60 (1H, d, J=2.4 Hz, H-b), 8.16 (1H, d, J=9.8 Hz, H-4). FABMS (positive) m/z 589 $[M+H]^+$.

3.2.3. MTPA ester of dihydroxybergamottin (4). A solution of **4** (4.0 mg) in THF (1.0 mL) was treated with (*S*)-MTPA chloride (6.1 μL) in the presence of DMAP (5.3 mg) at room temperature for 32 h. After evaporation, the residue was purified by silica gel chromatography (hexane/EtOAc, 3:1) and ODS HPLC (82% MeOH/H_2O) to afford (*R*)-MTPA ester (**4r**, 2.0 mg). (*S*)-MTPA ester (**4 s**, 1.5 mg) was prepared by the same procedure as that for (*R*)-MTPA ester (**4 s**).

4r: ^{1}H NMR ($CDCl_3$) δ 1.15 (3H, s, H_3-19), 1.20 (3H, s, H_3-20), 1.61 (3H, s, H_3-14), 1.62 (1H, m, H-16), 1.75 (1H, m, H-16), 1.97 (2H, t, J=7.3 Hz, H_2-15), 4.92 (2H, d, J=6.8 Hz, H_2-11), 4.97 (1H, dd, J=10.3, 2.4 Hz, H-17), 5.47 (1H, t, J=6.8 Hz, H-12), 6.28 (1H, d, J=9.8 Hz, H-3), 6.94 (1H, d, J=2.4 Hz, H-c), 7.17 (1H, s, H-8), 7.60 (1H, d, J=2.4 Hz, H-b), 8.16 (1H, d, J=9.8 Hz, H-4). FABMS (positive) m/z 589 $[M+H]^+$.

4s: ^{1}H NMR ($CDCl_3$) δ 1.14 (3H, s, H_3-19), 1.17 (3H, s, H_3-20), 1.66 (3H, s, H_3-14), 1.72 (1H, m, H-16), 1.85 (1H, m, H-16), 2.07 (2H, t, J=7.8 Hz, H_2-15), 4.94 (2H, d, J=6.8 Hz, H_2-11), 4.97 (1H, dd, J=9.8, 2.0 Hz, H-17), 5.51 (1H, t, J=6.8 Hz, H-12), 6.27 (1H, d, J=9.8 Hz, H-3), 6.95 (1H, d, J=2.4 Hz, H-c), 7.17 (1H, s, H-8), 7.60 (1H, d, J=2.4 Hz, H-b), 8.17 (1H, d, J=9.8 Hz, H-4). FABMS (positive) m/z 589 $[M+H]^+$.

3.2.4. Preparation of (*R*)-17-epoxybergamottin (17*R*-5). To a solution of $K_3Fe(CN)_6$ (156.4 mg, 3.0 mol equiv.), K_2CO_3 (65.6 mg, 3.0 mol equiv.), $CH_3SO_2NH_2$ (15.1 mg, 1.0 mol equiv.), and $(DHQ)_2$-PHAL (3.1 mg, 0.025 mol equiv.) in water (3.5 mL) at 0°C was added bergamottion (**7**, 58.9 mg) and OsO_4 (5.3 μL of 3.8% aqueous solution, 0.005 mol equiv.) in *t*-BuOH (3.5 mL). The mixture was stirred at 7°C for 21 h. To this was added Na_2SO_3 (250 mg, 12 mol equiv.) and stirred at this temperature for 30 min. The product was extracted with EtOAc (15 mL×3), and the organic layer was washed with brine, dried over Na_2SO_4, and concentrated to afford a residue (51.9 mg). Purification over silica gel using hexane/EtOAc (1:1) gave 17*S*,18-dihydroxybergamottin (17*S*-**4**, 30.2 mg, 86% ee) of which solution in EtOAc gave colorless needles (17*S*-**4**, 7.0 mg, 92% ee). Enantiomeric excess (ee) was analyzed by a chiral HPLC.

17*S*-**4**: mp 60–68°; ^{1}H NMR ($CDCl_3$) δ 1.17 (3H, s, H_3-19), 1.21 (3H, s, H_3-20), 1.46 (1H, m, H-16), 1.60 (1H, m, H-16), 1.70 (3H, s, H_3-14), 2.16 (1H, m, H-15), 2.37 (1H, m, H-15), 3.22 (1H, d, J=9.3 Hz, H-17), 4.94 (2H, d, J=6.8 Hz, H_2-11), 5.59 (1H, t, J=6.8 Hz, H-12), 6.26 (1H, d, J=9.8 Hz, H-3), 6.95 (1H, d, J=2.4 Hz, H-c), 7.13 (1H, s, H-8), 7.60 (1H, d, J=2.4 Hz, H-b), 8.15 (1H, d, J=9.8 Hz, H-4). FABMS (positive) m/z 373 $[M+H]^+$.

A solution of 17*S*-**4** (20.0 mg) in pyridine (200 μL) was added to MsCl (5.0 μL, 1.2 mol equiv.). The mixture was stirred at room temperature for 3 h, and to this was added additional MsCl (5.0 μL, 1.2 mol equiv.). After being stirred at room temperature for 4 h, a solution of DBU (16.2 μL, 2.0 mol equiv.) in CH_2Cl_2 (200 μL) was added. The mixture was stirred at room temperature for 1 h, and to this was added additional DBU (16.2 μL, 2.0 mol equiv.). The mixture was stirred at room temperature for 2 h, and the solution was applied on silica gel chromatography ($CHCl_3$), followed by HPLC with gel filtration column ($CHCl_3$) to afford (*R*)-epoxybergamottin (17*R*-**5**, 4.5 mg).

17*R*-**5**: $[\alpha]_D$=+4.6° (*c* 0.18, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.27 (3H, s, H_3-19), 1.31 (3H, s, H_3-20), 1.59–1.72 (2H, m, H_2-16), 1.73 (3H, s, H_3-14), 2.16–2.31 (2H, m, H_2-15), 2.70 (1H, dd, J=7.3, 5.4 Hz, H-17), 4.96 (2H, d, J=6.8 Hz, H_2-11), 5.60 (1H, t, J=6.8 Hz, H-12), 6.27 (1H, d, J=9.8 Hz, H-3), 6.95 (1H, d, J=2.4 Hz, H-c), 7.15 (1H, s, H-8), 7.60 (1H, d, J=2.4 Hz, H-b), 8.15 (1H, d, J=9.8 Hz, H-4). EI-MS (positive) m/z 354 $[M]^+$.

3.3. CYP inhibition assay

CYP activity was based on nifedipine oxidation. Various amounts (0–10 μM, final concentration) of samples in 1 μL of DMSO were added to 192 μL of solution containing 100 mM phosphate buffer (pH 7.4) containing 50 μM nifedipine (Wako Pure Chemical Industries, Ltd (Osaka, Japan)), 5 mM glucose-6-phosphate (Oriental Yeast Co., Ltd (Tokyo, Japan)), 0.5 mM β-$NADP^+$ (Oriental Yeast Co., Ltd), 0.5 mM $MgCl_2$, and 4.3 μg/mL glucose-6-phosphate dehydrogenase (Oriental Yeast Co., Ltd) and incubated at 37°C for 5 min. CYP3A4 (Gentest Co. (Woburn, MA, USA)) was also preincubated in 7 μL of the buffer at 37°C for 5 min and added to the sample solution. After the incubation at 37°C for 1 h, the reaction was quenched by the addition of 100 μL of MeOH. After

adding 3.7 μg of 6-methoxycarbonyl-5-methyl-7-(2-nitrophenyl)-4,7-dihydrofuro[3,4-*b*]pyridin-1-(3*H*)-one in 1 μL of DMSO as an internal standard, the reaction mixture was extracted with 1 mL of ether, and the ether layer was evaporated. The residue was dissolved in 100 μL of MeOH, and an aliquot (20 μL) was analyzed by reverse phase HPLC (column, TSK-gel ODS-120T, 4.6 mm i.d.×150 mm; mobile phase, 64% MeOH/H_2O; flow rate, 1.0 mL/min; detection, UV 254 nm); retention times: 2.9 min for the internal standard, 4.0 min for the nifedipine metabolite (nifedipine pyridine), and 5.5 min for nifedipine. The value of IC_{50}, the concentration required for 50% inhibition of CYP3A4 activity, was calculated from the data of duplicate measurements.

Acknowledgments

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References

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