JOC Article

Atropisomeric Myristinins: Selective COX-2 Inhibitors and Antifungal Agents from *Myristica cinnamomea*

Supaporn Sawadjoon,[†] Prasat Kittakoop,*[,‡] Kanyawim Kirtikara,[‡] Vanicha Vichai,[‡] Morakot Tanticharoen,[‡] and Yodhathai Thebtaranonth[†,‡]

Department of Chemistry, Faculty of Science, Mahidol University, Bangkok 10400, Thailand; and National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 73/1, Rama VI Road, Rajdhevee, Bangkok 10400, Thailand

prasat@biotec.or.th

Received January 18, 2002

The first naturally occurring atropisomeric flavans, myristinins B (**2**), C (**2a**), E (**4**), and F (**4a**), together with their corresponding *trans*-isomers, myristinins A (**1**) and D (**3**), were isolated from the CH_2Cl_2 extract of *Myristica cinnamomea* fruits. Compounds **1**, the mixture of **2** and **2a**, and the mixture of **4** and **4a**, exhibited antifungal activity against *Candida albicans* with IC_{50} values ranging from 5.9 to 8.8 μg/mL, and they selectively inhibited the enzyme cyclooxygenase-2 (COX-2).

Introduction

Myristica cinnamomea (Myristicaceae) is commonly found in the south of Thailand, whose arils and seeds have a spicy odor resembling that of *M. fragrans* (a nutmeg tree). While chemical constituents of *Myristica fragrans* have been extensively investigated due to their pharmacological activities (e.g., antiinflammatory, antiaggregating, psychotropic, antibacterial),[1] *M. cinnamomea* has never been chemically explored. As part of our continuing search for naturally occurring biologically active compounds,[2] we have screened for biological activities of many plants and fungi, and among those screened, the CH_2Cl_2 extract of *M. cinnamomea* fruits exhibited antifungal activity with IC_{50} values of 10−20 μg/mL. Chemical analysis led to the identification of six flavans, myristinins A−F (**1**, **2**, **2a**, **3**, **4**, and **4a**), each not only possessed antifungal activity but also selectively inhibited the enzyme cyclooxygenase-2 (COX-2). Myristinins B (**2**), C (**2a**), E (**4**), and F (**4a**) are first examples of atropisomeric flavans isolated from natural environments.

Results and Discussion

A CH_2Cl_2 extract of *M. cinnamomea* fruits was purified by column chromatography (Sephadex LH-20 and silica gel) and HPLC to furnish six chromans named myristinin A (**1**), myristinin B (**2**), myristinin C (**2a**), myristinin D (**3**), myristinin E (**4**), and myristinin F (**4a**). Known compounds, hinokinin,[3] dodecanoylphloroglucinol,[4] and 1-(2,4,6-trihydroxyphenyl)-9-phenylnonan-1-one,[5] were also isolated.

Myristinin A (**1**) (also referred to as YM 26567−1)[6a] was previously isolated from the fruits of *Horsfieldia amygdaline* and claimed to possess potent antiinflammatory activity through direct inhibition of the enzyme phospholipase A2.[6] However, its protons and carbons have not yet been assigned. Analyses of ^{1}H, ^{13}C, DEPT135, ^{1}H−^{1}H COSY, NOESY, HMQC, and HMBC spectral data (DMSO-d_6) of myristinin A (**1**) led to the complete assignment of protons and carbons in **1** (Table 1). It should be noted that a methine C-5′ in **1** exhibited a broad peak on the ^{13}C NMR spectrum, and the HMQC technique was employed to assign this carbon.

An inseparable mixture of myristinin B (**2**) and myristinin C (**2a**) was obtained as a brown solid in a ratio of 1.2:1. The ESI-TOF mass spectrum of the mixture showed a single prominent ion peak at *m*/*z* 549 $(M + H)^+$, and an accurate mass from the ESI-TOF mass spectrum revealed a molecular formula $C_{33}H_{40}O_7$ (observed *m*/*z*

* To whom correspondence should be addressed at the BIOTEC. Phone: +662−6448103. FAX: +662−6448107.
[†] Department of Chemistry, Mahidol University.
[‡] National Center for Genetic Engineering and Biotechnology.

(1) (a) Kim, Y. B.; Park, I. Y.; Shin, K. H. *Arch. Pharm. Res.* **1991**, *14* (1), 1−6. (b) Dorman, H. J.; Deans, S. G. *J. Appl. Microbiol.* **2000**, *88* (2), 308−316. (c) Orabi, K. Y.; Mossa, J. S.; el-Feraly, F. S. *J. Nat. Prod.* **1991**, *54* (3), 856−859. (d) Hussain, S. P.; Rao, A. R. *Cancer Lett.* **1991**, *56* (3), 231−234. (e) Ozaki, Y.; Soedigdo, S.; Wattimena, Y. R.; Suganda, A. G. *Jpn. J. Pharmacol.* **1989**, *49* (2), 155−163. (f) Kalbhen, D. A. *Angew. Chem., Int. Ed. Engl.* **1971**, *10* (6), 370−374.

(2) (a) Vongvanich, N.; Kittakoop, P.; Kramyu, J.; Tanticharoen, M.; Thebtaranonth, Y. *J. Org. Chem.* **2000**, *65*, 5420−5423. (b) Kittakoop, P.; Wanasith, S.; Watts, P.; Kramyu, J.; Tanticharoen, M.; Thebtaranonth, Y. *J. Nat. Prod.* **2001**, *64* (3), 385−388. (c) Boonphong, S.; Kittakoop, P.; Isaka, M.; Palittapongarnpim, P.; Jaturapat, A.; Danwisetkanjana, K.; Tanticharoen, M.; Thebtaranonth, Y. *Planta Med.* **2001**, *67*, 279−281. (d) Chinworrungsee, M.; Kittakoop, P.; Isaka, M.; Rungrod, A.; Tanticharoen, M.; Thebtaranonth, Y. *Bioorg. Med. Chem. Lett.* **2001**, *11* (15), 1965−1969. (e) Boonphong, S.; Kittakoop, P.; Isaka, M.; Pittayakhajonwut, D.; Tanticharoen, M.; Thebtaranonth, Y. *J. Nat. Prod.* **2001**, *64* (7), 965−967.

(3) (a) Gonzalez, M. J.; Pinto, M. M. M.; Kijjoa, A.; Tantisewie, B. *Fitoterapia* **1988**, *59* (6), 486-487. (b) Cavalcante, S. H.; Yoshida, M.; Gottlieb, O. R. *Phytochemistry* **1985**, *24* (5), 1051−1055.

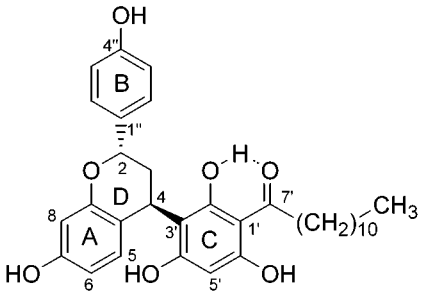
(4) Gunatilaka, A. A. L.; De Silva, A. M. Y. J.; Sotheeswaran, S.; Tillekeratne, L. M. V. *Phytochemistry* **1982**, *21* (11), 2719−2723.

(5) Pinto, M. M. M.; Kijjoa, A.; Tantisewie, B.; Yoshida, M.; Gottlieb, O. R. *Phytochemistry* **1988**, *27* (12), 3988−3989.

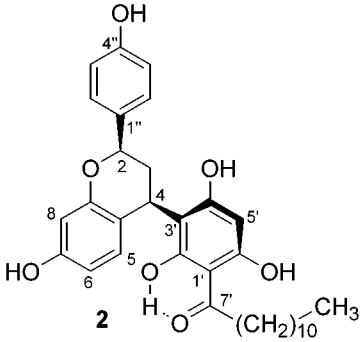
(6) (a) Katuhiko, H.; Akio, K.; Hirokazu, Y.; Akira, M.; Akira, I.; Akinori, S.; Shengji, P.; Yanhui, L.; Chun, W. Patent no. WO 9208712, 1992. (b) Akira, M.; Hirokazu, Y.; Takebayashi, Y.; Harumitsu, I.; Kazuo, H. *J. Pharmacol. Exp. Ther.* **1992**, *263* (3), 1302−1307.

10.1021/jo020045d CCC: $22.00
Published on Web 07/04/2002

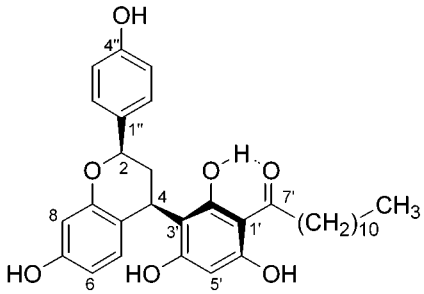
TABLE 1. ^{1}H and ^{13}C NMR Data (DMSO-d_6) of Compounds 1, 2, and 2a



1



2



2a

	1		**2**		**2a**	
	δ^{13}C	δ^1H, multiplicity, $J_{H,H}$ (Hz)	δ^{13}C	δ^1H, multiplicity, $J_{H,H}$ (Hz)	δ^{13}C	δ^1H, multiplicity, $J_{H,H}$ (Hz)
2	74.6, d	5.38, dd, 4.3, 3.9	78.0, d[b]	5.00, dd, 11.4, 7.0	78.1, d[b]	5.00, dd, 11.4, 7.0
3	32.1, t	2.07, m (H_{ax}) 2.59,m (H_{eq})	34.0, t	1.79, m (H_{eq}) 2.61, ddd (H_{ax}), 12.4, 11.9, 11.4	33.6, t	1.79, m (H_{eq}) 2.71, ddd (H_{ax}), 12.4, 11.9, 11.4
4	25.5, d	4.17, dd, 8.0, 7.0	30.4, d	4.69, dd, 11.9, 5.8	30.8, d	4.62, dd, 11.9, 5.8
4a	116.8, s		117.0, s		117.0, s	
5	127.9, d	6.38, d, 8.4	127.6, d[c]	6.42, d, 8.7	127.5, d[c]	6.42, d, 8.7
6	107.5, d	6.12, dd, 8.3, 2.1	107.8, d[d]	6.15, m	107.8, d[d]	6.15, m
7	155.9, s		155.7, s[e]		155.8, s[e]	
8	102.5, d	6.24, d, 2.2	102.7, d[f]	6.15, m	102.6, d[f]	6.15, m
8a	154.6, s		155.6, s[e]		155.5, s[e]	
1′	103.5, s		103.2, s		104.0, s	
2′	164.3, s		164.1, s		164.6, s	
3′	108.1, s		107.6, s[d]		107.3, s	
4′	163.0, s		163.1, s[g]		162.8, s[g]	
5′	94.5, d	5.97, s	95.1, d	5.92, s	93.9, d	6.09, s
6′	160.4, s		160.6, s[h]		160.3, s[h]	
7′	205.5, s		205.5, s[i]		205.6, s[i]	
8′	43.2, t	2.97, t, 7.3	43.2, t	3.05, t, 8.3	43.2, t	2.97, m
9′	24.6, t	1.56, m	24.5, t[j]	1.60, m	24.6, t[j]	1.54, m
10′	28.7, t[a]	1.25, m	28.7, t[k]	1.23, m	28.7, t[k]	1.23, m
11′	28.7, t[a]	1.25, m	28.9, t[k]	1.23, m	28.9, t[k]	1.23, m
12′	29.0, t[a]	1.25, m	28.9, t[k]	1.23, m	28.9, t[k]	1.23, m
13′	29.0, t[a]	1.25, m	29.0, t[k]	1.23, m	29.0, t[k]	1.23, m
14′	29.0, t[a]	1.25, m	29.0, t[k]	1.23, m	29.0, t[k]	1.23, m
15′	29.0, t[a]	1.25, m	29.0, t[k]	1.23, m	29.0, t[k]	1.23, m
16′	31.3, t	1.25, m	31.3, t	1.23, m	31.3, t	1.23, m
17′	22.1, t	1.25, m	22.1, t	1.23, m	22.1, t	1.23, m
18′	13.9, q	0.84, t, 6.5	13.9, q	0.84, t, 5.8	13.9, q	0.84, t, 5.8
1″	132.3, s		132.1, s		132.1, s	
2″	126.7, d	7.10, br d, 8.4	127.4, d[c]	7.24, br d, 7.8	127.5, d[c]	7.24, br d, 7.8
3″	115.0, d	6.71, br d, 8.4	115.0, d	6.76, br d, 8.4	115.0, d	6.76, br d, 8.4
4″	156.3, s		156.9, s		156.9, s	
5″	115.0, d	6.71, br d, 8.4	115.0, d	6.76, br d, 8.4	115.0, d	6.76, br d, 8.4
6″	126.7, d	7.10, br d, 8.4	127.4, d[c]	7.24, br d, 7.8	127.5, d[c]	7.24, br d, 7.8
7-OH		9.00, br s		9.00, br s		9.00, br s
2′-OH		14.23, br s		14.52, br s		14.10, br s
4′-OH		10.18, br s		10.07, br s		10.57, br s
6′-OH		10.57, br s		10.57, br s		10.57, br s
4″-OH		9.32, br s		9.42, br s		9.42, br s

[a−k] These assignments may be interchanged.

549.2852 $(M + H)^+$, Δ 0 mmu). The IR spectrum of the mixture showed absorption bands at 3315 cm^{-1} (OH) and 1618 cm^{-1} (C═O). ^{1}H NMR spectrum (DMSO-d_6) of the mixture (Figure 1) was similar to that of myristinin A (**1**), revealing the presence of a *para*-disubstituted aromatic ring B (two broad doublets at δ_H 6.76, J = 8.4 Hz and δ_H 7.24, J = 7.8 Hz), a dodecanoylphloroglucinol (a singlet aromatic protons at δ_H 5.92 for **2** and at δ_H 6.09 for **2a**, a triplet methyl at δ_H 0.84, and methylene protons at δ_H 3.05, 1.54−1.60 and 1.23), and an aromatic ring A (at δ_H 6.15−6.42). Significant differences between the mixture of **2** and **2a** and myristinin A (**1**) were revealed by absorptions of H-2 (δ_H 5.00, dd, J = 11.4 and 7 Hz for both **2** and **2a**) and H-4 (δ_H 4.69, dd, J = 11.9 and 5.8 Hz for **2**, and δ_H 4.62, dd, J = 11.9 and 5.8 Hz for **2a**). Coupling constants of H-2/H-3 and H-4/H-3 indicated a *cis*-orientation between H-2 and H-4. An intense cross-peak between H-2/H-4 observed in the NOESY spectrum of myristinins B (**2**) and C (**2a**) mixture also supported the relative *cis*-configuration mentioned above. Analyses of ^{13}C, DEPT, ^{1}H−^{1}H COSY, NOESY, and HMQC NMR spectral data revealed that the material under investigation is a mixture of isomers of myristinin A (**1**), and that **2** and **2a** exist as atropisomers. In general, the chemical

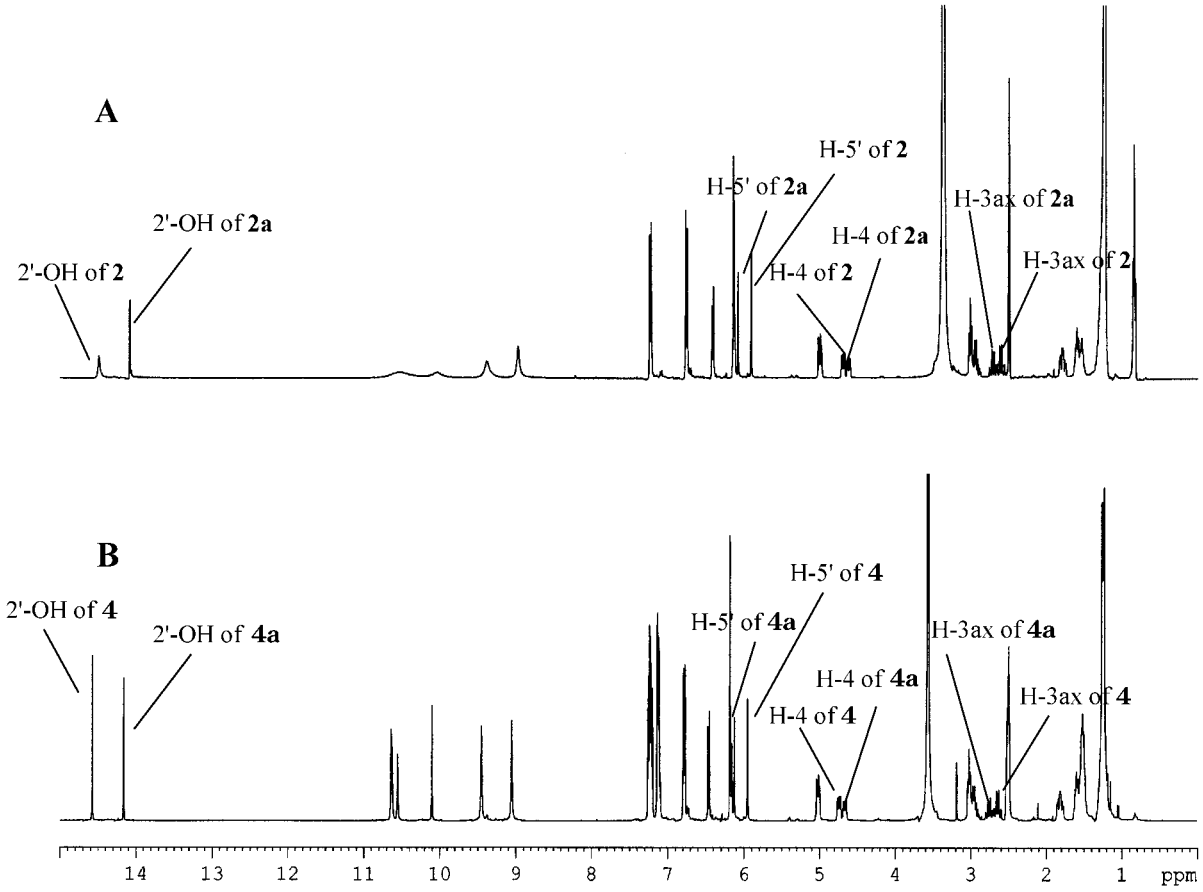


FIGURE 1. ^{1}H NMR spectra (DMSO-d_6) of (A) the mixture of myristinins B (**2**) and C (**2a**) and (B) the mixture of myristinins E (**4**) and F (**4a**).

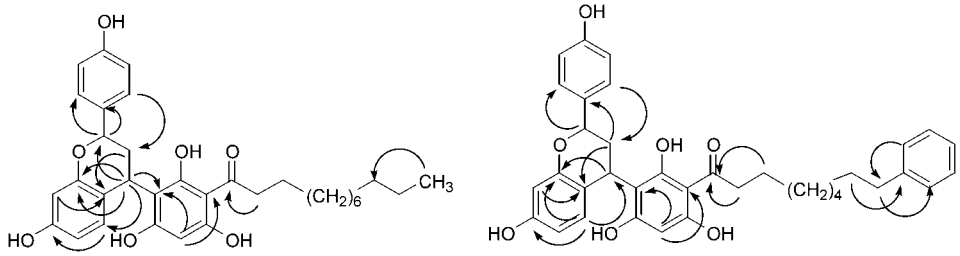
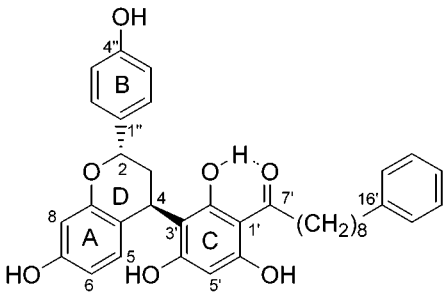


FIGURE 2. Selected HMBC correlations of compounds **1**, **2**, **2a**, **3**, **4**, and **4a**.

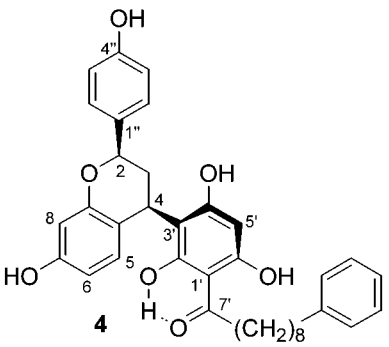
shifts of protons in **2** and **2a** were almost identical (Figure 1) except at positions adjacent to the C-4/C-3′ axis (e.g., H-3β, H-4, H-5′, and the chelated hydroxyl 2′-OH), where the molecules possessed restricted rotation. The marked differences of these ^{1}H resonances conclusively confirmed an atropisomeric existence of myristinins B (**2**) and C (**2a**). It is worth noting that the corresponding *trans*-isomer, myristinin A (**1**), does not exhibit such restricted rotation along the C-4/C-3′ axis as that of its *cis*-isomers, **2** and **2a**. Indeed, inspection of molecular models revealed *pseudo* equatorial orientations of both rings B and C in **2** and **2a** and a larger steric interaction between rings A and C (particularly between hydroxyl 2′-OH (or 4′-OH) in ring C and H-5 in ring A) as compared to that of **1**. The NOESY spectral data were very useful to establish the configuration of ring C in **2** and **2a**; a downward chelated hydroxyl 2′-OH of **2** (the major isomer) showed a cross-peak with the *pseudo* axial H-4, but such correlation was not observed in **2a**. This readily indicated configurations of myristinin B (**2**) and myristinin C (**2a**) as shown in figures. Analyses of the ^{1}H−^{1}H COSY and HMBC spectra assisted in the assignment of protons and carbons in **2** and **2a** (Table 1). Key HMBC correlations are depicted in Figure 2.

Myristinin D (**3**) was obtained as a brown solid. The ESI-TOF mass spectrum deduced a molecular formula of **3** as $C_{36}H_{38}O_7$ (observed *m/z* 583.2701 $(M + H)^+$, Δ +0.5 mmu). The IR spectrum of **3** revealed the presence of a hydroxyl group (at 3408 cm^{-1}) and a carbonyl (at 1616 cm^{-1}). The ^{1}H NMR spectrum (DMSO-d_6) of myristinin D (**3**) (Table 2)was similar to that of myristinin A (**1**); however, detailed analyses of the ^{1}H, ^{13}C, ^{1}H−^{1}H COSY, DEPT, and HMQC spectral data of **3** revealed that the dodecanoylphloroglucinol residue in myristinin A (**1**) was

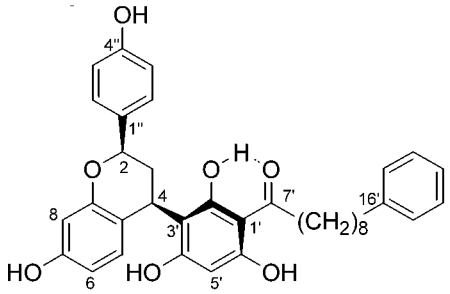
TABLE 2. ^{1}H and ^{13}C NMR Data (DMSO-d_6) of Compounds 3, 4, and 4a



3



4



4a

	3		4		4a	
no.	δ^{13}C	δ^1H, multiplicity, $J_{H,H}$ (Hz)	δ^{13}C	δ^1H, multiplicity, $J_{H,H}$ (Hz)	δ^{13}C	δ^1H, multiplicity, $J_{H,H}$ (Hz)
2	74.6, d	5.38, dd, 4.3, 4.1	78.3, d[b]	5.02, dd, 11.3, 7.0	78.2, d[b]	5.02, dd, 11.3, 7.0
3	32.2, t	2.04, m (H_{ax}) 2.59, m (H_{eq})	34.2, t	1.82, m (H_{eq}) 2.65, ddd (H_{ax}), 12.2, 11.7, 11.3	33.7, t	1.82, m (H_{eq}) 2.76, ddd (H_{ax}), 12.2, 11.7, 11.3
4	25.6, d	4.17, dd, 8.0, 7.0	30.6, d	4.74, dd, 11.7, 5.8	31.0, d	4.67, dd, 11.7, 5.8
4a	116.9, s		117.2, s[c]		117.1, s[c]	
5	128.0, d	6.38, d, 8.4	127.8, d[d]	6.47, d, 8.1	127.6, d[d]	6.47, d, 8.1
6	107.6, d	6.12, dd, 8.4, 2.2	107.9, d[e]	6.16−6.19, br d, 8.1	107.9, d[e]	6.16−6.19, br d, 8.1
7	156.0, s		155.9, s[f]		155.9, s[f]	
8	102.6, d	6.25, d, 2.3	102.8, d	6.19, br s	102.8, d	6.19, br s
8a	154.6, s		155.7, s[f]		155.7, s[f]	
1′	103.6, s		103.4, s		104.2, s	
2′	164.4, s		164.3, s		164.8, s	
3′	108.2, s		107.7, s[e]		107.4, s	
4′	163.0, s		162.9, s[g]		163.2, s[g]	
5′	94.6, s	5.97, s	95.2, d	5.96, s	94.0, d	6.13, s
6′	160.4, s		160.5, s[h]		160.7, s[h]	
7′	205.5, s		205.7, s[i]		205.6, s[i]	
8′	43.2, t	2.97, t, 7.3	43.3, t	3.03, t, 7.2	43.3, t	2.97, m
9′	24.6, t	1.54, m	24.7, t[j]	1.60, m	24.6, t[j]	1.60, m
10′	28.7, t[a]	1.26, m	28.7, t[k]	1.25, m	28.7, t[k]	1.25, m
11′	28.8, t[a]	1.26, m	28.9, t[k]	1.25, m	28.9, t[k]	1.25, m
12′	28.9, t[a]	1.26, m	29.1, t[k]	1.25, m	29.0, t[k]	1.25, m
13′	28.9, t[a]	1.26, m	29.1, t[k]	1.25, m	29.0, t[k]	1.25, m
14′	31.0, t	1.54, m	31.1, t	1.52, m	31.1, t	1.52, m
15′	35.2, t	2.53, t, 7.7	35.3, t	2.50, t, 7.7	35.3, t	2.50, t, 7.7
16′	142.4, s		142.4, s		142.4, s	
17′	128.3, d	7.15, m	128.3, d	7.12, m	128.3, d	7.12, m
18′	128.2, d	7.25, m	128.3, d	7.24, m	128.3, d	7.24, m
19′	125.6, d	7.15, m	125.6, d	7.12, m	125.6, d	7.12, m
20′	128.2, d	7.25, m	128.3, d	7.24, m	128.3, d	7.24, m
21′	128.3, d	7.15, m	128.3, d	7.12, m	128.3, d	7.12, m
1″	132.3, s		132.3, s		132.3, s	
2″	126.7, d	7.09, br d, 8.4	127.6, d[d]	7.24, br d, 8.4	127.7, d[d]	7.24, br d, 8.4
3″	115.1, d	6.71, br d, 8.4	115.2, d	6.79, br d, 8.4	115.2, d	6.79, br d, 8.4
4″	156.3, s		157.1, d		157.1, d	
5″	115.1, d	6.71, br d, 8.4	115.2, d	6.79, br d, 8.4	115.2, d	6.79, br d, 8.4
6″	126.7, d	7.09, br d, 8.4	127.6, d[d]	7.24, br d, 8.4	127.7, d[d]	7.24, br d, 8.4
7-OH		9.04, br s		9.06, br s		9.06, br s
2′-OH		14.33, br s		14.58, br s		14.17, br s
4′-OH		10.23, br s		10.11, br s		10.56, br s
6′-OH		10.61, br s		10.65, br s		10.63, br s
4″-OH		9.36, br s		9.46, br s		9.46, br s

[a−k] These assignments may be interchanged.

replaced by a 1-(2,4,6-trihydroxyphenyl)-9-phenylnonan-1-one unit in myristinin D (**3**). Analyses of coupling constants and NOESY spectral data of **3** indicated a *trans*-relationship of H-2 and H-4 (H-2 showed an intense cross-peak to H-3eq, while H-4 exhibited a correlation to H-3ax).

A mixture of myristinin E (**4**) and myristinin F (**4a**) was obtained as a brown solid with a ratio of **4**/**4a** = 1.2:1. A molecular formula $C_{36}H_{38}O_7$ was obtained from the ESI-TOF mass spectrum (accurate mass observed at *m*/*z* 583.2703 $(M + H)^+$, Δ +0.7 mmu). The IR spectrum of the mixture showed the presence of hydroxyl group (at 3275 cm^{-1}) and a carbonyl (at 1617 cm^{-1}). The ^{1}H NMR spectrum (DMSO-d_6) (Figure 1) of the mixture of **4** and **4a** was similar to that of myristinin D (**3**), except for resonances at H-2 and H-4. Analyses of coupling constants and NOESY spectral data revealed a *cis*-relationship between these two protons (an intense cross-peak

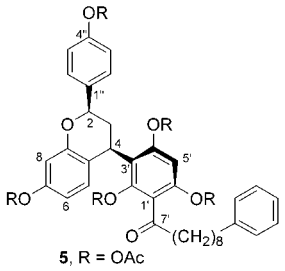
TABLE 3. Biological Activities of Myristinins A−F

compounds	antifungal activity (IC_{50}, μg/mL)	antiinflammatory activity (IC_{50}, μg/mL) COX-1	COX-2	cytotoxicity to Vero cells (IC_{50}, μg/mL)
myristinin A (**1**)	8.8	*a*	16.9	17.7
myristinins B+C (**2** and **2a**)	6.0	*a*	2.1	16.4
myristinin D (**3**)	inactive at 20 μg/mL	*a*	4.5	13.6
myristinins E+F (**4** and **4a**)	5.9	*a*	1.4	8.9
aspirin	nd[b]	2.0	3.5	nd
amphotericin B	0.01	nd	nd	nd

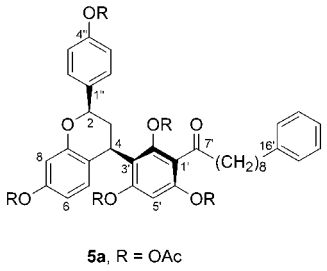
[a] Inhibition toward COX-1 isozyme was less than 40% at the concentration of 10 μg/mL. [b] Not determined.

of H-2/H-4 observed). Again, the NOESY spectral data demonstrated the correlation between a downward chelated hydroxyl 2′-OH and H-4 in **4** (the major isomer), but no such correlation was observed in **4a**, which established the orientation of ring C in **4** (downward chelated OH) and **4a** (upward chelated OH). In a fashion similar to that for myristinin B (**2**) and myristinin C (**2a**), analyses of ^{13}C, DEPT, $^1H-^1H$ COSY, NOESY, and HMQC NMR spectral data of the mixture suggested that **4** and **4a** are atropisomers. Complete assignment of protons and carbons in myristinins E (**4**) and F (**4a**) was successfully established by analyses of $^1H-^1H$ COSY and HMBC spectra (Table 1). Long ranged heteronuclear correlations ($^1H-^{13}C$) are shown in Figure 2.

Efforts to demonstrate the interconversions between **2** and **2a** and between **4** and **4a** by heating failed due to degradation after heating (i.e., in DMSO solution at 70 °C). However, after acetylation[7] of **4** and **4a**, the equilibrium ratio changed from 1.2:1 in **4**/**4a** to 1:2 in **5**/**5a**. Further studies on interconversions of **5** and **5a** by heating again had met with failure owing to degradation.



5, R = OAc



5a, R = OAc

Compounds **1**, the mixture of **2** and **2a** and the mixture of **4** and **4a**, exhibited antifungal activity against *C. albicans* with IC_{50} values ranging from 5.9 to 8.8 μg/mL, while **3** was inactive at 20 μg/mL (Table 3). Further, myristinin A (**1**), the mixture of myristinins B (**2**) and C (**2a**), myristinin D (**3**), and the mixture of myristinins E (**4**) and F (**4a**), showed preferential inhibition of COX-2 over COX-1. The IC_{50} values for COX-2 isozyme of myristinin A (**1**), a mixture of myristinins B (**2**) and C (**2a**), myristinin D (**3**), and a mixture of myristinins E (**4**) and F (**4a**) were 16.9, 2.1, 4.5, and 1.4 μg/mL, respectively, while these compounds inhibited COX-1 isozyme less than 40% at the concentration of 10 μg/mL (Table 3). Although the COX-1 IC_{50} values could not be determined due to their limited solubility in stock solutions, the results suggested that myristinin D (**3**), the mixture of myristinins B (**2**) and C (**2a**), and the mixture of myristinins E (**4**) and F (**4a**) are at least 10-fold more active against COX-2 than COX-1. It is interesting to note that all isolated flavans inhibited COX enzymes at comparable activities regardless to *cis*- or *trans*-configurations of rings B and C, as well as atropisomeric conformations. The cytotoxicity IC_{50} values of myristinin A (**1**), the mixture of myristinins B (**2**) and C (**2a**), myristinin D (**3**), and the mixture of myristinins E (**4**) and F (**4a**) against Vero cells were 17.7, 16.4, 13.6, and 8.9 μg/mL, respectively (Table 3).

In the present study, dodecanoylphloroglucinol and 1-(2,4,6-trihydroxyphenyl)-9-phenylnonan-1-one, were also isolated from the CH_2Cl_2 extract of *M. cinnamomea* fruits, and these phloroglucinol derivatives might be precursors of myristinins. To our knowledge, myristinins B (**2**) and C (**2a**) or myristinins E (**4**) and F (**4a**) are the first atropisomeric flavans isolated from natural environments.

Experimental Section

General. The ^1H, ^{13}C, $^1H-^1H$ COSY, NOESY, and DEPT NMR spectra were recorded at 400 MHz for proton and 100 MHz for carbon. Proton-detected heteronuclear correlations were measured using HMQC (optimized for $^1J_{HC} = 145$ Hz) and HMBC (optimized for $^nJ_{HC} = 4.0$ Hz) pulse sequences.

Extraction and Isolation. Dried fruits (whole fruit; 500 g) of *M. cinnamomea* were soaked with CH_2Cl_2 at room temperature for 2 days. A CH_2Cl_2 crude extract (103.5 g) was chromatographed on Sephadex LH-20, eluted with MeOH, yielding 14 fractions (A−N). Fraction E was rechromatographed on Sephadex LH-20 (MeOH as eluent) to yield fractions E_1-E_{10}. Fraction E_7 was further purified by reversed-phase preparative HPLC (gradient of 10−20% H_2O in MeOH) to afford myristinin A (**1**) (76 mg). Fraction E_9 was subjected to preparative HPLC (reversed-phase C_{18} column, MeCN−H_2O, 60:40) followed by silica gel column ($CH_2Cl_2-Me_2CO$, 7:3) furnishing myristinin D (**3**) (40 mg) and a mixture (30 mg) of myristinins B (**2**) and C (**2a**). Fraction E_{10} was purified by HPLC (reversed-phase C_{18} column, MeCN−H_2O, 65:35) and finally with silica gel column ($CH_2Cl_2-Me_2CO$, 7:3) yielding a mixture (80 mg) of myristinins E (**4**) and F (**4a**). Fraction F was rechromatographed on Sephadex LH-20 (MeOH as eluent) to yield fractions F_1-F_8. Fraction F_4 was rechromatographed on Sephadex LH-20 (MeOH as eluent) followed by HPLC (reversed-phase C_{18} column, MeOH−H_2O, 80:20) to yield hinokinin (40 mg) and 1-(2,4,6-trihydroxyphenyl)-9-phenylnonan-1-one (30 mg). Fraction F_5 was rechromatographed on Sephadex LH-20 (MeOH as eluent) followed by repeated silica

(7) The mixture of myristinins E (**4**) and F (**4a**) (25 mg) was dissolved in dry pyridine (0.25 mL) and treated with acetic anhydride (0.10 mL). A reaction mixture was stirred and allowed to stand overnight at room temperature. After evaporating reaction mixture to dryness, EtOAc (8 mL) was added to the dry mixture, which was subsequently washed with $NaHCO_3$ (5 × 5 mL) followed by H_2O (5 × 5 mL). The EtOAc layer was evaporated, yielding 22 mg of **5** and **5a** mixture. ESITOF MS *m/z* 815.3082 *m/z* $(M + Na)^+$, calcd for $C_{46}H_{48}O_{12}Na$ 815.3043; ^1H NMR ($CDCl_3$) δ_H 7.50 (2H, brd, $J = 8.4$ Hz, H-2″ and H-6″), 7.30 (2H, m, H-18′ and H-20′), 7.15−7.21 (5H, m, H-3″, H-5″, H-17′, H-19′, and H-21′), 7.02 (1H, s, H-5′ of **5a**), 7.00 (1H, s, H-5′ of **5**), 6.86 (1H, d, $J = 8.4$ Hz, H-5 of **5a**), 6.79 (1H, d, $J = 8.4$ Hz, H-5 of **5**), 6.69 (1H, d, $J = 2.3$ Hz, H-8 of **5a**), 6.67 (1H, d, $J = 2.3$ Hz, H-8 of **5**), 6.54 (1H, dd, $J = 8.4$ and 2.3 Hz, H-6), 5.13 (1H, brd, $J = 11.4$ Hz, H-2), 4.57 (1H, dd, $J = 11.7$ and 6.4 Hz, H-4 of **5**), 4.45 (1H, dd, $J = 11.7$ and 6.4 Hz, H-4 of **5a**), 2.79−2.30 (5H, m, H-3_{ax}, H-8′, and H-15′), 2.26−2.34 (15H, s, 5 x CH_3CO), 1.63−1.77 (5H, m, H-3_{eq}, H-9′ and H-14′), and 1.28−1.35 (8 H, s, H-10′, H-11′, H-12′and H-13′). The ratio of **5**/**5a** (1: 2) was calculated from peak integration of H-4 (at δ_H 4.57 for **5** and δ_H 4.45 for **5a**).

gel column (CH_2Cl_2−EtOAc, 9:1) to furnish dodecanoylphloroglucinol (35 mg).

Antifungal Activity Assay. The isolated compounds were tested for their antifungal activity against a clinical isolate of *Candida albicans* using a method modified from the soluble formazan assay.[8] Briefly, 100 μL of 2×10^6 CFU/ml *C. albicans* in RPMI 1640 medium, containing 34.53 g/mL 3-(*N*-morpholino)propanesulfonic acid (MOP) was added to each well of 96-well microculture plates containing 100 μL of test compound diluted in 10% dimethyl sulfoxide (DMSO). Plates were incubated at 37 °C for 4 h before adding 50 μL of a solution containing 1 mg/mL 2,3-bis(2-methoxy-4-nitro-5-sulfonylphenyl)-5-[(phenylamino)carbonyl]-2*H*-tetrazolium hydroxide (XTT tetrazolium) and 0.025 mM *N*-methylphenazolium methosulfate (PMS). After an additional 4 h incubation at 37 °C, the number of living cells was determined by measuring the absorbance of XTT formazan at 450 nm. Amphotericin B and 10% DMSO were used as a positive and a negative control, respectively. In our system, the IC_{50} value of the standard drug, Amphotericin B, was 0.01 μg/mL.

Antiinflammatory Activity Assay. Immortalized COX-$1^{-/-}$ and COX-$2^{-/-}$ mouse lung fibroblast cells were plated at 1×10^5 cells/mL in complete Dulbelcco's Modified Eagle Medium (DMEM) containing 0.1 mM nonessential amino acids, 292 mg/mL L-glutamine, 50 mg/mL ascorbic acid, and 10% fetal bovine serum, in 96-well flat-bottomed tissue culture plates at 83 μL/well. Cells were incubated at 37 °C for 72 h in a humidified incubator with 5% CO_2. Subsequently, cells were washed with phosphate buffer saline solution and incubated for 30 min in 83 μL serum-free DMEM medium containing test compounds. DMEM media containing drug vehicle, DMSO (0.1%), and aspirin were used as a control for 100% COX activities and a positive control, respectively. The medium was then replaced with serum-free DMEM containing the same amount of drugs or DMSO and 2 μM of calcium ionophore A23187, and cells were incubated for 30 min. Culture supernatants were collected at the end of incubation time and assayed for prostaglandin E_2 (PGE_2) concentrations by the radioimmunoassay method previously described by Kirtikara and co-workers.[9] The inhibition of COX activity was determined from the percent reduction of PGE_2 produced by drug-treated cells relative to PGE_2 produced by cells treated with DMSO alone. IC_{50} values of COX-1 and COX-2 were determined using the SOFTmax software (Molecular Devices, Sunnyvale, CA). Aspirin was used as a positive control and almost equally effective against COX-1 and COX-2. IC_{50} values of aspirin for COX-1 and COX-2 are 2.06 μg/mL and 3.57 μg/mL, respectively.

Cytotoxic Assay. The colorimetric cytotoxicity assay employed was modified from an anticancer screening method developed by Skehan and co-workers.[10] Test compounds were diluted in 10% DMSO and added to each well of 96-well tissue culture plates, 10 μL/well, followed by 190 μL of 1×10^5 cells/ml Vero cells. Plates were incubated at 37 °C for 72 h in a humidified incubator with 5% CO_2. Afterward, culture medium was discarded, and cells were fixed in 100 μL of 50% (W/V) trichloroacetic acid (TCA) at 4 °C for 30 min. Then, the plates were washed four times with tap water, air-dried at room temperature, and stained with 100 μL of 0.057% (W/V) sulforhodamine B (SRB) in 1% acetic acid for 30 min at room temperature. SRB solution was then discarded, and the plates were washed with 1% acetic acid four times and air-dried. To each well, 200 μL of 10 mM Tris-base (pH 10) was added, and plates were shaken for 5 min. The absorbance of SRB was measured at 515 nm by a microtiter plate reader. A solution of 10% DMSO was used as a negative control. Ellipticine was used as the reference substance, exhibiting the IC_{50} value of 0.3 μg/mL.

Myristinin A (1). Brown amorphous solid; $[\alpha]^{28}_D$ +39.1° (*c* 0.460, MeOH); UV (MeOH) λ_{max} (log ϵ) 226 (4.6), 288 (4.4) nm; IR ($CHCl_3$) ν_{max} 3404, 2928, 2856, 1616, 1506, 1438, 1264, 1166, 1027, 835 cm^{-1}; ESITOF MS *m*/*z* 549.2835 $(M + H)^+$, calcd for $[C_{33}H_{40}O_7 + H]$ 549.2852; ^1H and ^{13}C NMR see Table 1.

A Mixture of Myristinins B (2) and C (2a). Brown amorphous solid; $[\alpha]^{28}_D$ −280° (*c* 0.125, MeOH); UV (MeOH) λ_{max} (log ϵ) 226 (4.5), 287 (4.3) nm; IR (KBr) ν_{max} 3315, 2925, 2854, 1618, 1508, 1439, 1242, 1156, 1022, 835 cm^{-1}; ESITOF MS *m*/*z* 549.2852 $(M + H)^+$, calcd for $[C_{33}H_{40}O_7 + H]$ 549.2852; ^1H and ^{13}C NMR see Table 1.

Myristinin D (3). Brown amorphous solid; $[\alpha]^{28}_D$ +87.5° (*c* 0.160, MeOH); UV (MeOH) λ_{max} (log ϵ) 226 (4.3), 288 (4.1) nm; IR ($CHCl_3$) ν_{max} 3408, 3010, 2931, 2857, 1616, 1506, 1439, 1264, 1160, 1027, 835, 701 cm^{-1}; ESITOF MS *m*/*z* 583.2701 $(M + H)^+$, calcd for $[C_{36}H_{38}O_7 + H]$ 583.2696; ^1H and ^{13}C NMR see Table 2.

A Mixture of Myristinins E (4) and F (4a). Brown amorphous solid; $[\alpha]^{28}_D$ −32.2° (*c* 0.615, MeOH); UV (MeOH) λ_{max} (log ϵ) 226 (4.4), 288 (4.1) nm; IR ($CHCl_3$) ν_{max} 3275, 3011, 2931, 2857, 1617, 1506, 1441, 1244, 1166, 1027, 836, 701 cm^{-1}; ESITOF MS *m*/*z* 583.2703 $(M + H)^+$, calcd for $[C_{36}H_{38}O_7 + H]$, 583.2696; ^1H and ^{13}C NMR see Table 2.

Acknowledgment. We are indebted to the Biodiversity Research and Training Program (BRT) for financial support. Y.T. thanks the National Center for Genetic Engineering and Biotechnology for the Senior Research Fellowship Award. S.S. thanks the Thailand Graduate Institute of Science and Technology (TGIST) for a student's grant.

Supporting Information Available: ^1H and ^{13}C NMR spectra of compounds **1**, the mixture of **2** and **2a**, **3**, and the mixture of **4** and **4a**; NOESY spectra of the mixture of **2** and **2a** and the mixture of **4** and **4a**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO020045D

(8) Scudiero, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Tierney, S.; Nofziger, T. H.; Currens, M. J.; Seniff, D.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 8 (17), 4827−4833.

(9) Kirtikara, K.; Morham, S. G.; Raghow, R.; Laulederkind, S. J.; Kanekura, T.; Goorha, S.; Ballou, L. R. *J. Exp. Med.* **1998**, *187* (4), 517−523.

(10) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.