New Coumarins and Triterpenes from *Calophyllum inophyllum*

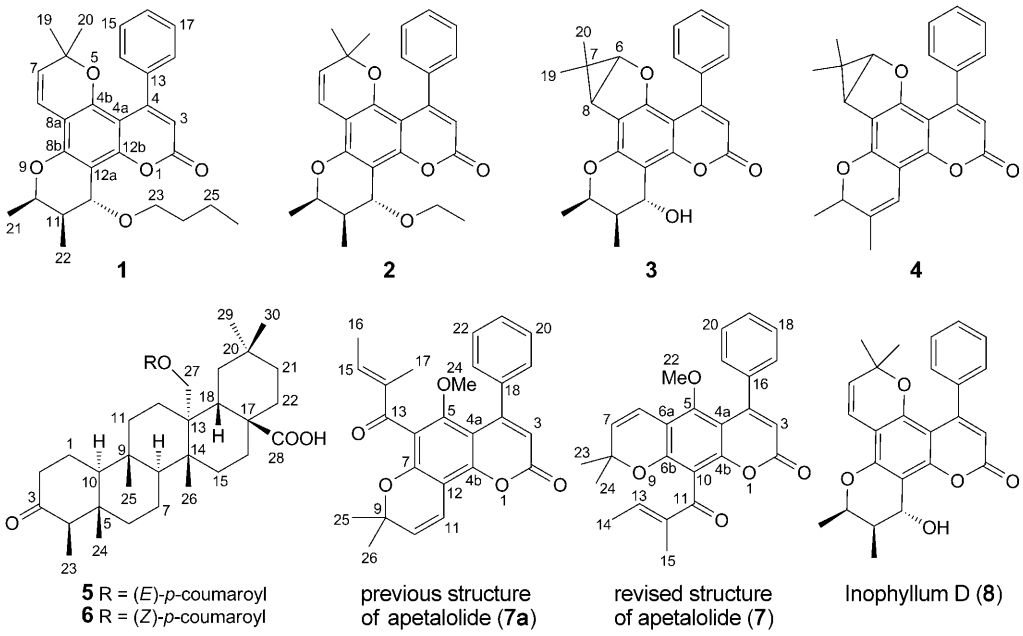
by **Jian Zou**, **Jian Wu**, **Shuang-Zhu Liu**, and **Wei-Min Zhao***

Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 201203, P. R. China
(phone/fax: +86-21-50806052; e-mail: wmzhao@mail.shcnc.ac.cn)

Four new coumarins, **1**–**4**, and two new triterpenes, **5** and **6**, along with nine known compounds have been identified from the leaves of *Calophyllum inophyllum* on the basis of spectroscopic analyses and chemical methods. The structure of apetalolide (**7**), previously reported from *C. apetalum*, was revised by means of 2D-NMR, and the absolute configuration of the known compound inophyllum D (**8**) was established by a modified *Mosher*'s method.

Introduction. – *Calophyllum inophyllum* (Guttiferae) is an arbor widely distributed in the southeast of Asia, and it has been used as herb medicine for the treatment of rheumatism, arthritis, lumbago, and wounds in the south of China [1]. Previous phytochemical investigations have revealed *C. inophyllum* to be a rich source of secondary metabolites, including xanthones [2], coumarins [3–5], and triterpenes [6], and some of them have been found to exhibit antimicrobial [2], cytotoxic [2], piscicidal [4], and anti-HIV activities [5]. To the best of our knowledge, no systematical investigation has been undertaken on the chemical constituents of *C. inophyllum* distributed in China. Since the same plant species grows in different regions, they may contain different constituents. Phytochemicals of the leaves of *C. inophyllum* collected in Hainan Island of China were investigated. We herein report the isolation and structural characterization of six new compounds, named 12-*O*-butylinophyllum D (**1**), 12-*O*-ethylinophyllum D (**2**), inophyllum H (**3**), inophyllum I (**4**), 27-[(*E*)-*p*-coumaroyloxy]friedelin-28-carboxylic acid (**5**), and 27-[(*Z*)-*p*-coumaroyloxy]friedelin-28-carboxylic acid (**6**), along with nine known compounds. The structure of the known compound apetalolide (**7**), identified previously from *C. apetalum*, was revised according to 2D-NMR spectral analysis, and the absolute configuration of inophyllum D (**8**), a known compound isolated from *C. inophyllum*, was established by a modified *Mosher*'s method.

Results and Discussion. – Compound **1** was obtained as a yellow amorphous powder with the molecular formula $C_{29}H_{32}O_5$, determined by HR-EI-MS and NMR analyses. The IR spectrum of **1** revealed the existence of OH (3438 cm^{-1}) and α,β-unsaturated lactone C=O (1739 cm^{-1}) groups, and an olefinic C=C bond (1637 cm^{-1}). Its UV spectrum exhibited absorption maxima at 332, 286, 278, and 236 nm, which were similar to those of inophyllum D (**8**), a known coumarin previously reported from the same species [4]. The ^{13}C-NMR spectrum of **1** (*Table 1*) showed 29 C-atom signals



corresponding to five Me, three sp^3 CH_2, three sp^3 CH, eight sp^2 CH groups, and one sp^3 and nine sp^2 quaternary C-atoms. The ^{1}H-NMR spectrum of **1** (*Table 2*) exhibited two Me *singlet*s at δ(H) 0.91 (Me(19)) and 0.94 (Me(20)), two Me *doublet*s at δ(H) 0.83 ($J = 7.3$, Me(21)), 1.44 ($J = 6.6$ Hz, Me(22)), one Me *triplet* at δ(H) 0.93 ($J = 7.4$, Me(26)), signals corresponding to three sp^3 CH_2 groups (δ(H) 3.78–3.80 (*m*, CH_2(23)), 1.58–1.62 (*m*, CH_2(24)), and 1.40–1.44 (*m*, CH_2(25))), three olefinic H-atom signals (δ(H) 5.36 (*d*, $J = 10.0$, H–C(7)), 6.58 (*d*, $J = 10.0$, H–C(8)), and 5.98 (*s*, H–C(3))), signals due to a mono-substituted benzene ring (δ(H) 7.38–7.40 (*m*, H–C(14,16,18)), 7.20–7.24 (*m*, H–C(15,17))), and three sp^3 CH signals (δ(H) 4.59 (*dq*, $J = 6.6$, 2.0, H–C(10)), 4.51 (*d*, $J = 2.2$, H–C(12)), and 2.05 (*ddq*, $J = 7.3$, 2.0, 2.2, H–C(11)). The ^{1}H-NMR data of **1** were similar to those of **8** except three additional sp^3 CH_2 signals and one Me *triplet* [4], and the *doublet* due to H–C(12) was shifted upfield from δ(H) 4.93 ($J = 2.1$) to 4.51 ($J = 2.2$), thus it was assumed that a BuO group was at C(12) of **1**. The planar structure of **1** was established by HMBC spectrum, in which the following ^{13}C,^{1}H long-range correlation signals were observed: C(4a)/H–C(3), C(4b)/Me(19,20), C(6)/H–C(8), C(7)/Me(19,20), C(8a)/H–C(7), C(8b)/H–C(10), C(10)/H–C(12) and Me(22), C(11)/Me(21), C(12)/Me(22) and CH_2(23), C(13)/H–C(3,15,17), C(19,20)/H–C(7), C(22)/H–C(10,12), C(23)/H–C(12), and CH_2(25), C(24)/Me(26), C(25)/CH_2(23), C(26)/CH_2(24). Hydrolysis of **1** yielded **8**, which was identified by co-TLC with authentic sample and optical-rotation data. Thus, compound **1** was assigned as 12-*O*-butylinophyllum D.

The absolute configuration of **8** was established by a modified *Mosher*'s method [7]. Both (*R*)- and (*S*)-MTPA (MTPA = α-methoxy-α-(trifluoromethyl)phenylacetic

Table 1. *^{13}C-NMR Data* (100 MHz, in $CDCl_3$) *of Compounds* **1**–**4**

C-Atom	**1**	**2**	**3**	**4**	Inophyllum D (**8**) [8]
C(2)	160.8 (*s*)	160.5 (*s*)	160.8 (*s*)	160.9 (*s*)	160.5 (*s*)
C(3)	112.3 (*d*)	111.9 (*d*)	111.4 (*d*)	111.8 (*d*)	111.9 (*d*)
C(4)	156.2 (*s*)	155.9 (*s*)	154.9 (*s*)	154.6 (*s*)	156.3 (*s*)
C(4a)	103.7 (*s*)	103.4 (*s*)	98.9 (*s*)	98.8 (*s*)	103.4 (*s*)
C(4b)	151.4 (*s*)	151.1 (*s*)	158.6 (*s*)	158.3 (*s*)	151.1 (*s*)
C(6)	77.2 (*s*)	76.8 (*s*)	72.7 (*d*)	73.0 (*d*)	77.1 (*s*)
C(7)	127.3 (*d*)	127.0 (*d*)	12.9 (*s*)	13.8 (*s*)	127.2 (*d*)
C(8)	116.3 (*d*)	115.9 (*d*)	29.2 (*d*)	30.9 (*d*)	115.9 (*d*)
C(8a)	106.1 (*s*)	105.8 (*s*)	111.5 (*s*)	113.0 (*s*)	106.0 (*s*)
C(8b)	154.2 (*s*)	153.9 (*s*)	155.1 (*s*)	152.6 (*s*)	153.9 (*s*)
C(10)	71.3 (*d*)	70.9 (*d*)	70.9 (*d*)	112.0 (*d*)	71.2 (*d*)
C(11)	34.3 (*d*)	34.2 (*d*)	37.4 (*d*)	132.1 (*s*)	37.2 (*d*)
C(12)	72.3 (*d*)	71.8 (*d*)	64.8 (*d*)	76.1 (*d*)	64.6 (*d*)
C(12a)	102.3 (*s*)	101.8 (*s*)	104.3 (*s*)	103.9 (*s*)	103.9 (*s*)
C(12b)	155.1 (*s*)	154.7 (*s*)	153.7 (*s*)	152.4 (*s*)	154.6 (*s*)
C(13)	140.4 (*s*)	140.1 (*s*)	137.5 (*s*)	137.8 (*s*)	139.9 (*s*)
C(14)	127.6 (*d*)	127.3 (*d*)	127.7 (*d*)	127.6 (*d*)	127.3 (*d*)
C(15)	127.6 (*d*)	127.3 (*d*)	127.7 (*d*)	127.9 (*d*)	127.4 (*d*)
C(16)	127.7 (*d*)	127.4 (*d*)	128.8 (*d*)	128.8 (*d*)	127.6 (*d*)
C(17)	127.6 (*d*)	127.3 (*d*)	127.7 (*d*)	127.9 (*d*)	127.4 (*d*)
C(18)	127.6 (*d*)	127.3 (*d*)	127.7 (*d*)	127.6 (*d*)	127.3 (*d*)
C(19)	27.2 (*q*)	26.9 (*q*)	22.6 (*q*)	22.7 (*q*)	27.0 (*q*)
C(20)	27.0 (*q*)	26.7 (*q*)	12.9 (*q*)	12.8 (*q*)	27.0 (*q*)
C(21)	18.0 (*q*)	17.4 (*q*)	17.7 (*q*)	19.6 (*q*)	17.1 (*q*)
C(22)	9.4 (*q*)	9.0 (*q*)	9.2 (*q*)	19.4 (*q*)	9.1 (*q*)
C(23)	69.5 (*t*)	64.7 (*t*)			
C(24)	32.5 (*t*)	15.8 (*q*)			
C(25)	19.8 (*t*)				
C(26)	14.2 (*q*)				

acid) esters of inophyllum D were prepared according to a usual method, and the $\Delta\delta$ values ($\Delta\delta = \delta_S - \delta_R$) of their ^{1}H-NMR data were calculated (*Fig.*). In this way, the absolute configuration at C(12) was determined to be (*R*). Since the relative configuration of **8** had already been determined [5], **8** was thus identified to be (10*R*,11*S*,12*R*)-12-hydroxy-11,12-dihydro-6,6,10,11-tetramethyl-4-phenyl-2*H*,6*H*,10*H*-dipyrano[2,3-*f*:2′,3′-*h*]chromen-2-one, and, accordingly, compound **1** was established as

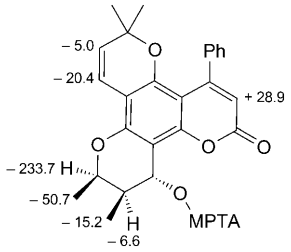


Figure. *Δδ Values* ($\Delta\delta = \delta_S - \delta_R$) *of ^{1}H-NMR data* (600 MHz, *J* in Hz) *of the MPTA esters of inophyllum D*

Table 2. *^{1}H-NMR Data* (400 MHz, in $CDCl_3$, *J* in Hz) *of* **1**–**4** *and Inophyllum D* (**8**)

	1	**2**	**3**	**4**	**8** [8]
H–C(3)	5.98 (*s*)	5.98 (*s*)	6.01 (*s*)	5.98 (*s*)	5.98 (*s*)
H–C(6)			4.21 (*d*, *J* = 5.7)	4.25 (*d*, *J* = 5.7)	
H–C(7)	5.36 (*d*, *J* = 10.0)	5.34 (*d*, *J* = 10.0)			5.36 (*d*, *J* = 10.0)
H–C(8)	6.58 (*d*, *J* = 10.0)	6.56 (*d*, *J* = 10.0)	2.42 (*d*, *J* = 5.7)	2.41 (*d*, *J* = 5.7)	5.66 (*d*, *J* = 10.0)
H–C(10)	4.59 (*dq*, *J* = 6.6, 2.0)	4.58 (*dq*, *J* = 6.7, 2.0)	4.39 (*dq*, *J* = 6.6, 2.0)	4.93 (*q*, *J* = 6.5)	4.56 (*dq*, *J* = 6.7, 2.0)
H–C(11)	2.05 (*ddq*, *J* = 7.3, 2.0, 2.2)	2.05 (*ddq*, *J* = 7.3, 1.9, 2.2)	2.04 (*ddq*, *J* = 7.2, 2.0, 2.2)		1.99 (*ddq*, *J* = 7.2, 2.0, 2.0)
H–C(12)	4.51 (*d*, *J* = 2.2)	4.56 (*d*, *J* = 2.2)	4.95 (*d*, *J* = 2.3)	6.68 (*s*)	4.95 (*d*, *J* = 2.0)
H–C(14,16,18)	7.38–7.40 (*m*)	7.37–7.39 (*m*)	7.40–7.42 (*m*)	7.39–7.41 (*m*)	7.31–7.33 (*m*)
H–C(15,17)	7.20–7.24 (*m*)	7.18–7.22 (*m*)	7.34–7.38 (*m*)	7.24–7.28 (*m*)	7.29–7.30 (*m*)
Me(19)	0.91 (*s*)	0.90 (*s*)	0.75 (*s*)	0.70 (*s*)	0.95 (*s*)
Me(20)	0.94 (*s*)	0.92 (*s*)	1.02 (*s*)	1.04 (*s*)	0.95 (*s*)
Me(21)	1.44 (*d*, *J* = 6.6)	1.41 (*d*, *J* = 6.6)	1.46 (*d*, *J* = 6.5)	1.40 (*d*, *J* = 6.5)	1.45 (*d*, *J* = 6.7)
Me(22)	0.83 (*d*, *J* = 7.3)	0.79 (*d*, *J* = 7.1)	0.81 (*d*, *J* = 7.2)	1.90 (*s*)	0.83 (*d*, *J* = 7.2)
CH_2(23)	3.78–3.80 (*m*)	3.81 (*q*, *J* = 7.0)			
CH_2(24) or Me(24)	1.58–1.62 (*m*)	1.23 (*t*, *J* = 7.0)			
CH_2(25)	1.40–1.44 (*m*)				
Me(26)	0.93 (*t*, *J* = 7.4)				

(10*R*,11*S*,12*R*)-12-butoxy-11,12-dihydro-6,6,10,11-tetramethyl-4-phenyl-2*H*,6*H*,10*H*-dipyrano[2,3-*f*:2′,3′-*h*]chromen-2-one.

Compound **2** was obtained as a yellow amorphous powder with the molecular formula $C_{27}H_{28}O_5$, determined by HR-EI-MS. The IR, UV, and ^{13}C-NMR spectra of **2** were similar to those of **1**, except with two sp^3 CH_2 signals less in its ^{13}C-NMR spectrum. Hydrolysis of **2** also yielded **8**. Further analysis of 2D-NMR spectra allowed the characterization of compound **2** as (10*R*,11*S*,12*R*)-12-ethoxy-11,12-dihydro-6,6,10,11-tetramethyl-4-phenyl-2*H*,6*H*,10*H*-dipyrano[2,3-*f*:2′,3′-*h*]chromen-2-one.

Compounds **1** and **2** were confirmed to be natural compounds by re-extraction of the plant material and preparative TLC purification without using BuOH and EtOH, and further LC/MS analyses.

Compound **3** was obtained as a yellow amorphous powder with the molecular formula $C_{25}H_{24}O_5$, determined by HR-EI-MS and NMR analyses. The IR spectrum of **3** revealed presence of OH (3426 cm^{-1}) and α,β-unsaturated lactone C=O (1708 cm^{-1}) functionalities, and a monosubstituted aromatic ring (700, 748 cm^{-1}). The ^{13}C-NMR spectrum of **3** (*Table 1*) showed 25 C-atom signals including those for four Me, five sp^3 CH, six sp^2 CH groups, and one sp^3 and nine sp^2 quaternary C-atoms. The ^{1}H-NMR spectrum of **3** (*Table 2*) exhibited two Me *singlet*s (δ(H) 0.75 (Me(19)), 1.02 (Me(20))), two Me *doublet*s (δ(H) 1.46 (*d*, *J* = 6.5, Me(21)), 0.81 (*J* = 7.2, Me(22))), one sp^2 H-atom *singlet* (δ(H) 6.01 (H−C(3)))), signals due to a monosubstituted aromatic ring (δ(H) 7.40–7.42 (*m*, H−C(14,16,18)), 7.34–7.38 (*m*, H−C(15,17)))), and five sp^3 CH signals (δ(H) 4.21 (*d*, *J* = 5.7, H−C(6)), 2.42 (*d*, *J* = 5.7, H−C(8)), 4.39 (*dq*, *J* = 6.6, 2.0, H−C(10)), 4.95 (*d*, *J* = 2.3, H−C(12)), and 2.04 (*ddq*, *J* = 7.2, 2.0, 2.2, H−C(11))). The ^{1}H-NMR spectrum of **3** was thus similar to that of **8** [4]; the difference between the two spectra consists in an upfield shift of H−C(7) and H−C(8), the *cis*-coupled olefinic *doublet* from δ(H) 5.35 (*d*, *J* = 10.0, H−C(7)), 6.52 (*d*, *J* = 10.0, H−C(8)) in **8** to 4.21 (*d*, *J* = 5.7, H−C(6)), 2.42 (*d*, *J* = 5.7, H−C(8)) in **3**, indicating the loss of the C(7)=C(8) bond and presence of a dimethylcyclopropane ring in ring *A* of **3** as in inophyllum G_1 and G_2 [5]. The observed upfield shift of the geminal Me *singlet* resonance (**8**: δ(H) 0.95 (*s*, Me(19,20)); **3**: δ(H) 0.75 (*s*, Me(19)), 1.02 (*s*, Me(20))) also supported the presence of a cyclopropane ring in ring *A* of **3**. The constitutional formula of **3** was established *via* the HMBC spectra, in which the following long-range ^{1}H,^{13}C-correlation signals were observed: C(4a)/H−C(3), C(4b)/H−C(6,8), C(6)/Me(19,20), C(8)/Me(19,20), C(8a)/H−C(6), C(8b)/H−C(8), C(10)/H−C(12), C(11)/Me(21), C(12)/H−C(10) and Me(22), C(13)/H−C(3,15,17), C(19)/H−C(6,8), C(20)/H−C(6,8), C(21)/H−C(11), C(22)/H−C(10,12). Since the chemical shifts and the coupling constants of H−C(10), H−C(11), and H−C(12) are almost the same as those of **8** [5], it can be assumed that the relative configuration of the chromanol ring is the same as that of **8**. To the best of our knowledge, compound **3** is a new compound, and has been assigned the name inophyllum H. It was difficult to completely specify the relative configuration of **3**, since it possesses two additional stereogenic centers, and the H-atoms in the cyclopropane ring are too far from the chromanol ring H-atoms to display a NOE-based relationship between the two spin systems, thus it was not possible to assign the configuration of the cyclopropane ring in **3** without an X-ray analysis.

Compound **4** was obtained as a yellow amorphous powder with the molecular formula of $C_{25}H_{22}O_4$, determined by HR-EI-MS and NMR analysis. The IR spectrum

and UV spectra were similar to those of **3**. The ^{13}C-NMR spectrum of **4** showed 25 C-atom signals including those for four Me, three sp^3 CH, and seven sp^2 CH groups, and one sp^3 and ten sp^2 quaternary C-atoms. The ^{1}H-NMR spectrum of **4** exhibited four Me signals (δ(H) 0.70 (*s*, Me(19)), 1.04 (*s*, Me(20)), 1.90 (*s*, Me(22)), and 1.40 (*d*, $J = 6.5$, Me(21))), two sp^2 H-atom signals (δ(H) 5.98 (*s*, H–C(3)) and 6.68 (*s*, H–C(12))), signals due to a monosubstituted aromatic ring (δ(H) 7.39–7.40 (*m*, H–C(14,16,18)), 7.24–7.26 (*m*, H–C(15,17))), and three sp^3 CH signals (δ(H) 4.25 (*d*, $J = 5.7$, H–C(6)), 2.41 (*d*, $J = 5.7$, H–C(8)), and 4.93 (*q*, $J = 6.5$, H–C(10))). Compared with the ^{1}H-NMR spectra of 11,12-dehydroxyinophyllum P [4], the presence of two sp^3 CH signals at δ(H) 4.20 (*d*, $J = 5.7$, H–C(6)), 2.41 (*d*, $J = 5.7$, H–C(8)) and the disappearance of the *cis*-coupled olefinic *doublet* indicated the loss of the C(7)=C(8) bond, and the presence of a dimethylcyclopropane ring in ring *A* of **4** as in **3**. The planar structure of **4** was established on the HMBC spectra, in which the following long-range ^{1}H,^{13}C-correlation signals were observed: C(4)/H–C(14,18), C(4a)/H–C(3), C(4b)/H–C(6,8), C(6)/Me(19,20), C(8)/Me(19,20), C(8a)/H–C(6), C(8b)/H–C(12), C(10)/H–C(12) and Me(22), C(11)/Me(21), C(12)/Me(22), C(13)/H–C(3), C(19)/H–C(6,8), C(20)/H–C(6,8), C(22)/H–C(10). Compound **4** is a new compound, and has been named inophyllum I.

Compound **5** was obtained as a white amorphous powder with the molecular formula of $C_{39}H_{54}O_6$, determined by HR-EI-MS and NMR analysis. The IR spectrum of **5** revealed the existence of a OH (3415 cm^{-1}) and a C=O group (1701 cm^{-1}), and of an aromatic ring (1604, 1514, 1450 cm^{-1}). The presence of the (*E*)-*p*-coumaroyl group was established by characteristic ^{1}H- and ^{13}C-NMR data (*Table 3*) [7], and characteristic peaks at *m*/*z* 164.0470 ($C_9H_8O_3$), 163.0401 ($C_9H_7O_3$), 147.0441 ($C_9H_7O_2$), and 119.0498 (C_8H_7O) in the HR-EI-MS. In the HR-EI-MS spectra of **5**, the peak at *m*/*z* 409.3447 ($C_{29}H_{45}O$, [*M* – coumaroyloxy group – COOH]$^+$) implied the presence of a free COOH group in the triterpene part of **5**. In the HMBC spectra of **5**, the following long-range ^{13}C,^{1}H correlation signals were observed: C(3)/CH$_2$(1) and Me(23), C(5)/Me(23), C(6)/H–C(8) and Me(24), C(8)/Me(25,26), C(9)/CH$_2$(12), C(10)/CH$_2$(2) and Me(24,25), C(11)/H–C(8) and Me(25), C(12)/CH$_2$(27), C(13)/Me(26), C(14)/CH$_2$(27), C(15)/Me(26), C(17)/CH$_2$(19), C(18)/CH$_2$(16,27), C(19)/Me(29,30), C(20)/CH$_2$(22), C(21)/CH$_2$(19) and Me(29,30), C(22)/H–C(18), C(24)/H–C(4), C(25)/H–C(8,10), C(26)/CH$_2$(15), C(27)/CH$_2$(12) and H–C(18), C(28)/CH$_2$(22), C(29,30)/CH$_2$(19,21), C(1′)/H–C(3′,5′,8′), C(9′)/CH$_2$(27) and H–C(7′). Thus, a friedelin skeleton was suggested for **5**, with the free COOH group at C(28) and the (*E*)-*p*-coumaroyloxy group at C(27), which was confirmed by NOE correlation signals observed between Me(23), and CH$_2$(2,6) and Me(24); Me(24) and CH$_2$(1,7) and Me(25); Me(25), and CH$_2$(1,11,12) and Me(26); Me(26), and CH$_2$(7) and H–C(18); H$_b$–C(27), and CH$_2$(15,12) and Me(29); H$_a$–C(27) and CH$_2$(19,22); Me(29), and CH$_2$(22) and Me(30); and Me(30) and CH$_2$(19,22). Compound **5** was finally characterized as 27-[(*E*)-*p*-coumaroyloxy]friedelin-28-carboxylic acid.

Compound **6** was obtained as a white amorphous powder with the molecular formula of $C_{39}H_{54}O_6$ determined by HR-EI-MS and NMR analysis. The IR spectrum of **6** revealed the presence of a OH (3392 cm^{-1}) and a C=O group (1701 cm^{-1}), and an aromatic ring (1604, 1514, 1451 cm^{-1}). The ^{13}C-NMR spectrum showed 39 C-atom signals including those for six Me groups, and ten aromatic and conjugated C=C bond

Table 3. *^1H- (400 MHz) and ^{13}C-NMR (100 MHz) Data of* **5** *and* **6** (C_5D_5N)

	5		**6**	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	1.56–1.80 (*m*)	22.3 (*t*)	1.54–1.78 (*m*)	22.4 (*t*)
CH_2(2)	2.18–2.32 (*m*)	41.4 (*t*)	2.08–2.30 (*m*)	41.5 (*t*)
C(3)		211.7 (*s*)		211.7 (*s*)
H–C(4)	2.12 (*q*, *J* = 6.6)	57.7 (*d*)	2.16 (*q*, *J* = 6.5)	57.7 (*d*)
C(5)		41.8 (*s*)		41.9 (*s*)
H–C(26)	1.58–1.62 (*m*)	40.7 (*t*)	1.60–1.64 (*m*)	40.8 (*t*)
CH_2(7)	1.57–1.59 (*m*)	18.7 (*t*)	1.57–1.63 (*m*)	18.5 (*t*)
H–C(8)	1.59–1.62 (*m*)	53.3 (*t*)	1.62–1.66 (*m*)	53.3 (*t*)
C(9)		37.8 (*s*)		37.7 (*s*)
H–C(10)	1.54–1.56 (*m*)	59.0 (*d*)	1.56–1.60 (*m*)	59.0 (*d*)
CH_2(11)	1.60–1.64 (*m*)	36.7 (*t*)	1.63–1.70 (*m*)	36.7 (*t*)
CH_2(12)	2.30–2.36 (*m*)	25.5 (*t*)	2.35–2.39 (*m*)	25.5 (*t*)
C(13)		43.4 (*s*)		43.2 (*s*)
C(14)		38.4 (*s*)		38.5 (*s*)
CH_2(15)	1.23–1.27 (*m*)	32.1 (*t*)	1,27–1.31 (*m*)	32.1 (*t*)
CH_2(16)	2.90 (*dd*, *J* = 14.7, 11.6)	30.3 (*t*)	2.88 (*dd*, *J* = 14.5, 11.2)	30.3 (*t*)
C(17)		45.1 (*s*)		45.1 (*s*)
H–C(18)	3.00 (*d*, *J* = 13.9)	38.7 (*d*)	2.98 (*d*, *J* = 13.6)	38.7 (*d*)
CH_2(19)	1.60–1.66 (*m*)	36.4 (*t*)	1.58–1.62 (*m*)	36.0 (*t*)
C(20)		28.8 (*s*)		28.8 (*s*)
CH_2(21)	1.30–1.34 (*m*)	33.1 (*t*)	1.27–1.33 (*m*)	33.2 (*t*)
CH_2(22)	1.70–1.80 (*m*)	36.5 (*t*)	1.70–1.80 (*m*)	36.5 (*t*)
Me(23)	0.91 (*d*, *J* = 6.6)	7.2 (*q*)	0.90 (*d*, *J* = 6.6)	7.2 (*q*)
Me(24)	0.61 (*s*)	14.7 (*q*)	0.60 (*s*)	14.6 (*q*)
Me(25)	0.80 (*s*)	17.5 (*q*)	0.78 (*s*)	17.5 (*q*)
Me(26)	1.18 (*s*)	21.8 (*q*)	1.15 (*s*)	21.8 (*q*)
CH_2(27)	4.90 (*d*, *J* = 12.3), 5.08 (*d*, *J* = 12.3)	25.5 (*t*)	4.90 (*d*, *J* = 12.5), 5.08 (*d*, *J* = 12.5)	25.5 (*t*)
C(28)		181.2 (*s*)		181.2 (*s*)
Me(29)	1.38 (*s*)	30.0 (*q*)	1.36 (*s*)	30.0 (*q*)
Me(30)	1.02 (*s*)	34.9 (*q*)	1.00 (*s*)	34.9 (*q*)
(*E*)-*p*-Coumaroyl:				
C(1′)		126.1 (*s*)		126.6 (*s*)
H–C(2′,6′)	7.71 (*d*, *J* = 8.7)	130.8 (*d*)	8.15 (*d*, *J* = 8.6)	133.7 (*d*)
H–C(3′,5′)	7.20 (*d*, *J* = 8.7)	116.9 (*d*)	7.20 (*d*, *J* = 8.6)	116.0 (*d*)
C(4′)		161.6 (*s*)		160.7 (*s*)
H–C(7′)	8.10 (*d*, *J* = 15.8)	145.3 (*d*)	7.08 (*d*, *J* = 12.8)	144.1 (*d*)
H–C(8′)	6.85 (*d*, *J* = 15.8)	115.6 (*d*)	6.18 (*d*, *J* = 12.8)	116.5 (*d*)
C(9′)		167.8 (*s*)		167.2 (*s*)

C-atoms. In the ^{1}H-NMR spectra of **6** (*Table 3*), the presence of a (*Z*)-coumaroyl group was revealed supported by the ^{13}C-NMR signals (*Table 3*) [9] and characteristic peaks at *m*/*z* 164.0473 ($C_9H_8O_3$), 147.0446 ($C_9H_7O_2$), and 119.0497 (C_8H_7O) in the HR-EI-MS of **6**. Analysis of ^{1}H, ^{13}C, DEPT, and EI-MS data indicated that **6** possesses also a triterpene skeleton and a (*Z*)-*p*-coumaroyl group. Since the ^{13}C-NMR spectral data (*Table 3*) of the triterpene skeleton in **6** are resembled closely to those of **5**, it can be

assumed that compound **6** is an isomer of **5** with a (*Z*)-*p*-coumaroyloxy group at C(27). As a result, the structure of **6** was elucidated as 27-[(*Z*)-coumaroyloxy]friedelin-28-carboxylic acid.

Compound **7** was obtained as a white amorphous powder with the molecular formula of $C_{26}H_{24}O_5$, determined by HR-EI-MS. Its NMR data were identical to those of **7a** previously identified by *Nigam et al.* under the name apetalolide [10]. *Palmer et al.* reported the total synthesis of **7a** in 1995; however, ^{1}H-NMR data of the synthetic and the natural product were different, and the structure **7** was proposed for apetalolide [8]. To confirm the structure of apetalolide, a series of 2D-NMR analyses were performed. In the ROESY spectrum, two NOE correlation were observed between the MeO signal (Me(22)) and that of H–C(6), and also between the MeO signal and the H-atom signal in the benzene ring at C(4), which excluded the possibility of the structure **7a**. Furthermore, the following long-range ^{13}C,^{1}H correlation signals were observed in the HMBC spectrum: C(4)/H–C(17,21), C(4a)/H–C(3), C(5)/H–C(6) and Me(22)O, C(6a)/H–C(7), C(6b)/H–C(6), C(7)/Me(23,24), C(8)/H–C(6), C(11)/H–C(13) and Me(15), C(12)/Me(14), C(13)/Me(15), and C(16)/H–C(3,18,20). Thus, the structure of apetalolide had to be revised to 5-methoxy-10-[(*E*)-2-methylcrotonoyl]-8,8-dimethyl-4-phenyl-8*H*-pyrano[3,2-*g*]chromen-2-one (**7**).

Besides compounds **1**–**7**, inophyllum A [4], 12-methoxyinophyllum D [11], inophyllum C [4], inophyllum D (**8**) [4], inophyllum E [4], calophyllolide [5], calophyllic acid [5], and astragalin [12] were also isolated and characterized by comparison of their spectroscopic data with those in the literature.

Experimental Part

General. HPTLC Plates (*Yantai Chemical Industrial Institute*, Yantai, P. R. China). Column chromatography (CC): silica gel H_{60} (SiO_2; *Qingdao Haiyang Chemical Group Corporation*, Qingdao, P. R. China), *MCI-gel CHP-20P* (*Mitsubishi Chemical*, Japan), and *Sephadex LH-20* (*Pharmacia Biotech AB*, S-Uppsala) as packing materials. Prep. HPLC: *Varian SD-1* instrument, equipped with a *Merck NW25* C_{18} column (10 m, 20 mm × 250 mm), and *ProStar 320* UV/VIS detector. Optical rotations: *Horiba Sepa-300* polarimeter. UV Spectra: *Beckman DU-7* spectrometer; λ_{max} in nm. IR Spectra: *Perkin-Elmer 577* spectrometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker AM-300*, *AM-400*, and *AM-600* spectrometers in $CDCl_3$ or C_5D_5N; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. EI- and HR-EI-MS: *Finnigan MAT 95* instrument; in *m*/*z* (rel. %). LC/MS: *Bruker esquir3000plus* instrument equipped with an *Varian Dynmax* C_{18} column (5 μm, 4.6 mm i.d. × 25 cm), and ionization mode, ESI; column flow, 0.5 ml/min; eluting program, solvent *A*/solvent *B* 100% : 0 → 0 : 100%; solvent *A:* H_2O + 0.05% HCOOH, solvent *B:* MeCN + 0.05% HCOOH; stop time, 15 min.

Plant Material. The leaves of *C. inophyllum* were collected in Hainan Island, P. R. China, in June 2003, and identified by Prof. *Ding Fang* of Guangxi Institute of Chinese Traditional Medicine. A voucher specimen (No. SIMM20001201) was deposited with the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and Isolation. Powdered air-dried leaves of *C. inophyllum* (1.2 kg) were extracted three times (3 × 3 l) with MeOH/$CHCl_3$ 1:1 at 70°, and then concentrated *in vacuo* to give a crude extract (28.5 g). The extract was subjected to an *MCI gel* column (7 × 15 cm) and eluted with a mixture of 10, 30, 50, 75, 90, and 100% MeOH and H_2O (each 500 ml) successively to give *Fractions A* (4.75 g), *B* (3.80 g), *C* (2.05 g), *D* (1.25 g), *E* (2.10 g), *F* (2.35 g), and *G* (10.3 g). *Fr. F* (2.35 g) was subjected to CC (SiO_2; petroleum ether (PE)/AcOEt gradient 15 : 1 → 4 : 1) to give **1** (84.0 mg), **2** (72.3 mg), **3** (11.0 mg), and inophyllum A (1.05 g). *Fr. E* (2.10 g) was subjected to CC (SiO_2; PE/AcOEt gradient 8 : 1 → 2 : 1) to give inophyllum A (350.5 mg), 12-methoxyinophyllum D (145.0 mg), and **8** (12.8 mg). *Fr. D* (1.25 g) was

subjected to CC (SiO_2; PE/AcOEt gradient $10:1 \rightarrow 3:1$) to give inophyllum A (250.6 mg), calophyllolide (564.0 mg), inophyllum C (80.3 mg), **8** (123.0 mg), and *Fr. D1* (13.3 mg). *Fr. D1* was subjected to HPTLC (hexane/AcOEt 3:1) to give **4** (7.2 mg) and **5** (1.2 mg). *Fr. C* (1.05 g) was subjected to prep. HPLC (MeOH/H_2O gradient $3:1 \rightarrow 20:1$) to give isocalophyllic acid (132.5 mg), calophyllic acid (120.8 mg), inophyllum E (110.6 mg), and *Fr. C1* (80.3 mg). *Fr. C1* was subjected to prep. HPLC (MeCN/H_2O 19:1) to give **6** (32.1 mg) and **7** (34.8 mg). *Fr. B* (3.80 g) was passed through a *Sephadex LH-20* column with 95% EtOH to give astragalin (3.06 g).

12-O-Butylinophyllum D (=*(10*R*,11*S*,12*R*)-12-Butoxy-11,12-dihydro-6,6,10,11-tetramethyl-4-phenyl-2*H*,6*H*,10*H*-dipyrano[2,3*-f*:2′,3′*-h*]chromen-2-one*; **1**). Yellow amorphous powder. $[\alpha]_D^{20} = +23.0$ ($c = 0.07$, $CHCl_3$). UV (MeOH): 236, 278, 286, 332 nm. IR (KBr): 3438, 2931, 1739 (C=O), 1637, 1587, 1463, 1373, 1315, 1181, 1128,1085, 865, 748, 702. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. EI-MS: 460 (24, M^+), 445 (100), 417 (4), 387 (57, $[M - C_4H_9OH]^+$), 371 (25), 333 (20), 149 (25). HR-EI-MS: 460.2254 (M^+, $C_{29}H_{32}O_5^+$; calc. 460.2250).

12-O-Ethylinophyllum D (=*(10*R*,11*S*,12*R*)-12-Ethoxy-11,12-dihydro-6,6,10,11-tetramethyl-4-phenyl-2*H*,6*H*,10*H*-dipyrano[2,3*-f*:2′,3′*-h*]chromen-2-one*; **2**). Yellow amorphous powder. $[\alpha]_D^{20} = -4.0$ ($c = 0.075$, $CHCl_3$). UV (MeOH): 236, 279, 285, 335. IR (KBr): 3435, 2971, 1736 (C=O), 1637, 1587, 1444, 1373, 1315, 1181, 1141, 1128, 1078, 1012, 858, 748, 702. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. EI-MS: 432 (20, M^+), 417 (100), 403 (65, $[M - C_2H_5]^+$), 387 (21, $[M - C_2H_5OH]^+$), 371 (25), 333 (16), 178 (6). HR-EI-MS: 432.1944 (M^+, $C_{27}H_{28}O_5^+$; calc. 432.1937).

Inophyllum H (=*(2*R**,3*R**,4*R**)-3,4,10,10a-Tetrahydro-4-hydroxy-2,3,10,10-tetramethyl-8-phenyl-2*H*,6*H*,9a*H*-cyclopropa[4,5]furo[2,3-f]pyrano[2,3-h]chromen-6-one*; **3**). White amorphous powder. $[\alpha]_D^{20} = +136.6$ ($c = 0.20$, $CHCl_3$). UV ($CHCl_3$): 226, 256, 337. IR (KBr): 3426 (OH), 2971, 1708, 1604, 1568, 1421, 1384, 1324, 1228, 1139, 1099, 1031, 748, 700. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. EI-MS: 404 (15, M^+), 389 (100), 371 (65), 334 (44), 317 (19), 178 (8), 164 (4), 84 (12). HR-EI-MS: 404.1630 (M^+, $C_{25}H_{24}O_5^+$; calc. 404.1624).

Inophyllum I (=*10,10a-Dihydro-2,3,10,10-tetramethyl-8-phenyl-2*H*,6*H*,9a*H*-cyclopropa[4,5]furo[2,3-f]pyrano[2,3-h]chromen-6-one*; **4**). White amorphous powder. $[\alpha]_D^{20} = -4.0$ ($c = 0.005$, $CHCl_3$). UV (MeOH): 228, 255, 338. IR (KBr): 3404 (OH), 2924, 1701, 1631, 1572, 1458, 1419, 1363, 1198, 1074, 1020, 903, 764, 698. EI-MS: 386 (21, M^+), 371 (100), 333 (41), 317 (3), 178 (5), 149 (6). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-EI-MS: 386.1502 (M^+, $C_{25}H_{22}O_4^+$; calc. 386.1518).

*27-[(*E*)-Coumaroyloxy]friedelin-28-carboxylic Acid* (=*(4a*S*,6a*R*,6b*S*,8a*S*,9*R*,12a*S*,12b*S*,14a*R*,14b*S*)-14a-({[(2*E*)-3-(4-Hydroxyphenyl)prop-2-enoyl]oxy}methyl)-2,2,6a,8a,9,12b-hexamethyl-10-oxo-icosahydropicene-4a(2*H*)-carboxylic Acid*; **5**). White amorphous powder. $[\alpha]_D^{20} = +49.4$ ($c = 0.37$, $CHCl_3$). UV (MeOH): 315, 231, 210. IR (KBr): 3415, 2948, 1701, 1604, 1514, 1451, 1392, 1267, 1169, 831, 733. ^{1}H- and ^{13}C-NMR: *Tables 3*. ESI-MS: 619 ($[M + H]^+$). EI-MS: 618 (8, M^+), 455 (4), 454 (10), 441 (12), 409 (6), 395 (45), 164 (40), 163 (9), 147 (100), 119 (31). HR-EI-MS: 618.3925 (M^+, $C_{39}H_{54}O_6^+$; calc. 618.3920).

27-[(Z)-Coumaroyloxy]friedelin-28-carboxylic Acid (=*(4a*S*,6a*R*,6b*S*,8a*S*,9*R*,12a*S*,12b*S*,14a*R*,14b*S*)-14a-({[(2*Z*)-3-(4-Hydroxyphenyl)prop-2-enoyl]oxy}methyl)-2,2,6a,8a,9,12b-hexamethyl-10-oxo-icosahydropicene-4a(2*H*)-carboxylic Acid*; **6**). White amorphous powder. $[\alpha]_D^{20} = -17.0$ ($c = 0.05$, $CHCl_3$). UV (MeOH): 310, 228, 212. IR (KBr): 3392, 2949, 2870, 1701, 1604, 1514, 1450, 1390, 1168, 983, 841, 733. ESI-MS: 619 ($[M + H]^+$). ^{1}H- and ^{13}C-NMR: *Tables 3*. EI-MS: 618 (14, M^+), 454 (9), 441 (20), 409 (17), 396 (15), 395 (48), 189 (17), 177 (25), 164 (12), 163 (17), 147 (100), 119 (19). HR-EI-MS: 618.3928 (M^+, $C_{39}H_{54}O_6^+$; calc. 618.3920).

Apetalolide (=*5-Methoxy-8,8-dimethyl-10-[(2*E*)-2-methylbut-2-enoyl]-4-phenyl-2*H*,8*H*-pyrano[3,2-g]chromen-2-one*; **7**). White amorphous powder. UV (MeOH): 352, 272, 237. IR (KBr): 3435, 2927, 2852, 1737, 1641, 1577, 1463, 1344, 1135, 1016, 864, 749, 700. EI-MS: 416 (19, M^+), 402 (24), 401 (100), 389 (49), 371 (74), 333 (27), 317 (5), 178 (4), 77 (5). HR-EI-MS: 416.1628 (M^+, $C_{26}H_{24}O_5^+$; calc. 416.1623).

*Preparation of the (*R*)- and (*S*)-MTPA Esters of Inophyllum D* (**8**). A soln. of (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid ((*R*)-MTPA) chloride (4.0 mg in 80 µl of benzene) was added to a soln. of **8** (4 mg) in dry benzene (3 ml). A 0.05-mg aliquot of 4-(dimethylamino)pyridine (DMAP) and Et_3N (15 µl) were added, and the mixture was refluxed. After 3 h, a second portion of (*R*)-MTPA chloride (4.0 mg) was added, and the mixture was refluxed for an additional 2 h. When the mixture was

cooled, benzene (10 ml) was added, and the org. phase was succesively washed with 10% HCl (5 ml), 1N $NaHCO_3$ (10 ml), and H_2O (20 ml). The soln. was evaporated to dryness, and subjected to HPTLC (PE/AcOEt 4:1) to give (*R*)-MTPA ester of inophyllum D (2.2 mg). (*S*)-MTPA Ester of inophyllum D (2.3 mg) was produced in the same way.

(S)-MTPA Ester of Inophyllum D. ^{1}H-NMR (600 MHz, $CDCl_3$): 7.62 (*m*, 2 arom. H of MPTA); 7.40 (*m*, 3 arom. H of MPTA); 7.22–7.39 (*m*, H–C(14,15,16,17,18)); 6.47 (*d*, *J* = 9.8, H–C(8)); 5.97 (*s*, H–C(3)); 5.34 (*d*, *J* = 9.8, H–C(7)); 4.06 (*dq*, *J* = 2.1, 6.0, H–C(10)); 3.55 (*s*, MeO of MPTA); 2.21 (*ddq*, *J* = 2.1, 1.9, 6.5, H–C(11)); 6.14 (*d*, *J* = 1.9, H–C(12)); 0.93 (*s*, Me(19,20)); 1.33 (*d*, *J* = 6.0, Me(21)); 0.93 (*d*, *J* = 6.5, Me(22)). ESI-MS: 621.2 ($[M+H]^+$).

(R)-MTPA Ester of Inophyllum D. ^{1}H-NMR (600 MHz, $CDCl_3$): 7.63 (*m*, 2 arom. H of MPTA); 7.39 (*m*, 3 arom. H of MPTA); 7.20–7.36, (*m*, H–C(14,15,16,17,18)); 6.51 (*d*, *J* = 10.0, H–C(8)); 5.93 (*s*, H–C(3)); 5.35 (*d*, *J* = 9.8, H–C(7)); 4.46 (*dq*, *J* = 2.0, 6.6, H–C(10)); 3.55 (*s*, MeO of MPTA); 2.22 (*ddq*, *J* = 2.0, 1.9, 6.9, H–C(11)); 6.25 (*d*, *J* = 1.9, H–C(12)); 0.93 (*s*, Me(19,20)); 1.44 (*d*, *J* = 6.6, Me(21)); 0.96 (*d*, *J* = 6.9, Me(22)). ESI-MS: 621.2 ($[M+H]^+$).

Hydrolysis of **1**, **2**, *and* **3**. A soln. of **1** (5.0 mg) in THF/H_2O 2:1 (2 ml) was treated with 6N HCl (40 µl). The mixture was stirred at r.t. for 72 h, and then $CHCl_3$ (20 ml) was added. The org. phase was washed with 1N $NaHCO_3$ and evaporated to dryness to give **8** (2.5 mg), which was confirmed by co-TLC. $[\alpha]_D^{20} = +40.0$ (*c* = 0.10, $CHCl_3$; $[\alpha]_D^{20} = +35.0$ (*c* = 1.86, $CHCl_3$) [4]). The same procedure was repeated with **2** and **3** to give **8** with $[\alpha]_D^{20} = +29.0$ (*c* = 0.06, $CHCl_3$) and $[\alpha]_D^{20} = +33.0$ (*c* = 0.08, $CHCl_3$), resp.

This work was financially supported by the *National Science & Technology Major Project 'Key New Drug Creation and Manufacturing Program'*, P. R. China (No. 2009ZX09301-001). The authors also thank Prof. *Ding Fang* at the Guangxi Institute of Chinese Traditional Medicine for identification of the plant material.

REFERENCES

[1] Chinese Traditional Medicine Corporation, 'Resource of Chinese Traditional Medicine', Science Press, Beijing, 1994, p. 419.
[2] M. C. Yimdjo, A. G. Azebaze, A. E. Nkengfack, A. M. Meyer, B. Bodo, Z. T. Fomum, *Phytochemistry* **2004**, *65*, 2789.
[3] K. Kawazu, H. Ohigashi, T. Mitsui, *Tetrahedron Lett.* **1968**, *9*, 2383.
[4] K. Kawazu, H. Ohigashi, N. Takahashi, T. Mitsui, *Bull. Inst. Chem. Res.* **1972**, *50*, 160.
[5] A. D. Patil, A. J. Freyer, D. S. Eggleston, R. C. Haltiwanger, M. F. Bean, P. B. Taylor, M. J. Caranfa, A. L. Breen, H. R. Bartus, R. K. Johnson, R. P. Hertzberg, J. W. Westley, *J. Med. Chem.* **1993**, *36*, 4131.
[6] T. R. Govindachari, N. Viswanathan, B. R. Pai, U. Ramadas Rao, M. Srinivasan, *Tetrahedron* **1967**, *23*, 1901.
[7] Y. Kashman, K. R. Gustafson, R. W. Fuller, J. H. Cardellina II, J. B. McMahon, M. J. Currens, R. W. Buckheit Jr., S. H. Hughes, G. M. Cragg, M. R. Boyd, *J. Med. Chem.* **1992**, *35*, 2735.
[8] C. J. Palmer, J. L. Josephs, *J. Chem. Soc., Perkin Trans. 1* **1995**, 3135.
[9] C.-I. Chang, Y.-H. Kuo, *Chem. Pharm. Bull.* **1998**, *46*, 1627.
[10] S. K. Nigam, C. R. Mitra, G. Kunesch, B. C. Das, J. Polonsky, *Tetrahedron Lett.* **1967**, *18*, 2633.
[11] S. P. Joshi, V. B. Deodhar, U. D. Phalgune, *Indian J. Chem., Sect. B* **2000**, *39*, 560.
[12] K. R. Markham, B. Ternai, R. Stanley, H. Geiger, T. J. Mabry, *Tetrahedron* **1978**, *34*, 1389.

Received December 28, 2009