Siamenols A—D, Four New Coumarins from *Mammea siamensis*

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Further investigation of the dichloromethane extract of the twigs of *Mammea siamensis* led to the isolation of four novel coumarins, named siamenols A—D (1—4) together with three known xanthones. The structures of the new coumarins were elucidated by spectroscopic analysis, including 2D NMR spectroscopic data. In addition, the absolute stereochemistry of hydroxyl group of siamenol C (3) was determined to be *S* configuration by using modified Mosher's method.

Key words *Mammea siamensis*; Clusiaceae; coumarin; xanthone; alcohol absolute stereochemistry

Mammea siamensis (Clusiaceae) is a plant widely distributed throughout Thailand, Myanmar, Laos and Vietnam whose Thai name is 'sarapee'. Its flowers have been used as a heart tonic in Thai traditional medicine. Recently, our group reported the isolation and structure elucidation of mammea coumarins from the flowers and twigs of this plant.[1—3] As a continuation of our studies on this plant, four new coumarins, siamenols **1**—**4**, together with three xanthones were isolated from the dichloromethane extract. The structures of these known xanthones were determined by comparing their spectral data with those of the literature values of 1,7-dihydroxyxanthone (**5**),[4,5] 5-hydroxy-1-methoxyxanthone (**6**),[5] and 5-hydroxy-1,3-dimethoxyxanthone (**7**).[4,5]

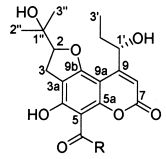
Results and Discussion

Siamenol A (**1**) was obtained as colorless oil whose molecular formula was $C_{21}H_{26}O_7$ as established by HR-FAB-MS showing $[M+H]^+$ at *m/z* 391.1756 compared with the calculated value of 391.1757. Its IR spectrum indicated the presence of a chelated hydroxyl group (3437 cm^{-1}), an α,β-unsaturated lactone (1724 cm^{-1}) and a chelated acyl group (1607 cm^{-1}). The UV spectrum of **1** showed maximum absorptions at 221 and 297 nm in EtOH and at 239 and 372 nm in 0.1 N KOH in EtOH suggesting that the structure of **1** is similar to those in a series of mammea B/B cyclo F.[6]

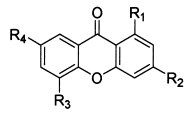
The ^{1}H-NMR spectrum (Table 1) showed a signal for an olefinic proton of C-8 at δ_H 6.42 as a singlet typically encountered in the coumarin structure, where the position at C-9 was substituted. This was in agreement with the 1-hydroxypropyl group showing the resonances at δ_H 5.16 (1H, dd, J=8.2, 2.0 Hz, H-1′), 1.46 (1H, m, H-2a′), 1.81 (1H, m, H-2b′) and 1.09 (3H, t, J=7.3 Hz, H-3′) and its position of attachment was further confirmed by the HMBC correlations (Fig. 1) between H-1′ and C-8 (δ_C 105.62), C-9a (δ_C 97.11) and between H-8 and C-1′ (δ_C 71.47) and C-9a. In addition, the low field ^{1}H-NMR peak at δ_H 14.12 indicated a strong intramolecular hydrogen bonded phenolic proton (4-OH) located at the peri position to a carbonyl group. Signals at δ_H 3.80 (1H, hept, J=6.7 Hz, H-2‴), 1.14 (3H, d, J=6.7 Hz, H-3‴) and 1.20 (3H, d, J=6.7 Hz, H-4‴) was assignable to be an isopropyl group. The remaining signals of two methyl groups on a carbinol carbon side chain at δ_H 1.25 (H-2″) and 1.51 (H-3″) and the ABX-type aliphatic protons at δ_H 3.13 (1H, dd, J=15.4, 9.9 Hz, H-3a), 3.27 (1H, dd, J=15.4, 8.7 Hz, H-3b) and 4.86 (1H, t, J=9.0 Hz, H-2) were attributed to the hydroxyisopropyldihydrofuran substituent which was further verified by the characteristic mass fragment at *m/z* 331 of the $[M-59]^+$. The position of the hydroxyisopropyldihydrofuran moiety was supported by HMBC correlations of C-3a (δ_C 110.68) to both H-3 and 4-OH. Furthermore, the bathochromic shift in alkaline solution to the long wavelength bands in UV spectrum confirmed the position of the acyl group at C-8.[6] From these results, the structure of siamenol A (**1**) was deduced to be 9-(1-hydroxypropyl)-4-hydroxy-5-(2-methyl-1-oxopropyl)-2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuro[2,3-f]chromene-7-one.

Siamenol B (**2**) was found to be an isomer of **1** by HR-FAB-MS. This compound exhibited its IR, UV and NMR spectra similar to those of **1**. The ^{1}H- and ^{13}C-NMR data (Tables 1, 2) of **2** indicated that this compound differed from **1** in the pattern of its ketonic side chain. A butyryl side chain was characterized in **2** from the signals at δ_H 2.93 (1H, dt, J=18.0, 7.5 Hz, H-2a‴), 3.02 (1H, dt, J=18.0, 7.5 Hz, H-2b‴), 1.68 (2H, m, H-3‴) and 1.00 (3H, t, J=7.4 Hz, H-4‴) and carbon signals at δ_C 46.30 (C-2‴), 17.59 (C-3‴), 13.53 (C-4‴) and 205.57 (C-1‴) ppm. Compound **2**, named siamenol B, was proposed to be 9-(1-hydroxypropyl)-4-hydroxy-5-(1-oxobutyl)-2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuro[2,3-f]chromene-7-one.

The molecular formula of siamenols C and D (**3**, **4**) was assigned to be $C_{22}H_{28}O_7$ on the basis of HR-FAB-MS, which indicated the presence of an additional methylene group with respect to **1**. Their NMR data also differed only in the signals of its acyl substitutents. A 3-methyl-1-oxobutyl chain in **3** and 2-methyl-1-oxobutyl chain in **4** could be characterized from their corresponding ^{1}H-, ^{13}C-NMR data (see Tables 1, 2). Therefore, the structures of siamenols C (**3**) and D (**4**) were proposed to be 9-(1-hydroxypropyl)-4-hydroxy-5-(3-methyl-1-oxobutyl)-2-(1-hydroxy-1-methylethyl)-2,3-di-



1: R=CH(CH$_3$)$_2$
2: R=CH$_2$CH$_2$CH$_3$
3: R=CH$_2$CH(CH$_3$)$_2$
4: R=CH(CH$_3$)CH$_2$CH$_3$



5: R$_1$=OH, R$_2$=H, R$_3$=H, R$_4$=OH
6: R$_1$=OCH$_3$, R$_2$=H, R$_3$=OH, R$_4$=H
7: R$_1$=OCH$_3$, R$_2$=OCH$_3$, R$_3$=OH, R$_4$=H

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Table 1. ^{1}H-NMR Data for Compounds **1**—**4** (400 MHz, $CDCl_3$, *J* in Hz)

Position	**1**	**2**	**3**	**4**
H-2	4.86 t (9.0)	4.87 t (9.0)	4.80 t (9.3)	4.85 t (9.5)
H-3	3.13 dd (15.4, 9.9)	3.12 dd (15.4, 9.9)	3.04 dd (15.4, 9.9)	3.09 dd (15.4, 9.9)
	3.27 dd (15.4, 8.7)	3.29 dd (15.4, 8.6)	3.26 dd (15.4, 8.8)	3.30 dd (15.4, 9.1)
H-8	6.42 s	6.37 s	6.27 s	6.40 s
4-OH	14.12 s	14.08 s	13.96 s	14.08 s
H-1′	5.16 dd (8.2, 2.0)	5.14 br dd (8.1, 1.5)	5.04 dd (8.1, 2.6)	5.15 dd (8, 1.9)
H-2′	1.46 m	1.39 m	1.27 m	1.42 m
	1.81 m	1.74 m	1.62 m	1.78 m
H-3′	1.09 t (7.3)	1.08 t (7.3)	1.01 t (7.2)	1.08 t (7.2)
H-2″	1.25 s	1.28 s	1.22 s	1.27 s
H-3″	1.51 s	1.53 s	1.49 s	1.54 s
H-2‴	3.80 hept (6.7)	2.93 dt (18.0, 7.5)	2.74 dd (16.7, 6.8)	3.64 sext (6.5)
		3.02 dt (18.0, 7.5)	2.79 dd (16.7, 6.8)	
H-3‴	1.14 d (6.7)	1.68 m	2.04 m	1.27 m
				1.67 m
H-4‴	1.20 d (6.7)	1.00 t (7.4)	0.84 d (6.6)	0.87 t (7.4)
H-5‴	—	—	0.92 d (6.7)	1.17 d (6.5)

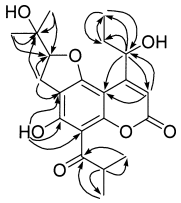


Fig. 1. Significant Correlations Observed in the HMBC Spectrum of **1**

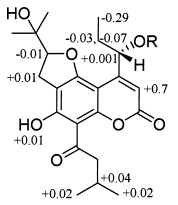
Table 2. ^{13}C-NMR Data for Compounds **1**—**4** (100 MHz, $CDCl_3$)[a]

Position	**1**	**2**	**3**	**4**
2	93.49 (d)	93.55 (d)	93.61 (d)	93.75 (d)
3	26.40 (t)	26.31 (t)	26.24 (t)	26.20 (t)
3a	110.68 (s)	110.62 (s)	110.66 (s)	110.88 (s)
4	163.46 (s)	163.10 (s)	162.99 (s)	163.33 (s)
5	103.99 (s)	104.76 (s)	104.80 (s)	104.18 (s)
5a	156.55 (s)	156.68 (s)	156.32 (s)	156.23 (s)
7	160.46 (s)	160.78 (s)	160.98 (s)	160.69 (s)
8	105.62 (d)	105.27 (d)	104.95 (d)	105.24 (d)
9	160.46 (s)	161.05 (s)	161.74 (s)	160.96 (s)
9a	97.11 (s)	97.04 (s)	96.93 (s)	96.94 (s)
9b	161.05 (s)	161.16 (s)	160.98 (s)	161.15 (s)
1′	71.47 (d)	71.22 (d)	70.95 (d)	71.17 (d)
2′	30.52 (t)	30.59 (t)	30.68 (t)	30.57 (t)
3′	10.38 (q)	10.45 (q)	10.53 (q)	10.36 (q)
1″	71.10 (s)	71.13 (s)	71.06 (s)	70.79 (s)
2″	24.69 (q)	24.55 (q)	24.45 (q)	24.70 (q)
3″	26.96 (q)	27.29 (q)	27.53 (q)	27.27 (q)
1‴	210.27 (s)	205.57 (s)	205.13 (s)	209.69 (s)
2‴	40.31 (d)	46.30 (t)	52.88 (t)	46.41 (d)
3‴	19.68 (q)	17.59 (t)	24.95 (d)	27.36 (t)
4‴	18.45 (q)	13.53 (q)	22.50[b] (q)	11.13 (q)
5‴	—	—	22.56[b] (q)	14.92 (q)

a) Multiplicities were deduced from DEPT experiments. *b*) Assignments were not absolute and the values may interchange.

hydrofuro[2,3-f]chromene-7-one and 9-(1-hydroxypropyl)-4-hydroxy-5-(2-methyl-1-oxobutyl)-2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuro[2,3-f]chromene-7-one, respectively.

The configuration of secondary alcohol at C-1′ position was determined using modified Mosher's method.[7] Due to the small quantities of all isolated siamenols A—D (**1**—**4**), only siamenol C (**3**) was studied for its absolute stereochemistry by converting it to the corresponding methoxyphenylacetate ester. Siamenol C (**3**) was esterified with (*R*)-(−)- and (*S*)-(+)-α-methoxyphenylacetic acid (MPA), DCC and DMAP in CH_2Cl_2 to give 1′-*O*-ester of (*R*)-(−)-MPA ester **3a** and 1′-*O*-ester of (*S*)-(+)-MPA ester **3b**. Analysis of the chemical shift differences between *R*- and *S*-MPA, (δ_R–δ_S) is shown in Fig. 2 indicating that the absolute stereochemistry at C-1′ of siamenol C was *S*-configuration. For siamenols A, B and D, the absolute stereochemistry at C-1′ was also inferred to have an *S*-configuration, since they exhibited the same sign of optical rotation as **3**. The *S*-configuration at C-1′ position for siamenols A—D is identical to those of therapins A—D, isolated from *Kayea assamica*,[8] whose structures possess the 1(*S*)-hydroxypropyl substituted coumarin skeleton.



3a: R = (*R*)-(-)-MPA
3b: R = (*S*)-(+)-MPA

Fig. 2. $\Delta\delta$ (δ_R−δ_S) Values Obtained from the (*R*)- and (*S*)-MPA Esters of **3** in ppm

Experimental

General Procedures Optical rotations were measured in chloroform, using a digital polarimeter (JASCO, DIP-370). UV spectra were recorded on a Shimadzu UV-vis 2001s spectrophotometer. IR spectra were obtained using Perkin-Elmer System 2000 FTIR spectrophotometer. NMR spectra were recorded on Bruker AM-400 (400 MHz) instruments using $CDCl_3$ as solvent with TMS as internal standard. Mass spectra were determined using Finnigan Mat 90 and Finnigan Polaris instruments. High performance liquid chromatography (HPLC) was performed using a LUNA 10 μm C8 100A stainless steel column (250×21.20 mm, cat no. 00G-4093-P0). Column chromatography was carried out using silica gel 60 (0.063—0.200 mm) and silica gel 60 (particle size less than 0.063 mm). TLC and preparative TLC were carried out on Si gel 60 F254 plates (Merck).

Plant Material The twigs of *M. siamensis* were collected from the Botanical Garden of Saraburi province, Thailand, in November 1996. This plant was identified by Dr. Thawatchai Santisuk, the Forest Herbarium, Royal Forestry Department, Bangkok, Thailand.

Extraction and Isolation Air-dried twigs of *M. siamensis* (6.5 kg) were extracted successively with hexane and dichloromethane to afford 74 g of hexane extract and 32 g of dichloromethane extract after removal of solvent under vacuum. The dichloromethane fraction (32 g) of the plant extract was fractionated by column chromatography on silica gel, using hexane with increasing proportions of ethyl acetate and ethyl acetate with increasing proportions of methanol and finally with pure methanol to obtain 14 fractions. Fraction 5 was separated on preparative TLC, using 0.5% MeOH–CH_2Cl_2 as a mobile phase to afford 1,7-dihydroxyxanthone (**5**, 25.0 mg). Fraction 11 was submitted to further column chromatography on silica gel, using hexane with increasing proportions of acetone and acetone with increasing proportions of methanol and finally with pure methanol to give subfractions A—H. Subfraction D was further separated on preparative TLC with 30% acetone–hexane as a mobile phase to give 5-hydroxy-1-methoxyxanthone (**6**, 6.0 mg). Subfraction E was rechromatographed on silica gel with a CH_2Cl_2–MeOH gradient system, and then purified on preparative TLC with 0.5% MeOH–CH_2Cl_2 as a mobile phase to give 5-hydroxy-1,3-dimethoxyxanthone (**7**, 10.0 mg). Subfraction G was separated by reverse phase HPLC (LUNA C-8) with MeOH–H_2O (6 : 3.5) as eluent at a flow rate of 10 ml/min to give **1** (4.4 mg), **2** (8.3 mg), **3** (23.7 mg) and **4** (32.1 mg).

Siamenol A (**1**): Colorless oil. ^{1}H-, ^{13}C-NMR, see Tables 1 and 2. IR ($CHCl_3$) cm^{-1}: 3437, 2931, 1724, 1631, 1607, 1391, 1129. UV λ_{max} (EtOH) nm (log ε): 221 (4.39), 297 (4.43) nm. UV λ_{max} (0.1 N KOH in EtOH) nm (log ε): 239 (4.19), 372 (4.07). HR-FAB-MS *m/z* 391.1756 (Calcd for $C_{21}H_{27}O_7$ $[M+H]^+$, 391.1757). EI-MS *m/z* (rel. int): 390 (17), 364 (20), 345 (4), 332 (95), 331 (57), 329 (30), 319 (18), 303 (36), 271 (17), 257 (27). $[\alpha]_D^{29} = -53.33°$ (c=0.135, $CHCl_3$).

Siamenol B (**2**): Colorless oil. ^{1}H-, ^{13}C-NMR, see Tables 1 and 2. IR ($CHCl_3$) cm^{-1}: 3430, 2930, 1723, 1631, 1608, 1399, 1382, 1132. UV λ_{max} (EtOH) nm (log ε): 221 (4.14), 295 (4.19). UV λ_{max} (0.1 N KOH in EtOH) nm (log ε): 237 (4.03), 372 (3.93). HR-FA-BMS *m/z* 391.1755 (Calcd for $C_{21}H_{27}O_7$ $[M+H]^+$, 391.1757). EI-MS *m/z* (rel. int): 390 (14), 372 (11), 364 (17), 347 (11), 332 (100), 331 (88), 313 (18), 299 (19), 275 (17), 271 (25), 257 (16). $[\alpha]_D^{29} = -86.10°$ (c=0.295, $CHCl_3$).

Siamenol C (**3**): Colorless oil; ^{1}H-, ^{13}C-NMR, see Tables 1 and 2. IR ($CHCl_3$) cm^{-1}: 3440, 2965, 1719, 1640, 1605, 1378, 1130, 1050, 988, 953. UV λ_{max} (EtOH) nm (log ε): 221 (4.49), 296 (4.5). UV λ_{max} (0.1 N KOH in EtOH) nm (log ε): 239 (4.33), 372 (4.25). HR-FAB-MS *m/z* 405.1913 (Calcd for $C_{22}H_{29}O_7$ $[M+H]^+$, 405.1913). FAB-MS *m/z* (rel. int): 405 $(M+H)^+$ (100), 388 (8), 347 (10), 315 (10), 281 (5), 207 (6), 185 (11), 106 (19). $[\alpha]_D^{29} = -114.77°$ (c=0.474, $CHCl_3$).

Siamenol D (**4**): Colorless oil. ^{1}H-, ^{13}C-NMR, see Tables 1 and 2. IR ($CHCl_3$) cm^{-1}: 3441, 2978, 2947, 1718, 1641, 1605, 1381, 1129, 1049, 963, 854. UV λ_{max} (EtOH) nm (log ε): 222 (4.19), 297 (4.23); UV λ_{max} (0.1 N KOH in EtOH) nm (log ε): 239 (4.34), 374 (4.25). HR-FAB-MS *m/z* 405.1907 (Calcd for $C_{22}H_{29}O_7$ $[M+H]^+$, 405.1913). EI-MS *m/z* (rel. int): 405 $(M+H)^+$ (100), 388 (10), 347 (11), 185 (6), 106 (13). $[\alpha]_D^{29} = -103.74°$ (c=0.624, $CHCl_3$).

***(R)*-MPA Ester (3a)** Compound **3** (3.2 mg, 0.0079 mmol) was treated with (*R*)-(−)-α-methoxy phenylacetic acid (4 mg, 0.0241 mmol) and *N*-[3-(dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) (4.5 mg, 0.0234 mmol) in the presence of a catalytic amount of *N*,*N*-(dimethylamino)pyridine in CH_2Cl_2 at room temperature for 24 h. The reaction mixture was purified by TLC (hexane : EtOAc, 7 : 3) to give (*R*)-MPA ester **3a** (1.7 mg): ^{1}H-NMR ($CDCl_3$, 400 MHz); δ 0.61 (3H, t, J=7.3 Hz, H-3′), 0.95 (3H, d, J=6.6 Hz, H-4‴), 0.96 (3H, d, J=6.6 Hz, H-5‴), 1.19 (3H, s, H-2″), 1.35 (3H, s, H-3″), 1.55 (1H, m, H-2a′), 1.73 (1H, m, H-2b′), 2.19 (1H, m, H-3‴), 3.04 (2H, m, H-2‴), 3.09 (1H, dd, J=15.5, 9.6 Hz, H-3a), 3.14 (1H, dd, J=15.5, 8.1 Hz, H-3b), 4.80 (1H, t, J=8.8 Hz, H-2), 6.10 (1H, br d, J=0.7 Hz, H-8), 6.443 (1H, dd, J=8.5, 3.0 Hz, H-1′), 14.18 (s, 4-OH), the α-methoxyphenyl acetic acid part had δ 7.41 (2H, m, aromatic proton), 7.33 (3H, m, aromatic proton), 4.79 (1H, s), 3.35 (3H, s, OCH_3); HR-FAB-MS *m/z* 553.2435 (Calcd for $C_{22}H_{29}O_7$ $[M+H]^+$, 553.2438).

***(S)*-MPA Ester (3b)** Compound **3** (1.7 mg, 0.0042 mmol) was treated with (*S*)-(+)-α-MPA (2 mg, 0.0120 mmol) and EDC (2.5 mg, 0.0131 mmol) in the presence of a catalytic amount of *N*,*N*-(dimethylamino)pyridine in CH_2Cl_2 at room temperature for 24 h. The reaction mixture was purified by TLC (hexane : EtOAc, 7 : 3) to give (*S*)-MPA ester **3b** (1 mg): ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.90 (3H, t, J=7.3 Hz, H-3′), 0.93 (3H, d, J=6.6 Hz, H-4‴), 0.94 (3H, d, J=6.6 Hz, H-5‴), 1.19 (3H, s, H-2″), 1.33 (3H, s, H-3″), 1.58 (1H, m, H-2a′), 1.80 (1H, m, H-2b′), 2.15 (1H, m, H-3‴), 2.97 (1H, dd, J=15.3, 6.7 Hz, H-2a‴), 3.02 (1H, dd, J=15.3, 6.6 Hz, H-2b‴), 3.08 (1H, dd, J=15.3, 9.6 Hz, H-3a), 3.13 (1H, dd, J=15.3, 8.1 Hz, H-3b), 4.81 (1H, t, J=8.4 Hz, H-2), 5.40 (1H, s, H-8), 6.442 (1H, dd, J=8.5, 3.2 Hz, H-1′), 14.17 (s, 4-OH), the α-methoxyphenyl acetic acid part had δ 7.36 (5H, s, aromatic proton), 4.81 (1H, s), 3.35 (3H, s, OCH3); HR-FAB-MS *m/z* 553.2438 (Calcd for $C_{22}H_{29}O_7$ $[M+H]^+$, 553.2436).

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References

1) Prachyawarakorn V., Mahidol C., Ruchirawat S., *Pharm. Biol.*, **38**, 58—62 (2000).
2) Kaweetripob W., Mahidol C., Prawat H., Ruchirawat S., *Pharm. Biol.*, **38**, 55—57 (2000).
3) Mahidol C., Kaweetripob W., Prawat H., Ruchirawat S., *J. Nat. Prod.*, **65**, 757—760 (2002).
4) Bandaranayake W. M., Selliah S. S., Sultanbawa M. U. S., Games D. E., *Phytochemistry*, **14**, 265—269 (1975).
5) Bandaranayake W. M., Karunanayake S., Sotheeswaran S., Sultanbawa M. U. S., *Indian J. Chem.*, **19B**, 463—467 (1980).
6) Crombie L., Games D. E., Haskins N. J., Reed G. F., *J. Chem. Soc. Perkin I*, **1972**, 2241—2247 (1972).
7) Latypov S. K., Seco J. M., Quiñoá E., Riguera R., *J. Org. Chem.*, **61**, 8569—8577 (1996).
8) Lee K.-H., Chai H.-B., Tamez P. A., Pezzuto J. M., Cordell G. A., Win K. K., Tin-Wa M., *Phytochemistry*, **64**, 535—541 (2003).