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Pyranocoumarins from the twigs of *Mammea siamensis*

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Abstract

Four unusual pyranocoumarins (**1**–**4**) have been isolated from the dried twigs of *M. siamensis*. The structures were determined by spectroscopic data, especially 1D and 2D NMR experiments.

Keywords: *Mammea siamensis*; Clusiaceae; Pyranocoumarin

1. Introduction

Mammea siamensis T. Anders (Clusiaceae) (Thai name "Sarapee") is distributed throughout Thailand, Myanmar, Laos, and Vietnam. The flowers have been used in traditional medicine as a heart tonic and an extract of the flowers has been reported to be effective against oral *Streptococcus* spp. (Apirasiyakul et al., 1991). Previous phytochemical studies of the flowers of *M. siamensis* have led to the isolation of coumarins (Kaweetripob et al., 2000; Mahidol et al., 2002; Thebtaranonth et al., 1981), while the leaves and twigs yielded proanthocyanidin polymers (Balza et al., 1989) and xanthones (Poobrasert et al., 1998), respectively. We have previously reported the isolation and structure determination of seven *mammea* coumarins from the twigs of this species (Prachyawarakorn et al., 2000). In a continuing phytochemical study on this plant, the hexane extract of the twigs of the *M. siamensis* was further examined to afford minor components of four new coumarins (**1**–**4**).

2. Results and discussion

The dried twigs of *M. siamensis* (6.7 kg) were extracted with hexane, CH_2Cl_2, and MeOH, successively. The hexane extract was repeatedly subjected to silica gel chromatography, followed by HPLC, to afford compounds **1** (6 mg), **2** (12 mg), **3** (2 mg), and **4** (15 mg) (Fig. 1).

Compound **1** was obtained as colorless needles with mp 126–127.5 °C. The molecular formula, $C_{22}H_{26}O_5$, was confirmed by positive HRFABMS measurement (*m*/*z* 371.1861 $[M+H]^+$, calcd. 371.1860) and its IR spectrum showed absorption bands at 1732 cm^{-1} (δ-lactone) and 1633 cm^{-1} (chelated aryl keto group). The UV spectrum exhibited absorptions at 221, 293, and 327 nm, similar to those of a 5,7-dioxygenated coumarin (Crombie et al., 1987a).

The ^1H NMR spectrum of compound **1** (Table 1) showed a distinct peak for the chelated hydroxyl region at δ_H 14.57 (1H, s) and a broad singlet for the olefinic proton of H-3 in the coumarin structure at δ_H 5.93, indicating that C-3a (coumarin C-4) was substituted. The 2-methylpyran moiety in **1** was revealed from the ^1H-^1H-COSY correlations of the C-5 oxygenated methine proton at δ_H 4.36 (1H, m) with the C-4 methylene protons at δ_H 2.78 (1H, ddd, J = 16.8, 11.0, 1.7 Hz) and 2.90 (1H, ddd, J = 16.8, 3.0, 0.7 Hz) and the methyl proton doublet at δ_H 1.54 (3H, d, J = 6.2 Hz). The

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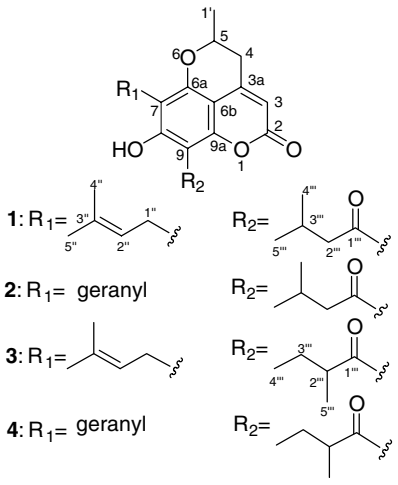

Fig. 1. Structures of mammea coumarins **1**–**4**.

attachment of the side chain at C-3a of the coumarin nucleus was evidenced from the HMBC correlations ($^{n}J_{HC} = 2.0$ Hz) from H-3 to C-4 (δ_C 35.0) and C-6b (δ_C 99.6); H-4 to C-3a (δ_C 149.0) and C-6b (δ_C 99.6); and H-5 to C-6a (δ_C 156.6) (Fig. 2). The ^{1}H NMR resonances at δ_H 3.34 (2H, d, J = 7.3 Hz), 5.20 (1H, m), 1.68 (3H, s, CH_3), and 1.79 (3H, s, CH_3) together with the ^{13}C signals at δ_C 17.8, 21.4, 25.8, 121.3, and 132.3 ppm indicated the presence of an isoprenyl group, which was also confirmed by the mass spectrum showing an important fragment at *m/z* 315 $[M - CH{=}C(Me)_2]^+$. The remaining signals were deduced to be a 3-methyl-1-oxobutyl group [δ_H 3.14 (2H, d, J = 6.7 Hz), 2.27 (1H, m, J = 6.7 Hz), and 1.04 (6H, d, J = 6.7 Hz)]. The HMBC spectrum of **1** assisted in placing the positions of isoprenyl and 3-methyl-1-oxobutyl moieties with the following key correlations: H-5 to C-6a; H-1″ to C-6a, C-7, and C-8; 8-OH proton to C-7, C-8, and C-9; and H-2‴ to C-1‴ and C-9 (Fig. 2). The proposed locations of 8-OH and 3-methyl-1-oxobutyl groups were further supported by the UV bathochromic shift in alkaline solution (Carpenter et al., 1971; Crombie et al., 1987b). On the basis of the above evidence, therefore, **1** was 8-hydroxy-5-methyl-7-(3-methyl-but-2-enyl)-9-(3-methyl-1-oxobutyl)-4,5-dihydropyrano[4,3,2-*de*]chromen-2-one.

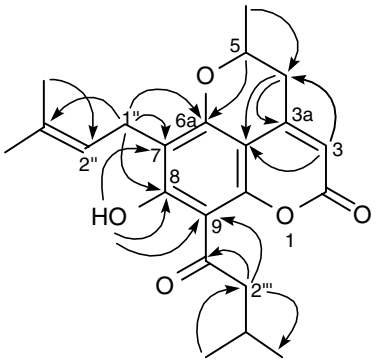

Fig. 2. Selected HMBC correlations for compound **1**.

Compound **2** was isolated as colorless needles with the molecular formula of $C_{27}H_{34}O_5$, which was determined by positive-ion HRFABMS. This compound showed IR, UV, and NMR spectra similar to those of **1**. An extensive NMR spectroscopic analysis (Tables 1 and 2) of **2** revealed that this coumarin exhibited substituents identical to those of **1**, except for the presence of a geranyl residue instead of an isoprenyl group. These data led us to assign the structure of **2** as 8-hydroxy-5-methyl-7-(3,7-dimethyl-octa-2,6-dienyl)-9-(3-methyl-1-oxobutyl)-4,5-dihydropyrano[4,3,2-*de*]chromen-2-one.

Compounds **3** and **4** were obtained as colorless needles. The molecular formulae of compounds **3** and **4** were each identified by their high resolution FABMS data to be $C_{22}H_{26}O_5$ and $C_{27}H_{34}O_5$, which were the same as that of compounds **1** and **2**, respectively. Comparison of the ^{1}H (Table 1) and ^{13}C (Table 2) NMR spectra of compounds **3** and **4** with **1** and **2** then suggested that they were closely related, differing only in the nature of their acyl substituents. A 2-methyl-1-oxobutyl side chain in **3** and **4** was

Table 1
^{1}H NMR (400 MHz) spectroscopic data of compounds **1**–**4** in $CDCl_3$ (ppm, J in Hz)

H	1	2	3	4
3	5.93, s	5.93, s	5.94, s	5.94, s
4	2.78, ddd (16.8, 11.0, 1.7)	2.77, ddd (16.8, 10.9, 1.7)	2.78, ddd (16.7, 11.0, 1.4)	2.78, ddd (16.7, 11.0, 1.0)
	2.90, ddd (16.8, 3.0, 0.7)	2.90, dd (16.8, 2.5)	2.91, ddd (16.7, 2.6, 0.6)	2.91, dd (16.7, 2.6)
5	4.36, m	4.36, m	4.37, m	4.36, m
8-OH	14.57, s	14.57, s	14.65/14.63, s	14.62/14.64, s
1′	1.54, d (6.2)	1.54, d (6.3)	1.55, d (6.2)	1.54, d (6.3)
1″	3.34, d (7.3)	3.34, d (7.2)	3.38, d (7.2)	3.35, d (7.1)
2″	5.20, m	5.20, m	5.21, m	5.20, t (6.8)
4″	1.68, s	1.96, m	1.68, s	1.95, m
5″	1.79, s	2.06, m	1.79, s	2.05, m
6″		5.06, m		5.06, t (6.8)
8″		1.57, s		1.57, s
9″		1.81, s		1.78, s
10″		1.64, s		1.63, s
2‴	3.14, d (6.7)	3.14, d (6.6)	3.92, sext (6.6),	3.93, sext (6.5)
3‴	2.27, m (6.7)	2.28, m (6.6)	1.46, m; 1.89, m	1.46, m; 1.90, m
4‴	1.04, d (6.7)	1.05, d (6.6)	0.99, t (7.6)/1.01, t (7.6)	0.99, t (7.5)/1.01, t (7.5)
5‴	1.04, d (6.7)	1.05, d (6.6)	1.24, d (6.6)/1.25, d (6.6)	1.24, d, (6.5)/1.25, d (6.5)

Table 2
^{13}C NMR (100 MHz) spectroscopic data of compounds **1**–**4** in $CDCl_3$ (ppm)

Carbon	1	2	3	4
2	159.6	159.6	159.7	159.7
3	105.8	105.8	105.8	105.8
3a	149.0	149.0	149.2	149.2
4	35.0	35.0	35.0	35.0
5	72.7	72.6	72.6	72.6
6a	156.6	156.7	156.6	156.6
6b	99.6	99.5	99.6	99.6
7	113.1	113.2	113.2	113.3
8	167.3	167.3	167.6	167.6/167.5
9	104.1	104.0	103.6	103.6/103.5
9a	154.5	154.5	154.3	154.3
1′	20.7	20.6	20.7	20.6
1″	21.4	21.3	21.4	21.3
2″	121.3	121.2	121.3	121.2
3″	132.3	135.7	132.3	135.7
4″	25.8	39.7	25.8	39.7
5″	17.8	26.6	17.8	26.6
6″		124.3		124.3
7″		131.3		131.3
8″		17.6		17.6
9″		16.1		16.1
10″		25.6		25.7
1‴	205.7	205.6	210.1	210.0
2‴	53.2	53.2	46.7/46.6	46.6/46.7
3‴	25.6	25.5	27.0/27.1	27.0/27.1
4‴	22.7	22.6	11.8/11.7	11.7/11.8
5‴	22.7	22.6	16.44	16.43

characterized from the ^{1}H and ^{13}C NMR spectroscopic data. However, the ^{1}H and ^{13}C NMR data of these two compounds showed two sets of signals, suggesting that they were isolated as an inseparable mixture of diastereomers in a ratio of approximately 1:1. The structures of 8-hydroxy-5-methyl-7-(3-methyl-but-2-enyl)-9-(2-methyl-1-oxobutyl)-4,5-dihydropyrano[4,3,2-*de*]chromen-2-one and 8-hydroxy-5-methyl-7-(3,7-dimethyl-octa-2,6-dienyl)-9-(2-methyl-1-oxobutyl)-4,5-dihydropyrano[4,3,2-*de*]chromen-2-one were proposed for compounds **3** and **4**, respectively.

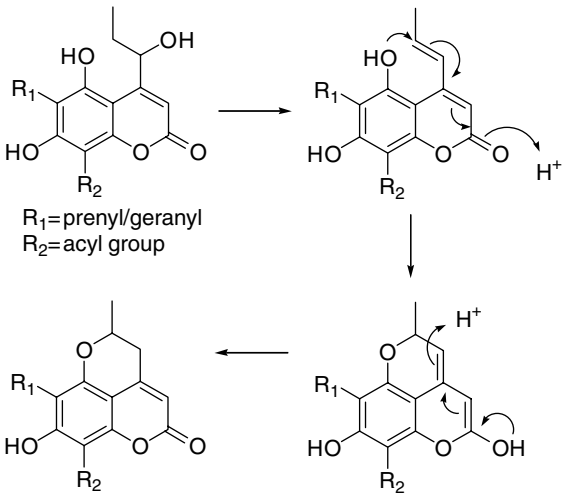

Fig. 3. Proposed biogenesis of pyranocoumarin derivatives.

The absolute stereochemistry of the methyls at C-5 and C-2‴ could not be established from the available data.

It is of interest to consider the biogenesis of pyranocoumarins **1**–**4** and our proposed biosynthetic route is illustrated in Fig. 3. Coumarins **1**–**4** could be derived from surangin C (Mahandru and Ravindran, 1986) and therapins A-C (Lee et al., 2003) both of which possess the 1-hydroxypropyl with either prenyl or geranyl group substituted at C-4 and C-6 of the coumarin skeleton, respectively. These compounds were first isolated from *M. longifolia* and *Kayea assamica*, respectively, which belong to the same family as *M. siamensis* from which coumarins **1**–**4** were isolated. A possible mechanism for the formation of the pyran nucleus could proceed via dehydration of a secondary alcohol to the corresponding conjugated double bond followed by cyclization through the intramolecular 1,6-addition reaction of phenolic group to furnish the pyranocoumarin skeleton (Fig. 3).

3. Concluding remarks

Plants in the genus *Mammea* are rich in secondary metabolites, e.g. coumarins with cytotoxic, antioxidant, and antifungal properties from *M. americana*, *M. harmandii*, and *M. longifolia* (Yang et al., 2005; Reutrakul et al., 2003; Deng and Nicholson, 2005); proanthocyanidins with antioxidant and radical scavenging activities from *M. longifolia* (Jagan Mohan Rao et al., 2004); flavonol monoglycosides from *M. longifolia* (Jagan Mohan Rao et al., 2002); and xanthones from *M. acuminata* (Tosa et al., 1997; Iinuma et al., 1996).

Several types of coumarins have been isolated from *Mammea* species; e.g. a series of mammeas A–C isolated from *M. americana* L. and *M. africana* (Crichton and Waterman, 1978; Crombie et al., 1967; Crombie et al., 1972). More recently, mammearin A, a furanocoumarin was isolated from *M. harmandii* (Reutrakul et al., 2003). A different type of pyranocoumarins has been found in many of plants of genus *Calophyllum* (McKee et al., 1998) which is in the same sub-family with genus *Mammea* (Guilet et al., 2001). These coumarins are derived from the cyclization of α,β-unsaturated acyl moiety with an *ortho*-phenol group. It is interesting to note that while coumarins containing α,β-unsaturated acyl moiety have been isolated from *Calophyllum* plants (McKee et al., 1998), at present in the genus *Mammea*, only coumarins containing the saturated acyl moiety are known. Pyranocoumarins **1**–**4** are the first representatives of this new type of pyranocoumarin in which the pyran ring is formed by linking of the oxygen of the phenol group with C-5 of the propyl side chain.

4. Experimental

4.1. General

Melting points were measured on a digital Electrothermal 9100 Melting Point Apparatus and reported without

correction. 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 2000 FT–IR spectrophotometer. NMR spectra were recorded on a Bruker AM-400 instrument (operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C) using $CDCl_3$ as solvent with TMS as internal standard. HMBC technique was performed with a Bruker AVANCE 600 NMR spectrometer. Mass spectra were determined using Finnigan MAT 90 and Finnigan Polaris instruments. High performance liquid chromatography (HPLC) was performed using an Exsil 100-10ODS HICHROM stainless steel column (250 × 21.20 mm, cat no. EXODS-10-250P).

4.2. Plant material

The twigs of *M. siamensis* were collected from the botany garden of Saraburi province, Thailand, in November 1996. This plant was identified by Dr. Thawatchai Santisuk, the Forest Herbarium, Royal Forestry Department, Bangkok, Thailand. A voucher specimen (NPCRI 286-39) has been deposited at the Chulabhorn Research Institute.

4.3. Extraction and isolation

Air-dried twigs of *M. siamensis* (6.5 kg) were ground and extracted three times with hexane (16 L × 7 days) at room temperature to afford a hexane extract (74 g) after removal of solvent in vacuo. The hexane fraction (72 g) was first subjected to silica gel cc, using hexane with increasing proportions of EtOAc, then EtOAc with increasing proportions of MeOH, and finally with MeOH to provide 14 fractions.

Fractions 10 and 11, which were eluted by 5–15% hexane/EtOAc, were combined and evaporated in vacuo to give yellow oil (10 g). Further separation by flash column chromatography over silica gel using a gradient mixture of hexane and EtOAc as eluents gave subfractions A–G. Subfraction E was further separated by preparative TLC using hexane:EtOAc (7:3) as eluent and subsequently purified by reversed phase HPLC [ODS 20 × 250 mm, flow rate of 10 mL min^{-1}, UV detector operating at 280 nm] using MeOH-H_2O (9:1) to give compounds **1** (6 mg), **2** (12 mg), **3** (2 mg), and **4** (15 mg).

Compound **1**; Colorless needles; mp 126–127.5 °C; $[\alpha]_D^{27}$ −1.3° (c 0.33, $CHCl_3$); IR ($CHCl_3$)ν_{max} cm^{-1}: 3026, 2969, 2929, 1732, 1633, 1600, 1403, 1293, 1235, 1192, 1120; UV (EtOH) λ_{max} nm (log ε): 221 (4.28), 293 (4.20), 327 (4.00), λ_{max} (0.1 N NaOH) 257 (4.86), 389 (4.67); EIMS *m/z* 370 ([M]$^+$, 47), 355 (19), 327 (58), 315 (94), 257 (83), 133 (20), 115 (20), 77 (26), 55 (27), 43 (100), 28 (74); HRFABMS *m/z* 371.1861 (M + H)$^+$, calcd. for $C_{22}H_{27}O_5$, 371.1860; for ^{1}H and ^{13}C NMR spectra, see Tables 1 and 2.

Compound **2**; Colorless needles; mp 85.5–86.5 °C; $[\alpha]_D^{27}$ −6.3° (c 0.17, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3031, 3028, 2928, 2856, 1733, 1633, 1600, 1456, 1404, 1383, 1294, 1121 cm^{-1}; UV (EtOH) λ_{max} nm (log ε): 220 (4.17), 293 (4.08), 321 (3.85), λ_{max} (0.1 N NaOH) 238 (4.82), 388 (4.77); EIMS *m/z* 438 ([M]$^+$, 19), 369 (20), 351 (19), 315 (80), 257 (24), 69 (55), 55 (22), 41 (100), 28 (76); HRFABMS *m/z* 439.24861 (M + H)$^+$, calcd. for $C_{27}H_{35}O_5$, 439.2485; for ^{1}H and ^{13}C NMR spectra, see Tables 1 and 2.

Compound **3**; Colorless needles; mp 143–146 °C; $[\alpha]_D^{23}$ −19.4° (c 0.33, $CHCl_3$); IR ($CHCl_3$)ν_{max} 3028, 2969, 2928, 2855, 1733, 1633, 1600, 1462, 1403, 1384, 1132 cm^{-1}; UV (EtOH) λ_{max} nm (log ε): 221 (3.54), 293 (3.47), 324 (3.23), λ_{max} (0.1 N NaOH) 237 (3.68), 389 (3.57); EIMS *m/z* 370 ([M]$^+$, 30), 327 (15), 315 (23), 313 (59), 257 (100), 133 (15), 55 (20), 41 (45); HRFABMS *m/z* 371.18603 [M + H]$^+$, calcd. for $C_{22}H_{27}O_5$, 371.1860; for ^{1}H and ^{13}C NMR spectra, see Tables 1 and 2.

Compound **4**; Colorless needles; mp 105.5–108 °C; $[\alpha]_D^{23}$ −17.7° (c 0.71, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3027, 2969, 2930, 1732, 1633, 1599, 1453, 1290, 1192, 1131 cm^{-1}; UV (EtOH) λ_{max} nm (log ε): 222 (4.37), 293 (4.33), 323 (4.10), λ_{max} (0.1 N NaOH) 238 (4.14), 388 (4.09); EIMS *m/z* 438 ([M]$^+$, 19), 381 (26), 369 (18), 351 (25), 315 (73), 257 (100), 69 (57), 41 (80); HRFABMS *m/z* 439.2487 [M + H]$^+$, calcd. for $C_{27}H_{35}O_5$, 439.2485; for ^{1}H and ^{13}C NMR spectra, see Tables 1 and 2.

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