Table 4 ^{13}C-NMR spectral data of compounds **4**-**5** and **6** (100 MHz, $CDCl_3$, solution, δ in ppm)†

Carbon	**4**	**5**	**6**
2	159.7(C)	159.5(C)	159.7(C)
3	107.0(CH)	107.0(CH)	107.0(CH)
4	161.0(C)	160.8(C)	161.1(C)
4a	101.2(C)	101.2(C)	101.1(C)
5	155.6(C)	155.5(C)	155.6(C)
6	106.1(C)	106.2(C)	106.1(C)
7	162.9(C)	163.1(C)	163.0(C)
8	104.5(C)	104.5(C)	104.5(C)
8a	157.1(C)	157.5(C)	156.9(C)
1-hydroxypropyl			
1′	71.7(CH)	71.8(CH)	71.7(CH)
2′	30.7(CH_2)	30.6, 30.7(CH)	30.7(CH_2)
3′	10.3(CH_3)	10.2, 10.3(CH_3)	10.3(CH_3)
Chromene moiety			
2″	80.1(C)	80.1(C)	80.1(C)
3″	126.4(CH)	126.4(CH)	126.4(CH)
4″	115.9(CH)	116.0(CH)	115.8(CH)
5″	28.2(CH_3)	28.2, 28.3(CH_3)	28.2(CH_3)
6″	28.2(CH_3)	28.0, 28.1(CH_3)	28.2(CH_3)
8-acyl moiety			
1‴	206.2(C)	210.7(C)	206.1(C)
2‴	46.7(CH_2)	46.9, 47.0(CH)	53.6(CH_2)
3‴	18.0(CH_2)	16.5(CH_3)	25.5(CH)
4‴	13.7(CH_3)	27.1, 27.11(CH_2)	22.5(CH_3)
5‴	-	11.68, 11.7(CH_3)	22.5(CH_3)

† Chemical shifts are relative to the TMS signal (δ 0.00 ppm). Multiplicities were determined by the DEPT pulse sequence.

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Output ที่ได้จากโครงการ

จากดอกของสารภีบ้านได้สารที่บริสุทธิ์ใหม่ 6 ตัว คาดว่าจะได้ตีพิมพ์ในวารสารวิชาการระดับนานาชาติ คือ Phytochemistry or Heterocycles

จากลำต้นของชะเอมเหนือ ได้สารที่บริสุทธิ์ใหม่ 2 ตัว และสารทั้ง 2 ตัวสามารถยับยั้งการเจริญเติบโตของเซลมะเร็งเม็ดเลือดขาวของหนู(P-388) ได้ ผลงานนี้ได้ตีพิมพ์แล้วในวารสารวิชาการระดับนานาชาติ คือ Chulabron Mahidol, Hunsa Prawat, Wirongrong Kaweetripob and Somsak Ruchirawat, Two new pyranoflavanones from the stems of *Derris reticulata*, *Heterocycles*, Vol. 57, No. 7, **2002**, pp. 1287-1291.

ผลงานวิจัยที่ได้สามารถนำไปใช้พัฒนาการเรียนการสอนและเป็นแบบอย่างสำหรับนักวิจัยใหม่ ๆ

ภาคผนวก

6- and 8-Acylcoumarins from the Flowers of *Mammea siamensis*

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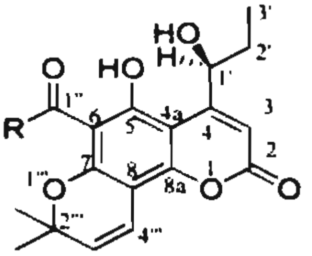
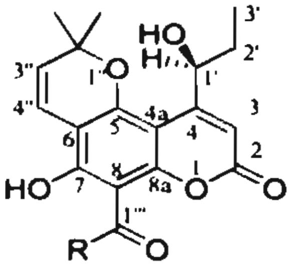
Abstract:- The hexane extract of the flower of *Mammea siamensis* afforded six news compounds, deacetylmammea E/AC cyclo D (**1**), deacetylmammea E/AA cyclo D (**2**), deacetylmammea E/AB cyclo D (**3**), deacetylmammea E/BC cyclo D (**4**), deacetylmammea E/BB cyclo D (**5**), deacetylmammea E/BA cyclo D (**6**). Their structures were elucidated by spectroscopic methods. The absolute stereochemistry of compounds **1**, **4**, **5**, **6** were established as [1'S] using the modified Mosher's method.

The genus Mammea is the same subfamily (Calophyllilide) as Calophyllum. Plants in subfamily are known to contain abundant amounts of coumarins[1-9] and xanthones[10,11]. Potent anti-HIV activity has recently been reported for such coumarins[12] and other bioactivities (e.g. antileukaemic[13] antimicrobial[14] have been reported for the xanthones. In the present work, six new mammea coumarins (**1-6**) were isolated and characterized from flowers of *Mammea siamensis* (Miq.) T. Anders. The structures of these new coumarins were determined using 1D, 2D NMR techniques (^{1}H, ^{13}C NMR, COSY, HMQC and HMBC).

Six compounds were isolated from fraction E-3 of hexane extract of the flowers of *Mammea siamensis* by successive silica gel column chromatography and preparative HPLC. Six new compounds, deacetylmammea E/AC cyclo D (**1**), deacetylmammea E/AA cyclo D (**2**), deacetylmammea E/AB cyclo D (**3**), deacetylmammea E/BC cyclo D (**4**), deacetylmammea E/BB cyclo D (**5**), and deacetylmammea E/BA cyclo D (**6**) were identified by means of spectroscopic studies.





1 R = $\underset{2''}{CH_2}\underset{3''}{CH_2}\underset{4''}{CH_3}$

2 R = $\underset{2''}{CH_2}\underset{3''}{CH}(\underset{4''}{CH_3})_{\underset{5''}{2}}$

3 R = $\underset{2''}{CH}(\underset{3''}{CH_3})\underset{4''}{CH_2}\underset{5''}{CH_3}$

4 R = $\underset{2'''}{CH_2}\underset{3'''}{CH_2}\underset{4'''}{CH_3}$

5 R = $\underset{2'''}{CH}(\underset{3'''}{CH_3})\underset{4'''}{CH_2}\underset{5'''}{CH_3}$

6 R = $\underset{2'''}{CH_2}\underset{3'''}{CH}(\underset{4'''}{CH_3})_{\underset{5'''}{2}}$

Coumarin **1** was isolated as a yellow gum which was shown to be optically active ($[\alpha]^{31}_D$ −44.2°, *c* 1.7, $CHCl_3$). The compound gave a parent ion by HRFABMS (positive ion) at *m/z* 373.1651 $[M+H]^+$, corresponding to a molecular formula of $C_{21}H_{24}O_6$. The EIMS showed the molecular ion at *m/z* 372 and fragment ions at *m/z* 357 ($[M-CH_3]^+$, 339, and 329. The UV spectrum (λ_{max} 202, 225, 286 nm) suggested the presence of 6-acylcoumarin.[2,15,16] Its IR spectrum of compound **1** shown absorption at 1729 cm^{-1} (pyrone carbonyl). The NMR spectrum revealed signals at δ 5.32 (1H,br d), 1.92 (1H, ddq, *J* = 14.0, 7.4, 3.3 Hz), 1.50 (1H, m), 1.09 (3H, t, *J* = 7.3 Hz) and 2.71 (1H, br s), which are due to the presence of an 1-hydroxypropyl group. The signal at δ 15.80 (1H, s) was ascribed to a phenolic group hydrogen bonded to an acyl group. Two singlets of three hydrogens each at δ 1.55 and 1.57 and the presence of two doublets of one hydrogen each at δ 5.61 (*J* = 10.1 Hz) and 6.84 (*J* = 10.1 Hz) established the presence of a 2,2-dimethyl-Δ^3-pyran ring. Substitution at C-4 of the coumarin nucleus was apparent from the C-3 proton broad singlet at δ 6.48 (1H). The nature of this substituent was deduced to be a butyryl chain from the triplet of two hydrogens at δ 3.10 (J = 7.4 Hz), a sextet of two protons at δ 1.76 (J = 7.4 Hz), and triplet of three protons at δ 1.03 (J = 7.3 Hz). The DEPT spectra (DEPT 90 and 135) of **1** gave rise to the existence of four methyl carbon atoms at δ 10.6 (C-3′), 13.8 (C-4″), 28.2 (C-5‴) and 28.3 C-6‴), three methylene carbon atoms at δ 30.1 (C-2′), 46.8 (C-2″) and 18.1 (C-3″), three olefinic methine carbon atoms at δ 107.6 (C-3), 126.5 (C-3‴) and 115.5 (C-4‴), one methine carbon atom at δ 72.6 (C-1′), and ten quaternary carbon atoms at δ 160.2 (C-2), 160.5 (C-4), 101.9 (C-4a), 163.9 (C-5), 106.9 (C-6), 157.7 (C-7), 101.9 (C-8), 155.0 (C-8a), 207.8 (C-1″) and 79.8 (C-2‴).

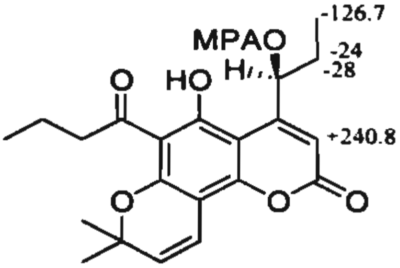
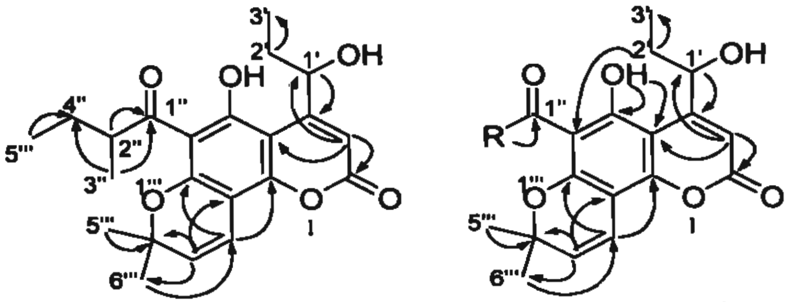


Figure 1 ^{1}H NMR Δδ (Δδ = δ_R-δ_S in Hz at 400 MHz) for (S)- and (R)-MPA esters of **1**

The position of the phenolic group at C-5 in **1** was established by the HMBC NMR spectral data (Figure 2) of the phenolic proton O*H*-5 to C-4a, C-5 and C-6, thereby locating the pyran ring of **1** between C-7 and C-8. Additionally, the proton signal of H-3 at δ 6.48 showed a cross peak with the carbon signals of C-2, C-4a and C-1′, and a cross peak of the H-1′ signal at δ 5.32 with the C-4 carbon signal was also observed. The stereochemistry at C-1′ of **1** was established by converting the secondary alcohol to the corresponding methoxyphenylacetate (MPA) ester as described by Latypov et al.[17] Acetylation of **1** with (R)-(-)- and (S)-(+)-methoxyphenyl acetic acid (MPAA) yielded the (R)-ester (**1R**) and (S)-ester (**1S**). The chemical shift differences (Δδ = δ_R-δ_S) of the individual protons of **1R** and **1S** are shown in Figure 1. From the systematic arangement of negative and positive values indicated that the absolute configuration of C-1′ is S. Based on the above evidence, therefore, compound **1** was characterized as 1′S-deacetylmammea E/AC cyclo D.



3

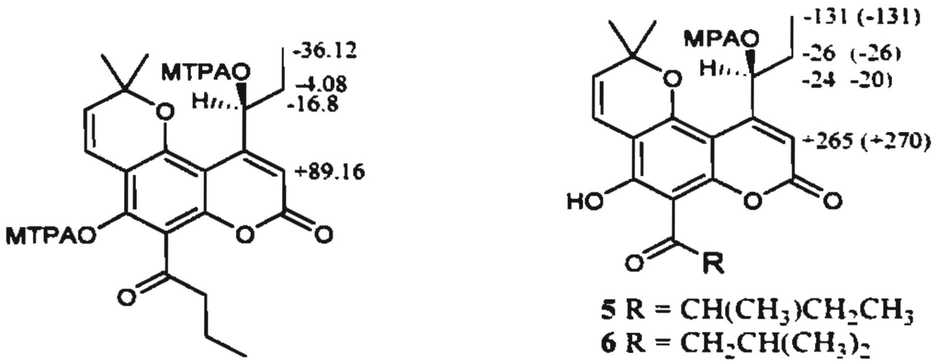
1 R = $CH_2CH_2CH_3$
2 R = $CH_2CH(CH_3)_2$

Figure 2. HMBC correlations for **1**, **2**, **3**

Coumarin **2** was isolated as a yellow gum which was shown to be optically active ($[\alpha]^{29}_{D}$ −34.8°, *c* 0.26, $CHCl_3$). Compound **2** has a molecular formula of $C_{22}H_{26}O_6$ determined from its positive-ion HRFABMS (*m/z* 387.1803, $[M+H]^+$). The UV (λ_{max} 203, 221, 285 nm), IR (ν_{max} 1728 cm^{-1}), and ^1H and ^{13}C NMR spectra of **2** were almost identical with those of compound **1**. However, coumarin **2** showed different ^1H and ^{13}C NMR spectral data from those of **1** only in the signals of the 6-acyl group. Compound **2**, which has a 3-methylbutyryl group, showed proton signals at δ 3.02 (2H, d, J = 6.9 Hz, H-2''), 2.28 (1H, heptet, J = 6.7 Hz, H-3'') and 1.01 (6H, d, J = 6.6 Hz, H-4'' and H-5'') and carbon signals at δ 207.5 (C-1''), 53.6 (C-2''), 25.1 (C-3''), and 22.7 (C-4'' and C-5''). The HMBC (Figure 2) and UV spectra supported the position of the acyl substituent in compound **2** at C-6.[2,15,16] The stereochemistry at C-1′ of **2** was tentatively assigned as S because the sign of rotation of **1** and **2** were the same. Based on the above evidence, therefore, compound **2** was characterized as 1′S-deacetylmammea E/AA cyclo D.

Coumarin **3** was isolated as a yellow gum which was shown to be optically active ($[\alpha]^{28}_{D}$ −30°, *c* 0.16, $CHCl_3$). Compound **3** showed different ^1H and ^{13}C NMR spectral data from those of **1** and **2** only in the signals of the 6-acyl group. Compound **3**, which has a 2-methylbutyryl group, showed proton signals at δ 3.76 (1H, sextet, J = 6.6 Hz, H-2''), 1.21 (3H, d, J = 6.6 Hz, H-3'') 1.78 and 1.46 (each 1H, m, H-4''), and 0.94 (3H, t, J = 7.3 Hz, H-5'') and carbon signals at δ 212.2 (C-1''), 46.7 (C-2''), 16.6, 16.7 (C-3''), 26.6, 26.7 (C-4''), and 11.8 (C-5''). The HMBC (Figure 2) supported the position of the 1-hydroxypropyl substituent in compound **3** at C-4. The stereochemistry at C-1′ of **3** was tentatively assigned as S because the sign of rotation of **1**, **2** and **3** were the same. Based on the above evidence, therefore, compound **3** was characterized as 1′S-deacetylmammea E/AB cyclo D.

Compound **4** was isolated as a yellow gum which was shown to be optically active ($[\alpha]^{28}_{D}$ −92.9°, *c* 4.28, $CHCl_3$). The IR spectrum of **4** showed band, which was ascribed to a *α,β*-unsaturated-*δ*-lactone (1729 cm^{-1}) group. The molecular formula **4** was determined to be $C_{21}H_{24}O_6$ by positive HRFABMS (calcd 373.1651 for $C_{21}H_{25}O_6$, found 373.1650) moreover, EIMS of **4** showed the fragmentation similar to those of compound **1**. Extensive NMR analysis of **4** showed that this coumarin had the same substituents as **1** since an 1-hydroxypropyl, a butyryl and 2,2-dimethyl Δ^3 pyran ring were revealed on its ^{1}H (Table 3) and ^{13}C NMR (Table 4) spectra. However, **1** and **4** exhibited quite different in their UV spectra (see experiment). Furthermore, the absolute stereochemistry of **4** was determined by the modified Mosther's method[18,19] The technique utilized anisotropic shifts induced ^{1}H NMR spectra of α-methoxy-α-(trifluoromethyl)phenylacetate (MTPA) esters of secondary alcohols to define the absolute stereochemistry. Both *R*- and *S*- MTPA esters of compound **4** were prepared and Δδ values (Figure 3) from their 400 MHz ^{1}H NMR spectra were calculated ($\Delta\delta = \delta_S - \delta_R$). By this method, the absolute configuration of C-1′ was determined to be 1′S in compound **4**. It was then deduced that **4** was a regioisomer of mammea **1**. The appearance of HMBC spectral data of **4** (Figure 4) revealed 3- and 2- bond correlation between the O*H*-7 proton with the C-6 (106.1) and C-7 (162.9). Based on the above evidence, therefore, compound **4** was characterized as 1′S-deacetylmammea E/AC cyclo D.

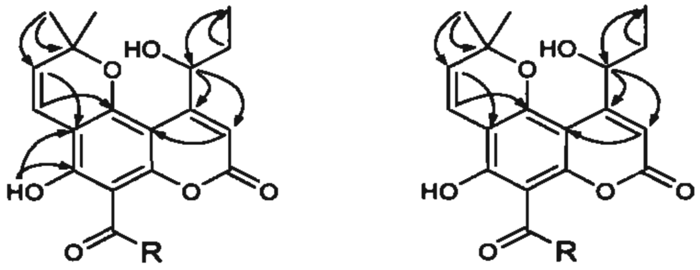


5 R = $CH(CH_3)CH_2CH_3$
6 R = $CH_2CH(CH_3)_2$

Figure 3 ^{1}H NMR Δδ (Δδ = δ_S-δ_R in Hz at 400 MHz) for (R)- and (S)-MTPA esters of **4** and ^{1}H NMR Δδ (Δδ = δ_R-δ_S in Hz at 400 MHz) for (R)- and (S)-MPA esters of 5 and 6 (in parenthesis)

Compound **5** was isolated as a yellow gum which was shown to be optically active ($[\alpha]^{29}_D$ −91.6°, *c* 0.50, $CHCl_3$). The IR spectrum of **5** showed band, which was ascribed to a *α,β*-unsaturated-*δ*-lactone (1729 cm^{-1}) group. The molecular formula **5** was determined to be $C_{22}H_{26}O_6$ by positive HRFABMS (calcd 387.1808 for $C_{22}H_{27}O_6$, found 387.1806). Extensive NMR analysis of **5** showed that this coumarin had the same substituents as **3** since an 1-hydroxypropyl, a 2-methylbutyryl and 2,2-dimethyl Δ^3 pyran ring were revealed on its ^{1}H (Table 3) and ^{13}C NMR (Table 4) spectra. However, 3 and **5** exhibited quite different in their UV spectra (see experiment). Both *R*- and *S*- MPA esters (**5R, 5S**) were prepared and Δδ values (Figure 3) from their 400 MHz ^{1}H NMR spectra were calculated (Δδ = δ_R-δ_S). The systematic arrangement of negative and positive values indicated that the absolute configuration of C-1′ was determined to be 1′S in compound **5**. The appearance of HMBC spectral data of **5** (Figure 4) revealed 3- and 2- bond correlation between the H-1′ proton with the C-3 (107.0), C-4 (160.8), C-2′ (30.7, 3.06) and C-3′. It was then deduced that **5** was a regioisomer of mammea **3**. Based on the above evidence, therefore, compound **5** was characterized as 1′S-deacetylmammea E/AC cyclo D.

Compound **6** was isolated as a yellow gum which was shown to be optically active ($[\alpha]^{29}_D$ −98.8°, *c* 1.21, $CHCl_3$). The molecular formula of **6** was deduced as $C_{22}H_{26}O_6$ by HRFABMS, identical to that of **2**. The NMR analysis of **6** showed that this coumarin had the same substituents as **2** since an 1-hydroxypropyl, a 3-methylbutyryl and 2,2-dimethyl Δ^3 pyran ring were revealed on its ^{1}H (Table 3) and ^{13}C NMR (Table 4) spectra. However, **2** and **6** exhibited quite different in their UV spectra (see experiment). Thus, compound **6** was deduced as regioisomer of **2**. The appearance of HMBC spectral data of **6** (Figure 4) revealed 3- and 2- bond correlation between the O*H*-7 proton with the C-6 (106.1) and C-7 (163.0). The differences ($\Delta\delta = \delta_R - \delta_S$) of indevidual proton of **6R** and **6S** (Figure 3) indicated that the absolute configuration of C-1' is S in **6**. Based on the above evidence, therefore, compound **6** was characterized as 1'S-deacetylmammea E/BA cyclo D.



4 R = $CH_2CH_2CH_3$ **5** R = $CH(CH_3)CH_2CH_3$

6 R = $CH_2CH(CH_3)_2$

Figure 4. HMBC correlations for **4**, **5**, and **6**

Table 1 ^{1}H NMR spectral data of compounds **1**, **2** and **3** (400 MHz, CDCl$_3$, solution, *J* in Hz, δ in ppm)

Proton	**1**	**2**	**3**
3	6.48, br s	6.47, br s	6.48, s
O*H*-5	15.80, s	15.86, s	15.80, s
1-hydroxypropyl			
1′	5.32, br d	5.32, m	5.32, br d
O*H*-1′	2.71, br s	2.62, br s	2.73, br s
2′a	1.92, ddq(14.0,7.4,3.3)	1.93, dd (14.0,7.4,3.3)	1.93, m
2′b	1.50, m	1.58, m	1.55, m
3′	1.09, t(7.3)	1.09, t(7.4)	1.04, t(7.3)
6-acyl moiety			
2″	3.10, t(7.4)	3.02, d(6.9)	3.76, sextet(6.6)
3″	1.76, sexter(7.4)	2.28, heptet(6.7)	1.21, d(6.6)
4″	1.03, t(7.4)	1.01, d(6.6)	1.87(1H, m), 1.46(1H, m)
5″		1.01, d(6.6)	0.94, br t(7.3)
chromene moiety			
3‴	5.61, d(10.1)	5.62, d(10.0)	5.62, d(10.1)
4‴	6.84, d(10.1)	6.85, d(10.0)	6.85, d(10.1)
5‴	1.55, s	1.55, s	1.55, s
6‴	1.57, s	1.56, s	1.56, s

Table 2 ^{13}C-NMR spectral data of compounds **1**-**3**. (100 MHz, CDCl$_3$, solution, δ in ppm)[†]

carbon	**1**	**2**	**3**
2	160.2(C)	160.9(C)	160.2(C)
3	107.6(CH)	107.8(CH)	107.6(CH)
4	160.5(C)	160.9(C)	160.4(C)
4a	101.9(C)	102.0(C)	102.0(C)
5	163.9(C)	164.0(C)	164.1(C)
6	106.9(C)	106.8(C)	106.8(C)
7	157.7(C)	158.0(C)	157.4(C)
8	101.9(C)	102.0(C)	102.0(C)
8a	155.0(C)	nd	155.0(C)
1-hydroxypropyl			
1′	72.6(CH)	72.7(CH)	72.7(CH)
2′	30.1(CH$_2$)	30.0(CH$_2$)	30.0, 30.1(CH$_2$)
3′	10.6(CH$_3$)	10.7(CH$_3$)	10.6(CH$_3$)
6-acyl moiety			
1″	207.8(C)	207.5(C)	212.2(C)
2″	46.8(CH$_2$)	53.6(CH$_2$)	46.7(CH)
3″	18.1(CH$_2$)	25.1(CH)	16.6, 16.7(CH$_3$)
4″	13.8(CH$_3$)	22.7(CH$_3$)	26.7, 26.67(CH$_2$)
5″		22.7(CH$_3$)	11.8(CH$_3$)
chromene moiety			
2‴	79.8(C)	79.9(C)	79.8(C)
3‴	126.5(CH)	126.5(CH)	126.5(CH)
4‴	115.5(CH)	115.6(CH)	115.6(CH)
5‴	28.2(CH$_3$)	28.3(CH$_3$)	28.1(CH$_3$)
6‴	28.3(CH$_3$)	28.4(CH$_3$)	28.2(CH$_3$)

[†] Chemical shifts are relative to the TMS signal (δ 0.00 ppm). Multiplicities were determined by the DEPT pulse sequence.

Table 3 ^{1}H NMR spectral data of compounds **4**, **5** and **6** (400 MHz, $CDCl_3$, solution, *J* in Hz, δ in ppm)

Proton	**4**	**5**	**6**
3	6.62, br s	6.62, 6.63 s	6.62, s
O*H*-7	14.49, s	14.44, 14.45, s	14.48, s
1-hydroxypropyl			
1′	5.46, dd(8.2, 2.8)	5.44, m	5.43, dd(8.4,2.7)
O*H*-1′	2.57, br s	2.13, s	2.66, br s
2′a	1.96, ddq(14.2,7.4,2.8)	1.95, m	1.94,ddq(14.2,7.4,2.7)
2′b	1.52, m	1.48, m	1.49, m
3′	1.15, t(7.3)	1.11, 1.12, t(7.4)	1.13, t(7.3)
Chromene moiety			
3″	5.61, d(10.1)	5.56, 5.57, d(10.0)	5.58, d(10.0)
4″	6.72, d(10.1)	6.73, 6.74, d(10.0)	6.71, d(10.0)
5″	1.57, s	1.52, 1.53, s	1.55, s
6″	1.57, s	1.56, 1.57, s	1.55, s
8-acyl moiety			
2‴	3.25, br t	3.89, 3.90, sextet(6.7)	3.14, 1H, dd(15.5, 6.7) 3.06, 1H, dd(15.5, 6.7)
3‴	1.79, sextet(7.3)	1.26, 1.25, d(6.6)	2.27, m
4‴	1.05, t(7.3)	1.87, 1H, m; 1.48,1H, m	1.03, d(6.6)
5‴	-	0.97, 0.98, t(7.4)	1.03, d(6.6)

Table 4 ^{13}C-NMR spectral data of compounds **4-5**. (100 MHz, CDCl$_3$, solution, δ in ppm) [†]

C	4	5	6
2	159.7(C)	159.5(C)	159.7(C)
3	107.0(CH)	107.0(CH)	107.0(CH)
4	161.0(C)	160.8(C)	161.1(C)
4a	101.2(C)	101.2(C)	101.1(C)
5	155.6(C)	155.5(C)	155.6(C)
6	106.1(C)	106.2(C)	106.1(C)
7	162.9(C)	163.1(C)	163.0(C)
8	104.5(C)	104.5(C)	104.5(C)
8a	157.1(C)	157.5(C)	156.9(C)
1-hydroxypropyl			
1′	71.7(CH)	71.8(CH)	71.7(CH)
2′	30.7(CH$_2$)	30.6, 30.7(CH)	30.7(CH$_2$)
3′	10.3(CH$_3$)	10.2, 10.3(CH$_3$)	10.3(CH$_3$)
Chromene moiety			
2″	80.1(C)	80.1(C)	80.1(C)
3″	126.4(CH)	126.4(CH)	126.4(CH)
4″	115.9(CH)	116.0(CH)	115.8(CH)
5″	28.2(CH$_3$)	28.2, 28.3(CH$_3$)	28.2(CH$_3$)
6″	28.2(CH$_3$)	28.0, 28.1(CH$_3$)	28.2(CH$_3$)
8-acyl moiety			
1‴	206.2(C)	210.7(C)	206.1(C)
2‴	46.7(CH$_2$)	46.9, 47.0(CH)	53.6(CH$_2$)
3‴	18.0(CH$_2$)	16.5(CH$_3$)	25.5(CH)
4‴	13.7(CH$_3$)	27.1, 27.11(CH$_2$)	22.5(CH$_3$)
5‴	-	11.68, 11.7(CH$_3$)	22.5(CH$_3$)

[†] Chemical shifts are relative to the TMS signal (δ 0.00 ppm). Multiplicities were determined by the DEPT pulse sequence.

Experimental Section

General Experimental Procedures. Optical rotations were measured in chloroform solution with the sodium D line (589 nm) on a JASCO P-1020 polarimeter. UV spectra were measured with Shimadzu UV-VIS 2001S spectrophotometer. Infrared spectra were obtained from Perkin Elmer System 2000 FT-IR spectrometers. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM 400, $CDCl_3$ was used as the solvent and TMS as an internal standard. Chemical shifts are given in parts per million downfield from TMS, and coupling constants are measured in Hz. DEPT, HMQC, COSY, HMBC and COSY experiments were obtained using standard Bruker software. Mass spectra were determined using a Finnigan GC-MS MAT 90 and GCQ Polaris instruments. HPLC was performed on the Thermo Separation Products, San Jose, CA, USA. (pump, P4000; detector, UV6000LP for analysis, UV2000 for preparative). The HPLC conditions were: (a) LUNA 5 µm C_8 stainless steel column, 150 × 4.60 mm, cat. no. 00F-4040-E0 for analytical applications. (b) LUNA 10 µm C_8 100 A stainless steel column, 250 × 21.20 mm, cat. no. 00G-4093-P0 for preparative applications. Compounds were purified by isocratic separation using H_2O-MeOH as mobile phase; scanning wave lengths from 190 to 420 nm. Column chromatography was carried out using Si gel 60 (0.063-0.200 mm) and Si gel 60 (particle size less than 0.063 mm). TLC was carried out on Si gel 60 F_{254} plates (cat. no. 7747 E. Merck). Compounds were detected by their UV absorbance at 254 and 366 nm. All commercial grade solvents were distilled prior to use and spectral grade solvents were used for spectroscopic measurements.

Plant Material. Dried flowers of *Mammea siamensis* were purchased from a local traditional drug store in Bangkok, in October 1995. The plant materials were further identified by Dr. Wongsatit Chuakul, Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University, Thailand.

Extraction and Isolation. The dried flowers (8.5 kg) of *Mammea siamensis* were extracted exhaustively with hexane at room temperature, followed by filtration. The filtrates were combined and evaporated under reduced pressure to afford a dark brown gum (428 g). The dried extract (300 g) was submitted to Si gel column chromatography and eluted with a gradient of hexane-EtOAc (0%-100%) to afford six fractions (A-F). A portion of fraction E (131 g) was then separated by column chromatography over Si gel with mixture of EtOAc in hexane of increasing polarity to give eight fractions (E-1-E-8). Fraction E-3 was further separated by column chromatography on a Si gel column with a hexane-EtOAc gradient and produced seven further fractions (f-1-f-7). Fraction f-2 was subjected to column chromatography on Si gel with hexane-EtOAc (18%) and further purified by preparative reversed-phase HPLC, run isocratically using 80% MeOH-H_2O with UV detection at 275 nm, with a flow rate of 10 mL/min, affording 5.6 mg of compound **1** (t_R 16.04 min) and 4.3 mg of **2** (t_R 19.92 min). Fraction f-3 was subjected to column chromatography on Si gel with hexane-EtOAc 20%) and further purified by preparative reversed-phase HPLC, run isocratically using 70% MeOH-H_2O with UV detection at 275 nm, with a flow rate 10 mL/min, affording 3.1 mg of compound **3** (t_R 17.3 min), 13.6 mg of **4** (t_R 18.3 min), 14.0 mg of **5** (t_R 22.3 min), and 4.9 mg of **6** (t_R 23.5 min).

Preparation of the (R)-(-)-α-Methoxyphenyl acetate ester of **1.**

Compound **1** (2.2 mg, 0.0059 mmol) was treated with (R)-(-)-α-MPAA (1 mg, 0.006 mmol) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) (1.12 mg, 0.006 mmol) in the presence of a catalytic amount of 4-dimethylamino pyridine in CH_2Cl_2 (1 mL), and the mixture was stirred for 4 h at room temperature. The (R)-(-)-α-methoxyphenyl acetate ester (**1R**, 2.6 mg) was obtained after purification by PLC (hexane-EtOAc; 85:15).

Preparation of the (S)-(+)-α-methoxyphenyl acetate ester of **1.**

Compound **1** (3.6 mg, 0.0097 mmol) was treated with (S)-(+)-α-MPAA (1.61 mg, 0.0097 mmol) and EDC (1.85 mg, 0.0097 mmol) in the presence of a catalytic amount of 4-dimethylaminopyridine in CH_2Cl_2 (1 mL), and the mixture was stirred for 4 h at room temperature. Work-up of the reaction product as described above furnished the (S)-(+)-α-methoxyphenyl acetate ester (**1S**, 5.4 mg).

Preparation of (R)-and (S)-MTPA esters of compound **4**

A solution of compound **4** (7 mg, 0.0188 mmol), dimethylaminopyridine (9.2 mg, 0.075 mmol), and triethylamine (3 drops) and (R)-α-methoxy-α-(trifluoromethyl) phenylacetylchloride (7.5 μL, 0.040 mmol) then was allowed to stand at room temperature for 3.5 h, then the solvent was evaporated. The obtained residue was applied to PLC using ethyl acetate-hexane (18:82) as developing solvent to give (S)-

MTPA ester **4S**. Similarly, (R)-MTPA ester **4R** was obtained using (S)-MTPA chloride.

Preparation of the (R)-(-)-α-Methoxyphenyl acetate ester of **5**.

Compound **5** (3.6 mg, 0.0093 mmol) was treated with (R)-(-)-α-MPAA (1.5 mg, 0.0093 mmol) and EDC (1.8 mg, 0.0093 mmol) in the presence of a catalytic amount of 4-dimethylaminopyridine in CH_2Cl_2 (1 mL), and the mixture was stirred for 24 h at room temperature. The (R)-(-)-α-methoxyphenyl acetate ester (**5R**, 3.1 mg) was obtained after purification by PLC (hexane-EtOAc; 80:20).

Preparation of the (S)-(+)-α-Methoxyphynyl acetate ester of **5**.

Compound **5** (3.5 mg, 0.0091 mmol) was treated with (S)-(+)-α-MPAA (1.5 mg, 0.0091 mmol) and EDC (1.7 mg, 0.0091 mmol) in the presence of a catalytic amount of 4-dimethylaminopyridine in CH_2Cl_2 (1 mL), and the mixture was stirred for 24 h at room temperature. The (S)-(+)-α-Methoxyphenyl acetate ester (**5S**, 4.2 mg) was obtained after purification by PLC (hexane-EtOAc; 80:20)

Preparation of the (R)-(-)-α-Methoxyphenyl acetate ester of **6**

Compound **6** (3.2 mg, 0.0082 mmol) was treated with (R)-(-)-α-MPAA (1.4 mg, 0.0084 mmol) and EDC (1.6 mg, 0.0083 mmol) in the presence of a catalytic amount of 4-dimethylaminopyridine in CH_2Cl_2 (1 mL), and the mixture was stirred for 24 h at room temperature. The (R)-(-)-α-Methoxyphenyl acetate ester (**6R**, 3.1 mg) was obtained after purification by PLC (hexane-EtOAc; 80:20).

Preparation of the (S)-(+)-α-Methoxyphenyl acetate ester of **6**.

Compound **6** (3.9 mg, 0.0101 mmol) was treated with (S)-(+)-α-MPAA (1.7 mg, 0.0102 mmol) and EDC (1.9 mg, 0.0101 mmol) in the presence of a catalytic amount of 4-dimethylaminopyridine in CH_2Cl_2 (1 mL), and the mixture was stirred for 24 h at room temperature. The (S)-(+)-α-Methoxyphenhyl acetate ester (**6S**, 3 mg) was obtained after purification by PLC (hexane-EtOAc; 80:20).

Deacetylmammea E/AC cyclo D (**1**): obtained as a yellow gum; $[\alpha]^{28}_{D}$ –44.2° (*c* 1.69, $CHCl_3$); UV λ_{max} MeOH (log *ε*) 204 (3.34), 225 (3.29), 286 (3.41) nm; IR ($CHCl_3$) ν_{max} 3473, 2957, 1728, 1615, 1585, 1464, 1117. cm^{-1}; ^1H and ^{13}C NMR see Table 1 and 2 respectively; EIMS *m/z* 372 $[M]^+$ (60), 357 $[M-CH_3]^+$ (100), 339 (65), 329 (33), 315 (48), 311 (35), 293 (12), 273 (10); HRFABMS (positive ion) *m/z* 373.1651 (calcd for $C_{21}H_{25}O_6$, 373.1652).

Deacetylmammea E/AA cyclo D (**2**): yellow gum; $[\alpha]^{28}_{D}$ –34.8° (*c* 0.26, $CHCl_3$); UV λ_{max} MeOH (log ε) 203(3.36), 221(3.22), 285(3.14) nm; IR ($CHCl_3$) ν_{max} 2928, 1728, 1620, 1585, 1470, 1261, 1117 cm^{-1}; ^1H and ^{13}C NMR see Table 1 and 2 respectively; EIMS *m/z* 386 $[M]^+$ (55), 371 $[M-CH_3]^+$ (100), 353 (87), 335 (22), 329 (55), 311 (23), 287 (9), 269 (12); HRFABMS (positive ion) *m/z* 387.1803 (calcd for $C_{22}H_{27}O_6$, 387.1808).

Deacetylmammea E/AB cyclo D (**3**): yellow gum; $[\alpha]^{28}_{D}$ –30.1° (*c* 0.16, $CHCl_3$); UV λ_{max} (from UV 6000) 225, 286 nm; ^1H and ^{13}C NMR see Table 1 and 2 respectively; EIMS *m/z* 386 $[M]^+$ (55), 371 $[M-CH_3]^+$ (100), 353 (87), 335 (22), 329 (55), 311 (23), 287 (9), 269 (12); HRFABMS (positive ion) *m/z* 387.1803 (calcd for $C_{22}H_{27}O_6$, 387.1808).

Deacetylmammea E/BC cyclo D (**4**): yellow gum; $[\alpha]^{28}_{D}$ –92.9° (*c* 4.28, $CHCl_3$); UV λ_{max} MeOH (log ε) 222 (4.00), 264 (4.15), 270 (4.19), 304 (4.14) nm; IR ($CHCl_3$) ν_{max} 3610 (sharp), 3465 (br), 2970, 2930, 2855, 1729, 1656, 1571, 1463, 1390, 1317, 1284, 1047, 980 cm^{-1}; ^1H and ^{13}C NMR see Table 3 and 4 respectively; EIMS *m/z* 372 $[M]^+$ (37), 357 $[M-CH_3]^+$ (100), 339 (24), 329 (29), 315 (49), 311 (16), 297 (14), 293 (8), 273 (11); HRFABMS (positive ion) *m/z* 373.1650 (calcd for $C_{21}H_{25}O_6$, 387.1651).

Deacetylmammea E/BB cyclo D (**5**): yellow semi-solid; $[\alpha]^{29}_{D}$ –91.6° (*c* 0.50, $CHCl_3$); UV λ_{max} MeOH (log ε) 222 (4.00), 265 (4.20), 271 (4.22), 305 (4.18) nm; IR ($CHCl_3$) ν_{max} 2927, 2855, 1729, 1607, 1578, 1463, 1385, 1142 cm^{-1}; ^1H and ^{13}C NMR see Table 3 and 4 respectively; EIMS *m/z* 386 $[M]^+$ (53), 371 $[M-CH_3]^+$ (100), 359 (31), 329 (72), 311 (27), 283 (12), 273 (24); HRFABMS (positive ion) *m/z* 387.1806 (calcd for $C_{22}H_{27}O_6$, 387.1808).

Deacetylmammea E/BB cyclo D (**6**): yellow semi-solid; $[\alpha]^{29}_{D}$ –98.8° (*c* 1.21, $CHCl_3$); UV λ_{max} MeOH (log ε) 218 (4.04), 265 (4.28), 271 (4.31), 305 (4.25) nm; IR ($CHCl_3$) ν_{max} 3607, 3460, 2964, 2931, 2874, 1733, 1608, 1583, 1464, 1400, 1362, 1286, 1128 cm^{-1}; ^1H and ^{13}C NMR see Table 3 and 4 respectively; EIMS *m/z* 386 $[M]^+$ (33), 371 $[M-CH_3]^+$ (100), 353 (31), 343 (23), 329 (58), 311 (20), 269 (8); HRFABMS (positive ion) *m/z* 387.1812 (calcd for $C_{22}H_{27}O_6$, 387.1808).

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TWO NEW PYRANOFLAVANONES FROM THE STEMS OF *DERRIS RETICULATA*

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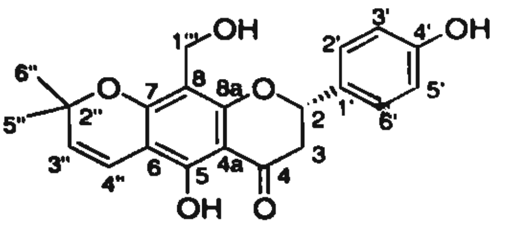
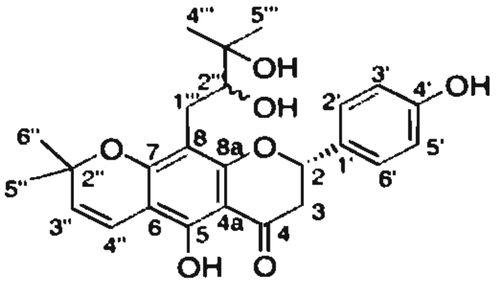
Abstract- Two new flavonoids, 4′,5-dihydroxy-8-hydroxymethyl-6″,6″-dimethylpyrano[2″,3″:7,6]flavanone (**1**), and 2″,3″-dihydroxylupinifolin (**2**) were isolated from the stems of *Derris reticulata*. Both compounds showed cytotoxic activity in the P-388 cell line. The structures were established by spectroscopic analysis and chemical transformations. The biosynthetic origin and the mechanism of formation of the hydroxymethyl group in compound (**1**) are proposed.

e Leguminosae is known to be a rich source of flavonoids and most of the prenyl derivatives have been
und in this family.[1,2] The flavonoids exhibit diverse biological activities and recent interest has been
cussed on their medicinal and nutritional values.[3] Recently, some biologically active prenylated
avonoids have been reported[4-6] and, significantly, it was found that the prenyl groups on the flavonoid
eleton play an important role in anti-HIV activity.[7] *Derris reticulata* (Leguminosae) is a well known
hai herbal medicine used for the relief of thirst and as an expectorant. Previously, we have reported the
olation and structural characterization of four prenylated flavanones, lupinifolin, 2‴,3‴-
poxylupinifolin, dereticulatin,[8] and 1‴-hydroxy-2‴,3‴-epoxylupinifolin from the stems *of Derris*
eticulata.[9] Further investigation of this herb has led to the isolation of two new cytotoxic flavonoids,
′,5-dihydroxy-8-hydroxymethyl-6″,6″-dimethylpyrano[2″,3″:7,6]flavanone (**1**), and 2″,3″-dihydroxy-
upinifolin (**2**). In this report we present the isolation, structure determination, and biological evaluation
f these two new compounds.

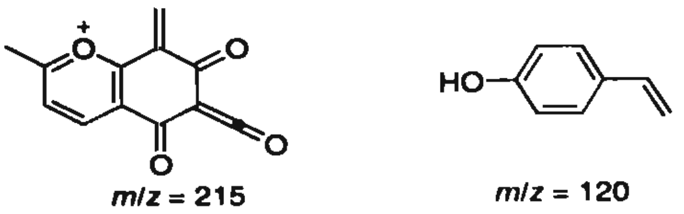
Compound (**1**) was obtained as a yellow solid, mp 141-142 °C, $[\alpha]_D$ −18.2°(*c* 0.12, $CHCl_3$). The UV ectrum (λ_{max} 226, 273, 298 nm) suggested the presence of a pyranoflavanone chromophore.[10] HREIMS f gave a molecular ion at m/z 368.1260, with the calculated value for $C_{21}H_{20}O_6$ being 368.1260. Its IR pectrum (EXPERIMENTAL) showed absorptions typical of hydroxyl, substituted aromatic ring, and bonyl groups. The ^{1}H NMR spectrum of **1** showed the typical pattern of protons at C-2 and C-3 in a vanone skeleton as three one-proton doublet of doublets at δ 5.34 (J = 12.9, 3.0 Hz), 3.07 (J = 17.2, .9 Hz), and 2.77 (J = 17.2, 3.0 Hz). In the aromatic region of the ^{1}H NMR spectrum of **1**, two doublets pearing at δ 7.27 and 6.83 (each 2H, J = 8.6 Hz) were assigned to the protons of *para*-substituted ring The signal at δ 12.32 (1H, s) was ascribed to a phenolic group hydrogen bonded to an acyl group and e signals at δ 4.54 and 4.59 (each 1H, d, ^2J = 11.7 Hz) were assigned to methylene protons of the enzylic alcohol. The signals that could be assigned to the 2,2-dimethylpyran group were at δ 1.43 and .45 (each 3H, s, 2 × Me), δ 5.51 (d, J = 10.0 Hz), and 6.58 (d, J = 10.0 Hz). No more protons in the romatic region was observed in the NMR spectrum, suggesting the absence of aromatic protons on ring A. Therefore, the three groups mentioned above were linked to ring A. The ^{13}C NMR spectrum of **1** howed signals of four oxygenated aromatic carbons (160.1, 160.2, 157.2, 157.4) and a carbonyl carbon 196.6). The ^{1}H and ^{13}C NMR (Table 1) spectra of **1** showed that the phenolic groups were located at C-5, nd C-4′. HMBC (Figure 1) experiments showed long-range C-H correlation in which two methylene rotons of the benzylic alcohol (δ 3.07 and 2.77) correlated with C-7, C-8, and C-8a. Furthermore, the tructure of compound (**1**) was confirmed by the EIMS spectrum which showed ions at m/z 215, and 120, resulting from the retro Diels-Alder cleavage of the ion at m/z 353 [M$^+$-CH$_3$], 335 [M$^+$-CH$_3$ -H$_2$O] (Scheme 1). The presence of an ion at m/z 120 confirmed that one phenolic group was located in ring B. Based on the above spectroscopic evidence, the structure of **1** is proposed to be 4′,5-dihydroxy-8-hydroxymethyl-6″,6″-dimethylpyrano[2″,3″:7,6]flavanone.[11]



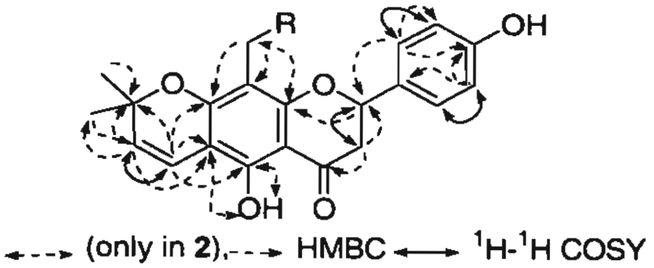
1



2



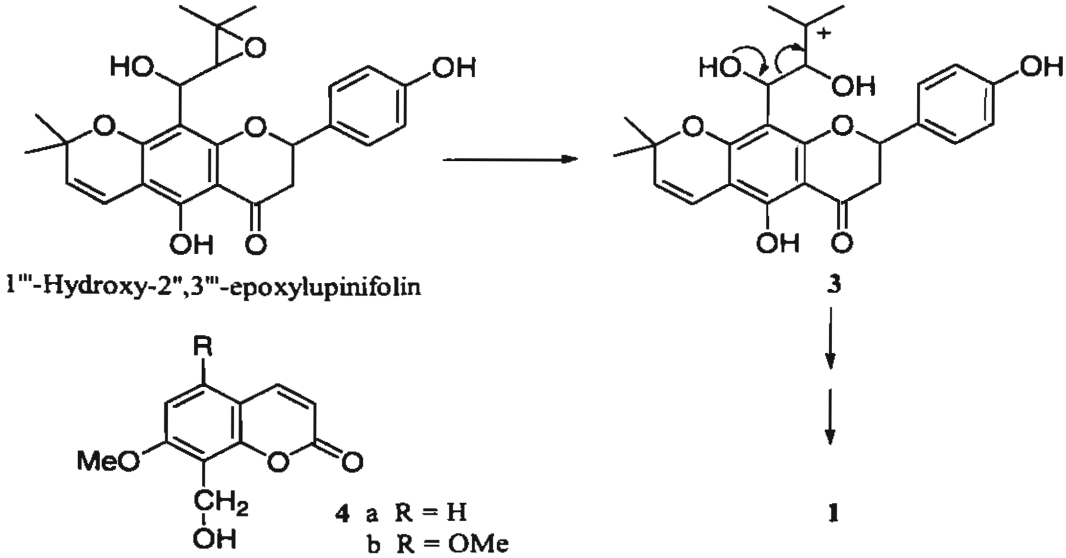
Scheme 1. Mass fragmentation of compound (1).



1 R = OH
2 R = CH(OH)COH(CH$_3$)$_2$

Figure 1. Summary of important connectivities observed in **1** and **2** by HMBC and COSY.

Compound (**2**), $[\alpha]_D$ −26.89°(*c* 0.10, CHCl$_3$) and the UV absorptions at 225, 266, 274, 300, 312, 363 nm were indicative of a pyranoflavanone chromophore.[5] HRFABMS (positive mode) exhibited $[M^++1]$ *m/z* 441.1913 corresponding to a molecular formula of $C_{25}H_{28}O_7$. The ^{1}H and ^{13}C NMR spectral data of **2** (Table 1) exhibited two sets of signals with partial overlapping suggesting the presence of two forms of the vicinal diol. The ^{1}H and ^{13}C NMR spectra of flavanone (**2**) were nearly identical with those of 2‴,3‴-epoxylupinifolin previously isolated from this plant.[8] Comparison of the molecular formula of **2** and 2‴,3‴-epoxylupinifolin suggested an additional H$_2$O molecule for **2**. The structure was also confirmed by reaction of epoxylupinifolin with 1% sulfuric acid in THF at room temperature for 24 h. From this reaction compound (**2**) was isolated as yellow solid. The structure of this compound was further confirmed by various spectroscopic methods including the correlations of the ^{13}C-^{1}H and ^{1}H-^{1}H in HMBC and COSY respectively as shown in Figure 1. Thus, from the spectroscopic studies and from semisynthesis, **2** was deduced to be 2‴,3‴-dihydroxylupinifolin. The stereochemistry at C-2 in compounds (**1**) and (**2**) was assigned to be *S* configuration by correlation with other related derivatives[8,10] isolated from this plant.



1‴-Hydroxy-2″,3‴-epoxylupinifolin

3

4 a R = H
b R = OMe

1

Scheme 2. The proposed biosynthesis of compound (**1**).

The co-occurrence of compound (**1**) with 1‴-hydroxy-2‴,3‴-epoxylupinifolin and compound (**2**) lends support to the proposal in Scheme 2 that the hydroxymethyl group in compound (**1**) could be derived om this compound by acid catalyzed opening of the epoxide ring or protonation of the hydroxyl group in mpound (**2**) to give the corresponding stable carbonium ion followed by carbon-carbon bond cleavage shown in **3** to give the aldehyde, an immediate precursor to compound (**1**). This biosynthetic route parently could be applied to rationalize the biosynthesis of the extremely rare hydroxymethyl oumarins, murrayacarpin-A and –B (**4a** and **4b**) which also co-occur with the prenyl derivatives.[12]

vitro bioassay evaluation of compounds (**1**) and (**2**) showed cytoxic activity in the P-388 cell line with C_{50} values of 6.4 and 1.3 μg/mL respectively, but were inactive against the KB cell line.

able 1: ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR Spectral Data for **1** and **2**.

position	**1**		**2**	
	δH (mult., *J* in Hz)	δC (mult.)	δH (mult., *J* in Hz)	δC (mult.)
2	5.34 (dd, *J* = 12.9, 3.0)	78.8 (d)	5.27, 5.33 (dd, *J* = 13.1, 3.0)	78.9, 79.0^{a} (d)
3	3.07 (dd, *J* = 17.2, 12.9)	42.5 (t)	2.99, 2.97 (dd, *J* = 17.1, 13.1)	43.0, 43.1 (t)
	2.77 (dd, *J* = 17.2, 3.0)		2.73, 2.71 (dd, *J* = 17.1, 3.0)	
4		196.6 (s)		196.4 (s)
4a		102.4 (s)		102.7, 102.8 (s)
5	OH 12.32 (s)	157.2^{a} (s)	OH 12.18 (s)	157.0, 157.1^{b} (s)
6		102.2 (s)		102.9 (s)
7		160.2^{b} (s)		159.8, 159.7^{c} (s)
8		107.4 (s)		105.6 (s)
8a		160.1^{b} (s)		159.6, 159.5^{c} (s)
1'		128.8 (s)		129.9, 129.8 (s)
2', 6'	7.27 (d, *J* = 8.5)	127.4 (d)	7.19 (d, *J* = 8.3)	127.6, 127.7 (d)
3', 5'	6.83 (d, *J* = 8.5)	115.1 (d)	6.76, 6.75 (d, *J* = 8.3)	115.8, 115.7 (d)
4'		157.4^{a} (s)		156.7, 156.6^{b} (s)
2''		78.4 (s)		78.8 (s)
3''	5.51 (d, *J* = 10)	126.0 (d)	5.44 (d, *J* = 10.0)	125.9, 125.8 (d)
4''	6.58 (d, *J* = 10)	114.9 (d)	6.57 (d, *J* = 10.0)	115.6, 115.5 (d)
5''	1.43 (s)	27.8 (q)	1.40, 1.38^{a} (all s)	28.6, 28.5^{d} (all s)
6''	1.45 (s)	27.6 (q)	1.37, 1.36^{a} (all s)	28.4, 28.3^{d} (all s)
1'''	4.59 (d, *J* = 11.7)	52.3 (t)	2.76, 2.75 (dd, *J* = 14.0, 1.9)	25.3, 25.2 (t)
	4.54 (d, *J* = 11.7)		2.53, 2.51 (dd, *J* = 14.0, 10.4)	
2'''			3.45, 3.42 (dd, *J* = 10.4, 1.9)	79.2, 79.1^{a} (d)
3'''				73.2 (s)
4''', 5'''	-		1.14, 1.13, 1.23, 1.12 (all s)	25.9, 23.4 (all s)

[a-d] Assignments may be interchangeable

PERIMENTAL

eneral: Melting points were determined on a Buchi 535 and are uncorrected. Optical rotations were easured with a JASCO P-1020 Digital Polarimeter. UV (MeOH) spectra were measured on a Shimadzu V-2100S spectrophotometer. IR (KBr) spectra were recorded on a Perkin-Elmer system 2000 FT-IR ectrophotometer. The NMR spectra were recorded on Bruker AM 400 (400 MHz for ^{1}H and 100 MHz or ^{13}C) spectrometer and chemical shifts were recorded in δ (ppm) using TMS as internal standard. MS pectra were determined on Finnigan Mat 90 and Finnigan Polaris instruments. HPLC was performed on ne Thermo Separation Products, San Jose, CA, U. S. A. (pump, P4000; detector, UV6000LP for analysis, JV2000 for preparative isolation).

Plant material: Dry stems of *Derris reticulata* were purchased from a local traditional drug store in Bangkok, Thailand (June 1998). Botanical identification was achieved through comparison by Prof. Nijsiri Ruangrungsi with the authentic specimen in the Bangkok Herbarium (BK 36776), Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand.

Extraction and Isolation: The dichloromethane extract (20 g) of dry powdered stems of *Derris reticulata* was separated by VLC (Vacuum Liquid Chromatography) using hexane and increasing amounts of ethyl acetate to give 8 fractions. Fraction 5 (200 mg) was resolved by preparative HPLC using Luna C_8 and UV detection at 274 nm. Elution with MeOH: H_2O (77:23), flow rate 8 mL/min, afforded compound (**1**) (3 mg); and 1‴-hydroxy-2‴,3‴-epoxylupinifolin (15 mg). Fraction 6 (429 mg) was further purified by repeated preparative TLC developed twice with a mixture of methanol:acetone:hexane (1:16:33) as eluent to yield 2 bands. The final purification of band 1 was achieved by preparative HPLC using Luna C_8 and UV detection at 280 nm, eluted with MeOH:H_2O (62.5:37.5) to furnish compound (**2**) (10 mg).

4′,5-Dihydroxy-8-hydroxymethyl-6″,6″-dimethylpyrano[2″,3″:7,6]flavanone (1): yellow solid (3 mg); mp 141-142 °C; $[\alpha]^{26}_D$ -18.2° (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 226 (4.16), 267 (4.41), 273 (4.47), 298 (3.94), 312 (3.92), 360 (3.29) nm; IR (KBr) ν_{max} 3300 (OH), 2925, 1647 (C=O), 1600, 1520, 1460, 1380, 1133, 830 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 1; EIMS *m/z* 368 $[M]^+$ (73), 353 $[M-CH_3]^+$ (37), 350 $[M-H_2O]^+$ (36), 335 $[M-CH_3, -H_2O]^+$ (87), 215 (100), 120 (12); HREIMS *m/z* 368.1260 (calcd for $C_{21}H_{20}O_6$, 368.1260).

2″,3″-Dihydroxylupinifolin (2): as yellow solid, $[\alpha]_D$ –26.89° (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 225 (4.14), 266 (4.48), 274 (4.56), 300 (3.91), 312 (3.93), 363 (3.36) nm; IR (KBr) ν_{max} 3411, 3244, 2974, 1620, 1521, 1451, 1382, 832 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 1; EIMS *m/z* 440 $[M]^+$

), 425 $[M-CH_3]^+$ (70), 407 (12), 381 (60), 351 (78), 335 (20), 305 (5), 287 (23), 261 (83), 231 (100), 5 (48), 120 (13); HRFABMS (positive ion) *m/z* 441.1913 (calcd for $C_{25}H_{29}O_7$, 441.1913).

eparation of 2''',3'''-dihydroxylupinifolin from 2''',3'''-epoxylupinifolin: A solution of 2''',3'''-oxylupinifolin (100 mg) in 1% H_2SO_4 in THF (5 mL) was stirred at rt for 24 h. The reaction mixture as poured into water (25 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was aporated to give crude product which was separated by silica gel preparative TLC using 1% MeOH in H_2Cl_2 to give 2''',3'''-dihydroxylupinifolin (53%).

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