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6-Acylcoumarins from *Mesua racemosa*

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Abstract

Two new 6-acylcoumarins, racemosol **1** and mammea A/AC cyclo F **2** were isolated by fractionation of a leave extract of *Mesua racemosa* (Planch. ex Triana and Planch.) Kostermans. Their structures were solved by extensive spectroscopic analysis as 5,7-dihydroxy-8-(2‴-hydroxy-3‴-methylbut-3‴-ene)-6-(1″-oxobutyl)-4-phenyl-2*H*-benzo[*b*]pyran-2-one **1** and 5-hydroxy-8-(1‴-hydroxy-1‴-methylethyl)-6-(1″-oxobutyl)-4-phenyl-8,9-dihydro-2*H*-furo [2′,3′:5,6]benzo[1,2-b]pyran-2-one **2**. Five known compounds namely mammea A/AC **3**, mammea A/AC cyclo D **4**, mammea A/AD cyclo D **5**, mammea A/BB **6** and mammea A/AA **7** were also isolated from the same source.

Keywords: *Mesua racemosa*; Clusiaceae; Guttiferae; Leaves; 6-Acylcoumarins; Chromene; 5,7-Dihydroxy-8-(2‴-hydroxy-3‴-methylbut-3‴-ene)-6-(1″-oxobutyl)-4-phenyl-2*H*-benzo[*b*]pyran-2-one; 5-Hydroxy-8-(1‴-hydroxy-1‴-methylethyl)-6-(1″-oxobutyl)-4-phenyl-8,9-dihydro-2*H*-furo[2′,3′:5,6]benzo[1,2-b]pyran-2-one; Racemosol; Mammea A/AC cyclo F

1. Introduction

Mesua racemosa (Clusiaceae) is a large tree widely distributed in tropical Asia. Previous phytochemical studies have already reported on the presence of xanthones (Sumitra, 1993), coumarins (Bhattacharyya, Chakrabartty, & Chowdhuty, 1988), flavonoids and triterpenes (Gunasekera & Sultanbawa, 1977) in this genus. In a continuation to a general survey of Malaysian flora as a source of new biologically active compounds (Benosman et al., 1995, 1997), an extract of *M. racemosa*, collected in Gua Musang, was selected for further phytochemical investigations due to its significant cytotoxic activity against P388 (100% for a concentration of 10 $\mu g \cdot ml^{-1}$) and KB cells (83 and 19% for concentrations of 10 and 1 $\mu g \cdot ml^{-1}$, respectively). This study led to the isolation of new 6-acylcoumarins **1** and **2** which were identified by means of extensive spectroscopic analysis.

2. Results and discussion

Dried leaves of *M. racemosa* were extracted with EtOAc in a Soxhlet apparatus for 72 h. This extract was then repeatedly chromatographied on silica gel by MPLC to give seven coumarins: mammea A/AC **3**, mammea A/AC cyclo D **4**, mammea A/AD cyclo D **5**, mammea A/BB **6** and mammea A/AA **7** together with new compounds **1** and **2**.

The molecular formula $C_{24}H_{24}O_6$ of **1** was established by high-resolution EI mass measurement of its molecular ion at *m/z* 408.1591 (calculated 408.1573). The UV spectrum of **1** (EtOH) supported a 6-acyl-5,7-dihydroxycoumarin type with absorptions at λ_{max} 237, 284 and 339 nm (Crombie, Games, & McCormick, 1967). The IR (KBr) spectrum showed absorptions at υ_{max} 3290 (OH), 1734 (δ-lactone), 1622 (C═O) and 1582 cm^{-1} (aromatic). As indicated in Table 1, two phenolic hydroxyls were in evidence on the ^{1}H NMR spectrum of **1** as two low field singlets at δ_H 14.38 and 10.20 ppm. This spectrum also exhibited signals at δ_H 5.96 (1H, s, H-3), 7.32 (2H, m, H-2′ and H-6′) and 7.41 ppm (3H, m, H-3′ to H-5′).

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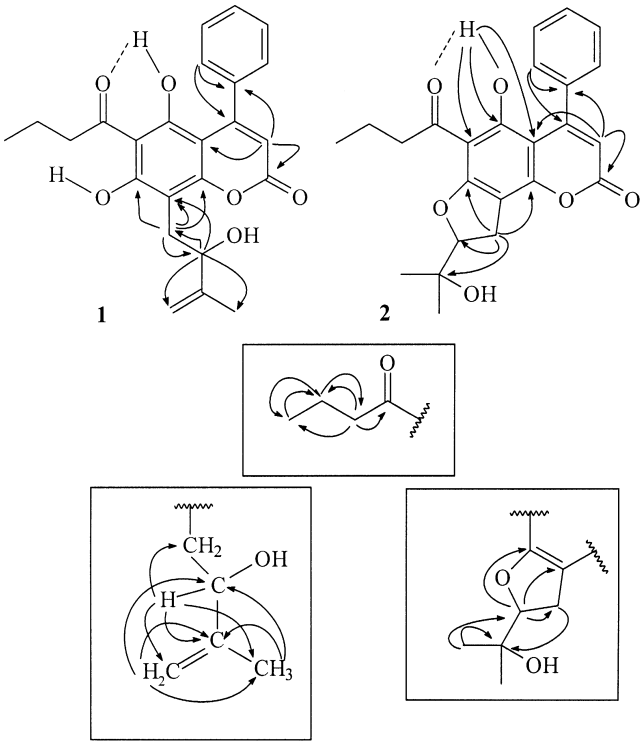
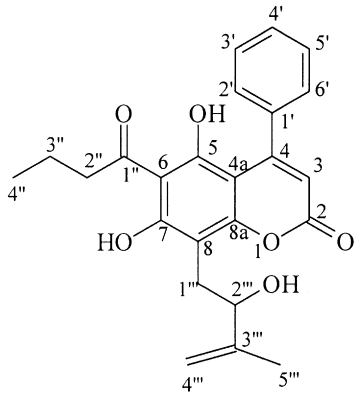


Fig. 1. Long-range correlations observed in the HMBC spectrum ($J = 6$ Hz) of **1** and **2**.

Considering these informations, **1** was thus identified as a 4-phenyl-5,7-dihydroxycoumarin derivative. The nature of the substituents at C-6 and C-8 was then deduced from a detailed interpretation of the NMR data of **1**. Indeed, the cross correlations deduced from a DQF–COSY spectrum revealed the presence of a *n*-propyl spin system with signals at δ_H 0.98 (3H, t, $J=7.5$ Hz, H-4″), 1.70 (2H, m, H-3″) and 3.13 ppm (2H, t, $J=7.5$ Hz, H-2″). In the HMBC spectrum of **1** (Fig. 1), the latter methylene protons caused cross-peaks with a keto function at δ_C 208.0 ppm. This way, an oxobutyryl chain was identified as one of the substituents of the coumarin heterocycle and location of this substituent at the C-6 position on the aromatic ring was inferred from the aforementioned UV data (Crombie et al., 1967). From an HMQC experiment, two olefinic protons belonging to a single CH_2 unit (δ_C 111.1 ppm) were characterized at δ_H 4.92 (1H, s, H-4b‴) and 5.03 ppm (1H, s, H-4a‴). In the HMBC spectrum, these vinylidene protons were correlated with a sp^2 quaternary carbon (δ_C 145.9 ppm), a secondary alcohol function (δ_C 77.2 ppm) and a methyl carbon (δ_C 18.7 ppm). These elements thus allowed us to identify a (2-hydroxy-3-methylbut-3-ene) chain as the C-8 substituent of the coumarin nucleus (Fig. 1). These data were confirmed by the base peak at m/z 337 ($[M]^+$ - C_4H_7O) on the mass spectrum of **1**, corresponding to the fragmentation of the 2-hydroxy-3-methylbut-3-ene moiety, as in the case of omphamurin (Wu, 1981). Therefore, **1** was identified as a new coumarin, namely the 5,7-dihydroxy-8-(2‴-hydroxy-3‴-methylbut-3‴-ene)-6-(1″-oxobutyl)-4-phenyl-2*H*-benzo[*b*]pyran-2-one which we have named racemosol.

Compound **2** cristallized in hexane–EtOAc (9:1) as yellow prisms (mp = 119°C) and had the molecular formula $C_{24}H_{24}O_6$ supported by its molecular ion at m/z 408.1591 (calculated 408.1573). Analysis of its ^{1}H NMR spectrum suggested again that **2** had a 4-phenylacylcoumarin skeleton with characteristic signals at 14.39 (1H, s, OH-5), 5.95 (1H, s, H-3), 7.30 (2H, m, H-2′ and H-6′) and 7.40 ppm (3H, m, H-3′ to H-5′). This hypothesis was confirmed by the UV absorbances of **2** (λ_{max} 230, 279 and 349 nm) whereas the IR spectrum exhibited main absorptions at 3566 (OH), 1734 (δ-lactone), 1704 (C=O) and 1614 cm^{-1} (aromatic). Close comparison of the ^{1}H NMR data of **1** and **2** then revealed the presence of the same oxobutyryl substituent in both compounds (δ_H 0.99 (3H, t, $J=7.5$ Hz, H-4″), 1.68 (2H, m, H-3″) and 3.00 ppm (2H, m, H-2″)). As expected, the methylene protons H-2″ of this acyl chain correlated with a keto function (δ_C 205.4 ppm) on the HMBC spectrum (Fig. 1). Furthermore, the chelated OH at δ_H 14.39 ppm caused a cross-peak with the carbon C-4a, thus indicating that the coumarin nucleus was substitued by this acyl chain at C-6. The absence of any other phenolic OH function and the presence of a quaternary carbon at δ_C 164.3 ppm (C-6a) then suggested the presence of an oxygenated ring system in the 7,8-position. Indeed in the HMBC spectrum (Fig. 1), the methyl protons at δ_H 1.44 and 1.32 ppm correlated with a tertiary alcohol function (δ_C 71.6 ppm) and with the tertiary carbon C-8 at δ_C 92.8 ppm. The H-8 (δ_H 4.92 ppm) also caused cross-peaks with two aromatic carbons at δ_C 105.1 and 164.3 ppm which were then respectively identified as C-9a and C-6a, and with the methylenic carbon C-9 at δ_C 26.8 ppm. These signals were thus associated with the presence of an hydroxyisopropyldihydrofuran ring system. This identification was confirmed by an examination of the EI mass spectrum of **2** where fragments at $[M]^+$ -43 ($[M]^+$ - $CH_2CH_2CH_3$) and $[M]^+$ -115 ($[M]^+$ -43–72) could be associated to the elimination of an isobutene oxide moiety from the $[M]^+$ -43 fragments (Carpenter, McGarry, & Scheinmann, 1970; Crombie, Games, Haskins, & Reed, 1972). Total assignments of the protons and carbons resonances of this compound were finally completed through exhaustive HMQC and HMBC data analysis (Table 1). Therefore, **2** was identified as the 5-hydroxy-8-(1‴-hydroxy-1‴-methylethyl)-6-(1″-oxobutyl)-4-phenyl-8,9-dihydro-2*H*-furo[2′,3′:5,6]benzo[1,2-b]pyran-2-one or, according to the nomenclature of Crombie and Games (1966), to mammea A/AC cyclo F.

As already reported in the literature in the case of 4-phenylcoumarins belonging to the mammea series, coumarin **1** did not present any optical activity ($[\alpha]_D$ and CD) (Crombie et al., 1967; Crombie, Games, & McCormick,



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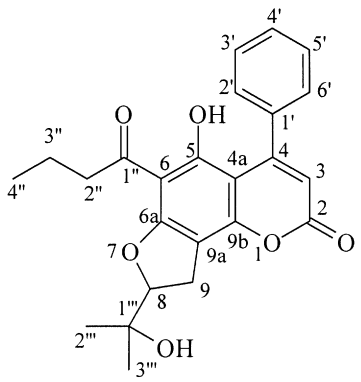

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Table 1
^{1}H ($CDCl_3$, 270 MHz) and ^{13}C ($CDCl_3$, 67.5 MHz) NMR spectra data of compounds **1** and **2**.

	1		**2**	
No.	δ_H	δ_C	δ_H	δ_C
2		160.0		159.7
3	5.96 (s)	112.1	5.95 (s)	112.1
4		156.7		156.5
4a		101.9		102.2
5		163.4		163.8
OH-5	14.38 (s)		14.39 (s)	
6		107.6		103.0
6a				164.3
7		162.6		
OH-7	10.20 (s)			
8		104.6	4.92 (t, $J=9.0$)	92.8
8a		157.7		
9			3.32 (dd, $J=6.0$, 3.0)	26.8
9a				105.1
9b				155.3
10				
1′		139.3		139.1
2′ and 6′	7.32 (m)	127.2	7.30 (m)	127.2
3′ and 5′	7.41 (m)	127.7	7.40 (m)	127.6
4′	7.41 (m)	128.3	7.40 (m)	128.3
1″		208.0		205.4
2″	3.13 (t, $J=7.5$)	46.7	3.00 (m)	45.2
3″	1.70 (m)	18.0	1.68 (m)	17.9
4″	0.98 (t, $J=7.5$)	13.9	0.99 (t, $J=7.5$)	13.8
1a‴	3.30 (d, $J=13.5$)	28.7		71.6
1b‴	3.04 (dd, $J=7.5$;15.0)			
2‴	4.50 (d, $J=7.5$)	77.2	1.32 (s)	24.8
3‴		145.9	1.44 (s)	26.2
4a‴	5.03 (s)	111.1		
4b‴	4.92 (s)			
5‴	1.93 (s)	18.7		

Coupling constants (J in Hz) are given in parentheses.

1967). However, a highly concentrated solution of **2** in chloroform exhibited a weak $[\alpha]_D$ of -2.6° ($c=4.52$). This result is therefore in agreement with the previously reported optical activities of closely related natural or synthetic 4-alkylcoumarins in similar measurement conditions (Finnegan, Merkel, & Back, 1972; Begley, Crombie, Jones, & Palmer, 1987).

Preliminary biological evaluation of **1** and **2** have revealed a weak cytotoxicity of both compounds against KB cells: 49 and 23% for **1** and 32 and 17% for **2** at 10 and 1 $\mu g \cdot ml^{-1}$, respectively.

In the same study and on the basis of their spectroscopic data, compounds **3**–**7** were respectively identified as known coumarins mammea A/AC (**3**)

(Thebtaranonth, Imraporn, & Padungkul, 1981), mammea A/AC cyclo D (**4**) (Thebtaranonth et al., 1981), mammea A/AD cyclo D (**5**) (Bala & Seshadri, 1971), mammea A/BB (**6**) (Crombie, Games, & McCormick, 1966) and mammea A/AA (**7**) (Bala & Seshadri, 1971). An interpretation of their ^{13}C NMR spectrum is however given in the experimental part since these data have not been previously reported in the literature.

3. Experimental

3.1. General

HREIMS (70 eV) were determined on a Varian Mat 311 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL GSX 270 MHz instrument. 2-D NMR experiments (DQF–COSY, HMQC, HMBC) were recorded on a Bruker Avance DRX 500 MHz spectrometer. IR spectra were obtained on a Perkin-Elmer 580 spectrometer and UV spectra were recorded on a Schimadzu UV-1601 spectrophotometer.

3.2. Plant material

The leaves of *M. racemosa* (Clusiaceae) were collected in August 1995 at Gua Musang, Malaysia. An herbarium specimen (No. KL 4524) is deposited at the laboratoire de Phanérogamie, MNHN, Paris and at the University of Malaya, Kuala-Lumpur.

3.3. Extraction and isolation

Powdered leaves of *M. racemosa* (0.5 kg) were extracted with EtOAc for 72 h in a Soxhlet apparatus. The extract was evaporated to dryness to yield a residue (11 g) which was chromatographied over silica gel. Elution was with hexane gradually enriched with EtOAc. Compounds obtained from *M. racemosa* were: racemosol **1** (90 mg), mammea A/AC cyclo F **2** (230 mg), mammea A/AC **3** (20 mg), mammea A/AC cyclo D **4** (40 mg), mammea A/AD cyclo D **5** (10 mg), mammea A/BB **6** (15 mg) and mammea A/AA **7** (20 mg).

3.4. Compound 1 (racemosol)

Yellow prisms (C_6H_{14}–EtOAc, 9:1). mp 140.8°C, $[\alpha]_D = 0°$ ($c = 0.8$, $CHCl_3$), IR υ_{max} cm^{-1}: 3290, 1734, 1622, 1582. UV (EtOH) λ_{max} nm ($\log \varepsilon$): 237 (4.17), 284 (4.38), 339 (3.96). ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Table 1. HREIMS m/z 408.1591 (calculated 408.1573 for $C_{24}H_{24}O_6$). EI-MS m/z: 408 ([M]$^+$, 14), 390 (13), 375 (15), 364 (21), 349 (16), 347 (17), 339 (11), 338 (41), 337 (100), 321 (24), 319 (24), 309 (14), 295 (22), 105 (13), 43 (25).

3.5. Compound 2 (mammea A/AC cyclo F)

Yellow prisms (C_6H_{14}–EtOAc, 9:1). mp 119.0°C, $[\alpha]_D = -2.6°$ ($c = 4.5$, $CHCl_3$), IR υ_{max} cm^{-1}: 3566, 1734, 1704, 1614. UV λ_{max} nm ($\log \varepsilon$): 230 (3.76), 279 (4.03), 349 (3.64). ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Table 1. HREIMS m/z 408.1591 (calculated 408.1573 for $C_{24}H_{24}O_6$). EI-MS m/z: 409 (23), 408 ([M]$^+$, 100), 375 (14), 365 (40), 351 (12), 350 (54), 349 (48), 337 (19), 322 (25), 321 (25), 307 (50), 305 (12), 294 (14), 293 (50), 279 (12), 105 (11), 59 (22), 43 (21).

3.6. Compound 3 (mammea A/AC)

^{13}C NMR ($CDCl_3$): δ 13.7 (C-4″), 17.6 (C-3″), 18.0 (C-4‴), 21.7 (C-1‴), 25.8 (C-5‴), 46.8 (C-2″), 101.3 (C-4a), 107.5 (C-8), 107.9 (C-6), 112.7 (C-3), 120.6 (C-2‴), 127.4 (C-2′ and C-6′), 129.1 (C-3′ and C-5′), 129.7 (C-4′), 135.4 (C-3‴), 137.2 (C-1′), 154.3 (C-5), 156.6 (C-7), 159.2 (C-2), 159.5 (C-4), 163.3 (C-8a), 207.4 (C-1″).

3.7. Compound 4 (mammea A/AC cyclo D)

^{13}C NMR ($CDCl_3$): δ 14.3 (C-4″), 18.7 (C-3″), 28.7 (C-1‴-2‴), 47.3 (C-2″), 80.3 (C-8), 101.9 (C-10a), 102.6 (C-4a), 107.4 (C-6), 113.1 (C-3), 116.0 (C-9), 126.8 (C-10), 127.6 (C-2′ and C-6′), 128.1 (C-3′ and C-5′), 128.7 (C-4′), 139.7 (C-1′), 155.2 (C-6a), 156.8 (C-4), 158.7 (C-10b), 160.1 (C-2), 164.8 (C-5), 207.5 (C-1″).

3.8. Compound 5 (mammea A/AD cyclo D)

^{13}C NMR ($CDCl_3$): δ 19.3 (C-3a″–C-3b″), 28.2 (C-1‴-2‴), 39.9 (C-2″), 79.9 (C-8), 101.5 (C-10a), 102.6 (C-4a), 106.4 (C-6), 112.7 (C-3), 115.5 (C-9), 126.3 (C-10), 127.2 (C-2′ and C-6′), 127.7 (C-3′ and C-5′), 128.3 (C-4′), 139.3 (C-1′), 154.8 (C-6a), 156.5 (C-4), 157.8 (C-10b), 159.7 (C-2), 164.6 (C-5), 211.5 (C-1″).

3.9. Compound 6 (mammea A/BB)

$[\alpha]_D = 0°$ ($c = 0.1$, $CHCl_3$), ^{13}C NMR ($CDCl_3$): δ 11.8 (C-4‴), 16.6 (C-2‴a), 17.9 (C-4″), 21.6 (C-1″), 25.6 (C-5″), 27.2 (C-3‴), 47.0 (C-2‴), 100.5 (C-4a), 104.1 (C-8), 112.1 (C-3), 112.6 (C-6), 127.5 (C-2′ and C-6′), 129.5 (C-3′ and C-5′), 130.2 (C-4′), 120.8 (C-2″), 134.1 (C-3″), 136.8 (C-1′), 154.1 (C-4), 155.7 (C-8a), 157.0 (C-5), 166.9 (C-7), 210.1 (C-1‴).

3.10. Compound 7 (mammea A/AA)

^{13}C NMR ($CDCl_3$): δ 18.0 (C-4a‴), 21.7 (C-1‴), 22.6 (C-4″), 24.7 (C-3″), 25.8 (C-4b‴), 53.6 (C-2″), 100.8 (C-4a), 107.3 (C-6), 108.0 (C-8), 112.7 (C-3), 120.6 (C-2‴), 127.4 (C-2′ and C-6′), 129.1 (C-3′ and C-5′), 129.7 (C-

4′), 135.5 (C-3‴), 137.2 (C-1′), 154.4 (C-4), 156.6 (C-8a), 159.2 (C-2), 159.5 (C-5), 163.3 (C-7), 207.1 (C-1″).

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