NEW COUMARINS FROM MESUA RACEMOSA: ISOLATION AND SYNTHESIS

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Abstract- The isolation and characterization of four new coumarins (racemosone, furanoracemosone, mammea A/BC, isoracemosol) isolated from the leaves of Mesua racemosa are reported. Their structures were established by means of spectroscopic studies and synthesis.

INTRODUCTION

Mesua representatives belong to a family of Clusiaceae including the Mammea and Calophyllum genus.[1] Plants in this subfamily are known as a rich source of xanthones,[2] coumarins[3] and biflavanoids.[4] In a previous paper,[5] we described the structures of several coumarins isolated from the leaves of Mesua racemosa. In a further search for biologically active compounds, we now report on the identification of new minor coumarins isolated from the same source.

RESULTS and DISCUSSION

Purification of an ethyl acetate extract of the leaves of Mesua racemosa through repeated silica gel column chromatographies resulted in the isolation of four new coumarins: racemosone (**1**), furanoracemosone (**2**), mammea A/BC (**3**), and isoracemosol (**4**).

Compound (**1**), mp 149-150°C, was obtained as white crystals (hexane/ethyl acetate) and its HREIMS showed the molecular ion at m/z 366.1100 associated with the molecular formula $C_{21}H_{18}O_6$. The UV and IR spectra of **1** suggested an acylcoumarin[6] while, in its ^{1}H-NMR spectrum, two chelated hydroxyl groups

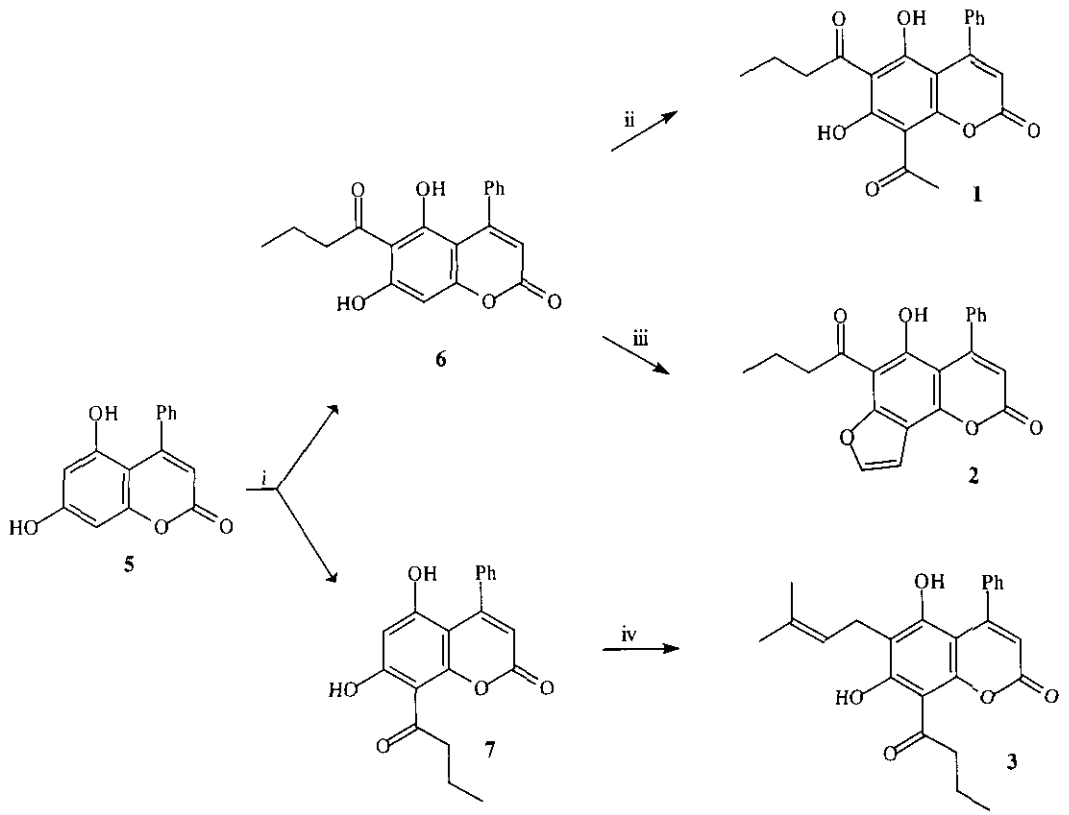
appeared as sharp singlets exchangeable with D_2O at δ_H 16.08 and 16.59 ppm. Both associated carbonyl functions were then revealed on the ^{13}C-NMR spectrum of **1** at δ_C 204.0 and 208.2 ppm. Substitution at the C-4 position of the coumarin was deduced from the resonance of the H-3 proton as a singlet at δ_H 6.05 ppm. In addition the ^1H-NMR spectrum of **1** showed the presence of a phenyl substituent in the molecule [δ_H 7.30 (2H, m), 7.40 ppm (3H, m) and δ_C 126.9, 127.8, 128.5 and 139.0 ppm] (Table 1). In the HMBC spectrum, the H-3 proton was correlated with the phenyl carbon at δ_C 139.0 ppm, which indicated a 4-phenylcoumarin skeleton for **1** (Figure 1). The cross correlations observed in the DQF-COSY experiment then revealed the presence of a n-propyl moiety in the molecule which was characterized as chemical shifts at δ_H 0.98 (3H, t, J = 7.5 Hz, H-4''), 1.72 (2H, m, H-3'') and 3.15 ppm (2H, t, J = 7.5 Hz, H-2''). This structural element was confirmed in the HREIMS spectrum which exhibited the base peak at m/z 323 $[M-C_3H_7]^+$. Finally, an acetyl methyl signal appeared at δ_H 2.98 ppm (3H, s). In the HMBC spectrum of **1** (Figure 1), the two protons at δ_H 3.15 ppm of the n-propyl moiety and the methyl protons at δ_H 2.98 ppm showed long-range correlations with carbonyl groups resonating at δ_C 208.2 and 204.0 ppm, respectively. From these data, it was concluded that this coumarin was substituted with two acyl chains i.e. a 1-butanoyl and an acetyl group.

In the HMBC spectrum of **1**, the chelated hydroxyl group at δ_H 16.08 ppm exhibited three cross peaks with three quaternary carbons at δ_C 101.5, 106.1 and 170.4 ppm respectively, one of these quaternary carbons (C-4a at δ_C 101.5 ppm) being also correlated with H-3 (δ_H at 6.05 ppm). These results thus allowed to locate this hydroxyl group at the C-5 position of the coumarin nucleus. The methyl group at δ_H 2.98 ppm correlated with a quaternary carbon at δ_C 102.7 ppm. The latter carbon also showed a long-range coupling with the other chelated hydroxyl (δ_H 16.59) at C-7. From these results, the acetyl group was firmly located at C-8. Compound (**1**) was thus characterized as the 5,7-dihydroxy-6-(1-butanoyl)-8-acetyl-4-phenyl-2H-[1]benzopyran-2-one which we have named racemosone and a total assignment of its ^{13}C and ^1H-NMR resonances is proposed in Table 1.

The structure of racemosone was finally confirmed through its total synthesis using the 5,7-dihydroxy-4-phenyl-2H-[1]benzopyran-2-one (**5**) as starting material (Scheme 1).[7]

In this preparation, a mixture of 6- and 8-acylcoumarins (**6** and **7**) was obtained according to a previously reported Friedel-Crafts acylation of **5**,[8] which was then separated by chromatography to give pure **6** and **7**. Further Friedel-Crafts acylation with CH_3COCl yielded **1** (11 %) and 58 % recovered starting material (**6**) (Scheme 1). This low yield can be explained by an electron withdrawing effect of the 1-butanoyl side

chain of **6**. Comparison of the spectral data of natural compound and synthetic product confirmed the structure of racemosone (**1**).



i) $CH_3CH_2CH_2COCl$ / $AlCl_3$ / CS_2, CH_3NO_2 / reflux

ii) CH_3COCl / $AlCl_3$ / CS_2, CH_3NO_2 / reflux

iii) 4-chloro-1,3-dioxolan-2-one / reflux

iv) $(CH_3)_2C{=}CHCH_2Br$ / 10% KOH / 0°C

Scheme 1

The molecular formula $C_{21}H_{16}O_5$ was assigned to furanoracemosone (**2**) on the basis of HREIMS data (M^+ at m/z 348.0973, Calcd 348.0998). The IR spectrum of **2** exhibited several absorptions due to hydroxyl (3447 cm^{-1}), lactone (1742 cm^{-1}) and carbonyl (1612 cm^{-1}) groups whereas its UV spectrum was characteristic of an acylcoumarin.[5, 6] The ^{1}H-NMR spectrum of **2** revealed the presence of a 4-phenyl substituent [δ_H 7.36 (2H, m) and 7.43 ppm (3H, m)] and a chelated hydroxyl group [δ_H 14.53 ppm (1H, s)]. As for racemosone (**1**), a 1-butanoyl chain [δ_H 3.25 (2H, t, J = 7.0 Hz), 1.79 (2H, m) and 1.05 ppm (3H, t, J = 7.0 Hz)] was thus present in the molecule. In the HMBC spectrum of **2** (Figure 1), the proton at δ_H 6.17 ppm showed correlations with carbons resonating at δ_C 104.7 (C-4a) and 138.9 ppm (C-1').

Furthermore, an hydroxyl group at δ_H 14.53 ppm showed C-H long-range correlations with carbons at δ_C 103.7 (C-6), 104.7 (C-4a) and 162.7 ppm (C-5). These results thus indicated that the 1-butanoyl chain was located at C-6. Two low-field doublets (J = 2.0 Hz) at δ_H 7.16 and 7.68 ppm were associated with a furan ring from the HMBC experiment (Figure 1). Therefore furanoracemosone was identified as the 5-hydroxy-6-(1-butanoyl)-4-phenyl-2H-furo[2',3':5,6]benzo[1,2-b]pyran-2-one.

Both coumarins (**1**) and (**2**) were considered as 6-(1-butanoyl)-5,7-dihydroxycoumarin derivatives. As a consequence, the prepared acylcoumarin (**6**) may also be used towards synthesis of **2**. The two carbons required to complete the furan ring synthesis were introduced using a 4-chloro-1,3-dioxolan-2-one reagent.[9] Under the reaction conditions given in Scheme 1, cyclization occurred leading in one step to the desired furanoracemosone (**2**) in 22 % yield.

Compound (**3**) had the molecular formula $C_{24}H_{24}O_5$ (HRLSIMS : $[M-H]^+$ at m/z 391.1526, Calcd 391.1545) and its UV and IR spectra suggested that it was an 8-acyl-5,7-dihydroxycoumarin. The ^{1}H-NMR spectrum of **3** also showed the presence of a 1-butanoyl chain [δ_H 3.30 (2H, m), 1.83 (2H, m) and 1.03 ppm (3H, t, J = 7.0 Hz)], a phenyl group [δ_H 7.44 (2H, m) and 7.57 ppm (3H, m)] and two exchangeable hydroxyl groups [δ_H 5.96 (1H, s) and 14.58 ppm (1H, s)]. In the HMBC spectrum of **3** (Figure 1), the hydroxyl proton at δ_H 5.96 ppm appeared to be ^{2}J or ^{3}J coupled with carbons at δ_C 157.0, 112.1 and 100.4 ppm. The H-3 singlet at δ_H 6.01 ppm was also correlated to C-4a at δ_C 100.4 ppm.
This spectral evidence thus showed that this hydroxyl group was located at the C-5 position of the coumarin skeleton, so the remaining hydroxyl group had to be located at the C-7 position and consequently the 1-butanoyl chain at C-8. In the ^{1}H-NMR spectrum of **3**, an isopentenyl substituent at the C-6 position was finally characterized by typical signals at δ_H 1.66 (3H, s), 1.71 (3H, s), 3.33 (2H, m) and 5.09 ppm (1H, t, J = 1.0 Hz). Compound (**3**), the 5,7-dihydroxy-6-(3-methylbut-2-enyl)-8-(1-butanoyl)-4-phenyl-2H-[1]benzopyran-2-one, is thus a regioisomer of the described mammea A/AC coumarin.[10]
Therefore, compound (**3**) isolated for the first time from a natural source was identified as mammea A/BC according to Crombie's nomenclature.[11]
Preparation of different acylprenylcoumarin analogs has already been developped by Thebtaranonth[10] and Crombie.[8] However, to the best of our knowledge, synthesis of mammea A/BC has never been described. Following Crombie procedure, C-prenylation of acylcoumarin (**7**) occurred in aqueous KOH leading to **3** in 15 % yield (Scheme 1). The spectral data (^{1}H-NMR) of natural and synthetic compounds were superimposable.

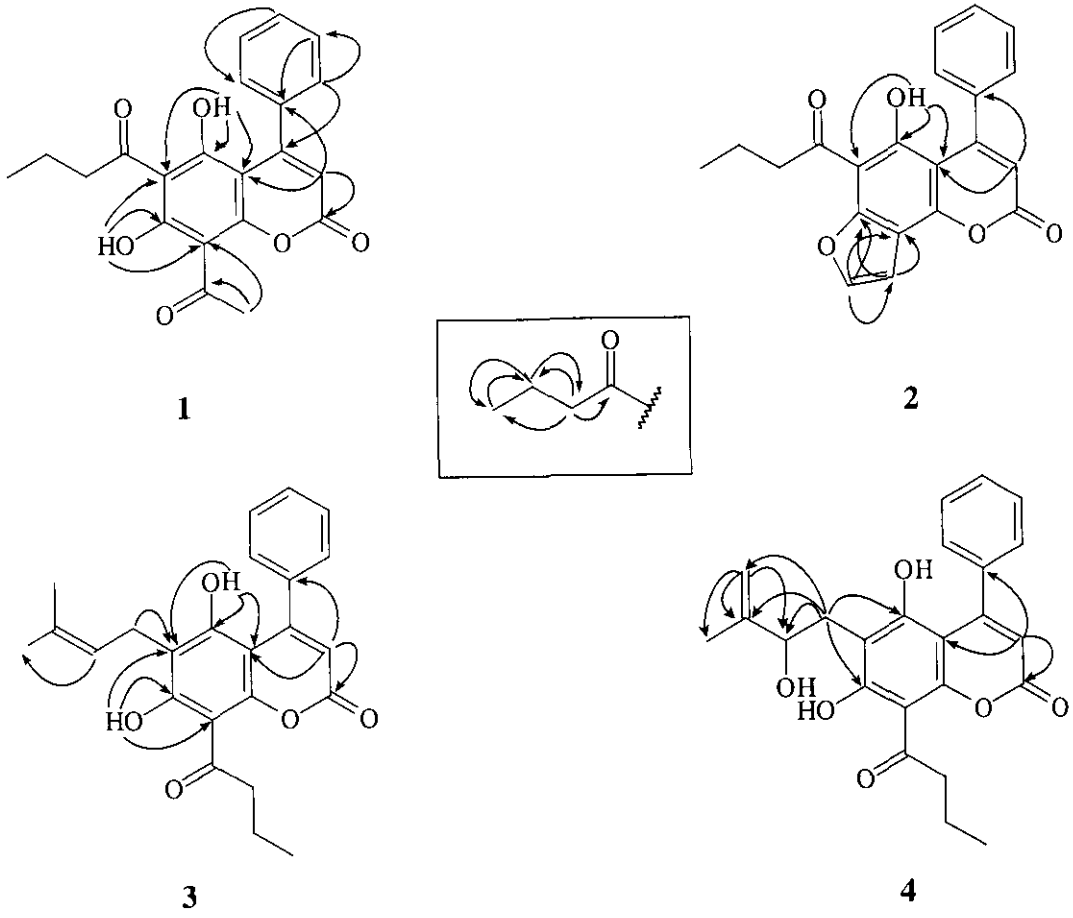
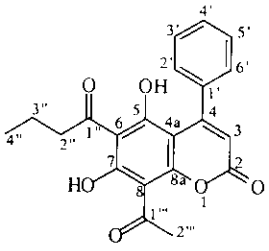
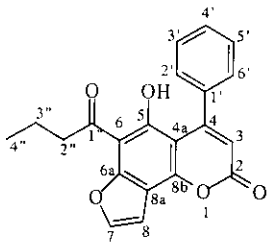


Figure 1. Long-range correlations observed in the HMBC spectrum (J = 6 Hz) of **1, 2, 3** and **4**

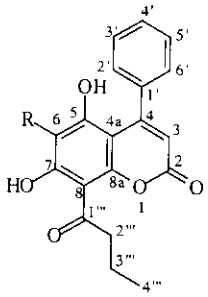
The HREIMS of the 8-acyl-5,7-dihydroxycoumarin (**4**) gave a molecular ion $[M]^+$ at m/z 409.1647 (Calcd 409.1651 for $C_{24}H_{25}O_6$). The ^{1}H-NMR spectrum of **4** displayed signals for a chelated hydroxyl [δ_H 14.77 ppm (1H, s)], five aromatic protons [δ_H 7.30 (2H, m) and 7.40 ppm (3H, m)], an butanoyl chain [δ_H 1.07 (3H, t, J = 7.5 Hz), 1.78 (2H, m) and 3.34 ppm (2H, t, J = 7.0 Hz)] and an olefinic proton [δ_H 6.03 ppm (1H, s)]. Comparison of the ^{1}H and the ^{13}C-NMR spectra of **3** and **4** showed that these compounds only differed in the nature of their side chain at C-6. Concerning the ^{1}H-NMR spectrum of **4**, signals for two olefinic protons at δ_H 4.86 (s) and 4.93 ppm (s) (δ_C 110.6 ppm), an allylic methyl group at δ_H 1.81 ppm (s) (δ_C 18.5 ppm) and a secondary hydroxyl group at δ_H 4.32 ppm (δ_C 77.4 ppm) were then associated with a 2-hydroxy-3-methylbut-3-enyl chain. Isoracemosol (**4**), 5,7-dihydroxy-6-(2-hydroxy-3-methylbut-3-enyl)-



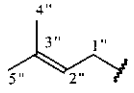
1



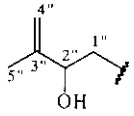
2



3 R =



4 R =



	1		2		3		4	
N°	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	-	158.1	-	159.3	-	158.7	-	159.3
3	6.05 (s)	112.3	6.17 (s)	114.3	6.01 (s)	112.5	6.03 (s)	112.1
4	-	156.6	-	156.8	-	154.1	-	156.6**
4a	-	101.5	-	104.7	-	100.4	-	102.2
5	-	170.4	-	162.7	-	157.0	-	160.3*
5-OH	16.08 (s)	-	14.53 (s)	-	5.96 (s)	-	-	-
6	-	106.1	-	103.7	-	112.1	-	109.8
6a	-	-	-	156.0	-		-	-
7	-	172.1	-	-	-	166.7	-	166.8*
7-OH	16.59 (s)	-	-	-	14.58 (s)	-	14.77 (s)	-
8	-	102.7	7.68 (d, J = 2.0)	143.9	-	104.5	-	104.0
8a	-	161.3	-	-	-	156.0	-	156.4**
9			7.16 (d, J = 2.0)	104.7				
9a				109.7				
9b	-	-	-	153.3	-	-	-	-
1'	-	139.0	-	138.9	-	136.8	-	139.8
2' and 6'	7.30 (m)	126.9	7.36 (m)	127.2	7.44 (m)	127.5	7.30 (m)	127.1
3' and 5'	7.40 (m)	127.8	7.43 (m)	127.7	7.57 (m)	129.6	7.40 (m)	127.8
4'	7.40 (m)	128.5	7.43 (m)	128.4	7.57 (m)	130.2	7.40 (m)	128.2
1''	-	208.2		204.5	3.33 (m)	21.5	2.78 (dd, J = 2/15) 3.10 (dd, J = 2/15)	28.5
2''	3.15 (t, J = 7.5)	46.6	3.25 (t, J = 7.0)	44.9	5.09 (t, J = 1.0)	120.8	4.32 (d, J = 7.5)	77.4
3''	1.72 (m)	17.7	1.79 (m)	17.5	-	134.1	-	146.4
4''	0.98 (t, J = 7.5)	13.8	1.05 (t, J = 7.0)	13.8	1.66 (s)	25.7	4.86 (s) 4.93 (s)	110.6
5''	-	-	-	-	1.71 (s)	17.9	1.81 (s)	18.5
1'''	-	204.0	-	-	-	206.3	-	206.3
2'''	2.98 (s)	33.6	-	-	3.30 (m)	46.7	3.34 (t, J = 7.0)	46.6
3'''	-	-	-	-	1.83 (m)	18.1	1.78 (m)	18.1
4'''	-	-	-	-	1.03 (t, J = 7.0)	13.8	1.07 (t, J = 7.5)	13.8

* and ** resonances may be interchangeable

Table 1 : ^1H ($CDCl_3$, 270 MHz) and ^{13}C ($CDCl_3$, 67.5 MHz) NMR data taken in $CDCl_3$ of compounds (**1-4**)

8-(1-butanoyl)-4-phenyl-2H-[1]benzopyran-2-one, is thus a new natural isomer of racemosol isolated from the same plant.[5]

EXPERIMENTAL

General. HREIMS were recorded on a Varian MAT 311 spectrometer at 70 eV. NMR spectra were recorded in $CDCl_3$ on JEOL GSX WB 270 MHz and Bruker Avance DRX 500 MHz instruments using TMS as an internal standard. IR spectra were recorded on a Perkin Elmer 580 spectrophotometer and UV spectra on a Schimadzu UV-1601 spectrophotometer. Melting points were determined with a Electrothermal 8100 melting point apparatus and are uncorrected.

Plant material. The leaves of Mesua racemosa 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.

Extraction and isolation. Powdered leaves of Mesua racemosa (0.5 kg) were extracted with ethyl acetate (4 L) for 72 h in a Soxhlet apparatus. The extract was evaporated to dryness to yield a residue (11 g) which was chromatographed over silica gel. Elution was hexane gradually enriched with ethyl acetate. Compounds obtained from Mesua racemosa were racemosone (**1**) (10 mg), furanoracemosone (**2**) (10 mg), mammea A/BC (**3**) (15 mg) and isoracemosol (**4**) (5 mg).

Racemosone (**1**), 5,7-dihydroxy-6-(1-butanoyl)-8-acetyl-4-phenyl-2H-[1]benzopyran-2-one, mp 149-150°C (hexane/ethyl acetate : 9/1). HREIMS : $[M]^+$ 366.1100 (Calcd 366.1103 for $C_{21}H_{18}O_6$), m/z (rel. Int.) : 367 (9), 366 (40), 351 (6), 338 (20), 324 (21), 323 (100). UV (EtOH + HCl) λ_{max} (log ε) : 325 (3.88), 269 (4.12), 216 nm (3.82), (EtOH + NaOH) λ_{max} (log ε) : 398 (3.76), 335 (3.94), 291 (4.01), 254 nm (4.00). IR υ_{max} : 3447, 1753, 1618, 1589, 1153, 769, 700 cm^{-1}. The ^1H and ^{13}C-NMR are listed in Table 1.

Furanoracemosone (**2**), 5-hydroxy-6-(1-butanoyl)-4-phenyl-2H-furo[2',3':5,6]benzo[1,2-b]pyran-2-one, white amorphous solid. HREIMS : $[M]^+$ 348.0973 (Calcd 348.0997 for $C_{21}H_{16}O_5$), m/z (rel. Int.) : 349 (13), 348 (53), 333 (6), 306 (12), 305 (100), 277 (10), 221 (7), 71 (6), 69 (10), 57 (10), 55 (6), 43 (9), 28 (12), 18 (8). UV (EtOH + HCl) λ_{max} (log ε) : 289 (3.66), 238 nm (3.54), (EtOH + NaOH) λ_{max} (log ε) : 377 (3.17), 279 nm (3.64). IR υ_{max} : 3447, 2958, 1742, 1612, 1161, 1136, 762, 698 cm^{-1}. The ^1H and ^{13}C-NMR are listed in Table 1.

Mammea A/BC (**3**), 5,7-dihydroxy-6-(3-methylbut-2-enyl)-8-(1-butanoyl)-4-phenyl-2H-[1]benzopyran-2-one, mp 123-124°C (hexane/ethyl acetate : 9/1). HRLSIMS : $[M-H]^+$ 391.1526 (Calcd 391.1545 for $C_{24}H_{24}O_5$). UV (EtOH + HCl) λ_{max} (log ε) : 331 (3.85), 294 (4.03), 225 nm (4.09), (EtOH + NaOH) λ_{max} (log ε) : 384 (3.73), 334 (4.17), 235 nm (4.21). IR υ_{max} : 3486, 1742, 1705, 1616, 1595, 1389, 1132, 769, 704 cm^{-1}. The ^1H and ^{13}C-NMR are listed in Table 1.

Isoracemosol (**4**), 5,7-dihydroxy-6-(2-hydroxy-3-methylbut-3-enyl)-8-(1-butanoyl)-4-phenyl-2H-[1]benzopyran-2-one : $[\alpha]_D$ = 0° (0.1, $CHCl_3$), yellow amorphous solid. HREIMS : $[M]^+$ 409.1647 (Calcd 409.1651 for $C_{24}H_{25}O_6$), m/z (rel. Int.) : 409 (55), 391 (66), 337 (72). UV (EtOH + HCl) λ_{max} (log ε) : 326 (3.66), 292 (3.89), 225 nm (3.89), (EtOH + NaOH) λ_{max} (log ε) : 335 (4.03), 229 (4.03), 257 nm (3.88). IR υ_{max} : 3447, 1734, 1707, 1597, 1387, 1134, 771, 702 cm^{-1}. The ^1H and ^{13}C-NMR are listed in Table 1.

Preparation of **1**

A solution of CH_3COCl (19 mg, 17 μL, 0.25 mmol) in CH_3NO_2 (1.2 mL) was added to a mixture of **6** (70 mg, 0.21 mmol) and $AlCl_3$ (92 mg, 0.69 mmol) in CS_2 (15 mL) and refluxed for 1 h. The reaction mixture was poured into ice-water and extracted with ethyl acetate (3x10 mL). The organic extracts were combined, washed with H_2O (2x15 mL), dried over Na_2SO_4 and evaporated. After chromatography (SiO_2, hexane/ethyl acetate : 7/3), the residue obtained was recrystallized from EtOH to give **1** (7 mg, 11 %).

Preparation of **2**

A mixture of 4-chloro-1,3-dioxolan-2-one (190 mg, 126 μL, 1.5 mmol) and 5,7-dihydroxy-6-(1-butanoyl)-4-phenyl-2H-[1]benzopyran-2-one (**6**) (50 mg, 0.15 mmol) was heated at 150°C for 4 h and then at 165 °C for 30 min. The residue was directly chromatographed on a silica gel column eluting with hexane/ethyl acetate (9/1) to give **2** (7 mg, 10 %).

Preparation of **3**

3-Methylbut-2-enyl bromide (477 mg, 370 μL, 3.20 mmol) was added to a solution of coumarin (**7**) (300 mg, 0.92 mmol) in 5 mL of 10% KOH at 0 °C. After stirring for 1.5 h, the reaction mixture was poured into 10% HCl (15 mL) and then extracted with CH_2Cl_2 (3x10 mL). The organic phase was separated, dried over Na_2SO_4, and evaporated to leave 70 mg of crude material. After chromatography on SiO_2 (hexane/ethyl acetate : 9/1) 45 mg (15 %) of compound (**3**) was recovered.

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