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DIMERIC CHALCONE DERIVATIVES FROM *MALLOTUS PHILIPPENSIS*

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Key Word Index—*Mallotus philippensis*; Euphorbiaceae; kamala; dimeric chalcones; kamalachalcones A and B.

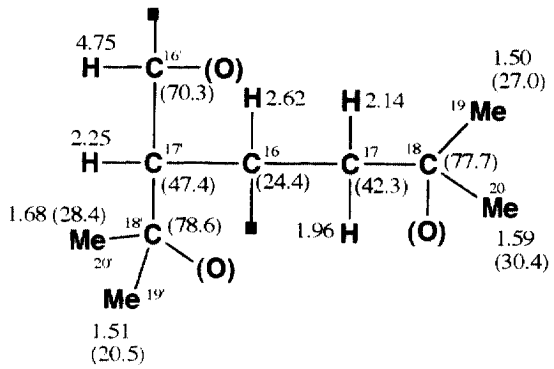
Abstract—Two new chalcone derivatives with a unique ring system caused by dimerization between a dimethylchromene ring and a phenoxyl group, were isolated from kamala (*Mallotus philippensis*). The structures were determined by spectral analysis.

INTRODUCTION

Granular hairs on the surface of fruits of *Mallotus philippensis* Muell. are covered with exudate of reddish substances, which is called kamala, and has been used as a drug and dye. In previous papers, the occurrence of many novel flavonoids has been reported [1–5]. Some of them have *iso*prenyl(s) and *C*-methyl groups, and others are condensed with an acetophenone moiety though a methylene group such as rottlerin (**4**). The cognates were also found in pericarps of *M. japonicus* Muell [6, 7], and showed cytotoxic activity in the KB system or the L-5178Y cell line; and inhibitory effect of rottlerin on protein kinase C with specificity was also reported [8]. The constituents in the genus *Mallotus* are thus drawing our attention to search for highly biological active constituents from natural sources. Nevertheless few reports have dealt with the bioactivity of kamala and with detailed phytochemistry. In our continuous research of bioactive compounds in natural resources, an acetone-soluble extract of kamala was examined and two novel chalcone dimers, kamalachalcones A (**1**) and B (**2**), were isolated. In the present paper, we report the isolation and structural elucidation of these compounds.

RESULTS AND DISCUSSION

Kamala was successively extracted with acetone and MeOH at room temperature. The acetone extract was chromatographed on silica gel eluted with $CHCl_3$-MeOH mixtures. The $CHCl_3$-MeOH (6:1) fraction was further purified by silica gel chromatography and recrystallization to give **1** and **2**, in addition to a large amount of rottlerin.



Scheme 1. Slide-chain structure of kamalachalcone A (**1**).

Compound **1** (kamalachalcone A), a yellow powder, gave a $[M]^+$ at *m/z* 671 in the negative ion FAB-mass spectrum and *m/z* 672 in the EI-mass spectrum, which corresponds to the molecular formula $C_{42}H_{40}O_8$. The ^1H NMR spectrum, including HH COSY, showed the presence of a sequence of aliphatic methines and a methylene proton coupled successively in this order [δ 4.75 (1H, *d*, $J = 5$ Hz, H-16′), 2.25 (1H, *t*, $J = 5$ Hz, H-17′), 2.62 (1H, *dt*, $J = 13$, 6H, H-16), 2.14 and 1.96 [(1H, *dd*, $J = 13$, 5 Hz, H-17β) and (1H, *t*, $J = 13$ Hz, H-17α)]. Four methyl groups [δ 1.50 (3H, *s*, H-19), 1.51 (3H, *s*, H-19′), 1.59 (3H, *s*, H-20), 1.68 (3H, *s*, H-20′) were also observed in the ^1H NMR spectrum. The carbons signals in the ^{13}C NMR spectrum attached to these protons are shown in Scheme

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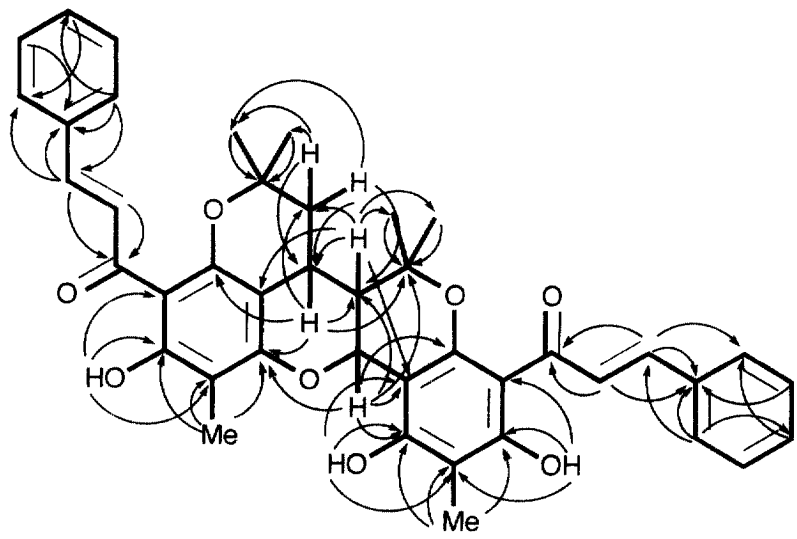
Table 1. ^{13}C NMR spectral data of kamalachalcones A (**1**) and B (**2**)

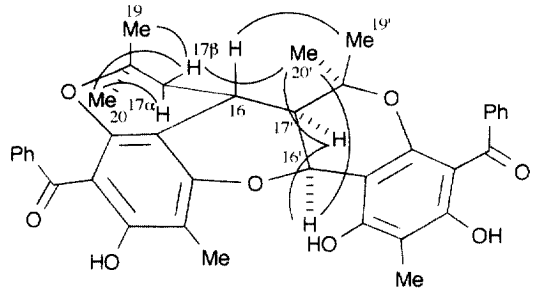
No	1	2	No	1	2
1	107.7	107.3	5′	105.4	103.1
2	154.4	155.6	6′	166.4	166.5
3	101.0	102.6	7′	193.5[a]	193.2
4	158.5	156.6[b]	8′	127.8[b]	127.6
5	105.9	109.1	9′	142.1[c]	142.3
6	162.9	161.4	10′	135.6[d]	135.5
7	193.0[a]	193.7	11′,15′	128.3[e]	128.3
8	127.8[b]	126.7	12′,14′	129.0[f]	129.0[i]
9	141.9[c]	143.9	13′	130.0[g]	130.2[j]
10	135.6[d]	135.2	16′	70.3	71.0
11,15	128.2[e]	128.5	17′	47.4	46.9
12,14	129.0[f]	129.1[i]	18′	78.6	78.8
13	130.1[g]	130.6[j]	19′	20.5	20.8
16	24.4	25.0	20′	28.4	28.6
17	42.3	41.8	21′	7.3	7.2
18	77.7	78.5	1″		104.0
19	27.0	30.4	2″		159.2[h]
20	30.4	27.1	3″		105.5
21	7.5	16.3	4″		162.3
1′	106.0	106.1	5″		104.4
2′	153.8	154.7	6″		160.0
3′	98.2	97.2	7″		204.1
4′	160.5	159.5	8″		33.2
					7.7

Measured in $CDCl_3$. a-j: interchangeable between same letters.

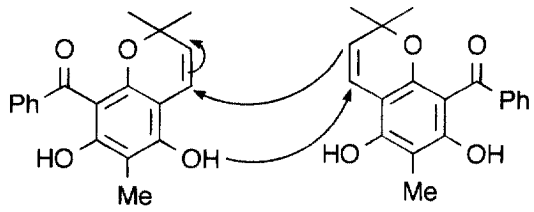


Scheme 2. CH long-range correlations of **1** in HMBC spectrum.

1 and the assignment confirmed by the analysis of HMQC spectra listed in Table 1. In the HMBC spectrum (Scheme 2), significant CH long-range correlations were observed between H-19/C-18 (δ 77.67), H-20/C-18, H-19′/C-18′ (δ 78.55), H-20′/C-18′, H-17α/C-19 (δ 27.01), H-17α/C-20 (δ 30.36), H-17β/C-19, H-17β/C-20, H-16/C-18′, H-17′/C-17, H-17′/C-19′ (δ 20.5) and H-17′/C-20′ (δ 28.4), which indicated the presence of a C_{10}-alkyl side-chain in the structure (Scheme 1). The ^1H NMR spectrum further exhibited the presence of two sets of *trans*-olefinic protons [δ 7.98, 8.0 (1H each, *d*, J=16 Hz, H-8,8′); δ 7.78, 7.79 (1H each, *br d*, J=16 Hz, H-9,9′)], two mono-substituted benzene rings [δ 7.61 (4H, *m*, H-11,11′,15,5′), 7.40 (6H, *m*, H-12-14 and 12′-14′)], two methyl groups attached to an aromatic ring [δ 2.13 (3H, *s*, H-21′), 2.16 (3H, *s*, H-21)] and three hydroxyl groups [δ 6.87 (1H, *br s*, C-4′-OH), 13.94 (1H, *s*, che-



Scheme 3. Significant NOE interactions in NOESY and NOE difference spectrum of **1** and **2**.



Scheme 4. Plausible biogensis of **1** and **2**.

lated OH, C-6-OH) and 14.37 (1H, *s*, chelated OH, C-6′-OH)]. The ^{13}C NMR spectrum showed the presence of two carbonyl groups at δ 193.0 and 193.5 (C-7,7′). Significant CH long-range correlations in the HMBC spectrum were observed between H-9(9′)/C-7(7′) and H-11,11′(15.15′)/C-9(9′) through ^3J (Scheme 2). Therefore, two cinnamoyl groups existed in **1**. Taking into consideration the UV spectrum and the presence of two chelated hydroxyl groups, **1** had two 2′-hydroxychalcone moieties in its structure. As the unsaturation number of **1** is 23 all oxygens were substituted with different carbons and a three-ring system is formed. In the ^{13}C NMR spectrum, six oxygenated quaternary aromatic carbons were observed at δ 166.4, 162.9, 160.5, 158.5, 154.4 and 153.8. Three of them were assignable to hydroxylated carbons [δ 166.4 (C-6′), 162.9 (C-6) and 160.5 (C-4′)] by ^3J CH long-range correlation in the HMBC spectrum (Scheme 1). Significant CH long-range correlations in the HMBC spectrum were observed between H-16′/C-2′ (δ_C 153.8), H-16′/C-4 (δ_C 158.5), H-16′/C-4′, H-16/C-4 (δ_C 158.5), H-16/C-2 (δ_C 154.4), H-21 (δ_H 2.16)/C-4, H-21/C-6, H-21′ (δH 2.1)/C-4′, H-21′/C-6′ H-17′/C-3 (δ_C 101.0), H-17′/C-3′ (δ_C 98.2), C6-OH/C-1 (δ_C 107.7) and C6′-OH/C-1′ (δ_C 106.0). Thus, the planar structure could be illustrated as shown in Scheme 2. In the difference NOE and NOESY spectrum, important NOE interactions were observed between H-16′/H-17′, H-16′/H-20′, H-20′/H-17β, H-19′/H-16, H-19/H-17β and H-20/H-17α. Therefore, the relative stereochemistry of **1** could be drawn as shown as Scheme 3 (H-16′/H-17′ *cis*; H-17′/H-16 *trans*).

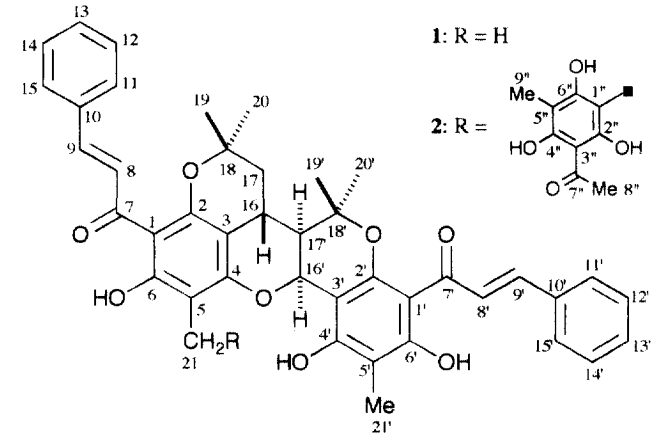
Compound **2** (kamalachalcone B), an orange powder, gave a [M-H]$^-$ at *m/z* 851 in the negative ion FAB-mass spectrum corresponding to the molecular formula $C_{51}H_{48}O_{12}$. The ^{1}H NMR spectrum showed the presence of two sets of *trans*-olefinic protons [δ 8.04 (1H, *d*, *J* = 15 Hz, H-8), 7.86 (1H, *br d*, *J* = 15 Hz, H-9); δ 7.93 (1H, *d*, *J* = 15 Hz, H-8′), 7.79 (1H, *br d*, *J* = 15 Hz, H-9′), two mono-substituted benzene rings [δ 7.61 (4H, *m*, H-11, 11′, 15, 15′), 7.42 (6H, *m*, H-12-14 and 12′-14′) and two chelated hydroxyl groups [δ 15.80 (1H, *s*, C-6-OH), 14.50 (1H, *s*, C-6′-OH)] attributable to two 2′-hydroxychalcone moieties, the same as in **1**. The ^{1}H NMR spectrum also exhibited the presence of a sequence of aliphatic methines and a methylene protons coupled successively in this order [δ 4.93 (1H, *d*, *J* = 4 Hz, H-16′), 2.27 (1H, *dd*, *J* = 4, 5 Hz, H-17′), 2.61 (1H, *dt*, *J* = 13, 5 H-16), 1.98 (1H, *t*, *J* = 13 Hz, H-17a), 2.11 (1H, *dd*, *J* = 13, 5 Hz, H-17β)] and four methyl groups attached to oxygenated quaternary carbons [δ 1.47 (3H, *s*, H-19), 1.49 (3H, *s*, H-19′), 1.62 (3H, *s*, H-20′), 1.67 (3H, *s*, H-20)]. All protonated carbons were assigned by the HMQC spectrum and are listed in Table 1. HMBC analysis showed that spectrum **2** had a similar structure to **1**. However, one of *C*-methyl groups (H-21) in **2** was changed to a benzylmethylene group, which was supported by the NMR spectrum [δ_H 3.78, 3.90 (1H each, *d*, *J* = 16 Hz); δ_C 16.3]. The ^{1}H and ^{13}C NMR spectra further showed the presence of a *C*-methyl group [δ_H 1.97 (3H, *s*, H-9″), δ_C 7.7 (C-9″)], an acetyl group [δ_H 2.51 (3H, s, H-8″); δ_C 33.2 (C-8″), 204.1 (C-7″)] and six quaternary carbons [δC 104.0 (C-1″), 159.2 (C-2″), 105.5 (C-3″), 162.3 (C-4″), 104.4 (C-5″) and 160.0 (C-6″)]. Among the quaternary carbons, three oxygenated carbons were assignable to phlorogucinol nucleus carbons. In the HMBC spectrum, CH long-range correlations were observed between H-21/C-5, H-21/C-6, H-21/C-4, H-21/C-1″, H-21/C-6″, H-9″/C-4″ and H-9″/C-6′. An acetophenone unit (2,4,6-trihydroxy-3-methyl-acetophenone) was then connected with the chalcone moiety through a methylene group. The relative stereochemistry of **2** was determined by NOE experiments. The results are shown in Scheme 2, which are the same as those of **1**.

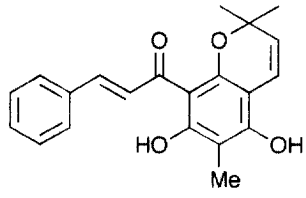
Kamalachalcones A and B have a unique ring system. A plausible biogenetic ring formation undergoes, in the case of **1**, between identical chalcones (**3**) and, in **2**, between **3** and rottlerin (**4**) (Scheme 4). Dimerization by phenol coupling and Diels-Alder reaction are sometimes found in flavonoid compounds [5]. Nevertheless, the dimerization such as between two dimethyl chromene rings and between dimethyl chromene ring and a phenolic hydroxyl group appears to be novel in naturally occurring compounds.

EXPERIMENTAL

Plant material

Kamala was purchased from Caear and Loretz GmBh (Caelo).





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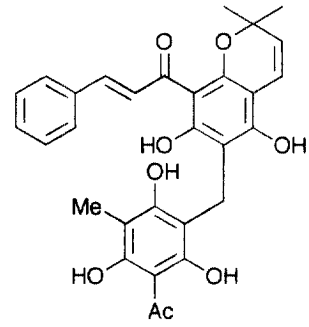

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Extraction and isolation

Kamala (2 kg) was extracted with Me_2CO and MeOH, successively. A part (120 g) of the Me_2CO extract (800 g) was chromatographed on silica gel eluted with $CHCl_3$-MeOH mixts. The $CHCl_3$-MeOH (6:1) fr. gave rottlerin (25 g) by filtration. The filtrate was chromatographed on Sephadex LH 20 eluted with MeOH and the frns with a yellow colour were repeatedly recrystallized from MeOH to give **1** (350 mg) and **2** (65 mg) in a pure form.

Kamalachalcone A (1)

Yellow powder. $[\alpha]_D$ −51° (MeOH, c = 0.06). UV (nm, MeOH): 276, 305sh, 344. Negative ion FABMS: $[M-H]^-$ m/z 671. EIMS m/z (rel. int.): 672 (33, $[M]^+$), 657 (35), 375 (7), 337 (47), 335 (60), 321 (90), 233 (15), 217 (100). UV (nm, MeOH): 276, 305sh, 344. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.50 (3H, *s*, Me, H-19), 1.51 (3H, *s*, Me, H-19′), 1.59 (3H, *s*, Me, H-20), 1.68 (3H, *s*, Me, H-20′), 1.96 (1H, *t*, J = 13 Hz, H-17α), 2.14 (1H, *dd*, J = 13, 5 Hz, H-17β), 2.13 (3H, *s*, Me, H-21′), 2.16 (3H, *s*, Me, H-21), 2.25 (1H, *t*, J = 5 Hz, H-17′), 2.62 (1H, *dt*, J = 13, 5 Hz, H-16), 4.75 (1H, *d*, J = 5 Hz, H-16′), 6.87 (1H, *br s*, C-4′-OH), 7.40 (6H, *m*, H-12-14 and 12′-14′), 7.61 (4H, *m*, H-11,11′,15,15′), 7.78, 7.79 (1H, *br d*, J = 16 Hz, H-9,9′), 7.98, 8.00 (1H each, *d*, J = 16 Hz, H-8,8′), 13.94 (1H, *s*, C-6-OH), 14.37 (1H, *s*, C-6′-OH). ^{13}C NMR (125 MHz): Table 1.

Kamalachalcone B (2)

Orange powder. $[\alpha]_D$ −60° (MeOH, c = 0.12). Negative ion FAB-MS: $[M-H]^-$ m/z 851. UV (nm, MeOH): 240, 296, 345. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.47 (3H, *s*, Me, H-19), 1.49 (3H, *s*, Me, H-19′), 1.62 (3H, *s*, Me, H-20′), 1.67 (3H, *s*, Me, H-20), 1.97 (3H, *s*, Me, H-9″), 1.98 (1H, *t*, J = 13 Hz, H-17α), 2.11 (1H, *dd*, J = 13, 5 Hz, H-17β), 2.19 (3H, *s*, Me, H-21′), 2.27 (1H, *dd*, J = 4, 5 Hz, H-17′), 2.51 (3H, *s*, Me, H-8″), 2.61 (1H, *dt*, J = 13, 5 Hz, H-16), 3.78, 3.90 (1H each, *d*, J = 16 Hz, H-21), 4.93 (1H, *d*, J = 4 Hz, H-16′), 7.42 (6H, *m*, H-12-14 and 12′-14′), 7.61 (4H, *m*, H-11,11′,15,15′), 7.79 (1H, *br d*, J = 15 Hz, H-8), 7.86 (1H, *br d*, J = 15 Hz, H-9), 7.93 (1H, *d*, J = 15 Hz, H-8′), 8.04 (1H, *d*, J = 15 Hz, H-8), 6.17, 8.46, 8.64, 13.72

(1H each, *br s*, OH), 14.50 (1H, *s*, C-6′-OH), 15.80 (1H, *s*, C-6-OH). ^{13}C NMR (125 MHz): Table 1.

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