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Isolation of new monoterpene coumarins from *Micromelum minutum* leaves and their cytotoxic activity against *Leishmania major* and cancer cells

A. Sakunpak [a], K. Matsunami [b], H. Otsuka [b], P. Panichayupakaranant [a,c,*]

[a] Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand
[b] Department of Pharmacognosy, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minamiku, Hiroshima 734-8553, Japan
[c] Phytomedicine and Pharmaceutical Biotechnology Research Center, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand

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ABSTRACT

On the basis of a leishmanicidal assay-guided isolation, two new monoterpene coumarins, minutin A and minutin B, were purified from *Micromelum minutum* leaves together with four known coumarins, 8,4″-dihydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol, 8-hydroxyisocapnolactone-2′,3′-diol, 8-hydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol, and clauslactone E. Among these compounds, minutin A, minutin B, 8-hydroxyisocapnolactone-2′,3′-diol and clauslactone E showed a significant cytotoxic activity against *Leishmania major* with IC_{50} values of 26.2, 20.2, 12.1, and 9.8 μM, respectively, while 8,4″-dihydroxy-3″4″-dihydrocapnolactone-2′,3′-diol and 8-hydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol were not active. However, all these compounds exhibited some inhibitory activity against one or more lung adenocarcinoma (SBC3 and A549) and leukaemia (K562, and K562/ADM) cell lines. Amongst these, clauslactone E, minutin B and 8-hydroxyisocapnolactone-2′,3′-diol possessed the strongest cytotoxic activity against SBC3, A549, K562, and K562/ADM cell lines, with IC_{50} values of 3.7, 10.4, 12.1, and 10.8 μM for clauslactone E; 9.6, 17.5, 8.7 and 6.7 μM for minutin B; 8.8, 10.1, 16.9, and 10.1 μM for 8-hydroxyisocapnolactone-2′,3′-diol, respectively.

1. Introduction

Lung cancer is now the leading cause of cancer deaths in industrialised as well as in developing countries (Jemal et al., 2011). Dietary chemoprevention using vegetables and fruits as an economical, practical, and effective approach for reducing the risk of cancers has been reported (Pan & Ho, 2008; Tan & Spivack, 2009; Vainio & Weiderpass, 2006). Also protection against lung cancer by having a higher dietary intake of vegetables and fruits has been reported in several epidemiological studies (Riboli & Norat, 2003).

Leishmaniasis, a zoonotic protozoan disease caused by *Leishmania*, is still considered a major health problem in the rural areas of the Middle East, Africa, Asia, Europe and Central and South America. Treatment of leishmaniasis remains problematic in developing countries, where it is most often found. Many of the available drugs against the disease are expensive and in certain cases parasite drug resistance has developed (El-Ona, Ozer, Gopas, Sneir, & Golan-Goldhirsh, 2009; Ozer, El-On, Golan-Goldhirsh, & Gopas, 2010; Santos et al., 2008). The development of a new, cheap, effective anti-leishmanial treatment would be extremely beneficial for the treatment and control of the disease.

There is a huge biodiversity in Thai vegetables used in foods and it is possible that some could be a great resource for detecting new anticancer and anti-leishmanial agents. *Micromelum minutum* (G. Forst.) Wight and Arn. (Rutaceae) is an edible plant belonging to the family Rutaceae. In Thailand, it is commonly known as "Mui" and its shoots are often used as fresh vegetables. The leaves of this plant are also traditionally used for the treatment of fever and dizziness (Bulkill, 1966). It has also been reported that the leaves of *M. minutum* contain two coumarin derivatives, 2′,3′-epoxyisocapnolactone and 8-hydroxyisocapnolactone-2′,3′-diol with strong cytotoxic activity against cancer cells (Susidarti et al., 2009; Tan, Alitheen, Yeap, Ali, & Mawardi, 2009). Many other chemicals, some with potential biological activities, have also been isolated, including five other coumarin derivatives, 3″,4″-dihydrocapnolactone, 8-hydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol, 8,4″-dihydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol, 8-methoxycapnolactone; two triterpenes and stigmasterol (Rahmani et al., 2003; Susidarti et al., 2006, 2007, 2009). In a previous investigation we reported antibacterial activity for a methanol extract of *M. minutum* leaves against *Helicobacter pylori*, *Escherichia coli*, *Salmonella typhimurium*, *Salmonella typhi* and *Shigella sonnei* (Sakunpak & Panichayupakaranant, 2012).

* Corresponding author at: Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand. Tel./fax: +66 74 428220.
E-mail address: pharkphoom.p@psu.ac.th (P. Panichayupakaranant).

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In this study, we report a phytochemical study of *M. minutum* leaves based on a leishmanicidal assay-guided isolation, and also report on the cytotoxic activity of some isolated compounds against lung adenocarcinoma (A549 and SBC3) and leukaemia (K562 and K562/ADM) cell lines.

2. Materials and methods

2.1. Plant materials

M. minutum leaves were collected from the Hat-Yai District, Songkhla Province, Thailand, in June 2008. Voucher specimens were deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. The leaves were dried at 50 °C for 24 h in a hot air oven and were then reduced to a coarse powder using a grinder, and the powder was passed through a sieve (No. 45).

2.2. Preparations of plant extracts

The dried leaf powder of *M. minutum* (1 kg) was extracted three times with methanol (3 L × 3) under reflux conditions for 1 h. The extracts were combined and concentrated under reduced pressure to produce the crude methanol extract (250 g).

2.3. Anti-Leishmania major activity

The anti-*Leishmania major* activity against promastigotes was determined by the colorimetric cell viability MTT assay (Macahig, Matsunami, & Otsuka, 2011). The promastigotes obtained from a culture in its logarithmic growth phase in M199 medium supplemented with 10% heat-inactivated foetal bovine serum and 100 μg/mL of kanamycin were used for the assay. In a 96-well plate, 1 μL of the sample solutions (concentration ranges from 0.5 to 100 μg/mL) and *L. major* cells (1×10^5 cells/well) in 100 μL of medium were added to each well and the plate incubated at 27 °C in a 5% CO_2 atmosphere for 48 h. A solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (100 μL) was then added to each well and the incubation was continued for a further 24 h. The formazan product of the MTT reduction was then dissolved in DMSO and an absorbance was measured using a Molecular Devices Versamax tunable microplate reader (Molecular Devices, LLC, Sunnyvale, CA). DMSO was used as a negative control and amphotericin B as a positive control. The experiment was performed in triplicate. The anti-*Leishmania major* activity was quantified as the percentage of the control absorbance of the reduced dye at 540 nm. The inhibitory activity was calculated as:

$$\% \text{ inhibition} = [1 - (A_{test} - A_{blank})/(A_{control} - A_{blank})] \times 100$$

where $A_{control}$ is the absorbance of the control (DMSO) well, A_{test} is the absorbance of the test wells and A_{blank} is the absorbance of the cell-free wells.

2.4. Cytotoxic activity against lung adenocarcinoma and leukaemia cells

The cytotoxic activity against lung adenocarcinoma and leukaemia cell lines was determined by the MTT colorimetric cell viability assay (Macahig et al., 2011). Four lung adenocarcinoma cell lines, A549, SBC3, K562, and K562/ADM, were kindly provided from the JCRB cell bank, Osaka, Japan. SBC3 and A549 cells were cultured in DMEM medium supplemented with 10% heat inactivated FCS, kanamycin (100 μg/mL) and amphotericin B (5.6 μg/mL), while the K562 and K562/ADM cells were cultured in RPMI-1690 medium supplemented with 10% heat-inactivated FCS, and kanamycin (100 μg/mL) and amphotericin B (5.6 μg/mL). In a 96-well plate, 1 μL of the sample solutions (concentration range from 0.5 to 100 μg/mL) and the cancer cells (5×10^3 cells/well) in 100 μL medium were added to each well and the plate incubated at 37 °C in a 5% CO_2 atmosphere for 72 h. One hundred microlitres of the MTT solution were then added to each well and incubation was continued for a further 1 h. The absorbance of each well was measured at 540 nm using a Molecular Devices Versamax tunable microplate reader. DMSO was used as a negative control and doxorubicin as a positive control. The cytotoxic activity was calculated as:

$$\% \text{ inhibition} = [1 - (A_{test} - A_{blank})/(A_{control} - A_{blank})] \times 100$$

where $A_{control}$ is the absorbance of the control (DMSO) well, A_{test} is the absorbance of the test wells and A_{blank} is the absorbance of the cell-free wells.

2.5. Bioassay guided isolation

The crude methanol extract of *M. minutum* leaves was subjected to silica gel vacuum chromatography. The extract (250 g), pre-adsorbed on silica gel, was applied to the top of the silica gel column (13 cm in diameter and 6 cm in height), and the column was subsequently eluted with 500 ml of solvent with the aid of a vacuum pump. Mixtures of hexane and ethyl acetate were used for column elution, using a step-gradient elution starting from 100% hexane to 100% ethyl acetate, followed by mixtures of ethyl acetate and methanol, starting from 100% ethyl acetate to 100% methanol, respectively. Based on the TLC chromatograms of each fraction (500 mL) fifteen pooled fractions (fractions 1–15) were obtained. The fractions were then tested by the leishmanicidal assay. The leishmanicidal active fraction (fractions 4 and 5) were subjected to further purification processes as follows.

Fraction 4 was purified by a reversed-phase ODS column eluted with mixtures of water and methanol, using a step-gradient elution starting from 10% methanol to 100% methanol, to give nine pooled fractions (fractions I–IX). Compounds **1** (21 mg) and **2** (15 mg) were obtained from the leishmanicidal active fraction (fraction VII) after being purified using a semi-preparative RP-C_{18} HPLC column and a mixture of water and methanol (35:65 v/v) as eluent, with a flow rate of 3.0 mL/min. The retention times of compounds **1** and **2** were 40 and 42 min, respectively.

Fraction 5 was purified by a reversed-phase ODS column eluted with mixtures of water and methanol, using a step-gradient elution starting from 10% methanol to 100% methanol, to give thirteen pooled fractions (fractions A–M). Compound **3** (17 mg) was obtained from fraction D after being purified using a semi-preparative RP-C_{18} HPLC column and a mixture of water and acetone (62:38 v/v) as eluent with a flow rate of 3.0 mL/min. The retention time of compound **3** was 30 min.

Compounds **4** (22 mg), **5** (17 mg) and **6** (14 mg) were obtained from fraction E after being purified using a semi-preparative RP-C_{18} HPLC column and a mixture of water and acetone (60:40 v/v) as eluent with a flow rate of 3.0 mL/min. The retention times of compounds **4**, **5** and **6** were 30, 35, and 40 min, respectively.

2.6. Identification of compounds *1* and *2*

Compound **1**; Yellow powder; UV λ_{max}(EtOAc) nm: 251 and 324; IR υ_{max}(KBr) cm^{-1}: 3351 (br) (OH), 2928 (CH_2=CH_2), 1699 (C=O), 1608 (C=C, Ar), 1499–1367 (C=C), 1120 (C–O); HRESI-MS *m*/*z*: 347.1494 $[M+H]^+$ (calc. 347. 1489 for $C_{19}H_{23}O_6$), ^{13}C and ^1H NMR: Table 1.

Compound **2**; Yellow powder; UV λ_{max}(EtOAc) nm: 251 and 324; IR υ_{max}(KBr) cm^{-1}: 3370 (br) (OH), 2927 (CH_2=CH_2), 1694

Table 1
^{13}C NMR (100 MHz) and ^{1}H NMR (400 MHz) data of compounds **1** and **2**.

Position	Compound **1**		Compound **2**	
	$^{\delta}$C ($CDCl_3$)	$^{\delta}$H ($CDCl_3$)	$^{\delta}$C ($CDCl_3$)	$^{\delta}$H ($CDCl_3$)
2	163.0		162.8	
3	109.7	6.10 *d* (9.6)	109.8	6.13 *d* (9.6)
4	140.0	8.01 *d* (9.6)	139.9	8.02 *d* (9.6)
4a	103.9		104.0	
5	156.6		156.7	
6	96.6	6.31 *d* (1.8)	96.5	6.32 *d* (1.9)
7	161.4		161.2	
8	95.8	6.59 *d* (1.8)	95.8	6.59 *d* (1.9)
8a	156.5		156.5	
1′	65.8	4.60 *d* (6.4)	65.8	4.61 *d* (6.2)
2′	119.8	5.50 *t* (6.4)	119.3	5.49 *t* (6.2)
3′	140.5		141.1	
4′	42.2	2.81 *d* (6.1)	35.4	2.13 *m*
5′	128.3	5.70 *overlapped*	28.7	1.74 *overlapped*
6′	135.8	5.70 *overlapped*	89.0	4.32 *m*
7′	82.3		143.4	
8′	24.3 (CH_3)	1.35 *s*	114.5	5.00 *s* and 5.03 *m*
9′	24.3 (CH_3)	1.35 *s*	17.2	1.74 *overlapped*
10′	16.8 (CH_3)	1.63 *s*	16.4	1.74 *overlapped*

The coupling constants (*J* in Hz) are given in parentheses.

(C=O), 1611 (C=C, Ar), 1462–1368 (C=C); HRESI-MS *m*/*z*: 347.1494 $[M+H]^+$ (calc. 347.1489 for $C_{19}H_{23}O_6$); ^{13}C and ^{1}H NMR: Table 1.

3. Results and discussion

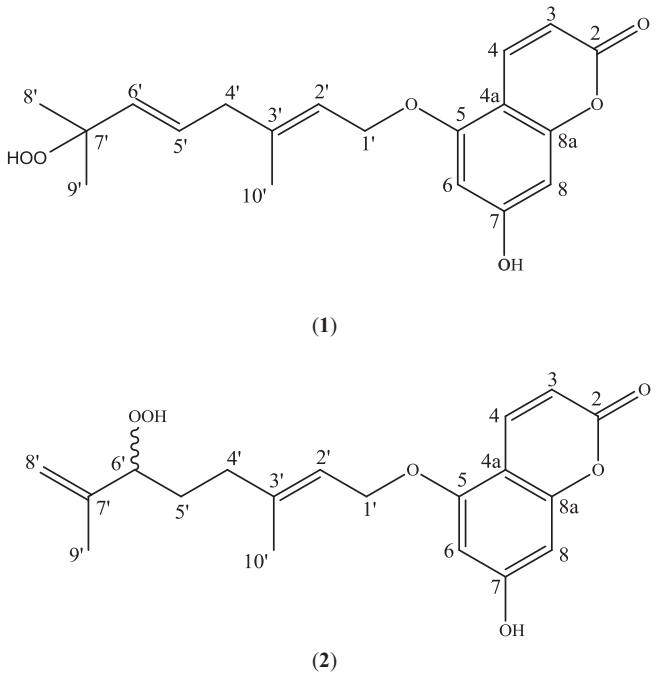
Evaluation of the leishmanicidal activity of the methanol extract of *M. minutum* leaves revealed that the crude extract at 100 μg/mL killed 68% of *L. major*. Further purification of the methanol extract using the leishmanicidal assay-guided isolation, produced two new monoterpene coumarins (compounds **1** and **2**) and four known coumarins (compounds **3**–**6**).

Compound **1** was identified as minutin A or 5-(7′-peroxyl-3′,7′-dimethyl-5′-octaenyloxy)-7-hydroxycoumarin, a novel monoterpene coumarin (Fig. 1) by the following spectroscopic data. The results of the high resolution mass spectrum (HRESIMS) gave a molecular formula of $C_{19}H_{22}O_6$ (*m*/*z*: 347.1494 $[M+H]^+$; calc. 347.1489 for $C_{19}H_{23}O_6$). The unsaturated degree of nine was calculated from the molecular formula. The IR absorption bands at 3400, 1650 and 1493 cm^{-1} confirmed the presence of a hydroxyl group, an ester carbonyl functionality and a conjugated sdouble bond (C=C), respectively. The ^{1}H-NMR spectrum (Table 1) exhibited three aliphatic methyl signals [δ_H 1.35 (3H, *s*); 1.35 (3H, *s*); 1.63 (3H, *s*)], three olefinic signals [δ_H 5.70 (1H, *overlapped*); 5.70 (1H, *overlapped*); 5.50 (1H, *t*)], and two methylene signals [δ_H 2.81 (2H, *d*, *J* = 6.1 Hz); 4.60 (2H, *d*, *J* = 6.4 Hz)]. In addition, there were four sp2 signals, including two doublet signals at δ_H 6.31 and 6.59, with a *m*-coupling (*J* = 1.8 Hz), each with one integrated proton, as well as two doublet signals at 6.10 and 8.01, with an *o*-coupling (*J* = 9.6 Hz), each with one integrated proton. The proton noise decoupled ^{13}C-NMR spectrum (Table 1) showed 19 carbon signals, including one ester carbonyl (δ_C 163.0), seven quaternary carbons, seven methines, two methylene and three methyl carbons. These spectral data indicated a coumarin nucleus substituted with an oxygenated functional group at C-5 and a hydroxyl at C-7.

Interpretation of the ^{1}H–^{1}H COSY indicated that the cross peaks in the aliphatic methylene region gave three partial structures, including fragment **A** [δ_H 2.81 (2H, *d*, *J* = 6.1 Hz, H-4′), 5.70 (1H, *overlapped*, H-5′) and δ_H 5.70 (1H, *overlapped*, H-6′)]; fragment **B** [δ_H 4.60 (2H, *d*, *J* = 6.4 Hz, H-1′), 5.50 (1H, *t*, H-2′)]; and fragment **C**, were the core structures, a coumarin nucleus oxygenated at the C-5 position and bearing a hydroxyl substitute at C-7 (Fig. 2).

The crucial HMBC correlations (Fig. 3) included those from the methyl proton at δ_H 1.63 (3H, *s*, H-10′) to the olefinic proton at C2′ and C4′; therefore, the methyl was substituted on the C-3′. Based on the HMBC data, the correlations between the methylene protons (δ_H 4.60, H-1′) that occurred in the lower magnetic field led to the proposal that C-1′ should be attached with an oxygen atom substituted on the C-5. There were two methyl proton signals that were substituted on the quaternary carbon (C-7′) and showed correlations to C-6′. Hence, the monoterpenoid side chain was proven (fragment D) (Fig. 2). In addition, the peroxide (OOH) contained in the structure of compound **1** was confirmed by the characteristic chemical shift of C-7′ (δ_C 95.8) and a positive result (pink colour) when tested with *N*,*N*-dimethyl-1,4-phenylenediammonium dichloride.

The molecular formula of compound **2** was assigned as $C_{19}H_{22}O_6$ on the basis of the HRESI-MS data (*m*/*z*: 347.1494 $[M+H]^+$; calc. 347.1489 for $C_{19}H_{23}O_6$). The IR spectrum showed absorption bands of a hydroxyl (3370 cm^{-1}) and a carbonyl (1694 cm^{-1}) group, and a carbon–carbon double bond (1611 cm^{-1}), indicating the presence of a 5,7,-dioxygenated coumarin nucleus. The ^{1}H-NMR and ^{13}C-NMR data (Table 1) for the 5,7,-dioxygenated coumarin nucleus were almost identical with those of compound **1**. The data of the long-range correlation between the proton signal at δ_H 4.61 (H-1′) and the carbon signal at δ_C 156.7 (C-5) indicated that there was a side chain substituted at C-5. After assignment of all protons directly bonded with carbon atoms by the ^{1}H–^{13}C shift-correlated measurements (HMQC spectrum), it was possible to assume from the ^{1}H–^{1}H COSY and HMBC that compound **2** was a monoterpene coumarin. The ^{1}H–^{1}H COSY experiment showed the cross peak in the aliphatic methylene region that led to three partial structures, including fragment **A** [δ_H 2.13 (2H, *m*, H-4′), 1.74 (1H, *overlapped*, H-5′), and 4.32 (1H, *m*, H-6′), fragment **B** [δ_H 4.61 (2H, *d*, *J* = 6.2 Hz, H-1′), 5.49 (1H, *t*, *J* = 6.2 Hz, H-2′)]; and fragment **C** (Fig. 4). The HMBC correlations showed correlations between the methyl proton at δ_H 1.74 (3H, *overlapped*, H-9′) and carbon signals at δ_C 89.0 (C-6′) and 114.5 (C-8′), respectively, and between the methyl protons at δ_H 1.74



(**1**)

(**2**)

Fig. 1. Chemical structures of minutin A (**1**) and minutin B (**2**).

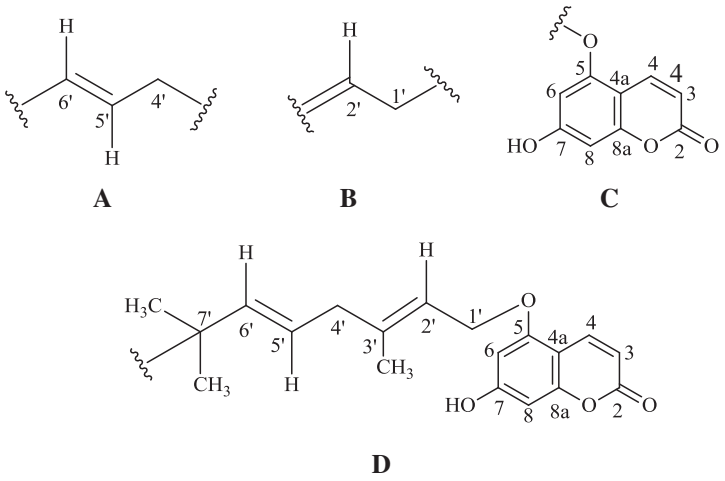


Fig. 2. Proposed partial fragments and structure of compound **1**.

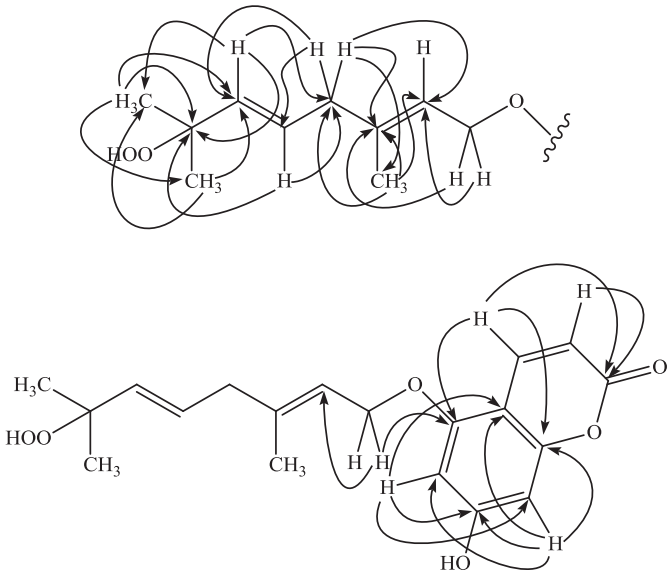


Fig. 3. Importance HMBC correlations of compound **1**.

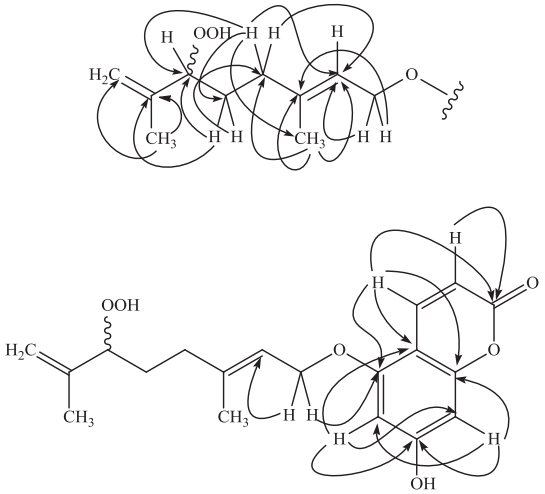


Fig. 5. Importance HMBC correlations of compound **2**.

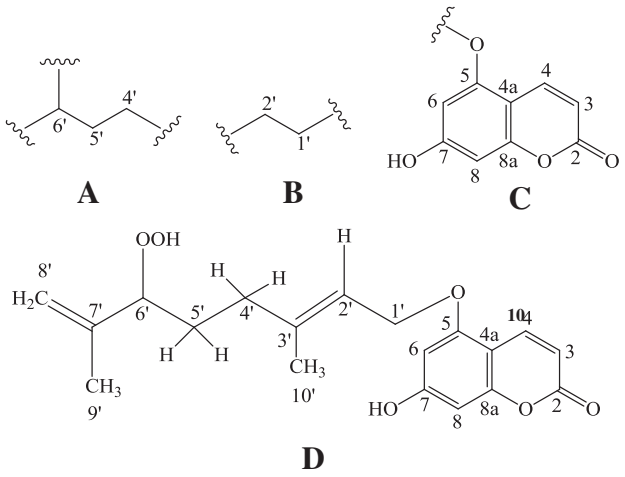
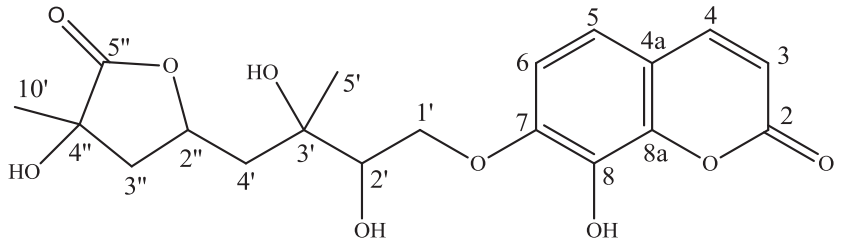


Fig. 4. Proposed partial fragments and structure of compound **2**.

(3H, *overlapped*, H-10′) and carbon signals at δ_C 119.3 (C-2′), 141.1 (C-3′) and 35.4 (C-4′), respectively (Fig. 5). The methyl groups were therefore substituted on C-7′ and C-3′, respectively. The olefinic proton at H-8′ also showed a correlation signal to the carbon at δ_C 89.0 (C-6′). Based on the HMBC data, the methylene proton at δ_H 4.61 (H-1′) that occurred in the lower magnetic field led to a proposal that C-1′ should be attached to an oxygen atom substituted on C-5. On the basis of the NMR, the carbon at position C-6′ showed a lower magnetic field signal at δ_C 89.0. From this it was presumed that this carbon was attached to oxygen and the proposed molecular formula ($C_{19}H_{22}O_6$) required six oxygen atoms. Therefore, in this case probably the peroxide (OOH) was present and connected with the quaternary carbon (C-6′). In addition, the peroxide (OOH) containing structure of compound **2** was also confirmed by a positive result (pink colour) when tested with *N*,*N*-dimethyl-1,4-phenylenediammonium dichloride. The compound **2** was therefore determined to be a novel coumarin, namely minutin B or 5-(6′-peroxyl-3′-methylene-2′-octaenyloxy)-7-hydroxycoumarin (Fig. 1).

Compounds **3** to **6** were identified as the known coumarins 8,4″-dihydroxy-3″,4″-dihydrocapnolactone-2′,3′diol (**3**), 8-hydroxyisocapnolactone-2′,3′- diol (**4**), 8-hydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol (**5**), and clauslactone E (**6**) (Fig. 6) by ^{1}H NMR, ^{13}C



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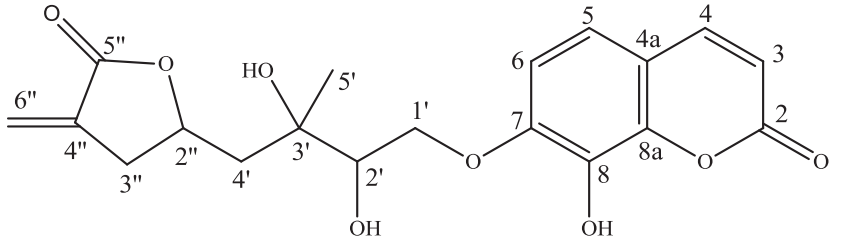

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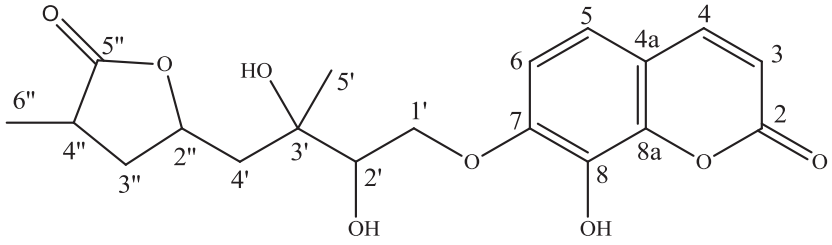

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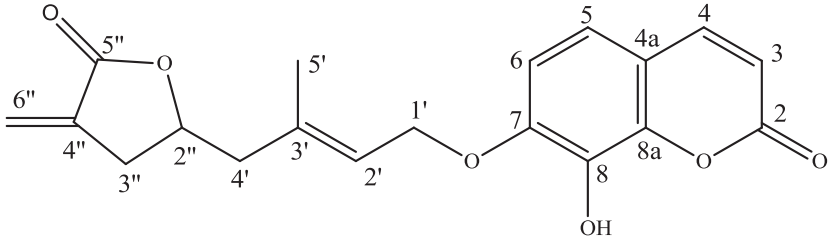

(**6**)

Fig. 6. Chemical structures of 8,4″-dihydroxy-3″,4″-dihydrocapnolactone-2′,3′diol (**3**), 8-hydroxyisocapnolactone-2′,3′- diol (**4**), 8-hydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol (**5**) and clauslactone E (**6**).

NMR, IR and ESI-MS and comparison with data in the literature (Ito, Otsuka, Ruangrungsi, & Furukawa, 2000; Rahmani et al., 2003; Susidarti et al., 2006).

The leishmanicidal activities of these six isolated compounds and amphotericin B are shown in Table 2. Minutin A, minutin B, 8-hydroxyisocapnolactone-2′,3′-diol and clauslactone E showed a significant cytotoxic activity against *Leishmania major*, while 8,4″-dihydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol and 8-hydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol were not active. In contrast all these compounds exhibited inhibitory activity against one or more lung adenocarcinoma and leukaemia cell lines (Table 3). Amongst these, clauslactone E, minutin B and 8-hydroxyisocapnolactone-2′,3′-diol possessed the strongest cytotoxic activity against all tested cancer cell lines, including SBC3, A549, K562, and K562/ADM, with IC_{50} values of 3.7, 10.4, 12.1, and 10.8 μM for clauslactone E; 9.6, 17.5, 8.7 and 6.7 μM for minutin B; 8.8, 10.1, 16.9, and 10.1 μM for 8-hydroxyisocapnolactone-2′,3′-diol,

Table 2
Cytotoxic activity of the isolated compounds against *L. major*.

Compounds	IC_{50} ± S.D. (μM)
Minutin A	26.2 ± 1.36
Minutin B	20.2 ± 2.08
Clauslactone E	9.8 ± 0.49
8-Hydroxyisocapnolactone-2′,3′-diol	12.1 ± 1.01
8,4-Dihydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol	>100
8-Hydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol	>100
Amphotericin B	14.6 ± 1.25

Table 3
Inhibitory effect of the isolated compounds against cancer cells lines.

Compounds	Cancer cell line IC_{50} ± S.D. (μM)			
	SBC3	A549	K562	K562/ADM
Minutin A	23.7 ± 1.06	18.2 ± 1.16	13.2 ± 1.03	9.5 ± 0.38
Minutin B	9.6 ± 0.47	17.5 ± 1.56	8.7 ± 0.48	6.7 ± 0.70
Clauslactone E	3.7 ± 0.89	10.4 ± 1.32	12.1 ± 2.36	10.8 ± 0.41
8-Hydroxyisocapnolactone-2′,3′-diol	8.8 ± 0.22	10.1 ± 0.22	16.9 ± 0.83	10.1 ± 1.41
8,4″-Dihydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol	92.6 ± 4.65	212.9 ± 5.76	n.a	246.6 ± 6.54
8-Hydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol	n.a.	n.a.	n.a.	97.50 ± 1.74
Doxorubicin	<0.4	0.68 ± 0.01	0.68 ± 0.04	>5.5

n.a. = Not active at concentration of 100 μg/mL.

respectively. It has also been reported that 8-hydroxyisocapnolactone-2′,3′-diol was also strongly active against T-lymphoblastic leukaemia (CEM-SS), promyelocytic leukaemia (HL60), cervical cancer (HeLa) and liver cancer (HepG2) cell lines, with IC_{50} values of 2.9, 2.5, 6.9, and 5.9 μg/mL, respectively (Susidarti et al., 2009). In contrast, 8-hydroxyisocapnolactone-2′,3′-diol was inactive when evaluated against the normal mouse fibroblast (3T3) cell line. Hence it could serve as a valuable lead for the further design and synthesis of more active analogues. In addition, clauslactone E, minutin A and minutin B are also potential nutraceutical chemopreventive agents and are worthy of further investigation for their *in vivo* activity and mechanistic effects.

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