# SECONDARY METABOLITES FROM *PEPEROMIA SUI*

*MING-JEN CHENG[1,2*], AND IH-SHENG CHEN[1*]*

*[1]School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan 807, R.O.C.*
*Correspondence: Professor I. S. Chen Ph. D. School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan 807, R.O.C.*
*[2]Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan, R.O.C.*
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## ABSTRACT

One chromone, peperosuione (**1**), together with eighteen known compounds were identified from the whole plant of *Peperomia sui* (Piperaceae). Peperovulcanone A (**2**) isolated firstly from the *P. vulcanica* also obtained from this study, its structure was revised on the basis of spectroscopic evidences. All of the isolates constituents were determined by means of spectral analyses.

**Keywords:** *Peperomia sui*; Piperaceae; Chromone; Peperosuione; Peperovulcanone A.

## INTRODUCTION

The *Peperomia* is the second largest genus in Piperaceae. About 1000 species widely distributed in tropical and subtropical regions; five species are native to Taiwan, namely *P. japonica*, *P. nakaharai*, *P. reflexa*, *P. rubrivenosa*, and *P. sui*, mostly growing on trees or moss-covered rocks. *P. sui* Lin & Lu, an endemic species in Taiwan, is a succulent herb and distributed in forests from low to medium altitudes[1]. Less than 11 species of *Peperomia* has been undergone phytochemical studies in previously researches. Its common constituents are phenylpropanoid, benzopyran, chromone, prenylated quinone, secolignan, and acylcyclohexane-1,3-dione[2–7]. The methanolic extracts of the whole plant of this species showed significant cytotoxicity on high-throughput screening against HONE-1 and NUGC-3 cancer cell lines *in vitro*. In the previous study from the whole plant of this plant, thirty-four compounds including three new polyketides, one new acylresorcinol and thirty known compounds were reported[5]. Carefully examination on this plant has resulted in the isolation of nineteen compounds as additional constituents, including one new compound. We herein reported the isolation, structural elucidation of a new chromone name peperosuione (**1**) and the structural revision of peperovulcanone A (**2**), a previously reported new constituent obtained from *P. vulcanica*.

## EXPERIMENTAL

### General experimental procedures

Melting points were determined with a YANACO micro-melting point apparatus and were uncorrected. IR spectra were taken on a Hitachi 260-30 spectrophotometer. UV spectra were obtained on a JASCO UV-240 spectrophotometer. EIMS spectra were recorded on a VG Biotech Quattro 5022 spectrometer. HREIMS were recorded on a JEOL JMX-HX 110 mass spectrometer. $^1$H NMR and $^{13}$C NMR spectra were measured on a Varian Gemini 200, and Varian Unity Plus 400 spectrometers, and are given in parts per million (δ) downfield from internal TMS. Si gel 60 (Merck 70-230 mesh, 230-400 mesh) was used for column chromatography, and Si gel 60 $F_{254}$ (Merck) for TLC.

### Plant material

Whole plants of *P. sui* were collected from Wutai, Pingtung County, Taiwan, in May 2001. A voucher specimen (Chen 6100) was deposited in the Herbarium of the School of Pharmacy, Kaohsiung Medical University, Taiwan, Republic of China.

### Extraction and separation of compounds

Dried whole plants (8.9 kg) were extracted with MeOH at room temperature, and concentrated in vacuo to leave a brownish viscous residue. The MeOH extract was partitioned between *n*-hexane-$H_2O$ to afford a *n*-hexane extract (fraction A, 50 g) and $H_2O$ layer (fraction C, 350 g). The $H_2O$ layer was then partitioned with EtOAc to give EtOAc extract (fraction B, 25 g), and $H_2O$ layer (fraction C, 350 g)[5].

A part of fraction A (30 g) was chromatographed over Si gel, eluting with a *n*-hexane-EtOAc gradient, to obtain 15 fractions (A1-A15). Fraction A1 (100 mg, *n*-hexane-EtOAc, 50:1) was subjected to Si gel chromatography, eluting with *n*-hexane-EtOAc (20:1) enriched gradually with EtOAc to obtain 5 fractions (A1-1-A1-5). Fraction A1-3 (10 mg) was purified by preparative TLC to give linoleic acid (**11**) (1.9 mg), 2,6-dimethoxy-*p*-quinone (**14**) (2.1 mg), peperosuione (**1**) (1.1 mg), and α-tocopherol (**12**) (3.1 mg). Fraction A3 (950 mg, *n*-hexane-EtOAc, 40:1) was subjected to Si gel chromatography, eluting with *n*-hexane-$Me_2CO$ (20:1) enriched gradually with $Me_2CO$ to obtain 7 fractions (A3-1-A3-7). Fraction A3-4 (110 mg) was purified by preparative TLC to give benzaldehyde (**16**) (12.1 mg), peperovulcanone A (**2**) (1.4 mg), a mixture of ficaprenol-10 and ficaprenol-11 (**19**) (12.9 mg). Fraction A5 (3.5 g, *n*-hexane-EtOAc, 25:1) was subjected to Si gel chromatography, eluting with *n*-hexane-EtOAc (10:1) enriched gradually with EtOAc to obtain 10 fractions (A5-1-A5-10). Fraction A5-8 (100 mg, *n*-hexane-EtOAc, 3:1) was purified by preparative TLC to give 1,2,3-trimethoxy-4,5-dioxo-6a,7-dehydroaporphine (**3**) (1.9 mg). Fraction A7 (2.7 g, *n*-hexane-EtOAc, 20:1) was subjected to Si gel chromatography, eluting with *n*-hexane-$Me_2CO$ (5:1) enriched gradually with $Me_2CO$ to obtain five fractions (A7-1-A-7-5). Fraction A7-3 (50 mg, *n*-hexane-$Me_2CO$, 5:1) was purified by preparative TLC to yield pheophytin-a (**6**) (3.5 mg), pheophytin-b (**7**) (1.7 mg), caryophyllene oxide (**10**) (12.1 mg). Fraction A15 (3.7 g, *n*-hexane-EtOAc, 2:1) was chromatographed over Si gel, eluting with a $CHCl_3$-EtOAc gradient, to obtain 10 fractions (A15-1-A15-10). Fraction A15-5 (1.1 g, *n*-hexane-EtOAc, 1.5:1) was resubjected to Si gel chromatography, eluting with $CHCl_3$-MeOH (10:1) enriched gradually with MeOH to obtain methyl asterrate (**18**) (2.1 mg), and isofraxidin (**4**) (3.1 mg).

Fraction B (25 g) was chromatographed over Si gel, eluting with a $CHCl_3$-MeOH gradient, to obtain 8 fractions (B1-B8). Fraction B3 (5.8 g, $CHCl_3$-MeOH, 50:1) was resubjected to Si gel, eluting with *n*-hexane-EtOAc (50:1) enriched gradually with EtOAc to obtain 10 fractions (B3-1-B3-10). Fraction B3-7 (500 mg, *n*-hexane-EtOAc, 5:1) was purified by preparative TLC to yield peperomin A (**5**) (2.0 mg), and pyropheophorbide (**8**) (2.7 mg). Fraction B8 (4.8 g, $CHCl_3$-MeOH, 10:1), when chromatographed over silica gel with $CHCl_3$ and $CHCl_3$/MeOH solvent mixtures, was followed by recrystallization to give 5-(acetoxymethyl)furfural (**13**).

Part (25 g) of fraction C (350 g) was chromatographed on Diaion HP-20 eluting with $H_2O$, gradually decreasing the polarity with MeOH to afford 10 fractions (C-1-C-10). Fraction C-4 (273 mg) was chromatographed on Si gel, eluting with $CH_2Cl_2$-MeOH (12:1) to afford methyl-α-D-glucopyranoside (**15**) (13.1 mg), and succinic acid (**17**) (2.0 mg).

### Spectroscopic data

**Peperosuione (1)**: Colorless oil. $[\alpha]_D^{25}$: ±0° (*c* 0.09, $CHCl_3$). UV (MeOH)$\lambda_{max}$ (log ε): 239 (3.74), 275 (3.72) nm. IR (Neat) $\nu_{max}$: 3480 (OH), 1650 (C=O), 1615, 1580 (benzene ring) cm$^{-1}$. $^1$H NMR ($CDCl_3$, 400 MHz): δ 0.88 (3H, t, *J* = 6.8 Hz, $CH_3$-17'), 1.25~1.33 (16H, m, H-3'~9', 16'), 1.35 (4H, m, H-10', 15'), 1.47 (2H, m, H-2'), 1.74 (1H, dddd, *J* = 14.0, 5.4, 5.4, 5.4, Hz, H-1'), 1.90 (1H, m, H-1'), 2.01 (4H, m, H-11', 14'), 2.72 (1H, dd, *J* = 17.2, 4.0 Hz, H-3), 2.79 (1H, dd, *J* = 17.2, 11.2 Hz, H-3), 4.50 (1H, ddd, *J* = 11.2, 5.4, 4.0 Hz, H-2), 4.93 (1H, s, OH-8, $D_2O$ exchangeable), 5.35 (2H, br t, *J* = 4.6 Hz, H-12', 13'), 6.42, 7.08 (each 1H, d, *J* = 8.8 Hz, H-6, 7), 10.95 (1H, s, OH-5, $D_2O$ exchangeable). $^{13}$C NMR ($CDCl_3$, 100 MHz): 14.0 (C-17'), 22.4 (C-16'), 24.9 (C-2'), 26.9 (C-11'), 27.2 (C-14'), 29.3~29.8 (C-3'~10'), 32.0 (C-15'), 34.7 (C-1'), 42.6 (C-3), 78.8 (C-2), 108.0 (C-4a), 108.4 (C-6), 124.2 (C-7), 129.8, 129.9 (C-12', 13'), 136.6 (C-8), 146.8 (C-8a), 154.6 (C-5), 197.8 (C-4). EI-MS *m/z* (rel. int): 416 $[M]^+$ (87), 389 (4), 387 (11), 318 (5), 291 (3), 245 (7), 217 (13), 203 (3.5), 181 (10), 179 (83), 153 (100), 125 (2). HRESIMS *m/z* 439.2820 (calcd for $C_{26}H_{40}O_4Na$, 439.2824).

e-mail: m635013@kmu.edu.tw

**Peperovulcanone A (2)**: Colorless oil. UV (MeOH)$\lambda_{max}$ (log ε): 231 (4.91), 327 (4.15) nm. IR (Neat) $\nu_{max}$: 3400 (OH), 1652 (C=O) $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz): δ 0.88 (3H, t, *J* = 6.8 Hz, $CH_3$-19'), 1.25~1.36 (20H, m, H-3'~11', 18'), 1.39 (4H, m, H-12', 17'), 1.73 (2H, q, *J* = 7.8 Hz, H-2'), 2.02 (4H, m, H-13', 16'), 2.61 (2H, t, *J* = 7.8 Hz, H-1'), 5.35 (2H, br t, *J* = 4.8 Hz, H-14', 15'), 6.11 (1H, s, H-3), 6.77 (1H, d, *J* = 8.2 Hz, H-8), 6.86 (1H, dd, *J* = 8.2, 0.4 Hz, H-6), 7.50 (1H, t, *J* = 8.2 Hz, H-7), 12.59 (1H, s, OH-5, $D_2O$ exchangeable). $^{13}C$ NMR ($CDCl_3$, 100 MHz): 14.0 (C-19'), 22.4 (C-18'), 26.8 (C-2'), 26.9 (C-16'), 27.2 (C-13'), 29.0~29.7 (C-3'~11'), 32.0 (C-12', 17'), 34.4 (C-1'), 106.9 (C-6), 108.4 (C-3), 110.1 (C-8a), 111.1 (C-8), 129.8, 129.9 (C-14', 15'), 135.1 (C-7), 156.8 (C-4a), 160.8 (C-5), 171.4 (C-2), 183.1 (C-4). EI-MS *m/z* (rel. int): 426 $[M]^+$ (2), 398 (11), 329 (13), 189 (100), 176 (27), 137 (20), 43 (19). HREIMS *m/z* 426.3133 (calcd for $C_{28}H_{42}O_3$, 426.3136).

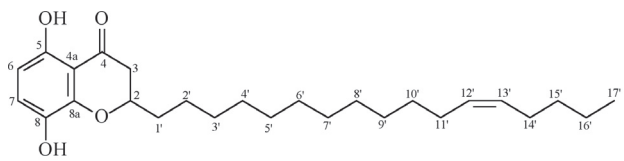
## RESULTS AND DISCUSSION

Peperosuione (**1**) was isolated as colorless oil, and its molecular formula, $C_{26}H_{40}O_4$, was determined by EIMS ($[M]^+$, *m/z* 416) and HRESI mass spectrometry. UV absorption bands at 239 and 275 nm demonstrated that **1** was structurally related to a 2,3-dihydrochromone group[3]. The IR spectrum showed a hydroxyl absorption at 3480 $cm^{-1}$, a conjugated carbonyl at 1650 $cm^{-1}$, and benzene ring at 1615, 1580 $cm^{-1}$. The $^1H$ NMR spectrum for **1** was very similar to that of the 2,3-dihydrochromone, proctorione A[3], except that a C-2 side chain of heptadec-12-enyl group in **1** was in place of a pentadecyl group in proctorione A. Signals for the olefinic protons appeared at δ 5.35 as a broad triplet (*J* = 4.6 Hz), and the signals at δ 129.8 and 129.9 in the $^{13}C$ NMR spectrum also confirmed the presence of a double bond, and its position at C-12' was further confirmed by the mass spectrum which showed the allylic cleavage fragments at *m/z* 43 and 319 (Fig. 2). The presence of main prominent ions at *m/z* 125, 153, 179, 203, 217, 245 can be explained and illustrated in the structural formula (Fig. 2). However, the geometry of the double bond can determine from the dissimilarity in shielding of chemical shifts to allylic carbons in $^{13}C$ NMR spectrum. Usually, the chemical shifts of allylic carbons of linear olefins of *cis*-isomers (*Z*) (δ<28) resonate at about 3 ppm higher field than those of *trans*-isomers (*E*) (δ>31)[8,9]. Because of the chemical shift of δ 26.9 (C-11') and δ 27.2 (C-14'), the geometry of this isolated double bond was determined to be the *cis*-isomers (*Z*). From the above evidence, the structure of **1** was elucidated as 2-((*Z*)-heptadec-12-enyl)-5,8-dihydroxy-chroman-4-one, named peperosuione, which was further confirmed by COSY, NOESY (Fig. 1), HSQC and HMBC (Fig. 1) plot.

Peperovulcanone A (**2**) was obtained as colorless oil. Its molecular formula was established as $C_{28}H_{42}O_3$ by EI-MS ($[M]^+$, *m/z* 426) and HREIMS. The IR spectrum displayed strong absorptions at 3400 $cm^{-1}$ (hydroxyl group) and 1652 $cm^{-1}$ (carbonyl group), which were characteristic of 5-hydroxy-2-alkyl-chromone moiety[10,11] and supported by the UV spectrum ($\lambda_{max}$ at 231 and 327 nm). The structure of 5-hydroxy-2-alkyl-chromone moiety was confirmed by the $^1H$ NMR spectrum of **2**, which contained three mutually coupling protons belong an ABC-type system at δ 6.77 (1H, d, *J* = 8.2 Hz), 6.86 (1H, dd, *J* = 8.2, 0.4 Hz) and 7.50 (1H, t, *J* = 8.2 Hz) corresponding to H-8, 6 and 7, one deshielding olefinic proton at δ 6.11 (1H, s) and a singlet at δ 12.59 (1H, s) ascribable to a chelated hydroxyl group at C-5. A 14'-nonadecenyl group [δ 0.88 (3H, t, *J* = 6.8 Hz, H-19'), 1.25~1.36 (20H, m, H-3'~11', 18'), 1.39 (4H, m, H-12', 17'), 1.73 (2H, quin, *J* = 7.5 Hz, H-2'), 2.02 (4H, m, H-13', 16'), 2.61 (2H, t, *J* = 7.8 Hz, H-1'), 5.53 (2H, br t, *J* = 4.8 Hz, H-14', 15')] was observed in the high field aliphatic region. The COSY spectrum revealed that the deshielded methylene δ 2.61 (H-1') was coupled into the methylene envelope via a $CH_2$ group at δ 1.73 (H-2'), then coupled with the signals at δ 1.25~1.36 (H-3'~11'). The signals at δ 1.39 (4H, m) of H-12' and 17' were coupled with the signal at δ 1.25~1.36 of H-11' and H-18', and the signals at δ 2.02 (4H, m) of H-13' and 16'. Two olefinic signals at δ 129.8 and 129.9 in the $^{13}C$ NMR spectrum proved the presence of a double bond along with the long chain, and its position at C-14' was further confirmed by the mass fragmentation (Fig. 2) which showed the fragments *m/z* 43, 329 through allylic cleavage at C-14'-15'. However, the *cis*-geometry of a double bond could also be deduced from chemical shifts of δ 27.2 (C-13') and δ 26.9 (C-16'). The alkyl chain suggested to be located at C-2 by the NOESY correlations between H-1'/H-3 (Fig. 1) and the key correlations of H-1'/C-2, 3 were also observed in the HMBC experiment (Fig. 1) can further support the position of 14'-nonadecenyl group was placed at C-2. The fragment at *m/z* 161 in **2** also confirmed the basic moiety as 5-hydroxy-2-methyl-chromone (Fig. 2). The structure was further confirmed by COSY, NOESY (Fig. 1), HSQC, and HMBC (Fig. 1) experiments. Compound **2** was identical with peperovulcanone A, which was reported as a new compound with a *E*-geometry from *P. vulcanica* by Tane et al[4]. According to the above spectroscopic evidences and the $^{13}C$-NMR chemical shift of C-13' and C-16', peperovulcanone A [5-hydroxy-2-(14'-(*E*)-nonadecenyl)-4*H*-chromen-4-one] must be revised as the 5-hydroxy-2-(14'-(*Z*)-nonadecenyl)-4*H*-chromen-4-one.

The known compounds included one aporphine alkaloid: 1,2,3-trimethoxy-4,5-dioxo-6a,7-dehydroaporphine (**3**)[12]; one coumarin: isofraxidin (**4**)[13]; one secolignan: peperomin A (**5**)[14]; three chlorophylls: pheophytin-a (**6**)[15], pheophytin-b (**7**)[16], pyropheophorbide (**8**)[17]; one diterpene: phytol acetate (**9**)[18]; one sesquiterpene: caryophyllene oxide (**10**)[19]; and nine other compounds: linoleic acid (**11**)[20], α-tocopherol (**12**)[21], 5-(acetoxymethyl)furfural (**13**)[22], 2,6-dimethoxy-*p*-quinone (**14**)[23], methyl-α-D-glucopyranoside (**15**)[24], benzaldehyde (**16**)[25], succinic acid (**17**)[26], methyl asterrate (**18**)[27], mixture of ficaprenol-10 and ficaprenol-11 (**19**)[28]. These compounds were identified by comparison of their spectral data (UV, IR, $^1H$ NMR, MS) with the data from the corresponding values in the literature, or with authentic samples. Among them, all known compounds except **3** and **5,** were isolated for the first time from this plant.

Many bioactive compounds, including ployketides, lignans and chromenes, isolated from *Peperomia* species have been reported in recent studies[2–7]. Most compounds possessed cytotoxicity activity. It's to deserve to be mentioned, a rare type of secolignan only appeared in *Peperomia* species like peperomins A~C, also isolated in this study, were evaluated for their inhibitory activities against HIV-1 in infect C8166 cells in recent studies by Li's group[29].



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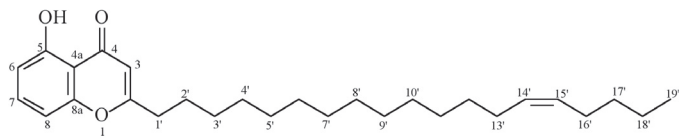

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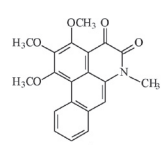

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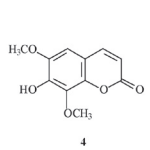

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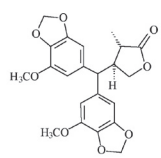

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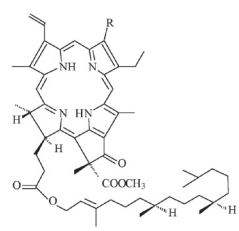
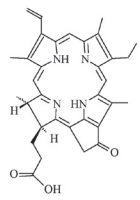


**6** R=$CH_3$
**7** R=CHO



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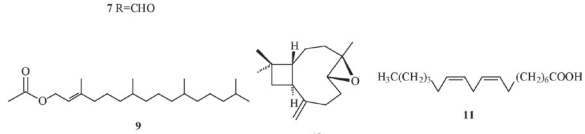


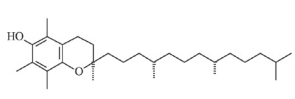
**9**



**10**



**11**



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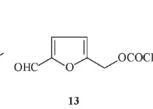

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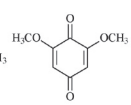

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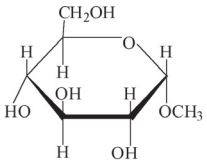

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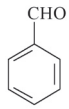

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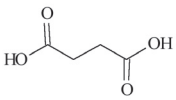

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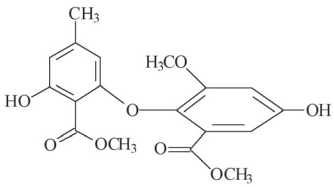

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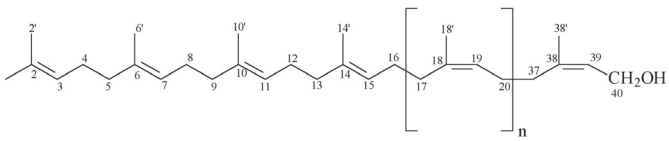
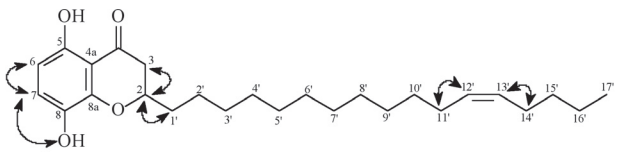


ficaprenol-10 n=4
ficaprenol-11 n=5 (**19**)



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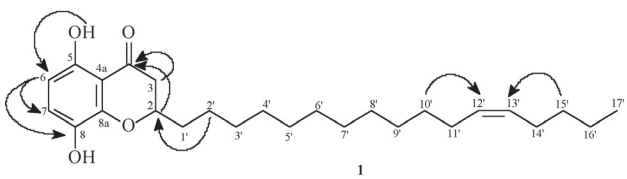

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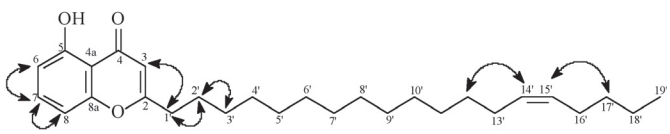
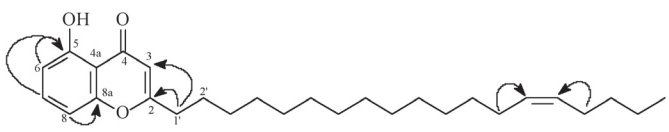
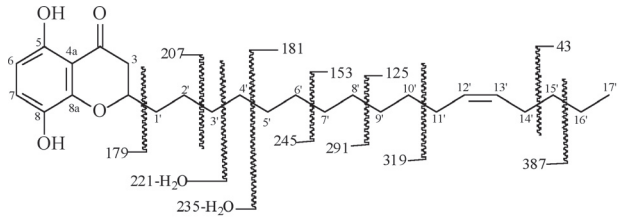


**2**

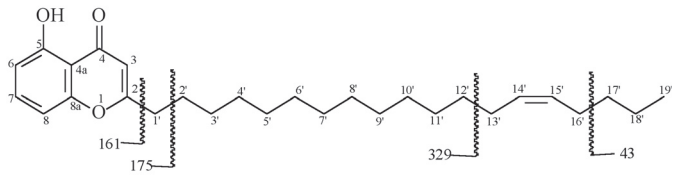


**2**

Fig. 1. NOESY ( ) and HMBC ( ) correlations of **1** and **2**.



**1**



**2**

Fig. 2. Mass fragments ions of **1** and **2**.

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