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Phytochemistry 63 (2003) 603–608
PHYTOCHEMISTRY
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Chemical and cytotoxic constituents from *Peperomia sui*

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Received 10 December 2002; received in revised form 20 February 2003

Abstract

Three polyketide compounds, surinone A, surinone B, surinone C and one acylresorcinol, suranone, along with thirty known compounds, were isolated from the whole plant of *Peperomia sui*. Their structures were elucidated from spectral analysis. Several compounds showed cytotoxic activity against HONE-1 and NUGC-3 cell lines in vitro.

Keywords: *Peperomia sui*; Piperaceae; Whole plant; Polyketide; Surinone A; Surinone B; Surinone C; Acylresorcinol; Suranone

1. Introduction

Peperomia sui Lin & Lu (Piperaceae), an endemic species in Taiwan, is a succulent herb growing on wet rocks and trees in forests from low to medium altitudes (Lin and Lu, 1996). No previous phytochemical study has been made on this species, although the genus *Peperomia* has undergone some phytochemical studies. Its common constituents are: phenylpropanoid, benzopyran, chromone, prenylated quinone and acylcyclohexane-1,3-dione (Monache and Compagnone, 1996; Govindachari et al., 1998; Bayma et al., 2000; Seeram et al., 2000; Villegas et al., 2001). 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. Investigation of the whole plant led to the isolation of four new compounds, surinone A (**1**), surinone B (**2**), surinone C (**3**) and suranone (**4**), along with thirty known compounds. The isolation, structural elucidation, and cytotoxicity of the isolated compounds are described herein.

2. Results and discussion

Surinone A (**1**) was isolated as a yellowish oil, whose molecular formula, $C_{28}H_{40}O_6$, was established by EIMS ($[M]^+$, m/z 472) and HREI mass spectrometry. Infrared absorptions at 3438; 1657; 1564; 1590, 1494; 1037 and 940 cm^{-1} provided evidence for hydroxyl, conjugated carbonyl, conjugated chelated carbonyl, benzene ring and methylenedioxy functionalities (Azevedo et al., 1997). The UV spectrum exhibited maxima at 234 and 270 nm, indicating the presence of the cyclic polyketone group, 'β-triketone' (Azevedo et al., 1997). Addition of KOH caused enhancement of the peak at 270 nm and disappearance of the peak at 234 nm, similar to that of acylcyclohexane-1,3-dione (Azevedo et al., 1997). The ^1H NMR spectrum showed a typical cyclohexane-1,3-dione moiety [δ 4.09 (1H, *dd*, $J=13.2$, 5.4 Hz, H-4), 1.82 (1H, *m*, H-5a), 2.38 (1H, *m*, H-5b), 2.79 (2H, *m*, H-6)]. An α-methylene resonance of a ketone [δ 2.97 (1H, *ddd*, $J=16.0$, 8.5, 6.5 Hz, H-2′a), 3.07 (1H, *ddd*, $J=16.0$, 8.5, 6.5, H-2′b)], signals of β-methylene protons of ketone and homobenzylic methylene [δ 1.52–1.66 (4H, *m*, H-3′ and H-14′)], a resonance of a benzylic proton [δ 2.51 (2H, *t*, $J=8.0$ Hz, H-15′), and signals of alkyl long chain moiety [δ 1.25–1.37 (20H, *m*, H-4′-13′)] were also observed, in addition to a 3,4-methylenedioxyphenyl group [δ 5.91 (2H, *s*, OCH_2O), 6.62 (1H, *dd*, $J=7.8$, 1.5 Hz, H-21′), 6.67 (1H, *d*, $J=1.5$ Hz, H-17′), 6.71 (1H, *d*, $J=7.8$ Hz, H-20′)]. The signal of a H-bonded hydroxyl group appeared at δ 18.27 (1H, *s*, OH-1). According to the above data, and with a negative $[\alpha]_D$ value −15.8° (*c* 0.070, $CHCl_3$), the structure of **1** was elucidated as (−)-2-(15-benzo[1,3]dioxol - 5 - yl - pentadecanoyl) - 3,6 - dihydroxy-cyclohex-2-enone. To further confirm its structure, sequential correlations of the NOESY and

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doi:10.1016/S0031-9422(03)00183-3

HMBC plots were successfully established as shown in Figs. 1 and 2. The stereochemistry of **1** was determined by large coupling constant ($J=13.2$ Hz) between H-5 (ψ-axial) and H-4, implying that OH-4 is ψ-equatorial (Azevedo et al., 1997).

Surinone B (**2**) was isolated as a yellowish oil, when molecular formula, $C_{25}H_{36}O_3$ was determined by EIMS ($[M]^+$, m/z 384) and HREI mass spectrometry. UV absorption bands at 233 and 271 nm demonstrated that **2** was also structurally related to a cyclic polyketone group (Azevedo et al., 1997). The IR spectrum showed a hydroxyl absorption at 3453 cm^{-1}, a conjugated carbonyl at 1664 cm^{-1}, and a conjugated chelated carbonyl group at 1576 cm^{-1}. The ^1H NMR spectrum for **2** was similar to that of a polyketide, oleiferinone (Azevedo et al., 1997), also isolated in this study, except that an oxymethine proton [δ 3.95 (1H, *dd*, $J=13.0$, 5.5 Hz, H-4)] and a hydroxyl group [δ 4.03 (1H, *br s*, OH-4)] in oleiferinone were replacing a methylene group [δ 2.49 (2H, *t*, $J=6.6$ Hz, H-4)] in **2**. Thus, the structure of **2** was elucidated as 3-hydroxy-2-(13-phenyltridecanoyl)-cyclohex-2-enone, which was further confirmed by NOESY experiment (Fig. 2).

Surinone C (**3**) was isolated as a colourless gum, and its molecular formula, $C_{26}H_{44}O_4$, was determined by EIMS ($[M]^+$, m/z 420) and HRFAB mass spectrometry. UV absorption bands at 233 and 271 nm demonstrated that **3** was also structurally related to a cyclic polyketone group (Azevedo et al., 1997). The IR spectrum was similar to that of surinone A (**1**) and showed a hydroxyl absorption at 3453 cm^{-1}, a conjugated carbonyl at 1665 cm^{-1}, and a conjugated chelated carbonyl group at 1576 cm^{-1}. The ^1H NMR spectrum for **3** was similar to that of the polyketide, proctorione C (**5**) (Seeram et al., 2000), also isolated in this study, except that a C-2 side chain of eicos-14-en-1-one group in **3** was in place of an octa-11-en-1-one in proctorione C (**5**). Two olefinic signals at δ 129.8 and 129.9 in the ^{13}C NMR spectrum suggested the presence of a double bond, and its position at C-14′ was further confirmed by the mass spectrum (Fig. 3) which showed fragments at m/z 43, 57, 153, 167, 181, 209, and 309. The fragments at m/z 127, and 155 also confirmed the basic moiety as 2-acylcyclohexane-1,3-dione (Fig. 3). However, the *cis*-geometry of a double bond could also be deduced from chemical shifts of δ 27.12, 27.18 (C-16′ and C-13′) (Mizutani et al., 1988). The stereochemistry of **3** was also determined by a large coupling constant ($J=12.9$ Hz) between H-5 (ψ-axial) and H-4, implying that OH-4 is ψ-equatorial. From the above evidence and with a negative [α_D] value −29.1° (*c* 0.075, $CHCl_3$), the structure of **3** was elucidated as (*Z*)-(−)-3,6-dihydroxy-2-icos-14-enoyl-cyclohex-2-enone, which was further confirmed by COSY, NOESY (Fig. 1), and a HSQC plot.

Suranone (**4**) was isolated as a yellowish oil, and its molecular formula, $C_{26}H_{44}O_4$, was determined by EIMS ($[M]^+$, m/z 374) and HREI mass spectrometry. The UV spectrum exhibited maxima at 223, 270 and 342 nm, indicating the presence of an acylresorcinol skeleton (Gonzalez et al., 1996; Kato et al., 1985). The IR spectrum showed absorptions due to hydroxyl group at 3368 cm^{-1}, a conjugated carbonyl group at 1627 cm^{-1}, and a benzene group at 1590, 1513 cm^{-1}. The ^1H NMR spectrum showed a resorcinol unit (Gonzalez et al., 1996) [δ

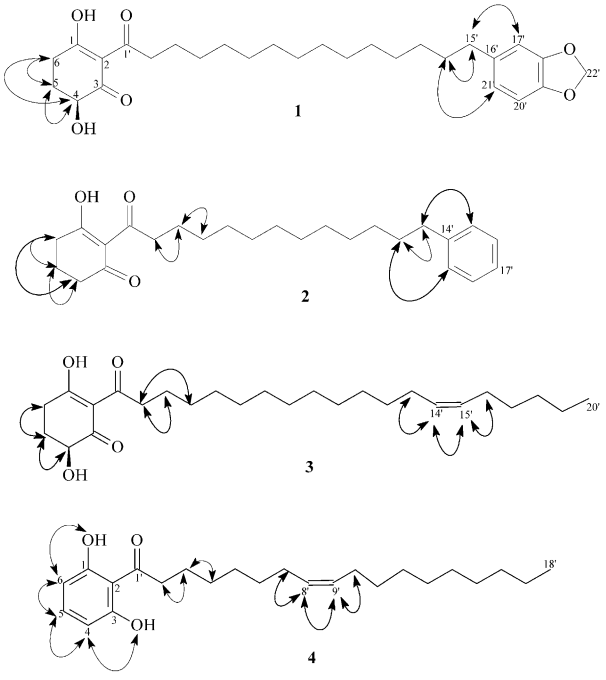


Fig. 1. NOESY correlations of compounds **1–4**.

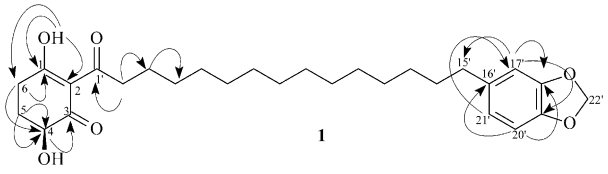


Fig. 2. HMBC correlations of compound **1**.

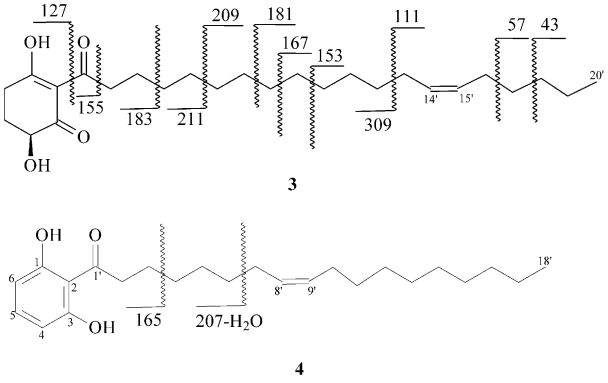


Fig. 3. Mass fragment ions of compounds **3** and **4**.

7.22 (1H, *t*, *J* = 8.4 Hz, H-5), 6.39 (2H, *d*, *J* = 8.4 Hz, H-4, 6)] with two chelatable hydroxyl groups [δ 9.60 (2H, *br s*, OH)]. An α-methylene signal of a ketone was observed at δ3.13 (2H, *t*, *J* = 7.2 Hz, H-2′), and resonances of β-methylene protons of a ketone were assigned at δ 1.70 (2H, *q*, *J* = 7.2 Hz, H-3′). Additional signals at δ 5.35 (2H, m, H-8′, 9′) were attributed to a double bond in a long aliphatic chain [δ 1.25–1.33 (20H, *m*, CH_2-4′–6′ and 11′–17′)]. Fragment at *m*/*z* 189 [207–H_2O]$^+$ (Fig. 3) due to allylic cleavage of C 8′-9′ confirmed the nature of the side chain and the position of the double bond. Based on the earlier data, the structure of **4** was elucidated as 1-(2,6-dihydroxyphenyl)-octadec-8-en-1-one and was further confirmed by ^{13}C NMR, DEPT, COSY, NOESY1D (Fig. 1) and HSQC experiments.

The remaining compounds were all of previously known structures: eugenol (Pouchert and Behnke, 1993a–c), phytol (Tsai et al., 2001), β-bisabolol (Cheng et al., 2002), proctorione C (Seeram et al., 2000), peperomin B (Chen et al., 1989; Jan, 1984; Sibi et al., 2001), peperomin E (Govindachari et al., 1998), methyl hexdecanoate (Seki et al., 1994), 5-hydroxymethylfurfural (Pouchert and Behnke, 1993a–c), peperomin C (Chen et al., 1989), oleiferinone (Azevedo et al., 1997), (+)-pinoresinol (Katayama et al., 1993), a mixture of *β*-sitosterol and stigmasterol (Cheng et al., 2001), vanillic acid (Cheng et al., 2001), (+)-sesamin (Cheng et al., 2001), methylparaben (Wu et al., 2000), 5-hydroxy-4′,7,8-trimethoxyflavone (Savona et al., 1982), 5-hydroxy-3′,4′,7,8-tetramethoxyflavone (Shaw et al., 1988), 5-hydroxy-4′,7-dimethoxyflavone (Achari et al., 1990), cepharadione B (Desai et al., 1988), phaeophorbide-a-methyl ester (Nakatani et al., 1981), salidroside (Hase et al., 1995), betulinic acid (Peng et al., 1998), methyl-21-hydroxy-(21*S*)-pheophorbide-b (Nakatani et al., 1981), *N-cis*-feruloyltyramine (Chang et al., 2001), *N-trans*-feruloyltyramine (Chang et al., 2001), (+)-ascorbic acid (Pouchert and Behnke, 1993a–c), a mixture of β-sitosteryl-3-*O*-β-D-glucoside and β-stigmasteryl-3-O-β-D-glucoside (Cheng et al., 2001), and (+)-hinokinin (Chen et al., 1994). These compounds were identified by comparison of their spectral (UV, IR, ^1H NMR, and MS) and/or mp data with those of corresponding authentic samples or data from the literature.

The cytotoxic activity of the following 13 compounds: **1**, **3**, **4**, peperomin C, oleiferinone, (+)-pinoresinol, peperomin B, proctorione C (**5**), peperomin E (**6**), methyl hexdecanoate, 5-hydroxymethylfurfural, 5-hydroxy-4′,7,8-trimethoxyflavone, and (+)-hinokinin were tested in vitro against HONE-1 and NUGC-3 cell lines. Preliminary biological study revealed that polyketide compounds **1**, **3**, and **5**, a secolignan **6** and a sesquiterpenoid β-bisabolol (**7**) (Cheng et al., 2002) showed significant cytotoxicity (31, 38; 39, 27; 0, 0; 5, 1; 0, 0%) interpreted by percentage of cell growth, respectively) at concentrations of 50 μM, whereas other compounds were ineffective against these two cell lines. Among these five compounds, **5** and **7** (Cheng et al., 2002) showed marginal activity, the former with IC_{50} values of 8.08, 10.3 μg/ml.

3. Experimental

3.1. General

Mps are uncorr. ^1H NMR (600, 500, 400 and 200 MHz) and ^{13}C NMR (150, 125, 100 and 50 MHz) were taken in $CDCl_3$. Chemical shifts are given in δ with TMS as int. standard. EI-mass spectra were recorded on a VG Biotech Quattro 5022 spectrometer. HR-mass spectra were recorded on a Jeol JMX-HX 110 spectrometer. Optical rotations were measured using a Jasco P-1020 polarimeter in $CHCl_3$. All melting points were determined on a Yanaco micro-melting point apparatus and were uncorrected. IR spectra were taken on a Genesis II FTIR spectrophotometer. UV spectra were obtained on a Shimadzu UV-160A spectrophotometer in EtOH. Silica gel (60–230, 230–400 mesh) (Merck) was used for CC and silica gel 60F-254 (Merck) for prep. TLC.

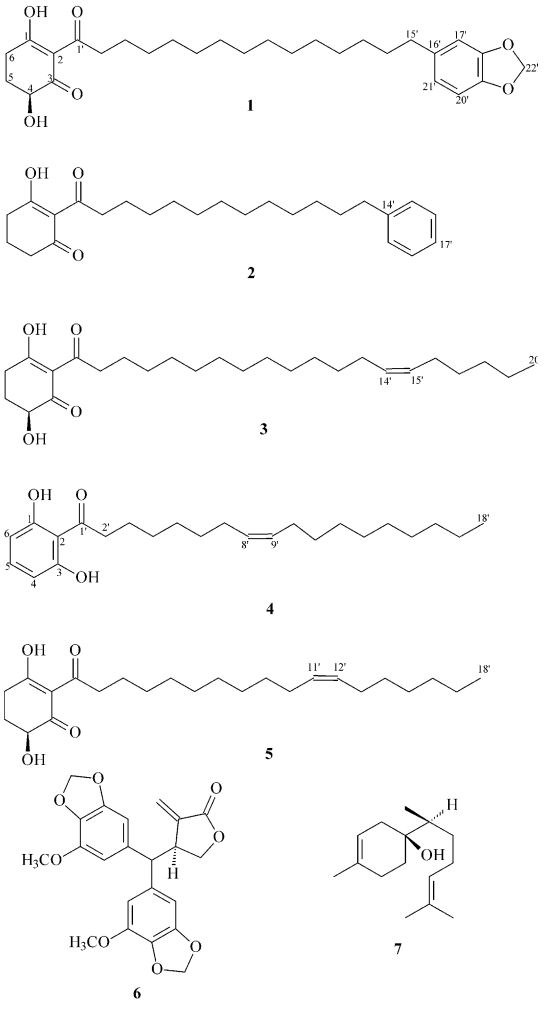
3.2. Plant material

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.

3.3. Extraction and isolation

Dried whole plants (8.9 kg) were extracted with MeOH (40 L) at room temperature (14 days), and concentrated in vacuo to leave a brownish viscous residue. The MeOH extracts was partitioned between *n*-hexane–H_2O and EtOAc–H_2O. The organic layers were concentrated under reduced pressure to yield a *n*-hexane extract (fraction A, 50 g) and an EtOAc extract (fraction B, 25 g) respectively. The *n*-hexane and EtOAc extracts showed significant cytotoxicity against HONE-1 and NUGC-3 cancer cell lines. A part of fraction A (30 g) applied to a Si gel column, eluted 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 five fractions (A1-1–A1-5). Fraction A1-3 (10 mg, *n*-hexane–EtOAc, 20:1) was purified by preparative TLC to give phytol (7 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 seven fractions (A3-1–A3-7). Fraction A3-4 (110 mg, *n*-hexane–Me_2CO, 15:1) was purified by preparative TLC to give eugenol (13 mg), phytol (11.3 mg), and β-bisabolol (20 mg). Fraction A5 (3 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 A5-3 (220 mg, *n*-hexane–EtOAc, 10:1) was purified by preparative TLC to give proctorione C (50 mg), peperomin B (**5**) (20 mg), peperomin E (10 mg), methyl hexdecanoate (12 mg) and 5-hydroxymethylfurfural (3 mg). Fraction A5-8 (100 mg, *n*-hexane–EtOAc, 3:1) was purified by preparative TLC to give surinone A (**1**) (5.1 mg), surinone C (**3**) (5.5 mg) and peperomin C (4.3 mg). Fraction A7 (2 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–A7-5). Fraction A7-3 (50 mg, *n*-hexane–Me_2CO, 5:1) was purified by preparative TLC to yield oleiferinone (5.3 mg), surinone B (**2**) (1.2 mg) and (+)-pinoresinol (10 mg). Fraction A15 (3.7 g, *n*-hexane–EtOAc, 2:1) was applied to a Si gel column, 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 mixture of β-sitosterol and β-stigmasterol (50 mg), vanillic acid (5.1 mg), suranone (**4**) (5.6 mg) and (+)-sesamin (10.2 mg). Fraction B (25 g) applied was Si gel column, eluting with a $CHCl_3$–MeOH gradient, to obtain eight fractions (B1–B8). Fraction B-1 (2.0 g, $CHCl_3$–MeOH, 50:1) was resubjected to Si gel chromatography, eluting with *n*-hexane–EtOAc (30:1) enriched gradually with EtOAc to obtain 10 fractions (B1-1–B1-10). Fraction B1-3 (100 mg, *n*-hexane–EtOAc, 10:1) was resubjected to Si gel CC and purified by preparative TLC (*n*-hexane–Me_2CO, 8:1) to yield proctorione C (25 mg), peperomin B (**5**) (5.1 mg), methyl hexdecanoate (3.1 mg) and 5-hydroxymethylfurfural (4.1 mg). Fraction B3 (5 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 B3-2 (1.1 g, *n*-hexane–EtOAc, 20:1) was resubjected to Si gel CC and purified by preparative TLC (cyclohexane–EtOAc, 3:1) to yield peperomin E (2.0 mg), methylparaben (2.6 mg), 5-hydroxy-4′,7,8-trimethoxyflavone (2.5 mg) and 5-hydroxy-3′,4′,6,7-tetramethoxyflavone (1.6 mg). Fraction B3-7 (500 mg, *n*-hexane–EtOAc, 5:1) was purified by preparative TLC to yield 5-hydroxy-4′,7-dimethoxyflavone (2.1 mg), cepharadione B (1.1 mg) and pheophorbide-a-methyl ester (5.1 mg). Fraction B8 (4.8 g, $CHCl_3$–MeOH, 10:1), when applied to a silica gel column, eluted with $CHCl_3$ and $CHCl_3$/MeOH solvent mixtures, was followed by recrystallization to give salidroside (8.2 mg), betulinic acid (5.7 mg), methyl-21-hydroxy-(21*S*)-pheophorbide-b (5.1 mg), *N*-*cis*-feruloyl-tyramine (1.3 mg), *N*-*trans*-feruloyltyramine (10.3 mg), (+)-ascorbic acid (30 mg), β-sitosteryl-3-O-D-glucoside, β-stigmasteryl-3-O-β-D-glucoside (9.5 mg) and (+)-hinokinin (4.6 mg).



3.4. Surinone A (*1*)

Yellowish oil; $[\alpha]_D^{25}$ −15.8° (*c* 0.070, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 234 (4.10), 270 (4.67) nm, +KOH (log ε) 268 (4.78) nm; IR (Neat) ν_{max} 3438 (OH), 1657 (conjugated C=O), 1564 (conjugated chelated C=O), 1590, 1494 (benzene ring), 1037, 940 (OCH_2O) cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): δ1.25–1.37 (20H, *m*, H-4′–13′), 1.52–1.66 (4H, *m*, H-3′, 14′), 1.82 (1H, *m*, H-5a), 2.38 (1H, *m*, H-5b), 2.51 (2H, *t*, *J*=7.2 Hz, H-15′), 2.79 (2H, *m*, H-6), 2.97 (1H, *ddd*, *J*=16.0, 8.5, 6.5 Hz, H-2′a), 3.07 (1H, *ddd*, *J*=16.0, 8.5, 6.5 Hz, H-2′b), 4.03 (1H, *br s*, OH-4, D_2O exchangeable), 4.09 (1H, *dd*, *J*=13.2, 5.4 Hz, H-4), 5.91 (2H, *s*, OCH_2O), 6.62 (1H, *dd*, *J*=7.8, 1.5 Hz, H-21′), 6.67 (1H, *d*, *J*=1.5 Hz, H-17′), 6.71 (1H, *d*, *J*=7.8

Hz, H-20′), 18.27 (1H, *s*, OH-1, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 150 MHz): 24.5 (C-3′), 27.1 (C-5), 29.2–29.6 (C-4′-13′), 31.3 (C-6), 31.8 (C-14′), 35.7 (C-15′), 40.3 (C-2′), 71.6 (C-4), 100.7 (OCH_2O), 108.0 (C-20′), 108.8 (C-17′), 110.3 (C-2), 121.0 (C-21′), 136.8 (C-16′), 145.3 (C-18′), 147.4 (C-19′), 195.6 (C-3), 197.9 (C-1), 206.1 (C-1′); EIMS *m*/*z* 472 $[M]^+$ (4.7), 444 (14.0), 183 (12.0), 148 (11.4), 137 (15.3), 136 (27.1), 135 (100), 105 (11.0), 91 (26.7), 85 (12.8), 84 (13.5), 77 (16.8); HREIMS *m*/*z* 472.2819 (calc. for $C_{28}H_{40}O_6$ 472.2813).

3.5. Surinone B (2)

Yellowish oil; UV (MeOH) λ_{max} (log ε) 233 (4.10), 271 (4.44) nm; IR (Neat) ν_{max} 3026 (OH), 1667 (conjugated C=O), 1546 (conjugated chelated C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ1.26 (16H, *br s*, H-4′–11′), 1.61 (4H, *quint*, H-3′, 12′), 1.98 (2H, *quint*, *J*=6.6 Hz, H-5), 2.49 (2H, *d*, *J*=6.6 Hz, H-4), 2.60 (2H, *t*, *J*=7.6 Hz, H-13′), 2.66 (2H, *d*, *J*=6.6 Hz, H-6), 3.02 (2H, *t*, *J*=7.6 Hz, H-2′), 7.17–7.27 (5H, *m*, ArH), 18.30 (1H, *s*, OH-1, D_2O exchangeable);^{13}C NMR ($CDCl_3$, 100 MHz): 19.1 (C-5), 24.7 (C-3′), 29.3-29.6 (C-4′-11′), 31.5 (C-12′), 33.3 (C-6), 36.0 (C-13′), 38.8 (C-4), 40.6 (C-2′), 113.0 (C-2), 125.5 (C-17′), 128.2 (C-15′ and 19′), 128.4 (C-16′ and 18′), 143.0 (C-14′), 195.3 (C-1), 206.4 (C-3), 207.2 (C-1′); EIMS *m*/*z* 384 $[M]^+$ (18.4), 168 (12.9), 167 (100), 154 (50.0), 139 (33.4), 91 (35.8), 83 (11.9); HREIMS *m*/*z* 384.2660 (calc. for $C_{25}H_{36}O_3$ 384.2656).

3.6. Surinone C (3)

Colourless gum; $[\alpha]_D^{25}$ −29.1° (*c* 0.075, $CHCl_3$); UV(MeOH) λ_{max} (log ε) 230 (4.11), 271 (4.77) nm, +KOH (log ε) 267 (4.81) nm; IR (Neat) ν_{max} 3461 (OH), 1665 (conjugated C=O), 1558 (conjugated chelated C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): δ 0.88 (3H, *t*, *J*=7.5 Hz, H-20′), 1.25–1.40 (22H, *m*, H-4′–12′, 17′–18′), 1.36 (2H, *m*, H-19′), 1.63 (2H, *m*, H-3′), 1.83 (1H, *ddt*, *J*=15.6, 12.9, 11.0 Hz, H-$5_{ax.}$), 2.01 (4H, *m*, H-13′, 16′), 2.40 (1H, *m*, H-$5_{eq.}$), 2.76 (2H, *m*, H-6), 2.97 (1H, *ddd*, *J*=16.0, 8.5, 6.5 Hz, H-2′a), 3.07 (1H, *ddd*, *J*=16.0, 8.5, 6.5 Hz, H-2′b), 4.05 (1H, *br s*, OH-4, D_2O exchangeable), 4.08 (1H, *dd*, *J*=12.9, 5.8 Hz, H-4), 5.35 (2H, *m*, H-14′, 15′), 18.28 (1H, *s*, OH-1, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 150 MHz): 14.0 (C-20′), 22.3 (C-19′), 24.5 (C-3′), 26.9 (C-5), 27.1 (C-16′), 27.2 (C-13′), 29.3–29.8 (C-4′–12′, 17′), 31.3 (C-18′), 32.0 (C-6), 40.3 (C-2′), 71.6 (C-4), 110.3 (C-2), 129.8, 129.9 (C-14′,15′), 195.6 (C-3), 197.9 (C-1), 206.1 (C-1); EIMS *m*/*z* 420 $[M]^+$ (88.4), 309 (2.9), 209 (2.2), 182 (65.6), 167 (9.2), 155 (13.1), 153 (11.4), 127 (2.7), 85 (10.6), 57 (14.7), 43 (31.1); HRFABMS *m*/*z* 421.3307 (calc. for $C_{26}H_{45}O_4$, 421.3308).

3.7. Suranone (4)

Yellowish oil; $[\alpha]_D^{25}$ ±0° (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 223 (4.14), 270 (4.08), 342 (3.48) nm; IR (Neat) ν_{max} 3368 (OH), 1627 (C=O), 1590, 1513 (benzene ring) cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): δ 0.90 (3H, *t*, *J*=7.2 Hz, CH_3-18′), 1.25–1.33 (20H, *m*, CH_2-4′–6′, 11′–17′), 1.70 (2H, *q*, *J*=7.2 Hz, H-3′), 2.02 (4H, *m*, H-7′, 10′), 3.13 (2H, *t*, *J*=7.2 Hz, H-2′), 5.35 (2H, *m*, H-8′, 9′), 6.39 (2H, *d*, *J*=8.1 Hz, H-4, 6), 7.22 (1H, *t*, *J*=8.1 Hz, H-5), 9.60 (2H, *br s*, 2×OH, D_2O exchangeable);^{13}C NMR ($CDCl_3$, 150 MHz): 14.0 (C-18), 22.3 (C-17′), 24.4 (C-3′), 26.9 (C-7′), 27.2 (C-10′), 29.3–29.8 (C-4′–6′, 11′–15′), 32.0 (C-16′), 44.8 (C-2′), 108.4 (C-4, 6), 110.0 (C-2), 129.8, 129.9 (C-8′, 9′), 135.6 (C-5), 161.1 (C-1, 3), 207.9 (C=O); EIMS *m*/*z* 374 $[M]^+$ (4.8), 189 (14.2), 175 (11.1), 164 (56.4), 152 (56.8), 137 (95.1), 136 (100), 95 (2.78), 43 (8.3); HREIMS *m*/*z* 374.2815 (calc. for $C_{24}H_{28}O_3$ 374.2809).

3.8. Cytotoxicity assay

Human HONE-1 cells (nasopharyngeal carcinoma, from FIRDI, Taiwan), and NUGC-3 cells (gastric adenocarcinoma) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and nonessential amino acid (Life Technologies, Inc.) and maintained at 37 °C in a humidified incubator with 5% CO_2.

Human cancer cells were seeded in 96-well microtiter plates at a density of 6000/well in 100 μl culture medium. After an overnight adaptation period, 50 μg/ml (final concentration) of test compounds in serum-free medium were added to individual wells. Cells were treated with test compounds for 3 days. Cell viability was determined by the 5-(3-carboxymethoxyphenyl)-2-(4,5-dimethylthiazoyl)-3-(4-sulfophenyl) tetrazolium salt (MTS) reduction assay. Actinomycin D 5 μM (final concentration) and DMSO 0.3% (final concentration) were used as positive and vehicle controls. Results were expressed as a percentage of DMSO control.

Acknowledgements

This work was generously supported by the National Science Council of the Republic of China (NSC 90-2323-B-037-002).

References

Achari, B., Chaudhuri, C., Saha, C.R., Dutta, P.K., Pakrashi, S.C., 1990. A clerodane diterpene and other constituents of *Clerodendron inerme*. Phytochemistry 29, 3671–3673.

Azevedo, N.R., Santos, S.C., De Miranda, E.G., Ferri, P.H., 1997. A 2-acylcyclohexane-1,3-dione from *Virola oleifera*. Phytochemistry 46, 1375–1377.

Bayma, J. de C., Arruda, M.S.P., Müller, A.H., Arruda, A.C., Canto, W.C., 2000. A dimeric ArC_2 compound from *Peperomia pellucida*. Phytochemistry 55, 779–782.

Chang, Y.C., Chen, C.Y., Chang, F.R., Wu, Y.C., 2001. Alkaloids from *Lindera glauca*. Journal of the Chinese Chemical Society 48, 811–815.

Chen, C.M., Jan, F.Y., Chen, M.T., Lee, T.J., 1989. Peperomins A, B and C, novel secolignans from *Peperomia japonica*. Heterocycles 29, 411–414. {The chemical shift of methoxy groups at δ 3.91 (9H, *s*, OCH_3) on peperomin B was not identical with the ^{1}H NMR plot[δ3.84 (3H, *s*, OCH_3), 3.91 (6H, *s*, OCH_3)] in author's thesis (Jan, 1984)}.

Chen, I.S., Wu, S.J., Tsai, I.L., 1994. Chemical and bioactive constituents from *Zanthoxylum simulans*. Journal of Natural Products 57, 1206–1211.

Cheng, M.J., Jayaprakasam, B., Ishikawa, T., Seki, H., Tsai, I.L., Wang, J.J., Chen, I.S., 2002. Chemical and cytotoxic constituents from the stem of *Machilus zuihoensis*. Helvetica Chimica Acta 85, 1909–1914.

Cheng, M.J., Tsai, I.L., Chen, I.S., 2001. Chemical constituents from *Strychnos cathayensis*. Journal of the Chinese Chemical Society 48, 235–239.

Desai, S.J., Prabhu, B.R., Mulchandani, N.B., 1988. Aristolactams and 4,5-dioxoaporphines from *Piper longum*. Phytochemistry 27, 1511–1515.

Gonzalez, M.J.T.G., DeOliveira, C.J.C., Fernandes, J.O., Kijjoa, A., Herz, W., 1996. Further alkyl and alkenylphenols of *Knema laurina* and *Knema austrosiamensis*: location of the double bond in the alkenyl side chains. Phytochemistry 43, 1333–1337.

Govindachari, T.R., Krishna Kumari, G.N., Partho, P.D., 1998. Two secolignans from *Peperomia dindigulensis*. Phytochemistry 49, 2129–2131.

Hase, T., Kawamoto, Y., Ohtani, K., Kasai, R., Yamasaki, K., Picheansoonthon, C., 1995. Cyclohexylethanoids and related glucosides from *Millingtonia hortensis*. Phytochemistry 39, 235–241.

Jan, F.Y., 1984. MS Thesis, National Tsing Hua University, Hsinchu, Taiwan, pp. 35–36.

Katayama, T., Davin, L.B., Chu, A., Lewis, N.G., 1993. Novel benzylic ether reductions in lignan biogenesis in *Forsythia intermedia*. Phytochemistry 33, 581–591.

Kato, M.J., Lopes, L.M.X., Paulino Fo, H.F., Yoshida, M., Gottlieb, O.R., 1985. Acylresorcinols from *Virola sebifera* and *Virola elongata*. Phytochemistry 24, 533–536.

Lin, T.T., Lu, S.Y., 1996. Piperaceae in Flora of Taiwan, 2nd ed. Editorial Committee of the Flora of Taiwan, Taipei. Vol. II, pp. 624–631.

Mizutani, K., Fukunaga, Y., Tanaka, O., Takasugi, N., Saruwatari, Y., Fuwa, T., Wang, J., Jia, M.R., Li, F.Y., Ling, Y.K., 1988. Amides from Huajiao, pericarps of *Zanthoxylum bungeanum* Maxim. Chemical and Pharmaceutical Bulletin 36, 2362–2365.

Monache, F.D., Compagnone, R.S., 1996. A secolignan from *Peperomia glabella*. Phytochemistry 43, 1097–1098.

Nakatani, Y., Ourisson, G., Beck, J.P., 1981. Chemistry and biochemistry of Chinese drugs. VII. Cytostatic pheophytins from silkworm excreta and derived photocytotoxic pheophorbides. Chemical and Pharmaceutical Bulletin 29, 2261–2262.

Peng, C., Bodenhausen, G., Qiu, S., Fong, H.H.S., Farnsworth, N.R., Yuan, S., Zheng, C., 1998. Computer-assisted structure elucidation: application of CISOC-SES to the resonance assignment and structure generation of betulinic acid. Magnetic Resonance in Chemistry 36, 267–278.

Pouchert, C.J., Behnke, J., 1993a. The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra. Vol. I, p1149C. Aldrich Chemical Company, Inc, USA.

Pouchert, C.J., Behnke, J., 1993b. The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra. Vol. II, p278B. Aldrich Chemical Company, Inc, USA.

Pouchert, C.J., Behnke, J., 1993c. The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra. Vol. III, p26A. Aldrich Chemical Company, Inc, USA.

Savona, G., Piozzi, F., Rodriguez, B., Servettaz, O., 1982. Galangustin, a new flavone from *Galeopsis angustifolia*. Heterocycles 19, 1581–1584.

Seeram, N.P., Lewis, A.W., Jacobs, H., Nair, M.G., McLean, S., Reynolds, W.F., 2000. Proctoriones A-C: 2-acylcyclohexane-1,3-dione derivatives from *Peperomia proctorii*. Journal of Natural Products 63, 399–402.

Seki, K., Tomihari, T., Haga, K., Kaneko, R., 1994. Iristectorenes A and C-G, monocyclic triterpene esters from *Iris tectorum*. Phytochemistry 36, 425–431.

Shaw, S.C., Azad, R., Mandal, S.P., Gandhi, R.S., 1988. Syntheses of 6-hydroxyluteolin and sinensetin by Wessely-Moser rearrangement. Journal of Indian Chemical Society 107–109.

Sibi, M.P., Johnson, M.D., Punniyamurthy, T., 2001. Enantioselective synthesis of peperomins A, S, D, and analogs-Examination of diastereoselective cuprate conjugate additions to *N*-enoyl-4-diphenylmethyl-2-oxazolidinones. Canadian Journal of Chemistry 79, 1546–1555.

Tsai, I.L., Jeng, Y.F., Duh, C.Y., Chen, I.S., 2001. Cytotoxic constituents from the leaves of *Litsea akoensis*. The Chinese Pharmaceutical Journal 53, 291–301.

Villegas, L.F., Marcalo, A., Martin, J., Fernandez, I.D., Maldonado, H., Vaisberg, A.J., Hammond, G.B., 2001. (+)-*epi*-α-Bisabolol is the wound-healing principle of *Peperomia galioides*: investigation of the in vivo wound-healing activity of related terpenoids. Journal of Natural Products 64, 1357–1359.

Wu, T.S., Leu, Y.L., Chan, Y.Y., 2000. Aristolochic acids as a defensive substance for the aristolochiaceous plant-feeding swallowtail butterfly, *Pachliopta aristolochiae interpositus*. Journal of the Chinese Chemical Society 47, 221–226.