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Lignans, an amide and anti-platelet activities from *Piper philippinum*

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Abstract

Investigation of the stem extract of *Piper philippinum* led to isolation of eight compounds, piperphilippinins I–VI (**1**–**6**), philippinamide (**7**), and (+)-bornyl caffeate (**8**), together with 26 known compounds. Among the isolates, (−)-3′,4′-*O*,*O*-demethylenehinokinin (**10**) and 3,4-methylenedioxycinnamaldehyde (**23**) showed anti-platelet activities *in vitro*.

Keywords: *Piper philippinum*; Piperaceae; Stem; Lignan; Piperphilippinins I–VI; Amide; Philippinamide; (+)-Bornyl caffeate; Anti-platelet activity

1. Introduction

Piper philippinum Miq. (*P. kwashoense* Hayata) (Piperaceae) is a woody climber distributed throughout the Philippines and Lanyu and Lutao Islands in Taiwan (Lin and Lu, 1996). The Tau (Yami) aborigines on Lanyu use the older stems in place of pistillate inflorescences of betel for betel quid chewing, together with lime and catechu (Cheng and Lu, 2000). A new lignan, (2*R*,3*R*)-2-(3-methoxy-4-hydroxybenzyl)-3-(3,4-dihydroxybenzyl)butyrolactone, and a known compound, 4′,7-di-*O*-methyl apigenin 8-glucosyl-6″-*O*-β-D-glucoside, have been isolated from the leaves of this plant (Shih, 1991). The chemical constituents and biological activities of the stem of this species have never been studied. In a screening program of Formosan plants, the MeOH extract of the stem of this plant showed significant activity, effecting anti-platelet aggregation *in vitro*. Subsequent investigation of the *n*-hexane- and chloroform-soluble fractions of the stem extract led to the isolation of eight new compounds, piperphilippinins I–VI (**1**–**6**), philippinamide (**7**) and (+)-bornyl caffeate (**8**), together with 26 known compounds (**9**–**34**). The isolation and structural elucidation of these new compounds and the anti-platelet activities of the isolates are described herein.

2. Results and discussion

Piperphilippinin I (**1**) was obtained as a colorless oil. Its molecular formula was established as $C_{19}H_{20}O_6$ by EIMS ([M]$^+$, *m*/*z* 344) and HREIMS. The presence of a phenolic dibenzylbutyrolactone lignan skeleton was suggested by the UV spectrum, showing absorptions at 224 and 283 nm and a bathochromic shift upon the addition of alkali, along with a γ-lactone carbonyl absorption at 1752 cm^{-1} and a hydroxy group absorption at 3338 cm^{-1} in the IR spectrum (Lopes et al., 1983). The ^{1}H NMR spectrum of **1** was similar to that of (−)-matairesinol (**11**) (Gözler et al., 1992), except that one methoxy group at C-3′ in **11** was replaced by a hydroxy group in **1**. The ^{1}H NMR spectrum of **1** showed eight aliphatic protons at δ 2.45–4.05, typical of dibenzylbutyrolactone lignans. Six aromatic protons [δ 6.56 (2H, *dd*, *J* = 8.0, 2.0 Hz, H-6 and H-6′), 6.68 (1H, *d*, *J* = 2.0 Hz, H-2), 6.73 (1H, *d*, *J* = 8.0 Hz, H-5), 6.77 (1H, *d*, *J* = 8.0 Hz, H-5′), 6.77 (1H, *d*, *J* = 2.0 Hz, H-2′)], one methoxy singlet at δ 3.80 (3H, *s*, MeO-3), and three hydroxy groups at δ 7.50, 7.91, 7.93 (each 1H, *br s*, OH-4, OH-3′ and OH-4′,

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exchangeable with D_2O) established the presence of 4-hydroxy-3-methoxybenzyl and a 3′,4′-dihydroxybenzyl moieties. The EI-mass spectrum of **1** displayed a fragment at *m/z* 123 ($C_7H_7O_2$) and a fragment at *m/z* 207 (**35**), which were in accordance with a 3′,4′-dihydroxybenzyl group on the lactone carbon C-8′ (Lopes et al., 1983). This was further supported by H-7′ (δ 2.79, 2.87) showing correlations with C-9′ (δ 179.8), C-1′ (δ 131.4), C-8 (δ 42.9), and C-8′ (δ 47.4) in the LR-HETCOR spectrum (Fig. 1). Furthermore, another fragment at *m/z* 137 in the EI-mass spectrum corresponded to a 4-hydroxy-3-methoxybenzyl group. The NOESY spectrum of **1** (Fig. 1), showing correlations between H-7 (δ 2.55) and H-9 (δ 3.87, 4.05), H-2 (δ 6.68) and between H-2 and MeO-3 (δ 3.80), suggested a 4-hydroxy-3-methoxybenzyl group at C-8. H_2-9 were non-equivalent in ^{1}H NMR [δ 3.87 (1H, *t*, $J = 8.8$ Hz) and 4.05 (1H, *dd*, $J = 8.8$, 7.2 Hz)], and the two benzyl groups in **1** were in the *trans*-configuration (Lopes et al., 1983). The CD spectrum of **1** showed negative Cotton effects around 288 and 232 nm and $[\alpha]_D^{24}$: −45.3 (MeOH; *c* 4.98), similar to those reported for (−)-matairesinol (**11**) (Gözler et al., 1992) and (−)-kusunokinin (**13**) (Gözler et al., 1984), of which C-8 and C-8′ were in the *R*-form. According to the above data, the structure of **1** was elucidated as (−)-(3*R*,4*R*)-3-(3,4-dihydroxybenzyl)-4-(4-hydroxy-3-methoxybenzyl)dihydrofuran-2(3*H*)-one, named piperphilippinin I, which was further confirmed by COSY, NOESY (Fig. 1), ^{13}C NMR, DEPT, HETCOR and LR-HETCOR (Fig. 1) experiments.

Piperphilippinin II (**2**) was obtained as a colorless oil. Its molecular formula was established as $C_{19}H_{18}O_6$ by EIMS ($[M]^+$, *m/z* 342) and HREIMS. The presence of a phenolic dibenzylbutyrolactone lignan skeleton was also suggested by the UV spectrum showing absorptions at 234 and 286 nm and a bathochromic shift observed upon addition of alkali along with a γ-lactone carbonyl absorption at 1745 cm^{-1} and a hydroxy group absorption at 3391 cm^{-1} in the IR spectrum (Lopes et al., 1983). The ^{1}H NMR spectrum of **2** was similar to that of 3′,4′-*O*,*O*-demethylenehinokinin (**10**) (Kuo et al., 2002) and showed eight aliphatic protons at δ 2.43–4.09, typical of dibenzylbutyrolactone lignans, together with the presence of a 3,4-methylenedioxybenzyl moiety [δ 5.93, 5.94 (each 1H, *d*, $J = 1.4$ Hz, OCH_2O), 6.47 (1H, *d*, $J = 1.6$ Hz, H-2), 6.48 (1H, *dd*, $J = 8.0$, 1.6 Hz, H-6), 6.71 (1H, *d*, $J = 8.0$ Hz, H-5),] and a 3′,4′-dihydroxybenzyl moiety [δ 5.37, 5.69 (each 1H, *br s*, OH-3′ and OH-4′, exchangeable with D_2O), 6.57 (1H, *dd*, $J = 8.4$, 2.0 Hz, H-6′), 6.68 (1H, *d*, $J = 2.0$ Hz, H-2′), 6.79 (1H, *d*, $J = 8.0$ Hz, H-5′)]. However, the EI-mass spectrum of **2** displayed a fragment at *m/z* 123 ($C_7H_7O_2$) and a fragment at *m/z* 206 ($C_{11}H_{10}O_4$) (**36**) (Lopes et al., 1983) these being in accordance with a 3′,4′-dihydroxybenzyl group on the lactone carbon C-8′. Another fragment at *m/z* 135 ($C_8H_7O_2$) corresponded to a 3,4-methylenedioxybenzyl group. The CD spectrum of **2** showed two negative Cotton effects around 292 and 234 nm and $[\alpha]_D^{21}$: −24.1 ($CHCl_3$; *c* 0.11), similar to those reported for (−)-matairesinol (**11**) (Gözler et al., 1992) and (−)-kusunokinin (**13**) (Gözler et al., 1984); therefore, C-8 and C-8′ were in the *R*-form. According to the above data, the structure of **2** was elucidated as (−)-(3*R*,4*R*)-3-(3,4-dihydroxybenzyl)-4-((benzo[*d*][1,3]dioxol-6-yl)methyl)-dihydrofuran-2(3*H*)-one, named piperphilippinin II, which was further confirmed by COSY, NOESY, ^{13}C NMR, DEPT, HMQC and HMBC (Fig. 2) experiments.

Piperphilippinin III (**3**) was obtained as colorless prisms. Its molecular formula was established as $C_{23}H_{26}O_8$ by EIMS ($[M]^+$, *m/z* 430) and HREIMS analyses. The presence of a benzylidenebenzyl-γ-butyrolactone lignan skeleton was suggested by the UV spectrum showing absorptions at 250 (sh), 265 (sh) and 336 nm, along with a γ-lactone carbonyl absorption at 1743 cm^{-1} in the IR spectrum (Estévez-Reyes et al., 1992). A strong bathochromic shift observed upon addition of alkali was due to the phenolic function present in the molecule, also evidenced by the strong IR absorption at 3391 cm^{-1}. The fragment of the EI-mass spectrum at *m/z* 249 and the ^{1}H NMR spectrum [δ 3.86 (1H, *m*, H-8), 3.92 (6H, *s*, MeO-3′ and MeO-5′), 4.29 (1H, *dd*, $J = 9.2$, 2.4 Hz, H-9a), 4.32 (1H, *dd*, $J = 9.2$, 6.4 Hz, H-9b), 5.86 (1H, *br s*, OH-4′, exchangeable with D_2O), 6.84 (2H, *s*, H-2′ and H-6′), 7.53 (1H, *d*, $J = 1.6$ Hz, H-7′)] indicated the presence of a 4′-hydroxy-3′,5′-dimethoxybenzylidene moiety on the lactone carbon C-8′. The existence of a 3,4,5-trimethoxybenzyl moiety

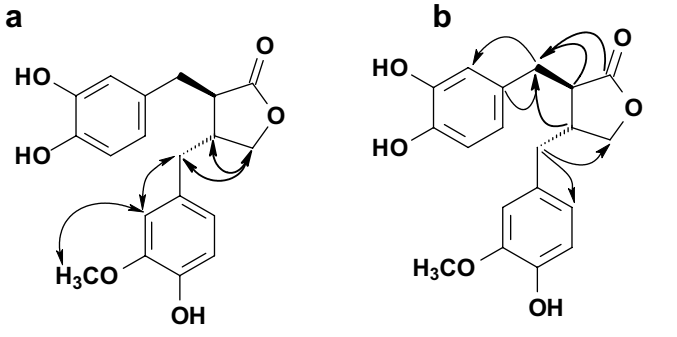


Fig. 1. Key NOESY contacts (a) and LR-HETCOR connectivites (b) for compound **1**.

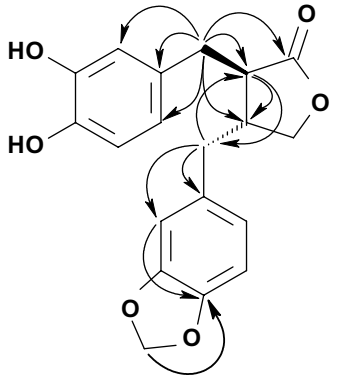


Fig. 2. Key HMBC connectivities for compound **2**.

was proven by the EI-mass base peak at *m/z* 181 ($C_{10}H_{13}O_3$) and the analysis of ^{1}H NMR spectrum [δ 2.66 (1H, *dd*, $J = 14.4$, 10.4 Hz, H-7a), 3.10 (1H, *dd*, $J = 14.4$, 4.4 Hz, H-7b), 3.81 (3H, *s*, MeO-4), 3.83 (6H, *s*, MeO-3 and MeO-5), 6.38 (2H, *s*, H-2 and H-6)]. Because no NOESY interactions could be observed between H-7 and H-7′ (Fig. 3), the geometry at C-7′ and C-8′ was indicated to be in the *E*-form. According to the above evidence, the structure of **3** was elucidated as (*E*)-4-(3,4,5-trimethoxybenzyl)-3-(4-hydroxy-3,5-dimethoxybenzylidene)dihydrofuran- 2(3*H*)-one, which was further confirmed by COSY, NOESY (Fig. 3), ^{13}C NMR, DEPT, HMQC and HMBC (Fig. 3) experiments. The spectroscopic data were also consistent with those of a synthetic (*S*)-(*E*)-2-(4-hydroxy-3,5-dimethoxybenzylidene)-3-(3,4,5-trimethoxybenzyl)butanolide with $[\alpha]_D^{24}$: +78.3 ($CHCl_3$; *c* 1.13) (Tanaka et al., 1995). Compound **3** exhibited levorotatory optical activity {$[\alpha]_D^{22}$: −52.0 ($CHCl_3$; *c* 0.15)}; therefore, C-8 was in the *R*-form in **3**.

Piperphilippinin IV (**4**) was obtained as a yellowish oil. Its molecular formula was established as $C_{19}H_{16}O_6$ by EIMS ($[M]^+$, *m/z* 340) and HREIMS. The presence of a benzylidenebenzyl-γ-butyrolactone lignan skeleton was suggested by the UV spectrum showing absorptions at 237, 295 and 338 nm, along with a γ-lactone carbonyl absorption at 1728 cm^{-1} in the IR spectrum (Estévez-Reyes et al., 1992). A bathochromic shift observed upon the addition of alkali was due to the phenolic function present in the molecule, which was also evidenced by the IR absorption at 3358 cm^{-1}. The ^{1}H NMR spectrum of **4** was similar to that of guamarol (Estévez-Reyes et al., 1992), except that the MeO-4′ group in guamarol was replaced by a hydroxy group in **4**. The fragment of the EI-mass spectrum at *m/z* 205 and the ^{1}H NMR spectrum [δ 3.95 (1H, *m*, H-8), 4.21 (1H, *dd*, $J = 8.8$, 2.4 Hz, H-9a), 4.26 (1H, *dd*, $J = 8.8$, 7.2 Hz, H-9b), 6.97 (1H, *d*, $J = 8.0$ Hz, H-5′), 7.15 (1H, *dd*, $J = 8.0$, 2.0 Hz, H-6′), 7.28 (1H, *d*, $J = 2.4$ Hz, H-2′), 7.35 (1H, *d*, $J = 2.0$ Hz, H-7′), 8.35 (1H, *br s*, OH, exchangeable with D_2O), 8.56 (1H, *br s*, OH, exchangeable with D_2O)] indicated the presence of a 3′,4′-dihydroxybenzylidene moiety on the lactone carbon C-8′. The existence of a 3,4-methylenedioxyphenyl moiety was established by the EI-mass base peak at *m/z* 135 ($C_8H_7O_2$) and the analysis of ^{1}H NMR spectrum [δ 2.62 (1H, *dd*, $J = 14.0$, 10.0 Hz, H-7a), 3.04 (1H, *dd*, $J = 14.0$, 4.0 Hz, H-7b), 5.97 (2H, *s*, OCH_2O), 6.77 (2H, *s*, H-5 and H-6), 6.86 (1H, *s*, H-2)]. No correlation existed between H-7 and H-7′ in the NOESY spectrum (Fig. 4); the double bond between C-7′ and C-8′ was thus indicated as being in the *E*-form. According to the above data, the structure of **4** was elucidated as (*E*)-3-(3,4-dihydroxybenzylidene)-4-((benzo[*d*][1,3]dioxol-6-yl)methyl)dihydrofuran-2-(3*H*)-one, which was further confirmed by COSY, NOESY (Fig. 4), ^{13}C NMR, DEPT, HMQC and HMBC (Fig. 4) experiments. Compound **4** showed levorotatory optical activity as $[\alpha]_D^{23}$: −7.0 (MeOH;*c* 0.195); therefore, the configuration of C-8 was in the *R*-form, the same as for **3**.

Piperphilippinin V (**5**) was obtained as a colorless oil. Its molecular formula was established as $C_{19}H_{20}O_5$ by EIMS ($[M]^+$, *m/z* 328) and HREIMS. The presence of a dibenzylbutyrolactone lignan skeleton was suggested by the UV spectrum showing absorptions at 226 and 280 nm, along with a γ-lactone carbonyl absorption at 1749 cm^{-1} in the IR spectrum (Lopes et al., 1983). A bathochromic shift observed upon the addition of alkali was due to the phenolic function present in the molecule, which was also supported by the IR absorption at 3394 cm^{-1}. The ^{1}H NMR spectrum showed eight aliphatic protons at δ 2.43–4.12, typical of dibenzylbutyrolactone lignans. One set of ABX system phenyl protons [δ 6.44 (1H, *d*, $J = 1.6$ Hz, H-2), 6.51 (1H, *dd*, $J = 8.0$, 1.6 Hz, H-6), 6.81 (1H, *d*, $J = 8.0$ Hz, H-5)], one set of A_2B_2 system phenyl protons [δ 6.75 (2H, *d*, $J = 8.4$ Hz, H-3′ and H-5′), 6.99 (2H, *d*, $J = 8.4$ Hz, H-2′ and H-6′)], one methoxy singlet at δ 3.83 (3H, *s*, MeO-3), and two hydroxy groups at δ 5.21, 5.56 (each 1H, *br s*, OH-4 and OH-4′, exchangeable with D_2O) indicated the presence of a 4-hydroxy-3-methoxyphenyl moiety and a 4′-hydroxyphenyl moiety. The EI-mass spectrum of **5** showed a fragment at *m/z* 107 (C_7H_7O) and a fragment at *m/z* 164 (**37**) (Lopes et al., 1983); those are in accordance with a 4′-hydroxyphenyl group on the lactone carbon C-8′. Another fragment at *m/z* 137 corresponded to a 4-hydroxy-3-methoxybenzyl group. Compound **5** showed levorotatory optical activity with

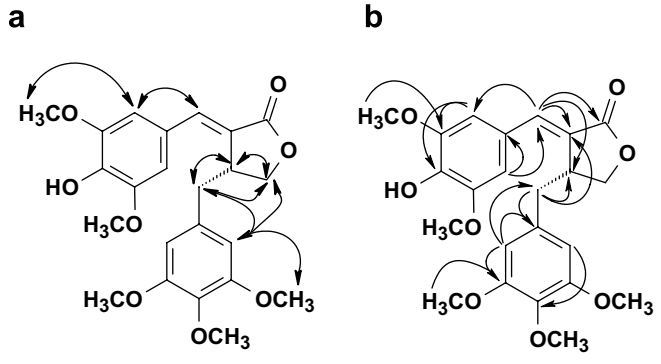


Fig. 3. Key NOESY contacts (a) and HMBC connectivites (b) for compound **3**.

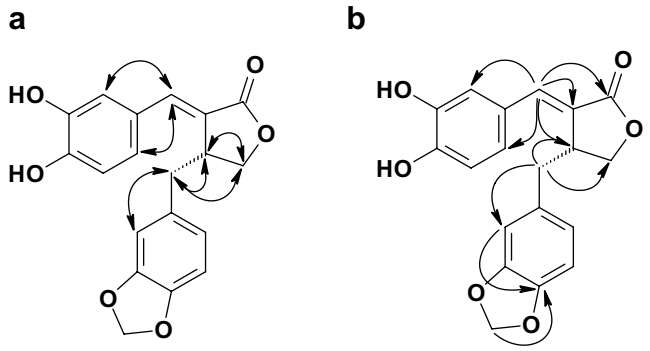


Fig. 4. Key NOESY contacts (a) and HMBC connectivites (b) for compound **4**.

$[\alpha]_D^{20}$: −34.9 ($CHCl_3$; *c* 0.22), and the CD spectrum of **5** showed two negative Cotton effects around 288 and 232 nm, similar to those reported for (−)-matairesinol (**11**) (Gözler et al., 1992) and (−)-kusunokinin (**13**) (Gözler et al., 1984); therefore, the stereochemistry of C-8 and C-8′ were in the *R*-form. According to the above data, the structure of **5** was elucidated as (−)-(3*R*,4*R*)-4-(4-hydroxy-3-methoxybenzyl)-3-(4-hydroxybenzyl)-dihydrofuran-2(3*H*)-one, named piperphilippinin V, which was further confirmed by COSY, NOESY (Fig. 5), ^{13}C NMR, DEPT, HMQC and HMBC (Fig. 5) experiments.

Piperphilippinin VI (**6**) was obtained as a colorless oil. Its molecular formula was established as $C_{20}H_{24}O_6$ by EIMS ($[M]^+$, *m/z* 360) and HREIMS. The presence of a diarylbutanediol lignan skeleton with a phenolic function was suggested by the UV spectrum showing absorptions at 229 (sh) and 283 nm, along with the strong IR absorption at 3394 cm^{-1} and a bathochromic shift observed upon the addition of alkali (Xie et al., 2003b). The ^1H NMR spectrum of **6** was similar to that of dihydrocubebin (Satyanarayana and Venkateswarlu, 1991), except for a 4-hydroxy-3-methoxyphenyl moiety [δ 3.85 (3H, *s*, OCH_3-3), 5.48 (1H, *br s*, OH-4, exchangeable with D_2O), 6.63 (1H, *br s*, H-2), 6.64 (1H, *dd*, *J* = 8.0, 2.0 Hz, H-6), 6.82 (1H, *d*, *J* = 8.0 Hz, H-5)] in **6** substituted for one 3,4-methylenedioxyphenyl moiety in dihydrocubebin. According to the above evidence, the structure of **6** was elucidated as 4-(4-(benzo[*d*][1,3]dioxol-5-yl)-2,3-bis(hydroxymethyl)butyl)-2-methoxyphenol, which was further confirmed by COSY, NOESY (Fig. 6), ^{13}C NMR, DEPT, HMQC and HMBC (Fig. 6) experiments. The *R*-configurations at C-8 and C-8′ of **6** were supported by the following observations: (i) the optical rotation of the reported (8*R*,8′*R*)-diarylbutanediol lignans was negative (Xie et al., 2003b), as in **6**; (ii) all the other lignans isolated with this plant had the configurations 8*R*,8′*R*.

Philippinamide (**7**) was obtained as colorless needles. Its molecular formula was established as $C_{22}H_{31}NO_2$ by EIMS ($[M]^+$, *m/z* 341) and HREIMS. The presence of an amide group was suggested by the IR absorptions at 3304 (NH) and 1658 (C=O) cm^{-1}. The ^1H NMR spectrum of **7** was similar to that of pipercide (Miyakado et al., 1979; Rotherham and Semple, 1998), except a 4-methoxyphenyl moiety [δ 3.80 (3H, *s*, MeO-4), 6.84 (2H, *d*, *J* = 8.8 Hz, H-3 and H-5), 7.27 (2H, *d*, *J* = 8.8 Hz, H-2 and H-6)] in **7** replaced a 3,4-methylenedioxyphenyl moiety in pipercide. According to the above data, the structure of **7** was elucidated as (2*E*,4*E*,10*E*)-*N*-isobutyl-11-(4-methoxyphenyl)undeca-2,4,10-trienamide, named philippinamide, which was further confirmed by COSY, NOESY (Fig. 7), ^{13}C NMR, DEPT, HMQC and HMBC (Fig. 7) experiments.

(+)-Bornyl caffeate (**8**) was obtained as colorless oil. All of the spectra confirmed **8** as bornyl caffeate (Bohlmann and Zdero, 1976; Maldonado et al., 1998). The NOESY experiment (Fig. 8) showed that H-2′ at δ 4.99 correlated with H-10′ at δ 0.88 and H-9′ at δ 0.94. The above data supported the assignments of H-2′ as *exo* and the caffeic group as *endo*. Thus, the bornyl group had two possible absolute configurations: (1*R*,2*S*,4*R*) (**8**) or (1*S*,2*R*,4*S*) (**8a**). (1*R*,2*S*,4*R*)-borneol showed positive optical rotation {$[\alpha]_D^{25}$: +37.5 (neat)} and (1*S*,2*R*,4*S*)-borneol was negative {$[\alpha]_D^{25}$: −37.3 (neat)} (Hirata et al., 2000). Thus, **8** was elucidated as (+)-(1*R*,2*S*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl-3-(3,4-dihydroxyphenyl)acrylate. Though (−)-bornyl caffeate {$[\alpha]_D^{24}$: −19.8 ($CHCl_3$; *c* 0.6); $[\alpha]_D^{24}$: −24.8 ($CHCl_3$; *c* 0.25)}

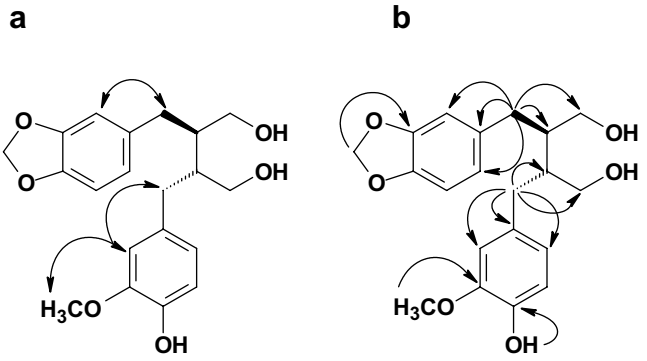


Fig. 6. Key NOESY contacts (a) and HMBC connectivites (b) for compound **6**.

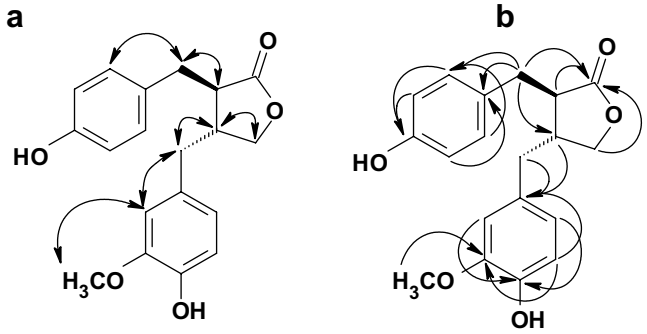


Fig. 5. Key NOESY contacts (a) and HMBC connectivites (b) for compound **5**.

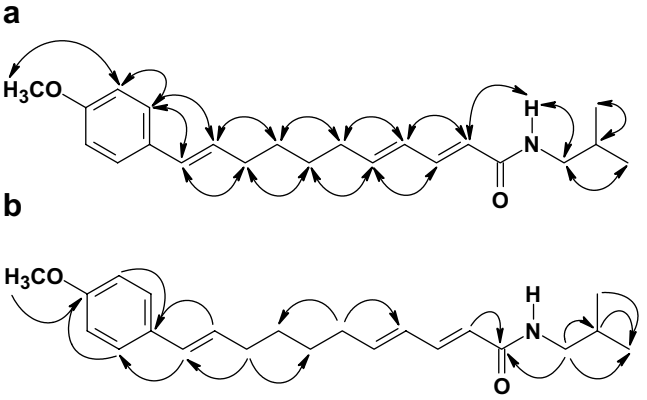


Fig. 7. NOESY contacts (a) and HMBC connectivites (b) for compound **7**.

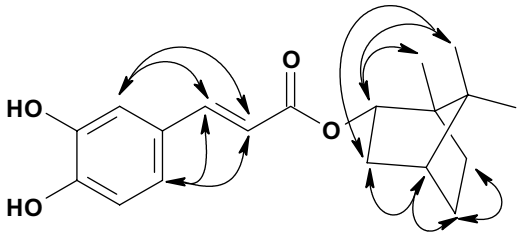


Fig. 8. Key NOESY contacts for compound **8**.

had been reported previously (Bohlmann and Zdero, 1976; Maldonado et al., 1998), (+)-bornyl caffeate (**8**) was first isolated from nature.

Twenty-six known compounds, including (−)-(2*R*,3*R*)-2-(3′,4′-dihydroxybenzyl)-3-(3″,4″-dimethoxybenzyl)butyrolactone (**9**) (Xie et al., 2003a), (−)-3′,4′-*O*,*O*-demethylenehinokinin (**10**) (Kuo et al., 2002), (−)-matairesinol (**11**) (Gözler et al., 1992), (−)-haplomyrfolin (**12**) (Evcim et al., 1986), (−)-kusunokinin (**13**) (Gözler et al., 1984), (−)-hinokinin (**14**) (Lopes et al., 1983), *N*-(*m*-methoxycinnamoyl)pyrrolidine (**15**) (Achenbach and Karl, 1970), 3′,5′-dimethoxycinnamic acid pyrrolidide (**16**) (Achenbach et al., 1986), *N*-*trans*-feruloy-3-*O*-methyldopamine (**17**) (Suzuki et al., 1981; Chiji et al., 1984), piperolactam A (**18**) (Desai et al., 1988), 5-hydroxy-3,7,4′-trimethoxyflavone (**19**) (Rossi et al., 1997), caryophyllene oxide (**20**) (Thebtaranonth et al., 1995), 4-methoxycinnamaldehyde (**21**) (Bellassoued and Majidi, 1993), 4-methoxy-*trans*-cinnamic acid (**22**) (Miyazawa et al., 1998), 3,4-methylenedioxycinnamaldehyde (**23**) (Schobert et al., 2001), 3,4-methylenedioxycinnamic acid (**24**) (Jackson and Dewick, 1984), methyl 3,4-methylenedioxycinnamate (**25**) (Schobert et al., 2001), *p*-anisaldehyde (**26**) (Baillargeon and Stille, 1986), *p*-anisic acid (**27**) (Kamaya and Ageta, 1990), piperonal (**28**) (Adesina et al., 1997), 3,4-methylenedioxybenzoic acid (**29**) (Koike and Ohmoto, 1990), the mixture of (24*R*)-stigmast-4-en-3-one (**30**) (Wu et al., 1987) and (22*E*,24*S*)-stigmast-4,22-dien-3-one (**31**) (Wu et al., 1987), the mixture of β-sitosterol (**32**) (Kojima et al., 1990) and stigmasterol (**33**) (Kojima et al., 1990), and ergosta-4,6,8(14),22-tetraen-3-one (**34**) (Gan et al., 1998), were readily identified by comparison of physical and spectroscopic data (UV, IR, ^{1}H NMR, $[\alpha]_D$, and mass spectrometry data) with values found in the literature. Compound **9** was first isolated from a higher plant, although it has been formed from arctiin by human intestinal bacteria (Xie et al., 2003a). Piperphilippinin I (**1**) was the major constituent of this plant.

The anti-platelet aggregation effects of the isolates are shown in Table 1. In this study, **4**, **10**, and **23** at 100 μM showed complete or nearly complete inhibition of platelet aggregation induced by arachidonic acid (AA). Except for **3** and **12**–**14**, most compounds at 100 μM showed inhibition of platelet aggregation induced by AA or collagen. Compound **18** has previously been reported to possess marked anti-platelet aggregation effects (Chen et al., 2004).

2.1. Concluding remarks

In addition, from the results of the anti-platelet aggregation tests four conclusions can be drawn as follows: (a) compared with the 3′,4′-methylenedioxy dibenzylbutyrolactone lignans, **10,** which has a 3,4-dihydroxy group, was more potent than **12**–**14**, which have either a methoxy or methylenedioxy group on the 3- and 4-position, in inhibiting the effect of AA- or collagen-induced platelet aggregation; (b) compared with the 3′,4′-dihydroxy dibenzylbutyrolactone lignans, the inhibitory potency of AA-induced platelet aggregation was of the order **1** (with 4-hydroxy-3-methoxy) >**9** (with 3,4-dimethoxy) >**2** (with 3,4-methylenedioxy), but the inhibitory potency of collagen-induced platelet aggregation was of the order **2** > **9** > **1**; (c) compared with the 4-hydroxy-3-methoxy dibenzylbutyrolactone lignans, the inhibitory potency of AA-induced platelet aggregation was of the order **5** (with 4′-hydroxy) >**1** (with 3′,4′-dihydroxy) >**11** (with 4′-hydroxy-3′-methoxy) >**12** (with 3′,4′-methylenedioxy), and the inhibitory potency of collagen-induced platelet aggregation was of the order **11** > **1** > **5** > **12**; (d) compared with **2** and **10**, the 3,4-dihydroxy group was more important than 3′,4′-dihydroxy group on the inhibitory effect of AA-induced platelet aggregation.

In our previous research on several Formosan piperaceous plants, piperidine amides and pyrrolidine amides were the major isolates in *P. sintenense* (Chen et al., 2002; Chen et al., 2003); 4-allylcatechol was the major component in *P. taiwanense* (Chen et al., 2004), and cyclobutanoid amides were the major constituents in *P. arborescens* (Lee et al., 2004; Tsai et al., 2005). In this study, dibenzylbutyrolactone lignans were the major isolates in *P. philippinum*. The above observations indicate a great biodiversity of the major constituents in Formosan Piper species. Such major constituents of Formosan piperaceous plants except for *P. sintenense* all belong to phenylpropanoids which are biosynthesized via the shikimic acid pathway.

3. Experimental

3.1. General

All melting points were determined on a Yanaco micro-melting point apparatus and were uncorrected. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were obtained in $CDCl_3$. Chemical shifts are given as δ with TMS as internal 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 in $CHCl_3$ or MeOH using a Jasco P-1020 polarimeter. IR spectra were recorded on a Genesis II FTIR spectrophotometer, whereas UV spectra in MeOH were obtained on a Shimadzu UV-160A spectrophotometer. Silica gel (60-230, 230–400 mesh) (Merck) and Sepha-

Table 1
Inhibitory effects[a] of compounds on the aggregation of washed rabbit platelets induced by thrombin (Thr), arachidonic acid (AA), collagen (Col) and platelet-activating factor (PAF)

Compound	Concentration (μM)	Aggregation (%)			
		Thr (0.1 U/mL)	AA (100 μM)	Col (10 μg/mL)	PAF (2 ng/mL)
Control		82.5 ± 3.3 (3)	81.6 ± 2.6 (3)	83.2 ± 0.8 (3)	83.9 ± 2.4 (3)
Piperphilippinin I (**1**)	100	81.3 ± 3.5 (3)	35.5 ± 5.2 (3)[b]	52.3 ± 7.3 (3)[b]	80.5 ± 5.9 (3)
	50		82.5 ± 6.5 (3)	79.3 ± 5.8 (3)	
Piperphilippinin II (**2**)	100	81.8 ± 2.7 (3)	75.9 ± 6.7 (3)	35.4 ± 2.1 (3)[b]	73.7 ± 8.3 (3)
	50			76.2 ± 6.3 (3)	
Piperphilippinin III (**3**)	100	80.3 ± 6.5 (3)	77.5 ± 8.5 (3)	65.3 ± 4.3 (3)[c]	86.5 ± 6.4 (3)
	50			79.3 ± 5.8 (3)	
Piperphilippinin IV (**4**)	100	81.3 ± 6.2 (3)	15.5 ± 9.2 (3)[b]	35.3 ± 4.3 (3)[b]	83.5 ± 6.9 (3)
	50		62.5 ± 2.4 (3)[c]	69.3 ± 5.8 (3)	
	20		77.5 ± 8.5 (3)		
Piperphilippinin V (**5**)	100	61.3 ± 3.5 (3)[c]	25.5 ± 5.2 (3)[b]	55.3 ± 2.3 (3)[b]	84.5 ± 5.9 (3)
	50	81.3 ± 6.3 (3)	62.5 ± 2.5 (3)[b]	59.3 ± 5.8 (3)[c]	
	20		85.5 ± 8.2 (3)	79.3 ± 4.8 (3)[c]	
Philippinamide (**7**)	100	75.3 ± 6.5 (3)	62.5 ± 3.5 (3)[b]	33.3 ± 4.3 (3)[b]	82.5 ± 7.6 (3)
	50		71.5 ± 1.1 (3)	55.3 ± 8.9 (3)[b]	
	20		81.5 ± 5.1 (3)	69.5 ± 1.1 (3)	
	10			82.5 ± 6.1 (3)	
(+)-Bornyl caffeate (**8**)	100	80.3 ± 6.5 (3)	20.5 ± 7.5 (3)[b]	25.3 ± 4.3 (3)[b]	80.5 ± 6.4 (3)
	50		55.5 ± 5.1 (3)[c]	69.3 ± 3.8 (3)[c]	
	20		79.5 ± 6.5 (3)	79.3 ± 5.4 (3)	
(−)-(2*R*,3*R*)-2-(3′,4′-Dihydroxybenzyl)-3-(3″,4″-dimethoxybenzyl)butyrolactone (**9**)	100	79.3 ± 2.5 (3)	51.5 ± 6.5 (3)[c]	42.3 ± 17.3 (3)[b]	83.5 ± 2.9 (3)
	50		81.5 ± 1.5 (3)	56.7 ± 9.3 (3)[c]	
	20			76.3 ± 0.8 (3)	
(−)-3′,4′-*O*,*O*-Demethylenehinokinin (**10**)	100	82.8 ± 2.7 (3)	0.0 ± 0.0 (3)[d]	35.3 ± 2.1 (3)	71.7 ± 18.3 (3)
	50		74.5 ± 6.1 (3)	75.1 ± 7.6 (3)	
(−)-Matairesinol (**11**)	100	71.3 ± 2.5 (3)	51.5 ± 5.2 (3)[b]	42.3 ± 11.3 (3)[b]	86.5 ± 3.9 (3)
	50	82.3 ± 1.3 (3)	81.5 ± 1.5 (3)	76.3 ± 3.8 (3)	
(−)-Haplomyrfolin (**12**)	100	77.9 ± 6.8 (3)	80.8 ± 1.3 (3)	78.1 ± 6.5 (3)	81.6 ± 2.5 (3)
(−)-Kusunokinin (**13**)	100	83.7 ± 2.8 (3)	79.8 ± 5.3 (3)	79.9 ± 5.5 (3)	80.8 ± 5.6 (3)
(−)-Hinokinin (**14**)	100	81.7 ± 2.8 (3)	76.8 ± 2.3 (3)	60.9 ± 3.5 (3)[c]	60.8 ± 5.6 (3)[c]
	50		80.4 ± 0.8 (3)	76.2 ± 4.6 (3)	73.7 ± 11.6 (3)
N-*trans*-Feruloy-3-*O*-methyldopamine (**17**)	100	78.3 ± 4.5 (3)	52.5 ± 6.5 (3)[b]	13.3 ± 4.6 (3)[b]	82.5 ± 7.6 (3)
	50		81.5 ± 5.1 (3)	45.3 ± 5.9 (3)[b]	
	20			82.5 ± 6.1 (3)	
5-Hydroxy-3,7,4′-trimethoxyflavone (**19**)	100	75.3 ± 6.5 (3)	60.5 ± 7.5 (3)[b]	45.3 ± 4.3 (3)[b]	81.5 ± 5.6 (3)
	50		75.5 ± 8.1 (3)	75.3 ± 8.9 (3)	
3,4-Methylenedioxycinnamaldehyde (**23**)	100	78.3 ± 4.5 (3)	11.5 ± 5.2 (3)[d]	43.3 ± 5.5 (3)[b]	81.5 ± 3.8 (3)
	50		51.5 ± 6.1 (3)[b]	80.5 ± 3.1 (3)	
	20		76.5 ± 2.5 (3)		
p-Anisic acid (**27**)	100	78.3 ± 4.5 (3)	81.5 ± 5.1 (3)	53.3 ± 6.6 (3)[c]	86.5 ± 2.6 (3)
	50			82.5 ± 6.1 (3)	
Aspirin[e]	200	80.3 ± 1.8 (4)	0.0 ± 0.0 (3)	10.1 ± 2.1 (3)	84.9 ± 4.3 (3)
	100		5.2 ± 0.3 (3)	30.5 ± 5.2 (3)	

[a] Platelets were pre-incubated with each compound or DMSO (0.5%, control) at 37 °C for 3 min, then the inducer arachidonic acid (AA), collagen, thrombin or PAF was added. Values are presented as mean ± s.e.m. (*n*).
[b] $P < 0.01$.
[c] $P < 0.05$.
[d] $P < 0.001$, as compared with the respective control.
[e] Positive control.

dex LH-20 gel (Pharmacia, Fine Chemicals AB, Uppsala) were used for CC and silica gel 60F-254 (Merck) for preparative TLC. Further purification was performed by HPLC [column: Merck LichroCART 250 × 10 Cat. 50850 Lichrospher Si-60 (10 μm), refractive index].

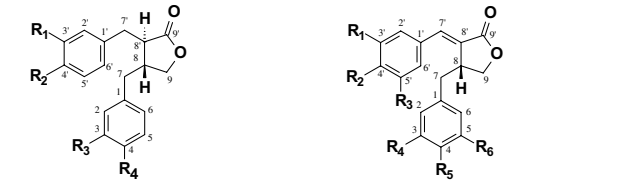
3.2. Plant material

The stems of *P. philippinum* were collected from Lanyu Island, Taitung County, Taiwan, in July 2000 and identified by Dr. Ih-Sheng Chen, College of Pharmacy, Kaohsiung Medical University. A voucher specimen (Chen 6102) has been deposited in the Herbarium of the School of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan, ROC.

3.3. Extraction and isolation

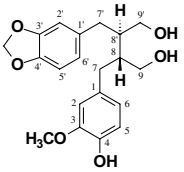
Dried stems (4.0 kg) of *P. philippinum* were sliced and extracted with cold MeOH (30 L) three times. After removal of the solvent in vacuo, the extract was partitioned into a *n*-hexane (fraction A, 22 g), $CHCl_3$ (fraction B, 60 g), *n*-BuOH (fraction C, 60 g), and water (fraction D, 120 g) soluble fractions.

Fraction A (22 g) was subjected to silica gel CC (880 g), eluting initially with *n*-hexane and gradually enriching with EtOAc to give 16 fractions. Fraction A-6 (660 mg, *n*-hexane–EtOAc, 95:5) was applied to a silica gel column (20 g), eluting with *n*-hexane and gradually increasing the polarity with EtOAc, to obtain 13 fractions. Fraction A-6-2 (8.6 mg, *n*-hexane–EtOAc, 99:1) was subjected to TLC (benzene) to afford **20** (1.8 mg). Fraction A-6-4 (161 mg, *n*-hexane–EtOAc, 99:1) was subjected to TLC (*n*-hexane–EtOAc, 10:1) to afford **28** (1.1 mg). Fraction A-8 (2.7 g, *n*-hexane–EtOAc, 90:10) was applied to a silica gel column (81 g), eluting with CH_2Cl_2 to give 11 fractions. Fraction A-8-4 (491 mg) was subjected to silica gel CC (14.8 g), eluting with *n*-hexane–acetone (20:1) to obtain 4 fractions. Fraction A-8-4-3 (332 mg) was applied to silica gel CC (10 g), eluting with *n*-hexane–$CHCl_3$ (1:4), to obtain five fractions. Fraction A-8-4-3-1 (38.8 mg) was further purified by TLC (CH_2Cl_2–MeOH, 20:1) to afford **28** (2.9 mg) and a mixture (2.7 mg) of **21** and **23**. Fraction A-8-5 (534 mg) was subjected to silica gel CC (81 g), eluting with *n*-hexane–acetone (10:1), to obtain 7 fractions. Fraction A-8-5-4 (5.9 mg) was further purified by TLC (*n*-hexane–acetone, 10:1) to give **23** (3.2 mg). Fraction A-8-9 (9.5 mg) was washed with MeOH to obtain a mixture (44 mg) of **32** and **33**. The mother liquid (51 mg) was further purified by TLC (*n*-hexane–EtOAc, 6:1) to give **34** (5.6 mg) and a mixture (2.2 mg) of **30** and **31**. Fraction A-11 (1.1 g, *n*-hexane–EtOAc, 80:20) was washed with MeOH and *n*-hexane to obtain **19** (50 mg). Fraction A-16 (525 mg, *n*-hexane–EtOAc, 70:30) was applied to a silica gel column (880 g), eluting with CH_2Cl_2 and gradually increasing the polarity with acetone to obtain 8 fractions. Fraction A-16-3 (58.8 mg, CH_2Cl_2–acetone, 50:1) was further purified by TLC (*n*-hexane–acetone, 2:1) to afford **15** (1.5 mg), **16** (1.3 mg), and **18** (1.6 mg). Fraction A-16-6 (300 mg, CH_2Cl_2–acetone, 1:1) was washed with Et_2O to obtain **24** (0.9 mg).



1 $R_1 = R_2 = R_4 = OH$, $R_3 = OCH_3$
2 $R_1 = R_2 = OH$, $R_3 + R_4 = OCH_2O$
5 $R_1 = H$, $R_2 = R_4 = OH$, $R_3 = OCH_3$
9 $R_1 = R_2 = OH$, $R_3 = R_4 = OCH_3$
10 $R_1 + R_2 = OCH_2O$, $R_3 = R_4 = OH$
11 $R_1 = R_3 = OCH_3$, $R_2 = R_4 = OH$
12 $R_1 + R_2 = OCH_2O$, $R_3 = OCH_3$, $R_4 = OH$
13 $R_1 + R_2 = OCH_2O$, $R_3 = R_4 = OCH_3$
14 $R_1 + R_2 = OCH_2O$, $R_3 + R_4 = OCH_2O$

3 $R_1 = R_3 = R_4 = R_5 = R_6 = OCH_3$, $R_2 = OH$
4 $R_1 = R_2 = OH$, $R_3 = R_6 = H$, $R_4 + R_5 = OCH_2O$



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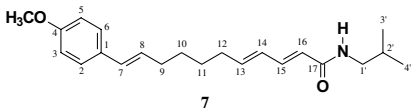

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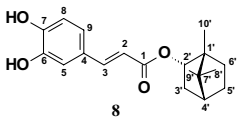

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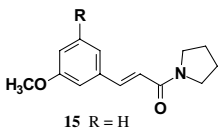

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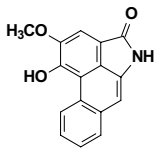
16 R = OCH_3



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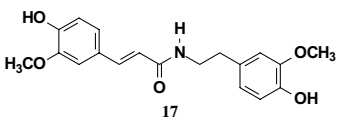

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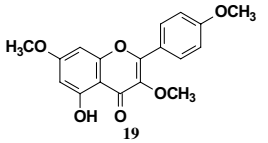

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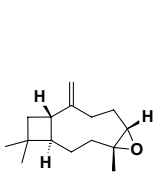

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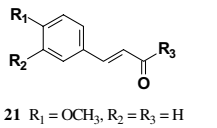
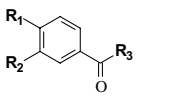
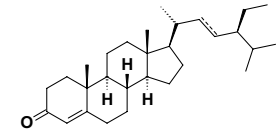


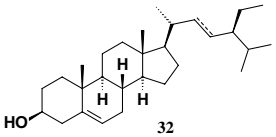
21 $R_1 = OCH_3$, $R_2 = R_3 = H$
22 $R_1 = OCH_3$, $R_2 = H$, $R_3 = OH$
23 $R_1 + R_2 = OCH_2O$, $R_3 = H$
24 $R_1 + R_2 = OCH_2O$, $R_3 = OH$
25 $R_1 + R_2 = OCH_2O$, $R_3 = OCH_3$



26 $R_1 = OCH_3$, $R_2 = R_3 = H$
27 $R_1 = OCH_3$, $R_2 = H$, $R_3 = OH$
28 $R_1 + R_2 = OCH_2O$, $R_3 = H$
29 $R_1 + R_2 = OCH_2O$, $R_3 = OH$



30
31 Δ^{22}



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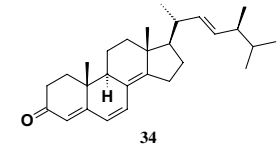

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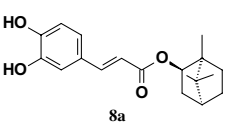

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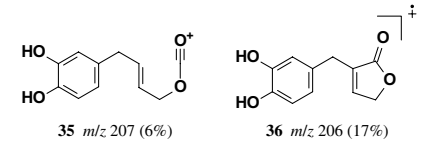

35 *m/z* 207 (6%)
36 *m/z* 206 (17%)
37 *m/z* 164 (27%)

Fraction B (60 g) was subjected to silica gel CC (1.8 kg), eluting with $CHCl_3$ and gradually enriching with acetone to give 12 fractions. Fraction B-3 (1.3 g, $CHCl_3$–acetone, 99:1) was applied to a silica gel column (40 g), eluting with $CHCl_3$ and gradually increasing the polarity with acetone to obtain 14 fractions. Some of these fractions were subjected to preparative HPLC (*n*-hexane–EtOAc, 10:1, flow rate = 1 mL/min), with the following results: fraction B-3-1 (76.6 mg, $CHCl_3$) gave **23** (2.6 mg), **28** (2.7 mg), and **29** (0.9 mg); fraction B-3-2 (265 mg, $CHCl_3$) gave **25** (1.4 mg), **26** (8.5 mg), and **28** (11.9 mg); fraction B-3-3 (335 mg, $CHCl_3$) gave **14** (147 mg), **19** (13.7 mg), **22** (0.9 mg), and **23** (14.7 mg). Fraction B-5 (570 mg, $CHCl_3$–acetone, 98:2) was subjected to preparative HPLC (*n*-hexane–EtOAc, 3:1, flow rate = 2 mL/min) to give 8 fractions. Several of these were further purified by TLC: fraction B-5-4 (47.6 mg; $CHCl_3$–EtOAc, 3:1) gave a mixture (3.2 mg) of **21** and **23**; fraction B-5-5 (30.6 mg; *n*-hexane–acetone, 3:1) gave **7** (1.3 mg); fraction B-5-6 (71.2 mg; $CHCl_3$–EtOAc, 5:1) gave **13** (32.1 mg); and fraction B-5-7 (54.3 mg; $CHCl_3$–acetone, 10:1) gave **12** (24 mg). Fraction B-7 (4.5 g, $CHCl_3$–acetone, 95:5) was applied to a Sephadex LH-20 gel column (300 g), eluting with MeOH to give 7 fractions. Fraction B-7-3 (3.5 g) was applied to a silica gel column (105 g), eluting with $CHCl_3$–acetone (100:1) and gradually increasing the polarity with acetone to obtain 11 fractions. Fraction B-7-3-2 (25.3 mg) was further purified by TLC (*n*-hexane–CH_2Cl_2, 1:3) to afford **19** (4.3 mg). Fraction B-7-3-6 (2.4 g) was subjected to silica gel CC (72 g) eluting with CH_2Cl_2 to give 8 fractions: fraction B-7-3-6-2 (931 mg) was applied to a preparative HPLC (*n*-hexane–EtOAc, 3:1, flow rate = 4 mL/min) to give **11** (19.2 mg) and fraction B-7-3-6-7 (18.1 mg) was further purified by TLC ($CHCl_3$–acetone–MeOH, 40:1:1) to give **3** (3.0 mg) and **16** (12.9 mg). Fraction B-9 (21.5 g, $CHCl_3$–acetone, 80:20) was subjected to silica gel CC (645 g), eluting with $CHCl_3$ and gradually increasing the polarity with MeOH to obtain 19 fractions. Fraction B-9-9 (4.2 g, $CHCl_3$–MeOH, 98:2) was applied to a Sephadex LH-20 gel column (300 g), eluting with MeOH to give 5 fractions. Fraction B-9-9-3 (730 mg) was subjected to silica gel CC (22 g), eluting with *n*-hexane–EtOAc (2:1) and gradually increasing the polarity with EtOAc to obtain 10 fractions. Fraction B-9-9-3-1 (11.6 mg, *n*-hexane–EtOAc, 2:1) was further purified by TLC ($CHCl_3$–MeOH, 10:1) to give **8** (8.4 mg). Fraction B-9-9-3-3 (92.4 mg, *n*-hexane–EtOAc, 1:1) was developed by TLC ($CHCl_3$–EtOAc, 1:1) to obtain four fractions, of which fraction B-9-9-3-3-2 (40.7 mg) was purified by TLC ($CHCl_3$–MeOH, 10:1) and then developed by TLC (*n*-hexane–EtOAc, 1:1) to afford 2 fractions-fraction B-9-9-3-3-2-1 (24.1 mg) was developed 20 times by TLC (*n*-hexane–EtOAc, 2:1) to give **2** (2.7 mg) and **10** (5.1 mg) and fraction B-9-9-3-3-2-2 (6.5 mg) was further purified by TLC (*n*-hexane–EtOAc, 2:3) to obtain **5** (4.4 mg). Fraction B-9-9-3-6 (124 mg) was developed by TLC (CH_2Cl_2–EtOAc, 1:2) and then purified by TLC (*n*-hexane–acetone, 1:1) to give **17** (2.5 mg). Fraction B-9-10 (388 mg, $CHCl_3$–MeOH, 98:2) was developed by TLC (*n*-hexane–EtOAc, 1:1) to obtain 10 fractions: fractions B-9-10-2 (12.7 mg), B-9-10-3 (10.4 mg), and B-9-10-7 (30.9 mg) were further purified by TLC (*n*-hexane–acetone, 1:1) to give **27** (7.1 mg), **22** (2.1 mg), and **9** (10.8 mg), respectively; fraction B-9-10-5 (20.5 mg) was developed by TLC (*n*-hexane–acetone, 1:1) and then purified by TLC (CH_2Cl_2–EtOAc, 1:1) to afford **4** (3.9 mg). Fraction B-9-11 (474 mg, $CHCl_3$–MeOH, 95:5) was applied to a silica gel column (14.2 g), eluting with *n*-hexane–acetone (2:1) and gradually increasing the polarity with acetone to obtain 14 fractions: fraction B-9-11-3 (50.8 mg, *n*-hexane–acetone, 2:1) was purified by TLC ($CHCl_3$–EtOAc, 2:1) to give **27** (7.0 mg); fraction B-9-11-6 (42.7 mg, *n*-hexane–acetone, 2:1) was developed by TLC (CH_2Cl_2–MeOH, 10:1) and then purified by TLC (CH_2Cl_2–EtOAc, 1:2) to afford **4** (6.7 mg) and **6** (1.4 mg). Fraction B-9-12 (11.9 g, $CHCl_3$–MeOH, 95:5) was subjected to silica gel CC (14.2 g), eluting with $CHCl_3$ and gradually increasing the polarity with MeOH to obtain 11 fractions: fraction B-9-12-8 (1.2 g, $CHCl_3$–MeOH, 98:2) was applied to a Sephadex LH-20 gel (300 g) eluting with MeOH to give 3 fractions-part (358 mg) of fraction B-9-12-8-2 (1.1 g) was further purified by TLC ($CHCl_3$–MeOH, 10:1) to obtain **1** (185 mg); fraction B-9-12-10 (320 mg, $CHCl_3$–MeOH, 95:5) was washed with CH_2Cl_2 to give **24** (20 mg).

3.4. Piperphilippinin I (*1*)

Colorless oil: $[\alpha]_D^{24}$: −45.3 (MeOH; *c* 4.98); UV λ_{max}^{MeOH} nm (log ε): 224 (4.51), 283 (4.23); UV $\lambda_{max}^{MeOH+KOH}$ nm (log ε): 244 (sh, 4.45), 290 (4.25), 406 (3.45); IR ν_{max}^{neat} cm^{-1}: 3338 (OH), 1752 (C=O); for ^1H and ^{13}C NMR spectra, see Tables 2 and 3; EIMS *m/z* (rel. int.): 344 $[M]^+$ (32), 207 (6), 138 (100), 137 (82), 131 (22), 123 (51); HREIMS *m/z*: 344.1263 (calc. for $C_{19}H_{20}O_6$, 344.1260); CD (MeOH): $\Delta\varepsilon_{288}$ −0.30, $\Delta\varepsilon_{232}$ −3.19 ($dm^3\,mol^{-1}\,cm^{-1}$).

3.5. Piperphilippinin II (*2*)

Colorless oil: $[\alpha]_D^{21}$: −24.1 ($CHCl_3$; *c* 0.11); UV λ_{max}^{MeOH} nm (log ε): 234 (sh, 4.01), 286 (3.94); UV $\lambda_{max}^{MeOH+KOH}$ nm (log ε): 240 (sh, 4.05), 291 (3.97); IR ν_{max}^{neat} cm^{-1}: 3391 (OH), 1745 (C=O), 1036 and 926 (OCH_2O); for ^1H and ^{13}C NMR spectra, see Tables 2 and 3; EIMS *m/z* (rel. int.): 342 $[M]^+$ (29), 206 (17), 161 (28), 136 (100), 135 (67), 131 (20), 123 (40), 77 (29); HREIMS *m/z*: 342.1106 (calc. for $C_{19}H_{18}O_6$, 342.1103); CD (MeOH): $\Delta\varepsilon_{292}$ −0.22, $\Delta\varepsilon_{234}$ −3.14 ($dm^3\,mol^{-1}\,cm^{-1}$).

3.6. Piperphilippinin III (*3*)

Colorless prisms (*n*-hexane–EtOAc); mp 90–91 °C; $[\alpha]_D^{22}$: −52.0 ($CHCl_3$; *c* 0.15); UV λ_{max}^{MeOH} nm (log ε): 250

Table 2
^{1}H NMR data (400 MHz) of compounds **1**–**5**

H	**1**[a]	**2**[b]	**3**[b]	**4**[a]	**5**[b]
2	6.68 (*d*, *J* = 2.0)	6.47 (*d*, *J* = 1.6)	6.38 (*s*)	6.86 (*s*)	6.44 (*d*, *J* = 1.6)
5	6.73 (*d*, *J* = 8.0)	6.71 (*d*, *J* = 8.0)		6.77 (*s*)	6.81 (*d*, *J* = 8.0)
6	6.56 (*dd*, *J* = 8.0, 2.0)	6.48 (*dd*, *J* = 8.0, 1.6)	6.38 (*s*)	6.77 (*s*)	6.51 (*dd*, *J* = 8.0, 1.6)
7	2.45–2.64 (*m*)	2.43–2.65 (*m*)	2.66 (*dd*, *J* = 14.4,10.4)	2.62 (*dd*, *J* = 14.0, 10.0)	2.43–2.61 (*m*)
8			3.10 (*dd*, *J* = 14.4, 4.4)	3.04 (*dd*, *J* = 14.0, 4.0)	
8′			3.86 (*m*)	3.95 (*m*)	
9	3.87 (*t*, *J* = 8.8)	3.85 (*dd*, *J* = 9.2, 7.2)	4.29 (*dd*, *J* = 9.2, 2.4)	4.21 (*dd*, *J* = 8.8, 2.4)	3.87 (*dd*, *J* = 9.2, 7.2)
	4.05 (*dd*, *J* = 8.8, 7.2)	4.09 (*dd*, *J* = 9.2, 6.8)	4.32 (*dd*, *J* = 9.2, 6.4)	4.26 (*dd*, *J* = 8.8, 7.2)	4.12 (*dd*, *J* = 9.2, 7.2)
2′	6.77 (*d*, *J* = 2.0)	6.68 (*d*, *J* = 2.0)	6.84 (*s*)	7.28 (*d*, *J* = 2.4)	6.99 (*d*, *J* = 8.4)
3′					6.75 (*d*, *J* = 8.4)
5′	6.77 (*d*, *J* = 8.0)	6.79 (*d*, *J* = 8.0)		6.97 (*d*, *J* = 8.0)	6.75 (*d*, *J* = 8.4)
6′	6.56 (*dd*, *J* = 8.0, 2.0)	6.57 (*dd*, *J* = 8.4, 2.0)	6.84 (*s*)	7.15 (*dd*, *J* = 8.0, 2.0)	6.99 (*d*, *J* = 8.4)
7′	2.79 (*dd*, *J* = 13.8, 6.4)	2.86 (*dd*, *J* = 14.0, 6.0)	7.53 (*d*, *J* = 1.6)	7.35 (*d*, *J* = 2.0)	2.88 (*dd*, *J* = 14.4, 7.2)
	2.87 (*dd*, *J* = 13.8, 5.2)	2.90 (*dd*, *J* = 14.0, 5.2)			2.97 (*dd*, *J* = 14.4, 5.2)
MeO-3	3.80 (*s*)		3.83 (*s*)		3.83 (*s*)
MeO-4			3.81 (*s*)		
MeO-5			3.83 (*s*)		
MeO-3′			3.92 (*s*)		
MeO-5′			3.92 (*s*)		
OCH_2O		5.93 (*d*, *J* = 1.4)		5.97 (*s*)	
		5.94 (*d*, *J* = 1.4)			
OH	7.50[c], 7.91[c], 7.93[c]	5.37[c], 5.69[c]	5.86[c]	8.35[c], 8.56[c]	5.21[c], 5.56[c]

Chemical shifts δ in ppm relative to TMS, *J* in Hz.
[a] In acetone-d_6.
[b] In $CDCl_3$.
[c] *br s*, exchangeable with D_2O.

(sh, 4.25), 265 (sh, 4.04), 336 (4.22); UV $\lambda_{max}^{MeOH+KOH}$ nm (log ε): 265 (4.22), 406 (4.34); IR ν_{max}^{KBr} cm^{-1}: 3391 (OH), 1743 (C=O); for ^{1}H and ^{13}C NMR spectra, see Tables 2 and 3; EIMS *m*/*z* (rel. int.): 430 $[M]^+$ (7), 249 (9), 182 (13), 181 (100), 148 (9); HREIMS *m*/*z*: 430.1632 (calc. for $C_{23}H_{26}O_8$, 430.1628).

3.7. Piperphilippinin IV (**4**)

Yellowish oil: $[\alpha]_D^{23}$: −7.0 (MeOH; *c* 0.195); UV λ_{max}^{MeOH} nm (log ε): 237 (4.15), 295 (4.11), 338 (4.24); UV $\lambda_{max}^{MeOH+KOH}$ nm (log ε): 279 (4.05), 392 (4.32); IR $\nu_{max}^{neat}$$cm^{-1}$: 3358 (OH), 1728 (C=O), 1037 and 926 (OCH_2O); for ^{1}H and ^{13}C NMR spectra, see Tables 2 and 3; EIMS *m*/*z* (rel. int.): 341 (18), 340 $[M]^+$ (86), 295 (15), 268 (24), 181 (16), 136 (23), 135 (100), 77 (27); HREIMS *m*/*z*: 340.0946 (calc. for $C_{19}H_{16}O_6$, 340.0947).

3.8. Piperphilippinin V (**5**)

Colorless oil: $[\alpha]_D^{20}$: −34.9 ($CHCl_3$; *c* 0.22); UV λ_{max}^{MeOH} nm (log ε): 226 (4.07), 280 (3.59); UV $\lambda_{max}^{MeOH+KOH}$ nm (log ε): 245 (4.09), 290 (3.62); IR $\nu_{max}^{neat}$$cm^{-1}$: 3394 (OH), 1749 (C=O); for ^{1}H and ^{13}C NMR spectra, see Tables 2 and 3; HREIMS *m*/*z*: 328.1310 (calc. for $C_{19}H_{20}O_5$, 328.1310); CD (MeOH): $\Delta\varepsilon_{288}$ −0.56, $\Delta\varepsilon_{232}$ −5.03 (dm^3 mol^{-1} cm^{-1}).

Table 3
^{13}C NMR data (100 MHz) of compounds **1**–**5**

C	**1**[a]	**2**[b]	**3**[b]	**4**[a]	**5**[b]
1	131.7	131.6	133.5	133.7	129.8
2	113.6	108.9	105.8	110.8	111.0
3	148.9	146.3	153.4	149.5	146.5
4	145.2	147.8	137.1	148.0	144.3
5	116.4	108.4	153.4	109.6	114.5
6	122.5	121.6	105.8	123.9	121.3
7	38.8	38.2	38.2	38.2	38.2
8	42.9	41.0	39.5	40.8	41.2
9	72.2	71.3	69.7	70.3	71.3
1′	131.4	130.3	125.4	127.9	129.6
2′	117.9	116.1	107.4	118.1	130.4
3′	146.5	143.7	147.2	147.0	115.4
4′	146.5	142.7	137.1	149.0	154.6
5′	116.7	115.3	147.2	117.3	115.4
6′	122.3	121.9	107.4	125.3	130.4
7′	35.3	34.1	137.9	138.0	34.1
8′	47.4	46.5	125.7	127.1	46.5
9′	179.8	179.0	172.5	173.3	178.9
MeO-3	56.8		56.4		55.8
MeO-4			60.9		
MeO-5			56.4		
MeO-3′			56.1		
MeO-5′			56.1		
OCH_2O		101.0		102.6	

Chemical shifts δ in ppm relative to TMS.
[a] In acetone-d_6.
[b] In $CDCl_3$.

3.9. Piperphilippinin VI (6)

Colorless oil: $[\alpha]_D^{24}$: −17.0 ($CHCl_3$; *c* 0.07); UV λ_{max}^{MeOH} nm (log ε): 229 (sh, 3.88), 283 (3.66); UV $\lambda_{max}^{MeOH+KOH}$ nm (log ε): 232 (sh, 3.87), 287 (3.66); IR ν_{max}^{neat} cm^{-1}: 3362 (OH), 1037 and 926 (OCH_2O); ^1H NMR (400 MHz, $CDCl_3$): δ 1.86 (2H, *m*, H-8 and H-8′), 2.64 (2H, *dd*, *J* = 13.6, 6.4 Hz, H-7a and H-7′a), 2.75 (2H, *br s*, OH-9 and OH-9′, exchangeable with D_2O), 2.76 (2H, *dd*, *J* = 13.6, 8.8 Hz, H-7b and H-7′b), 3.53 (2H, *dt*, *J* = 11.2, 4.4 Hz, H-9a and H-9′a), 3.81 (2H, *dt*, *J* = 11.2, 2.0 Hz, H-9b and H-9′b), 3.85 (3H, *s*, MeO-3), 5.48 (1H, *br s*, OH-4, exchangeable with D_2O), 5.92 (2H, *s*, OCH_2O), 6.60 (1H, *dd*, *J* = 8.0, 1.6 Hz, H-6′), 6.63 (2H, *br s*, H-2 and H-2′), 6.64 (1H, *dd*, *J* = 8.0, 2.0 Hz, H-6), 6.71 (1H, *d*, *J* = 8.0 Hz, H-5′), 6.82 (1H, *d*, *J* = 8.0 Hz, H-5); ^{13}C NMR (100 MHz, $CDCl_3$): δ 35.86 (C-7 or C-7′), 35.91 (C-7 or C-7′), 44.06 (C-8 or C-8′), 44.09 (C-8 or C-8′), 55.85 (MeO-3), 60.55 (C-9 or C-9′), 60.67 (C-9 or C-9′), 100.8 (OCH_2O), 108.1 (C-5′), 109.3 (C-2′), 111.3 (C-2), 114.1 (C-5), 121.6 (C-6), 121.9 (C-6′), 132.3 (C-1), 134.3 (C-1′), 143.8 (C-4), 145.7 (C-4′), 146.4 (C-3), 147.6 (C-3′); EIMS *m*/*z* (rel. int.): 361 (8), 360 $[M]^+$ (41), 342 (5), 206 (7), 192 (4), 189 (6), 188 (3), 187 (5), 175 (3), 163 (4), 161 (4), 151 (5), 150 (4), 138 (18), 137 (100), 136 (19), 135 (100), 131 (6), 123 (3), 122 (6), 77 (6); HREIMS *m*/*z*: 360.1576 (calc. for $C_{20}H_{24}O_6$, 360.1573).

3.10. Philippinamide (7)

Colorless needles (MeOH); mp 135.5-136.5 °C; UV λ_{max}^{MeOH} nm (log ε): 261 (3.62); IR ν_{max}^{KBr} cm^{-1}: 3304 (NH), 1658 (C=O); ^1H NMR (400 MHz, $CDCl_3$): δ 0.92 (6H, *d*, *J* = 6.8 Hz, H-3′ and H-4′), 1.47 (4H, *br s*, H-10 and H-11), 1.80 (1H, *sept*, *J* = 6.8 Hz, H-2′), 2.18 (4H, *br s*, H-9 and H-12), 3.16 (2H, *t*, *J* = 6.8 Hz, H-1′), 3.80 (3H, *s*, MeO-4), 5.45 (1H, *br s*, NH), 5.74 (1H, *d*, *J* = 14.8 Hz, H-16), 6.06 (1H, *dt*, *J* = 15.6, 6.8 Hz, H-8), 6.07 (1H, *dt*, *J* = 15.2, 6.0 Hz, H-13), 6.14 (1H, *dd*, *J* = 15.2, 10.0 Hz, H-14), 6.32 (1H, *d*, *J* = 15.6 Hz, H-7), 6.84 (2H, *d*, *J* = 8.8 Hz, H-3 and H-5), 7.19 (1H, *dd*, *J* = 14.8, 10.0 Hz, H-15), 7.27 (2H, *d*, *J* = 8.8 Hz, H-2 and H-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.1 (C-3′ and C-4′), 28.3 (C-2′), 28.6 (C-10 or C-11), 29.0 (C-10 or C-11), 32.7 (C-9 or C-12), 32.8 (C-9 or C-12), 46.9 (C-1′), 55.3 (MeO-4), 113.9 (C-3, 5), 121.8 (C-16), 127.0 (C-2, 6), 128.4 (C-13), 128.5 (C-8), 129.3 (C-7), 130.6 (C-1), 141.2 (C-15), 142.8 (C-14), 158.6 (C-4), 166.3 (C-17); EIMS *m*/*z* (rel. int.): 342 (18), 341 $[M]^+$ (15), 241 (19), 240 (15), 226 (17), 220 (24), 173 (14), 159 (22), 152 (23), 147 (30), 135 (26), 134 (20), 122 (18), 121 (100), 115 (25), 91 (54), 79 (19), 77 (25); HREIMS *m*/*z*: 341.2358 (calc. for $C_{22}H_{31}NO_2$, 341.2355).

3.11. (+)-Bornyl caffeate (8)

Colorless oil: $[\alpha]_D^{24}$: +19.8 ($CHCl_3$; *c* 0.33); UV λ_{max}^{MeOH} nm (log ε): 215 (sh, 4.07), 243 (sh, 3.93), 301 (sh, 3.93), 328 (3.99); UV $\lambda_{max}^{MeOH+KOH}$ nm (log ε): 256 (sh, 3.88), 306 (sh, 3.72), 371 (4.08); IR ν_{max}^{neat} cm^{-1}: 3339 (OH), 1675 (C=O); ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (3H, *s*, H-10′), 0.90 (3H, *s*, H-8′), 0.94 (3H, *s*, H-9′), 1.06 (1H, *dd*, *J* = 13.6, 3.6 Hz, H-3′b), 1.28 (1H, *m*, H-5′b), 1.35 (1H, *m*, H-6′b), 1.71 (1H, *t*, *J* = 4.4 Hz, H-4′), 1.79 (1H, *m*, H-5′a), 2.03 (1H, *m*, H-6′a), 2.42 (1H, *m*, H-3′a), 4.99 (1H, *ddd*, *J* = 10.0, 3.6, 2.0 Hz, H-2′), 6.19 (1H, *br s*, OH, exchangeable with D_2O), 6.29 (1H, *d*, *J* = 16.0 Hz, H-2), 6.49 (1H, *br s*, OH, exchangeable with D_2O), 6.88 (1H, *d*, *J* = 8.0 Hz, H-8), 7.01 (1H, *dd*, *J* = 8.0, 2.0 Hz, H-9), 7.12 (1H, *d*, *J* = 2.0 Hz, H-5), 7.57 (1H, *d*, *J* = 16.0 Hz, H-3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.5 (C-10′), 18.9 (C-9′), 19.7 (C-8′), 27.2 (C-6′), 28.0 (C-5′), 36.8 (C-3′), 44.9 (C-4′), 47.9 (C-7′), 48.9 (C-1′), 80.4 (C-2′), 114.5 (C-8), 115.4 (C-2), 116.0 (C-5), 122.3 (C-9), 127.5 (C-4), 143.9 (C-3), 144.7 (C-6), 146.5 (C-7), 168.4 (C-1); EIMS *m*/*z* (rel. int.): 316 $[M]^+$ (15), 180 (22), 164 (13), 163 (100), 145 (20), 136 (9), 135 (9), 134 (7), 117 (12), 91 (6), 89 (17), 79 (6), 77 (11), 63 (5).

3.12. Anti-platelet aggregation test

Blood was collected from the rabbit marginal vein, anticoagulated with EDTA (6 mM) and centrifuged for 10 min at 90*g* at room temperature to obtain platelet-rich plasma (PRP). A platelet suspension was prepared from this EDTA-anticoagulated PRP according to the washing procedures described previously (Teng et al., 1987). Platelet numbers were counted by a Coulter counter (Model ZM) and adjusted to 4.5×10^8 platelets/mL. The platelet pellets were finally suspended in Tyrode's solution of the following composition (mM): NaCl (136.8), KCl (2.8), $NaHCO_3$ (11.9), $MgCl_2$ (2.1), NaH_2PO_4 (0.33), $CaCl_2$ (1.0) and glucose (11.2), containing bovine serum albumin (O'Brien, 1962) using a Payton aggregometer. The platelet suspensions were stirred at 900 rpm. All tested compounds were dissolved in dimethyl sulfoxide (DMSO). In order to eliminate the effect of the solvent on the aggregation, the final concentration of DMSO was fixed at 0.5%, and did not affect the aggregation measured. Percentages of aggregation were calculated using the absorbance of the platelet suspension to represent 0% aggregation and the absorbance of Tyrode's solution to represent 100% aggregation. Aspirin was used as a positive control. Data were analyzed using Student's "*t*"-test.

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References

Achenbach, H., Karl, W., 1970. Über die Isolierung von zwei neuen Pyrrolididen aus Rauschpfeffer (*Piper methysticum* Forst.). Chem. Ber. 103, 2535–2540.

Achenbach, H., Fietz, W., Wörth, J., Waibel, R., Portecop, J., 1986. Constituents of tropical medicinal plants, IXX GC/MS-investigations of the constituents of *Piper amalago* – 30 new amides of the piperine-type. Planta Med. 52, 12–18.

Adesina, S.K., Olugbade, T.A., Akinwusi, D.D., Bergenthal, D., 1997. Extractives from *Zanthoxylum lemairie* root and stem. Pharmazie 52, 720–724.

Baillargeon, V.P., Stille, J.K., 1986. Palladium-catalyzed formylation of organic halides with carbon monoxide and tin hydride. J. Am. Chem. Soc. 108, 452–461.

Bellassoued, M., Majidi, A., 1993. A simple and highly stereoselective route to *E*-α,β-unsaturated aldehydes. J. Org. Chem. 58, 2517–2522.

Bohlmann, F., Zdero, C., 1976. Neue Terpen-Inhaltsstoffe aus *Verbesina*-Arten. Phytochemistry 15, 1310–1311.

Chen, J.J., Huang, Y.C., Chen, Y.C., Huang, Y.J., Wang, S.W., Peng, C.Y., Teng, C.M., Chen, I.S., 2002. Cytotoxic amides from *Piper sintenense*. Planta Med. 68, 980–985.

Chen, J.J., Duh, C.Y., Huang, H.Y., Chen, I.S., 2003. Cytotoxic constituents of *Piper sintenense*. Helv. Chim. Acta 86, 2058–2064.

Chen, Y.C., Chen, J.J., Chang, Y.L., Teng, C.M., Lin, W.Y., Wang, E.C., Chen, I.S., 2004. A new aristolactam alkaloid and anti-platelet aggregation constituents from *Piper taiwanense*. Planta Med. 70, 174–177.

Cheng, H.W., Lu, S.Y., 2000. Botel Tabaco, Yami & Plants. Lamper Enterprises, Taipei.

Chiji, H., Giga, T., Izawa, M., Kiriyama, S., 1984. Two phenolic amides in the seed balls of sugar beet (*Beta vulgaris* L. var. *saccharifera* Alefeld). Agric. Biol. Chem. 48, 1653–1654.

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

Estévez-Reyes, R., Estévez-Braun, A., González, A.G., 1992. Lignanolides from *Bupleurum salicifolium*. Phytochemistry 31, 2841–2845.

Evcim, U., Gözler, B., Freyer, A.J., Shamma, M., 1986. Haplomyrtin and (−)-haplomyrfolin: two lignans from *Haplophyllum myrtifolium*. Phytochemistry 25, 1949–1951.

Gan, K.H., Kuo, S.H., Lin, C.N., 1998. Steroidal constituents of *Ganoderma applanatum* and *Ganoderma neo-japonicum*. J. Nat. Prod. 61, 1421–1422.

Gözler, T., Gözler, B., Patra, A., Leet, J.E., Freyer, A.J., Shamma, M., 1984. Konyanin: a new lignan from *Haplophyllum vulcanicum*. Tetrahedron 40, 1145–1150.

Gözler, B., Arar, G., Gözler, T., Hesse, M., 1992. Isodaurinol, an arylnaphthalene lignan from *Haplophyllum cappadocicum*. Phytochemistry 31, 2473–2475.

Hirata, T., Tamura, Y., Yokobatake, N., Shimoda, K., Ashida, Y., 2000. A 38 kDa allylic alcohol dehydrogenase from the cultured cells of *Nicotiana tabacum*. Phytochemistry 55, 297–303.

Jackson, D.E., Dewick, P.M., 1984. Biosynthesis of *Podophyllum* lignans—i. Cinnamic acid precursors of podophyllotoxin in *Podophyllum hexandrum*. Phytochemistry 23, 1029–1035.

Kamaya, R., Ageta, H., 1990. Fern constituents: cheilanthenetriol and cheilanthenediol. Sesterterpenoids isolated from the leaves of *Aleuritopteris khunii*. Chem. Pharm. Bull. 38, 342–346.

Koike, K., Ohmoto, T., 1990. Quassinoids from *Picrasma javanica*. Phytochemistry 29, 2617–2621.

Kojima, H., Sato, N., Hatano, A., Ogura, H., 1990. Sterol glucosides from *Prunella vulgaris*. Phytochemistry 29, 2351–2355.

Kuo, Y.H., Chen, C.H., Lin, Y.L., 2002. New lignans from the heartwood of *Chamaecyparis obtusa* var. *formosana*. Chem. Pharm. Bull. 50, 978–980.

Lee, F.P., Chen, Y.C., Chen, J.J., Tsai, I.L., Chen, I.S., 2004. Cyclobutanoid amides from *Piper arborescens*. Helv. Chim. Acta 87, 463–468.

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

Lopes, L.M.X., Yoshida, M., Gottlieb, O.R., 1983. Dibenzylbutyrolactone lignans from *Virola sebifera*. Phytochemistry 22, 1516–1518.

Maldonado, E., Apan, M.T.R., Pérez-Castorena, A.L., 1998. Anti-inflammatory activity of phenylpropanoids from *Coreopsis mutica* var. *mutica*. Planta Med. 64, 660–661.

Miyakado, M., Nakayama, I., Yoshioka, H., Nakatani, N., 1979. The Piperaceae amides I: structure of pipercide, a new insecticidal amide from *Piper nigrum* L. Agric. Biol. Chem. 43, 1609–1611.

Miyazawa, M., Okuno, Y., Nakamura, S., Kameoka, H., 1998. Suppression of SOS-inducing activity of chemical mutagens by cinnamic acid derivatives from *Scrophularia ningpoensis* in the *Salmonella typhimurium* TA1535/pSK1002 *umu* test. J. Agric. Food Chem. 46, 904–910.

O'Brien, J.R., 1962. Platelet aggregation II. Some results from a new method of study. J. Clin. Path. 15, 452–455.

Rossi, M.H., Yoshida, M., Maia, J.G.S., 1997. Neolignans, styrylpyrones and flavonoids from an *Aniba* species. Phytochemistry 45, 1263–1269.

Rotherham, L.W., Semple, J.E., 1998. A practical and efficient synthetic route to dihydropipercide and pipercide. J. Org. Chem. 63, 6667–6672.

Satyanarayana, P., Venkateswarlu, S., 1991. Isolation, structure and synthesis of new diarylbutane lignans from *Phyllanthus niruri*: synthesis of 5′-desmethoxy niranthin and an antitumour extractive. Tetrahedron 47, 8931–8940.

Schobert, R., Siegfried, S., Gordon, G.J., 2001. Three-component synthesis of (*E*)-α,β-unsaturated amides of the piperine family. J. Chem. Soc., Perkin Trans. 1, 2393–2397.

Shih, M.H., 1991. Ph.D. Thesis. National Tsing Hua University, Hsinchu, Taiwan, pp. 216–224.

Suzuki, T., Holden, I., Casida, J.E., 1981. Diphenyl ether herbicides remarkably elevate the content in *Spinacia oleracea* of (*E*)-3-(4-hydroxy-3-methoxyphenyl)-*N*-[2-(4-hydroxy-3-methoxyphenyl)ethyl]-2-propenamide. J. Agric. Food Chem. 29, 992–995.

Tanaka, M., Ikeya, Y., Mitsuhashi, H., Maruno, M., Wakamatsu, T., 1995. Total syntheses of the metabolites of schizandrin. Tetrahedron 51, 11703–11724.

Teng, C.M., Chen, W.Y., Ko, W.C., Ouyang, C., 1987. Antiplatelet effect of butylidenephthalide. Biochim. Biophys. Acta 924, 375–382.

Thebtaranonth, C., Thebtaranonth, Y., Wanauppathamkul, S., Yuthavong, Y., 1995. Antimalarial sesquiterpenes from tubers of *Cyperus rotundus*: structure of 10,12-peroxycalamenene, a sesquiterpene endoperoxide. Phytochemistry 40, 125–128.

Tsai, I.L., Lee, F.P., Wu, C.C., Duh, C.Y., Ishikawa, T., Chen, J.J., Chen, Y.C., Seki, H., Chen, I.S., 2005. New cytotoxic cyclobutanoid amides, new furanoid lignan and anti-platelet aggregation constituents from *Piper arborescens*. Planta Med. 71, 535–542.

Wu, T.S., Jong, T.T., Tien, H.J., Kuoh, C.S., Furukawa, H., Lee, K.H., 1987. Annoquinone-A, an antimicrobial and cytotoxic principle from *Annona montana*. Phytochemistry 26, 1623–1625.

Xie, L.H., Ahn, E.M., Akao, T., Abdel-Hafez, A.A.M., Nakamura, N., Hattori, M., 2003a. Transformation of arctiin to estrogenic and antiestrogenic substances by human intestinal bacteria. Chem. Pharm. Bull. 51, 378–384.

Xie, L.H., Akao, T., Hamasaki, K., Deyama, T., Hattori, M., 2003b. Biotransformation of pinoresinol diglucoside to mammalian lignans by human intestinal microflora, and isolation of *Enterococcus faecalis* strain PDG-1 responsible for the transformation of (+)-pinoresinol to (+)-lariciresinol. Chem. Pharm. Bull. 51, 508–515.