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Bioactive composition of *Reevesia formosana* root and stem with cytotoxic activity potential†

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Six new compounds including three lignanoids: reevesiacoumarin (**1**), reevesic acid (**2**), and reevesilignan (**3**), and three terpenoids: reevesiterpenol A (**4**), reevesiterpenol B (**5**), and 3α,27-di-*O*-*trans*-caffeoylbetulinic acid (**6**), along with 40 known compounds were isolated from the root and stem of *Reevesia formosana* (Sterculiaceae). The structures of **1–6** were determined by spectroscopic techniques. Bioassays for the cytotoxicities of MCF-7, NCI–H460, and HepG2 cancer cell lines led to finding three cardenolides: strophanthojavoside (**31**) and ascleposide (**32**) with IC_{50} < 1 μM and strophalloside (**33**) displayed selective cytotoxicity to NCI–H460 with IC_{50} 0.62 ± 0.06 μM as well. 3α,27-Di-*O*-*trans*-caffeoylbetulinic acid (**6**) and secoisolariciresinol (**13**) also showed weak but selective cytotoxicity to NCI–H460 and HepG2 cancer cell lines, respectively.

Introduction

For decades, the role of cardenolides had transformed from the traditional use, treatment of anti-arrhythmia and heart failure, into the new prospect of anticancer. *Reevesia formosana* Sprague (Sterculiaceae) was found to be cytotoxic in the root, stem, and fruits, and also as the most bioactive one among 1400 species of Formosan plants through the cytotoxic assay for MCF-7, NCI–H460, and HepG2 *in vitro*. With our previous investigation of the root of *R. formosana*, individual cardenolides have been isolated,[1] including reevesiosides A–I and *epi*-reevesiosides F–I. Continuing these rigorous studies, three cardenolides: reevesioside J, reevesioside K, and *epi*-reevesioside K, three sesquiterpenoids: reevesiterpenols C–E, and two glycosides: reevesianins A and B, along with 46 known compounds were also isolated from the fruits of *R. formosana*.[2] Among these isolates, all cardenolides showed significant cytotoxicity against MCF-7, NCI–H460, and HepG2 cancer cell lines and reevesiterpenol E also exhibited the best selective cytotoxicity to the NCI–H460 cell line. Furthermore, reevesioside A,[3] reevesioside F,[4] and *epi*-reevesioside F[5] had already been discussed for the mechanism of several cancer cells. In this study, we investigated the stem of *R. formosana* and the remaining fractions of the root of *R. formosana*. From these two parts led to the isolation of six new compounds including three lignanoids: reevesiacoumarin (**1**), reevesic acid (**2**), and reevesilignan (**3**), and three terpenoids: reevesiterpenol A (**4**), reevesiterpenol B (**5**), and 3α,27-di-*O*-*trans*-caffeoylbetulinic acid (**6**) (Fig. 1), along with 40 known compounds.

The bioassay indicated three cardenolides: strophanthojavoside (**31**) and ascleposide (**32**) with IC_{50} < 1 μM and strophalloside (**33**) displayed selective cytotoxicity to NCI–H460 with IC_{50} 0.62 ± 0.06 μM as well. 3α,27-Di-*O*-*trans*-caffeoylbetulinic acid (**6**) and secoisolariciresinol (**13**) also showed weak but selective cytotoxicity to NCI–H460 and HepG2 cancer cell lines, respectively. All the structures were elucidated and confirmed through the 1D and 2D spectroscopic techniques.

Results and discussion

The root and stem of *R. formosana* were extracted with methanol, and the produced extracts were partitioned into the EtOAc and H_2O soluble layers. Both of the EtOAc layers were purified by conventional chromatographic techniques to obtain forty-six compounds (**1–46**), and the structures were elucidated by 1D and 2D NMR spectra and comparison with literature data.

Compound **1** was isolated as a yellowish powder with a molecular formula of $C_{20}H_{18}O_9$ as determined by positive-ion HRESIMS, showing a $[M + Na]^+$ ion at *m*/*z* 425.0845 (calcd for $C_{20}H_{18}O_9Na$, *m*/*z* 425.0848). The presence of hydroxy and

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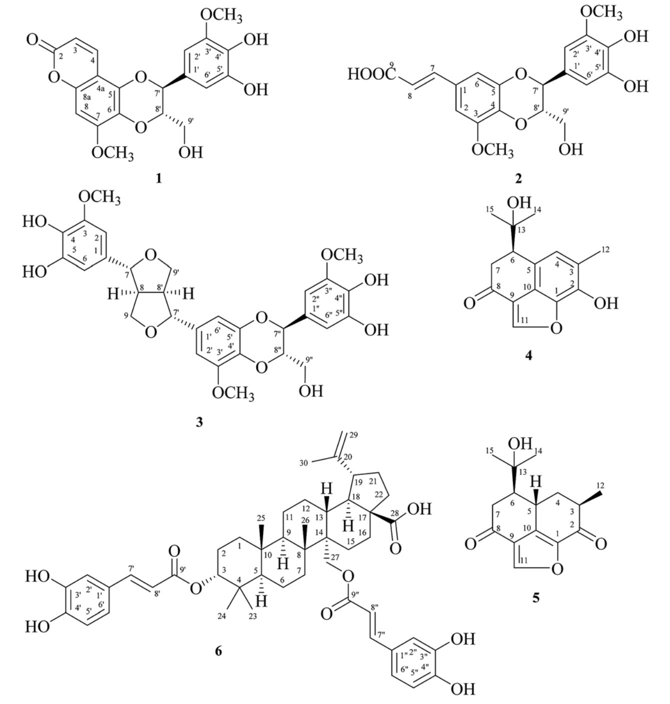

Fig. 1 Chemical structures of new compounds **1–6**.

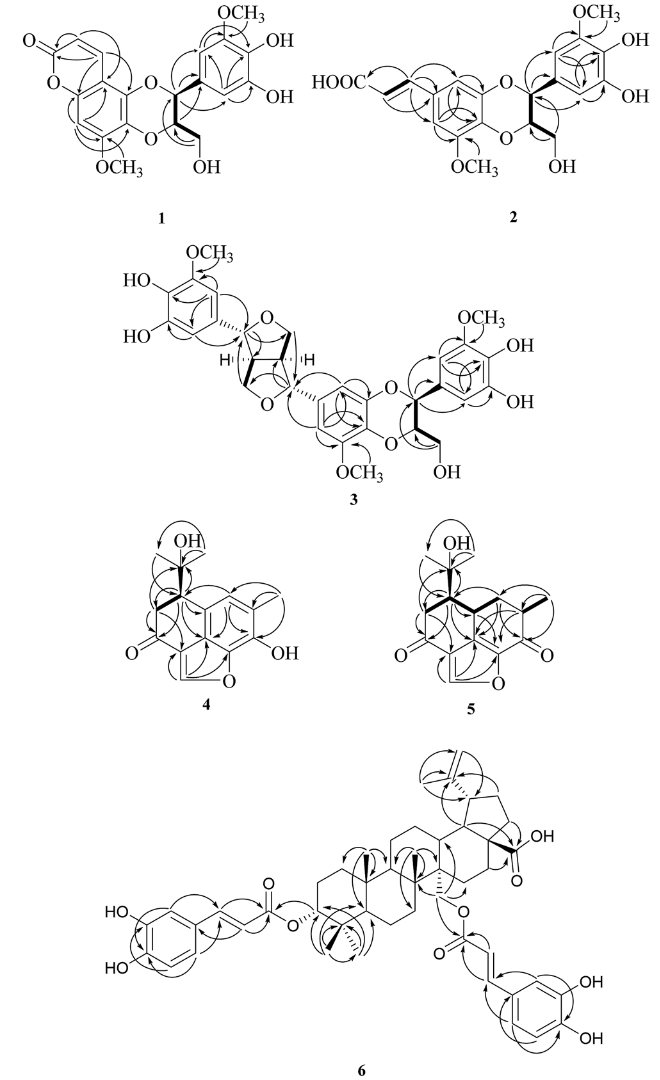

Fig. 2 Key HMBC (H → C), COSY (bold line) of compounds **1–6**.

carbonyl groups in **1** was shown by the bands at 3420 and 1708 cm^{-1}, respectively, in the IR spectrum. The ^{1}H NMR spectrum showed two *meta*-coupled protons of an aromatic ring at δ_H 6.70 (1H, dd, $J = 2.0$, 0.6 Hz, H-6′) and 6.73 (1H, d, $J = 2.0$ Hz, H-2′), one singlet proton of another aromatic ring at δ_H 6.61 (1H, s, H-8), two oxymethine protons at δ_H 4.08 (1H, ddd, $J = 8.0$, 6.4, 3.6 Hz, H-8′) and 5.08 (1H, d, $J = 8.0$ Hz, H-7′), two non-equivalent oxymethylene protons at δ_H 3.57 (1H, dd, $J = 12.0$, 3.6 Hz, H-9′b) and 3.85 (1H, dd, $J = 12.0$, 6.4 Hz, H-9′a), two methoxy groups at δ_H 3.84 (3H, s, OCH_3-3′) and 3.96 (3H, s, OCH_3-7). Also, a pair of mutually coupled protons at δ_H 6.15 (1H, d, $J = 9.6$ Hz, H-3) and 7.96 (1H, dd, $J = 9.6$, 0.6 Hz, H-4), assigned to the vinylic protons. The HMBC correlations from H-3 to C-2 (δ_C 161.8) and C-4a (δ_C 104.5), from H-4 to C-2, C-5 (δ_C 141.4), and C-8a (δ_C 151.1), from H-8 to C-4a, C-6 (δ_C 131.3), C-7 (δ_C 154.2), and C-8a and from OCH_3-7 to C-7 were further confirmed the 5,6-dioxo-7-methoxycoumarin moiety.[6] Furthermore, the location of the another methoxy group of a tetrasubstituted aromatic ring at C-3′ (δ_C 149.8) was further confirmed by the HMBC cross-peaks of H-2′ to C-3′ and C-4′ (δ_C 136.1), H-6′ to C-4′ and C-5′ (δ_C 147.1), and OCH_3-3′ to C-3′. The fragments of C-7′ (δ_C 78.8)-C-8′ (δ_C 79.9)-C-9′ (δ_C 62.3) were observed by COSY analysis (Fig. 2) as well as the phenylpropanoid moiety (C-1′–C-9′) was confirmed by correlations in the HMBC spectrum from H-7′ to C-1′, C-2′, and C-6′. According to the molecular formula of **1** with 12 indices of hydrogen deficiency (IHD) indicated the presence of a 1,4-dioxane ring between the 5,6-dioxo-7-methoxycoumarin moiety and the phenylpropanoid moiety (C-1′–C-9′). The O-linkages between C-5–O–C-7′ and C-6–O–C-8′ were confirmed by the NOESY spectrum (Fig. 3) showed correlations between H-9′ and OCH_3-7. The coupling constant ($J =$ 8.0 Hz) between H-7′ and H-8′ approved the *trans*-form.[7] The absolute configurations at C-7′ and C-8′ were determined as 7′*S*,8′*S* by CD spectral comparison with the analogous neolignan 7*S*,8*S*-nitidanin.[8] By the above data, the structure of **1** was further confirmed by DEPT, HSQC, COSY, NOESY, and HMBC experiments and named reevesiacoumarin.

Compound **2** was obtained as an optically colorless oil with $[\alpha]_D^{25}$ −8.1 (*c* 0.14, MeOH), and the molecular formula was calculated as $C_{20}H_{20}O_9$ by ESIMS and HRSIMS analyses with 11 degrees of unsaturation. UV and IR spectra were similar to those of simplidin (**7**)[8] also isolated in this study, except one additional carbonyl (1731 cm^{-1}) was appeared in IR spectrum. Analyses of 1D and 2D NMR [COSY (Fig. 2), HSQC, and HMBC (Fig. 2)] data established a neolignan-based gross structure, which was also closely related to simplidin (**7**).[9] The difference was attributed to a carboxylic acid (δ_C 173.8) at C-8 of **2** to replace a hydroxy group of simplidin (**7**), as evident from the ^{3}J-correlation of HMBC between H-7 to a carbonyl carbon (δ_C

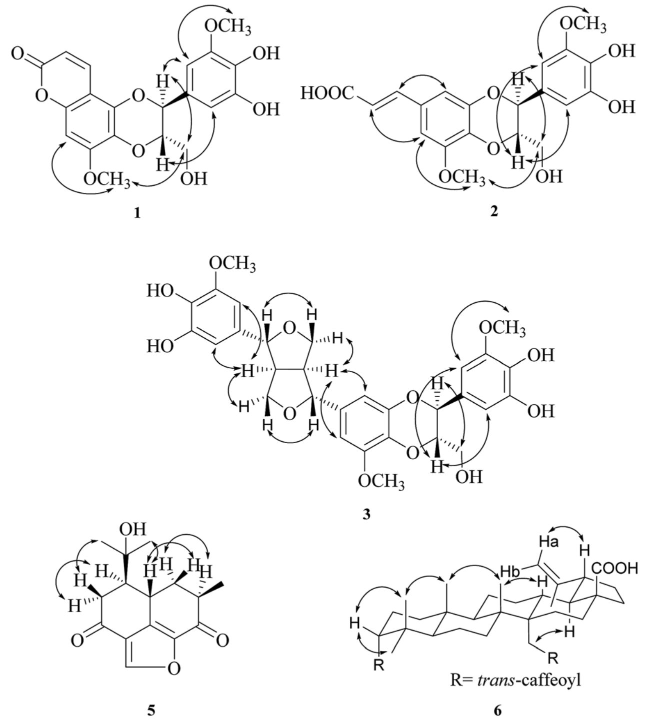

Fig. 3 Key NOESY (H ↔ H) correlations of compounds **1–6**.

173.8, C-9) and IR plot. Thus, the structure of **2** was determined and named reevesic acid.

Compound **3** was yielded as a colorless oil, with $[\alpha]_D^{25}$ −10.5 (*c* 0.06, MeOH), and the ESIMS and HRESIMS established the molecular formula as $C_{30}H_{32}O_{12}$, and the phenolic moiety was present by the bathochromic shift of UV spectrum. From the ^{1}H NMR spectrum, four methines [δ_H 3.11 (2H, m, H-8, H-8′)] including two oxygen-bearing [δ_H 4.64 (1H, br d, J = 4.2 Hz, H-7′) and 4.71 (1H, dd, J = 4.8, 1.8 Hz, H-7)], two oxymethylene groups [δ_H 3.86 (2H, m, H-9b, H-9′b) and 4.25 (2H, m, H-9a, H-9′a)], two pairs of *meta*-coupled aromatic protons [δ_H 6.49 (1H, br d, J = 1.8 Hz, H-6), 6.51 (1H, br d, J = 1.8 Hz, H-2)/δ_H 6.60 (1H, br t, J = 1.8 Hz, H-6′), 6.64 (1H, br t, J = 1.8 Hz, H-2′)], and the connection of two methoxy groups (δ_H 3.85, 3.88) to C-3 and C-3′, respectively, by HMBC (Fig. 2) correlations, pointed out the existence of 4′,5′-dioxo-5-hydroxypinoresinol moiety. While the rest of the ^{1}H NMR signals of **3** were identical to a phenylpropanoid moiety [δ_H 3.51 (1H, dd, J = 12.6, 4.2 Hz, H-9″b), 3.71 (1H, dd, J = 12.6, 2.4 Hz, H-9″a), 3.98 (1H, ddd, J = 7.8, 4.2, 2.4 Hz, H-8″), 4.80 (1H, d, J = 7.8 Hz, H-7″), 6.55 (1H, br d, J = 2.4 Hz, H-6″), and 6.58 (1H, br d, J = 1.8 Hz, H-2″)] alike C-1′–C-9′ of **1**. The coupling constant (J = 7.8 Hz) between H-7″ and H-8″ of **3** approved the *trans*-form.[7] The H-7″ showed correlation with H-9″ and showed no correlation to H-8″ also confirmed the *trans*-form of H-7″ and H-8″. Furthermore, 1,4-dioxane ring between the 4′,5′-dioxo-5-hydroxypinoresinol moiety and the phenylpropanoid moiety (C-1″–C-9″) was also confirmed the same as **1**. Thus, the planar structure of **3** was decided and the relative configuration was determined by NOESY (Fig. 3) correlations. According to the above evidence, compound **3** as a new substance named reevesilignan.

Compound **4** was obtained as an optically active colorless oil, with $[\alpha]_D^{25}$ +20.0 (*c* 0.10, $CHCl_3$). The molecular formula was obtained as $C_{15}H_{16}O_4$ with ESIMS and HRESIMS analyses, with the observation of HSQC and DEPT spectra, the substance was suggested to be sesquiterpenoid. The UV spectrum displayed the maxima absorptions at 211, 223 sh, and 249 sh nm then with the bathochromic shift by the addition of KOH aqueous solution further provided the presence of phenolic moiety. The ^{1}H NMR spectrum showed three singlet methyl groups at δ_H 1.16, 1.21, and 2.42, one methylene group [δ_H 2.84 (1H, dd, J = 16.6, 6.9 Hz, H-7b), 3.06 (1H, dd, J = 16.6, 1.7 Hz, H-7a)], one methine [δ_H 3.31 (1H, dd, J = 6.9, 1.7 Hz, H-6)], one aromatic proton [δ_H 7.08 (1H, s, H-4)], one oxoolefinic proton [δ_H 7.99 (1H, s, H-11)], and two broad singlets of hydroxy group at δ_H 3.60 and 5.60 as well. As eight degrees of unsaturation, the indication of conjugated carbonyl group (1682 cm^{-1}) and phenolic moiety, and the oxoolefinic proton (H-11) presented the $^{2,3}J$-correlations to δ_C 118.8 (C-9), 128.2 (C-10), 141.6 (C-1), suggested the presence of a furan ring, thus the structure of **4** was further confirmed as a furanosesquiterpenoid. The above ^{1}H NMR and physical data of **4** resembled hibiscone D[10] while the downfield shift of the quaternary carbon [δ_C 73.5 (C-13)] proposed a hydroxyisopropyl group [δ_H 1.16 (3H, s, H-14), 1.21 (3H, s, H-15); δ_C 73.5 (C-13), 27.2 (C-14), and 27.7 (C-15)] in **4** replaced an isopropyl group in hibiscone D. This was also proved by the HRESIMS *m/z* 283.0947 [M + Na]$^+$ (calcd for $C_{15}H_{16}O_4Na$, 283.0946). Therefore, the planar structure of **4** was determined and its relative configuration of **4** is the same as hibiscone D[10] according to the positive optical rotation ($[\alpha]_D^{25}$ +20.0), similar to hibiscone D ($[\alpha]_D^{26}$ +37). Compound **5**, as an optically active colorless oil with $[\alpha]_D^{25}$ −6.9 (*c* 0.05, $CHCl_3$). The molecular formula calculated for $C_{15}H_{18}O_4$ by HRESIMS, then further combined to the observation of ^{13}C and DEPT spectra, **5** was suggested to share the similar skeletone with **4** as furanosesquiterpenoid. Comparison of **5** to hibiscone C,[10] isolated from *Hibiscus elatus*, showed similarities in both the physical data and the ^{1}H NMR spectra while the difference appeared at the HRESIMS analysis for one more oxygen atom. The disappearance of one methine and presence of a quaternary carbon at δ_C 73.1 (C-13) were implied that the hydroxyisopropyl group [δ_H 1.34 (3H, s, H-14), 1.35 (3H, s, H-15); δ_C 73.1 (C-13), 24.9 (C-14), and 30.7 (C-15)] at C-6 in **5** was in place of isopropyl group at C-6 in hibiscone C. The relative configuration of **5** was confirmed with the NOESY correlations and the optical rotation ($[\alpha]_D^{25}$ −6.9), similar to hibiscone C ($[\alpha]_D^{27}$ −23). As determined by the above observations, **4** and **5** were recommended as the structures in Fig. 1 and named reevesiterpenol A and reevesiterpenol B, respectively, which were further confirmed by DEPT, HSQC, COSY (Fig. 2), and HMBC (Fig. 2) experiments.

Compound **6** was obtained as a yellowish oil. ESIMS and HRESIMS (*m/z* 819.4089 [M + Na]$^+$) analyses established the molecular formula of **6** as $C_{48}H_{60}O_{10}$. The IR absorption bands suggested the presence of hydroxy (3335 cm^{-1}), conjugated carbonyl ester (1697, 1683 cm^{-1}), and ^{13}C NMR data supported the presences of carboxylic (δ_C 179.9) and ester carbonyl (δ_C 169.5 and 168.9) groups. The ^{1}H NMR spectrum of **6** indicated five methyl singlets at δ_H 0.86, 0.93, 0.96, 1.06, and 1.73; the presence of two typical *trans*-caffeoyl groups were deduced by four olefinic protons at δ_H 6.287 (1H, d, $J = 16.0$ Hz, H-8″), 6.291 (1H, d, $J = 16.0$ Hz, H-8′), 7.56 (1H, d, $J = 16.0$ Hz, H-7″), 7.58 (1H, d, $J = 16.0$ Hz, H-7′) and by two 1,3,4-trisubstituted benzene rings at δ_H 6.75 (1H, d, $J = 8.4$ Hz, H-5′), 6.80 (1H, d, $J = 8.4$ Hz, H-5″), 6.90 (1H, dd, $J = 8.4$, 2.0 Hz, H-6′), 7.015 (1H, dd, $J = 8.4$, 2.0 Hz, H-6″), 7.018 (1H, d, $J = 2.0$ Hz, H-2′), and 7.11 (1H, d, $J = 2.0$ Hz, H-2″). The ^{13}C NMR data of **6** resembles 27-*O*-*trans*-caffeoylcylicodiscic acid with lupane type skeleton.[11] The major differences between **6** and 27-*O*-*trans*-caffeoylcylicodiscic acid were one additional *trans*-caffeoyl group at C-3 in **6** instead of the hydroxy group at C-3 in 27-*O*-*trans*-caffeoylcylicodiscic acid. The HMBC correlations from H-3 (δ_H 4.69) to C-9′ (δ_C 168.9); from H-27a (δ_H 4.88) and H-27b (δ_H 4.52) to C-9″ (δ_C 169.5) suggested two *trans*-caffeoyl groups linkage at C-3 and C-27, respectively. Moreover, the HMBC (Fig. 2) correlations from H-18 (δ_H 1.80) to C-28 (δ_C 179.9) indicated that a carboxylic group is attached to C-17. The 3α-configuration of the *trans*-caffeoyl group was deduced from the H-3 signal pattern at the downfield shifts at δ_H 4.69 (br s) and its ^{13}C NMR signal at δ_C 79.5.[12,13] The relative configurations of **6** were determined through inspection of the NOESY spectrum (Fig. 3). The several key NOESY correlations (H-3/H-23; H-3/H-24; H-24/H-25; H-25/H-26; H-13/H-26; H-18/H-27) suggested that the α-equatorial orientation of H-3 in *trans* A/B ring junction (Fig. 3). As a result, **6** was established as 3α,27-di-*O*-*trans*-caffeoylbetulinic acid and was further confirmed by DEPT, HSQC, COSY, and HMBC (Fig. 2) experiments.

The known compounds, simplidin (**7**),[9] 5-*O*-demethylbilagrewin (**8**),[14] malloapelin C (**9**),[15] syringaresinol (**10**),[16] pinoresinol (**11**),[16] 3-(α,4-dihydroxy-3-methoxy-benzyl)-4-(4-hydroxy-3-methoxybenzyl)tetrahydrofuran (**12**),[17] secoisolariciresinol (**13**),[18] rosmarinic acid (**14**),[19] clinopodic acid A (**15**),[19] *cis*-7-hydroxycalamenene (**16**),[20] *trans*-7-hydroxycalamenene (**17**),[20] 7-hydroxycadalene (**18**),[21] 4,5-dihydroblumenol A (**19**),[22] scopoletin (**20**),[23] fraxetin (**21**),[23] isofraxidin (**22**),[24] *trans*-ferulic acid (**23**),[18] vanillic acid (**24**),[25] a mixture of β-sitosterol (**25**) & stigmasterol (**26**),[26] a mixture of (24*R*)-stigmast-4-en-3-one (**27**) & (22*E*,24*S*)-stigmast-4,22-dien-3-one (**28**),[27] Q10 (**29**),[28] proanthocyanidin A2 (**30**),[29] strophanthojavoside (**31**),[30] ascleposide (**32**),[31] and strophalloside (**33**)[30] from the root of *R. formosana*, and **7**, **8**, **10**, **20**, a mixture of **25** & **26**, 3β-*trans*-caffeoylbetulinic acid (**34**),[32] 3β-*trans*-caffeoylbetulin (**35**),[33] 27-*O*-*trans*-caffeoylcylicodiscic acid (**36**),[11] 3-*epi*-betulinic acid (**37**),[34] 3-*epi*-betulinic acid acetate (**38**),[35] betulonic acid (**39**),[36] lupeol (**40**),[37] oleanolic acid (**41**),[38] 3β-hydroxysitost-5-en-7-one (**42**),[39] ergosterol peroxide (**43**),[40] reevesioside A (**44**),[1] and a mixture of reevesioside G (**45**), and *epi*-reevesioside G (**46**)[1] from the stem of *R. formosana* were identified by comparison of their physical and spectroscopic data with values reported in the literatures.

Among the 46 compounds isolated, 25 compounds (**1–10**, **12–15**, **19–24**, and **29–33**) had been tested for their cytotoxicity against the MCF-7, NCI–H460, and HepG2 cancer cell lines. The results for the active compounds are listed in Table 1. The results indicated that 3α,27-di-*O*-*trans*-caffeoylbetulinic acid (**6**) and secoisolariciresinol (**13**) displayed weak but selective cytotoxicity toward NCI–H460 and HepG2 cancer cell lines, respectively. While three cardenolides: strophanthojavoside (**31**) and ascleposide (**32**) with $IC_{50} < 1$ μM and strophalloside (**33**) displayed selective cytotoxicity to NCI–H460 with IC_{50} 0.62 ± 0.06 μM as well. The bioactive compounds were provided as cardenolides, with the results corresponded to the previous reports of the root[1] and fruits,[2] suggested that cardenolides played an important role and contributed mainly to the cytotoxicity of this species as the major component.

Through the bioassay screening among 1400 species of Formosan plants, *R. formosana* was found to be the most active one with the discovery to the new cytotoxic cardenolides. The phytochemistry of genus "*Reevesia*" hasn't been studied before our studies from the root[1] and fruits[2] of *R. formosana*, except for a report with five known compounds isolated from *R. longipetiolata*.[41] The results of the investigation this time were coherent with the previous reports, both led to the isolation and identification of cytotoxic cardenolides. So far, 27 new compounds including 16 cardenolides (reevesiosides A–K and *epi*-reevesiosides F–I, K), five sesquiterpenoids (reevesiterpenols A–E), two glycosides (reevesianins A and B), three lignanoids, (reevesiacoumarin, reevesic acid, and reevesilignan), and one triterpenoid (3α,27-di-*O*-*trans*-caffeoylbetulinic acid), along with 65 known compounds were identified from the root, stem, and fruits of *R. formosana*. Three new sugar moieties 4,6-dideoxy-2,3-methylenedioxy-β-D-allopyranosyl, 4,6-dideoxy-2-*O*-methyl-β-D-allopyranosyl, and 6-deoxy-2,3-methylenedioxy-β-D-glucopyranosyl together with some rare sugar moieties are also found as the glycones of cardenolides in this species. Among these isolates, all cardenolides presented prominent cytotoxicities against the MCF-7, NCI–H460, and HepG2 cancer cell lines, and particularly, reevesiosides A, F, and *epi*-reevesioside F were in the nanomolar level.[1] Reevesiterpenol E also displayed the best selective cytotoxicity to the NCI–H460 cell line.[2]

Therefore, the cardenolides and furanosesquiterpenoids from *R. formosana* are hopeful to be candidates for the discovery of anticancer compounds, primarily, the anticancer mechanisms had been studied by our research group. Such as reevesioside A induced G1 arrest and suppressed the expression of *c*-myc of human hormone-refractory prostate cancer,[3] and the anti-proliferative activity of reevesioside F was confirmed to be Na^+/K^+-ATPase α3 subunit-dependent[4] whereas the function of *epi*-reevesioside F was further identified to be PI3-kinase/Akt pathway related.[5] The new compounds, reevesiterpenols A–D were isolated from *R. formosana* in our previous study[2] and this study, were identified as furanosesquiterpenoids, which type was commonly found in genus *Hibiscus* (Malvaceae) before, and occurred in Sterculiaceae for the first time. Though the family of Sterculiaceae shared an intimate relationship with Malvaceae in plant taxonomy, there were no cardenolides revealed in Malvaceae. With entirely studied on the constituents of *R. formosana*, this species was standing as a unique one in the family of Sterculiaceae for the presence of cardenolides.

Table 1 The cytotoxicity (IC_{50} values) against the MCF-7, NCI–H460, and HepG2 cancer cell lines

	IC_{50} (μM)		
Compounds	MCF-7	NCI–H460	HepG2
3α,27-Di-*O*-*trans*-caffeoylbetulinic acid (**6**)	13.20 ± 0.90	7.60 ± 1.70	29.00 ± 0.80
5-*O*-Demethylbilagrewin (**8**)	21.20 ± 1.10	39.80 ± 1.00	41.8 ± 2.20
Secoisolariciresinol (**13**)	>50	>50	31.94 ± 0.93
Strophanthojavoside (**31**)	0.77 ± 0.03	0.17 ± 0.01	0.65 ± 0.06
Ascleposide (**32**)	0.16 ± 0.02	0.03 ± 0.01	0.37 ± 0.02
Strophalloside (**33**)	3.46 ± 0.13	0.62 ± 0.06	2.59 ± 0.13
Actinomycin D[a]	0.01 ± 0.001	0.02 ± 0.005	0.10 ± 0.015

[a] Positive control.

Experimental

General experimental procedures

The UV spectra were measured on a Jasco V-530 UV/VIS spectrophotometer; the IR spectra were recorded on a Jasco FTIR-4200 spectrophotometer (KBr or neat or ATR); optical rotations data were obtained with a JASCO P-2000 polarimeter; CD experiments were performed by a Jasco J-810 circular dichroism spectrophotometer. Silica gel (70–230 or 230–400 mesh, Merck) were used for column chromatography; TLC was carried out on precoated silica gel 60 F_{254} (Merck) for analytics and preparation; a spherical C18 100 Å (particle size: 20–40 μm) (Silicycle) was used for medium-pressure liquid chromatography. The NMR spectra were used methanol-d_4 (^{1}H, δ 3.31; ^{13}C, δ 49.0), acetone-d_6 (^{1}H, δ 2.05; ^{13}C, δ 30.5) or $CDCl_3$ (^{1}H, δ 7.26; ^{13}C, δ 77.0) as solvent were recorded on Varian Gemini 2000–200 (200 MHz for ^{1}H and 50 MHz for ^{13}C NMR), Varian Unity Plus 400 (400 MHz for ^{1}H and 100 MHz for ^{13}C NMR) and Varian VNMRS-600 (600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR) spectrometers. The EIMS data obtained on a VG-Biotech Quatro-5022 mass spectrometer: in m/z (rel.%). The HREIMS data were recorded on a Finnigan/Thermo Quest NAT mass spectrometer. The ESI/HRESIMS data obtained from a Bruker APEX-II mass spectrometer; in m/z.

Plant material

The root and stem of *R. formosana* were collected from the Mudan Village, Pingtung County, Taiwan, in September 2009 and August 2010, respectively. They were identified by Prof. Ih-Sheng Chen, one of the authors, Kaohsiung Medical University, Kaohsiung, Taiwan. A voucher specimen (Chen 6117) was deposited in the Herbarium of the School of Pharmacy, College of Pharmacy, Kaohsiung Medical University.

Extraction and isolation

The dried root of *R. formosana* (6.5 kg) was sliced and extracted with MeOH (30 L) at room temperature repeated three times, three days for each time. Evaporated *in vacuo* to obtain the methanolic extract (150 g), then partitioned into the EtOAc-soluble (45 g) and H_2O-soluble parts (100 g). As the bioassay shown cytotoxicity toward both parts, the EtOAc-soluble part (45 g) eluted with *n*-hexane–EtOAc by silica gel CC (70–230 mesh) in the gradient to 12 fractions (A-1–A-12). The bioactive fractions are A-9–A-12 mentioned previously.[1] Fraction A-2 (217 mg) was subjected to MPLC with *n*-hexane–EtOAc (20 : 1) to afford seven fractions (A-2-1–A-2-7). Fraction A-2-4 (9.2 mg) purified with PTLC (RP-18, MeOH–CH_2Cl_2, 2 : 1) to obtain **29** (2.2 mg, R_f 0.29). Fraction A-2-6 (10.0 mg) treated through PTLC (*n*-hexane–acetone, 10 : 1) for three times then afforded a mixture of **16**&**17** (1.0 mg, R_f 0.33) and **18** (2.2 mg, R_f 0.57). Fraction A-3 (410 mg) subjected to MPLC (*n*-hexane–CH_2Cl_2–EtOAc, 20 : 1 : 1) to yield 12 fractions (A-3-1–A-3-12), and fraction A-3-7 (164 mg) was purified by MPLC (RP-18, acetone–MeOH, 1 : 3) to obtain a mixture of **27** & **28** (56 mg). Fraction A-5 (1.8 g) was crystallized from MeOH and afforded a mixture of **25** & **26** (722 mg). Fraction A-9 (3.6 g) went through MPLC (RP-18, MeOH–H_2O, 1 : 1) and provided 10 fractions (A-9-1–A-9-10). Fraction A-9-2 (214 mg) eluted with CH_2Cl_2–MeOH (25 : 1) by MPLC to gain 10 fractions (A-9-2-1–A-9-2-10), and fraction A-9-2-4 (48.4 mg) further purified by PTLC (CH_2Cl_2–MeOH, 10 : 1) to give **23** (3.0 mg, R_f 0.32) and **24** (4.9 mg, R_f 0.45). Fraction A-9-2-5 (14.2 mg) further purified by PTLC (acetone–H_2O, 1 : 2) to give **21** (3.0 mg, R_f 0.32). The application of PTLC (CH_2Cl_2–EtOAc, 40 : 1) to fraction A-9-4 (119 mg), then repeated four times to yield **20** (4.9 mg, R_f 0.59) and **22** (8.5 mg, R_f 0.43), respectively. Eluting with *n*-hexane–CH_2Cl_2–acetone (4 : 1 : 1) by MPLC, fraction A-9-5 (67.7 mg) afforded six fractions (A-9-5-1–A-9-5-6). Fraction A-9-5-4 (13.7 mg) purified with PTLC (CH_2Cl_2–EtOAc, 6 : 1) to give **5** (4.3 mg, R_f 0.26) and **19** (4.2 mg, R_f 0.15). Fraction A-9-5-6 (39.3 mg) eluted with MeOH–H_2O (1 : 2) through MPLC to afford **10** (1.4 mg) and **12** (1.4 mg). Fraction A-9-6 (344 mg), eluted with *n*-hexane–CH_2Cl_2–acetone (2 : 1 : 1) by MPLC to gain nine fractions (A-9-6-1–A-9-6-9), and fraction A-9-6-7 (27.4 mg) further purified by PTLC (CH_2Cl_2–EtOAc, 5 : 1) to give **4** (2.0 mg, R_f 0.21). Fraction A-10 (3.6 g) went through Sephadex LH-20 column eluted with MeOH and separated into 13 fractions (A-10-1–A-10-13). Fraction A-10-4 (680 mg) through the elution of MeOH–H_2O (3 : 2) with MPLC (RP-18) was parted into 10 fractions (A-10-4-1–A-10-4-10), and with the further purification of MPLC under the same solvent system to give **9** (2.4 mg), **11** (2.0 mg) and **13** (2.1 mg). Fraction A-10-7 (521 mg) separated to nine fractions *via* the application of MPLC (RP-18, MeOH–H_2O, 1 : 1). Fraction A-10-7-1 (38.5 mg) was applied to PTLC (RP-18, acetone–H_2O, 1 : 2) for three times repeatedly to yield **14**

(19.0 mg, R_f 0.46) and fraction A-10-7-4 (143 mg) further followed the same steps of purification to obtain **2** (2.9 mg, R_f 0.25). As for fraction A-10-7-5 (49 mg) was purified by PTLC (RP-18) with solvent system MeOH–H_2O (1 : 1) to provide **8** (14.6 mg, R_f 0.14) and **15** (3.6 mg, R_f 0.25). Fraction A-10-9 (26.9 mg) treated with PTLC (RP-18, acetone–H_2O, 1 : 2) then **30** (11.6 mg, R_f 0.38) was yielded. Fraction A-11 (9.0 g) submitted to Sephadex LH-20 and eluted with MeOH to gain nine fractions (A-11-1–A-11-9). Fraction A-11-2 (741.1 mg) through the elution of MeOH–H_2O (1 : 1) with MPLC (RP-18) was parted into 14 fractions (A-11-2-1–A-11-2-14), and fraction A-11-2-6 was further purified by MPLC under the same solvent system to give **31** (3.9 mg), **32** (34 mg), and **33** (2.0 mg). Fraction A-11-4 (1.5 g) was applied to MPLC (RP-18, MeOH–H_2O, 1 : 2) and further purified by PTLC (RP-18, acetone–MeOH–H_2O, 1 : 1 : 2) to provide **7** (13.1 mg, R_f 0.26), and continuing *via* PTLC (CH_2Cl_2–EtOAc, 1 : 3) to afford **3** (4.4 mg, R_f 0.37) eventually.

The dried stem of *R. formosana* (7.0 kg) was sliced and extracted with MeOH (20 L) at room temperature repeated three times, three days for each time. Evaporated *in vacuo* to obtain the methanolic extract (160 g), then partitioned into the EtOAc-soluble (30 g) and H_2O-soluble parts (100 g). As the bioassay shown cytotoxicity toward both parts, the EtOAc-soluble part (45 g) eluted with *n*-hexane–EtOAc by silica gel CC (70–230 mesh) in gradient to 19 fractions (B-1–B-19). The bioactive fractions B-7, B-12–B-19 tended to be polar and against the cancer cell lines mentioned previously. Fraction B-6 (3.0 g) was subjected to MPLC with *n*-hexane–acetone (12 : 1) to yield 11 fractions (B-6-1–B-6-11). Fraction B-6-5 (1.2 g) was crystallized from MeOH to afford a mixture of **25** & **26** (1.0 g). Fraction B-7 (531 mg) subjected to MPLC (*n*-hexane–EtOAc, 5 : 1) to yield nine fractions (B-7-1–B-7-9). Fraction B-7-4 (47.3 mg) purified with PTLC (CH_2Cl_2–EtOAc, 30 : 1) to obtain **38** (5.8 mg, R_f 0.69) and **40** (2.0 mg, R_f 0.26). Fraction B-7-5 (250 mg) eluted with *n*-hexane–acetone, 10 : 1 by MPLC to gain six fractions (B-7-5-1–B-7-5-6), and fraction B-7-5-3 (44.7 mg) purified with PTLC (CH_2Cl_2–EtOAc, 80 : 1) to obtain **39** (14.9 mg, R_f 0.50), and fraction B-7-5-4 (49 mg) purified with PTLC (CH_2Cl_2–EtOAc, 60 : 1) to give **37** (10.5 mg, R_f 0.66). Eluting with *n*-hexane–EtOAc (3 : 1) by MPLC to fraction B-9 (409 mg) afforded 10 fractions (B-9-1–B-9-10). Fraction B-9-3 (155 mg) went through MPLC (CH_2Cl_2–EtOAc, 30 : 1) and provided nine fractions (B-9-3-1–B-9-3-9). Fraction A-9-3-9 was to obtain **36** (13.5 mg). Fraction B-9-4 (42.7 mg) treated with PTLC (CH_2Cl_2–acetone, 15 : 1) then **41** (8.8 mg, R_f 0.24) was yielded. Fraction B-9-6 (48.4 mg) purified with PTLC (CH_2Cl_2–acetone, 15 : 1) to give **42** (5.4 mg, R_f 0.24) and **43** (7.5 mg, R_f 0.32). Fraction B-12 (1.64 g) went through MPLC (*n*-hexane–EtOAc, 3 : 1) and provided eight fractions (B-12-1–B-12-8). Fraction B-12-4 (233 mg) eluted with CH_2Cl_2–EtOAc (20 : 1) through MPLC to afford 10 fractions (B-12-4-1–B-12-4-10). Fraction B-12-4-5 (37.5 mg) further purified by PTLC (*n*-hexane–EtOAc, 2 : 1) to give **34** (15.2 mg, R_f 0.26). Fraction B-12-4-6 (93 mg) separated to seven fractions with the application of MPLC (*n*-hexane–EtOAc, 2 : 1), then fraction B-12-4-6-4 (38.8 mg) was applied to PTLC (*n*-hexane–acetone, 1 : 1) to yield **35** (7.7 mg, R_f 0.53). Fraction B-12-5 (441 mg) was subjected to MPLC with CH_2Cl_2–acetone (5 : 1) to afford 11 fractions (B-12-5-1–B-12-5-11). Fraction B-12-5-3 (78.9 mg) eluted with CH_2Cl_2–acetone (3 : 1) by MPLC to gain nine fractions (B-15-5-3-1–B-12-5-3-9), and fraction B-12-5-3-4 (9.0 mg) further purified by PTLC (CH_2Cl_2–acetone, 6 : 1) to give **20** (3.2 mg, R_f 0.48). Fraction B-13 (1.7 g) went through MPLC (CH_2Cl_2–acetone, 8 : 1) and provided 10 fractions (B-13-1–B-13-10). Fraction B-13-8 (36.4 mg) subjected to MPLC (CH_2Cl_2–MeOH, 20 : 1) to yield eight fractions (B-13-8-1–B-13-8-8). Fraction B-13-8-1 was to obtain **10** (15.7 mg). Fraction B-13-10 (1.4 g) eluted with CH_2Cl_2–MeOH (20 : 1) by MPLC to gain eight fractions (B-13-10-1–B-13-10-8), then fraction B-13-10-2 (271 mg) further purified by MPLC (RP-18, H_2O–acetone, 1 : 1) to yield 10 fractions (B-13-10-2-1–B-13-2-10-10), then fraction B-13-10-2-8 was to afford a mixture of **45** and **46** (92.7 mg) and fraction B-13-10-2-9 was to give **44** (83 mg). Fraction B-14 (813 mg) submitted to Sephadex LH-20 eluted with MeOH and six fractions (B-14-1–B-14-6) were separated. Fraction B-14-2 were further applied to MPLC (RP-18, H_2O–acetone, 2 : 1) to provide **6** (7.0 mg, R_f 0.24). Fraction B-15 (712 mg) submitted to Sephadex LH-20 with seven fractions (B-15-1–B-15-7). Fraction B-15-4 (145 mg) separated to nine fractions with the application of MPLC (RP-18, H_2O–MeOH, 1.5 : 1) to afford **1** (9.5 mg, R_f 0.20). Fraction B-16 (1.63 g) went through Sephadex LH-20 column eluted with MeOH and separated into seven fractions (B-16-1–B-16-7). Fraction B-16-6 (600 mg) eluted with H_2O–MeOH–acetone (1 : 1) by MPLC (RP-18) to gain eight fractions (B-16-6-1–B-16-6-10), and fraction B-16-6-1 was to afford **8** (32 mg, R_f 0.38). Fraction B-16-9 (210 mg) further purified by MPLC (RP-18, H_2O–acetone, 2 : 1) to give **7** (3.0 mg, R_f 0.51).

Reevesiacoumarin (1). Yellowish powder; $[\alpha]_D^{25}$ −16.8 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ε) 237 sh (4.32), 320 (4.10) nm; UV (MeOH + KOH) λ_{max} (log ε) 322 (4.11) nm; CD (MeOH, $\Delta\varepsilon$) 224 (−0.59), 236 (+0.39), 286 (+0.60) nm; IR (KBr) ν_{max} 3420 (OH), 1708 (C=O) cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) δ 3.57 (1H, dd, J = 12.0, 3.6 Hz, H-9′b), 3.84 (3H, s, OCH_3-3′), 3.85 (1H, dd, J = 12.0, 6.4 Hz, H-9′a), 3.96 (3H, s, OCH_3-7), 4.08 (1H, ddd, J = 8.0, 6.4, 3.6 Hz, H-8′), 5.08 (1H, d, J = 8.0 Hz, H-7′), 6.15 (1H, d, J = 9.6 Hz, H-3), 6.61 (1H, s, H-8), 6.70 (1H, dd, J = 2.0, 0.6 Hz, H-6′), 6.73 (1H, d, J = 2.0 Hz, H-2′), 7.79 (1H, br s, OH, D_2O exchangeable), 7.96 (1H, dd, J = 9.6, 0.6 Hz, H-4); ^{13}C NMR (acetone-d_6, 100 MHz) δ 57.2 (OCH_3-3′), 57.4 (OCH_3-7), 62.3 (C-9′), 78.8 (C-7′), 79.9 (C-8′), 94.1 (C-8), 104.5 (C-4a), 104.6 (C-2′), 110.1 (C-6′), 113.1 (C-3), 128.6 (C-1′), 131.3 (C-6), 136.1 (C-4′), 139.4 (C-4), 141.4 (C-5), 147.1 (C-5′), 149.8 (C-3′), 151.1 (C-8a), 154.2 (C-7), 161.8 (C-2); ESIMS m/z 403 $[M + H]^+$; HRESIMS m/z 425.0845 $[M + Na]^+$ (calcd for $C_{20}H_{18}O_9Na$, 425.0848).

Reevesic acid (2). Colorless oil; $[\alpha]_D^{25}$ −8.1 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.09), 229 sh (3.93), 299 (3.63) nm; UV (MeOH + KOH) λ_{max} (log ε) 220 (4.73), 305 (3.66) nm; IR (neat) ν_{max} 3483 (OH), 1731 (C=O) cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) δ 3.52 (1H, dd, J = 12.2, 5.7 Hz, H-9′b), 3.73 (1H, dd, J = 12.2, 2.7 Hz, H-9′a), 3.86 (3H, s, OCH_3-3′), 3.91 (3H, s, OCH_3-3), 4.03 (1H, ddd, J = 7.8, 5.7, 2.7 Hz, H-8′), 4.83 (1H, d, J = 7.8 Hz, H-7′), 6.37 (1H, d, J = 15.6 Hz, H-8), 6.57 (1H, dd, J = 1.8, 0.6 Hz, H-6′), 6.59 (1H, d, J = 1.8 Hz, H-2′), 6.79 (1H, dd, J = 1.8, 0.6 Hz, H-6), 6.84 (1H, d, J = 1.8 Hz, H-2), 7.37 (1H, d, J = 15.6 Hz, H-7); ^{13}C NMR (CD_3OD, 150 MHz) δ 56.68 (OCH_3-3′), 56.73 (OCH_3-3), 62.0

(C-9′), 77.7 (C-7′), 80.3 (C-8′), 104.0 (C-2′), 104.9 (C-2), 109.3 (C-6′), 110.9 (C-6), 122.8 (C-8), 128.5 (C-1′), 129.4 (C-1), 135.9 (C-4 and C-4′), 142.7 (C-7), 145.9 (C-5), 146.8 (C-5′), 149.8 (C-3′), 150.4 (C-3), 173.8 (C=O); ESIMS m/z 427 $[M + Na]^+$; HRESIMS m/z 427.09977 $[M + Na]^+$ (calcd for $C_{20}H_{20}O_9Na$, 427.09995).

Reevesilignan (3). Colorless oil; $[\alpha]_D^{25}$ −10.5 (c 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 214 (4.75), 239 sh (4.26), 277 (3.61) nm; UV (MeOH + KOH) λ_{max} (log ε) 223 (4.90), 262 (4.03) nm; IR (neat) ν_{max} 3407 (OH) cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) δ 3.11 (2H, m, H-8, H-8′), 3.51 (1H, dd, J = 12.6, 4.2 Hz, H-9″b), 3.71 (1H, dd, J = 12.6, 2.4 Hz, H-9″a), 3.84 (3H, s, OCH_3-3″), 3.85 (3H, s, OCH_3-3), 3.86 (2H, m, H-9b, H-9′b), 3.88 (3H, s, OCH_3-3′), 3.98 (1H, ddd, J = 7.8, 4.2, 2.4 Hz, H-8″), 4.25 (2H, m, H-9a, H-9′a), 4.64 (1H, br d, J = 4.2 Hz, H-7′), 4.71 (1H, br dd, J = 4.8, 1.8 Hz, H-7), 4.80 (1H, d, J = 7.8 Hz, H-7″), 6.49 (1H, br d, J = 1.8 Hz, H-6), 6.51 (1H, br d, J = 1.8 Hz, H-2), 6.55 (1H, br d, J = 2.4 Hz, H-6″), 6.58 (1H, br d, J = 1.8 Hz, H-2″), 6.60 (1H, br t, J = 1.8 Hz, H-6′), 6.64 (1H, br t, J = 1.8 Hz, H-2′); ^{13}C NMR (CD_3OD, 150 MHz) δ 55.4 (C-8), 55.5 (C-8′), 56.63 (OCH_3-3″), 56.67 (OCH_3-3), 56.7 (OCH_3-3′), 62.1 (C-9″), 72.7 (C-9), 72.8 (C-9′), 77.8 (C-7″), 80.0 (C-8″), 87.3 (C-7′), 87.6 (C-7), 102.6 (C-2), 103.6 (C-2′), 104.0 (C-2″), 107.8 (C-6), 108.5 (C-6′), 109.3 (C-6″), 128.6 (C-1′ and C-1″), 133.1 (C-1), 133.9 (C-4′), 134.8 (C-4), 135.9 (C-4″), 145.7 (C-5′), 146.6 (C-5), 149.7 (C-3), 146.8 (C-5″), 149.8 (C-3″), 150.3 (C-3′); ESIMS m/z 607 $[M + Na]^+$; HRESIMS m/z 607.1787 $[M + Na]^+$ (calcd for $C_{30}H_{32}O_{12}Na$, 607.1791).

Reevesiterpenol A (4). Colorless oil; $[\alpha]_D^{25}$ +20.0 (c 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 211 (4.30), 223 sh (4.01), 249 sh (3.88) nm; UV (MeOH + KOH) λ_{max} (log ε) 219 (4.57), 236 sh (4.07), 274 sh (3.85) nm; IR (neat) ν_{max} 3417 (OH), 1682 (C=O), 1557, 1538, 1516 (aromatic ring) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.16 (3H, s, H-14), 1.21 (3H, s, H-15), 2.42 (3H, s, H-12), 2.84 (1H, dd, J = 16.6, 6.9 Hz, H-7b), 3.06 (1H, dd, J = 16.6, 1.7 Hz, H-7a), 3.31 (1H, dd, J = 6.9, 1.7 Hz, H-6), 3.60 (1H, br s, OH-13, D_2O exchangeable), 5.60 (1H, br s, OH-2, D_2O exchangeable), 7.08 (1H, s, H-4), 7.99 (1H, s, H-11); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 15.7 (C-12), 27.2 (C-14), 27.7 (C-15), 43.9 (C-7), 50.9 (C-6), 73.5 (C-13), 118.8 (C-9), 120.9 (C-5), 121.7 (C-3), 126.9 (C-4), 128.2 (C-10), 137.8 (C-2), 141.6 (C-1), 142.4 (C-11), 193.8 (C-8); ESIMS m/z 283 $[M + Na]^+$; HRESIMS m/z 283.0947 $[M + Na]^+$ (calcd for $C_{15}H_{16}O_4Na$, 283.0946).

Reevesiterpenol B (5). Colorless oil; $[\alpha]_D^{25}$ −6.9 (c 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 230 (4.49), 264 (4.42) nm; IR (neat) ν_{max} 3440 (OH), 1679 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.34 (3H, s, H-14), 1.35 (3H, s, H-15), 1.36 (3H, d, J = 7.6 Hz, H-12), 2.06 (1H, ddd, J = 13.6, 11.2, 3.2 Hz, H-6), 2.16 (1H, ddd, J = 14.9, 11.4, 4.4 Hz, H-4b), 2.36 (1H, dd, J = 16.8, 13.6 Hz, H-7b), 2.64 (1H, ddd, J = 14.9, 4.6, 2.4 Hz, H-4a), 2.76 (1H, dd, J = 16.8, 3.2 Hz, H-7a), 2.77 (1H, qdd, J = 7.6, 4.4, 2.4 Hz, H-3), 3.22 (1H, ddd, J = 11.4, 11.2, 4.6 Hz, H-5), 8.12 (1H, s, H-11); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.1 (C-12), 24.9 (C-14), 30.6 (C-5), 30.7 (C-15), 38.8 (C-4), 42.9 (C-3), 43.7 (C-7), 52.4 (C-6), 73.1 (C-13), 123.0 (C-9), 143.9 (C-10), 145.1 (C-1), 147.5 (C-11), 189.0 (C-2), 192.3 (C-8); ESIMS m/z 285 $[M + Na]^+$; HRESIMS m/z 285.1102 $[M + Na]^+$ (calcd for $C_{15}H_{18}O_4Na$, 285.1103).

3α,27-Di-*O-trans*-caffeoylbetulinic acid (6). Yellowish oil; $[\alpha]_D^{25}$ −99.9 (c 0.24, MeOH); UV (MeOH) λ_{max} (log ε) 215 (4.49), 243 (4.32), 300 (4.43), 327 (4.55) nm; UV (MeOH + KOH) λ_{max} (log ε) 258 (4.25), 308 (4.17), 369 (4.64) nm; IR (neat) ν_{max} 3335 (OH), 1697 (OCOCH), 1683 (COOH) cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 0.86 (3H, s, H-23), 0.93 (3H, s, H-24), 0.96 (3H, s, H-25), 1.02 (1H, m, H-12b), 1.06 (3H, s, H-26), 1.29 (1H, m, H-11b), 1.30 (1H, m, H-1b), 1.34 (1H, m, H-16b), 1.41 (1H, m, H-5), 1.42 (1H, m, H-21b), 1.43 (1H, m, H-6b), 1.45 (1H, m, H-22b), 1.46 (1H, m, H-7b), 1.47 (1H, m, H-15b), 1.48 (1H, m, H-6a), 1.54 (1H, m, H-1a), 1.56 (1H, m, H-11a), 1.58 (1H, m, H-7a), 1.61 (1H, m, H-9), 1.64 (1H, m, H-2b), 1.73 (3H, s, H-30), 1.79 (1H, m, H-18), 1.80 (1H, m, H-12a), 1.88 (1H, m, H-15a), 1.95 (1H, m, H-22a), 1.96 (1H, m, H-21a), 2.00 (1H, m, H-2a), 2.32 (1H, br d, J = 12.4 Hz, H-16a), 2.54 (1H, td, J = 12.6, 3.6 Hz, H-13), 3.07 (1H, td, J = 10.4, 4.8 Hz, H-19), 4.52 (1H, d, J = 12.8 Hz, H-27b), 4.62 (1H, br s, H-29b), 4.69 (1H, br s, H-3), 4.75 (1H, br s, H-29a), 4.88 (1H, d, J = 12.8 Hz, H-27a), 6.287 (1H, d, J = 16.0 Hz, H-8″), 6.291 (1H, d, J = 16.0 Hz, H-8′), 6.75 (1H, d, J = 8.4 Hz, H-5′), 6.80 (1H, d, J = 8.4 Hz, H-5″), 6.90 (1H, dd, J = 8.4, 2.0 Hz, H-6′), 7.015 (1H, dd, J = 8.4, 2.0 Hz, H-6″), 7.018 (1H, d, J = 2.0 Hz, H-2′), 7.11 (1H, d, J = 2.0 Hz, H-2″), 7.56 (1H, d, J = 16.0 Hz, H-7″), 7.58 (1H, d, J = 16.0 Hz, H-7′); ^{13}C NMR (CD_3OD, 100 MHz) δ 16.9 (C-25), 17.2 (C-26), 19.2 (C-6), 19.6 (C-30), 22.0 (C-11), 22.2 (C-24), 24.0 (C-2), 25.3 (C-15), 26.6 (C-12), 28.5 (C-23), 31.6 (C-21), 33.8 (C-16), 35.5 (C-1), 36.5 (C-7), 37.9 (C-22), 38.1 (C-4), 38.7 (C-10), 40.3 (C-13), 42.9 (C-8), 46.9 (C-14), 48.4 (C-19), 50.6 (C-18), 52.0 (C-5), 53.2 (C-9), 57.3 (C-17), 64.4 (C-27), 79.5 (C-3), 110.5 (C-29), 115.1 (C-2″), 115.2 (C-2′), 115.2 (C-8′), 115.7 (C-8″), 116.5 (C-5′, C-5″), 123.1 (C-6′), 123.4 (C-6″), 127.6 (C-1′), 127.8 (C-1″), 146.7 (C-7″), 146.8 (C-3″), 147.2 (C-3′), 147.2 (C-7′), 149.5 (C-4″), 149.7 (C-4′), 151.7 (C-20), 168.9 (C-9′), 169.5 (C-9″), 179.9 (C-28); ESIMS m/z 797 $[M + H]^+$; HRESIMS m/z 819.4089 $[M + Na]^+$ (calcd for $C_{48}H_{60}O_{10}Na$, 819.4089).

Cytotoxicity assay

HepG2 (liver hepatocellular cells, [ATCC HB-8065]), NCI–H460 (nonsmall-cell lung cancer, [ATCCHTB-177]), and MCF-7 (human breast adenocarcinoma, [ATCC HTB-22]) cancer cells were seeded in 96-well microtiter plates in 100 μL culture medium per well at cell numbers of 10 000, 2500, and 6500, respectively. HepG2 and MCF-7 were cultured in Dulbeccos modified Eagles medium (Hyclone Laboratory Inc.), NCI–H640 was cultured in RPMI-1640 medium (GIBCO-Life Technologies, Inc.), supplemented with 10% fetal calf serum (Biological Industries Inc.) and nonessential amino acid (Biological Industries, Inc.) and maintained at 37 °C in a humidified incubator with an atmosphere of 5% CO_2. The cytotoxicity assay was performed as described.

Conclusions

In summary, we investigated the stem of *R. formosana* and the remaining fractions of the root of *R. formosana* led to the isolation of six new compounds including three lignanoids: reevesiacoumarin (**1**), reevesic acid (**2**), and reevesilignan (**3**), and three terpenoids: reevesiterpenol A (**4**), reevesiterpenol B (**5**), and 3α,27-di-*O-trans*-caffeoylbetulinic acid (**6**), along with 40 known compounds. In our serious studies found that all cardenolides presented prominent cytotoxicities against the MCF-7, NCI–H460, and HepG2 cancer cell lines and some terpenoids and lignans showed selective cytotoxic activities. Therefore, compounds isolated from *R. formosana* could potentially support the development of anticancer therapies.

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