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Composition and antifungal activity of balsam from *Liquidambar formosana* Hance

Abstract: Formosan sweet gum (*Liquidambar formosana* Hance) is a tree species endemic in Taiwan. In this study, the composition of balsam from *L. formosana* has been determined by several chromatographic and spectroscopic techniques. Among the 26 compounds identified, three new triterpenoids were detected, namely, 2α,3α-dihydroxyolean-12-en-28-al (**1**), 3α-hydroxyolean-12-en-30-ol (**2**), and 3α-hydroxyolean-2-oxo-12-en-28-al (**3**). The most abundant volatile compounds were β-caryophyllene (22.7%), α-pinene (23.3%), and β-pinene (19.6%), and the most abundant nonvolatile compounds were 3α,25-dihydroxyolean-12-en-28-oic acid (**12**, 19.1%), oleanonic aldehyde (**9**, 14.0%), and betulonic acid (**15**, 13.4%). The compounds 3α,25-dihydroxyolean-12-en-28-oic acid and bornyl cinnamate were found to be inhibitory for white rot (*Lenzites betulina*) and brown rot (*Laetiporus sulphureus*) fungi.

Keywords: antifungal activity, bornyl cinnamate, Formosan sweet gum, *Liquidambar formosana*, resina liquidambaris, triterpenoids

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Introduction

Research on wood and bark extractives have increasing importance as they are environmentally benign sources of antimicrobial compounds and are useful chemicals with a variety of medical and industrial applications (Lim et al. 2007; Mburu et al. 2007; Binbuga et al. 2008; Borges et al. 2008; Gao et al. 2008; Smeds et al. 2011).

Tree exudates are also known as sources of phytomedicines, perfumes, and flavors for more than seven centuries (Öztürka et al. 2008). They have potentially a great economic value. Commercially exploited tree exudates include damar from Dipterocarpaceae, rosin and turpentine from *Pinus* spp., and frankincense from *Boswellia* spp. (Rijkers et al. 2006). According to the classification of Hillis (1987), tree exudates can be divided into nine groups: resin, gum, kino, latex, manna, amber, balsam, maple sugar, and crystalline compounds. Trees, particularly conifers, contain oleoresins composed of sesquiterpenoids and diterpenoids, which occur in resin ducts or blisters in the bark (Hillis 1987; Byun-McKay et al. 2003; Holmbom et al. 2008).

Balsam as a resinous exudate can be obtained by damaging the tree bark of various shrubs and trees including *Liquidambar* spp. or *Myroxylon* spp.; it has a fragrant aroma and pungent taste. Storax is a well-known balsam since ancient times; it is produced by *Liquidambar orientalis* (Hamamelidaceae) and used as an aromatic fixative. Formosan sweet gum (*Liquidambar formosana* Hance) is a deciduous tree native to Taiwan. Its leaves are downy and violet-red when the tree is young, and turn to delicate shades of rose in autumn (Fordham 1961). The stem belongs to the economically important heartwood species, which can be used for construction, furniture, and mushroom cultivation, just to mention a few. Like *L. orientalis*, *L. formosana* also produces a balsamic exudate, named resina liquidambaris), which has numerous medical applications in Asian folk medicine, such as promoter of blood circulation, alleviator of blood stasis, analgesic, and anti-inflammatory and wound-healing agent (Kan 1977). Yang et al. (2011) identified 10 pentacyclic triterpenoids, but it can be presumed that there are still interesting and valuable undetected compounds in resina liquidambaris.

In the present study, both volatile and nonvolatile compounds of resina liquidambaris and their antifungal effects will be further elucidated and quantified by means of modern analytical techniques.

Materials and methods

Instruments: Optical rotation (PerkinElmer 241 polarimeter, Foster, CA, USA), UV (Bio-Tek μQuant MQX200 ELISA reader, Winooski, Vermont, USA), and infrared data (PerkinElmer Spectrum 100 FT-IR spectrometer, Foster, CA, USA). ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, heteronuclear single-quantum coherence (HSQC), heteronuclear multiple-bond correlation (HMBC), ^{1}H-^{1}H correlation spectroscopy (COSY), and distortionless enhancement by polarization transfer (DEPT) spectra were obtained on a Varian Unity Inova 600 MHz and 400 MHz spectrometer with $CDCl_3$ as the solvent. High-resolution electrospray ionization mass spectrometry (ESI-MS) data were obtained on a Thermo/Finnigan Quest MAT 95XL mass spectrometer. High-performance liquid chromatography (HPLC) was carried out over a Phenomenex column (5 μm, 250 mm×10 mm, Phenomenex); Shodex RI-101 detector. Open column chromatography was carried out over silica gel (4×30 cm; 60–80 mesh; Merck).

Detection of volatile compounds by SPME

The solid-phase microextraction (SPME) method was applied. The SPME holder and carboxen-polydimethylsiloxane (75 μm) were purchased from Supelco (Bellefonte, PA, USA). Before use, SPME fibers were conditioned by heating in the hot injection port of a gas chromatograph at 250°C for 20 min to remove contaminants. One milligram of balsam was placed in a 20-ml sample vial sealed with parafilm. The vial was placed in a water bath (27±2°C) and conditioned for 20 min without fiber. Then, an SPME fiber was introduced into the vial and exposed to the headspace above the wood meal for 10 min. After this, the fiber was immediately inserted into the injection port of the gas chromatograph equipped with a heated SPME liner (250°C for 10 s).

Fragrance analysis by GC/MS

Instrument: ITQ Series gas chromatography-mass spectrometry (GC/MS) system (Thermo) equipped with a DB-5 column (30 m×0.25 mm inside diameter, 0.25 μm film thickness, J & W Scientific). Temperature program: 50°C for 1 min, heated at 3°C min^{-1} to 200°C and held for 4 min. Injector temperature, 230°C; ion source temperature, 250°C; electron impact, 70 eV; carrier gas, He at 1 ml min^{-1}; and mass range, 45–425 *m*/*z*. Quantification: percentage peak area with a response factor of 1.0. MS library: Wiley/NBS Registry of Mass Spectral Data, National Institute of Standards and Technology (NIST) search; authentic reference compounds were also used.

NMR analyses of nonvolatile compounds

The solvent and internal standards were $CDCl_3$ and tetramethylsilane (TMS), respectively. ^{1}H, ^{13}C, COSY, nuclear Overhauser effect spectroscopy (NOESY), HSQC, and HMBC NMR spectra were recorded with standard pulse sequences of the instrument. For ^{1}H NMR, the chemical shift (δ) is reported in parts per million (ppm) relative to TMS. The δ values were referenced to $CDCl_3$ (7.24 ppm). First-order behavior was assumed in the analysis of ^{1}H NMR spectra; the multiplicities are indicated by the usual abbreviations (s, singlet; d, doublet; t, triplet; m, multiplet; and br, broad). For ^{13}C NMR, the δ values were referenced to $CDCl_3$ (77.0 ppm).

Balsam collection, isolation, and identification of its components

Balsam of Formosan sweet gum (1.45 g) was collected in September 2009 at the campus of National Chung-Hsing University, Taichung, Taiwan. The solution in n-hexane gave 0.62 g of solvent and an n-hexane-insoluble fraction. The latter was dissolved in ethyl acetate (EtOAc) and gave 0.77 g of soluble fraction and an insoluble fraction (0.16 g). The n-hexane-soluble fraction was further separated by HPLC on a Luna silica column eluted with an n-hexane/EtOAc solvent system and led to the compounds junenol (**4**, 10.2 mg) (Niwa et al. 1976), bornyl cinnamate (**5**, 7.4 mg) (Wu et al. 2002), and cinnamyl cinnamate (**6**, 32.2 mg) (Li et al. 2010) (Figure 1). The EtOAc-soluble fraction was further separated by HPLC (Luna silica column) eluted with an n-hexane/EtOAc solvent system, which led to 13 compounds: 2α,3α-dihydroxyolean-12-en-28-al (**1,** 30.5 mg), 3α-hydroxyolean-12-en-30-ol (**2,** 23.2 mg), 3α-hydroxyolean-2-oxo-12-en-28-al (**3,** 32.6 mg), 3α,28-dihydroxyolean-12-ene (**7**, 21.0 mg) (Zhang et al. 2008), 28-hydroxy-β-amyrone (**8,** 23.7 mg) (Yuerueker et al. 1998), oleanonic aldehyde (**9**, 15.2 mg) (Kuddus et al. 2011), 3-oxoolean-12-en-28-oic acid (**10,** 23.4 mg) (Nguyen et al. 2005), 3-oxoolean-12-en-28-yl acetate (**11,** 19.7 mg) (Honda et al. 1997), 3α,25-dihydroxyolean-12-en-28-oic acid (**12,** 25.7 mg) (Sakai et al. 2004), 3,28-dioxobetulin (**13,** 32.7 mg) (Nunez et al. 2005), 3-oxobetulin (**14,** 18.2 mg) (Kim et al. 2002), betulonic acid (**15,** 12.3 mg) (Kitajima and Tanaka 1989), and 3-oxoursa-12-en-28-al (**16**, 14.3 mg) (Nasini and Piozzi 1981) (Figure 1). The structures of these compounds were elucidated and confirmed by spectroscopic analyses.

Trimethylsilylation and GC/MS analysis of balsam

The compounds of the balsam (4.0 mg), **1**–**16** (1.0 mg each), were weighed with analytical precision and were dissolved in 50 μl of pyridine and 25 μl MSTFA in 2-ml Reacti vials. Vials were heated in a water bath at 50°C for 1 h. The solution was blow-dried with N_2 and the residual was dissolved in EtOAc at a suitable concentration. The injector block temperature was 280°C; heating program of the column: 50°C, → 260°C at 8°C min^{-1}, → 280°C at 0.5°C min^{-1}.

Evaluation of antifungal activities

The antifungal activity of the balsam and its derivatives was tested with *Lenzites betulina* (BCRC35296, white rot fungus) and *Laetiporus sulphureus* (BCRC35305, brown rot fungus). The tests were performed in triplicate and the data were averaged. Different concentrations of the balsam (50, 100, 200 μg ml^{-1}) and compounds (30 μg ml^{-1}) were added

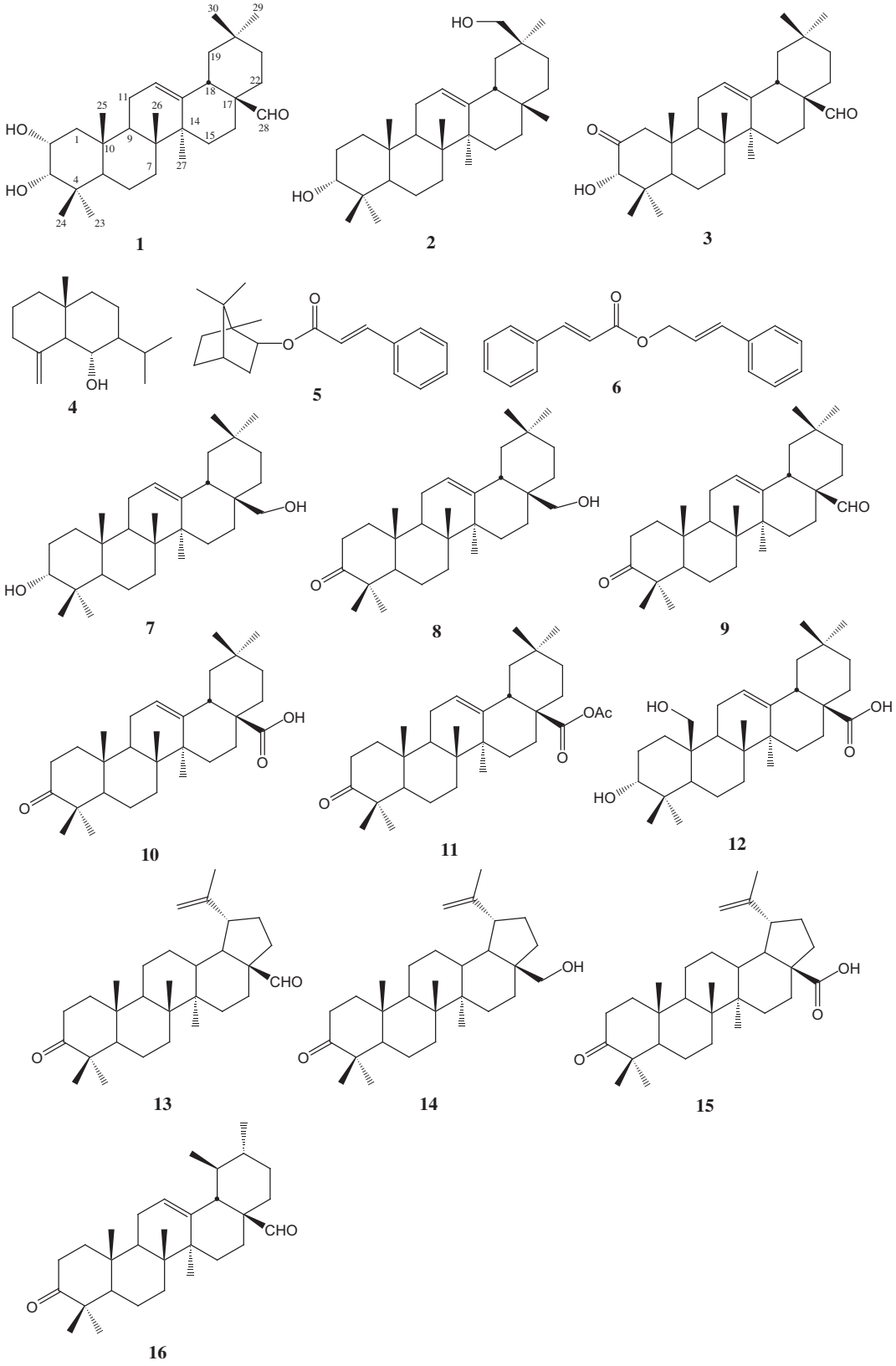

Figure 1 Structure of compounds isolated from balsam of Formosan sweet gum. **1**: 2α,3α-dihydroxyolean-12-en-28-al; **2**: 3α-hydroxyolean-12-en-30-ol; **3**: 3α-hydroxyolean-2-oxo-12-en-28-al; **4**: junenol; **5**: bornyl cinnamate; **6**: cinnamyl cinnamate; **7**: 3α,28-dihydroxyolean-12-ene; **8**: 28-hydroxy-β-amyrone; **9**: oleanonic aldehyde; **10**: 3-oxoolean-12-en-28-oic acid; **11**: 3-oxoolean-12-en-28-yl acetate; **12**: 3α,25-dihydroxyolean-12-en-28-oic acid; **13**: 3,28-dioxobetulin; **14**: 3-oxobetulin; **15**: betulonic acid; **16**:3-oxoursa-12-en-28-al.

to sterilized potato dextrose agar. The testing plates were incubated at 27°C. When the mycelium of fungi reached the edge of the control plate the antifungal index (AI) was calculated: AI (%)=(1-D_a/D_b)×100, where D_a is the diameter of the growth zone in the experimental dish (cm), and D_b is the diameter of the growth zone in the control dish (cm).

Results and discussion

The balsam fractionation and analysis gave rise to 26 compounds, which were partly volatile and nonvolatile.

Identification of volatile compounds

The volatile compounds of the balsam liquidambaris resina were collected by the SPME technique and 10 of them were identified (Table 1). The balsam is rich in α-pinene (23.3%), β-caryophyllene (22.7%), and β-pinene (19.6%). Liu et al. (2010) found by means of *in vitro* and *in vivo* assays that β-caryophyllene and α-pinene possess strong inhibitory activity in *Aphis gossypii* against acetylcholine esterase, polyphenol oxidase, carboxylesterase, and glutathione *S*-transferase. The quoted authors concluded that β-caryophyllene and α-pinene are important insecticidal compounds with characteristic diversity of action mechanisms. β-Caryophyllene and α-pinene play probably the key role in the insecticide mechanism.

Identification of nonvolatile compounds

In this study, 16 nonvolatile compounds (Figure 1) were isolated and purified from liquidambaris resina by semi-HPLC. Among them, compounds **1**–**3** are new. The spectral data of the known compounds **4**–**16** are in agreement with those reported in the literature.

RT (min)	Compounds	KI[a]	Conc. (%)	Identification by[b]
7.90	α-Pinene	936	23.27	KI, MS, ST
8.39	Camphene	950	2.99	KI, MS, ST
9.44	β-Pinene	977	19.62	KI, MS, ST
9.99	β-Myrcene	990	10.90	KI, MS, ST
10.98	α-Terpinen	1016	0.86	KI, MS, ST
11.29	β-Cymene	1024	1.51	KI, MS
11.48	Limonene	1029	8.39	KI, MS, ST
12.75	δ-Terpene	1059	2.05	KI, MS, ST
14.03	Terpinolene	1087	1.21	KI, MS, ST
28.51	β-Caryophyllene	1418	22.67	KI, MS, ST

Table 1 Volatile compounds from balsam of Formosan sweet gum found by SPME analysis.
[a]Kovats index (KI) relative to n-alkanes on a DB-5 MS column.
[b]MS: NIST and Wiley libraries and literature. RT, retention time; ST, authentic standard compounds.

The detailed structural elucidation of the new compounds is as follows.

Compound **1** was obtained as an amorphous solid. Its molecular formula was established as $C_{30}H_{48}O_3$ by ESI-MS at *m*/*z* 456 and ^{13}C as well as DEPT spectra. Seven singlet methyl groups and one singlet aldehyde signal as well as one olefinic resonance but no germinal olefinic signals were found in the ^1H and ^{13}C spectra (Table 2). Accordingly, compound **1** is an oleanane-type terperpenoid. Its HMBC (Figure 2a) correlations of oxygenated carbon [δ 78.9 (C-3)] with H-23 (δ 1.14) and H-24 (δ 0.85) and NOESY (Figure 2b) correlations of δ 3.42 (H-3) with H-23 and H-24 are interpreted as a 3α-axial hydroxy orientation. The coupling constant of H-2 (br d, *J*=12.0 Hz) is a hint to 2α-equatorial hydroxy orientation. The position of the aldehyde was established by HMBC (Figure 2a) correlations of C-17 (δ 49.1) and C-18 (δ 40.3) with H-28 (δ 9.39) and NOESY (Figure 2b) correlation between H-18 (δ 2.62) and H-28. The HMBC (Figure 2a) correlations of C-9 (δ 47.2) with H-12 (δ 5.34) and C-18 (40.3) with H-12 confirmed that the olefinic proton at C-12 is the usual oleananoid one. 2D NMR (HSQC, HMBC, COSY, and NOESY) experiments allowed us to make an unambiguous and complete assignment of the ^1H and ^{13}C NMR spectra of **1**. The structure of compound **1** was thus assigned as 2α,3α-dihydroxyolean-12-en-28-al, which is a new triterpenoid.

Compound **2** is a 12-oleanene derivative with seven singlet methyl groups [δ 0.85, 0.87, 0.89, 0.93, 0.94, 0.96 1.17 (s)] as well as one hydroxymethyl group [δ 3.21, 3.55 (d, *J*=11.0 Hz)] and an olefinic proton [δ 5.19 (t, *J*=3.0 Hz)]. The HMBC (Figure 2c) correlations of oxygenated carbon [δ 76.1 (C-3)] with H-23 (δ 0.96) and H-24 (δ 0.85) and NOESY (Figure 2d) correlations of δ 3.41 (H-3) with H-23 and H-24 demonstrated the 3α-axial hydroxy orientation. The HMBC (Figure 2c) correlations of C-20 (δ 30.6) with H-29 (δ 0.87) and H-30 (δ 3.21, 3.55) and NOESY (Figure 2d) correlations of H-30 with H-18 (δ 1.96) confirmed the presence of the 30-hydroxymethyl group. This is an evidence for the structure of compound **2** as 3α-hydroxyolean-12-en-30-ol, which is not yet described. HSQC, HMBC, COSY, and NOESY techniques also confirmed the assigned structure.

Compound **3** was obtained as an amorphous solid; its molecular formula was established as $C_{30}H_{46}O_3$ by ESI-MS at *m*/*z* 454 and ^{13}C NMR and DEPT spectra. The ^1H and ^{13}C NMR spectral data (Table 2) show some resemblance to compound **1** except the relatively low field chemical shifts of C-2 (δ 211.0). The 3α-hydroxy

No.	Compound 1		Compound 2		Compound 3	
	δ_H (ppm), / (Hz)	δ_C (ppm)	δ_H (ppm), / (Hz)	δ_C (ppm)	δ_H (ppm), / (Hz)	δ_C (ppm)
1	1.20[a], 1.62[a]	41.7	1.28 m, 1.32[a]	33.0	2.06 d (16.0) 2.46 d (16.0)	53.1
2	3.99 br d (12.0)	66.5	1.0 m, 1.69 m	25.4		211.0
3	3.42 d (2.4)	78.9	3.41 br s	76.1	3.89 s	82.8
4		38.3		37.3		49.1
5	1.19 [a]	48.0	1.24 d (12.0, 2.4)	48.9	1.44 m	54.4
6	1.45 [a]	17.9	1.44 m	18.2	1.66[a]	18.5
7	1.08 m, 1.64[a]	26.6	1.34[a], 1.52[a]	31.0	1.10 m, 1.66[a]	26.7
8		39.7		39.9		40.0
9	1.62[a]	47.2	1.67 t (8.4)	47.3	1.83 m	47.5
10		38.2		36.9		43.6
11	1.91 m	23.3	1.87 m	23.4	1.59[a], 1.98 td (13.2, 3.6)	22.0
12	5.34 (3.0)	123.0	5.19 t (3.0)	122.4	5.35 t (3.6)	122.4
13		143.0		144.1		143.2
14		41.7		41.7		41.8
15	1.19[a], 1.47[a]	27.7	1.53[a], 1.93 m	25.2	1.21[a], 1.48 m	27.6
16	1.56 m 1.98 td (13.8, 4.2)	22.0	1.18[a], 1.29[a]	34.0	1.78 m, 1.92 m	23.3
17		49.1		36.9		45.7
18	2.62 dd (13.8, 3.6)	40.3	1.96 m	42.3	2.64 dd (13.2, 4.8)	40.3
19	1.20[a], 1.66[a]	45.5	1.06 m, 1.72 m	46.4	1.20[a], 1.69[a]	45.5
20		30.6		30.9		30.6
21	1.26 m	33.1	1.30[a], 1.54[a]	32.4	1.26[a], 1.31 m	33.1
22	1.29 m, 1.47 m	32.4	1.18[a], 1.89[a]	21.9	1.37 m, 1.59[a]	32.3
23	1.14 s	28.5	0.96 s	28.2	1.19 s	29.4
24	0.85 s	21.8	0.85 s	22.3	0.69 s	16.6
25	0.95 s	16.3	0.94 s	15.3	0.87 s	16.2
26	0.71 s	17.0	0.93 s	16.6	0.73 s	16.6
27	1.14 s	25.7	1.17 s	26.1	1.19 s	25.5
28	9.39 s	207.7	0.89 s	33.2	9.39 s	207.3
29	0.91 s	33.1	0.87 s	23.6	0.92 s	33.0
30	0.92 s	23.4	3.21 d (11.0) 3.55 d (11.0)	69.8	0.91 s	23.4

Table 2 ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) spectroscopic data of compounds **1**, **2**, and **3** (in $CDCl_3$) (δ, ppm; *J*, Hz).
[a]Signals are in interference with others.

orientation and 28-aldehyde were confirmed by HMBC and NOESY spectra. The AB-type geminal protons [δ 2.06, d (*J*=16.0 Hz) and 2.46, d (*J*=16.0 Hz)] ascribed to the carbonyl group and the HMBC (Figure 2e) correlations of carbonyl carbon [δ 211.0 (C-2)] with H-1 (δ 2.06, 2.46) and H-3 (δ 3.89) established the position of the carbonyl group. HSQC, HMBC, COSY, and NOESY experiments were also run to assign all proton and carbon signals for compound **3**, which was identified as 3α-hydroxyolean-2-oxo-12-en-28-al.

For qualitative and quantitative analysis of liquidambaris resina, the collected balsam and its isolated compounds were converted to TMS derivatives. GC-MS analysis revealed that the most abundant components were 3α,25-dihydroxyolean-12-en-28-oic acid (**12**, 19.1%), oleanonic aldehyde (**9**, 14.0%), and betulonic acid (**15**, 13.4%) (Table 3).

Evaluation of antifungal activities

Two representative fungal strains of *L. betulina* (BCRC35296, white rot fungus) and *L. sulphureus* (BCRC35305, brown rot fungus) were selected to test the antifungal activity of the balsam and its derivatives. The 50% growth inhibition concentrations (IC_{50}) for *L. betulina* and *L. sulphureus* were 159.7 and 181.1 μg ml^{-1}, respectively. The AIs of the dominant compounds at the dosage of 30

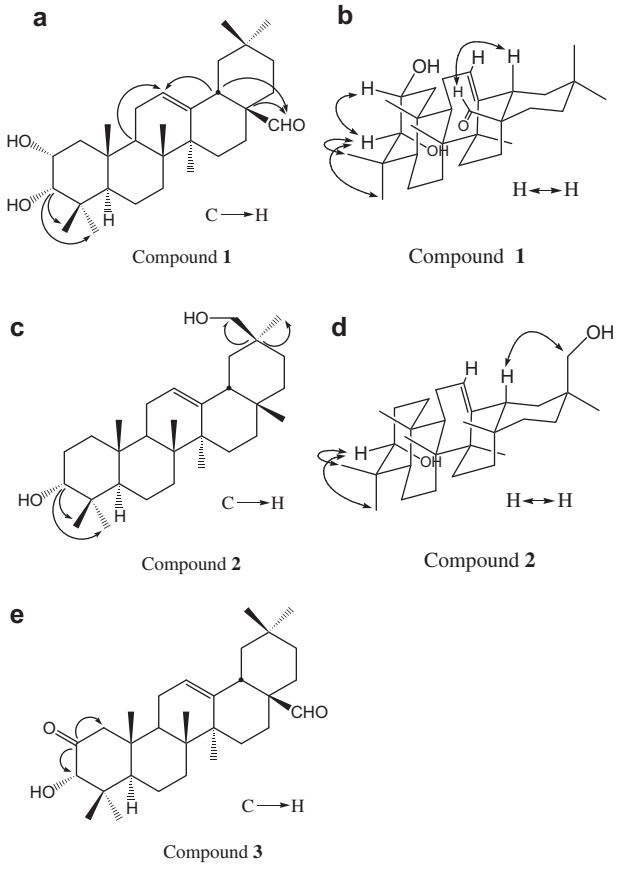

Figure 2 (a) Key HMBC correlations of compound **1**. (b) Key NOESY correlations of compound **1**. (c) Key HMBC correlations of compound **2**. (d) Key NOESY correlations of compound **2**. (e) Key HMBC correlations of compound **3**.

μg ml^{-1} are listed in Table 4. Among these compounds, bornyl cinnamate exhibited the strongest antifungal activity. The AIs for *L. betulina* and *L. sulphureus* were 70% and 47%, respectively. 3α,25-Dihydroxyolean-12-en-28-oic acid also expressed significant activity against both fungi with AIs of 62% and 63%, respectively.

Compound	RT (min)	Abundance (%)
Junenol (**4**)	14.96	1.2
Bornyl cinnamate (**5**)	25.43	8.87
Cinnamyl cinnamate (**6**)	27.33	1.09
3α,28-Dihydroxyolean-12-ene (**7**)	40.22	1.13
3-Oxoolean-12-en-28-oic acid (**10**)	47.23	6.27
3-Oxoursa-12-en-28-al (**16**)	50.21	3.75
3-Oxoolean-12-en-28-yl acetate (**11**)	50.67	1.92
3,28-Dioxobetulin (**13**)	51.99	2.52
Oleanonic aldehyde (**9**)	52.68	13.96
28-Hydroxy-β-amyrone (**8**)	53.75	6.71
3α,25-Dihydroxyolean-12-en-28-oic acid (**12**)	56.13	19.12
Betulonic acid (**15**)	57.35	13.41
3-Oxobetulin (**14**)	58.71	3.74

Table 3 Qualitative and quantitative analysis of balsam from Formosan sweet gum.

Tested compounds	AI (%)	
	L. betulina	*L. sulphureus*
Betulonic acid (**15**)	24	35
28-Hydroxy-β-amyrone (**8**)	30	29
Oleanonic aldehyde (**9**)	42	35
3-Oxoolean-12-en-28-oic acid (**10**)	35	48
3,28-Dioxobetulin (**13**)	54	48
3-Oxobetulin (**14**)	40	38
3α,25-Dihydroxyolean-12-en-28-oic acid (**12**)	62	63
Bornyl cinnamate (**5**)	70	47
Cinnamyl cinnamate (**6**)	55	39

Table 4 Antifungal activities of compounds (30 μg ml^{-1}) against white rot fungus *L. betulina* and the brown rot fungus *L. sulphureus*.

Conclusion

The bark of Formosan sweet gum (*Liquidambar formosana* Hance) produces a colorless, fragrant gum-like exudate upon mechanical damage. In this study, 26 volatile and nonvolatile compounds, among them 13 triterpenoids, 2 sesquiterpenoids, 9 monoterpenoids, and 2 cinnamyl derivatives were identified. Three of these compounds, 2α,3α-dihydroxyolean-12-en-28-al (**1**), 3α-hydroxyolean-12-en-30-ol (**2**), and 3α-hydroxyolean-2-oxo-12-en-28-al (**3**), were not yet described. The most abundant volatile compounds in *L. formosana* balsam were caryophyllene (22.7%), α-pinene (23.3%), and β-pinene (19.6%), whereas the nonvolatile fraction of the balsam was rich in 3α,25-dihydroxyolean-12-en-28-oic acid (19.1%), oleanonic aldehyde (14.0%), and betulonic acid (13.4%). The antifungal activity tests against *L. betulina* and *L. sulphureus* IC_{50} revealed values of 159.7 and 181.1 μg ml^{-1}, respectively. Bornyl cinnamate and 3α,25-dihydroxyolean-12-en-28-oic acid showed the most significant antifungal activity.

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References

Binbuga, N., Ruhs, C., Hasty, J.K., Henry, W.P., Schultz, T.P. (2008) Developing environmentally benign and effective organic wood preservatives by understanding the biocidal and non-biocidal properties of extractives in naturally durable heartwood. Holzforschung 62:264–269.

Borges, L.M.S., Cragg, S.M., Bergot, J., Williams, J.R., Shayler, B., Sawyer, G.S. (2008) Laboratory screening of tropical hardwoods for natural resistance to the marine borer *Limnoria quadripunctata*: The role of leachable and non-leachable factors. Holzforschung 62:99–111.

Byun-McKay, S.A., Hunter, W.L., Godard, K.A., Wang, S.X., Martin, D.M., Bohlmann, J., Plant, A.L. (2003) Insect attack and wounding induce traumatic resin duct development and gene expression of (–)-pinene synthase in Sitka spruce. Plant Physiol. 133:368–378.

Fordham, A.J. (1961) Liquidambar. Arnoldia 21:59–66.

Gao, H., Obanda, D.N., Shupe, T.F., Hse, C.Y., Ring, D.R. (2008) Antifungal activities of heartwood extracts of Port-Orford cedar extractives. Holzforschung 62:620–623.

Hillis, W.E. Heartwood and Tree Exudates. Springer-Verlag, New York, 1987.

Holmbom, T., Reunanen, M., Fardim, P. (2008) Composition of callus resin of Norway spruce, Scots pine, European larch and Douglas fir. Holzforschung 62:417–422.

Honda, T., Finlay, H.J., Gribble, G.W. (1997) Partial synthesis of krukovines A and B, triterpene ketones isolated from the Brazilian medicinal plant *Maytenus krukovii*. J. Nat. Prod. 60:1174–1177.

Kan, W.S. Pharmaceutical Botany. National Research Institute of Chinese Medicine, Taipei, Taiwan, 1977.

Kim, D.K., Nam, I.Y., Kim, J.W., Shin, T.Y., Lim, J.P. (2002) Pentacyclic triterpenoids from *Ilex macropoda*. Arch. Pharm. Res. 25:617–620.

Kitajima, J., Tanaka, Y. (1989) Two new triterpenoid glycosides from the leaves of *Schefflera octophylla*. Chem. Pharm. Bull. 37:2727–2730.

Kuddus, M.R., Rumi, F., Kaisar, M.A., Rahman, M.S., Hasan, C.M., Rashid, M.A., Hassan, M.A. (2011) Secondary metabolites from *Melocanna baccifera* (Roxb.). Asian J. Chem. 23:85–88.

Li, F., Awale, S., Tezuka, Y., Kadota, S., Esumi, H. (2010) Study on the constituents of Mexican propolis and their cytotoxic activity against PANC-1 human pancreatic cancer cells. J. Nat. Prod. 73:623–627.

Lim, Y.W., Chedgy, R.J., Amirthalingam, S., Breuil, C. (2007) Screening fungi tolerant to Western red cedar (*Thuja plicata* Donn) extractives. Part 2. Development of a feeder strip assay. Holzforschung 61:195–200.

Liu, Y.Q., Xue, M., Zhang, Q.C., Zhou, F.Y., Wei, J.Q. (2010) Toxicity of β-caryophyllene from *Vitex negundo* (Lamiales: Verbeaceae) to *Aphis gossypii* Glover (Homotera: Aphididae) and its action mechanism. Acta Entomol. Sin. 53:396–404.

Mburu, F., Dumarçay, S., Gérardin, P. (2007) Evidence of fungicidal and termicidal properties of *Prunus africana* heartwood extractives. Holzforschung 61:323–325.

Nasini, G., Piozzi, F. (1981) Pterocarpol and triterpenes from *Daemonorops draco*. Phytochemistry 20:514–516.

Nguyen, A.T., Fontaine, J., Malonne, H., Claeys, M., Luhmer, M., Duez, P. (2005) A sugar ester and an iridoid glycoside from *Scrophularia ningpoensis*. Phytochemistry 66:1186–1191.

Niwa, M., Masanobu, I., Yamamura, S. (1976) Chemical correlations of α-santonin with (+)-junenol and acolamone. Bull. Chem. Soc. Jpn. 49:3145–3147.

Nunez, M.J., Reyes, C.P., Jimenez, I.A., Moujir, L., Bazzocchi, I.L. (2005) Lupane triterpenoids from *Maytenus* species. J. Nat. Prod. 68:1018–1021.

Öztürka, M., Çelikb, A., Güvensena, A., Hamzaoğluc, E. (2008) Ecology of tertiary relict endemic *Liquidambar orientalis* Mill. forests. For. Ecol. Manage. 4:510–518.

Rijkers, T., Ogbazghi, W., Wessel, M., Bongers, F. (2006) The effect of tapping for frankincense on sexual reproduction in *Boswellia papyrifera*. J. Appl. Ecol. 43:1188–1195.

Sakai, K., Fukuda, Y., Matsunaga, S., Tanaka, R., Yamori, T. (2004) New cytotoxic oleanane-type triterpenoids from the cones of *Liquidamber styraciflua*. J. Nat. Prod. 67:1088–1093.

Smeds, A.I., Eklund, P.C., Monogioudi, E., Willför, S.M. (2011) Chemical characterization of polymerized products formed in the reactions of matairesinol and pinoresinol with the stable radical 2,2-diphenyl-1-picrylhydrazyl. Holzforschung 66:283–294.

Wu, D., Nair, M.G., DeWitt, D.L. (2002) Novel compounds from *Piper methysticum* Forst (Kava Kava) roots and their effect on cyclooxygenase enzyme. J. Agric. Food Chem. 50:701–705.

Yang, N.Y., Chen, J.H., Zhou, G.S., Tang, Y.P., Duan, J.A., Tian, L.J.T., Liu, X.H. (2011) Pentacyclic triterpenes from the resin of *Liquidambar formosana*. Fitoterapia 82:927–931.

Yuerueker, A., Orjala, J., Sricher, O., Rali, T. (1998) Triterpenes from *Rhus taitensis*. Phytochemistry 48:863–866.

Zhang, Y.N., Zhang, W., Hong, D., Shi, L., Shen, Q., Li, J.Y., Li, J., Hu, L.H. (2008) Oleanolic acid and its derivatives: New inhibitor of protein tyrosine phosphatase 1B with cellular activities. Bioorg. Med. Chem. 16:8697–8705.