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Tetracyclic triterpenoids and terpenylated coumarins from the bark of *Ailanthus altissima* ("Tree of Heaven")

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ABSTRACT

Tetracyclic triterpenoids (named as altissimanins A–E, **1**–**5**) and a terpenylated coumarin (denominated as altissimacoumarin G, **6**), along with fifteen known compounds (**7**–**21**) were isolated from the bark of *Ailanthus altissima*. Structures of compounds **1**–**6** were established by spectroscopic methods and chemical transformations. Altissimanin A (**1**) is a tirucallane-type triterpenoid bearing an uncommon oxetane ring in the side-chain, while altissimanins D (**4**) and E (**5**) are two unprecedented dimers each consisting of one tirucallane-type and one dammarane-type triterpenoid moiety. All the isolates were evaluated for their cytotoxic effects against a small panel of human cancer cell lines.

1. Introduction

Ailanthus altissima (Miller) Swingle (family Simaroubaceae), commonly known as the 'Tree of Heaven', is a deciduous tree indigenous to China. The bark of this tree, called 'chu-bai-pi' in Mandarin, has been used for centuries as an astringent traditional Chinese medicine for treatment of diarrhea and bleeding (Jiangsu New Medical College, 1986; De Feo et al., 2003). This plant has been well-documented to be rich in quassinoids with various biological activities (Kundu and Laskar, 2010); however, the occurrence of tetracyclic triterpenes in 'chu-bai-pi' was seldom reported (Zhou et al., 2011; Wang et al., 2010). By focusing one of our research efforts on new cytotoxic tetracyclic triterpenoids from higher plants, *Melia toosendan* (Wu et al., 2010) and *Melia azedarach* (Wu et al., 2011) were recently studied. As a continuation of this approach, the tetracyclic triterpenoids from 'chu-bai-pi' were therefore reinvestigated. In this study, seven tirucallane-type (**1**, **2**, **7**–**11**) and seven dammarane-type (**3**, **12**–**17**) triterpenoids together with two triterpene dimers (**4**, **5**) were identified. In addition, three terpenylated coumarins (**6**, **18**, **19**), one quassinoid (**20**), and one steroid (**21**) were also encountered. We herein report the isolation and structure elucidation of the new compounds and their cytotoxicities.

2. Results and discussion

2.1. Structure elucidation

Our conventional natural product research procedures (Wu et al., 2011, 2010) applied to the methanol extract of 'chu-bai-pi' led to the isolation of six (**1**–**6**) new and 15 (**7**–**21**) known compounds (Fig. 1). Comparing their spectroscopic data and physicochemical properties with those reported in the literature, the known compounds were identified to be (24*S*)-24,25-dihydroxytirucall-7-en-3-one (**7**) (Mulholland et al., 1998), niloticin (**8**) (Su et al., 1990), piscidinol A (**9**) (McChesney et al., 1997), bourjotinolone B (**10**) (Wang et al., 2008), (23*E*)-3β,25-dihydroxytirucalla-7,23-diene (**11**) (Takahashi et al., 2007; Luo et al., 2000), isofouquierone (**12**) (Waterman and Ampofo, 1985), (20*S*)-hydroxy-25-methoxy-dammar-23-en- 3-one (**13**) (Xiong et al., 2011), 24,25-dihydroxydammar-20-en-3-one (**14**) (Boar and Damps, 1977), cabralealactone (**15**) (Phongmaykin et al., 2008), betulafolienediolone (**16**) (Asakawa et al., 1977), (20*S*,24*R*)-12β,25-dihydroxy-20,24-epoxydammaran-3-one (**17**) (Valverde et al., 1985), altissimacoumarin B (**18**) (Hwang et al., 2005), altissimacoumarin D (**19**) (Dao et al., 2012),

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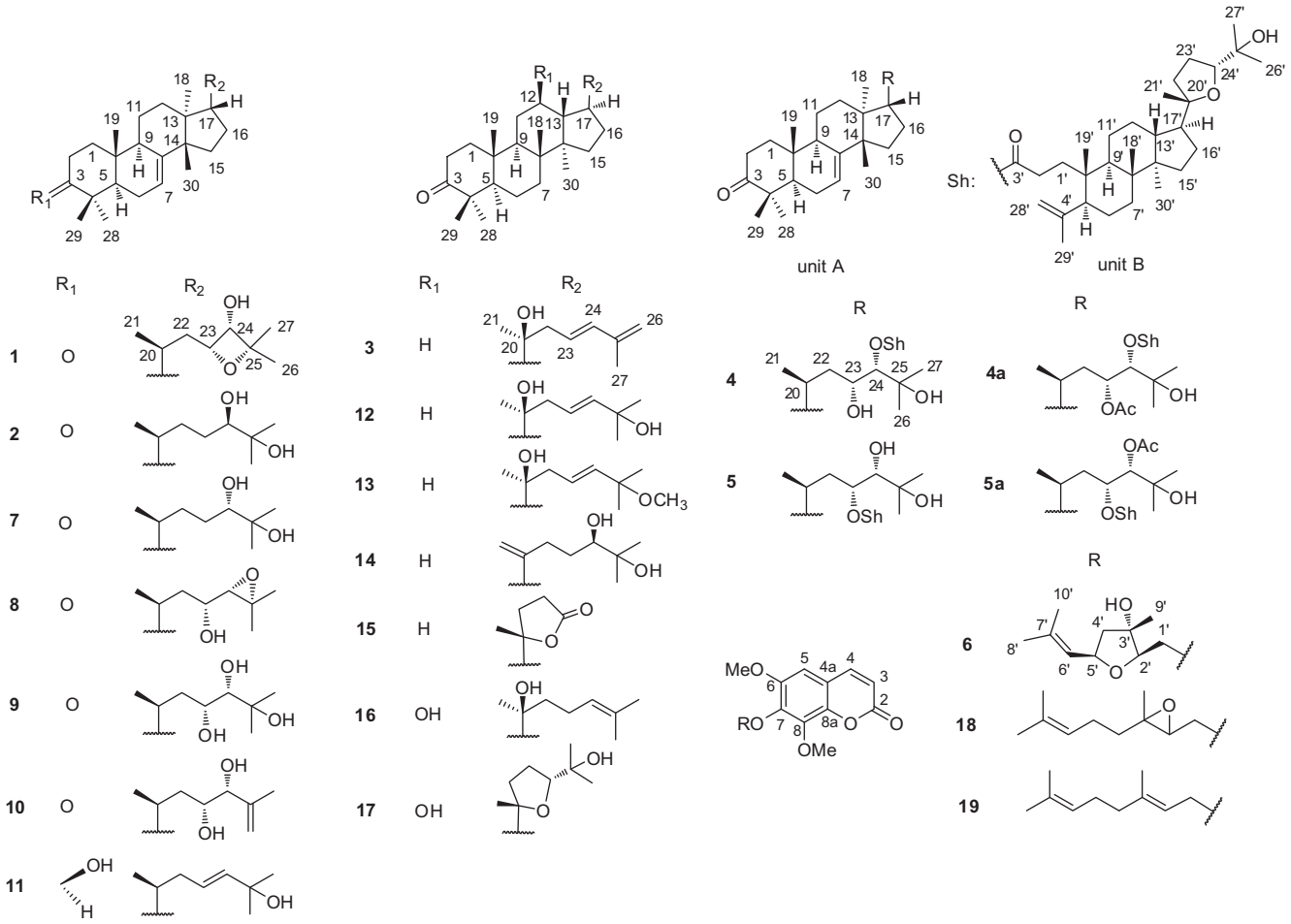

Fig. 1. Chemical structures of compounds **1**–**19**.

shinjudilactone (**20**) (Kubota et al., 1996), and 7β-hydroxy-sitosterol (**21**) (Chaurasia and Wichtl, 1987), respectively.

The molecular weight of compound **1** and its chemical formula, $C_{30}H_{48}O_3$, was determined from HR-ESIMS, which gave an intense quasi-molecular ion peak at *m/z* 479.3497 $[M+Na]^+$ (calcd. 479.3496). The ^{1}H NMR spectrum (Table 1) of **1** displayed signals attributed to seven tertiary methyl groups [δ 0.79 (3H, s, Me-18), 1.00 (3H, s, Me-19), 1.32 (3H, s, Me-26), 1.42 (3H, s, Me-27), 1.04 (3H, s, Me-28), 1.11 (3H, s, Me-29), 1.01 (3H, s, Me-30)], one secondary methyl group [δ 0.92 (3H, d, J = 6.3 Hz, Me-21)], two oxymethines [δ 4.35 (1H, d, J = 6.3 Hz, H-24) and 4.77 (1H, brq, J = 6.3 Hz, H-23)], and an olefinic proton [δ 5.30 (1H, brd, J = 2.8 Hz, H-7)]. The ^{13}C NMR spectrum of **1** exhibited thirty carbon resonances, which were classified by DEPT and HSQC NMR experiments as eight sp^3 methyls, eight sp^3 methylenes, six sp^3 methines (two oxygenated at δ 73.9 and 79.5), five sp^3 quaternary (one oxygenated at δ 86.1), a ketonic carbonyl (δ 217.0) and two olefinic carbons [δ 117.8 (CH) and 145.9 (C)]. The above NMR spectroscopic data of **1** closely resembled those of niloticin (**8**) (Fig. 1), a known tirucall-7-en-3-one triterpenoid derivative previously isolated from the fruits of *Phellodendron chinense* (Su et al., 1990). Compounds **1** and **8** were found to have the same molecular formula, but the proton chemical shifts assigned to H-23 and H-24 and the carbon chemical shifts of C-22 to C-27 (Table 1) in **1** were different from those of **8** (Su et al., 1990), implying both have a different internal ether linkage in the C-17 side-chain (Fig. 1). Unlike **8**, which has an epoxy group at C-24/C-25, compound **1** has an ether linkage between C-23 and C-25. This oxetane linkage in **1** was established by COSY correlations between OH-24 [δ 1.88 (1H, br s, D_2O-exchangeable)] and H-24 (δ 4.35), between H-24 and H-23 (δ 4.77), between H-23 and H_2-22 (δ 1.36/1.98), between H_2-22 and H-20 (δ 1.45), and between H-20 and Me-21 (δ 0.92) (Fig. 2). The ketone at C-3, the double bond at C-7, the secondary hydroxy group at C-24, and the ether linkage at C-23/C-25 were all further supported by HSQC and HMBC NMR experiments.

The relative stereochemistry of the chiral centers in the C-17 side-chain of **1** was determined by a NOESY NMR experiment. Clear NOE correlations (Fig. 2) were observed between Me-21 and H-23, between H-23 and H-24, between H-23 and Me-27, and between H-24 and Me-27, thereby establishing that Me-21, H-23, H-24 and Me-27 were all β-oriented. Furthermore, the NOE between Me-18 and Me-21 was consistent with those of reported tirucallane-type triterpenes (Liu and Abreu, 2006 Wang et al., 2003). The absolute configuration at C-24 in **1** was established by application of the modified Mosher's method (Li et al., 2011; Ukiya et al., 2003b). Briefly, treatment of **1** with (*S*)- or (*R*)-α-methoxy-α-trifluoromethyl-phenyl acetic acid (MTPA) in the presence of 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride (EDC·HCl) and 4-dimethylaminopyridine (4-DMAP) gave rise to its 24-*O*-(*R*)-MTPA ester (1*R*) and 24-*O*-(*S*)-MTPA ester (1*S*), respectively. As shown in Fig. 3, the $\Delta\delta$ (δ_S–δ_R) values for Me-26 ($\Delta\delta$ = 0.08) and Me-27 ($\Delta\delta$ = 0.01) were found to be positive, whereas those for the H-23 ($\Delta\delta$ = −0.02), Me-21 ($\Delta\delta$ = −0.03) and Me-18 ($\Delta\delta$ = −0.07) were negative. These data unequivocally demonstrated that **1** possesses an *S* configuration at C-24, and the configuration at C-23 was therefore accordingly deduced to be *R*.

Table 1
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectroscopic data of compounds **1–3** (in $CDCl_3$).

	1		2		3	
No.	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	1.45, m	38.5	1.47, m	38.5	1.50, m	39.9
	1.99, m		2.00, m		1.99, m	
2	2.23, m	34.9	2.23, m	34.9	2.44, m	34.1
	2.74, dt (14.5, 5.5)		2.75, dt (14.5, 5.0)		2.48, m	
3		217.0		217.0		218.1
4		47.9		47.8		47.4
5	1.71, dd (9.2, 8.3)	52.3	1.72, dd (9.2, 8.2)	52.3	1.35, m	55.4
6	2.08, m	24.3	2.09, m	24.3	1.55, m	19.6
7	5.30, brd (2.8)	117.8	5.31, brd (2.8)	117.8	1.31, m	34.5
					1.58, m	
8		145.9		145.9		40.3
9	2.26, m	48.4	2.29, m	48.4	1.41, m	50.2
10		35.0		35.0		36.8
11	1.55, m	18.3	1.58, m	18.3	1.30, m	22.0
					1.54, m	
12	1.63, m	33.6	1.66, m	33.6	1.31, m	27.5
	1.80, m		1.81, m		1.92, m	
13		43.5		43.5	1.73, m	42.5
14		51.1		51.1		50.3
15	1.47, m	34.0	1.49, m	34.0	1.09, m	31.1
	1.49, m				1.52, m	
16	1.28, m	28.3	1.34, m	28.1	1.56, m	24.9
	2.00, m		1.98, m		1.74, m	
17	1.52, m	53.6	1.50, m	53.0	1.75, m	50.1
18	0.79, s	21.9	0.81, s	21.9	1.01, s	15.2
19	1.00, s	12.8	1.01, s	12.8	0.95, s	16.0
20	1.45, m	34.2	1.40, m	36.4		75.3
21	0.92, d (6.3)	19.2	0.89, d (6.4)	18.5	1.15, s	26.1
22	1.36, m	36.8	1.00, m	33.4	2.29, m	43.8
	1.98, m		1.62, m			
23	4.77, q (6.3)	79.5	1.21, m	28.8	5.71, dt (15.6, 7.5)	125.7
			1.51, m			
24	4.35, d (6.3)	73.9	3.29, brd (9.9)	79.5	6.20, d (15.6)	136.6
25		86.1		73.2		141.9
26	1.32, s	23.1	1.16, s	23.2	4.91, brs	115.2
27	1.42, s	27.9	1.22, s	26.5	1.86, s	18.7
28	1.04, s	24.5	1.05, s	24.5	1.08, s	26.7
29	1.11, s	21.6	1.12, s	21.6	1.04, s	21.0
30	1.01, s	27.4	1.01, s	27.4	0.89, s	16.3
24-OH	1.88, br s					

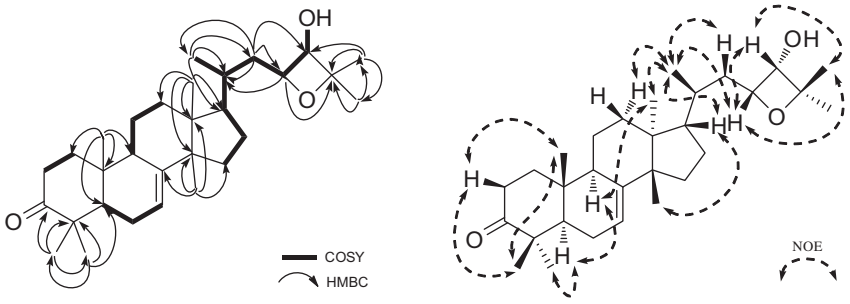

Fig. 2. Observed key COSY, HMBC and NOE correlations for compound **1**.

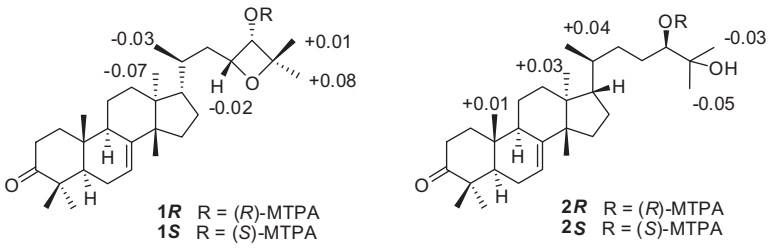

Fig. 3. Chemical shift differences ($\Delta\delta$) between (*S*)-MTPA and (*R*)-MTPA esters of 1 and 2.

Meanwhile, the *S* configuration at C-20 was also unambiguously confirmed (the *S* configuration at C-20 was previously proposed based upon biogenetic consideration) (McChesney et al., 1997). Thus, the structure of **1** was elucidated to be (20*S*,23*R*,24*S*)-24-hydroxy-23,25-epoxytirucall-7-en-3-one (altissimanin A). Naturally occurring triterpenoids with an oxetane ring in the C-17 side-chain are quite rare. To our knowledge, only cumingianoside O, a tirucallane glucoside from the leaves of *Dysoxylum cumingianum*, was previously reported to possess such a four-membered ring unit (Fujioka et al., 1997).

The HR-ESIMS of compound **2** exhibited a *pseudo*-molecular ion peak at *m/z* 481.3662 $[M+Na]^+$ corresponding to its molecular formula $C_{30}H_{50}O_3$. The NMR spectra (Table 1) of **2** showed general features similar to those of **1**, implying that **2** was also a tirucall-7-en-3-one derivative. Moreover, the NMR spectroscopic data of **2** were found to be almost identical with those of 24(*S*)-24,25-dihydroxytirucall-7-en-3-one (**7**) (both **2** and **7** have the same molecular formula), a known compound previously obtained from the plant *Owenia cepiodora* (Mulholland et al., 1998). The distinct differences between compounds **2** and **7** were the chemical shifts of H-24 (**2**: δ 3.29; **7**: δ 3.32) and C-24 (**2**: δ 79.5, **7**: δ 78.6) (Mulholland et al., 1998), suggesting that **2** might be a C-24 epimer of **7**. The absolute configuration at C-24 of **7** has been previously ascertained to be *S* by using a lanthanide-shifted [e.g. adding the $Eu(dpm)_3$ complex] NMR procedure (Mulholland et al., 1998). An attempt to determine the absolute configuration of the 24,25-diol moiety in compound **2** using induced circular dichroism spectra by Snatzke's method (Di Bari et al., 2001) failed. Similar to **1**, the absolute configuration at C-24 in **2** was finally established to be *R* by application of the modified Mosher's method (Fig. 3). Therefore, **2** was deduced to be (24*R*)-24,25-dihydroxytirucall-7-en-3-one (altissimanin B). It is worth pointing out that the most remarkable differences between **2** and **7** are the chemical shifts of H-24 and C-24, which are in full agreement with those of 24*R* and 24*S* epimers of 24,25-dihydroxyhelianol (rearranged 3,4-*seco*-tirucallane-type triterpenoids) (Ukiya et al., 2003a, 2003b).

Compound **3** was assigned a molecular formula of $C_{30}H_{48}O_2$ as determined by HR-ESIMS data. The ^1H, ^{13}C and DEPT NMR spectra (Table 1) of **3** displayed signals for seven methyl singlets (one vinylic methyl at δ_H 1.86; δ_c 18.7), a terminal double bond [δ_H 4.91 (2H, brs); δ_c 115.2 (CH_2) and 141.9 (qC)], a disubstituted double bond [δ_H 5.71 (dd, *J* = 15.6, 7.5 Hz) and 6.20 (brd, *J* = 15.6 Hz); δ_c 125.7 (CH) and 136.6 (CH)], and a ketonic carbonyl group [δ_c 218.1 (qC)]. Comparison of its NMR spectroscopic data with those of the known dammarane-type triterpene isofouquierone (**12**) (Waterman and Ampofo, 1985) suggested that both **3** and **12** had the same dammar-3-one skeleton (Fig. 1). The obvious difference was that the tertiary hydroxy group at C-25 in **12** was replaced by a $\Delta^{25,26}$ double bond in the C-17 side-chain of **3**. In the COSY NMR spectrum of **3**, a spin system of $CH_2CH{=}CHC(CH_3){=}CH_2$ was found between H_2-22 (δ 2.29) and H-23 (δ 5.71), between H-23 and H-24 (δ 6.20), allylic couplings between H-24 and H-26 (δ 4.91), as well as between H-26 and Me-27 (δ 1.86). These data clearly indicated that the conjugated double bonds were located at C-23 and C-25 in **3**, which was supported by the UV absorption band at 228 nm ($\log\varepsilon$ 4.05). The geometry of Δ^{23} was assigned as *E* on the basis of the large coupling constant (15.6 Hz) between H-23 and H-24. The *S* configuration at C-20 was determined based on the carbon chemical shift (δ 26.1) of Me-21 (the Me-21 carbon chemical shift is generally around δ 25.5 in 20*S*-isomers and about δ 23.6 in 20*R*-isomers) (Yamashita et al., 1998). Consequently, compound **3** was elucidated to be (20*S*,23*E*)-20-hydroxydammar-23,25-dien-3-one (altissimanin C).

Compounds **4** and **5** were obtained as a mixture in a ratio of about 3:1 (**4**/**5**) by analysis of the ^1H NMR spectrum (see Supporting Information). Due to the difficulties encountered in the separation of **4** and **5**, the mixture was treated with acetic anhydride in anhydrous pyridine to afford the corresponding monoacetylated derivatives **4a** and **5a** (Fig. 1). Successful separation of **4a** and **5a** was then achieved. The molecular formula of **4a** was determined to be $C_{62}H_{100}O_8$ according to a *pseudo*-molecular ion peak at *m/z* 995.7316 $[M+Na]^+$ in its HR-ESIMS, implying thirteen degrees of unsaturation. In the highfield of the ^1H NMR spectrum (Table 2) of **4a**, 13 tertiary methyls (δ 0.80, 0.86, 0.92, 0.98, 0.99, 1.01, 1.05, 1.11, 1.13, 1.14, 1.16, 1.22, 1.23), a methyl doublet at δ 0.95, a vinylic methyl at δ 1.74 (brs), and an acetyl methyl (δ 2.07) were presented. In addition to two characteristic signals for the acetoxy group [δ 170.4, 21.5], the ^{13}C NMR spectrum of **4a** displayed sixty resonances, including a ketonic carbonyl (δ 216.8), an ester carbonyl (δ 173.8), and two pairs of olefinic [δ 118.0 (CH), 145.7 (qC)]; [113.4 (CH_2), 147.6 (qC)] carbons in the low field. Detailed analysis of the 1D NMR data of **4a** with the aid of 2D NMR techniques (COSY, HSQC and HMBC) (Fig. 4) indicated that **4a** was a dimeric triterpene composed of one tirucallane-type (unit A) and one dammarane-type (unit B) moiety. The NMR spectroscopic data of unit B resembled those of shoreic acid, a known 20,24-epoxy-25-hydroxy-3,4-*seco*-dammar-4(28)-en-3-oic acid previously reported by one of the authors (Xiong et al., 2011). The distinct difference between unit B and shoreic acid was the chemical shift of the C-3′ carbonyl carbon (**4a**: δ 173.8, shoreic acid: δ 179.5) (Roux et al., 1998), indicating that the carboxylic acid group in unit B was esterified.

Meanwhile, the NMR data of unit A resembled those of compound **1** (Tables 1 and 2). Careful comparison of the NMR spectroscopic data of unit A in **4a** with those of compound **1** and another related known tirucall-7-en-3-one analogue (piscidinol A, **9**) (McChesney et al., 1997) established that unit A was a derivative of **9** with 23,24-dihydroxy groups both esterified, which could be confirmed by 2D (COSY, HSQC, HMBC) NMR experiments. Correlations between H-24 at δ 4.88 and H-23 at δ 5.42, between H-23 and H_2-22 (δ 1.17/1.64), between H_2-22 and H-20 (δ 1.42), as well as between H-20 and Me-21 (δ 0.95) were observed in the COSY spectrum of **4a**. A diagnostic ^3J correlation was then found between H-24 (δ 4.88) and C-3′ (δ 173.8) in the HMBC spectrum of **4a** (Fig. 4). The above data unequivocally indicated that unit A was conjugated with unit B by an ester bridge at the acyl residue as depicted in Fig. 1 and Fig. 4. Thus, the structure of **4a** was established as a dimer of 23-acetyl piscidinol A (unit A) and shoreic acid (unit B), which was also supported by ESI-MS-MS spectrum performed on the $[M+Na]^+$ adduct (*m/z* 995.7; see Supporting information). The predominant fragmentations occurred by cleavage of the acetyl group at C-23 (*m/z* 935.7 $[M-CH_3COOH+Na]^+$) and the cleavage of ester bond at C-24 (*m/z* 497.3 [M − unit A+Na]$^+$). The configuration of **4a** was determined by comparing the NMR data with those of shoreic acid and piscidinol A (**9**). In fact, the configurations of these two naturally occurring compounds have been previously ascertained by single crystal X-ray diffraction analysis (Roux et al., 1998; McChesney et al., 1997). For **4a**, the vicinal coupling constant between H-23 and H-24 was close to zero, indicating a *syn* configuration between H-23 and H-24 (Toume et al., 2011). Finally, altissimanin D (**4**) was deduced to be a dimer of (23*R*,24*S*)-23,24,25-trihydroxy-tirucall-7-en-3-one (piscidinol A) and (20*S*,24*R*)-3,4- *seco*-25-hydroxy-20,24-epoxydammar-3-oic acid (shoreic acid) linked by an ester bond between C-24 and C-3′ (Fig. 1).

Compound **5a** has the same molecular formula ($C_{62}H_{100}O_8$) as **4a**. Comparison of the ^1H and ^{13}C NMR spectroscopic data of **5a** with those of **4a** (Table 2) showed that the structures of these two compounds were quite similar. The most obvious difference was the proton chemical shift of the acetyl methyl group (**4a**: δ 2.07; **5a**: δ 2.19), indicating that the positions of the acetyl group and unit B (shoreic acid) were probably interchanged in these two dimers. This was further confirmed by the HMBC NMR examination (Fig. 4). Differing from **4a**, H-24 (δ 4.88, brs) was found to

Table 2
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectroscopic data of compounds **4a** and **5a** (in $CDCl_3$).

No.	**4a**		**5a**		No.	**4a**		**5a**	
	δH (*J* in Hz)	δC	δH (*J* in Hz)	δC		δH (*J* in Hz)	δC	δH (*J* in Hz)	δC
1	1.45, m	38.4	1.46, m	38.5	1′	1.63, m	34.7	1.59, m	34.5
	1.98, m		1.98, m						
2	2.26, m	34.9	2.23, m	34.9	2′	2.23, m	28.6	2.10, m	29.1
	2.74, dt (14.5, 5.3)		2.75, dt (14.5, 5.5)			2.51, m		2.35, m	
3		216.8		216.8	3′		173.8		173.6
4		47.8		47.9	4′		147.6		147.5
5	1.72, m	52.2	1.73, m	52.2	5′	2.01, m	50.8	1.97, m	50.7[d]
6	2.09, m	24.3[a]	2.08, m	24.4[d]	6′	1.39, m	24.5[a]	1.34, m	24.5[e]
						1.84, m		1.80, m	
7	5.30, brs	117.9	5.30, brs	118.0	7′	1.28, m	33.9[b]	1.23, m	33.9
						1.38, m		1.44	
8		145.7		145.7	8′		39.9		40.0
9	2.25, m	48.4	2.26, m	48.4	9′	1.52, m	41.2	1.49, m	41.2
10		35.0		35.0	10′		39.1		39.1
11	1.54, m	18.3	1.54, m	18.3	11′	1.28, m	22.1	1.39, m	22.1
12	1.66, m	33.8[b]	1.79, m	33.8[e]	12′	1.28, m	27.4[c]	1.24, m	27.3
	1.74, m		1.64, m			1.82, m		1.78, m	
13		43.6		43.6	13′	1.60, m	43.0	1.58, m	43.0
14		51.1		51.2	14′		50.4		50.4
15	1.50, m	33.9[b]	1.50, m	34.0[e]	15′	1.09, m	31.5	1.09, m	31.5
	1.54, m		1.53, m			1.47, m		1.47, m	
16	1.85, m	28.0	1.88, m	27.9	16′	1.46, m	25.7	1.48, m	25.7
						1.80, m		1.80, m	
17	1.40, m	53.6	1.43, m	53.7	17′	1.81, m	49.5	1.81, m	49.5
18	0.80, s	21.9	0.80, s	22.0	18′	0.92, s	16.5	0.89, s	16.4
19	0.99, s	12.8	1.00, s	12.8	19′	0.86, s	20.2	0.85, s	20.2
20	1.42, m	33.1	1.45, m	33.1	20′		86.3		86.3
21	0.95, d (5.5)	18.3	0.95, d (5.3)	18.5	21′	1.14, s	23.6	1.14, s	23.5
22	1.17, m	37.9	1.14, m	38.1	22′	1.66, m	35.6	1.64, m	35.8
	1.64, m		1.65, m						
23	5.42, brdd (8.9, 5.0)	70.3	5.40, brdd (8.9, 4.8)	70.2	23′	1.82, m	26.1	1.80, m	26.1
						1.88, m		1.88, m	
24	4.88, brs	76.3	4.88, brs	76.7	24′	3.74, dd (7.6,7.3)	83.3	3.73, dd (7.6,7.4)	83.3
25		72.5		72.5	251		71.4		71.4
26	1.16, s	27.1	1.19, s	27.1	26′	1.22, s	27.5[c]	1.21, s	27.5
27	1.23, s	26.3	1.23, s	26.3	27′	1.13, s	24.3	1.12, s	24.3
28	1.05, s	24.5	1.05, s	24.6	28′	4.70, brs	113.4	4.68, brs	113.5
						4.85, brs		4.85, brs	
29	1.11, s	21.6	1.11, s	21.6	29′	1.74, brs	23.1	1.74, brs	23.2
30	0.98, s	27.4[c]	0.99, s	27.5	30′	1.01, s	15.3	1.00, s	15.3
OAc	2.07, s	21.5	2.19, s	20.9					
		170.4		170.8					

[a–e]Assignments might be interchangeable within the same superscript.

have a ^{3}J HMBC correlation with the acetyl carbonyl (δ_c 170.8). Thus, the acetoxy group was located at C-24, whereas unit B was attached to C-23. Based on the above evidence, altissimanin E (**5**) was assigned as a dimer with piscidinol A and shoreic acid linked by an ester bond between C-23 and C-3′ (Fig. 1).

The molecular weight of compound **6** and its chemical formula of $C_{21}H_{26}O_7$ were deduced from its positive mode HR-ESIMS, which resulted in an $[M+Na]^+$ ion peak at *m*/*z* 413.1561. The IR spectrum of **6** showed absorption bands (ν_{max}) at 3430 (hydroxy group), 1730 (ester carbonyl), and 1639, 1565, 1461 (aromatic ring) cm^{-1}. Its UV spectrum exhibited absorption maxima at 225, 298 and 340 nm. Analysis of the ^{1}H NMR spectrum established the presence of a pair of doublets at δ 6.35 and 7.61 (each 1H, d, *J* = 9.5 Hz)], an aromatic proton singlet at δ 6.67 (1H, s), and two methoxy signals [δ 3.88 and 4.02 (each 3H, s)] (See Experimental section). These data indicated that **6** was an isofraxidin-type derivative related to altissimacoumarin B (**18**), a known terpenylated coumarin previously isolated from the bark of *A. altissima* by Park and coworkers (Hwang et al., 2005). The molecular formula of **6** implied nine degrees of unsaturation in the molecule. Apart from seven in the coumarin framework and one olefinic bond [δ_H 5.19 (1H, brd, *J* = 8.5 Hz); δ_C 125.4 (CH) and 136.9 (qC)] in the C-7 oxygenated terpenyl group, the remaining degree of unsaturation must be a ring system in the side-chain. The tetrahydrofuran ring at C-2′ and C-5′ and the tertiary hydroxy group at C-3′ in **6** were determined by analysis of its 1D and 2D (COSY, HSQC, and HMBC) NMR data. The COSY spectrum of **6** showed two proton spin systems for the C-7 side-chain: $-OCH_2-CH(O)-$ (H_2-1′/H-2′) and $-CH_2CH(O)-CH{=}C(Me)_2$ (H_2-4′, H-5′, H-6′, and allylic couplings between H-6′ and Me-8′/Me-10′) (Fig. 5). A key ^{3}J correlation between H-2′ (δ 3.98) and C-5′ (δ 74.4) was observed in the HMBC NMR experiment of **6**.

The relative configurations of the chiral centers at C-2′, C-3′ and C-5′ in the tetrahydrofuran ring were determined by a NOESY NMR experiment (Fig. 5). Clear NOE correlations were observed between H_2-1′ (δ 4.42/4.10) and Me-9′ (δ 1.47), between Me-9′ and H_b-4′ (δ 1.74), between H-2′ (δ 3.98) and H-5′ (δ 4.86), between H-5′ and H_a-4′ (δ 2.20)/Me-10′ (δ 1.73), and between H_a-4′ and Me-10′. These data suggested that Me-9′ and H_b-4′ were in the same side of the tetrahydrofuran ring, while H-2′, H_a-4′, and H-5′ adopted the opposite orientation. Consequently, compound **6** was characterized as 7-[(2,5)-epoxy-3-hydroxy-3,7-dimethyl-6- octenoxy]-6,8-dimethoxycoumarin. Compound **6** was named altissimacoumarin G, whose trivial name was sequentially derived from the work of two Korean groups (Hwang et al., 2005; Dao et al., 2012). In our lab, a naturally occurring coumarin bearing a tetrahydrofuran ring in the monoterpenyl side-chain has been previously obtained from the rhizomes of *Notopterygium incisum* (Wu et al., 2008).

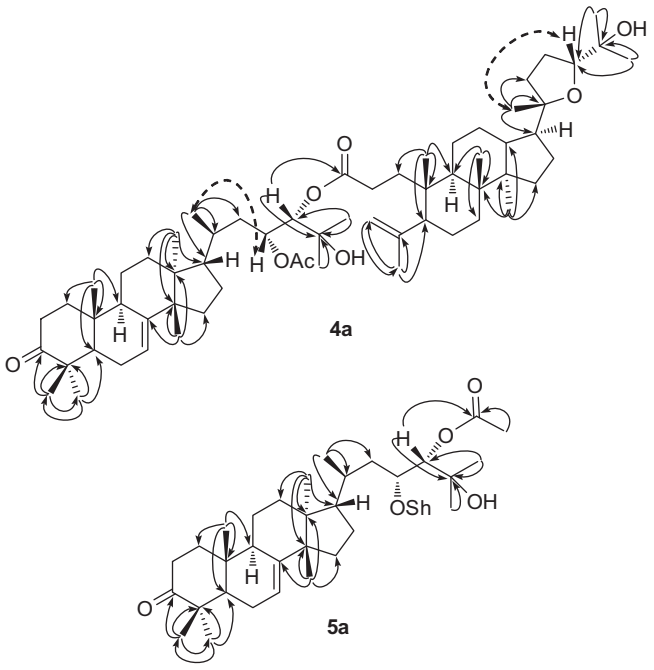

Fig. 4. Key HMBC (H → C) and NOE (H⇢H) correlations of **4a** and **5a**.

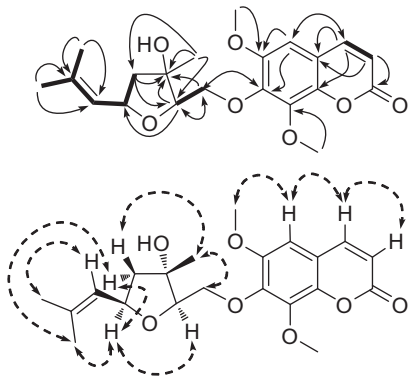

Fig. 5. Key COSY (—), HMBC (H → C), and NOE (H⇢H) correlations of **6**.

The molecular formula of compound **19** was determined to be $C_{21}H_{26}O_5$ based on a *pseudo*-molecular ion peak at *m/z* 381.1675 $[M+Na]^+$ in its positive mode HR-ESIMS. The ^1H and ^{13}C NMR data of **19** resembled those of **18** (See Experimental section). Detailed analysis of the 1D NMR data of **19** with the aid of 2D NMR techniques (COSY, HSQC, HMBC and NOESY), the structure of **19** was determined to be 7-geranyloxy-6,8-dimethoxycoumarin. Interestingly, a Korean group has also reported the discovery of this compound with a trivial name of altissimacoumarin D (Dao et al., 2012). It is worth mentioning that the ^1H and ^{13}C NMR data of the latest reported altissimacoumarin F (Dao et al., 2012) were found to be the same as our compound **6** (altissimacoumarin G). The assigned structure for altissimacoumarin F by Dao et al is suspect, even though this structure was reported to be assured by the Mosher's method (Dao et al., 2012).

2.2. Cytotoxicity

All the isolated compounds (except for 7β-hydroxy-sitosterol, **21**) were evaluated for their cytotoxic effects against human cancer cell lines of BGC-823 and KE-97 (gastric carcinoma), KB (nasopharynx carcinoma), Huh-7 (liver cancer) and jurkat (T lymphoma) using the CellTiter Glo^{TM} luminescent cell viability assay (Wu et al., 2011). Staurospirine was used as the positive control (IC_{50s} ranging from 0.14 to 0.58 μM). In this study, some of the compounds could not be tested in the cytotoxicity assay due to their poor solubility in culture medium, and only tirucallane-type triterpenoids niloticin (**8**) and piscidinol A (**9**) were found to exhibit moderate cytotoxicities against the above BGC-823, KE-97, KB, Huh-7 and jurkat cancer cell lines (for **8**: IC_{50} = 51.8, 46.5, 31.0, 38.1 and 26.4 μM, resp.; for **9**: IC_{50} = 67.5, 70.0, 35.4, 40.3 and 25.8 μM, resp.). Interestingly, these two compounds previously showed comparable cytotoxicities against murine leukemia P388 (IC_{50} = 3.3 and 2.5 μM, resp.) and KB (IC_{50} = 18.2 and 10.5 μM, resp.) cells (Itokawa et al., 1992).

3. Concluding remarks

The occurrence of tetracyclic triterpenes was seldom reported from the genus *Ailanthus*. Through a comprehensive investigation, three new tetracyclic triterpenes and two new dimeric triterpenes were identified from the dried bark of *A. altissima*. Natural occurring triterpenoids possessing an oxetane ring in the C-17 side-chain (e.g. **1**) are quite rare. Altissimanins D (**4**) and E (**5**) are the first examples of triterpene dimers each composed of one tirucallane-type and one dammarane-type triterpenoid moiety. Meanwhile, the known tirucallane-type triterpenoids (**7**, **8**, **10**, **11**) and the dammarane-type triterpenoids (**12**–**15**, **17**) were reported herein from the genus *Ailanthus* for the first time. The isolated triterpenoids and terpenylated coumarins (e.g. **6**, **18**, and **19**) could stimulate future phytochemical/genomics studies.

4. Experimental

4.1. General experimental procedures

Optical rotations were determined using a Perkin-Elmer 341 polarimeter. UV absorptions were obtained on a Libra S35 PC UV-VIS recording spectrophotometer, whereas IR spectra were measured on a Nicolet NEXUS-670 FT-IR spectrophotometer. NMR spectra were recorded on a Bruker Avance DRX-500 spectrometer (500 MHz). Chemical shifts are expressed in δ (ppm), and are referenced to the residual solvent signals. Electrospray ionization mass spectra (ESI-MS) were measured on a Bruker Daltonics micrOTOF-QII mass spectrometer. Semi-preparative HPLC was performed on a Beckman system consisting of a Beckman Coulter System Gold 508 autosampler, Gold 126 gradient HPLC pumps with a Beckman System Gold 168 UV detector, a Sedex 80 (SEDERE, France) evaporative light-scattering detector (ELSD), and a YMC-Pack ODS-A column (250 × 10 mm, dp 5 μM). Column chromatography (CC) was performed using silica gel (200-300 mesh, Ji-Yi-Da Silysia Chemical Ltd., Qingdao, China), MCI gel CHP20P (75-150 μ, Mitsubishi Chemical Industries, Tokyo, Japan), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Silica gel-precoated plates (GF_{254}, 0.25 mm, Kang-Bi-Nuo Silysia Chemical Ltd, Yantai, China) were used for TLC detection. Spots were visualized using UV light (254 and/or 365 nm) and by spraying with 5% (v/v) H_2SO_4-EtOH followed by heating to 120C.

4.2. Plant material

The dried bark of *A. altissima* was purchased from Shanghai Jiu-Zhou-Tong Medicine Co. Ltd, and was originally collected in May 2009 from Nanjing, Jiangsu province of China. The plant was identified by Prof. Bao-Kang Huang (Department of Pharmacognosy, the Second Military Medical University of China). A voucher specimen (No.100114) was deposited at the Herbarium of the Shanghai

Key Laboratory of Brain Functional Genomics, East China Normal University.

4.3. Extraction and isolation

The dried bark of *A. altissima* (10.0 kg) was extracted with MeOH (3 × 15 L) at room temperature. The solvent was removed under vacuum to give a crude extract (*ca* 980 g), which was then subjected to silica gel CC with a petroleum ether (PE)-EtOAc gradient (15:1–1:1–0:1, v/v) and EtOAc-MeOH (15:1–1:1–0:1) to yield twelve fractions (Fr.1–Fr.12). Fr.3 (15.5 g) was applied to a silica gel column eluting with a CH_2Cl_2-EtOAc gradient (8:1–0:1) to afford eight subfractions Fr.3A-Fr.3H. Fr.3B (0.8 g) was then subjected to repeated silica gel CC (CH_2Cl_2–EtOAc, 12:1; PE-EtOAc, 8:1) to afford compound **3** (3.2 mg), which was further purified by gel permeation chromatography (GPC) on Sephadex LH-20 (MeOH). Fr.3D (1.2 g) was applied repeatedly to a silica gel column (PE-acetone, 10:1; PE-EtOAc, 3:1), followed by MCI gel (MeOH) to furnish **15** (33.0 mg). Compound **19** (9.5 mg) was isolated from Fr.3E (1.5 g) by MCI gel (MeOH) and subsequently purified by preparative TLC (PE-EtOAc, 3:1). Fr.4 (55.8 g) was separated by silica gel CC using a step gradient of CH_2Cl_2-EtOAc (5:1–0:1) to give six subfractions Fr.4A–Fr.4F. Fr.4C (0.7 g) was subjected to silica gel CC (PE-EtOAc, 3:1), and was then purified by semi-preparative reversed phase HPLC (CH_3CN-H_2O, 90:10; flow rate, 3.0 mL/min; t_R = 23.2 min) to afford **13** (4.0 mg). Compounds **12** (11.6 mg), **16** (13.5 mg) and **21** (3.5 mg) were isolated from Fr. 4D (2.8 g) through silica gel CC with PE-EtOAc (5:1) and were further purified by Sephadex LH-20 (MeOH). Fr.5 (32.1 g) was subjected to silica gel CC with a CH_2Cl_2-EtOAc gradient (4:1–0:1) to provide Fr.5A–5D. The purification of Fr.5B (4.2 g) yielded compounds **1** (10.1 mg), **8** (25.2 mg), **10** (17.8 mg), and **11** (12.2 mg) via silica gel CC with PE-EtOAc (3:1), MCI-gel using a stepwise gradient elution with MeOH-H_2O (from 80% to 100% MeOH), and Sephadex LH-20 in MeOH. Fr.6 (31.7 g) was subjected to silica gel CC (CH_2Cl_2-EtOAc, 2:1) to afford Fr.6A-Fr.6D. Repeated silica gel CC (CH_2Cl_2-acetone 10:1; PE-EtOAc, 3:1) and then Sephadex LH-20 (MeOH) of Fr.6C (7.5 g) furnished compounds **4** and **5** (mixture, 27.3 mg), **17** (58.0 mg), and **18** (12.7 mg). Fr.6D (9.4 g) was applied to MCI-gel using MeOH-H_2O (80% MeOH) to yield Fr.6 Da- Fr.6Df. Compounds **2** (30.0 mg), **7** (13.3 mg), and **14** (10.1 mg) were isolated from Fr.6Db (1.7 g) by silica gel CC (PE-EtOAc, 2:1), and each was further purified by Sephadex LH-20 (MeOH). Compound **9** (7.7 mg) was obtained by semi-preparative HPLC (MeOH-H_2O, 86:14; flow rate, 3.0 mL/min; t_R = 27.0 min) from Fr.6De (0.3 g). Fr.7 (6.8 g) was subjected to silica gel (CH_2Cl_2-MeOH, 20:1) to yield Fr.7A–Fr.7G. Compound **6** (10.5 mg) was purified from Fr.7D (40.5 mg) by HPLC (MeOH-H_2O, 69:31; flow rate, 3.0 mL/min; t_R = 28.5 min). Fr.8 (30.9 g) was applied to a silica gel column (CH_2Cl_2-MeOH, 15:1) to provide Fr.8A–Fr.8H. Crystallization of Fr.8D (1.2 g) from MeOH yielded **20** (42.6 mg), which was further purified by Sephadex LH-20 (CH_2Cl_2-MeOH, 2:1).

4.3.1. Preparation of (R)- and (S)-MTPA esters of **1** and **2** (Li et al., 2011).

A solution of **1** (1.5 mg) in dried CH_2Cl_2 (1.5 mL) was treated with (*R*)-MTPA (35 mg) [or (*S*)-MTPA (35 mg)] in the presence of EDC·HCl (50 mg) and 4-DMAP (50 mg), and the mixture was stirred at room temperature overnight. The residue obtained after removal of solvent was subjected to preparative TLC (PE/EtOAc = 3:1) and then Sephadex LH–20 in CH_2Cl_2/MeOH (2:1) to give pure (*R*)-MTPA ester and (*S*)-MTPA ester of **1**, respectively. Treatment of **2** with (*R*)-MTPA [or (*S*)-MTPA] in the same manner as above gave the corresponding esters of **2**.

4.3.2. Altissimanin A (**1**)

White amorphous powder; $[\alpha]_D^{15}$ –123 (*c* 0.1, MeOH); IR (KBr) ν_{max}: 3413 (*br*), 2967, 2927, 1708, 1634, 1454, 1384, 1317, 1150, 1112, 1088, 1050, 949 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; (+) ESI-MS *m/z* 479.3 $[M+Na]^+$, 935.7 $[2\,M+Na]^+$; (+) HR-ESI-MS *m/z* 479.3497 (calcd for $C_{30}H_{48}O_3Na$, 479.3496).

4.3.3. (R)-MTPA (**1R**) and (S)-MTPA (**1S**) esters of **1**.

1R:

$[\alpha]_D^{15}$ –71 (*c* 0.3, MeOH); ^1H NMR data ($CDCl_3$, 500 MHz) δ 0.75 (3H, s, CH_3-18), 0.88 (3H, d, *J* = 6.2 Hz, CH_3-21), 0.99 (3H, s, CH_3-19), 1.00 (3H, s, CH_3-30), 1.05 (3H, s, CH_3-28), 1.12 (3H, s, CH_3-29), 1.22 (3H, s, CH_3-26), 1.53 (3H, s, CH_3-27), 3.53 (3H, s, OCH_3), 4.87 (1H, q, *J* = 5.7 Hz H-23), 5.29 (1H, d, *J* = 5.7 Hz, H-24), 5.30 (1H, s, H-7), 7.41-7.42 (3H, m), 7.52-7.54 (2H, m); (+) ESI-MS *m/z* 673.3 $[M+H]^+$, 695.2 $[M+Na]^+$. **1S**: $[\alpha]_D^{15}$ -61 (*c* 0.3, MeOH); ^1H NMR data ($CDCl_3$, 500 MHz) δ 0.68 (3H, s, CH_3-18), 0.85 (3H, d, *J* = 6.2 Hz, CH_3-21), 0.99 (3H, s, CH_3-19), 1.00 (3H, s, CH_3-30), 1.05 (3H, s, CH_3-28), 1.12 (3H, s, CH_3-29), 1.22 (3H, s, CH_3-26), 1.54 (3H, s, CH_3-27), 3.58 (3H, s, OCH_3), 4.85 (1H, q, *J* = 6.3 Hz, H-23), 5.29 (1H, d, *J* = 6.3 Hz, H-24), 5.31 (1H, s, H-7), 7.41-7.43 (3H, m), 7.52-7.54 (2H, m); (+) ESI-MS *m/z* 673.3 $[M+H]^+$.

4.3.4. Altissimanin B (**2**)

White amorphous powder; $[\alpha]_D^{22}$ –73 (*c* 0.4, MeOH); IR (KBr) ν_{max}: 3431 (*br*), 2970, 2882, 1709, 1635, 1459, 1385, 1316, 1266, 1162, 1077, 1050, 967 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; (+) ESI-MS *m/z* 481.4 $[M+Na]^+$, 939.7 $[2\,M+Na]^+$; (+) HR-ESI-MS *m/z* 481.3662 (calcd for $C_{30}H_{48}O_2Na$, 463.3552).

4.3.5. (R)-MTPA (**2R**) and (S)-MTPA (**2S**) Esters of **2**.

2R:

$[\alpha]_D^{15}$–61 (*c* 0.6, MeOH); ^1H NMR data ($CDCl_3$, 500 MHz) δ 0.76 (3H, s, CH_3-18), 0.83 (3H, d, *J* = 6.6 Hz, CH_3-21), 0.99 (3H, s, CH_3-19), 1.00 (3H, s, CH_3-30), 1.05 (3H, s, CH_3-28), 1.12 (3H, s, CH_3-29), 1.17 (3H, s, CH_3-26), 1.23 (3H, s, CH_3-27) 3.56 (3H, s, OCH_3), 4.95 (1H, d, *J* = 7.9 Hz H-23), 5.30 (1H, s, H-7), 7.40-7.42 (3H, m), 7.62-7.63 (2H, m); (+) ESI-MS *m/z* 657.3 $[M+H-H_2O]^+$, 675.3 $[M+H]^+$, 697.3 $[M+Na]^+$. **2S:** $[\alpha]_D^{15}$ -53 (*c* 0.2, MeOH); ^1H NMR data ($CDCl_3$, 500 MHz) δ 0.79 (3H, s, CH_3-18), 0.87 (3H, d, *J* = 6.5 Hz, CH_3-21), 1.00 (3H, s, CH_3-19), 1.00 (3H, s, CH_3-30), 1.05 (3H, s, CH_3-28), 1.12 (3H, s, CH_3-29), 1.14 (3H, s, CH_3-26), 1.18 (3H, s, CH_3-27), 3.59 (3H, s, OCH_3-18), 4.95 (1H, q, *J* = 7.7 Hz, H-23), 5.31 (1H, d, *J* = 2.9 Hz, H-7), 7.37-7.41 (3H, m), 7.60-7.61 (2H, m); (+) ESI-MS *m/z* 657.3 $[M+H-H_2O]^+$, 675.3 $[M+H]^+$, 697.3 $[M+Na]^+$.

4.3.6. Altissimanin C (**3**)

White amorphous powder; $[\alpha]_D^{15}$ + 22 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 228 (4.05); IR (KBr) ν_{max}: 3430 (*br*), 2926, 2855, 1703, 1634, 1455, 1384, 1030 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; (+) ESI-MS *m/z* 463.4 $[M+Na]^+$, 903.7 $[2\,M+Na]^+$; (+) HR-ESI-MS *m/z* 463.3526 (calcd for $C_{30}H_{48}O_2$-Na, 463.3547).

4.3.7. Altissimanins D (**4**) and E (**5**)

Obtained as a mixture; (+) ESI-MS *m/z* 953.7 $[M+Na]^+$, 1885.8 $[2\,M+Na]^+$; (+) HR-ESI-MS *m/z* 953.7200 (calcd for $C_{60}H_{98}O_7Na$, 953.7205).

4.3.8. Acetylation of **4** and **5**

The mixture (27.3 mg) of **4** and **5** were treated with dry pyridine (2 mL) and Ac_2O (2 mL) overnight at room temperature. The reaction mixture was diluted with EtOAc and washed with H_2O three times. After removal of the solvent, the obtained residue was sub-

jected to silica gel CC (PE-EtOAc, 4:1) to afford **4a** (9.7 mg) and **5a** (3.5 mg), and each was further refined by Sephadex LH–20 (MeOH).

*4.3.9. Compound **4a***

White amorphous powder; $[\alpha]_D^{22}$ −82 (*c* 0.1, MeOH); IR (KBr) ν_{max}: 3429 (*br*), 2972, 2931, 1738, 1711, 1633, 1454, 1384, 1313, 1245, 1162, 1085, 1051 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 2; (+) ESI-MS *m*/*z* 995.7 $[M+Na]^+$, 1969.7 $[2\,M+Na]^+$; (+) HR-ESI-MS *m*/*z* 995.7316 (calcd for $C_{62}H_{100}O_8Na$, 995.7310).

*4.3.10. Compound **5a***

White amorphous powder; $[\alpha]_D^{22}$ −76 (*c* 0.1, MeOH); IR (KBr) ν_{max}: 3431 (*br*), 2986, 2931, 1736, 1711, 1636, 1458, 1384, 1315, 1237, 1171, 1113, 1082, 1049 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 2; (+) ESI-MS *m*/*z* 995.7 $[M+Na]^+$, 1969.5 $[2\,M+Na]^+$; (+) HR-EI-MS *m*/*z* 995.7255 $[M+Na]^+$ (calcd for $C_{62}H_{100}O_8Na$, 995.7310).

*4.3.11. Altissimacoumarin G (**6**)*

White amorphous powder, $[\alpha]_D^{15}$ + 20 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 225 (3.99), 298 (3.54), 340 (3.40) nm; IR (KBr) ν_{max}: 3430 (*br*), 2975, 2941, 1730, 1639, 1565, 1461, 1409, 1384, 1292, 1266, 1155, 1126, 1092, 1043, 851 cm^{-1}; ^1H NMR (500 MHz, in $CDCl_3$) δ 1.47 (3H, brs, Me-9′), 1.73 (3H, brs, Me-10′), 1.74 (3H, brs, Me-8′), 1.74 (1H, dd, overlapped, H_a-4′), 2.20 (1H, dd, *J* = 12.5, 7.0 Hz, H_b-4′), 3.88 (3H, s, OMe-6), 3.98 (1H, dd, *J* = 8.0, 4.5 Hz, H-2′), 4.02 (3H, s, OMe-8), 4.10 (1H, dd, *J* = 10.0, 8.0 Hz, H_a-1′), 4.42 (1H, dd, *J* = 10.0, 4.5 Hz, H_b-1′), 4.86 (1H, brq, *J* = 8.5 Hz, H-5′), 5.19 (1H, brd, *J* = 8.5 Hz, H-6′), 6.35 (1H, d, *J* = 9.5 Hz, H-3), 6.67 (1H, s, H-5), 7.61 (1H, d, *J* = 9.5 Hz, H-4); ^{13}C NMR (125 MHz, in $CDCl_3$) δ 18.2 (C-10′), 23.6 (C-9′), 25.8 (C-8′), 47.5 (C-4′), 56.2 (OMe-6), 62.0 (OMe-8), 73.1 (C-1′), 74.4 (C-5′), 79.4 (C-3′), 84.7 (C-2′), 103.9 (C-5), 114.3 (C-4a), 115.3 (C-3), 125.4 (C-6′), 136.9 (C-7′), 140.7 (C-8), 143.2 (C-8a), 143.4 (C-4), 144.8 (C-7), 149.6 (C-6), 160.4 (C-2); (+) ESI-MS *m*/*z* 413.2 $[M+Na]^+$, 803.3 $[2\,M+Na]^+$; (+) HR-ESI-MS *m*/*z* 413.1561 (calcd for $C_{21}H_{26}O_7Na$, 413.1571).

*4.3.12. Altissimacoumarin D (**19**)*

Colorless oil; $[\alpha]_D^{22}$ 0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 225 (4.02), 297 (3.76), 339 (3.63) nm; IR (KBr) ν_{max}: 2967, 2927, 2854, 1730, 1609, 1564, 1485, 1457, 1422, 1409, 1382, 1347, 1291, 1273, 1229, 1195, 1152, 1126, 1087, 1042, 983 cm^{-1}; ^1H NMR (500 MHz, in $CDCl_3$) δ 1.59 (3H, brs, Me-10′), 1.67 (3H, brs, Me-8′), 1.70 (3H, brs, Me-9′), 2.05 (2H, m, H-4′), 2.08 (2H, m, H-5′), 3.89 (3H, s, OMe-6), 4.03 (3H, s, OMe-8), 4.68 (2H, d, *J* = 7.0, Hz, H-1′), 5.06 (1H,, brt, *J* = 7.0 Hz, H-6′), 5.56 (1H, brt, *J* = 7.0 Hz, H-2′), 6.34 (1H, d, *J* = 9.5 Hz, H-3), 6.66 (1H, s, H-5), 7.62 (1H, d, *J* = 9.5 Hz, H-4); ^{13}C NMR (125 MHz, in $CDCl_3$) δ 16.4 (C-9′), 17.6 (C-10′), 25.6 (C-8′), 26.3 (C-5′), 39.6 (C-4′), 56.2 (OMe-6), 61.7 (OMe-8), 70.2 (C-1′), 103.5 (C-5), 114.4 (C-4a), 115.1 (C-3), 119.6 (C-2′), 123.8 (C-6′), 131.7 (C-7′), 141.8 (C-8), 142.5 (C-3′), 143.5 (C-8a), 143.5 (C-4), 144.9 (C-7), 150.7 (C-6), 160.5 (C-2); (+) ESI-MS *m*/*z* 381.2 $[M+Na]^+$, 739.3 $[2\,M+Na]^+$; (+) HR-ESI-MS *m*/*z* 381.1675 (calcd for $C_{21}H_{26}O_5Na$, 381.1672).

4.4. Cytotoxicity assay

The human gastric carcinoma cell lines BGC-823 and KE-97, human nasopharynx carcinoma cell line KB, Huh-7 human liver cancer cell line, and the jurkat human T lymphoma cancer cell line were purchased from the cell bank of the Shanghai Institute of Cell Biology. The BGC-823, KB, KE-97 and Jurkat cell lines were cultured in the RPMI-1640 medium, while the Huh-7 cell line was cultured in the DMEM medium. All media were supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 units/mL streptomycin (Invitrogen). The cells were maintained at 37 °C in a humidified environment with 5% CO_2. The cell viability was determined by using the CellTiter Glo™ luminescent cell viability assay (Promega) (Wu et al., 2011). In order to exclude phototoxicity (Colombain et al., 2001), the operation process was kept away from bright light and the cells were incubated in a dark incubator. The cells were also incubated in fresh cell culture medium and washed carefully to avoid false positive results (Bruggisser et al., 2002). Briefly, the cancer cells were seeded into 384-well plates at an initial density of 1000 cells/well in 45 μL of medium. Then the cells were treated with compounds at varying concentrations. Staurosporine (Sigma-Aldrich, catalog No. S6942-200UL) was used as a positive control. After incubation for 72 h, 10% of CellTiter Glo™ reagent was added, and luminescent signals were read on a VeriScan reader (Thermo Fisher Scientific). The IC_{50} value was calculated from the curves generated by plotting the percentage of the viable cells versus test concentrations on a logarithmic scale using SigmaPlot 10.0 software.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytochem.2012.10.008.

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