Cytotoxic Terpenoids from *Turraea pubescens*

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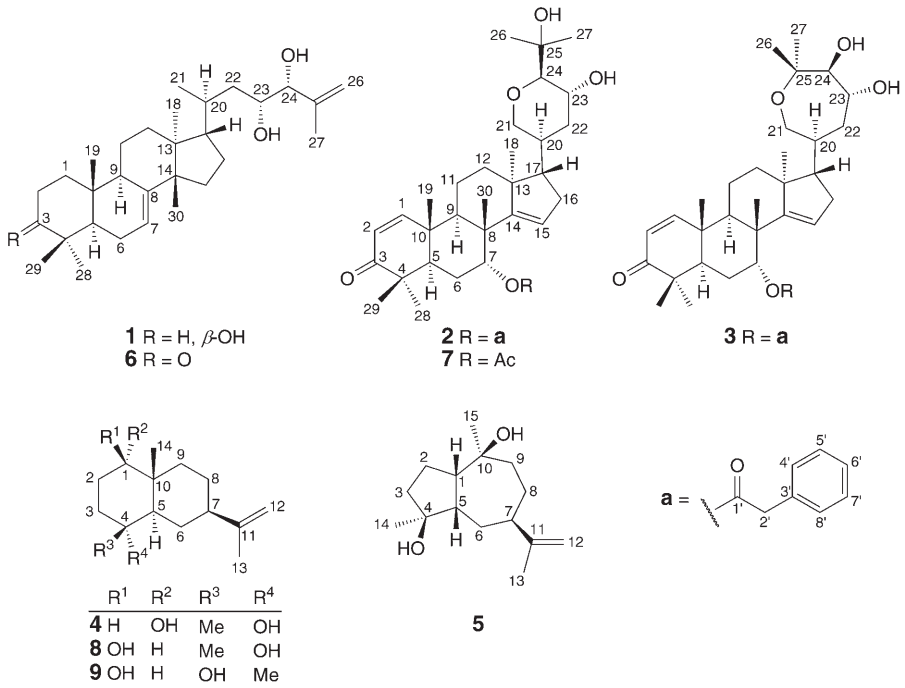
Three new triterpenoids, turrapubesols A–C (**1**–**3**), and one new sesquiterpenoid, 1α,4α-dihydroxyeudesman-11-ene (**4**), along with 12 known terpenoids were isolated from *Turraea pubescens*. The structures of the terpenoids were established on the basis of extensive spectroscopic analyses and by a chemical transformation. The previous assignment of the NMR data for guaidiol (**5**) was corrected. Some of the triterpenoids exhibited cytotoxicity against the P-388 cell line.

Introduction. – The genus *Turraea* (Meliaceae) comprises approximately 90 species of small trees and shrubs widely distributed in the tropical and subtropical areas of Asia, Australia, and Southern Africa [1]. A number of triterpenoids, limonoids, and other kinds of secondary metabolites have been isolated from this genus previously [2]. *Turraea pubescens* HELLEN is a wild shrub found mainly in South and Southeast Asia and Western Australia. In traditional Chinese medicine, the twigs and leaves of *T. pubescens* are applied to treat dysentery, pharyngolaryngitis, and traumatic hemorrhage [1]. We have reported the isolation and characterization of three pregnane steroids [3] and twelve ring B-*seco* limonoids [4][5] from *T. pubescens* collected in Hainan Island of the P. R. of China. In the present work, three new triterpenoids, turrapubesols A–C (**1**–**3**), and one new sesquiterpenoid, 1α,4α-dihydroxyeudesman-11-ene (**4**), along with 12 known compounds were isolated from the twigs and leaves of *T. pubescens*. Herein, we report the isolation, structure elucidation, and cytotoxicity of these terpenoids.

Results and Discussion. – Turrapubesol A (**1**) was obtained as colorless plates. The molecular formula was determined to be $C_{30}H_{50}O_3$ by HR-EI-MS (*m*/*z* 458.3769 (M^+; calc. 458.3760)) and NMR data (*Table 1*). The IR spectrum showed absorption bands for a OH group (3406 cm^{-1}) and double bonds (1647 cm^{-1}). The ^{1}H-NMR data of **1** were very similar to those of bourjotinolone B (**6**) [6], a known compound also obtained in the present study, indicating that they are closely related. The ^{1}H- and ^{13}C-NMR data of both **1** and **6** were assigned by HSQC experiments (*Table 1*). The only difference between them was that the CO group (δ(C) 217.0) in **6** was replaced by an oxygenated CH [δ(H) 3.23 (*dd*, J = 11.0, 4.0 Hz, H–C(3)); δ(C) 79.2] in **1**. The resonance for H$_\beta$–C(2) in **1** (δ(H) 1.65–1.70 (*m*)) was shielded by *ca.* 1 ppm as compared to that in **6** (δ(H) 2.75 (*td*, J = 14.6, 5.5 Hz)). The above analysis indicated that a OH group was present at C(3), which was confirmed by HMBC correlations



	R^1	R^2	R^3	R^4
4	H	OH	Me	OH
8	OH	H	Me	OH
9	OH	H	OH	Me

5

from Me(28) and Me(29) to C(3). The OH–C(3) group was established as β-oriented (equatorial) from the ROESY correlations of H–C(3)/H_α–C(1), H–C(5), and Me(28) and its vicinal coupling constants with CH_2(2) ($^3J = 11.0$, 4.0). The configurations at the remaining chiral centers was determined to be the same as that of **6** by a ROESY experiment. Reduction of **6** with $NaBH_4$ gave **1** in high yield, which unambiguously confirmed the above conclusions.

Turrapubesol B (**2**) was obtained as a white amorphous solid. The molecular formula was determined to be $C_{38}H_{52}O_6$ on the basis of HR-EI-MS (*m/z* 604.3764 (M^+; calc. 604.3764)) and NMR data (*Table 2*). The IR absorptions at 3431, 1728, 1670, and 1456 cm^{-1} indicated the presence of OH, CO, and an aromatic ring. The ^{13}C-NMR data displayed signals for 38 C-atoms, which were classified as seven Me groups, seven CH_2 groups (one oxygenated, at δ(C) 69.7), seven sp^3 CH groups (three oxygenated, at δ(C) 64.0, 74.7, and 86.1), five quaternary sp^3 C-atoms (one oxygenated, at δ(C) 73.6), one 1,2-disubstituted double bond, one trisubstituted double bond, one monosubstituted benzene ring, and two CO groups (δ(C) 204.4 and 170.1). The ^{1}H- and ^{13}C-NMR data in combination with the HMBC spectrum (*Fig. 1*) revealed the gross structure of **2**, which is similar to that of sapelin C and grandifoliolenone (**7**) [7], except for the substituent at C(7). Instead of the hydroxy group for sapelin C, a phenylacetoxy functionality was present at C(7) of **2** as deduced from the HMBC correlation of H–C(7)/C(1′).

As far as the stereochemistry of **2** is concerned, the ROESY correlations (*Fig. 2*) of Me(19)/Me(29), Me(30), and H_β–C(6); Me(29)/H_β–C(6); H–C(7)/Me(30) and

Table 1. *^{1}H- and ^{13}C-NMR Data for* **1** *and* **6**. At 400/100 MHz, resp., in $CDCl_3$; δ in ppm, J in Hz.

	1		**6**	
	δ(H)	δ(C)	δ(H)	δ(C)
H_α–C(1)	1.09–1.18 (*m*)	37.2	1.44–1.47 (*m*)	38.5
H_β–C(1)	1.67–1.70 (*m*)		1.99–2.03 (*m*)	
H_α–C(2)	1.55–1.59 (*m*)	27.6	2.20–2.24 (*m*)	35.0
H_β–C(2)	1.65–1.70 (*m*)		2.75 (*td*, *J* = 14.6, 5.5)	
H–C(3)	3.23 (*dd*, *J* = 11.0, 4.0)	79.2	–	217.0
C(4)	–	38.9	–	47.9
H–C(5)	1.30 (*dd*, *J* = 12.0, 5.8)	50.6	1.70–1.73 (*m*)	52.3
H_α–C(6)	2.09–2.15 (*m*)	23.9	2.06–2.09 (*m*)	24.3
H_β–C(6)	1.89–1.94 (*m*)		2.08–2.12 (*m*)	
H–C(7)	5.23–5.28 (*m*)	117.9	5.28–5.33 (*m*)	117.9
C(8)	–	145.7	–	145.8
H–C(9)	2.18–2.23 (*m*)	48.9	2.24–2.29 (*m*)	48.4
C(10)	–	34.9	–	34.9
CH_2(11)	1.48–1.54 (*m*)	18.1	1.54–1.59 (*m*)	18.3
H_α–C(12)	1.62–1.65 (*m*)	33.8	1.63–1.68 (*m*)	33.7
H_β–C(12)	1.78–1.84 (*m*)		1.78–1.86 (*m*)	
C(13)	–	43.5	–	43.5
C(14)	–	51.1	–	51.1
CH_2(15)	1.26–1.42 (*m*)	34.0	1.47–1.52 (*m*)	34.0
H_α–C(16)	1.23–1.27 (*m*)	28.4	1.27–1.32 (*m*)	28.4
H_β–C(16)	1.94–1.97 (*m*)		1.95–1.99 (*m*)	
H–C(17)	1.51–1.55 (*m*)	53.7	1.54–1.58 (*m*)	53.7
Me(18)	0.81 (*s*)	21.8	0.81 (*s*)	21.9
Me(19)	0.74 (*s*)	13.1	0.99 (*s*)	12.8
H–C(20)	1.46–1.50 (*m*)	34.5	1.50–1.53 (*m*)	34.5
Me(21)	0.97 (*d*, *J* = 6.0)	19.5	0.97 (*d*, *J* = 6.0)	19.5
H_a–C(22)	1.17–1.23 (*m*)	39.6	1.19–1.24 (*m*)	39.5
H_b–C(22)	1.67–1.73 (*m*)		1.72–1.74 (*m*)	
H–C(23)	3.67–3.76 (*m*)	70.8	3.70–3.77 (*m*)	70.8
H–C(24)	3.86–3.91 (*m*)	77.3	3.87–3.92 (*m*)	76.7
C(25)		145.1		154.1
H_a–C(26)	5.05 (*s*)	112.9	5.05 (*s*)	113.0
H_b–C(26)	4.99 (*s*)		4.99 (*s*)	
Me(27)	1.75 (*s*)	18.7	1.75 (*s*)	18.7
Me(28)	0.96 (*s*)	27.6	1.04 (*s*)	24.5
Me(29)	0.85 (*s*)	14.7	1.11 (*s*)	21.6
Me(30)	0.96 (*s*)	27.2	1.01 (*s*)	27.4

H–C(15); H_β–C(12)/H–C(17); H–C(9)/H–C(5) and Me(18); and Me(18)/H–C(20) showed that Me(19), Me(29), Me(30), H–C(7), and H–C(17) were all β-oriented, whereas H–C(5), H–C(9), and Me(18) were α-oriented, as in sapelin C and in **7** [7]. The ROESY correlations of H_α–C(21)/H_α–C(22); H–C(23)/H_β–C(16) and H–C(17); and H–C(24)/H_α–C(21) and H_α–C(22), in combination with the small coupling constants (< 2 Hz) between H–C(20) and both H-atoms at C(21), and the large coupling constant between H–C(23) and H–C(24) (J = 8.8 Hz) established the

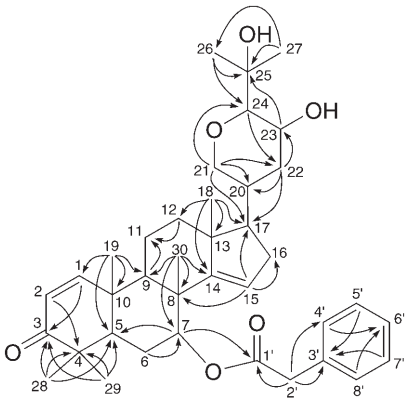


Fig. 1. *Selected HMBC correlations* (H → C) *of* **2**

configuration of the side chain as depicted [7]. Thus, compound **2** was determined to be sapelin C 7-*O*-phenylacetate[1]).

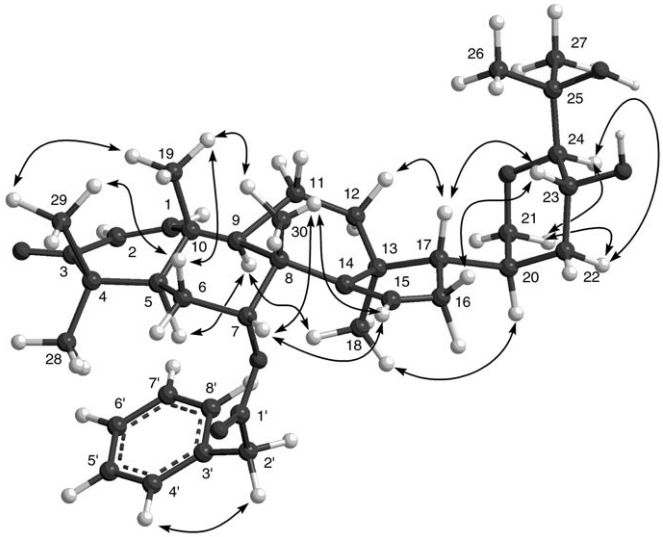


Fig. 2. *Key ROESY correlations* (H ↔ H) *of* **2**

Turrapubesol C (**3**) was obtained as a white amorphous solid. The EI-MS gave a molecular ion peak (*m*/*z* 604) of very low intensity and the molecular formula $C_{38}H_{52}O_6$ was determined from the $[M - H_2O]^+$ peak at *m*/*z* 586.3649 (calc. 586.3658) in the HR-EI-MS. The UV, IR, and ^{1}H- and ^{13}C-NMR (*Table 2*) data of **3** showed close resemblance to those of **2**, implying that **3** was also a tetracyclic triterpenoid. The notable difference was that the side chain at C(17) of **3** was established as a substituted oxepane ring by comparison with the ^{1}H- and ^{13}C-NMR data described for sapelin E

[1]) For systematic names, see *Exper. Part.*

Table 2. *^{1}H- and ^{13}C-NMR Data for* **2** *and* **3**. At 400/100 MHz, resp., in $CDCl_3$; δ in ppm, J in Hz.

	2		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	7.12 (*d*, *J* = 10.2)	158.0	7.12 (*d*, *J* = 10.2)	158.4
H–C(2)	5.81 (*d*, *J* = 10.2)	124.9	5.81 (*d*, *J* = 10.2)	125.3
C(3)	–	204.4	–	204.8
C(4)	–	43.6	–	44.0
H–C(5)	1.95–1.99 (*m*)	45.4	1.91–2.00 (*m*)	45.8
H_α–C(6)	1.68–1.72 (*m*)	23.2	1.65–1.72 (*m*)	23.5
H_β–C(6)	1.82–1.85 (*m*)		1.81–1.85 (*m*)	
H–C(7)	5.22 (br. *s*)	74.7	5.21 (br. *s*)	74.9
C(8)	–	42.2	–	42.5
H–C(9)	2.15 (*dd*, *J* = 11.7, 5.8)	38.1	2.14 (*dd*, *J* = 12.2, 5.9)	38.3
C(10)	–	39.3	–	39.7
H_α–C(11)	1.89–1.92 (*m*)	16.3	1.85–1.89 (*m*)	16.6
H_β–C(11)	1.59–1.65 (*m*)		1.65–1.72 (*m*)	
H_α–C(12)	1.88–1.91 (*m*)	34.2	1.85–1.89 (*m*)	33.8
H_β–C(12)	1.58–1.62 (*m*)		1.53–1.61 (*m*)	
C(13)	–	46.0	–	46.3
C(14)	–	158.4	–	158.9
H–C(15)	5.28–5.32 (*m*)	118.9	5.24–5.29 (*m*)	118.9
H_α–C(16)	1.92–1.96 (*m*)	34.4	1.87–1.92 (*m*)	34.9
H_β–C(16)	2.25 (*ddd*, *J* = 15.0, 7.0, 3.6)		2.20–2.26 (*m*)	
H–C(17)	1.98–2.03 (*m*)	51.9	1.86–1.92 (*m*)	54.1
Me(18)	0.92 (*s*)	19.7	0.93 (*s*)	19.7
Me(19)	1.12 (*s*)	18.6	1.12 (*s*)	18.9
H–C(20)	1.85–1.89 (*m*)	35.4	1.87–1.92 (*m*)	36.3
H_α–C(21)	3.41 (*dd*, *J* = 11.8, 2.0)	69.7	3.60 (br. *d*, *J* = 12.8)	64.2
H_β–C(21)	3.96 (br. *d*, *J* = 11.8)		3.48–3.53 (*m*)	
H_α–C(22)	1.49–1.58 (*m*)	35.8	1.61–1.65 (*m*)	37.9
H_β–C(22)	1.98–2.03 (*m*)		1.91–1.95 (*m*)	
H–C(23)	3.82–3.90 (*m*)	64.0	3.75–3.82 (*m*)	67.9
H–C(24)	2.89 (*d*, *J* = 8.8)	86.1	3.41 (*d*, *J* = 8.8)	80.7
C(25)	–	73.6	–	76.2
Me(26)	1.26 (*s*)	23.6	1.15 (*s*)	22.4
Me(27)	1.30 (*s*)	28.0	1.30 (*s*)	26.3
Me(28)	0.78 (*s*)	26.2	0.77 (*s*)	26.6
Me(29)	1.00 (*s*)	20.8	0.99 (*s*)	21.2
Me(30)	1.18 (*s*)	27.0	1.16 (*s*)	27.4
C(1′)	–	170.1	–	170.5
CH_2(2′)	3.49 (*s*)	41.6	3.48 (*s*)	41.9
C(3′)	–	133.6	–	134.0
H–C(4′,8′)	7.16–7.20 (*m*)	128.7	7.15–7.20 (*m*)	129.1
H–C(5′,7′)	7.21–7.25 (*m*)	128.1	7.22–7.25 (*m*)	128.5
H–C(6′)	7.19–7.22 (*m*)	126.7	7.19–7.22 (*m*)	127.1

and melianin B [7][8]. The structure of this moiety was confirmed by the HMBC correlations from CH_2(21) to C(22) and C(25), and from H–C(24) to C(22) and C(25). The stereochemistry of the backbone of **3** was determined to be the same as that of **2** by the ROESY spectrum. The proton and carbon chemical shift values for the oxepane

side chain of **3** were almost identical to those of melianin B [8], suggesting that **3** contained the same stereochemistry as melianin B in this part of the molecule. These findings were supported by the ROESY correlations of H_α–C(21)/H_α–C(22), H–C(24), and Me(27); H–C(23)/H–C(17) and Me(26); and H–C(24)/H_α–C(22) and Me(27) (*Fig. 3*). Therefore, **3** was structurally determined to be sapelin E 7-*O*-phenylacetate[1]).

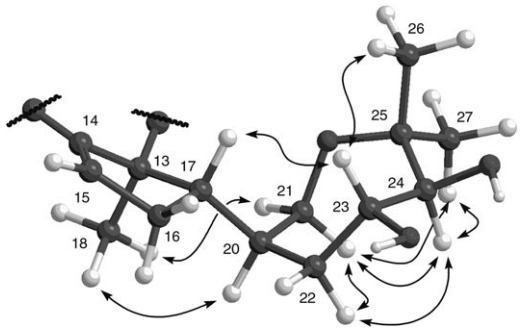


Fig. 3. *Key ROESY correlations* (H ↔ H) *for the side chain part of* **3**

Compound **4** was isolated as colorless needles. The molecular formula $C_{15}H_{26}O_2$ was determined from the pseudomolecular ion peak at *m*/*z* 261.1839 ($[M+Na]^+$; calc. 261.1831) in the HR-ESI-MS. The IR spectrum showed absorption bands for OH groups (3319 cm^{-1}) and a C=C bond (1645 and 1450 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra (*Table 3*) showed resonances for three tertiary Me (δ(H) 1.74 (*s*, Me(13)), 0.90 (*s*, Me(14)), and 1.09 (*s*, Me(15)); δ(C) 21.7, 20.3, and 23.0, resp.), five CH_2, three CH (one oxygenated), a double bond, and two quaternary C-atoms. The ^{1}H,^{1}H-COSY spectrum, in combination with the HMBC experiment, suggested **4** being an eudesmane derivative (*Fig. 4*,*a*). A literature search revealed that **4** has the same constitution as cyperusol C (**8**) [9], lairdinol A [10], and 1β,4β-dihydroxyeudesman-11-ene (**9**) [11]. However, the OH–C(1) and OH–C(4) groups were both α-oriented according to the ROESY correlations of Me(14)/H–C(1), H_β–C(2), Me(15), H_β–C(6), and H_β–C(8); Me(15)/H_β–C(2) and H_β–C(6); and H–C(5)/H_α–C(3), H–C(7), and H–C(9) (*Fig. 4*,*b*). As OH–C(1) was α-oriented, no γ-gauche effect of OH–C(1) to C(14) was observed. Consequently, C(14) was considerably downfield shifted compared to **8** (δ(C) 20.3 in **4** *vs.* 13.0 in **8**), which also supported the above conclusion. Therefore, compound **4** was determined as 1α,4α-dihydroxyeudesman-11-ene[1]).

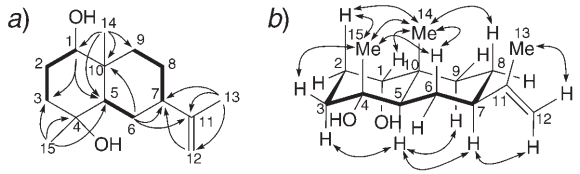


Fig. 4. a) *Key* ^{1}*H,*^{1}*H-COSY* (—) *and HMBC correlations* (H → C) *of* **4**. b) *Key ROESY correlations* (H ↔ H) *of* **4**.

Table 3. *^{1}H- and ^{13}C-NMR Data for* **4** *and* **5**. At 400/100 MHz, resp., δ in ppm, J in Hz.

	4[a])		**5**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	3.30 (br. *s*)	74.8	2.69–2.78 (*m*)	52.1
H_α–C(2)	1.61–1.67 (*m*)	28.5	1.44–1.49 (*m*)	26.1
H_β–C(2)	1.86–1.90 (*m*)		1.90–1.97 (*m*)	
H_α–C(3)	1.86–1.89 (*m*)	37.5	1.69–1.75 (*m*)	39.2
H_β–C(3)	1.42–1.47 (*m*)		1.69–1.75 (*m*)	
C(4)	–	73.4	–	83.6
H–C(5)	1.73 (*dd*, J = 12.7, 2.7)	48.7	1.98–2.01 (*m*)	49.2
H_α–C(6)	1.88–1.94 (*m*)	27.6	1.52–1.57 (*m*)	29.2
H_β–C(6)	1.16–1.27 (*m*)		1.75–1.80 (*m*)	
H–C(7)	1.88–1.96 (*m*)	48.1	2.04–2.11 (*m*)	43.0
H_α–C(8)	1.54–1.61 (*m*)	28.5	1.61–1.68 (*m*)	32.1
H_β–C(8)	1.42–1.48 (*m*)		1.80–1.85 (*m*)	
H_α–C(9)	1.80–1.86 (*m*)	39.2	1.61–1.68 (*m*)	36.5
H_β–C(9)	1.13–1.19 (*m*)		1.75–1.80 (*m*)	
C(10)		40.5		75.1
C(11)		152.4		151.1
H_a–C(12)	4.70 (br. *s*)	109.1	4.70 (br. *s*)	108.3
H_b–C(12)	4.66 (br. *s*)		4.64 (br. *s*)	
Me(13)	1.74 (*s*)	21.7	1.71 (*s*)	20.7
Me(14)	0.90 (*s*)	20.3	1.29 (*s*)	25.3
Me(15)	1.09 (*s*)	23.0	1.24 (*s*)	32.2

[a]) Recorded in CD_3OD. [b]) Recorded in $CDCl_3$.

Guaidiol (**5**) is a sesquiterpene previously isolated from *Curcuma zedoaria*, the structure of which has been determined by X-ray crystallography and the NMR data were assigned in (D_6)acetone [12]. However, perhaps due to overlapping of the solvent signals with some signals of **5**, some of the signals were erroneously assigned and were inconsistent with analogous compounds in the literature [13]. Therefore, we recorded the HMBC and HSQC of **5** in $CDCl_3$ and reassigned the NMR data (*Table 3*).

The known triterpenoids, bourjotinolone B (**6**) [6], grandifoliolenone (**7**) [7], piscidinol A [14], hispidone [15], bourjotinolone A [15][16], hispidol B [17], and 3-episapelin A [16], along with the known sesquiterpenoids cyperusol C (**8**) [9], 1β,4β-dihydroxyeudesman-11-ene (**9**) [11], clovandiol [18][19], and caryolane-1,9β-diol [19] were identified by comparison of the ^{1}H- and ^{13}C-NMR as well as MS data with those reported in the literature. All of these compounds were isolated from this species for the first time.

The cytotoxicity of the obtained triterpenoids against the P-388 (murine leukemia) and A-549 (human lung adenocarcinoma) cell lines were evaluated with pseudolaric acid B as positive control (IC_{50} = 0.74 and 0.30 μM against P-388 and A-549, resp.). Turrapubesol B (**2**), turrapubesol C (**3**), hispidone, bourjotinolone A, and hispidol B showed growth inhibitory activities against the P-388 cell line with IC_{50} values of 7.06, 6.98, 6.98, 6.89, and 4.07 μM, respectively. All of the isolated compounds were inactive against the A-549 cells.

Experimental Part

General. All solvents used were of anal. grade (*Shanghai Chemical Plant*, Shanghai, P. R. China). Thin-layer chromatography (TLC): pre-coated silica gel GF_{254} plates (*Qingdao Haiyang Chemical Co. Ltd.*, Qingdao, P. R. China). Column chromatography (CC): silica gel (200–300 mesh), silica gel *H60*, C_{18} reversed-phase (*RP-18*) silica gel (150–200 mesh; *Merck*), *Sephadex LH-20* gel (*Amersham Biosciences*), and *MCI* gel (*CHP20P*, 75–150 μm, *Mitsubishi Chemical Industries Ltd.*). Semi-preparative HPLC was performed on a *Waters 515* pump equipped with a *Waters 2487* detector (254 nm) and a *YMC-Pack* ODS-A column (250 × 10 mm, S-5 μm, 12 nm). Melting points were measured with an *SGW X-4* melting point apparatus and are uncorrected. Optical rotations were measured on a *Perkin-Elmer 341* polarimeter. UV spectra were obtained on a *Shimadzu UV-2550* spectrophotometer; λ_{max} (log ε) in nm. IR spectra were obtained on a *Perkin-Elmer 577* spectrometer with KBr discs; in cm^{-1}. NMR Spectra were recorded on a *Bruker AM-400* spectrometer; δ in ppm rel. to Me_4Si, *J* in Hz. Electron impact-ionization mass spectrometry (EI-MS) was performed at 70 eV with a *Finnigan MAT-95* mass spectrometer; in *m*/*z* (rel. %). Low-resolution electrospray-ionization mass spectrometry (LR-ESI-MS) was carried out on a *Finnigan LC* Q^{DECA} instrument, and high-resolution ESI-MS (HR-ESI-MS) was carried out on a *Waters-Micromass Q-TOF* mass spectrometer; in *m*/*z* (rel. %).

Plant Material. The twigs and leaves of *T. pubescens* were collected in August of 2003 from Hainan Province of the P. R. China. The plant was authenticated by Prof. *Shi-Man Huang*, Department of Biology, Hainan University of the P. R. China. A voucher specimen has been deposited in Shanghai Institute of Materia Medica, SIBS, Chinese Academy of Sciences (accession number: TP-2003-1Y).

Extraction and Isolation. The air-dried powder of the plant (5 kg) was percolated with 95% EtOH, and 600 g of crude extract was subsequently extracted successively with petroleum ether, AcOEt and BuOH. The AcOEt-soluble fraction (211 g) was separated by silica gel CC eluted with petroleum ether (PE)/Me_2CO (10:1 → 0:1) to give six fractions (*Fr. A–F*). *Fr. E* (50 g) was then separated on an *MCI* gel column by elution with MeOH/H_2O (5:5 → 9:1) to give six subfractions (*Fr. E1- E6*). *Fr. E2* (4 g) was chromatographed over silica gel (PE/Me_2CO, 6:1 → 1:1) to afford six sub-subfractions (*Fr. E2a–E2f*). *Fr. E2b* (0.4 g) was first subjected to CC (SiO_2; CH_2Cl_2/MeOH, 50:1), and then purified by CC (*Sephadex LH-20*; EtOH) to afford **8** (8 mg). *Fr. E2c* (1.0 g) was subjected to CC (SiO_2; CH_2Cl_2/MeOH, 75:1 → 30:1) to give two the *Fractions E2c-1* and *E2c-2*, *Fr. E2c-1* (0.3 g) was further purified over silica gel (PE/Me_2CO, 6:1) and then recrystallized from acetone to afford **4** (8 mg) and **9** (20 mg). *Fr. E2c-2* (0.5 g) was subjected to semi-preparative HPLC by elution with MeCN/H_2O (50:50, 3 ml/min) to give **5** (7 mg), clovandiol (10 mg), and caryolane-1,9β-diol (5 mg). *Fr. E3* (7 g) was chromatographed through a column of *RP-18* silica gel eluted with MeOH/H_2O (50:50 → 100:0) to afford seven sub-subfractions (*Fr. E3a–E3g*). *Fr. E3b* (0.31 g) gave hispidol B (90 mg), as colorless crystals after filtration. *Fr. E3c* (0.30 g) first gave the crystals of piscidinol A (90 mg), and the mother liquor was subjected to *RP-18* silica gel (MeOH/H_2O, 80:20) and *Sephadex LH-20* (EtOH) to afford 3-episapelin A (31 mg). *Fr. E3d* (2.20 g) was first chromatographed over silica gel ($CHCl_3$/MeOH, 50:1) and then subjected to semi-preparative HPLC to give **2** (100 mg) and **3** (80 mg). *Fr. E3f* (1.30 g) was subjected to repeated CC of silica gel (PE/AcOEt, 2:1 and $CHCl_3$/MeOH, 50:1) and *RP-18* (MeOH/H_2O, 70:30) to give **7** (60 mg). *Fr. E4* (4 g) was subjected to CC (SiO_2; PE/acetone 6:1 → 1:1) to give seven subfractions (*Fr. E4a–E4g*). *Fr. E4a* (20 mg) was purified by *Sephadex LH-20* (EtOH) to afford **6** (10 mg). *Fr. E4b* (1.4 g) was chromatographed over silica gel eluted with pure $CHCl_3$ to give hispidone (100 mg) and bourjotinolone A (60 mg). *Fr. E4c* (0.1 g) was purified by CC (SiO_2; $CHCl_3$) and CC (*Sephadex LH-20*; EtOH) to afford **1** (12 mg). *Fr. E4d* (1.1 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 50:1) to afford another part of piscidinol A (50 mg) and **3** (20 mg).

$NaBH_4$ Reduction of **6** *to* **1**. A soln. of bourjotinolone B (**6**) (7 mg) and $NaBH_4$ (20 mg) in MeOH (3 ml) was stirred at r.t. for 2 h. The mixture was diluted with H_2O, acidified with HCl (2M), and extracted with AcOEt. The AcOEt layer was evaporated and purified by CC (SiO_2; PE/acetone 6:1) to give a compound (5 mg), which was identical to turrapubesol A (**1**) by its ^{1}H-NMR spectrum and TLC.

*Turrapubesol A (=(3β,13α,14β,17α,20*S*,23*R*,24*R*)-Lanosta-7,25-diene-3,23,24-triol*; **1**). Colorless crystals. M.p. 204–205°. $[\alpha]_D^{20} = -33.0$ ($c = 0.110$, MeOH). IR (KBr): 3406, 2960, 2927, 1647, 1442,

1383, 1097, 1034, 908, 823. ^{1}H- and ^{13}C-NMR: see *Table 1*. EI-MS: 458 (22, M^+), 443 (32), 425 (52), 407 (44), 385 (40), 369 (95), 325 (72), 187 (44), 121 (60), 95 (64), 72 (100). HR-EI-MS: 458.3769 (M^+, $C_{30}H_{50}O_3^+$; calc. 458.3760).

Turrapubesol B (= *(5α,7α,13α,17α,20*S*,23*R*,24*R*)-21,24-Epoxy-23,25-dihydroxy-4,4,8-trimethyl-3-oxocholesta-1,14-dien-7-yl Benzeneacetic Acid Ester*; **2**). White, amorphous solid. $[\alpha]_D^{20} = +31.0$ (c = 0.035, MeOH). UV (MeOH): 204 (3.88), 225 (sh, 3.70). IR (KBr): 3431, 2976, 2937, 1728, 1670, 1456, 1381, 1259, 1174, 1074, 1007, 756. ^{1}H- and ^{13}C-NMR: see *Table 2*. EI-MS: 604 (2, M^+), 586 (17), 568 (16), 515 (12), 444 (36), 308 (90), 293 (28), 150 (56), 91 (100). HR-EI-MS: 604.3764 (M^+, $C_{38}H_{52}O_6^+$; calc. 604.3764).

Turrapubesol C (= *(5a,7a,13a,20*S*,23*R*,24*S*)-21,25-Epoxy-23,24-dihydroxy-4,4,8-trimethyl-3-oxocholesta-1,14-dien-7-yl Benzeneacetic Acid Ester*; **3**). White, amorphous solid. $[\alpha]_D^{20} = +60.0$ (c = 0.035, MeOH). UV (MeOH): 203 (4.08), 225 (sh, 3.87). IR (KBr): 3433, 2935, 1728, 1670, 1456, 1381, 1259, 1157, 1078, 1020, 723. ^{1}H- and ^{13}C-NMR: see *Table 2*. EI-MS: 586 (4, $[M - H_2O]^+$), 568 (2), 515 (10), 444 (12), 395 (17), 308 (28), 295 (16), 150 (52), 91 (100). HR-EI-MS: 586.3649 ($[M - H_2O]^+$, $C_{38}H_{50}O_5^+$; calc. 586.3658).

1α,4α-Dihydroxyeudesman-11-ene (= *(1*R*,4*S*,4a*R*,7*R*,8a*R*)-Decahydro-1,4a-dimethyl-7-(1-methylethenyl)naphthalene-1,4-diol*; **4**). Colorless crystals. M.p. 149–150°. $[\alpha]_D^{20} = -10.0$ (c = 0.080, MeOH). IR (KBr): 3319, 3240, 2918, 1645, 1450, 1383, 1165, 1082, 1057, 885, 671. ^{1}H- and ^{13}C-NMR: see *Table 3*. EI-MS: 220 (22, $[M - H_2O]^+$), 202 (26), 162 (100), 159 (60), 147 (44), 107 (76), 93 (72), 72 (88). ESI-MS (pos.): 261 (16, $[M + Na]^+$), 203 (68, $[M + H - 2\ H_2O]^+$), 147 (100). HR-ESI-MS (pos.): 261.1839 ($[M + Na]^+$, $C_{15}H_{26}NaO_2^+$; calc. 261.1831).

Guaidiol (= *(1*S*,3a*S*,4*S*,7*R*,8a*S*)-Decahydro-1,4-dimethyl-7-(1-methylethenyl)azulene-1,4-diol*; **5**). ^{1}H- and ^{13}C-NMR data in $CDCl_3$: see *Table 3*. ^{1}H-NMR ((D$_6$)acetone, 400 MHz): 4.65–4.69 (*m*, H_b–C(12)); 4.55–4.59 (*m*, H_a–C(12)); 2.72–2.81 (*m*, H–C(1)); 1.67 (*dd*, J = 1.3, 0.8, Me(13)); 1.21 (*s*, Me(14)); 1.17 (*s*, Me(15)). ^{13}C-NMR ((D$_6$)acetone, 100 MHz): 152.3 (C(11)); 107.8 (C(12)); 82.6 (C(4)); 74.1 (C(10)); 52.1 (C(1)); 49.3 (C(5)); 43.9 (C(7)); 39.7 (C(3)); 36.8 (C(9)); 32.7 (C(8)); 32.3 (C(15)); 30.1 (C(6)); 26.5 (C(2)); 25.1 (C(14)); 20.3 (C(13)).

Bourjotinolone B (= *(13α,14β,17α,20*S*,23*R*,24*R*)-23,24-Dihydroxylanosta-7,25-dien-3-one*; **6**). Colorless crystals. M.p. 200–201°. ^{1}H- and ^{13}C-NMR: see *Table 1*. EI-MS: 456 (12, M^+), 441 (14), 423 (42), 405 (24), 385 (48), 369 (100), 325 (76), 271 (20), 187 (24), 121 (36), 95 (42), 72 (90).

Cytotoxicity Assay. The MTT [20] and SRB [21] methods have been adapted for the assay of cytotoxicity of the triterpenoids against the P-388 (murine leukemia) and A-549 (human lung adenocarcinoma) cell lines, respectively, according to methods described in the literature. Pseudolaric acid B [22] was used as a positive control.

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