Terpenoids from *Eupatorium fortunei* TURCZ

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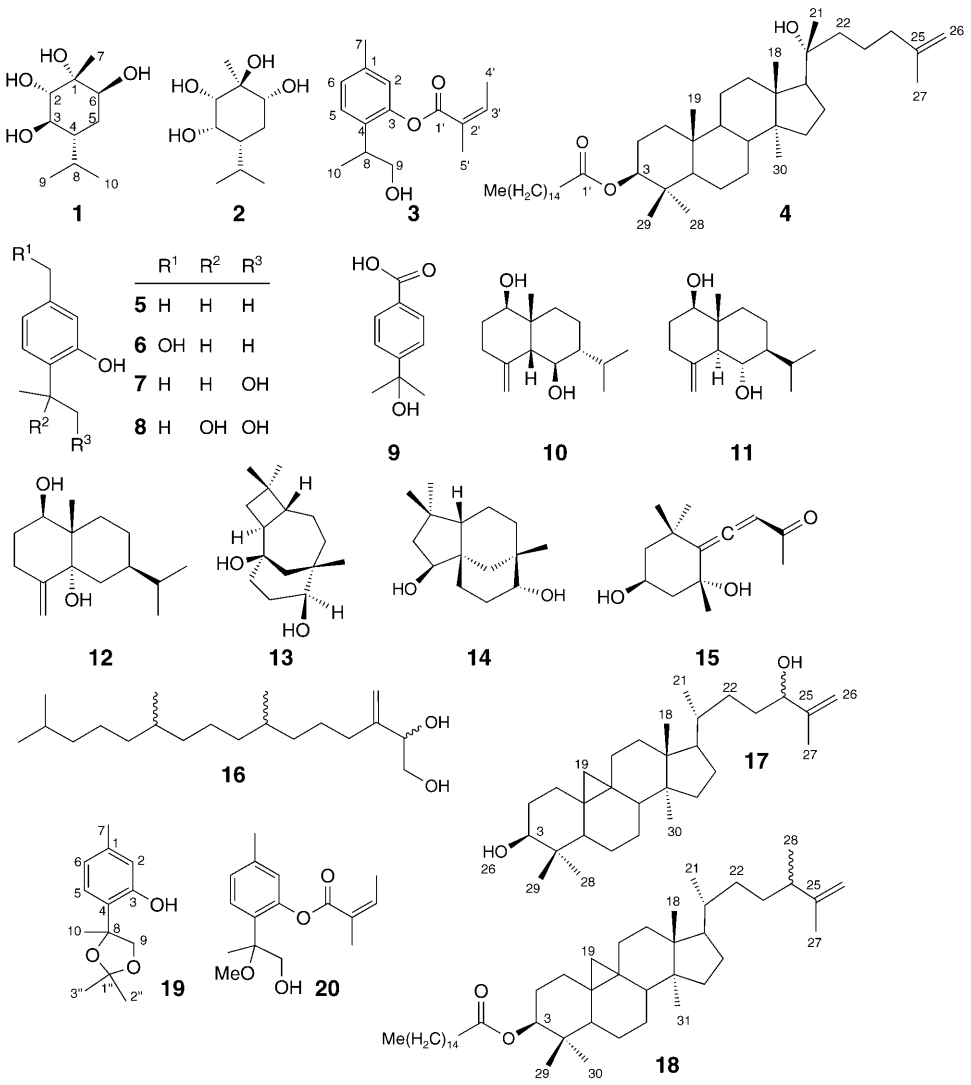
Four new terpenoids, namely, *rel*-(1*R*,2*S*,3*R*,4*R*,6*S*)-*p*-menthane-1,2,3,6-tetrol (**1**), *rel*-(1*R*,2*R*,3*R*,4*S*,6*S*)-*p*-menthane-1,2,3,6-tetrol (**2**), 9-hydroxythymol 3-*O*-angelate (**3**), and (3*β*,20*R*)-20-hydroxylanost-25-en-3-yl palmitate (**4**), together with fourteen known compounds, were isolated from the AcOEt part of the MeOH extracts of *Eupatorium fortunei*. In addition, two other monoterpenoids, 'acetone thymol-8,9-diyl ketal' (**19**) and 8-methoxy-9-hydroxythymol 3-*O*-angelate (**20**) were also obtained which were probably artifacts but have never been reported in the literature. The structures of the new compounds, including their relative configurations, were established by an extensive study of their spectral data, especially 1D- and 2D-NMR. The cytotoxic activity of the new compounds against human hepatoma (SMMC-7721), human leukemia (HL-60), and human hepatocyte (LO_2) cells was investigated.

1. Introduction. – The genus *Eupatorium* (Compositae) consists of about 1200 species, with 14 species widely distributed in China [1], of which 8 species have long been used as Chinese folk medicines [2], especially *Eupatorium fortunei*. This species has been used as a diuretic and detoxifying drug [3] in China for the treatment of a dropsy, chill, and fever. In continuation of our search for bioactive compounds from species of the family of Compositae [4][5], we studied the whole plant of *E. fortunei*. The petroleum ether, AcOEt, and BuOH extracts of the initially obtained MeOH extract of *E. fortune* were firstly tested for their antitumor activities against human hepatoma (SMMC-7721) and human leukemia (HL-60) cells, establishing cytotoxicity of the AcOEt extract (see below, *Table 3*). Therefore, we investigated the AcOEt extract and succeeded isolating of four new terpenoids **1**–**3** and **4**, together with the fourteen known compounds **5**–**18**. Furthermore, two new monoterpenoids, **19** and **20**, were also obtained which were probably artifacts. These new compounds were investigated for the cytotoxic activities against SMMC-7721, HL-60, and human hepatocyte (LO_2) cells, revealing that compounds **1**, **2**, **19**, and **20** were cytotoxic against HL-60 cell. We report here on the isolation and structural elucidation of these compounds and the cytotoxicity-assay results.

2. Results and Discussion. – The known compounds were identified by comparing their spectral data (MS, IR, ^{1}H- and ^{13}C-NMR) with those reported in the literature, *i.e.*, as thymol (=5-methyl-2-(1-methylethyl)phenol; **5**) [6], 7-hydroxythymol (=5-(hydroxymethyl)-2-(1-methylethyl)phenol; **6**) [7][8], 9-hydroxythymol (=2-(2-hy-



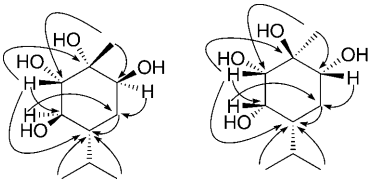
droxy-1-methylethyl)-5-methylphenol; **7**) [9], 8,9-dihydroxythymol (=2-(2-hydroxy-4-methylphenyl)propane-1,2-diol; **8**) [10], 4-(1-hydroxy-1-methylethyl)benzoic acid (**9**) [11], (1β,6β)-5,7-epieudesm-4(14)-ene-1,6-diol[1]) (=(1*R*,2*R*,4a*R*,5*R*,8a*R*)-decahydro-4a-methyl-8-methylene-2-(1-methylethyl)naphthalenene-1,5-diol; **10**) [12], (1β,6α)-eudesm-4(14)-ene-1,6-diol[1]) (=(1*S*,2*S*,4a*R*,5*R*,8a*S*)-decahydro-4a-methyl-8-methylene-2-(1-methylethyl) naphthalenene-1,5-diol; **11**) [12][13], (1β,5α)-eudesm-4(14)-ene-1,5-diol[1]) (=(1*R*,4a*R*,6*R*,8a*S*)-octahydro-8a-methyl-4-methylene-6-(1-methyl-

[1]) IUPAC Atom numbering; such compounds were also named as 'eudesm-4(15)-ene' instead of 'eudesm-4(14)-ene' derivatives.

ethyl)naphthalene-1,4a(2*H*)-diol; **12**) [14], (1*β*,9*β*)-caryolane-1,9-diol (=(1*R*,2*S*,5*R*,8*S*,9*S*)-4,4,8-trimethyltricyclo[6.3.1.$0^{2,5}$]dodecane-1,9-diol; **13**) [15], (2*β*,9*α*)-clovane-2*β*,9*α*-diol (=(3*S*,3a*S*,6*R*,7*R*,9a*S*)-decahydro-1,1,7-trimethyl-3a,7-methano-3a*H*-cyclopentacyclooctene-3,6-diol; **14**) [15], (3*S*,5*R*,8*R*)-3,5-dihydroxymegastigma-6,7-dien-9-one (=(3*R*)-4-((2*R*,4*S*)-2,4-dihydroxy-2,6,6-trimethylcyclohexylidene)but-3-en-2-one; **15**) [16][17], 7,11,15-trimethyl-3-methylidenehexadecane-1,2-diol (**16**) [18][19], (3*β*,24*RS*)-cycloart-25-ene-3,24-diol (=(3*β*)-9,19-cyclolanost-25-ene-3,24-diol; **17**) [20][21], and cycloaudenyl palmitate (=hexadecanoic acid (3*β*)-24-methyl-9,19-cylcolanost-25-en-3-yl ester; **18**) [22][23].

Compound **1** was obtained as colorless oil. Its HR-SI-MS showed a quasi-molecular-ion peak $[M+H]^+$ at *m*/*z* 205.0536 ($[C_{10}H_{20}O_4+H]^+$), and the EI-MS gave a molecular-ion peak at *m*/*z* 204 and fragment-ion peaks at *m*/*z* 186 ($[M-H_2O]^+$), 168 ($[M-2\ H_2O]^+$), 153 ($[M-2\ H_2O-Me]^+$), 125 ($[M-2\ H_2O-\text{isopropyl}]^+$), and 107 ($[M-3\ H_2O-\text{isopropyl}]^+$), corresponding to a molecular formula $C_{10}H_{20}O_4$. The IR spectrum of **1** showed an obvious absorption band for OH groups at 3422 cm^{-1}. From further spectral data, the structure of **1** was deduced to be *rel*-(1*R*,2*S*,3*R*,4*R*,6*S*)-*p*-menthane-1,2,3,6-tetrol.

The ^{1}H-NMR, ^{13}C-NMR and DEPT spectra (*Tables 1* and *2*) showed signals for 1 C, 5 CH, 1 CH_2, and 3 Me groups. In the ^{1}H-NMR spectrum, the three Me groups appeared at *δ*(H) 1.39 (*s*), 0.92 (*d*, *J*=7.2 Hz), and 0.78 (*d*, *J*=7.2 Hz), and out of the five methine protons, three were OCH signals at *δ*(H) 3.69 (*d*, *J*=9.3 Hz), 3.95 (*dd*, *J*=9.3, 11.4 Hz), and 3.77 (*t*, *J*=2.4 Hz). The signal of the quaternary C-atom was at *δ*(C) 74.6 in the ^{13}C-NMR spectrum. Thus, compound **1** was a menthane monoterpene derivative with four OH groups [24]. The ^{1}H,^{1}H-COSY cross-peaks H–C(3) (*δ* 3.95)/H–C(2) (*δ* 3.69), and H–C(4) (*δ* 1.97), H–C(4)/H–C(3), H–C(5) (*δ* 1.59 and 1.77), and H–C(8) (*δ* 2.30), and H–C(6) (*δ* 3.77)/H–C(5), and the HMBC correlations (*Fig. 1*) of a Me *s* (*δ* 1.39) with C(1) (*δ* 74.6), C(2) (*δ* 76.7), and C(6) (*δ* 72.9) as well as other HMBC long-range correlations indicated that the four OH groups were located at C(1), C(2), C(3), and C(6), and the ^{i}Pr group at C(4), thus establishing the *p*-menthane-1,2,3,6-tetrol structure. The relative configuration of **1** was determined by the ^{1}H,^{1}H-coupling pattern of the ring protons. The large *J*s of H–C(3) with H–C(2) and H–C(4) (*J*(3,2)=9.3 Hz, *J*(3,4)=11.4 Hz) showed that H–C(2), H–C(3), and H–C(4) were axial protons, and the small *J*s of H–C(6) with H_{ax}–C(5) and H_{eq}–C(5) (*J*(6,5ax)=*J*(6,5eq)=2.4 Hz) were characteristic for an equatorial H–C(6) (*Fig. 2*). The relative configuration at C(1) was determined from NOE experiments: irradiation of the Me signal at *δ* 1.39 (Me(7)) enhanced the signal at *δ* 3.69 (H–C(2)). Therefore, the configuration *rel*-(1*R*,2*S*,3*R*,4*R*,6*S*) was deduced.



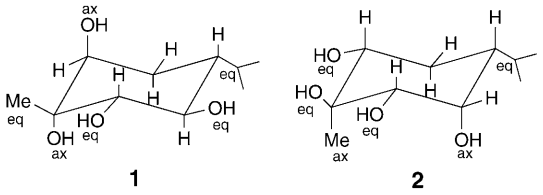
1 **2**

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

Compound **2** was obtained as colorless oil. Its HR-SI-MS showed $[M+H]^+$ at *m*/*z* 205.0521 ($[C_{10}H_{20}O_4+H]^+$), and the EI-MS revealed the M^+ peak at *m*/*z* 204 and fragment-ion peaks at *m*/*z* 189 ($[M-Me]^+$), 186 ($[M-H_2O]^+$), 161 ($[M-\text{isopropyl}]^+$), 143 ($[M-H_2O-\text{isopropyl}]^+$), and 125 ($[M-2\ H_2O-\text{isopropyl}]^+$), corresponding to a

Table 1. *^{1}H-NMR Data* (300 MHz, $CDCl_3$) *of Compounds* **1**–**3**, **19**, *and* **20**. δ in ppm, J in Hz. Trivial numbering.

	1	**2**	**3**	**19**	**20**
H–C(2)	3.69 (*d*, *J* = 9.3)	3.81 (*d*, *J* = 2.7)	6.87 (br. *s*)	6.69 (br. *s*)	6.85 (br. *s*)
H–C(3)	3.95 (*dd*, *J* = 11.4, 9.3)	4.15 (*t*, *J* = 2.7)			
H–C(4)	1.97 (*ddt*, *J* = 11.7, 11.4, 3.0)	2.06 (*dq*, *J* = 13.5, 2.7)			
CH_2(5) or	1.75–1.79 (*m*),	1.68–1.72 (*m*),	7.21 (*d*,	6.74 (*d*, *J* = 7.8)	7.32 (*d*, *J* = 8.1)
H–C(5)	1.56–1.61 (*m*)	1.76–1.80 (*m*)	*J* = 7.8)		
H–C(6)	3.77 (*t*, *J* = 2.4)	4.29 (*dd*, *J* = 10.8, 2.4)	7.09 (br. *d*, *J* = 7.8)	6.65 (br. *d*, *J* = 7.8)	7.06 (br. *d*, *J* = 8.1)
Me(7)	1.39 (*s*)	1.45 (*s*)	2.33 (*s*)	2.27 (*s*)	2.35 (*s*)
H–C(8)	2.24–2.34 (*m*)	1.64–1.69 (*m*)	3.04–3.12 (*m*)		
Me(9) or	0.92 (*d*, *J* = 7.2)	0.97 (*d*, *J* = 6.6)	3.67–3.69 (*m*)	4.30 (*d*, *J* = 9),	3.85 (*d*, *J* = 10.5),
CH_2(9)				4.16 (*d*, *J* = 9)	3.61 (*d*, *J* = 10.5)
Me(10)	0.78 (*d*, *J* = 7.2)	0.97 (*d*, *J* = 6.6)	1.23 (*d*, *J* = 6.9)	1.58 (*s*)	1.60 (*s*)
H–C(3′)			6.28 (*qq*, *J* = 6.9, 1.2)		6.27 (*qq*, *J* = 7.2, 1.2)
H–C(4′)			2.08 (*dq*, *J* = 6.9, 1.0)		2.07 (*dq*, *J* = 7.2, 1.0)
H–C(5′)			2.07 (*dq*, *J* = 1.2, 1.0)		2.06 (*dq*, *J* = 1.2, 1.0)
Me(2″)				1.37 (*s*)	
Me(3″)				1.55 (*s*)	
MeO					3.13 (*s*)



1 **2**

Fig. 2. *Stable conformers of compounds* **1** *and* **2**

molecular formula $C_{10}H_{20}O_4$, the same as that of compound **1**. The IR spectrum of **2** was very similar to that of **1**. In addition, **2** and **1** had similar chemical shifts in the ^{1}H-NMR spectra but obviously different coupling constants (*Table 1*), and only small differences of their chemical shifts were observed in the ^{13}C-NMR spectra (*Table 2*), suggesting that compound **2** was a stereoisomer of **1**. The structure of **2** was established as *rel*-(1*R*,2*R*,3*R*,4*S*,6*S*)-*p*-menthane-1,2,3,6-tetrol.

The protons and C-atoms of **2** were easily assigned by HMBC experiments (*Fig. 1*), with the aid of the ^{1}H,^{1}H-COSY plot. The coupling constants of H–C(4) with H_{ax}–C(5) (J(4,5ax) = 13.5 Hz) of H–C(6) with H_{ax}–C(5) (J(6,5ax) = 10.8 Hz), and of H–C(3) with H–C(2) and H–C(4) (J(3,2) = J(3,4) = 2.7 Hz) showed that H–C(4) and H–C(6) were axial protons and H–C(3) was an equatorial proton (*Fig. 2*). In

Table 2. *^{13}C-NMR and DEPT Data* (75 MHz, $CDCl_3$) *of Compounds* **1**–**3**, **19**, *and* **20**. δ in ppm. Trivial numbering.

	1	**2**	**3**	**19**	**20**
C(1)	74.6 (C)	74.3 (C)	137.6 (C)	138.8 (C)	139.1 (C)
C(2)	76.7 (CH)	77.8 (CH)	123.3 (CH)	118.0 (CH)	125.1 (CH)
C(3)	68.9 (CH)	70.4 (CH)	148.9 (C)	154.5 (C)	148.6 (C)
C(4)	41.4 (CH)	43.5 (CH)	127.4 (C)	125.2 (C)	130.0 (C)
C(5)	26.8 (CH_2)	32.7 (CH_2)	127.4 (CH)	125.2 (CH)	129.0 (CH)
C(6)	72.9 (CH)	66.9 (CH)	127.4 (CH)	120.5 (CH)	126.7 (CH)
C(7)	23.9 (Me)	20.6 (Me)	20.9 (Me)	20.9 (Me)	20.8 (Me)
C(8)	27.8 (CH)	27.8 (CH)	37.4 (CH)	84.6 (C)	79.4 (C)
C(9)	20.9 (Me)	21.2 (Me)	67.9 (CH_2)	75.2 (CH_2)	69.1 (CH_2)
C(10)	14.9 (Me)	21.2 (Me)	17.3 (Me)	28.8 (Me)	20.2 (Me)
C(1′)			166.8 (C)		166.4 (C)
C(2′)			132.3 (C)		127.1 (C)
C(3′)			141.1 (CH)		141.2 (CH)
C(4′)			16.0 (Me)		15.9 (Me)
C(5′)			20.7 (Me)		20.7 (Me)
C(1″)				110.6 (C)	
C(2″)				25.4 (Me)	
C(3″)				27.4 (Me)	
MeO					50.9 (Me)

the NOE experiments, enhancement between H–C(6) and H–C(2) could be observed but not between Me(7) at δ 1.45 and H–C(6) or H–C(2), indicating that Me(7) was at the opposite side of H–C(2) and H–C(6) and confirming the configuration *rel*-(1*R*,2*R*,3*R*,4*S*,6*S*).

Compound **3** was obtained as colorless oil. Its HR-ESI-MS showed $[M+Na]^+$ at *m*/*z* 271.1302 ($[C_{15}H_{20}O_3+Na]^+$) corresponding to a molecular formula $C_{15}H_{20}O_3$. The IR spectrum showed absorption bands for an OH group (3417 cm^{-1}), a substituted benzene moiety (1647, 1505 cm^{-1}) and a typical α,β-unsaturated carboxylic ester group (1733, 1647 cm^{-1}). The absorption band at 221 nm in the UV spectrum also indicated an aromatic character. By comparing all spectral data of **3** with those of the known compound **7** [9], the structure of **3** was deduced to be an angelate (=(2*Z*)-2-methylbut-2-enoate) derivative of thymol, named 9-hydroxythymol 3-*O*-angelate.

The ^{1}H-NMR and ^{13}C-NMR spectra of **3** (*Tables 1* and *2*) exhibited a typical pattern of a 1,2,4-trisubstituted benzene moiety (δ(H) 7.21 (*d*, *J* = 7.8 Hz, 1 H), 7.09 (br. *d*, *J* = 7.8 Hz, 1 H), and 6.87 (br. *s*, 1 H)), and of an angeloyloxy group (δ(H) 6.28 (*qq*, *J* = 6.9, 1.2 Hz, 1 H), 2.08 (*dq*, *J* = 6.9, 1.0 Hz, 3 H), 2.07 (*dq*, *J* = 1.2, 1.0 Hz, 3 H) and δ(C) 166.8 (*s*), 141.1 (*d*), 132.3 (*s*), 20.7 (*q*) and 16.0 (*q*)) [25]. Two of the three substituents at the benzene moiety were easily characterized as a Me group (δ(H) 2.33 (*s*) and δ(C) 20.9 (*q*)) and an angeloyloxy group. The third was deduced to be an oxygenated isopropyl group on the basis of the EI-MS, ^{1}H-NMR, ^{13}C-NMR, and DEPT spectra: the presence of a primary alcohol function was established by the fragment peak at *m*/*z* 217 ($[M-CH_2OH]^+$), the two-proton signal at δ(H) 3.68 (*m*, 2 H), and a C signal at δ(C) 67.9. The structure was confirmed by the HMBC correlations Me(7) (δ 2.33)/C(1) (δ 137.6), C(2) (δ 123.3), and C(6) (δ 127.4), Me(10) (δ 1.23)/C(4) (δ 127.4), C(8) (δ 37.4), and C(9) (δ 67.9), H–C(5) (δ 7.21)/C(3) (δ 148.9), C(4) (δ 127.4), and C(8) (δ 37.4).

Compound **19** was obtained as a white powder. Its HR-ESI-MS showed $[M-H]^+$ at *m*/*z* 221.1176 ($[C_{13}H_{18}O_3-H]^+$), corresponding to a molecular formula $C_{13}H_{18}O_3$. The

IR spectrum showed absorption bands for an OH group (3327 cm^{-1}) and a substituted benzene moiety (3040, 1618, and 1586 cm^{-1}). The band at 222 nm in the UV spectrum also indicated an aromatic character. The NMR data of **19** were similar to those of 8,9-dihydroxythymol (**8**) [10] except for the signals of C(8) and C(9) which were shifted downfield compared with those of **8**. Thus, compound **19** was deduced to be acetone thymol-8,9-diyl ketal, which is probably an artifact due to the chromatographic operations (see *Exper. Part*).

The signals of **19** at δ(H) 1.55 and 1.37 (*s*, each 3 H) in the ^{1}H-NMR spectrum (*Table 1*) and δ(C) 110.6 (*s*), 27.4 (*q*) and 25.4 (*q*) in the ^{13}C-NMR and DEPT spectra (*Table 2*) showed that compound **19** was an acetone ketal of compound **8** at its 8,9-position. This was further confirmed by the HMBC correlations Me(10) (δ 1.58)/C(4) (δ 125.2), C(8) (δ 84.6), and C(9) (δ 75.2), H–C(5) (δ 6.74)/C(8) (δ 84.6), and H–C(9) (δ 4.30 and 4.16)/C(4) (δ 125.2), C(1″) (δ 110.6), and C(10) (δ 28.8).

Compound **20** was obtained as colorless oil. Its HR-ESI-MS showed $[M+Na]^+$ at *m*/*z* 301.1416 ($[C_{16}H_{22}O_4+Na]^+$), corresponding to a molecular formula $C_{16}H_{22}O_4$. The IR, NMR, and UV spectra showed that **20** was also a thymol derivative. Its ^{1}H-NMR and ^{13}C-NMR data (*Tables 1* and *2*) were similar to those of compound **3**, except for the presence of a MeO group (δ(H) 3.13 (*s*, 3 H); δ(C) 50.9 (*q*)). The MeO group was determined to be at the 8-position by the HMBC correlation Me/C(8), thus establishing the structure of **20** as 8-methoxy-9-hydroxythymol 3-*O*-angelate. Compound **20** was optically inactive, which suggested that **20** may exist as a racemic mixture and may be an artifact formed during the extraction with MeOH (see *Exper. Part*) [26]. Some thymol derivatives isolated from *Eupatorium stoedhadosmum* showed no optical rotation, although they had stereogenic centers [27].

Compound **4** was obtained as a white powder. Its FAB-MS showed $[M+Na]^+$ at *m*/*z* 705.9 and $[M+Li]^+$ at *m*/*z* 689.9, and the HR-ESI-MS showed $[M+Na]^+$ at *m*/*z* 705.6161 ($[C_{46}H_{82}O_3+Na]^+$), corresponding to a molecular formula $C_{46}H_{82}O_3$. In the IR spectrum of **4**, absorptions for an OH group (3385 cm^{-1}), a C=C bond (3059, 1648 cm^{-1}), a carbonyl (1728 cm^{-1}), and CH_2 group (2917, 2849 cm^{-1}) were present. The NMR data of **4** suggested that it was a tetracyclic triterpenoid incorporating a fatty acid ester moiety. A comparison of the NMR data with those of closely related compounds [28][29] indicated that **4** possessed a lanostane-type configuration and that an OH group was present at C(20) of the triterpene skeleton [29]. The structure of **4** was established as (3β,20*R*)-20-hydroxylanost-25-en-3-yl palmitate.

The ^{1}H-NMR, ^{13}C-NMR, and DEPT spectra of **4** displayed signals of seven tertiary Me groups (δ(H) 1.73 (*s*, 3 H), 1.14 (*s*, 3 H), 0.94 (*s*, 3 H), 0.87 (*s*, 6 H), 0.84 (*s*, 6 H)), a tertiary OH group (δ(C) 75.1 (*s*)), a terminal C=C bond (δ(H) 4.95 and 4.83 (br. *s*, each 1 H), δ(C) 147.6 (*s*), 111.0 (*t*)), and a long-chain fatty acid ester (Me *t* at δ(H) 0.86, two-proton *t* at δ(H) 2.29 (α-methylene), strong absorption at δ(H) 1.25, and COO at δ(C) 173.7 (*s*)). A *dd* at δ(H) 4.47 (J=9.3 and 6.0 Hz, 1 H) and an OCH *d* at δ(C) 80.5 were characteristics of the axial H_α–C(3) of the triterpene moiety geminal to an ester function. This was further confirmed by the cross-peaks between H–C(3) (δ 4.47, *dd*, 1 H) and C(1′) (δ 173.7) and C(29) (δ 16.5) in the HMBC experiment. The correlations of the olefinic protons (δ 4.95 and 4.83, br. *s*, 1 H each) with C(25) (δ 147.6) and C(27) (δ 17.7), of Me(27) (δ 1.73, *s*, 3 H) with C(25) (δ 147.6) and C(26) (δ 111.0), and of Me(21) (δ 1.14, *s*, 3 H) with C(17) (δ 50.1), C(20) (δ 75.1), and C(22) (δ 36.6) confirmed that the exocyclic C=C bond was at C(25) and the OH group at C(20). The (20*R*) con-

figuration was also suggested by a comparison of the Me(21) signal of **4** (δ 1.14 (*s*, 3 H)) with that of (20*S*)- and (20*R*)-20-hydroxycholesterol (δ 1.28 (*s*, 3 H) and δ 1.13 (*s*, 3 H) [29–31] in their ^{1}H-NMR spectra.

The testing of *in vitro* antitumor activities for compounds **1**–**4**, **19**, and **20**, and of the AcOEt extract against SMMC-7721, HL-60, and LO_2 cells were carried out by the method of the cells stained with sulforhodamine B (SRB). As shown in *Table 3*, compounds **1**, **2**, **19**, and **20** showed cytotoxic activity against HL-60 cells.

Table 3. *Cytotoxocity of Compounds* **1**–**4**, **19**, *and* **20** *and of the AcOEt Extract.* IC_{50} in μg/ml.

	SMMC-7721	HL-60	LO_2
1	193.4	42.20	>200
2	>200	49.83	>200
3	>200	197.67	>200
4	>200	>200	>200
19	170.64	52.45	>200
20	137.59	48.16	167.04
AcOEt extract	86.1	69.5	
10-Hydroxycamptothecin[a])	0.0157	0.0084	0.0006

[a]) The 10-hydroxycamptothecine (HCPT) was purchased from *Hainan Weikang Pharmaceutical Co.*, *Ltd.*, Hainan, China.

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Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh; *Qingdao Marine Chemical Factory*). TLC: silica GF_{254} (10–40 μ; *Qingdao Marine Chemical Factory*). Optical rotations: *Perkin-Elmer 341* polarimeter. IR Spectra: *Nicolet NEXUS-670 FT-IR* spectrometer. UV Spectra: *Shimadzu UV-260* spectrometer. NMR Spectra: *Varian Mercury-300BB* (300 MHz) instrument with $SiMe_4$ as the internal standard; $CDCl_3$ as solvent. EI-MS: *VG ZAB-HS* instrument at 70 eV. FAB-MS: *ZAB-HS* instrument. HR-ESI-MS and HR-SI-MS: *Bruker APEX-II* instrument; glycerol as the matrix.

Plant Material. The air-dried whole plants of *E. fortunei* were purchased in a traditional Chinese medicine market in Lanzhou, China, in September 2002, and identified by Prof. *Chengyi Li* from the Gansu College of Traditional Chinese Medicine.

Extraction and Isolation. The dried and powdered whole plants of *Eupatorium fortunei* (5.7 kg) were extracted three times with MeOH (each for 7 days) at r.t. The combined extracts were evaporated. The residue (654.0 g) was suspended in H_2O and partitioned successively with petroleum ether, AcOEt, and BuOH. The AcOEt extract (175 g) was prefractionated by CC (silica gel) yielding *Fr. A* (petroleum ether/AcOEt 60:1 → 40:1), *B* (petroleum ether/AcOEt 30:1 → 10:1), *C* (petroleum ether/AcOEt 2:1), and *D* (petroleum ether/AcOEt 1:1 → 0:1). *Fr. A* contained paraffin wax and volatile oil and was not further separated. From *Fr. B*, coumarin (*ca.* 300 mg), β-sitosterol (*ca.* 200 mg), and stigmasterol (*ca.* 400 mg) were obtained. The residue of *Fr. B* (1.5 g) was subjected to CC (silica gel, petroleum ether/Me_2CO 20:1 and petroleum ether/AcOEt 20:1): **18** (1 mg). *Fr. C* (22 g) was subjected to CC (silica gel, $CHCl_3$/Me_2CO 80:1, 70:1, 60:1, 50:1, 10:1, 0:1): *Fr. C.1*–*C.3*. *Fr. C.1* (3.7 g) was purified by recrystallization: **17** (7 mg). The residue of *Fr. C.1* (500 mg) was separated by CC (silica gel, petroleum ether/Me_2CO 10:1, 7:1) and purified by prep. TLC (petroleum ether/Me_2CO 6:1 and petroleum ether/AcOEt 5:1): **10** (3 mg), **3** (3 mg), and **20** (5 mg). *Fr. C.2* (2.9 g) was separated by CC (silica gel,

$CHCl_3/Me_2CO$ 80:1, 60:1, 30:1, 10:1, 0:1): **11** (17 mg), **7** (27 mg), **4** (5 mg), **12** (8 mg), and **16** (4 mg). *Fr. C.3* (2.5 g) was separated by CC (silica gel, $CHCl_3$/MeOH 50:1, 30:1, 20:1, 10:1, 0:1 and petroleum ether/Me_2CO 30:1, 20:1, 10:1, 5:1, 2:1, 0:1): **5** (1 mg), **8** (4 mg), **6** (4 mg), **9** (5 mg), and **19** (10 mg). *Fr. D* (38 g) was separated by CC (silica gel, $CHCl_3$/MeOH 20:1 → 5:1): **1** (39 mg), **2** (3 mg), **15** (6 mg), and β-daucosterol (*ca.* 180 mg). The residue of *Fr. D* was purified by CC (silica gel, petroleum ether/AcOEt) and separated by prep. TLC ($CHCl_3$/AcOEt 4:1): **13** (9 mg) and **14** (11 mg).

Cytotoxicity Assays. SMMC-7721, HL-60, and LO_2 cells were cultured with 10% bovine serum at 37° and with 5% CO_2. The survival rates were determined with sulforhodamine B (SRB) method: Cells were cultured at 37° under a humidified atmosphere of 5% CO_2 in RPMI 1640 medium supplemented with 10% fetal calf serum and dispersed in replicate 96-well plates with $4 \cdot 10^3$ cells/well for 24 h. Compounds **1**–**4**, **19**, and **20** and the AcOEt extract or HCPT (=10-hydroxycamptothecin; used as a positive control) were then added. After 48-h exposure to the toxins, the cultures were fixed at 4° for 1 h by addition of ice-cold 50% CCl_3COOH. Fixed cells were rinsed 5 times with deionized H_2O and stained for 10 min with 0.4% sulforhodamine B dissolved in 0.1% AcOH. The wells were washed 5 times with 0.1% AcOH and left to dry overnight. Cell proliferation was assessed by the sulforhodamine B (SRB) cytotoxicity assay by measuring the absorbance at 515 nm with a microplate reader (*Bio-Rad*). Each test was performed in triplicate.

rel-*(1*R,*2*S,*3*R,*4*R,*6*S)-p-*Menthane-1,2,3,6-tetrol* (=rel*(1*R,*2*S,*3*R,*4*S,*5*R*)-2-Methyl-5-(1-methylethyl)-cyclohexane-1,2,3,4-tetrol*; **1**): Colorless oil. $[\alpha]_D^{17} = +4$ ($c = 0.15$, CH_2Cl_2). IR (KBr): 3422, 1705, 1464, 1370, 1248, 1088, 1061, 1038, 941. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS (70 eV): 204 (2, M^+), 186 (2, $[M - H_2O]^+$), 168 (5, $[M - 2\,H_2O]^+$), 161 (8), 153 (3, $[M - 2\,H_2O - Me]^+$), 143 (19), 125 (55, $[M - 2\,H_2O$ – isopropyl$]^+$), 123 (6), 113 (36), 107 (9, $[M - 3\,H_2O$ – isopropyl$]^+$), 99 (23), 74 (48), 71 (23), 55 (26), 43 (100), 41 (40). HR-SI-MS: 205.0536 ($[M+H]^+$, $C_{10}H_{21}O_4^+$; calc. 205.1434).

rel-*(1*R,*2*R,*3*R,*4*S,*6*S)-p-*Menthane-1,2,3,6-tetrol* (=rel-*(1*R,*2*S,*3*S,*4*S,*5*R*)-2-Methyl-5-(1-methylethyl)-cyclohexane-1,2,3,4-tetrol*; **2**): Colorless oil. $[\alpha]_D^{17} = -36$ ($c = 1.4$, CH_2Cl_2). IR (KBr): 3423, 1714, 1465, 1376, 1287, 1125, 1059, 977. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS (70eV): 204 (4, M^+), 189 (2, $[M - Me]^+$), 186 (0.8, $[M - H_2O]^+$), 168 (1), 161 (13, $[M$ – isopropyl$]^+$), 151 (4), 143 (4, $[M - H_2O$ – isopropyl$]^+$), 125 (42, $[M - 2\,H_2O$ – isopropyl$]^+$), 112 (77), 107 (10, $[M - 3\,H_2O$ – isopropyl$]^+$), 99 (14), 83 (15), 74 (39), 71 (35), 55 (22), 43 (100), 41 (38). HR-SI-MS: 205.0521 ($[M+H]^+$, $C_{10}H_{21}O_4^+$; calc. 205.1434).

9-Hydroxythymol 3-O-*Angelate* (=*(2Z)-2-Methylbut-2-enoic Acid 2-(2-Hydroxy-1-methylethyl)-5-methylphenyl Ester*; **3**): Colorless oil. $[\alpha]_D^{28} = -4$ ($c = 0.25$, MeOH). UV (MeOH): 221 (2.15). IR (KBr): 3417, 1733, 1647, 1505, 1460, 1378, 1226, 1131, 1038. ^{1}H- and ^{13}C-NMR: *Tables 1* and 2. EI-MS (70 eV): 248 (0.5, M^+), 221 (1), 217 (0.4, $[M - CH_2OH]^+$), 194 (0.6), 177 (0.7), 166 (0.7), 159 (1), 148 (68), 135 (21), 83 (100), 55 (57), 43. HR-ESI-MS: 271.1302 ($[M+Na]^+$, $C_{15}H_{20}NaO_3^+$; calc. 271.1305).

'Acetone Thymol 8,9-diyl Ketal' (=*5-Methyl-2-(2,2,4-trimethyl-1,3-dioxolan-4-yl)phenol*; **19**): White powder. M.p. 69–70°. $[\alpha]_D^{29} = -1$ ($c = 1.07$, MeOH). UV (MeOH): 222 (2.33), 275 (1.00), 280 (0.96). IR (KBr): 3327, 3040, 2992, 2938, 2894, 1618, 1586, 1043. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS (70 eV): 222 (11, M^+), 207 (6), 191 (13), 164 (32), 149 (100), 135 (77), 121 (61), 91 (35), 77 (39), 43 (83). HR-ESI-MS: 221.1176 ($[M-H]^+$, $C_{13}H_{17}O_3^+$; calc. 221.1183).

9-Hydroxy-8-methoxythymol 3-O-*Angelate* (=*(2Z)-2-Methylbut-2-enoic Acid 2-(2-Hydroxy-1-methoxy-1-methylethyl)-5-methylphenyl Ester*; **20**): Colorless oil. $[\alpha]_D^{29} = 0$ ($c = 1$, MeOH). UV (MeOH): 220 (1.80). IR (KBr): 3736, 3449, 2930, 2829, 1733, 1646, 1620, 1456, 1380, 1225, 1134, 1069, 1036. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS (70 eV): 278 (0.17, M^+), 247 (44), 165 (68), 83 (100), 55 (55). HR-ESI-MS: 301.1416 ($[M+Na]^+$, $C_{16}H_{22}NaO_4^+$; calc. 301.1410).

*(3β,20*R*)-20-Hydroxylanost-25-en-3-yl Palmitate* (=*Hexadecanoic Acid (3β,20*R*)-20-Hydroxylanost-25-en-3-yl Ester*; **4**): White powder. M.p. 99–100°. $[\alpha]_D^{28} = +12$ ($c = 0.77$, CH_2Cl_2). IR (KBr): 3385, 3300, 3059, 2917, 2849, 1728, 1648, 1459, 1420, 1371, 1309, 1242, 1172, 1094, 1030, 1005, 975, 887. ^{1}H-NMR (300 MHz, $CDCl_3$): 4.95 (br. *s*, H_a–C(26)); 4.83 (br. *s*, H_b–C(26)); 4.47 (*dd*, $J = 9.3, 6.0$, H–C(3)); 2.29 (*t*, $J = 7.5$, CH_2 (2′)); 1.73 (*s*, Me(27)); 1.58–1.65 (*m*, CH_2(3′)); 1.25 (br. *s*, CH_2(4′) to CH_2(15′)); 1.14 (*s*, Me(21)); 0.94 (*s*, Me(18)); 0.87 (*s*, Me(30)); 0.87 (*s*, Me(19)); 0.86 (*t*, $J = 7.5$, Me(16′)); 0.84 (*s*, Me(29)); 0.84 (*s*, Me(28)). ^{13}C-NMR (75 MHz,$CDCl_3$): 173.7 (C(1′)); 147.6 (C(25)); 111.0 (C(26)); 80.5 (C(3)); 75.1 (C(20)); 55.9 (C(5)); 50.5 (C(9)); 50.3 (C(14)); 50.1 (C(17)); 42.3 (C(8)); 40.3 (C(13));

38.6 (C(15)); 38.6 (C(24)); 37.9 (C(4)); 37.0 (C(10)); 36.6 (C(22)); 35.1 (C(12)); 34.9 (C(2′)); 31.9 (C(14′)); 31.1 (C(1)); 29.3–29.2 (C(4′) to C(13′)); 28.0 (C(28)); 27.4 (C(7)); 25.4 (C(21)); 25.2 (C(16)); 24.8 (C(3′)); 23.7 (C(2)); 22.7 (C(15′)); 22.7 (C(23)); 21.5 (C(11)); 18.1 (C(6)); 17.7 (C(27)); 16.5 (C(29)); 16.4 (C(19)); 16.3 (C(30)); 15.4 (C(18)); 14.1 (C(16′)). FAB-MS: 705.9 ($[M+Na]^+$), 689.9 ($[M+Li]^+$). HR-ESI-MS: 705.6161 ($[M+Na]^+$, $C_{46}H_{82}NaO_3^+$; calc. 705.6156).

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