New Taxane Diterpenoids from the Roots of *Taxus mairei*

Ya-Ching Shen,* Hung-Ru Tai, and Ching-Yeu Chen

Institute of Marine Resources, National Sun Yat-Sen University, 70 Lien-Hai Road, Kaohsiung, Taiwan, Republic of China

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Two new taxoids, taxumairols A (**1**) and B (**2**), have been isolated from extracts of the roots of Formosan *Taxus mairei* (Lemee & Levl.) S. Y. Hu. The structures of **1** and **2** were identified as 5α,7*β*,9α,10*β*,13α-pentaacetoxy-20-(benzoyloxy)-2α,4α-dihydroxytax-11-ene and 2α,5α,10*β*,-13α-tetraacetoxy-1*β*,7*β*,9α-trihydroxy-4*β*,20-epoxytax-11-ene, primarily on the basis of 1D- and 2D-NMR techniques including DEPT, COSY, and HMBC experiments, as well as chemical correlation with known compounds. Taxumairol A (**1**) exhibited significant cytotoxicities against murine P-388 and human KB-16, A-549, and HT-29 tumor cell lines.

The clinical application of paclitaxel (Taxol) for the treatment of ovarian and breast cancer has stimulated a great interest in the isolation of bioactive taxoids from various parts of *Taxus* species.[1−6] Paclitaxel has a novel diterpene skeleton and possesses a unique mode of action involving binding to polymerized tubulin, leading to inhibition of mitosis. Paclitaxel's poor water solubility and its limited source are two urgent problems that need to be addressed.[7] Paclitaxel is presently isolated from the bark of the Pacific yew *Taxus brevifolia*[8] or its European relative *Taxus baccata*.[9] Because removal of the bark destroys the trees, phytochemical investigations for new sources of paclitaxel have grown exponentially in the past few years. As a result, many taxoids were discovered, and many papers on the isolation and structural elucidation of taxoids have appeared. More than 100 taxoids were found in nature and hundreds of structurally related products were synthesized and characterized before March 1992.[10−12] An important review dealing with the phytochemistry of the yew tree collected another 100 new taxoids published between 1992 and 1994.[13]

Previous studies on diterpenoids in the bark and the heartwood of *Taxus mairei* (Lemee & Levl.) S. Y. Hu (*Taxaceace*) have resulted in the isolation of many new taxoids such as 1*β*-acetoxy-5α-deacetylbaccatin I and 1*β*-dehydroxy-4α-deacetylbaccatin IV from the bark [14,15] and 1*β*-dehydroxybaccatin IV, 1*β*-dehydroxybaccatin VI, and 5α-(cinnamoyloxy)-9α,10*β*,13α-triacetoxytaxa-4(20),-11-diene from the heartwood.[16,17] Recently, the two new taxoids 5α,7*β*,9α,10*β*,13α-pentaacetoxy-2α-(benzoyloxy)-4α,20-dihydroxytax-11-ene (**3**) and 7*β*,9α,10*β*,13α,20-pentaacetoxy-2α-(benzoyloxy)-4α,5α-dihydroxytax-11-ene (**4**), each with an opened oxetane ring, were isolated from the heartwood of this plant.[18] Very few studies, however, have been carried out on the constituents of the roots.[19−21] The roots of *T. mairei* have been used in Chinese folk medicine for the treatment of diabetes,[22] but no chemical investigations have been reported. As part of a program searching for practical and renewable sources of taxol and other potentially useful taxoids, we now report the isolation and structure elucidation of two novel taxoids from the roots of this species.

As described in the Experimental Section, an EtOH extract of the roots of *T. mairei* was added to an equal volume of MeOH and H_2O, and the suspension was stirred overnight. Extraction of the MeOH/H_2O solution with $CHCl_3$ gave a residue that was fractionated by extensive column chromatography on Sephadex LH-20 and Si gel and by preparative TLC to yield taxumairols A (**1**) and B (**2**).

Taxumairol A (**1**), [α] +46° (MeOH), had the composition $C_{37}H_{48}O_{14}$ as deduced by a combination of low resolution mass and ^{13}C-NMR spectroscopy. Its UV and IR bands indicated the presence of benzoyl (226 nm, 1705 cm^{-1}), hydroxyl (3488 cm^{-1}), and acetyl (1720 cm^{-1}) groups. This finding was also supported by fragment ions at *m*/*z* 594 $(M - C_6H_5COOH)^+$ and *m*/*z* 576 $(M - C_6H_5COOH - H_2O)^+$ in its EIMS spectrum. The presence of a taxene skeleton was inferred from the observation of characteristic resonances, such as four methyl singlets (*δ* 1.12, 1.16, 1.70, and 2.27) and corresponding carbon signals.[10] Detailed analysis of the ^{1}H- and ^{13}C-NMR spectra (Table 1) revealed that **1** is a 6/8/6 taxene with an opened oxetane ring.[18] The relationships between all the protons in **2** were established by a COSY spectrum; a correlation between H-2 and C2-OH (one-proton doublet at 3.80 ppm) was observed,[23] but the correlation with H-1 could not be assigned. A comparison with literature data indicated that taxumairol A (**1**) is an isomer of compounds **3** and **4**, isolated from *T. mairei* by Liang and Kingston.[18] These isomeric compounds had similar ^{1}H- and ^{13}C-NMR spectra; the major difference between **1** and **3** was in the chemical shifts of the C-2 and C-20 protons. In compound **1**, these occurred at *δ* 4.24 and 5.28/4.64 ppm, respectively, but were at *δ* 5.21 and 3.43/3.71 ppm in compound **3**. The chemical shifts of the C-20 protons in **1** were also different from those of compound **4**. The C-20 protons of the latter appeared at 4.02 and 4.23 ppm, respectively, because compound **4** had an acetoxyl group at C-20. These findings clearly indicate that taxumairol A (**1**) has a free hydroxy group at C-2 and a benzoyl group at C-20. Detailed comparison of the ^{13}C-NMR spectrum of **1** with that of **3** also suggested the location of the benzoyl moiety to be at C-20 in **1** rather than at C-2, as in **3**. Indeed, an HMBC study (Table 1) revealed that correlation of the signal due to the benzoyl carbonyl at *δ* 166.0 with those of the C-20 protons and the *ortho* aromatic protons unambiguously assigned the location of the benzoate group to C-20. Other correlations were also in agreement with the structure **1**.

The stereochemistry of **1** was assigned on the basis of NOE studies and comparison of the observed coupling

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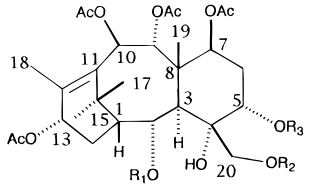
Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of Taxumairol A (**1**)

position	^{13}C	carbon type[a]	^{1}H[b]	COSY	HMBC
1	51.1	D	2.00 m		Me16, Me17, H3
2	71.4	D	4.24 (dd, 11.7, 3.9)	H1, H3	H3
3	45.6	D	2.78 (d, 3.9)	H2	H5, H20A
4	77.2	S			H3, H5, H20
5	71.9	D	5.26 (brs)	H6	H20
6	31.1	T	2.06 m, 1.87 m	H5	
7	68.7	D	5.58 (dd, 12, 4.8)	H6	H5, H9, Me19
8	45.8	S			H3, H9
9	75.3	D	5.82 (d, 11.4)	H10	H10, Me19
10	71.0	D	6.17 (d, 11.4)	H9	H9
11	134.2	S			H10, Me16 Me17, Me18
12	137.3	S			H10, H14β
13	70.8	D	5.85 m	H14, Me18	H14
14	29.7	T	2.60 m (β), 2.02 m		H13
15	37.9	S			H10, H14 Me16, Me17
16	26.9	Q	1.70 s		Me17
17	31.7	Q	1.12 s		Me16
18	15.0	Q	2.27 s	H13	
19	14.7	Q	1.16 s		H3, H7
20 A	66.7	T	5.28 (d, 11.8)	H20	H3
20 B			4.64 (d, 11.8)	H20	
5-OAc	172.0	S	2.31 s		H-5
7-OAc	169.6	S	2.11 s[c]		H-7
9-OAc	170.2	S	2.08 s[c]		H-9
10-OAc	169.1	S	2.05 s[c]		H-10
13-OAc	170.2	S	1.98 s[c]		
$OCOC_6H_5$	166.0	S			H20, *o*-C_6H_5
i	129.6	S			
o	129.7	D	8.06 (d, 7.5)	*m*-C_6H_5	*p*-C_6H_5, *m*-C_6H_5
m	128.6	D	7.47 (t, 7.5)	*o*,*p*-C_6H_5	
p	133.4	D	7.59 (t, 7.5)	*m*-C_6H_5	*o*-C_6H_5
2α-OH			3.80 (d, 11.7)	H2	

[a] S = C, D = CH, T = CH_2, Q = CH_3. Multiplicities and assignments were made by the DEPT and HMBC techniques. [b] Multiplicities and coupling constants in Hz are in parentheses. [c] Data may be interchanged.



1 R_1= H, R_2= COC_6H_5, R_3= Ac
3 R_1= COC_6H_5, R_2= H, R_3= Ac
4 R_1= COC_6H_5, R_2= Ac, R_3= H

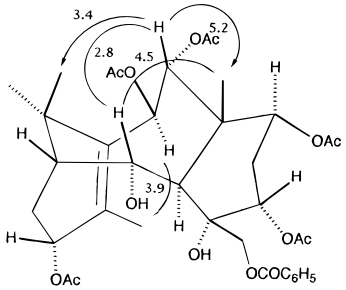


Figure 1. NOE studies and proposed conformation of taxumairol A (**1**).

constants with those of compound **3**. Irradiation of H-10 at δ 6.17 enhanced the intensity of Me-18 (3.9%) but did not increase the intensity of H-9, clearly determining the orientation of H-10 as α and H-9 as β. The close spatial relationship between Me-16, Me-19, H-2, and H-9 was observed by irradiation of H-9 at δ 5.82. The result (3.4%, 5.2%, and 2.8% increases, respectively) established that Me-16, Me-19, and H-2 all have a β-orientation. The coupling between H-2 and H-3 also agreed with α-configurations for the hydroxy group and for H-3. The coupling pattern of H-7 at δ 5.58 and the broad singlet for H-5 at δ 5.26, as well as coupling between H-9 and H-10 (J = 11.4 Hz) of **1**, were similar to those of compound **3**, indicating identical chirality among them. Thus, the structure of taxumairol A (**1**) was elucidated as 5α,7β,9α,10β,13α-pentaacetoxy-20-(benzoyloxy)-2α,4α-dihydroxytax-11-ene. Although we recognize that the conformation of ring B in **1** is flexible, a proposed model consistent with the results of NOE studies is given in Figure 1. The coupling constant for H-9/H-10 suggested that the conformation of ring B is in the twist-boat conformation with H-9β and H-10α pseudoaxial.[24]

Taxumairol B (**2**), [α] +15° (MeOH), had the composition $C_{28}H_{40}O_{12}$, as derived from EIMS, ^{13}C-NMR, and DEPT spectral data. Its UV and IR bands indicated the presence of hydroxyl (3488 cm^{-1}) and acetyl (215 nm, 1725 cm^{-1}) groups. This was also supported by the fragment ions at m/z 508 $(M - AcOH)^+$, m/z 490 $(M - AcOH - H_2O)^+$ and m/z 448 $(M - 2AcOH)^+$ in the mass spectrum. The presence of four acetates and three hydroxyls was verified by the observation of ^{1}H- and ^{13}C-NMR spectral data (Table 2). As in **1**, the characteristic resonances of four methyl singlets at δ 1.24 (17-Me), 1.39 (19-Me), 1.53 (16-Me), and 2.18 (18-Me) indicates that compound **2** is a taxene analogue. A pair of doublets at δ 2.33 (J = 5.4 Hz) and 3.48 (J = 5.4 Hz) accounted for the C-20 methylene protons of the oxetane ring.[10] The C-3 proton appeared as a doublet at δ 3.08 coupled with a doublet at δ 5.33, which was assigned

Table 2. ^{1}H- and ^{13}C-NMR Spectral Data of Taxumairol B (**2**)

position	^{13}C	carbon type[a]	^{1}H[b]	HMBC
1	76.0	S		H13, Me16
2	71.2	D	5.33 (d, 3.3)	H3
3	40.2	D	3.08 (d, 3.9)	
4	58.4	S		H3, H5, H20
5	78.2	D	4.21 (brs)	H20
6	33.1	T		
7	69.6	D	4.26 (dd, 11.7, 5.1)	H5
8	46.5	S		H2, H3, H9
9	78.3	D	4.58 (d, 10.3)	H10
10	74.0	D	6.05 (d, 10.3)	H9
11	136.2	S		H10, Me16 Me17, Me18
12	140.1	S		H10, H13, Me18
13	72.6	D	6.06 m	
14	38.6	T	2.52 m (β)	H2
15	43.4	S		
16	21.7	Q	1.53 s	H14β
17	15.5	Q	1.24 s	Me16
18	28.4	Q	2.18 s	
19	13.5	Q	1.39 s	H3, H7
20	49.9	T	3.48 (d, 5.4) 2.33 (d, 5.4)	
OAc	169.9	S	2.14 s	
	21.4	Q		
OAc	169.8	S	2.10 s	
	21.2	Q		
OAc	169.2	S	2.06 s	
	20.9	Q		
OAc	170.1	S	2.19 s	
	22.1	Q		

[a] S = C, D = CH, T = CH_2, Q = CH_3. Multiplicities and assignments were made by the DEPT and HMBC techniques. [b] Multiplicities and coupling constants in Hz are in parentheses.

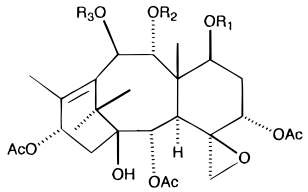
to H-2. The isolated spin system composed of two doublets at δ 4.58 and δ 6.05 was attributed to H-9 and H-10, respectively, with the large vicinal coupling (J = 10.3 Hz) indicative of a trans-oriented configuration. The ^{13}C-NMR spectrum of **2** also supported the assigned structure. Comparison of the ^{1}H- and ^{13}C-NMR spectra with those of 1β-hydroxy baccatin I (**5**),[25,26] a compound previously isolated from *T. mairei* by Yeh *et al.*[25] and from *T. wallichiana* by Miller *et al.*,[26] revealed that the only difference between them was that **2** contains three hydroxyl groups instead of one, as in **5**. The signals of H-5 and H-7 in **2** at δ 4.21 and 4.26 ppm seemed to imply that two hydroxyls were attached to C-5 and C-7. Upon acetylation, however, compound **2** yielded the product **5**, which showed the signal of H-7 at δ 5.24 and the unchanged signal of H-5 at δ 4.22 in its ^{1}H-NMR spectrum, suggesting that the first hydroxy group is at the C-7 position. The second hydroxy group must be located at either the C-1 position or the C-5 position, and the latter was assigned on the basis of ^{1}H-NMR data of 1β-acetoxy-5α-deacetylbaccatin I (**8**), a compound isolated from *T. mairei* by Liang et al.[14] The third hydroxyl group was tentatively assigned to either the C-9 or the C-10 position.

On the basis of this spectral evidence, there were four possible structures for taxumairol B. One of them was 1β-acetyl-5,7,10-deacetylbaccatin I (**9**), previously isolated from the leaves and stems of *Taxus canadensis*,[27] and the other three were structures **2**, **6**, and **7**. An HMBC experiment (Table 2) showed connectivities between H-10 (δ 6.05) and C-11 (δ 136.2) and between H-10 and C-12 (δ 140.1). This unambiguously established the third hydroxy at the C-9 position and excluded structures **6** and **9** from consideration. Structure **7**, with a free C-5 hydroxyl group, was excluded because acetylation did not take place at C-5, and this fact led to the assignment of structure **2.** This assignment was supported by a comparison of the chemical shift of H-5 in **2** with those in similar taxoids, which, in turn, supported the presence of an acetyl group at C-5 in **2**.[23,25,26] The unusual upfield shift of H-5 was caused by the anisotropic effect of the neighboring C-4(20) epoxide ring. The stereochemistry of **2** was completely determined by observation of its coupling constants and by chemical correlation with known compounds. Therefore, compound **2** was established as 2α,5α,10β,13α-tetraacetoxy-1β,7β,9α-trihydroxy-4β,20-epoxy-tax-11-ene. This structure was proposed as a revision of structure **9** by Zamir *et al.* in a brief note without any spectral or chemical evidence.[28] Because the ^{1}H-NMR spectrum of compound **5** was completely identical to that of the proposed 1β-acetoxy-5α-deacetylbaccatin I (**8**), the structure of **8** must be revised to that of 1β-hydroxybaccatin I (**5**).

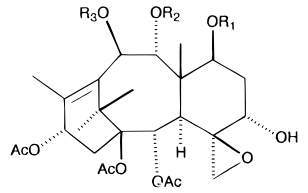
Table 3. Cytotoxicities of Taxumairols A (**1**), B (**2**), and **3** (IC_{50}, μg/mL)[a]

cell lines	1	2	3
P-388 (leukemia)	0.05	>50	2.4
KB-16 (nasopharynx)	0.4	1.4	1.0
A-549 (lung)	1.7	>50	4.8
HT-29 (colon)	1.0	>50	1.4

[a] The concentation of compound that inhibits 50% (IC_{50}) of the growth of human and murine tumor cell line, P-388 (murine lymphocytic leukemia), KB-16 (human nasopharyngeal carcinoma), A-549 (human lung adenocarcinoma), or HT-29 (human colon adenocarcinoma), after 72 h of drug exposure according to the method developed by Mosmann.[29]



2 $R_1 = R_2 = H$, $R_3 = Ac$
5 $R_1 = R_2 = R_3 = Ac$
6 $R_1 = H$, $R_2 = Ac$, $R_3 = H$



7 $R_1 = R_2 = H$, $R_3 = Ac$
8 $R_1 = R_2 = R_3 = Ac$
9 $R_1 = H$, $R_2 = Ac$, $R_3 = H$

The cytotoxicities of the new taxoids **1**, **2**, and **3** were evaluated in vitro against human and murine tumor cell lines. As shown in Table 3, compounds **1** and **3** exhibited significant cytotoxicities against human lung, colon, and nasopharyngeal cancers, and murine lymphocytic leukemia cells. It is noteworthy that **1** showed better activity than **3** for P-388 murine leukemia and human lung and nasopharyngeal carcinomas, even though compounds **1** and **3** share the common feature of an opened oxetane ring system.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR and UV spectra were measured Hitachi T-2001 and on Hitachi V-3210 spectrophotometers, respectively. EIMS and FABMS spectra were recorded on a VG Quattro 5022 mass spectrometer. The ^{1}H and ^{13}C-NMR and NOE spectra were recorded on a Varian FT-300 instrument, and HMBC spectra were recorded on a Bruker 300-AC spectrometer.

Plant Material. The roots of *Taxus mairei* (Lemee & Levl.) S. Y. Hu were purchased in Kaohsiung, Taiwan, in 1993. A voucher specimen is kept in Institute of Marine Resources, National Sun Yat-sen University.

Extraction And Isolation. The dried roots (4 kg) were ground and repeatedly extracted with EtOH (10 L). The combined extracts were concentrated to a brown tar, which was added with a mixture of MeOH (1 L) and H_2O (1 L) and stirred overnight. The MeOH/H_2O-soluble fraction was extracted exhaustively with $CHCl_3$. The lower layer ($CHCl_3$/MeOH) was concentrated under vacuum to give a residue (35 g) that was applied on a Sephadex LH-20 and eluted with MeOH to afford a taxoid-containing fraction (25 g). The taxoid fraction was chromatographed on a Si gel column (200 g) and eluted with a solvent mixture of $CHCl_3$/Me_2CO in increasing polarity to provide 12 fractions. Further separation of fraction 7 (0.7 g) by reversed-phase column chromatography (C-18, developed with gradient CH_3CN/H_2O solution) and preparative TLC (Si gel, *n*-hexane/$CHCl_3$/MeOH, 5:5:1) yielded taxumairol A (**1**, 10 mg) and compound **3** (30 mg). Fraction 8 (0.4 g) was chromatographed on a C-18 column (gradient CH_3CN/H_2O) and further purified by preparative TLC (Si gel, *n*-hexane/$CHCl_3$/MeOH, 4:4:1) to furnish taxumairol B (**2**) (11 mg). Compound **3** showed identical spectral data (^{1}H-, ^{13}C-NMR, EIMS, and [α]) to those reported for 5α,7*β*,9α,10*β*,13α-pentaacetoxy-2α-(benzoyloxy)-4α,20-dihydroxytax-11-ene.[18]

Taxumairol A (1): isolated as an amorphous powder; $[\alpha]^{25}_D$ +46° (*c* 0.1, MeOH); IR (neat) υ_{max} 3488, 1720, 1705, 1606, 1426, 1370 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 2.26 (4.1) nm; ^{1}H- and ^{13}C-NMR ($CDCl_3$) spectral data are listed in Table 1; EIMS *m*/*z* (rel int) 596 (0.3), 576 (0.1), 534 (0.2), 474 (0.9), 271 (1.0), 221 (5.3), 133 (14.3), 105 (45), 77 (12), 43 (100).

Taxumairol B (2): isolated as an amorphous powder; $[\alpha]^{25}_D$ +15 (*c* 0.6, MeOH); IR (neat) υ_{max} 3488, 1725, 1606, 1426, 1370 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 215 (4.0) nm; ^{1}H- and ^{13}C-NMR ($CDCl_3$) spectral data are listed in Table 2; EIMS *m*/*z* (rel int) 508 (0.2), 490 (0.4), 448 (0.7), 430 (0.5), 195 (26), 181 (15), 153 (16), 137 (16), 105 (13), 93 (11), 55 (16), 43 (100).

Acetylation of Taxumairol B (2). Acetylation (Ac_2O/Py; 1:1; room temperature) of **2** (5 mg) gave, after workup, a solid (3 mg) that showed identical spectral data (^{1}H-NMR, EIMS, and [α]) with those of 1*β*-hydroxybaccatin I (**5**).[25,26]

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References and Notes

(1) Tong, X. J.; Fang, W. S.; Zhou, J. Y.; He, C. H.; Chen, W. M.; Fang, Q. C. *J. Nat. Prod*. **1995**, *58*, 233−238.
(2) Velde, D. G. V.; Georg, G. I.; Gollapudi, S. R.; Jampani, H. B.; Liang, X. Z.; Mitscher, L. A.; Ye, Q. M. *J. Nat. Prod*. **1994**, *57*, 862−867.
(3) Yue, Q.; Fang, Q. C.; Liang, X. T.; He, C. H. *Phytochemistry* **1995**, *39*, 871−873.
(4) Kitagawa, I.; Mahmud, T.; Kobayashi, M.; Shibuta, H. *Chem. Pharm. Bull*. **1995**, *43*, 365−367.
(5) Tong, X. J.; Liu, Y. C.; Chang, C. J. *Nat. Prod. Lett*. **1995**, *6*, 197−202.
(6) Chattopadhyay, S. K.; Sharma, R. P.; Appendino, G.; Gariboldi, P. *Phytochemistry* **1995**, *39*, 869−870.
(7) Kingston, D. G. I.; Samaranayake, G.; Ivey, C. A. *J. Nat. Prod*. **1990**, *53*, 1−12.
(8) Kingston, D. G. I.; Hawkins, D. D.; Ovington, L. *J. Nat Prod*. **1982**, *45*, 466−470.
(9) Appendino, G.; Gariboldi, P.; Pisetta, A; Bombardelli, E..; Gabetta, B. *Phytochemistry* **1992**, *31*, 4253−4257.
(10) Kingston, D. G. I.; Molinero, A. A.; Rimoldi, J. M. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Tamm, C., Eds.; Springer Verlag: New York, 1993; Vol. 62, pp 1−188.
(11) Ojima, I.; Habus, I.; Zhao, M.; Zucco, M.; Park, Y. H.; Sun, C. M.; Brigaud, T. *Tetrahedron* **1992**, *48*, 6985−7012.
(12) Klein, L. L.; Maring, L.; Li, C. J.; Yeung, C. M.; Thomas, S. A.; Grampovnik, D. J.; Plattner, J. J. *J. Med. Chem*. **1995**, *38*, 1482−1492.
(13) Appendino, G. *Nat. Prod. Rep*. **1995**, *12*, 349−360.
(14) Liang, J. Y.; Min, Z. D.; Mizuno, M.; Tanaka, T.; Inuma, M. *Phytochemistry* **1988**, *27*, 3674−3675.
(15) Liang, J. Y.; Min, Z. D.; Niwa, M. *J. Acta Chim. Sin*. **1988**, *46*, 1053.
(16) Min, Z. D.; Jiang, H.; Liang, J. Y. *Acta Pharm. Sin*. **1989**, *24*, 673−677.
(17) Yeh, M. K.; Wang, J. S.; Liu, L. P.; Chen, F. C. *Phytochemistry* **1988**, *27*, 1534−1536.
(18) Liang, J. Y.; Kingston, D. G. I. *J. Nat. Prod*. **1993**, *56*, 594−599.
(19) Appendino, G.; Enriu, G. C. R.; Gariboldi, P.; Barboni, L.; Torregiani, E.; Gabetta, B.; Zini, G.; Bombardelli, E. *J. Nat. Prod*. **1994**, *57*, 607−613.
(20) Vidensek, N.; Lin, P.; Campbell, A.; Carlson, C. *J. Nat. Prod*. **1990**, *53*, 1609.
(21) Barboni, L.; Gariboldi, P.; Torregiani, E.; Appendino, G.; Gabetta, B.; Bombardelli, E. *Phytochemistry* **1994**, *36*, 987−990.
(22) Kan, W. S. *Pharmaceutical Botany*; National Research Institute of Chinese Medicine: Taipei, 1975; p 140.
(23) Zhang, Z. P.; Wiedenfeld, H.; Roder, E. *Phytochemistry* **1995**, *38*, 667−670.
(24) Yue, Q.; Fang, Q. C.; Ling, X. T.;He, C. H. *Phytochemistry* **1995**, *39*, 871−873.
(25) Yeh, M. K.; Wang, J. S.; Liu, L. P.; Chen, F. C. *J. Chin. Chem. Soc*. **1988**, *35*, 309−313.
(26) Miller, R. W.; Powell, R. G.; Smith, Jr., C. R. *J. Org. Chem*. **1981**, *46*, 1469−1474.
(27) Zamir, L. O.; Nedea, M. E.; Belair, S.; Sariol, F.; Mamer, O.; Jacqmain, E.; Jean, F. I.; Garneau, F. X. *Tetrahedron Lett*. **1992**, *33*, 5173−5176.
(28) Zamir, L. O.; Nedea, M. E.; Belair, S.; Sariol, F.; Mamer, O.; Jacqmain, E.; Jean, F. I.; Garneau, F. X. *Tetrahedron Lett*. **1992**, *33*, 6548.
(29) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55−63.

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