Taxane Diterpenoids from the Stem Bark of *Taxus mairei*

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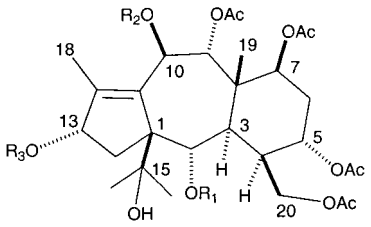
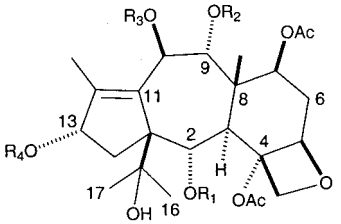
Three new 11(15→1)-*abeo*-taxanes, taxumairols U−W (**1**−**3**), have been isolated from extracts of the stem bark of Formosan *Taxus mairei*. The structures of **1**−**3** were identified as 5α,7*β*,9α,13α,20-pentaacetoxy-2α,10*β*,15-trihydroxy-11(15→1)-*abeo*-taxene, 5α,7*β*,9α,20-tetraacetoxy-2α,10*β*,13α,15-tetrahydroxy-11-(15→1)-*abeo*-taxene, and 2α,4α,7*β*,10*β*-tetraacetoxy-5*β*,20-epoxy-9α,13α,15-trihydroxy-11(15→1)-*abeo*-taxene, respectively, on the basis of 2D NMR techniques including COSY, HSQC, HMBC, and NOESY experiments as well as chemical reactions of compounds **1**−**3** to give **4** (5α,7*β*,9α,10*β*,13α,20-hexaacetoxy-2α,15-dihydroxy-11(15→1)-*abeo*-taxene) and **5** (4α,7*β*,10*β*-triacetoxy-9α,13α-dibenzoxy-5*β*,20-epoxy-2α,15-dihydroxy-11(15→1)-*abeo*-taxene), which are also novel taxane derivatives. Taxumairols U (**1**) and V (**2**) exhibited significant cytotoxicities against human hepatoma tumor cells, while taxumairol W (**3**) was inactive.

More than 100 taxoids have been isolated from the Taiwanese yew *Taxus mairei* (Lemee & Levl.) S. Y. Hu. Previous studies on diterpenoids of *T. mairei* have resulted in the isolation of 13-deacetylcanadensene and 7-deacetylcanadensene and novel taxachitrienes from the leaves,[1] taxumairols N and O from the roots,[2] and taxumairol R from the root bark.[3] Two new *abeo*-taxanes, taxumains A and B, both with an opened oxetane ring, were isolated from the twigs of this plant.[4] Recently, taxumairol M and taxumairone A were isolated from its seeds.[5,6] As part of searching for practical and renewable sources of Taxol and useful taxoids for SAR study,[7,8] we now report the isolation and structure elucidation of three novel taxoids (**1**−**3**) from the stem bark of *T. mairei*.

The EtOH extract of the stem bark of the Taiwanese yew *T. mairei* yielded compounds **1**−**3**. Taxumairol U (**1**) had a molecular formula of $C_{30}H_{44}O_{13}$ as deduced from high-resolution FABMS. Its IR bands indicated the presence of hydroxyl (3462 cm^{-1}) and acetyl (1734 cm^{-1}) groups. The ^1H NMR data of **1** showed five acetyl singlets (*δ* 2.04, 2.05, 2.06, 2.10, 2.13), four typical methyl singlets (*δ* 0.97, 1.12, 1.41, 1.85), a double doublet at *δ* 2.53 (H-3, J = 7.5, 4.5 Hz), a doublet at *δ* 5.05 (H-5, J = 2.5 Hz), and two pairs of coupled systems at *δ* 3.92, 4.37 (H-20), 4.63, and 5.57 (H-10, 9). Detailed analysis of the ^1H and ^{13}C NMR, COSY, and HSQC spectra revealed that **1** is a 5/7/6 taxene with an opened oxetane ring.[9] This 11(1→15)-*abeo*-taxane skeleton bearing a dimethyl carbinol group in C-1 was confirmed from the observation of adjacent quaternary sp^3 carbons at *δ* 69.0 (C-1) and 76.3 (C-15) and cross-peaks from Me-16 (*δ* 1.41) and Me-17 (*δ* 1.12) to C-1 and C-15 as well as correlations of geminal dimethyl (16/17) in the HMBC spectrum. The remaining two hydroxyl groups were determined at C-2 and C-10 by observation of HMBC correlation of H-2 (*δ* 4.64) to C-3 (*δ* 41.0), C-8 (*δ* 43.2), and C-15 (*δ* 76.3) and H-10 (*δ* 4.63) to C-9 (*δ* 79.8) and C-11 (*δ* 140.7). A comparison with literature data indicated that taxumairol U (**1**) is an isomer of taxayuntin J (**6**), isolated from *T. yunnanensis*. Upon acetylation, compound **1** yielded a novel compound **4**, which showed only an additional acetyl singlet at *δ* 1.94 in the ^1H NMR spectrum, while the C-2 hydroxyl group could not be acetylated. Also, the overlapping H-10 was shifted from 4.63 ppm in **1** to 6.24 ppm in **2**. Detailed assignments of protons and corresponding carbons were completed by COSY and HSQC experiments. The NOESY correlations of H-2/H-9, Me-16, Me-19, and H-9/Me-19 in **1** suggested that H-2, H-9, Me-19, and the dimethyl carbinol group were in *β*-orientation. Correlations between H-3/H-7 and H-10/Me-18 agreed with the α-configuration of H-3, H-7, and H-10. A coupling constant between H-9 and H-10 of 9.0 Hz indicated a trans-relationship.



1 R_1= R_2= H, R_3= Ac
2 R_1= R_2= R_3 = H
4 R_1= H, R_2= R_3 = Ac
6 R_1= H, R_2= Ac, R_3 = H



3 R_1= Ac, R_2= R_4= H, R_3= Ac
5 R_1= H, R_2= R_4 = COPh, R_3= Ac
7 R_1= R_2= R_3= Ac, R_4= H
8 R_1= R_2= Ac, R_3= R_4= H

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Table 1. Cytotoxicity of Taxoids **1**−**5** against Human Tumor Cells (IC_{50}, μg/mL)[a]

	KB	Hepa
taxumairol U (**1**)	10.3	0.3
taxumairol V (**2**)	3.9	1.6
taxumairol W (**3**)	>20	7.0
4	>20	2.7
5	>20	>20
paclitaxel	<0.1	<0.1

[a] The concentration of compound that inhibits 50% (IC_{50}) of the growth of human tumor cell line, KB (oral epidermoid carcinoma) and Hepa (human hepatoma), after 72 h exposure according to the method described in the Experimental Section.

Taxumairol V (**2**) had the composition $C_{28}H_{42}O_{12}$ as derived from negative HRFABMS. Its IR bands indicated the presence of hydroxyl (3424 cm^{-1}) and acetyl (1732 cm^{-1}) groups. Analysis of ^1H and ^{13}C NMR spectra of **2** revealed that it was an analogue of **1**. Characteristic peaks included two methylene protons (CH_2OAc) at δ 4.43 and 3.92, and C-1 and C-15 at δ 68.8 and 76.5, respectively. The assignment was further confirmed by COSY, HSQC, and HMBC experiments. Comparison of ^1H NMR data with those of **1** revealed that the only difference between them was that **2** contains four hydroxyl groups instead of three. The signal of H-13 in **2** at δ 4.60 indicated that the additional hydroxyl was attached to C-13. The NOESY correlations of **2** were similar to those of compound **1**. Observation of cross-peaks H-7/H-10 and Me-19/CH_2-20 confirmed H-4 to be in the α-configuration. Upon acetylation, **2** yielded a diacetate identical with compound **4**. As in **1**, acetylation could not take place at the C-2 hydroxyl group due to steric hindrance in the 5/7/6 taxane ring.

Taxumairol W (**3**) had the composition $C_{28}H_{40}O_{12}$, as determined by high-resolution negative FABMS. Its IR bands indicated the presence of hydroxyl (3425 cm^{-1}) and acetyl (1732 cm^{-1}) groups. The presence of hydroxyls, acetoxyls, and a taxane skeleton was verified from the ^1H and ^{13}C NMR data of **3**. The overlapped H-13 and H-20A (δ 4.47) signals were noticed by their correlations with H-14 (δ 1.48 and 2.12) and H-20B (δ 4.38), respectively, in the COSY spectrum. The signals of δ 5.88 (H-2), 5.35 (H-7), and 5.70 (H-10) suggested that they were connected with acetoxyl groups, while signals of δ 4.53 (H-9) and 4.47 (H-13) had hydroxyl groups attached. This structural assignment was similar to the reported data of taxayuntin H (**7**), which has one more acetoxyl group at C-9 than **3**.[10] Detailed analysis of the ^1H and ^{13}C NMR spectra revealed that compound **3** seems to be identical to 10,13-deacetylabeobaccatin IV (**8**), isolated from *T. wallichiana* because they had similar ^1H and ^{13}C NMR spectra in the same solvent (DMSO-d_6).[11] However, long-rage correlations of H-9/C-19, H-7/C-19, and H-3/C-19 in the HMBC spectrum clearly indicated that C-9 was hydroxylated. The NOESY correlations of H-9/Me-19/H-2/H-20β, H-2/Me-16, and H-3/H-7 not only unambiguously assigned the acetoxy at C-10 and the hydroxy at C-9 but also established the relative stereochemistry of **3**. Upon benzoylation, compound **3** yielded dibenzoate **5**. It was not predicted in this reaction that the acetyl group at C-2 was missing and led to the upfield shift of H-2 from δ 5.88 in **3** to δ 4.86 in **5**. Because the ^1H and ^{13}C NMR data of compound **3** were completely identical with those of 10,13-deacetylabeobaccatin IV (**8**), the structure of **8** should be revised to taxumairol W (**3**) accordingly.

The cytotoxicities of the new taxoids **1**−**5** were evaluated in vitro against human tumor cell lines. As shown in Table 1, compounds **1**, **2**, and **4** exhibited significant and selective cytotoxicity against human hepatoma cells with IC_{50}'s at 0.3, 1.6, and 2.7 μg/mL, respectively.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-1000 polarimeter. IR and UV spectra were recorded with a HORIBA FT-720 and a HITACHI U-3210 spectrophotometer, respectively. EIMS, FABMS, and HRFABMS were measured with a VG Quattro 5022 and JEOL JMS-SX 102 mass spectrometers. ^1H and ^{13}C NMR, COSY, HSQC, HMBC, and NOESY spectra were recorded using a Bruker FT-300 (AVANCE) or a Varian FT-500 (ANOVA) NMR instrument.

Plant Material. The stem bark of *Taxus mairei* was collected in Tai-chung county in October 1997. A voucher specimen (TPG8-4) was deposited in the Institute of Marine Resources, National Sun Yat-sen University, Kaohsiung, Taiwan.

Extraction and Isolation. Dried stem bark (2.5 kg) was ground and extracted with EtOH to afford a crude extract (65 g), which was partitioned between H_2O (1.5 L) and $CHCl_3$ (1.5 L) to yield a $CHCl_3$-soluble fraction (34.5 g). This fraction was defatted with *n*-hexane (800 mL) and 25% aqueous MeOH (800 mL) to yield a 25% aqueous MeOH-soluble residue (29 g). Part of the residue (20 g) was applied on a silica gel column (500 g) and eluted with a solvent mixture of *n*-hexane/$CHCl_3$/MeOH (5:5:1 and 3:3:1) to afford 13 fractions, A (15 mg), B (1.03 g), C (40 mg), D (1.0 g), E (1.0 g), F (0.97 g), G (0.73 g), H (0.81 g), I (1.6 g), J (1.02 g), K (1.78 g), L (0.76 g), and M (6.02 g). Fraction E was chromatographed on a silica gel (20 g) column and eluted with $CHCl_3$/MeOH (49:1) to give a residue (84 mg), which was applied on a preparative TLC plate (RP-C_{18}) developed with MeOH/H_2O (4:1) to yield taxumairol U (**1**, 9.5 mg). Fraction F was chromatographed on a silica gel (25 g) column eluted with $CHCl_3$/MeOH (24:1) to give a residue (34 mg), which was applied on a preparative TLC plate (RP-C_{18}) and developed with MeOH/H_2O (85:15) to yield taxumairol W (**3**, 12 mg). Fraction H was chromatographed on a silica gel (15 g) column eluted with $CHCl_3$/MeOH (94:6) to give a residue (22 mg), which was applied on a preparative TLC plate (RP-C_{18}) developed with MeOH/H_2O (75:25) to yield taxumairol V (**2**, 3.5 mg).

Taxumairol U (1): amorphous solid; $[\alpha]^{25}_D$ −18° (*c* 0.05, CH_2Cl_2); IR (neat) ν_{max} 3462, 2920, 1734, 1437, 1373, 1244, 1206 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.64 (1H, overlap, H-2), 2.53 (1H, dd, J = 7.5, 4.5 Hz, H-3), 2.23 (1H, m, H-4), 5.05 (1H, d, J = 2.5 Hz, H-5), 1.87 (2 H, m, H-6), 5.23 (1H, dd, J = 10, 5.5 Hz, H-7), 5.57 (1H, d, J = 9.0 Hz, H-9), 4.63 (1H, d, J = 9.0 Hz, H-10), 5.60 (1H, t, overlap, H-13), 1.66 (1H, dd, J = 8, 14.7 Hz, H-14α), 2.20 (1H, m, H-14β), 1.41 (3H, s, H-16), 1.12 (3H, s, H-17), 1.85 (3H, s, H-18), 0.97 (3H, s, H-19), 4.37 (1H, d, J = 10.3 Hz, H-20a), 3.92 (1H, dd, J = 10.3, 10 Hz, H-20b), 2.04, 2.06 × 2, 2.10, 2.13 (s, $OCOCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 69.0 (s, C-1), 65.0 (d, C-2), 41.0 (d, C-3), 41.2 (s, C-4), 70.4 (d, C-5), 29.7 (t, C-6), 69.4 (d, C-7), 43.2 (s, C-8), 79.8 (d, C-9), 66.5 (d, C-10), 140.7 (s, C-11), 141.5 (s, C-12), 79.8 (d, C-13), 36.9 (t, C-14), 76.3 (s, C-15), 26.8 (q, C-16), 27.7 (q, C-17), 11.0 (q, C-18), 14.3 (q, C-19), 63.9 (t, C-20), 169.6, 170.0, 170.7, 171.5, 172.1 (s, $OCOCH_3$), 20.9, 21.0, 21.3, 21.5 × 2 (q, $OCOCH_3$); HMBC (300 MHz, $CDCl_3$) [C-1, H-3, H-14, H-16, H-17], [C-2, H-3, H-14], [C-3, H-2], [C-4, H-3, H-20], [C-5, H-20], [C-6, H-7], [C-7, H-9, H-19], [C-8, H-3, H-6, H-7], [C-9, H-10, H-19], [C-10, H-9], [C-11, H-10, H-13, H-14, H-18], [C-13, H-14, H-18], [C-15, H-16, H-17], [C-16, H-17], [C-17, H-16], [C-19, H-3, H-7], [C-20, H-3], [$COCH_3$, H-5, H-7, H-9, H-13, H-20]; NOESY (500 MHz, $CDCl_3$): [H-2, H-19], [H-2, H-9], [H-9, H-19], [H-3, H-7], [H-5β, H-6β], [H-10, H-18], [H-13, H-18]; FABMS *m/z* 635 $[M + Na]^+$; EIMS *m/z* (rel int) 595 ($[M - OH]^+$, 0.1), 535 ($[M - AcOH - OH]^+$, 1), 475 ($[M - 2AcOH - OH]^+$,1.5), 434 (5), 416 (8), 374 (12), 356 (11), 314 (9), 296 (8), 254 (11), 236 (25), 221 (15), 149 (39), 121 (38), 105 (51), 91 (31), 79 (24), 59 (77); negative HRFABMS *m/z* 611.2715 ([M − H], calcd for $C_{30}H_{43}O_{13}$, 611.2704).

Acetylation of Taxumairol U (1). Taxumairol U (3 mg) was acetylated with acetic anhydride and pyridine (1:1, each 0.5 mL) and after usual workup furnished a taxumairol U

monoacetate (**4**, 3 mg): $[\alpha]^{25}_D$ −32.4° (*c* 0.05, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.66 (1H, d, J = 8.1 Hz, H-2), 2.50 (1H, m, H-3), 2.25 (1H, m, H-4), 5.06 (1H, d, J = 2.0 Hz, H-5), 1.90 (2 H, m, H-6), 5.34 (1H, t, J = 7.4 Hz, H-7), 5.66 (1H, d, J = 10.5 Hz, H-9), 6.24 (1H, d, J = 10.5 Hz, H-10), 5.59 (1H, t, J = 6.9 Hz, H-13), 1.75, 2.40 (2H, m, H-14), 1.35 (3H, s, H-16), 1.30 (3H, s, H-17), 1.88 (3H, s, H-18), 1.02 (3H, s, H-19), 4.41 (1H, d, J = 10.4 Hz, H-20a), 4.04 (1H, dd, J = 10.4, 9.9 Hz, H-20b), 2.17, 2.08, 2.07, 1.98, 1.94 (s, $OCOCH_3$); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 69.2 (s, C-1), 65.7 (d, C-2), 40.6 (d, C-3), 41.1 (s, C-4), 70.3 (d, C-5), 29.7 (t, C-6), 69.2 (d, C-7), 43.5 (s, C-8), 76.3 (d, C-9), 68.4 (d, C-10), 136.7 (s, C-11), 146.4 (s, C-12), 79.0 (d, C-13), 37.1 (t, C-14), 76.7 (s, C-15), 27.0 (q, C-16), 27.5 (q, C-17), 11.8 (q, C-18), 14.1 (q, C-19), 63.8 (t, C-20), 167.9, 169.5, 169.6, 169.9, 170.6, 171.6 (s, $OCOCH_3$), 21.4, 21.2, 21.1 × 2, 21.0, 20.8 (q, $OCOCH_3$); FABMS *m/z* 677 $[M + Na]^+$; EIMS *m/z* (rel int) 653 ($[M - H]^+$, 0.2), 635 (0.1), 593 ($[M - AcOH]^+$, 0.7), 577 (2), 532 ($[M - 2AcOH]^+$, 1), 517 (2), 474 ($[M - 3AcOH]^+$, 2), 416 (5), 356 (20), 314 (5), 296 (14), 281 (9), 236 (20), 221 (17), 149 (35), 105 (55), 95 (31), 81 (30), 69 (35), 59 (59).

Taxumairol V (2): amorphous powder; $[\alpha]^{25}_D$ −13 (*c* 0.05, CH_2Cl_2); IR (neat) ν_{max} 3424, 2933, 1732, 1435, 1371, 1246, 1030 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.60 (1H, overlap, H-2), 2.57 (1H, dd, J = 8.5 Hz, H-3), 2.26 (1H, m, H-4), 5.05 (1H, d, J = 2.5 Hz, H-5), 1.86 (2 H, m, H-6), 5.25 (1H, dd, J = 10.5, 6.0 Hz, H-7), 5.51 (1H, d, J = 10.5 Hz, H-9), 4.60 (2H, overlap, H-10, H-13), 1.63(1H, m, H-14a), 2.11 (1H, m, H-14b), 1.45 (3H, s, H-16), 1.08 (3H, s, H-17), 1.93 (3H, s, H-18), 0.99 (3H, s, H-19), 4.43 (1H, d, J = 11 Hz, H-20a), 3.92 (1H, dd, J = 11, 10 Hz, H-20b), 2.07, 2.08, 2.09, 2.10 (s, $OCOCH_3$); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 68.8 (s, C-1), 65.3 (d, C-2), 40.9 (d, C-3), 41.2 (s, C-4), 69.4 (d, C-5), 29.7 (t, C-6), 68.8 (d, C-7), 43.2 (s, C-8), 79.5 (d, C-9), 66.9 (d, C-10), 138.4 (s, C-11), 145.3 (s, C-12), 76.5 (d, C-13), 39.6 (t, C-14), 76.5 (s, C-15), 27.3 (q, C-16), 27.7 (q, C-17), 11.0 (q, C-18), 14.2 (q, C-19), 63.9 (t, C-20), 169.9, 170.2, 171.7, 171.8 (s, $OCOCH_3$), 20.9, 21.1, 21.3, 21.5 (q, $OCOCH_3$); FABMS *m/z* 593 $[M + Na]^+$; EIMS *m/z* (rel int) 552 (1), 537 (1), 523 (1.2), 509 (1), 449 (0.3), 433 (4), 389 (1), 368 (3), 314 (3), 285 (2.4), 236 (9), 149 (15), 105 (31), 91 (18), 69 (25), 57 (37); negative HRFABMS *m/z* 569.2604 ([M − H], calcd for $C_{28}H_{41}O_{12}$, 569.2598).

Acetylation of Taxumairol V (2). Acetylation (Ac_2O/Py; 1:1; rt) of **2** (1 mg) gave after workup a solid (1.1 mg), which showed identical spectral data (^{1}H NMR, EIMS, and [α]) with those of taxumairol U monoacetate (**4**).

Taxumairol W (3): amorphous solid; $[\alpha]^{25}_D$ −54° (*c* 0.05, CH_2Cl_2); IR (neat) ν_{max} 3425, 1732, 1705, 1606, 1426, 1370 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.88 (1H, d, J = 6.5 Hz, H-2), 3.06 (1H, d, J = 7.5 Hz, H-3), 4.91 (1H, d, J = 7.5 Hz, H-5), 2.60 (1H, m, H-6α), 1.81 (1 H, m, H-6β), 5.35 (1H, m, H-7), 4.53 (1H, d, J = 9.0 Hz, H-9), 5.70 (1H, d, J = 9.0 Hz, H-10), 4.47 (1H, overlap, H-13), 1.48 (1H, m, H-14a), 2.12 (1H, m, H-14b), 1.18 (3H, s, H-16), 1.01 (3H, s, H-17), 1.91 (3H, s, H-18), 1.62 (3H, s, H-19), 4.47 (1H, overlap, H-20a), 4.38 (1H, d, J = 7.0 Hz, H-20b), 1.96, 2.08 × 2, 2.14 (s, $OCOCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 66.8 (s, C-1), 68.3 (d, C-2), 43.9 (d, C-3), 79.8 (s, C-4), 85.1 (d, C-5), 34.7 (t, C-6), 70.3 (d, C-7), 43.3 (s, C-8), 66.4 (d, C-9), 79.5 (d, C-10), 138.0 (s, C-11), 146.1 (s, C-12), 77.6 (d, C-13), 39.3 (t, C-14), 76.2 (s, C-15), 25.5 (q, C-16), 27.5 (q, C-17), 11.1 (q, C-18), 12.8 (q, C-19), 74.9 (t, C-20), 170.1, 170.6, 171.3 × 2 (s, $OCOCH_3$), 21.2, 21.5 × 2, 22.3, (q, $OCOCH_3$); HMBC (300 MHz, $CDCl_3$) [C-1, H-3, H-14, H-16, H-17], [C-2, H-3], [C-3, H-2], [C-4, H-3, H-20], [C-5, H-3, H-20], [C-7, H-6, H-19], [C-8, H-3, H-6], [C-9, H-10, H-19], [C-10, H-9], [C-11, H-14, H-18], [C-12, H-14, H-18], [C-13, H-14, H-18], [C-14, H-2], [C-15, H-2], [C-16, H-17], [C-17, H-16], [C-19, H-3, H-7]; NOESY (500 MHz, $CDCl_3$) [H-2, H-19], [H-2, H-9], [H-9, H-19], [H-3, H-7], [H-5, H-6α], [H-7, H-6α], [H-10, H-18], [H-13, H-18]; FABMS *m/z* 591 $[M + Na]^+$; EIMS *m/z* (rel int) 569 ($[M + H]^+$, 0.6), 551 ($[M - OH]^+$, 1), 533 (2), 509 ($[M - Ac]^+$, 0.5), 491 (2), 473 (2), 447 (1), 433 (4), 390 (38), 373 (20), 330 (14), 313 (18), 297 (58), 270 (23), 241 (29), 241 (29), 223 (33), 105 (29), 93 (25), 59 (55), 43 (100); negative HRFABMS *m/z* 567.2440 ([M − H], calcd for $C_{28}H_{39}O_{12}$, 567.2442).

Benzoylation of Taxumairol W (3). A solution of taxumairol W (**3**, 7 mg) in dry pyridine (1 mL) was treated with benzoyl chloride (1 mL) and stirred for 16 h at room temperature. After workup the residue was purified by a silica gel column using $CHCl_3$ as eluent to give **5** (3.2 mg) as a white powder: $[\alpha]^{25}_D$ −12.6 (*c* 0.05, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.86 (1H, d, J = 6.7 Hz, H-2), 2.75 (1H, d, J = 6.9 Hz, H-3), 5.14 (1H, brs, H-5), 1.90 (2H, m, H-6), 5.58 (1H, dd, J = 9.6, 6.9 Hz, H-7), 6.55 (1H, d, J = 10.5 Hz, H-9), 6.07 (1H, d, J = 10.5 Hz, H-10), 5.80 (1H, m, H-13), 2.10 (1H, m, H-14a), 2.50 (1H, m, H-14b), 1.47 (3H, s, H-16), 1.37 (3H, s, H-17), 1.70 (3H, s, H-18), 1.32 (3H, s, H-19), 4.62 (1H, d, J = 12 Hz, H-20a), 4.71 (1H, d, J = 12 Hz, H-20b), 2.15, 2.02, 2.04 (s, $OCOCH_3$), 8.04 (2H, d, J = 7.2 Hz, OBz), 7.97 (2H, d, J = 7.5 Hz, OBz), 7.59 (2H, t, J = 7.5 Hz, OBz), 7.45 (4H, t, J = 7.5 Hz, OBz); EIMS *m/z* (rel int) 638 ($[M - AcOH - 2H_2O]^+$, 0.2), 596 ($[M - 2AcOH]^+$, 0.2), 551 (0.3), 537 (0.4), 523 (0.4), 495 (0.4), 474 (0.8), 432 (1), 414 (1), 357 (5), 339 (2), 269 (3), 221 (6), 122 (58), 105 (100), 81 (11), 77 (75), 43 (81).

Cytotoxicity Assay. Bioassay against KB (oral epidermoid carcinoma) and Hepa (hepatoma) tumor cells was based on reported procedures.[12] The cells for assay were cultured in RPMI-1640 medium supplemented with a 5% CO_2 incubator at 37° C. The cytotoxicity assay depends on the binding of methylene blue to fixed monolayers of cells at pH 8.5, washing the monolayer, and releasing the dye by lowering the pH value. In summary, samples and control standard drugs were prepared at a concentration of 1, 10, 40, and 100 μg/mL. After seeding 2880 cells/well in a 96-well microplate for 3 h, 20 μL of sample or standard agent was placed in each well and incubated at 37° C for 3 days. After removing the medium from the microplates, the cells were fixed with 10% formal saline for 30 min, then dyed with 1% (w/v) methylene blue in 0.01 M borate-buffer (100 μL/well) for 30 min. The 96-well plate was dipped into a 0.01 M borate-buffer solution four times in order to remove the dye. Then, 100 μL/well ethanol/0.1 M HCl (1:1) was added as a dye eluting solvent, and the absorbance was measured on a microtiter plate reader (Dynatech, MR 7000) at a wavelength of 650 nm. The IC_{50} value was defined by a comparison with the untreated cells as the concentration of test sample resulting in 50% reduction of absorbance. Mytomycin C and actinomycin D were used as standard compounds, which both exhibited an IC_{50} value of 0.01 μg/mL under the above conditions.

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