New Taxane Diterpenoids from the Leaves and Twigs of *Taxus sumatrana*

Ya-Ching Shen,*,† Shih-Sheng Wang,† Yu-Ling Pan,† Kuang-Liang Lo,† Rupak Chakraborty,† Ching-Te Chien,‡ Yao-Haur Kuo,§ and Yu-Chi Lin†

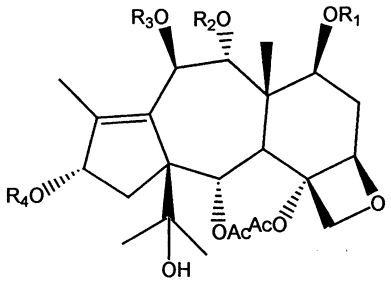
Institute of Marine Resources, National Sun Yat-sen University, 70 Lien Hai Road, Kaohsiung, Taiwan 80424, Republic of China, Taiwan Forestry Research Institute, Taiwan, Republic of China, and National Research Institute of Chinese Medicine, Taipei, Taiwan, Republic of China

Received May 15, 2002

Two new taxoids, taxumairol Q (**1**) and 13-*O*-acetyl wallifoliol (**2**), have been isolated from the leaves and twigs of *Taxus sumatrana*. Taxuspine F and wallifoliol (**10**) have been isolated for the first time from the yew *T. sumatrana*. Seventeen known taxoid diterpenoids have also been isolated. The new derivatives, 9,13-diacetyltaxumairol W (**3**), 10,13-dibenzoyltaxacustin (**4**), 7,13-diacetylwallifoliol (**5**), 7,13-dibenzoylwallifoliol (**6**), and 7,9-dibenzoyltaxumairol P (**7**), have been prepared by acylation of a crude mixture of taxoids. All structures were established primarily on the basis of 1D and 2D NMR techniques, including DEPT, COSY, and HMBC experiments, as well as chemical correlation with known compounds. Wallifoliol (**10**) exhibited significant cytotoxicities against both Hepa 59 T/VGH (human liver carcinoma) and KB (human oral epidermoid carcinoma) tumor cells. Taxuspine F and compound **5** possessed moderate activity against Hepa cells only, while **3**, **6**, **7**, and 10-deacetylbaccatin III showed only marginal activity against Hepa cells.

Reports on the phytochemistry, semisynthesis, and biosynthesis of paclitaxel and related taxoids have proliferated, and several review articles have been published.[1−4] Recently a number of new taxoids have been isolated from different *Taxus* species,[5,6] and some of them have activity as modulators of multidrug-resistant tumor cells.[7] Although more than 400 taxane diterpenes have been isolated to date, there are still new taxoids awaiting isolation and structural characterization.

Taxus sumatrana (Miq.) de Laub. (Taxaceae) is a rare plant growing at a high altitude (2600 m) in central parts of Taiwan.[8] In our continuing search for taxoid diterpenoids with novel skeletal constitution and bioactivity[9−15] we have isolated two new taxoids along with some taxoids that have been isolated for the first time from male *Taxus sumatrana* trees. We have also prepared some new taxoid derivatives.

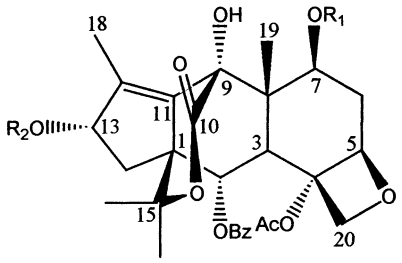


1 $R_1 = R_2 = R_3 = R_4 = H$
3 $R_1 = R_2 = R_3 = R_4 = Ac$
4 $R_1 = R_2 = Ac$, $R_3 = R_4 = COPh$
8 $R_1 = R_3 = Ac$, $R_2 = R_4 = H$
9 $R_1 = COPh$, $R_2 = R_3 = R_4 = H$

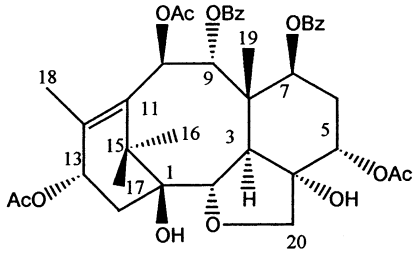
Results and Discussion

The acetone and ethyl acetate extracts of *Taxus sumatrana* were fractionated by repeated column chromatography and preparative TLC or HPLC to furnish taxumairol Q (**1**) and 13-*O*-acetylwallifoliol (**2**). Five new taxoid derivatives (**3**−**7**) have been prepared via acetylation and benzoylation, namely, 9,13-diacetyltaxumairol W (**3**), 10,13-dibenzoyltaxacustin (**4**), 7,13-diacetylwallifoliol (**5**), 7,13-dibenzoylwallifoliol (**6**), and 7,9-dibenzoyltaxumairol P (**7**).

Taxumairol Q (**1**) had a molecular formula $C_{24}H_{36}O_{10}$, as deduced from EIMS *m*/*z* 466 $[M - H_2O]^+$, ^1H, ^{13}C NMR, and DEPT spectral data. The IR spectrum indicated the presence of OH (3500 cm^{-1}) and $OCOCH_3$ (1743 cm^{-1}) groups. The ^{13}C NMR (Table 1) and DEPT spectra showed the presence of eight quaternary, seven methine, three methylene, and six methyl carbons. The ^1H NMR spectrum of **1** showed four methyl and two acetate singlets at δ 1.15, 1.05, 1.91, and 1.80 and δ 2.14 and 2.00. The two protons at C-20 appeared as an AB doublet of doublets at δ 4.36 (d, J = 7.5 Hz) and 4.48 (d, J = 7.5 Hz). The 5α-H gave a



2 $R_1 = H$, $R_2 = Ac$
5 $R_1 = R_2 = Ac$
6 $R_1 = R_2 = COPh$
10 $R_1 = R_2 = H$



7

* To whom correspondence should be addressed. Tel: 886-7-5252000, ext. 5058. Fax: 886-7-5255020. E-mail: ycshen@mail.nsysu.edu.tw.
† National Sun Yat-sen University.
‡ Taiwan Forestry Research Institute.
§ National Research Institute of Chinese Medicine.

10.1021/np0202273 CCC: $22.00
Published on Web 10/26/2002

Table 1. ^{13}C NMR Data ($CDCl_3$, 75 MHz) of Compounds **1**−**7**[a]

carbon	**1**[b]	**2**	**3**	**4**	**5**	**6**	**7**[b]
1	67.1 s	60.6 s	67.8 s	68.0 s	60.3 s	60.8 s	79.2 s
2	68.8 d	68.2 d	68.2 d	68.4 d	68.1 d	68.1 d	82.4 d
3	44.2 d	43.8 d	43.6 d	44.6 d	42.9 d	43.0 d	47.7 d
4	80.4 s	80.2 s	79.4 s	79.5 s	81.1 s	81.7 s	81.5 s
5	85.2 d	84.2 d	84.8 d	84.8 d	83.1 d	83.1 d	69.3 d
6	37.4 t	37.5 t	36.8 t	34.9 t	35.8 t	35.9 t	31.0 t
7	72.4 d	71.2 d	70.6 d	70.7 d	71.4 d	71.9 d	70.8 d
8	42.5 s	48.5 s	44.7 s	43.8 s	48.0 s	48.5 s	45.7 s
9	68.8 d	85.1 s	78.7 d	79.8 d	84.3 s	84.5 s	77.5 d
10	80.6 d	174.4 s	78.7 d	68.7 d	173.7 s	173.7 s	72.3 d
11	137.1 s	133.7 s	136.0 s	136.2 s	133.7 s	136.0 s	135.2 s
12	147.1 s	136.6 s	147.0 s	148.1 s	137.1 s	149.9 s	137.8 s
13	77.6 d	81.1 d	70.6 d	75.7 t	79.6 d	79.6 d	70.5 d
14	39.7 t	34.1 t	34.8 t	37.7 t	34.2 t	34.4 t	37.3 t
15	76.5 s	90.1 s	74.6 s	74.9 s	89.7 s	89.6 s	41.6 s
16	25.0 q	24.9 q	12.5 q	25.5 q	24.9 q	25.0 q	27.4 q
17	27.8 q	22.5 q	11.8 q	27.8 q	22.5 q	22.5 q	22.3 q
18	11.3 q	11.2 q	27.6 q	12.1 q	11.7 q	11.9 q	14.9 q
19	12.0 q	10.2 q	25.1 q	12.6 q	11.1 q	11.3 q	15.7 q
20	74.8 t	74.1 t	75.3 t	73.9 t	74.3 t	74.4 t	74.4 t
$OCOC_6H_5$		164.8 s		170.0 s	165.0 s	165.6 s	165.4 s
				170.3 s		166.7 s	167.3 s
						169.1 s	
i		129.7 s		129.8 s	129.7 s	133.2 s	132.6 s
				130.1 s		133.5 s	132.7 s
						133.7 s	
o		129.5 d		129.6 d	129.4 d	129.5 d	129.6 d
				129.7 d		129.6 d	130.2 d
						129.9 d	
m		128.7 d		128.7 d	128.7 d	128.6 d	127.8 d
				128.9 d		128.7 d	128.0 d
						128.9 d	
p		133.4 d		133.5 d	137.1 d	130.0 d	128.8 d
				133.6 d		130.3 d	129.3 d
						130.6 d	
OAc	170.7 s	169.2 s	168.8 s	168.7 s	168.9 s	171.0 s	169.5 s
	21.7 q	21.2 q	20.7 q	20.7 q	21.1 q	21.1 q	20.6 q
OAc	171.2 s	171.0 s	169.1 s	169.8 s	169.7 s		169.6 s
	22.4 q	21.2 q	20.8 q	21.5 q	21.1 q		21.5 q
OAc			169.7 s	169.9 s	171.1 s		169.9 s
			21.1 q	21.8 q	21.5 q		21.6 q
OAc			169.8 s	170.4 s			
			21.4 q	25.4 q			
OAc			170.3 s				
			21.7 q				
OAc			170.7 s				
			22.0 q				

[a] Assignments were made using HMQC and HMBC techniques. [b] 125 MHz.

signal at δ 4.91 (d, J = 8.5 Hz). Three signals at δ 80.4 (C-4), 85.2 (C-5), and 74.8 (C-20) were assigned to the three carbons of the oxetane ring. The protons at C-2 and C-3 coupled with each other to give signals at δ 5.79 (d, J = 8.0 Hz) and 2.91 (d, J = 8.0 Hz), respectively. In the HMBC spectrum, δ 5.79 (H-2) had a strong correlation with C-1 (δ 67.1), C-4 (δ 80.4), and C-15 (δ 76.5). The chemical shift of the C-15 indicated that it is oxygenated, which thus requires an 11(15→1)-*abeo*-taxane skeleton. The two ^{13}C signals at δ 137.1 (s, C-11) and 147.1 (s, C-12) of the olefinic bond matched with the same signals of 2α,4α-diacetoxy-9α,10β,13α,15-tetrahydroxy-5β(20)-epoxy-7β-benzoyloxy-11(15→1)-*abeo*-tax-11-ene (**9**).[17] In the ^1H NMR spectrum of **9**, the H-7 signal was downfield (δ 5.52) compared to δ 4.18 in compound **1**, due to the 7-benzoyloxy group. All these spectral data confirm the structure of the new taxoid taxumairol Q (**1**).

13-*O*-Acetylwallifoliol (**2**), $[\alpha]_D^{25}$ −24° (CH_2Cl_2), had the molecular formula $C_{33}H_{36}O_{11}$ as derived from quasi-molecular ions at *m*/*z* 585 $[M + H]^+$ and *m*/*z* 607 $[M + Na]^+$ in its FAB mass spectrum. Its UV indicated the presence of a benzoyloxy group in the molecule. Its IR spectrum showed the presence of OH (3300 cm^{-1}) and OCOPh (1714 cm^{-1}) groups. The four methyl signals and two acetyl signals were at δ 1.25, 1.34, 1.69, 2.10, and δ 1.74, 2.01, respectively, in its ^1H NMR spectrum. In addition, four oxygenated methine protons appeared at δ 5.85 (H-2), 4.86 (H-5), 4.35 (H-7), and 5.55 (H-13). The ^{13}C NMR (Table 1) and DEPT spectra of **2** showed the presence of three methylene carbons and five methine carbons. However, signals for H-9 and H-10 were missing. The COSY spectrum indicated the correlation of H-2/H-3 and H-13/H-14 and therefore assigned the acetoxyl group at C-13 and the benzoyloxyl group at C-2. This finding was supported by long-range correlation of H-2 with the benzoyl carbonyl (*O*COPh, δ 164.8) in the HMBC spectrum of **2**. All these data along with ^{13}C signals at δ 85.1 (C-8), 174.4 (C-9), and 90.1 (C-15) suggested its structural similarity to wallifoliol (**10**).[22] Furthermore, the HMBC spectrum of **2** showed the following correlations of H-2/C-1, 3, 8, 15; H-3/C-7, 8; H-5/C-4; H-6/C-4, 8; H-7/C-9; H-14/C-1, 13; H-16 and 17/C-1, 15; H-18/C-11, 12, 13; H-19/C-3, 7, 8, 9; and H-20/C-3, 5, confirming the structure of **2**.

Compound **3** was obtained from acetylation of a partially purified fraction 13, and its structure was assigned by comparison of its NMR data with those of **1** and **8**. In the

^{1}H NMR spectrum of **3**, apart from four methyl and six acetate signals at δ 1.12, 1.15, 1.65, 1.82 and δ 1.95, 2.00, 2.02, 2.08, 2.10, 2.11, the two protons of the C-20 methylene group gave an AB doublet of doublets at δ 4.47 (d, J = 7.4 Hz) and 4.38 (d, J = 7.4 Hz). The oxymethine protons shifted downfield from δ 4.18 (H-7), 4.23 (H-9), 4.50 (H-13), and 4.51 (H-10) in **1** to δ 5.49, 5.61, 6.06, and 6.26 in **3**, respectively, suggesting that the hydroxyl groups were at C-7, C-9, C-10, and C-13. In the HMBC spectrum of **3**, H-2 (δ 6.00) exhibited correlations with C-1 (δ 67.8), C-4 (δ 79.4), and C-15 (δ 74.6). Moreover, both the C-9 and C-13 positions in **3** carry acetyloxy groups, as evidenced by the downfield shift of H-9 (δ 6.06) and H-13 (δ 5.61) as compared to the corresponding protons of **8** (δ 4.53 and 4.47, respectively).[15] Compound **4** was obtained from benzoylation of fraction 13, and its structure was confirmed by comparison of its spectral data with those of **3**. The ^{1}H and ^{13}C NMR signals for **4** and **3** were very similar, including the oxymethylene protons (δ 4.36, 4.50, H-20) and the oxymethine protons (δ 4.97, H-5; δ 5.30, H-7; δ 5.64, H-2; δ 5.66, H-9) except those signals in the aromaic region. Compound **4** had 10 more aromatic protons and two fewer acetyl singlets in its ^{1}H NMR spectrum than **3**. In addition, the protons at δ 6.64 (H-10) and 6.20 (H-13) were shifted downfield compared to δ 6.27 (H-10) and 5.61 (H-13) in **3**.

Compound **5** was obtained from acetylation of fraction 13. It had molecular formula $C_{33}H_{38}O_{12}$ as derived from EIMS data. The ^{1}H NMR spectrum of **5** exhibited four methyl singlets (δ 1.27, 1.32, 1.79, 2.00) and three acetyl singlets (δ 1.68, 2.08, 2.16) and other characteristic signals similar to those of **2**. The ^{13}C NMR spectrum of **5** exhibited characteristic carbon signals at δ 60.8 (C-1), 173.7 (C-10), 133.7 (C-11), 137.1 (C-12), and 89.7 (C-15), which were similar to those of **2**. The only differences between them were chemical shifts of C-5 (δ 83.1, −1.1 ppm), C-6 (δ 35.8, −1.8 ppm), and C-9 (δ 84.3, −0.8 ppm), suggesting that the acetoxyl group is at C-7 in **5**. Furthermore, the H-7 proton appeared at δ 5.30 in **5** relative to that in **2** at δ 4.35, confirming the structure of **5**.

Compound **6** was obtained from benzoylation of fraction 13. It contained 15 aromatic proton signals in the region δ 7.46−8.19. The spectral data of **6** were compared with that of wallifoliol (**10**).[18] The ^{13}C NMR (Table 1) of **6** exhibited characteristic tertiary carbons at δ 60.8 (C-1), 89.6 (C-15), 84.5 (C-9), and 173.7 (C-10), similar to those of **10**. H-7 and H-13 in **6** gave signals that were downfield at δ 5.65 (dd, J = 5.4, 5.3 Hz) and 5.83 (t, J = 6.1 Hz) compared to δ 4.35 (t, J = 8.1 Hz) and 4.58 (t, J = 6.6 Hz), respectively, in **10**, due to the presence of two extra benzoyl groups in **6**.

Compound **7** was also obtained by benzoylation of fraction 13. It had molecular formula $C_{40}H_{46}O_{13}$ as derived from FABMS, ^{1}H, ^{13}C NMR, and DEPT spectral data. Its IR spectrum showed the presence of OH (3540 cm^{-1}), $OCOCH_3$ (1734 cm^{-1}), and OCOPh (1650 cm^{-1}) groups. Its ^{13}C NMR (Table 1) and DEPT spectra showed the presence of 13 quaternary, 17 methine, three methylene, and seven methyl carbons in the molecule. In its ^{1}H NMR spectrum the four methyl and three acetate singlets appeared at δ 1.16, 1.65, 1.65, 2.26 and 1.57, 2.12, 2.26, respectively. The compound had 10 aromatic proton signals in the region δ 6.91−7.78, signifying the presence of two benzoyl groups. The protons at δ 2.44 (H-6)/5.06 (H-5)/5.81 (H-7), δ 2.59 (H-14)/5.87 (H-13), δ 2.73 (H-3)/4.46 (H-2), and δ 6.34 (H-9)/6.43 (H-10) had COSY correlations with each other. Compound **7** thus possesses a 6−8−6 ring skeleton along with a tetrahydrofuran moiety. This type of taxoid, such as taxuspine K, has been found in *Taxus cuspidata* but is rare.[19] In its HMBC spectrum δ 3.58 (H-20) had a correlation with δ 82.4 (C-2), which indicated that C-2, C-4, and C-20 formed part of a tetrahydrofuranyl system. The protons at δ 6.43 (H-10), 5.06 (H-5), and 5.87 (H-13) correlated with δ 169.9, 169.6, and 169.5 (all OAc carbons) in the HMBC of **7**, indicating the presence of acetate groups on C-10, C-5, and C-13. The protons at δ 6.34 (H-9) and 5.81 (H-7) gave HMBC correlations with δ 167.3 and 165.4 (O*C*OPh), which indicated the presence of benzoyloxy groups at C-7 and C-9. Finally, in a NOESY spectrum (Figure 1) the protons H-2/H-9, H-2/17-Me, H-3/H-20α, H-5/H-20β, H-3/H-7, H-10/H-7, and H-13/17-Me showed correlations with each other, which indicated that H-2, -9, -13, and 17-Me have β orientations, while H-3, -7, and -10 have α orientations. These data confirmed the structure of the new derivative **7**.

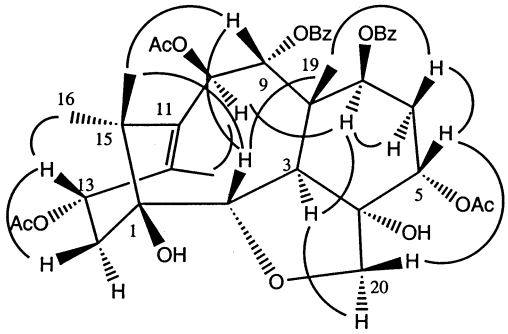


Figure 1. Key NOESY correlations for **7**.

Table 2. Cytotoxicity of Taxoids against Human Tumor Cells (IC_{50}, μg/ML)[a]

	KB	Hepa 59T/VGH
1	16.25	14.52
2	2.91	13.92
3	4.92	6.02
4	9.69	>20
5	8.96	3.09
6	15.43	4.83
7	12.80	7.02
taxuspine F	16.90	3.31
wallifoliol (**10**)	0.56	0.10
paclitaxel	0.001	0.001

[a] The concentration of compound that inhibits 50% (IC_{50}) of the growth of human tumor cell line, KB (oral epidermoid carcinoma) and Hepa 59 T/VGH (liver carcinoma), after 72 h exposure according to the method described in the Eperimental Section.

An additional 17 taxoids were also isolated. They are taxuspine F,[20] taxinine M,[21] Taxol,[2] wallifoliol (**10**),[17] taxumairol C,[10] taxumairol V,[15] 19-hydroxybaccatin III,[22] 10-deacetylbaccatin III,[22] 10-deacetyltaxol C,[20] taxin B,[23] taxinine B,[24] taxumairol B,[9] taxumairol U,[15] cephalomannine,[25] baccatin III,[25] taxumairol W (**8**),[15] and 10-deacetyl-10-oxo-7-epitaxol.[26] Structures of known compounds were confirmed by comparison of spectral data with literature reports.

As shown in Table 2, the cytotoxicities of the taxoids (**1**−**7**) and taxuspine F (**10**) were evaluated in vitro against human liver carcinoma (Hepa 59T/VGH) and oral epidermoid carcinoma (KB) cells. Among them, compound **2** was weakly active against KB cells and compound **5** and taxuspine F were weakly active against Hepa tumor cells. The only compound with significant cytotoxicity was wallifoliol (**10**), which was much less cytotoxic than standard paclitaxel.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-1000 polarimeter. IR and

UV spectra were recorded with HORIBA FT-720 and HITACHI U-3210 spectrophotometers, respectively. EIMS, FABMS, and HRFABMS were measured with VG Quattro 5022 and JEOL JMS-SX 102 spectrometers. ^{1}H and ^{13}C NMR, COSY, HSQC, HMBC, and NOESY spectra were recorded using a Bruker FT-300 (AVANCE) or a Varian FT-500 (INOVA) NMR instrument. HPLC was performed using Hitachi L-6250, intelligent pump Hitachi L-4000H, Hitachi integrator D-7500, Lichrosorb Si-60 (7 μm, 250 nm × 10 mm), and Lichrosorb RP-18 (7 μm, 250 mm × 10 mm) columns. All chemicals were procured from E. Merck (Germany) and were used without further purification.

Plant Material. The leaves and twigs with male flowers of *Taxus sumatrana* (Miq.) de Laub. were collected from Nantou County (central part of Taiwan) at an altitude of 2600 m in March 2001. This material was identified by one of the authors (C.-T.C.). A voucher specimen was deposited in the Institute of Marine Resources, National Sun Yat-sen University.

Extraction and Isolation. Dried leaves and twigs (7.8 kg) of *T. sumatrana* were extracted with *n*-hexane (40 L) to give a crude extract (123 g). The marc was then successively extracted with 40 L of EtOAc, 40 L of acetone, and 20 L of MeOH, to give EtOAc (300 g), acetone (350 g), and MeOH (250 g) extract. The acetone extract (350 g) was first chromatographed over LH-20 (MeOH) to give Tax-A (130 g). Further column chromatography of Tax-A over silica gel using *n*-hexane−CH_2Cl_2−MeOH (100:100:1−2:2:1) as eluent gave 17 fractions, 1−17. Fraction 11 (2.1 g) was chromatographed over a silica gel column using *n*-hexane−CH_2Cl_2−MeOH (100:100:1−50:50:1) to afford 11A (157 mg) and 11B (466.7 mg). Fraction 11A (60 mg) was subjected to PTLC on a reversed-phase plate using MeOH−H_2O (7:3) to give taxuspine F (7 mg). Fraction 11B (60 mg) on preparative TLC (PTLC) over a reversed-phase plate using MeOH−CH_3CN−H_2O (6:1:3) furnished taxinine M (18.3 mg). Fraction 12 (3.8 g) was column chromatographed over a LH-20 column using MeOH to give 12A (2.5 g), which on further column chromatography over LH-20 using CH_2Cl_2−MeOH (1:2) as eluent afforded $12A_1$ (1.5 g). Part of $12A_1$ (1.44 g) was chromatographed on a RP-C18 column (MeOH−CH_3CN−H_2O, 30:5:65) to furnish wallifoliol (81 mg), taxumairol C (12 mg), and Taxol (7 mg). Fraction 13 (530 mg) on a RP column (MeOH−H_2O, 2:8−1:1) gave 13A (175 mg). Fraction 13A (60 mg) on RP-PTLC using MeOH−H_2O (57:43) afforded $13A_1$ (6 mg), which on further PTLC on normal-phase silica gel using *n*-hexane−CH_2Cl_2−MeOH (43:43:10) afforded taxumairol Q (**1**, 1.5 mg). Fraction 14 (3 g) was chromatographed on a LH-20 column using MeOH, to give taxumairol V (5 mg) and 14A (230 mg). Fraction 14A (230 mg) on RP-C18 column chromatography using MeOH−H_2O (3:7) afforded 19-hydroxybaccatin III (1.5 mg), taxumairol Q (**1**, 1.5 mg), 10-deacetylbaccatin III (3 mg), and 10-deacetyltaxol C (2 mg). Fraction 15 (7.67 g) gave 15A (2 g) on a LH-20 column (MeOH); this fraction (60 mg) on RP-PTLC using MeOH−H_2O (1:1) afforded 10-deacetylbaccatin III (19 mg).

The EtOAc extract (300 g) was chromatographed over a silica gel column using *n*-hexane−CH_2Cl_2−MeOH (100:100:1−1:1:1) to give F-1 (34 g) and F-2 (60 g). Fraction F-1 (34 g) was separated on a silica gel column using hexane−acetone (100:1−1:1) to give F-1a (1.7 g), and this fraction on further LH-20 column chromatography (CH_2Cl_2−MeOH, 1:3) followed by RP-PTLC (MeOH−H_2O, 7:3) gave taxin B (4 mg) and taxinine B (1.0 mg). Fraction F-2 (60 g) on silica gel CC using *n*-hexane−CH_2Cl_2−MeOH (50:50:1−1:1:1) gave F-2a (2 g), F-2b (1.01 g), F-2c (3.5 g), and F-2d (500 mg). Fraction F-2a on an LH-20 column (CH_2Cl_2−MeOH, 1:3) followed by reversed-phase PTLC (RP-C18) using MeOH−CH_3CN−H_2O (60:10:30) gave 13-*O*-acetylwallifoliol (**2**, 11 mg). Fraction F-2b (1.01 g) on an LH-20 column (MeOH) followed by PTLC using *n*-hexane−CH_2Cl_2−MeOH (5:5:1) as eluent gave taxinine M (20 mg). Fraction F-2c (3.5 g) on an LH-20 column (MeOH) gave $F\text{-}2c_1$ (701 mg) and $F\text{-}2c_2$ (422 mg). Fraction $F\text{-}2c_1$ (60 mg) on reversed-phase PTLC (MeOH−H_2O, 40:60) gave taxumairol B (4 mg), taxumairol U (1.5 mg), and cephalomannine (3 mg). F-2d (500 mg) on a LH-20 column (MeOH) and further on reversed-phase PTLC (MeOH−H_2O, 7:3) gave taxumairol W (**8**, 3 mg) and 10-deacetyl-10-oxotaxol (2 mg).

Taxumairol Q (1): white amorphous solid; R_f 0.23 (*n*-hexane−CH_2Cl_2−MeOH, 5:5:1); $[\alpha]_D^{25}$ −23.5° (*c* 0.075, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} cm^{-1} 3500, 3430, 3850, 1743, 1243; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.05 (3H, s, H-17), 1.15 (3H, s, H-16), 1.53 (1H, m, H-14), 1.80 (3H, s, H-19), 1.85 (1H, m, H-6), 1.91 (3H, s, H-18), 2.00, 2.14 (6H, s, 2 × OAc), 2.10 (1H, m, H-14), 2.58 (1H, m, H-6), 2.91 (1H, d, J = 7.5 Hz, H-3), 4.18 (1H, t, J = 8.5 Hz, H-7), 4.23 (1H, m, H-9), 4.36 (1H, d, J = 7.5 Hz, H-20), 4.48 (1H, d, J = 7.5 Hz, H-20), 4.50 (1H, m, H-13), 4.51 (1H, d, J = 9.0 Hz, H-10), 4.91 (1H, d, J = 8.5 Hz, H-5), 5.79 (1H, d, J = 8.0 Hz, H-2); ^{13}C NMR (in Table 1); HMBC (300 MHz, $CDCl_3$) [C-1, H-2, H-14, H-16, H-17], [C-2, H-3, 2-OAc], [C-3, H-2, H-6], [C-4, H-2, H-3, H-5, H-20], [C-7, H-6], [C-8, H-2], [C-9, H-3, H-10], [C-11, H-18], [C-13, H-14], [C-15, H-2, H-16, H-17]; EIMS *m/z* 466 $[M - H_2O]^+$ (0.1), 447 (0.5), 410 (1), 367 (2), 306 (2.5), 288 (5.3); FABMS *m/z* 485 $[M + H]^+$, 507 $[M + Na]^+$.

13-*O*-Acetylwallifoliol (2): yellow solid; R_f 0.56 (*n*-hexane−acetone, 1:1); $[\alpha]_D^{25}$ −24.0° (*c* 0.3, CH_2Cl_2); UV (MeOH) λ_{max} nm (log ϵ) 231 (4.15), 274 (3.56); IR (neat) ν_{max} cm^{-1} 1452, 1508, 1602, 1674, 1714, 1739, 3200, 3300; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.25 (3H, s, H-17), 1.34 (3H, s, H-16), 1.69 (3H, s, H-19), 1.74 (3H, s, OAc), 1.85 (1H, m, H-6), 2.01 (3H, s, OAc), 2.10 (3H, s, H-18), 2.11 (1H, m, H-14), 2.29 (1H, m, H-14), 2.73 (1H, d, J = 12.0 Hz, H-3), 2.80 (1H, m, H-6), 4.22 (1H, d, J = 8.6 Hz, H-20), 4.35 (1H, t, J = 8.0 Hz, H-7), 4.63 (1H, d, J = 8.6 Hz, H-20), 4.86 (1H, d, J = 7.9 Hz, H-5), 5.55 (1H, t, J = 6.4 Hz, H-13), 5.85 (1H, d, J = 12.0 Hz, H-2), 7.48 (2H, t, J = 7.3 Hz, OCOPh, *m*-H), 7.62 (1H, t, J = 7.3 Hz, OCOPh, *p*-H), 7.92 (2H, d, J = 7.3 Hz, OCOPh, *o*-H); ^{13}C NMR (in Table 1); HMBC (300 MHz, $CDCl_3$) [C-1, H-2, H-14, H-16, H-17], [C-3, H-2, H-6, H-20], [C-4, H-5, H-6], [C-5, H-4], [C-7, H-3, H-19], [C-8, H-2, H-3, H-6, H-19], [C-9, H-7, H-19], [C-11, H-18], [C-12, H-18], [C-13, H-14, H-18], [C-15, H-2, H-16, H-17]; EIMS *m/z* 343 (0.1), 333 (0.1), 298 (0.2), 283 (0.2), 253 (0.2), 225 (0.4), 211 (0.4), 185 (0.5), 165 (0.7), 147 (1.2), 105 (23.4), 77 (13.4); FABMS *m/z* 585 $[M + H]^+$, 607 $[M + Na]^+$.

Preparation of 3 and 5. To a solution of fraction 13 (600 mg) in anhydrous pyridine (5 mL) was added acetic anhydride (2 mL), and the mixture was stirred for 16 h at room temperature. The reaction mixture was poured into ice H_2O and extracted with ethyl acetate, the organic layer washed with aqueous $NaHCO_3$ and water and dried over anhydrous $MgSO_4$, and the solvent removed under vacuum. The crude product was purified by column chromatography over silica gel using *n*-hexane−CH_2Cl_2−MeOH (100:100:1) as eluent to give F-13B (284 mg) and F-13C (175 mg). F-13B (120 mg) on HPLC using MeOH−CH_3CN−H_2O (65:5:30) gave 7,13-diacetylwallifoliol (**5**, 43 mg). F-13C (70 mg) on HPLC (MeOH−CH_3CN−H_2O, 65:5:30) gave $F\text{-}13C_1$ (38.5 mg), which on further PTLC using *n*-hexane−CH_2Cl_2−MeOH (5:5:1) gave 9,13-diacetyltaxumairol W (**3**, 9 mg).

9,13-Diacetyltaxumairol W (3): white amorphous solid; R_f 0.46 (*n*-hexane−CH_2Cl_2−MeOH, 5:5:1); $[\alpha]_D^{25}$ −66° (*c* 0.05, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} cm^{-1} 3450, 1731, 1450, 1243; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.12 (3H, s, H-17), 1.15 (3H, s, H-16), 1.65 (3H, s, H-19), 1.70 (1H, m, H-14), 1.82 (3H, s, H-18), 1.91 (1H, m, H-6), 1.95, 2.00, 2.02, 2.08, 2.10, 2.11 (18H, s, 6 × OAc), 2.25 (1H, m, H-14), 2.53 (1H, m, H-6), 2.92 (1H, d, J = 7.8 Hz, H-3), 4.38 (1H, d, J = 7.5 Hz, H-20), 4.47 (1H, d, J = 7.5 Hz, H-20), 4.98 (1H, d, J = 7.2 Hz, H-5), 5.49 (1H, t, J = 8.7 Hz, H-7), 5.61 (1H, t, J = 7.5 Hz, H-13), 6.00 (1H, d, J = 7.8 Hz, H-2), 6.06 (1H, d, J = 10.5 Hz, H-9), 6.26 (1H, d, J = 10.5 Hz, H-10); ^{13}C NMR (in Table 1); EIMS *m/z* 609 $[M - OAc]^+$ (3.6), 593 (3.4), 533 (1.9), 515 (1.7), 473 (2.6), 441 (5.2), 413 (3.1), 384 (1.1), 353 (1.2), 323 (1.4); FABMS *m/z* 653 $[M + H]^+$.

7,13-Diacetylwallifoliol (5): white amorphous solid; R_f 0.48 (*n*-hexane−CH_2Cl_2−MeOH, 5:5:1); $[\alpha]_D^{25}$ −27° (*c* 0.2, CH_2Cl_2); UV (MeOH) λ_{max} nm 230; IR (CH_2Cl_2) ν_{max} cm^{-1} 3645, 2647, 1715, 1546, 1243; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.27 (3H, s, H-19), 1.32 (3H, s, H-17), 1.68, 2.08, 2.16 (9H, s, 3 × OAc), 1.79 (3H, s, H-16), 2.01 (1H, m, H-6), 2.01 (3H, s, H-18), 2.34

(2H, m, H-14), 2.82 (1H, d, J = 11.8 Hz, H-3), 3.01 (1H, m, H-6), 4.28 (1H, d, J = 8.4 Hz, H-20), 4.69 (1H, d, J = 8.4 Hz, H-20), 4.84 (1H, d, J = 6.5 Hz, H-5), 5.30 (1H, dd, J = 5.4, 9.4 Hz, H-7), 5.55 (1H, t, J = 6.3 Hz, H-13), 5.80 (1H, d, J = 11.9 Hz, H-2), 7.48 (2H, t, J = 7.3 Hz, OCOPh, *m*-H), 7.63 (1H, t, J = 7.3 Hz, OCOPh, *p*-H), 7.92 (2H, d, J = 7.3 Hz, OCOPh, *o*-H); ^{13}C NMR (in Table 1); EIMS *m*/*z* 626 $[M]^+$ (0.3), 566 (0.8), 506 (1.2), 446 (0.7), 402 (1.6), 374 (1.8), 332 (1.5), 298 (2); FABMS *m*/*z* 627 $[M + H]^+$.

Preparation of 4, 6, and 7. To a solution of fraction 13 (1 g) in anhydrous pyridine (10 mL) was added benzoyl chloride (3 mL), and the mixture was stirred for 2 min at room temperature. After usual workup as for the preparation of compounds **3** and **5**, the crude product was purified by column chromatography over silica gel using CH_2Cl_2 as eluent to give F-13D (293 mg), F-13E (157 mg), and F-13F (58 mg). F-13D (80 mg) on PTLC (*n*-hexane−CH_2Cl_2−MeOH, 5:5:1) afforded 7,13-dibenzoylwallifoliol (**6**, 49 mg). F-13E (50 mg) on PTLC (*n*-hexane−CH_2Cl_2−MeOH, 5:5:1) furnished 10,13-dibenzoyltaxacustin (**4**, 13 mg). F-13F (58 mg) on preparative TLC (MeOH−H_2O, 77:33) gave 7,9-dibenzoyl taxumairol P (**7**, 4 mg).

10,13-Dibenzoyltaxacustin (4): pale yellow solid; R_f 0.52 (*n*-hexane−CH_2Cl_2−MeOH, 5:5:1); $[\alpha]_D^{25}$ −6° (*c* 0.2, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} cm^{-1} 3500, 1743, 1705, 1246; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.21 (3H, s, H-17), 1.24 (3H, s, H-16), 1.51 (1H, m, H-14), 1.67 (3H, s, H-19), 1.72 (3H, s, H-18), 1.76, 1.99, 2.07, 2.10 (12H, s, 4 × OAc), 2.30 (1H, m, H-14), 2.57 (1H, m, H-6), 3.07 (1H, d, J = 7.8 Hz, H-3), 4.36 (1H, d, J = 7.7 Hz, H-20), 4.50 (1H, d, J = 7.7 Hz, H-20), 4.97 (1H, d, J = 8.0 Hz, H-5), 5.30 (1H, brs, H-7), 5.64 (1H, d, J = 7.9 Hz, H-2), 5.66 (1H, d, J = 10.8 Hz, H-9), 6.20 (1H, dd, J = 11.1, 8.1 Hz, H-13), 6.64 (1H, d, J = 10.8 Hz, H-10), 7.42 (2H, m, OCOPh, *m*-H), 7.47 (2H, m, OCOPh, *m*-H), 7.53 (1H, m, OCOPh, *p*-H), 7.58 (1H, m, OCOPh, *p*-H), 7.87 (2H, d, J = 7.3 Hz, OCOPh, *o*-H), 8.13 (2H, d, J = 7.3 Hz, OCOPh, *o*-H); ^{13}C NMR (in Table 1); EIMS *m*/*z* 717 $[M - OAc]^+$ (4.3), 673 (3.8), 657 (2.3), 597 (1.4), 584 (1.7), 540 (3.1), 507 (1.2), 430 (1.4), 409 (1.1), 365 (1.3); FABMS *m*/*z* 777 $[M + H]^+$.

7,13-Dibenzoylwallifoliol (6): pale yellow solid; R_f 0.6 (*n*-hexane−CH_2Cl_2−MeOH, 5:5:1); $[\alpha]_D^{25}$ −19° (*c* 0.2, CH_2Cl_2); UV (MeOH) λ_{max} nm 231; IR (CH_2Cl_2) ν_{max} cm^{-1} 3440, 1730, 1690, 1272; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.34 (3H, s, H-17), 1.37 (3H, s, H-16), 1.55 (3H, s, H-19), 1.99 (1H, m, H-6), 2.02 (3H, s, H-18), 2.10 (3H, s, OAc), 2.33 (1H, dd, J = 5.4, 15.4 Hz, H-14), 2.52 (1H, dd, J = 7.4, 7.4 Hz, H-14), 3.01 (1H, m, H-6), 3.06 (1H, d, J = 12.0 Hz, H-3), 4.76 (1H, d, J = 8.5 Hz, H-20), 4.75 (1H, d, J = 8.5 Hz, H-20), 4.91 (1H, d, J = 6.6 Hz, H-5), 5.65 (1H, dd, J = 5.4, 5.3 Hz, H-7), 5.83 (1H, t, J = 6.1 Hz, H-13), 5.92 (1H, d, J = 12.0 Hz, H-2), 7.46 (2H, m, OCOPh, *m*-H), 7.48 (2H, m, OCOPh, *m*-H), 7.52 (2H, m, OCOPh, *m*-H), 7.54 (1H, m, OCOPh, *p*-H), 7.56 (1H, m, OCOPh, *p*-H), 7.58 (1H, m, OCOPh, *p*-H), 7.91 (2H, d, J = 7.2 Hz, OCOPh, *o*-H), 8.10 (2H, d, J = 7.2 Hz, OCOPh, *o*-H), 8.19 (2H, d, J = 7.2 Hz, OCOPh, *o*-H); ^{13}C NMR (in Table 1); EIMS *m*/*z* 691 $[M - OAc]^+$ (3.1), 672 (8.4), 642 (1.0), 610 (2.5), 566 (1.2), 533 (1.4), 489 (4.3), 473 (1.1), 456 (1.3), 396 (1.0); FABMS *m*/*z* 751 $[M + H]^+$.

7,9-Dibenzoyltaxumairol P (7): pale yellow solid; R_f 0.25 (*n*-hexane−CH_2Cl_2−MeOH, 5:5:1); $[\alpha]_D^{25}$ +30° (*c* 0.2, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} cm^{-1} 3540, 1734, 1650; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.16 (3H, s, H-17), 1.57 (3H, s, OAc), 1.65 (3H, s, H-16), 1.65 (3H, s, H-19), 1.93 (1H, m, H-6α), 2.07 (1H, m, H-14α), 2.12 (3H, s, OAc), 2.26 (3H, s, H-18), 2.26 (3H, s, OAc), 2.44 (1H, t, J = 11.6, 11.7 Hz, H-6β), 2.59 (1H, m, H-14β), 2.73 (1H, d, J = 9.8 Hz, H-3), 3.58 (2H, ABq, J = 9.1 Hz, H-20), 4.46 (1H, d, J = 9.5 Hz, H-2), 5.06 (1H, brs, H-5), 5.81 (1H, dd, J = 4.5, 4.7 Hz, H-7), 5.87 (1H, m, H-13), 6.34 (1H, d, J = 11.0 Hz, H-9), 6.43 (1H, d, J = 11.0 Hz, H-10), 6.91, 7.10 (4H, t, J = 7.7, 7.8 Hz, OCOPh, *m*-H), 7.20 (2H, t, J = 7.6, 7.7 Hz, OCOPh, *p*-H), 7.49, 7.78 (4H, d, J = 7.2 Hz, OCOPh, *o*-H); ^{13}C NMR (in Table 1); HMBC (300 MHz, $CDCl_3$) [C-1, H-14], [C-2, H-14, H-20], [C-3, H-5, H-20], [C-5, H-6], [C-7, H-9, H-19], [C-9, H-10, H-19], [C-10, H-9], [C-11, H-10], [C-12, H-10, H-18], [C-13, H-14, H-18], [5-OAc, H-5], [9-OBz, H-9], [10-OAc, H-10]; EIMS *m*/*z* 675 $[M - OAc]^+$ (2.1), 631 (2.2), 615 (1.9), 603 (1.4), 559 (1.1), 526 (1.2), 510 (1.7), 582 (1.1), 428 (1.3), 384 (1.2); FABMS *m*/*z* 757 $[M + Na]^+$.

Cytotoxicity Assay. Bioassay against KB (oral epidermoid carcinoma) and Hepa (hepatoma) tumor cells was based on reported procedures.[16] 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. 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% formaldehyde in 0.9% 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 EtOH−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.

Acknowledgment. The authors thank the National Institute of Health, Republic of China (NHRI-GT-EX 90-8809BL), for financial support. We acknowledge Ms. Chao-Lein Ho and Shin-Ching Yu of NSC Southern NMR and MS Instrument Center for high-resolution NMR (500 MHz) and MS data.

References and Notes

(1) Baloglu, E.; Kingston, D. G. I. *J. Nat. Prod.* **1999**, *62*, 1448−1472.
(2) Kingston, D. G. I. *J. Nat. Prod.* **2000**, *63*, 726−734.
(3) Parmar V. S.; Jha A.; Bisht K. S.; Taneja P.; Singh S. K.; Kumar A.; Poonam J. R.; Olsen C. E. *Phytochemistry* **1999**, *50*, 1267−1304.
(4) Kingston, D. G. I.; Xuan, H.; Jagtap, P. G.; Samada, L. *Prog. Chem. Org. Nat. Prod.* **2002**, *35*, in press.
(5) Li, S. H.; Zhang, H. J.; Yao, Ping, Sun, H. D.; Fong, H. H. S. *J. Nat. Prod.* **2000**, *63*, 1488−1491.
(6) Zhang, J.; Sauriol, F.; Mamer, O.; You, X. L.; Jamali, M. A. A.; Batist, G.; Zamir, L. O. *J. Nat. Prod.* **2001**, *64*, 450−455.
(7) Sakai, J. I.; Sasaki, H.; Kosugi, K.; Zhang, S.; Hirata, N.; Hirose, K.; Tomida, A.; Tsuruo, T.; Ando, M. *Heterocycles* **2001**, *54*, 999−1009.
(8) Li, H. L.; Keng, H. *Flora of Taiwan*, Vol. 1, 2nd ed.; Huang, T. C., Ed.; Nat. Sci. Coun., R. O. C., Taipei, 1994; pp 550−552.
(9) Shen, Y. C.; Tai, H. R.; Chen, C. Y. *J. Nat. Prod.* **1996**, *59*, 173−176.
(10) Shen, Y. C.; Chen, C. Y. *Phytochemistry* **1997**, *44*, 1527−1533.
(11) Shen, Y. C.; Chen, C. Y.; Kuo, Y. H. *J. Nat. Prod.* **1998**, *61*, 838−848.
(12) Shen, Y. C.; Chen, C. Y.; Chen, Y. J. *Planta Med.* **1999**, *65*, 582−584.
(13) Shen, Y. C.; Chen, C. Y.; Hung, M. C. *Chem. Pharm. Bull.* **2000**, *48*, 1344−1346.
(14) Shen, Y. C.; Lo, K. L.; Chen, C. Y.; Kuo, Y. H.; Hung, M. C. *J. Nat. Prod.* **2000**, *63*, 720−722.
(15) Shen, Y. C.; Prakash, C. V. S.; Chen, Y. Z.; Hwang, J. F.; Kuo, Y. H.; Chen, C. Y. *J. Nat. Prod.* **2001**, *64*, 950−952.
(16) Shen, Y. C.; Tai, H. R.; Hsieh, P. W.; Chen, C. Y. *Chin. Pharm. J.* **1996**, 48, 207−217.
(17) Shi, Q. W.; Oritani, T.; Sugiyama, T.; Kiyota, H.; Horiguchi, T. *Heterocycles* **1999**, *51*, 841−850.
(18) Chauviere, G.; Guenard, D.; Picot, F.; Senilh, V.; Potier, P. *Acad. Sci. Paris, Ser. II* **1981**, *293*, 501−503.
(19) Wang, X.-X.; Shigemori, H.; Kobayash:, J. *Tetrahedron* **1996**, *52*, 2337−2342.
(20) Kobayashi, J.; Inubushi, A.; Hosoyama, H.; Yoshida, N.; Sasaki, T.; Shigemori, H. *Tetrahedron* **1995**, 51, 5971−5978.
(21) Beutler, J. A.; Chmurny, G. M.; Look, S. A.; Witherp, K. M. *J. Nat. Prod.* **1991**, *54*, 893−897.
(22) VanderVelde, D. G.; George, G. I.; Gollapudi, S. R.; Jampani, H. B.; Liang, X. Z.; Mitscher, L. A.; Ye, Q. M. *J. Nat. Prod.* **1994**, *57*, 862−867.
(23) Ettouati, L.; Ahond, A.; Poupat, C.; Potier, P. *J. Nat. Prod.* **1991**, *54*, 1455−1458.
(24) Woods, M. C.; Chiang, H. C.; Nakadaira, Y.; Nakanishi, K. *J. Am. Chem. Soc.* **1968**, *90*, 522−523.
(25) Senilh, V.; Blechert, S.; Colin, M.; Guenard, D.; Picot, F.; Potier, P.; Varenne, P. *J. Nat. Prod.* **1984**, *47*, 131−137.
(26) Huang, C. H. O.; Kingston, D. G. I.; Magri, N. F.; Samaranayke, G. *J. Nat. Prod.* **1986**, *49*, 665−669.

NP0202273