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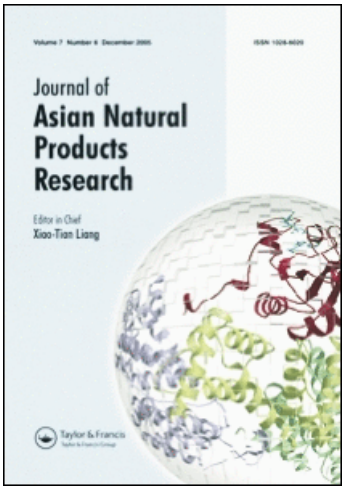

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
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Qing-Wen Shi[a]; Takayuki Oritani[a]; Ji-Shun Gu[b]; Qing-Zhi[b]; Ren-Lin Liu[c]
[a] Laboratory of Applied Bioorganic Chemistry, Division of Life Science, Graduate School of Agricultural Science, Tohoku University, Aoba-ku, Sendai, Japan [b] Pharmaceutical Department, China [c] Jiangxi Forestry School, China

To cite this Article Shi, Qing-Wen , Oritani, Takayuki , Gu, Ji-Shun , Qing-Zhi and Liu, Ren-Lin(2000) 'Three New Taxane Diterpenoids from the Seeds of the Chinese Yew, *Taxus Chinensis* var *Mairei*', Journal of Asian Natural Products Research, 2: 4, 311 — 319
To link to this Article: DOI: 10.1080/10286020008041371
URL: http://dx.doi.org/10.1080/10286020008041371

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JANPR, Vol. 2, pp. 311–319
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THREE NEW TAXANE DITERPENOIDS FROM THE SEEDS OF THE CHINESE YEW, *TAXUS CHINENSIS* VAR *MAIREI**

QING-WEN SHI[a,‡], TAKAYUKI ORITANI[a,†], JI-SHUN GU[b], QING-ZHI MENG[b] and REN-LIN LIU[c]

[a]*Laboratory of Applied Bioorganic Chemistry, Division of Life Science, Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumidori-Amamiya, Aoba-ku, Sendai 981-8555, Japan;* [b]*Pharmaceutical Department, Cangzhou Health Bureau, Hebei Province, China;* [c]*Jiangxi Forestry School, Jiangxi Province, China*

(*Received 9 July 1999; Revised 14 September 1999; In final form 4 January 2000*)

Three new taxoids were isolated from the seeds of the Chinese yew, *Taxus chinensis* var *mairei*, their structures were established as 9α,13α-diacetoxy-5α-cinnamoyloxy-11(15→1)-*abeo*-taxa-4(20),11-diene-10β,15-diol, 2α,9α,10β-triacetoxy-taxa-4(20),11-diene-5α,13α-diol and 2α,7β,10β-triacetoxy-5α-cinnamyloxy-9α-hydroxy-taxa-4(20),11-diene-13-one on the basis of 1D, 2D NMR, and MS spectral analyses.

Keywords: *Taxus chinensis* var *mairei*; Taxaceae; Taxoids; 11(15→1)-*Abeotaxane*; Seeds

INTRODUCTION

During the course of our studies on the taxane diterpenoids of yew trees, we have reported the isolation and characterization of several bicyclic and rearranged taxoids from the needles and bark of the Chinese yew, *Taxus chinensis* var *mairei* [1–4]. In view of only a few studies on the constituents of the seeds of *T. chinensis* var *mairei* that were carried out [5], we recently investigated the components of the seeds of *T. chinensis* var *mairei*, and this

*This paper is dedicated to memory of the late associate professor Takeyoshi Sugiyama.

†Corresponding author. Tel./Fax: 081-22-717-8783. E-mail: oritani@biochem.tohoku.ac.jp.
‡E-mail: shi@biochem.tohoku.ac.jp.

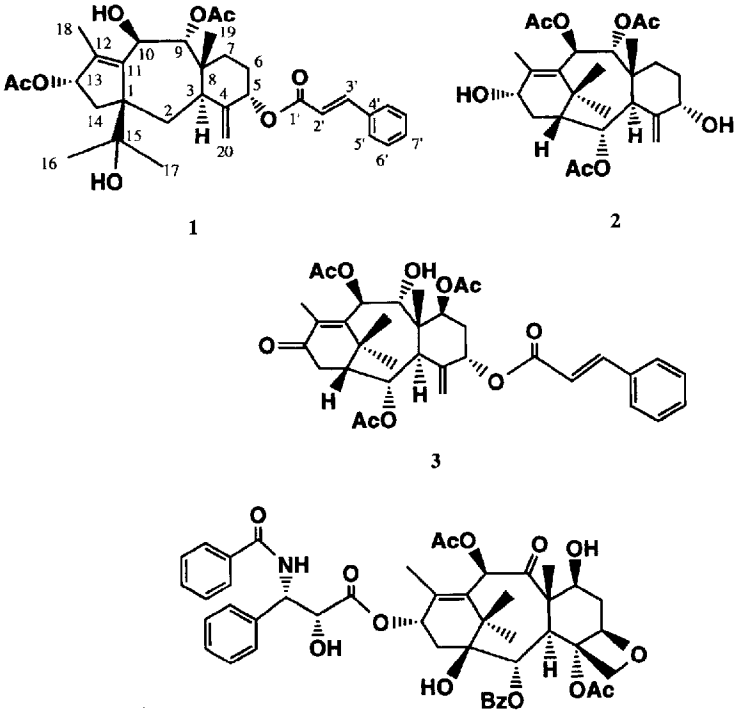

FIGURE 1 The structures of taxol and new taxoids from *Taxus chinensis* var *mairei*.

led to the isolation of three new taxane diterpenoids. This communication deals with the isolation and structure elucidation of three new taxoids from the seeds of *T. chinensis* var *mairei* (Fig. 1).

RESULTS AND DISCUSSION

A methanolic extract of the seeds of *T. chinensis* var *mairei* was processed as described in the Experimental section to afford three new taxane diterpenoids (**1**, **2** and **3**). Compound **1** was isolated as a colorless gummy substance in a yield of 0.001% on the dry material. FAB-MS produced a protonated ion peak at *m/z* 567 ($[M+H]^+$) and an ion peak at *m/z* 605 ($[M+K]^+$). The molecular formula of compound **1**, $C_{33}H_{42}O_8$, was deduced from combined analysis of HR-FAB-MS at *m/z* 605.2510 ($[M+K]^+$) and ^{13}C-NMR spectrum. Intensive absorptions at 3400, 1730, 1710, and

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TABLE I ^{1}H- and ^{13}C-NMR spectral data of **1** and **2** (300 MHz for ^{1}H, 125 MHz for ^{13}C, $CDCl_3$)

Position	**1**			**2**		
	^{13}C	^1H	*J*	^{13}C	^1H	*J*
1	61.66			47.67	1.75 brd	6.9
2a	27.65	2.00 m		71.26	5.41 dd	1.9, 5.8
2b		1.33 m				
3	40.63	2.78 d	7.9	41.73	3.46 d	5.8
4	147.21			145.12		
5	75.00	5.47 brs		77.20	4.30 brs	
6a	27.16	1.81 m		32.06	1.70 m	
6b		1.78 m				
7	28.99	1.80 m		26.56	1.95 m	
8	41.50			44.72		
9	80.93	5.60 d	9.7	77.01	5.76 d	10.5
10	67.37	4.51 d	9.7	73.34	6.05 d	10.5
11	141.79			132.99		
12	140.34			142.21		
13	80.17	5.22 brt	7.0	68.03	4.35 m	
14α	44.01	1.10 m		31.85	2.70 m	
14β		2.38 dd	6.6, 13.5		1.56 m	
15	76.75			36.96		
16	25.39	1.34 s		25.64	1.66 s	
17	27.63	1.12 s		32.43	0.93 s	
18	11.20	1.90 brs		16.62	2.25 brs	
19	16.99	0.78 s		17.26	0.85 s	
20a	112.17	4.78 brs		115.29	4.84 brs	
20b		5.22 brs			5.21 brs	
2-OAc				21.53	2.01 s	
				169.49		
9-OAc	21.17	2.10 s		20.81	2.04 s	
	171.54			170.05		
10-OAc				21.14	2.05 s	
				170.08		
13-OAc	20.46	1.50 s				
	171.10					
1′	165.63					
2′	118.59	6.39 d	16.2			
3′	144.29	7.65 d	16.2			
4′	134.30					
5′	128.84	7.50 m				
6′	127.85	7.36 m				
7′	130.30	7.36 m				

1630 cm^{-1} in the IR spectrum implied that **1** possesses hydroxyl, ester, α,β-unsaturated ester groups and α,β-unsaturated double bond, respectively. The ^{1}H-NMR spectrum of **1**, shown in Table I, exhibited the proton signals due to the four methyl groups at δ 0.78, 1.12, 1.34, and 1.90, two acetyl groups resonated at δ 2.10 and 1.50, which was confirmed by the observation of ^{13}C-NMR signals at δ 171.54, 171.10, 20.46 and 21.17. These signals suggested that **1** had a taxane-type skeleton. Proton signals due to a cinnamoyl

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group were observed at δ 7.50 (2H, m), 7.36 (3H, m), 7.65 (1H, d, $J=$ 16.2 Hz), and 6.39 (1H, d, $J=16.2$ Hz; *trans*-oriented). UV absorption at 278 nm and prominated fragment peak at *m/z* 131 (C_9H_7O) in FAB-MS supported the presence of a cinnamoyl group in **1**. The connectivities of the protons at the taxane skeleton of **1** were determined by the analysis of the $^1H-^1H$ COSY spectrum. Interpretation of ^1H-, ^{13}C-NMR, HMQC and HMBC spectra permitted the positional assignment of functional groups. The ^1H-NMR signals at δ 5.22 (1H, brs), 4.78 (1H, brs) and 2.78 (1H, d, $J=7.9$ Hz) are characteristic of an exocyclic methylene and C-3 ring junction proton in a taxa-4(20), 11-diene, respectively [6]. Additionally, four oxymethine protons appeared at lower field, they were assigned as H-5β, H-9β, H-10α, and H-13β on the basis of $^1H-^1H$ COSY and HMBC spectra. Large vicinal coupling indicated a *trans*-oriented configuration of the H-9β and H-10α. The spin system derived from 18-CH_3, H-13β, H-14α, and H-14β was readily interpreted. The C-11 and C-12 carbon signals showed cross-peaks with the H-14β resonance indicating that both C-11 and C-12 are three bonds apart from H-14β. This means that the A ring was a cyclopentene as in an 11(15 → 1)-*abeo*taxane structure [7,8]. The carbon signal at δ 76.75, assigned to the hydroxyl-bearing C-15, displayed a cross-peak with the C-16 and C-17 methyl resonances at δ 1.34 and 1.12. The C-1 signal (δ 61.66), apart from H-10α, also showed three-bond coupling with the H-3α and C-16, C-17 methyl signals. Since no cross-peak was observed between C-16, C-17 (methyl) signals and the C-11 olefinic carbon in the HMBC spectrum, it further supported the 11(15 → 1)-*abeotaxane* skeleton for **1**. The location of the acetyl and cinnamoyl groups were deduced at C-13, C-9 and C-5 from the HMBC spectrum (Fig. 2). The relative stereochemistry

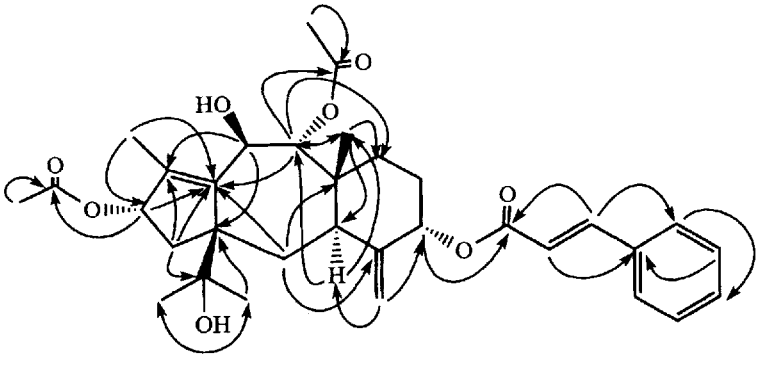

FIGURE 2 Selected H–C long-range correlations observed from the HMBC spectrum of **1** (500 MHz). Most protons have been omitted for clarity.

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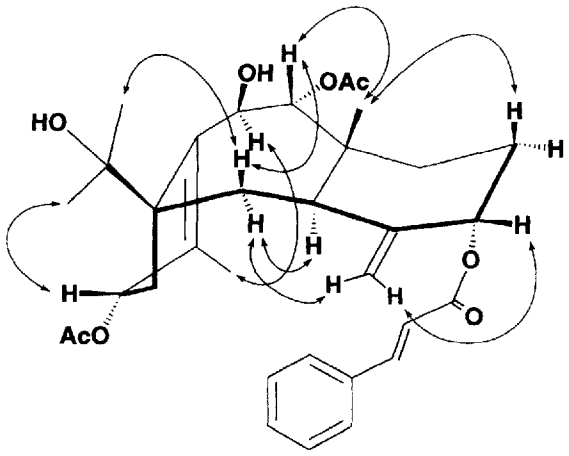

FIGURE 3 Relative stereochemistry of 1, proposed by NOESY experiment (500 MHz).

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of the terpenoid skeleton of **1** was determined from the chemical shifts, coupling constants and NOESY experiment. A coupling constant between H-9 and H-10 of $J = 9.7$ Hz indicated that the B-ring was the chair-boat conformation. The NOESY experiment established the relative stereochemistry of **1** at all the positions, and the results are shown in Fig. 3. Thus the structure of **1** was established as 9α,13α-diacetoxy-5α-cinnamoyloxy-11(15 $\rightarrow$ 1)-*abeo*-taxa-4(20),11-diene-10β,15-diol.

Compound **2**, which was isolated as a colorless gummy substance, had a composition of $C_{26}H_{38}O_8$ as derived from a molecular ion peak at *m/z* 478.2571 in its HR-EI-MS. The IR spectrum had bands at 3410 cm^{-1} (hydroxy), and 1735 cm^{-1} (ester). The ^{1}H-NMR and ^{13}C-NMR spectra showed well-dispersed characteristic signals for a taxa-4(20),11-diene with signals for the exocyclic methylene at δ 4.84 (1H, brs), 5.21 (1H, brs), and carbon signals at δ 115.29 and 145.12. In addition, the presence of four methyl signals (δ 0.85, 0.93, 1.66, and 2.25) and three acetyl groups were verified by observation of the ^{1}H- and ^{13}C-NMR spectral data (Table I). The proton connectivities were established by the analysis of ^{1}H–^{1}H COSY spectrum. Interpretation of ^{1}H-, ^{13}C-NMR, HMQC and HMBC spectra permitted the positional assignment of functional groups. A methine proton at δ 5.41 (1H, dd, $J = 1.9, 5.8$ Hz) was assigned to H-2 because it correlated with H-3 at δ 3.46 (1H, d, $J = 5.8$ Hz) and H-1 at δ 1.75 (1H, brd, $J =$ 6.9 Hz). A pair of doublets at δ 5.76 and 6.05 with a large coupling constant ($J = 10.5$ Hz) were atributed to H-9β and H-10α, respectively. Lower field chemical shifts suggested three acetyl groups attached to C-2, C-9, and

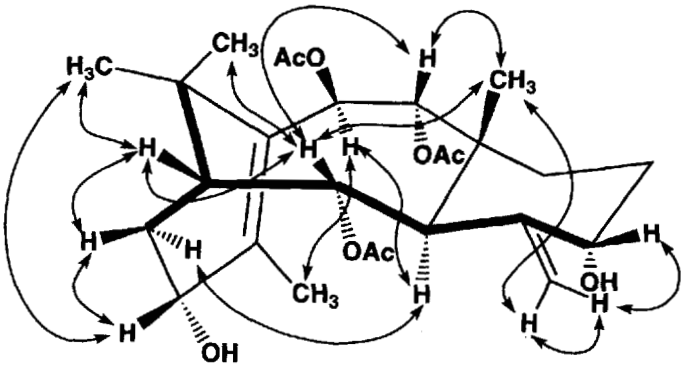

FIGURE 4 Relative stereochemistry of 2, proposed by NOESY experiment (500 MHz).

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C-10, which was verified by HMBC spectrum. Therefore, the structure of **2** was determined to be 2α,9α,10β-triacetoxy-taxa-4(20),11-diene-5α,13α-diol. The relative stereochemistry of **2** was deduced from the chemical shifts, coupling constants and NOESY experiment, the results are shown in Fig. 4.

Compound **3** was shown to have the molecular formula of $C_{35}H_{42}O_{10}$ (*m/z* 622.2782 M^+, Δ +0.4 mmu) by HR-EI-MS analysis. The ^{1}H-NMR spectrum of **3** suggested that **3** has a taxane-type skeleton. Proton signal due to a cinnamyl group appeared at δ 7.77 (2H, d, $J = 6.87$ Hz), 7.45 (3H, m), 7.68 (1H, d, $J = 15.9$ Hz), and 6.44 (1H, d, $J = 15.9$ Hz). The ^{1}H-NMR signals at δ 5.41 (1H, brs), 4.93 (1H, brs) and 3.22 (1H, brd, $J = 5.8$ Hz) are characteristic of an exocyclic methylene and C-3 ring junction proton in a taxa-4(20),11-diene, respectively. The fact that 18-CH_3 was a sharp singlet suggested that C-13 did not have a hydrogen attached, but instead bore a ketone function. In accordance with this, H_2-14 displayed a large $J_{gem} = 20.1$ Hz. By means of ^{1}H–^{1}H COSY spectrum, the complete connection network was established for H-14α-H-14β-H-1β-H-2β-H-3α-H-20a-H-20b-H-5β-H_2-6-H-7α. H-3α and H-5β had allylic coupling with H-20a and H-20b. Lower field chemical shift of H-2α and H-7α indicated acetoxy groups located at C-2 and C-7. Remaining acetate group was connected to C-10, the H-10α (δ 6.12, 1H, d, $J = 10.2$ Hz) was deshielded by neighboring acetoxy group and C-11,12 double bond. The signal at δ 4.29, which correlated with H-10α and a hydroxyl signal at δ 2.58 (1H, d, $J =$ 8.2 Hz), was attributed to H-9β. The cinnamate group was located at C-5α, in accordance with what is observed in the other taxinine derivatives [9,10]. Thus, the structure of **3** was elucidated as 2α,7β,10β-triacetoxy-5α-cinnamyloxy-9α-hydroxy-taxa-4(20),11-diene-13-one (9α-deacetyl taxinine B).

The relative stereochemistry of **3** was deduced from the NOESY experiment, and the results are described in the experimental section.

EXPERIMENTAL SECTION

General Experimental Procedures

Optical rotations were recorded on a Horiba SEPA-300 digital polarimeter. UV spectrum was run on a Shimadzu UV-1600 spectro-photometer. IR spectra were obtained on a Jasco IR-810 instrument. MS were measured on a Jeol JMS-700 spectrometer using EI and FAB modes. ^{13}C-NMR spectra were obtained on Varian Unity Inova 500 spectrometers operating at 125 MHz, ^{1}H- and ^{1}H–^{1}H COSY spectrum were measured on Varian GEMINI 2000/300 spectrometer at 300 MHz, in $CDCl_3$ at 20°C, chemical shifts are expressed in parts per million scale relative to that of tetramethylsilane (TMS, $\delta = 0$) as an internal standard, and coupling constants are given in Hertz. ^{1}H ^{13}C HETCOR and HMBC experiments were performed on the same spectrometer, using standard Varian pulse sequences. Open column chromatography was performed using Merck silica gel 60 (100–200 mesh). Thin layer chromatography (TLC) was carried out with the precoated Merck silica gel 60 F_{254} plates. Preparative TLC was performed with the same type of plate as used for TLC but with 0.85 mm thickness (dried for 24 h at room temperature and activated for 4 h at 120°C, developed in an unsaturated tank). The spots were detected under UV (254 nm) and/or by spraying with 10% sulfuric acid and then heating on a hot plate.

Plant Material

The seeds of *T. chinensis* var *mairei* were collected in Jinggangshan, in the south-east of China, in October 1995. The botanical identification was made by Prof. R.L. Liu. A voucher specimen has been deposited in our laboratory.

Extraction and Isolation

Air dried seeds (1.1 kg) were crushed and extracted with *n*-hexane three times at room temperature to remove the major part of nondesired neutral components. The residue was extracted twice with methanol (MeOH),

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the MeOH extracts were condensed to a residue under reduced pressure. Subsequently, this residue was diluted with water and then extracted five times with EtOAc. The combined EtOAc layer was further extracted with 5% HCl. The EtOAc layer, upon evaporation, yielded 5.5 g of yellowish syrup, which was subjected to column chromatography (CC), which was repeatly eluted with *n*-hexane : acetone (v/v 4 : 1, 2 : 1, 1 : 1, and 2 : 3). Each fraction was then applied to preparative TLC with different developing solvents ($CHCl_3$–MeOH, 100 : 4.5; *n*-hexane–EtOAc, 2 : 3; *n*-hexane–acetone, 3 : 2), and finally compound **1** (*n*-hexane–acetone, 3 : 2, R_f 0.37, 12.5 mg), **2** (*n*-hexane–EtOAc, 2 : 3, R_f 0.40, 3 mg), and **3** (*n*-hexane–acetone, 3 : 2, R_f 0.52, 1 mg) were separated.

*9α,13α-Diacetoxy-5α-cinnamoyloxy-11(15 → 1)-abeo-taxa-4(20),11-diene-10β,15-diol (**1**)* Gum, $[\alpha]_D^{24}$ −23° (*c* 0.01, $CHCl_3$); IR (film, $CHCl_3$) ν_{max}: 3400, 2930, 1730, 1710, 1630, 1440, 1370, 1240, 1170, 1020, and 750 cm^{-1}; UV (MeOH) λ_{max} (log ε) 278 (4.1) nm; FAB-MS *m/z*: 605 ($[M+K]^+$), 567 ($[M+H]^+$), 549, 461, 369, 277, 223, 185, 131, 105, and 93. HR-FAB-MS: 605.2910 (calcd. for $C_{33}H_{42}O_8K$, 605.2514); ^1H- and ^{13}C-NMR spectral data, see Table I.

*2α,9α,10β-Triacetoxy-taxa-4(20),11-diene-5α,13α-diol (**2**)* Colorless gum; $[\alpha]_D^{25}$ +30 (*c* 0.01, $CHCl_3$); IR (film, $CHCl_3$) ν_{max}: 3410, 2930, 1735, 1440, 1370, 1240, 1170, 1020, and 750 cm^{-1}; EI-MS *m/z* (rel. int.): 478 (8) ($[M]^+$), 460 (9) ($[M-H_2O]^+$), 386 (7), 358 (6), 284 (30), 256 (100), 82 (90), and 43 (100). HR-EI-MS *m/z*: 478.2571 (calcd. for $C_{26}H_{38}O_8$, 478.2565); ^1H-NMR and ^{13}C-NMR, see Table I.

*2α,7β,10β-Triacetoxy-5α-cinnamyloxy-9α-hydroxy-taxa-4(20),11-diene-13-one (**3**)* $[\alpha]_D^{24}$ +17° (*c* 0.02, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 277 (4.1) nm; IR (film, $CHCl_3$) ν_{max}: 3450, 3005, 2930, 2820, 1730, 1710, 1670, 1630, 1450, 1430, 1370, 1240, 1160, 1030, and 760 cm^{-1}; EI-MS *m/z*: 622 ($[M]^+$), 580, 562 ($[M-AcOH]^+$), 502 ($[M-2AcOH]^+$), 283, 131 and 43; HR-EI-MS: 622.2782 (calcd. for $C_{35}H_{42}O_{10}$, 622.2775); ^1H-NMR ($CDCl_3$): 2.14 (1H, m, H-1), 5.51 (1H, dd, J = 5.8, 2.1 Hz, H-2), 3.22 (1H, brd, J = 5.8 Hz, H-3), 5.39 (1H, brs, H-5), 1.75 (1H, m, H-6a), 2.15 (1H, m, H-6b), 5.55 (1H, m, H-7), 4.29 (1H, brt, J = 10.2 Hz, H-9), 6.12 (1H, d, J = 10.2 Hz, H-10), 2.36 (1H, d, J = 20.1 Hz, H-14α), 2.84 (1H, dd, J = 20.1, 7.7 Hz, H-14β), 1.53 (3H, s, 16-CH_3), 1.68 (3H, s, 17-CH_3), 2.31 (3H, s, 18-CH_3), 1.16 (3H, s, 19-CH_3), 2.07 (6H, s, CH_3CO–), 2.11 (3H, s, CH_3CO–), 6.44 (1H, d, J = 15.9 Hz, 2′-H), 7.68 (1H, d, J = 15.9 Hz, 3′-H), 7.45 (3H, m, Ph-H), 7.77 (2H, d, J = 6.9 Hz, Ph-H); NOESY correlations ($CDCl_3$, H/H): 1/2, 1/14β, 1/16, 1/17, 2/9, 2/16, 2/19, 3/7, 3/10, 3/14α, 3/18, 5/6a, 5/6b, 5/20b, 9/16, 9/19, 14α/18, 19/20a.

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Acknowledgments

The authors are grateful to Mrs. Teiko Yamada for measuring the HR-MS data. The financial support for the work described here was provided by the Ministry of Education, Science, Sports, and Culture of Japan through a grant-in-aid for scientific research.

References

[1] Q.W. Shi, T. Oritani, T. Sugiyama and H. Kiyota. *Planta Med.* 1999, **64**, 766–769.
[2] Q.W. Shi, T. Oritani, T. Sugiyama and H. Kiyota. *J. Nat. Prod.* 1998, **61**, 1437–1440.
[3] Q.W. Shi, T. Oritani and T. Sugiyama, *Phytochemistry* 1999, **50**, 633–636.
[4] Q.W. Shi, T. Oritani, T. Sugiyama, H. Kiyota and T. Horiguchi, *Heterocycles* 1999, **51**, 841–850.
[5] Y. Chen, C. Chen and Y. Shen, *J. Nat. Prod.* 1999, **62**, 149–151.
[6] G. Appendino, in *The Chemistry and Pharmacology of Taxol and Its Derivatives*, Eds. Farina, V., Amsterdam, 1995, **22**, 55–101.
[7] K. Fuji, K. Tanaka, B. Li, T. Shingu, T. Yokoi, H.D. Sun and T. Taga, *Tetrahedron* 1995, **51**, 10175–10188.
[8] S. Zhang, C.T. Lee, T. Che, Y. Kashiwad, D. Zhe, A. McPhail and K. Lee, *J. Chem. Soc., Chem. Commun.* 1994, 1561–1563.
[9] D.G.I. Kingston, A.A. Molinero and J.M. Rimoldi, in *Progress in the Chemistry of Organic Natural Products*, Eds. Herz, W., Kirby, G.W., Moore, R.E., Steglich, W. and Tamm, C.H., Springer, 1993, **61**, 1–206.
[10] G. Appendino, *Nat. Prod. Rep.* 1995, **12**, 349–360.

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