A New *pseudo*-Alkaloid Taxane and a New Rearranged Taxane from the Needles of *Taxus canadensis*

Manli Zhang[a], Changhong Huo[a], Mei Dong[a], Ligeng Li[a], Françoise Sauriol[b], Qingwen Shi[a], Yucheng Gu[c], Hiromasa Kiyota[d], and Bin Cong[a]

[a] School of Pharmaceutical Sciences & College of Basic Medical Sciences, Hebei Medical University, 361 Zhongshan East Road, 050017, Shijiazhuang, Hebei Province, PR China
[b] Department of Chemistry, Queen's University, Kingston, Ontario, K7L 3N6, Canada
[c] Syngenta Jealott's Hill International Reasearch Centre, Bracknell, Berkshire RG42 6EY, United Kingdom
[d] Department of Bioscience and Biotechnology for Future Bioindustry, Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumidori-Amamiya, Aoba-ku, Sendai, 981-8555 Japan

Reprint requests to Dr. Qingwen Shi. E-mail: shiqingwen@hebmu.edu.cn or Dr. Bin Cong. E-mail: congbin@263.net

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A new taxane with an amino side chain on C-5 and a new 11(15→1)*abeo*taxane having a tetrahydrofuran ring along carbon atoms C-2, C-3, C-4, C-20 identified for the first time from the needles of the Canadian yew, *Taxus canadensis*. Their structures were characterized as 2α,7β,9α,10β,13-pentaacetoxy-11β-hydroxy-5α-(2′-hydroxy,3′-*N,N*-dimethylamino-3′-phenyl)-propionyloxytaxa-4(20),12-diene (**1**) and 13α,20β-diacetoxy-5α,7β,9α,10β-tetrahydroxy-2α,20-epoxy-11(15→1)*abeo*taxa-11,15-diene (**2**) on the basis of 1D, 2D NMR spectroscopy and high-resolution FABMS analysis. Taxane **1** contains a rare C-12, C-13 double bond and a basic side chain, while taxane **2** bears a rare isopropenyl group at C-1.

Key words: Taxaceae, *Taxus canadensis*, Taxane

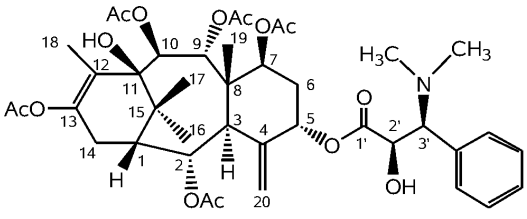
Introduction

The discovery of Taxol® (paclitaxel) has stimulated great renewed interest in the analysis of the various *Taxus* species with the aim to find alternative sources for this or taxol-related compounds with improved activities. Although in total more than 500 taxanes have been isolated to date [1], several new taxanes have been reported only recently [2 – 14]. Apparently, there are still new ones awaiting isolation and structural characterization. Structure-activity relationship and synthetic modification studies of taxanes have been aimed at increasing activity and solubility of new analogs [15, 16]. Recently, several non-taxol-type taxanes were also reported to show interesting activities other than cytotoxic ones. In particular, certain non-taxol-type taxanes reduced the $CaCl_2$-induced depolymerization of microtubles, and others increased the cellular accumulation of vincristine in multidrug-resistant tumor cells [17, 18]. The Canadian yew *Taxus canadensis* Marsh (Taxaceae), a low-trailing bush very common in Quebec, has been thoroughly investigated since 1992 and has been shown to be an interesting plant containing unusual taxanes specific to this yew [19 – 22]. In the present publication we are reporting the characterization of a new *pseudo*-alkaloid taxane and a new 11(15→1)*abeo*taxane from the needles of *T. canadensis*. Their chemical structures were rigorously characterized using 1D and 2D NMR data and were further confirmed by high- resolution fast atom bombardment mass spectrometry (HR-FABMS).

Results and Discussion

A methanolic extract of the needles of *T. canadensis* was processed as described in the Experimental Section to provide a new taxane containing nitrogen on the C-5 side chain (**1**) and a new 11(15→1)*abeo*taxane having a tetrahydrofuran ring along carbon atoms C-2, C-3, C-4, C-20 and an isopropenyl group at C-1 (**2**) (Fig. 1).

Compound **1** was obtained as a white amorphous powder. Its molecular composition, $C_{41}H_{55}NO_{14}$, was established from the combined analysis of HR-



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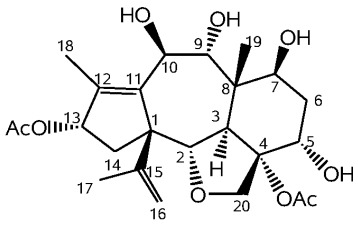

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Fig. 1. The structures of taxanes **1** and **2**.

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data of **1** in $CDCl_3$.

No.	δ (^{1}H) mult[a]	*J* (Hz)	^{1}H-^{1}H COSY	δ (^{13}C)	HMBC (H→C)	NOESY
1	1.80 (br d)	7.7	H-2, 14a, 14b	50.1	2, 11, 13, 14, 3/15, 16	2, 14a, 16, 17
2	5.64 (br d)	6.8	H-1, 3	68.1	3, 8, 14, 170.3	1, 3, 7, 17, 19
3	3.55 (d)	6.8	H-2, 20a, 20b	40.6	1, 4, 5, 8, 9, 19, 20	2, 7, 14b, 20a
4	–			140.9		
5	5.01 (o m)		H-6a, 6b	68.5	169.0	
6a	1.55 (m)		H-5, 6b, 7	31.8		6b, 5
6b	1.22 (m)		H-5, 6a, 7			
7	4.57 (dd)	10.2, 8.2	H-6a, 6b	70.0	8, 19, 169.8	3
8	–			42.6		
9	4.73 (d)	4.8	H-10	74.4	3, 8, 10, 19, 169.1	10, 19
10	5.52 (d)	4.8	H-9	76.5	8, 9, 11, 12, 15, 169.2	9, 18
11	–			78.3		
12	–			124.0		
13	–			143.9		
14a	2.51 (ddd)	18.7, 7.7, 2.3	H-1, 14b, 18	25.7		1, 14b, 17
14b	2.28 (d)	18.7	H-1, 14a			3, 14a, 20a
15	–			40.9		
16	1.13 (s)		H-17	31.3	1, 11, 15, Me-17	1, 14a, 17
17	1.42 (s)		H-16	23.8	1, 11, 15, Me-16	1, 2, 16
18	1.52 (br s)		H-14a	11.5	11, 12, 13	10
19	1.35 (s)			14.6	3, 7, 8, 9	2, 9, 5/20
20a	5.00 (s)		H-3, 20b	111.2	3, 4, 5	3, 14b
20b	4.96 (s)		H-3, 20a			
2-OAc	1.87 (s)			21.3	170.3	
				170.3		
7-OAc	1.91 (s)			21.0	169.8	
				169.8		
9-OAc	2.14 (s)			21.0	169.1	
				169.1		
10-OAc	1.94 (s)			20.5	169.2	
				169.2		
13-OAc	2.19 (s)			20.5	168.4	
				168.4		
1′	–			169.0		
2′	4.83 (d)	9.7	H-3′	69.8	1′, 3′	
3′	4.30 (br d)	9.7	H-2′	71.1		2′, N-Me
3′-Ph				137.5		
o	7.33 (br)		H-*m*	130.4	3′	2′, 3′, N-Me
m	7.42 (br)		H-*o*, *p*	130.4		
p	7.42 (br)		H-*m*	129.2		
NMe_2	2.61 (br s)			40.8		

[a] o = overlapped.

FABMS and 2D NMR data. The ^{1}H and ^{13}C NMR data of **1** (Table 1) showed the characteristic signals of taxanoids such as four tertiary methyl groups, two of the methyl signals at δ = 1.13 and 1.42 being geminally correlated, five acetyl groups, an exomethylene and a ring junction proton H-3 at δ = 3.55 (1H, d,

Table 2. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data of **2** in $CDCl_3$.

No.	δ (H) mult	J (Hz)	^{1}H-^{1}H COSY	δ (C)	HMBC	NOESY
1	–			59.4		
2	4.62 (d)	7.4	H-3	81.2	1, 11, 15	2, 3, 9, 16, 17, 19, 20b
3	2.27 (d)	7.4	H-2	50.0	1, 2, 4, 7, 8	2, 7
4	–			95.1		
5	4.18 (br t)	9.8	H-6a, 6b	72.2		9
6a	2.02 (m)		H-5, 6b	32.9		
6b	1.92 (m)		H-5, 6a			
7	3.93 (dd)	2.6, 11.3	H-6a, 6b	69.2	3, 5, 8, 9	3, 10
8	–			44.3		
9	3.91 (br d)	4.6	H-10	74.6		2, 10
10	5.04 (d)	4.6	H-9	73.1	1, 8, 11, 12	7, 9, 18
11	–			139.3		
12	–			147.7		
13	5.65 (br t)	~7.4	H-14a, 14b	81.1	18	18
14a	2.21 (m)		H-13, 14b	36.8		
14b	2.04 (m)		H-13, 14a			
15	–			153.0		
16a	4.82 (s)		H-16b	109.7	1	13, 17
16b	4.80 (s)		H-16a			2, 17
17	1.92 (s)			21.2	1, 15, 16	2, 16ab
18	1.80 (s)			12.2	11, 12, 13	10, 13
19	1.26 (s)			13.5	3, 7, 8, 9	2, 6b/17, 9, 20b
20a	4.38 (d)	11.5	H-20b	72.8		20b
20b	3.70 (d)	11.5	H-20a		4	2, 19, 20a
OAc	2.06 (s)			21.2	170.5	
	2.03 (s)			21.8	172.1	
OH	4.44 (br s)					

J = 6.8 Hz). The 6/8/6 membered ring skeleton was verified by the HMBC correlations of H-3 to C-8, C-9, C-19; Me-16 and Me-17 to C-1 and C-11, and H-2 to C-8 and C-14. The presence of a 3′-*N,N*-dimethylamino-3′-phenylisoseryloxyl moiety was suggested by the ^{1}H NMR data: H-2′ resonated as a doublet at δ = 4.83 (1H, d, J = 9.7 Hz); H-3′ resonated as a broad doublet at δ = 4.30 (1H, br d, J = 9.7 Hz); two magnetically equivalent NMe groups at δ = 2.61 (6H, br s, NMe_2) and five aromatic protons between δ = 7.33 – 7.42 (5H, m, Ph-H). This conclusion was further verified by the fragment ion peaks at *m/z* = 134 $[C_9H_{12}N]^+$ and 210 $[C_{11}H_{16}NO_3]^+$ in the mass spectra of **1**, which are characteristic fragmental ions of 3′-*N,N*-dimethylamino-3′-phenylisoseryloxyl moieties in the *pseudo*-alkaloid-type taxanes [23]. The protons on the skeleton were assigned on the basis of a ^{1}H-^{1}H COSY spectrum using H-3 as a reference. Based on the HMBC correlations, an AB system resonating at δ = 4.73 and δ = 5.52 was assigned to H-9 and H-10, respectively. The connectivities from H-3 to H-2 to H-1 to H-14, and from H-5 to H-7 through H-6 were revealed by detailed ^{1}H-^{1}H COSY analysis. The carbon signals of the skeleton were assigned with the aid of HSQC and HMBC spectral correlations. The locations of acetyl groups and the side chain were deduced from the HMBC correlations of carbonyl carbons with corresponding protons. The signal at δ = 78.3, which showed long-range correlations with H-1, H-10, Me-16 and Me-17, was assigned to C-11, and a hydroxyl group attached to C-11 was suggested by its chemical shift value. The olefinic carbon at δ = 124.0, which showed HMBC correlations with H-10 and Me-18, was assigned to C-12 [24, 25]. The olefinic carbon signal at δ = 143.9, which exhibited long-range correlations with H-1, Me-18, was assigned to C-13. This conclusion is in good agreement with the observed chemical shifts of H-14a and H-14b in the ^{1}H NMR spectrum as well as with the exhibited long-range ^{1}H-^{1}H COSY relationship between H-14a and Me-18. The chemical shifts of these olefinic carbons implied the presence of a rare alkenyl acetate moiety in ring A [25, 26]. The NOESY experiment (Table 1) established the relative stereochemistry of **1**. Hence the structure of **1** was unrevealed to be 2α,7β,9α,10β,13-pentaacetoxy-11β-hydroxy-5α-(2′-hydroxy,3′-*N,N*-dimethylamino-3′-phenyl)-propionyloxytaxa-4(20),12-diene. This is

the second examples of a taxane with a C-12, C-13 double bond and a C5-amino side chain found in the needles of the Canadian yew [1].

The taxane **2** was obtained as an amorphous solid from the methanol extract of the *Taxus canadensis* needles. The molecular composition of **2**, $C_{24}H_{34}O_9$, was deduced from combined analysis of high-resolution FABMS at *m/z* = 489.2097 $[M+Na]^+$ and ^{13}C NMR spectroscopic data. Eight indices of hydrogen deficiency were calculated from the molecular formula. The ^1H NMR spectrum of **2**, tabulated in Table 2, exhibited three three-proton signals due to the three methyl groups at δ = 1.92, 1.80 and δ = 1.26; two acetyl groups at δ = 2.03 and 2.06, which were verified by the observation of ^{13}C NMR signals of carbonyl groups at δ = 172.1 and 170.5 as well as corresponding methyl groups of acetates at δ = 21.8 and 21.2. These signals together with the plant origin consideration suggested that **2** was a taxane derivative [27]. Analysis of the ^1H, ^{13}C and 2D NMR spectral data disclosed 24 carbon signals due to two acetyls, six oxymethines, one methine, two methylenes, one methyleneoxyl, six quaternary carbons including three sp^2 (three fully substituted olefinic carbons) and three sp^3 hybrid carbons, three methyl groups, and a sp^2 hybrid methylene carbon. These observations indicated that **2** contained 4 rings. The chemical shift of C-1 at δ = 59.4, an unusually downfield chemical shift for an unoxygenated quaternary carbon, together with absent long-range correlations between C-11 and Me-16, Me-17, which are always observed in the HMBC experiment in the 6/8/6-membered ring taxanes, strongly indicated that taxane **2** possessed a 11(15→1) rearranged skeleton, *i. e.*, it is a brevifoliol analog [27 – 29]. This conclusion was further verified by long-range correlations between H-10 and C-1 as well as between H-2 and C-11 observed in the HMBC experiment. The assignments of functional groups on the taxane skeleton were made on the basis of analyses of the ^1H-^1H COSY and HMBC correlations. Starting with the signal at δ = 2.27 (1H, d, *J* = 7.4 Hz), which is typical for a H-3α ring junction proton in the taxane diterpenes, the signal at δ = 4.62 (1H, d, *J* = 7.4 Hz) was assigned to H-2. The chemical shift of H-2 revealed that no acetyl group is located at C-2. Similarly, the signal at δ = 4.18 (1H, br t, *J* = 9.8 Hz) is the characteristic signal of H-5β of taxane [27]. The chemical shift of H-5β implied that a free hydroxyl group was connected to C-5. Using H-5β as reference, the spin systems from H-5 to H-6 and H-7 were readily interpreted. The chemical shift of H-7 (δ = 3.93, 1H) demonstrated that C-7 bears a free hydroxyl function. The signal at δ = 5.65 (1H, br t, *J* = 7.4 Hz) was assigned to H-13. The corresponding carbon atom exhibited long-range C-H correlations with Me-18 in the HMBC spectra, and H-13 exhibited cross-peaks with geminal protons of a methylene group at δ = 2.21 (1H, m, H-14a) and 2.04 (1H, m, H-14b) in the ^1H-^1H COSY map. The chemical shift of H-13 suggested that an acetyl group was positioned at C-13 [27]. The remaining three sets of coupling systems at δ = 3.91 (1H, br d, *J* = 4.6 Hz) and 5.04 (1H, d, *J* = 4.6 Hz); 4.38 (1H, d, *J* = 11.5 Hz) and 3.70 (1H, d, *J* = 11.5 Hz); and 4.82 (1H, s) and 4.80 (1H, s), were attributed to H-9 and H-10, H-20a and H-20b, and H-16a and H-16b, respectively, with the help of ^1H-^1H COSY and HMBC experiments. The chemical shifts of H-9 and H-10 implied that two free hydroxyl groups are located at C-9 and C-10. The chemical shifts of C-4 (δ = 95.1) and C-2 (δ = 81.2) indicated that the remaining acetyl group was positioned at C-4, and a tetrahydrofuran ring along C-2, C-3, C-4, C-20 was formed [30]. Repeated attempts to obtain an HMBC correlation between H-2 and/or H-20 and C-20 and/or C-2 carbon resonance, which would unequivocally confirm the formation of a tetrahydrofuran ring along C-2, C-3, C-4, C-20, proved unsuccessful, however. However, given the other evidence for the structure assignement, the lack of such a correlation is not considered crucial. The relative stereochemistry of taxane **2** was deduced using the information contained in the NOESY spectrum and by comparing the coupling constants with those of its analog [30]. Taking all above arguments into account, the structure of **2** was characterized as 13α,20β-diacetoxy-5α,7β,9α,10β-tetrahydroxy-2α,20-epoxy-11(15→1)*abeo*taxa-11,15-diene (Fig. 1). This is the first example of a taxane with a tetrahydrofuran ring along C-2, C-3, C-4, C-20 and an isopropenyl moiety at C-1 reported for the needles of the Canadian yew [1], although such kinds of natural taxanes have been isolated from the roots of *Taxus mairei* [31].

Taxane **1** is a 6/8/6-membered ring taxane with a rare alkenyl acetate group at C-13. Of such substituted taxanes, a total of only six compounds were isolated from the stems and seeds of the Japanese yew, *Taxus cuspidata*, and the needles of the Canadian yew, *T. canadensis*. These are taxuspines D [26], P [27], taxezopidine K [32], 5-decinnamoyltaxuspine D [33], 7-deacetoxyl taxuspine D [34] and 2α,7β,9α,13-tetra-

acetoxyraxa-4(20),12-diene-5α,10β,11β-triol [14]. It should be noted that both yew trees grow in the cold regions of the northern hemisphere. These taxanes can possibly serve as a chemotaxonomic mark of these yews. Beside 6/8/6-membered ring taxanes, alkenyl acetate groups are also encountered in many bicyclic taxanes [35 – 39] and other compounds such as 2-acetoxy-1-cyclohexene [40]. Of them, taxachitriene B was characterized by X-ray diffraction which confirmed the alkenyl acetate group [35]. Compound **1** is the first example of an alkenyl acetate taxane with a 3′-*N,N*-dimethylamino-3′-phenylisoseryloxyl substituent at C-5, although it should be noted that a taxane with a 3′-*N,N*-dimethylamino-3′-phenylpropanoyl moiety at C-5 has been reported from stems of *T. cuspidata* in 1996 [27]. The failure to crystallize, the limitation of the available material and the scarcity of the sample source prevented compound **1** from being characterized by X-ray diffraction. Likewise, chemical conversions or enzymatic hydrolysis to further corroborate its structure have not been possible as yet. Nevertheless, the unambiguous rationalization of all of the ^{1}H and ^{13}C NMR resonances using a combination of ^{1}H-^{1}H COSY, HMQC, HMBC, and NOESY techniques and confirmation by HR-FABMS analysis make us confident that the structural assignments are correct.

Experimental Section

General

Optical rotations were recorded on a JASCO DIP-370 digital polarimeter. Flash chromatography was performed on Silica gel 60 (230 – 400 mesh EM Science). Thin layer chromatography was conducted on Silica Gel 60 F_{254} pre-coated TLC plates (0.25 mm or 0.5 mm, EM Science). The compounds were visualized on TLC plates with 10 % sulfuric acid in ethanol and heating on a hot plate. Na_2SO_4 was the drying agent used in all work-up procedures. All the reagents and solvents were of the best available commercial quality and were used without further purification.

Plant material

The needles of *T. canadensis* were collected in September 1997 at St-Jean, Quebec, Canada. Several specimens (under accession voucher number lz97-03) have been deposited in the herbarium of the Montreal Botanical Garden, Montreal, Canada.

Extraction and isolation

Air-dried needles of *T. canadensis* were ground (4.0 kg) and extracted with 24 L of methanol for one day at r. t. The ground plants were filtered and extracted again with fresh solvent for another three times (each time with 8 L solvent, total 24 L) in three days. The combined organic extracts were evaporated under reduced pressure. Water (3 L) was added, and lipids were removed by stirring the mixture with hexane (3 × 3 L). The aqueous phase was then salted (NaCl, 200 g) and extracted with CH_2Cl_2 (4 × 3 L). The combined CH_2Cl_2 extracts were dried with anhydrous Na_2SO_4, filtered and evaporated yielding 115 g of a dark green extract.

A portion of the CH_2Cl_2 extract (50 g) was absorbed onto 110 g silica gel and subjected to column chromatography (silica gel 230 – 400 mesh, 1320 g). Successive elution with a CH_2Cl_2-MeOH gradient with increasing amounts of MeOH from 5 % to 45 % (total 15 L) yielded 45 fractions (Fr_{D-1} to Fr_{D-45}). Fractions Fr_{D-38} to Fr_{D-41} were combined (24 g) according to their TLC behavior, chromatographed over silica gel (770 g), eluted with hexane-acetone (3 : 2, 3000 mL; 1 : 1, 3000 mL and 2 : 3, 3000 mL) and yielded 28 fractions (Fr_{D-38-1} to $Fr_{D-38-28}$). Fraction $Fr_{D-38-22}$ (2.5 g) was rechromatographed over silica gel (180 g), eluted with hexane-acetone (4 : 3, 1000 mL; 1 : 1, 1000 mL, and 3 : 4, 1000 mL) to afford 15 fractions ($Fr_{D-38-22-1}$ to $Fr_{D-38-22-15}$). Fraction $Fr_{D-38-2-13}$ (80 mg) was subjected to a preparative TLC (3 × 20 × 20 cm, thickness 0.50 mm) developed with CH_2Cl_2-CH_3CN = 5 : 8, the zone located at R_f = 0.41 under UV light was scraped, harvested, and washed with acetone and further purified by preparative TLC (1 × 20 × 20 cm, thickness 0.25 mm) developed with hexane-acetone = 5 : 6. Finally 2.0 mg of **1** was provided (R_f = 0.53). Fraction $Fr_{D-38-22-14}$ (59 mg) was separated by preparative HPLC (Whatman partisil 10 ODS-2 Mag-20 prep. Column, 22 × 500 mm, eluting solvent: a linear gradient of CH_3CN in water from 25 % to 100 % in 50 min at a flow rate of 18 mL min^{-1}) and offered 1.5 mg of **2** (t_R = 20.04 min).

*2α,7β,9α,10β,13-Pentaacetoxy-11β-hydroxy-5α-(2′-hydroxy-3′-N,N-dimethylamino-3′-phenyl)-propanoyloxytaxa-4(20),12-diene (**1**)*

Amorphous solid; $[\alpha]_D^{22}$ = +37° (c = 0.3, $CHCl_3$). – ^{1}H and ^{13}C NMR spectral data see Table 1. – HR-FABMS: *m/z* = 824.3260 (calcd. 824.3253 for $C_{41}H_{55}NO_{14}K$, $[M + K]^+$), 808.3520 (calcd. 808.3513 for $C_{41}H_{55}NO_{14}Na$, $[M + Na]^+$), 786.3701 (calcd. 786.3703 for $C_{41}H_{55}NO_{14}H$, $[M + H]^+$), 134.0984 (calcd. 134.0970 for $C_9H_{12}N$), 210.1116 (calcd. 210.1130 for $C_{11}H_{16}NO_3$).

*13α,20β-Diacetoxy-5α,7β,9α,10β-tetrahydroxy-2α,20-epoxy-11(15→1)-abeotaxa-11,15-diene (**2**)*

Amorphous solid; $[\alpha]_D^{22}$ = +23° (c = 0.1, $CHCl_3$). – ^{1}H and ^{13}C NMR spectral data see Table 2. – HR-FABMS: *m/z* = 489.2097 (calcd. 489.2101 for $C_{24}H_{34}O_9Na$, $[M + Na]^+$).

NMR spectra

All NMR spectra were recorded on a Bruker Avance DRX-500 NMR spectrometer equipped with 5 mm probes at ambient temperature in $CDCl_3$ solution. The ^1H and ^{13}C chemical shifts were referenced relative to the corresponding residual solvent signals (δ ^1H = 7.26 and δ ^{13}C = 77.7, respectively). Typical one-dimensional (1D) ^1H and ^{13}C spectra were acquired under standard conditions on the spectrometer operated at 500.13 and 125.77 MHz, respectively. The ^1H and ^{13}C chemical shifts were expressed on δ as parts per million (ppm) scale and coupling constants (J) expressed in Hz; splitting patterns have been designated as following: s (singlet), d (doublet), m (multiplet), br (broad), and dd (double doublet). Two-dimensional (2D) hydrogen-detected ^1H, ^{13}C heteronuclear chemical shift correlation spectra (HMQC) and long-range correlation spectra (HMBC) were recorded using the standard Bruker pulse program [^1J(C, H) = 150 Hz, f_2 32000 Hz and f_1 8000 Hz, relaxation delay = 1.0 s; MJ (the delay used for J modulation) = 70 ms, ^nJ(C, H) = 8 Hz, f_2 32000 Hz and f_1 8000 Hz, relaxation delay = 1.0 s, respectively].

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[1] E. Baloglu, D. G. I. Kingston, *J. Nat. Prod.* **1999**, *62*, 1448.
[2] S. H. Li, H. J. Zhang, X. M. Niu, P. Yao, H. D. Sun, H. H. S. Fong, *Tetrahedron* **2003**, *59*, 37.
[3] H. Morita, I. Machida, Y. Hirasawa, J. Kobayashi, *J. Nat. Prod.* **2005**, *68*, 935.
[4] Y. C. Shen, Y. C. Lin, Y. B. Cheng, K. C. Cheng, A. T. Khalil, J. H. Guh, Y. H. Kuo, C. T. Chien, Y. C. Lin, *Tetrahedron* **2005**, *61*, 1345.
[5] Y. C. Shen, K. C. Cheng, Y. C. Lin, Y. B. Cheng, A. T. Khalil, J. H. Guh, C. T. Chien, C. M. Teng, Y. T. Chang, *J. Nat. Prod.* **2005**, *68*, 90.
[6] Y. C. Shen, S. S. Wang, Y. L. Pan, K. L. Lo, R. Chakraborty, C. T. Chien, Y. H. Kuo, Y. C. Lin, *J. Nat. Prod.* **2002**, *65*, 1848.
[7] Y. C. Shen, Y. T. Chang, Y. C. Lin, C. L. Lin, Y. H. Kuo, C. Y. Chen, *Chem. Pharm. Bull.* **2002**, *50*, 781.
[8] Z. Xia, L. Peng, Y. Zhao, G. Xua, Q. Zhao, H. Sun, *Chem. Biodiv.* **2005**, *2*, 1316.
[9] T. L. Petzke, Q. W. Shi, F. Sauriol, O. Mamer, L. O. Zamir, *J. Nat. Prod.* **2004**, *67*, 1864.
[10] H. Morita, I. Machda, Y. Hirasawa, J. Kobayashi, *J. Nat. Prod.* **2005**, *68*, 935.
[11] Q. W. Shi, F. Sauriol, O. Mamer, O. L. Zamir, *Bioorg. Med. Chem.* **2003**, *11*, 293.
[12] Q. W. Shi, F. Sauriol, O. Mamer, O. L. Zamir, *J. Chem. Soc., Chem. Commun.* **2003**, 68.
[13] L. G. Li, C. M. Cao, C. H. Huo, M. L. Zhang, Q. W. Shi, H. Kiyota, *Mag. Res. Chem.* **2005**, *43*, 475.
[14] Q. W. Shi, F. Sauriol, Y. Park, V. H. Smith, G. Lord, L. O. Zamir, *Mag. Res. Chem.* **2005**, *43*, 798.
[15] F. Gueritte, *Current Pharmaceutical Design* **2001**, *7*, 1229.
[16] D. G. I. Kingston, *J. Chem. Soc., Chem. Commun.* **2001**, 867.
[17] J. Kobayashi, H. Hosoyama, X. X. Wang, H. Shigemori, Y. Koiso, S. Iwasaki, T. Sasaki, M. Naito, T. Tsuruo, *Bioorg. Med. Chem. Lett.* **1997**, *7*, 393.
[18] K. Kosugi, J. Sakai, S. Zhang, Y. Watanabe, H. Sasaki, T. Suzuki, H. Hagiwara, N. Hirata, K. Hirose, M. Ando, A. Tomida, T. Tsuruo, *Phytochemistry* **2000**, *54*, 839.
[19] L. O. Zamir, M. E. Nedea, S. Bélair, F. Sauriol, O. Mamer, E. Jacqmain, F. I. Jean, F. X. Garneau, *Tetrahedron Lett.* **1992**, *33*, 5173.
[20] G. P. Gunawardana, U. Premachandran, N. S. Burres, D. N. Whittern, R. Henry, S. Spanton, J. McAlpine, *J. Nat. Prod.* **1992**, *55*, 1686.
[21] L. O. Zamir, M. E. Nedea, Z. H. Zhou, S. Bélair, G. Caron, F. Sauriol, E. Jacqmain, F. I. Jean, F. X. Garneau, O. Mamer, *Can. J. Chem.* **1995**, *73*, 655.
[22] J. Zhang, F. Sauriol, O. Mamer, X. L. You, M. Alaoui-Jamali, G. Batist, L. O. Zamir, *J. Nat. Prod.* **2001**, *64*, 450, and refs. therein.
[23] A. Nikolakakis, G. Caron, A. Cherestes, F. Sauriol, O. Mamer, L. O. Zamir, *Bioorg. Med. Chem.* **2000**, *8*, 1269.
[24] G. Appendino, H. C. Ozen, I. Fenoglio, P. Gariboldi, B. Gabetta, E. Bombardelli, *Phytochemistry* **1993**, *33*, 1521.
[25] J. A. Beutler, G. M. Churny, S. A. Looks, K. M. Witherup, *J. Nat. Prod.* **1991**, *54*, 893.
[26] J. Kobayashi, H. Hosoyama, H. Shigemori, Y. Koiso, S. Iwasaki, *Experienta* **1995**, *51*, 592.
[27] J. Kobayashi, H. Hosoyama, T. Katsui, N. Yoshida, H. Shigemori, *Tetrahedron* **1996**, *52*, 5391.
[28] G. Appendino, in *The Chemistry and Pharmacology of Taxol and Its Derivatives*, (Ed.: V. Farina), Elsevier, Amsterdam **1995**, p. 7.
[29] K. Fuji, K. Tanaka, B. Li, T. Shingu, T. Yokoi, H. D. Sun, T. Taga, *Tetrahedron* **1995**, *51*, 10175.
[30] Q. W. Shi, T. Oritani, T. Sugiyama, T. Horiguchi, R. Murakami, *Tetrahedron* **1999**, *55*, 8365.

[31] Y. C. Shen, Y. T. Chang, Y. C. Lin, C. L. Lin, Y. H. Kuo, C. Y. Chen, *Chem. Pharm. Bull*. **2002**, *50*, 781.
[32] L. O. Zamir, J. Z. Zhang, J. H. Wu, F. Sauriol, O. Mamer, *J. Nat. Prod*. **1999**, *62*, 1268.
[33] Q. W. Shi, F. Sauriol, O. Mamer, L. O. Zamir, *J. Nat. Prod*. **2003**, *66*, 470.
[34] Q. W. Shi, F. Sauriol, Y. Park, V. H. Smith, Jr., G. Lord, L. O. Zamir, *Magn. Reson. Chem*. **2005**, *43*, 798.
[35] W. S. Fang, Q. C. Fang, X. T. Liang, Y. Lu, Q. T. Zheng, *Tetrahedron* **1995**, *51*, 8483.
[36] H. Shigemori, X. X. Wang, N. Yoshida, J. Kobayashi, *Chem. Pharm. Bull*. **1997**, *45*, 1205.
[37] L. O. Zamir, Z. H. Zhou, G. Carron, M. E. Nedea, F. Sauriol, O. Mamer, *J. Chem. Soc., Chem. Commun*. **1995**, 529.
[38] Y. C. Shen, S. M. Hsu, Y. S. Lin, K. C. Cheng, C. T. Chien, C. H. Chou, Y. B. Chen, *Chem. Pharm. Bull*. **2005**, *53*, 808.
[39] Q. W. Shi, T. Oritani, T. Horiguchi, T. Sugiyama, T. Yamada, *Biosci. Biotech. Biochem*. **1999**, *63*, 756.
[40] R. A. Jones, K. J. Stokes, *Tetrahedron* **1984**, *40*, 1051.