New Taxane Diterpenoids from Taiwanese *Taxus sumatrana*

Ya-Ching SHEN,* Yu-Ling PAN, Kuang-Liang LO, Shih-Sheng WANG, Yao-To CHANG, Li-Tang WANG, and Yu-Chi LIN

Institute of Marine Resources, National Sun Yat-sen University; 70 Lien-Hai Rd., Kaohsiung, Taiwan, Republic of China.
Received January 27, 2003; accepted March 10, 2003

Two new taxane diterpenoids, tasumatrols A (1) and B (2), have been isolated from extracts of the leaves and twigs of Taiwanese *Taxus sumatrana*. Tasumatrol A is a rare 5/6/6 taxene system, having a novel γ-lactone at C-10 and C-19. The structures of compounds 1 and 2 were determined on the basis of two dimensional (2D)-NMR techniques, including correlation spectroscopy (COSY), ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC) experiments.

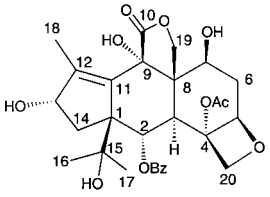
Key words *Taxus sumatrana*; Taxaceae; taxane diterpenoid; tasumatrol

Several review articles about natural taxoids have been published in recent years.[1—4] A number of new taxoids have been isolated from different *Taxus* species.[5—7] Some of them possess interesting bioactivity as modulators of multidrug-resistant tumor cells.[8] Although more than 400 taxane diterpenoids have been isolated to date, there are still new taxoids awaiting to be discovered. *Taxus sumatrana* (MIQ.) de LAUB. (Taxaceae) is a rare plant growing at a high altitude (2600 m) in central Taiwan.[9] In our continuing search for new taxane diterpenoids with novel skeleton,[10—15] we herein report the isolation of two new taxoids along with some known taxoids from the male tree of *T. sumatrana*.

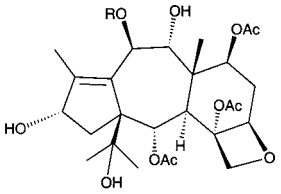
Results and Discussion

The acetone extract of *T. sumatrana* was fractionated by repeated column chromatography and preparative TLC to furnish tasumatrols A (**1**) and B (**2**) in addition to wallifoliol,[16] taxumairol V,[15] 7-*epi*-10-deacetyl-10-oxobaccatin V[17] and 10-deacetylbaccatin III,[18] 19-hydroxybaccatin III[18] and 10-deacetyltaxol C.[19] Structures of known compounds were confirmed by comparison of spectral data with literature values.

Tasumatrol A (**1**), $[\alpha]$ −12° (MeOH), had a molecular formula of $C_{29}H_{34}O_{11}$ as derived from FAB-MS and distortionless enhancement by polarization transfer (DEPT) spectra. IR indicated the presence of benzoyl (1710, 1454, 1370 cm^{-1}), hydroxyl (3460 cm^{-1}) and acetyl (1740 cm^{-1}) and γ-lactone (1772 cm^{-1}) groups. The ^{1}H-NMR data of **1** (Table 1) indicated a benzoyl group (δ 7.98, d, J=7.5 Hz; δ 7.45, t, J=7.5 Hz; δ 7.54, t, J=7.5 Hz), an acetyl singlet (δ 2.18), three methyl singlets (δ 1.05, 1.23, 2.31), two pairs of coupled doublets at δ 5.03, 4.95 (J=10 Hz, H-19), 4.37 and 4.85 (J=8.8 Hz, H-20), and four oxygenated methine protons at δ 6.19 (d, J=11.5 Hz, H-2), δ 4.76 (d, J=7.5 Hz, H-5), δ 4.47 (m, H-7), and δ 4.60 (m, H-13). However, the signals of H-9 and H-10 were missing. This finding was supported by the correlation spectroscopy (COSY) spectrum of **1**, which showed only the connectivities between H-2/H-3, H-5/H-6/H-7, and H-13/H-14. Analysis of the ^{13}C-NMR (Table 1) and DEPT spectra revealed that **1** contains four methylene carbons at δ 35.8 (C-6), 41.1 (C-14), 66.8 (C-19) and 74.3



1



2 R = H
3 R = Ac

Table 1. ^{1}H- and ^{13}C-NMR ($CDCl_3$) Spectral Data of Tasumatrol A (**1**)

Position	^{13}C (ppm)[a]	^{1}H[b]	COSY	HMBC
1	61.4 s			Me16, Me17, H2 H14
2	69.0 d	6.19 (d, 11.5)	H3	H3, H14
3	41.6 d	2.63 (d, 11.5)	H2, H20A	H5, H2, H20 H19
4	80.2 s			H3, H5, H20
5	84.3 d	4.76 (d, 7.5)	H6	H3, H6, H20
6	35.8 t	2.75 m, 1.86 m	H5, H7	
7	69.5 d	4.47 m	H6	H3, H5, H6, H19
8	50.5 s			H3, H6, H19
9	80.4 s			H3
10	177.0 s			H19
11	134.5 s			H13, H14 Me18
12	148.0 s			H13, H14b Me18
13	79.2 d	4.60 m	H14	Me18
14	41.1 t	2.16 m	H13	
15	76.5 s			H14, H2 Me16, Me17
16	26.7 q	1.05 s		Me17
17	26.8 q	1.23 s		Me16
18	14.5 q	2.31 s		
19A	66.8 t	5.03 (d, 10.0)		H3, H7
19B		4.95 (d, 10.0)		
20A	74.3 t	4.37 (d, 8.8)	H20B	
20B		4.85 (d, 8.8)	H20A	
4-OAc	169.7 s	2.18 s		
	20.8 q			
$OCOC_6H_5$	164.6 s			H2, *o*-C_6H_5
i	134.5 s			
o	129.5 d	7.98 (d, 7.5)	*m*-C_6H_5	*p*-C_6H_5, *m*-C_6H_5
m	128.6 d	7.45 (t, 7.5)	*o*, *p*-C_6H_5	
p	133.4 d	7.54 (t, 7.5)	*m*-C_6H_5	*o*-C_6H_5
OH		3.55 (br s), 4.50 (br s)		

a) S=C, D=CH, T=CH_2, Q=CH_3. Multiplicities and assignments made by HMQC and HMBC. *b*) Multiplicities and coupling constants in Hz in parentheses.

* To whom correspondence should be addressed. e-mail: ycshen@mail.nsysu.edu.tw

(C-20), and four oxygenated methine carbons at δ 69.0 (C-2), 84.3 (C-5), 69.5 (C-7) and 79.2 (C-13). Three oxygenated quaternary carbons appeared at δ 80.2 (C-4), 80.4 (C-9) and 76.5 (C-15). Comparison of the ^{1}H- and ^{13}C-NMR spectra of **1** with those of wallifoliol revealed that they had similar data at C-13, C-20 and from C-1 to C-8, as well as the data of their attached protons. However, the data from C-9 to C-12 and from C-14 to C-19 were quite different. It was suggested that the lactone ring in wallifoliol should be opened in **1**. This result led to a conclusion that one more ring is required to account for the unsaturation degree of 13. In the heteronuclear multiple bond connectivity (HMBC) spectrum, both methyl protons at δ 1.05 (H-16) and 1.23 (H-17) exhibited cross peaks with each of the carbon signals at δ 76.5 (C-15) and δ 61.4 (C-1), which proved that the dimethyl carbinol group is linked to C-1. Moreover, long range correlations among H-19/C-10 and C-19/H-3, H-7, and H-3/C-8, C-9 indicated that compound **1** contains a γ-lactone ring between the C-8 and C-9 positions. Other HMBC correlations (Table 1), such as H-2/C-1, C-15, C-3, H-3/C-2, C-4, C-7, C-20 and Me-18/C-11, C-12, H-19/C-3 and H-6/C-4, C-5, C-7 and H-14/C-1, C-2, C-12, C-15 fully supported the proposed structure of **1** as having a rearranged 5/6/6-membered skeleton with a γ-lactone ring. The benzoyl group was attached to C-2, as evidenced from the HMBC correlation of H-2 (δ 6.19) with the benzoyl carbonyl signal (δ 164.6). The relative stereochemistry of **1** was assumed to be the same as that of wallifoliol and 13-*O*-acetyl wallifoliol due to the similar coupling constants and spin pattern in the ^{1}H-NMR spectra.[20]

Tasumatrol B (**2**), $[\alpha]$ −7.6° (MeOH), had the molecular formula $C_{26}H_{38}O_{11}$, as determined by a combination of low resolution FAB-MS and NMR spectra. Its IR bands indicated the presence of hydroxyl (3430 cm^{-1}) and acetyl (1735, 1725 cm^{-1}) groups. The presence of hydroxyls and acetoxyls was verified from the ^{1}H- and ^{13}C-NMR spectral data of **2**. A taxene skeleton was inferred from the observation of characteristic resonances, such as four methyl singlets (δ 1.10, 1.04, 1.86, 1.94) and three acetoxyls (δ 2.15, 2.06, 2.00). Its COSY spectrum determined the connectivity of H-2/H-3, H-5/H-6/H-7, H-9/H-10, H-13/H-14 in **2**. The signals at δ 5.81 (H-2) and δ 5.28 (H-7) suggested that they were connected with acetoxyl groups, while signals of δ 4.20 (H-9), 4.55 (H-10) and δ 4.54 (H-13) had attached hydroxyl groups. Furthermore, HMBC correlations of H-9/C-19, H-7/C-19 and H-3/C-19, H-2/C-15, H-16/C-1, C-15, Me-17/C-1, C-15 and H-3/C-2, C-4, C-5, C-8, C-19, C-20 confirmed the structure of **2**. Consequently, the NMR data unambiguously assigned the acetoxyl groups at C-2 and C-7, and thus the hydroxyl groups at C-9, C-10 and C-13. The relative stereochemistry of **2** was determined by comparison of the chemical shifts and coupling constants of **2** with those of taxumairol W (**3**).[15] The multiplicities and coupling constants of H-2 (d, 7.5 Hz), H-3 (d, 7.5 Hz), H-5 (d, 7.6 Hz), H-7 (t, 8.0 Hz), H-9 (d, 10.0 Hz), H-10 (d, 10.0 Hz) and H-13 (t, overlapped) were in good agreement with those of **3**, suggesting that **2** had the same chirality as **3**.

Among the isolated taxoids, compound **1** is a novel diterpene having a γ-lactone ring at the C-8 and C-9 positions. This is the first report in taxane chemistry. The occurrence of tasumatrols A and B in Taiwanese *T. sumatrana* may be of chemotaxonomic significance.

Experimental

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. FAB-MS were measured with VG Quattro 5022 and JEOL JMS-SX 102 spectrometers. ^{1}H- and ^{13}C-NMR, COSY, ^{1}H-detected heteronuclear multiple quantum coherence (HMQC), HMBC and nuclear Overhauser effect spectroscopy (NOESY) spectra were recorded using a Bruker FT-300 (AVANCE) or a Varian FT-500 (ANOVA) NMR instrument. HPLC were performed using HITACHI L-6250, intelligent pump HITACHI L-4000H, HITACHI integrator D-7500, Lichrosorb Si-60 and Lichrosorb RP-C_{18} column. All chemicals were procured from E. Merck (Germany), and were used without further purification.

Plant Material Leaves and twigs with male flowers of *Taxus sumatrana* (Miq.) de Laub. were collected from Nan-tou County at an altitude of 2600 m in March, 2001. A voucher specimen (TPG 8-5) was deposited in the Institute of Marine Resources, National Sun Yat-sen University.

Extraction and Isolation Dried leaves and twigs (7.8 kg) of *Taxus sumatrana* were extracted with 40 l of *n*-hexane to give a crude extract (123 g). This marc was then successively extracted with 40 l of EtOAc, 40 l of acetone and 20 l of MeOH, to give an EtOAc extract (300 g), acetone extract (350 g) and MeOH extract (250 g). The acetone extract (350 g) was chromatographed over LH-20 (MeOH) to give Tax-A (130 g). Further column chromatography over silica gel using *n*-hexane–CH_2Cl_2–MeOH (100 : 100 : 1—2 : 2 : 1) as an eluent gave seventeen fractions 1—17. Fraction 14 (3 g) was applied on an LH-20 column using MeOH as eluent to give taxumairol V (5 mg), 14A (230 mg) and 14B (313 mg). Fraction 14B was chromatographed on a RP-C_{18} column using MeOH–H_2O (35 : 65, 45 : 55, 55 : 45, 60 : 40), and further by PTLC (Si gel, *n*-hexane/acetone, 2 : 1) to afford the known 7-*epi*-10-deacetyl-10-oxobaccatin V (1 mg), 10-deacetylbaccatin III and wallifoliol, and the new compound **1** (1 mg). Fraction 14A (230 mg) was chromatographed on a RP-C_{18} column using MeOH/H_2O (3 : 7) as eluent to give compound **2** (2.5 mg).

Tasumatrol A (**1**): Isolated as an amorphous solid: $[\alpha]_D^{25}$ −12° (c=0.1, MeOH); IR (neat) ν_{max} 3460, 1772, 1740, 1710, 1620, 1454 and 1370 cm^{-1}; ^{1}H- and ^{13}C-NMR ($CDCl_3$): Table 1; FAB-MS *m/z*: 559 $[M+H]^+$, 581 $[M+Na]^+$.

Tasumatrol B (**2**): Isolated as an amorphous powder: $[\alpha]_D^{25}$ −7.6° (c=0.2, MeOH); IR (neat) ν_{max} 3430, 1735, 1725, 1610, 1425, 1372 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ: 5.81 (1H, d, J=7.5 Hz, H-2), 3.00 (1H, d, J=7.5 Hz, H-3), 4.92 (1H, d, J=7.6 Hz, H-5), 2.60 (1H, m, H-6a), 1.85 (1H, m, H-6b), 5.28 (1H, t, J=8.0 Hz, H-7), 4.20 (1H, d, J=10.0 Hz, H-9), 4.55 (1H, d, J=10.0 Hz, H-10), 4.54 (1H, overlap, H-13), 1.52 (1H, m, H-14a), 2.15 (1H, m, H-14b), 1.04 (3H, s, H-16), 1.10 (3H, s, H-17), 1.94 (3H, s, H-18), 1.86 (3H, s, H-19), 4.52 (1H, J=7.5 Hz, H-20a), 4.38 (1H, d, J=7.5 Hz, H-20b), 2.00, 2.06, 2.15 (s, $OCOCH_3$); ^{13}C-NMR (125 MHz, acetone-d_6) δ: 68.3 (s, C-1), 69.2 (d, C-2), 45.5 (d, C-3), 80.2 (s, C-4), 85.6 (d, C-5), 37.1 (t, C-6), 70.5 (d, C-7), 43.0 (s, C-8), 78.8 (d, C-9), 69.5 (d, C-10), 138.3 (s, C-11), 147.4 (s, C-12), 77.4 (d, C-13), 38.5 (t, C-14), 76.3 (s, C-15), 25.0 (q, C-16), 28.4 (q, C-17), 11.5 (q, C-18), 12.5 (q, C-19), 75.0 (t, C-20), 170.0, 170.5, 171.6 (s, $O\underline{C}OCH_3$), 21.9, 22.0, 22.7 (q, $OCO\underline{C}H_3$); FAB-MS *m/z*: 527 $[M+H]^+$, 549 $[M+Na]^+$.

Acknowledgments The authors thank the National Institute of Health, Republic of China (NHRI-EX-90-8809BL), for the financial support. We thank Ms. Chao-Lein Ho and Ms. Shiu-Ching Yu of NSC Southern NMR and MS Instrument Center for high-resolution NMR (500 MHz) and MS data. We also thank Dr. Ching-Te Chien, Taiwan Forestry Research Institute, for his collection and identification of male Yew *Taxus sumatrana*.

References

1) Baloglu E., Kingston D. G. I., *J. Nat. Prod.*, **62**, 1448—1472 (1999).
2) Kingston D. G. I., *J. Nat. Prod.*, **63**, 726—734 (2000).
3) Parmar V. S., Jha A., Bisht K. S., Taneja P., Singh S. K., Kumar A., Poonam Jain R., Olsen C. E., *Phytochemistry*, **50**, 1267—1304 (1999).
4) Kingston D. G. I., Xuan H., Jagtap P. G., Samada L., *Prog. Chem. Org. Nat. Prod.*, **35**, in press (2002).
5) Li S. H., Zhang H. J., Yao P., Sun H. D., Fong H. H. S., *J. Nat. Prod.*, **63**, 1488—1491 (2000).
6) Sakai J. I., Sasaki H., Kosugi K., Zhang S., Hirata N., Hirose K., Tomida A., Tsuruo T., Ando M., *Heterocycles*, **54**, 999—1009 (2001).
7) Zhang J., Sauriol F., Mamer O., You X. L., Jamali M. A. A., Batist G., Zamir L. O., *J. Nat. Prod.*, **64**, 450—455 (2001).
8) Li H. L., Keng H., "Flora of Taiwan," 2nd ed., Vol. 1, ed. by Huang T. C., Nat. Sci. Coun., R.O.C., Taipei, 1975, pp. 550—552.

9) Shen Y. C., Tai H. R., Chen C. Y., *J. Nat. Prod.*, **59**, 173—176 (1996).
10) Shen Y. C., Chen C. Y., *Phytochemistry*, **44**, 1527—1533 (1997).
11) Shen Y. C., Chen C. Y., Kuo Y. H., *J. Nat. Prod.*, **61**, 838—840 (1998).
12) Shen Y. C., Chen C. Y., Chen Y. J., *Planta Med.*, **65**, 582—584 (1999).
13) Shen Y. C., Chen C. Y., Hung M. C., *Chem. Pharm. Bull.*, **48**, 1344—1346 (2000).
14) Shen Y. C., Lo K. L., Chen C. Y., Kuo Y. H., Hung M. C., *J. Nat. Prod.*, **63**, 720—722 (2000).
15) Shen Y. C., Prakash C. V. S., Chen Y. J., Hwang J. F., Kuo Y. H., Chen C. Y., *J. Nat. Prod.*, **64**, 950—952 (2001).
16) VanderVelde D. G., George G. I., Gollapudi S. R., Jampani H. B., Liang X. Z., Mitscher L. A., Ye Q. M., *J. Nat. Prod.*, **57**, 862—867 (1994).
17) Fuji K., Tanaka K., Li B., Shingu T., Sun H., Taga T., *J. Nat. Prod.*, **56**, 1520—1531 (1993).
18) Chauviere G., Guenard D., Picot F., Senilh V., Potier P., *Acad. Sci. Paris*, *Ser. II*, **293**, 501—503 (1981).
19) Kobayashi J., Inubushi A., Hosoyama H., Yoshida N., Sasaki T., Shigemori H., *Tetrahedron*, **51**, 5971—5978 (1995).
20) Shen Y. C., Wang S. S., Pan Y. L., Lo K. L., Chakraborty R., Chien C. T., Kuo Y. H., Lin Y. C., *J. Nat. Prod.*, **65**, 1848—1852 (2002).