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TWO NEW TAXOIDS FROM THE NEEDLES AND YOUNG STEMS OF *TAXUS CUSPIDATA*

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Abstract – A new basic taxoid, taxine NA-13 (**1**) and a new neutral taxoid, 3α,11α-cyclotaxinine NN-2 (**2**) were isolated from Japanese yew tree, *Taxus cuspidata*. The structures of two new taxoids **1** and **2** were established as shown in structure **1** and structure **2** by spectroscopic analysis.

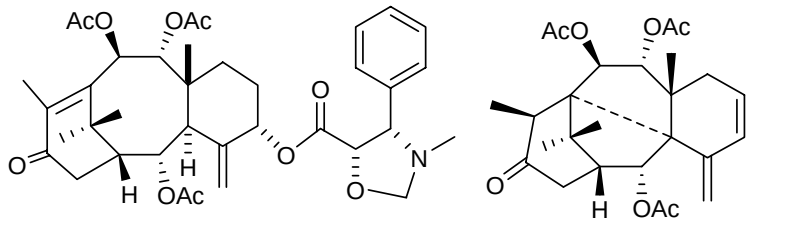
INTRODUCTION

Since the discovery of the anticancer activity of paclitaxel (Taxol®) against various cancers as well as HIV-associated Kaposi's sarcoma, much attention has been paid to the isolation of new taxoids from various species of yews.[1] The needles and young stems of Japanese yew, *Taxus cuspidata* contain an impressive array of taxoids.[2,3,4] However, the content of the antitumor paclitaxel in the needles and young stems of this species is generally low. Taxane derivatives occurring in consistently large amounts are taxinine[5] as a neutral taxoid, and 2'-hydroxytaxine II (taxine NA-1)[6] and taxine II[7] as basic taxoids. As a part of study on taxoids, we conducted an investigation on the needles and young stems of *T. cuspidata* as a source of taxoids.

RESULTS AND DISCUSSION

Air-dried needles and young stems of *Taxus cuspidata* were extracted successively with hexane, EtOAc,

and MeOH. The MeOH-soluble portion was further extracted with $CHCl_3$. The $CHCl_3$ extract gave a new basic taxoid, taxine NA-13 (**1**) and EtOAc extract gave 3α,11α-cyclotaxinine NN-2 (**2**) (Figure 1).



taxine NA-13 (**1**) 3α,11α-cyclotaxinine NN-1 (**2**)

Figure 1

Taxine NA-13 (**1)** was obtained as a colorless grain crystal. Its molecular formula was determined to be $C_{37}H_{48}O_{10}N$ $[M+H]^+$ from the peak at m/z 666.3279 $[M+H]^+$ (calcd for $C_{37}H_{48}O_{10}N$ 666.3278), in the HREIMS. The IR spectrum indicated the presence of ester carbonyl (1744 cm^{-1}) and α,β-unsaturated carbonyl (1674 cm^{-1}) groups. Its ^{13}C NMR spectrum displayed 35 carbon resonances. One keto carbonyl carbon and four ester carbonyl carbons resonated at δ_C 198.8, and 171.2, 169.8, 169.7, and 169.4, respectively. Four olefin carbon resonances were located at δ_C 149.0 (s), 141.1 (s), 138.0 (s), and 118.6 (t). Five resonances for carbons bearing oxygen were observed at δ_C 82.3 (d), 78.8 (d), 75.7 (d), 73.1 (d), and 69.5 (d) in addition to those for a carbon bearing nitrogen at δ_C 73.5 (d), an *N*-Me carbon at δ_C 36.5 (q), a methylene carbon connecting nitrogen and oxygen atoms at δ_C 89.3 (t), and three acetyl methyl carbons at δ_C 24.4 (q), 20.8 (q), and 20.7 (q). From the DEPT and HMQC spectra, the remaining carbon resonances were four methyl carbons, three methylene carbons, two methine carbons, and two quaternary carbons. The ^1H NMR spectra showed four methyl singlets (δ 0.85, 1.04, 1.54 and 1.65). The connectivity of the protonated carbons (C-1 through C-3; C-5 through C-7; C-9 and C-10, and C-14 and C-1) was determined by the ^1H-^1H COSY spectrum. An HMBC experiment was used to determine the carbon-carbon connection through the nonprotonated carbon atoms [HMBC correlations: C-4 (δ_C 141.1) with H-3, H_2-20; C-8 (δ_C 44.4) with H-2, H-3, H_3-19; C-11 (δ_C 149.0) with H-1, H-9, H-10, H_3-16, H_3-17, H_3-18; C-12 (δ_C 138.0) with H-10, H_2-14, H_3-18; C-13 (δ_C 198.8) with H-1, H_2-14, H_3-18. HMBC correlations [C-1 (δ_C 48.4) with H-2, H-3, H_2-14, H_3-16, H_3-17] indicated the connections of C_1 with C_{14}, C_1 through C_3, C_1 to C_{16} and C_{17} through C_{15}. HMBC correlations [C-7 (δ_C 27.3) with H-3, H-5, H_3-19] indicated the connections of C_7 to C_3 through C_8, C_7 to C_{19} through C_8, and C_7 to C_5 through C_6. HMBC correlations [C-20 (δ_C 118.6) with H-3, H-5] were used to place an exo-methylene at C-4. Interpretation of these results suggests that compound **1** has taxoidal A, B, and C rings bearing an α,β-unsaturated carbonyl group at C-13. The HMBC correlations of three acetoxy carbonyl groups [(δ_C 169.4) with H-2; (δ_C 169.8) with H-9; (δ_C 169.7) with H-10] were used to place acetoxy groups at C-2, C-9, and C-10. The correlation of signal due to *N*-methyl-*N*,*O*-methylene-3-phenylisoseryl carbonyl C-1' (δ_C 171.2) with

those of H-5 (δ 5.31), H-2' (δ 4.22) and H-3' (δ 3.48) indicated the location of the (*N*-methyl-*N*, *O*-methylene-3-phenylisoseryl) oxy group at C-5. The presence of methylene bridge between oxygen and nitrogen atoms and a methyl group connecting with nitrogen atom in isoseryloxy group at C-5 was indicated by HMBC correlation [C-4' (δ_C 89.3) with H-2', H-3', and *N*-methyl protons; *N*-methyl carbon with H-3', H_2-4'; C-3' with H-2', H_2-4' , and *N*-methyl protons.

The full stereostructure of the taxane skeleton of **1** was determined by NOESY experiments as well as by a consideration of vicinal coupling constants. The NOE correlation of compound **1** determined by the NOESY experiment is summarized in (Figure 2). The results are in good agreement with the relative stereostructure in taxane skeleton of compound **1**. The existence of NOESY correlation of H-2' with H-3' of the cyclic phenyl isoseryloxy side chain at C-5 indicated H-2' and H-3' to be *cis*-orientation. Considering the C-3' stereochemistry of isophenylalanyloxy side chain and 3-phenylisoseryloxy side chain of known taxanes of *T. cuspidata*, the C-3' stereochemistry of **1** was assumed to have (3'*S*)-stereochemistry. Thus, **1** was assumed to have (2'*S*,3'*S*) stereochemistry. This assumption was supported by the observed coupling constant ($J_{2',3'}$ = 7.6 Hz).

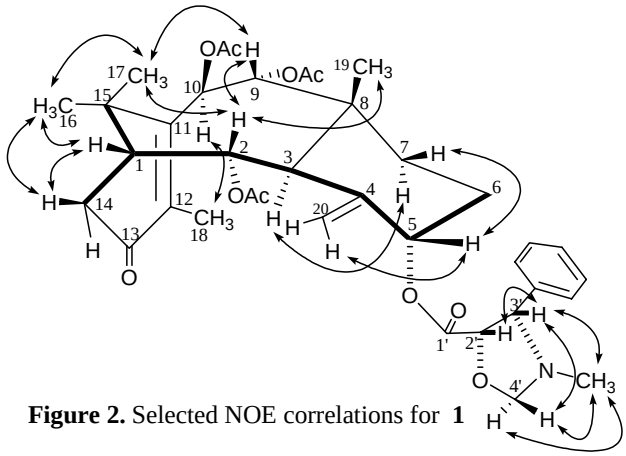
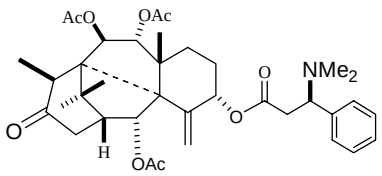


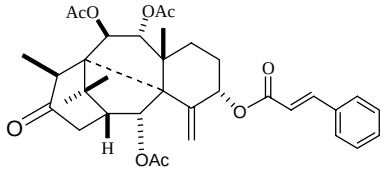
Figure 2. Selected NOE correlations for **1**

3α,11α-cyclotaxinine NN-2 (**2**) was obtained as a white amorphous powder with molecular formula $C_{26}H_{34}O_7$ deduced from HREIMS at *m/z* 458.2306 (calc. 458.2306, $C_{26}H_{34}O_7$). The IR spectrum indicated the presence of ester carbonyl (1746 cm^{-1}) and saturated keto carbonyl (1706 cm^{-1}) groups. The ^{13}C NMR spectrum displayed 26 carbon resonances. One saturated carbonyl carbon at δ_C 214.1 and three ester carbonyl carbons resonated at δ_C 170.9, 169.8, and 169.6. Four olefin carbon resonances were located at δ_C 143.2, 134.4, 131.5, and 117.0. Three resonances for carbons bearing oxygen were observed at δ_C 83.3, 80.3, and 76.3, and three acetyl methyl carbons at δ_C 21.4, 21.1, and 21.0. From the DEPT and HMQC spectra, the remaining carbon resonances were four methyl carbons, two methylene carbons, two methine carbons, and four quaternary carbons. The ^1H NMR spectra showed four methyl resonances [δ 1.19 (d, *J* = 7.3 Hz), and 1.19 (s), 1.63, 1.37]. The connectivity of the protonated carbons (C-1 and C-2; C-5 through C-7; C-9 and C-10, C-12 and C-18, and C-14 and C-1) was determined by the ^1H-^1H COSY

spectrum. An HMBC experiment was used to determine the carbon-carbon connection through the nonprotonated carbon atoms [HMBC correlations: C-3 (δ_C 66.9) with H-1, H-2, H-7α, H-12, H_3-19, and H_2-20; C-8 (δ_C 49.3) with H-2, H-7α, H-10, H_3-19; C-11 (δ_C 57.8) with H-1, H-10, H-12, H_3-16, H_3-17, and H_3-18; C-13 (δ_C 214.1) with H-1, H-12, H_2-14, and H_3-18; C-15 (δ_C 42.1) with H-1, H-10, H-12, H-14α, H_3-16, and H_3-17]. HMBC correlation [C-4 (δ_C 143.2) with H-2, H-20a; C-5 (δ_C 134.4) with H-6, H_2-7, and H_2-20; C-6 (δ_C 131.5) with H-5, and H_2-7; C-7 (δ_C 34.5) with H-9, and H_3-19] suggests the existence of an *exo*-conjugated diene in D-ring. Interpretation of these results suggests that compound **2** has 3,11-cyclotaxane A, B, C and D ring structure bearing a conjugated *exo*-double bond at 4(20), 5 positions as shown in structure **2**. The HMBC correlations [(δ_C 169.6) with H-2; (δ_C 169.8) with H-9; (δ_C 170.9) with H-10] were used to place acetoxy groups at C-2, C-9, and C-10. Although the NOESY experiment of **2** did not succeed because of the instability and limited amounts of **2**, the full stereostructure of **2** was deduced as following. The δ values of C-1 through C-3 and C-9 through C-19, the corresponding resonances of A, B, and C rings of **2**, are superimposible with those of taxuspine H (**3**)[3] and taxuspine C (**4**)[4,8] in the comparison of their ^{13}C NMR spectra (Table 1). This observation strongly



taxuspine H (**3**)



taxuspine C (**4**)

Table 1. ^{13}C NMR Data of the Taxane Skeletone of **2**, **3**, and **4** (δin ppm, in $CDCl_3$)[a]

Positio	**2**	**3**	**4**
1	48.1 (d)	47.7(d)	47.8 (d)
2	76.3 (d)	76.5(d)	76.7 (d)
3	66.9 (s)	65.8 (d)	65.9 (s)
4	143.2 (s)	141.8(s)	142.2 (s)
5	134.4 (d)	76.6 (d)	76.5 (d)
6	131.5 (d)	25.3 (t)	25.9 (t)
7	34.5 (t)	31.1(t)	31.2 (t)
8	49.3 (d)	44.4 (s)	44.5 (s)
9	80.3 (d)	79.6 (d)	79.6 (d)
10	83.3 (d)	82.3 (d)	82.3 (d)
11	57.8 (s)	57.7 (s)	57.8 (s)
12	52.2 (d)	52.3 (s)	52.3 (d)
13	214.1 (s)	214.2 (s)	214.2 (s)
14	38.7 (t)	38.8 (t)	38.8 (t)
15	42.1 (s)	42.6 (s)	42.7 (s)
16	28.1 (q)	28.7 (q)	28.8 (q)
17	26.7 (q)	26.7 (q)	26.5 (q)
18	15.8 (q)	15.6 (q)	15.7 (q)
19	26.9 (q)	26.5(q)	26.7 (q)
20	117.0 (t)	129.4 (t)	129.5 (t)

[a]Signals were assigned from the HMQC and HMBC spectra

suggests that the stereochemistry of A, B, and C rings of **2** is same with that of **3** and **4**. The full stereostructure of 3α,11α-cyclotaxane structures of **3** and **4** had already been established by photochemical transformations[3,4] of taxine II and taxinine to **3** and **4**, respectively as well as by the NOESY correlations[4] of **4**. The observed coupling constants ($J_{1,2}$ = 5.1 Hz and $J_{9,10}$ = 9.0 Hz) of **2** are also in good agreement with 2α-OAc, 9α-OAc, and 10β-OAc stereochemistries in structure **2**. Thus, the full stereostructure of **2** is 3α,9α,10β-triacetoxy-3α,11α-cyclotaxa-4(20),5-dien-13-one.

EXPERIMENTAL

General Experimental Procedures. Melting points were determined by Yanagimoto micro-melting point instrument and uncorrected. Optical rotations were measured using a Horiba Sepa-200 polarimeter. IR spectra were recorded on a Shimadzu FTIR-4200 infrared spectrometer. ^{1}H and ^{13}C NMR spectra were measured with a Varian Unity-plus instrument at 500 and 125 MHz. ^{1}H NMR assignments were determined by ^{1}H-^{1}H COSY experiments. ^{13}C NMR assignments were determined using DEPT, HMQC, and HMBC experiments. HREIMS was recorded on a JEOL JMS-HX110 instrument. Silica gel (200-400 mesh) was employed for column chromatography. HPLC separations were performed on a Hitachi L-6200 HPLC instrument monitored by a Hitachi L-7400 UV detector and a Shodex SE-61 RI detector.

Plant Material. The stems and needles of *T. cuspidata* were collected from trees of 3-m hight in Aobayama, Sendai, Japan on October 1, 2002. The plant was identified by Dr. K. Yonekura, Department of Biology, Faculty of Science, Tohoku University, Sendai, Japan. A voucher specimen (2002-10-1) was deposited at the Department of Chemistry and Chemical Engineering, Niigata University.

Extraction and isolation. Fresh needles and young stems of *T. cuspidata* (4.2 Kg) were extracted with hexane, EtOAc and MeOH. The MeOH-soluble portion was extracted with $CHCl_3$. The extract with $CHCl_3$ (71.8 g) was separated by silica gel column chromatography and normal phase HPLC, repeatedly. Finally, compound **1** was isolated by HPLC [25 × 2 cm i.d. stainless steel column packed with Inertsil Prepsil; EtOAc–hexane (7:3); 5 mL/min; t_R, 6.5 min]. The yield of **1** was 30.4 mg (0.00064%).

The extract with EtOAc (9.26 g) was separated by silica gel column chromatography and normal phase HPLC, repeatedly. Finally, compound **2** was isolated by HPLC [linearly connected (25 cm × 1 cm i.d. stainless steel column packed with Inertsil Prep-ODS) and (25 × 1 cm i.d. stainless steel column packed with Shodex-ODS); MeOH–0.05 M NH_4OAc buffer (pH 4.8)–MeCN (1:2:2); 5 mL/min; t_R, 24.1 min]. The yield of **2** was 7.7 mg (0.00018%).

Taxine NA-13 (1): colorless grain crystal, mp 96-100 °C, $[\alpha]^{20}_D$ +34.2° ($CHCl_3$, *c*, 0.15); ^{1}H NMR ($CHCl_3$) δ 7.42–7.41 (2H, m, *m*-Ph), 7.29–7.36 (3H, m, *p*-, *o*-Ph),, 5.80 (1H, br s, H-9), 5.80 (1H, br s, H-10), 5.46 (1H, dd, *J* = 6.6, 2.0 Hz, H-2), 5.40 (1H, br s, H-20a), 5.31 (1H, br s, H-5), 4.87 (1H, d, *J* =

2.9 Hz, H-4'α), 4.85 (1H, br s, H-20b), 4.28 (1H, d, *J* = 2.7 Hz, H-4'β), 4.22 (1H, d, *J* = 7.6 Hz, H-2'), 3.48 (1H, d, *J* = 7.6 Hz, H-3'), 2.95 (1H, br d, *J* = 6.6 Hz, H-3), 2.71 (1H, dd, *J* = 19.8, 7.1 Hz, H-14β), 2.25 (3H, s), 2.19 (1H, d, *J* = 20.0 Hz, H-14α), 2.12 (1H, dd, *J* = 6.8, 1.7 Hz, H-1), 2.05 (3H, s), 2.04 (3H, s), 2.02 (3H, s), 1.71 (1H, m, H-6β), 1.65 (3H, s, H_3-17), 1.64 (1H, m, H-7α), 1.64 (1H, m, H-6α), 1.54 (3H, s, H_3-18), 1.37 (1H, m, H-7β), 1.04 (3H, s, H_3-16), 0.85 (3H, s, H_3-19); ^{13}C NMR ($CHCl_3$) δ 198.8 (C-13), 171.2 (C-1'), 169.8 (9-OAc), 169.7 (10-OAc), 169.4 (2-OAc), 149.0 (C-11), 141.1 (C-4), 138.0 (C-12), 137.6 (*q*-Ph), 128.6 (*o*-Ph), 128.4 (*m*-Ph), 128.0 (*p*-Ph), 118.6 (C-20), 89.3 (C-4'), 82.3 (C-2'), 78.8 (C-5), 75.7 (C-9), 73.5 (C-3'), 73.1 (C-10), 69.5 (C-2), 48.4 (C-1), 44.4 (C-8), 43.1 (C-3), 37.3 (C-15), 37.2 (C-16), 36.5 (*N*-methyl), 35.8 (C-14), 28.3 (C-6), 27.3 (C-7), 25.1 (C-17), 24.4, 20.8, 20.7 (acetyl methyl), 17.5 (C-19), 13.2 (C-18); IR ($CHCl_3$) ν_{max} 1744, 1674 cm^{-1}; HREIMS *m/z* 666.3279 ([M+H]$^+$, calcd for $C_{37}H_{48}O_{10}N$, 666.3278).

3α,11α-Cyclotaxinine NN-2 (2): white amorphous powder, mp 45-47°C; $[\alpha]^{20}_D$ –108.1° ($CHCl_3$, *c*, 0.06); ^{1}H NMR ($CHCl_3$) δ 6.26 (1H, dd, *J* = 9.0, 2.0 Hz, H-5), 6.14 (1H, d, *J* = 5.1 Hz, H-2), 6.10 (1H, ddd, *J* = 9.2, 6.6, 2.7 Hz, H-6), 5.56 (1H, d, *J* = 1.0 Hz, H-20a), 5.54 (1H, d, *J* = 9.0 Hz, H-9), 5.23 (1H, d, *J* = 9.0 Hz, H-10), 5.10 (1H, br s, H-20b), 2.68 (1H, d, *J* = 20.3 Hz, H-14α), 2.68 (1H, d, *J* = 7.3 Hz, H-12), 2.49 (1H, dd, *J* = 20.3, 7.3 Hz, H-14β), 2.20 (1H, dd, *J* = 14.7, 7.1 Hz, H-7α), 2.17 (1H, m, H-1), 2.10 (3H, s), 2.06 (3H, s), 2.00 (3H, s), 1.66 (1H, ddd, *J* = 14.7, 2.7, 2.0 Hz, H-7β), 1.63 (3H, s, H_3-16), 1.37 (3H, s, H_3-19), 1.19 (3H, d, *J* = 7.3 Hz, H_3-18), 1.19 (3H, s, H_3-17); ^{13}C NMR ($CHCl_3$) δ 214.1 (C-13), 170.9 (10-OAc), 169.8 (9-OAc), 169.6 (2-OAc), 143.2 (C-4), 134.4 (C-5), 131.5 (C-6), 117.0 (C-20), 83.3 (C-9), 80.3 (C-10), 76.3 (C-2), 66.9 (C-3), 57.8 (C-11), 52.2 (C-12), 49.3 (C-8), 48.1 (C-1), 42.1 (C-15), 38.7 (C-14), 34.5 (C-7), 28.1 (C-16), 26.9 (C-19), 26.7 (C-17), 21.4, 21.1, 21.0 (acetyl methyl), 15.8 (C-18); IR ($CHCl_3$) ν_{max} 1746, 1706 cm^{-1}; HREIMS *m/z* 458.2306 ([M]$^+$, calcd for $C_{26}H_{34}O_7$ 458.2306).

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REFERENCES AND NOTES

1. D. G. I. Kingston, A. A. Molinero, and J. M. Rimoldi, *Prog. Chem. Org. Nat. Prod.*, 1993, **61**, 1; E. Balogle and D. G. I. Kingston, *J. Nat. Prod.*, 1999, **62**, 1448.
2. J. Kobayashi, A. Ogiwara, H. Hosoyama, H. Sigemori, Y. Koiso, and S. Iwasaki, *Experimentia*, 1995, **51**, 592; K. Kosugi, J. Sakai, S. Zhang, Y. Watanabe, H. Sasaki, T. Suzuki, H. Hagiwara, K.

Hirose, M. Ando, A. Tomida, and T. Turuo, *Pytochemistry,* 2000, **54**, 839; J. Sakai, H. Sasaki, K. Kosugi, S. Zhang, N. Hirata, K. Hirose, A. Tomida, and T. Turuo, *Heterocycles*, 2001, **54**, 999.

3. J. Kobayashi, A. Inubushi, H. Hosoyama, N. Yoshida, T. Sasaki, and H. Shigemori, *Tetrahedron*, 1995, **51**, 5971.
4. J. Kobayashi, A. Ogiwara, H. Hosoyama, H. Shigemori, N. Yoshida, T. Sasaki, Y. Li, S. Iwasaki, M. Naito, and T. Tsuruo, *Tetrahedron*, 1994, **50**, 7401.
5. M. Shiro, T. Sato, H. Koyama, Y. Maki, K. Nakanishi, and S. Uyeo, *Chem. Comm.*, 1966, 97; M. Dukes, D. H. Eyre, J. W. Harison, and B. Lythogoe, *Tetrahedron Lett.*, 1965, 4765; D. H. Eyre, J. W. Harison, and B. Lythgoe, *J. Chem. Soc.*, *(C)*, 1967, 452.
6. M. Ando. J. Sakai, S. Zhang, Y. Watanabe, K. Kosugi, T. Suzuki, and H. Hagiwara, *J. Nat. Prod.*, 1997, **60**, 499.
7. F. Yoshizaki, M. Madarame, C. Takahashi, and S. Hisamichi, *Shoyakugaku Zashi*, 1986, **40**, 429.
8. G. Appendino, P. Lusso, P. Gariboldi, E. Bombardelli, and B. Gabetta, *Phytochemistry*, 1992, **31**, 4259; T. Kobayashi, M. Kurono, H. Sato, and K. Nakanishi, *J. Am. Chem. Soc.*, 1972, **19**, 2863.