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A taxoid from the needles of the Japanese yew, *Taxus cuspidata*

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Abstract

5α-Hydroxy-2α,9α,10β-triacetoxy-11,12-epoxy-taxa-4(20)-en-13-one (taxinine A 11,12-epoxide) was isolated from the needles of the Japanese yew, *Taxus cuspidata* Sieb et Zucc, together with 10 other taxoids. Its structure was established on the basis of spectroscopic analysis. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Taxus cuspidata*, Taxaceae; Needles; Taxane diterpenoid

1. Introduction

Taxus cuspidata, is an evergreen tall tree or shrub mainly found in mountainous areas; it is also a popular garden shrub in Japan. Previous studies on taxane diterpenes in its bark, leaves, branches and seed resulted in isolation of more than 50 taxane diterpenoids (Ando et al., 1997; Kobayashi & Shigemori, 1998; Morita, Wei, Takeya & Itokawa, 1997; Morita et al., 1997, 1998a, 1998b; Wang, Shigemori & Kobayashi, 1998; Yoshizaki, Madarame, Takahashi & Hisamichi, 1986). In this course of our study, we investigated the constitutions of its needles: eleven taxoids were isolated, one of which is new compound (**1**). In this paper, we report its isolation and structure elucidation.

2. Results and discussion

Compound **1** was obtained as a white solid in a yield of 0.00008% by weight of fresh needles. HRFABMS revealed the molecular formula was $C_{26}H_{36}O_9$ [(m/z493.2439($[M+H]^+$), $\Delta + 0.1$ mmu], and IR absorptions at 3460, 1740 and 1720 cm^{-1} implied that **1** possessed hydroxy, ester and carbonyl groups. The ^1H NMR spectrum of **1** showed four tertiary methyl signals (δ 0.80, 0.94, 1.83 and 1.94, each 3H, s), three acetate signals (δ 2.03, 2.05 and 2.07, each 3H, s) and four oxymethine signals (δ 4.30, brs; 5.38, d; 5.74, dd and 5.96, d). The proton signals at δ 5.14 and δ 5.29 and those at δ 116.6 and δ 145.6 in the ^{13}C spectrum NMR suggested presence of an exomethylenic moiety. The ^{13}C NMR signal at δ 208.5 suggested the presence of C-13 carbonyl moeity. However, the downfield chemical shift of C-13 and the lack of further olefinic carbons from the ^{13}C NMR spectrum suggested that the endocyclic double bond was either oxidized or reduced.

Detailed analysis of the ^1H–^1H COSY spectrum implied connectivities of C-1 to C-3, C-5 to C-7, C-9 to C-10, C-14 to C-1. The assignments of all protons and carbons of **1** were achieved by analysis of ^1H–^1H COSY, ^{13}C NMR, HMBC and HMQC spectra. In the HMBC spectrum of **1**, H_2-14 showed long-range C/H correlations with C-1, C-12, C-13 and C-15, and Me-18 showed cross-peaks with C-11, C-12, and C-13. The cross-peaks of Me-16 and Me-17 to C-1, C-11 C-15 indicated that Me-16 and Me-17 were attached at C-15. These correlations showed the presence of an

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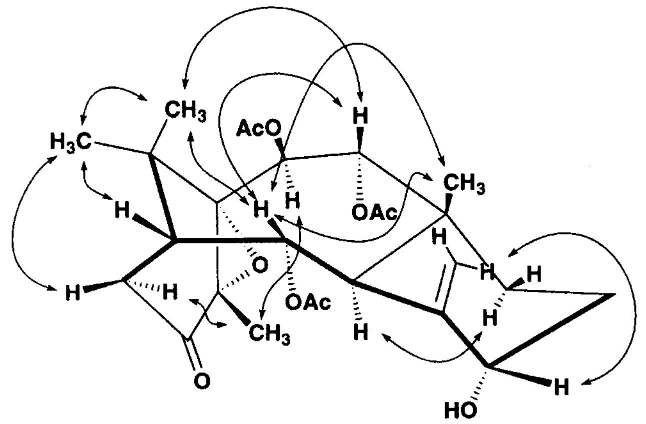

Fig. 1. Relative stereochemistry of **1**, proposed from NOESY correlations (500 MHz).

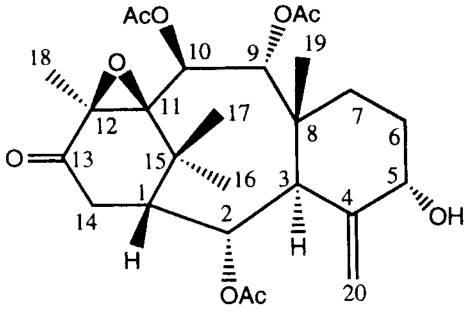

Fig. 2. Compound **1** from needles of *Taxus cuspidata*.

cyclohexane moiety (ring A). Cross-peaks of H-2 to C-3, C-8, C-15, H-3 to C-1, C-2, C-7, C-8, C-9, C-19, H-9 to C-8, C-10, C-19, and H-10 to C-9, C-11, C-15 showed the presence of an eight member ring (ring B). Cross-peaks of H-3 to C-4, C-5, C-7, C-8, C-19, C-20, H_2-20 to C-3, C-4, C-5, H-5 to C-3, C-7, C-20, and Me-19 to C-3, C-7, C-8 showed the presence of a cyclohexane moiety (ring C). Three acetoxy groups were attached at C-2, C-9, C-10 as indicated by the HMBC correlations from acetoxy carbonyl carbons at δ 169.8, 169.5, 169.2 to H-2, H-9, H-10, respectively. The ^{13}C NMR spectrum of **1** showed signals of both C-11 and C-12 at δ 64.2 and 59.8, respectively, this being confirmed from its HMBC spectrum. Judging from the ^{13}C NMR chemical shift and molecular formula, the presence of an epoxide at C-11 and C-12 was suggested. Usually, H-10α always resonates downfield relative to that of H-9β in the 9,10-dihydroxy or 9,10-diacetoxy substituted taxoids (Appendino, 1995; Kingston, Molinero & Rimoldi, 1993). Due to the magnetic anisotropy of the 11,12-epoxy ring, H-10α was observed at an unusual upfield resonance to that of H-9β. Accordingly, taxane-11,12-oxide maybe assigned incorrectly (Yue, Fang & Liang, 1996), since the assignment of C-11,12 in taxane-11,12-oxide should be exchanged with each other (Yue et al., 1996).

The relative stereochemistry of **1** was established from analysis of the NOESY spectrum data (Fig. 1) and its coupling constant. The coupling constant between H-9 and H-10 (10.8 Hz) and NOESY correlations of H-2/H-9 and H-9/Me-16 established a boat–chair conformation for ring B, which is the typical taxane conformation. The β-orientations of H-2, H-5 and H-9 were assigned by NOESY correlations of H-2/H-16, H-5/H-20a and H-9/H-16. The α-orientation of H-10 was assigned by NOESY correlation of H-10/H-18. The β-orientation of the epoxide group at C-11 and C-12 was established by NOESY correlation of H-14α/Me-18. The upfield chemical shift of Me-17 due to the presence of C-11, C-12 epoxide near Me-17 also established the β-orientation of the epoxide group. From these data, the structure of **1** was determined to be 5α-hydroxy-2α,9α,10β-triacetoxy-11,12-epoxy-taxa-4(20)-en-13-one (taxinine A 11,12-epoxide) as shown in Fig. 2. This was the second example of taxoid with an 11,12-epoxide group (Yue et al., 1996).

Other known compounds were identified as taxinine (Brawn & Bell, 1963; Shiro et al., 1966; Tanida, Tsuji & Irie, 1966), 5-cinnamoyltaxicin-I triacetate (Brawn & Bell, 1963), taxinine A (Chiang, Woods, Nakadaira & Nakanishi, 1967), taxinine B (Yoshizaki et al., 1986), taxinine M (Beutler, Chmurny, Look & Witherup, 1991), taxuspine J (Kobayashi & Shigemori, 1998), 2′-hydroxytaxinine (Ando et al., 1997), 13-decinnamoyltaxchinin B (Appendino et al., 1993), cephalomannine (Miller, Powell & Smith, 1981) and taxol (Wani, Taylor, Wall, Coggon & McPhail, 1971) by means of spectroscopic analysis and by comparing with the spectral data described in the literature. Since taxinine was isolated in 0.2% yield on the fresh needles, this suggested that the needles of T. cuspidata is a good source of this compound.

3. Experimental

3.1. General

Mp was measured on a MRK micro-melting point apparatus and are uncorr. Optical rotation was recorded on a Horiba-SEPA-300 digital polarimeter. The UV spectrum was obtained using a Shimadzu UV-1600 spectrophotometer.. IR spectrum was recorded on a Jasco IR-810 instrument. MS spectrum was obtained on a JMS-700 mass spectrometer, using the FAB method and glycerol as a matrix. NMR spectra were recorded on Varian GEMINI 2000/300 (300 MHz for ^{1}H) and Varian Unity INOVA 500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometers with the compound of interest dissolved in $CDCl_3$ at 20°C. Chemical shifts are reported in ppm scale relative to that of tetramethylsilane (TMS, $d = 0$) as an internal

Table 1
^{1}H and ^{13}C NMR spectral data of **1** in $CDCl_3$

Position	^{1}H	J	^{1}H–^{1}H COSY	^{13}C	HMBC
1	1.91 m		H-2β, 14β	51.0	H-3, 16, 17
2	5.74 dd	5.4, 1.8	H-1β, 3α	70.3	H-14
3	3.28 d	5.5	H-2β	41.2	H-1, 19, 20
4				145.4	H-2, 3, 6
5	4.30 brs		H-6α, 6β	76.5	H-3, 20
6α	1.78 m		H-6β, 7	29.5	H-5
6β	1.72 m		H-6α, 7		
7α	1.93 m		H-7β, 6	25.9	H-3, 5, 19
7β	1.72 m		H-7α, 6		
8				43.7	H-2, 10
9	5.96 d	10.8	H-10α	76.7	H-3, 10, 19
10	5.38 d	10.8	H-9β	71.6	H-9
11				64.2	H-1, 9, 16, 17, 18
12				59.8	H-10, 14, 18
13				208.4	H-1, 18
14α	2.48 d	19.8	H-14β	37.9	H-2
14β	2.65 dd	19.8, 8.8	H-14α, 1β		
15				38.2	H-2, 10, 14
16	1.83 s			25.0	H-1, 17
17	0.80 s			28.7	H-1, 16
18	1.94 s			14.5	
19	0.94 s			17.5	H-3, 7, 9
20a	5.29 s		H-20b	116.4	H-3, 5
20b	5.14 s		H-20a		
AcO	2.05 s			20.4	
	2.06 s			20.6	
	2.07 s			21.2	
AcO				169.0	
				169.3	
				169.6	

standard and coupling constants are given in Hertz. Open column chromatography was performed using Merck silica gel 60 (100–200 mesh). Thin layer chromatography was carried out with precoated Merck silica gel 60 F_{254} plates. Preparative TLC were of 0.75 mm thickness, with detection by UV (254 nm) and/or spraying with 10% sulfuric acid and then heating on a hot plate.

3.2. Plant material

Needles of *Taxus cuspidata* Sieb et Zucc were collected from Yagishiri island, Hokkaido, in the summer of 1997. The botanical identification was made by Professor Takashi Oritani of Junior College, Toyama Prefectural University, Japan. A voucher specimen is deposited in our laboratory at Graduate School of Agricultural Science, Tohoku University, Japan

3.3. Extraction and isolation

Needles (2.5 kg) were extracted twice with MeOH at room temperature, with MeOH extracts partitioned between EtOAc and H_2O. The EtOAc soluble portions were evaporated under reduced pressure to give a dark syrup. This was subjected to silica gel column chromatography, with hexane–EtOAc (3:1, 2:1, 1:1 and EtOAc) as eluent to give three fractions (fractions a, b and c). Fractions b and c were subjected to column chromatography and preparative TLC repeatedly, eluted or developed with hexane–acetone, $CHCl_3$–MeOH, and diisopropyl ether–CH_3CN, respectively. In our way compounds **1** (2 mg) and **2**–**11** were individually purified.

5α-Hydroxy-2α,9α,10β-triacetoxy-11,12-epoxy-taxa-4(20)-en-13-one (taxinine A 11,12-epoxide): white amorphous solid: mp 162–163°C: $[\alpha]_D^{17}$ +7.5 (c 0.06, MeOH): UV(MeOH) λ_{max} 210 (ϵ 1650), 276 (ϵ 810) nm: IR (film) ν_{max} 3460, 2920, 2850, 1740, 1720, 1370, 1240, 1200, 1050 and 1020 cm^{-1}: FABMS m/z $[(M+H)^+]$ 493: HRFABMS m/z 493.2439 ($[M+H]^+$ calcd. for $C_{26}H_{36}O_9$, 493.2438): ^{1}H and ^{13}C NMR data see Table 1.

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