A Novel Taxatetraene Framework Formed From a New Natural Taxane from *Taxus cuspidata*

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Sinenxan H [2α,14β-diacetoxytaxa-4(20),11-diene-5α, 10β-diol, **1**] was isolated from the heartwood of *Taxus cuspidata*, for the first time from natural sources. Compound **1** was found to be readily converted into 2α-acetoxytaxa-4(20),10,12(18),13-tetraen-5α-ol (**2**), the first example of a taxane with 10,11- and/or 13,14-double bond(s) and a conjugated triene system, in $CDCl_3$.

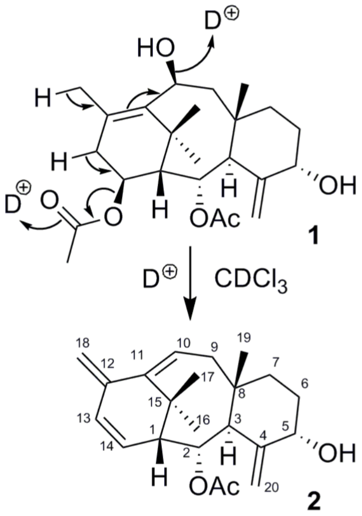
Key words: Taxus cuspidata, Taxanes, Taxatetraene, Structure Elucidation

Introduction

Paclitaxel (Taxol®), one of the most potent anticancer drugs, has encouraged many groups of scientists to conduct research work on plants of the *Taxus* genus. As a result, more than 500 taxane-type diterpenes have been discovered from various *Taxus* plants to date [1 – 3]. Chemical investigations on the Japanese yew, *T. cuspidata*, have led to the identification of more than 120 taxanes of various skeletons [4, 5]. As a part of our project to search for bioactive taxanes, we detected a taxane with a new tetraene system from the conversion of a known taxane.

Results and Discussion

From the methanol extract of the heartwood of *T. cuspidata*, 2α,14β-diacetoxytaxa-4(20),11-diene-5α,10β-diol (sinenxan H, **1**, Scheme 1), was obtained as an amorphous solid. This compound was reported as a product of microbial hydrolysis of the corresponding tetraacetate [6]. Unexpectedly, **1** was converted gradually into a product **2** in $CDCl_3$ during NMR measurements. After recording a 2D NMR spectrum of **1** kept for another day, most of **1** was converted into **2**, whose molecular formula was determined to be $C_{22}H_{30}O_3$ by HRMS ((+)-FAB). The calculated index of hydrogen deficiency of the molecule is eight. The ^{1}H NMR spectrum of **2** (Table 1) displayed signals of one acetyl methyl (δ_H = 2.05), three quaternary methyls (δ_H = 1.09, 1.55, and 0.96), and two oxygenated methines (δ_H = 5.51 and 4.21). The former (δ_H = 5.51), which exhibited H-C correlations with C-1, C-3, C-8, and C-14, as well as with a carbonyl carbon at δ_C = 169.8, was assigned to H-2, and an acetoxy group was attached to C-2. The latter one (δ_H = 4.21) displayed long-range correlations with C-3, C-7 and C-20 in the HMBC experiment and was assigned to H-5β. Two sets of exo-olefinic protons, δ_H = 5.11 and 4.34, were attributed to H-20 and H-18, respectively,



Scheme 1. Conversion of **1** into **2**.

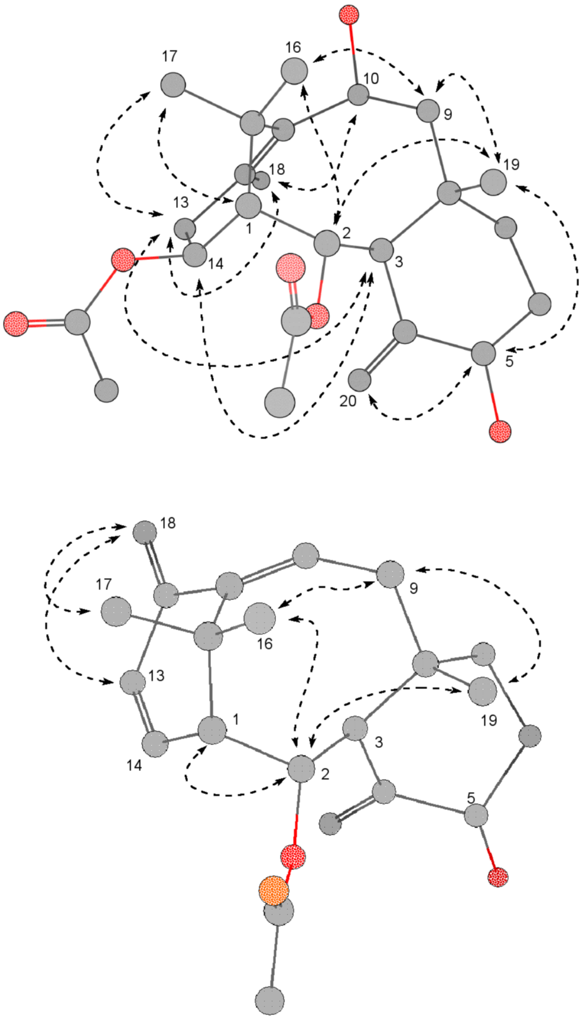


Fig. 1 (color online). The relative configurations of **1** (above) and **2** (below). Dashed arrows gave the key NOE correlations. A conformational search was carried out by minimizing the energy using standard MM2 constants based on the structure elucidated by the NOESY data [7].

by the HMBC data. The ^{13}C NMR spectrum displayed the signals of one acetyl (δ_C = 21.0 and 169.8), two oxygenated carbons (δ_C = 71.3 and 74.4) and eight sp^2 carbons (δ_C = 148.1, 125.7, 147.5, 150.4, 135.0, 126.6, 107.1, and 112.1). To accommodate eight degrees of unsaturation, **2** appears to contain three rings besides the above functional groups. Taking all these data into account and by comparing them with the spectroscopic data of **1**, we postulated that the hydroxy group at C-10 and the acetoxy group at C-14 in **1** were eliminated to form two double bonds. In the HMBC experiment, H-10 showed long-range correlations with C-12; H-18 with C-11 and C-13; H-13 with C-11, C-12 and C-18; and H-14 with C-1 and C-15. The relative configuration was elucidated by the NOESY data and the coupling constants as shown in Fig. 1. The foregoing data show unambiguously that the structure of **2** is 2α-acetoxytaxa-4(20),10,12(18),13-tetraen-5α-ol. **2** is the first example of a taxane with a double bond at C-10 and/or C-13 (a new *anti*-Bredt compound) in a conjugated triene system, and also the least two substituents at C-2 and C-5. Although **2** was an unexpected product in this experiment, it is of interest and may inspire us when we consider the stability of **1** and its analogs, especially when a free hydroxy group is located at C-10. This result also provides new information for taxane derivatization.

Experimental Section

General. Optical rotation: Jasco DIP-370. NMR: Bruker Avance-500. FAB-MS: Vacuum Generators ZAB-HS. Flash chromatography: Silica gel 60 (230 – 400 mesh EM Science). TLC: Silica gel 60 F_{254} (0.25 mm or 0.5 mm, EM Science). Preparative HPLC: Waters Delta Prep 3000, UV 486 (210 nm), Whatman partisil 10 ODS-2 Mag-9 (9.4 × 250 mm^2).

Plant material. The heartwood of *T. cuspidata* was collected in the autumn of 1997 in Toyama Prefecture in Japan and was botanically identified by Professor T. Oritani (Toyama Prefectural University, Japan). Several voucher specimens have been preserved in the laboratory of Applied Bioorganic Chemistry, Graduate School of Agricultural Sciences, Tohoku University, Japan.

Extraction and isolation. Air-dried heartwood of *T. cuspidata* was chipped (5.74 kg) and extracted with 18 L of MeOH for two weeks. The plant residue was filtered and extracted again with fresh 12 L of MeOH for one week. The combined organic solution was evaporated under reduced pressure. The residue was suspended in water (3 L), and lipids were removed by partition 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 solution was dried with anhydrous Na_2SO_4, filtered and evaporated to give a dark extract (55 g). A portion of the CH_2Cl_2 extract (35 g) was chromatographed on silica gel (50 g). Elution with CH_2Cl_2-MeOH (95 : 5 – 55 : 45, total 15 L) yielded 40 fractions ($Fr_{D\text{-}1}$ to $Fr_{D\text{-}40}$). Fractions $Fr_{D\text{-}30}$ to $Fr_{D\text{-}33}$ were combined (4 g) and chromatographed on silica gel (150 g). Elution with hexane-acetone yielded 12 fractions ($Fr_{D\text{-}30-1}$ to $Fr_{D\text{-}30-12}$). $Fr_{D\text{-}30-10}$ and $Fr_{D\text{-}30-11}$ (0.57 g) were subjected to a preparative HPLC, eluted with a 50 min linear gradient of MeCN (25 to 100 %) in H_2O at 18 mL min^{-1}, yielding **1** (5 mg, t_R = 24.1 min); $[\alpha]_D^{22}$ = +29° (c = 0.050, $CHCl_3$). – HRMS ((+)-

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

Position	δ_C[a]	δ_H (J in Hz)	HMBC	NOESY[b]
1	58.8	2.40, dd (5.4, 3.1)		2^m
2	71.3	5.51, dd (8.3, 3.1)	1, 3, 8, 14, 169.8	1^m, 16^s, 19^m
3	42.6	3.17, d (8.3)		
4	**148.1**	–		
5	74.4	4.21, t (3.3)	3, 7, 20	
6a	31.9	1.71, o. m		
6b		1.63, m		
7a	34.3	2.10, m		
7b		1.02, br. ddd (13.9, 5.1, 1.7)		
8	**46.1**	–		
9α	40.0	1.70, o. m		$9\beta^s$
9β		2.81, dd (13.4, 12.7)		$9\alpha^s$, 16^s, 19^m
10	125.7	5.68, dd (12.7, 6.6)	12	
11	**147.5**	–		
12	**150.4**	–		
13	135.0	6.35, d (9.6)	11, 12, 18	14,w 18bw
14	126.6	5.58, dd (9.6, 5.4)	1, 15	13^w
15	**37.4**	–		
16	25.4	1.55, s	1, 11, 15, 17	2^s, $9\beta^s$
17	30.8	1.09, s	1, 11, 15, 16	
18a	107.1	4.89, br. s	11	
18b		4.71, br. s	13	13^w
19	21.0	0.96, s	3, 7, 8, 9, 10	2^m, $9\beta^m$
20a	112,1	5.11, br. s	3, 5	
20b		4.34, br. s	3, 4, 5	
OAc	21.0	2.05, s	169.8	
	169.8			

[a] The ^{13}C chemical shifts were extracted from the HMQC experiment (±0.2 ppm). The numbers in bold character represent quaternary carbons whose chemical shifts were obtained from the HMBC experiment (±0.2 ppm); [b] NOESY intensities are marked as strong (s), medium (m), or weak (w).

FAB): m/z = 381.1828 (calcd. 381.1832 for $C_{22}H_{30}O_3K$, $[M+K]^+$).

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