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A new taxane composed of two *N*-formyl rotamers from *Taxus canadensis*

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Abstract

A taxane with an amino-side chain on C-5 was identified for the first time from rooted cuttings of the Canadian yew, *Taxus canadensis*. The structure was characterized as 7β,9α,10β,13α-tetraacetoxy-5α-[3′-(*N*-formyl-*N*-methylamino)-3′-phenylpropanoyl]oxytaxa-4(20),12-diene (**1**) on the basis of 1D-, 2D-NMR data, and HR-FABMS analyses. The spectra revealed that in $CDCl_3$ solution **1** was composed of two rotamers (**1a** and **1b**) in a ratio of approximately 2:1.

1. Introduction

Nature has been the primary source for most of the drugs presently on the market. Pharmaceutical companies succeed in improving their biological activity by judicious choice of substituents. Paclitaxel (Taxol®), the anticancer wonder drug of the 1990s, firstly isolated from the pacific yew *Taxus brevifolia* Nutt (Taxaceae)[1] and docetaxel obtained by semi-synthesis are such examples.[2] Extensive investigation of different *Taxus* species led to isolate a large number of taxane diterpenoids.[3–7] Indeed, more than 400 taxanes are presently known. *Taxus canadensis* is a low trailing shrub ubiquitous to the Quebec region and its composition has been shown to be very different from other species.[8] This yew was developed for ornamental purposes and is popular as landscape material. Extensive cultivations exist in the nurseries. In order to compare the components of rooted cuttings with the mature yew, we carried out a chemical investigation on the components of the rooted cuttings of *T. canadensis* and resulted in the isolation of a new taxane. In the present publication, we are reporting for the first time the characterization of a taxane with an amino-side chain on C-5 from the rooted cuttings of *T. canadensis* (Fig. 1). We have rigorously elucidated their chemical structures using 1D- (^{1}H NMR, ^{13}C NMR) and 2D-NMR (^{1}H–^{1}H COSY, HMQC or HSQC, HMBC, and NOESY) methods and confirmed them by HR-FABMS.

2. Results and discussion

Taxane **1** was obtained as a colorless amorphous solid. Its molecular composition ($C_{39}H_{51}NO_{11}$) was determined by its HR-FABMS data [$(M+K)^+$ and $(M+Na)^+$]. The ^{1}H and ^{13}C NMR spectral data showed signals in duplicate

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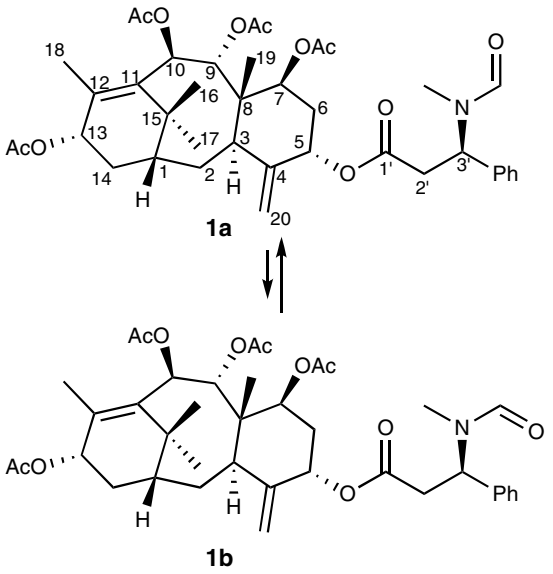

Fig. 1. The structures of **1a** and its minor rotamer **1b**.

(Fig. 2 and Table 1) in $CDCl_3$, especially for the side chain. This spectrum revealed that **1** existed as two rotameric isomers (**1a** and **1b**) in a ratio of approximately 2:1. Therefore, each of the signals of two rotamers was assigned individually. Structure elucidation is described for the main rotamer **1a**. The ^1H and ^{13}C NMR spectra showed signals due to four tertiary methyl groups (δ_H 0.84, 1.11, 1.62, and 2.18 ppm), four acetyl groups (δ_H 2.09, δ_C 21.3, and 169.9; δ_H 2.03, δ_C 20.7, and 170.2; δ_H 1.98, δ_C 20.9, and 169.2; and δ_H 1.79, δ_C 21.1, and 169.8 ppm), and an exocyclic methylene group [δ_H 5.00 (1H, s), 5.30 (1H, s), δ_C 116.4, and 145.8 ppm]. These signals suggested that **1a** has a taxane skeleton.[9,10] The signal at δ_H 2.87 ppm (1H, br d, $J = 5.5$ Hz) was characteristic of the C-3 ring junction proton in a taxa-4(20),11-dienes.[9,10] In addition, five proton signals attached to oxygenated carbons are detected from the chemical shift data and HMBC correlations. AB type protons resonating at δ_H 5.92 ppm correlated with C-7, C-8, C-10, C-11, C-19, and a carbonyl carbon (δ_C 170.2 ppm); and at δ_H 6.20 ppm with C-9, C-11, C-12, C-15, and a carbonyl carbon (δ_C 169.2 ppm) were assigned to H-9 and H-10, respectively. Thus, two acetoxy groups were located at C-9 and C-10. The trans-orientation of H-9 and H-10 was suggested by the large vicinal coupling constant ($J = 11.1$ Hz). By using H-3 as a starting point, the spin system derived from H-3 to H-2 to H-1 to H-14 to H-13 was readily interpreted. The chemical shifts of H-13 at δ_H 5.88 ppm implied that an acetoxy group was attached to C-13. The spin system composed of H-5, H-6, and H-7 was assigned by ^1H–^1H COSY and HMBC spectral data. The chemical shifts of H-5 at δ_H 5.44 (1H, t, $J = 2.7$ Hz) and H-7 at δ_H 5.57 (1H, dd, $J = 9.8$, 6.1 Hz) indicated that both of them were acylated. The presence of a modified Winterstein acid [3′-(*N*-formyl-*N*-methylamino)-3′-phenylpropanoyl] moiety in **1a** was suggested from the signals at δ_H 2.69 (3H, s, NCH_3), 3.20 (1H, dd, $J = 14.9$, 5.7 Hz, H-2′a), 2.05 (1H, dd, $J = 14.9$, 10.0 Hz, H-2′b), 5.23 (1H, dd, $J = 10.0$, 5.7 Hz, H-3′), 7.24–7.39 (5H, m, Ph), and 8.37 (1H, s, NCHO). The location of 3′-(*N*-formyl-*N*-methylamino)-3′-phenylpropanoyl group was deduced to be at C-5 as observed from the HMBC correlation between H-5 and C-1′ (δ_C 168.8 ppm). The relative stereochemistry was determined from chemical shifts, coupling constants, and the NOESY experiment. The coupling constant between H-9 and H-10 (11.1 Hz) indicated that the B-ring was in a chair-boat conformation.[11] 7β,9α,10β,13α-Orientation of the four acetoxy groups was deduced by NOESY correlations of H-3/H-7, H7/H10/H_3-18, H-9/H_3-19, H-9/H-2β, H-9/H_3-17, and H-13/H-16. α-Orientation of 5-acyloxy group was inferred by analogy to the related compound.[9,10] Thus, the structure was established as 7β,9α,10β,13α-tetraacetoxy-5α-[3′-(*N*-methyl-*N*-formylamino)-3′-phenylpropanoyl]oxytaxa-4(20),12-diene.

The spectral data for the minor rotamer **1b** were consistent of the structure elucidation described above for **1a**. The conformation of each formyl group was deduced by the NOESY correlations (NCHO/3′ for **1a** and NCHO/NCH_3 for **1b**).

Growth inhibitory activities of **1** against human tumor cells were tested.[12–14] **1** showed moderate growth inhibitory activities against HMV-1 (IC_{50} 22), KT (88), T-98 (~100), and MM1-CB (~100), while inactive against HeLa, HEC-1, SHIN3, HOC-21, and HAC-2, U251-SP cells.

Taxane **1** is the first example of a taxane with a *N*-formyl group composed of cis- and trans-rotamers.

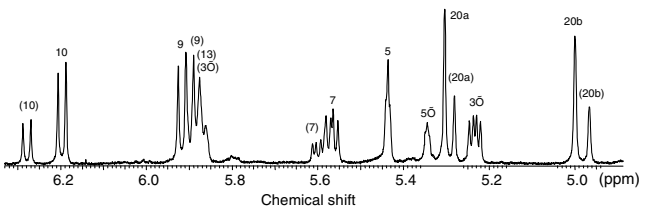

Fig. 2. Part of ^1H NMR chart of **1a** and **1b**. The positional numbers in parentheses are of the minor rotamer **1b**.

3. Experimental

3.1. General

Optical rotation, JASCO DIP-370; NMR, Bruker Avance-500; FABMS, a 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, a Waters Delta Prep 3000 & UV 486 Tunable Absorbance detector set at 227 nm and 210 nm (Waters, Montreal, Quebec, Canada) and a partisil 10 ODS-2 MAG-20 preparative column (22 × 500 mm).

Table 1
The ^{1}H and ^{13}C NMR data of taxanes **1a** and **1b** (500 MHz for ^{1}H, 125 MHz for ^{13}C in $CDCl_3$)

Position	1a					1b				
	δ (^{1}H) mult	J (Hz)	δ (^{13}C)[a]	HMBC	NOESY[b]	δ (^{1}H) mult	J (Hz)	δ (^{13}C)	HMBC	NOESY
1	1.88 (o m)		40.2		16^{m}	1.88 (o m)		40.2		
2α	1.75 (o m)		27.2			1.75 (o m)		27.2		
2β	1.87 (o m)				$9,^{s}$ 16^{m}	1.87 (o m)				9^{s}
3	2.87 (br d)	5.5	37.7	1, 2, 7, 8, 19, 20	$7,^{s}$ $14\alpha,^{s}$ 18^{w}	2.87 (br d)	5.5	37.7		$7,^{s}$ $14\alpha,^{s}$ 18^{w}
4	—		**145.8**			—		**145.8**		
5	5.44 (t)	2.7	75.4	3, 4, 20, 1′	$6,^{s}$ $20a^{s}$	5.34 (dd)	3.2, 2.8	75.2		$6\beta,^{m}$ $20a^{s}$
6α	1.82 (o m)		34.1		$5,^{s}$ 7^{m}	1.92 (o m)		34.1		5^{m}
6β						1.75 (o m)				
7	5.57 (dd)	9.8, 6.1	69.9	6, 8, 9, 19, 7-Ac	$3,^{s}$ $6,^{m}$ $10,^{s}$ 18^{s}	5.60 (dd)	11.5, 5.3	69.9		$3,^{s}$ $10,^{s}$ 18^{s}
8	—		**46.1**			—				
9	5.92 (d)	11.1	76.4	7, 8, 10, 11, 19, 9-Ac	$2\beta,^{s}$ $17,^{s}$ 19^{s}	5.90 (d)	11.0	76.4		$2\beta,^{s}$ $17,^{s}$ 19^{s}
10	6.20 (d)	11.1	71.1	9, 11, 12, 15, 10-Ac	$7,^{s}$ 18^{s}	6.28 (d)	11.0	71.6	9, 11, 12, 15, 169.2	$7,^{s}$ 18^{s}
11	—		**134.8**			—		**136.7**		
12	—		**136.6**			—		**134.6**		
13	5.88 (o m)		70.7		$14\beta,^{s}$ $16,^{s}$ 18^{m}	5.88 (o m)		70.5		$14\beta,^{s}$ $16,^{s}$ 18^{m}
14α	0.99 (dd)	14.0, 7.7	31.3		$3,^{s}$ $14\beta^{s}$	2.65 (o m)		31.3		$3,^{s}$
14β	2.65 (o m)	14.0, 7.7			$13,^{s}$ $14\alpha,^{s}$ 16^{w}	0.99 (o.dd)				13^{s}
15	—		**39.9**			—		**39.8**		
16	1.11 (s)		31.0	1, 11, 15, 17	$1/2b,^{m}$ $13,^{s}$ $14\beta,^{w}$ 17^{s}	1.10 (s)		31.0	1, 11, 15, 17	$13,^{s}$ 17^{s}
17	1.62 (s)		27.4	1, 11, 15, 16	$9,^{s}$ 16^{s}	1.61 (s)		27.4	1, 11, 15, 16	$9,^{s}$ 16^{s}
18	2.18 (br.s)		15.0	11, 12, 13	$3,^{w}$ $7,^{s}$ $10,^{s}$ 13^{m}	2.18 (br.s)		15.0	11, 12, 13	$3,^{w}$ $7,^{s}$ $10,^{s}$ 13^{m}
19	0.84 (s)		13.3	3, 7, 8, 9	9^{s}	0.82 (s)		13.3	3, 7, 8, 9	9^{s}
20a	5.30 (s)		116.4	3, 4, 5	$5,^{s}$ $20b^{s}$	5.28 (s)		115.9		$5,^{s}$ $6a^{s}$
20b	5.00 (s)			3, 4, 5	$20a^{s}$	4.97 (s)				
7-OAc	2.09		21.3	169.9		2.08		21.3		
			169.9			2.02		20.7		
9-OAc	2.03		20.7	170.2		1.97		20.9		
			170.2			1.89		21.1		
10-OAc	1.98		20.9	169.2						
			169.2							
13-OAc	1.79		21.1	169.8	$2'a^{w}$					
			169.8							
1′			**168.8**					**169.3**		
2′a	3.20 (dd)	14.9, 5.7	36.6	1′, 3′, Ph-*ipso*	$2'b,^{s}$ $3',^{m}$ Ph-$o,^{s}$ 13-OAc^{w}	3.11 (d)	7.9	35.6	169.3	
2′b	2.05 (dd)	14.9, 10.0		1′, 3′, Ph-*ipso*	$2'a^{s}$					
3′	5.23 (dd)	10.0, 5.7	58.1	1′, 2′, Ph-*o*, Ph-*ipso*, NMe, NCHO	$2'a,^{m}$ $NCHO^{m}$	5.89 (o m)		52.5	136.8, 127.5	
Ph-*ipso*			**136.7**					**136.8**		
Ph-*o*	7.24 (o m)		126.6	Ph-*ipso*, Ph-*m*/*p*, 3′	$2'a^{s}$	7.29 (o m)		127.5		
Ph-*m,p*	7.39 (t)	7.5	128.5			7.39 (o m)				
						7.39 (o m)				
NMe	2.69 (s)		25.8	3′, Ph-*ipso*, NCHO		2.79 (s)		31.2	3′, NCHO	$NCHO^{m}$
NCHO	8.37 (s)		**162.8**	3′, NMe	$3'^{s}$	8.07 (s)		**162.3**	3′, NMe	NMe^{m}

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

3.2. Plant material

The rooted cuttings of *T. canadensis* Marsh were collected in May of 2002 at St-Jean, Quebec, Canada. Several specimens have been deposited in the herbarium of the Montreal Botanical Garden, Montreal, Canada.

3.3. Extraction and isolation

Air-dried rooted cuttings of *T. canadensis* were ground (1.0 kg) and extracted with 6 L MeOH for four times at ambient temperature. The combined organic extracts were evaporated under reduced pressure. H_2O was added and lipids were removed by stirring the mixture with hexane. The aqueous phase was then salted and extracted with CH_2Cl_2. The combined CH_2Cl_2 extract was dried with $MgSO_4$, filtered, and evaporated yielding a dark green extract (25 g). The CH_2Cl_2 extract was chromatographed on a silica gel (40 g) eluting with a CH_2Cl_2–MeOH (95:5 $\sim$ 55:45) yielded 25 fractions ($Fr_{D\text{-}1}$ to $Fr_{D\text{-}25}$). Fractions $Fr_{D\text{-}18}$ to $Fr_{D\text{-}22}$ were combined (3 g) and chromatographed on a silica gel eluting with hexane–acetone yielded 15 fractions ($Fr_{D\text{-}18\text{-}1}$ to $Fr_{D\text{-}18\text{-}15}$). Fraction $Fr_{D\text{-}18\text{-}10}$ (240 mg) was purified with HPLC. The material (t_R 58.24 min) eluted with a 50 min linear gradient of MeCN–H_2O (25:75–100:0) at 18 mL/min was further purified by a preparative TLC developed with hexane–ethyl acetate (40:65) yielded **1** (2.5 mg, $R_f = 0.24$) as an amorphous solid; $[\alpha]_D^{22}$ +69 (*c* 0.05, $CHCl_3$); HR-FABMS *m/z* 748.3105 $[M+K]^+$ (calcd for $C_{39}H_{51}NO_{11}K$, 748.3098) and 732.3349 $[M+Na]^+$ (calcd for $C_{39}H_{51}NO_{11}Na$, 732.3359).

3.4. Biological evaluation

Human tumor cell lines used were as follows: HeLa (cervical cancer cell line), HEC-1 (endometrial adenocarcinoma cell line), SHIN3 (ovarian clear-cell cystadenocarcinoma cell line), HOC-21 (ovarian clear-cell cystadenocarcinoma cell line), HAC-2 (Ovarian clear-cell carcinoma cell line), HLE (hepatoma cell line), U251-SP (glioma cell line), T-98 (glioma cell line), MM1-CB (melanoma cell line), HMV-1 (melanoma cell line), and KT (breast carcinoma cell line).[12,13] Cells were cultured in Eagle's minimal essential medium (EMEM) (GIBCO/BRL, Grand Island, NY, USA), containing 10% (v/v) calf serum (Intergen, NY, USA) and antibiotics (100 μg/ml of streptomycin and 100 units/ml of penicillin G) (Meiji Seika, Tokyo, Japan), at 37 °C in a humidified atmosphere containing 5% CO_2. Cell survival was estimated by MTT assay, as described elsewhere.[14] Briefly, logarithmically proliferating cells were plated into 96-well plates (1×10^4 cells/well) with the medium containing the test compounds at the indicated doses, followed by being cultured for 2 days. After the culture, the activity of mitochondrial succinic dehydrogenase was measured by further incubation of the cells with 0.5 mg/ml MTT (Sigma) for 4 h, followed by the estimation of absorbance at 570 nm with a reference wavelength of 655 nm. Cell viability was calculated by absorbance as a percentage of the survival cells.

Acknowledgments

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