Two New Phytoecdysteroids from the Needles of *Taxus canadensis*

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Two new phytoecdysteroids with a 20,22-acetal group were identified for the first time from the needles of the Canadian yew, *Taxus canadensis*. Their structures were characterized as ponasterone A 20,22-*p*-hydroxybenzylidene acetal (**1**) and ponasterone A 20,22-acetonide (**2**) on the basis of 1D, 2D NMR evidence and high-resolution FABMS analysis.

Key words: Taxus canadensis, Taxaceae, Ponasterone 20,22-*p*-Hydroxybenzylidene Acetal, Ponasterone 20,22-Acetonide, Structure Elucidation

Introduction

Ecdysteroids are steroids of 27, 28, or 29 carbon atoms with a skeleton characterized by hydroxy groups at least at C-2, C-3 and C-14α, a keto function at C-6 conjugated with a C-7–C-8 double bond and multiple alcohol functions on the side chain, which belong to the large group of polyhydroxylated steroids. Since the first isolation and identification of phytoecdysteroid [1], which is structurally related to the insect moulting hormone ecdysone from a plant, a considerable effort to ascertain their possible significance and role in the plant-insect chemical interaction has been expended [2]. In spite of this effort, there is no direct evidence that these compounds can take part in any kind of known protection mechanism [3 – 5]. However, the idea that phytoecdysteroids may play a role in plant defense against phytophagous insects seems to be generally accepted. On the other hand, ecdysteroids and their analogs are also of great interest for medicinal application [2]. To date, although more than 300 phytoecdysteroids and zooecdysteroids have been identified in plants and in invertebrates [6], only six phytoecdysteroids from the plants of genus *Taxus* were reported [7 – 9].

Taxus canadensis Marsh (Family: Taxaceae) is a low-trailing bush very common in the Quebec region of Canada. Previous phytochemical studies on this species led to the isolation of taxanes with various skeleton, and other classes of compounds [10, 11]. In the present publication we are reporting the characterization of two new phytoecdysteroids from the needles of *T. canadensis*. Their chemical structures were characterized using 1D and 2D NMR data and were further confirmed by high-resolution fast atom bombardment mass spectrometry (HR-FABMS).

Results and Discussion

In our further investigation on the needles of *T. canadensis*, we report herein the isolation and characterization of two phytoecdysteroids with a 20,22-acetal unit, namely, ponasterone A 20,22-*p*-hydroxybenzylidene acetal (**1**) and ponasterone A 20,22-acetonide (**2**) (Fig. 1) from high-polarity fractions. Compounds **1** and **2** are new phytoecdysteroids with a rare substituted side chain.

Compound **1** was obtained as a colorless amorphous solid from air-dried needles of *T. canadensis*. The molecular composition of **1**, $C_{34}H_{48}O_7$, was estab-

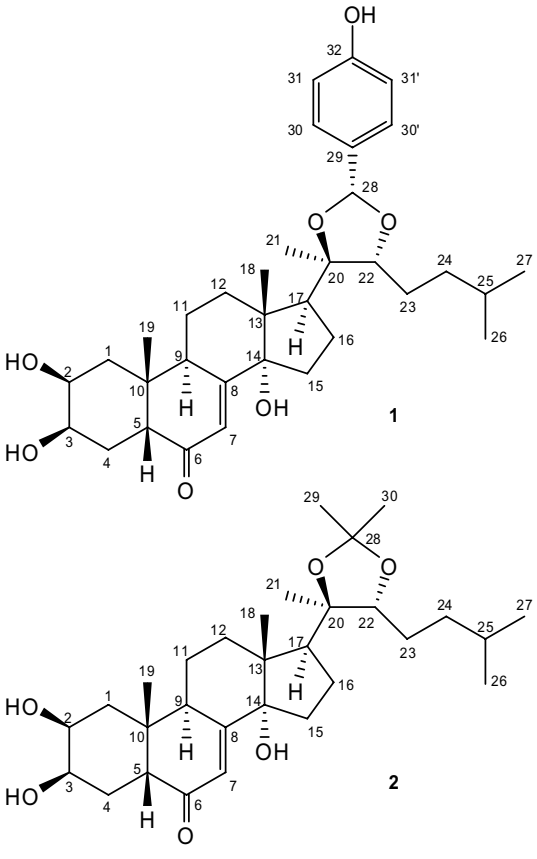

Fig. 1. Ponasterone A 20,22-*p*-hydroxybenzylidene acetal (**1**) and ponasterone A 20,22-acetonide (**2**) isolated from *Taxus cuspidata*.

lished from the combined analysis of high-resolution FABMS and 2D NMR spectral data. The proton signals of the acetal proton at δ = 5.73 (1H, s), an AA'XX' spin system of the aromatic protons at δ = 6.79 (2H, d, J = 8.6 Hz), 7.31 (2H, d, J = 8.6 Hz) and carbon signals at δ = 105.23, 129.47, 115.83, and 159.38 indicated the existence of an unusual *p*-hydroxylbenzylidenedioxy group [12, 13]. Other spectroscopic data of **1** closely resemble those of ponasterone A, which has been isolated from the bark of *Taxus brevifolia* [14], *Taxus cuspidata* [9, 15 – 17] and *Taxus yunnanensis* [8]. Furthermore, the HMBC correlations from the acetal proton (δ_H = 5.73) to C-20 (δ_C = 85.52) and C-22 (δ_C = 85.75) proved the presence of the *p*-hydroxybenzylidene acetal group in the 20,22-position. Based on these features, it was concluded that the connectivity of **1** corresponded to ponasterone A 20,22-*p*-hydroxybenzylidene acetal. Key HMBC and ^{1}H-^{1}H COSY correlations are depicted in Fig. 2.

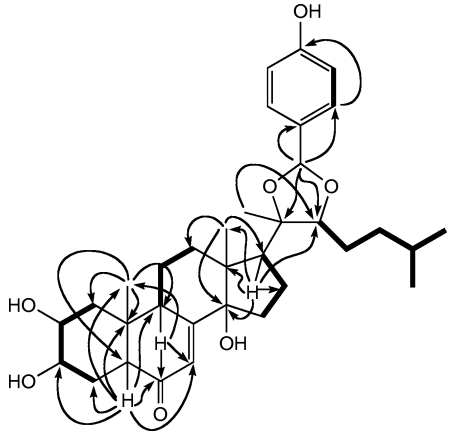

Fig. 2. Key HMBC (H→C) correlations of **1**. The bold lines denote ^{1}H-^{1}H COSY correlations.

The relative configuration of **1** was deduced from NOESY data. From the NOESY correlations of H-1(ax) to H_3-19, H-2(ax) to H-9(ax), H-4(ax) to H-9(ax), and H-5(ax) to H_3-19, the *cis* junction of rings A/B was unambiguous. Furthermore, the hydroxy groups at C-2 and C-3 were judged to be β-oriented from the broad singlet of H-3 at δ = 3.97. The H-12(eq)/H_3-18 and H-12(ax)/H-17 NOESY cross peaks confirmed not only the *trans* junction of rings C/D, but also the β-orientation of the side chain C-20–C-27. C-20-OH, C-22-OH and the hydroxyphenyl ring were assigned to be β- and α-oriented, respectively, on the basis of H-17/H-21, H-21/H-30 and H-22/H-28 NOESY correlations, and by comparing the NMR data with those of an analogous compound [12, 13]. Consequently, compound **1** was determined to be ponasterone A 20,22-*p*-hydroxybenzylidene acetal, as depicted in Fig. 3. Full assignments of protons and carbons were achieved on the basis of 1D and 2D spectroscopic analyses including ^{1}H-^{1}H COSY, HMQC and HMBC experiments.

With the structure of **1** established, the structure elucidation of **2** was relatively straightforward because its spectral data closely resemble those of compound **1** except that a dimethyl acetal in **2** (δ_H = 1.40, 1.31, each s; δ_C = 106.9, 28.9 and 26.7) replaces the *p*-hydroxylbenzylidene acetal in **1**. Thus the structure of **2** was determined to be ponasterone A 20,22-acetonide Compound **2** was not an artifact formed by reaction of the corresponding diol. Although we used a hexane-acetone solvent system for the later stages of chromatography, TLC behavior of the compounds was the same before and after using this solvent system.

Table 1. ^{1}H and ^{13}C NMR data of **1** in CD_3OD (500 MHz for ^{1}H, 125 MHz for ^{13}C).

Position	δ_H (mult)[a]	J (Hz)	δ_C	HMBC	NOESY[b]
1ax	1.45 (t)	13.0	37.37		5ax,^{s} 19^s
1eq	1.81 (m)				
2ax	3.85 (m)		68.72		3eq,^{s} 9ax^s
3eq	3.97 (br.s)		68.51		2ax,^{s} 4ax^s
4ax	1.73 (m)		32.88		3eq,^{s} 9ax^s
4eq	1.76 (m)				5ax,^{s} 19^s
5ax	2.38 (dd)	12.6, 4.3	51.79	3, 4, 6, 7, 9, 10, 19	1ax,^{s} 4eq,^{s} 19^s
6	–		206.41		
7	5.84 (d)	2.0	122.18		15β,^{s}
8	–		167.54		
9ax	3.18 (m)		35.17	6, 7, 8, 10, 19	2ax,^{s} 4ax, ^{s}11eq^s
10	–		39.24		
11eq	1.83 (m)		21.51		9ax^s
11ax	1.72 (m)				18,^{s} 19^s
12ax	2.16 (td)	13.4, 4.8	32.19		17^s
12eq	1.87 (br.dd)	13.4, 2.6			18^s
13	–		48.23		
14	–		85.28		
15β	2.02 (m)		31.78		7,^{s} 18,^{s} 28^s
15α	1.68 (m)				
16α	2.09 (m)		22.80		
16β	1.99 (m)				18,^{s} 28^s
17	2.44 (t)	9.0	51.55	13, 14,16, 18, 21	12ax,^{s} 21,^{s} 23a,^{s} 27^s
18	0.90 (s)		17.67	12, 13, 14, 17	11ax,^{s} 12eq,^{s}15β,^{s} 16β,^{s} 21^s
19	0.98 (s)		24.45	1, 5, 9, 10, 2	1ax,^{s} 4eq,^{s} 5ax,^{s} 11ax^s
20	–		85.52		
21	1.29 (s)		23.58	17, 20, 22	17,^{s} 18,^{s} 30^s
22	3.85 (m)		85.75		28^s
23a	1.54 (m)		27.72		17^s
23b	1.61 (m)				
24a	1.49 (m)		37.60		
24b	1.32 (m)				
25	1.63 (m)		29.25		
26	0.95 (d)	6.6	22.95	24, 25, Me-27	
27	0.95 (d)	6.6	22.87	24, 25, Me-26	17,^{s} 30^s
28	5.73 (s)		105.23	20, 22, 29, 30	15β/16β,^{s} 22,^{s} 30^s
29	–		131.03		
30 (30′)	7.31 (d)	8.6	129.47	28, 30′, 32	21,^{s} 27,^{s} 28,^{s} 31^s
31 (31′)	6.79 (d)	8.6	115.83	29, 31′, 32	30^s
32	–		159.38		

[a] Mutiplicity: s, singlet; d, doublet; dd, doublet of doublets; m, mutiplet; t, triplet; br., broad; [b] NOESY intensities are marked as strong (s), medium (m), or weak (w).

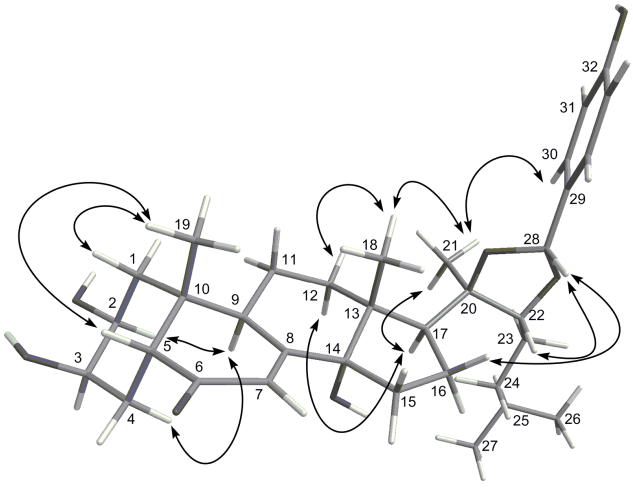

Fig. 3. Relative stereochemistry of **1**. The arrows denote selected NOESY correlations. The 3D structure was calculated with MM2 (CHEM3D program version 10.0, Cambridge Soft, Cambridge, MA (USA)).

Compounds **1** and **2** are two new phytoecdysteroids with a 20,22-acetal group, and they are the first of phytoecdysteroids from *T. canadensis*. To the best of our knowledge, phytoecdysteroids with such functional groups have never been reported from other yew trees. Therefore, the occurrence of these two phytoecdysteroids can be considered as a chemotaxonomic marker for *T. canadensis*. Phytoecdysteroids participate in the defense of plants against non-adapted phytophagous invertebrates [18, 19]. The fact that yew trees exhibit a very strong resistance to insect pests was previously considered to be caused by taxanes such as 10-deacetylbaccatin III and 10-deacetylbaccatin V [20]. The occurrence of phytoecdysteroids such as **1** and **2** in the needles of the *T. canadensis* may also be responsible for insecticidal activities [21].

Experimental Section

General

NMR spectra: Bruker Avance DRX-500 NMR (500 MHz for ^{1}H and 125 MHz for ^{13}C). Optical rotations: JASCO DIP-370. Flash chromatography: Silica gel 60 (230 – 400 mesh EM Science). Thin layer chromatography: Silica gel 60 F254 (0.25 mm or 0.5 mm, EM Science). Preparative HPLC: Waters Delta Prep.

Plant material

The needles of *T. canadensis* were collected in September 1997 at St.-Jean, Quebec, Canada. Several specimens (under accession voucher number lz97-03) have been deposited in the herbarium of the Montreal Botanical Garden, Montreal, Canada.

Extraction and isolation

Air-dried needles (4.0 kg) of *T. canadensis* were ground and submerged in 24 L of MeOH and allowed to stand for one day at r. t. The ground plants were filtered and extracted again with fresh solvent another three times (each time with 8 L solvent, total 24 L) in three days. The combined extracts were concentrated *in vacuo*. H_2O (3 L) was added, and lipids were removed by stirring the mixture with hexane (3 × 3 L). The volume of the hexane fraction was reduced to 1500 mL and extracted four times with 80 % MeOH (each 500 mL). The 80 % MeOH extract, after re-extraction with hexane two times (each 300 mL), was evaporated under reduced pressure, and 1 L of H_2O was added and the mixture extracted with EtOAc three times (each 700 mL). The aqueous phase was then extracted with CH_2Cl_2 (4 × 3 L). The combined CH_2Cl_2 extracts were dried with anhydrous Na_2SO_4, filtered and evaporated yielding a dark-green extract (115 g). A portion of the CH_2Cl_2 extract (50 g) was absorbed onto 110 g silica gel and packed on an open wet column (1320 g). Successive elution with CH_2Cl_2-MeOH (95 : 5 to 55 : 45) yielded 45 major fractions designated $Fr_{D\text{-}1}$ to $Fr_{D\text{-}45}$. $Fr_{D\text{-}39}$ to $Fr_{D\text{-}42}$ were pooled (316 mg), adsorbed onto 360 mg silica gel and packed on a wet column (silica gel 230 – 400 mesh, 25 g). Successive elution with a gradient of hexane-acetone (1 : 1 and 2 : 3) yielded 10 subfractions ($Fr_{D\text{-}39\text{-}1}$ to $Fr_{D\text{-}39\text{-}10}$). The combination of $Fr_{D\text{-}39\text{-}5}$ and $Fr_{D\text{-}39\text{-}6}$ (65 mg) was further purified by preparative HPLC (Whatman partisil 10 ODS-2 Mag-20 prep. column, 22 × 500 mm^2, eluting solvent: a linear gradient of CH_3CN in water from 25 % to 100 % in 50 min at a flow rate of 18 mL min^{-1}) and yielded **1** (3.0 mg, t_R = 19.36 min) and **2** (2.5 mg, t_R = 21.12 min).

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

Position	δ_H (mult)	J (Hz)	δ_C
1ax	1.41 (m)		36.8
1eq	1.85 (m)		
2ax	3.90 (∼dt)	11.1, 3.6	67.6
3eq	4.06 (br.s)		67.3
4ax	1.88 (m)		31.3
4eq	1.65 (m)		
5ax	2.44 (dd)	13.5, 4.4	49.8
6	–		206.4
7	5.86 (d)	1.9	121.6
8	–		167.9
9ax	2.99 (t)	9.1	33.6
10	–		38.2
11eq	1.78 (m)		20.2
11ax	1.66 (m)		
12ax	2.02 (m)		30.8
12eq	1.84 (m)		
13	–		48.2
14	–		85.5
15β	2.05 (m)		31.4
15α	1.56 (m)		
16α	2.04 (m)		20.9
16β	1.80 (m)		
17	2.16 (m)		48.7
18	0.80 (s)		17.0
19	0.99 (s)		23.8
20	–		84.5
21	1.12 (s)		21.8
22	3.73 (d)	11.3	78.8
23a	1.49 (m)		33.2
23b	1.04 (m)		
24ab	1.58 (m)		35.2
25	1.60 (m)		32.8
26	0.83 (d)	6.6	17.7
27	0.89 (d)	6.6	20.3
28	–		106.9
29	1.40 (s)		28.9
30	1.31 (s)		26.7

*Ponasterone 20,22-p-hydroxybenzylidene acetal (**1**)*

Amorphous solid. – $[\alpha]_D^{22}$ = +64° (c = 0.10, MeOH). – HRMS ((+)-FAB): m/z = 569.3473 (calcd. 569.3473

for $C_{34}H_{49}O_7$, $[M+H]^+$), 551.3374 (calcd. 551.3367 for $C_{34}H_{47}O_6$, $[M+H-H_2O]^+$).

*Ponasterone 20,22-acetonide (**2**)*

Amorphous solid. – $[\alpha]_D^{22}$ = +67° (*c* = 0.10, MeOH). – HRMS ((+)-FAB): *m/z* = 505.3528 (calcd. 505.3529 for $C_{30}H_{49}O_6$, $[M+H]^+$).

Acknowledgements

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