DOI: 10.1002/ejoc.200800587

Taxpropellane: A Novel Taxane with an Unprecedented Polycyclic Skeleton from the Needles of *Taxus canadensis*

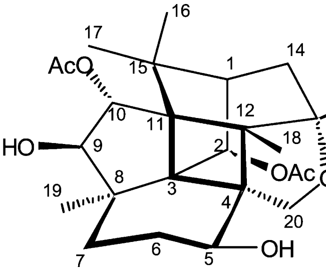
Man-Li Zhang,[a] Mei Dong,[b] Chang-Hong Huo,[a] Li-Geng Li,[a] Françoise Sauriol,[c] Qing-Wen Shi,*[a] Yu-Cheng Gu,[d] Hidefumi Makabe,[e] and Hiromasa Kiyota*[f]

Keywords: Propellanes / Structure elucidation / Terpenoids / Fused-ring systems

Taxpropellane, the first example of a taxane with an unprecedented 6/5/5/6/4/5-membered ring hexacyclic skeleton ([3.3.2]propellane), was isolated from the needles of *Taxus canadensis*. This compound would be derived from a normal 6/8/6 taxane by a [2+2] cycloaddition reaction between the 3,4- and 11,12-double bonds.

Introduction

The Canadian yew (*Taxus canadensis*), a small rambling bush with a maximum height of one metre, is a rich source of taxane diterpenoids with great chemical and structural diversity, especially those with novel carbon frameworks.[1] In our continuing search for bioactive taxanes, we isolated new taxanes with different skeletons from the needles of the Canadian yew.[2,3] Further studies on this plant have now led to the isolation of a novel taxane with a unique 6/5/5/6/4/5-membered ring carbon skeleton ([3.3.2]propellane), named taxpropellane (**1**).[3,4]



1

Results and Discussion

Compound **1** was obtained as a colourless amorphous solid. The molecular composition of **1** ($C_{24}H_{34}O_8$) was established from combined HRMS (FAB) and ^{13}C NMR spectroscopic analysis (Table 1). The calculated indices of hydrogen deficiency of the molecule are eight. In the ^1H NMR spectrum of **1**, there are six methyl signals at δ_H = 1.07, 1.15, 1.26, 1.28, 2.10, 2.12 ppm. The ^1H–^1H COSY related signals (δ_H = 1.28 and 1.15 ppm) were assigned to the geminal methyl groups (Me-16 and Me-17). The presence of the two acetyl groups was verified by the HMBC related sets of signals (δ_C = 21.34, 169.81 ppm and δ_C = 21.16, 171.96 ppm). Comprehensive analysis of the ^1H and ^{13}C and 2D NMR spectroscopic data revealed the existence of 24 carbon atoms. These signals were generated by the carbon atoms of two acetyl, one methine, four oxymethine, three methylene, one oxymethylene and four methyl groups as well as those at a hemiacetal and six quaternary centres. This information also supported the conclusion that **1** contains six rings. No signal was observed in the olefinic region in the ^{13}C NMR spectrum, suggesting that the double bonds between C-11 and C-12, and between C-4 and C-20, which are present in most natural taxanes, are pyramidalized.[1,5] This conclusion was in accordance with the upfield position of the protons in the 18-methyl group at δ_H = 1.26 ppm. In addition, the characteristic ^1H signals due to geminal protons in a C-4,20-epoxide or 5(20)-oxetane ring were absent.[5] Further detailed analysis of the ^1H and ^{13}C NMR, ^1H–^1H COSY and HMBC spectra (Figure 1) permitted the assignment of the positions of the functional groups and quaternary carbon atoms. A pair of isolated AB system protons resonating at δ_H = 5.91 and 4.10 ppm with a large coupling constant (10.1 Hz) were attributed to 10-H and 9-H, respectively. Their chemical shifts suggested that an acetyl group was attached to C-10 and one free hydroxy group was connected to C-9.[5]

---

[a] School of Pharmaceutical Sciences, Hebei Medical University,
361 Zhongshan East Road, 050017, Shijiazhuang, Hebei Province, P. R. China
E-mail: shiqingwen@hebmu.edu.cn
[b] Division of Forensic Medicine, Hebei Medical University,
361 Zhongshan East Road, 050017, Shijiazhuang, Hebei Province, P. R. China
[c] Department of Chemistry, Queen's University,
Kingston, Ontario K7L 3N6, Canada
[d] Syngenta, Jealott's Hill International Research Centre,
Bracknell, Berkshire RG42 6EY, UK
[e] Graduate School of Agriculture, Shinshu University,
8304 Minami-Minowa, Kami-Ina, Nagano 399-4598, Japan
[f] Graduate School of Agricultural Science, Tohoku University,
1-1 Tsutsumidori-Amamiya, Aoba-ku, Sendai 981-8555, Japan

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

Position	δ_H [ppm]	δ_C [ppm]	HMBC	NOESY[a]
1	2.13 (m)	48.72		2 (s), 20a (s)
2	5.74 (d, J = 5.3 Hz)	78.48	1, 3, 8, 11, 14, 169.81	1 (s), 17 (s), 19 (s)
3	–	56.85		
4	–	53.92		
5	3.76 (dd, J = 12.2, 5.4 Hz)	77.36	4, 6, 12, 20	6 (w), 7a (s), 20b (s)
6ab	1.86 (m)	29.59		5 (w)
7a	2.20 (m)	30.80		5 (s)
7b	1.02 (m)		5, 6, 8, 19	
8	–	40.80		
9	4.10 (d, J = 10.1 Hz)	85.13	7, 8, 10, 19	17 (s), 19 (s)
10	5.91 (d, J = 10.1 Hz)	80.76	9, 11, 12, 15, 171.96	18 (s)
11	–	53.28		
12	–	54.25		
13	–	105.60		
14a	2.39 (m)	36.03		
14b	2.18 (m)			
15	–	39.42		
16	1.28 (s)	28.34	1, 11, 15, 17	17 (s)
17	1.15 (s)	20.85	1, 11, 15, 16	2 (s), 9 (s), 16 (s)
18	1.26 (s)	16.78	4, 11, 12, 13	10 (s)
19	1.07 (s)	25.85	3, 7, 8, 9	2 (s), 9 (s)
20a	4.91 (d, J = 10.5 Hz)	70.14	3, 5	1 (m), 20b (s)
20b	3.65 (d, J = 10.5 Hz)		3, 4, 5, 12, 13	5 (s), 20a (s)
2-Ac	2.10 (s)	21.34	169.81	
		169.81		
10-Ac	2.12 (s)	21.16	171.96	
		171.96		

[a] NOESY intensities are marked as strong (s), medium (m) or weak (w).

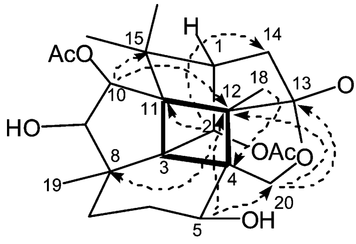


Figure 1. Selected HMBC correlations (H→C) of **1**. Most protons are omitted for clarity.

The above assignments were further supported by the correlations between 10-H and a carbonyl carbon atom at δ_C = 171.96 ppm in the HMBC spectrum, whereas 9-H exhibited long-range correlations with C-7, C-8, C-10 and C-19. The signal resonating at δ_H = 5.74 ppm, which correlated with C-1, C-3, C-8, C-11, C-14 and a carbonyl carbon atom at δ_C = 169.81 ppm was attributed to 2-H. Thus, the second acetoxy group was positioned at C-2. Diagnostic long-range correlations between 2-H and C-11, as well as between 20-H and C-12, clearly suggested that C-3 is connected to C-11 and that C-4 is connected to C-12 to form a four-membered ring. The signal appearing at δ_H = 3.76 ppm correlated with C-4, C-6, C-12 and C-20 and was assigned to 5-H; the attachment of a hydroxy group to C-5 looked likely from its ^{1}H chemical shift. In addition, the spin system from 5-H to 7-H through 6-H was established by using 5-H as a reference in the ^{1}H–^{1}H COSY spectrum. Both 16-H_3 and 17-H_3 correlated with C-1, C-11 and C-15 in the HMBC spectrum, which revealed that they were linked to C-15. The long-range proton–carbon couplings of 19-H_3 to C-3, C-7, C-8 and C-9 implied that Me-19 is attached to C-8. A hemiacetal carbon signal appeared at a relatively downfield position (δ_C = 105.60 ppm). The 20a-H and 20b-H protons resonated at δ_H = 4.91 and 3.65 ppm with a coupling constant of J = 10.5 Hz. A three-bond long-range correlation was also observed between 20b-H and the hemiacetal carbon atom at δ_C = 105.60 ppm in the HMBC experiment, which strongly suggested that the C-20 oxymethylene was linked to the C-13 hemiacetal carbon atom to form a five-membered ring. The structure of **1** was, therefore, elucidated as 2α,10β-diacetoxy-13α,20-epoxy-3α,11α;4α,12α-dicyclotaxane-5α,9α,13β-triol. To address the presence of a propellane and emphasize the plant origin of the compound, the trivial name taxpropellane is proposed for this novel taxane diterpenoid.

The relative stereochemistry of **1** (Figure 2) was defined on the basis of the chemical shifts, coupling constants and the NOESY correlations. The magnitudes of the vicinal coupling constant (J = 10.1 Hz) between 9-H and 10-H indicated that 9-H and 10-H adopt the *trans* orientation, as in other taxanes.[3] The 2-H proton displays strong NOE correlations with 17-H_3, 19-H_3 and 1-H, a pattern similar to that observed in other taxanes, which indicates that these protons are all on the same face of the molecule. The observation of NOESY correlations between 9-H/17-H and 9-H/19-H defined the β orientation of 9-H. The stereochemistry of the C-13 hemiacetal carbon atom was unequivocally elucidated from the propellane structure.

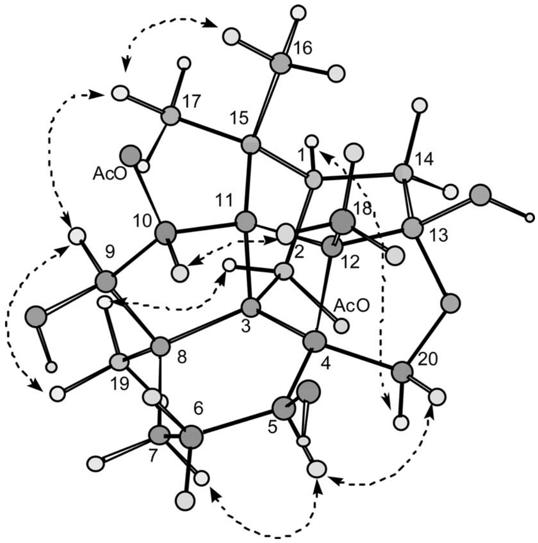
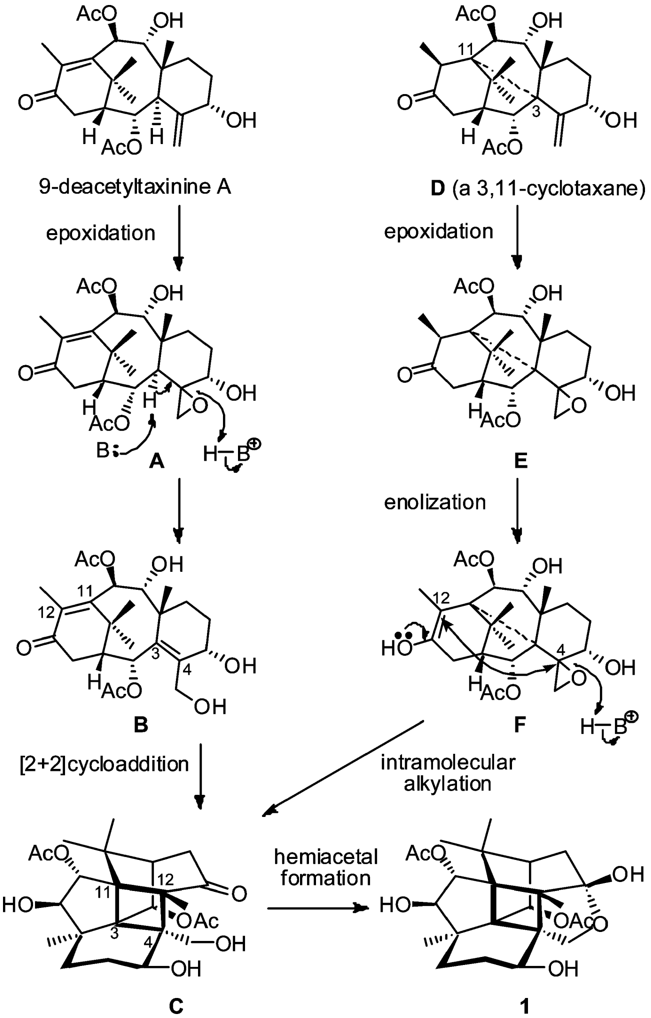


Figure 2. Relative stereochemistry of **1**, calculated by MM2 (Chem3D program V4.0, Cambridge Soft, Cambridge, MA). Dotted arrows show key NOESY correlations. Acetyl groups are omitted for clarity.



Scheme 1. Hypothetical biogenetic routes from 9-deacetyltaxinine A to compound **1**.

Although more than 500 natural taxanes have been reported so far,[1] compound **1** is the first taxane to have the novel 6/5/5/6/4/5-membered ring system and the most complex skeleton discovered to date.[1] A putative biogenetic pathways for **1** is proposed in Scheme 1. 9-Deacetyltaxinine A, isolated from the needles of the Canadian yew,[2c] is supposed to be the precursor. The first step would be epoxidation to form **A**, which arranges to allylic alcohol **B**.[6] The very unusual four-membered ring **C** is then formed by a [2+2] cycloaddition reaction between the C-11,12 and the C-3,4 olefinic bonds. The final hemiacetal ring formation results in the stable hexacyclic structure containing a unique [3.3.2]propellane.[3,4] Another plausible pathway starts from 3,11-cyclotaxane **D**. Epoxidation (**E**) followed by intramolecular alkylation by attack of the enol on the epoxy ring (**F**) forms **C**. The proposed structure is consistent with its spectral properties and is also reasonable from a chemical and biogenetic point of view. Because taxa-3,11-dienes like **B** or 4,20-epoxy-3,11-cyclotaxanes like **E** have not been found in nature, taxpropellane (**1**) should not be an artefact.

Taxpropellane (**1**) showed no cytotoxicity against human breast cancer MCF-7 cell line and HAC-2 ovarian clear-cell carcinoma cell line.

Conclusions

The 6/5/5/6/4/5-membered ring hexacyclic skeleton containing a [3.3.2]propellane of taxpropellane (**1**) represents a new addition to the architectural diversity of structures in the taxane family, and is also of interest because of its biogenetic pathway. Its occurrence serves as a useful chemotaxonomic marker for *T. canadensis.*

Experimental Section

General: Flash chromatography was performed on silica gel 60 (230–400 mesh EM Science). Thin-layer chromatography was conducted on silica Gel 60 F254 precoated TLC plates (0.25 or 0.5 mm, EM Science). The compounds were visualized on TLC plates with 10% sulfuric acid in ethanol and heating on a hot plate. Preparative HPLC was carried out on a Waters Delta Prep 3000 instrument coupled to a UV 486 Tunable Absorbance detector set at 227, 210 or 287 nm (Waters, Montreal, Quebec, Canada). Analytical HPLC was performed with two Whatman partisil 10 ODS-2 analytical columns (4.6 × 250 mm) in series. Semipreparative HPLC was performed with two Whatman partisil 10 ODS-2Mag-9 semipreparative columns (9.4 × 250 mm) in series. Preparative HPLC was performed with one partisil 10 ODS-2MAG-20 preparative column (22 × 500 mm). The products were eluted with a 50 min linear gradient of acetonitrile (25–100%) in water at a flow rate of 18 mL min^{-1} (preparative HPLC). NMR spectroscopic data were obtained at room temperature with a Bruker Avance-500 spectrometer operating at 500 MHz for ^{1}H and at 125 MHz for ^{13}C by using $CDCl_3$ as an internal reference (δ = 7.25 ppm for ^{1}H and 77.0 ppm for ^{13}C). The various 2D spectra were acquired and processed by using standard procedures. For phase-sensitive 2D experiments (NOESY, HMBC and HSQC), the data were acquired by using the TPPI phase mode. The NOESY experiment was obtained by using a mixing time of 0.3 s and a relaxation delay of 1.5 s. Fast atom bombardment mass spectra (FABMS) were obtained with a Vacuum Generators ZAB-HS double-focusing instrument by using a xenon beam having 8 kV energy at 1 mA equivalent neutral current. Samples were dissolved in 0.2 mL DMSO before addition of 0.5 mL glycerol.

Extraction, Isolation and Purification:[7] Air-dried needles of *Taxus canadensis* were ground (4.0 kg) and extracted with MeOH (24 L) for 1 d at room temperature. The ground plants were filtered and extracted again with MeOH (3 × 8 L solvent, total 24 L) over 3 d. The combined organic extract was evaporated under reduced pressure. Water (3 L) was added and lipids were removed by stirring the mixture with hexane (3 × 3 L). The hexane fraction was condensed to 1500 mL and extracted with 80% aq. MeOH (4 × 500 mL). The 80% aq. MeOH extract, after being reextracted with hexane (2 × 300 mL), was evaporated under reduced pressure, and 1 L of water was added. The aqueous layer was extracted with EtOAc (3 × 700 mL). The combined EtOAc extract was dried with anhydrous Na_2SO_4, filtered and evaporated to yield a dark-brown extract (25 g). The aqueous phase was then salted (NaCl, 200 g) and extracted with CH_2Cl_2 (4 × 3 L). The combined CH_2Cl_2 extract was dried with anhydrous Na_2SO_4, filtered and evaporated to yield a dark-green extract (115 g). A portion of the CH_2Cl_2 extract (50 g) was chromatographed on silica gel (110 g). Gradient elution with CH_2Cl_2/MeOH (95:5–55:45, total 15 L) yielded 45 fractions (Fr_{D-1} to Fr_{D-45}). The Fr_{D-38} to Fr_{D-41} fractions were combined (24 g) and rechromatographed on silica gel (770 g) with hexane/acetone as the elution solvent. This yielded 28 fractions (Fr_{D-38-1} to $Fr_{D-38-28}$). The $Fr_{D-38-15}$ (1.8 g) fraction was rechromatographed on silica gel (60 g; CH_2Cl_2/CH_3CN, 8:3) to give 15 fractions ($Fr_{D-38-15-1}$ to $Fr_{D-38-15-15}$). The $Fr_{D-38-15-13}$ and $Fr_{D-38-15-15}$ fractions were combined (2.09 g) and subjected to column chromatography (180 g silica gel; CH_2Cl_2/CH_3CN, 3:2–1:1) to give 25 subfractions. Subfraction 8 was purified by preparative HPLC to give **1** (1.3 mg, t_R = 17.37 min). The overall yield was 0.000075%. $[\alpha]_D^{22}$ = –21 (c = 0.1, $CHCl_3$). HRMS (FAB): calcd. for $C_{24}H_{34}O_8K$ [M + K]$^+$ 489.1891; found 489.1888.

Acknowledgments

We thank the Scientific Research Foundation for the Returned Overseas Chinese Scholars, Hebei Province, the State Education Ministry of China and the Foundation for RND (No: 2003AA2Z3527). We also appreciate financial support from Syngenta Ltd. (2005-Hebei Medical University-Syngenta-01) and the Grant-in-aid from Japan Society for the Promotion of Science (19580120). We thank Dr. John Clough of Syngenta Jealott's Hill International Research Centre for his proof reading.

---

[1] a) E. Baloglu, D. G. I. Kingston, *J. Nat. Prod.* **1999**, *62*, 1448–1472; b) V. S. Parmer, A. Jha, K. S. Bish, P. Taneja, S. K. Sight, A. Kumar, J. R. Poonam, C. E. Olsen, *Phytochemistry* **1999**, *50*, 1267–1304.

[2] a) Q. W. Shi, Y. F. Wang, H. Kiyota in *Chemistry of Natural Products: Recent Trends & Development 2006* (Ed.: G. Brahmachari), Research Signpost, India, **2006**, pp. 43–59; b) M. Dong, M.-L. Zhang, Q.-W. Shi, Y.-C. Gu, H. Kiyota in *Natural Products: Chemistry, Biochemistry and Pharmacology* (Ed.: H. Brahmachari), Narosa Publishing House, New Delhi, India, **2008**, pp. 247–269; c) Q. W. Shi, F. Sauriol, O. Mamer, L. O. Zamir, *J. Nat. Prod.* **2003**, *66*, 470–476; d) Q. W. Shi, M. Dong, C. H. Huo, X. H. Su, C. F. Li, X. P. Zhang, Y. F. Wang, H. Kiyota, *Biosci. Biotechnol. Biochem.* **2007**, *71*, 1777–1780; e) C. H. Huo, Y. M. Zhao, X. T. Si, L. G. Li, M. L. Zhang, Q. W. Shi, H. Kiyota, *Biochem. System. Eco.* **2007**, *35*, 909–913.

[3] Isolation of propellane-type taxanes from *Taxus canadensis*: a) Q. W. Shi, F. Sauriol, O. Mamer, L. O. Zamir, *Chem. Commun.* **2003**, 68–69; b) Q. W. Shi, F. Sauriol, A. Lesimple, L. O. Zamir, *Chem. Commun.* **2004**, 544–545; c) C. H. Huo, X. H. Su, Y. F. Wang, X. P. Zhang, Q. W. Shi, H. Kiyota, *Tetrahedron Lett.* **2007**, *48*, 2721–2724.

[4] Isolation of other natural propellanes: a) L. H. Zalkow, R. N. Harris, D. Van Derveer, *J. Chem. Soc., Chem. Commun.* **1978**, 420–421; b) F. Bohlmann, C. Zdero, R. Bohlmann, R. King, M. H. Robinson, *Phytochemistry* **1980**, *19*, 579–582.

[5] a) D. G. I. Kingston, A. A. Molinero, J. M. Rimoldi in *Progress in the Chemistry of Organic Natural Products*, (Eds.: W. Herz, G. W. Kirby, R. E. Moore, W. Steglich, C. H. Tamm), Springer, New York, **1993**, vol. 61, pp. 1–206; b) G. Appendino in *The Chemistry and Pharmacology of Taxol and Its Derivatives* (Ed.: V. Farina), Elsevier, Amsterdam, **1995**, vol. 22, pp. 1–53 and pp. 55–101.

[6] The 9-*O*-Acetyl-12-hydro-11-hydroxy derivative of **B**, taxezopidine B, was isolated from the seeds of the Japanese yew: X. X. Wang, H. Shigemori, J. Kobayashi, *J. Nat. Prod.* **1998**, *61*, 474–479.

[7] Q. W. Shi, F. Sauriol, O. Mamer, L. O. Zamir, *Bioorg. Med. Chem.* **2003**, *11*, 293–303.

Received: June 18, 2008