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First three examples of taxane-derived di-propellanes in *Taxus canadensis* needles†

Qing Wen Shi,[a] Françoise Sauriol,[b] Alain Lesimple[c] and Lolita O. Zamir*[a]

[a] *Human Health Research Center, INRS-Institut Armand-Frappier, Université du Quebec, 531 Boulevard des Prairies, Laval, Quebec, Canada H7V 1B7*

[b] *Department of Chemistry, Queen's University, Kingston, Canada K7L 3N6*

[c] *Biomedical Mass Spectrometry Unit, McGill University, 1130 Pine Avenue west, Montreal, Quebec, Canada H3A 1A3*

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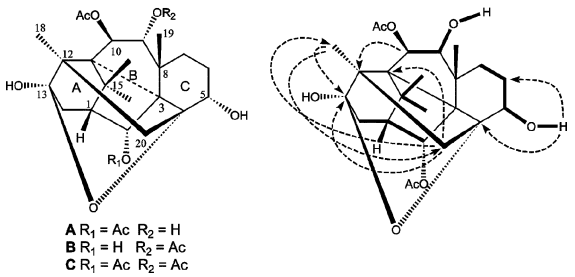
The first three examples of taxane-derived [3.3.3][3.4.5] di-propellanes isolated from the needles of a yew tree are reported. They differ in their acetylation pattern and their biogenesis from a putative taxane precursor is proposed.

The term [a.b.c]-propellane was coined by David Ginsburg[1,2] for tricyclic saturated [a.b.c] hydrocarbons. These structures attracted the imagination of synthetic organic chemists and very elegant syntheses were published.[3,4] Very few natural products with this propeller structure have been reported. The first [3.3.3]-propellane natural product was the carbocyclic sesquiterpenoid modhephene, which was isolated from the roots of *Liabrum eggersii* or from the leaves and stems of the toxic plant *Isacoma wrightii*.[5,6] We have recently reported the first example of a taxane-derived propellane in *Taxus canadensis* needles.[7]

Here we report three taxane-derived di-propellanes **A**–**C** differing only in their acetylation pattern: **A** is diacetylated on C-2 and C-10; **B** is diacetylated on C-9 and C-10 whereas **C** has three acetyls on C-2, C-9 and C-10. The two propellanes forming these di-propellane structures are shown in Fig. 1: in one of them the bond 3–11 is shared by three cyclopentanes ([3.3.3]) whereas in the second one the bond 3–4 is common to a cyclopentane, a cyclohexane and an oxacyclohexane ([3.4.5]). It seems that these two propellanes are attached together by the bond C-12–C-13. The isolation procedure for **A**, **B** and **C** is described in ESI.†

The molecular composition of **A** was established as $C_{24}H_{34}O_8$ from both HRFABMS *m*/*z* 489.1892 $[M + K]^+$ and ^{13}C NMR spectroscopic data (ESI†). In the NMR spectra of **A** two acetyl groups were observed at δ_H 2.01 and 2.07 and confirmed by ^{13}C NMR signals at δ_C 20.2, 169.7 and δ_C 20.3 and 169.4. The ^{13}C NMR spectrum of **1** did not display any signal in the olefinic carbon range. These observations together with the degrees of unsaturation required by its molecular formula suggested that **A** had a skeleton with six rings. The connectivities of the protons were determined by analysis of the ^1H–^1H COSY spectrum. Interpretation of the ^1H, ^{13}C NMR, HSQC and HMBC spectral data permitted the positional assignments of the functional groups.



A R_1 = Ac R_2 = H
B R_1 = H R_2 = Ac
C R_1 = Ac R_2 = Ac

Fig. 1 The structures of **A**, **B** and **C** (left). Dotted arrows denote selected key HMBC correlations (H → C) of **A** (right). The bold bonds show the ^1H–^1H COSY correlations.

† Electronic supplementary information (ESI) available: experimental and NMR data. See http://www.rsc.org/suppdata/cc/b3/b316051c/

The ^1H NMR spectrum of **A** revealed four singlets corresponding to the four methyl groups at δ 1.14, 1.20, 1.23, and 1.34; two of them were weakly correlated in COSY implying a geminal relationship which was proven by HMBC. Those two geminal methyls, at positions 16 and 17 (δ 1.23 and 1.34), are correlated in HMBC to two quaternary carbons identified as C-11 (δ 63.5) and C-15 (δ 39.5) and to the protonated carbon C-1 (δ 47.8). Methyl 18 (δ_H 1.20) is also correlated to C-11 and to three other carbons: C-12 (δ 55.1), C-13 (δ 100.4) and a methylene carbon assigned as C-20 (δ 46.8). The ^1H NMR of **A** in acetone-D_6 allowed us to observe the hydroxyl groups directly. One of these hydroxyl protons, the OH-13 (δ 4.75) located on the hemiacetal carbon C-13, is correlated to C-13, C-12 and to a methylene carbon C-14 (δ 33.4). In the ^1H–^1H-COSY, the H-14 protons showed correlation with H-1, allowing us to close the first ring (ring A) as a six-membered ring. The H-1 proton is also COSY correlated to a deshielded proton H-2 (δ_H 5.60). The ring A closure is further confirmed by the observation of HMBC correlation of the H-14b proton (δ_H 1.90) with C-1, C-2, C-12, C-13 and C-15. The ^1H NMR of **A** in acetone-D_6 showed four signals due to protons attached to oxygenated carbons and three signals due to three free hydroxyl groups. The H-2 resonating at δ_H 5.60 (1H, d, J = 5.3 Hz) showed HMBC correlations with C-1, C-3, C-4, C-8 and C-14 as well as a carbonyl acetyl carbon allowing us to attach one of the acetyl groups to C-2. A pair of AB doublets resonating at δ_H 5.47 and δ_H 4.13 with a large coupling constant (J = 9.0 Hz) was attributed to H-10 and H-9, their chemical shifts requiring an acetyl and a hydroxyl group to be attached to C-10 and C-9, respectively. This was confirmed by the HMBC experiment where the H-10 proton shows correlation to an acetyl carbonyl. The H-10 is also correlated in HMBC to C-11, C-12 and C-15 on ring A and to C-8 (δ 41.2) and C-9 (δ 85.0). The H-9 proton in HMBC is correlated to C-10 (δ 80.4), C-8, a methylene carbon assigned to C-7 (δ 27.0) and a methyl carbon C-19 (δ 22.1). The methyl-19 singlet (δ_H 1.14) is HMBC correlated to C-9, C-8, C-7 and C-3 (δ 65.1), allowing the closure of the eight-membered ring (ring B). The signal resonating at δ_H 4.23 was assigned to H-5 according to its ^1H–^1H COSY correlations with H-6a and H-6b and its long-range correlations with C-4, C-6, C-7, and C-20 in the HMBC spectrum. These HMBC correlations allowed us to close ring C. The chemical shift of H-5 implied that a hydroxyl group was bound to C-5. A COSY correlation was observed between H-5 and its hydroxyl proton which was observed at δ_H 3.48 as a broad doublet. This 5-OH proton has HMBC correlations with C-4, C-5 and C-6 confirming the shifts of the neighboring C-5. The closure of six-membered ring C is further supported by the observation that one of the protons on C-7 (δ_H 1.27) has HMBC correlations with C-3, C-8 and methyl C-19 as in the other 6/8/6-membered taxanes.[8] Long-range correlations of H-20 to C-11, C-12, C-13 and C-18 as well as H-18 to C-20 strongly suggested that C-20 is connected to C-12 to form a new five-membered ring; this is confirmed with the observed chemical shifts and geminal coupling constant of H-20a (δ_H 2.11, d, J = 11.4 Hz) and H-20b (δ_H 2.00, d, J = 11.4 Hz). The chemical shifts of the two quaternary carbons C-3 and C-11 suggested that **A** was C-3,11 cyclotaxane.[9] The much deshielded oxygenated carbon of C-4 at δ_C 87.4 and the fact that no free

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hydroxyl group was free (the three hydroxyl groups observed in the ^{1}H NMR spectrum are attached to C-5, C-9 and C-13) together with one remaining unsaturation degree requirement suggested that the hemiacetal was formed between C-13 and C-4. Thus, the structure of **A** was rigorously characterized as shown in Fig. 1. The relative stereochemistry of **A** (Fig. 2) was elucidated from analysis of the NOESY experiment and the coupling constants. The large coupling constant between H-9 and H-10 (J = 9.0 Hz) suggested that H-9 and H-10 adopted *trans*-orientations. The β-orientations of H-2 and H-9 were assigned by NOESY correlations of H-2 with H1, H-17, and H-19, and H-9 with H-17 and H-19. The α-orientation of H-10 was assigned by the observation of NOESY correlations between H-10 with H-18. The β-orientation of H-5 was indicated by the observation of NOESY correlations between H-5 and H-19, indicating that ring C has some boat character.

B was isolated as an amorphous solid; its elemental composition was determined to be $C_{24}H_{34}O_8$ on the basis of high-resolution FABMS analysis, which displayed a pseudo-molecular ion at *m*/*z* 473.2164 $[M + Na]^+$ and 489.1888 $[M + K]^+$. The double bond equivalents of **B** were calculated to be eight. The ^{1}H NMR spectrum of **C** exhibited four signals due to the protons which correlated with acetyl carbonyl carbons and three hydroxyl groups (when recorded in acetone-D_6) in addition to four signals for four tertiary methyls. The ^{13}C NMR spectrum of **C** demonstrated a hemiacetal carbon (δ_C 101.6), five oxygenated carbons, and two acetyl groups. As in **A**, the ^{13}C NMR spectrum of **B** did not reveal any olefinic carbon signals. Thus, six rings must form the molecular skeleton of **B**, taking into consideration the subtraction of only two double bond equivalents for two carbonyl groups. After careful comparison of the NMR data of **A** and **B** we found that both ^{1}H and ^{13}C NMR spectral data of **B** closely resembled those of **A** except that H-9 is shifted to down field δ_H 5.42 in **B** (from δ_H 4.13 in **A**) while H-2 is shifted to the up field δ_H 4.60 in **B** (from δ_H 5.60 in **A**). Therefore, the structure of **B** was unravelled as 2-deacetyl-9-acetyl **A**. The complete assignments of all the ^{1}H and ^{13}C signals were achieved with the help of ^{1}H–^{1}H COSY, HSQC and HMBC experiments as described for **A**.

C exhibits very similar spectral data when compared with **A** and **B** except for the presence of three acetyl groups (δ_H 2.01 (s), 2.03 (s), 2.04 (s)). After examining the deshielded positions of H-2 (δ_H 5.63), H-9 (δ_H 5.43) and H-10 (δ_H 5.59) we concluded that the three acetyl groups were located at these positions. HMBC allowed us to confirm this finding with the observation that these three protons were correlated to acetyl carbonyl carbons.

A putative biogenesis of the dipropellanes is proposed in Scheme 1 with taxinine A, previously isolated from the needles of the Canadian yew,[10] as a starting material. The first step would be a rearrangement of the 4(20) double bond to a more stable 3,4-tetra-substituted compound that we named 20-deoxytaxezopidine B, by analogy with taxezopidine B with a C-20-hydroxyl group which was found in the seeds of the Japanese yew.[11] Abstraction of an allylic hydrogen from C-20 would lead to a 3,11-cyclic intermediate with an enol-form on C12–C13. The three-dimensional structure of taxanes assures the proximity of the C4–C20 and C12–C13 double bonds, causing the cyclization to form a bond between C20 and C12 restoring a keto-group on C-13. This reaction is promoted by oxidation of the 4–20 double bond by an oxygenase (OH^+). The last cycle to be formed will be generated by attack of the hydroxyl group on C-4 on the carbon-13 ketone to form a stable complex molecule as shown in Scheme 1. These compounds are the

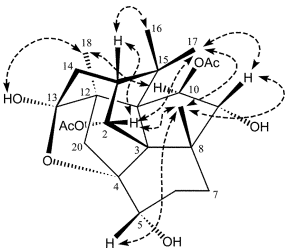
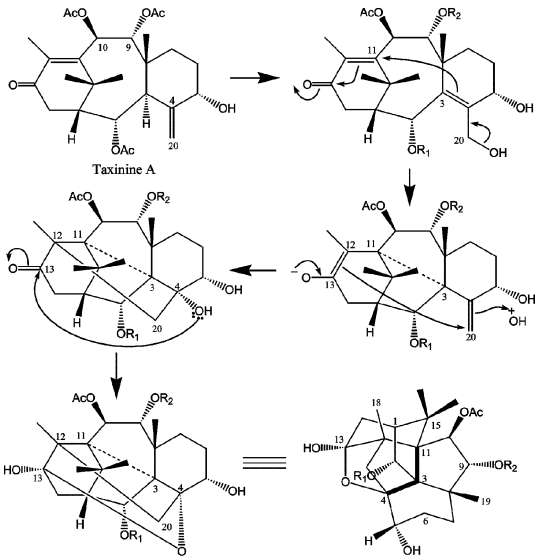


Fig. 2 Relative stereochemistry of **A** showing the NOESY correlations in dotted arrows.



Scheme 1 Proposed mechanism for the biosyntheses of the three dipropellanes **A**, **B**, **C**.

first di-propellanes isolated from a natural source. One of these [3.3.3] propellanes is based on the 3–11 bond which is shared by three cyclopentanes. The second one ([3.4.5]) has the 3–4 bond shared by a cyclopentane, a cyclohexane and an oxacyclohexane.

The biological activities of these taxanes were evaluated by the research group of Professor Richard Momparler at the Saint-Justine Hospital in Montreal. The samples were tested for *in vitro* antileukemic activity against human HL-60 myeloid leukemic cells and in the L1210 leukemia mouse model but showed no growth inhibition. The controls taxol® and taxotère® were very active and showed growth inhibition of 86% at 72 hours. These results could have been expected since these taxane derivatives do not have any of the bioactivities associated with the parent compound Taxol®. Indeed, none of the required chemical groups associated with this activity[12] (C-2-benzoyl, C4-C5-oxetane and C-13-side chain) are found in these metabolites.

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