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Taxanes Isolated from Taxus canadensis

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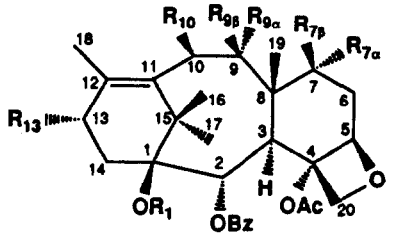
Abstract : Eight taxanes isolated from *Taxus canadensis* have been rigorously characterized by spectroscopic techniques. Their relative amounts differ from other *Taxus* species. Three of these metabolites had not been reported in other *Taxus* species.

Taxol the unusual diterpene natural product, isolated in 1971[1], has recently received an enormous publicity both in the scientific[2] and local media[3]. The unusual anticancer activity of taxol[4], its unique mode of action[5], its scarcity[6] and the desperate need of the populations for the "magic pill" against cancer might be some of the reasons. Taxol is presently isolated from the bark of *Taxus brevifolia*, the pacific yew tree or of *Taxus baccata*, its European relative. Since removal of the bark destroys the trees and endangers the survival of the species, the National Cancer Institute report of its isolation from stems and needles of various *Taxus* species including *Taxus canadensis*, offered the hope that taxol supply will be unlimited. This is however still a long-range objective since all the *Taxus* species grow very slowly. *Taxus canadensis* is a ramping evergreen bush which is commonly found in all shady woods in Quebec.

In this publication, we report the detection and characterization of eight taxanes from *Taxus canadensis*, three of them have never been reported before. We compare the relative amounts of these taxanes with those reported in other *Taxus* species. Most of the taxanes that we isolated have similar overall structures (differing in substituents) to the ones described in other *Taxus* species. The relative amounts of taxanes, however differ. For example, the major metabolite in *Taxus baccata* and *Taxus brevifolia*[8], 10-deacetylbaccatin III is not abundant in the Canadian species. We found taxanes with the three major structural groups detected by Potier's research group[9]. The structure characterizations were performed using spectroscopic techniques (UV, ^{1}H nmr, ^{13}C nmr, 2D and NOEd experiments, M.S. and High resolution M.S.).

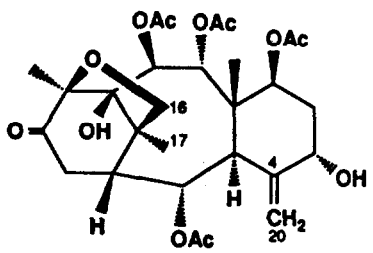
The eight taxane structures rigorously identified in *T. canadensis* are shown in scheme 1. The most abundant taxane is metabolite 5[10] which had never been reported previously. We found 10-deacetylbaccatin III (3 in scheme 1 where $R_{7\beta}=R_{10}=R_{13}=OH$; $R_{9\alpha\beta}=O$; $R_1=R_{7\alpha}=H$) to be a minor metabolite. The taxol (1, scheme 1 where $R_{7\beta}=OH$; $R_{7\alpha}=R_1=H$; $R_{9\alpha\beta}=O$; $R_{10}=OAc$; $R_{13}=$PhCONH-CH(Ph)CH (OH)-COO) was isolated in the same yield as reported[7]. Cephalomannine (2 where $R_{7\beta}$ = OH; $R_{7\alpha}$ = R_1 =H; $R_{9\alpha\beta}$ = O; R_{10} = OAc; R_{13} = $CH_3C{:}C(CH_3)CONHCH(Ph)CH(OH)$-COO); 10-deacetylbaccatin III and taxol were identified by NMR, M.S. HRMS, comparison with literature [11,12] and by spiking with standards (we gratefully acknowledge the supply of standards by Ms Nancita Lomex and Dr Kathleen H. Groover, National Cancer Institute, Maryland USA). Comparison of the spectral data of metabolite 4[13] with that of closely related

taxane diterpenes, reveals that it belongs to the type C defined by Potier[9], having an oxetane ring at positions 4 -5 and a C-9 ketone. It resembles very much to 10-deacetylbaccatin III except that at C-1 there is an acetyl instead of a hydroxyl group. This conclusion was reached from the proton nmr. H-2 was deshielded by the presence of the benzoyl group on C-2. All the other protons were not deshielded by the presence of an ester group and appeared at usual chemical shifts for hydroxylated positions. Since we observed two isolated acetyls on quaternary carbons (one of them being C-4) the only other hydroxylated carbon was C-1. Interestingly enough this metabolite had not been reported previously in any *Taxus sp.* All the spectroscopic data [13] agree with this structure. Although an ion having m/z 587 corresponding to MH^+ was not prominent in the FAB mass spectra of 4, fragments were apparent that support this assignment and high resolution measurements confirmed the empirical formula : $MH^+ - H_2O$: 569.23776: $C_{31}H_{37}O_{10}$ requires 569.23867; MH^+ - AcOH, 527.22835: $C_{29}H_{35}O_9$ requires 527.22811.



Metabolites 1 - 5

4 : $R_{7\beta} = R_{10} = R_{13} = OH$; $R_{7\alpha} = H$; $R_{9\alpha,\beta} = O$; $R_1 = Ac$
5 : $R_{7\beta} = R_{9\alpha} = OH$; $R_{7\alpha} = R_{9\beta} = R_1 = H$; $R_{10} = R_{13} = OAc$



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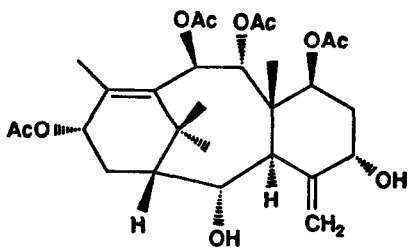

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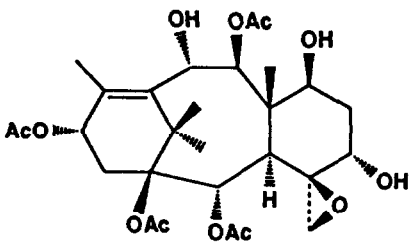

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Scheme 1 Taxanes in *Taxus canadensis*: the metabolites 4, 5, 8 are new natural products.

In order to confirm the assignments of the new metabolite **5**, it was acetylated. The ^1H NMR of acetylated **5**[14] (two acetyls had been introduced) was identical to that of baccatin VI a natural product which was reported in *Taxus baccata*.[15] Analysis [14,16] of the NMR's revealed that metabolite **5** was 7,9-deacetyl baccatin VI. The natural product **5** differed from 10-deacetylbaccatin III by two acetyl groups at C-10 and C-13 and a hydroxyl group at C-9. Low resolution FAB mass spectra as well as high resolution confirmed the formulas of both metabolite **5** and its acetylated derivative. Indeed, MH^+ for metabolite **5** was found to be 631.27567. $C_{33}H_{43}O_{12}$ requires 631.27545. The acetylated derivative of **5** MH^+ was 715.29679 with $C_{37}H_{47}O_{14}$ requiring 715.29658. Metabolite **6** showed spectroscopic properties[17] which agree with 2-deacetyl-5-decinnamate taxagifine[18] but has an acetyl group at C-2. The authors[18] reported that the 2-acetyl derivative (which could be identical to **6**) had been published in a local chinese publication[19]. It could belong to the structural group B[9] because of the substitution pattern at C4-C5, however, ring A is strikingly different . Metabolite **7** first time isolated in *T. canadensis* was determined from spectroscopic data to be identical to 2-deacetyl-5-decinnamoyltaxinine J a taxane previously isolated in *Austrotaxus spicata*[20]. Metabolite **8** was a novel taxane never reported previously in other yews. The same skeleton however with an OH at C-1, an OAc at C-10 and at C-7 has been identified in *Taxus yunnanensis*[21]. In the ^1H nmr of **8**[22] we first note the presence of three singlet methyl groups shielded as usual taxanes (Me-16, Me-17, Me-19). In the 2 ppm area we observe four acetyls and 1 methyl doublet (Me-18) (very small coupling) embedded in this area. From the COSY experiment we observe that the deshielded proton assigned to H-2 is coupled to a shielded doublet (H-3). The AB observed normally for the C-20 oxetane is absent and is replaced by a methylene (two doublets with coupling typical for a three membered ring) from an epoxide at a shielded position (3.4 ppm, 2.3 ppm). H-5, H-6 and H-7 are connected together as shown in the COSY, and from the shielding observed for H-5 and H-7 (4.1 - 4.2 ppm) we can conclude that these positions are hydroxylated (no acetyls). The 9 and 10 positions appear as a pair of doublets, one very deshielded (acetyl) and the other hydroxylated (no acetyl). The deshielded proton is at 6.0 ppm which is too shielded to be adjacent to a double bond (this was also confirmed by NOEd experiments[23]). The last spin system composed of H-13 and H-14 (connected from COSY) shows that H-13 is deshielded by both an acetyl and a double bond. We observe four acetyl groups and only three of them could be localized from their deshielding effect, we can therefore conclude that the last acetyl has to be on a quaternary carbon. The only quaternary carbon is C-1. The relative stereochemistry of the substituents was confirmed by NOEd experiments. The eight taxanes isolated and characterized from *T. canadensis* show all the structure varieties found in other yews with the advantages that : i) they are isolated from the needles and stems and ii) the relative amounts of the various taxanes differ from other yews. We are therefore presently synthesizing various substituted taxanes.

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REFERENCES AND NOTES :

1. Wani, M.C.; Taylor, H. L.; Wall, M.E.; Coggon, P.; McPhail, A.T. *J. Am. Chem. Soc.* **1971** *93*, 2325-2326.
2. Kingston, D.C.I.; Samaranayake, G.; Ivey, C.A. *J. Nat. Prod.* **1990**, *53*, 1-12.
3. The New York Times, May 13, 1991, pp A1; The Wall Street Journal, April 9, 12 **1991** pA.
4. Suffness, M.; Cordell, G.A. In the *Alkaloids*; Brossi, A. Ed.; Academic Press; New York, 1985, Vol. 25, Chapter 1; pp. 1-368.
5. Manfredi, J.J.; Horwitz, S.B. *Pharmac. Ther.* **1984**, *25*, 83-125.
6. Denis, J.N.; Greene, A.E.; Guénard, D.; Guéritte-Voegelein, F.; Mangatal, L.; Potier, P. *J. Am. Chem. Soc.* **1988**, *110*, 5917-5919.
7. Witherup, K.M.; Look, S.A.; Stasko, M.W.; Ghiorri, T.J.; Muschik, G.M.; Cragg, G.M. *J. Nat. Prod.* **1990** *53*, 1249-1255.

8. Kingston, D.G., Hawkins, D.R.; Ovington, L. *J. Nat. Prod.* **1982**, *45*, 466-470.
9. Guéritte-Voegelein, F.; Guénard, D.; Potier, P. *J. Nat. Prod.* **1987**, *50*, 9-18.
10. The yields of the purified eight taxanes isolated from dried needles and stems of *T. canadensis* are the following: **1** (taxol): 0.006%; **2** (cephalomannine): 0.002%; **3** (10-deacetylbaccatin III): 0.004%; **4** (1-acetyl, 10-deacetylbaccatin III); 0.01%; **5** (7, 9-deacetylbaccatin VI): 0.014%; **6** (5-decinnamate taxagifine): 0.004%; **7** (2-deacetyl-5-decinnamoyltaxinine J): 0.005%; **8** (1ß-acetyl, 5,7,10-deacetylbaccatin I): 0.003%.
11. Senilh, V.; Blechert, S.; Colin, M.; Guénard, D.; Picot, F.; Potier, P.; Varenne, P. *J. Nat. Prod.* **1984**, *47*, 131-137.
12. Miller, R.W. *J. Nat. Prod.* **1980** *43*, 425-437.
13. ^{1}H nmr of **4** in ppm: H-2 (d): 5.742 J= 6.6 Hz; H-3 (d): 4.113 J= 6.6 Hz; H-5 (dd): 5.081 J= 7.3 Hz; H-6a (ddd): 2.706 J= 6.6, J= 9.2, J=14.5 Hz; H6b (ddd): 1.929 J=2.0, J= 11.2, J=14 Hz; H-7 (dd): 4.388, J= 11.2, J= 7.3 Hz; H-10 (s): 5.353; H-13 (brt): 4.985 J= 7.9 Hz; H-14ab (m): 2.366; H-16/H-17: 1.199; H-18: 2.181; H-19: 1.849; H-20a (d): 4.428 J= 7.9 Hz; H-20b (d): 4.274 J= 7.9Hz; benzoyl - ortho (d) (2H): 8.206, J= 7.2, -meta (t) (2H): 7.583 J= 7.3 Hz, -para (t) (1H): 7.715 J= 7.9 Hz Acetyls (s) 2.393, (s) 2.110.
14. ^{1}H nmr of acetylated **5**: H-2 (d): 5.849 J= 5.9 Hz; H-3 (d): 3.161 J= 6.0 Hz; H-5 (d): 4.948 J= 7.9 Hz; H-6a (ddd): 2,482 J= 8.8 J= 14.9 J= 15.1 Hz; H-6b (ddd): 1.848 J= 1.3 J= 9.9 J= 15.0 Hz; H-7 (dd): 5.525 J= 9.9, J= 8.8 Hz; H-9 (d): 5.979 J= 11.2 Hz; H-10 (d): 6.196 J= 11.2 Hz; H-13 (brt): 6.149; H-14a/H-14b (m): 2.202; H-16 (s): 1.763; H-17 (s): 1.21; H18 (d): 2.010 J= 1.3; H-19 (s): 1.58; H-20a (d): 4.312 J= 8.6 Hz; H-20b (d): 4.104 J= 8.6 Hz; Benzoyl - ortho (d) 8.076 J= 7.1, - meta (t) 7.591, -para (t) 7.458; OAc 4 (s) for 5 acetyls: (3H): 2.270; (6H): 2.088; (3H): 2.176; (3H): 1.978.
15. Della Casa de Marcano, D.P.; Halsall, T.G. *J. Chem. Soc. Chem Comm* **1975**, 365-366.
16. ^{1}H nmr of metabolite **5** in ppm: H-2 (d): 5.734 J= 6.0 Hz; H-3 (d): 3.028 J= 6.0 Hz; H-5 (d): 4.934 J= 7.9Hz; H-6a (ddd): 2,526 J= 7.9, J= 9.2 J= 15.2 Hz; H-6b: 1.86; H-7/H-9, 4.414; H-10/H-13, 6.15; H-14a/H-14b: 2.2 - 2.0; H-16 (s): 1.657; H-17 (s): 1.232; H-18 (d): 1.925 J= 1.3 Hz; H-19 (s): 1.798; H-20a (d): 4.291 J= 8.6 Hz; H-20b (d): 4.140 J= 8.0 Hz.
17. ^{1}H nmr of **6** (from *T. canadensis*)in ppm: : H-1 (dd): 2.342 $J_{2,1}$ 1,5 Hz $J_{1,14}$ = 11.4 Hz; H-2 (dd): 5.494 $J_{2,3}$ = 9.1 $J_{2,1}$ 2.0 Hz; H-3 (d): 3.517 $J_{2,3}$: 9.1 Hz; H-5 (t): 4.318 $J_{5,6ab}$ = 3.3 Hz; H-6a (m): 2.06; H-6b (m): 1.53; H-7 (dd): 5.371 $J_{7,6a}$ = 6.3 $J_{7,6b}$ = 10.7 Hz; H-9 (d): 4.90 $J_{9,10}$ = 3.5 Hz; H-10 (d): 5.335 $J_{9,10}$ = 3.91 Hz; H-14a (dd): 3.018 J_{ab}= 18.2 Hz $J_{14a,1}$= 11.6 Hz; H-14b (d): 2.619 J_{ab} = 18.4 Hz; H-16a (d): 4.166 J_{ab} =8.2 Hz; H-16b (d): 3.669 J_{ab} 8.3 Hz; H-17 (s): 1.504; H-18 (s): 1.153; H-19 (s): 1.033; H-20a (s): 5.238; H-20b (s): 4.476; four OAc: 2.093(s), 2.081(s), 2.009(s), 1.940(s).
18. Zhang, Z.P.; Jia, Z. *Phytochemistry* **1991** *30*, 2345-2348.
19. Zhang, Z.P.; Jia, Z.J.; Zhu, Z.O.; Cui, Y.X.; Cheng J.L.; Wang Q.G. Kexue Tongbao **1989** *21*, 1630.
20. Ettouati, L.; Ahond, A.; Convert, O.; Laurent, D.; Poupar, C.; Potier, P. *Bull. Soc. Chim. Fr.* **1988**, 749-755.
21. Zhang, Z.; Jia, Z. *Phytochemistry* **1990** *29*, 3673-3675.
22. Assignments of the ^{1}H nmr of **8** isolated from *T. canadensis*: H-2 (d): 5.314 $J_{2,3}$ = 3.5 Hz; H-3 (d): 3.054; H-5 (t): 4.186 $J_{5\text{-}6a}$= $J_{5\text{-}6b}$ = 3.0 Hz; H-6a,b: 2.1 - 1.95; H-7 (dd): 4.235 $J_{6a,7}$= 11.7, $J_{6b,7}$ = 4.7 Hz; H-9 (d): 6.016 $J_{9,10}$ = 10.5 Hz; H-10 (d): 4.576; H-13 (om): 6.03; H-14a (dd): 2.492, J_{ab} = 14.9 $J_{13,14a}$= 9.3; H-14b: 1.8; H-16: 1.211; H-17: 1.509; H-18 (d): 2.140 $J_{13,18}$ = 1.3; H-19 (s): 1.373; H-20a (d): 3.458 $J_{20a,20b}$ = 5.3; H-20b (d): 2.306 Acetyls 2.167 (s); 2,118 (s); 2.082 (s); 2.035 (s).
23. Results of NOEd on **8**. The symbols W will represent weak enhancement, M medium and S strong; the irradiated protons will be in brackets {}. {Me-16} led to H-13 (S); {Me-19) led to H-2 (W) and H-10 (W); {Me-17} led to H-2 (S), H-10 (S) and Me-16 (M); {Me-18} led to H-10 (S); {H-20B} led to H-20A (S) and H-5 (S); {H-14A} led to H-13 (M) and H-14B (M); {H-3} led to H-2 (W), H-7 (W) and H-9 (W); {H-20A} led to H-20B (S), H-9 (W) and H-2 (W); {H-5) led to H-20B (S), Me-16 (W) and Me-19 (W); {H-7} led to H-9 (S), H-3 (S) and H-20A (W); {H-10} led to H-2 (S), H-9 (M), Me-17 (M), Me-19 (M-W) and H-3 (W); {H-2} led to H-10 (S), Me-17 (M) and Me-19 (M); {H-9 & H-13} led to H-7 (S), Me-18 (S) and Me-16 (S).

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