Pergamon

0031-9422(95)00706-7

Phytochemistry, Vol. 41, No. 3, pp. 803–805, 1996

0031-9422/96 $15.00 + 0.00

ISOLATION AND SEMI-SYNTHESIS OF A BIOACTIVE TAXANE FROM *TAXUS CANADENSIS*

LOLITA ORA ZAMIR, MARIA ELENA NEDEA, ZHEN-HUA ZHOU, GAÉTAN CARON, FRANÇOISE SAURIOL* and ORVAL MAMER†

Université du Québec, Institut Armand-Frappier, 531, boul. des Prairies, Laval-des-Rapides, Québec, Canada H7N 4Z3; *Department of Chemistry, McGill University, 801 Sherbrooke Street West, Montreal, Québec, Canada H3A 2K6; †Biomedical Mass Spectrometry Unit, McGill University, 1130 Pine Avenue West, Montreal, Québec, Canada H3A 1A3

(*Received in revised form* 21 *June* 1995)

Key Word Index—Taxanes; *Taxus canadensis*; Taxaceae; taxcultine.

Abstract—A minor bioactive taxane, isolated from the needles of *Taxus canadensis* and semi-synthesized, was shown to be taxcultine.

INTRODUCTION

Taxus canadensis, a small ramping bush abundant in Quebec, seems to differ from other yews by the content of its taxanes. 9-Dihydro-13-acetylbaccatin III is found in amounts three to seven times the content of taxol [1]. Various derivatives of 9-dihydro-13-acetylbaccatin III and an uncyclized taxane (canadensene) were also found in the needles of the canadian yew [2, 3]. This paper reports the isolation of taxcultine (**1**) from *T. canadensis* needles and its semi-synthesis. This taxane was previously obtained from cell cultures of *T. baccata* (hence the given name of taxcultine) [4, 5].

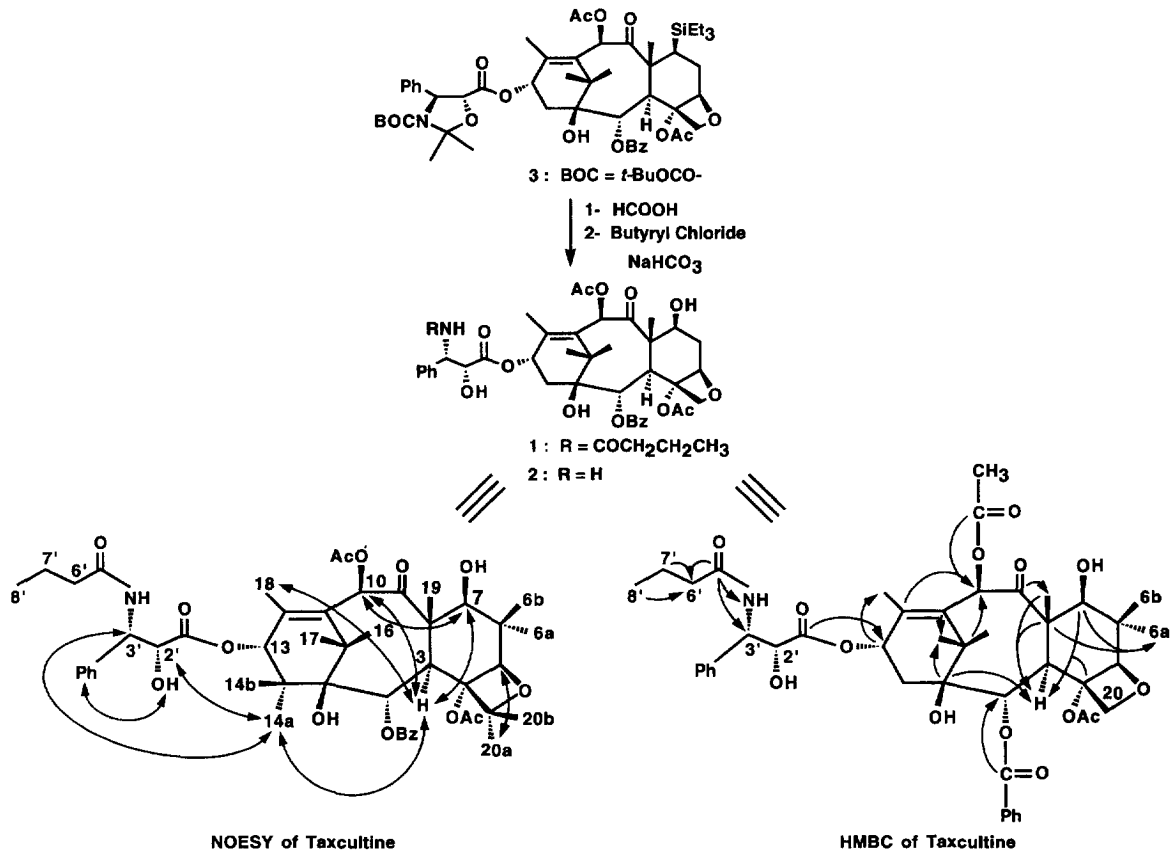
RESULTS AND DISCUSSION

Compound **1** was a minor metabolite (0.0008% yield from *T. canadensis* needles) which was found in the same flash chromatography fraction as taxol, 9-dihydro-13-acetylbaccatin III and numerous minor taxanes. Semi-preparative HPLC using a reversed phase column and a gradient solvent system of water–acetonitrile allowed the isolation of a mixture containing **1** and 10-hydroxyacetylbaccatin VI, a new taxane recently identified [3]. The final purification of **1** was achieved by analytical HPLC.

A detailed high resolution NMR analysis (500 MHz ^{1}H NMR, HMQC and HMBC) allowed the assignments of the protons and the carbons. The two-dimensional ^{1}H–^{13}C correlations (HMBC) obtained for **1** are shown on the lower right side of the figure: the arrows indicate carbon–hydrogen connectivities with the arrow head indicating location of the proton. The NOESY experiment enabled us to confirm the stereochemistry of the diterpenoid core skeleton and that the 'U-shape' of taxol is retained in **1** as can be seen by the long range correlation between methyl-18 and H-3. The α-side correlations are shown on the lower left side of the figure. Absolute confirmation of the structure of **1** was obtained by semi-synthesis. 10-Deacetylbaccatin III isolated from *T. canadensis* needles was protected [6] and coupled with *N,O*-protected β-phenylisoserine to give **3** and hydrolysed [7] to give **2**. Acylation with butyryl chloride yielded a compound, which, after purification (53% yield), was identical to **1**. High resolution NMR and mass spectra of the semi-synthetic and naturally occurring **1** were identical. In addition, HPLC chromatograms of the natural and semi-synthetic products under three different mobile phase conditions were identical. Compound **1** promotes the assembly of microtubules, and its activity is slightly higher than that of taxol, but lower than that of taxotere. The semi-synthesis can now provide large quantities of **1** for further testing.

EXPERIMENTAL

General. NMR: 500 MHz (^{1}H), and 125 MHz (^{13}C) in $CDCl_3$. Low resolution Xe fast atom bombardment mass spectra were obtained in glycerol. The samples were dissolved in DMSO before addition of 0.5 ml glycerol. High resolution mass spectra were made similarly in glycerol–DMSO, at a resolving power of 12000. Semi-prep. HPLC was performed on a Waters Delta Prep 3000 instrument coupled with a Model 481 variable wavelength detector with two ODS-2 MAG-9 reversed phase semi-prep. column (Whatman) connected in series (9.4 × 500 mm), at 3 ml min^{-1}. Detection was at 227 nm. For all semi-prep. HPLC sepns, a gradient of acetonitrile (25–100%) in H_2O over 50 min was used unless otherwise stated. The analyt. HPLC system was a Perkin–Elmer instrument equipped with



an isocratic and binary LC pump (Model 250). The sepns were achieved on two ODS-2 reversed phase columns (Whatman) connected in series (4.6 × 300 mm), at 1 ml min^{-1}.

Plant material. Taxus canadensis was collected in Quebec, during the autumn season. The plants were stored at 4° in sterilized sand and peatmoss and were dried before grinding.

Extraction, isolation and purification. The extraction procedure is the same as described in ref. [3]. Semi-prep. HPLC allowed isolation of a peak (R_t = 38 min) which contained a mixt. of **1** and 10-hydroxyacetylbaccatin VI [3]. The final purification of **1** was achieved by analyt. HPLC (isocratic conditions, eluent H_2O–MeCN (29:21; R_t = 49.2 min) and yielded 4 mg **1** as a powder. UV λ_{max} MeOH 230 nm (ε12800). FAB-MS *m/z*: 820.35424, $C_{44}H_{54}NO_{14}$ requires 820.35443. ^{1}H NMR: δ 0.90 (3H, *t*, *J* = 7.3 Hz, H-8′), 1.15 (3H, *s*, H-17), 1.26 (3H, *s*, H-16), 1.62 (2H, *m*, H-7′), 1.68 (3H, *s*, H-19), 1.82 (3H, *s*, H-18), 1.88 (1H, *dd*, *J* = 10.7, 15.6 Hz, H_b-6), 2.18 (2H, *t*, *J* = 7.3 Hz, H-6′), 2.25 (3H, *s*, Ac-10), 2.28–2.32 (2H, *m*, H-14), 2.34 (3H, *s*, Ac-4), 2.46 (1H, *brs*, OH-2′), 2.54 (1H, *ddd*, *J* = 15.6, 9.3, 6.5 Hz, H_a-6), 3.79 (1H, *d*, *J* = 7.0 Hz, H-3), 4.19 (1H, *d*, *J* = 8.3 Hz, H_b-20), 4.30 (1H, *d*, *J* = 8.3 Hz, H_a-20), 4.40 (1H, *dd*, *J* = 10.7, 6.3 Hz, H-7), 4.68 (1H, *brs*, H-2′), 4.94 (1H, *d*, *J* = 9.3 Hz, H-5), 5.57 (1H, *dd*, *J* = 9.3, 2.4 Hz, H-3′), 5.67 (1H, *d*, *J* = 7.0 Hz, H-2), 6.20 (1H, *d*, *J* = 9.3 Hz, NH-4′), 6.21 (1H, *m*, H-13), 6.28 (1H, *s*, H-10), 7.35–7.40 (5H, *m*, CHPh), 7.51–8.11 (5H, *m*, COPh). ^{13}C NMR: δ 9.16 (C-19), 13.53 (C-8′), 14.70 (C-18), 18.79 (C-7′), 20.54 (Ac-10), 21.70 (C-17), 22.28 (Ac-4), 26.66 (C-16), 35.41 (C-6), 35.41 (C-14), 38.33 (C-6′), 43.02 (C-15), 45.33 (C-3), 54.37 (C-3′), 58.39 (C-8), 72.16 (C-7), 72.16 (C-13), 73.04 (C-2′), 74.79 (C-2), 75.37 (C-10), 76.25 (C-20), 78.90 (C-1), 81.03 (C-4), 84.12 (C-5), 127.00, 128.17, 129.04, 137.84 (CHPh), 128.75, 130.21, 133.71, 137.84, 166.88 (COPh), 133.14 (C-11), 142.11 (C-12), 170.30 (Ac-4), 171.16 (Ac-10), 172.86 (C-1′), 172.86 (C-5′), 203.62 (C-9).

Biological activity: in vitro *polymerization.* A modification of the method in ref. [8] was used to measure the ability of taxol, **1** and taxotere to stimulate the assembly of tubulin into microtubules. Microtubule protein was isolated from fresh bovine brain by two assembly–disassembly cycles similar to the method of ref. [9], but with some modifications [10]. Initial rates and final values of absorbance at 350 nm were for taxotere: 0.149 AU min^{-1}; 0.56 AU; **1**: 0.068 AU min^{-1}; 0.40 AU; taxol: 0.058 AU min^{-1}; 0.28 AU; control: 0.003 AU min^{-1}; 0.06 AU.

Semi-synthesis: *the coupling compound* (**3**). The side chain of **3** was prepd according to ref. [7]; FAB-MS of the methyl ester *m/z*: 336.1812, $C_{18}H_{26}NO_5$ requires 336.1811; FAB-MS of the acid-CH_3 *m/z*: 306.1341, $C_{16}H_{20}NO_5$ requires 306.1342. The coupling procedure of ref. [6] was used. After work up, the product was purified by semi-prep. HPLC (R_t = 63.1 min). The product (**3**) was obtained as a solid (85% yield). Starting material (10%; R_t = 50.8 min) was recovered. Compound **3**: FAB-MS *m/z* $[MH]^+$ 1004, $[MH - AcOH]^+$ 944.

^{1}H NMR: (two rotamers present; second downfield signals represented by an asterisk; integrations of split signals are half indicated value) δ 0.56 (6H, *m*, $SiCH_2$), 0.91 (9H, *m*, $SiCH_2CH_3$), 1.55 (9H, *s*, *t*-Bu), 1.80 (1H, *m*, H_a-6), 2.48 (1H, *m*, H_b-6), 2.14 (2H, *m*, H-14), 3.74 (1H, *d*, J = 7.0 Hz, H-3), 3.77 (*1H, *d*, J = 7.1 Hz, H-3), 4.09 (1H, *d*, J = 8.3 Hz, H_b-20), 4.10 (*1H, *d*, J = 8.1 Hz, H_a-20), 4.23 (1H, *d*, J = 8.0 Hz, H_a-20), 4,24 (*1H, *d*, J = 8.3 Hz, H_b-20), 4.46 (1H, *m*, H-7), 4.86 (1H, *d*, J = 7.3 Hz, H-5), 4.87 (*1H, *d*, J = 8.0 Hz, H-5), 5.06 (1H, *br s*, H-2′), 5.55 (1H, *br s*, H-3′), 5.64 (1H, *d*, J = 7.1 Hz, H-2), 5.65 (*1H, *d*, J = 6.9 Hz, H-2), 6.24 (1H, *t*, J = 8.8 Hz, H-13), 6.43 (1H, *s*, H-10), 6.44 (*1H, *s*, H-10), 7.3–7.4 (5H, *m*, CHPh), 7.50–8.04 (5H, *m*, COPh).

Semi-synthetic taxcultine (**1**). The coupling compound (**3**) (4.0 mg, 0.0040 mmol) was stirred in 0.3 ml 95–97% HCO_2H at room temp. The reaction was monitored by TLC and after 4 h, HCO_2H was removed by a flow of N_2. After drying under vacuum, the residue was dissolved in EtOAc (0.5 ml), and $NaHCO_3$ (10 mg) and butyryl chloride (5 μl, 0.047 mmol) were added. After stirring for 1 h, EtOAc (10 ml) was added to the soln, which was washed with brine and dried over $MgSO_4$. After filtration and evapn, the residue was purified by semi-prep. HPLC (R_t = 31.7 min). Compound **1** was obtained as a solid [1.8 mg, 53% yield based on compound **3**, R_f = 0.25, EtOAc–hexane (7:3)]; FAB-MS *m/z*: 820.35423, $C_{44}H_{54}NO_{14}$ requires 820.35443. NMR values were identical to that of the naturally occurring **1**. Co-elution of the semi-synthetic and naturally occurring samples of **1** on analyt. HPLC showed identical R_t values in 3 different conditions: 1—linear gradient of AcCN (25–100%) in H_2O over 50 min, R_t = 31.7 min; 2—linear gradient of AcCN (25–100%) in H_2O over 140 min, R_t = 57.1 min; 3—isocratic conditions with AcCN (43%) in H_2O, R_t = 45.9 min.

Acknowledgements—We thank the Natural Sciences and Engineering Research Council of Canada and the Canadian Breast Cancer Research Initiative for support of this work via operating grants to L.O.Z. The Fondation Armand-Frappier is gratefully acknowledged for a post-doctoral fellowship to G.C.

REFERENCES

1. Zamir, L. O., Nedea, M. E., Bélair, S., Sauriol, F., Mamer, O., Jacqmain, E., Jean, F. I. and Garneau, F. X. (1992) *Tetrahedron Letters* **33**, 5173.
2. Zamir, L. O., Zhou, Z. H., Caron, G., Nedea, M. E., Sauriol, F. and Mamer, O. (1995) *J. Chem. Soc., Chem. Commun.* 529.
3. Zamir, L. O., Nedea, M. E., Zhou, Z. H., Bélair, S., Sauriol, F., Jacqmain, E., Jean, F., Garneau, F. X. and Mamer, O. (1995) *Can. J. Chem.* **73**, 655.
4. Ma, W., Park, G. L., Gomez, G. A., Nieder, M. H., Adams, T. L., Aynsley, J. S., Sahai, O. P., Smith, R. J., Stahlhut, R. W. and Hylands, P. J. (1994) *J. Nat. Prod.* **57**, 116.
5. Appendino, G., Cravotto, G., Enriù, R., Gariboldi, P., Barboni, L., Torregiani, E., Gabetta, B., Zini, G. and Bombardelli, E. (1994) *J. Nat. Prod.* **57**, 607.
6. Denis, J.-N., Greene, A. E., Guénard, D., Guéritte-Voegelein, F., Mangatal, L. and Potier, P. (1988) *J. Am. Chem. Soc.* **110**, 5917.
7. Commerçon, A., Bézard, D., Bernard, F. and Bourzat, J. D. (1992) *Tetrahedron Letters* **33**, 5185.
8. Brunner, M., Albertini, S. and Wurgler, F. E. (1991) *Mutagenesis* **6**, 65.
9. Shelanski, M. L., Gaskin, F. and Cantor, C. R. (1973) *Proc. Natl Acad. Sci. U.S.A.* **70**, 765.
10. Williams, R. C., Jr. and Lee, J. C. (1982) *Meth. Enzymol.* **85**, 376.