Pergamon

Tetrahedron Letters 42 (2001) 3787–3789

TETRAHEDRON LETTERS

Biotransformation of taxol

Tom S. Chen,[a,*] Xiaohua Li,[a] Dan Bollag,[a] Yeuh-chuen Liu[b] and Ching-jer Chang[b]

[a]*Merck Research Laboratories, Rahway, NJ 07065, USA*
[b]*Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, W. Lafayette, IN 47907, USA*

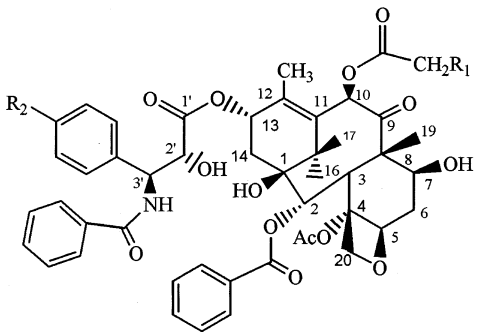
Received 22 December 2000; accepted 12 March 2001

Abstract—Bioconversion of taxol/cephalomannine by *Streptomyces* sp. MA 7065 resulted in hydroxylation on the 10-acetyl methyl group in 60% yield and on the benzene ring at the *para* position of the phenylisoserine side chain in 10% yield. This culture could also hydroxylate the allylic methyl group of the phenylisoserine side chain of cephalomannine quantitatively. All three metabolites were cytotoxic toward human lung, breast and colon tumor cell lines. © 2001 Elsevier Science Ltd. All rights reserved.

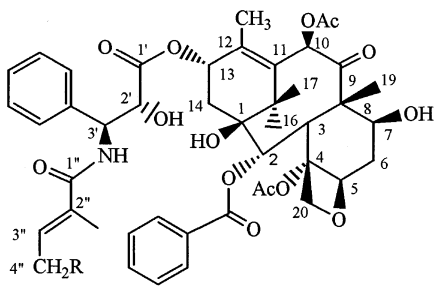
Taxol was first isolated from the stem bark of the Western yew, *Taxus brevifolia*[1] and its structure reported by Wani et al. in 1971. The biological activity of taxol is mainly related to its inhibitory effect on cell division. Taxol promotes formation of microtubules that form the mitotic spindle during cell division and induces apoptosis. Taxol has been effectively used for the treatment of ovarian, breast and lung cancers.[2,3] However, it is administered with Cremophor, which often induced allergic reactions. Thus, further structural modifications of taxol to generate new analogs with increased water solubility and improved bioavailability may provide high utility in cancer treatment.[3]

Taxol is a complicated molecule. Structurally, it can be viewed as the *N*-benzoyl-β-phenylisoserine ester of diterpenoid baccatin III with a very characteristic oxetane ring. Cephalomannine is the minor component of the taxol sample we used for microbial transformation studies. It has a *N*-tigloyl group replacing the *N*-benzoyl group of taxol. Because of the complexity of their structures, we initiated a microbial transformation program primarily aimed at producing more polar taxol/cephalomannine derivatives.

More than 500 microorganisms were screened for their ability to achieve useful biotransformation of taxol/cephalomannine.[4] Of the active cultures identified *Streptomyces* sp. MA 7065 was selected for a two-stage fermentation process scale-up. After incubation of taxol/cephalomannine (80/20) for 72 h, three biotransformation products were detected by HPLC. The crude ethyl acetate extract was initially separated by silica gel chromatotron and further purified by HPLC. The first



R_1=H, R_2=H Taxol
R_1=H, R_2=OH **1**
R_1=OH, R_2=H **2**



R=H Cephalomannine
R=OH **3**

* Corresponding author.

PII: S0040-4039(01)00557-3

product (rt: 39.7 min) displayed the typical UV spectrum of taxol, indicating that none of the phenyl components was biomodified. Its FAB-MS revealed the pseudomolecular ion at m/z 871,[5] suggestive of the structural identity of a hydroxyl taxol. A ^{1}H NMR spectrum[5] showed the disappearance of the 10-acetyl singlet, which was replaced by a two-proton AB-quartet at 4.43 and 4.31 ppm ($J=17.5$ Hz), suggesting that the 10-acetyl group was transformed into a 10-hydroxyacetyl group. The appearance of a new methylene carbon peak at 60.6 ppm further confirmed the presence of the hydroxymethyl group.[5] Extensive COSY NMR analysis substantiated the structure of 10-hydroxyacetyl-10-deacetyltaxol **1** for the first biotransformed product of taxol.

The second biotransformed product also appeared to be a hydroxyl taxol analog on the basis of its FAB-MS pseudomolecular ion at m/z 871.[6] In contrast with the ^{1}H NMR spectrum of **1**, the ^{1}H NMR spectrum of the second product displayed two singlets at 2.38 and 2.24 ppm for 4-acetyl and 10-acetyl groups.[6] In addition, the ^{1}H NMR also showed new A_2X_2 four-spin signals at 7.35 and 6.85 ppm ($J=8.5$ and 2.0 Hz[6]) in lieu of the characteristic five-spin signals [7.47 (*o*-H, dd, $J=7.5$ and 1.5 Hz), 7.40 (*m*-H, t, $J=7.5$ Hz) and 7.34 (*p*-H, tt, $J=7.5$ and 1.5 Hz)] for the 3′-phenyl group of the phenylisoserine side chain. These spectral data strongly indicated that this microbial transformed product was identical to the known metabolite of taxol in humans[7] and rats[8] 3′-(4-hydroxyphenyl)-3′-dephenyltaxol **2**, which was prepared from baccatin III by Park et al.[9]

The UV spectral features of the third product disclosed the skeleton of cephalomannine. The FAB mass spectrum of **3** gave a pseudomolecular ion at m/z 848 that corresponds to an increase of 16 mass units from cephalomannine, indicating that it is a hydroxylated derivative. The ^{1}H NMR spectral data[10] indicated that both phenyl and acetyl groups of cephalomannine were intact. The most distinct spectral changes were centered around the tigloyl amide group of the phenylisoserine side chain. The 4″-allylic methyl proton signal (3H; dq, $J=6.5$ and 1.5 Hz) was replaced by a signal at 4.22 ppm (2H; dq, $J=6.0$ and 1.5 Hz), indicative of hydroxylation of 4″-methyl group. The corresponding coupling pattern changes for 2″-methyl proton (pentet, $J=1.5$ Hz→q, $J=1.5$ Hz) and 3″-olefinic proton (qq, $J=6.5$ and 1.5 Hz→tq, $J=6.0$ and 1.5 Hz) were consistent with the proposed modification. Thus, the third microbial transformation product was assigned as 4″-hydroxycephalomannine **3**. All three microbial transformed products exhibited cytotoxicity against human lung, breast and colon tumor cell lines although they were relatively less potent than the parent compounds (Table 1).

Table 1. Antitumor cytotoxicity of taxanes against human tumor cell lines GI_{50} (μg/mL)

Compound	A-549	MCF-7	HT-29
Taxol	4×10^{-7}	2×10^{-6}	1×10^{-7}
Cephalomannine	1×10^{-4}	8×10^{-4}	2×10^{-4}
1	4×10^{-5}	1×10^{-5}	8×10^{-5}
2	$<10^{-5}$	4×10^{-5}	1×10^{-5}
3	3×10^{-2}	5×10^{-2}	2×10^{-2}

A-549, lung carcinoma; MCF-7, breast adenocarcinoma; HT-29, colon adenocarcinoma.

We have uncovered three microbial biotransformation pathways for taxol/cephalomannine. This is the first time that the hydroxylation of the 10-acetyl group of taxol is reported. The second oxidative transformation occurred at the *para*-position of the 3′-phenyl group in the C-13 side chain, which was detected as the major metabolite of taxol in rats.[8] On the other hand, this hydroxylation product was only a minor metabolite in human.[7] The major hydroxyl metabolite in human took place at C-6.[11] In rats, hydroxylations of the *meta*-position of the 2-benzoyl group and C-19 were detected as well.[7] These results demonstrated the unique differences in the biotransformation of taxol by microorganisms, rats and human. The efficient transformation of taxol into the hydroxyphenyl product **2** by *Streptomyces* species may provide an alternative approach for the preparation of this human metabolite. It is also interesting to note that the oxidative hydroxylation only occurred at the non-aromatic acyl group of the 2′-amide moiety of cephalomannine **3**, reminiscent of the metabolism of docetaxel in humans and rats.[12]

Acknowledgements

We are grateful for the finacial support from the National Cancer Institute (R01 CA55118).

References

1. Wani, M. C.; Taylor, H.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325.
2. Rowinsky, E. K.; Donehower, R. C. *New Engl. J. Med.* **1995**, *332*, 1004.
3. For review: (a) Suffness, M. *Taxol Science and Application*; CRC: Boca Raton, Fl, 1995. (b) Georg, G. I.; Chen, T. T.; Ojima, I.; Vyas, D. M. *Taxane Anticancer Agents: Basic Science and Current Status*; American Chemical Society: Washington, DC, 1995. (c) Farina, V. *The Chemistry and Pharmacology of Taxol and Its Derivatives*; Elsevier: Amsterdam, 1995.
4. A two stage fermentation procedure was used. Frozen vegetative mycelium was used to inoculate a 250 mL baffled flask containing 50 mL seed medium. The seed flasks were incubated on a rotary shaker (220 rpm) at 27°C for 1–2 days. A 1 mL aliquot of the seed culture was used to inoculate a 50 mL non-baffled shake flask containing 10 mL of transformation medium for the screening experiments. Taxol was dissolved in DMSO and added to the fermentation at 0 h to achieve a final concentration of 0.05 mg/mL. The shake flasks were subsequently incubated for 1–4 days. Following incubation, 0.5 mL acetonitrile was added to 0.5 mL whole broth and vortexed for 1 min. The resulting solution was

centrifuged and subjected to HPLC analysis of the biotransformation products. HPLC analytical system: (column: Econosphere C-18, 5μ, 46×150 mm, with guard column; mobile phase: 37.5% acetonitrile in water, flow rate: 1 mL/min). Seed medium consisted of (in g/L) dextrin 10.0, glucose 1.0, beef extract 3.0, Ardamine PH (Yeast Products, Inc.) 5.0, N-Z Amine type E 5.0, $MgSO_4 \cdot 7H_2O$ 0.05, KH_2PO_4 0.37 and $CaCO_3$ 0.5. The pH of the seed medium was adjusted to 7.1 before autoclaving. Product medium consisted of (in g/L) glucose 20.0, soya meal 5.0, yeast autolysate 5.0, and NaCl 5.0. The pH was adjusted to 7.0 before autoclaving.

5. Selective spectral data for **1**: FAB-MS m/z 871 (M+1); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.11 (dd, J=7.5, 1.0 Hz, 2H, o-H of OBz), 7.72 (dd, J=7.5, 1.0 Hz, 2H, o-H of NBz), 7.60 (tt, J=7.5, 1.0 Hz, 1H, p-H of OBz), 7.50 (t, J=7.5 Hz, 2H, m-H of OBz), 7.48 (tt, J=7.0, 1.0 Hz, 1H, p-H of NBz), 7.47 (dt, J=7.5, 1.5 Hz, 2H, o-H of 3′Ph), 7.40 (td, J=7.5, 1.5 Hz, 2H, m-H of 3′-Ph), 7.39 (dd, J=7.5, 7.0 Hz, 2H, m-H of NBz), 7.34 (tt, J=7.5, 1.5 Hz, 1H, p-H of 3′-Ph), 6.94 (d, J=8.5 Hz, 1H, NH), 6.37 (s, 1H, H-10), 6.21 (br. t, J=9.0 Hz, 1H, H-13), 5.76 (dd, J=8.5, 2.5 Hz, 1H, H-3′), 5.66 (d, J=7.0 Hz, 1H, H-2), 4.92 (dd, J=10.0, 1.0 Hz, 1H, H-5), 4.77 (br. s, 1H, H-2′), 4.43 (d, J=17.5 Hz, 1H, 10-OCOC$\underline{H}_2$OH), 4.38 (dd, J=10.5, 8.5 Hz, 1H, H-7), 4.31 (d, J=17.5 Hz, 1H, 10-OCOC$\underline{H}_2$OH), 4.29 (d, J=8.5 Hz, 1H, H-20), 4.17 (d, J=8.5 Hz, 1H, H-20), 3.77 (d, J=7.0 Hz, 1H, H-3), 3.64 (br. s, 1H, 10-OCOCH$_2$O$\underline{H}$), 3.52 (d, J=5.0 Hz, 1H, 2′-OH), 2.55 (ddd, J=15.0, 10.0, 8.5 Hz, 1H, H-6), 2.37 (s, 3H, 4-COC$\underline{H}_3$), 2.32 (dd, J=15.0, 9.0 Hz, 1H, H-14), 2.22 (dd, J=15.0, 9.0 Hz, 1H, H-14), 1.94 (br. s, 1H, 1-OH), 1.86 (ddd, J=15.0, 10.5, 1.0 Hz, 1H, H-6), 1.79 (d, J=1.0 Hz, 3H, H-18), 1.22 (s, 3H, H-19), 1.21 (s, 3H, H-16), 1.10 (s, 3H, H-17). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 173.23 (10-$\underline{C}$OCH$_2$OH), 172.63 (C-1′), 170.36 (4-$\underline{C}$OCH$_3$), 166.89 (1-$\underline{C}$OCH$_3$ of), 142.51 (C-12), 137.83 (quaternary C of NBz), 137.73 (p-C of OBz), 133.50 (m-C of 3′-Ph), 133.49 (C-11), 131.96 (p-C of 3′-Ph), 130.15 (o-C of OBz), 129.00 (m-C of NBz), 128.99 (quaternary C of Obz), 128.70 (m-C of Obz), 128.66 (m-C of 3′-Ph), 128.36 (p-C of NBz), 126.97 (o-C of 3′-Ph), 84.27 (C-5), 81.07 (C-4), 78.92 (C-1), 76.47 (C-20), 76.29 (C-10), 74.76 (C-2), 73.12 (C-2′), 72.28 (C-3), 72.07 (C-7), 60.62 (10-OCOC$\underline{H}_2$OH), 58.60 (C-8), 55.08 (C-3′), 45.75 (C-13), 43.12 (C-15), 35.89 (C-14), 35.67 (C-6), 26.94 (C-17), 22.68 (4-CO$\underline{C}$H$_3$), 21.80 (C-16), 14.95 (C-18), 9.68 (C-19).
6. ^{1}H NMR spectral data for **2** ($CDCl_3$, 500 MHz): δ 8.13 (dd, J=7.5, 0.5 Hz, 2H, o-H of OBz), 7.74 (dd, J=8.0, 1.0 Hz, 2H, o-H of NBz), 7.62 (tt, J=7.5, 0.5 Hz, 1H, p-H of OBz), 7.51 (t, J=7.5, 2H, m-H of OBz), 7.48 (tt, J=7.5, 1.0 Hz, 1H, p-H of NBz), 7.40 (dd, J=8.0, 7.5 Hz, 2H, m-H of NBz), 7.35 (dd, J=8.5, 2.0 Hz, 2H, o-H of 3′-Ph), 6.94 (d, J=8.5 Hz, 1H, NH), 6.85 (dd, J=8.5, 2.0 Hz, 2H, m-H of 3′-Ph), 6.27 (s, 1H, H-10), 6.22 (br. t, 1H, J=9.5 Hz, H-13), 5.71 (dd, J=8.5, 2.5 Hz, 1H, H-3′), 5.67 (d, J=7.0 Hz, 1H, H-2), 5.06 (br. s, 1H, OH), 4.95 (dd, J=10.0, 1.0 Hz, 1H, H-5), 4.75 (dd, J=5.0, 2.5 Hz, 1H, H-2′), 4.40 (dd, J=10.5, 8.5 Hz, 1H, H-7), 4.36 (d, J=8.5 Hz, 1H, H-20), 4.20 (d, J=8.5 Hz, 1H, H-20), 3.80 (d, J=7.0 Hz, 1H, H-3), 3.54 (d, J=5.0, 1H, 2′-OH), 2.54 (ddd, J=15.0, 10.0, 8.5 Hz, 1H, H-6), 2.38 (s, 3H, 4-OCOC$\underline{H}_3$), 2.30 (dd, J=15.0, 9.5 Hz, H-14), 2.24 (s, 3H, 10-OCOC$\underline{H}_3$), 2.22 (dd, J=15.0, 9.5 Hz, H-14), 1.94 (br. s, 1-OH), 1.86 (ddd, J=15.0, 10.5, 1.0 Hz, H-6), 1.79 (d, J=1.0 Hz, H-18), 1.69 (s, H-19), 1.24 (s, H-16), 1.14 (s, H-17).
7. (a) Rowinsky, E. K.; Wright, M.; Monsarrat, B.; Lesser, G. J.; Donehower, R. C. *Cancer Surveys* **1993**, *17*, 283; (b) Vuilhorgne, M.; Gaillard, C.; Sanderink, G. J.; Royer, I.; Monsarrat, B.; Dubois, J.; Wright, M. In *Taxane Antitumor Agents*: *Basic Science and Current Status*; Georg, G. I.; Chen, T. T.; Ojima, I.; Vyas, D. M., Eds.; American Chemical Society: Washington, DC, 1995; pp. 98–110.
8. Monsarrat, B.; Mariel, E.; Cros, S.; Gares, M.; Guenard, D.; Gueritte-Voegelein, F.; Wright, M. *Drug Metab. Dispos.* **1990**, *18*, 895.
9. (a) Park, H.; Hepperle, M.; Boge, T. C.; Himes, R. H.; Georg, G. I. *J. Med. Chem.* **1996**, *39*, 2705; (b) Sonnichse, D. S.; Liu, Q.; Schuetz, J. D.; Pappo, A.; Relling, M. V. *J. Pharmacol. Exper. Ther.* **1995**, *275*, 266.
10. Selective spectral data for **3**: FAB-MS, m/z 848 (M+1); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.11 (dd, J=8.5, 1.0 Hz, 2H, o-H of OBz), 7.59 (tt, J=7.5, 1.0 Hz, 1H, p-H of OBz), 7.49 (dd, 8.5, 7.5 Hz, 2H, m-H of OBz), 7.39 (dt, J=7.5, 1.0 Hz, 2H, o-H of 3′-Ph), 7.39 (td, J=7.5, 1.0 Hz, 2H, m-H of 3′-Ph), 7.32 (tt, J=7.5, 1.0 Hz, 1H, p-H of 3′-Ph), 6.58 (d, J=9.0 Hz, 1H, NH), 6.35 (tq, J=6.0, 1.5 Hz, 1H, 3″-H), 6.25 (s, 1H, H-10), 6.22 (br. t, J=6.0 Hz, 1H, H-13), 5.65 (d, J=7.0 Hz, 1H, H-2), 5.60 (dd, J=9.0, 2.5 Hz, 1H, H-3′), 4.92 (dd, J=9.5, 2.0 Hz, 1H, H-5), 4.70 (d, J=2.5 Hz, 1H, H-2′), 4.38 (dd, J=10.5, 6.5 Hz, 1H, H-7), 4.32 (d, J=9.0 Hz, 1H, H-20), 4.22 (dq, J=6.0, 1.5 Hz, 2H, H-4″), 4.16 (d, J=9.0 Hz, 1H, H-20), 3.78 (d, J=7.0 Hz, 1H, H-3), 3.47 (br. s, 1H), 2.52 (ddd, J=15.0, 9.5, 6.5 Hz, 1H, H-6), 2.35 (s, 3H, 10-OCOC$\underline{H}_3$), 2.30 (dd, J=15.0, 6.0 Hz, 1H, H-14), 2.23 (s, 3H, 4-OCOC$\underline{H}_3$), 2.22 (dd, J=15.0, 6.0 Hz, 1H, H-14), 1.94 (br. s, 1H, 1-OH), 1.86 (ddd, J=15.0, 10.5, 2.0 Hz, 1H, H-6), 1.74 (d, J=1.0 Hz, 3H, H-18), 1.07 (pentet, J=1.5 Hz, 3H, 2″-CH$_3$), 1.65 (s, 3H, H-19), 1.24 (s, 3H, H-16), 1.13 (s, 3H, H-17).
11. (a) Harris, J. W.; Katki, A.; Anderson, L. W.; Chmurny, G. N.; Paukstelis, J. V.; Collins, J. M. *J. Med. Chem.* **1994**, *37*, 706; (b) Kumar, G. N.; Oatis, Jr., J. E.; Thornburg, K. R.; Heldrich, F. J.; Hazard, III, E. S.; Walle, T. *Drug Metab. Dispos.* **1994**, *22*, 177.
12. (a) Monegier, B.; Gaillard, C.; Sable, S.; Vuilhorgne, M. *Tetrahedron* **1994**, *35*, 3715; (b) Rosing, H.; Lustig, V.; Warmerdam, L. J. C.; Huizing, M. T.; Bokkel Huinink, W. W.; Schlellens, J. H. M.; Rodenhuis, S.; Bult, A.; Beijnen, J. H. *Cancer Chemother. Pharmacol.* **2000**, *45*, 213.