Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 273–276

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A Paclitaxel Analogue with a 2(3→20)*Abeo*taxane Skeleton: Synthesis and Biological Evaluation

Javier Soto, José L. Mascareñas and Luis Castedo*

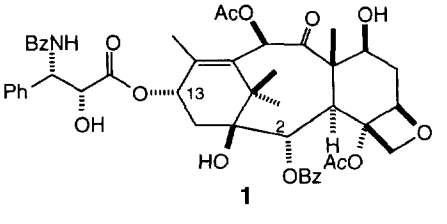
Departamento de Química Orgánica, Facultade de Química, Universidade de Santiago de Compostela e Unidade Asociada ó C.S.I.C., 15706 Santiago de Compostela, SPAIN, Fax 34-81-595012, qocasted@uscmail.usc.es.

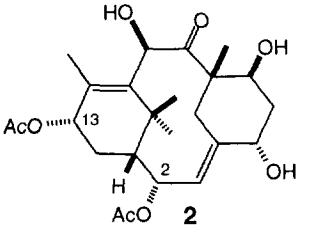
Received 7 October 1997; accepted 22 December 1997

Abstract. A paclitaxel analogue having an unusual tricyclic [9.3.1.1] hexadecane skeleton was synthesized from deaminoacyltaxine A, a 2(3→20) *abeo*taxane isolated in considerable amounts from the leaves of *Taxus baccata* L. In preliminary studies, this compound showed a much lower cytotoxicity than paclitaxel. © 1998 Elsevier Science Ltd. All rights reserved.

The anticancer agent paclitaxel (**1**, commonly named taxol®) is a structurally complex diterpenoid that was originally isolated from the bark of Pacific Yew (*Taxus brevifolia* Nutt.).[1] It has been approved for the treatment of ovarian and breast cancer by the US Food and Drug Administration, and has shown promise for the therapy of lung cancer, head and neck cancer, and esophageal carcinomas.[2] Owing to these extraordinary clinical expectations, there is great interest in the discovery of paclitaxel analogues with similar or greater antitumour activity but lower toxicity than paclitaxel, and that could be more easily synthesized than this taxoid.[3] In our search for such analogues, we investigated the taxoid content of the leaves of *Taxus baccata* L. of Galicia (NW Spain). Among the taxoids isolated were considerable amounts (approx. 100 mg/kg) of deaminoacyltaxine A (**2**), which has an unusual tricyclic [9.3.1.1] hexadecane skeleton.[4]

Scheme 1





The ^{1}H NMR NOESY spectrum of **2** in $CDCl_3$, in particular the NOEs between C18-CH_3 and H-7/H-10, H-2 and H-20, and H-2 and C16-CH_3 suggested this compound to have a rigid concave structure such as that shown in Figure 1A. This structure closely agrees with the lowest energy conformation calculated for **2** by molecular modelling using a MMX force field (Figure 1B),[5] and is similar to the cup-like conformation of paclitaxel.[6]

E-mail: qocasted@usc.es; Fax: Int. +34.81.594404

PII: S0960-894X(98)00005-5

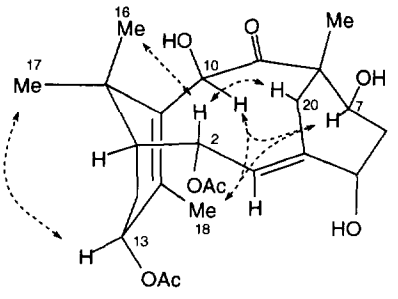


Figure 1A. Selected NOEs

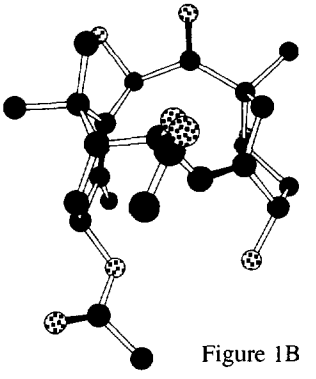


Figure 1B

We envisaged that introduction into **2** of further structural features of **1** considered essential to its biological activity would provide a more interesting analogue for biological evaluation. We accordingly set about adding an isoserine side chain at C13 and a benzoate ester at C2, noting that these key pharmacophores have similar orientations in **1**[7] and the energy-minimized conformation of the resulting analogue **3** (Figure 2). This novel type of anticancer taxoid could prove more active and easier to synthesize than paclitaxel and its congeners, and its biological evaluation should provide further insight into the structure-activity relationships of taxanes.

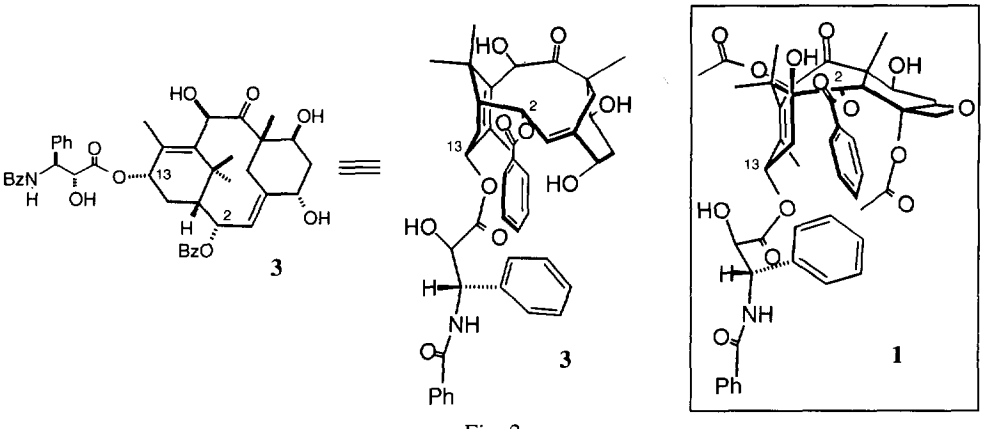


Fig. 2

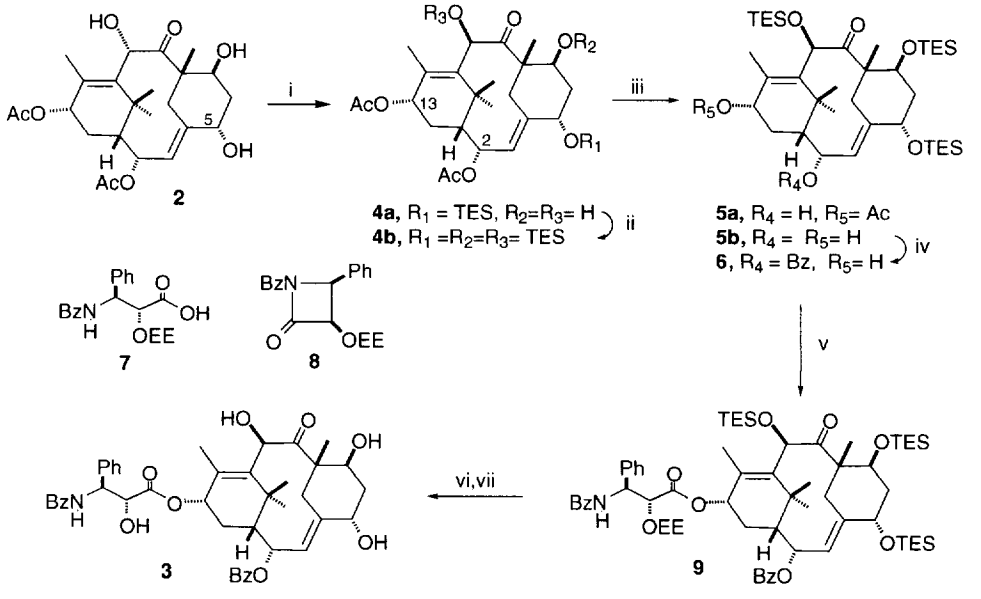
The conversion of **2** to **3** required initial protection of the three free hydroxyl groups followed by deacylation and selective manipulation of the hydroxyl groups at C13 and C2. The protection step was first attempted using conditions developed for the silylation of secondary alcohols of 10-deacetylbaccatine III (TESCl in pyridine, room temperature).[8] Under these conditions, only the monosilylated derivative of deaminoacyltaxine A (**4a**) was obtained;[9] however, when silver nitrate was added as activating agent,[10] the trisilylated derivative **4b** could be obtained in 83% from **4a**, or in 86% yield directly from **2**.

Attempts at deacylation of **4b** by methanolysis at room temperature exclusively afforded the monodeacetylated product **5a** (58% yield), while use of methanolysis at reflux caused desilylation of the hydroxyl group at C5, as well as the desired bisdeacylation. After testing several other sets of conditions, we found that treatment of **4b** with $LiAlH_4$ in THF at 0°C gave the desired dihydroxy product **5b**.

The differential reactivity of the C2 and C13 hydroxyls was confirmed in the benzoylation step in which treatment of **5b** with benzoic acid, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and DMAP in DMF exclusively gave the 2-monobenzoylated product **6** (86% yield). Attempts at introduction of the isoserine side chain by reacting **6** with the acid **7** under conditions described previously for the preparation of baccatine derivatives[11] failed to give the desired product. Fortunately, however, coupling of **6** with the β-lactam **8** (2.2 equiv.) in THF at 0°C, using $LiN(TMS)_2$ as base, gave the isoserine ester derivative **9** in 22% yield.[12]

Removal of the protecting groups of **9** was easily effected by treating it with ethanolic HCl in THF at 0°C, and then HF in pyridine, thus affording the desired final product **3**[9] in 54% yield.

Scheme 2



i) TESCl (6 equiv.), Py, rt, 18h, 87%; ii) TESCl (4 equiv.), $AgNO_3$ (4 equiv.), Py, rt, 24h, 83%; iii) $LiAlH_4$ (2 equiv.), THF, 0°C, 4h, 95%; iv) BzOH (6 equiv.), EDC (6 equiv.), DMAP (2.2 equiv.), DMF, rt, 20h, 86%; v) **8** (2 equiv.), $LiN(TMS)_2$ (1 equiv.), THF, 0°C, 4h, 22%; vi) 1% HCl, EtOH-THF, 0°C, 24h; vii) HF-Py, 0°C, 10h, 54%.

The cytotoxicity of taxoid **3** was evaluated against P388 mouse lymphoma cells, A649 human mammary carcinoma cells, HT29 human colon adenocarcinoma cells and MEL28 melanoma cells. As can be deduced from the results summarized in Table 1, the new taxoid is more cytotoxic than deaminoacyltaxine A against all the human and mouse cancer cell lines, but much less cytotoxic than paclitaxel.

Table 1. Cytotoxicities (IC_{50} μg/ml) of **3**, deaminoacyltaxine A and Paclitaxel.

Taxoid	P388	A649	HT29	MEL28
Paclitaxel	0.2	0.002	0.002	0.002
Deaminoacyltaxine A	>10	>10	>10	>10
3	6	6	6	6

Although these results suggest that the taxane-type skeleton is necessary for biological activity, confirmation of this will require biological evaluation of *abeo*taxanes incorporating further essential structural features of paclitaxel, such as the acetate at C4 and the oxetane ring. Work in this direction is in progress.

Acknowledgments: We are grateful to the Xunta de Galicia for financial support under project 20906B96. The plant material was kindly provided by Prof. Antonio Mouriño, the late Dr. José Luis González Mantilla and the Town Council of Santiago de Compostela. We thank Prof. Andrew Greene for providing β-lactam **8** and Dr. María Dolores García Grábalos (Pharma Mar) for biological evaluation.

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.
2. a) Rowinsky, E. K.; Donehower, R. C. *J. Natl. Cancer Inst.* **1991**, *83*, 1778. b) Arbuck, S. G.; Blaylock, B. A. *Taxol, Science and Applications* **1995**, Ed: Suffness, M.; CRC Press, Inc., Ch. 14, p. 380.
3. The synthesis of the paclitaxel nucleus is an extremely difficult task. See: a) Holton, R. A.; Kim, H.-B.; Somoza, C.; Liang, F.; Biediger, R. J.; Boatman, P. D.; Shindo, M.; Smith, C. C.; Kim, S.; Nadizadeh, H.; Suzuki, Y.; Tao, C.; Vu, P.; Tang, S.; Zhang, P.; Murthi, K. K.; Gentile, L. N.; Liu, J. H. *J. Am. Chem. Soc.* **1994**, *116*, 1599. b) Nicolaou, K. C.; Ueno, H.; Liu, J.-J.; Nantermet, P. G.; Yang, Z.; Renaud, J.; Paulvannan, K.; Chadha, R. *J. Am. Chem. Soc.* **1995**, *117*, 653. c) Masters, J. J.; Link, J. T.; Snyder, L. B.; Young, W. B.; Danishefsky, S. J. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 16.
4. This compound has been recently isolated from *Taxus baccata* L.: Appendino, G.; Cravotto, G.; Enriù, R.; Jakupovic, J.; Gariboldi, P.; Gabetta, B.; Bombardelli, E. *Phytochemistry* **1994**, *36*, 407.
5. Computer calculations were performed by a Silicon Graphics Indigo computer running the Biosym Technologies program, Discover MSP version 2.90.
6. Georg, G. I.; Bobe, T. C.; Cheruvallath, Z. S.; Clowers, J. S.; Harriman, G. C. B.; Hepperle, M.; Park, H. *Taxol, Science and Applications* **1995**, Ed: Suffness, M.; CRC Press, Inc., Ch. 13, p. 365.
7. There is a general agreement that a hydrophobic collapse between the C2 benzoate and the C13 side chain is essential for biological activity: a) Vander Velde, D. G.; Georg, G. I.; Grunewald, G. L.; Gunn, C. W.; Mitscher, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 11650. b) Paloma, L. G.; Guy, R. K.; Wrasidlo, W.; Nicolaou, K. C. *Chem. & Biol.* **1994**, *1*, 107. c) Ojima, I.; Kuduk, S. D.; Chakravarty, S.; Ourevitch, M.; Bégué, J.-P. *J. Am. Chem. Soc.* **1997**, *119*, 5519.
8. Denis, J.-N.; Greene, A. E.; Guénard, D.; Guéritte-Voegelein, F.; Mangatal, L.; Potier, P. *J. Am. Chem. Soc.* **1988**, *110*, 5917.
9. All new compounds were fully characterized. Analytical data for **3**: **^{1}H-NMR** ($CDCl_3$, 500 MHz): δ 8.02 (d, *J*=7 Hz, 2H, Ph); 7.73 (d, *J*=7 Hz, 2H, Ph); 7.53 (m, 11H, Ph); 7.21 (d, $J_{3'\text{-NH}}$=9.3 Hz, 1H, NH); 6.00 (d, $J_{2\text{-}3}$=10 Hz, 1H, H-2); 5.94 (d, $J_{2\text{-}3}$= 10 Hz, 1H, H-3); 5.90 (dd, $J_{3'\text{-NH}}$=9.3 and $J_{2'\text{-}3'}$= 4 Hz, 1H, H-3'); 5.67 (d, $J_{13\text{-}14}$=10.4 Hz, 1H, H-13); 5.28 (d, $J_{10\text{-OH}}$=2.6 Hz, 1H, H-10); 4.66 (t, $J_{2'\text{-}3'}$=$J_{2'\text{-OH}}$=4 Hz, 1H, H-2'); 4.45 (m, 1H, H-5); 4.23 (d, *J*=4.9 Hz, 1H, OH); 4.21 (d, $J_{10\text{-OH}}$=2.6 Hz, 1H, OH); 4.10 (bd, 1H, H-7); 4.01 (d, $J_{2'\text{-OH}}$=4.4 Hz, 1H, OH); 2.80 (d, $J_{20\text{-}20'}$=15.2 Hz, 1H, H-20); 2.71 (m, 1H, H-14); 2.40 (m, 1H, H-6'); 2.10 (d, $J_{14\text{-}14'}$=16.7 Hz, 1H, H-14'); 2.00 (dd, $J_{6\text{-}6'}$=14.4 and *J*=3.4 Hz, 1H, H-6); 1.88 (d, $J_{20\text{-}20'}$=15.2 Hz, 1H, H-20'); 1.82 (d, $J_{1\text{-}14}$=6.9 Hz, 1H, H-1); 1.74 (s, 3H, H-18); 1.31 (s, 3H, H-16); 1.28 (s, 3H, H-17); 1.19 (s, 3H, H-19). **^{13}C-NMR** ($CDCl_3$, 125 MHz): δ 214.3 s (C-9); 171.7 s (C-1'); 167.6 s (Bz); 165.5 s (BzN); 138.9 s (C-4);138.2 s (C-12); 135.2 s (BzN); 133.6 s (Ph-3'); 133.1 s (C-11); 133.0 d (Ph); 132.1 d (Ph); 130.5 s (Bz); 129.5 d (Ph, 2C); 128.9 d (Ph, 2C); 128.8 d (Ph, 2C); 128.3 d (Ph, 2C); 127.1 d (Ph, 2C); 126.9 d (Ph, 2C); 121.6 d (C-3); 77.2 d (C-10); 74.6 d (C-2'); 71.6 d (C-2); 71.0 d (C-13); 68.8 d (C-5); 67.6 d (C-7); 54.9 d (C-3'); 53.0 s (C-8); 46.9 d (C-1); 38.9 t (C-6); 37.3 s (C-15); 35.0 t (C-14); 34.5 c (C-19); 26.1 t (C-20); 24.0 (C-17); 18.5 c (C-16); 16.7 c (C-18). **FABMS** *m/z* (rel. %.): 760 $[M+Na]^+$ (6), 453 (14), 286 (45).
10. Kunz, H.; Waldmann, H. *Comprehensive Organic Synthesis* **1991**, Eds: Trost, B. M.; Fleming, I.; Vol 6, Ch. 3.1, p. 656.
11. Chaudhary, A. G.; Gharpure, M. M.; Rimoldi, J. M.; Chordia, M. D.; Gunatilaka, A. A. L.; Kingston, D. G. I.; Grover, S.; Lin, C. M.; Hamel, E. *J. Am. Chem. Soc.* **1994**, *116*, 4097.
12. a) Ojima, I.; Sun, C. M.; Zucco, M.; Park, Y. H.; Duclos, O.; Kuduk, S. *Tetrahedron Lett.* **1993**, *34*, 4149. b) Li, L.; Thomas, S. A.; Klein, L. L.; Yeung, C. M.; Maring, C. J.; Grampovnik, D. J.; Lartey, P. A.; Plattner, J. J. *J. Med. Chem.* **1994**, *37*, 2655.