Pergamon

PII: S0031-9422(97)00712-7

Phytochemistry, Vol. 47, No. 7, pp. 1325–1329, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

PACLITAXEL ANALOGUES FROM *TAXUS BACCATA*

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(*Received in revised form* 11 *July* 1997)

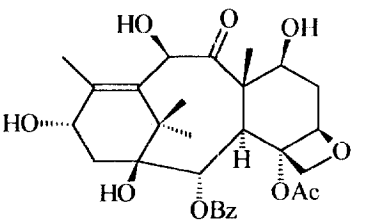
Key Word Index—*Taxus baccata*; Taxaceae; *N*-debenzoyl-*N*-butanoyl-10-deacetylpaclitaxel; *N*-debenzoyl-*N*-propanoyl-10-deacetylpaclitaxel.

Abstract—*N*-Debenzoyl-*N*-butanoyl- and *N*-debenzoyl-*N*-propanoyl-10-deacetylpaclitaxel were identified as minor constituents of *T. baccata* by HPLC-MS. The structure of these compounds was confirmed by comparison with synthetic samples prepared from 7-triethylsilylbaccatin III. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Over the past few years, the needles of the yew tree (*Taxus* spp. vv.) have become an important commodity for the pharmaceutical industry, serving as a renewable source of 10-deacetylbaccatin III (**1**) [1]. Thanks to improvements in synthetic methodologies [2], commercially viable methods for the conversion of **1** to the anticancer drug paclitaxel (=Taxol®) (**2a**) have been developed [2]. As a result, semisynthetic paclitaxel obtained from **1** was approved for clinical use in December 1994 [3], effectively solving the ecological issues raised by its isolation from the bark of the Pacific yew (*T. brevifolia* Nutt.), a non renewable source [4]. Compound **1** has also been extensively used in studies aimed at unravelling the structure-activity relationship within antitumor taxoids [5], serving as a starting material for the synthesis of first generation [6] and second generation [7] chemical analogues of paclitaxel.

10-Deacetylbaccatin III can be isolated from yew more easily than paclitaxel, owing to a higher concentration and a very poor solubility in a variety of organic solvents. As part of an investigation aimed at the identification of minor paclitaxel analogues in yew tissues, we examined the mother liquors of a batch of **1** prepared from *T. baccata* L.



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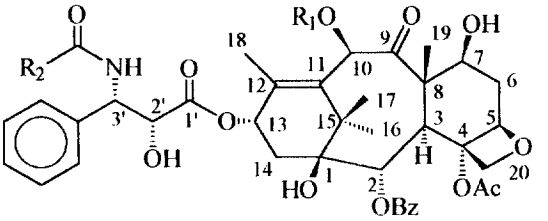

	R_1	R_2
2a	Ac	Ph
2b	H	*n*-Pr
2c	H	Et
2d	Ac	*n*-Pr
2e	Ac	Et

RESULTS AND DISCUSSION

In previous studies, the coupling of high performance liquid chromatography with mass spectrometry using a thermospray (TSP) interface proved very useful for the on-line analysis of taxanes in crude plant extracts [8]. Paclitaxel and its analogues give positive thermospray mass spectra showing, beside the protonated molecule ion MH^+, an extensive and characteristic fragmentation pattern [8]. The main

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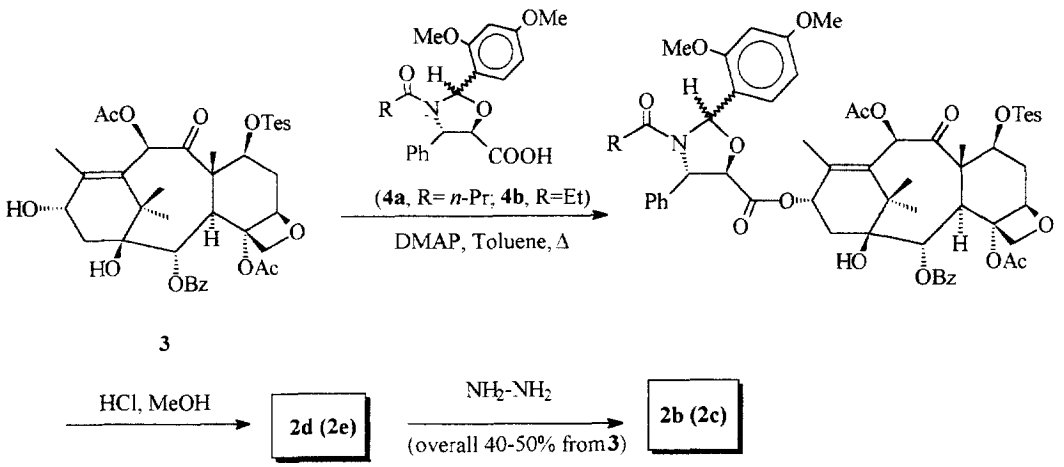


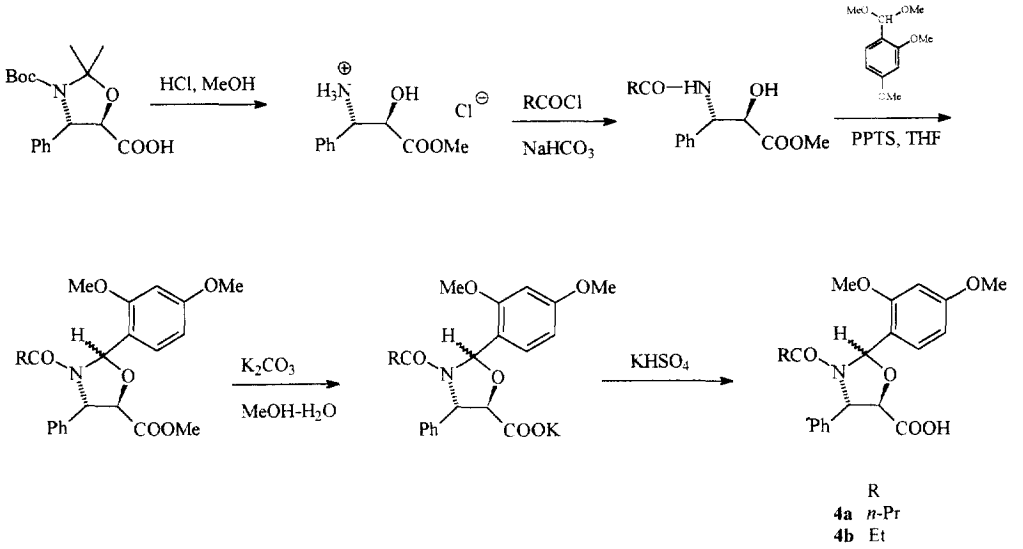
Scheme 1. Synthesis of **2b** and **2c** from 7-Tesbaccatin III (**3**).

ions correspond to the phenylisoserine chain, ($[ScH+H]^+$, base peak), and to the terpenoid moiety (ions $[MH—ScH]^+$ and $[MH–ScH–PhCOOH]^+$). The presence of these fragments allows a straightforward identification of the terpenoid core and the *N*-acylation pattern of the side chain. The HPLC TSP-MS analysis of the mother liquors of a methanol crystallization of **1** revealed two minor constituents **2b** (< 1%, $[MH^+$ at *m/z* 778]) and **2c** ($[MH^+$ at *m/z* 764]). The presence of an ion at *m/z* 527 in both compounds showed that they were esters of 10-deacetylbaccatin III, whereas the ions at *m/z* 252 and *m/z* 238 (100% relative intensity) suggested the presence of side chains where the *N*-benzoyl moiety of paclitaxel was replaced by a *N*-butanoyl and a *N*-propanoyl residue, respectively. Mass spectra thus suggested that **2b** was *N*-debenzoyl-*N*-butanoyl-10-deacetylpaclitaxel and **2c** was *N*-debenzoyl-*N*-propanoyl-10-deacetylpaclitaxel. The very low concentration of these metabolites precluded their isolation and thus an examination of their stereochemical features, which cannot be deduced by mass spectral analysis alone. Therefore, compounds corresponding to these structures were synthesized, in order to compare their chromatographic and mass spectral properties with those of the natural products. The synthesis of **2b** and **2c** starts from 7-triethylsilylbaccatin III (**3**) [9] (Scheme 1). The baccatin III derivative **3** was preferred over diprotected 10-deacetylbaccatin III derivatives (7,10-bistriethylsilyl-7,10-bistrichloroethoxycarbonyl-derivatives [5]) since also the corresponding compounds of the paclitaxel series (10-acetyl) would have been available in this way. The oxazolidine carboxylic acids **4a** and **4b** were used as compact synthons of the phenylisoserine side chains of **2b** and **2c**, respectively, and were prepared as shown in Scheme 2. Both syntheses proceeded uneventfully, giving the paclitaxel analogues **2d** and **2e**, which were then deacetylated with hydrazine in ethanol [10]. The synthetic products showed HPLC profiles and mass spectra identical to those of the natural products, confirming that **2b** and **2c** were indeed *N*-debenzoyl-*N*-butanoyl and *N*-debenzoyl-*N*-propanoyl-10-deacetylpaclitaxel, respectively. The proton and carbon resonances of **2b** and **2c** are reported in Tables 1 and 2. An examination of extracts from various yews, showed that **2b** and **2c** are present as trace constituents (< 0.0001%) in the European and the Himalayan yews (*T. baccata* L. and *T. wallichiana* Zucc., respectively).

The identification of minor compounds structurally related to paclitaxel is interesting in the context of the biogenesis of these products. Indeed, the isolation of **2c**, with a *N*-propanoyl group, makes it unlikely that compounds like taxol C [11] and taxol D [12], having a *N*-hexanoyl and a *N*-butanoyl group, are synthesized by homologation of an hitherto unreported *N*-acetyl starter, since only acyl residues with an even number of carbon atoms would be expected. It is thus more likely that the various *N*-alkanoyl residues of the natural taxols are introduced as such, in what is considered the final step of their biosynthesis [13].

EXPERIMENTAL

Liquid chromatography thermospray mass spectrometry. The HPLC-MS system used included a Waters 600-MS pump/system controller and Waters 486 tunable UV/Vis detector. Injections were performed with a Waters 717 plus autosampler. A Zorbax SB-CN column (250 × 4.6 mm I.D., 5 μm) was used. The mobile phase composition was eluent A (H_2O—MeOH 8:2), eluent B (MeCN—MeOH 8:2), in gradient conditions (eluent A from 99% to 35% in 65 min). The flow rate was 1 ml min^{-1} and the UV detector was set at 227 nm. The liquid chromatography system was connected to a Finnigan-MAT TSQ triple quadrupole mass spectrometer equipped with a TSP-2 thermospray interface and a



Scheme 2. Synthesis of the side chain synthons **4a** (R = C_3H_7) and **4b** (R = C_2H_5). PPTS = pyridinium *p*-toluenesulphonate.

Table 1. ^{1}H NMR spectral data of **2b** and **2c** (200 MHz, $CDCl_3$, TMS as reference, *J* in Hz)

H	2b	2c
2	5.67 *d*(7.0)	5.68 *d*(7.2)
3	3.86 *d*(7.0)	3.89 *d*(7.2)
5	4.91 *dd* (9.6, 1.9)	4.92 *dd* (9.5, 2.1)
6α	2.53 *ddd*(14.8, 8.9, 5.5)	2.56 *ddd*(14.5, 11.5, 4.9)
6β	1.83 *m*	1.80 *m*
7	4.22 *m*	4.22 *m*
10	5.21 *s*	5.19 *s*
13	6.16 *dd*(8.9, 9.0)	6.18 *dd*(8.9, 9.0)
14a,b	2.26 *m*	2.28 *m*
16	1.11 *s*	1.12 *s*
17	1.22 *s*	1.23 *s*
18	1.80 *br s*	1.81 *br s*
19	1.73 *s*	1.75 *s*
20a	4.29 *d* (8.6)	4.22 *m*
20b	4.19 *d* (8.6)	
2′	4.67 *dd*(4.5, 2.7)	4.76 *dd*(4.5, 2.5)
3′	5.56 *dd*(9.0, 2.7)	5.56 *dd*(9.1, 2.5)
NH-COCH_2R	2.16 *t* (7.7)	2.23 *q* (7.7)
NH-COCH$_2$CH_2R	1.59 *m*	1.10 *t* (7.6)
NH-COCH$_2$CH$_2$CH_3	1.20 *t* (7.5)	
4-OAc	2.32 *s*	2.34 *s*
3′NH	6.44 *d*(9.0)	6.36 *d*(9.1)
Bz	8.14 *d*(8.0)	8.10 *d*(8.0)
	7.62 *t* (8.0)	7.62 *t* (8.0)
	7.52 *t* (8.0)	7.52 *t* (8.0)
Ph	7.34–7.56 *m*	7.34–7.56 *m*

5100 DEC Station with ICIS data system. Mass spectrometer conditions were optimized in order to achieve maximum sensitivity. Typical values were as follows: source block temp. 230°C, vaporizer temp. 70°C, repeller voltage 30 V, discharge 1800 V, filament off-mode. The electron multiplier and dynode voltages were set to 2000 V and 15 kV, respectively. Pre-amplifier sensitivity was 10^{-8} A/V. Positive ther-

Table 2. ^{13}C NMR spectral data of **2b** and **2c** (50 MHz, $CDCl_3$, TMS as reference)

C	2b	2c
1	78.2 *s*	78.2 *s*
2	74.1 *d*	74.1 *d*
3	46.0 *d*	45.9 *d*
4	80.6 *s*	80.6 *s*
5	83.7 *d*	83.7 *d*
6	35.3 *t*	35.3 *t*
7	71.5 *d*	71.5 *d*
8	57.2 *s*	57.2 *s*
9	210.9 *s*	211.0 *s*
10	74.4 *d*	74.3 *d*
11	135.6 *s*	135.7 *s*
12	137.8 *s*	137.8 *s*
13	72.7 *d*	72.7 *d*
14	36.4 *t*	36.5 *t*
15	42.6 *s*	42.6 *s*
16	20.2 *q*	20.2 *q*
17	26.0 *q*	26.0 *q*
18	13.8 *q*	13.8 *q*
19	9.3 *q*	9.3 *q*
20	76.1 *t*	76.2 *t*
1′	172.5 *s*	172.3 *s*
2′	71.9 *d*	71.9 *d*
3′	54.0 *d*	54.0 *d*
3′-Ph	137.7 *s*	137.6 *s*
	126.5 *d*	126.5 *d*
	128.5 *d*	128.5 *d*
	127.7 *d*	127.8 *d*
4-OAc	169.9 *s*	169.9 *s*
	22.0 *q*	22.0 *q*
NH-*C*OR	172.5 *s*	173.2 *s*
NH-CO*C*H_2R	38.0 *t*	29.1 *t*
NH-$COCH_2CH_2$R	18.6 *t*	9.3 *q*
NH-$COCH_2CH_2CH_3$	13.2 *q*	
Bz	166.5 *s*	166.5 *s*
	128.8 *s*	128.8 *s*
	129.7 *d*	129.7 *d*
	128.3 *d*	128.3 *d*
	133.3 *d*	133.3 *d*

mospray mass spectra from *m/z* 200 to 1000 (scan time 1.5 sec) were obtained scanning the Q3 analyzer and acquired in centroid mode.

N-Debenzoyl-N-butanoyl-10-deacetylpaclitaxel (**2b**). Mp 244° (dec.); IR ν_{max}^{KBr} cm^{-1}: 3428 (OH), 1724 (ester C=O), 1654 (amide C=O). CI-MS 140 eV, *m/z* (rel. int.): 795 $[C_{42}H_{51}NO_{13}+NH_4]^+$ $[M+NH_4]^+$ (50), 562 $[MNH_4-(ScH-H_2O)]^+$ (55), 544 $[MNH_4-ScH]^+$ (15), 269 $[ScHNH_4]^+$ (35), 105 $[PhCO]^+$ (100). HPLC relative retention time (RR_t): 1.70 (compared to 10-deacetylbaccatin III).

N-Debenzoyl-N-propanoyl-10-deacetylpaclitaxel (**2c**). Mp 245° (dec.). IR ν_{max}^{KBr} cm^{-1}: 3407 (OH), 1725 (ester C=O), 1655 (amide C=O). CI-MS 140 eV, *m/z* (rel. int.): 781 $[C_{41}H_{49}NO_{13}+NH_4]^+$ $[M+NH_4]^+$ (35), 544 $[MNH_4-ScH]^+$ (15), 255 $[ScHNH_4]^+$ (100). HPLC relative retention time (RR_t): 1.58 (compared to 10-deacetylbaccatin III).

Synthesis of **2b**. To a solution of 7-triethylsilylbaccatin III **3** [9] (650 mg, 0.99 mmol) in toluene (60 ml), (4*S*,5*R*) *N*-(butanoyl)-2-(2,4-dimethoxyphenyl)-4-phenyl-5-oxazolidine-carboxylic acid (**4a**) (804 mg, 2.0 mmol, 2 mol. equiv.; prep [15] from (4*S*,5*R*) *N*-(*t*-butoxycarbonyl)-2,2-dimethyl-4-phenyl-5-oxazolidine-carboxylic acid [14], see Scheme 2), DCC (1.5 g, 7.3 mmol, 7.4 mol. equiv.) and 4-dimethylamminopyridine (250 mg, 2.0 mmol, 2.0 mol. equiv.) were added. The mixture was heated at 70 C for 3 hr and then worked up by filtration and evapn. To remove the protecting groups, the residue was dissolved in a soln of dry HCl in MeOH (prepared by dissolving AcCl (350 μl) in 50 ml of MeOH). After stirring 2 hr at room temp., the soln was evapd. The residue was taken up in CH_2Cl_2 and washed with sat. $NaHCO_3$. After drying (Na_2SO_4) and evapn, crude **2d** was obtained. The latter was dissolved in EtOH (abs., 2.5 ml) and treated with 2.6 ml of a soln prepared dissolving 1 ml 85% aq. hydrazine in 10 ml abs. EtOH. After stirring 2 hr at room temp., the reaction was worked up by dilution with dil. HCl and extracted with EtOAc. The residue was purified by CC (CH_2Cl_2—MeOH 98:2) to give 340 mg of **2b** (44% from 7-triethylsilylbaccatin III).

Synthesis of **2c**. The same procedure used for the synthesis of **2b** was employed, but (4*S*,5*R*) N-(propanoyl)-2-(2,4-dimethoxyphenyl)-4-phenyl-5-oxazolidine-carboxylic acid **4b** was used for the coupling step.

REFERENCES

1. Appendino, G., *Nat. Prod. Rep.*, 1995, **12**, 349.
2. Kant, J., in *The Chemistry and Pharmacology of Taxol*® and its Derivatives, ed. Farina, V., Elsevier, 1995, pp. 255–300.
3. Perlman, D., *San Francisco Chronicle*, 1994, Dec. 14.
4. Cragg, G. M., Schepartz, S. A., Suffness, M. and Grever, M., *J. Nat. Prod.*, 1993, **56**, 1657.
5. Chen, S.-H. and Farina, V., in *The Chemistry and Pharmacology of Taxol*® and its Derivatives, ed. Farina, V., Elsevier, 1995, pp. 165–253.
6. Guénard, D., Guéritte-Voegelein, F. and Potier, P., *Acc. Chem. Res.*, 1993, **26**, 160.
7. Ojima, I., Slater, J. C., Michaud, E., Kuduk, S. D., Bounaud, P.-Y., Vrignaud, P., Bissery, M.-C., Veith, J. M., Pera, P. and Bernacki, R. J., *J. Med. Chem.*, 1996, **39**, 3889.
8. Griffini, A., Peterlongo, F., De Bellis, P. and Pace, R., *Fitoterapia*, 1993, **64**(Suppl. 1), 53.
9. Denis, J.-N., Greene, A. E., Guénard, D., Guéritte-Voegelein, F., Mangatal, L. and Potier, P., *J. Am. Chem. Soc.*, 1998, **110**, 5917.
10. Johnson, R. A., Nidy, E. G., Dobrowolski, P. J., Gebhard, I., Qualls, S. J., Wicniensky, N. A. and Kelly, R. C., *Tetrahedron Lett.*, 1994, **35**, 7893.
11. Barboni, L., Gariboldi, P., Torregiani, E.,

Appendino, G., Gabetta, B. and Bombardelli, E., *Phytochemistry*, 1994, **36**, 987.
12. Appendino, G., Cravotto, G., Enriù, R., Gariboldi, P., Barboni, L., Torregiani, E., Gabetta, B., Zini, G. and Bombardelli, E., *J. Nat. Prod.*, 1994, **57**, 607.
13. Fleming, P. E., Knaggs, A. R., He, X.-G., Mocek, U. and Floss, H. G., *J. Am. Chem. Soc.*, 1994, **116**, 4137.
14. Commerçon, A., Bézard, D., Bernard, F. and Bourzat, J. D., *Tetrahedron Lett.*, 1992, **33**, 5185.
15. Didier, E., Fouque, E., Taillepied, I. and Commerçon, A., *Tetrahedron Lett.*, 1994, **35**, 2349.