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TAXANES FROM *TAXUS* × *MEDIA**

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IN HONOUR OF PROFESSOR ANTONIO G. GONZALEZ

Key Word Index—*Taxus* × *media* cv; Hicksii; Taxaceae; taxoids.

Abstract—The roots of *Taxus* × *media* gave two new taxoids, the structures of which were established as 10-deacetyl-10-dehydro-7-acetyl taxol A and 10-deacetylyunnanxane on the basis of spectroscopic data.

INTRODUCTION

Taxols are *N*-acyl(*N*-alkyl)phenylisoserine esters of baccatin III-type taxoids [1]. Four major structural types are known; these differ from each other in the nature of the *N*-acyl group on the side-chain (taxols A–D) [1, 2]. Modification of the diterpenoid core occurs at C-7 (epimerization, xylosidation) and C-10 (dehydrogenation, hydrolysis and esterification with β-hydroxybutyric acid) [3]. The combinations of the various changes to the side-chain and the terpenoid core gives a series of over 20 compounds, the most important of which is taxol A (**1a**), also known under the proprietary name of Taxol® and the generic name of paclitaxel [4]. None of the natural modifications lead to a substantial improvement of the biological activity of **1a** [4], but the isolation of new and minor taxols is important in the context of the plant biosynthesis and metabolism of these compounds.

We report here on the isolation of the new taxol **1b** from the roots of *Taxus* × *media* Rehd. cv. Hicksii. This hybrid yew can withstand severe winter climates and is a popular evergreen in northern and central U.S.A. More than one million plants of the above mentioned hybrid were found in a survey on cultivated yews in nurseries, making it one of the more abundant cultivars for the supply of yew biomass [5]. Besides **1b**, a new taiwanxan-type taxoid was also isolated (**5**). Based on its NMR features, the structural revision of some α-methylbutyrates of taxoids from the European yew [6] is suggested.

RESULTS AND DISCUSSION

The CI-mass spectrum of **1b** showed a negative parent ion $[M]^-$ at *m/z* 851, and a peak at *m/z* 566, corresponding to the loss of the amino acidic side chain $[M - 285]^-$ [7]. The corresponding fragments in the CI-mass spectrum of **1a** are two amu higher at *m/z* 853 and 568, respectively [7], showing a further unsaturation in the terpenoid core of **1b**. Comparison of the ^{1}H NMR spectra of **1a** [8] and **1b** showed the disappearance of H-10 and a downfield shift of H-7 ($\Delta\delta$0.79 in $CDCl_3$) in **1b**. This, coupled with the detection of a further carbonyl resonance at δ193.0, suggested that in **1b** the 7-hydroxyl group is acetylated, and a keto group is present at C-10. A thorough analysis of the ^{1}H and ^{13}C NMR spectra via 1D and 2D techniques (DEPT, HETCOR, ROESY and FLOCK) confirmed that **1b** is 10-deacetyl-10-dehydro-7-acetyltaxol A, and allowed us to assign all resonances (Tables 1 and 2). Taxoids of the 10-deacetyl-10-dehydrobaccatin III-type are very easily epimerized at C-7, presumably because of the stabilization of the intermediate enolate ion by conjugation with the 10-oxo group [9]. However, the 7-acyloxy group of **1b** was β-oriented, as shown by the ROESY spectrum (ROE effects H-7/H-3 and H-7/H-6α) and by the upfield chemical shift of the angular methyl C-19 (δ9.56) [8]. Taxoids of the 10-deacetyl-10-dehydrobaccatin III-type are also acid-sensitive, and undergo Wagner–Meerwein rearrangement of the AB ring system with closure of a hemiacetal ring between C-9 and C-15. This is shown by the formation of **3** and **4** on acidic treatment of **2** (Scheme 1). The presence of a conjugated carbonyl (δ195.7 in **3**, δ197.1 in **4**) showed that the ring closure had taken place at C-9. The chemoselectivity of this acetalization is interesting, since formation of a C-10 ether bridge had been reported in a related compound [10], and the 10-keto group is sterically hindered [4]. The formation of only the 20-acetate

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*Part 22 in the series 'The Chemistry and Occurrence of Taxane Derivatives'. For part 21 see ref. [9].

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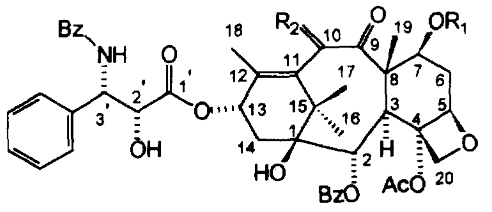
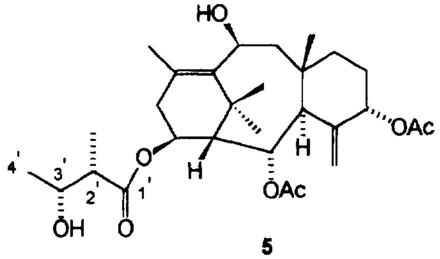


	R_1	R_2
1a	H	β-OAc, α-H
1b	Ac	=O



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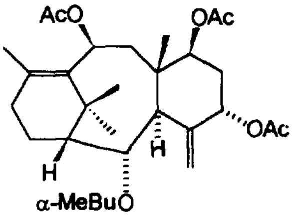
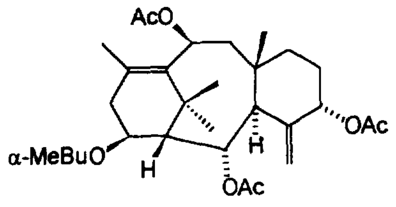
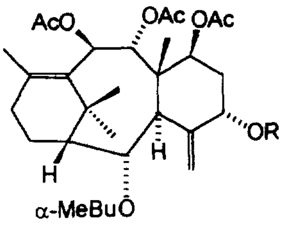


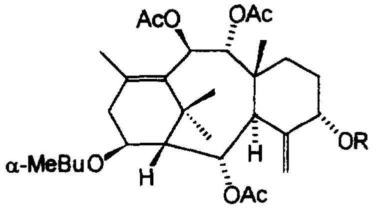
6a



6b



	R
7a	H
8a	Ac



	R
7b	H
8b	Ac

is also noteworthy, since the acetate-assisted opening of the oxetane ring generally gives a mixture of 5- and 20-acetates [11].

The NMR spectra of the taxane **5** (Tables 1 and 2) were very close to those of yunnanxane [12], the only difference being the absence of an acetyl resonance and an upfield shift of H-10 ($\Delta\delta$ $-$ 0.95 in $CDCl_3$). This suggested that **5** is 10-deacetylyunnanxane, and this was confirmed by the complete analysis of the spectra by 1D and 2D techniques (see above). The similarity of the resonances of the α-methyl-β-hydroxybutyrate moiety makes it likely that the configuration of the side-chain is the same as in yunnanxane (2*S*,3*R*) [12]. Taiwanxan-type taxoids like **5** are characterized by a C-2, C-5, (C-9), C-10, C-14 oxygenation pattern. Their most remarkable spectroscoipic feature is the ^{13}C NMR resonance of the C-1 doublet around δ60 [13], a rather unsual chemical shift value for a non-oxygenated methine. This downfield resonance is presumably related to the deshielding effects of the two oxygen functions at C-2 and C-14, since the chemical shift of C-1 in related taxanes lacking the C-14 oxygen is normal (δ*ca* 50, *cf.* taxinine [14]). Inspection of the literature data shows that a similar resonance was detected in three taxanes isolated from the European yew

Table 1. ^{1}H NMR spectral data for compounds **1b**, **3**, **4** and **5** (300 MHz, $CDCl_3$, TMS as int. standard, *J* in Hz)

H	**1b***	**3**†	**4**‡	**5**§
1		—	—	1.89 *d* (2.4)
2	5.79 *d* (6.4)	6.20 *d* (11.3)	6.25 *d* (11.3)	5.36 *dd* (6.7, 2.4)
3	3.75 *d* (6.4)	3.15 *d* (11.3)	3.05 *d* (11.3)	2.92 *d* (6.7)
5	4.91 *d* (9.0)	4.95 *br d* (7.3)	4.09 *br d* (2.5)	5.28 *br s*
6α	2.61 *ddd* (14.4, 7.2, 9.0)	‖	2.14 *m*¶	1.80 *m*
6β	1.82 *m*	‖	2.04 *m*¶	1.80 *m*
7α	5.19 *dd* (7.2, 10.5)	—	—	1.24 *m*¶
7β	—	4.25 *dd* (10.4, 6.1)	3.75 *t* (2.7)	1.92 *m*¶
9α	—	—	—	1.68 *dd* (14.9, 5.7)
9β	—	—	—	2.38 *dd* (14.9, 11.6)
10	—	—	—	5.11 *dd* (11.6, 5.7)
13α	—	—	—	2.84 *dd* (18.9, 9.1)
13β	6.15 *t* (8.9)	4.45 *br t* (7.8)	4.46 *br d* (7.0)	2.38 *m*
14α	2.43 *dd* (15.0, 8.9)	‖	1.86 *dd* (7.02, 15.6)¶	5.02 *dd* (9.1, 4.6)
14β	2.37 *dd* (15.0, 8.9)	‖	1.66 *d* (15.6)¶	—
16	1.18 *s*	1.05 *s*	1.03 *s*	1.73 *s*
17	1.22 *s*	1.57 *s*	1.20 *s*	1.18 *s*
18	1.84 *s*	2.26 *s*	2.35 *s*	1.98 *d* (0.9)
19	1.83 *s*	1.62 *s*	1.55 *s*	0.84 *s*
20α (a)	4.33 *d* (8.4)	4.35 *d* (8.0)	4.18 *s*	5.26 *br s*
20β (b)	4.19 *d* (8.4)	4.43 *d* (8.0)	4.18 *s*	4.82 *br s*

*Ester groups: δ2.37 (*s*, 4-Ac), 2.01 (*s*, 7-Ac). 4.80 (*dd*, *J* = 5.1, 2.4 Hz, H-2′), 5.77 (*dd*, *J* = 8.8, 2.6 Hz, H-2′), 7.42 (*m*, *ortho*- and *meta*-Ph), 7.35 (*m*, *para*-Ph), 7.71 (*br d*, *J* = 7.6 Hz, *ortho*-NHBz), 7.39 (*m*, *meta*-NHBz), 7.48 (*m*, *para*-NHBz), 8.01 (*br d*, *J* = 7.6 Hz, *ortho*-OBz), 7.51 (*m*, *meta*-OBz), 7.65 (*m*, *para*-OBz). Exchangeable signals: δ 7.03 (*d*, *J* = 8.9 Hz, NH), 2.8 (*s*, 1-OH), 3.72 (*d*, *J* = 5.1 Hz, 2′-OH).

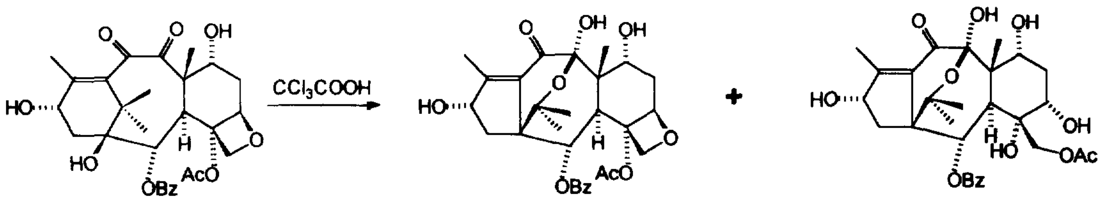
†Ester groups: δ2.14 (*s*, 4-Ac), 8.16 (*m*, *ortho*-Bz), 7.64 (*m*, *meta*-Bz), 7.48 (*m*, *para*-Bz). Exchangeable signals: δ3.80 (*d*, *J* = 10.4 Hz, 7-OH), 4.80 (*s*, 9-OH).

‡Ester groups: δ1.91 (*s*, 20-Ac), 8.20 (*m*, *ortho*-Bz), 7.65 (*m*, *para*-Bz), 7.51 (*m*, *meta*-Bz).

§Ester groups: δ2.18 (*s*, Ac), 2.02 (*s*, Ac), 2.38 (*m*, H-2′), 3.85 (*m*, H-3′), 1.21 (*d*, *J* = 6.4 Hz, H-4′), 1.16 (*d*, *J* = 6.4 Hz, 2′-Me).

¶Interchangeable signals.

‖Could not be assigned because of overlapping.



2 **3** **4**

Scheme 1. Acidic rearrangement of 10-deacetyl-10-dehydrobaccatin V (**2**).

and reported to have a 2,5,7(9),10-oxygenation pattern (**6a**–**8a**) [6, 15]. The structure of **6a** was recently revised to **6b** [16], but it is also likely that the structures of **7a** and **8a** should be revised to **7b** and **8b**, respectively.

The isolation of the nex taxanes **1b** and **5** from *T.* × *media* cv. Hicksii highlights the importance of cultivated varieties of yew as a source of novel taxoids [2, 17, 18].

EXPERIMENTAL

CC: silica gel, Merck, 70–230 mesh; MPLC: silica gel, Merck LiChroprep Si60, 15–25 mm, 5–15 bar.

Plant material. Roots of *T.* × *media* Rehd. cv. Hicksii were identified by U. Boni (Indena S.p.A.). A voucher specimen is kept at the Indena Laboratories, Settala, Milano, Italy.

Isolation of constituents. Dried powdered roots were extracted as previously reported [17]. The mother liquors from the crystallization of taxol C were concd to give a semi-solid residue (50 g). A portion (5 g) of this material was subjected to MPLC (hexane–EtOAc, 3:2) to give, in order of elution, 150 mg **1b** (yield 0.0015%) and 140 mg crude **5**, which was further purified by MPLC (CHC_3) to give 15 mg pure **5** (yield 0.00015%).

Table 2. ^{13}C NMR spectral data of compounds **1b**, **3**, **4** and **5** (75 MHz, $CDCl_3$, TMS as int. standard, *J* in Hz)

C	**1b***	**3**†	**4**‡	**5**§
1	80.6 *s*	62.6 *s*	64.3 *s*	59.2 *d*
2	74.1 *d*	70.0 *d*‖	72.5 *d*	70.6 *d*
3	46.2 *d*	38.4 *d*	40.8 *d*	41.9 *d*
4	78.8 *s*	78.4 *s*¶	78.3 *s*	142.3 *s*
5	83.9 *d*	83.6 *d*	72.6 *d*	78.3 *d*
6	32.9 *t*	37.3 *t***	29.7 *t*	28.9 *t*
7	69.7 *d*	70.4 *d*‖	71.0 *d*	33.9 *t*
8	56.7 *s*	49.6 *s*	50.3 *s*	39.6 *s*
9	204.3 *s*	99.0 *s*	99.3 *s*	47.0 *t*
10	192.9 *s*	195.7 *s*	197.1 *s*	67.2 *d*
11	141.8 *s*	135.0 *s*	134.2 *s*	138.7 *s*
12	144.7 *s*	155.0 *s*	156.6 *s*	132.2 *s*
13	71.9 *d*	79.4 *d*	79.4 *d*	39.5 *t*
14	35.5 *t*	36.1 *t***	37.8 *t*	70.9 *d*
15	40.8 *s*	80.6 *s*¶	75.6 *s*	37.4 *s*
16	23.2 *q*	24.4 *q*††	24.5 *q*‖	25.3 *q*
17	26.7 *q*	26.5 *q*††	27.0 *q*‖	32.0 *q*
18	13.9 *q*	12.0 *q*‡‡	16.0 *q*¶	21.0 *q*
19	9.6 *q*	13.6 *q*‡‡	14.1 *q*¶	22.5 *q*
20	76.1 *t*	74.1 *t*	65.4 *t*	116.8 *t*

*Ester groups: δ 170.2 (*s*, 4-COAc), 22.5 (*q*, 4-Ac); 169.4 (*s*, 7-COAc), 20.8 (*q*, 7-Ac); 172.5 (*s*, C-1′), 72.9 (*d*, C-2′), 55.0 (*d*, C-3′), 137.8 (*s*, *ipso*-Ph), 127.0 (*d*, *ortho*-Ph),129.0 (*d*, *meta*-Ph), 128.4 (*d*, *para*-Ph), 167.3 (*s*, CONHBz). 133.5 (*s*, *ipso*-NHBz), 127.0 (*d*, *ortho*-NHBz), 128.8 (*d*, *meta*-NHBz), 132.0 (*d*, *para*-NHBz); 166.7 (*s*, (2)COBz), 128.8 (*s*, *ipso*-OBz), 130.2 (*d*, *ortho*-OBz), 128.7 (*d*, *meta*-OBz), 133.9 (*d*, *para*-OBz).

†Ester groups: δ 171.1 (*s*, COAc), 21.6 (*q*, Ac); 1651.1 (*s*, COBz), 128.9 (*s*, *ipso*-Bz), 129.9 (*d*, *ortho*-Bz), 128.7 (*d*, *meta*-Bz), 133.9 (*d*, *para*-Bz).

‡Ester groups: δ 170.9 (*s*, COAc), 20.5 (*q*, Ac); 165.4 (*s*, COBz), 129.3 (*s*, *ipso*-Bz), 130.0 (*d*, *ortho*-Bz), 128.9 (*d*, *meta*-Bz), 133.8 (*d*, *para*-Bz).

§Ester groups: δ 169.9 (*s*, COAc), 169.8 (*s*, COAc), 21.4 (*q*, Ac), 21.9 (*q*, Ac); 174.8 (*s*, C-1′), 46.9 (*d*, C-2′), 69.3 (*d*, C-3′), 20.7 (*q*, C-4′), 14.0 (*q*, C-2′-Me).

¶,‖,**,††,‡‡Interchangeable signals.

10-*Deacetyl*-10-*dehydro*-7-*acetyltaxol A* (**1b**). Crystals, mp 226–230°, $[\alpha]_D^{25}$ − 57° (*c* 2.8, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3435, 1732, 1667, 1603, 1518, 1375, 1243, 1107,1069, 712; UV λ_{max}^{EtOH} nm: 274, 232, 220; negative-ion MS 140 eV, *m*/*z* (rel. int.): 851 $[M]^-$ (12), 566 $[M - \text{side-chain}]^-$ (1).

10-*Deacetylyunnanxane* (**5**). Amorphous, $[\alpha]_D^{25}$ − 41° ($CHCl_3$, *c* 1.3). IR ν_{max}^{nujol} cm^{-1}: 3420, 1700, 1630, 750; UV λ_{max}^{EtOH} nm 202; CIMS 140 eV, *m*/*z* (rel. int.): 538 $[M + NH_4]^+$ (100).

Acidic rearrangement of 10-*deacetyl*-10-*dehydrobaccatin V* (**2**). To a soln. of **2** (380 mg, 0.70 mmol) in dry CH_2Cl_2 (10 ml), TCA (1.145 g, 7.0 mmol, 10 mol. equiv.) was added. After stirring at room temp. for 6 hr, the reaction was worked up by addition of satd aq. $NaHCO_3$ and extracted with CH_2Cl_2. After washing with brine and removal of the solvent, the residue was purified by CC (hexane–EtOAc, 1 : 1) to give 105 mg starting material, 165 mg **3** and 48 mg **4**. Compound **3**, amorphous, $[\alpha]_D^{25}$ − 92° (MeOH, *c* 1.8). IR ν_{max}^{nujol} cm^{-1}: 3390, 1690, 1630, 1250, 1010, 970; CIMS 140 eV, *m*/*z* (rel. int.): 562 $[M + NH_4]^+$ (100). Compound **4** powder, mp 132°, $[\alpha]_D^{25}$ − 77° (MeOH, *c* 0.90). IR ν_{max}^{nujol} cm^{-1}: 3350, 1695, 1285, 1010, 950; CIMS 140 eV, *m*/*z* (rel. int.): 580 $[M + NH_4]^+$ (100).

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