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J. Nat. Prod., **1994**, 57 (1), 116-122• DOI:
10.1021/np50103a016 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 1, pp. 116-122, January 1994

NEW BIOACTIVE TAXOIDS FROM CELL CULTURES OF *TAXUS BACCATA*

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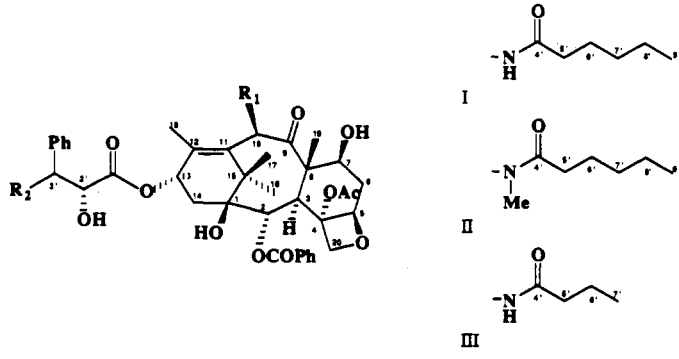
ABSTRACT.—Four new taxoids were isolated from cell cultures of *Taxus baccata*. Their structures were elucidated by spectroscopic analyses. Two were the aglycones corresponding to previously isolated 7-*O*-xylosides of taxol C [**1**] and 10-deacetyltaxol C [**2**]. The third [**3**] had an *N*-methylated side-chain, while the fourth, named taxcultine [**4**], contained an *n*-propyl group on the side-chain. All four compounds actively promoted tubulin assembly. Taxol C [**1**] showed potent and selective cytotoxicity in the NCI human cell line screen.

Taxol [**5**], a promising antineoplastic compound, is currently approved for the treatment of advanced ovarian cancer (1). This compound has a unique mechanism of action which results from specific binding to polymerized tubulin and consequent inhibition of mitosis (2). Taxol was originally isolated from the bark of the Pacific yew, *Taxus brevifolia*, at a yield of 0.02% w/w (3). Although various species of *Taxus* have been reported to contain taxol, large quantities of taxol for clinical trials were originally isolated from the bark of the Pacific yew. The needles of some *Taxus* species may become the preferred source (4,5). Semi-synthesis from natural taxoids and total synthesis are also being actively pursued (6). Cell culture may offer the advantage of reliable production of taxol using a renewable resource. The large-scale production of taxol by plant cell culture has also been shown to provide a rich source of bioactive and structurally unique taxoids which may serve as an alternative to taxol or as precursors for semisynthesis of taxol. These taxoids also provide information for better understanding of structure-activity relationships and biosynthesis, as well as improving the quality control of taxol production.



1 R_1=OAc, R_2=I
2 R_1=OH, R_2=I
3 R_1=OAc, R_2=II
4 R_1=OAc, R_2=III
5 R_1=OAc, R_2=$NHCOC_6H_5$

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RESULTS AND DISCUSSION

The ^{1}H-nmr spectrum of **1** (Table 1) was similar to that of taxol (7). However, only one doublet of doublets at 8.11 ppm was observed and the total integration of the aromatic range was five protons less than that of taxol, which indicated that **1** does not have a benzoyl group at C-3. The ^{1}H-nmr spectrum of **1** further showed at least 6 more alphatic protons at 1.2 ppm for methylene groups and a triplet at 0.84 ppm attributed to a methyl group. These results suggested an *n*-pentyl group attached to C-4′, which was confirmed by the mass spectral fragments at *m/z* 280, 262, and 234 (Figure 1) and

TABLE 1. ^{1}H- and ^{13}C-nmr Data of **1** in $CDCl_3$.

Atom	δ_H (Multiplicity, *J* Hz)	δ_C
1		78.89
2	5.67 (d, 7.0)	74.88
3	3.78 (d, 6.9)	45.52
4		81.04
5	4.93 (bd, 9.5)	84.32
6	2.54 (m)	35.55
	1.88 (m)	
7	4.40 (dd, 6.5, 10.7)	72.12
8		58.54
9		203.49
10	6.28 (s)	75.50
11		133.59
12		141.93
13	6.22 (m)	72.37
14	2.30 (m)	35.60
	2.20 (m)	
15		43.18
16-CH_3	1.15 (s)	21.89
17-CH_3	1.27 (s)	26.80
18-CH_3	1.82 (s)	14.84
19-CH_3	1.68 (s)	9.56
20	4.18 (d, 8.5)	76.42
	4.29 (d, 8.5)	
1′		172.70
2′	4.67 (d, 2.5)	73.05
3′	5.57 (dd, 2.6, 8.9)	54.48
5′	2.20 (t, 7.1)	36.58
6′	1.24 (m)	25.36
7′	1.24 (m)	31.28
8′	1.24 (m)	22.29
9′	0.84 (t, 6.7)	13.85
NH	6.21 (d, 8.4)	
4-OAc	2.35 (s)	22.61
		170.08
10-OAc	2.24 (s)	20.86
		171.70
2-COPh		129.02
	8.11 (dd, 1.2, 8.2)	130.10
	7.61 (t, 7.3)	128.87
	7.51 (t, 7.6)	133.03
		166.80
3′-Ph		137.90
	7.40 (m)	126.85
	7.34 (m)	128.59
		128.18

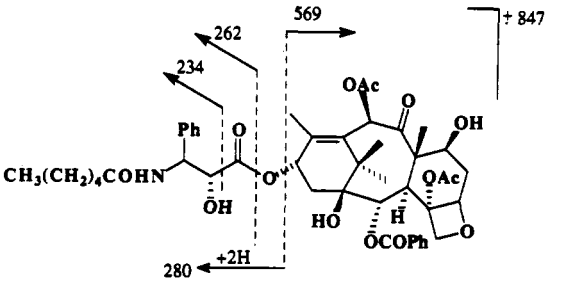


FIGURE 1. Esms fragmentation of **1**.

the ^{13}C-nmr signals at 13.85, 22.29, 25.36, 31.28, and 36.58 ppm (Table 1). High-resolution mass measurement of **1** showed *m/z* 848.3857, consistent with the formula $C_{46}H_{58}NO_{14}$. Its electrospray mass spectral (esms) fragments at *m/z* 770, 569, 551, and 509 indicated that the taxol skeleton was present. An inverse-detection one-bond heteronuclear correlation (HMQC) nmr experiment indicated that C-5′ (36.58 ppm) correlated with the H-5′ protons (2.20 ppm) which were overlapped by the protons of C-14 and -OAc and C-10 in the ^{1}H-nmr spectrum. Details of the ^{1}H- and ^{13}C-nmr data of **1** are presented in Table 1. These data show that the compound is taxol C, which has not previously been isolated from plant materials, although its 7-*O*-xyloside has been shown to be present in *T. baccata* (8).

The esms spectrum of **2** showed a protonated molecular ion at *m/z* 806 (high-resolution mass measurement at *m/z* 806.3753 corresponding to a formula $C_{44}H_{56}NO_{13}$). The esms fragments of *m/z* 527, 509, and 280 indicated that this compound was a deacetyl derivative of **1**. The ^{1}H-nmr spectrum of **2** (Table 2) was also similar to that of **1**, but only one OAc signal was observed. The C-10 proton signal shifted from 6.3 ppm to 5.2 ppm, the C-19 methyl signal shifted to 1.78 ppm, and the C-7 proton signal shifted to 4.2 ppm. These results indicated that an OH, not an OAc group, was present at C-10. Therefore, the structure of **2** is 10-deacetyltaxol C, the xyloside of which has been previously found in *T. baccata* (8).

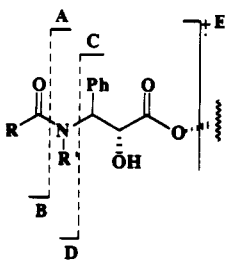
The high-resolution mass measurement of **3** gave a protonated molecular ion at *m/z* 862.3753, corresponding to the formula, $C_{47}H_{60}NO_{14}$. Esms fragments at *m/z* 569, 551, and 509 indicated the presence of the taxol skeleton. However, the fragment at *m/z* 294 suggested an additional methyl group in the side-chain of **3**. As shown by Bitsch *et al.* (9), the side-chain structure of taxoids can be partially elucidated by ms/ms of the C-13 side-chain fragments produced by electrospray ionization. Comparing the C-13 side chain ion at *m/z* 294 of **3** with that of the C-13 side-chain ion at *m/z* 280 of **1**, the daughter ions at *m/z* 196 and 130 from *m/z* 296 clearly indicated that the methyl group is on the nitrogen of the side-chain (Figure 2). The ^{1}H-nmr spectrum of **3** (Table 2) showed a CH_3 signal at 2.89 ppm. The chemical shift of this methyl group ruled out the possibility of methyl substitution at the C-2′-OH or at the terminal aliphatic group of the C-13 side-chain. The substitution of the CH_3 at the nitrogen was also strongly supported by the disappearance of the NH proton, which showed as a doublet at 6.2 ppm in the ^{1}H-nmr spectrum of **1**. Thus, the structure of **3** is *N*-methyltaxol C which, in contrast with **1** and **2**, has not been previously reported either in a free or glycoside form.

The structure elucidation of taxcultine [**4**] was simplified by direct comparison of its esms/ms and nmr spectra with those of **1**. The high-resolution mass spectrum of **4** showed a protonated molecular ion at *m/z* 820.3559, which indicated the composition $C_{44}H_{54}NO_{14}$. The esms fragments at *m/z* 569, 551, and 509 indicated the presence of the taxol skeleton. The fragment at *m/z* 252 indicated a shorter aliphatic group on the C-13

TABLE 2. ^{1}H-Nmr Data of **2–4** in $CDCl_3$.

Proton	δ_H (Multiplicity, J Hz)		
	2	**3**	**4**
2	5.68 (d, 7.1)	5.67 (d, 7.1)	5.67 (d, 7.1)
3	3.45 (d, 5.3)	3.80 (d, 7.0)	3.79 (d, 7.1)
5	4.94 (bd, 9.3)	4.89 (m)	4.93 (bd, 9.5)
6	2.57 (m)	2.55 (m)	2.54 (m)
		2.10 (m)	1.88 (m)
7	4.21 (m)	4.42 (dd, 6.5, 10.7)	4.42 (m)
10	5.19 (s)	6.31 (s)	6.29 (s)
13	6.19 (t, 7.3)	6.19 (t, 7.7)	6.21 (t, 7.3)
14	2.28 (m)	2.28 (m)	2.28 (m)
	2.19 (m)		2.20 (m)
16-CH_3	1.13 (s)	1.16 (s)	1.16 (s)
17-CH_3	1.24 (m)	1.28 (s)	1.27 (s)
18-CH_3	1.76 (s)	1.88 (s)	1.89 (s)
19-CH_3	1.58 (s)	1.67 (s)	1.68 (s)
20	4.21 (m)	4.17 (d, 8.4)	4.19 (d, 8.4)
	4.30 (d, 8.3)	4.27 (d, 8.4)	4.28 (d, 8.4)
2′	4.66 (dd, 2.4, 2.6)	4.89 (m)	4.66 (dd, 2.8, 2.9)
3′	5.57 (dd, 2.6, 9.0)	5.77 (d, 3.8)	5.57 (dd, 2.6, 8.9)
5′	2.20 (m)	2.30 (m)	2.30 (m)
6′	1.24 (m)	1.25 (m)	1.25 (m)
7′	1.24 (m)	1.25 (m)	0.90 (t, 7.3)
8′	1.24 (m)	1.25 (s)	
9′	0.90 (t, 7.3)	0.86 (t, 7.0)	
NH	6.27 (d, 10.3)		6.14 (d, 8.9)
4-OAc	2.34 (s)	2.33 (s)	2.22 (s)
10-OAc		2.24 (s)	2.23 (s)
2-COPh	8.10 (dd, 1.3, 8.2)	8.11 (dd, 1.2, 8.1)	8.10 (dd, 1.2, 7.9)
	7.60 (t, 7.4)	7.60 (t, 7.4)	7.60 (t, 7.5)
	7.50 (t, 7.6)	7.50 (t, 7.6)	7.50 (t, 7.6)
3′-Ph	7.39 (m)	7.38 (m)	7.39 (m)
	7.34 (m)	7.35 (m)	7.34 (m)
N-CH_3		2.89 (s)	

side-chain than that of **1**. By comparing the ms/ms spectra of the ion *m/z* 252 with that of ion *m/z* 280 from **1**, it was clear that a propyl group is attached at C-4′ (Figure 2). The ^{1}H-nmr spectrum of **4** was very similar to that of **1**. However, the integration of the methylene protons at 1.2 ppm was 4 mass units less. A triplet for a methyl group was observed at 0.85 ppm. Complete ^{1}H-nmr assignments of **4** are listed in Table 2. These results are consistent with the presence of an *n*-propyl group at C-4′. Therefore, the



	R	R′	A	B	C	D	E
1	C_5H_{11}	H	182	99	164	116	280
3	C_5H_{11}	CH_3	196	99	164	130	294
4	C_3H_7	H	182	71	164	88	252

FIGURE 2. Esms/ms fragmentation of the C-13 side-chains of **1**, **3**, and **4**.

structure of taxcultine [**4**] can best be represented as having an *n*-propyl group on the side-chain as shown in structure **4**.

The stereochemistry of all four compounds is believed to be the same as that of taxol because of their nearly identical coupling constants and chemical shifts in the ^{1}H-nmr spectra. However, the similarity of their structures to that of taxol suggests a common intermediate in the biosynthetic pathway. The presence of these new compounds in *T. baccata* cell culture indicates that plant cell culture may serve as a new source for active natural product analogs. These taxoids are present in significantly lower concentrations than taxol, the yield of which was greater than that of the content found in bark (0.02% w/w) (3), in the particular cell culture investigated.

All four of these new taxoids showed activity close to that of taxol in the tubulin assembly assay (Table 3). Compound **1** was very active in the NCI human tumor panel in vitro test with an average GI_{50} value of 4.47×10^{-7} M. This compound was the most selective against cell lines of non-small-cell lung cancer (HOP-62 and NCI H-460), small-cell lung cancer (DMS 114), colon cancer (COLO 205), CNS cancer (SF-539 and SNB-75), and ovarian cancer (OVCAR-3) (Table 4). The other three taxoids [**2–4**] have not been tested in the NCI human cancer cell line panel. The major structural differences of these four new taxoids from taxol are in the *N*-acyl substitution of the C-13 side-chain confirming that the presence of a specific *N*-acyl substituent does not appear to be critical to the activity of taxol and its derivatives. In fact, the *N*-acyl group of the C-13 side-chain has been a target for the chemical modification of taxol (10–12).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Thomas Hoover melting point apparatus and are uncorrected. Optical rotations were measured in MeOH on a JASCO DIP-360 polarimeter. Uv spectra were recorded in MeOH on a Bausch & Lomb Spectronic 2000. Ir spectra were taken on a Perkin-Elmer 1600 series Ft-ir as films. ^{1}H-Nmr spectra were obtained in $CDCl_3$ on a Bruker AM-400 spectometer and a Nicolet NT 360 spectrometer. The HMQC nmr spectrum was recorded on a Varian VXR 400 spectrometer. ^{13}C-Nmr spectra were performed on a Varian VXR-500s spectrometer. Esms and esms/ms spectra were taken on a VG BioQ MS. High-resolution mass measurements were recorded by the fab method (Cs^+ as atom beam and *m*-nitrobenzyl alcohol as matrix) on either a VG ZAB2-EQ or JEOL HX110 instrument.

MATERIAL AND EXTRACTION.—Callus culture of *T. baccata* stem tissue was spread uniformly on a modified B5 medium (14) in culture dishes. After growth for 40-60 days, biomass was scraped from plates, filtered to remove free liquid, and frozen at −80°. Altogether, 5 kg of this biomass were used for the extraction and isolation of taxol. After taxol was removed, the fractions were combined and further separated over a Si gel column eluted with hexane/CH_2Cl_2/MeOH gradients followed by hplc with a C_{18} silica column

TABLE 3. Tubulin Assembly Assay Results of **1–4**, Taxol, and Cephalomannine.[a]

Compound	Tubulin concentration (mg/ml)	ED_{50}, μM	ED_{50}/ED_{50} of taxol
1	1.5	1.40	1.82
	1.0	1.00	1.05
2	1.0	2.96	2.57
3	1.0	1.91	1.66
4	1.0	2.35	2.04
Taxol	1.5	0.76	1.00
	1.0	0.95	1.00
	1.0	1.15	1.00
Cephalomannine	1.5	0.95	1.11
	1.0	0.85	1.05

[a]Bovine brain tubulin was used for tubulin assembly assay and experiment was performed at Professor R.H. Himes' laboratory, University of Kansas (13).

TABLE 4. Selected Results in the NCI In vitro Human Tumor Test of **1**[a] Compound with Taxol.[b]

Panel/Cell Line	1			Taxol		
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
Non-Small Cell Lung Cancer						
HOP-62	<1.00E-08	1.21E-08	3.20E-05	6.71E-08	>1.00E-06	>1.00E-06
NCI-H460	<1.00E-08	3.49E-07	6.98E-05	2.19E-08	>1.00E-06	>1.00E-06
Small Cell Lung Cancer						
DMS 114.....	<1.00E-08	2.38E-08	2.17E-05	1.23E-08	6.12E-08	>1.00E-06
Colon Cancer						
COLO 205	<1.00E-08	2.29E-08	2.33E-05	1.76E-09	4.32E-08	1.45E-07
CNS Cancer						
SF-539.....	<1.00E-08	<1.00E-08	NV[c]	3.66E-09	8.99E-08	>2.50E-05
SNB-75.....	<1.00E-08	1.92E-08	>1.00E-04	8.08E-09	7.73E-08	>1.00E-06
Ovarian Cancer						
OVCAR-3	<1.00E-08	<1.00E-08	>1.00E-04	1.97E-08	4.87E-08	6.08E-07

[a]Compound **1**, NCS D-655481-N/0-1.45, was tested by the Drug Synthesis & Chemistry Branch, NCI. Data for the cell lines against which **1** showed highest activity are listed.

[b]The data for taxol, NSC 125973-L/22, were provided by the Drug Synthesis & Chemistry Branch, NCI.

[c]Data not available.

eluted by CH_3CN/H_2O gradients. The compounds were crystallized from CH_2Cl_2 and hexane mixtures and recrystallized from MeOH.

Taxol C [**1**].—Yellowish amorphous solid (22 mg), mp 150° (from MeOH); $[\alpha]_D$ −107° (c=0.054, MeOH); uv λ max 227 nm (log ε 4.19, MeOH); ir ν max (film) 3425, 2923, 1731, 1456, 1372, 1241, and 1070 cm^{-1}; ms *m/z* 848, 847,770, 569, 551, 509, 280, 262, and 234; high-resolution mass measurement for $C_{46}H_{58}NO_{14}$: found 848.3857, calcd 848.3847, for $C_{46}H_{57}NO_{14}Na$: found 870.3668, calcd 870.3677; ^1H- and ^{13}C-nmr data, see Table 1.

10-Deacetyltaxol C [**2**].—Compound **2** was a white amorphous solid (5 mg); mp 157° (dec) (from MeOH); $[\alpha]_D$ −14.78° (c=0.115, MeOH); uv λ max 230 nm (log ε 4.20, MeOH); ir ν max (film) 3413, 2927, 1725, 1652, 1370, 1245, and 1070 cm^{-1}; ms *m/z* 806, 509, 280, 262, and 234; high-resolution mass measurement for $C_{44}H_{56}NO_{13}$: found 806.3753, calcd 806.3752; ^1H-nmr ($CDCl_3$, 360 MHz) data, see Table 2.

N-*Methyltaxol C* [**3**].—Compound **3** was as white needles (11 mg); mp 134° (dec) (from MeOH); $[\alpha]_D$ −16.52° (c=0.103, MeOH); uv λ max 223 nm (log ε 4.23, MeOH); ir ν max (film) 3415, 2929, 1725, 1629, 1371, 1242, and 1071 cm^{-1}; ms *m/z* 862, 569, 509, 294, and 276; high-resolution mass measurement for $C_{47}H_{60}NO_{14}$: found 862.3996, calcd 862.4014; ^1H-nmr ($CDCl_3$, 360 MHz) data, see Table 2.

Taxcultine [**4**].—Compound **4** was a white amorphous solid (8 mg); mp 155° (from MeOH); $[\alpha]_D$ −7.54° (c=0.106, MeOH); uv λ max 229 nm (log ε 4.23, MeOH); ir ν max (film) 3415, 2911, 1724, 1658, 1367, 1242, and 1067 cm^{-1}; ms *m/z* 821, 569, 551, 509, 252, and 234; high-resolution mass measurement for $C_{44}H_{54}NO_{14}$: found 820.3559, calcd 820.3544; ^1H-nmr ($CDCl_3$, 360 MHz) data, see Table 2.

ACKNOWLEDGMENTS

We are grateful to NCI for supporting the taxol cell culture project of ESCAgenetics Corp., the parent company of PHYTOpharmaceuticals, Inc. (Grant No. RO1 CA 55102-01). The electrospray mass spectrometric studies were partially supported by the NIH through grant DK34400 (to CHLS) and a shared instrument Grant RR06505. We appreciate the assistance of Dr. Matthew Suffness and Dr. Ken Snader for obtaining taxol and other taxoid standards, and Dr. Ven Narayanan of NCI for the in vitro human tumor panel test of taxol C. We acknowledge Professor Richard H. Himes of the University of Kansas for performing the tubulin assembly assay. We thank Dr. Julie Leary of the University of California, Berkeley, and Dr. Brian Musselman of JEOL USA, Boston, MA, for high-resolution esms measurements, Mr. Richard Mazarazi of the University of California, Berkeley, and Ms. Virginia Miner of Acorn NMR, Fremont California, for nmr spectra.

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Received 8 July 1993