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Ru Chen, and David G. I. Kingston

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ISOLATION AND STRUCTURE ELUCIDATION OF NEW TAXOIDS FROM *TAXUS BREVIFOLIA*

RU CHEN and DAVID G.I. KINGSTON*

Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0212

ABSTRACT.—An investigation of *Taxus brevifolia* extracts afforded two taxoids characterized by the A-nortaxol ring system. These were identified as 7,13-dideacetyl-9,10-debenzoyltaxchinin C [**1**] and 9-deacetyl-9-benzoyl-10-debenzoylbrevifoliol [**4**]. Structures were elucidated by spectroscopic methods, and in particular by comparison of nmr data with those of taxchinin C and brevifoliol.

The clinical activity of taxol (1) against breast and ovarian cancers (2) has spurred a worldwide search for better sources and improved analogues of this drug. Although over 100 taxoids have been isolated to date (3), new taxoids continue to be isolated, and many of these could serve as precursors to new analogues of taxol.

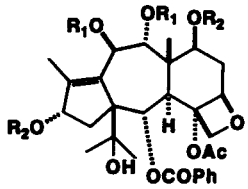
The western yew, *Taxus brevifolia*, was the original source of taxol (1), and it continues to be a source of other natural taxoids (4–6). In continuation of our work on this plant, we have examined a pre-taxol fraction from a large-scale isolation of taxol and have obtained two new taxoids with the uncommon brevifoliol (or taxchinin A) skeleton.[1]

The starting material for our work was a "pre-taxol" fraction from a previous large-scale isolation of taxol from *T. brevifolia* (4,5). Partitioning of this fraction between CH_2Cl_2 and H_2O, followed by cc on Si gel, and finally repeated prep. tlc, yielded the two new taxoids **1** and **4**.

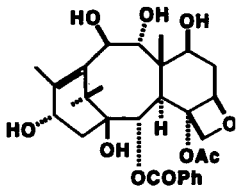
Compound **1** had a composition of $C_{29}H_{38}O_{10}$ as determined by hrfabms and ^{13}C-nmr spectroscopy. Its ^1H-nmr spectrum showed characteristic taxoid signals, including singlets for four methyl groups at 1.05, 1.08, 1.84, and 1.93 ppm, and a pair of doublets for oxetane protons at 4.08 and 4.43 ppm. The presence of an oxetane ring was further supported by a ^{13}C-nmr signal for the methylene carbon at 74.7 ppm and by ^1H decoupling and TOCSY (12) experiments to verify the coupling of the H-20α and H-20β protons. Characteristic signals for a benzoate group were observed in both the ^1H- (7.46, 7.60, and 7.99 ppm) and the ^{13}C-nmr spectra.

The basic ^1H- and ^{13}C-nmr assignments for **1** were made by a combination of DEPT (13), HETCOR (13), TOCSY (12), and HMBC (14) techniques, and are shown in Table 1. The doublet at 6.04 ppm was assigned to the C-2 proton, deshielded by the benzoate group, and this was coupled to the C-3 proton at 3.00 ppm. HMBC correlations of the *ortho*-benzoyl proton at 7.99 ppm with the carbonyl signal at 166.2 ppm assigned the latter as being due to the benzoate carbonyl group, and this signal in turn was correlated with the proton signal at 6.04 ppm, confirming the location of the benzoate group at C-2. The appearance of the C-2 and C-3 protons as an isolated spin system confirmed the fully substituted nature of C-1 and C-4.

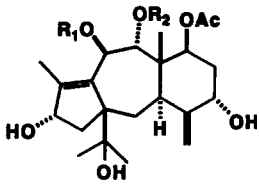
[1]The taxoid brevifoliol was the first natural taxoid with the A-nortaxane skeleton to be isolated (6), although it was initially assigned a normal taxane skeleton which was later corrected to the A-nortaxane skeleton (7,8); the diterpenoid taxchinin A was the first naturally occurring taxoid to be correctly assigned the A-nortaxane skeleton (9). The first example of a taxoid with this skeleton was a semisynthetic product from taxol (10), while the first example of a correctly assigned natural A-nortaxoid with an oxetane ring was taxchinin B (11). Because of this complicated chronology, we propose to name A-nortaxoids with a C-4(20) exocyclic double bond as brevifoliol derivatives and those with an oxetane ring as taxchinin B derivatives.



1 $R_1=R_2=H$
3 $R_1=PhCO$, $R_2=Ac$



2



4 $R_1=H$, $R_2=PhCO$
5 $R_1=PhCO$, $R_2=Ac$

The remaining key question was the assignment of the ring system as that of taxol and related compounds [**2**] or of the newer brevifoliol type [**1**]. Two key results supported the brevifoliol skeleton for **1**. The first was the assignment of the ^{13}C-nmr signal at 76.4 ppm to C-15, an assignment supported by HMBC correlations with the protons on C-2 and C-14. The downfield nature of this signal indicated that C-15 must be oxygenated, which is consistent with structure **1** but not with structure **2**. Second, the lack of a diagnostic three-bond correlation from H-16 and H-17 to C-11 (7) eliminated structure **2** from consideration. The structure of the new compound is thus established as a derivative of taxchinin C [**3**] (11) and specifically as 7,13-dideacetyl-9,10-didebenzoyltaxchinin C. It is noteworthy that an unusual 4-bond HMBC correlation was detected between H-14β and C-18; this is probably due to the W-effect. Correlations are not observed be-

TABLE 1. Nmr Data of 7,13-Deacetyl-9,10-debenzoyltaxchinin C [**1**].

Position	^1H[a]	^{13}C[b]	TOCSY	HMBC[c]
1		67.7		
2	6.04 (1H, d, 7.4)	68.8	H-3	C-3, C-4, C-8, C-15, $OCOC_6H_5$
3	3.00 (1H, d, 7.4)	44.5	H-2	C-1, C-2, C-4, C-8, C-19, C-20
4		80.4		
5	4.89 (1H, br d, 8.5)	85.1	H-6α	C-3, C-4, C-7
6	α 1.80 (1H, m)	37.2	H-5, H-6β, H-7	C-7, C-8
	β 2.55 (1H, ddd, 16.0, 8.2, 1.5)		H-6α, H-7	
7	4.20 (1H, br t, 8.2)	72.4	H-6α, H-6β	C-19
8		42.6		
9	4.30 (1H, d, 10.4)	80.7	H-10	
10	4.55 (1H, d, 10.4)	68.7	H-9	
11		137.2		
12		146.8		
13	4.58 (1H, br t)	77.6	H-14α, H-14β	
14	α 1.75 (1H, dd, 15, 7.4)	39.4	H-14α, H-13	C-1, C-2, C-13, C-15,
	β 2.25 (1H, dd, 15, 7.4)		H-14β, H-13	C-18
15		76.4		
16	1.08 (3H, s)	24.7		
17	1.05 (3H, s)	27.7		C-16
18	1.93 (3H, s)	11.4		C-11, C-12, C-13
19	1.84 (3H, s)	12.2		C-9
20	α 4.43 (1H, d, 7.9)[d]	74.7	H-20β	C-3, C-4, C-5
	β 4.08 (1H, d, 7.9)		H-20α	
$OCOCH_3$	2.20 (3H, s)	22.4		$OCOCH_3$
$OCOCH_3$		171.1		
$OCOC_6H_5$		166.2		
Ar i		129.9		
o	7.99 (2H, d, 7.2)	129.6	m-Ar	$OCOC_6H_5$
m	7.46 (2H, m)	128.6	o-Ar, p-Ar	
p	7.60 (1H, m)	133.9	m-Ar	

[a]Multiplicity and apparent coupling constant(s) (*J*) in Hz in parentheses.

[b]^{13}C-Nmr assignments of protonated carbons were confirmed by a HETCOR experiment, and carbon types were assigned by a DEPT experiment.

[c]The HMBC experiment was performed with the second delay in the *J* filter segment set for $J=8$ Hz and $J=10$ Hz. Data from both experiments are combined in this column.

[d]The assignments of 20α and 20β were made by comparison with data for taxol (18); the assignments for taxchinin B (11) are reversed.

tween H-14β and C-11 or C-12, but a similar failure to observe these correlations is seen in the HMBC spectrum of taxol (15).

The second new compound [**4**] had a composition of $C_{29}H_{38}O_8$ as deduced by hrfabms and ^{13}C-nmr spectroscopy. Its ^1H- and ^{13}C-nmr spectra (Table 2) were similar in many respects to those of compound **1**, with the significant difference that signals for an exocyclic methylene group (δ_H 4.82 and 5.10 ppm, δ_C 150.0, 111.8 ppm) replaced those for the oxetane ring. Signals for one acetate group and one benzoate group could also be identified.

One interesting feature of both ^1H- and ^{13}C-nmr spectra that was observed in the spectra of **4** but not in those of **1** was that the signals were very broad when the spectra were obtained at room temperature. Increasing the temperature to 57°, however, yielded normal narrow linewidths, indicating that line broadening was due to a slow equilibrium between two or more conformational isomers. This behavior has been observed previously in taxoids that were subsequently shown to have the brevifoliol skeleton (16,8), and appears to be characteristic of this structural type.

A comparison of the ^1H- and ^{13}C-nmr spectra of **4** with those of brevifoliol [**5**] (6–8) indicated that **4** differed from brevifoliol primarily in lacking one acetate group. The locations of the acetate and benzoate groups in **4** were confirmed by acetylation and by HMBC experiments. Acetylation of **4** yielded a triacetate derivative in which the signals for

TABLE 2. Nmr Data of 9-Deacetyl-9-benzoyl-10-debenzoylbrevifoliol [**4**].

Position	^1H[a]	^{13}C[b]	TOCSY	HMBC[c]
1		61.2		
2	α 1.58 (1H, d, 14.3)	28.9	H-2β	C-1, C-3, C-8, C-14, C-15
	β 2.30 (1H, dd, 14.3, 9.0)		H-2α, H-3	
3	2.84 (1H, d, 9.0)	37.1	H-2β	C-1, C-2, C-4, C-7, C-8, C-19, C-20
4		150.0		
5	4.36 (1H, br s)	72.9	H-6α	
6	α 1.70 (1H, m)	35.6	H-6β, H-7	
	β 1.99 (1H, br d, 11.5)		H-6α, H-7	
7	5.51 (1H, dd, 11.5, 5)	71.0	H-6α, H-6β	$OCOCH_3$, C-19
8		45.5		
9	5.70 (1H, d, 9.2)	80.2	H-10	$OCOC_6H_5$
10	4.85 (1H, d, 9.2)	68.0	H-9	C-1, C-12
11		137.4		
12		145.9		
13	4.43 (1H, br t, 7.0)	78.0	H-14α, H-14β	C-11, C-12
14	α 1.35 (1H, dd, 14.0, 7.0)	46.4	H-14α, H-13	C-11, C-12, C-13, C-15
	β 2.39 (1H, dd, 14.0, 7.0)		H-14β, H-13	
15		76.5		
16	1.07 (3H, s)	27.1		C-1
17	1.39 (3H, s)	25.9		C-1, C-15, C-16
18	1.75 (3H, s)	11.9		C-12
19	1.03 (3H, s)	12.8		
20	α 5.10 (1H, s)[d]	111.8		C-3, C-5
	β 4.80 (1H, s)			
$OCOCH_3$	1.87 (3H, s)	21.6		$OCOCH_3$
$OCOCH_3$		170.8		
$OCOC_6H_5$		167.0		
Ar i		130.6		
o	8.00 (2H, d, 7.9)	129.6	m-Ar	$OCOC_6H_5$
m	7.40 (2H, m)	128.2	o-Ar, p-Ar	
p	7.53 (1H, m)	132.7	m-Ar	

[a]Multiplicity and apparent coupling constant(s) (*J*) in Hz in parentheses.

[b]^{13}C-Nmr assignments of protonated carbons were confirmed by a HETCOR experiment, and carbon types were assigned by a DEPT experiment.

[c]The HMBC experiment was performed with the second delay in the *J* filter segment set for $J=8$ Hz and $J=10$ Hz. Data from both experiments are combined in this column.

[d]The assignments of 20α and 20β were made by comparison with data for taxol (18); the assignments for taxchinin B (11) are reversed.

C-5, C-10, and C-13 had shifted downfield by approximately 1 ppm, indicating that these positions in **4** carried secondary hydroxyl groups. HMBC experiments then related the benzoate carbonyl group both to the *ortho* protons at 8.00 ppm and to the C-9 proton at 5.70 ppm, and the acetate carbonyl group to the acetate methyl group at 1.87 ppm and the C-7 proton at 5.51 ppm. The assignment of the signal at 5.70 ppm to C-9 (rather than C-10) was made on the basis of HMBC correlations of the C-10 proton at 4.85 ppm with C-1 and C-12.

These results, taken in conjunction with the published spectra of brevifoliol (6–8) and the additional correlations summarized in Table 2, establish the structure of **4** as the brevifoliol analogue 9-deacetyl-9-benzoyl-10-debenzoyl-brevifoliol.

Compounds **1** and **4** are thus new additions to the rapidly-growing family of A-nortaxoids [or 11(15→1)-*abeo* taxoids, as they are more correctly described (10)]. In addition to brevifoliol (6–8) from *T. brevifolia* and the taxchinins from *T. chinensis* (9), compounds with this ring system have also been isolated from *T. baccata* (8,17) and *T. wallichiana* (16). The fact that the first observation of this structural type was as a rearrangement product of taxol (10) raises the question as to whether all these compounds are simply artifacts. Although this possibility cannot be completely excluded, the fact that the rearranged taxoids are being obtained from different species by different research groups, coupled with the fact that rearrangement of taxol requires rather vigorous conditions, suggests that some if not all of the isolated A-nortaxoids are genuine natural products.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Thomas-Hoover capillary melting-point apparatus and are uncorrected. Ft-ir spectra were recorded as KBr pellets on a Nicolet 710 Ft-ir spectrometer. Uv spectra were obtained on a Perkin-Elmer 330 spectrophotometer. Mass spectra were obtained on a Kratos MS50 mass spectrometer. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. Nmr spectra were recorded on a Varian Unity 400 spectrometer in $CDCl_3$. ^{1}H-Nmr chemical shifts were recorded in ppm from internal TMS and ^{13}C-nmr chemical shifts were based on the $CHCl_3$ signal at 77.0 ppm.

FRACTION FB10115-J.—*Taxus brevifolia* bark was processed as previously described (5) to yield a taxol-rich fraction which was crystallized to give crude taxol. The mother liquors from this crystallization were rechromatographed on a Florisil™ column with elution with Me_2CO-hexane (50:50) to yield additional crude taxol. Fraction FB10115-J eluted just before taxol on this column.

ISOLATION OF COMPOUNDS **1** AND **4**.—A 35 g portion of a dark brown syrupy residue of FB10115-J was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 extract (32 g dark brown syrup) was subjected to chromatography on Si gel (3 times) with elution by CH_2Cl_2-MeOH (93:7), EtOAc, and EtOAc-hexane (9:1), respectively. Fractions which eluted after 10-deacetylbaccatin III were combined and purified by repeated prep. tlc (Si gel F_{254}, 20×20 cm) with development by EtOAc/hexane. A total of 62.5 mg of compound **1** and 105.2 mg of compound **4** was obtained.

Compound **1**.—Mp 162°; $[\alpha]^{25}D$ −15° (c=10, $CHCl_3$), uv (EtOH) λ max (log ε) 240 nm (4.52); ir (KBr) ν max 3405, 2995, 2976, 2930, 2891, 1736, 1715, 1663, 1449, 1369, 1269, 1240, 1175, 1111, 1067, 1043, 1024, 731, 712 cm^{-1}; fabms *m/z* $[M+Na]^+$ 569 (51), 499 (35), 476 (43), 413 (32), 349 (10), 326 (18), 242 (6), 199 (26), 176 (100), 173 (60), 136 (8); hrfabms *m/z* $[M+Na]^+$ 569.23260, calcd for $C_{29}H_{38}O_{10}Na$ 569.23624; ^{1}H and ^{13}C nmr, see Table 1.

Compound **4**.—Mp 152°; $[\alpha]^{25}D$ +18° (c=10, $CHCl_3$); uv (EtOH) λ max (log ε) 228 nm (5.93); ir (KBr) ν max 3380, 2974, 2939, 2896, 1715, 1663, 1373, 1281, 1176, 1111, 1096, 1069, 1026, 730, 712 cm^{-1}; fabms *m/z* $[M+Na]^+$ 537 (58), $[M-H_2O-OH]^+$ 479 (20), $[M-COCH_3-2OH]^+$ 421 (43), 357 (14), 329 (68), 219 (5), 154 (100); hrfabms *m/z* $[M+Na]^+$ 537.24610, calcd for $C_{29}H_{38}O_8Na$ 537.24644; ^{1}H and ^{13}C nmr, see Table 2.

ACETYLATION OF COMPOUND **4**.—Compound **4** (20 mg) in dry CH_2Cl_2 (100 μl) and dry pyridine (20 μl) was mixed with Ac_2O (20 μl). The mixture was stirred at room temperature for 35 min, diluted with EtOAc, and extracted with H_2O. The organic layer was washed with dilute HCl and H_2O, dried, and evaporated. The crude products were isolated by prep. tlc with EtOAc-hexane (3:2). The 5,10,13-triacetate (2.7 mg) and the 5-monoacetate (1.3 mg) were obtained directly, and the 5,10-diacetate (4.4 mg) was obtained by further prep. tlc with EtOAc-hexane (7:3).

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