Journal of Natural Products
Vol. 58, No. 12, pp. 1906-1912, December 1995

TWO NEW TAXOIDS FROM THE STEM BARK OF *TAXUS BACCATA*

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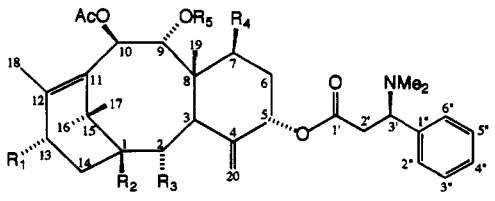
ABSTRACT.—Two new taxoids, 13-deoxo-13α-acetyloxy-7β,9α-diacetyl-1,2-dideoxytaxine B [**1**] and 7β-xylosyl-10-deacetyltaxol D [**7**], were isolated from the stem bark of *Taxus baccata* cv. stricta. Their structures were elucidated using spectroscopic methods and their bioactivity was evaluated using an in vitro microtubule assembly assay.

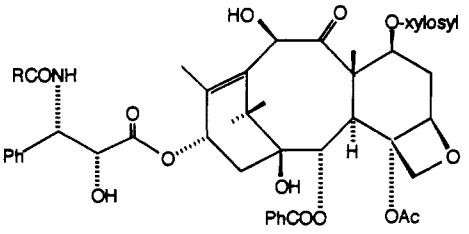
The European yew (*T. baccata* L.) is a very poisonous plant whose toxicity is due to its content of taxine, a mixture of toxic alkaloids. In 1968, Lythgoe reported the isolation of, among other taxoids, taxines A, B [**2**], and C, but their structure elucidation had not been achieved at that time (1). A decade later, Graf *et al.* established the structures of taxine A (2) and taxine B (3). The structure of taxine B was revised recently as **2** (4).

We reported previously the isolation, from the stem bark of *T. baccata* L. cv. stricta, of a new taxoid, 13-deacetyl-10-debenzoyltaxchinin C, together with taxol, cephalomannine, baccatin III, and taxol C (5). Further investigation has led to the isolation of five other taxoids, among which two were new derivatives, a taxine B derivative [**1**] and a xyloside of taxol D [**7**].

A crude MeOH extract of the plant material was separated by cc on Si gel, and the fraction eluted with $CHCl_3$-MeOH (98:2) was further purified by C_{18} cc to yield compound **1** which was crystallized from $MeOH/H_2O$. Its fabms exhibited an ion at *m/z* 696 corresponding to $[M+H]^+$ which was appropriate for the molecular formula $C_{39}H_{53}NO_{10}$. The nmr data are shown in Table 1. The proton-bearing carbons were assigned by a ^1H-^{13}C correlation spectrum (HMQC) and the quaternary carbons were assigned by a ^1H-^{13}C long-range correlation (HMBC) spectrum. The ^1H-nmr spectrum showed the presence of a C-4 exomethylene group and a Winterstein acid moiety, which were identical to those of taxine B and its derivatives (4,6). The lack of a ketone carbonyl at C-13 was confirmed by both ^1H- and ^{13}C-nmr spectra. The ^1H-nmr spectrum of **1** differed



	R_1	R_2	R_3	R_4	R_5
1	OAc	H	H	OAc	Ac
2	=O	OH	OH	H	H
3	OAc	H	OH	H	H



	R
4	C_6H_5
5	$CH_3CH=C-CH_3$
6	n-C_5H_{11}
7	n-C_3H_7

from that of 13-deoxo-13α-acetyloxy-1-deoxytaxine B [**3**] in the number and position of acetyl groups (6). The ^{1}H-^{1}H COSY nmr spectrum showed that the signals of H-5 and H-7, which appeared each as a 1H doublet of doublets at δ 5.39 and 5.40, were both coupled to the H-6α and H-6β ^{1}H-nmr resonances at δ 1.28 and 1.58. The 1H doublet of doublet of doublets at δ 0.97 and the 1H doublet of triplets at δ 2.62 were assigned to H-14α and H-14β, respectively, according to their geminal coupling ($J_{14\alpha,14\beta}$=14.5 Hz), their coupling to the H-13β multiplet at δ 5.87 ($J_{13\beta,14\beta}$=9.8 Hz, $J_{13\beta,14\alpha}$=7.6 Hz), and to the H-1β multiplet at δ 1.82. The C-2 methylene protons as multiplets at δ 1.69 and 1.82 were correlated with the multiplet at δ 2.80 assigned to H-3α and with the H-1β multiplet at δ 1.82. The isolated spin-system comprising doublets at δ 5.86 and 6.28 was attributed to H-9β and H-10α, with the large vicinal coupling ($J_{9\beta,10\alpha}$=11.1 Hz) indicative of a large dihedral angle between the two protons. The low-field signals of H-10α at δ 6.28, H-13β at δ 5.87, H-9β at δ 5.86, and H-7α at δ 5.40, and the HMBC data (Table 1) indicated the presence of acetate groups at C-10, C-13, C-9, and C-7. The HMBC spectrum also indicated the correlation of the H-5 resonance to the Winterstein acid carbonyl. The stereochemistry of **1** was determined by nOe difference spectroscopy. The results are shown in Figure 1. Thus, the structure of **1** was established as 13-deoxo-13α-acetyloxy-7β,9α-diacetyl-1,2-dideoxytaxine B.

An aliquot of the fraction eluted with $CHCl_3$-MeOH (95:5) from cc was first purified by passing it through a C_{18} cartridge, then by hplc. Four compounds [**4–7**] bearing xylosyl substituents were obtained. Three of these [**4–6**] were identified by ^{1}H-nmr spectroscopy as 7β-xylosyl-10-deacetyltaxol, 7β-xylosyl-10-deacetylcephalomannine, and 7β-xylosyl-10-deacetyltaxol C, respectively. These compounds were reported previously by Sénilh *et al.* (7). In addition, the ^{1}H-nmr data of **7** (Table 2) showed that it also exhibited a group of signals due to the xylosyl substituent at C-7 and the deacetylation of the hydroxyl at C-10. The difference between **7** and **4–6** was attributed to the substitution at C-13. Compound **7** had an *N*-butanoylphenylisoserine side-chain as in taxol D (8), which is identical to taxcultine isolated from cell cultures of *T. baccata* (9). The fabms of **7** showed an ion at *m/z* 910 corresponding to $[M+H]^+$ which is appropriate for the molecular formula $C_{47}H_{59}NO_{17}$. The mass fragment at *m/z* 659 corresponded to the loss of the *N*-butanoylphenylisoserine part of the molecule $[M-251]^+$ and the fragment at *m/z* 509 to the loss of the xylosyl group from this last fragment; both confirmed that this compound was a deacetyl derivative of taxol D. Therefore, the structure of **7** was assigned as 7β-xylosyl-10-deacetyltaxol D.

TABLE 1. Nmr Data for Compound **1**.

^{13}C nmr ($CDCl_3$)		^{1}H nmr ($CDCl_3$)			HMBC
δ	Position	δ, *J* in Hz	Position	^{1}H-^{1}H COSY	
40.69	C-1	1.82, 1H, m	H-1	H-14α, β	C-3, C-11, C-14
27.51	C-2	1.69, 1H, m	H-2α	H-3, H-2β	C-1, C-3, C-4, C-8, C-15
		1.82, 1H, m	H-2β	H-3, H-2α	C-1, C-3, C-8, C-14
37.91	C-3	2.80, 1H, m	H-3	H-2α, β, H-20a, b	C-1, C-2, C-4, C-7, C-8, C-20, Me-19
146.85	C-4				
74.24	C-5	5.39, 1H, dd, *J*=3.9, 2.3	H-5	H-6α, β	C-6, C-1′
34.43	C-6	1.28, 1H, ddd, *J*=14.4, 5.2, 2.5	H-6α	H-5, H-6β, H-7	C-4, C-5, C-7, C-8
		1.58, 1H, m	H-6β	H-5, H-6α, H-7	C-7
70.29	C-7	5.40, 1H, dd, *J*=11.7, 5.1	H-7	H-6α, β	C-6, C-8, Me-19, COMe
46.45	C-8				
77.10	C-9	5.86, 1H, d, *J*=11.1	H-9	H-10	C-7, C-8, C-10, C-11, Me-19, COMe
71.99	C-10	6.28, 1H, d, *J*=11.1	H-10	H-9	C-9, C-11, C-12, C-15, COMe
134.83	C-11				
137.54	C-12				
70.80	C-13	5.87, 1H, m	H-13	H-14α, β, Me-18	C-11, C-12, COMe
31.65	C-14	0.97, 1H, ddd, *J*=14.7, 7.6, 0.9	H-14α	H-1, H-13, H-14β	C-1, C-2, C-13, C-15
		2.62, 1H, dt, *J*=14.3, 9.8	H-14β	H-1, H-13, H-14α	C-2, C-12, C-13
39.67	C-15				
27.70	C-16	1.60, 3H, s	Me-16	Me-17	C-1, C-11, C-15, Me-17
31.31	C-17	1.10, 3H, s	Me-17	Me-16	C-1, C-11, C-15, Me-16
15.37	C-18	2.21, 3H, d, *J*=1.5	Me-18	H-13	C-11, C-12, C-13
13.53	C-19	0.77, 3H, s	Me-19	H-3, H-9	C-3, C-7, C-8, C-9
115.98	C-20	5.24, 1H, d, *J*=1.4	H-20a	H-3, H-20b	C-3, C-5
		4.91, 1H, d, *J*=1.7	H-20b	H-3, H-20a	C-3, C-4, C-5
170.90	C-1′				
39.74	C-2′	2.97, 1H, dd, *J*=13.8, 7.5	H-2′α	H-2′β, H-3′	C-1′, C-3′, C-1″
		2.83, 1H, dd, *J*=13.8, 8.3	H-2′β	H-2′α, H-3′	C-1′, C-3′, C-1″
67.25	C-3′	3.89, 1H, t, *J*=7.9	H-3′	H-2′α, β	C-1′, C-2′, C-1″, C-2″, C-6″, $N(Me)_2$

TABLE 1. Continued.

^{13}C nmr ($CDCl_3$)		^{1}H nmr ($CDCl_3$)			HMBC
δ	Position	δ, *J* in Hz	Position	^{1}H-^{1}H COSY	
42.35	N(*Me*)$_2$	2.14, 6H, s	N(Me)$_2$		C-3′, N(Me)$_2$
170.72	*C*OMe				
170.39	*C*OMe				
169.55	*C*OMe				
169.31	*C*OMe				
21.57	CO*Me*	2.05, 3H, s	COMe		*C*OMe
21.01	CO*Me*	2.01, 3H, s	COMe		*C*OMe
21.15	CO*Me*	1.96, 3H, s	COMe		*C*OMe
21.45	CO*Me*	1.95, 3H, s	COMe		*C*OMe
138.15	C-1″				
128.57	C-2″ and C-6″	7.20, 2H, m	H-2″ and H-6″	H-3″, H-5″	C-3′, C-3″, C-4″, C-5″
128.57	C-3″ and C-5″	7.30, 2H, m	H-3″ and H-5″	H-2″, H-4″, H-6″	C-1″, C-2″, C-4″, C-6″
127.73	C-4″	7.20, 1H, m	H-4″	H-3″, H-5″	C-2″, C-3″, C-5″, C-6″

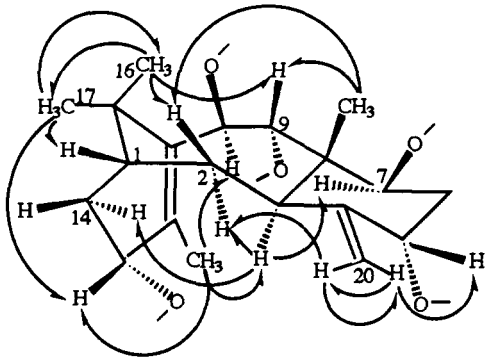


FIGURE 1. Conformation of **1** and observed nOes.

TABLE 2. ^{1}H-Nmr Data of 7β-Xylosyl-10-deacetyltaxol D [7].

Position	δ (CD_3OD) J in Hz
2	5.63, 1H, d, J=7.1
3	3.88, 1H, d, J=7.0
5	4.97, 1H, d, J=7.9
6	1.91, 1H, m
	2.67, 1H, m
7	4.19, 1H, m
10	5.37, 1H, s
13	6.15, 1H, t, J=8.2
14	2.06, 1H, m
	2.23, 1H, m
16	1.18, 3H, s
17	1.13, 3H, s
18	1.91, 3H, s
19	1.75, 3H, s
20	4.19, 2H, m
OAc	2.33, 3H, s
2′	4.58, 1H, d, J=4.6
3′	5.46, 1H, d, J=4.6
5′	2.26, 2H, t, J=6.5
6′	1.62, 2H, m
7′	0.92, 3H, t, J=7.3
1″	4.09, 1H, d, J=7.4
2″	3.04, 1H, m
3″	3.25, 1H, t, J=9.1
4″	3.42, 1H, m
5″eq	3.80, 1H, m
5″ax	3.17, 1H, m
2-COPh	8.09, 2H, dd, J=7.0, 1.5
	7.66, 1H, t, J=7.3
	7.55, 2H, t, J=7.0
3′-Ph	7.40, 4H, m
	7.27, 1H, m

The two new taxoids [**1**, **7**] were submitted to an in vitro microtubule assembly assay. Microtubule assembly was monitored by the increase of turbidity. Taxol promotes in vitro microtubule assembly and the microtubules polymerized in the presence of taxol are further resistant to $CaCl_2$-induced depolymerization (10). As shown in Figure 2, the kinetics of microtubule assembly were altered by the addition of taxol (0.1 or 10 μM). Under the same experimental conditions, **7** showed an activity closely related to that of taxol. Compound **1** exhibited a very low activity even at 10 μM. In contrast, taxol D was shown to be half as active as taxol in the tubulin assembly assay (9). The major structural differences between **7** and taxol D are the presence of a xylosyl moiety at the C-7 position and the absence of an acetate function at the C-10 position. The presence of the xylosyl moiety in **7** might improve its hydrophilicity and thus its interaction with microtubule protein.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The mps were determined in a Gallenkamp melting-point apparatus. The optical rotations were determined on a Perkin-Elmer 141 polarimeter. The uv spectra were taken on a Shimadzu UV-265 FS spectrophotometer, and the ir spectra recorded as KBr pellets on a Shimadzu IR-470 spectrophotometer. ^{1}H- and ^{13}C-nmr spectra were recorded on a Varian-Unit 600 instrument. Chemical shift data are in ppm downfield from TMS as internal reference. The DEPT sequence was used to distinguish the methylene carbon signals from those due to methine, Me, and quaternary carbons; one-bond ^{1}H-^{13}C and ^{1}H-^{1}H connectivities were determined via 2D proton-detected HMQC and COSY experiments, respectively; two- and three-bond ^{1}H-^{13}C correlations were determined using 2D proton-detected HMBC experiments optimized for J_{CH}=5

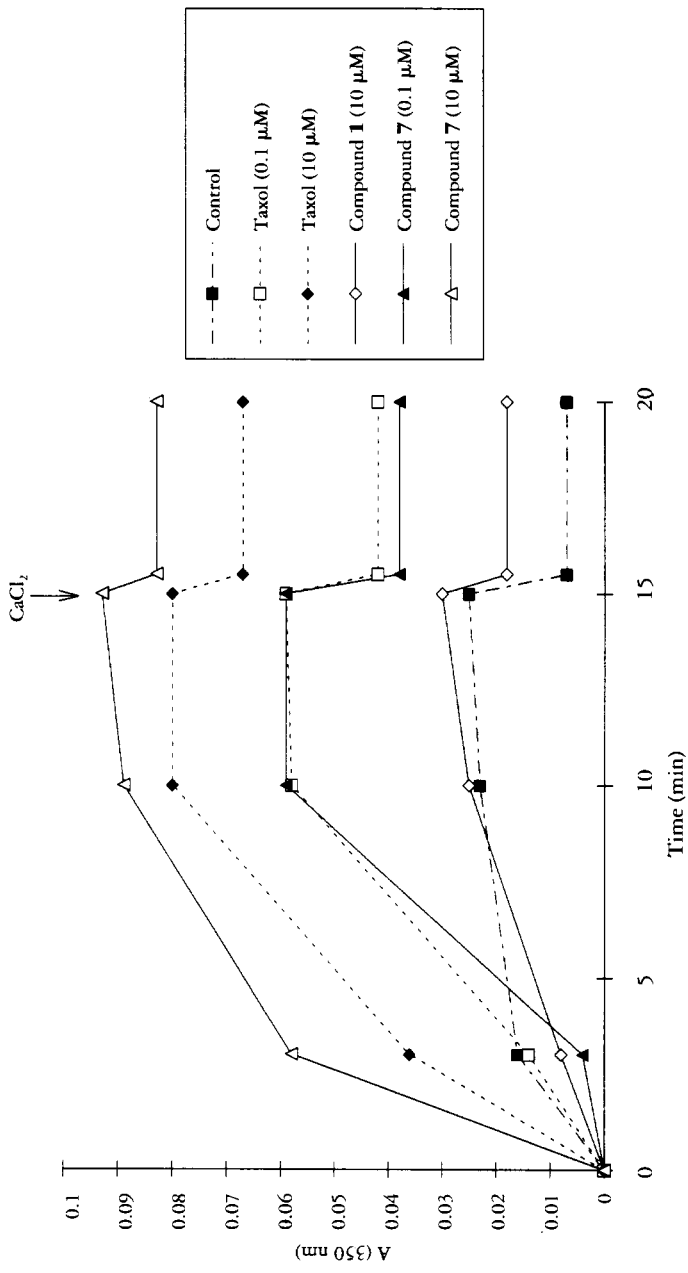


FIGURE 2. Kinetics of in vitro microtubule assembly and disassembly monitored by turbidity at 350 nm (10).

Hz and 10 Hz. The fabms were obtained on a Kratos MS9 spectrometer using glycerol as matrix. Si gel 60 and Lichroprep RP_{18} (Merck, mean particle size, 0.040–0.063 mm) were used for cc. Hplc was performed using a Hibar Lichrosorb® RP-18 pre-packed column (mean particle size 7 mm, 250 mm×4 mm i.d.), with a guard column (LiChrospher® 100, RP-18, 5 μm), and using MeCN-H_2O-MeOH (35:45:20) as mobile phase at a flow rate of 1 ml/min. The hplc system was equipped with a photodiode-array detector suitable for monitoring the collected fractions.

PLANT MATERIAL.—Stem bark samples of *T. baccata* L. cv. stricta (Taxaceae) were collected from trees growing in the Experimental Garden of Jean Massart of the Université Libre de Bruxelles, Belgium.

EXTRACTION AND ISOLATION.—Dried stem bark (880 g) was macerated for 48 h at room temperature in MeOH. The syrup remaining after evaporation (ca. 140 g) was extracted first with petroleum ether (4×200 ml) then with CH_2Cl_2 (4×200 ml). The CH_2Cl_2 residue (ca. 10 g) was absorbed on cellulose 2100 FF (Macherey-Nagel, Düren, Germany) (10 g) and submitted to Si gel (100 g) cc and eluted using stepwise gradients of $CHCl_3$ in petroleum ether (in increments of 10%) until pure $CHCl_3$, then with increased concentrations of MeOH in $CHCl_3$ until pure MeOH. The fractions eluted with $CHCl_3$-MeOH (98:2) were first purified by C_{18} cc using increased concentrations of MeOH in H_2O until pure MeOH, and **1** was then crystallized from fraction 4 with MeOH-H_2O (yield about 0.04%). An aliquot of fraction eluted from the Si gel cc with $CHCl_3$-MeOH (95:5) was purified by passing through a C_{18} cartridge, then by hplc. Four taxoids with the xylosyl substituent were isolated: **4–6**, and the new taxoid, 7β-xylosyl-10-deacetyltaxol D [7].

13-Deoxo-13α-acetyloxy-7β,9α-diacetyl-1,2-dideoxytaxine B [**1**].—White needle crystals (MeOH/H_2O), mp 162–164°; $[\alpha]^{20}D$ +76.9°, (c=1.01, MeOH); uv (MeOH) λ max (log ε) 221.9 (3.39), 277.6 (2.76) nm; ir (KBr) ν max 3430, 2940, 1741, 1628, 1429, 1362, 1235, 1150, 1015, 911, 799, 701 cm^{-1}; fabms m/z $[M+H]^+$ 696, [M−OCOMe] 636, [M−2OCOMe] 577; ^1H- and ^{13}C-nmr data, see Table 1.

7β-Xylosyl-10-deacetyltaxol D [**7**].—White amorphous powder, $[\alpha]^{20}D$ −14.3° (c=0.27, MeOH); uv (MeOH) λ max (log ε) 227.2 (3.79), 263.6 (2.97), 267.4 (2.97), 273.5 (2.98), 280.7 (2.91) nm; fabms m/z $[M+H]^+$ 910, [M−N−butanoylphenylisoserine]$^+$ 659, [M−251−xylosyl]$^+$ 509; ^1H-nmr data, see Table 2.

BIOLOGICAL TESTING.—Tubulin was extracted from pork brain following the procedure previously described by Shelanski *et al.* (11) and the microtubule assembly assay was performed according to the procedure of Schiff *et al.* (10).

ACKNOWLEDGMENTS

Y.-W. Guo thanks the "Fondation Universitaire David et Alice van Buuren" for financial support.

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Received 29 March 1995