

BREVITAXIN, A NEW DITERPENOLIGNAN FROM THE BARK OF *TAXUS BREVIFOLIA*

ROBERT L. ARSLANIAN,* DAVID T. BAILEY, MICHAEL C. KENT, STEVEN L. RICHHEIMER,
KELLY R. THORNBURG, DANIEL W. TIMMONS, and QUN Y. ZHENG

Hauser Chemical Research, Inc., 5555 Airport Boulevard, Boulder, Colorado 80301

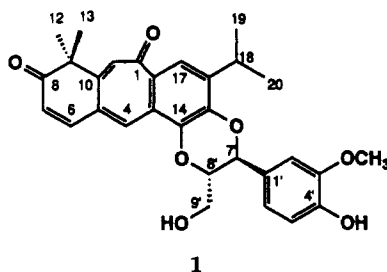
ABSTRACT.—The first terpenolignan, brevitaxin [1], has been isolated from the bark of *Taxus brevifolia*. Identification was carried out using spectral methods, and the regiochemistry and cytotoxicity of 1 are discussed.

The bark of *Taxus brevifolia* Nutt. (Taxaceae) has yielded a variety of diterpenes (1–6) and is the starting material for a commercial paclitaxel¹ production process. In the present work, a new diterpenolignan has been isolated from a crystallization sidestream. Although coumarinolignans (6), flavanolignans (7), and xanthanolignans (8) have been isolated previously, to our knowledge, this is the first report of a terpenolignan.

A yellow solid was isolated from a crystallization sidestream that was found to contain 1. Alumina chromatography, crystallization, and precipitation from CHCl₃/hexane gave 1 as a bright yellow powder.

Compound 1 has the molecular formula C₃₀H₃₀O₇, which was established by hrms (*m/z* 503.2048 for [M+H]⁺) and supported by both ¹H- and ¹³C-nmr data. Treatment with Ac₂O/pyridine gave the bis-acetylated derivative of 1, indicating the presence of two OH groups. DEPT and ¹³C-nmr spectra indicated the presence of 5 methyls, 1 methylene, 11 methines, and 13 quaternary carbons. COSY and ¹H-nmr data included a spin-system containing five protons. Signals at 5.06 and 4.30 ppm were assigned to H-7' and H-8' and were consistent with a substituted 1,4-dioxane ring system. Starting at H-7', correlations were observed that showed connectivities from H-7' to H-8' onto the H-9' methylene protons terminating at the H-9' OH.

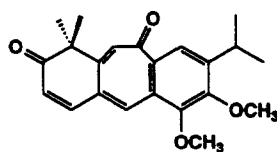
¹Also known in the scientific literature as taxol.



HMBC correlations from H-7' to C-2' and C-6' and ¹³C-nmr data confirmed the presence of the dioxyconiferyl alcohol moiety. ¹H-Nmr values for the dioxyconiferyl alcohol moiety were in good agreement with those for known xanthanolignoids (8).

The remainder of the molecule corresponded to a C₂₀H₁₈O₂ fragment. HMBC, HETCOR, and COSY nmr data showed the presence of one isopropyl, one *gem*-dimethyl, and two carbonyl groups. From the molecular formula of this fragment, we determined that these functional groups were imbedded within a tricyclic tropone ring system, very much reminiscent of taxamarin B [2] (9). Indeed, ¹H- and ¹³C-nmr data for 1 were in good agreement with values for the known diterpene 2. HMBC correlations on brevitaxin confirmed its structure as 1.

Regiochemistry was established us-



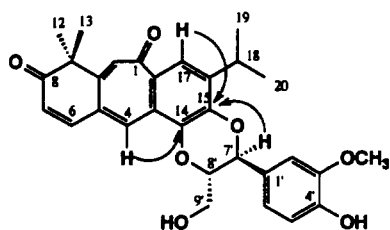


FIGURE 1. SINEPT nmr irradiation of H-7', H-17, and H-4 in **1**.

ing *J* selective INEPT (SINEPT) (10) nmr data that showed three-bond correlations from H-7' and H-17 to C-15 (Figure 1). Here, nmr data were measured using CDCl₃ as solvent because the C-14 and C-15 carbon signals overlapped in DMSO-*d*₆. Optical rotation measurements for **1** indicated a racemic mixture. The presence of **1** in a MeOH extract of bark was confirmed by lc/ms. Compound **1** showed cytotoxicity in the NCI 60-cell line assay. Selectivity within the prostate cancer panel was observed with the DU 145 cell line, with an ED₅₀ value of 6.8 μM.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured in CHCl₃ using a Rudolph-Re-

search Autopol® III polarimeter. The ¹³C-nmr spectrum was obtained using a Bruker-IBM WP-270 instrument at 63 MHz. The ¹H-nmr and COSY nmr experiments were carried out using a Bruker AM-500 instrument at 500 MHz. The ¹³C-nmr, DEPT, and HETCOR nmr data were collected using a Bruker ACE-300 instrument at 75 MHz. SINEPT nmr experiments were carried out on a JEOL Eclipse 400 instrument. Samples were prepared using DMSO-*d*₆ or CDCl₃. Lc/ms experiments were carried out on an HP-1050 lc coupled to a Fisons VG Platform system using a quadrupole mass spectrometer. Hrms data were obtained from the Midwest Center for Mass Spectrometry.

PLANT MATERIAL.—Commercially available bark from *Taxus brevifolia* was used. A voucher specimen is kept at Hauser Chemical Research, Inc., Boulder, Colorado.

EXTRACTION AND ISOLATION.—*Taxus brevifolia* bark was extracted with MeOH and the extract subjected to a series of purification steps terminating in the crystallization of paclitaxel. Second crop paclitaxel crystals (1.5 g) that had a noticeable yellow color were dissolved in 100 ml of MeOH and passed through a 2.5 × 15-cm column packed with decolorizing alumina (AL 5005, Scientific Adsorbents, Inc., Atlanta, GA). The column was first washed with 1100 ml of Me₂CO followed by 150 ml of a 1:1 mixture of MeOH-H₂O. Most of the MeOH from the MeOH/H₂O fraction was evaporated *in vacuo*, leaving a yellow solid that was collected by vacuum filtration and crystallized from hot MeOH. Approximately 50 mg of solids were obtained. The solids were dissolved in minimal hot CHCl₃ (20 ml), which was added to 200 ml of hexane while stirring. Upon

TABLE 1. ¹H-Nmr Data for **1** (DMSO-*d*₆).

Position	δ	Mult.	<i>J</i> _{H-H}	COSY	HMBC
4	8.15	s			150.1, 148.1, 141.0, 132.7
6	7.61	d	9.9	6.11	200.0, 150.1, 130.1
7	6.11	br d	9.5	7.61	49.7
11	6.81	s			130.1, 49.7
12	1.37	s			200.0, 150.1, 49.7, 27.3
13	1.33	s			200.0, 150.1, 49.7, 25.3
17	7.59	s			144.1, 121.9
18	3.25	qq	6.9, 6.9	1.23, 1.20	141.0, 118.1, 22.0
19	1.23	d	6.9	3.25	141.0, 27.1, 21.7
20	1.20	d	6.9	3.25	141.0, 27.1, 22.0
2'	7.06	d	1.9		147.2, 120.3, 75.9
5'	6.83	d	8.0	6.90	147.2
6'	6.90	dd	8.2, 1.9	6.83	147.2, 111.6, 75.9
7'	5.06	d	8.0	4.30, 3.51	126.9, 120.3, 111.6, 78.6
8'	4.30	m		5.06, 3.63, 3.51, 4.30	
9'a	3.63	ddd	12.6, 6.2, 2.4	5.14, 3.51	
9'b	3.51	ddd	12.6, 5.6, 5.6	5.14, 3.63, 4.30	
OMe-3'	3.77	s			147.7
OH-4'	9.18	s			
OH-9'	5.14	t	5.9	3.63, 3.51	

TABLE 2. ^{13}C -Nmr Data for **1** (DMSO- d_6).

Position	δ	Mult.	HETCOR	Position	δ	Mult.	HETCOR
1	186.6	s		17	118.1	d	7.59
2	132.7	s		18	27.1	d	3.25
3	144.1	s		19	21.7	q	1.20
4	133.3	d	8.15	20	22.0	q	1.23
5	130.1	s		1'	126.9	s	
6	148.1	d	7.61	2'	111.6	d	7.06
7	123.2	d	6.11	3'	147.7	s	
8	200.0	s		4'	147.2	s	
9	49.7	s		5'	115.4	d	6.83
10	150.1	s		6'	120.3	d	6.90
11	131.0	d	6.81	7'	75.9	d	5.06
12	27.3	q	1.33	8'	78.6	d	4.30
13	25.3	q	1.37	9'	60.0	t	3.63, 3.51
14,15	141.0 (2C)	s		OMe-3'	55.7	q	3.77
16	121.9	s					

drying the precipitate at 60° under vacuum, 40 mg of a bright yellow solid were obtained.

Brevitaxin [**1**].—Yellow powder, mp (dec) 280°; $[\alpha]_D^{20}$ ($c=1.0$, CHCl_3); uv λ max (MeOH) 315 (ϵ 24,000), 390 sh nm; hrfabms $[\text{M}+\text{H}]^+ m/z$ 503.2048, calcd 503.2070 for $\text{C}_{30}\text{H}_{31}\text{O}_7$; ^1H - and ^{13}C -nmr data, see Tables 1 and 2.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Ms. Carol Elkins for editing and technical writing support, the Midwest Center for Mass Spectrometry, University of Nebraska-Lincoln, partially supported by the National Science Foundation, Biology Division (Grant No. DIR9017262), and the National Cancer Institute for the 60-cell line assay screen. We also thank Dr. Christopher Rithner of Colorado State University, Department of Chemistry, for the 2D nmr data reported herein.

LITERATURE CITED

- M.C. Wani, H.L. Taylor, M.E. Wall, P. Coggon, and A.T. McPhail, *J. Am. Chem. Soc.*, **93**, 2325 (1971).
- D.G.I. Kingston, D.R. Hawkins, and L. Ovington, *J. Nat. Prod.*, **45**, 466 (1982).
- C.H.O. Huang, D.G.I. Kingston, N.F. Magri, G. Samaranyake, and F.E. Boettner, *J. Nat. Prod.*, **49**, 665 (1986).
- J.A. Beutler, G.M. Chmurny, S.A. Look, and K.M. Witherup, *J. Nat. Prod.*, **54**, 893 (1991).
- A. Chu, J. Zajicek, L.B. Davin, N.G. Lewis, and R.B. Croteau, *Phytochemistry*, **31**, 4249 (1992).
- A.B. Ray, S.K. Chattopadhyay, S. Kumar, C. Konno, Y. Kiso, and H. Hikino, *Tetrahedron*, **41**, 209 (1985).
- A. Pelter and R. Hänsel, *Tetrahedron Lett.*, 2911 (1968).
- M. Abou-Shoer, A.-A. Habib, C.-J. Chang, and J.M. Cassady, *Phytochemistry*, **28**, 2483 (1989).
- J.-Y. Liang, Z.-D. Min, M. Iinuma, T. Tanaka, and M. Mizuno, *Chem. Pharm. Bull.*, **35**, 2614 (1987).
- L.-J. Lin and G.A. Cordell, *J. Chem. Soc., Chem. Commun.*, 379 (1986).

Received 26 August 1994