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J. Nat. Prod., **1991**, 54 (3), 893-897 • DOI:
10.1021/np50075a028 • Publication Date (Web): 01 July 2004

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DC 20036

TAXININE M, A NEW TETRACYCLIC TAXANE FROM *TAXUS BREVIFOLIA*

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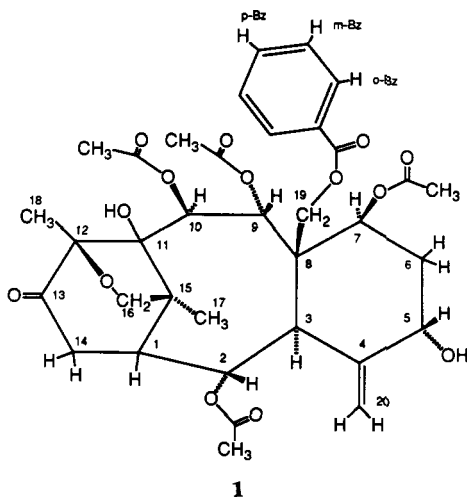
ABSTRACT.—The isolation of a novel tetracyclic taxane, taxinine M [**1**], from *Taxus brevifolia* bark and its structure elucidation by spectroscopic methods are reported.

The large-scale isolation of taxol (1–3) from *Taxus brevifolia* Nutt. (Taxaceae) bark has provided an opportunity to examine the many related taxanes present in abundance in this plant. We report here the structure elucidation of a novel taxane which elutes near taxol in the large-scale isolation process.

The fab mass spectrum of the title compound showed a sodium adduct ion at m/z 709, which on high resolution measurement corresponded to a formula of $C_{35}H_{42}O_{14}Na$. A second ion corresponding to loss of H_2O from the protonated molecular ion was measured as $C_{35}H_{41}O_{13}$. Thus the mol wt of the compound was determined as 686, and the molecular formula as $C_{35}H_{42}O_{14}$. The unsaturation number of 15 could be accounted for by a tetracyclic structure with one isolated double bond, a single unsubstituted benzene, and six carbonyls.

The ^{13}C -nmr spectrum showed one of these carbonyls to be a ketone and the rest to be esters. Close analysis led to the conclusion that there were four acetates present and one benzoate. A puzzling

point in the 1H -nmr spectrum was the paucity of methyl singlets compared to other known taxanes. From the COSY spectrum it was possible to discern structure fragments which accounted for several parts of the molecule. An inverse detection one-bond heteronuclear correlation experiment (HMQC) (4) connected most of these protons to specific proton-bearing carbons. The single benzoate accounted for all but two of the carbon lines in the range 110 to 150 ppm. Four singlets (91, 80, 49.7, 49.6 ppm) were also unaccounted for in the sp^3 hybridization range. The long-range heteronuclear experiment HMBC (4) was used to locate the positions and connectivity of these singlets, as well as the points of ester attachment. The most unusual features of the structure are the Me-19 benzoate and the cyclized Me-16 unit. Detailed results of the correlation experiments are presented in Tables 1 and 2 and Figure 1.



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TABLE 1. ^1H -nmr Data for Taxinine M [1] (CDCl_3 , 500 MHz).

Proton	ppm	J (Hz)	Integ.	COSY	nOe
<i>o</i> -Bz	8.16 ^a		2H	a	—
<i>p</i> -Bz	7.61 ^a		1H		—
<i>m</i> -Bz	7.51 ^a		2H	a	—
H-2	6.14 dd	10.4, 2.4	1H	b	8.16
H-7	5.51 dd	10.7, 6.2	1H	c	—
H-20-Z	5.41 s	—	1H	—	4.68
H-9	5.36 d	3.0	1H	d	1.29
H-10	5.31 d	3.0	1H	d	3.71
H-19a	5.14 d	12.2	1H	e	4.4
H-20-E	4.68 s	—	1H	—	5.41
H-5	4.45 brt	2-3	—	—	5.41
H-19b	4.42 d	12.2	1H	e	5.14
11-OH	4.10 brs	—	—	—	3.63, 1.29
H-16a	4.08 d	8.1	1H	f	—
H-3	3.71 d	10.4	1H	b	5.31, 6.14
H-16b	3.63 d	8.1	1H	f	4.10
H-14a	3.00 dd	11.6, 19.2	1H	g	2.48, 2.75
H-14b	2.75 d	19.2	1H	g	3.00
H-1	2.48 ddd	0.7, 2.4, 11.6	1H	g	3.00, 3.63, 6.14
H-6a	2.23 ddd	2.1, 6.1, 14.2	1H	c	1.70, 5.51
9-OAc	2.15 s		3H	—	—
10-OAc	2.11 s		3H	—	—
2,7-OAc	2.03 s		6H	—	—
H-6b	1.70 ddd	3.6, 10.7, 14.2	1H	c	2.23, 5.51
H-17	1.29 s		3H	—	4.08, 5.36, 6.14, 8.16
H-18	1.17 s		3H	—	—

^aThe aromatic protons form a magnetically nonequivalent AA'BB'C spin system with average $J_{\text{ortho}} = 7.9$ Hz, $J_{\text{meta}} = 1.2$ Hz, and $J_{\text{para}} = 0.9$ Hz.

To confirm the position and chemical shift of the tertiary alcohol carbon, we performed a ^{13}C -nmr exchange experiment using equal proportions of H_2O and D_2O . If conditions of exchange are

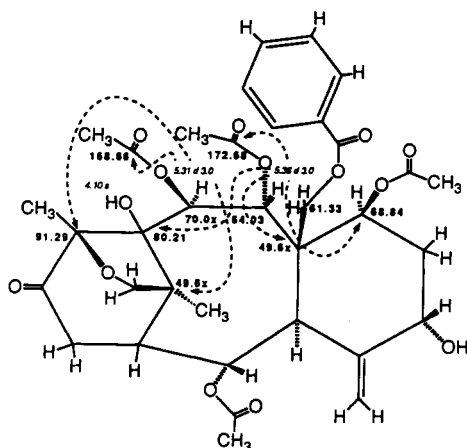


FIGURE 1. Long-range heteronuclear correlations of H-9 and H-10.

sufficiently slow, the hydroxyl-bearing carbon resonance will be doubled because both COH and COD species are present. Indeed, we found that the carbon resonance at 80.21 showed a doubling effect, with an isotope-induced shift of 12 Hz, compared to an α shift of 2.7 Hz for the carbon resonance at 91.29 ppm. Another α shift was seen for the line at 64.03 ppm, but the overlap of the two 49.6 ppm carbon signals made it impossible to observe an isotope shift for the third α carbon. No isotope shift was seen for the secondary hydroxyl carbon at 72.60 ppm, presumably because of faster exchange.

The above data led to structure **1**. It is notable that carbons 15, 11, 12, and 13 constitute a string of four carbons without attached protons, making interpretation of HMBC data difficult, since correlations can be observed for three-

TABLE 2. ^{13}C -nmr Data for Taxinine M [1] (CDCl_3 , 125 MHz).

Carbon	ppm	APT	$^1J_{\text{CH}}$ (Hz)	Hetcorr	HMBC (3 Hz)
Carbonyls					
C-13	204.45	s			2.75, 3.00, 2.48
9-OAc	172.68	s	6.9, 3.9		2.15, 5.36
2-OAc	169.98	s	6.7, 4.1		2.03, 6.14
10-OAc	168.66	s	ca. 6.5		2.11, 5.31
7-OAc	168.37	s	6.8, 3.7		2.03, 5.51
19-OBz	166.82	s			5.14, 8.16, 4.42 ^a
Unsaturated					
C-4	144.99	s	ca. 6.2		4.68, 3.71, 2.23, 5.41
<i>p</i> -Bz	133.59	d	161.4, 7.6	7.61	8.16
<i>o</i> -Bz	130.05	d × 2	167.5, 6.7	8.16	8.16, 7.61
Bz	129.18	s	8.4		7.51
<i>m</i> -Bz	128.63	d × 2	162.5, 7.7	7.51	7.51
C-20	113.28	t	157.8, 4.6	5.41, 4.68	6.14, 3.71
Alkyl					
C-12	91.29	s			3.63, 1.17, 4.10, 5.31
C-16	82.16	t	148.9, 6.4, 4.2	4.08, 3.63	1.29
C-11	80.21	s			1.29, 1.17, 3.63, 5.36, 2.48, 4.10
C-5	72.60	d	150.9, 5.1, 8.2	4.45	5.41, 4.68, 2.23
C-2, C-9	{ 70.09 70.04	{ d d	ca. 147	6.14, 5.36	3.00, 4.45, 2.75, 2.48, 4.42, 4.08, 3.71, 5.51, 5.14
C-7	68.84	d	150.6	5.51	5.36
C-10	64.03	d	137.5	5.31	—
C-19	61.33	t	150.5, ca. 5.2	5.14, 4.42	5.51, 3.71
C-8, C-15	{ 49.67 49.59	{ s s			5.36, 5.31, 2.75, 1.29, 3.71, 4.42, 5.14, 5.41, 3.63
C-1	48.36	d	131.0	2.48	1.29, 4.08, 3.63, 3.00, 2.75, 3.71
C-6	39.05	t	130.7	2.23, 1.70	—
C-3	38.71	d	124.3	3.71	5.41, 5.14, 4.68, 4.42, 5.51
C-14	33.72	t	126.9, 132.2, 5.3	2.75, 3.00	6.14, 2.48
10,11-OAc	{ 21.25 21.22	{ q q	129.8 129.5	2.03 2.03	
7,9-OAc	{ 20.69 20.66	{ q q	130.3 130.3	2.11 2.15	
C-17	15.52	q	127.5, 3.5	1.29	4.08
C-18	11.99	q	128.7	1.17	

^aValues in italics are correlations observed at 3 Hz.

two-, and occasionally four-bond couplings. The three- and four-bond couplings show a dihedral angle dependence; thus one cannot count on observing any particular long-range correlation. To account for unfavorable dihedral angles in three-bond couplings, we ran the HMBC experiment with settings optimized for both 8 Hz and 3 Hz.

The correlations observed at 3 Hz are shown in Table 2 in italics.

The stereochemistry was deduced by NOESY and difference nOe experiments (Table 1). The transannular interactions between the pairs H-3 and H-10, H-9 and H-17, and H-2 and H-17 were particularly helpful in determining the torsion of the 8-membered B ring. It was

not possible to define the relative stereochemistry of positions 5 and 7 with certainty, but those shown appear to fit best.

We have named the new compound taxinine M, because it bears a resemblance to the series of taxinines isolated by Nakanishi's group from *Taxus cuspidata* (5). It shares the carbon skeleton of taxagifine and two congeners isolated from *Taxus baccata* (6) and *Taxus chinensis* (7).

Because taxinine M lacks the taxol side chain, we expected it to show poor bioactivity compared to taxol. In a simple brine shrimp lethality model it was only weakly active, with an estimated LC_{50} of 620 $\mu\text{g/ml}$, compared to values of 0.28 $\mu\text{g/ml}$ for taxol and 0.81 $\mu\text{g/ml}$ for cephalomannine.

The crystal structure of taxagifine (6) was used as a basis for molecular modeling studies to explore the conformation of taxinine M. An energy-minimized conformation was generated which gave good correlations between predicted and observed scalar couplings and nOe's (Table 3, Figure 2). The model also predicted a hydrogen bond between the 11-OH proton and the 12, 16 ether oxygen, a situation which would be expected to lead to slower exchange in the deuterium shift experiment reported above.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—

Nmr spectra were acquired on Nicolet NT-300 and Varian VXR-500S spectrometers in CDCl_3 . Eims data were obtained on a VG ZAB-2F or 70-

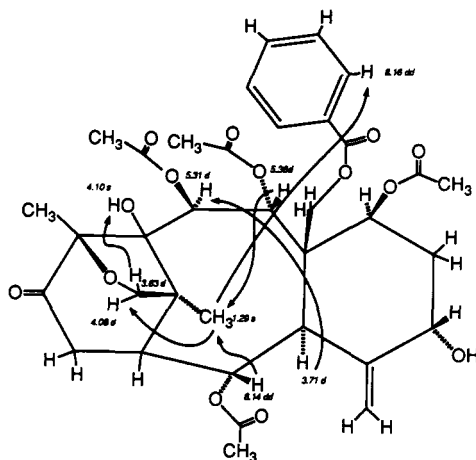


FIGURE 2. Key nOe relationships.

250. All solvents were of hplc grade, and CHCl_3 was hydrocarbon-stabilized. Energy minimizations were carried out using the program MacroModel v3.0 (8). Plant material was as previously described (1-3).

ISOLATION.—A crude MeOH extract of *T. brevifolia* was partitioned between equal volumes of CH_2Cl_2 and H_2O . The organic soluble material (40 g) was triturated in 100 ml each of hexane and Me_2CO with 2 g of celite, yielding 25.85 g of soluble material. This material was coated on 75 g of Florisil and chromatographed over 592 g of Florisil using a step gradient of Me_2CO in hexane (5% steps of 1 liter to 50%, then 25% steps to 100% Me_2CO). Taxol and the title compound eluted in the 45, 50, and 75% Me_2CO fractions, which were combined to give 7.01 g of a mixture of taxanes. This mixture (2.7 g) was then chromatographed on a 2.5 cm \times 27.5 cm silica column with increasing amounts of iPrOH in CH_2Cl_2 . Four fractions were collected at 3% iPrOH which contained taxol and several other similar compounds (total mass 0.9 g). These fractions were purified by preparative hplc on a Rainin Dynamax 8 μ CN bonded phase column (10 mm \times 250 mm) using a gradient solvent sys-

TABLE 3. Comparison of Selected Scalar Couplings and nOe's Between Taxinine M and Energy-minimized Model.

Scalar Coupling	Predicted	Observed	nOe Effect	Distance Predicted
$J_{1,14}$	9.6 Hz	11.3 Hz	H-2 to Me-17	2.18 Å
$J_{1,2}$	2.3 Hz	2.7 Hz	H-3 to H-10	2.11 Å
$J_{2,3}$	10.5 Hz	10.4 Hz	H-9 to Me-17	1.94 Å
$J_{5,6a}$	3.1 Hz	2.1 Hz	11-OH to H _B -16	2.24 Å
$J_{5,6b}$	3.4 Hz	3.6 Hz	H _A -16 to Me-17	2.79 Å
$J_{6a,7}$	5.4 Hz	6.2 Hz	<i>o</i> -Bz to Me-17	2.31 Å
$J_{6b,7}$	10.7 Hz	10.7 Hz		
$J_{9,10}$	5.1 Hz	3.0 Hz		

tem of MeCN 20% to 80% over 30 min, 20% MeOH, and H₂O.

TAXININE M.—Glassy white amorphous solid: ir (cm⁻¹, film from CHCl₃) 3431 (br), 3017, 2935, 1723, 1373, 1251, 1092, 1064, 1026, 756, 714; uv (MeOH) 227 nm (log ϵ = 4.73), 275 (4.04); [α]_D -24° (MeOH); fabms (positive ion mode) 819 (5%), 725 (5%), 709 (16%), 669 (27%), 609 (26%), 549 (8%), 445 (22%), 325 (9%), 104 (100%); ¹H nmr see Table 1; ¹³C nmr see Table 2.

BRINE SHRIMP BIOASSAY.—The brine shrimp lethality assay was as described by Meyer *et al.* (9). Counts of viability were made at 24 h. LC₅₀ values were determined as 0.28 ± 0.04 µg/ml for taxol and 0.81 ± 0.13 µg/ml for cephalomannine. Taxinine M had the following activity: 620 ppm, 5/10 alive; 125 ppm, 15/20 alive; 50 ppm, 16/20 alive; 15 ppm, 15/20 alive.

ACKNOWLEDGMENTS

We wish to thank Climaco Metral and John Roman for mass spectral analyses and Thomas McCloud for brine shrimp lethality experiments. Research supported by the National Cancer Institute, DHHS, under contract NO1-CO-74102 with Program Resources, Incorporated. The contents of this publication do not necessarily reflect the views or policies of the DHHS, nor does mention of trade names, commercial products, or or-

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Received 1 October 1990