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journal or publication title	Tohoku journal of agricultural research
volume	50
number	1/2
page range	33-46
year	1999-09-30
URL	http://hdl.handle.net/10097/30015

Four Novel Pseudoalkaloid Taxanes from the Seeds of the Chinese Yew, *Taxus chinensis* var. *mairei*

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(Received, June 29, 1999)

Summary

Four novel pseudoalkaloid taxane diterpenoids were isolated from the seeds of the Chinese yew tree *Taxus chinensis* var. *mairei*. The structures were established as $2\alpha, 9\alpha, 10\beta, 13\alpha$ -tetraacetoxy- 5α -(3'-methylamino-3'-phenyl)-propionyloxy-taxa-4(20), 11-diene (1) and $7\beta, 9\alpha, 10\beta, 13\alpha$ -tetraacetoxy- 5α -(3'-methylamino-3'-phenyl)-propionyloxy-taxa-4(20), 11-diene (2), $9\alpha, 10\beta, 13\alpha$ -triacetoxy- 5α -(3'-methylamino-3'-phenyl)-propionyloxy-taxa-4(20), 11-diene (3), and $2\alpha, 9\alpha, 10\beta, 13\alpha$ -tetraacetoxy- 5α -(3'-methylamino-3'-phenyl)-propionyloxy-taxa-4(20), 11-diene (4) with the aid of NMR spectral data analysis.

Introduction

Paclitaxel (Taxol®), isolated from various species of the genus *Taxus* and docetaxel (Taxotere®), a semisynthetic analog, are a new class of anticancer agents especially effective for the suffers with advanced ovarian and breast cancers. Paclitaxel is considered as a leading compound in the cancer chemotherapy, and is currently intensively investigated from a chemical, biological, pharmacological and clinical point of view. On the other hand, very few biosynthetic studies have been described and the mode of regulation of taxol metabolism remain unknown (1, 2). In the course of our studies on the yew tree, we have investigated the constitutions of the leave and bark of Chinese yew, *Taxus chinensis* var. *mairei* (3-

7). In view of no report on the constitutions of seeds of this plant, recently we investigated the component of the seed and resulted in the isolation of four novel pseudoalkaloid taxanes. In this communication we describe the isolation and characterization of these four novel taxane diterpenoids.

Materials and Methods

General experimental

Silica gel 60 (Merck 100–200 mesh) was used for conventional CC. Thin layer chromatography (TLC) were conducted on precoated silica gel Kieselgel 60 F₂₅₄ plates (0.2 mm thick), preparative TLC were performed using the same type of plates as used for TLC but with 0.85 mm thickness, developed in an unsaturated tank with n-hexane-acetone, chloroform-methanol, and n-hexane-EtOAc, the spots were detected by ultraviolet (UV) illumination and by spraying 10% H₂SO₄, and then heating on a hot plate. Optical rotations were recorded on a SEPA-300 polarimeter. The UV spectrum was recorded on a Shimadzu UV-1600 spectrophotometer. IR spectrum was recorded on a Jasco IR-810 spectrophotometer for chloroform solutions. Optical rotations were measured on a Horiba SEPA-300 polarimeter. MS were obtained on a JMS-Dx 305 HF mass spectrometer, using the FAB method and glycerol as a matrix. NMR spectra were taken on Varian GEMINI 2000/300 (300 MHz) and Varian Unity Inova 500 (500 MHz) spectrometers operating at 500 and 300 MHz for ¹H, and 125 MHz for ¹³C respectively in CDCl₃ at ambient temperature, coupling constants (*J* values) are listed in hertz (Hz). Splitting patterns have been designated as following s (singlet), d (doublet), t (triplet), and m (multiplet), dd (double doublet), ddd (double double doublet), and ¹H chemical shifts data were expressed as parts per million downfield from tetramethylsilane (TMS) as internal reference. ¹H-¹H connectivities were determined *via* COSY experiments. ¹³C shifts were based on the CDCl₃ signal at 77.0 ppm.

Plant material

The seeds of *T. mairei* were collected in Jinggangshan, in the south-east of China, in October of 1995. The botanical identification was made by Prof. R.L. Liu, Zhangzhou Forestry School, China. A voucher specimen has been deposited in our laboratory of Graduate School of Agricultural Science, Tohoku University.

Extraction and isolation

Air dried and crushed seeds (1.1 kg) were extracted with hexane three times at room temperature to remove major part of nondesired neutral component. The residue was extracted twice with methanol (MeOH), the MeOH extracts were condensed to residue under reduced pressure. Subsequently this residue was

diluted with water and was extracted five times with EtOAc. The combined EtOAc layer was further extracted with 5% HCl. After neutralization, the aqueous layer was extracted three times with EtOAc. The combined EtOAc extract, upon evaporation, yielded 3.0 g of yellowish syrup, which was subjected to preparative TLC repeatedly with the different developing solvents, and finally separated compounds **1**, **2**, **3**, and **4**.

Results and Discussion

A methanolic extract of the seeds of *T. chinensis* var. *mairii* was processed as described in the materials and methods section to afford four pseudoalkaloid taxane diterpenoids.

Compound **1** was isolated as a colorless gummy substance with a yield of 0.0027% on the dry material, $[\alpha]_D^{24} + 47^\circ$ (c 0.002, CHCl_3). FAB-MS produced a protonated ion peak at m/z 682 ($[\text{M}+\text{H}]^+$). The molecular formula of compound **1**, $\text{C}_{38}\text{H}_{51}\text{O}_{10}\text{N}$, was deduced from a combined analysis of HR-FAB-MS at m/z 682.3591 ($[\text{M}+\text{H}]^+$) ($\Delta - 0.0$ mmu) and ^{13}C NMR spectrum. Intensive absorptions at 1,740 and 1,720, and a weak absorption at $3,350\text{ cm}^{-1}$ in the IR spectrum implied that **1** possesses ester and amino groups, respectively. The ^1H -NMR spectrum of **1**, tabulated in Table 1, exhibited proton signals due to the four methyl groups at δ 0.86, 1.11, 1.74, and 2.05 ppm, which were the characteristic signals of a taxane skeleton. Four acetyl groups in a relatively lower field (δ 2.01, 2.05, 2.01 and 2.05 ppm), were verified by the observation of ^{13}C -NMR signals at δ 169.45, 170.03, 169.92 and 170.62 ppm. These signals suggested that **1** had a taxane-type skeleton. The connectivities of the protons in the taxane skeleton of **1** were determined by an analysis of the ^1H - ^1H COSY spectrum. Interpretation of ^1H -, ^{13}C -NMR and HMBC spectra permitted the positional assignment of

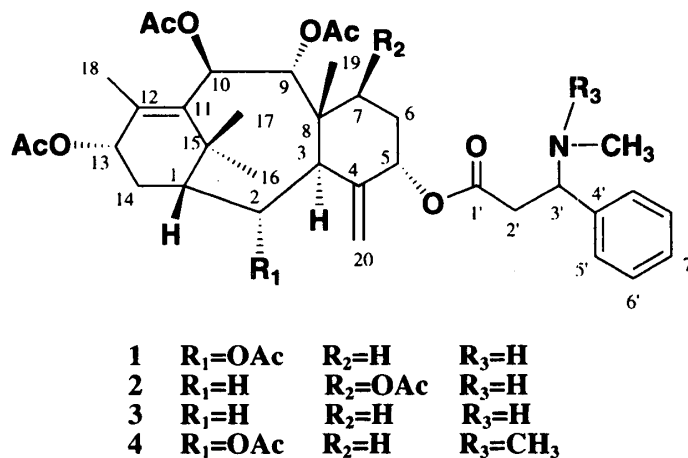


FIG. 1. Novel pseudoalkaloid taxanes from the seeds of *Taxus chinensis* var. *mairii*

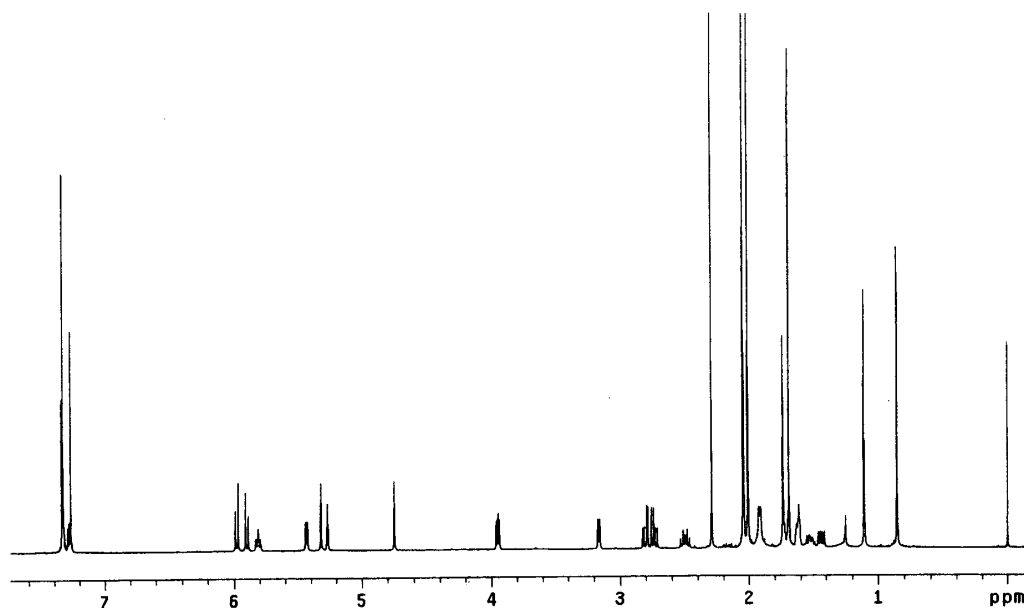


FIG. 2. ^1H NMR spectrum of compound 1 (500 MHz, CDCl_3).

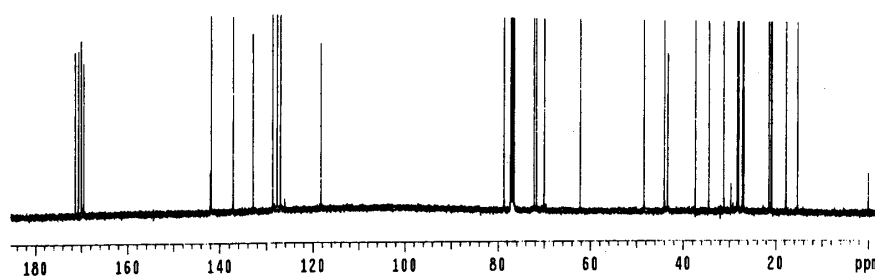


FIG. 3. ^{13}C NMR spectrum of compound 1 (125 MHz, CDCl_3).

functional groups. The ^1H -NMR signals at δ 5.32 (1H, br.s), 4.76 (1H, br.s) and 3.16 (1H, br.d, $J = 7.13$ Hz) are characteristic of an exocyclic methylene and C-3 ring junction proton in a taxa-4(20),11-diene, respectively (8, 9). Additionally, five oxygen-bearing one-proton signals appeared at the lower field, of which a pair of one-proton doublets at 5.99 and 5.90 ppm with a coupling constant of 10.71 Hz were assigned to H-10 α and H-9 β , respectively. Large vicinal coupling indicated a *trans*-oriented configuration of H-9 and H-10. The spin system derived from 18-CH₃, H-13 β , H-14 α , H-14 β , H-1 β , H-2 β , and H-3 α was readily interpreted. The signal of three protons as a doublet at δ 2.05 ppm was assigned to 18-CH₃ based on the long-range coupling with H-13 β ; the triplet at δ 5.82 ppm (1H, br. t, $J = 8.24$ Hz) was assigned to H-13 β ; the doublet of doublets of doublets at δ 2.50 ppm and the doublet of doublets at δ 1.44 ppm were assigned to the C-14 methylene protons, H-14 β and H-14 α , respectively, based on their germinal coupling and coupling to H-13 β . The signal at 5.27 ppm (1H, br.s) was a

TABLE 1. ^1H and ^{13}C NMR spectral data of **1** and ^1H NMR spectral data of **2** in CDCl_3 .

position	1				2		
	^{13}C	^1H	J (Hz)	^1H - ^1H COSY	^1H	J (Hz)	^1H - ^1H COSY
1	48.29	1.92 br.d	9.34	H-2, 14 β	1.80 m		H-2, 14 β
2	71.71	5.44 dd	2.47, 7.13	H-3	1.84 m		H-1, 3
3	44.07	3.16 d	7.13	H-2	2.81 br.d	9.12	
4	141.81						
5	78.70	5.27 br.s		H-6 α , 6 β	5.38 br.s		H-6, 7
6	27.17	1.58 m		H-5, 7	1.71 m		H-5, 7
7	28.30	1.63 m		H-6	5.45 br.t	9.06	H-6
8	44.04						
9	76.54	5.90 d	10.71	H-10	5.88 d	11.00	H-10
10	72.23	5.99 d	10.71	H-9	6.24 d	11.00	H-10
11	137.12						
12	132.56						
13	70.01	5.82 t	8.24	H-14 α , 14 β , 18	5.87 br.t	8.12	H-14 α , 14 β , 18
14 α	28.00	1.44 dd	14.90, 7.97	H-13, 14 β	0.96 m		H-13, 14 β
14 β		2.50 ddd	14.90, 9.34, 3.60	H-1, 13, 14 α	2.55 m		H-1, 13, 14 α
15	37.38						
16	31.26	1.11 s			1.10 s		
17	26.89	1.74 s			1.60		
18	15.29	2.05 br.s		H-13	2.13 br.s		H-13
19	17.80	0.86 s			0.81		
20a	118.28	4.76 br.s		20b	4.94 br.s		H-20b
20b		5.32 br.s		20a	5.26 br.s		H-20a
1'	171.32						
2'	43.55	2.81 dd	14.83, 5.77	H-2'', 3'	2.74 dd	11.27, 6.12	H-2'', 3'
2''		2.72 dd	14.83, 8.50	H-2', 3'	2.65 dd	11.27, 8.79	H-2', 3'
3'	62.19	3.95 dd	5.77, 8.50	H-2', 2''	3.95 dd	6.12, 8.79	H-2', 2''
4'	142.11						
5', 9'	128.66	7.34 m			7.32 m		
6', 8'	127.03	7.34 m			7.32 m		
7'	127.76	7.34 m			7.32 m		
2-AcO	21.48	2.01 s					
	169.45						
9-AcO	21.02	2.05 s			1.97 s		
	170.03						
10-AcO	20.81	2.01 s			2.02 s		
	169.92						
13-AcO	21.02	2.05 s			2.09 s		
	170.62						
N-CH ₃	34.48	2.29 s			2.26 s		

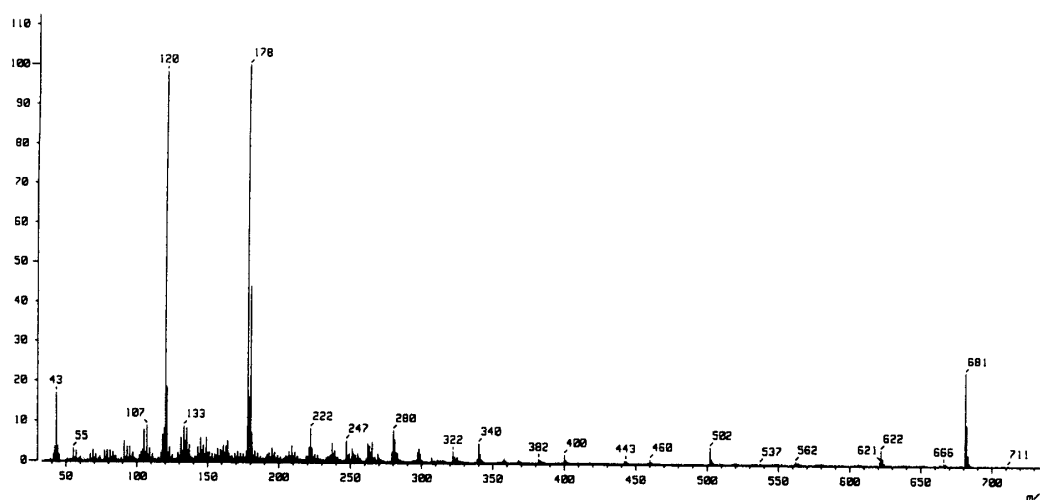


Fig. 4. EI-MS ion mode spectrum of compound 1 at 213.9°C. This technique provides a strong M^+ peak at m/z 681. All the characteristic ions of the side chain and skeleton are clearly observed.

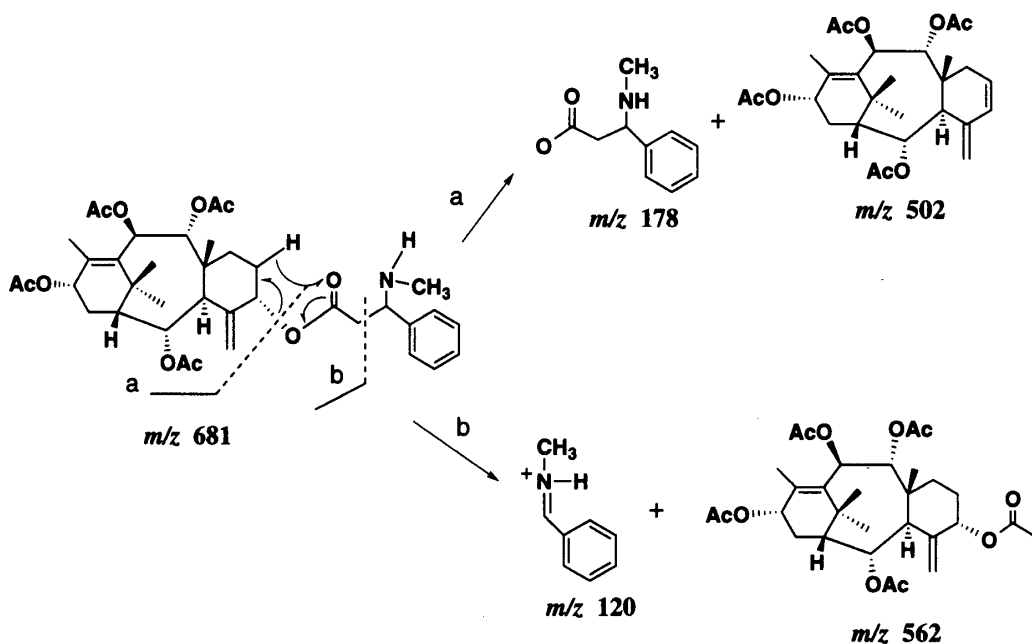


Fig. 5. Major fragmental fission patterns of 1 in the EI-MS spectrum.

characteristic signal of H-5 β . All of the proton-bearing carbons were assigned by an analysis of the HETCOR spectrum. Five oxygen-containing carbons (C-2, C-5, C-9, C-10, and C-13) were correlated with their corresponding proton signals. The presence of a nor-Winterstein acid [3-(N-methylamino)-3-phenylpropanoyl] moiety in 1 was suggested from the signals at δ 2.29 (3H, s), 2.81 (1H, dd, J = 14.83, 5.77 Hz), 2.72 (1H, dd, J = 14.83, 8.50 Hz), 3.95 (1H, dd, J = 5.77, 8.50 Hz), and 7.34 (5H, m) ppm in the ^1H NMR spectrum. Further support was provided

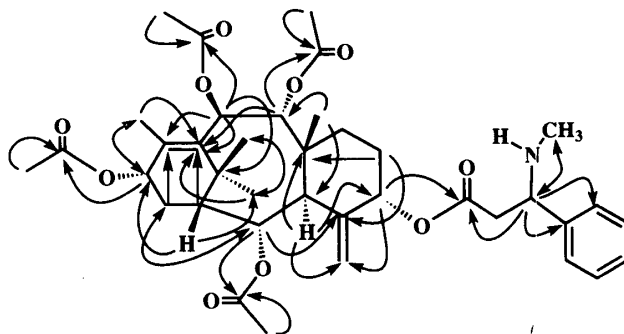


FIG. 6. H-C Long-range correlations observed from the HMBC spectrum of **1** (500 MHz), in which the most protons are omitted for clarity.

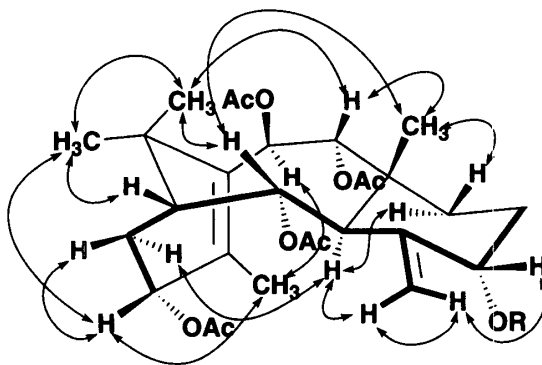


FIG. 7. Relative stereochemistry of **1**, proposed through NOESY experiment (500 MHz).

by the fragment ions in the FAB-MS at m/z 180.1027 and 120.0815 (base peaks) that analyzed for $C_{10}H_{14}O_2N$ ($\Delta + 0.3$ mmu) and $C_8H_{12}N$ ($\Delta + 0.2$ mmu) by HR-FAB-MS, respectively. It was reported that the removal of one methyl from the dimethylamino group of a Winterstein acid moiety caused a marked upfield shift (*ca* 8 ppm) of the remaining methyl in the ^{13}C NMR spectrum (10). The signal of N-CH₃ of **1** appeared at δ 34.48 ppm, which was in good agreement with this conclusion. The location of the nor-Winterstein acid moiety was deduced at C-5 from the HMBC spectrum. The three-proton signal at δ 2.29 ppm was assigned to N-CH₃. The remaining four three-proton signals at δ 2.01, 2.05, 2.01 and 2.05 ppm should be the acetyl groups connected to C-2, C-9, C-10, and C-13 as deduced from the chemical shifts of the corresponding protons attached to them. The relative stereochemistry of the terpenoid skeleton of **1** was determined from chemical shifts, coupling constants and the NOESY experiment. A coupling constant between H-9 and H-10 of 10.71 Hz indicated that the B-ring had a chair-boat conformation. The NOESY experiment established the relative stereochemistry of **1** at all positions, and the results are shown in Fig. 2. Thus the structure was determined as a 2α , 9α , 10β , 13α -tetraacetoxy- 5α -(3'-methylamino-

3'-phenyl)-propionyloxy-taxa-4(20), 11-diene.

Compound **2** was isolated as a colorless gummy substance with a yield of 0.0013% on the dry material, $[\alpha]_D^{25} + 10.3^\circ$ (*c* 0.005, CHCl₃). Intensive absorptions at 1,735 and 1,720, and a weak absorption at 3,330 cm⁻¹ implied that **2** possesses ester and amino groups. The ¹H NMR spectrum of **2** also showed four methyl groups in a relatively high field: δ 0.81, 1.10, 1.60, and 2.13 ppm, which were the characteristic signals of the taxane skeleton. The signals at δ 4.94 and 5.26 ppm (each 1H, br.s) in the ¹H NMR spectrum, which showed a weak correlation in the ¹H-¹H COSY spectrum, suggested the presence of an exomethylene moiety. Additionally, five oxygen-bearing one-proton signals appeared in the lower field, of which a pair of one-proton doublets at δ 6.24 and 5.88 ppm with a coupling constant of 11.00 Hz were assigned to H-10 α and H-9 β , respectively. Large vicinal coupling indicated a *trans*-oriented configuration of H-9 and H-10. The signal at δ 5.87 ppm (1H, br.t, *J* = 8.12 Hz), which showed a correlation with 18-CH₃ in the ¹H-¹H COSY spectrum, was assigned to H-13; the signal at 5.38 ppm (1H, br.s) was characteristic signal of H-5 β , which correlated with H-6. In turn, H-6 showed a cross peak with the signal at δ 5.45 ppm (1H, br.t, *J* = 9.06 Hz) in the ¹H-¹H COSY spectrum, and the latter one was assigned to H-7 α . Protonated molecular ion at *m/z* 682.3589 indicated the composition of **2** was C₃₈H₅₁O₁₀N, which was the same as that of **1**. Two daughter ions at *m/z* 502 and 562 clearly indicated that a nor-Winterstein acid group disappeared from the side-chain. A base peak at *m/z* 120.0812 that analyzed for C₈H₁₀N and a stronger fragment ion at *m/z* 180.1025 analyzed for C₁₀H₁₄O₂N, further supported that the side-chain was a nor-Winterstein acid. The presence of a nor-Winterstein acid [3-(*N*-methylamino)-3-phenylpropanoyl] moiety in **2** was also suggested from the signals at δ 2.26 (3H, s), 2.74 (1H, dd, *J* = 11.27, 6.12 Hz), 2.65 (1H, dd, *J* = 11.27, 8.79 Hz), 3.95 (1H, dd, *J* = 6.12, 8.79 Hz), and 7.32 (5H, m) ppm in the ¹H NMR spectrum. The location of a nor-Winterstein acid moiety was suggested at C-5 as in the case of all the other taxoids ever isolated (8-16). From the above analysis, the structure was established as 7 β , 9 α , 10 β , 13 α -tetraacetoxy-5 α (3'-methylamino-3'-phenyl)-propionyloxy-taxa-4(20), 11-diene. Unfortunately, we couldn't obtain a sufficient sample for running ¹³C NMR and HMBC spectra.

Compound **3** was isolated as colorless gum, $[\alpha]_D^{24} + 14.5^\circ$ (*c* 0.003, CHCl₃). The HR-EI-MS of compound **3** revealed a molecular formula of C₃₆H₄₉O₈N. Intensive absorptions at 1,735 and 1,720, and a weak absorption at 3,330 cm⁻¹ implied that **3** possesses ester and amino groups. The ¹H NMR spectrum of **3** showed four methyl groups in a relatively high field: 0.71, 1.08, 1.26, and 2.02 ppm, which were the characteristic signals of a taxane skeleton. The signals at 4.84 and 5.18 ppm (each 1H, br.s) in the ¹H NMR spectrum, which showed weak correlation in the ¹H-¹H COSY spectrum, suggested the presence of an exomethylene moiety. Additionally, four oxygen-bearing one-proton signals appeared at

lower field, of which a pair of one-proton doublets at 6.03 and 5.84 ppm with a coupling constant of 10.71 Hz were assigned to H-10 α and H-9 β , respectively. Large vicinal coupling indicated a *trans*-oriented configuration of the H-9 and H-10. The signal at 5.79 ppm (1H, br.t, $J = 7.14$ Hz), which showed a correlation with 18-CH₃ in the ¹H-¹H COSY spectrum, was assigned to H-13; the signal at 5.33 ppm (1H, br.s) was characteristic to H-5 α . The presence of a nor-Winterstein acid [3-(N-methylamino)-3-phenylpropanoyl] moiety in **3** was suggested from the signals at 2.29 (3H, s), 2.73 (2H, d, $J = 7.41$ Hz), 3.95 (1H, t, $J = 7.41$ Hz) and 7.33 (5H, m) ppm in the ¹H NMR spectrum, which was in good agreement with the literature values (2). Further support was provided by the fragment ions in the EI-MS at m/z 178 and 120 (both base peaks) which were analyzed for C₁₀H₁₂O₂N and C₈H₁₀N by HR-EI-MS, respectively. The location of a nor-Winterstein acid moiety was suggested at C-5, as in the case of all the other taxoids ever isolated (4), although HMBC data could not be obtained to provide direct evidence for the connecting position. The three-proton signal at 2.29 ppm was assigned as that of N-CH₃. The remaining three three-proton signals at 2.05, 2.01, and 1.73 ppm should be the acetyl groups connected to C-9, C-10, and C-13 as deduced from the chemical shifts of the corresponding protons attached to them. Thus the structure was determined as 9 α , 10 β , 13 α -triacetoxy-5 α -(3'-methylamino-3'-phenyl)-propionyloxy-taxa-4(20), 11-diene, *i.e.* a nor derivative of 7, 2'-bisdeacetyxaustrospicatine (10). It was reported that the removal of one methyl from the dimethylamino group of a Winterstein acid moiety caused a marked upfield shift (*ca* 8 ppm) of the remaining methyl (10). Unfortunately, we couldn't isolated the sample sufficiently enough for running a ¹³C NMR spectrum.

Compound **4** was isolated as a colorless gummy substance with a yield of 0.0032% on the dry material, $[\alpha]_D^{24} + 67^\circ$ (*c* 0.002, CHCl₃). FAB-MS produced a protonated ion peak at m/z 696 ($[M+H]^+$). The molecular formula of compound **4**, C₃₉H₅₃O₁₀N, was deduced from a combined analysis of HR-FAB-MS at

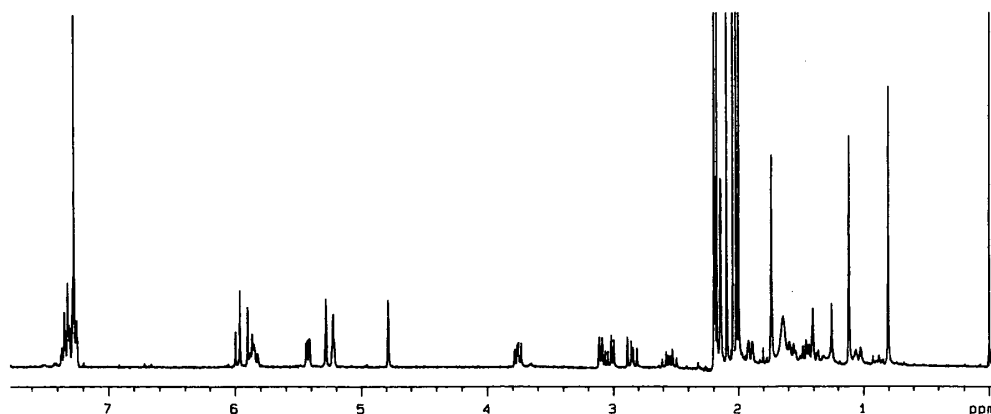


FIG. 8. ¹H NMR spectrum of compound **4** (300 MHz, CDCl₃).

m/z 6696.3746 ($[M+H]^+$) ($\Delta -0.1$ mmu) and ^{13}C NMR spectrum. Intensive absorptions at 1,740 and 1,720, and a weak absorption at $3,450\text{ cm}^{-1}$ in the IR spectrum implied that **4** possesses ester and amino groups, respectively. The ^1H -NMR spectrum of **4**, tabulated in Table 1, exhibited the proton signals due to the four methyl groups at δ 0.80, 1.11, 1.73, and 2.13 ppm, which were the characteristic signals of a taxane skeleton. Four acetyl groups at a relatively lower field (δ 2.03, 2.09, 2.01 and 1.99 ppm), which was verified by the observation of ^{13}C -NMR signals at δ 169.43, 170.04, 169.97 and 170.55 ppm. These signals suggested that **4** had a taxane-type skeleton. The connectivities of the protons at the taxane skeleton of **4** were determined by an analysis of the ^1H - ^1H COSY spectrum. Interpretation of ^1H -, ^{13}C -NMR and HMBC spectra permitted the positional assignment of functional groups. The ^1H -NMR signals at δ 5.27 (1H, br.s), 4.78 (1H, br.s) and 3.09 (1H, br.d, $J=6.87$ Hz) are characteristic of a exocyclic methylene and C-3 ring junction proton in a taxa-4(20), 11-diene, respectively (6, 7). Additionally, five oxygen-bearing one-proton signals appeared in the lower field, of which a pair of one-proton doublets at 5.97 and 5.88 ppm with a coupling constant of 10.44 Hz were assigned to H-10 α and H-9 β , respectively. Large vicinal coupling indicated a *trans*-oriented configuration of H-9 and H-10. The spin system derived from 18-CH₃, H-13 β , H-14 α , and H-14 β , H-1 β , H-2 β , and H-3 α was readily interpreted. The signal of three protons as a doublet at δ 2.13 ppm was assigned to 18-CH₃, based on the long-range coupling with H-13 β ; the triplet at δ 5.84 ppm (1H, br. t, $J=8.24$ Hz) was assigned to H-13 β ; the doublet of doublets of doublets at δ 2.54 ppm and the multiplet at δ 1.44 ppm were assigned to the C-14 methylene protons, H-14 β and H-14 α , respectively, based on their germinal coupling and coupling to H-13 β . The signal at 5.22 ppm (1H, br. s) was characteristic signal of H-5 β . All of the proton-bearing carbons were assigned by an analysis of the HETCOR spectrum. Five oxygen-containing carbons (C-2, C-5, C-9, C-10, and C-13) were correlated with their corresponding proton signals. The presence of a Winterstein acid moiety in **4** was suggested

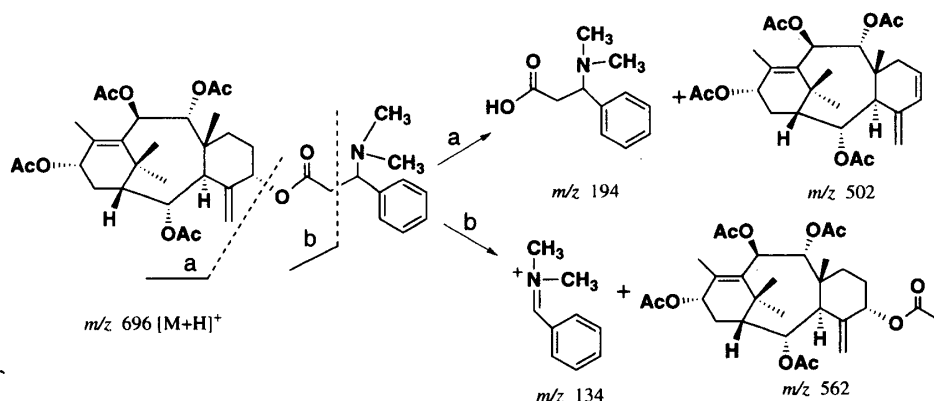


FIG. 9. Major fragmental fission patterns of **4** in the EI-MS spectrum.

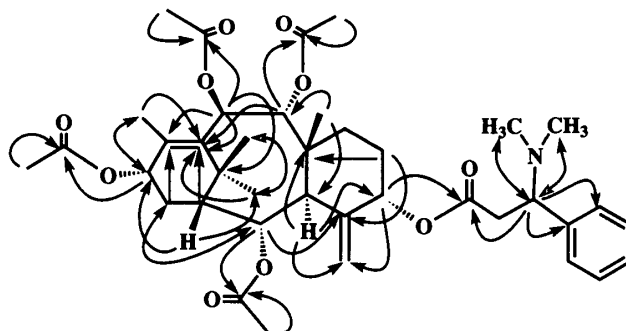


FIG. 10. H-C Long-range correlations observed from the HMBC spectrum of **4** (500 MHz), in which the most protons are omitted for clarity.

TABLE 2. ^1H NMR Spectral data of **3** (300 Hz, CDCl_3).

Position	^1H	J	Position	^1H	J
1	1.81 m		18	2.02 s	
2α	1.74 m		19	0.71 s	
2β	1.67 m		20a	5.18 br s	
3	2.89 br s	4.67	20b	4.84 br s	
5	5.33 br s		2'	2.73 d	7.41
6α	1.57 m		3'	3.94 d	7.41
6β	1.67 m		5', 9'	7.30 m	
7α	1.61 m		6', 8'	7.30 m	
7β	1.61 m		7'	7.30 m	
9	5.84 d	10.71	OAc	2.01 s	
10	6.03 d	10.71	OAc	1.73 s	
13	5.79 t	7.14	OAc	2.05 s	
14α	1.01 br dd	14.90, 8.00	N- CH_3	2.29 s	
14β	2.63 ddd	14.70, 9.30, 5.03			
16	1.08 s				
17	1.26 s				

from the signals at δ 2.18 (6H, s), 2.84 (1H, dd, $J = 13.74, 9.34$ Hz), 3.02 (1H, dd, $J = 13.74, 6.14$ Hz), 3.75 (1H, dd, $J = 9.34, 6.14$ Hz), and 7.27–7.37 (5H, m) ppm in the ^1H NMR spectrum. Further support was provided by the fragment ions in the FAB-MS at m/z 194.1183 and 134.0967 (base peaks) that analyzed for $\text{C}_{11}\text{H}_{16}\text{O}_2\text{N}$ ($\Delta + 0.2$ mmu) and $\text{C}_9\text{H}_{12}\text{N}$ ($\Delta - 0.2$ mmu) by HR-FAB-MS, respectively. The location of the Winterstein acid moiety was deduced at C-5 from the HMBC spectrum. The six protons signal at δ 2.18 ppm was assigned to N- CH_3 . The remaining four three-proton signals at δ 2.03, 2.09, 2.01 and 1.99 ppm should be the acetyl groups connected to C-2, C-9, C-10, and C-13 as deduced from the chemical shifts of the corresponding protons attached to them. The relative

TABLE 3. ^1H and ^{13}C NMR spectral data of **4** in CDCl_3 .

Position	^1H	J	^1H - ^1H COSY	^{13}C
1	1.89 br d	10.16	H-2 β , 14 β	48.36
2	5.41 dd	2.20, 6.87	H-1 β , 3 α	71.86
3	3.09 br dd	6.87	H-2 β , 5 α	43.97
4				141.89
5	5.22 br s		H-3 α , 20a	77.91
6a	1.57 m		H-6b, 1	
6b	1.39 m		H-6a, 7	27.03
7	1.44 m		H-6a, 6b	28.30
8				44.05
9	5.88 d	10.44	H-10 α	76.48
10	5.97 d	10.44	H-9 β	72.23
11				137.13
12				132.96
13	5.84 br t	8.24	H-14 α , 14 β , 18-CH ₃	70.18
14 α	1.44 m		H-13, 14 β	
14 β	2.54 ddd	15.11, 8.84, 5.49	H-1 β , 13, 14 α	28.13
15				37.39
16	1.73 s			31.31
17	1.11 s			28.30
18	2.13 br s		H-13	15.43
19	0.80 s			17.82
20a	5.27 br s		H-5, 20b	
20b	4.78 br s		20a	118.37
2-AcO	2.03 s			20.80
				169.43
9-AcO	2.09 s			21.40
				170.04
10-AcO	2.01 s			21.03
				169.97
13-AcO	1.99 s			21.47
				170.55
1'				170.87
2'a	3.02 dd	13.74, 6.14	H-2'b, 3'	40.36
2'b'	2.84 dd	13.74, 9.34	H-1'a, 3'	
3'	3.75 dd	6.14, 9.34	H-2'a, 2'b	67.96
N-CH ₃	2.18 s			42.90
Ph	7.27- 7.37 m			138.93
				127.22
				128.36
				128.22

stereochemistry of the terpenoid skeleton of **4** was determined from chemical shifts, coupling constants and the NOESY experiment. A coupling constant between H-9 and H-10 of 10.44 Hz indicated that the B-ring had a chair-boat conformation. The NOESY experiment established the relative stereochemistry of **4** at all positions, and the results are shown in Fig. 5. Thus the structure was determined as a $2\alpha, 9\alpha, 10\beta, 13\alpha$ -tetraacetoxy- 5α -(3'-methylamino-3'-phenyl)-propionyloxy-taxa-4(20), 11-diene.

In summary, to the best of our knowledge, the structures of **1**, **2** and **3**, with a nor-Winterstein acid side chain at C-5, have not been reported before. Both EI-MS and FAB-MS spectra can produce strong molecular or protonated molecular ions. Daughter ions, usually appearing as base peaks, can provide useful information about the side chain. The signal of N-CH₃ in the nor-Winterstein acid [3-(N-methylamino)-3-phenylpropanoyl] usually resonates at δ 34.5 ppm in the ¹³C NMR spectrum, which upfield shifts *ca* 8 ppm than in the Winterstein acid.

Acknowledgements

We extend our sincere appreciation to Mrs. Teiko Yamada and Mrs. Yuhko Sugiyama for measuring the NMR data, and to Mrs. Eiko Aoki for running the MS spectra. We are grateful to the Ministry of Education, Science and Culture of Japan for a Grant-in-Aid to Scientific Research.

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