Pergamon

0031-9422(94)00715-2

Phytochemistry, Vol. 38, No. 3, pp. 667–670, 1995

0031-9422/95 $9.50 + 0.00

TAXANES FROM *TAXUS CHINENSIS*

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(*Received* 1 *July* 1994)

Key Word Index—*Taxus chinensis*; Taxaceae; diterpenoid; taxol; taxanes; 2α,5α,9α-trihydroxy-10β,13α-diacetoxy-4β,20-epoxy-taxa-11-ene; 2α,5α,9α-trihydroxy-10β,13α-diacetoxy-taxa-4(20),11-diene; 1β,5α,7β,9α,10β,13α-hexaol-9α,10β-diacetate-7β-benzoate-taxa-4(20),11-diene; 2α,9α-dihydroxy-10β,13α-diacetoxy-5α-cinnamatetaxa-4(20),11-diene.

Abstract—Two new taxanes were isolated from the needles of *Taxus chinensis* and their structures were established as 2α,5α,9α-trihydroxy-10β,13α-diacetoxy-4β,20-epoxy-taxa-11-ene and 2α,5α,9α-trihydroxy-10β,13α-diacetoxy-taxa-4(20),11-diene.

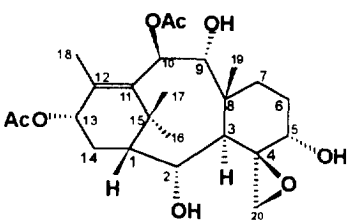
INTRODUCTION

Taxol has become an important new cancer chemotherapeutic agent since its discovery more than 20 years ago [1]. It has significant activity in drug-refractory ovarian cancer [2] and was approved for this disease by the U.S. Food and Drug Administration in 1992 and may also have broad spectrum antitumour activities. The shortage of a commercial supply of taxol coupled with its unique structural features, novel mode of action and low aqueous solubility have greatly stimulated the study of the total synthesis, biosynthesis, partial synthesis from abundant but inactive taxanes, structural modification and structure–activity relationship. There is also a search for new taxanes having similar activities and for the means of obtaining large quantities from the needles of various *Taxus* species which can be harvested without destroying the trees. At the start of this year the total synthesis of taxol was announced by the two groups of Nicolaou and Holton and this represents a landmark of taxol research [3–5].

As part of our continuing study on the constituents of *Taxus chinensis* [6–8], we report here the structure elucidation of four minor taxane diterpenes from the leaves and stems of this plant. Among them are two new taxane diterpenes: 2α,5α,9α-trihydroxy-10β,13α-diacetoxy-4β,20-epoxy-taxa-11-ene (**1**) and 2α,5α,9α-trihydroxy-10β,13α-diacetoxy-taxa-4(20),11-diene (**2**). Known compounds are 1β,5α,7β,9α,10β,13α-hexaol-9α,10β-diacetate-7β-benzoate-taxa-4(20),11-diene; 2α,9α-dihydroxy-10β,13α-diacetoxy-5α-cinnamate-taxa-4(20),11-diene [9, 10].

RESULTS AND DISCUSSION

Compound **1** was determined to have a molecular formula of $C_{24}H_{36}O_8$ by analysis of the ^{13}C NMR and FAB mass spectral data. IR bands at 1724.9 and 1630.2 cm^{-1} indicated carbonyl absorptions suggesting acetate substituents. The presence of two acetates was verified by the observation of ^{13}C NMR signals at δ170.2 (*s*), 170.5 (*s*), 21.1 (*q*) and 21.2 (*q*), and ^1H NMR signals at δ2.10 (*s*, 3H) and 2.15 (*s*, 3H). The connectivities of the protons in the taxane skeleton of **1** were determined by analysing the ^1H–^1H COSY spectrum. Interpretation of ^1H and ^{13}C NMR spectra permitted the positional assignment of functional groups. The ^1H NMR signals at 2.62 (*dd*, 1H, J = 4.4, 0.9 Hz) and 3.64 (*dd*, 1H, J = 4.4, 0.6 Hz) are characteristic of the C-20 methylene protons of the oxirane bridge. The 1H doublet of doublet of



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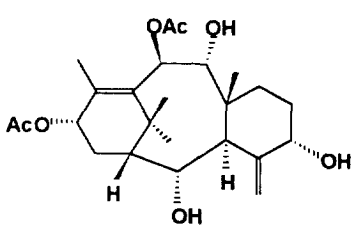

2

doublets at δ2.6 and the 1H doublet of doublets at δ1.52 were assigned to the C-14 methylene protons, H-14β and H-14α, respectively, based on their geminal coupling ($J_{14\alpha,14\beta}$ = 15.5 Hz), coupling ($J_{13\beta,14\beta}$ = 10.4 Hz, $J_{13\beta,14\alpha}$ = 4.8 Hz) to the H-13β doublet of doublet of doublets at δ5.80 ($J_{13\beta,18}$ = 1.4 Hz) and coupling ($J_{1\beta,14\beta}$ = 8.2 Hz) to the H-1β doublet of doublets at δ1.84 ($J_{1\beta,14\beta}$ = 8.2 Hz, $J_{1\beta,2\beta}$ = 1.6 Hz). The H-2β doublet of doublet of doublets at δ3.90 was correlated with the signal at δ4.38 assigned to 2α-OH ($J_{2\alpha-OH,2\beta-H}$ = 10.8 Hz), with the signal at δ3.19 assigned to H-3α ($J_{2\beta,3\alpha}$ = 4.5 Hz) and with the signal at δ1.84 assigned to H-1β ($J_{1\beta,14\beta}$ = 8.2 Hz, $J_{1\beta,2\beta}$ = 1.6 Hz). The high-field H-5β triplet at δ3.18 (J = 2.5 Hz) was coupled with the 2H multiplet at δ1.75 assigned to the C-6 methylene protons. The 2H-6 resonances were also coupled with two multiplets at δ1.84 and 2.12 assigned to the C-7 methylene protons. The isolated spin system comprised of doublets at δ4.04, and 5.82 was attributed to H-9β and H-10α, with the large vicinal coupling ($J_{9\beta,10\alpha}$ = 9.8 Hz) indicative of a *trans*-oriented configuration. The three singlets at δ1.02, 1.47 and 1.19 corresponded to methyl groups at C-16, C-17 and C-19, whereas the 3H doublet at δ2.09 corresponded to the C-18 methyl group based upon its long-range coupling with H-13β at δ6.03 ($J_{13\beta,18}$ = 1.4 Hz). The low-field signals of H-10α at δ5.82 and H-13β at δ5.80 indicated acetate groups at C-10 and C-13. Therefore, the structure of **1** was established as 2α,5α,9α-trihydroxy10β,13α-diacetoxy-4β,20-epoxy-taxa-11-ene.

Compound **2** was determined to have a molecular formula of $C_{24}H_{36}O_7$ by analysis of the ^{13}C NMR and FAB mass spectral data. IR bands at 1717.4 and 1631.3 cm^{-1} indicated carbonyl absorptions suggesting acetate substituents. The ^{1}H NMR spectrum of the upfield region (δ1.02–2.05) suggested the presence of four methyl and two acetoxy methyl groups, with a characteristic 3H doublet at δ2.08 assigned to the C-18 methyl group based upon its long-range coupling with H-13β ($J_{13\beta,18}$ = 1.5 Hz) as before. The presence of two acetates was verified by the observation of ^{13}C NMR signals at δ170.2 (*s*), 170.5 (*s*), 21.1 (*q*) and 21.2 (*q*). The ^{1}H NMR signals at δ5.27 (*d*, 1H, J = 1.7 Hz), 5.31 (*t*, 1H, J = 1.7 Hz) and 3.33 (*br d*, 1H, J = 6.0 Hz) are characteristic of an exocyclic methylene and C-3 ring junction

Table 1. ^{1}H NMR data for **1** and **2**

	1		**2**	
Proton	δ_H, J (Hz)	COSY	δ_H, J (Hz)	COSY
H-1β	1.84 (*dd*) J = 8.2, 1.6	H-14β, 2	1.91 (*dd*) J = 8.5, 1.8	H-14α, 2
H-2β	3.90 (*ddd*) J = 10.8, 4.5, 1.6	H-3, 1,2-OH	4.05 (*dd*) J = 6.0, 1.8	H-1, 3
H-3α	3.19 (*br d*) J = 4.5	H-2, 20	3.33 (*br d*) J = 6.0	H-2, 20
H-5β	3.18 (*br t*) J = 2.5	H-6, 7	4.22 (*t*) J = 1.7	H-6
H_2-6	1.75 (*m*)	H-5, 7	1.79, 1.60 (*m*)	H-5, 7
H_2-7	1.84, 2.12 (*m*)	H-5, 6, 9	1.69 (*m*)	H-6
H-9β	4.07 (*d*) J = 9.8	H-10	4.18 (*d*) J = 9.9	H-10
H-10α	5.82 (*d*) J = 9.8	H-9	5.83 (*d*) J = 9.9	H-9
H-13β	5.80 (*ddd*) J = 10.4, 4.8, 1.4	H-14α, 14β, 18	5.72 (*ddd*) J = 10.3, 5.0, 1.2	H-14α, 14β, 18
H-14α	1.52 (*dd*) J = 15.6, 4.8	H-14β, 13	1.38 (*dd*) J = 15.9, 5.0	H-14β, 13
H-14β	2.68 (*ddd*) J = 15.6, 10.4, 8.2	H-14α, 1, 13	2.60 (*ddd*) J = 15.9, 10.3, 8.5	H-14α, 1, 13
Me-16	1.02 (*s*)	Me-17	1.02 (*s*)	Me-17
Me-17	1.47 (*s*)	Me-16	1.48 (*s*)	Me-16
Me-18	2.11 (*d*) J = 1.4	H-13	2.08 (*d*) J = 1.2	H-13
Me-19	1.19 (*s*)	H-3	1.06 (*s*)	H-3
H_2-20	2.62 (*dd*) J = 4.4, 0.9 3.64 (*dd*) J = 4.4, 0.7	H-3	5.27 (*t*) J = 1.7 5.31 (*t*) J = 1.8	H-3 H-5
OAc	2.10 (*s*) 2.15 (*s*)		2.03 (*s*) 2.04 (*s*)	
2α-OH	4.38 (*d*) J = 10.8			

Table 2. ^{13}C NMR data for **1** and **2**

	1		2	
C	δ_C	DEPT	δ_C	DEPT
1	50.7	CH	50.9	CH
2	72.2	CH	69.4	CH
3	35.9	CH	43.7	CH
4	67.2	C	149.0	C
5	70.0	CH	69.9	CH
6	26.8	CH_2	28.3	CH_2
7	24.3	CH_2	25.4	CH_2
8	44.8	C	45.6	C
9	75.8	CH	76.1	CH
10	76.1	CH	76.9	CH
11	134.8	C	134.7	C
12	137.7	C	136.8	C
13	76.2	CH	79.9	CH
14	28.3	CH_2	31.3	CH_2
15	37.4	C	37.1	C
16	26.4	Me	26.4	Me
17	32.2	Me	32.5	Me
18	15.7	Me	15.8	Me
19	17.7	Me	17.6	Me
20	54.2	CH_2	115.5	CH_2
OAc	21.1	Me	21.2	Me
	21.2	Me	21.4	Me
	170.2	C	170.4	C
	170.5	C	170.6	C

proton in a taxa-4(20),11-diene, respectively. The connectivities of the protons in the taxane skeleton of **2** were also determined by analysing the ^{1}H–^{1}H COSY spectrum. As previously noted in **1**, the H-2β doublet of doublet of doublets at δ4.05 was correlated with the signal at δ3.33 assigned to H-3α (*br d*, $J_{2\beta,3\alpha}$ = 6.0 Hz) and with the signal at δ1.91 assigned to H-1β (*dd*, $J_{1\beta,14\beta}$ = 8.5 Hz, $J_{1\beta,2\beta}$ = 1.8 Hz). The 1H doublet of doublet of doublets at δ2.6 and the 1H doublet of doublets at δ1.38 were assigned to the C-14 methylene protons, H-14β and H-14α, respectively, based on their geminal coupling ($J_{14\alpha,14\beta}$ = 15.9 Hz), coupling ($J_{13\beta,14\beta}$ = 10.3 Hz, $J_{13\beta,14\alpha}$ = 5.0 Hz) to the H-13β doublet of doublet of doublets at δ5.72 ($J_{13\beta,18}$ = 1.2 Hz) and coupling with the H-1β at δ1.91 (*dd*, $J_{1\beta,14\beta}$ = 8.5 Hz, $J_{1\beta,2\beta}$ = 1.8 Hz). The H-5β resonance appeared as a 1H triplet at δ4.22 (J = 1.7 Hz), and was coupled to both H-6α and H-6β proton resonances at δ1.60 and 1.79. The 2H-6 resonances were also coupled with the multiplets at δ1.69 assigned to the C-7 methylene protons. The isolated spin system comprised of doublets at δ4.18, and 5.83 was attributed to H-9β and H-10α, with the large vicinal coupling ($J_{9\beta,10\alpha}$ = 9.8 Hz) indicative of a *trans*-oriented configuration. The low field signals of H-10α at δ5.83 and H-13β at δ5.72 indicated acetate groups at C-10 and C-13. The stereochemistry of **2** was determined by NOE difference spectroscopy. The results are shown in Fig. 1. Therefore, the structure of **2** was established as 2α,5α,9α-trihydroxy-10β,13α-diacetoxy-taxa-4(20),11-diene.

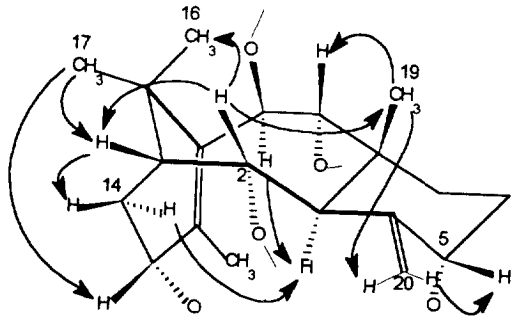

Fig. 1. Conformation of **2** and observed NOEs.

EXPERIMENTAL

Silica gel 60 (Merck 230–400 mesh) was used for conventional CC. Silica gel (Aldrich, TLC high purity grade) was used for medium pressure CC, and solvents were redistilled prior to use: ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}CNMR (100 MHz, $CDCl_3$) spectra were taken on a Bruker AM400 FT-NMR Spectrometer, and chemical shifts are given in δ(ppm) with $CDCl_3$–H signal as an int. standard. IR spectra were measured with a Perkin Elmer 1600 FTIR Spectrometer and KBr pellets.

Plant material. The leaves and stems of *Taxus chinensis* (Pilger) Rehd. were collected from the Xiao long mountainous region of southern Gansu Province of China in April 1988. A voucher specimen is kept at the Herbarium of the Biology Department, Lanzhou University.

Extraction and isolation. The air-dried stems and leaves of *Taxus chinensis* (29 kg) were ground to a fine powder. The resulting powder was extracted with 80% EtOH (5 ×) at room temp. The EtOH extracts were concd to a residue under red. pres. This residue was diluted with H_2O and the aq. soln was extracted with petrol (bp 60–90°) to remove lipid material and then was extracted with $CHCl_3$. The combined $CHCl_3$ extracts, upon evapn, yielded 300 g of a brown syrup. The $CHCl_3$ extract was mixed with 300 g silica gel (100 mesh), and then subjected to a silica gel column (2.5 kg, 12 × 170 cm), eluted with $CHCl_3$, and a gradient of $CHCl_3$–MeOH (100:0.5–1:1). Ten frs were obtained. Fr. 3 (22.8 g) was reconstituted in $CHCl_3$ (20 ml) and applied to a silica gel column (300 g, and 4 × 40 cm) eluted with a gradient of $CHCl_3$–MeOH (199:1–10:1) to give the mixt. of **3** and **4** (1.2 g) as a pale brown foam. Further medium pressure chromatography (1.5 kg cm^{-2}) of this mixt. on high purity TLC silica gel (50 g, 2 × 40 cm) eluted with hexane–Me_2CO (3:1) afforded 2 pale yellow foam frs (0.4 and 0.6 g). Medium pressure chromatography of these 2 frs (20 g, 1.2 × 25 cm) eluted with hexane–EtOAc (1:1–2:3), respectively, afforded pure taxanes **3** (83 mg) as an amorphous powder and **4** (120 mg) as needle crystals.

Fr. 5 (15.8 g) was reconstituted in $CHCl_3$ (20 ml) and applied to a silica gel column (300 g, 4 × 40 cm) eluted with hexane–*n*-BuOH (7:3) to give the mixt. of **1** and **2** (850 mg) as a pale brown foam. Further medium pressure chromatography (1.5 kg cm^{-2}) of this mixt. on high pur-

ity TLC silica gel (50 g, 2 × 40 cm) eluted with hexane–EtOAc (1:3) afforded pure taxanes **1** (12 mg) as crystals and **2** (53 mg) as an amorphous powder.

2α,5α,9α-*Trihydroxy*-10β,13α-*diacetoxy*-4β,20-*epoxy-taxa*-11-*ene* (**1**). Mp 216–218°. ^{1}H NMR (Table 1); ^{13}C NMR (Table 2). FABMS (*m/z*): 475 $[M + Na]^+$, 393 $[M - NaOAc]^+$, 333 $[M - NaOAc - HOAc]^+$, 315 $[333 - H_2O]^+$, 297 $[315 - H_2O]^+$, 435 $[M - NaOH]^+$, 375 $[435 - HOAc]^+$, 357 $[375 - H_2O]^+$; FABMS (*m/z*: 475 $[M + Na]^+$, 415 $[M + Na - HOAc]^+$, 355 $[415 - HOAc]^+$. FT-IR ν_{max} cm^{-1}: 3440, 2929, 1725, 1630, 1458, 1372, 1242, 1022.

2α,5α,9α-*trihydroxy*-10β,13α-*diacetoxy-taxa*-4(20), 11-*diene* (**2**). Mp 186–188°. ^{1}H NMR (Table 1); ^{13}C NMR (Table 2). FABMS (*m/z*): 419 $[MH - H_2O]^+$, 377 $[MH - HOAc]^+$, 299 $[419 - 2 \times HOAc]^+$, 281 $[299 - H_2O]^+$; FABMS (+ Na^+, *m/z*): 459 $[M + Na]^+$, 399 $[M + Na - HOAc]^+$, 339 $[399 - HOAc]^+$. FT-IR ν_{max} cm^{-1}: 3462, 2943, 1717, 1631, 1374, 1247, 1012.

Acknowledgement—Dr Zong Ping Zhang acknowledges the Alexander von Humboldt Foundation in Germany for a research fellowship.

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