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YUNNANXANE AND ITS HOMOLOGOUS ESTERS FROM CELL CULTURES OF *TAXUS CHINENSIS* VAR. *MAIREI*

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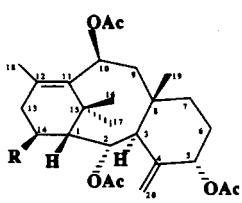
ABSTRACT.—From cell cultures of *Taxus chinensis* var. *mairei*, yunnanxane [2α,5α,10β-triacetoxy-14β-(2′-methyl-3′-hydroxyl)-butyryloxy-4(20),11-taxadiene, [**1**], and four new homologous esters, 2α,5α,10β,14β-tetra-acetoxy-4(20),11-taxadiene [**2**], 2α,5α,10β-triacetoxy-14β-propionyloxy-4(20),11-taxadiene [**3**], 2α,5α,10β-triacetoxy-14β-isobutyryloxy-4(20),11-taxadiene [**4**], and 2α,5α,10β-triacetoxy-14β-(2′-methyl)-butyryloxy-4(20),11-taxadiene [**5**] have been isolated. Their structures were determined by spectroscopic methods.

In a previous paper, we reported the isolation of four new bioactive taxoids from cell cultures of *Taxus baccata* (1). Encouraged by these findings, we have extended our studies to cell cultures of other *Taxus* species. The current report describes the isolation and structure elucidation of one known compound, yunnanxane [**1**], and four new homologous esters [**2–5**] from cultures of *Taxus chinensis* (Pilger) Rehd var. *mairei* (Lemee & Levl.) W.C. Chang & L.K. Fu (Taxaceae). This study give further support to the view that cell cultures are a potential means of discovering interesting compounds that have not been previously reported from intact plants.

Compound **1** was isolated as colorless prisms. Its ^{1}H-, ^{13}C-nmr, and ms data are identical with those of yunnanxane, a taxoid previously isolated from *Taxus yunnanensis* (2). X-ray crystallography of **1** also supported its relative stereochemistry, while COSY, HETCOR, and long-range HETCOR nmr experiments were helpful in making the assignments of ^{1}H- and ^{13}C-nmr data of **1** and its ester derivatives [**2–5**] that were isolated later.

The fabms of **2–5** contained several common fragments to that of **1** at *m/z* 265, 283, 325, 343, and 385, which suggested that all five compounds are closely related structurally. In the ^{1}H-nmr spectra of **3–5**, the presence of four methyl signals at about 0.85, 1.13, 1.66, and 2.09 ppm and two exocyclic methylene signals at about 4.86 and 5.27 ppm indicated that these compounds are similar to **1** with a C-4(20) exocyclic double bond, which was also supported by ob-



a: $-OCOCH(CH_3)CH(OH)CH_3$

b: $-OCOCH_3$

c: $-OCOCH_2CH_3$

d: $-OCOCH(CH_3)CH_3$

e: $-OCOCH(CH_3)CH_2CH_3$

1 R=a
2 R=b
3 R=c
4 R=d
5 R=e

served ^{13}C-nmr signals near 142.33 and 116.91 ppm.

The hrfabms of **2** showed an $[M+Na]^+$ fragment peak at *m/z* 527.2629 ($C_{28}H_{40}O_8Na$, calcd 527.2621), which was the same molecular formula as taxusin (3,4). The ^{1}H-nmr spectrum of **2** showed four acetyl signals at 2.01, 2.04, 2.05, and 2.17 ppm, and the H-10 signals appeared as a doublet of doublets at 6.06 ppm indicating the presence of a methylene group at C-9 and an acetyl group at C-10. The signal of H-14 at 5.00 ppm (dd, J=9.2 and 4.8 Hz) suggested the presence of an acetyl group at C-14. Furthermore, the appearance of the H-2 resonance at 5.36 ppm (dd, J=6.5 and 2.2 Hz), and those of H-13 at 2.85 (dd, 19.0 and 9.1 Hz) and 2.40 ppm (m) supported the presence of an acetyl group at C-2. An acetyl group at C-5 was indicated by the signal at 5.29 ppm (t, J=2.9 Hz). The proposed locations of the acetyl groups of **2** were also supported by ^{13}C-nmr signals at 70.60, 78.26, 70.10, and 70.60 ppm for C-2, C-5, C-10, and C-14, respectively, by direct comparison with the spectrum of **1**, whose assignment was determined by long-range HETCOR nmr. Therefore, compound **2** is an isomer of taxusin (wherein the four acetyl groups are at C-5, C-9, C-10, and C-13 instead).

Compound **3** has the composition $C_{29}H_{42}O_8$ (hrfabms for $[M+Na]^+$, found *m/z* 541.2757, calcd 541.2777). The ^{1}H-nmr spectrum indicated that its three acetyl groups were likely to be at C-2, C-5, and C-10 as a result of signals at 2.04, 2.05, and 2.17 ppm. This observation was also supported by the companion ^{13}C-nmr signals at 70.61, 78.24, and 70.12 ppm. The presence of a methylene quartet signal at 2.27 ppm (J=7.6 Hz) and its carbon signal at 27.96 ppm, a methyl triplet signal at 1.12 ppm (J=7.6 Hz) and a carbon signal at 9.17 ppm, as well as the H-14 signal at 5.01 ppm (dd, J=9.2 and 5.7 Hz) and a carbon signal at 70.37 ppm, all indicated that the propionyl group was affixed to C-14.

Compound **4** showed a molecular formula of $C_{30}H_{44}O_8$, by hrfabms ($[M+Na]^+$, found *m/z* 555.2944, calcd 555.2939). The presence of an isobutyryl group was supported by the observation of two methyl doublets at 1.13 and 1.14 ppm and one methine signal at 2.03 ppm in its ^{1}H-nmr spectrum. The isobutyryl group was placed at C-14 because of the upfield shift of H-14, compared with the corresponding signal in the structure of compound **1**, and the carbon signal of C-14 observed at 176.0 ppm. The singlets at 2.02, 2.05, and 2.18 ppm were assigned to the acetyl groups at C-2, C-5, and C-10, respectively.

Hrfabms of compound **5** showed an elemental composition of $C_{31}H_{46}O_8$ (found $[M+Na]^+$ *m/z* 569.3083, calcd 569.3090). The observed methyl triplet signal at 0.89 ppm and the methyl doublet signal at 1.11 ppm, along with multiplets for the methine signal at 2.35 ppm and the methylene signal at 1.47 ppm in the ^{1}H-nmr spectrum and the signals at 41.09, 26.71, 16.52, and 11.53 ppm in the ^{13}C-nmr spectrum of **5**, suggested the presence of a methylbutyrate ester functionality. The signals at 70.61, 78.18, and 70.12 (×2) ppm were assigned to C-2, C-5, C-10, and C-14, respectively.

Assignments of the ^{1}H- and ^{13}C-nmr spectra of compounds **2–5** are listed in Tables 1 and 2. Compounds **1–5** showed no activity in a tubulin assembly assay at a concentration as high as 10 μM. This is presumably due to their lack of the C-13 side-chain and oxetane ring which are necessary for tubulin binding (5).

A recent review has indicated that over one hundred taxoids have been isolated from the Taxaceae (6). Among them, taxoids with a C-4(20) exocyclic double bond comprise the most common group (6). However, unlike most of the taxoids in this group, yunnanxane [**1**] and its esters [**2–5**] also lack oxygen substituents at C-1, C-7, C-9, and C-13, but have, interestingly, an additional oxygen substituent at C-14.

TABLE 1. ^{1}H-Nmr Data of Compounds **2–5** ($CDCl_3$, 360 MHz).

Proton	δ_H (mult., J Hz)			
	2	**3**	**4**	**5**
1	1.89 (d, 2.2)	1.88 (d, 2.3)	1.87 (d, 2.1)	1.88 (d, 2.4)
2	5.36 (dd, 6.5, 2.2)	5.36 (dd, 6.5, 2.3)	5.36 (dd, 6.6, 2.3)	5.36 (dd, 6.6, 2.3)
3	2.93 (d, 6.5)	2.94 (d, 6.5)	2.94 (d, 6.5)	2.94 (d, 6.6)
5	5.29 (t, 2.9)	5.29 (t, 2.9)	5.29 (t, 2.9)	5.29 (t, 2.8)
6	1.81 (m)	1.81 (m)	1.81 (m)	1.81 (m)
7	1.24 (m), 1.98 (m)	1.24 (m), 1.98 (m)	1.24 (m), 1.98 (m)	1.24 (m), 1.98 (m)
9	2.39 (m), 1.65 (dd, 14.9, 5.6)	2.39 (m), 1.64 (dd, 14.9, 5.6)	2.38 (m), 1.65 (m)	2.39 (m), 1.65 (m)
10	6.06 (dd, 5.6, 12.1)	6.06 (dd, 5.7, 12.1)	6.06 (dd, 5.6, 12.1)	6.06 (dd, 5.7, 12.1)
13	2.82 (dd, 19.0, 9.2), 2.40 (m)	2.83 (dd, 19.0, 9.2), 2.39 (m)	2.82 (dd, 19.0, 9.2), 2.38 (m)	2.82 (dd, 19.0, 9.2), 2.40 (m)
14	5.00 (dd, 9.2, 4.8)	5.01 (dd, 9.2, 5.7)	4.98 (dd, 9.2, 4.8)	4.99 (dd, 9.1, 4.7)
16	1.66 (s)	1.66 (s)	1.67 (s)	1.66 (s)
17	1.13 (s)	1.13 (s)	1.13 (s)	1.13 (s)
18	2.09 (d, 0.6)	2.09 (d, 0.5)	2.09 (d, 0.8)	2.09 (d, 0.8)
19	0.84 (s)	0.85 (s)	0.85 (s)	0.84 (s)
20	5.27 (s), 4.86 (t, 1.1)	5.26 (s), 4.86 (t, 1.2)	5.26 (s), 4.84 (t, 1.3)	5.27 (s), 4.86 (t, 1.1)
2′	2.04 (s)	2.27 (q, 7.6)	2.03 (m)	2.35 (m)
3′		1.12 (t, 7.6)	1.14 (d, 7.1)[a]	1.47 (m)
4′			1.13 (d, 6.9)[a]	0.89 (t, 7.4)
5′				1.11 (d, 7.4)
2-$OCOCH_3$	2.01 (s)	2.04 (s)	2.02 (s)	2.01 (s)
5-$OCOCH_3$	2.05 (s)	2.05 (s)	2.05 (s)	2.05 (s)
10-$OCOCH_3$	2.17 (s)	2.17 (s)	2.18 (s)	2.18 (s)

[a]Interchangeable assignments.

TABLE 2. APT ^{13}C-Nmr Data of Compounds **2–5** ($CDCl_3$, 90 MHz).

Carbon	Compound			
	2	**3**	**4**	**5**
1	59.01	59.12	59.23	59.33
2	70.60	70.61	70.60	70.61
3	42.16	42.15	42.16	42.20
4	142.33	142.33	142.29	142.33
5	78.26	78.24	78.22	78.18
6	28.88	28.88	28.89	28.91
7	33.84	33.84	33.84	33.87
8	37.34	37.32	37.28	37.30
9	43.94	43.94	43.92	43.95
10	70.10	70.12	70.13	70.12
11	135.43	135.41	135.38	135.43
12	134.71	134.79	134.82	134.82
13	39.49	39.61	39.65	39.66
14	70.60	70.37	70.26	70.12
15	39.68	39.66	39.65	39.66
16	25.44	25.42	25.41	25.42
17	31.74	31.74	31.72	31.70
18	21.78	21.83	21.86	21.83
19	22.45	22.47	22.47	22.47
20	116.91	116.88	116.85	116.77
1′	169.88[a]	173.30	176.00	175.52
2′	21.38	27.96	34.02	41.01
3′		9.17	18.84	26.71
4′			18.84	11.53
5′				16.52
2-$OCOCH_3$	170.16, 21.38	170.16, 21.40	170.23, 21.39	170.12, 21.35
5-$OCOCH_3$	169.98, 20.91	169.98, 20.92	169.99, 20.93	169.87, 20.92
10-$OCOCH_3$	169.69[a], 21.38	169.72, 21.40	169.80, 21.35	169.69, 21.35

[a]Assignments may be reversed.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—See Ma *et al.* (1).

MATERIAL, EXTRACTION, AND ISOLATION.—A cell suspension culture initiated from stem tissue of *Taxus chinensis* var. *mairei* was grown using a modified Gamborgs B5 medium (7) in an 8-liter bioreactor. Cells were separated from this medium by filtration and stored at −80°. Biomass (86 g) was extracted with MeOH. The MeOH extract was then partitioned between CH_2Cl_2 and H_2O. After drying *in vacuo*, the CH_2Cl_2 extract was subjected to cc on Si gel with hexane/CH_2Cl_2/MeOH gradients and prep. hplc with CH_3CN/H_2O gradients following recrystallization to give compounds **1** (31 mg), **2** (12 mg), **3** (21 mg), **4** (25 mg), and **5** (34 mg).

Yunnanxane [**1**].—Prisms, mp 161°; [α]D +40.38° (*c*=0.104, MeOH); ir ν max (KBr) 3452, 2983, 1736, 1373, 1238, 1017, 961 cm^{-1}; fabms (NBA) *m/z* 585, 562, 503, 461, 444, 401, 385, 343, 325; hrfabms for $C_{31}H_{46}O_9Na$: found *m/z* 585.3047, calcd 585.3040; ^{1}H- and ^{13}C-nmr data and X-ray crystallography results are as previously reported (2).

2α,5α,10β,14β-Tetra-acetoxy-4(20),11-taxadiene [**2**].—Needles, mp 170°; [α]D +31.95° (*c*=0.338, MeOH); ir ν max (KBr) 3447, 2931, 1735, 1630, 1371, 1242, 1018, 953 cm^{-1}; fabms (NBA) *m/z* 527 (M^++Na), 504 (M^+), 445 (M^+−OAc), 385 (*m/z* 503−OAc), 343, 325, 265; hrfabms for $C_{28}H_{40}O_8Na$: found *m/z* 527.2629, calcd 527.2621; ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2.

2α,5α,10β-Triacetoxy-14β-propionyloxy-4(20),11-taxadiene [**3**].—Needles, mp 195°; [α]D +41.37° (*c*=0.087, MeOH); ir ν max (KBr) 3454, 2931, 1732, 1655, 1377, 1238, 1021, 960 cm^{-1}; fabms (NBA) *m/z* 541 (M^++Na), 518 (M^+), 459 (M^+−OAc), 445 (M^+−$OCOCH_2CH_3$), 385 (*m/z* 459−$OCOCH_2CH_3$), 343, 325, 265; hrfabms for $C_{29}H_{42}O_8Na$: found 541.2757, calcd 541.2777; ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2.

2α,5α,10β-Triacetoxy-14β-isobutyryloxy-4(20),11-taxadiene [**4**].—Needles, mp 183°; [α]D

+33.96° (*c*=0.157, MeOH); ir ν max (KBr) 3452, 2983, 1736, 1660, 1373, 1238, 1017, 961 cm^{-1}; fabms (NBA) *m/z* 555 (M^++Na), 445 (M^+−methylbutyrate), 385 (*m/z* 445−HOAc), 343, 325, 265; hrfabms for $C_{30}H_{44}O_8Na$: found *m/z* 555.2944, calcd 555.2934; ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2.

2α,5α,10β-Triacetoxy-14β-(2-methyl)-butyryloxy-4(20),11-taxadiene [**5**].—Prisms, mp 106°; [α]D +36.96° (*c*=0.339, MeOH); ir ν max (KBr) 3446, 2935, 1735, 1662, 1373, 1237, 1015, 961 cm^{-1}; fabms *m/z* 569 (M^++Na), 487 (M^+−OAc), 445 (M^+−$OCOCHCH_3CH_2CH_3$), 385 (*m/z* 445−HOAc), 343, 325, 265; hrfabms for $C_{31}H_{46}O_8Na$: found *m/z* 569.3083, calcd 569.3090; ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2.

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