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Three rearranged 2(3 → 20) *abeo*taxanes from the bark of *Taxus mairei*

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Abstract

Three new rearranged 2(3 → 20)*abeo*taxane diterpenoids were isolated from the bark of the Chinese yew, *Taxus mairei*. Their structures were established as 2α,5α,7β,13α-tetraacetoxy-10β-hydroxyl-2(3 → 20)*abeo*taxan-9-one; 7β,13α-diacetoxyl-2α,5α,10β-trihydroxyl-2(3 → 20)*abeo*taxan-9-one and 2α,7β-diacetoxy-5α,10β,13α-trihydroxyl-2(3 → 20)*abeo*taxane-9-one on the basis of 1D and 2D NMR data. This is the first example of rearranged 2(3 → 20)*abeo*taxanes were isolated from *Taxus mairei*. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Taxus mairei*; Taxaceae; 2(3 → 20)*abeo*taxanes; Bark; Taxane diterpenoids

1. Introduction

In view of the demonstrated clinical effectiveness of Taxol® (paclitaxel) in advanced ovarian, breast and other carcinomas, there has been an intensive effort to search for other members of the taxane group, and this situation has led to more than 250 taxoids having been isolated so far (Appendino, 1995a; Wu & Lu, 1996; Kingston, Molinero & Rimoldi, 1993). Of them, 12 rearranged 2(3 → 20)*abeo*taxoids were isolated from *Taxus baccata* (Graf, Kirfel, Wolff & Breimaier, 1982; Poupat, Ahond & Potier, 1994; Appendio et al., 1994; Barboni et al., 1995), *T.* ×*media* (Rao, Chandrasekhara & Juchum, 1996; Hall, Tong & Chang, 1997), *T. yunnanensis* Yue, Fang, Liang, He & Jing, 1995), and *T. cuspidata* (Hosoyama, Inubushi, Katsu, Shigemori & Kobayashi, 1996; Morita et al., 1998; Kobayashi et al., 1994). In the previous study, our group isolated a novel taxoid from the Japanese yew Sugiyama, Oritani & Oritani, 1994). As a continuation of this work, we investigated the composition of the bark of *Taxus mairei*.

Taxus mairei, indigenous to China, is a tall evergreen tree mainly distributed in Jiangxi, Taiwan and Fujian provinces in the southeast of the People's Republic of China. Previous studies on this plant led to the isolation of more than 20 new taxoids including normal 6/8/6 membered taxanes, 9(10 → 20)*abeo*abietanes, rearranged 5/7/6 membered 11(15 → 1)*abeo*taxanes and bicyclic taxanes (Liang & Kingston, 1993; Liang, Min & Niwa, 1988; Liang, Min, Mizuno, Tanaka & Iinuma, 1988a, 1988b; Min, Jiang & Liang, 1989; Shen, Tai & Chen, 1996; Shen, Tai, Hsieh & Chen, 1996; Shen & Chen, 1997; Yang, Fang & Cheng, 1996; Shi, Oritani, Sugiyama & Kiyota, 1998a, 1998b; Shi, Oritani & Sugiyama, 1999; Tanaka et al., 1996; Yek, Wang, Liu & Chen, 1988). Recently we re-examined the bark of this plant from trees growing in a different area from those examined by other groups, and three new rearranged 2(3 → 20)*abeo*taxane diterpenoids with a unique 6/10/6 skeleton were isolated. A discussion of the isolation and structure characterization of these new taxoids is presented in this communication.

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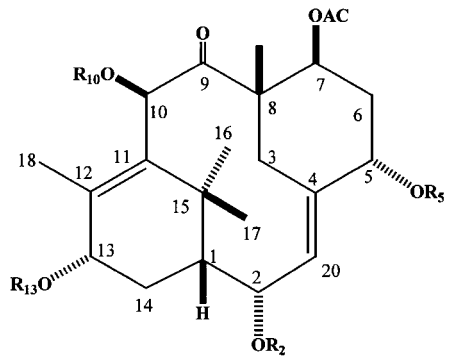

1 R_2=Ac R_5=Ac $R1_0$=H R_{13}=Ac
2 R_2=H R_5=H R_{10}=H R_{13}=Ac
3 R_2=Ac R_5=H R_{10}=H R_{13}=H
4 R_2=Ac R_5=Ac R_{10}=Ac R_{13}=Ac

Fig. 1. 2(3 → 20)*Abeo*taxanes from the bark of the Chinese yew, *Taxus mairei*.

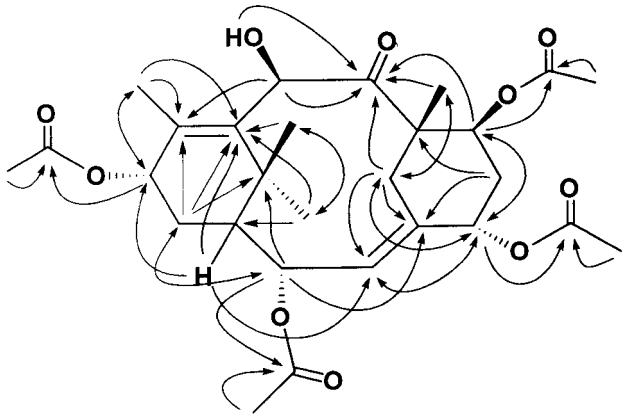

Fig. 2. Selected C/H correlations obtained from HMBC spectrum of **1** (most protons are not shown for clarity).

2. Result and discussion

Compound 1 was isolated as a colorless gum (Fig. 1). The IR absorptions at 3420, 1735 and 1700 cm^{-1} were attributed to hydroxyl, ester and ketone groups, respectively. FAB-MS gave ion peaks at *m/z*: 573 $(M+K)^+$, 557 $(M+Na)^+$, 475 $(M+H–AcOH)^+$. HR-FAB-MS analysis gave a peak at *m/z* 557.2380 $(M+Na)^+$ corresponding to the molecular formula $C_{28}H_{38}O_{10}Na$ (calcd. 557.2360). Thus, the molecular formula was established as $C_{28}H_{38}O_{10}$. The ^1H NMR spectrum of **1** showed some similarities to that of other taxoid diterpenes. The four distinctive methyl groups were seen in the upfield region (1.17, 1.19, 1.33 and 1.94 ppm), four acetate methyl groups were seen in the relative downfield (2.16, 2.24, 2.01 and 2.00 ppm) as is seen in all taxoids, and a doublet was detected in the downfield region (5.32 ppm, J = 2.75 Hz) which is similar to hydroxylated H-10 in other taxoid spectra (Liang et al., 1988a; Min et al., 1989). In the ^1H–^1H COSY spectrum, no proton corresponding to H-9 (also located downfield between 5.0 and 6.0 ppm) was identified, but a deuterium exchangeable doublet was seen at 4.21 ppm (J = 2.75 Hz). It was assumed that C-9 was a carbonyl and a free hydroxyl group was attached to C-10. This pattern is also indicative of a taxoid, and was verified by the HMBC spectrum because H-10 correlated with only one carbonyl signal at 211.98 ppm. A broad singlet was found at 5.52 ppm, the region of H-5. It is always oxygenated in naturally occurring taxoids where it is usually located, and its chemical shift suggested that an acetoxy group is attached to C-5. In the ^1H–^1H COSY spectrum, H-5 correlated with two signals at 2.19 (1H, m) and 1.98 ppm (1H, m), which were assignable to H-6α and H-6β, respectively. In turn, these two signals also coupled with the signal at 5.09 ppm (1H, dd, J = 3.34, 12.20 Hz), which was assigned to H-7. The downfield chemical shift implied an acetoxy group was attached to C-7, and this conclusion was further supported by the HMBC spectrum. Other protons also known to resonate downfield due to oxygenation of their shared carbons are H-13 and H-2. H-13 resonates at 5.49 ppm (1H, d, J = 10.34 Hz), which correlated with two alkene carbons at 132.25, 131.68 ppm and a carbonyl carbon at 170.47 ppm. In turn, these two alkene carbons correlated with H-10 in the HMBC spectrum, indicating an acetoxy located on C-13. H-13 correlated with two signals at 1.83 (1H, dd, J = 16.51, 3.30 Hz) and 2.72 ppm (1H, dd, J = 16.51, 8.15 Hz) in the ^1H–^1H COSY spectrum, which were assigned to H-14α and 14β. 14β coupled with the signal at 1.67 ppm (1H, dd, J = 2.20, 7.72 Hz), which was assigned to H-1β. In turn, H-1β coupled with H-2 at 5.72 ppm (1H, dd, J = 2.20, 9.86 Hz), and the chemical shift suggested an acetoxy group connected to C-2. Acetylation of compound **1** produced acetated **4** with five acetate signals, thus showing that compound 1 has one hydroxyl and four acetoxyl groups. H-2 coupled with the signal at 5.54 ppm (1H, br d, J = 10.32 Hz) instead of any of the signals at 2.8–4.0 ppm in the ^1H NMR spectrum, which is characteristic of H-3α in most taxoids (Appendino, 1995b). The ^1H NMR spectrum, however, lacked a pair of singlets corresponding to an AX system (chemical shift difference of about 0.30 ppm) of the exocyclic methylene protons, as seen in many other taxoids. Additionally, no AB quartet (at about 4.20 ppm with a coupling constant of about 9 Hz) corresponding to an oxetane ring was observed (Appendino, 1995b). An isolated spin system of doublets at 2.79 and 2.00 ppm with the coupling constant J = 15.40 Hz occurred instead.

In the ^{13}C NMR spectrum, the four peaks in the alkene region (136.07, 132.25, 131.68 and 124.55 ppm) revealed the existence of two double bonds, of which the one at 124.55 ppm carried a proton, while others

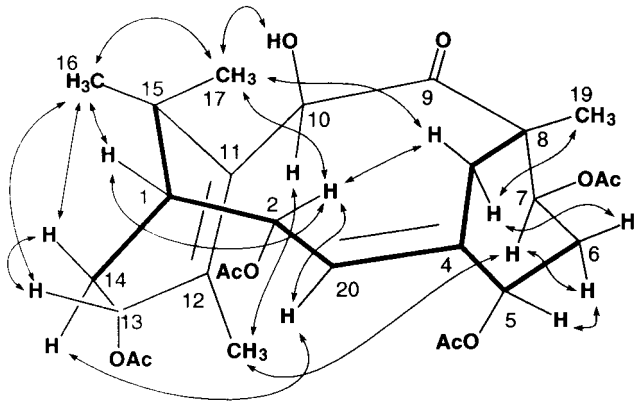

Fig. 3. Relative stereochemistry of **1**. Arrows denote NOESY correlations.

were quaternary as shown in the HMQC spectrum. The signals at 132.25 and 131.68 ppm were assigned as C-11 and C-12, respectively, based on the HMBC spectrum. Because the signal at 124.55 ppm was correlated only with the one-proton broad doublet at 5.54 ppm in the HMQC spectrum, the C-4/C-20 double bond might be endocyclic, instead of exocyclic which is common in many natural taxoids. Keeping the above observations in view, the skeleton of compound **1** was elucidated as consisting of a 6/10/6 membered ring with a C-4/C-20 endocyclic double bond, which belongs to a 2(3 → 20)*abeo*taxane derivative as in taxane A (Appendio et al., 1994; Graf et al., 1982), but without the N,N-dimethyl phenylisoserine side chain at C-5. The complete assignment of ^{1}H and ^{13}C data of compound **1** was made with the help of ^{1}H–^{1}H COSY, DEPT, HMBC (Fig. 2) and HMQC spectra. Based on the coupling constants and the NOESY spectra, the protons at 2, 5, 7, 10 and 13 were assigned to β, β, α, α and β, respectively, having the same configurations as found in taxine A, and in most natural taxoids. The relative stereochemistry of compound **1** was elucidated as shown in Fig. 3 by the results of the NOESY experiments. The NOESY spectrum showed NOE correlations between H-2 and H-3α, 17-CH_3, and between H-20 and H-14α, which indicated an *E*-configuration of the C-4/C-20 double bond. In addition, correlations between H-13β and 16-CH_3, H-7α and 18-CH_3, and H-3b and H-6β implied that both rings A and B had adopted boat conformations. This was in accordance with the conformation of taxine A. Therefore, compound **1** was characterized unambiguously as 2α,5α,7β,13α-tetraacetoxy-10β-hydroxyl-2(3 → 20)*abeo*-taxan-9-one.

Compound **2** was obtained in the form of colorless crystals (Fig. 1). The molecular formula $C_{24}H_{34}O_8$ was established by the combined analysis of FAB-MS and ^{13}C NMR spectroscopic data. Its ^{1}H NMR spectrum closely resembles that of compound **1** with the exception of H-5 upfield to 4.49 ppm and H-2 upfield to 4.64 ppm as shown in the ^{1}H NMR and ^{1}H–^{1}H COSY spectra. Detailed analysis of ^{1}H NMR, ^{1}H–^{1}H COSY, ^{13}C NMR, DEPT, HMBC and HMQC spectra allowed the structure of compound **2** to be assigned as 7β,13α-diacetoxy-2α,5α,10β-trihydroxyl-2(3 → 20)*abeo*-taxan-9-one.

Compound 3 was obtained as a colorless gum (Fig. 1). HR-FAB-MS analysis established the molecular formula as $C_{24}H_{34}O_8$. Its ^{1}H NMR spectrum closely resembled that of compound **2** except that H-13 was shifted upfield to 4.24 ppm and H-2 was shifted downfield to 5.72 ppm, as shown in the ^{1}H NMR and ^{1}H-^{1}H COSY spectra. ^{13}C NMR spectrum could not be obtained for compound **3** due to a lack of sample. The structure of compound **3** was proposed to be 2α,7β-diacetoxy-5α,10β,13α-trihydroxyl-2(3 → 20)*abeo*-taxane-9-one by analysis of its ^{1}H NMR, ^{1}H–^{1}H COSY spectra and by comparison with compound **2**.

This is the first report of the occurrence of 2(3 → 20)*abeo*taxanes in *Taxus mairei*.

3. Experimental

3.1. General

Silica gel 60 (Merck 100–200 mesh) was used for conventional CC. Precoated silica gel Kieselgel 60 F_{254} plates (0.2 mm thick) were used for thin layer chromatography, and the spots were detected by ultraviolet (UV) illumination and by spraying 10% H_2SO_4, followed by heating. Melting points were measured on an MRK micro-melting point apparatus and are uncorrected. Optical rotations were recorded on a SEPA-300 polarimeter. The IR spectrum was recorded on a Jasco IR-810 spectrometer. Optical rotation was measured on a Horiba SEPA-300 polarimeter. MS were obtained on a JMS-Dx 305 HF mass spectrometer, using the FAB method with glycerol as a matrix. NMR spectra were taken on Varian GEMINI 2000/300 and Varian Unity Inova 500 spectrometers operating at 300 and 500 MHz for ^{1}H and 125 MHz for ^{13}C in $CDCl_3$ at ambient temperature. Coupling constants are given in Hz, and ^{1}H chemical shifts data were recorded in ppm downfield from TMS as an internal reference. ^{1}H–^{1}H connectivities were determined via COSY experiments. ^{13}C shifts were based on the $CDCl_3$ signal at 77.0 ppm.

3.2. Plant material

The bark of *T. mairei* was collected from the Jiangxi province, in the southeast of the People's Republic of China, in the autumn of 1995. The plant material was authenticated by professor R. L. Liu, of Zhangzhou

Table 1
^{1}H-NMR spectral data of **1**, **2**, **3** and **4** ($CDCl_3$, ppm, 300 MHz and 500 MHz)

Position	**1**		**2**		**3**		**4**	
	^{1}H	*J*(Hz)	^{1}H	*J*(Hz)	^{1}H	*J*(Hz)	^{1}H	(Hz)
1	1.67 dd	7.72, 2.20	1.63 dd	7.70, 2.20	1.73 dd	1.90, 6.82	1.73 dd	8.80, 2.00
2	5.72 dd	9.86, 2.20	4.64 dd	10.09, 2.20	5.72 dd	9.62, 1.90	5.75 dd	9.62, 2.00
3a	2.79 d	15.40	2.57 d	15.38	2.75 d	15.66	2.75 d	15.36
3b	2.00 d	15.40	1.96 d	15.38	1.92 d	15.66	1.91 d	15.36
5	5.52 br s		4.49 br s		4.49 br s		5.51 br s	
6α	2.19 m		2.63 m		2.10 m		2.16 m	
6β	1.98 m		2.08 m		1.95 m		2.00 m	
7	5.09 dd	12.20, 3.34	5.08 dd	11.81, 4.10	5.13 dd	12.36, 5.37	5.24 dd	12.91, 4.12
10	5.32 d	2.75	5.47 d	2.75	5.32 d	3.00	6.30 s	
13	5.49 br d	10.34	5.37 d	10.16	4.24 m		5.47 br d	10.62
14α	1.83 dd	16.51, 3.30	2.07 m		2.10 m		2.75 br dd	18.23, 10.64
14β	2.72 br dd	16.51, 8.15	2.63 m		2.49 br dd	17.21, 9.07	1.85 dd	18.23, 2.20
16	1.17 s		1.15 s		1.16 s		1.12 s	
17	1.19 s		1.21 s		1.19 s		1.27 s	
18	1.94 d	1.1	1.94 br s		1.92 d	1.1	1.95 d	0.82
19	1.33 s		1.30 s		1.26 s		1.30 s	
20	5.54 br d	10.32	5.70 dt	10.09, 1,92	5.56 br d	9.62	5.45 br d	9.62
10-OH	4.21 d	2.75	4.19 d		4.18 d	3.00		
2-Oac	2.24 s				2.19 s		2.24 s[a]	
5-Oac	2.16 s						2.14 s[a]	
7-AcO	2.01 s		2.02 s		2.00 s		2.01 s[a]	
10-Oac							2.17 s[a]	
13-Oac	2.00 s		2.17 s				2.06 s[a]	

[a] Indicated these signals may be exchangable.

Forestry School, where a voucher specimen has been deposited. The material was stored at 0°C before extraction.

3.3. Extraction and isolation

Air dried bark (13.25 kg) was extracted with MeOH twice at room temp. The MeOH extracts were concd to residue under red. pres. This residue was diluted with water and the aq. soln was extracted with EtOAc three times. The combined EtOAc extract, upon evap, yielded 88 g of dark syrup. Part of this (65 g) was subjected to silica gel column chromatography, eluted with hexane and a gradient of hexane-EtOAc (2:1, 1:1, 1:2, 1:4 and EtOAc), and six fractions were obtained. Fr. 2 was repeatedly chromatographed on the silica gel column and plates, eluted or developed with hexane-acetone and chloroform-methanol, and finally afforded compounds **1** (11.4 mg), **2** (6.5 mg), and **3** (0.8 mg).

3.4. 2α,5α,7β,13α-Tetraacetoxy-10β-hydroxyl-2(3 → 20)abeotaxane-9-one (*1*)

Colorless gum, $[\alpha]_D^{25}$: −11.2°C (*c* 0. 002, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1}: 3420, 1735, 1700, 1370, 1230. FAB-MS *m/z*: 573 ($[M+K]^+$), 557 ($[M+Na]^+$), 475 ($[M+H–AcOH]^+$), 457 ($[M+H–AcOH–H_2O]^+$), 415 ($[M+H–2AcOH]^+$), 397 ($[M+H–2AcOH–H_2O]^+$), 355 ($[M+H–3AcOH]^+$), 295 ($[M+H–4AcOH]^+$), and 43 (100); HR-FAB-MS 557.2380 ($C_{28}H_{38}O_{10}Na$, calcd. 556.2360). ^{1}H and ^{13}C NMR spectra (300 MHz for ^{1}H, 125 MHz for ^{13}C, $CDCl_3$, TMS) see Tables 1 and 2.

3.5. 7β,13α-Diacetoxy-2α,5α,10β-trihydroxyl-2(3 → 20)abeotaxane-9-one (*2*)

Colorless crystal, mp 220–222°, $[\alpha]_D^{25}$: −28.5°C (*c* 0. 002, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1}: 3520, 3440, 1720, 1700, 1680, 1360, 1230. FAB-MS *m/z*: 433 ($[M+H–H_2O]^+$), 415 ($[M+H–2H_2O]^+$), 373 ($[M+H–H_2O–AcOH]^+$), 355 ($[M+H–2H_2O–AcOH]^+$), 313 ($[M+H–H_2O–2AcOH]^+$), 295 ($[M+H–2AcOH–2H_2O]^+$), 105, and 43 (100); HR-FAB-MS 433.2235 ($C_{24}H_{33}O_7$, calcd. 433.2224). ^{1}H and ^{13}C NMR spectra (300 MHz for ^{1}H, 125 MHz for ^{13}C, $CDCl_3$, TMS) see Table 1 and 2.

3.6. 2α,7β-Diacetoxy-5α,10β,13α-trihydroxyl-2(3 → 20)abeotaxane-9-one (*3*)

IR ν_{max} ($CHCl_3$) cm^{-1}: 3400, 1730, 1710, 1660, 1360, 1230. FAB-MS *m/z*: 451 ($[M+H]^+$), 433 ($[M+H–H_2O]^+$), 415 ($[M+H–2H_2O]^+$), 391 ($[M+H–AcOH]^+$), 373 ($[M+H–AcOH–H_2O]^+$), 331 ($[M+H–2AcOH]^+$), 313 ($[M+H–2AcOH–H_2O]^+$), 185 and 93

Table 2
^{13}C-NMR spectral data of **1**, **2** and **4** ($CDCl_3$, ppm, 125 MHz)

Position	**1**[a]	**2**[a]	**4**[b]
1	47.20	49.69	46.50
2	71.01	67.31	76.92
3	35.24	35.60	37.50
4	136.07	136.67	132.92
5	76.72	68.35	69.00
6	32.07	35.60	35.09
7	70.81	70.53	70.10
8	52.89	52.54	52.75
9	211.98	213.25	205.50
10	77.01	77.23	77.57
11	131.68	135.11	138.75
12	132.25	133.90	133.15
13	69.71	69.96	69.69
14	27.26	25.47	29.32
15	37.69	37.19	32.00
16	32.16	34.95	31.28
17	24.70	24.19	24.67
18	16.62	18.34	16.52
19	21.01	21.05	26.80
20	124.55	128.66	123.55
2-OAc	21.41		21.10[c]
	169.34		170.30[d]
5-OAc	20.59		20.41[c]
	170.02		170.06[d]
7-AcO	21.56	20.98	20.01[c]
	170.30	169.82	169.36[d]
10-OAc			21.12[c]
			170.23[d]
13-AcO	21.40	20.92	20.98[c]
	170.47	170.03	169.71[d]

[a] Assignment based on DEPT, HMBC and HMQC spectra.
[b] Assignment was according to the analogues of this compound.
[c] Indicated these signals may be exchangable.
[d] Indicated these signals may be exchangable.

(100); HR-FAB-MS 451.2336 ($C_{24}H_{35}O_8$, calcd. 451.2330). ^{1}H NMR spectrum (300 MHz, $CDCl_3$, TMS) see Table 1.

3.7. Acelatylation of compound *1*

10 mg **1** in 2 ml of pyridine was treated with 2 ml Ac_2O overnight. The mixture was poured into ice-H_2O and extracted with $CHCl_3$. The organic layer was washed with H_2O, dilute HCl, aqueous $NaHCO_3$ and H_2O, dried with $MgSO_4$, filtered, evaporated and separated to yielded 6 mg of **4**.

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