Two New Rearranged Taxoids from *Taxus wallichiana* Zucc.

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Two new rearranged taxane diterpenoids, 5α,7β,10β,13α-tetrahydroxy-2α,9α,15-triacetoxy-11(15→1)-*abeo*-taxa-4(20),11-diene (1) and 5α,9α,10β,13α-tetraacetoxy-15-hydroxy-11(15→1)-*abeo*-taxa-4(20),11-diene (2) have been isolated from the barks of *Taxus wallichiana*. The structures of these compounds have been confirmed by modern spectroscopic techniques.

Key words *Taxus wallichiana*; Taxaceae; rearranged taxoids

Taxus wallichiana Zucc. is a member of the family Taxaceae which is commonly known as the Himalayan yew.[1)] The plant is widely distributed in Pakistan and India, and used in the traditional systems of medicine.[2)] Its leaves are reported to have sedative, antispasmodic, and aphrodisiac properties and have also been used for the treatment of asthma, indigestion and epilepsy. The investigations on the genus *Taxus* is important mainly due to the presence of taxol and structurally-related taxanes which are clinically used against ovarian and breast cancers.[3,4)] Several comprehensive reviews have been published on the phytochemistry of *Taxus* species.[5—8)] Our previous work on this plant has resulted in the isolation of several new taxane derivatives.[9)]

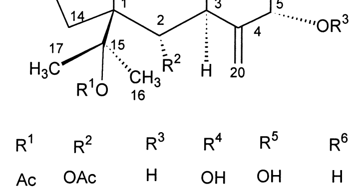
The barks of *T. wallichiana* were collected from Hainah in Leepa Valley, 2900 meters above the sea level, District Muzaffarabad, Azad Kashmir (Pakistan) in December, 1996. Column and thin-layer chromatographic separation of the methanolic extract of the barks yielded two new brevifoliol analogues **1** and **2**.

The compound **1** was isolated as colorless gum. The electron impact (EI)-MS of **1** showed the M^+-CH_3COOH ion at *m/z* 450. In the FAB-MS the $(M+1)^{+1}$ ion appeared at *m/z* 511. The high resolution (HR)-EI-MS showed the $M^+-CH_3COOH-H_2O$ ion at *m/z* 432.2087 ($C_{24}H_{32}O_7$, Calcd 432.2079). The molecular formula was therefore deduced to be $C_{26}H_{38}O_{10}$ corresponding to eight degrees of unsaturation in the molecule. The UV spectrum showed only the end absorption. The IR spectrum displayed intense absorptions at 1735 (CO), 3194 (C–H) and 3628 (OH) cm^{-1}.

The ^{1}H-NMR spectrum of **1** showed seven methyl singlets at δ 1.56 (Me-19), 1.70 (Me-18), 1.04 (Me-16), 1.19 (Me-17), 1.92 (–OCOMe), 1.93 (–OCOMe) and 1.95 (–OCOMe). The exomethylenic protons resonated as broad singlets at δ 4.90 (H-20a) and 5.40 (H-20b), while H-5β and H-7α appeared each as double doublets at δ 4.80 ($J_{5\beta,6\alpha}$=8.5 Hz, $J_{5\beta,6\beta}$=6.0 Hz, H-5β) and 4.59 ($J_{7\alpha,6\beta}$=9.5 Hz, $J_{7\alpha,6\alpha}$=7.5 Hz, H-7α). Furthermore, a triplet resonated at δ 4.52 was assigned to H-13β ($J_{13\beta,14}$=7.5 Hz, H-13β). The spectrum also exhibited three 1H doublets resonating at δ 5.83 ($J_{2\beta,3\alpha}$=9.5 Hz, H-2β), 4.98 ($J_{9\beta,10\alpha}$=4.0 Hz, H-9β) and 4.76 ($J_{10\alpha,9\beta}$=4.0 Hz, H-10α). H-2 (δ 5.83) and H-9β (δ 4.98) both geminal to acetoxy groups, showed strong heteronuclear multiple bond connectivity (HMBC) correlations with ester carbonyl carbons. The third acetoxy group has no geminal protons and it was accordingly placed at C-15 (δ 76.3). The coupling constants of H-9β and H-10α were found to be 4.0 Hz due to small dihedral angle which was supported by the Drieding model and the data of reported conformers of related skeleton.[10,11)] In addition, the signal of the H-3α methine appeared at δ 3.04 as a doublet ($J_{3\alpha,2\beta}$=9.0 Hz).

The correlation spectroscopy (COSY)-45° spectrum revealed the presence of four isolated spin systems in the molecule. The H-5β (δ 4.80) displayed a cross-peak with H-6β (δ 2.01) and H-6α (δ 1.71) which were in turn coupled with H-7α (δ 4.59). H-9β (δ 4.98) showed strong vicinal couplings with H-10α (δ 4.76), whereas H-13β (δ 4.52) showed couplings with H-14α (1.70) and H-14β (δ 2.30). The vicinal coupling of H-2β (δ 5.83) with H-3α (δ 3.04) indicated the presence of an acetoxy group at C-2.

The ^{13}C-NMR spectra (Broad Band and distortionless enhancement by polarization transfer (DEPT)) of **1** showed the resonances for all twenty six carbon atoms in the molecule corresponding to seven methyl, three methylene, seven methine and nine quaternary carbons (Table 1). 9-Deacetyl-9-benzoyl-10-debenzyol-5-cinnamoyl-taxchinin A, a compound that was isolated from *Taxus mairei* has spectral data closely comparable to our compound **1**.[10)] The HMBC spectrum was recorded to locate the quaternary carbons and confirm various chemical shift assignments. The spectrum showed that the proton resonating at δ 4.80 (H-5β) displayed heteronuclear shift correlation ($^3J_{C-H}$) with the C-3 at δ 45.9. This suggested the close proximity of the C-3 methine to the C-5 hydroxyl group. H-9β (δ 4.98) showed correlations with the C-8 (δ 43.0), C-3 (δ 45.9) and C-10 (δ 66.5) which allowed the placement of one of the three acetoxy groups at C-9 (δ 77.4). The exomethylene protons at δ 4.90 (H-20_a) and 5.40 (H-20_b) exhibited shift correlation with the C-4 (δ



	R^1	R^2	R^3	R^4	R^5	R^6
1	Ac	OAc	H	OH	OH	H
2	H	H	Ac	H	OAc	Ac

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149.3). The proton at δ 4.76 (H-10α) geminal to the hydroxy group displayed interactions with C-11 (δ 135.0), C-9 (δ 77.4) and C-1 which indicated that the C-11 is linked with C-10, C-12 and C-1. The proton at δ 4.52 (H-13β) showed long-range heteronuclear correlations with the carbons resonated at δ 148.8 (C-12), 135.0 (C-11) and 67.3 (C-1). In addition, H-2β (δ 5.83) exhibited couplings with the C-1 (δ 67.3) and C-3 (δ 45.9) which concluded that one acetoxy group located between C-1 and C-3.

A literature survey revealed that C-1 of an *abeo*-taxane generally resonates between δ 67.0—68.0 when an acetoxy group present at C-2, whereas the same carbon resonates downfield between δ 63.0—64.5 in the absence of an acetoxy group.[12,13] Another strong evidence of the presence of an *abeo*-taxane skeleton was provided by the long-range interactions of both Me-16 (δ 1.04) and Me-17 (δ 1.19) with C-15 (δ 76.3) and C-1 (δ 67.3). The relative stereochemistry at C-1 for compounds **1** and **2** is supported by the previously developed stereochemistry which is reported as β for all known compounds of the same skeleton.[5—8]

The relative stereochemistry at various chiral centers of taxoid **1** was established by nuclear Overhauser effect spectroscopy (NOESY) experiment and coupling constants. The protons resonating at δ 1.56 (Me-19) showed correlations with H-2 (δ 5.83), H-6β (δ 2.01) and H-9β (δ 4.98). Similarly, H-5β (δ 4.80) also exhibited strong vicinal coupling with H-6β (δ 2.02). Based on these studies, the structure of **1** was deduced to be 5α,7β,10β,13α-tetrahydroxy-2α,9α,15-triacetoxy-11(15→1)-*abeo*-taxa-4(20),11-diene.

The compound **2** was also isolated as colorless gum. The EI-MS of compound **2** showed the M^+-CH_3COOH ion at *m/z* 460. The HR-EI-MS showed the loss of three –OAc groups, affording an $M^+-(3\times OAc)$ ion at *m/z* 343.1949 ($C_{21}H_{27}O_4$, Calcd 343.1909). The FAB-MS afforded the $(M+1)^{+1}$ ion at *m/z* 521 which indicated the loss of one molecule of acetic acid in the EI-MS. On the basis of mass, ^{1}H- and ^{13}C-NMR studies the molecular formula of **2** was concluded to be $C_{28}H_{40}O_9$ with nine degrees of unsaturation. The UV spectrum of **2** showed only the end absorption. The IR spectrum ($CHCl_3$) displayed absorptions at 1742 (–CO–), 2953 (C–H) and 3589 (–OH) cm^{-1}.

Table 1. ^{1}H- and ^{13}C-NMR Chemical Shift Assignments of **1** and **2**

Carbon No.	**1** ^{1}H-NMR, 500 MHz, δ (ppm), J=Hz	**1** ^{13}C-NMR, 75 MHz, δ (ppm)	**2** ^{1}H-NMR, 500 MHz, δ (ppm), J=Hz	**2** ^{13}C-NMR, 75 MHz, δ (ppm)
1	—	67.3	—	64.1
2	5.83 (d, J=9.5)	71.7	1.90—2.01 (m)	30.5
3	3.04 (d, J=9.5)	45.9	2.74 (br d, J=8.2)	41.4
4	—	149.3	—	148.7
5	4.80 (dd, J=8.5, 6.0)	68.8	5.34 (dd, J=8.6, 6.4)	76.6
6	6β 2.02 (m) 6α 1.71 (m)	36.6	1.90—2.31 (m)	28.2
7	4.59 (dd, J=9.5, 7.5)	72.7	1.90—2.01 (m)	28.6
8	—	43.0	—	42.9
9	4.86 (d, J=4.0)	77.4	5.75 (d, J=10.5)	79.2
10	4.76 (d, J=4.0)	66.5	6.16 (d, J=10.5)	69.9
11	—	135.0	—	139.3
12	—	148.8	—	146.8
13	4.52 (t, J=7.5)	77.9	5.57 (t, J=7.3)	80.7
14	14α 1.70 (overlapping signal) 14β 2.30 (dd, J=14.5, 7.0)	41.7	14α 2.01 (overlapping signal) 14β 2.60 (dd, J=13.6, 7.1)	45.2
15	—	76.3	—	76.7
16	1.04 (s)	21.0	1.35 (s)	26.0
17	1.19 (s)	21.7	1.21 (s)	27.1
18	1.70 (s)	12.7	1.78 (s)	11.6
19	1.56 (s)	14.9	0.78 (s)	17.2
20	20a 4.90 (br s) 20b 5.40 (br s)	114.1	20a 4.80 (br s) 20b 5.15 (br s)	112.8
OCOMe	1.92 (s)	28.1[a)]	1.99 (s)	20.6
OCOMe	—	173.0[b)]	—	171.5[b)]
OCOMe	1.93 (s)	28.2[a)]	2.01 (s)	20.7[a)]
OCOMe	—	172.0[b)]	—	171.6[b)]
OCOMe	1.95 (s)	26.8[a)]	2.02 (s)	21.3[a)]
OCOMe	—	172.1[b)]	—	170.9[b)]
OCOMe	—	—	2.03 (s)	20.9[a)]
OCOMe	—	—	—	172.4[b)]

a, b) Exchangeable assignments. ^{1}H- and ^{13}C-NMR techniques were performed in CD_3OD.

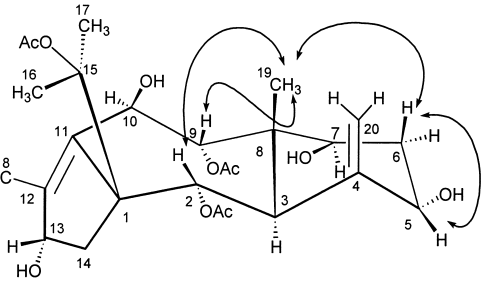

Fig. 1. Perspective Drawing of **1** with Some Important NOESY Interactions

The ^{1}H-NMR spectrum of **2** displayed eight 3H singlets for eight methyl groups at δ 0.78 (Me-19), 1.21 (Me-17), 1.35 (Me-16), 1.76 (Me-18), 1.99 (–OCOMe), 2.01 (–OCOMe), 2.02 (–OCOMe), and 2.03 (–OCOMe). The exomethylenic protons resonated at δ 4.80 and 5.15 as broad singlets, while H-5β appeared as double doublet at δ 5.34 ($J_{5\beta,6\alpha}$=8.6 Hz, $J_{5\beta,6\beta}$=6.4 Hz), whereas H-13β appeared as a triplet at δ 5.57 ($J_{13\beta,14}$=7.3 Hz). The spectrum also exhibited two 1H doublets at δ 5.75 ($J_{9\beta,10\alpha}$=10.5 Hz) and 6.16 ($J_{10\alpha,9\beta}$=10.5 Hz) which were assigned to H-9β and H-10α, respectively. The *trans*-orientation of H-9β and H-10α was inferred from large vicinal coupling constant ($J_{9\beta,10\alpha}$=10.5 Hz) between them. Furthermore, a characteristic signal of H-3α appeared at δ 2.74 as a broad doublet ($J_{3\alpha,2\beta}$=8.2 Hz). In the COSY-45° spectrum of **2**, the H-5β (δ 5.34) displayed a cross-peaks with the H-6α (δ 1.91) and H-6β (δ 2.31), which were in turn coupled with H-7α (δ 1.93) and H-7β (δ 2.30). Vicinal couplings were observed between H-9β (δ 5.75) and H-10α (δ 6.16), and between H-13β (δ 5.57) and H-14β (δ 2.60)/H-14α (2.01). H-13β also displayed a weak homoallylic coupling with Me-18 (δ 1.78). The couplings of H-2β (δ 2.01) and H-2α (δ 1.90) with the methine H-3α (δ 2.74) were also observed in the COSY-45° spectrum.

The ^{13}C-NMR data of **2** showed resonances for all twenty eight carbon atoms with eight methyl, five methylene, five methine and ten quaternary carbons (Table 1). 5,10,13-Acetyl-10-debenzoyl brevifoliol that had been isolated from *T. wallichiana* has spectral data closely comparable to our compound **2**.[13] On the basis of the above spectroscopic evidences, structure **2** was assigned to 5α,9α,10β,13α-tetraacetoxy-15-hydroxy-11(15→1)-*abeo*-taxa-4(20),11-diene.

Experimental

Optical rotations were measured on Jasco-DIP-360 digital polarimeter in MeOH. The IR spectra were recorded on a Jasco A-302 spectrophotometer. The UV spectra were measured on a Hitachi U-3200 spectrophotometer. The ^{1}H-NMR spectra were recorded on Bruker AM 300, AM 400 and AMX 500 spectrometers using UNIX data system at 300, 400 and 500 MHz, respectively, while ^{13}C-NMR spectra were recorded at 75, 100 and 125 MHz on the same instruments. The EI and HR-EI-MS were recorded on Jeol JMS HX 110 with the data system DA 5000 and on MAT 112S mass spectrometers.

Plant Material The bark of *T. wallichiana* Zucc. was collected from Hainah in Leepa Valley, 2900 m above the sea level, District Muzaffarabad, Azad Kashmir (Pakistan) in December, 1996. The plant was identified by Prof. Shafiq-ur-Rahman and a specimen was deposited in the Herbarium of the Botany Department, University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan.

Extraction and Isolation Powdered dried bark (23 kg) of *T. wallichiana* was soaked in methanol (40 l, 3 times) for one week. The methanolic extract was filtered and then evaporated under vacuum. The residue (1150 g) was partitioned between water (2 l) and *n*-hexane (5 l). The water layer was extracted with $CHCl_3$ (5 l, 3 times). The $CHCl_3$ extract (47 g) was subjected to column chromatography on silica gel (70—230 mesh size). The elution of the column with *n*-hexane–EtOAc and EtOAc–MeOH yielded three major fractions which were subjected to preparative TLC using $CHCl_3$–MeOH (9 : 1) and *n*-hexane–$CHCl_3$–EtOAc–NH_4OH (1 : 8 : 1 : 3 drops) as solvent systems which afforded compounds **1** and **2**, respectively.

5α,7β,10β,13α-Tetrahydroxy-2α,9α,15-triacetoxy-11(15→1)-*abeo*-taxa-4(20),11-diene (**1**): Oily compound (15.4 mg, yield 6.6×10^{-5} %); *Rf*=0.53; $[\alpha]_D^{20}$ 112 (MeOH); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1735 (–CO–), 3194 (C–H), 3628 (–OH); UV λ_{max}^{MeOH}: 206 nm (log ε=4.8); ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 75 MHz) δ: See Table 1; EI-MS *m*/*z*: 450 (6%, M^+-AcOH), 432 (9), 390 (75), 254 (100); FAB-MS *m*/*z* 511 $(M+1)^+$; HR-EI-MS *m*/*z* 432.2087 ($C_{24}H_{32}O_7$, Calcd 432.2079, $M^+-C_2H_6O_3$).

5α,9α,10β,13α-Tetraacetoxy-15-hydroxy-11(15→1)-*abeo*-taxa-4(20),11-diene (**2**): Oily compound (11.5 mg, yield 5.0×10^{-5} %); *Rf*=0.42; $[\alpha]_D^{20}$ 85 (MeOH); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1742 (–CO–), 2952 (C–H), 3589 (–OH); UV λ_{max}^{MeOH}: 201 nm (log ε=4.7); ^{1}H-NMR (CD_3OD, 400 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz) δ: See Table 1; EI-MS *m*/*z*: 460 (6%, M^+-HOAc), 400 (1), 343 (8), 300 (100), 240 (78); FAB-MS $(M+1)^+$ *m*/*z* 521; HR-EI-MS *m*/*z* 343.1949 ($C_{21}H_{27}O_4$, Calcd 343.1909, M^+-OAc×3).

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