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Studies on the Himalayan yew *Taxus wallichiana*: Part VII — The taxoids and phenolic constituents of the roots of *Taxus wallichiana*†

S K Chattopadhyay*, M Kulshrestha, V Tripathi, G C Saha, R P Sharma & V K Mehta
Central Institute of Medicinal and Aromatic Plants, P.O. CIMAP, Lucknow-226 015, India

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The systematic investigation on the roots of *Taxus wallichiana* has resulted in the isolation of nine taxoids-taxol **1** baccatin III **2**, baccatin IV **3**, taxusin **4**, a C-14 oxygenated taxoid **5**, 1β-hydroxybaccatin I **6**, pentaacetoxy taxadiene **7**, a dibenzoylated rearranged taxoid **8**, 7-xylosyl-10-deacetyl-taxol C **9** and three phenolic compounds (−) seco-isolariciresinol **11**, taxiresinol **12** and isotaxiresinol **13**. The compounds have been characterized on the basis of their spectral characteristics. The occurrence of taxoid **9** in the roots of the plant is quite significant. The distribution of the above compounds in other parts of the plant are also summarized.

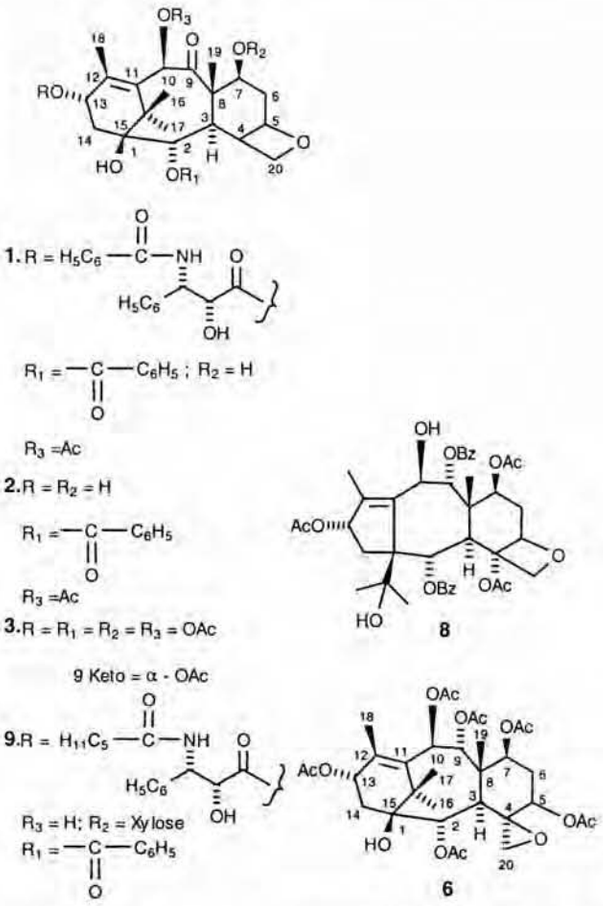
As a part of our ongoing studies on the systematic investigation of the chemical constituents of different parts of the Himalayan yew *Taxus wallichiana*, we have investigated the constituents of stem bark[1-6], leaves[1,7,8] and heart wood[9] of this plant. This search has resulted in the isolation of taxol[1], naturally occurring analogues and precursors of taxol and several regular and rearranged taxoids[1-9].

In this paper, we report the isolation and characterization of nine taxoids and three phenolic compounds from the roots of this plant.

Taxoid **1** was crystallized from aqueous methanol as needles, m.p. 199-200°C, $[\alpha]_D$−42° (c1, MeOH). The FAB-MS of the compound showed its $[M+H]^+$ peak at m/z 854 with characteristic fragments at m/z 569 and 509.

The ^{1}H-NMR (400 MHz) spectrum of the compound was found to be identical with the reported data of taxol[10]. On the basis of the physical and spectral properties of the compound, taxoid **1** was identified as taxol which was further verified by direct comparison with an authentic sample of taxol **1** previously isolated by us from the stem bark of this plant. This is the first report of isolation of taxol from the roots of *T. wallichiana*.

Taxoid **2** was obtained as crystalline compound from acetone, m.p. 230-32°C, $[\alpha]_{D+}$−50° (c 0.5, MeOH). The ^{1}H NMR (300 MHz) of the compound was found to be identical with those reported for baccatin III[11] and thus it was characterized as baccatin III **2**. Moreover, it was found to be identical with an authentic sample of baccatin III prepared from 10-deacetylbaccatin III. This is the first report of isolation of baccatin III from the roots of *T. wallichiana*.



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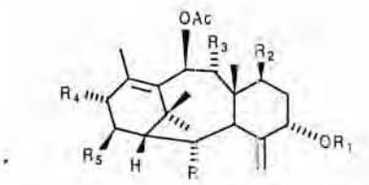
Taxoid **3** was crystallized from hexane-acetone mixture as needles, m.p. 198-99°C; $[\alpha]_D$+60° (c 0.4, $CHCl_3$). The molecular composition $C_{32}H_{44}O_{14}$ of the molecule was established from its FAB-MS which showed the $[M+Na]^+$ peak at m/z 675. The ^1H NMR (400 MHz) of the compound was identical with that of baccatin IV **3**. Finally it was directly compared with an authentic sample of baccatin IV previously isolated by us from the stem bark of the plant[8]. Thus, Taxoid-3 was characterized as baccatin IV **3**. This constitutes the first report of occurrence of this molecule in the roots of *T. wallichiana.*

Taxoid **4** was obtained as crystalline solid in the form of cubes, m.p. 130-31°C; $[\alpha]_D$+80° (c1, $CHCl_3$). The molecular composition of the molecule was established as $C_{28}H_{40}O_8$ by its FAB-MS which showed its $[M+Na]^+$ peak at m/z 527. The ^1H-NMR (300 MHz) of the compound showed the characteristic taxoid signals for its four methyl groups (δ 0.75, 1.11, 1.62, 2.17, 4s); two exocyclic methylene protons (δ 5.21, 4.85, br s); four methine protons [δ 5.36, t (*J*=3 Hz), 5.88, d (*J*=10.8 Hz), 6.05, d (*J*=10.8 Hz), 5.86, t (merged with the signal at δ 5.88)]. On the basis of the molecular formula and ^1H NMR spectrum, taxoid **4** was characterized as taxusin **4**[13] which was further verified by direct comparison with an authentic sample of taxusin isolated by us from the heartwood of the plant[9].

Taxusin **4** has been isolated previously from the heartwood of *T. baccata*[14], *T. mairei*[14], *T. cuspidata*[15] and *T. wallichiana*[9]. This is the first report of isolation of this molecule from the roots of *T. wallichiana.*

Taxoid **5** — Optically active molecule $[\alpha]_D$+35° (c 1.0, $CHCl_3$) was isolated as prisms, m.p. 107-10°C. On FAB-MS analysis, it showed a $[M+Na]^+$ peak at m/z 569 as the base peak thus confirming its molecular composition as $C_{31}H_{46}O_8$. The ^1H-NMR (300 MHz) spectrum of the molecule showed the presence of four characteristic taxoid methyl groups, three acetates, two exocyclic methylene protons and a characteristic one proton doublet of doublets at δ 6.06 (*J*=6, 12 Hz) and a methyl butyrate ester group [δ 0.89 (3H, t, *J*=6 Hz, CH_3CH_2), 1.11 (3H, d *J*=6 Hz, CH_3–CH(Et)–); 2.35 (1H, m, –CH(Me)(Et)); 1.45 (2H, m, CH_3CH_2–CH(Me)–)].

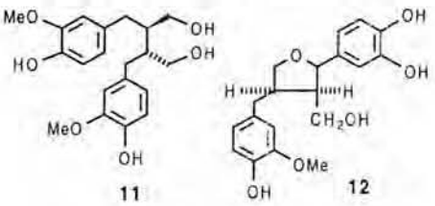


4. R = R_2 = R_5 = H; R_3 = R_4 = OAc

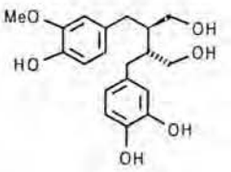
5. R_2 = R_3 = R_4 = H; R_1 = OAc; R_5 = –OCOCH(CH$_3$)–C_2H_5

7. R = R_5 = H; R_1 = R_2 = R_3 = R_4 = OAc

10. R = R_1 = R_5 = H; R_2 = R_3 = R_4 = OAxc



11 **12**



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On the basis of the molecular formula and diagnostic NMR data, taxoid **5** was characterized as 2α,5α,10β-triacetoxy-14β-(2′-methyl)-butysyloxy-4-(20),11-taxadiene **5**[15,9] which was further confirmed by direct comparison with an authentic sample previously isolated by us from the heartwood of the plant[9]. This molecule has been isolated recently from the heartwood of *T. cuspidata*[15], *T. wallichiana*[9] and cell cultures of *T. chinensis*[16]. The roots of *T. wallichiana* have now become the alternative source for this rare molecule.

Taxoid **6** ($C_{32}H_{43}O_{14}$) was obtained as a crystalline solid, m.p. 234-35°C, $[\alpha]_D$+90° (c1, $CHCl_3$). Its ^1H NMR (400 MHz) data was found to be identical with those reported for 1β-hydroxybaccatin I **6**[17]. In the ^1H NMR of the 1β-hydroxybaccatin I which has a β-epoxide system, there are two typical features which include the large chemical shift separation (>1ppm) between the two geminal oxirane protons [δ 3.56, d (*J*=5.3 Hz); 2.39, d (*J*=5.3 Hz)]; and the upfield shift of the H-5 protons (δ 4.21, t, *J*=3 Hz).

This is the first report of occurrence of 1β-hydroxybaccatin I in the roots of *T. wallichiana.*

Taxoid **7** was isolated as a crystalline solid, m.p. 203-04°C, $[\alpha]_D$+60° (c 0.5, $CHCl_3$); FAB-MS of the molecule showed its $[M+Na]^+$ peak at m/z 585 thus assigning its molecular formula as $C_{30}H_{42}O_{10}$. The ^1H NMR (400 MHz) of the compound was identical with a taxoid pentaacetoxy taxadiene **7** previously isolated from the heartwood of *T. baccata*[13] and *T. wallichiana*[9]. Furthermore, we had isolated a new taxoid 2′-deacetoxy decinnamoyl taxinine J **10** from the stem bark of *T. wallichiana*[2]. Taxoid **7** on acetylation with acetic anhydride and pyridine yielded a crystalline acetate which was found to be identical with taxadiene **7**. Thus, taxoid **7** was identified as pentaacetoxy taxadiene **7**[13]. This is the first report of occurrence of this taxoid in the roots of this plant.

Taxoid **8**, was isolated as an amorphous solid, $[\alpha]_D$ −88° (c 0.5, $CHCl_3$). Its molecular composition $C_{40}H_{46}O_{13}$ was established form its FAB-MS analysis which showed its $[M+Na]^+$ peak at m/z 757. The ^1H NMR (300 MHz) of the taxoid at room temperature showed that it exists as a mixture of two conformational isomers (conformer ratio 0.6:0.4) due to ring flip of the 7-membered ring. The molecular formula and the ^1H NMR of the molecule was found to be identical with 9-O-benzoyl-9,10-diacetyl-11(15→1) abeobaccatin VI **8**[18] which was further confirmed by direct comparison with an authentic sample previously isolated by us from the stem bark[5] and heartwood[9] of *T. wallichiana*.

Taxoid **9** — This amorphous taxoid, $[\alpha]_D$+4° (c1, pyridine) showed in its FAB-MS the $[M+K]^+$ peak at m/z 976 thus confirming its molecular composition as $C_{49}H_{63}O_{17}N$. The molecular weight of this compound corresponded with 7-xylosyl-10-deacetyl-taxol C[19]; ^1H NMR (300 MHz) of the molecule further substantiated it by showing characteristic signals that were reported for 7-xylosyl-10-deacetyl taxol C **9**[19]. Finally it was directly compared with an authentic sample of it previously isolated by us from the heartwood of this plant[9]. It is worthwhile to mention here that a mixture of three xylosides 7-xylosyl-10-deacetyl-taxol, 7-xylosyl-10-deacetyl-cephalomamine and 7-xylosyl-10-deacetyl-taxol C was first isolated from the stem bark of *T. baccata*[19]. In contrast to the above finding, the roots of *T. wallichiana* contains only this xyloside. This is the first report of presence of this rare xyloside in the roots of *T. wallichiana*.

In addition to the above nine taxoids that were isolated from the roots of *T. wallichiana*, three lignans **1**, **2** and **3** were also isolated and fully characterized.

Table I — Distribution of some of the compounds of roots in different parts of *T. wallichiana*

Compd	Plant part
Taxol **1**	Stem bark, needles
Baccatin IV **3**	Stem bark, needles
Taxusin **4**	Heartwood
C-14 oxygenated taxoid **5**	Heartwood
1β-Hydroxybaccatin I **6**	Needles, stem bark, heartwood
Pentaacetoxy taxadiene **7**	Heartwood
Rearranged taxoid **8**	Stem bark, heartwood
Xyloside **9**	Heartwood
(−) seco-Isolariciresinol **11**	Heartwood
Taxiresinol **12**	Heartwood
Isotaxiresinol **13**	Heartwood

Lignan-1, $[\alpha]_D$ −25° (c1, MeOH) gave positive ferric chloride test for phenol. The FAB-MS of the compound showed its $[M+K]^+$ peak at m/z 401. Thus, its molecular composition was $C_{20}H_{26}O_6$. The molecule was found to be identical with (−) seco-isolariciresinol **11** by direct comparison with an authentic sample[20]. This is the first report of isolation of (−) seco-isolariciresinol **11** from the roots of *T. wallichiana*.

Lignan-2 $[\alpha]_D$+45° (c1, MeOH) crystallized from acetone as needles, m.p. 156-57°C. The ^1H-NMR (80 MHz) of the compound was found to be identical with those reported for taxiresinol[21]. Finally, it was directly compared with an authentic sample of taxiresinol **12** previously isolated by us from the heartwood of this plant[21]. Taxiresinol **12** on acid treatment gave a product, $[\alpha]_D$+25° (c1, MeOH) which was found to be identical with lignan-3. Since taxiresinol under acidic condition rearranges into an isomeric compound isotaxiresinol **13**[21], lignan-3 was thus characterized as isotaxiresinol. Finally, lignan-3 was compared with an authentic sample of isotaxiresinol previously isolated by us from the heartwood of this plant[20].

It is worthwhile to mention here that some of the compounds that have now been isolated from the roots of *T. wallichiana* have also been isolated by us from other parts of the plant before. The distribution of the compounds in different parts of the plant is summarized in **Table I**.

Experimental Section

Melting points are uncorrected. ^1H-NMR spectra were recorded on a Bruker WM (400 MHz) and Bruker (300 MHz) instruments using TMS as internal standard (Chemical shifts in δ, ppm), and FAB-mass

spectra on a JEOL SX102 mass spectrometer with DA-6000 data system using argon (6 KV, 10 MA) as the FAB gas and *m*-nitrobenzyl alcohol as the matrix. Specific rotations were determined on a Jasco dip-181 digital polarimeter. Silica gel (60-120 mesh) was used for column chromatography and silica gel G for TLC using pet. ether-EtOAc and $CHCl_3$–MeOH as developing solvents. Spots were visualized on TLC under UV light, on exposure to iodine vapour in an iodine chamber and also by heating the chromato plates at 100°C in an oven after spraying with 10% H_2SO_4. The roots of *T. wallichiana* were collected in Bonera, Kashmir, and identified by V K Mehta of CIMAP, Bonera, Kashmir, India. A voucher specimen is deposited at the harbarium of CIMAP.

Extraction and isolation of compounds

Air dried and ground roots (1.0 kg) were extracted with MeOH. The MeOH extract was fractionated into $CHCl_3$ and EtOAc soluble fractions respectively. The column chromatography (SiO_2) of the $CHCl_3$ fraction gave the following compounds-**1** (10 mg), **2** (8 mg), **3** (9 mg), **4** (400 mg), **5** (120 mg), **6** (10 mg), **7** (50 mg), **8** (5 mg), **9** (20 mg), **11** (200 mg). Compounds **12** (4 g) and **13** (6 g) were isolated from the EtOAc fraction by column chromatography over silica gel.

Characterization of the compounds

The taxoids **1-9**, and the phenolic compounds **11-13** were characterized on the basis of their physical and spectral characteristics as well as by direct comparison with authentic samples as mentioned in the text.

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