

# Taxoids, Lignans, and Simple Phenolic Compounds from a Sample of the Needles of Himalayan *Taxus baccata*<sup>†</sup>

Biswanath Das\*, G. Anjani, A. Kashinatham,  
B. Venkataiah, and S. Padma Rao

Organic Chemistry Division-I, Indian Institute of Chemical Technology, Hyderabad 500 007, India

**Abstract** – Chemical investigation on a sample of the needles of Himalayan *Taxus baccata* has resulted in the isolation of several taxoids including taxol (1) 10-deacetyl-baccatin III (2) and 2-deacetoxytaxinine J (3) along with different lignans (6 and 7) and simple phenolics (8, 9, 10, 11 and 12). The occurrence of 4-(4'-hydroxyphenyl)-butane-2-one and 4-(4'-hydroxyphenyl)-*trans*-but 3-ene-2-one (8) in *Taxus* species is reported for the first time. The <sup>13</sup>C-NMR spectral data of two rearranged taxoid constituents, brevifoliol (4) and 13-decinnamoyltaxchinin B (5) are presented. The acid-catalyzed decomposition of taxol has been discussed. The synthesis of other two constituents, rhododendrol (10) and hibalactone (7) has been described.

**Key words** – *Taxus baccata*, Taxaceae, needles, taxoid, lignan, phenolic.

## Introduction

In continuation of our work (Das *et al.*, 1995a, 1995b, 1997a, 1997b, and Das, 1996) on taxol (Wani *et al.*, 1971), a promising antitumour compound and its related taxoids, we have recently chemically examined a sample of the needles of Himalayan *Taxus baccata* Linn (Taxaceae). Several taxoids [taxol (1), 10-deacetyl-baccatin-III (2), 2-deacetoxytaxinine J (3), brevifoliol (4) and 13-decinnamoyltaxchinin B (5)], lignans [suchilactone (6) and (-)-hibalactone (7)] and simple phenolic compounds [4-(4'-hydroxyphenyl)-*trans*-but-3-ene-2-one (8), 4-(4'-hydroxyphenyl)-butan-2-one (9), (-)-rhododendrol (10), (-)-rhododendrin (11) and 4-hydroxybenzaldehyde (12)] have been isolated. Here, we report the isolation and characterization of these constituents. The acid-ca-

talyzed decomposition of taxol and the synthesis of two constituents, rhododendrol and hibalactone in racemic form have been discussed.

## Experimental

Spectra were recorded with the following instruments: IR, Nicolet 740 FTIR spectrophotometer; UV, Shimadzu 240 spectrophotometer; <sup>1</sup>H- and <sup>13</sup>C-NMR, Varian Gemini 200 MHz; EIMS, VG Micromass 7070H (70 eV) and LSIMS, VG Autospec M. Optical rotations were determined with a Jasco DIP 360 digital polarimeter. Column chromatography was performed on silica gel (BDH, 100~200 mesh) and TLC with silica gel-G. HPLC was carried out with Shimadzu instrument using the reverse phase ODS C-18 column and UV detector (227 nm). The

\*Author for correspondence.

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mixture of MeOH-H<sub>2</sub>O-MeCN (5:12:8) was used as mobile phase and flow rate was kept at 1 ml/min.

**Plant material** – The needles of *Taxus baccata* were collected from Jammu. The voucher specimen (No. Tb-NJ) is deposited in our laboratory.

**Isolation of constituents** – Air dried needles of *T. baccata* (1 kg) were powdered and extracted thrice with CH<sub>2</sub>Cl<sub>2</sub>-MeOH (1:1) (3×2 l) at room temperature. Each extraction was continued for three days. The combined extract was filtered and the solvent removed under reduced pressure to yield a dark green residue (32 gm). This was chromatographed over silica gel and the column was eluted with solvents of increasing polarity using hexane and EtOAc. The eluates were collected in fractions of 50 ml each, resolution of the components in the mixture being monitored by TLC. Similar fractions were combined. A yellow solid consisting of a mixture of biflavonoids was obtained from the fractions eluting with hexane-C<sub>6</sub>H<sub>6</sub> (1:9). The other fractions were further purified by HPLC. The following compounds were isolated according to the increasing order of polarity; 4-hydroxybenzaldehyde (**12**) (48 mg), 4-(4'-hydroxyphenyl)-butan-2-one (**9**) (43 mg), 4-(4'-hydroxyphenyl)-*trans*-but-3-ene-2-one (**8**) (32 mg), taxol (**1**) (33 mg), 2-deacetoxytaxinine J (**3**) (42 mg), (-)-hibalactone (**7**) (46 mg), suchilactone (**6**) (34 mg), 13-decinnamoyltaxchinin B (**5**) (65 mg), (-)-rhododendrol (**10**) (256 mg), (-)-rhododendrin (**11**) (87 mg), brevifoliol (**4**) (167 mg) and 10-deacetylbaaccatin-III (**2**) (155 mg).

**Acid catalyzed decomposition of taxol**

– To a solution of taxol (15 mg) in CHCl<sub>3</sub> (20 ml) a drop of conc. HCl was added. The mixture was kept at room temperature for seven days. The TLC experiment showed a complex mixture of several compounds. The analytical HPLC experiment indicated the presence of baaccatin-III, 10-deacetylbaaccatin-III, 7-epitaxol and undecomposed taxol

along with several other compounds which could not be identified. An intense blue spot was observed under UV light with R<sub>f</sub> 0.52 when the TLC experiment was performed with a running solvent system consisting of 5% MeOH in EtOAc. The compound corresponding to this spot was purified by preparative TLC and crystallized from EtOAc to afford the product, **13**, m.p. 174-175°, [ $\alpha$ ]<sub>D</sub><sup>25</sup> -36.20° (EtOH, c: 0.8625), yield 3 mg; found in a dry sample: C, 78.68; H, 5.98 and N, 5.68% and calculated for C<sub>16</sub>H<sub>15</sub>N<sub>4</sub>: C, 78.36; H, 6.12 and N, 5.71%.

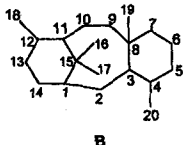
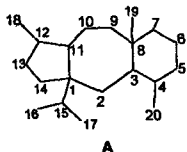
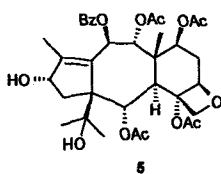
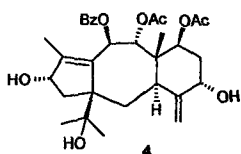
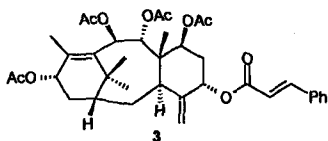
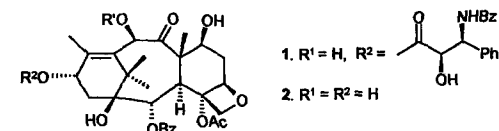
**Synthesis of (±) Rhododendrol** – (a)

Reaction of 4-hydroxybenzaldehyde with acetone: To a stirring mixture of acetone (50 ml) and aqueous NaOH solution (3 N, 10 ml) 4-hydroxybenzaldehyde (2 gm) was added. The stirring was continued for 24 hr. The reaction mixture was neutralized with HCl (3 N) and extracted with EtOAc (3×100 ml). The extract was washed with water (3×50 ml), dried and concentrated to a solid which was crystallized from EtOH to afford 4-(4'-hydroxyphenyl)-but-3-ene-2-one (Zemplen *et al.*, 1944), m.p. 113-114°, yield 1.84 gm; found in a dry sample: C, 73.69 and H, 6.23 % and calculated for C<sub>10</sub>H<sub>10</sub>O<sub>2</sub>: C, 74.04 and H, 6.17%.

(b) Reduction of 4-(4'-hydroxyphenyl)-but-3-ene-2-one with LiAlH<sub>4</sub>: A solution of 4-(4'-hydroxyphenyl)-but-3-ene-2-one (1 gm) in dry THF (50 ml) was added dropwise to a slurry of LiAlH<sub>4</sub> (500 mg) in THF (20 ml) at 0°C under dry conditions with vigorous stirring (6 hr). Excess reagent was decomposed with EtOAc and ice-cold water. The solution was filtered and the residue washed with hot EtOAc. The EtOAc solution was concentrated to form (±)-rhododendrol [(±)**10**] as a viscous mass, [ $\alpha$ ]<sub>D</sub><sup>25</sup> ± 0° (c 0.8256, MeOH), yield 0.85 gm; found in a dry sample: C, 72.08 and H, 8.56% and calculated for C<sub>10</sub>H<sub>14</sub>O<sub>2</sub>: C, 72.29 and H, 8.43%.

**Synthesis of (±) hibalactone** – (a)

Stobbe condensation of piperonal **14** with dimethyl succinate: Piperonal (2 gm) and di-



methyl succinate (20 ml, d: 1.4 g/ml) was added to a solution of NaOMe (0.5 g) in MeOH (50 ml). The mixture was heated to reflux for 2 hr, cooled and acidified with dil. HCl (1 N). The mixture was then extracted with ether (3×50 ml). The ethereal fraction was re-extracted with aqueous saturated NaHCO<sub>3</sub> solution (3×50 ml). Acidification of the NaHCO<sub>3</sub> extract with conc. HCl (12N) afforded an oily layer. This was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×50 ml). The extract was washed with water (3×20 ml), dried and concentrated. The concentrated mass was crystallized from MeOH to afford 4-(3,4-methylenedioxy-phenyl)-3-methoxycarbonylbut-3-enoic acid (**15**) as a yellow solid, m.p. 141-142°, yield 1.4 g; found in a dry sample: C, 59.23 and H, 4.38% and calculated for C<sub>13</sub>H<sub>12</sub>O<sub>6</sub>: C, 59.09 and H, 4.54%; IR:  $\nu_{\max}^{\text{KBr}}$  3070, 1632, 1499, 1485 cm<sup>-1</sup>, <sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  7.83 (1H, s, H-4), 6.93-6.78 (3H, m, Ar-H), 6.01 (2H, s, -OCH<sub>2</sub>O-), 3.84 (3H, s, -COOMe) and 3.60 (2H, s, H<sub>2</sub>-2); MS: m/z 264

(M<sup>+</sup>, 55%), 232 (12), 220 (60), 189 (18), 160 (100), 102 (48).

(b) Methylation of **15**: To an ice-cold solution of ethereal CH<sub>2</sub>N<sub>2</sub>, a solution of **15** (1 gm) in MeOH (50 ml) was added. The mixture was kept for 12 hr, filtered and concentrated to produce the diester **16** which was crystallized from MeOH as a white solid, m.p. 84-85°; yield 880 mg; found in a dry sample: C, 60.58 and H, 5.25% and calculated for C<sub>14</sub>H<sub>14</sub>O<sub>6</sub>: C, 60.43 and H, 5.04%; IR:  $\nu_{\max}^{\text{KBr}}$  1724, 1703, 1640, 1500 cm<sup>-1</sup>, <sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  7.82 (1H, s, H-4), 6.92-6.82 (3H, m, Ar-H), 6.02 (2H, s, -OCH<sub>2</sub>O-), 3.83 and 3.76 (3H each, s, 2× -COOMe), 3.57 (2H, s, H-2); MS: m/z 278 (M<sup>+</sup>, 100%), 274 (10), 218 (28), 189 (65), 159 (45), 102 (35).

(c) Stobbe condensation of **16** with piperonal: Compound **16** (500 mg) on Stobbe condensation (as discussed above) with piperonal (300 mg) in presence of NaOMe (200 mg) in MeOH (50 ml) afforded a light yellow solid which was purified by crystallization from MeOH to furnish the product, **17**, m.p. 166-168°; yield 525 mg; found in a dry sample: C, 63.42 and H, 4.17% and calculated for C<sub>21</sub>H<sub>16</sub>O<sub>8</sub>: C, 63.63 and H, 4.03%; IR:  $\nu_{\max}^{\text{KBr}}$  3410, 1700, 1690, 1505, 1500 cm<sup>-1</sup>, <sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  7.86 (1H, s, olefinic-H), 7.84 (1H, s, olefinic-H), 7.04-6.98 (3H, m, Ar-H), 6.88-6.62 (3H, m, Ar-H), 5.95 (4H, s, 2× -OCH<sub>2</sub>O-), 3.72 (3H, s, -COOMe); MS: m/z 396 (M<sup>+</sup>, 20%), 364 (85), 292 (42), 243 (45), 135 (82), 122 (100).

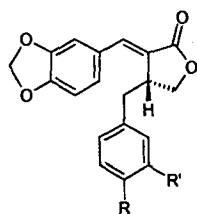
(d) Bouveault-Blanc reduction of **17**: To a solution of compound **17** (100 mg) in dry EtOH (50 ml) Na (1 gm) (cut into small pieces) was slowly added at room temperature. The mixture was kept for 6 hr, acidified with dilute HCl (1 N) and extracted with EtOAc (3×50 ml). The extract was washed with aqueous NaHCO<sub>3</sub> solution (3×20 ml) followed by water (3×50 ml). (±)-Hibac-tone [(±)-**7**] was isolated by preparative TLC and crystallized from C<sub>6</sub>H<sub>6</sub> to a white solid, m.p. 147-148°;  $[\alpha]_{\text{D}}^{25} \pm 0^\circ$  (c 0.7964, MeOH), yield

74 mg; found in a dry sample: C, 68.42 and H, 4.46% and calculated for  $C_{20}H_{16}O_6$ : C, 68.18 and H, 4.54%.

## Results and Discussion

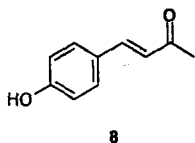
Chemical investigation on a sample of the needles of Himalayan *T. baccata* has resulted in the isolation of five taxoids, taxol (1) (Das, 1996 and Das *et al.*, 1997a, 1997b), 10-deacetylbaaccatin III (2) (Das *et al.*, 1997b), 2-deacetoxytaxinin J (3) (Das, 1996), brevifoliol (4) (Das *et al.*, 1995a) and 13-decinnamoyltaxchinin B (5) (Das *et al.*, 1995b) along with two lignans, suchilactone (6) (Das *et al.*, 1995a) and (-)-hibalactone (7) (Das *et al.*, 1995c) and five simple phenolic compounds, 4-(4'-hydroxyphenyl)-*trans*-but-3-ene-2-one (8) (Zemplen *et al.*, 1944), 4-(4'-hydroxyphenyl)-butan-2-one (9) (Zemplen *et al.*, 1944 and Prasad and Chaudhury, 1962), (-)-rhododendrol (10) (Das *et al.*, 1993a, 1993b) and (-)-rhododendrin (11) (Das *et al.*, 1993b) and 4-hydroxybenzaldehyde (12). Compounds 8 and 9 were not previously reported from a *Taxus* species. The isolated constituents were identified from their spectral data and by comparison of their physical and spectral properties with those reported for the compounds in the literature. A yellow solid isolated from the plant material was found to be a mixture of biflavonoids (Das *et al.*, 1994) which was not further purified.

During the structural elucidation of the constituents, the  $^{13}C$ -NMR spectra of bre-

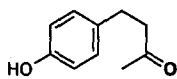


6. R = R' = OMe

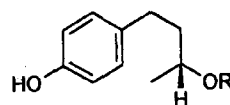
7. R, R' = -OCH<sub>2</sub>O-



8



9



10. R = H

11. R = Glc

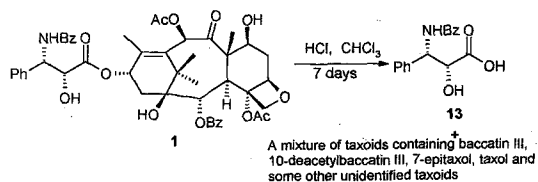
vifoliol (4) and 13-decinnamoyltaxchinin B (5), two representative members of rearranged taxoids (Das *et al.*, 1995d), have been studied in detail. The rearranged taxoids possess a 5,7,6-membered ring system (A) instead of a 6,8,6-membered ring system (B) present in a normal taxoid. Brevifoliol (4) contains an exocyclic double bond at C-4, C-20 while 13-decinnamoyltaxchinin B (5) contains an oxetane ring at C-5, C-20. Both the compounds were originally considered (Barboni *et al.*, 1993) as normal taxoids and later their correct rearranged structures, 4 and 5 were established (Appendino *et al.*, 1993). We have fully characterized the  $^{13}C$ -NMR spectra of these two compounds (Table 1) by using DEPT and HETCOR spectroscopic experiments. The C-15 of brevifoliol (4) and 13-decinnamoyltaxchinin B (5) appeared at  $\delta$  75.9 and 75.5, respectively while C-1 at  $\delta$  62.3 and 67.6, respectively suggesting the 11(15 $\rightarrow$ 1)-abeo structure (Das *et al.*, 1995d) for both of the compounds. The  $^{13}C$ -NMR spectral data of these two compounds will be useful to characterize other rearranged taxoids possessing exocyclic double bond or oxetane ring.

The isolation of taxol (1) from the needles of Himalayan *T. baccata* is significant as the source is indigenous, abundant and regenerable. 10-Deacetylbaaccatin III (2), which was obtained in larger quantity, could be utilized for semisynthesis of taxol. While we have studied the NMR spectra of taxol, we have observed that the compound when kept in  $CDCl_3$  for several days (one week or more) at room temperature it decomposed to different products. The  $^1H$ -NMR spectrum of the storage solution showed the presence of a complex mixture of taxoids possibly form-

**Table 1.**  $^{13}\text{C}$ -NMR (50 MHz,  $\text{CDCl}_3$ ) Spectral Data of Brevifoliol (4) and 13-Decinamoyltaxchinin B (5)

C	$^{13}\text{C}$ -NMR Chemical Shift (ppm) of	
	Brevifoliol (4)	13-Decinamoyltaxchinin B (5)
1	62.3	67.6
2	29.0	68.0
3	37.8	43.9
4	149.1	79.8
5	72.3	84.9
6	35.9	37.6
7	70.2	70.3
8	44.9	43.6
9	77.0	76.3
10	70.1	69.1
11	133.9	134.0
12	151.3	151.7
13	76.9	77.0
14	47.0	39.4
15	75.9	75.5
16	24.7	25.1
17	26.8	27.3
18	11.8	11.6
19	12.7	12.3
20	111.9	74.9
Ar-1'	129.3	129.2
Ar-2' and 6'	129.4	129.6
Ar-3' and 5'	128.6	128.8
Ar-4'	133.2	133.5
-OCOAr	164.3	164.3
-OCOMe	21.3	21.4, 21.2,
	20.6	22.1, 20.4
-OCOMe	170.4	171.3, 170.6,
	170.0	170.1, 170.0

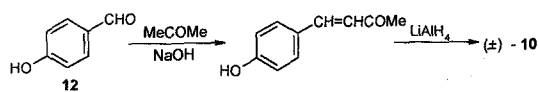
ed by hydrolysis of ester functionalities and epimerisation of hydroxyl groups by catalytic amount of hydrochloric acid usually present in  $\text{CDCl}_3$ . Such transformation is frequently observed (Miller *et al.*, 1981) in the family of taxoids. Similar conversion was also noted (by HPLC analysis of the reaction mixture) when taxol (1) was kept in chloroform for seven days with a trace of hydrochloric acid (Scheme 1). We were able to isolate successfully the side chain of taxol (phenylisoserine unit) from such reaction mixture. The taxoid portion was not purified due to paucity of the material but was found

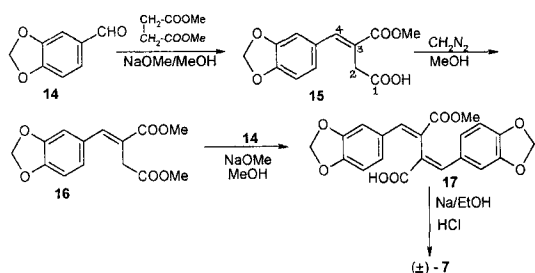
**Scheme 1.** Acid-catalyzed Decomposition of Taxol (1).

by HPLC analysis to be a mixture of baccatin III, 10-deacetylbaccatin III and 7-epitaxol along with unconverted taxol and some other unidentified taxoids. The phenylisoserine unit [N-benzoyl-(2R, 3S)-3-phenylisoserine (13)] was characterized by direct comparison with an authentic sample (Das *et al.*, 1997b).

The simple phenolic compounds isolated from the investigated sample of the plant constituted the major part of its constituents. The occurrence of 4-hydroxy benzaldehyde (12), 4-(4'-hydroxyphenyl)-*trans*-but-3-ene-2-one (8), 4-(4'-hydroxyphenyl)-butan-2-one (9), (-)-rhododendrol [(*-*)-4-(4'-hydroxyphenyl)-butan-2-ol] (10) and (-)-rhododendrin [(*-*)-4-(4'-hydroxyphenyl)-butan-2-O-glucoside] (11) in the same species is biogenetically significant. Rhododendrol (Das *et al.*, 1993a, 1993b), an important constituent possessing hepatoprotective activity, was synthesized from 4-hydroxybenzaldehyde in two steps (Scheme 2). The condensation of the latter with acetone in presence of sodium hydroxide afforded the known compound, 4-(4-hydroxyphenyl)-but-3-ene-2-one (Zemplen *et al.*, 1944) which was reduced with lithium aluminium hydride to produce rhododendrol in racemic form. The product [(+)-10] showed similar spectral properties (IR,  $^1\text{H}$ -NMR and MS) to those of natural (-)- rhododendrol (10).

Hibalactone (Das *et al.*, 1995c), a lignan

**Scheme 2.** Synthesis of (±)-Rhododendrol [(±)-10].



**Scheme 3.** Synthesis of (±)-Hibalactone [(±)-7].

constituent having insecticidal activity was also synthesized in racemic form (Scheme 3). Piperonal (**14**) was used as the starting material. Stobbe condensation of the compound (**14**) with dimethylsuccinate in presence of sodium methoxide in methanol afforded 4-(3',4'-methylenedioxyphenyl)-3-methoxycarbonylbut-3-enoic acid (**15**). The latter on methylation with diazomethane produced the diester **16**. A second Stobbe condensation of the latter with piperonal (**14**) produced the diene **17** which underwent Bouveault Blanc reduction to yield (±)-hibalactone [(±)-**7**]. The structures of all the reaction products were established from their spectroscopic data (vide Experimental). The spectral properties (IR, <sup>1</sup>H-NMR and MS) of the final products [(±)-hibalactone] were found to be identical to those of naturally occurring (-)-hibalactone (**7**).

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