Lignans of *Chamaecyparis obtusa*

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**Abstract** Heartwood of *Chamaecyparis obtusa* contains significant amounts of a dibenzylbutyrolactone lignan, hinokinin (8). This investigation demonstrated that the contents of 8 and a norlignan, hinokiresinol (12), were higher in the heartwood region than in the sapwood, indicating their nature of being heartwood extractives. Eleven lignans - xanthonylol (1), 7-oxohinokinin (2), savinin (3), dihydrosesamin (4), isouctifolin (5), sesamin (6), piperitol (7), hinokinin (8), pluviatolide (9), haplomyrolfalin (10), and matairesinol (11) - were isolated from young shoots of *Chamaecyparis obtusa* cv. Breviramea. Eight lignans (1, 2, 4, 5, 7, 9, 10, and 11) were isolated from this plant for the first time. Chiral high-performance liquid chromatographic analysis showed that 8, 9, 10, and 11, were found to be levorotatory and optically pure (>99% e.e.). Based on the chemical structures of the isolated lignans, possible biosynthetic pathways of 8 are discussed.

**Key words** Lignan - Stereochemistry - *Chamaecyparis obtusa* - *Chamaecyparis obtusa* cv. Breviramea - Heartwood

**Introduction**

Heartwood formation, which occurs in inner trunks of woody plants but not in herbaceous plants, is one of the metabolic events specific to woody plants. This metabolic event is accompanied by deposition of significant amounts of secondary metabolites, so-called heartwood extractives, including lignans and norlignans. The heartwood extractives encrust the cell walls and have marked effects on the physical and chemical properties of wood, such as acoustic properties, durability, impregnation of preservatives, origin of the fragrance of wood, and color of wood. Thus, heartwood formation is of interest from the standpoints of both basic plant science and wood technology, but little has been known about its biochemical mechanisms.

Elucidating the molecular mechanisms for biosynthesis of heartwood secondary metabolites would be a clue to the studies of heartwood formation mechanism, as the deposition of some secondary metabolites occurs specifically in heartwood. The heartwood of hinoki cypress (*Chamaecyparis obtusa*), which is one of major species used for wood construction materials in Japan, contains significant amounts of a dibenzylbutyrolactone lignan, hinokinin (8). In addition, two lignans, savinin (3) and sesamin (6), were isolated from its cultivar, *C. obtusa* cv. Breviramea (Chabohiba in Japanese). However, a detailed survey of lignans, especially possible biosynthetic precursor lignans of 8 in this species, has not yet been reported. Here we report the distribution of 8 and a norlignan, hinokiresinol (12), in a cross section of *C. obtusa* and a survey of lignans in young shoots of *C. obtusa* cv. Breviramea. We discuss possible biosynthetic pathways of lignans in *C. obtusa* cv. Breviramea.

**Experimental**

**Instruments and chromatography**

One- and two-dimensional nuclear magnetic resonance (NMR) spectra were obtained with a JNM-LA400MK FT-
NMR system (JEOL). Chemical shifts and coupling constants \((J)\) were expressed in \(\delta\) and hertz, respectively. Gas chromatography-mass spectrometry (GC-MS), low- and high-resolution electron impact mass spectrometry (EI-MS), and high-performance liquid chromatography (HPLC) were performed as previously reported. The reversed-phase column was a Waters Novapak C18 (150 x 3.9 mm), which was eluted with two solvent systems: (1) solvent system A, for isocratic elution at 1 ml/min by CH3CN-H2O (37:63); and (2) solvent system B for linear gradient elution at 1 ml/min by CH3CN-H2O (23:77) at \(t = 0\) to CH3CN-H2O (50:50) at 25 min. The elution conditions for chiral HPLC were as follows. 

### Synthesis of compounds

\((-\)-Matairesinols \([\pm]-11\) were prepared previously. \((-\)-Hinokinin \([\pm]-8\), \((-\)-Pluviatolides \([\pm]-9\), and \((-\)-Haployrufolins \([\pm]-10\) were synthesized by methods similar to those used for \((-\)-11) but with different starting materials: for \((-\)-8), piperonyl alcohol and methyl 2-carboxymethyl-3-(3,4-methylenedioxyphenyl)propiionate instead of vanillyl alcohol and methyl 2-carboxymethyl-3-(4-hydroxy-3-methoxyphenyl)propanoate as the starting materials, respectively; for \((-\)-9), methyl 2-carboxymethyl-3-(3,4-methylenedioxyphenyl)propiionate instead of methyl 2-carboxymethyl-3-(4-hydroxy-3-methoxyphenyl)propiionate as one of the starting materials; and for \((-\)-10), piperonyl alcohol instead of vanillyl alcohol as one of the starting materials.

### Plant material

Chamaecyparis obtusa wood with 36 annual rings harvested in Kamigamo Experimental Forest, Kyoto University was provided by Prof. M. Fujita, Kyoto University. Chamaecyparis obtusa cv. Breviramea plants were obtained from a local nursery and maintained in the experimental forest of the Wood Research Institute, Kyoto University, Japan. Young shoots with leaves of the plant, collected in February 1994 and April 1999, were used for lignan extraction.

### Isolation and structural determination of lignans

Chamaecyparis obtusa wood was pulverized using a Wiley mill, and the wood meal thus obtained was air-dried. The dried wood meal (61.70 g) was extracted with hot methanol. The methanol extracts (2.53 g) were subjected to successive purification by silica gel column chromatography to afford 8, 9, 10, and 11.
(1H, dt, J = 1.4, J = 10.3, H-9a), 6.06 (1H, ddd, J = 6.8, J = 10.3, J = 17.1, H-8), 6.21 (1H, dd, J = 6.8, J = 15.8, H-8'), 6.33 (1H, d, J = 16.1, H-7'), 6.74–6.79 (4H, m, Ar-H), 7.10–7.25 (4H, m, Ar-H); 13C-NMR (CDCl3): 51.55, 115.36, 115.40, 115.45, 127.64, 129.33, 129.79, 130.01, 130.51, 135.13, 140.54, 154.13, and 154.87; MS m/z [rel. int. (%)]: 252 (100, M+), 237 (24.1), 223 (9.4), 204 (11.2), 159 (13.4), 158 (48.7), 157 (14.2), 145 (36.7), 131 (26.4), 119 (15.2), 107 (43.8).

Freeze-dried C. obtusa cv. Breviramea young shoots with leaves (163.43g) were pulverized using a Waring blender and then extracted seven times with hot methanol (total 1300ml). The combined methanol extracts (47.76g) were suspended in distilled water (300ml), which was extracted with ethyl ether (200ml × 3). The combined ethyl ether extracts (13.26g) were submitted to repeated column chromatography (solvents: appropriate mixtures of methanol/dichloromethane, ethyl acetate/n-hexane, or acetone/dichloromethane). Each fraction obtained was submitted to repeated TLC (solvents: appropriate mixtures of methanol/dichloromethane, ethyl acetate/n-hexane, or acetone/dichloromethane) and reversed-phase HPLC (solvent system A) to afford 11 lignans: 1 (0.6mg), 2 (22.7mg), 4 (19.4mg), 5 (3.6mg), 6 (18.6mg), 7 (2.8mg), 8 (6.2mg), 9 (1.9mg), 10 (1.1mg), and 11 (3.3mg).

Xanthoxyll (1): 1H-NMR (CDCl3): 2.88 (1H, dd, J = 7.0, J = 14.5, H-8), 3.26–3.35 (2H, m, H-8' and H-9'), 3.80–3.84 (2H, m, H-9 and H-9'), 3.90 (3H, s, OMe), 4.10 (1H, broad d, J = 9.8, H-9), 4.40 (1H, d, J = 7.3, H-7), 4.83 (1H, d, J = 5.4, H-7'), 5.95 (2H, s, OCH2O), 6.77–6.90 (6H, m, Ar-H); 13C-NMR (CDCl3): 50.24, 54.62, 56.03, 69.70, 71.00, 82.13, 87.78, 101.06, 106.49, 108.23, 108.63, 114.31, 118.77, 119.31, 132.35, 133.07, 145.42, 146.64, 146.79, and 147.71; MS m/z [rel. int. (%)]: 356 (84.7, M+), 325 (85.82, 101.11, 106.52, 108.22, 119.39, 135.09, 147.46, and 174.99; MS m/z [rel. int (%): 354 (60.93, 219 (74.4), 203 (20.8), 178 (16.5), 161 (46.4), 150 (33.5), 149 (100), 148 (33.9), 135 (49.6), 131 (37.2), 122 (22.4); high-resolution MS m/z (M+): calculated for C29H40O9, 534.1103, found 534.1101.

Piperitol (7): 1H-NMR (CDCl3): 3.02–3.11 (2H, m, H-8 and H-9'), 3.84–3.88 (2H, m, H-9 and H-9'), 3.90 (3H, s, OMe), 4.20–4.26 (2H, m, H-9 and H-9'), 4.71 (1H, d, J = 4.6, H-7 or H-7'), 4.72 (1H, d, J = 4.4, H-7 or H-7'), 5.94 (4H, s, OCH2O × 2), 6.76–6.84 (6H, m, Ar-H); 13C-NMR (CDCl3): 54.36, 71.74, 85.82, 101.11, 106.52, 108.22, 119.39, 135.09, 147.85, 147.91, and 149.76; MS m/z [rel. int. (%): 252 (100, M+), 205 (14.2), 192 (28.0), 178 (16.1), 161 (46.4), 150 (33.5), 149 (100), 148 (33.9), 135 (49.6), 131 (37.2), 122 (22.4); high-resolution MS m/z (M+): calculated for C29H40O9, 534.1103, found 534.1101.
Quantitation of hinokinin (8) and hinokiresinol (12)
in a cross section of *C. obtusa*

A cross section about 1 cm thick was taken from a *C. obtusa* log with 36 annual rings. For analysis, 11 rectangular radial segments (1.2-1.4 g), each containing three to six annual rings, were cut, pulverized by a Wiley mill, and extracted with hot methanol. To each extract 1.0 mg of internal standard, 3-(4-ethoxy-3-methoxyphenyl)-1-propanol, was added. An aliquot of each methanol extract was subjected to HPLC (solvent system B) to quantify 8 and 12.

Isolation and enantiomeric composition of lignans from
*C. obtusa* cv. Breviramea

Eleven lignans were isolated from *C. obtusa* cv. Breviramea young shoots with leaves: xanthoxylol (1), 7-oxohinokinin (2), savinin (3), dihydrodesamin (4), isoactifolin (5), sesamin (6), piperitol (7), hinokinin (8), pluviatolide (9), haplomyrfolin (10), and matairesinol (11) (Fig. 1).

The structures of 1, 2, 3, 4, 6, and 7 were confirmed by comparing their one- and two-dimensional NMR and mass spectral data with those of the lignans and related compounds reported previously (1, 9, 10, 11, 12, 13, 14, 15, 16, and 17). As for compound (1), with a relative configuration of 7R*, 7'S*, 8S*, and 8'S*, the possibility of its regioisomer 7'S*, 7'R*, 8S*, and 8'R*, the possibility of its regioisomer 7'R*, 7'S*, 8R*, and 8'R*, pluviatolide, was eliminated by comparing the 1H-NMR data of the acetate of 1 with that of acetyl pluviatilol, as described by González et al. The lignans 8, 9, 10, and 11 were identified by directly comparing their 1H-NMR and mass spectral data with those of chemically synthesized authentic samples. The eight lignans 1, 2, 4, 5, 7, 9, 10, and 11, were isolated from *C. obtusa* cv. Breviramea for the first time, and isolation of a new compound (5) was reported elsewhere.

Chiral HPLC analysis indicated that all the dibenzylbutyrolactone lignans (8, 9, 10, 11) isolated from both *C. obtusa* and *C. obtusa* cv. Breviramea were levorotatory and optically pure (>99% e.e.) (Figs. 1, 3). Enantiomers of 8, 9, 10, and 11 are known to have the absolute configurations shown in Fig. 1. 20

**Discussion**

In 1933 Yoshiki and Ishiguro reported that *Chamaecyparis obtusa* heartwood contained large amounts of a dibenzylbutyrolactone lignan they named hinokinin (8), and later Keimatsu and Ishiguro proposed the structure of 8 shown in Fig. 1 for the lignan. They reported that hinokinin (8) accounted for about 30% of the resins extracted from the heartwood, which is a rather high yield among naturally occurring lignans. The present investigation confirmed this high yield of 8 from the heartwood.
Thus, as shown in Fig. 2, the highest content of 8 was about 1.2% based on the wood meal. In sharp contrast, the amount of 8 was negligible in the sapwood region. The norlignan (12) exhibited a similar distribution across the cross section (Fig. 2), unequivocally confirming the nature of 8 and 12 as heartwood substances.

Next, identification of possible biosynthetic precursors of 8 was attempted. Despite the high contents of 8 in heartwood, GC-MS analysis of methanol extracts of C. obtusa xylem wood meal did not show the presence of any lignans other than 8 (data not shown). However, it was reported that young leaves of C. obtusa and its cultivar, C. obtusa cv. Breviramea, contained 3, which is a dehydro derivative of 8; and that the cultivar leaves contained more 3 than did C. obtusa leaves.34 Also, the lignan (6) was isolated from C. obtusa cv. Breviramea.3

These earlier reports3-5,22 stimulated us to survey lignans in C. obtusa cv. Breviramea young shoots instead of C. obtusa xylem. Preliminary GC-MS analysis of methanol extracts from young leaves of C. obtusa cv. Breviramea strongly suggested the presence of 8 as well as the previously reported lignans, 33-6. In addition to the three lignans, the presence of matairesinol (11) was suggested by mass chromatographic analysis. However, possible precursors of 11 (piroresinol, lariciresinol, secoisolariciresinol) were not detected in this analysis.

To confirm the active lignan biosynthesis from monolignol in the young shoots of C. obtusa cv. Breviramea, [9,9-2H2,OC2H3]coniferyl alcohol was administered to the shoots. GC-MS analysis of the methanol extracts obtained following the administration indicated incorporation of deuterium atoms from [9,9-2H2,OC2H3]coniferyl alcohol into lariciresinol, secoisolariciresinol, and 11 (data not shown), indicating the occurrence of lignan biosynthesis in the tissue.

Recent studies of lignan biosynthesis with Forsythia plants have demonstrated the following enzymatic conversion giving rise to 11: coniferyl alcohol → pinoresinol → lariciresinol → secoisolariciresinol → matairesinol (11).23-24 Most of these reactions were also demonstrated enzymatically or by feeding experiments with some other plant species.25-31 These studies together with the present feeding experiment suggest that the conversion to 11 occurs generally in plants including C. obtusa cv. Breviramea.

In addition, the lignan (8) might be synthesized from 11 via dual methylenedioxy bridge formation involving the monomethylenedioxy lignans 9 and 10 as intermediates, as the formation of methylenedioxy bridges of piperonyl groups in alkaloid and lignan molecules from the correspondingly guaiacyl (4-hydroxy-3-methoxyphenyl) groups was known.31-32

Next, with these data in hand, we surveyed lignans in C. obtusa cv. Breviramea. Here particular attention was given to isolating 9, 10, and 11 (the possible precursors of 8) using the corresponding chemically synthesized authentic samples; the following 11 lignans including 9, 10, and 11 were isolated from the young shoots with leaves of C. obtusa cv. Breviramea: dibenzylbutyrolactone lignans 2, 3, 8, 9, 10, and 11; furan lignans 1, 6, and 7; furan lignans 4, and 5. The lignan (5) was a new lignan, and its isolation has been reported.35 In addition to 5, the seven lignans 1, 2, 4, 7, 9, 10, and 11 were isolated from this plant species for the first time. Recently, lignans 3, 8, and 9 as well as two other lignans, norlatrachelogenin and 7-hydroxymatairesinol, were isolated from Chamaecyparis formosensis.33

Isolation of 9 and 10 as well as 11 suggests that the first methylenedioxy bridge formation may take place in one of the aromatic rings of 11, giving rise to 9 and 10, which may be converted to 8 by a second methylenedioxy bridge formation (Fig. 4), although establishing the metabolic sequence awaits concrete evidence from biochemical experiments.
Recent studies on lignan biosynthesis have indicated that there is stereochemical diversity in lignan biosynthesis, emphasizing stereochemical characterization of key metabolic intermediates. In addition, all the dibenzylbutyrolactone lignans of which enantiomeric compositions have so far been determined were optically pure; most were levorotatory, but those isolated from Thymelaeaceae plants were dextrorotatory. Hence, we subjected the dibenzylbutyrolactone lignans (8 and its possible precursors 9, 10, and 11) to chiral HPLC using chemically synthesized racemic authentic samples as standards. All of the four dibenzylbutyrolactone lignans isolated from *C. obtusa* cv. Breviramea (Fig. 3) and 12 were found to be levorotatory and optically pure (>99% e.e.), which accorded well with previous reports on enantiomeric compositions of dibenzylbutyrolactone lignans.

In addition to the dibenzylbutyrolactone lignans, five furanofuran and furan lignans were isolated. Two of them (4 and 6) have two piperylon (methyleneoxyphenyl) groups, and the rest (1, 5, 7) have one piperylon and one guaiacyl group. The lignans 4, 6, and 7 might serve as precursors of 8 via an alternative pathway where 11 is not involved (Fig. 4).

In conclusion, the present study reported isolation of several lignans from *C. obtusa* cv. Breviramea, including possible biosynthetic precursors of *C. obtusa* heartwood lignan, hinokinin (8).

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References


