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ORIGINAL ARTICLE

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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 – xanthoxylol (**1**), 7-oxohinokinin (**2**), savinin (**3**), dihydrosesamin (**4**), isoactifolin (**5**), sesamin (**6**), piperitol (**7**), hinokinin (**8**), pluviatolide (**9**), haplomyrfolin (**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

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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.[1] 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**).[2] In addition, two lignans, savinin (**3**)[3,4] and sesamin (**6**),[5] 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 δ 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.[6] The reversed-phase column was a Waters Novapak C_{18} (150 × 3.9 mm), which was eluted with two solvent systems: (1) solvent system A, for isocratic elution at 1 ml/min by $CH_3CN—H_2O$ (37:63); and (2) solvent system B for linear gradient elution at 1 ml/min by $CH_3CN—H_2O$ (23:77) at t = 0 to $CH_3CN—H_2O$ (50:50) at 25 min. The elution conditions for chiral HPLC were as follows. Hinokinin (**8**): Chiralcel OD column (Daicel Chemical Co.; 250 × 4.6 mm) with ethanol—n-hexane (50:50) at 0.7 ml/min; pluviatolide (**9**): Chiralcel OD column with ethanol—1% acetic acid in n-hexane (15:85) at 0.6 ml/min; haplomyrfolin (**10**): Chiralcel OD column with ethanol—1% acetic acid in n-hexane (15:85) at 0.9 ml/min; matairesinol (**11**): Chiralcel OD column with ethanol—1% acetic acid in n-hexane (15:85) at 0.8 ml/min. The sign for optical rotation of each enantiomer of **8**, **9**, **10**, and **11**, was determined by a chiral detector (OR-990; JASCO). Silica gel column chromatography and silica gel thin-layer chromatography (TLC) employed Kieselgel 60 (70–230 mesh; Merck) and Kieselgel 60 F_{254} (20 × 20 cm, 0.5 or 0.25 mm; Merck).

Synthesis of compounds

(±)-Matairesinols [(±)-**11**] were prepared previously.[7] (±)-Hinokinins [(±)-**8**], (±)-pluviatolides [(±)-**9**], and (±)-haplomyrfolins [(±)-**10**] were synthesized by methods similar to those used for (±)-**11**[7] but with different starting materials: for (±)-**8**, piperonyl alcohol and methyl 2-carboxymethyl-3-(3,4-methylenedioxyphenyl)propionate instead of vanillyl alcohol and methyl 2-carboxymethyl-3-(4-hydroxy-3-methoxyphenyl)propionate as the starting materials, respectively; for (±)-**9**, methyl 2-carboxymethyl-3-(3,4-methylenedioxyphenyl)propionate instead of methyl 2-carboxymethyl-3-(4-hydroxy-3-methoxyphenyl) propionate as one of the starting materials; and for (±)-**10**, piperonyl alcohol instead of vanillyl alcohol as one of the starting materials.

Hinokinin (**8**): ^{1}H-NMR ($CDCl_3$): 2.41–2.62 (4H, m, H-7′, H-7′, H-8, and H-8′), 2.82 (1H, dd, J = 7.3, J = 14.1, H-7), 2.97 (1H, dd, J = 5.0, J = 14.0, H-7), 3.84 (1H, dd, J = 7.0, J = 9.1, H-9′), 4.11 (1H, dd, J = 6.8, J = 9.0, H-9′), 5.89–5.95 (4H, m, OCH_2O × 2), 6.44–6.73 (6H, m, Ar-H); ^{13}C-NMR ($CDCl_3$): 34.93, 38.46, 41.38, 46.58, 71.23, 101.12, 108.37, 108.45, 108.91, 109.53, 121.63, 122.32, 131.43, 131.70, 146.45, 146.57, 147.96, 147.99, and 178.51; MS m/z [rel. int. (%)]: 354 (42.6, M^+), 218 (11.0), 192 (4.8), 173 (4.5), 162 (10.2), 135 (100), 105 (5.7).

Pluviatolide (**9**): ^{1}H-NMR ($CDCl_3$): 2.40–2.63 (4H, m, H-7′, H-7′, H-8, and H-8′), 2.87 (1H, dd, J = 6.7, J = 14.0, H-7), 2.95 (1H, dd, J = 5.1, J = 14.1, H-7), 3.83 (3H, s, OMe), 3.84 (1H, dd, J = 7.1, J = 9.0, H-9′), 4.10 (1H, dd, J = 7.0, J = 9.1, H-9′), 5.91–5.93 (2H, m, OCH_2O), 6.43–6.84 (6H, m, Ar-H); ^{13}C-NMR ($CDCl_3$): 34.71, 38.38, 41.10, 46.68, 55.97, 71.30, 101.14, 108.40, 108.90, 111.61, 114.34, 121.65, 122.16, 129.53, 131.70, 144.62, 146.41, 146.77, 147.95, and 178.79; MS m/z [rel. int. (%)]: 356 (51.7, M^+), 220 (11.9), 162 (10.2), 138 (15.1), 137 (100), 136 (26.3), 135 (36.5), 131 (7.8), 122 (7.1).

Haplomyrfolin (**10**): ^{1}H-NMR ($CDCl_3$): 2.43–2.62 (4H, m, H-7′, H-7′, H-8, and H-8′), 2.84 (1H, dd, J = 7.0, J = 14.0, H-7), 2.95 (1H, dd, J = 5.2, J = 14.0, H-7), 3.83 (3H, s, OMe), 3.86 (1H, dd, J = 7.2, J = 9.1, H-9′), 4.13 (1H, dd, J = 7.0, J = 9.1, H-9′), 5.92–5.93 (2H, m, OCH_2O), 6.45–6.81 (6H, m, Ar-H); ^{13}C-NMR ($CDCl_3$): 34.84, 38.39, 41.39, 46.53, 55.90, 71.31, 101.10, 108.29, 109.56, 111.06, 114.56, 121.36, 122.35, 129.83, 131.47, 144.49, 146.52, 146.64, 147.94, and 178.61; MS m/z [rel. int. (%)]: 356 (66.4, M^+), 164 (12.0), 149 (3.1), 138 (34.1), 137 (52.5), 136 (19.1), 135 (100), 131 (11.7), 122 (7.2).

3-(4-Ethoxy-3-methoxyphenyl)-1-propanol was prepared by ethylation of coniferyl alcohol[6] [EtI, K_2CO_3, in N,N-dimethylformamide (DMF)] followed by catalytic hydrogenation (H_2, 10% Pd-C, in methanol).

3-(4-Ethoxy-3-methoxyphenyl)-1-propanol: ^{1}H-NMR ($CDCl_3$): 1.45 (3H, t, O—C—CH_3), 1.88 (2H, tt, J = 8.0, J = 6.5, C—CH_2—C—O), 2.66 (2H, t, J = 8.0, CH_2—C—C—O), 3.68 (2H, t, J = 6.5, C—C—CH_2—O), 3.86 (3H, s, OMe), 4.07 (2H, q, J = 7.0, O—CH_2—C), 6.70–6.85 (3H, m, Ar-H); MS m/z [rel. int. (%)]: 210 (100, M^+), 182 (13.8), 166 (26.3), 165 (39.0), 163 (14.7), 138 (36.0), 137 (86.2).

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** and **12**.

Hinokinin (**8**): ^{1}H-NMR ($CDCl_3$): 2.43–2.58 (4H, m), 2.83 (1H, dd, J = 7.1, J = 14.1), 2.97 (1H, dd, J = 6.3, J = 15.3), 3.85 (1H, dd, J = 7.2, J = 9.2), 4.11 (1H, dd, J = 6.8, J = 9.3), 5.91–5.96 (4H, m), 6.44–6.73 (6H, m); ^{13}C-NMR ($CDCl_3$): 34.90, 38.43, 41.35, 46.54, 71.21, 101.09, 108.35, 108.42, 108.87, 109.50, 121.61, 122.29, 131.38, 131.66, 146.41, 146.53, 147.92, 147.95, and 178.50; MS m/z [rel. int. (%)]: 354 (71.2, M^+), 218 (14.1), 192 (6.9), 173 (5.8), 162 (13.5), 135 (100), 105 (7.4).

Hinokiresinol (**12**): ^{1}H-NMR ($CDCl_3$): 4.12 (1H, broad t, J = 6.6, H-7), 5.07 (1H, dt, J = 1.5, J = 17.3, H-9_{trans}), 5.11

(1H, dt, $J = 1.4$, $J = 10.3$, H-9_{cis}), 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); ^{13}C-NMR ($CDCl_3$): 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 (163.43 g) were pulverized using a Waring blender and then extracted seven times with hot methanol (total 1300 ml). The combined methanol extracts (43.76 g) were suspended in distilled water (300 ml), which was extracted with diethyl ether (200 ml × 3). The combined diethyl ether extracts (13.26 g) 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** (5.6 mg), **2** (0.6 mg), **3** (22.7 mg), **4** (19.4 mg), **5** (3.6 mg), **6** (18.6 mg), **7** (2.8 mg), **8** (6.2 mg), **9** (1.9 mg), **10** (1.1 mg), and **11** (3.3 mg).

Xanthoxylol (**1**): ^{1}H-NMR ($CDCl_3$): 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, OCH_2O), 6.77–6.90 (6H, m, Ar-H); ^{13}C-NMR ($CDCl_3$): 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 (8.5), 205 (19.2), 203 (15.4), 178 (22.6), 163 (23.2), 161 (36.8), 151 (100), 150 (34.7), 149 (65.9), 137 (43.9), 135 (49.0), 131 (44.8), 122 (17.2), 103 (10.2); high-resolution MS *m/z* (M^+): calculated for $C_{20}H_{20}O_6$ 356.1260, found 356.1273.

7-Oxohinokinin (**2**): ^{1}H-NMR ($CDCl_3$): 2.76 (2H, dd, $J = 4.5$, $J = 7.5$, H-7′ and H-7′), 3.22–3.59 (1H, m, H-8′), 4.12 (1H, dd, $J = 5.5$, $J = 9.0$, H-9′), 4.18 (1H, d, $J = 5.5$, H-8), 4.51 (1H, dd, $J = 6.8$, $J = 8.8$, H-9′), 5.93 (2H, s, OCH_2O), 6.05 (2H, s, OCH_2O), 6.56–6.63 (2H, m, H-2′ and H-6′), 6.72 (1H, d, $J = 8.0$, H-5′), 6.81 (1H, d, $J = 8.0$, H-5), 7.28 (1H, d, $J = 1.8$, H-2), 7.41 (1H, dd, $J = 2.0$, $J = 8.0$, H-6); MS *m/z* [rel. int. (%)]: 368 (20.3, M^+), 233 (8.7), 178 (8.5), 161 (100), 149 (62.4), 135 (26.3), 131 (66.9), 121 (19.4); high-resolution MS *m/z* (M^+): calculated for $C_{20}H_{16}O_7$ 368.0896, found 368.0886.

Savinin (**3**): ^{1}H-NMR ($CDCl_3$): 2.58 (1H, dd, $J = 10.1$, $J = 14.3$, H-7′), 2.98 (1H, dd, $J = 4.4$, $J = 14.1$, H-7′), 3.70–3.76 (1H, m, H-8′), 4.21–4.27 (2H, m, H-9′ and H-9′), 5.92 (2H, dd, $J = 1.5$, $J = 3.7$, OCH_2O), 6.03 (2H, s, OCH_2O), 6.62 (1H, dd, $J = 1.8$, $J = 7.9$, H-6′), 6.65 (1H, d, $J = 1.5$, H-2′), 6.72 (1H, d, $J = 7.8$, H-5′), 6.87 (1H, d, $J = 8.1$, H-5), 7.03 (1H, d, $J = 1.7$, H-2), 7.07 (1H, dd, $J = 1.7$, $J = 8.1$, H-6), 7.48 (1H, d, $J = 2.0$, H-7); ^{13}C-NMR ($CDCl_3$): 37.63, 40.00, 69.60, 101.14, 101.83, 108.59, 108.76, 108.91, 109.25, 122.18, 125.91, 126.19, 128.27, 131.58, 137.38, 146.65, 148.04, 148.45, 149.29, and 172.63; MS *m/z* [rel. int. (%)]: 352 (15.8, M^+), 217 (20.2), 189 (4.1), 159 (7.6), 135 (100); high-resolution MS *m/z* (M^+): calculated for $C_{20}H_{16}O_6$ 352.0947, found 352.0955.

Dihydrosesamin (**4**): ^{1}H-NMR ($CDCl_3$): 2.30–2.37(1H, m, H-8), 2.52 (1H, dd, $J = 10.5$, $J = 13.4$, H-7′), 2.64–2.73 (1H, m, H-8′), 2.86 (1H, dd, $J = 5.2$, $J = 13.5$, H-7′), 3.71 (1H, dd, $J = 6.6$, $J = 8.5$, H-9′), 3.73 (1H, dd, $J = 6.8$, $J = 10.7$, H-9), 3.87 (1H, dd, $J = 7.0$, $J = 10.6$, H-9), 4.03 (1H, dd, $J = 6.6$, $J = 8.5$, H-9′), 4.78 (1H, d, $J = 6.1$, H-7), 5.92 (2H, s, OCH_2O), 5.93 (2H, s, OCH_2O), 6.61–6.82 (6H, m, Ar-H); ^{13}C-NMR ($CDCl_3$): 33.27, 42.35, 52.64, 60.88, 72.93, 82.88, 100.92, 101.02, 106.31, 108.09, 108.32, 108.96, 119.08, 121.45, 134.19, 137.10, 145.95, 146.91, 147.79, and 147.86; MS *m/z* [rel. int. (%)]: 356 (71.3, M^+), 192 (26.1), 178 (27.3), 173 (16.5), 162 (13.6), 151 (28.0), 149 (43.3), 148 (17.8), 136 (28.4), 135 (100), 122 (11.6), 77 (25.3); high-resolution MS *m/z* (M^+): calculated for $C_{20}H_{20}O_6$ 356.1260, found 356.1255.

Sesamin (**6**): ^{1}H-NMR ($CDCl_3$): 3.00–3.08 (2H, m, H-8 and H-8′), 3.86 (2H, dd, $J = 3.7$, $J = 9.3$, H-9 and H-9′), 4.22 (2H, dd, $J = 9.0$, $J = 6.8$, H-9 and H-9′), 4.70 (2H, d, $J = 4.4$, H-7 and H-7′), 5.94 (4H, s, OCH_2O × 2), 6.76–6.84 (6H, m, Ar-H); ^{13}C-NMR ($CDCl_3$): 54.36, 71.74, 85.82, 101.11, 106.52, 108.22, 119.39, 135.09, 147.14, and 147.99; MS *m/z* [rel. int. (%)]: 354 (50.9, M^+), 219 (7.4), 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 $C_{20}H_{18}O_6$ 354.1103, found 354.1101.

Piperitol (**7**): ^{1}H-NMR ($CDCl_3$): 3.02–3.11 (2H, m, H-8 and H-8′), 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 (2H, s, OCH_2O), 6.76–6.89 (6H, m, Ar-H); ^{13}C-NMR ($CDCl_3$): 54.20, 54.35, 55.98, 71.70, 71.74, 85.84, 85.89, 101.11, 106.54, 108.22, 108.59, 114.28, 119.01, 119.39, 132.91, 135.11, 145.27, 146.72, 147.13, and 147.99; MS *m/z* [rel. int. (%)]: 356 (78.4, M^+), 325 (11.5), 205 (15.8), 203 (21.0), 189 (16.2), 178 (17.5), 163 (35.1), 161 (44.5), 151 (85.4), 150 (54.8), 149 (100), 137 (36.3), 135 (60.3), 131 (65.0), 122 (23.6), 103 (13.0); high-resolution MS *m/z* (M^+): calculated for $C_{20}H_{20}O_6$ 356.1260, found 356.1254.

Hinokinin (**8**): ^{1}H-NMR and mass spectra coincided with those of **8** isolated from *C. obtusa* wood and of chemically synthesized one in all respects; high-resolution MS *m/z* (M^+): calculated for $C_{20}H_{18}O_6$ 354.1103, found 354.1103.

Pluviatolide (**9**): ^{1}H-NMR ($CDCl_3$): 2.36–2.68 (4H, m), 2.88 (1H, dd, $J = 5.3$, $J = 13.3$), 2.97 (1H, dd, $J = 4.5$, $J = 13.5$), 3.84 (3H, s), 3.85 (1H, dd, $J = 7.0$, $J = 9.0$), 4.11 (1H, dd, $J = 6.5$, $J = 9.3$), 5.93 (2H, s), 6.43–6.85 (6H, m); MS *m/z* [rel. int. (%)]: 356 (41.2, M^+), 220 (14.8), 162 (10.3), 138 (16.9), 137 (100), 136 (29.3), 135 (38.6), 131 (10.4), 122 (8.9); high-resolution MS *m/z* (M^+): calculated for $C_{20}H_{20}O_6$ 356.1260, found 356.1259.

Haplomyrfolin (**10**): ^{1}H-NMR ($CDCl_3$): 2.41–2.67 (4H, m), 2.85 (1H, dd, $J = 6.8$, $J = 14.0$), 2.97 (1H, dd, $J = 4.8$, $J = 13.8$), 3.84 (3H, s), 3.87 (1H, dd, $J = 7.0$, $J = 10.5$), 4.15

(1H, dd, J = 6.5, J = 9.0), 5.93 (2H, s), 6.45–6.83 (6H, m); MS m/z [rel. int. (%)]: 356 (46.5, M^+), 164 (9.5), 149 (13.1), 138 (38.3), 137 (57.9), 136 (20.2), 135 (100), 131 (16.0), 122 (9.2); high-resolution MS m/z (M^+): calculated for $C_{20}H_{20}O_6$ 356.1260, found 356.1274.

Matairesinol (**11**): ^{1}H-NMR ($CDCl_3$): 2.46–2.78 (4H, m, H-7′, H-7′, H-8, and H-8′), 2.86 (1H, dd, J = 6.0, J = 14.0, H-7), 2.96 (1H, dd, J = 5.0, J = 13.3, H-7), 3.805 (3H, s, OMe), 3.811 (3H, s, OMe), 3.88 (1H, dd, J = 6.5, J = 9.3, H-9′), 4.15 (1H, dd, J = 6.5, J = 9.0, H-9′), 6.39–6.83 (6H, m, Ar-H); MS m/z [rel. int. (%)]: 358 (38.8, M^+), 149 (11.1), 138 (36.5), 137 (100), 122 (10.5), 136 (29.3), 135 (38.6), 131 (10.4), 122 (8.9); high-resolution MS m/z (M^+): calculated for $C_{20}H_{22}O_6$ 358.1416, found 358.1424.

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**.

Results

Isolation of hinokinin (**8**) and hinokiresinol (**12**) from *C. obtusa* wood and their quantitation in a cross section of the trunk.

A dibenzylbutyrolactone lignan (**8**) and a norlignan (**12**) (Fig. 1) were isolated from hinoki wood, confirming the previous report.[8] Figure 2 shows the distribution of **8** and **12** in a cross section of *C. obtusa* wood. A heartwood–sapwood boundary was observed between the 22nd and 23rd annual rings by anatomical observation. The contents of the two compounds in the heartwood region were higher than those in the sapwood in order of magnitude.

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**), dihydrosesamin (**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] **2**,[11] **3**,[12,13] **4**,[14,15] **6**,[16] and **7**[17,18]). As for compound (**1**), with a relative configuration of 7*R**, 7′*S**, 8*S**, and 8′*S**, the possibility of its regioisomer with 7*R**, 7′*S**, 8*R**, and 8′*R**, pluviatilol, was eliminated by comparing the ^{1}H-NMR data of the acetate of **1** with that of acetyl pluviatilol, as described by Gonzaléz et al.[9] The lignans **8**, **9**, **10**, and **11** were identified by directly comparing their ^{1}H-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.[19]

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**),[2] and later Keimatsu and Ishiguro proposed the structure of **8** shown in Fig. 1 for the lignan.[21] They reported that hinokinin (**8**) accounted for about 30% of the resins extracted from the heartwood,[2,8] which is a rather high yield among naturally occurring lignans. The present investigation confirmed this high yield of **8** from the heartwood.

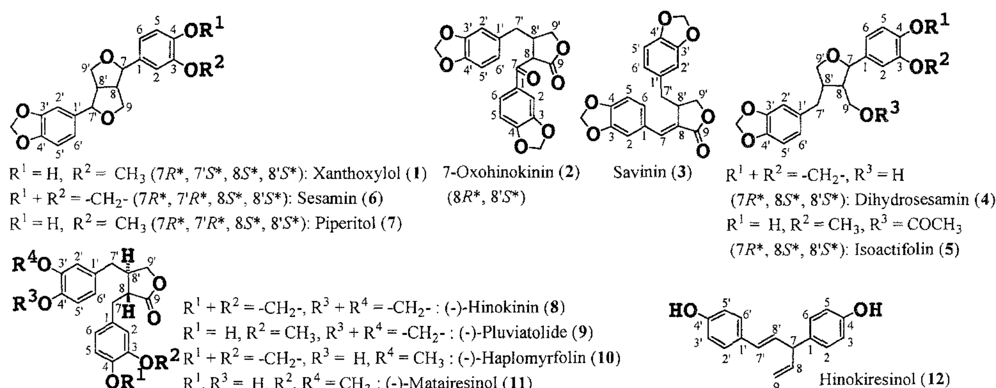

Fig. 1. Structures of lignans and a norlignan

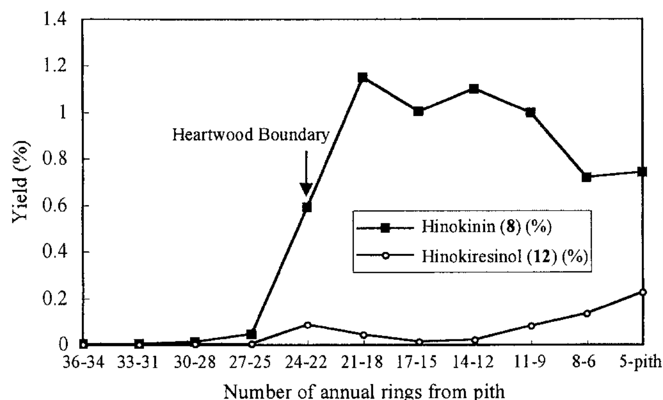

Fig. 2. Distribution of hinokinin (**8**) and hinokiresinol (**12**) in a cross section of *Chamaecyparis obtusa*

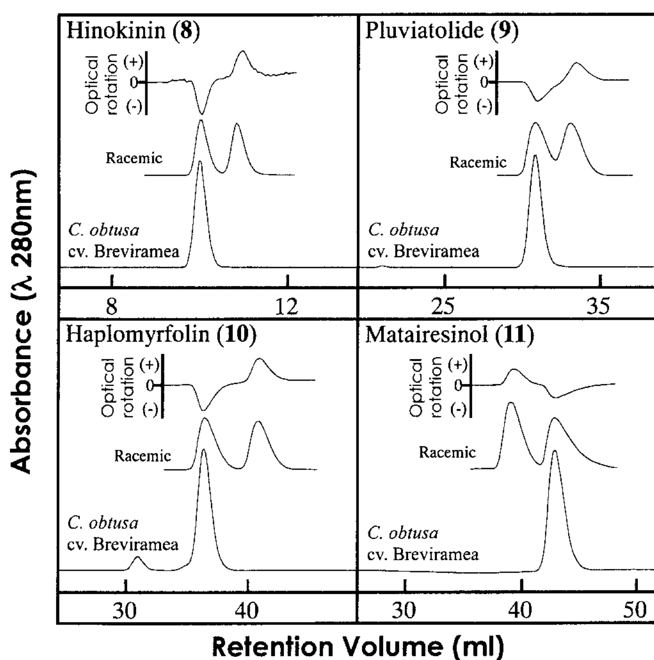

Fig. 3. Chiral high-performance liquid chromatograms of lignans isolated from *Chamaecyparis obtusa* cv. Breviramea. Elution details are described in the Experimental section. Optical rotation was detected by a chiral detector. *C. obtusa* cv. Breviramea: lignans isolated from *C. obtusa* cv. Breviramea; *Racemic*: racemic authentic sample; (+), (−), dextrorotatory and levorotatory enantiomers, respectively

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.[3,22] Also, the lignan (**6**) was isolated from *C. obtusa* cv. Breviramea.[5]

These earlier reports[3–5,22] 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, **3**[3,22] and **6**.[5] In addition to the three lignans, the presence of matairesinol (**11**) was suggested by mass chromatographic analysis. However, possible precursors of **11** (pinoresinol, 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-$^2H_2$,OC$^2H_3$]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-$^2H_2$,OC$^2H_3$]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.[25–30] These studies together with the present feeding experiment suggest that the conversion to **11** occurs generally in plants including *C. obtusa* cv. Breviramea.

In additon, 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 corresposponding 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**; furofuran lignans **1**, **6**, and **7**; furan lignans **4**, and **5**. The lignan (**5**) was a new lignan, and its isolation has been reported.[19] 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, nortrachelogenin 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.

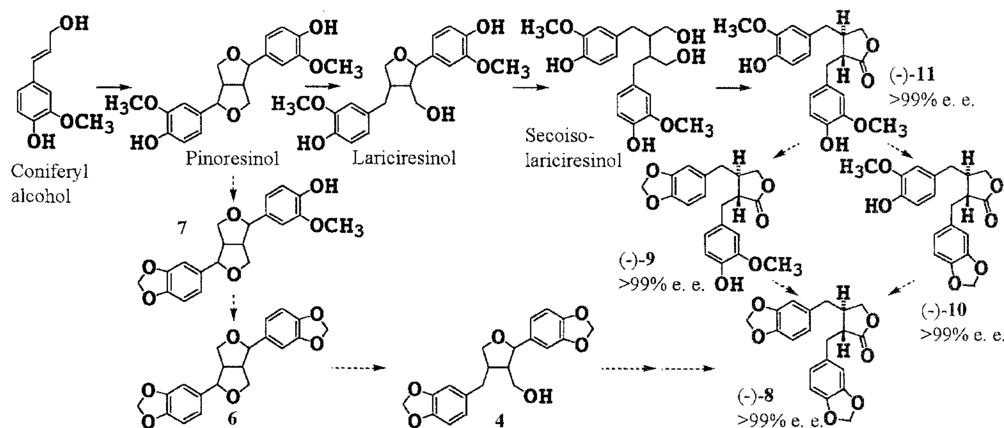

Fig. 4. Possible biosynthetic pathways for lignans in *Chamaecyparis obtusa* cv. Breviramea. *Arrows*, a presumed route based on the feeding experiment with deuterium-labeled coniferyl alcohol; *broken arrows*, a putative pathway based on consideration of the chemical structures

Recent studies on lignan biosynthesis have indicated that there is stereochemical diversity in lignan biosynthesis,[20,29,30] 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.[20] 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 *C. obtusa* were found to be levorotatory and optically pure (>99% e.e.), which accorded well with previous reports on enantiomeric compositions of dibenzylbutyrolactone lignans.[20]

In addition to the dibenzylbutyrolactone lignans, five furofuran and furan lignans were isolated. Two of them (**4** and **6**) have two piperonyl (methylenedioxyphenyl) groups, and the rest (**1**, **5**, **7**) have one piperonyl 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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