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Identification of *Thuja occidentalis* lignans and its biosynthetic relationship

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Abstract

Five lignans, (8*R*,8′*R*)-(−)-matairesinol, (8*R*,8′*R*)-(−)-thujaplicatin methyl ether, (8*S*,8′*S*)-(−)-wikstromol, 8-hydroxythujaplicatin methyl ether and *epi*-pinoresinol were isolated from *Thuja occidentalis* branch xylem, besides the previously identified (8*R*,8′*R*)-(−)-4-*O*-demethylyatein. Chiral HPLC analyses of matairesinol, thujaplicatin methyl ether, wikstromol and 4-*O*-demethylyatein indicated that these compounds were optically pure. A neolignan, dihydrodehydrodiconiferyl alcohol, was isolated from the acid-hydrolyzed extracts of *T. occidentalis* leaves. The lignans pinoresinol and secoisolariciresinol were also identified by GC–MS analysis of the extracts of xylem and leaves, respectively.

Feeding experiments of [9-$^2H_2$, OC^2H_3]coniferyl alcohol into *T. occidentalis* young shoots showed that two molecules of coniferyl alcohol were incorporated into pinoresinol and lariciresinol.

Keywords: Thuja occidentalis; Cupressaceae; Lignan biosynthesis; Gymnosperm lignin; 3,5-Dimethoxy-4-hydroxyphenyl group; Stereochemistry

1. Introduction

Gymnosperm *Thuja* species (Cupressaceae) accumulate characteristic lignans with a 3,5-dimethoxy-4-hydroxyphenyl (syringyl) group (Maclean, & Murakami, 1966a, 1966b; Murakami, 1970). However, these oxidative modifications are not present in gymnosperm lignin (Kutsuki, Shimada, & Higuchi, 1981) and it is of interest to know whether the syringyl group is formed before or after dimerization of phenylpropane units.

In the previous paper (Kawai, Hasegawa, Gotoh, & Ohashi, 1994), we searched for lignans with a syringyl group in the branch wood of *T. occidentalis* and isolated (−)-4-*O*-demethylyatein [(8*R*,8′*R*)-(−)-2-(3,5-dimethoxy-4-hydroxybenzyl)-3-(3,4-methylenedioxybenzyl)butyrolacotone (**1**)] from this tree.

In this paper, we report the isolation of lignans from the branch xylem and leaves of *T. occidentalis* mainly of guaiacyl type and chiral HPLC analyses of isolated dibenzylbutyrolactones. In addition, the bioconversion of deuterated coniferyl alcohols fed into the lignans of *T. occidentalis* young shoots was examined.

2. Results and discussion

To identify lignans presented in *T. occidentalis*, the branch xylem and leaves were extracted with MeOH and the extract was fractionated. In addition (−)-4-*O*-demethylyatein (**1**) (Kawai et al., 1994), (−)-matairesinol (**2**) and (−)-thujaplicatin methyl ether (**3**) were isolated from the xylem and the structures were identified by direct comparison with synthetic compounds, respectively. Furthermore, (−)-wikstromol (**4**) (Umezawa, & Shimada, 1996), 8-hydroxythujaplicatin methyl ether (**5**) (Maclean, & Murakami, 1966b) and *epi*-pinoresinol (**6**) (Katayama, Davin, Chu, & Lewis, 1993) from the xylem were isolated and identified by comparison of analytical data. A neolignan, dihydro-

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dehydrodiconiferyl alcohol (**7**) (as acetate) (Nabeta, Hiraata, Ohki, Samaraweera, & Okuyama, 1994) was also isolated from the acid-hydrolyzed extracts of *T. occidentalis* leaves. Pinoresinol (**8**) and 4-*O*-demethylyatein (**1**) in the xylem and secoisolariciresinol (**9**) (Ohashi, Kawai, Sakurai, & Yasue, 1992) in the leaves were confirmed by GC–MS analyses by comparison with authentic compounds.

To confirm the enantiomeric composition, four lignans, 4-*O*-demethylyatein (**1**), matairesinol (**2**), thujaplicatin methyl ether (**3**) and wikstromol (**4**), were analyzed by chiral HPLC. Fig. 1 shows the HPLC chromatograms of racemic and isolated lignans **1**–**4**, respectively, and the results clearly indicated that these lignans isolated from *T. occidentalis* were optically pure. This is a first report of chiral HPLC separation of **1** and **3**. The stereochemistry of lignan formation in *T. occidentalis* is at least regulated at the stage of dibenzylbutyrolactone type.

[9-$^2H_2$, OC$^2H_3$]Coniferyl alcohol was administered to excised *T. occidentalis* shoots which were allowed to metabolize for 24 h. The lignan fraction was analyzed by GC–MS and formation of pinoresinol (**8**) and lariciresinol (**10**) was confirmed (Fig. 2). The formed products **8** and **10** gave molecular ion peaks 10 mass units higher than those of unlabelled authentic compounds. The results demonstrated that two molecules of the administered penta deuterated coniferyl alcohols were incorporated into lignans **8** and **10**. However, attempts to isolate **8** and **10** failed and the enantiomeric compositions of the lignans could thus not be analyzed. This feeding experiment and the isolation of secoisolariciresinol (**9**) and matairesinol (**1**) insinuate that the pathway from coniferyl alcohol to matairesinol (**1**), which was first proven in *Forsythia* sp. (Oreaceae) (Chu, Dinkova, Davin, Bedgar, & Lewis, 1993; Dinkova-Kostova, Gang, Davin, Bedgar, & Lewis, 1996; Davin et al., 1997; Umezawa, 1997), exists also in this conifer. Very recently, Davin et al. demonstrated that the recombinant pinoresinol/lariciresinol reductase obtained from *T. plicata* cDNA caused the enantiospecific reduction of pinoresinol (**8**) to secoisolariciresinol (**9**) (Davin, Gang, Fujita, Anterola, & Lewis, 1997).

T. plicata and *T. standishii* accumulate the lignans, thujaplicatin methyl ether (**3**), dihydroxythujaplicatin methyl ether and so on, which have syringyl groups (Maclean, & Murakami, 1966a, 1966b; Murakami, 1970; Kawai et al., 1994). These oxidative modifications in the aromatic ring are not present in gymnosperm lignin, because of the oxidative pattern of gymnosperm lignin contain mostly 3-methoxy-4-hydroxyphenyl (guaiacyl) group (Kutsuki et al., 1981).

The possible formation pathway of syringyl lignans **1**, **3** and **5** is shown in Fig. 3. Although we could not detect in this tree thujaplicatin (**11**) which has 3,4-dihydroxy-5-methoxyphenyl group, it is likely that in *Thuja*

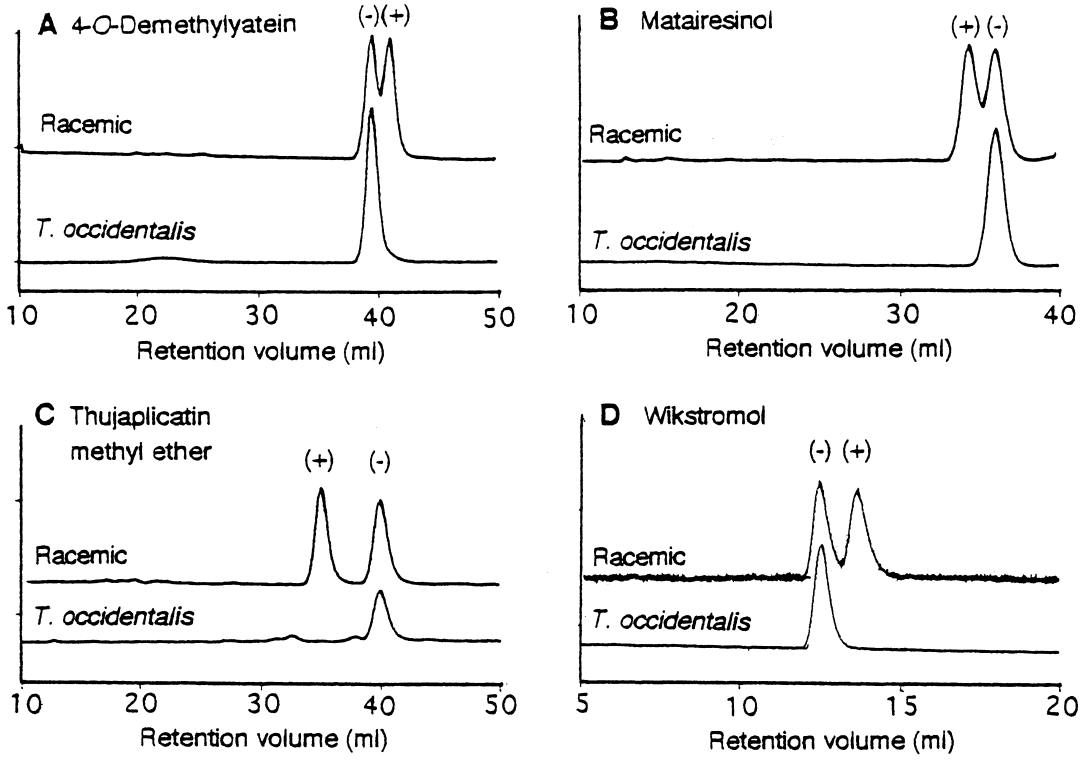

Fig. 1. Chiral HPLC chromatograms of (A) 4-*O*-demethylyatein, (B) matairesinol, (C) thujaplicatin methyl ether and (D) wikstromol isolated from *Thuja occidentalis*. Chiralcel OD for (A), (B) and (C) and chiralcel OC for (D) were used.

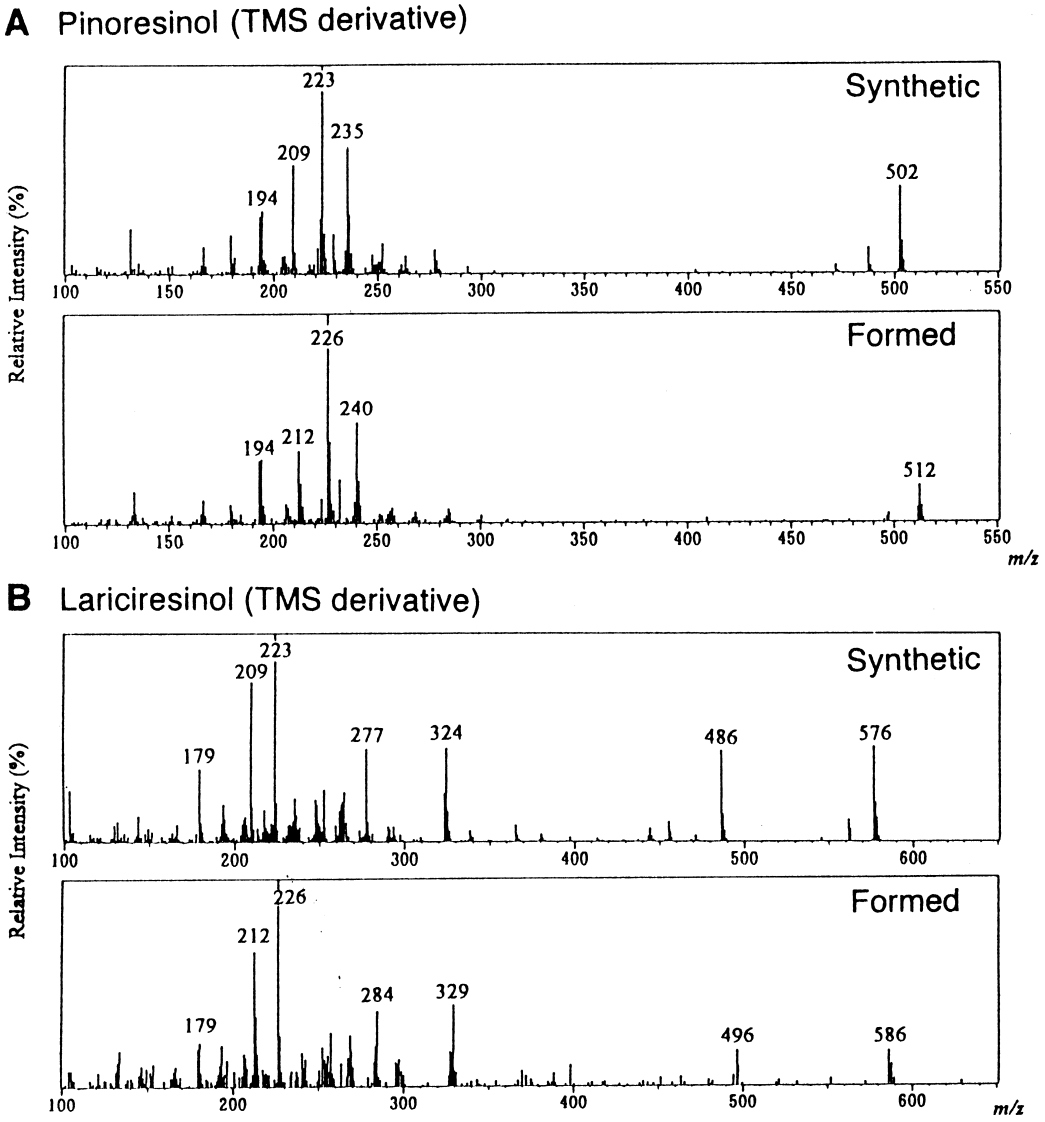

Fig. 2. Mass spectra of (A) pinoresinol and (B) lariciresinol obtained following administration of [9-$^2H_2$, OC$^2H_3$]coniferyl alcohol into *Thuja occidentalis* young shoots.

spp. matairesinol (**2**) is first biosynthesized and it was converted to syringyl lignans **1**, **3** and **5** via the hydroxylation, the methylation and so on. In angiosperm *Podophyllum hexandrum* (Berberidaceae), it was demonstrated that ferulic acid (Jackson, & Dewick, 1984) and matairesinol (**2**) (Broomhead, Rahman, Dewick, Jackson, & Lucas, 1991) were good precursors of *Podophyllum* lignans with syringyl and 3,4,5-trimethoxyphenyl groups, whereas sinapic acid was poorly utilized (Jackson, & Dewick, 1984).

3. Experimental

3.1. General

TLC and CC were performed on Kieselgel 60 F_{254} (Merck) and silica gel BW200 (Fuji Silysia). HPLC was carried out with detection at 280 nm using either a reversed phase (μ Bondasphere 5 μC_{18}-100 Å, 19 × 150 mm, Waters) or chiral (Chiralcel OD and OC, 4.6 × 250 mm, Daicel) columns. Sepn by reversed phase HPLC was performed with MeOH–H_2O (1:1) at a flow rate of 7 ml min^{-1}. Chiral HPLC was performed with 1% AcOH in EtOH–*n*-hexane (15:85) at a flow rate 1 ml min^{-1} for 4-*O*-demethylyatein (**1**), matairesinol (**2**) and thujaplicatin methyl ether (**3**) with Chiralcel OD and with EtOH–*n*-hexane (1:1) at a flow rate of 0.5 ml min^{-1} for wikstromol (**4**) with Chiralcel OC. ^1H NMR (400 MHz) spectra were taken with a Varian FT-NMR Unity Inova 400 using TMS as an internal standard. DI- and GC–MS were performed with a Shimadzu GCMS-QP 5000 gas chromatograph mass spectrometer (EI, 70 eV). GC–MS analyses were

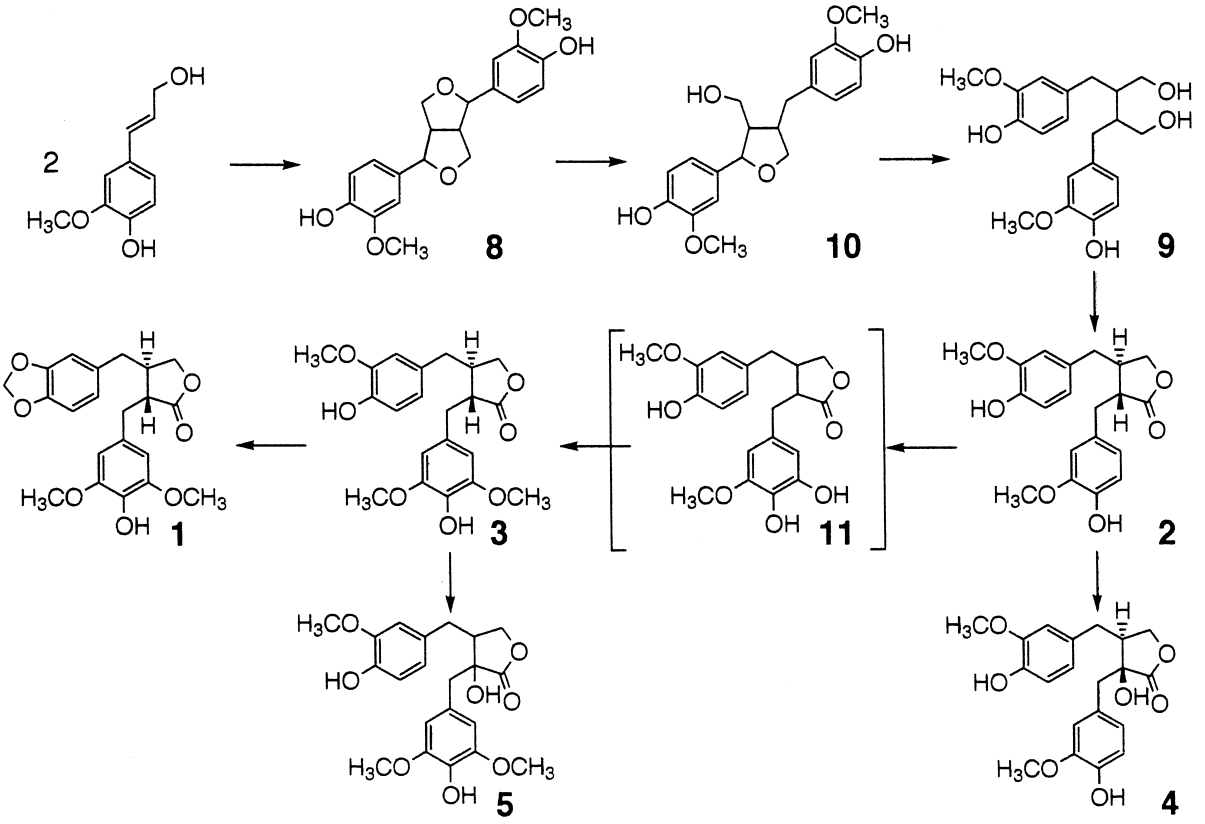

Fig. 3. Possible biosynthetic pathways to the lignans of *Thuja occidentalis*.

carried out on a capillary column (DB-1, 30 m × 0.25 mm i.d., film, 1 μm, J&W Scientific) at a rate of 5°C min^{-1} from 200 to 280°C using He as a carrier gas. Optical rotations were measured with a Union Automatic Digital Polarimeter.

3.2. Syntheses of authentic compounds

(±)-4-*O*-Demethylyatein (**1**), (±)-matairesinol (**2**) and (±)-thujaplicatin methyl ether (**3**) were synthesized by the methods of the previous papers (Umezawa, Davin, & Lewis, 1991; Kawai et al., 1994). (±)-Wikstromol (**4**) (Umezawa, & Shimada, 1996) and (±)-pinoresinol (**8**) were generous gifts of Associate Professor T. Umezawa, Kyoto University, and Professor M. Tanahashi, Gifu University, respectively. (−)-Secoisolariciresinol (**9**) was used as previously isolated from *Araucaria angustifolia* (Araucariaceae) (Ohashi et al., 1992). Lariciresinol (**10**) was obtained by the hydrogenation of pinoresinol (**8**). [9-$^2H_2$, OC^2H_3]Coniferyl alcohol was prepared as previously described (Umezawa et al., 1991).

3.3. Extraction and isolation

Branches of *T. occidentalis* L. grown on the campus of Gifu University were cut in June, 1996 and separated into the xylem and leaves parts. The air dried and powdered xylem (230 g) was extracted and fractionated by CC (EtOAc–*n*-hexane, 1:1) by the methods previously described (Kawai et al., 1994). The frozen and powdered leaves (100 g) were extracted with hot MeOH for 24 h. The MeOH solubles were extracted with *n*-hexane. The residual layer was concentrated and partitioned between EtOAc and water. The water solubles were hydrolyzed with 5% HCl and reextracted with EtOAc. The combined EtOAc solubles were dried and the residual part was fractionated by HPLC. The fractions from the xylem and leaves were analyzed by GC–MS and further purified by repeated preparative TLC (MeOH–CH_2Cl_2, 2:98 and/or 3:97) and HPLC.

(8*R*,8′*R*)-(−)-Matairesinol (**2**), $[\alpha]_D = -29.5°$ ($c = 2.4 \times 10^{-3}$, $CHCl_3$), (11.3 mg) and (8*R*,8′*R*)-(−)-thujaplicatin methyl ether (**3**), $[\alpha]_D = -29.5°$ ($c = 2.1 \times 10^{-3}$, $CHCl_3$), (11.0 mg) were isolated from the extractives of xylem and the structures were identified by direct comparison with authentic compounds, respectively.

Furthermore, (8*R*,8′*R*)-(−)-wikstromol (**4**), $[\alpha]_D = -23.9°$ ($c = 0.92 \times 10^{-3}$, $CHCl_3$), (6.3 mg) (Umezawa, & Shimada, 1996), 8-hydroxythujaplicatin methyl ether (**5**) (< 1 mg) (Maclean, & Murakami, 1966b) and *epi*-pinoresinol (**6**) (< 1 mg) (Katayama et

al., 1993) were isolated and identified by comparison of analytical data.

4-*O*-Demethylyatein (**1**) (Kawai et al., 1994) and pinoresinol (**8**) were confirmed by GC–MS analyses by direct comparison with authentic compounds.

Secoisolariciresinol (**9**) was confirmed in the leaf extracts by GC–MS analyses with the authentic sample (Ohashi et al., 1992). Since the presence of dihydrodehydrodiconiferyl alcohol (**7**) was also suggested, the fraction was acetylated and it was purified by preparative TLC (CH_2Cl_2). Triacetate of **7** (3.2 mg) was obtained and identified by comparison of analytical data (Nabeta et al., 1994).

3.4. Feeding experiment

[9-$^2H_2$, OC$^2H_3$]Coniferyl alcohol (25 mM in 0.1 M phosphate buffer (pH 7.0), 500 μl × 10) was administrated to 10 excised *T. occidentalis* shoots (cut in September, 1996) which were allowed to metabolized for 24 h. The leaves were removed from the resulting shoots and stems were frozen, powdered and extracted with hot MeOH for 24 h. The lignan fraction was separated from the extracts by TLC and it was analyzed by GC–MS as TMS derivatives.

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