J Wood Sci (2002) 48:440–445

NOTE

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Lignans of *Linum flavum* var. *compactum*

Received: July 11, 2001 / Accepted: November 14, 2001

Abstract A new dibenzylbutyrolactone lignan 7,6′-dihydroxybursehernin, together with six known lignans (pinoresinol, lariciresinol, secoisolariciresinol, α-peltatin, β-peltatin, 5-methoxypodophyllotoxin) were isolated from the methanol extracts of *Linum flavum* var. *compactum*. The enantiomeric analysis of pinoresinol and lariciresinol isolated from the species, which are upstream lignans in the lignan biosynthetic pathway, indicated that they are not optically pure, which is in accordance with our recent findings on lignans occurring in other plant species.

Key words Lignan · 7,6′-Dihydroxybursehernin · *Linum flavum* var. *compactum* · Linaceae · Biosynthesis

Introduction

An aryltetralin lignan, podophyllotoxin (Fig. 1), has been isolated from herbaceous perennial *Podophyllum* species and has long been reputed to have antitumor activity.[1–4] The lignan is exploited commercially as a source of a semisynthetic anticancer drug, etoposide, which is being applied successfully as cancer chemotherapy.[1–3] Because of the limited supply of *Podophyllum emodi*, much attention has been focused on the availability of the lignan in various plants and its biotechnological production.[3] Several woody plants (e.g., *Juniperus sabina*, *Thujopsis dolabrata*, *Callitrus drummondii*, *Hernandia ovigera*) produce podophyllotoxin, its congeners, or both.[1] Hence, production of the antitumor lignan by woody plants is a challenging subject in the field of wood chemistry and biochemistry. As the first step to accessing biotechnological production it is necessary to understand the biosynthetic mechanisms, but much remains unknown about the biosynthesis of this lignan.[4]

In addition to these woody plants and *Podophyllum* spp., some herbaceous plants[1] such as *Anthriscus sylvestris*[5] and *Linum* spp. (especially those belonging to the section *Syllinum* including *Linum flavum* and *Linum album*)[6–10] have been known to produce podophyllotoxin and related lignans. In addition, suspension, root, and hairy root cultures of *Linum* species producing significant amounts of 5-methoxypodophyllotoxin (Fig. 1) and related lignans have been established.[8,11–14] Thus, *Linum* plants are attractive as plant systems for elucidating the biosynthetic mechanisms of podophyllotoxin congeners, and the detailed knowledge of the mechanisms can be applied to biotechnological production of the lignans in woody plants as well as *Linum* spp. As for *A. sylvestris*, this species exhibits good growth behavior and contains large amounts of desoxypodophyllotoxin (deoxypodophyllotoxin, anthricin) and yatein (Fig. 1), which were reported to be precursors of podophyllotoxin.[15,16] Thus, the application of this species to the studies of podophyllotoxin biosynthesis is also of interest; we have reported the lignan analysis in *A. sylvestris* and formation of lignans by an enzyme preparation of the plant.[5]

Recently, enzymatic reactions of upstream steps in the lignan biosynthetic pathway in *L. flavum* have been reported: conversion of pinoresinol to 7′-hydroxymatairesinol (Fig. 1) via lariciresinol, secoisolariciresinol, and matairesinol.[17] However, the enantiomeric compositions of the upstream lignans and other possible precursors of aryltetralin lignans occurring in this *Linum* species remains unknown. Herein we report isolation of a new lignan, 7,6′-dihydroxybursehernin, and six known lignans (Fig. 1) from

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Parts of this report were presented at the 42nd Lignin Symposium, Sapporo, October 1997; and the 43rd Lignin Symposium, Fuchu, October 1998

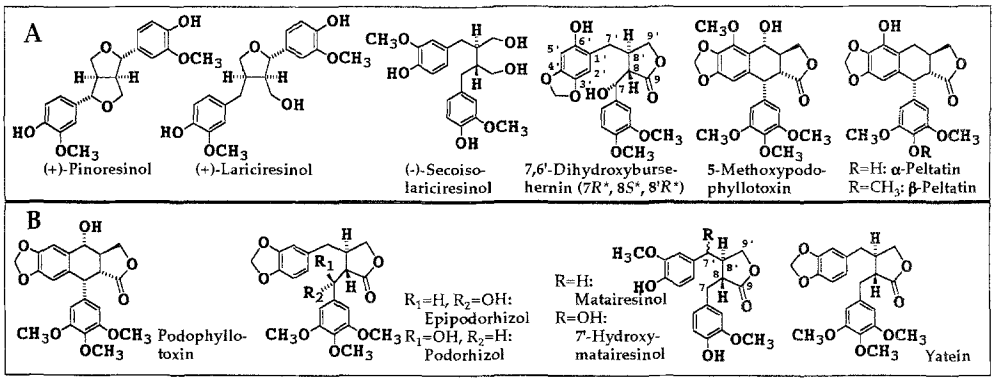

Fig. 1. Structures of lignans isolated from *Linum flavum* var. *compactum* (**A**) and related lignans (**B**)

L. flavum var. *compactum* and the stereochemical characterization of the upstream lignans.

Experimental

Plant material

Seeds of *Linum flavum* var. *compactum* (Harris Seeds, Rochester, NY, USA) were sowed in a gardening soil and grown in an incubator at 23°C under a day (ca. 20000lux)/night regime (14/10h) for 3 years.

General experimental procedures

One-dimensional and two-dimensional nuclear magnetic resonance (NMR) spectra were obtained with a JNM-LA400MK FT-NMR system (JEOL). Chemical shift and coupling constants (J) were expressed in δ and Hz, respectively. Low- and high-resolution electron impact-mass spectrometry (EI-MS), gas chromatography-mass spectrometry (GC-MS), and high-performance liquid chromatography (HPLC) were conducted as previously described.[18–24] The reversed-phase HPLC column used was a Waters Symmetry C18 (150 × 3.9mm), which was eluted with the following three solvent systems: (1) Solvent system A was for linear gradient elution at 1ml/min by CH_3CN–H_2O (25:75) at t = 0–15min and then to 50:50 at t = 20min, the latter composition being held for an additional 5min. (2) Solvent system B was for linear gradient elution at 1ml/min by CH_3CN–H_2O (20:80) at t = 0–12min and then to 50:50 at t = 18min, the latter being held for an additional 5min. (3) Solvent system C was for linear gradient elution at 1ml/min by CH_3CN–H_2O (20:80) at t = 0–10min and then to 50:50 at t = 16min, the latter being held for an additional 5min. The elution conditions for chiral HPLC were the same as reported previously: pinoresinol,[18] lariciresinol,[22] and secoisolariciresinol.[22] Infrared (IR) spectra were obtained by a JASCO IR-810 infrared spectrophotometer with solution cells. Silica gel column chromatography and silica gel thin-layer chromatography (TLC) employed Kieselgel 60 (Merck, 70–230 mesh) and Kieselgel 60 F_{254} (Merck, 20 × 20cm, 0.50 and 0.25mm), respectively. All chemicals used were reagent grade unless otherwise stated.

Extraction and isolation

Freeze-dried aerial parts (6.3g) of *L. flavum* var. *compactum* were pulverized and extracted six times with hot MeOH (total 260ml). To the suspension of the MeOH extracts (1.3g) in 100ml of 0.1M NaOAc buffer (pH 5.0) were added 500 units of β-glucosidase (Sigma G-0395, 5.0U/mg, from almond). Following incubation at 37°C for 24h, the reaction mixture was extracted with CH_2Cl_2. An aliquot (381mg) of the CH_2Cl_2 extracts (454mg) thus obtained was submitted to repeated column chromatography (solvents: appropriate mixtures of MeOH–CH_2Cl_2), TLC (solvents: appropriate mixtures of MeOH–CH_2Cl_2, EtOAc–n-hexane, or benzene–EtOAc), and reversed-phase HPLC (solvent system A, B, or C) to afford 7,6'-dihydroxybursehernin (8.0mg), α-peltatin (13.0mg), β-peltatin (13.8mg), pinoresinol (5.1mg), lariciresinol (2.3mg), and secoisolariciresinol (2.2mg).

Freeze-dried roots (7.0g) of the plant were pulverized and extracted with hot MeOH as above. The MeOH extracts (2.0g) thus obtained were treated with β-glucosidase and extracted with CH_2Cl_2 as above. An aliquot (ca. 500mg) of the CH_2Cl_2 extracts (954mg) was subjected to repeated TLC to afford 5-methoxypodophyllotoxin (15.0mg).

7,6'-Dihydroxybursehernin: ^{1}H- and ^{13}C-NMR, ^{1}H-^{1}H correlated spectrometry (COSY), and ^{1}H-detected heteronuclear multiple-bond quantum correlation (HMBC) are summarized in Table 1. MS m/z (%): 402 (2.1), 400 (3.1), 384 (19.6), 233 (15.6), 166 (13.4), 165 (11.0), 151 (100), 139 (10.0). High-resolution MS m/z (M^+), calculated for $C_{21}H_{22}O_8$: 402.1315, found: 402.1287.

5-Methoxypodophyllotoxin, α-peltatin, and β-peltatin: ^{1}H- and ^{13}C-NMR ($CDCl_3$) data coincided with data in the literature.[7,11,25] Pinoresinol, lariciresinol, and secoisolariciresinol: ^{1}H-NMR ($CDCl_3$) data were superimposable on those of chemically synthesized authentic samples, respectively.

Enantiomeric compositions of lignans

The enantiomeric composition of secoisolariciresinol isolated from *L. flavum* var. *compactum* was determined by chiral HPLC analysis, as previously described.[18] Enantiomeric compositions of pinoresinol and lariciresinol isolated

Table 1. NMR data for 7,6′-dihydroxybursehernin isolated from *L. flavum* var. *compactum* in $CDCl_3$

Carbon no.	^{13}C[a]	^{1}H[a]	H-H COSY[b]	HMBC[c]
1	132.5			H-5, 7
2	109.6	6.95 (1H, m)		H-6, 7
3	149.3[e]			H-2, 5, OCH_3
4	149.4[e]			
5	110.9	6.85 (1H, d, J = 8.1)	H-6	
6	119.1	6.95 (1H, m)	H-5	H-2, 7
7	74.4	4.92 (1H, d, J = 7.6)	H-8	H-2, 6, 8
8	51.1	2.69 (1H, dd, J = 7.6, 9.5)	H-7, 8′	H-9, 7′
9	178.8			H-7, 8, 9′
1′	115.2			H-5′, 7′, 7′
2′	109.7	6.25 (1H, s)		H-7′, 7′
3′	141.5[d]			H-2′, 5′, —CH_2O—
4′	147.0[d]			H-2′, 5′, —CH_2O—
5′	98.5	6.30 (1H, s)		
6′	148.1			H-2′, 5′, 7′
7′	31.9	2.14 (1H, dd, J = 4.9, 14.2)	H-7′, 8′	H-8, 2′, 8′, 9′
		2.37 (1H, dd, J = 8.5, 14.2)	H-7′, 8′	
8′	38.4	2.59 (1H, m)	H-7′, 7′, 8, 9′, 9′	H-7, 8, 7′, 7′, 9′
9′	71.6	3.95 (1H, t, J = 8.9)	H-8′, 9′	H-7′
		4.13 (1H, t, J = 8.7)	H-8′, 9′	H-7′
MeO	56.0	3.87 (3H, s)		
	56.0	3.88 (3H, s)		
—OCH_2O—	101.2	5.86 (2H, s)		

NMR, nuclear magnetic resonance; COSY, correlated spectroscopy; HMBC, ^{1}H-detected heteronuclear multiple-bond quantum correlation

[a] Chemical shifts are δ values; coupling constants (J in parentheses) are given in Hz

[b] Correlation between H-2 and H-6 was not observed clearly

[c] Protons correlated with carbon resonances

[d,e] May be interchanged

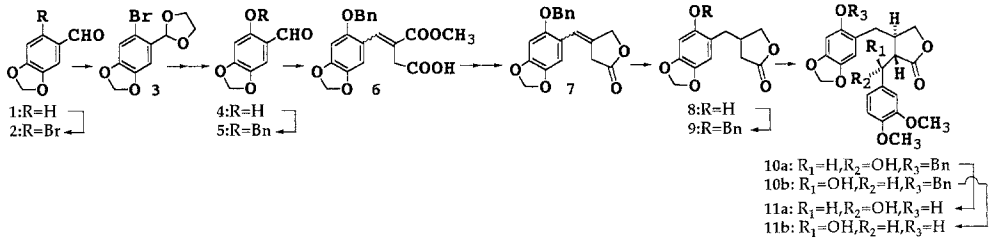

Fig. 2. Synthetic route for two epimers of (±)-7,6′-dihydroxybursehernins (**11a** and **11b**). Note that only one enantiomer of each compound is shown

from the plant were determined by chiral HPLC followed by GC-MS analysis with racemic (±)-[9,9,9′,9′-$^{2}H_4$]pinoresinols and (±)-[9,9,9′,9′-$^{2}H_4$]lariciresinols as internal standards, as previously described.[22–24]

Chemical synthesis of authentic lignans

(±)-[9,9,9′,9′-$^{2}H_4$]Pinoresinols,[23] (±)-pinoresinols,[18] (±)-[9,9,9′,9′-$^{2}H_4$]lariciresinols,[23] (±)-lariciresinols,[19] and (±)-secoisolariciresinols[18] were prepared previously.

Two diastereomers of (±)-7,6′-dihydroxybursehernins (**11a** and **11b**) were synthesized from piperonal (**1**) as follows (Fig. 2).

Compound (**3**). This was synthesized by refluxing a benzene solution of 6-bromopiperonal (**2**) prepared from **1**,[26] ethylene glycol, and *p*-toluenesulfonic acid with azeotropic removal of water. Yield, 96%. ^{1}H-NMR ($CDCl_3$): δ 4.03 (2H, m), 4.12 (2H, m), 5.96 (2H, s), 5.99 (1H, s), 6.98 (1H, s), 7.06 (1H, s).

6-Hydroxypiperonal (**4**). Compound (**3**) in dry Et_2O was lithiated with *n*-butyllithium (*n*BuLi) at −78°C. The resulting phenyllithium was treated with dry O_2 at 0°C to afford crude 2-(6-hydroxypiperonyl)-1,3-dioxolane, which was hydrolyzed with acetone/0.1 N HCl (30:1). The crude product was purified by silica gel column chromatography [eluent, CH_2Cl_2–*n*-hexane (1:2 then 2:1), stepwise elution] to give a mixture of **4** and a by-product **1**. The mixture was subjected to the next step without further purification. ^{1}H-NMR ($CDCl_3$): δ 6.00 (2H, s), 6.45 (1H, s), 6.85 (1H, s), 9.61 (1H, s), 11.77 (1H, s).

6-Benzyloxypiperonal (**5**). Compound (**4**) was benzylated with benzylbromide and K_2CO_3 in *N*,*N*-dimethylformamide at room temperature to afford **5**. Yield, 17% from compound (**3**). ^{1}H-NMR ($CDCl_3$): δ 5.11 (2H, s), 5.97 (2H, s), 6.57 (1H, s), 7.25 (1H, s), 7.36 (5H, m), 10.33 (1H, s).

Compound (**6**). 6-Benzyloxypiperonal (**5**) was converted to **6** by Stobbe condensation with dimethyl succinate and potassium *tert*-butoxide in *t*-butyl alcohol at reflux tempera-

ture. Yield, 84%. ^{1}H-NMR ($CDCl_3$): δ 3.50 (2H, s), 3.80 (3H, s), 5.04 (2H, s), 5.91 (2H, m), 6.54 (1H, s), 6.90 (1H, s), 7.35 (5H, m), 8.03 (1H, s).

Compound (**7**). Compound (**6**) was reduced to **7** with an ate complex[19] prepared at 0°C from *n*BuLi and diisobutyl aluminum hydride in dry THF. Yield, 40%. ^{1}H-NMR ($CDCl_3$): δ 3.34 (2H, m), 4.96 (2H, m), 5.00 (2H, s), 5.94 (2H, m), 6.58 (1H, s), 6.68 (2H, m), 7.37 (5H, m).

Compound (**8**). Catalytic reduction (10% palladium on carbon and H_2 in THF and MeOH) of **7** gave **8** (quantitative). ^{1}H-NMR ($CDCl_3$): δ 2.33 (1H, dd, J = 7.3, 17.6), 2.57 (1H, dd, J = 8.3, 17.6), 2.69 (2H, m), 2.87 (1H, m), 4.07 (1H, dd, J = 6.3, 9.0), 4.33 (1H, dd, J = 7.1, 9.0), 5.89 (2H, s), 6.36 (1H, s), 6.54 (1H, s).

Compound (**9**). Benzylation of **8** was conducted in the same way as the preparation of **5**, giving rise to **9**. Yield, 80%. ^{1}H-NMR ($CDCl_3$): δ 2.26 (1H, dd, J = 7.4, 17.4), 2.52 (1H, dd, J = 8.2, 17.4), 2.70 (2H, m), 2.83 (1H, m), 4.00 (1H, dd, J = 6.6, 9.0), 4.27 (1H, dd, J = 7.1, 9.0), 4.99 (2H, s), 5.89 (2H, s), 6.57 (1H, s), 6.58 (1H, s), 7.36 (5H, m).

Compounds (**10a**) and (**10b**). Compound (**9**) was converted to the corresponding lithium enolate at −78°C with lithium hexamethyldisilylamide prepared from *n*BuLi and hexamethyldisilazane in dry THF,[19,27] and the resulting enolate ion was condensed with 3,4-dimethoxybenzaldehyde to give a mixture of two diastereomers, **10a** and **10b**, which were separated by silica gel TLC [developing solvent, MeOH–CH_2Cl_2 (1:99), v/v] to afford **10a** (33%) and **10b** (33%). Compound (**10a**): ^{1}H-NMR ($CDCl_3$): δ 2.13 (1H, dd, J = 5.2, 13.5), 2.25 (1H, dd, J = 8.7, 13.6), 2.59 (2H, m), 3.82 (3H, s), 3.83 (3H, s), 3.87 (1H, dd, J = 8.3, 9.3), 4.07 (1H, dd, J = 7.6, 9.3), 4.73 (1H, d, J = 8.1), 4.89 (2H, s), 5.87 (2H, s), 6.28 (1H, s), 6.45 (1H, s), 6.72 (1H, d, J = 8.1), 6.77 (1H, dd, J = 1.7, 8.3), 6.90 (1H, d, J = 1.7), 7.36 (5H, m). Compound (**10b**): ^{1}H-NMR ($CDCl_3$): δ 2.41 (1H, dd, J = 8.2, 13.5), 2.49 (1H, dd, J = 7.2, 13.6), 2.67 (2H, dd, J = 3.2, 6.1), 2.83 (1H, m), 3.72 (3H, s), 3.83 (3H, s), 3.94 (1H, dd, J = 5.7, 8.9), 4.26 (1H, dd, J = 8.0, 8.6), 4.87 (2H, s), 5.20 (1H, broad s), 5.87 (2H, m), 6.25 (1H, s), 6.39 (1H, s), 6.63 (1H, d, J = 2.0), 6.68 (1H, d, J = 8.3), 6.73 (1H, dd, J = 2.0, 8.3), 7.36 (5H, m).

Compounds (**11a**) and (**11b**). Debenzylation of **10a** and **10b** was done in the same way as the preparation of **8**, giving rise to **11a** (yield, 90%) and **11b** (yield, 91%), respectively. Compound (**11a**): ^{1}H-NMR ($CDCl_3$): δ 2.14 (1H, dd, J = 4.9, 13.9), 2.38 (1H, dd, J = 8.6, 13.9), 2.57 (1H, m), 2.69 (1H, dd, J = 7.6, 9.5), 3.86 (3H, s), 3.87 (3H, s), 3.94 (1H, t, J = 9.0), 4.12 (1H, dd, J = 8.0, J = 9.3), 4.92 (1H, d, J = 7.3), 5.85 (2H, s), 6.24 (1H, s), 6.29 (1H s), 6.85 (1H, d, J = 8.0), 6.94 (2H, m). ^{13}C-NMR ($CDCl_3$): δ 31.9, 38.4, 51.1, 55.9, 56.0, 71.7, 74.3, 98.5, 101.2, 109.6, 109.7, 110.9, 115.3, 119.2, 132.5, 141.4, 146.9, 148.2, 149.2, 149.3, 178.9; IR ν_{max} (CH_2Cl_2) cm^{-1}: 1750 (C=O), 3510 (OH), 3590 (OH). Compound (**11b**): ^{1}H-NMR ($CDCl_3$): δ 2.43 (2H, m), 2.71 (1H, dd, J = 3.7, 6.6), 2.83 (1H, m), 3.80 (3H, s), 3.83 (3H, s), 3.99 (1H, dd, J = 6.1, 8.8), 4.32 (1H, t, J = 8.4), 5.17 (1H, broad s), 5.84 (2H, s), 6.20 (1H, s), 6.25 (1H, s), 6.76 (3H, m); ^{13}C-NMR ($CDCl_3$): δ 33.2, 36.1, 52.4, 55.8, 55.8, 72.5, 72.9, 98.1, 101.1, 108.7, 109.6, 110.9, 115.8, 117.7, 133.5, 141.1, 146.6, 148.3, 148.4, 148.9, 179.1; IR ν_{max} (CH_2Cl_2) cm^{-1}: 1762 (C=O), 3450 (OH), 3600 (OH).

Results and discussion

In the present study, a novel 7-hydroxydibenzylbutyrolactone lignan, 7,6′-dihydroxybursehernin, along with six known lignans (5-methoxypodophyllotoxin, α-peltatin, β-peltatin, pinoresinol, lariciresinol, secoisolariciresinol) were isolated from *Linum flavum* var. *compactum* (Fig. 1). The isolation of the known lignans from this species is in good accordance with previous reports; *Linum* spp., especially those of section *Syllinum* including *Linum flavum* and *Linum album*, produced 5-methoxypodophyllotoxin, podophyllotoxin, α-peltatin, and β-peltatin.[7-9] Also, production of pinoresinol and lariciresinol in *L. album*[12] and of pinoresinol in *L. flavum*[10] were reported.

The novel lignan was identified to be 7,6′-dihydroxybursehernin (Fig. 1) as follows. High-resolution EI-MS exhibited a molecular ion peak at *m/z* 402.1287, indicating a molecular formula of $C_{21}H_{22}O_8$. Its IR spectrum exhibited strong absorbance at 1750 cm^{-1}, showing the presence of an ester or a lactone ring. The ^{1}H-NMR spectrum (Table 1) gave two triplets, δ 3.95 (1H) and 4.13 (1H), indicating the presence of oxygenated methylene protons. The two dd signals at δ 2.14 and δ 2.37 are assigned to benzylic protons, and the two signals at δ 2.59 (m) and δ 2.69 (dd) are ascribed to two methine protons. The doublet at δ 4.92 is indicative of an oxygenated benzyl group. The singlet at δ 5.86 is indicative of an aromatic methylenedioxy group, and two singlets at δ 3.87 and 3.88 are assigned to two aromatic methoxyl groups. A group of signals between δ 6.85 and δ 6.97 are assigned to the 1,3,4-trisubstituted aromatic ring, and two singlet signals at δ 6.25 and δ 6.30 are ascribed to the 1,2,4,5-tetrasubstituted aromatic ring. The data together with ^{13}C-NMR (Table 1), H-H COSY (Table 1), C-H COSY (not shown), and HMBC (Table 1) results indicate that the compound is a new 7-hydroxydibenzylbutyrolactone lignan, 7,6′-dihydroxybursehernin. The structure was further confirmed by total synthesis; and the relative configuration of its C_7OH was determined by comparing the synthesized two C_7OH epimers (**11a** and **11b**) (Fig. 2).

The spectrometric data of 7,6′-dihydroxybursehernin isolated from *L. flavum* var. *compactum* coincided with those of **11a** in all respects but not with **11b**. A 7-hydroxydibenzylbutyrolactone, epipodorhizol (Fig. 1), which has intramolecular hydrogen bonding between C_7OH and lactone C=O and a larger $J_{7,8}$ value (6.6 Hz), was determined to be *7R,8S* isomer; and the other epimer (podorhizol) (Fig. 1), which does not show intramolecular hydrogen bonding and has a smaller $J_{7,8}$ value (2.2 Hz), was identified to be *7S,8S* (*7R*,8R**) isomer.[28,29] The IR spectra of the epimeric alcohols **11a** and **11b** showed striking differences with respect to the absorption range of OH and the lactone carbonyl groups as in the case of podorhizol and epipodorhizol.[28] Thus, the IR spectrum of **11a** in CH_2Cl_2

exhibited, in addition to a band at 3590 cm^{-1} (free OH group), a broad and concentration-independent band at 3510 cm^{-1}. In contrast, **11b** gave the corresponding absorption bands in 3600 cm^{-1} and 3450 cm^{-1}; and the latter band disappeared after dilution. The epimer **11b** exhibited typical γ-lactone carbonyl absorption at 1762 cm^{-1}, whereas in the case of **11a** a distinct shift to a longer wave length (1750 cm^{-1}) was observed. These data indicate that **11a** has hydrogen bonding between C_7OH and the lactone carbonyl, as in epipodorhizol, shown by Kuhn and von Wartburg.[28] In contrast, this is not the case for **11b**, like podorhizol.[28]

The presence of intramolecular hydrogen bonding in **11a** together with the H_7-H_8 coupling constant ($J_{7,8}$ = 7.6) of **11a** indicates that this compound (and 7,6′-dihydroxybursehernin isolated from *L. flavum* var. *compactum*) has the relative configuration of *7R*, 8S**, like epipodorhizol but not podorhizol.[28,29]

7-Hydroxydibenzylbutyrolactones formed by aldol condensation of the lithium enolates of β-benzyl-γ-butyrolactones with benzaldehydes have H_8-$H_{8'}$ *trans* configuration,[19,27,29–31] indicating that **10a,b** and **11a,b** are H_8-$H_{8'}$ *trans* diastereomers. This was further confirmed by NOE difference measurements; during irradiation of 7,6′-dihydroxybursehernin isolated from *L. flavum* var. *compactum* at δ 3.95 ($H_{9'a}$), the signals of H_8 and $H_{9'b}$ but not $H_{8'}$ were enhanced, whereas $H_{8'}$ and $H_{9'a}$ but not H_8 were enhanced when δ 4.13 ($H_{9'b}$) was irradiated, confirming the *trans* configuration of H_8 and $H_{8'}$ in terms of the γ-butyrolactone ring. Taken together, the relative configuration of 7,6′-dihydroxybursehernin isolated from *L. flavum* var. *compactum* (and **11a**) was determined to be *7R**, *8S**, and *8′R**(Fig. 1).

Recently, it has been found that there is a great stereochemical diversity in the upstream steps of lignan biosynthesis.[4,20–24,32] Therefore, characterization of enantiomeric compositions of the upstream lignans is of importance. Secoisolariciresinol isolated from *L. flavum* var. *compactum* in the present study is an optically pure (−)-enantiomer, whereas pinoresinol and lariciresinol are not, with 65% and 70% enantiomer excess (e.e.) in favor of (+)-enantiomers, respectively (data not shown). The result accords well with the general features of enantiomeric compositions of naturally occurring lignans[32]; pinoresinol and lariciresinol are not optically pure. In contrast, Xia et al. reported that optically pure (+)-lariciresinol and optically pure (−)-secoisolariciresinol were formed following incubation of racemic (±)-pinoresinols with a crude pinoresinol/lariciresinol reductase preparation from *L. flavum*.[17] However, Xia et al. did not compare the enantiomeric compositions of the enzymatically formed lignans with the corresponding lignans occurring in their *Linum* plant. The fact that lariciresinol isolated from *L. flavum* var. *compactum* in the present study is not optically pure can be accounted for by postulating that this plant has two pinoresinol/lariciresinol reductase isoforms that reduce the opposite enantiomers of pinoresinol, as in the case of the *Arctium lappa* enzymes[4,20,22] and *Thuja plicata* recombinant pinoresinol/lariciresinol reductase isozymes[33]; one has the same stereochemical selectivity in terms of pinoresinol enantiomers as the reductase reported by Xia et al.,[17] and the other has the opposite stereochemical selectivity affording (−)-lariciresinol as *Daphne genkwa* reductase.[24]

In conclusion, we have reported the phytochemical characterization of *L. flavum* var. *compactum* lignans, including isolation of the novel lignan.

Acknowledgments This research was supported partly by Grants-in-Aid for Scientific Research (nos. 06760160, 07660222, 08306021, 10660163, 12660150) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan; by the Sumitomo Foundation; and by a Grant-in-Aid from Interdisciplinary Research Institute of Environmental Science. The authors are grateful to Mr. Kanji Minato (Takii Seeds Co.) for supplying the seeds of *L. flavum* var. *compactum*.

References

1. Sackett DL (1993) Podophyllotoxin, steganacin and combretastatin: natural products that bind at the colchicine site of tubulin. Pharmacol Ther 59:163–223
2. Umezawa T (1996) Biological activity and biosynthesis of lignans. Mokuzai Gakkaishi 42:911–920
3. Canel C, Moraes RM, Dayan FE, Ferreira D (2000) Molecules of interest, podophyllotoxin. Phytochemistry 54:115–120
4. Umezawa T (2001) Biosynthesis of lignans and related phenylpropanoid compounds. Regul Plant Growth Dev 36:57–67
5. Suzuki S, Sakakibara N, Umezawa T, Shimada M (2002) Survey and enzymatic formation of lignans of *Anthriscus sylvestris*. J Wood Sci (in press)
6. Weiss SG, Tin-Wa M, Perdue RE Jr, Farnsworth NR (1975) Potential anticancer agents. II. Antitumor and cytotoxic lignans from *Linum album* (Linaceae). J Pharm Sci 64:95–98
7. Broomhead AJ, Dewick PM (1990) Aryltetralin lignans from *Linum flavum* and *Linum capitatum*. Phytochemistry 29:3839–3844
8. Van Uden W, Pras N, Woerdenbag HJ (1994) *Linum* species (flax): in vivo and in vitro accumulation of lignans and other metabolites. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry, vol 26: Medicinal and aromatic plants VI. Springer-Verlag, Berlin, pp 219–244
9. Konuklugil B (1996) Aryltetralin lignans from genus *Linum*. Fitoterapia 67:379–381
10. Konuklugil B (1997) Lignans from *L. flavum* var. *compactum* (Linaceae). Biochem Syst Ecol 25:75
11. Wichers HJ, Harkes MP, Arroo RRJ (1990) Occurrence of 5-methoxypodophyllotoxin in plants, cell cultures and regenerated plants of *Linum flavum*. Plant Cell Tissue Organ Cult 23:93–100
12. Smollny T, Wichers H, de Rijk T, van Zwam A, Shasavari A, Alfermann AW (1992) Formation of lignans in suspension cultures of *Linum album*. Planta Med 58:A622–A624
13. Oostdam A, Mol JNM, Van der Plas LHW (1993) Establishment of hairy root cultures of *Linum flavum* producing the lignan 5-methoxypodophyllotoxin. Plant Cell Rep 12:474–477
14. Konuklugil B, Schmidt TJ, Alfermann AW (1999) Accumulation of aryltetralin lactone lignans in cell suspension cultures of *Linum nodiflorum*. Planta Med 65:587–588
15. Jackson DE, Dewick PM (1984) Biosynthesis of *Podophyllum* lignans. II. Interconversions of aryltetralin lignans in *Podophyllum hexandrum*. Phytochemistry 23:1037–1042
16. Kamil WM, Dewick PM (1986) Biosynthetic relationship of aryltetralin lactone lignans to dibenzylbutyrolactone lignans. Phytochemistry 25:2093–2102
17. Xia ZQ, Costa MA, Proctor J, Davin LB, Lewis NG (2000) Dirigent-mediated podophyllotoxin biosyntheis in *Linum flavum* and *Podophyllum peltatum*. Phytochemistry 55:537–549
18. Umezawa T, Kuroda H, Isohata T, Higuchi T, Shimada M (1994) Enantioselective lignan synthesis by cell-free extracts of *Forsythia koreana*. Biosci Biotechnol Biochem 58:230–234
19. Umezawa T, Shimada M (1994) Syntheses of (±)-lariciresinols. Mokuzai Gakkaishi 40:231–235

20. Umezawa T, Shimada M (1996) Formation of the lignan (+)-secoisolariciresinol by cell-free extracts of *Arctium lappa*. Biosci Biotechnol Biochem 60:736–737
21. Umezawa T, Shimada M (1996) Enantiomeric composition of (−)-pinoresinol, (+)-matairesinol and (+)-wikstromol isolated from *Wikstroemia sikokiana*. Mokuzai Gakkaishi 42:180–185
22. Suzuki S, Umezawa T, Shimada M (1998) Stereochemical difference in secoisolariciresinol formation between cell-free extracts from petioles and from ripening seeds of *Arctium lappa* L. Biosci Biotechnol Biochem 62:1468–1470
23. Okunishi T, Umezawa T, Shimada M (2000) Enantiomeric compositions and biosynthesis of *Wikstroemia sikokiana* lignans. J Wood Sci 46:234–242
24. Okunishi T, Umezawa T, Shimada M (2001) Isolation and enzymatic formation of lignans of *Daphne genkwa* and *Daphne odora*. J Wood Sci 47:383–388
25. Perry NB, Foster LM (1994) Antitumour lignans and cytotoxic resin acids from a New Zealand gymnosperm, *Libocedrus plumosa*. Phytomedicine 1:233–237
26. Meyers AI, Flisak JR, Aitken RA (1987) An asymmetric synthesis of (−)-steganone: further application of chiral biaryl syntheses. J Am Chem Soc 109:5446–5452
27. Brown E, Daugan A (1987) The synthesis of (R)-(+)-β-vanillyl-γ-butyrolactone and of chiral lignans therefrom. Heterocycles 26:1169–1172
28. Kuhn M, von Wartburg A (1967) *Podophyllum*-Lignane: Struktur und Absolutkonfiguration von Podorhizol-β-D-glucosid (= Lignan F). Helv Chim Acta 50:1546–1565
29. Ziegler FE, Schwartz JA (1978) Synthetic studies on lignan lactones: aryl dithiane route to (±)-podorhizol and (±)-isopodophyllotoxone and approaches to the stegane skeleton. J Org Chem 43:985–991
30. Robin J-P, Dhal R, Brown E (1982) Syntheses totales et etudes de lignanes biologiquement actifs – II. Tetrahedron 38:3667–3671
31. Fujimoto H, Nakatsubo F, Higuchi T (1982) Synthesis of pinoresinol. Mokuzai Gakkaishi 28:555–562
32. Umezawa T, Okunishi T, Shimada M (1997) Stereochemical diversity in lignan biosynthesis. Wood Res 84:62–75
33. Fujita M, Gang DR, Davin LB, Lewis NG (1999) Recombinant pinoresinol-lariciresinol reductases from western red cedar (*Thuja plicata*) catalyze opposite enantiospecific conversions. J Biol Chem 274:618–627