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Preparation and properties of a coniferin enantiomer

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Abstract

L-Coniferin (**1L**), which is an enantiomer of natural coniferin (D-coniferin (**1D**)), was prepared from L-glucose according to the conventional method for compound **1D**. The reactivity of L-glucose and its derivatives was found to be almost same as that of the corresponding D-glucose and its derivatives during the preparation for compound **1L**. Compound **1L** showed resistance toward enzymatic hydrolysis by commercial β-glucosidase from Almond. However, unlike compound **1D**, compound **1L** was not transported across the membrane obtained from differentiating xylem of a hybrid poplar in the present assay. The result suggested for the first time that the D-/L-configuration of the glucose moiety of coniferin is an important factor affecting coniferin transport across the membrane.

Keywords: Coniferin, Enantiomer, D-Glucose, L-Glucose, Transporter

Introduction

Natural coniferin (hereafter "D-coniferin (**1D**)") is a D-glucoside consisting of D-glucose and coniferyl alcohol. It is considered to be a form of coniferyl alcohol that functions in storage and transport in lignin biosynthesis [1–4]. There are three important pieces of information known about coniferin transport. First, compound **1D** is found in the cambial sap of many tree species [1, 5]. Second, coniferin β-glucosidase is located in the cell wall of coniferous and broad-leaf trees [6, 7]. Third, the incorporation of radioactivity into cell wall lignin is observed when radiolabeled compound **1D** is fed to a variety of plants [8]. Taken together, this suggests that compound **1D** might be transported through the membrane to the cell wall and be subjected to enzymatic hydrolysis by β-glucosidase to form coniferyl alcohol for lignification. However, this hypothesis has not yet been proven and there is still debate as to whether coniferyl alcohol is transported in its free or glucoside form [3, 4].

Theoretically, there should be a non-natural coniferin, an enantiomer of compound **1D** (hereafter "L-coniferin (**1L**)"), which consists of L-glucose and coniferyl alcohol as shown in Fig. 1, because there is an enantiomer of D-glucose (L-glucose). To the best of our knowledge, compound **1L** was not found in nature. It is widely known that a pair of enantiomers has the same chemical and physical properties except for their optical properties such as specific rotation. In addition, L-glucoside is thought not to be hydrolyzed by β-glucosidase because of the substrate specificity of the enzyme [9], although the experimental data have not been found. Therefore, compound **1L** might be useful as a transport tracer in an administration experiment, because it might be transported to the site of lignification to a similar degree as compound **1D**, but would not be hydrolyzed by the enzyme as it accumulated. However, compound **1L** has not been available, because there is no report about the preparation of compound **1L**.

This research describes the first preparation of compound **1L** and its properties including its enzymatic hydrolysis and membrane transport ability.

Experimental

Materials

L-Glucose and β-glucosidase (from Almond, 121 U/mg) were purchased from Tokyo Chemical Industry Co. (Tokyo, Japan) and Oriental Yeast Co. (Tokyo, Japan), respectively. All other chemicals were purchased from

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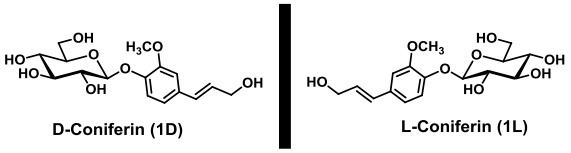


Fig. 1 Natural coniferin (**1D**) and its enantiomer (**1L**)

commercial sources and used without further purification unless otherwise noted.

Measurements

^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA300 MHz FT-NMR (300 MHz) spectrometer (Agilent Technologies, Santa Clara, CA, USA) using tetramethylsilane as an internal standard in DMSO-d_6 for compounds **1L** and **1D** or $CDCl_3$ for others. Chemical shift (δ) and coupling constant (J) are given in ppm (parts per million) and Hz, respectively. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) measurements were recorded on a Bruker MALDI-TOF MS REFLEX III (Bruker, Billerica, MA, USA) in the positive and linear ion modes. A nitrogen laser was used for the ionization of the samples. All spectra were obtained using 2,5-dihydroxybenzoic acid as a matrix. Specific rotations were recorded on a JASCO P-2200 polarimeter (JASCO, Hachioji, Japan) in H_2O for compounds **1L** and **1D** or $CHCl_3$ for others, and were determined as the average values of five measurements. Melting points (m.p.) were measured in a micro-melting point apparatus (Yanagimoto Seisakusho, Kyoto, Japan). UV–Vis spectra were recorded on a JASCO V-560 spectrophotometer (JASCO).

Preparation of L-coniferin (1L)

Compound **1L** was prepared from L-glucose (**2L**) according to the conventional method for compound **1D** [10] as shown in Fig. 2.

l-Glucose pentaacetate (3L)

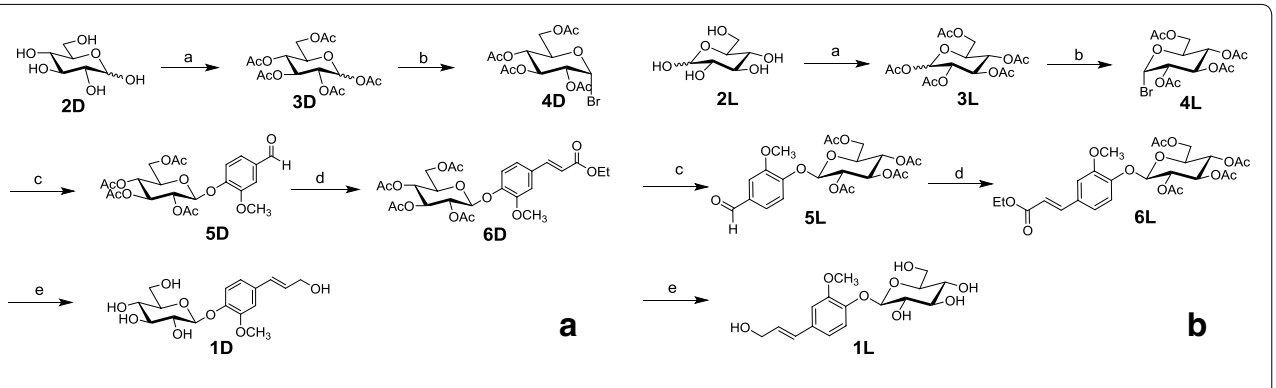
Compound **2L** (5 g, 27.8 mmol) and CH_3COONa (2.5 g, 30.5 mmol) were suspended in Ac_2O (25 mL). The suspension was stirred at 80 °C for 3.5 h, cooled to ambient temperature and extracted with EtOAc. The organic layer was washed with a saturated $NaHCO_3$ solution, distilled water, and brine, dried over Na_2SO_4 and evaporated to give a colorless residue. The residue was recrystallized from EtOH to give compound **3L** (9.63 g, 88.9% yield).

Compound **3L**: $[\alpha]_D^{25} = -$ 20.7° ($c = 1.03$, in $CHCl_3$); ^{1}H and ^{13}C NMR spectra data of compound **3L** were in agreement with the published data of L-glucose pentaacetate [11]; MALDI-TOF MS: m/z calcd. for $[M+Na]^+$ $C_{16}H_{22}NaO_{11}$: 413.34, found: 413.56.

2,3,4,6-Tetra-O-acetyl-α-l-glucopyranosyl bromide (4L)

Compound **3L** (1000 mg, 2.56 mmol) was dissolved in CH_2Cl_2 (2 mL). 33%-HBr in AcOH (2.5 mL, 14.4 mmol) was added to the solution at 0 °C. After stirring the reaction solution at ambient temperature for 2 h, distilled water (10 mL) was added to the solution at 0 °C. The reaction mixture was extracted with EtOAc. The organic layer was washed with distilled water, a saturated $NaHCO_3$ solution, and brine, dried over Na_2SO_4 and evaporated to give a colorless residue. The residue was recrystallized from EtOH to give compound **4L** (845 mg, 80.2% yield).

Compound **4L**: $[\alpha]_D^{25} = -$ 194.3° ($c = 1.04$, in $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.62 (d, 1H, $J = 4.2$, H-1), 5.56 (t, 1H, $J = 9.8$, H-3), 5.16 (t, 1H, $J = 9.8$, H-4), 4.84 (dd,



(a) CH_3COONa / (CH_3CO)$_2O$ / 80 °C / 3.5 h
(b) CH_2Cl_2 / HBr in AcOH / r.t / 2 h
(c) vanilln / Ag_2O / quinoline / r.t / 1.5 h
(d) Monoethyl malonic acid / pyridine / piperidine / 100 °C / 1.5 h
(e) DIBAL-H in toluene / dry toluene / 0 °C / 1 h

(a) CH_3COONa / (CH_3CO)$_2O$ / 80 °C / 3.5 h / 88.9%
(b) CH_2Cl_2 / HBr in AcOH / r.t / 2 h / 80.2%
© vanilln / Ag_2O / quinoline / r.t / 1.5 h / 72.9%
(d) Monoethyl malonic acid / pyridine / piperidine /100 °C / 1.5 h / 85.9%
(e) DIBAL-H in toluene / dry toluene / 0 °C / 1 h / 49.1%

Fig. 2 Synthetic route for D-conferin (**1D**) from D-glucose (**2D**) (**a**); L-conferin (**1L**) from L-glucose (**2L**) (**b**)

1H, $J=9.8$, 4.2, H-2), 4.33 (dd, 1H, $J=14.1$, 4.2, H-6a), 4.30 (ddd, $J=9.8$, 4.2, 1.5, H-5), 4.13 (dd, 1H, $J=14.1$, 1.5, H-6b), 2.11, 2.10, 2.06, 2.04 (s, 3H, acetyl CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.6, 170.0, 169.9, 169.6 (acetyl C=O), 86.7 (C-1), 72.2 (C-5), 70.7 (C-2), 70.3 (C-3), 67.3 (C-4), 61.0 (C-6), 20.8, 20.8, 20.7, 20.7 (acetyl CH_3); MALDI-TOF MS: *m/z* calcd. for $[M+Na]^+$ $C_{14}H_{19}BrNaO_9$: 433.20, found: 434.99.

(4-Formyl-2-methoxy)phenyl 2,3,4,6-tetra-O-acetyl-β-l-gluco pyranoside (5L)

Compound **4L**: (700 mg, 1.65 mmol) and vanillin (250 mg, 1.65 mmol) were dissolved in quinoline (4.5 mL). Ag_2O (390 mg) was added to the solution at 0 °C. The reaction mixture was stirred at ambient temperature for 1.5 h, filtered through Celite (535RVS, Nacalai Tesque (Kyoto, Japan)), and extracted with EtOAc. The organic layer was washed with a 1 M HCl solution, a saturated $NaHCO_3$ solution, and brine, dried over Na_2SO_4 and evaporated to give a light brown residue. The residue was recrystallized from EtOH to give compound **5L** (599 mg, 72.9% yield).

Compound **5L**: $[\alpha]_D^{25} = +42.9°$ ($c=0.53$, in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 9.90 (s, 1H, CHO), 7.44 (d, 1H, $J=2.1$, H-2′), 7.42 (dd, 1H, $J=8.7$, 2.1, H-6′), 7.22 (d, 1H, $J=8.7$, H-5′), 5.37–5.27 (m, 2H, H-2, H-3), 5.22–5.15 (m, 1H, H-4), 5.11 (d, 1H, $J=7.8$, H-1), 4.28 (dd, 1H, $J=12.3$, 5.4, 1H, H-6a), 4.19 (dd, 1H, $J=12.3$, 2.7, H-6b), 3.90 (s, 3H, OCH_3), 3.85 (ddd, 1H, $J=9.8$, 5.4, 2.7, H-5), 2.08, 2.08, 2.05, 2.05 (s, 3H, acetyl CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 191.1 (C-α), 170.7, 170.4, 169.5, 169.4 (acetyl C=O), 151.2 (C-4′), 151.1 (C-3′), 133.0 (C-1′), 125.5 (C-6′), 118.3 (C-5′), 110.8 (C-2′), 99.9 (C-1), 72.5 (C-3), 72.4 (C-5), 71.1 (C-2), 68.4 (C-4), 62.0 (C-6), 56.2 (OCH_3), 20.9, 20.9, 20.8, 20.8 (acetyl CH_3); MALDI-TOF MS: *m/z* calcd. for $[M+Na]^+$ $C_{22}H_{26}NaO_{12}$: 505.44, found: 505.35.

(4-Ethoxycarbonyl-2-methoxy)phenyl 2,3,4,6-tetra-O-acetyl-β-l-glucopyranoside (6L)

Compound **5L** (1000 mg, 2.07 mmol) and ethyl malonic acid (520 mg, 3.96 mmol) were dissolved in pyridine (16.5 mL). After the addition of piperidine (0.275 mL, 2.78 mmol), the reaction solution was stirred at 100 °C for 1.5 h and concentrated by azeotrope distillation with EtOH to give a colorless residue. The residue was recrystallized from EtOH to give compound **6L** (979 mg, 85.9% yield).

Compound **6L**: $[\alpha]_D^{25} = +27.2°$ ($c=0.56$, in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.62 (d, 1H, $J=16.2$, H-α), 7.10 (d, 1H, $J=8.4$, H-5′), 7.07 (d, 1H, $J=1.5$, H-2′), 7.05 (dd, 1H, $J=8.4$, 1.5, H-6′), 6.35 (d, 1H, $J=16.2$, H-β), 5.40–5.26 (m, 2H, H-2, H-3), 5.20–5.13 (m, 1H, H-4), 5.02–5.00 (m, 1H, H-1), 4.28 (dd, 1H, $J=12.5$, 5.1, H-6a), 4.26 (dd, 2H, $J=14.1$, 7.2, CH_2), 4.17 (dd, 1H, $J=12.5$, 2.4, H-6b), 3.85 (s, 3H, OCH_3), 3.80 (ddd, 1H, $J=10.2$, 5.1, 2.4, H-5), 2.08, 2.05, 2.05, 2.04 (s, 3H, acetyl CH_3), 1.34 (t, 3H, $J=7.2$, ethyl CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.5, 170.3, 169.4, 169.3 (acetyl C=O), 166.9 (C-ϒ), 150.7 (C-3′), 147.7 (C-4′), 143.9 (C-α), 131.0 (C-1′), 121.6 (C-6′), 119.5 (C-5′), 117.6 (C-β), 111.3 (C-2′), 100.3 (C-1), 72.4 (C-3), 72.0 (C-5), 71.0 (C-2), 68.3 (C-4), 61.8 (C-6), 60.5 (ethyl CH_2), 56.0 (OCH_3), 20.7, 20.6, 20.6, 20.6 (acetyl CH_3), 14.3 (ethyl CH_3); MALDI-TOF MS: *m/z* calcd. for $[M+Na]^+$ $C_{26}H_{32}NaO_{13}$: 575.53, found: 575.57.

(4-(3-Hydroxy-2-propenyl)-2-methoxy)phenyl β-l-glucopyranoside (l-coniferin) (1L)

Compound **6L** (900 mg, 1.63 mmol) was dissolved in toluene (22 mL). The 1.01 mol/L of DIBAL-H in toluene (15 mL, 15.1 mmol) was added dropwise to the solution at 0 °C for 10 min. The reaction mixture was stirred at 0 °C for 1 h and EtOH (20 mL) was added slowly to the mixture. The reaction mixture was stirred at 0 °C for another 30 min, concentrated and filtered with hot water. The filtrate was concentrated by azeotrope distillation with EtOH to give a colorless residue. The residue was purified by preparative thin-layer chromatography using a silica gel plate (silica gel $60F_{254}$, 2-mm thickness, Merck, Darmstadt, Germany) developed with 20% $MeOH/CH_2Cl_2$ (v/v) and recrystallized from water three times to give compound **1L** (295 mg, 49.1% yield).

Compound **1L**: m.p.: 179–182 °C; $[\alpha]_D^{25} = +65.8°$ ($c=0.5$, in $CHCl_3$); ^1H NMR (300 MHz, DMSO-d_6): δ 7.06 (d, 1H, $J=1.8$, H-2′), 7.02 (d, 1H, $J=8.4$, H-5′), 6.90 (dd, 1H, $J=8.4$, 1.8, H-6′), 6.48 (d, 1H, $J=15.9$, H-α), 6.28 (dt, 1H, $J=15.9$, 5.1, H-β), 5.25 (d, 1H, $J=4.8$, OH-3), 5.10 (d, 1H, $J=4.8$, OH-2), 5.04 (d, 1H, $J=4.8$, OH-4), 4.89 (d, 1H, $J=8.1$, H-1), 4.85 (d, 1H, $J=5.4$, OH-γ), 4.57 (t, 1H, $J=5.4$, OH-6), 4.10 (dd, 1H, $J=5.1$, 1.2, H-ϒa), 4.09 (dd, 1H, $J=5.1$, 1.2, H-ϒb), 3.79 (s, 3H, OCH_3), 3.68–3.65 (m, 1H, H-6a), 3.52–3.17 (m, 5H, H-2, H-3, H-4, H-5, H-6b); ^{13}C NMR (75 MHz, DMSO-d_6): δ 149.4 (C-3′), 146.4 (C-4′), 131.4 (C-1′), 129.4 (C-β), 128.9 (C-α), 119.5 (C-6′), 115.6 (C-5′), 110.2 (C-2′), 100.4 (C-1), 77.4 (C-3), 77.3 (C-5), 73.7 (C-2), 70.7 (C-4), 62.1 (C-ϒ), 61.1 (C-6), 56.0 (OCH_3); MALDI-TOF MS: *m/z* calcd. for $[M+Na]^+$ $C_{16}H_{22}NaO_8$: 365.34, found: 365.30.

Preparation of D-coniferin (1D)

Compound **1D** was also prepared by the conventional method as shown in Fig. 2a [10]. Compound **3D:** $[\alpha]_D^{25} = +21.4°$ ($c=1.34$, in $CHCl_3$); **4D**: $[\alpha]_D^{25} = +193.5°$ ($c=1.13$, in $CHCl_3$), **5D**: $[\alpha]_D^{25} = -43.7°$ ($c=0.52$, in $CHCl_3$), **6D**: $[\alpha]_D^{25} = -26.6°$ ($c=0.56$, in $CHCl_3$), **1D**:

m.p. 181–183 °C (literature: 183–185 °C [10], 185–188 °C [12]); $[\alpha]_D^{25} = -64.8°$ ($c = 0.43$, in $CHCl_3$).

Enzymatic hydrolysis of D- and L-coniferin (1D and 1L) by β-glucosidase

An aqueous solution of β-glucosidase (6×10^{-5} g/L, 5 mL) was added to an aqueous solution of coniferin (0.3 mmol/L, 10 mL). The reaction solution was stirred at 40 °C. An aliquot of the reaction solution (1 mL) was taken out at the prescribed time and poured into distilled water (3 mL) and then 0.1 mL of a 2.5 mM NaOH aqueous solution was immediately added. The mixed solution was subjected to UV–Vis measurement. The amount of coniferyl alcohol formed was determined using a calibration curve of coniferyl alcohol at 311 nm.

Transport assay of D- and L-coniferin (1D and 1L)

The preparation of microsomal fractions from differentiating xylem of poplar (*Populus sieboldii* × *P. grandidentata*) and the transport assay were carried out according to the methods used in previous studies [3, 13]. Uptake of coniferin by membrane vesicles was measured at 28 °C for 20 min in 100 µL of reaction mixture [50 mM HEPES–KOH (pH 7.5), 5 mM Mg/ATP, 50 µM substrate and membrane vesicles (ca. 10 µg protein)], unless otherwise stated. Data are reported as technical replicates.

Results and discussion

Preparation of D- and L-coniferin (1D and 1L)

Compound **1L** was prepared from compound **2L** according to the conventional method for compound **1D** [10] as shown in Fig. 2. Compound **1D** was also prepared as a control compound. All the reaction steps [(a) acetylation, (b) bromination, (c) glycosidation, (d) Knoevenagel condensation, (e) reduction with DIBAL-H], proceeded smoothly to afford the final compound **6L**. Indeed, the reactivity of the L-glucose/L-glucose derivative (compounds **2L** to **6L**) was found to be almost the same as the D-glucose/D-glucose derivative (compounds **2D** to **6D**) during the preparation of compound **1L**. Reports of similar reactivities among L-glucose and D-glucose derivatives are known in the literature for the synthesis of digitoxigenin glycoside [14]. All products (Compounds **1L**, **3L** to **6L**) were characterized by acquiring their ^{1}H and ^{13}C NMR and MALDI-TOF MS spectra, and their specific rotation. The ^{1}H and ^{13}C NMR spectra of all the L-glucose derivatives had the same peak pattern as those of the D-glucose derivatives, but the sign of the specific rotation of the L-glucose derivatives was opposite to that of the specific rotation of the D-glucose derivatives (Additional file 1: Figs. S1–S10).

As an example, the ^{1}H and ^{13}C NMR spectra of compounds **1L** and **1D** are shown in Fig. 3. The spectra of compound **1L** were the same as those of compound **1D**. The value of $[M+Na]^+$ for compound **1L** also corresponded to its calculated value from the MALDI-TOF–MS data of compound **1L**. The specific rotations of compounds **1L** and **1D** were + 65.8° and − 64.8°, respectively. These results clearly indicate that compound **1L** was an enantiomer of compound **1D**.

Enzymatic hydrolysis of D- and L-coniferin (1D and 1L) by β-glucosidase

The enzymatic hydrolysis of compounds **1L** and **1D** by the commercial β-glucosidase from Almond was evaluated based on the formation of coniferyl alcohol in the reaction. The determination of the coniferyl alcohol formed in the reaction mixture was performed by a UV–Vis method (detection wavelength: 311 nm), because the peak for coniferyl alcohol in an alkali solution at

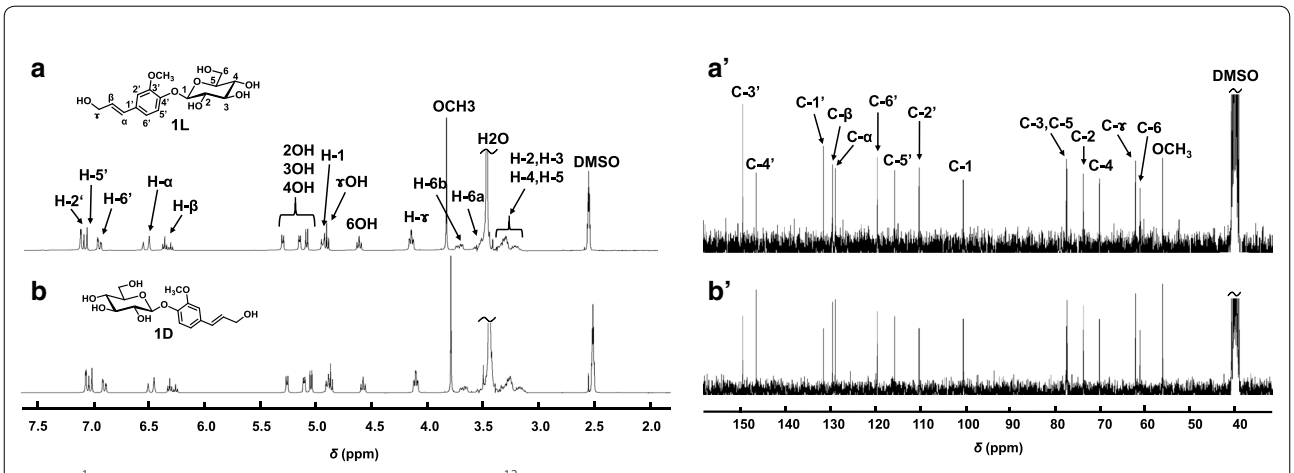


Fig. 3 ^{1}H NMR spectra of compounds **1L** (**a**) and **1D** (**b**); ^{13}C NMR spectra of compounds **1L** (**a′**) and **1D** (**b′**)

311 nm did not overlap with any peaks from coniferin or the β-glucosidase (Fig. 4a). The percent hydrolysis of compounds **1L** and **1D** is shown in Fig. 4b. The percent hydrolysis of compound **1D** increased with an increase of time and reached almost 100% after 24 h. By contrast, the hydrolysis of compound **1L** was minimal with only 5.4% hydrolyzed after 24 h. The results clearly show that compound **1L** was resistant to enzymatic hydrolysis by the β-glucosidase, although further investigation (for example, the experiments using other β-glucosidase) is required.

Transport assay of D- and L-coniferin (1D and 1L)

ATP-dependent compound **1D** transport in the lignifying tissues of woody plants was suggested from the transport assay results of compound **1D** [3]. That is, strong transport activity of compound **1D** was observed in the presence of ATP, whereas weak transport activity was observed in the absence of ATP. Compound **1L** was also tested in the transport assay. Figure 5 shows the uptake of compounds **1L** and **1D** into crude microsomal membrane vesicles derived from plasma membrane, vacuolar membrane, Golgi membrane and other endomembranes obtained from differentiating xylem of hybrid poplar. Surprisingly, only weak uptake activity was observed for compound **1L** in the presence of ATP and no uptake activity was observed in the absence of ATP. Thus, there was a significant difference of uptake activity between

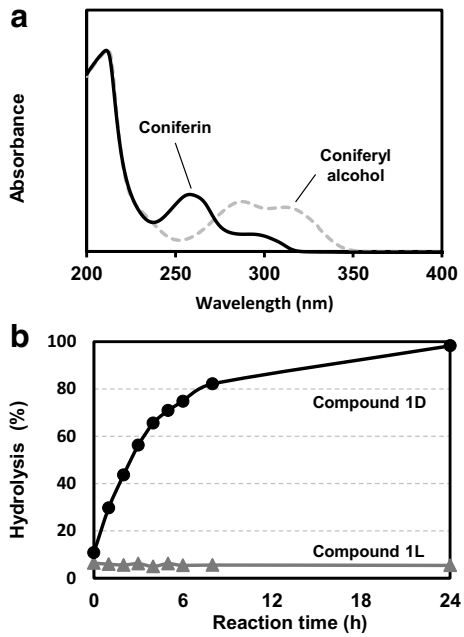


Fig. 4 UV–Vis spectra of coniferin and coniferyl alcohol in an alkali solution (**a**); hydrolysis of compounds **1D** and **1L** by β-glucosidase (**b**)

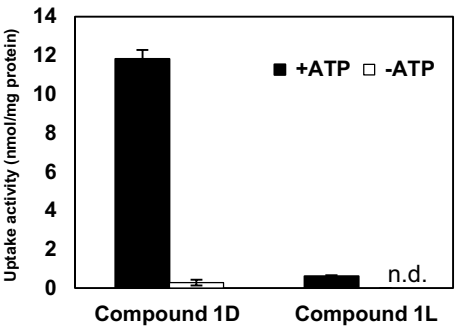


Fig. 5 Uptake of compounds **1D** and **1L** into membrane vesicles in the presence and absence of ATP over 20 min

compounds **1L** and **1D** in the presence of ATP, although compound **1L** is an enantiomer of compound **1D**. Previous study indicated that transport of compound **1D** is involved in vacuolar type H^+-ATPase which is localized not only vacuolar membrane but also endoplasmic reticulum, Golgi apparatus, and other endomembrane systems [3]. Although it is indistinct whether transport activity of compound **1D** shows transport into vacuole and/or other endomembrane systems, compound **1L** was not transported across the crude microsomal membranes containing plasma membrane, vacuolar membrane, Golgi membrane and other endomembranes obtained from differentiating xylem of hybrid poplar. Therefore, the D-/L-configuration of the sugar moiety of coniferin was strictly recognized during the coniferin transport process across the membrane in the present assay.

Conclusions

Compound **1L** was successfully prepared according to the conventional method for compound **1D**. Compound **1L** was not found to be useful as a transport tracer in an administration experiment, although compound **1L** was resistant toward enzymatic hydrolysis by commercial β-glucosidase from Almond. However, the D-/L-configuration of the sugar moiety of coniferin was found to be a factor that affected coniferin transport across the membrane. This new finding might provide supporting evidence that compound **1D** is a transport form of coniferyl alcohol.

Additional file

Additional file 1. Additional figures.

Abbreviations

MALDI-TOF MS: matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; m.p.: melting point.

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Authors' contributions
HM contributed to preparation of D- and L-coniferin and their enzymatic hydrolysis experiments. HK supported to HM's experiments. TT and KT contributed to transport assay. TT (corresponding author) designed this study and wrote this paper. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing interests.

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