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Semi-micro chiral HPLC analysis of lignans

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Abstract Chiral high-performance liquid chromatography (HPLC) separation of lignans with semi-micro columns of 1.0–2.0 mm inner diameter (i.d.) was established for the first time. Practical sensitivity was increased 5 to 20-fold compared with that of conventional chiral HPLC using analytical columns with 4.6 mm i.d. The semi-micro chiral HPLC system can be applied to high-sensitivity enantiomeric separation of many chiral organic compounds in addition to lignans.

Key words Lignan · Semi-micro chiral HPLC · Enantiomer

Introduction

Lignan biosynthesis involves strict stereochemical controls, and enantiomeric compositions of lignans have been determined at many stages of the stereochemical studies. Because chiral high-performance liquid chromatography (HPLC) is the only technique that allows us to determine enantiomeric compositions with less than a few milligrams of lignan specimens, it has played a significant role in studies of lignan biosynthesis. Thus, normal-phase chiral HPLC columns with 4.6 mm inner diameter (i.d.) using cellulose carbamate or amylose carbamate as packing materials have often been employed for chiral separation of lignans.[1–24] However, the amount of lignans obtained in these biosynthetic studies are often small (less than a few micrograms), and improvement of the HPLC sensitivity has been required. For this purpose, the use of semi-micro columns with 1–2 mm i.d. is beneficial because semi-micro HPLC has higher practical sensitivities in order of magnitude than conventional HPLC using wide-bore (e.g., 4.6 mm i.d.) columns. Although in the semi-micro HPLC system a flow cell volume of a ultraviolet (UV) detector and inner diameters of the connecting tubing must be sufficiently miniaturized, such a modification can be made easily.

Semi-micro chiral HPLC columns using amylose carbamate[25] and cellulose carbamate[26,27] as packing materials have been reported. However, the cellulose carbamate-based column (Chiralcel OD-RH)[26,27] is designed for reversed-phase use, and to our knowledge cellulose carbamete-based semi-micro chiral columns for normal-phase HPLC have not yet been reported. Herein, we report for the first time the chiral separation of lignans through semi-micro, cellulose carbamate-based HPLC columns for normal-phase use.

Experimental

Instruments

Semi-micro chiral HPLC was done with the HPLC system outlined in Fig. 1, composed of a Waters 600E system controller, a Waters 6000A solvent delivery system (or a Waters 60F fluid pump), a Rheodyne model 7125 syringe loading sample injector with a 5-μl sample loop, and a JEOL CAP-UV01 UV detector (flow cell volume 1 μl; flow cell light path 5 mm). The mobile phase at 1.0 and 0.7 ml/min obtained by the pump was split using a resistant column of 250 × 4.6 mm (Chiralcel OD; Daicel Chemical) to give flows of about 45 and 110 μl/min, which were applied to semi-micro chiral HPLC columns, a Daicel Chiralcel OD-H (250 × 1.0 mm; packing material particle size 5 μm) and a Daicel Chiralcel OC (250 × 2.0 mm; packing material particle size 10 μm), respectively. The mobile phases were as follows:

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pinoresinol, Chiralcel OD-H, ethyl alcohol; secoisolariciresinol, Chiarlcel OD-H, ethyl alcohol/*n*-hexane/glycerol (300:700:5); matairesinol, Chiralcel OD-H, ethyl alcohol/1% acetic acid in *n*-hexane (15:85); lariciresinol, Chiralcel OC, ethyl alcohol/1% acetic acid in *n*-hexane (80:20).

Chiral HPLC using 4.6 mm i.d. columns was conducted with an HPLC system comprising a Waters 600E system controller, a Waters 6000A solvent delivery system (or a Waters 60F Fluid Pump), a Waters model UK6 universal injector, and a Waters model 440 absorbance detector (flow cell volume 15.5 μl, flow cell light path 10 mm). The chiral columns used were Chiralcel OD (Daicel Chemical; 250 × 4.6 mm; packing material particle size 10 μm) and Chiralcel OC (Daicel Chemical; 250 × 4.6 mm; packing material particle size 10 μm). The elution conditions were as follows: pinoresinol, Chiralcel OD, ethyl alcohol at 0.4 ml/min; secoisolariciresinol, Chiarlcel OD, ethyl alcohol/*n*-hexane/glycerol (300:700:5) at 1.0 ml/min; matairesinol, Chiralcel OD, ethyl alcohol/1% acetic acid in *n*-hexane (15:85) at 1.0 ml/min; lariciresinol, Chiralcel OC, ethyl alcohol/1% acetic acid in *n*-hexane (80:20) at 0.5 ml/min.

Lignan specimens were dissolved in the mobile phase [(±)-secoisolariciresinols, (±)-matairesinols, and (±)-lariciresinols] or methyl alcohol [(±)-pinoresinols] (ca. 0.1–1.0 μg/μl). The solution (2.0 μl) was applied to both semi-micro and 4.6 mm i.d. columns at room temperature (23°–25°C). Detection was made at λ = 280 nm.

Preparation of compounds and chemicals

(±)-Pinoresinols,[11] (±)-secoisolariciresinols,[11] (±)-matairesinols,[5] and (±)-lariciresinols[28] (Fig. 2) were prepared previously. All the chemicals used were reagent or HPLC grade.

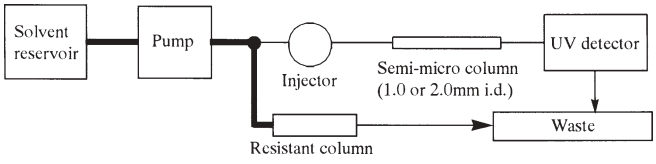

Fig. 1. Semi-micro chiral high-performance liquid chromatography (HPLC) system. See the text for a detailed description of the system. *Bold lines* and *solid lines* connecting each component represent stainless steel tubes with 0.25 and 0.10 mm inner diameters, respectively

Results and discussion

Chiral separation of lignans so far reported has employed conventional HPLC systems operated at solvent flows of 0.4–1.0 ml/min and wide-bore (4.6 or 3.9 mm i.d.) columns for use with normal-phase solvents. For example, enantiomers of matairesinol,[4,5,10,15,18,20,21,23] arctigenin,[5,10,23] hinokinin,[21] pluviatolide,[21] haplomyrfolin,[21] kusunokinin,[19] dimethylmatairesinol,[10] isoarctigenin,[10] 4-*O*-demthylyatein,[18] thujaplicatin methyl ether,[18] secoisolariciresinol,[2,4,5,7–9,11,12,14,16,17,19,20,23,24] pinoresinol,[3,5–7,9,11,13,15–17,20,22–24,29–31] epipinoresinol,[5] phylligenin,[5] piperitol,[29,30] monomethylpinoresinol,[31] eudesmin,[31] and syringaresinol[6] were separated through Chiralcel OD (packing material, cellulose dimethylphenylcarbamate, 4.6 mm i.d.); Chiralcel OC (packing material, cellulose phenylcarbamate; 4.6 mm i.d.) was used for the enantiomeric separation of wikstromol,[15,18] lariciresinol,[8,9,12,16,17,19,20,23,24] and syringaresinol.[22,32] These columns were also applied to chiral separation of some neolignans.[33,34] In addition, (+)- and (−)-pinoresinols were separated by another cellulose dimethylphenylcarbamate column (Waters, Opti-Pak XC; 300 × 3.9 mm),[35] and an amylose carbamate column (Chiralpak AD, Daicel; 4.6 mm i.d.) is effective for chiral separation of lariciresinol[11] and methyltrachelogenin.[19]

In the present study, the cellulose carbamates, which have been widely applied to chiral separation of lignans, were chosen to prepare the semi-micro chiral columns Chiralcel OD-H (1.0 mm i.d.) and Chiralcel OC (2.0 mm i.d.), which were submitted to enantiomeric separation of several lignans. Figure 1 outlines the semi-micro HPLC equipment. To operate the semi-micro columns near their optimum efficiency, the liquid chromatograph conditions for use with wide-bore columns were modified. First, the mobile phase (1 ml/min) from the pump was split by using a resistant column (Chiralcel OD, 4.6 mm i.d.), so semi-micro flows were obtained inversely related to the cross-sectional ratio between the resistant and semi-micro columns. Second, narrow-bore connecting tubing (0.1 mm i.d.), a narrow-bore injector with a 5-μl sample loop, and a UV detector with a micro-flow cell (volume 1 μl; light path 5 mm) were employed to minimize sample dispersion. Finally, each lignan specimen was dissolved in the mobile phase (lariciresinol, secoisolariciresinol, matairesinol) or methyl alcohol (pinoresinol) and applied to the column. Using methyl alcohol for secoisolariciresinol dissolution, a much more polar solvent than the mobile phase in this case, spoiled the enantiomeric separation.

Fig. 2. Structures of lignans analyzed by semi-micro chiral HPLC system. Note that only one enantiomer of each compound is shown

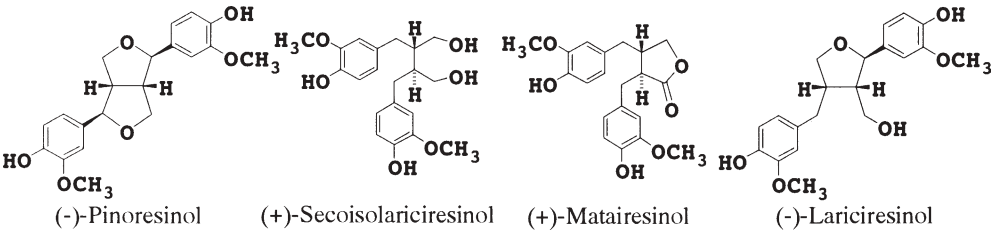

Figure 3 shows the semi-micro HPLC chromatograms in comparison with those obtained with the corresponding 4.6 mm i.d. columns. Dextrorotatory and levorotatory enantiomers of the racemic lignans (±)-pinoresinols, (±)-secoisolariciresinols, and (±)-matairesinols were separated efficiently through the semi-micro Chiralcel OD-H column, as were the (±)-lariciresinols through the semi-micro Chiralcel OC column. On the other hand, separation of (+)- and (−)-wikstromols were incomplete with the semi-micro Chiralcel OC column (data not shown), although they were separated completely with the 4.6 mm i.d. Chiralcel OC column.[15]

Because sample concentrations in column eluates are to be related inversely to cross sections of the columns when the same amounts of samples are applied to the columns, the chromatographic peak height at $\lambda = 280$ nm in the semi-micro system was expected to increase in the order of magnitude compared with the system for use with conventional 4.6 mm i.d. columns. As expected from the ratios of cross-sectional areas of columns, Fig. 3 shows that the peak heights in the semi-micro system, as raw data expressed in the absorbance at $\lambda = 280$ nm, were increased more than 5-fold (lariciresinol) to 20-fold (pinoresinol and secoisolariciresinol) compared with those obtained using the corresponding 4.6 mm i.d. columns. Because of the difference in the UV detectors including the flow cells between the semi-micro and wide-bore (4.6 mm i.d. column) systems, a strict comparison of sensitivities between the two systems cannot be made. However, the 20-fold increase is critically important to determine enantiomeric compositions of trace amounts (less than a few micrograms) of lignan specimens, which are often encountered when characterizing stereochemical properties of reactions catalyzed by lignan-synthesizing enzymes.

Next, the semi-micro chiral column (Chiralcel OD-H) was directly connected with a mass spectrometer, and matairesinol was subjected to the chiral liquid chromatography-mass spectrometry (LC-MS) system. The lignan gave clear mass spectra under APCI negative ionization conditions. This technique was applied to determine the enantiomeric composition of matairesinol formed following incubation of racemic (±)-secoisolariciresinols with a *Daphne odora* secoisolariciresinol dehydrogenase preparation.[36]

In summary, the chiral HPLC separation of lignans with semi-micro columns was reported for the first time. Because chiral HPLC columns with cellulose carbamates as packing materials can be applied successfully to enantiomeric separation of a number of chiral organic compounds as well as lignans,[37,38] the semi-micro chiral columns will be useful for highly sensitive chiral HPLC and LC-MS analyses of a wide range of compounds.

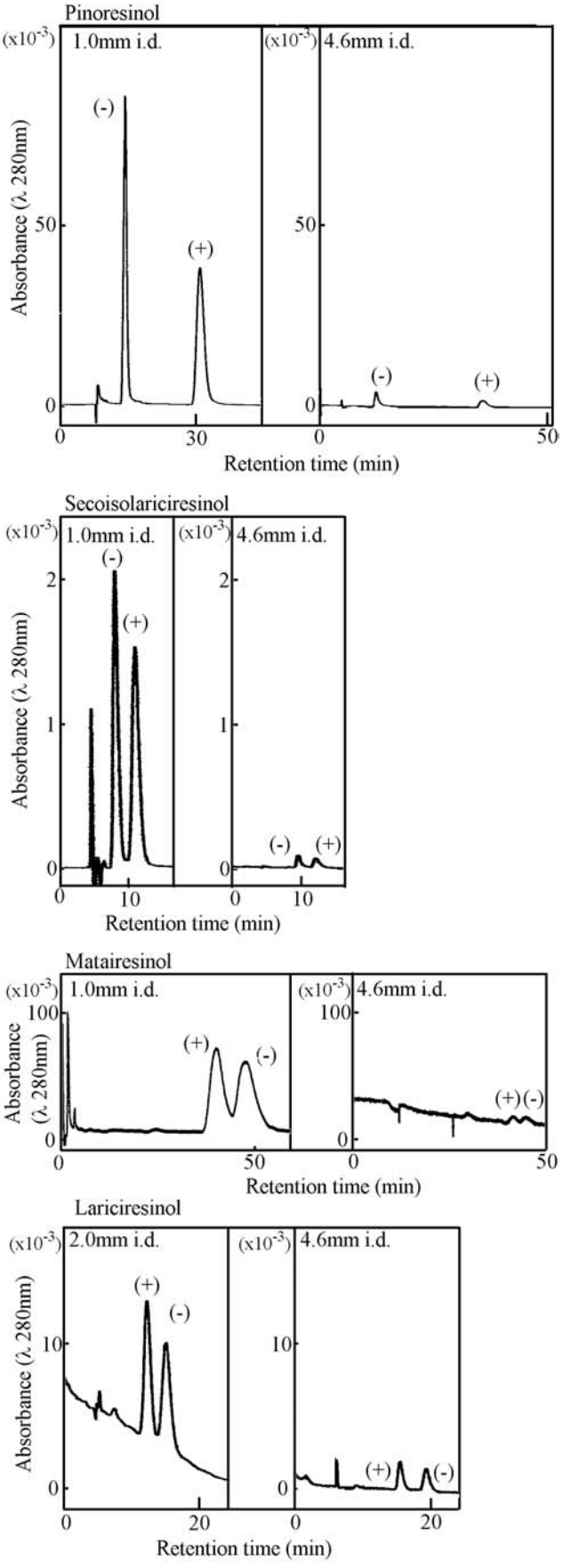

Fig. 3. Chiral high-performance liquid chromatograms of lignans. *4.6 mm i.d.*, *2.0 mm i.d.*, and *1.0 mm i.d.*, chromatograms obtained using columns whose inner diameters are 4.6, 2.0, and 1.0 mm, respectively; (+) and (−), (+)- and (−)-enantiomers, respectively. Elution conditions are described in the text. Note that the same volume (2 μl) of each lignan solution was applied to the columns

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