methods

Separation of polyprenol and dolichol by monolithic silica capillary column chromatography

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Abstract **We attempted an analysis of naturally occurring polyprenol and dolichol using a monolithic silica capillary column in HPLC. First, the separation of the polyprenol mixture alone was performed using a 250 × 0.2 mm inner diameter (ID) octadecylsilyl (ODS)-monolithic silica capillary column. The resolution of the separation between octadecaprenol (prenol 18) and nonadecaprenol (prenol 19) exceeded by ≥2-fold the level recorded when using a conventional ODS-silica particle-packed column (250 × 4.6 mm ID) under the same elution conditions. Next, the mixture of the prenol type (polyprenol) and dolichol type (dihydropolyprenol) was subjected to this capillary HPLC system, and the separation of each homolog was successfully achieved. During the analysis of polyprenol fraction derived from *Eucommia ulmoides* leaves, dolichols were found as a single peak, including all-*trans*-polyprenol and *cis*-polyprenol previously identified. This sensitive high-resolution system is very useful for the analysis of compounds that are structurally close to polyprenols and dolichols and that have a low content.**—Bamba, T., E. Fukusaki, H. Minakuchi, Y. Nakazawa, and A. Kobayashi. **Separation of polyprenol and dolichol by monolithic silica capillary column chromatography. *J. Lipid Res.* 2005.** 46: **2295–2298.**

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Polyprenol is the generic name for linear 1,4-polyprenyl alcohols. Naturally occurring polyprenols can be classified into four categories (**Fig. 1**): *I*) all *trans* form; only three such polyprenols are known, namely, solanesol (9-mer), spadicol (10-mer), and long-chain *trans*-polyprenol from *Eucommia ulmoides* Oliver; *II*) *trans-trans-trans*-poly*cis*-prenols of the ficaprenol type; *III*) *trans-trans*-poly*cis*-prenols, such as the bacteria prenol and beturaprenol types; *IV*) the dolichol type; the α terminal is saturated only in the dolichol type.

Since the first report of a polyprenol isolated from tobacco, named solanesol (1), further polyprenols have been isolated from animals, microbes, plants, etc. (2–4). The best-known dolichol plays an important role as a sugar-carrying intermediate in the biosynthesis of glycoproteins (5, 6). There are also many reports concerning the structure and chain-length distribution of polyprenols (4, 7–10).

For polyprenol analyses, reverse-phase HPLC using an octadecylsilyl (ODS)-silica particle-packed column has been widely used (8). This system of analysis is useful for separating polyprenol homologs based on the degree of polymerization. However, its resolution is insufficient for the baseline separation of geometric isomers and long-chain polyprenols, prompting us to develop recently a high-resolution analytical system for polyprenol, using supercritical fluid chromatography (SFC) (11, 12). In addition, the structure of polyprenols derived from *E. ulmoides* has been analyzed in detail using SFC, and the chain-length of *cis* and *trans* geometric isomers and their distribution in the harvest parts of *E. ulmoides* elucidated (13). We also succeeded in the separation of geometric isomers by connecting the monolithic silica column, which is the low backpressure in HPLC, as well as SFC (14).

With regard to the separation of the prenol type (polyprenol) and dolichol type (dihydropolyprenol), there has been no report concerning separation using a column chromatography such as HPLC until now; only the use of two-dimensional thin-layer chromatography (TLC) has been reported (15). Therefore, in this study, we tried to separate polyprenol and dolichol using a monolithic silica capillary column in HPLC. This monolithic silica column can be prepared in a fused-silica capillary using a sol-gel method developed by Nakanishi et al. (16, 17). This column facilitates increasing the resolution of separation (*Rs*) through extension of the column length because of the low backpressure (17, 18). Additionally, the capillary HPLC system using this column is useful for the analysis of the low-content natural product.

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Abbreviations: ODS, octadecylsilyl; SFC, supercritical fluid chromatography.

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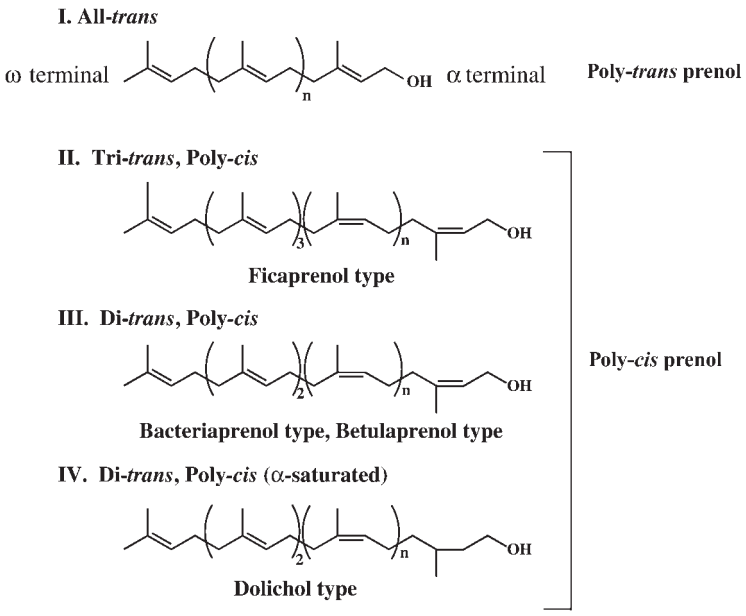

Fig. 1. Chemical structures of natural polyprenols.

MATERIALS AND METHODS

Materials

Polyprenol (prenol 16-21 from *Gingko biloba* leaves, which has the structure shown in Fig. 1III), and dolichol (dolichol 17-21 from bovine serum, which has the structure shown in Fig. 1IV), were purchased from Funakoshi Co. (Tokyo, Japan). Undecaprenol (C_{55}) from *Aianthus altissima*, which has the structure shown in Fig. 1II, was obtained from Sigma-Aldrich Japan (Tokyo, Japan). For the HPLC analyses, HPLC-grade methanol, 2-propanol, and N-hexane were used (Merck; Darmstadt, Germany), and water was purified using a Millipore Milli-Q system (Bedford, MA).

Samples of *E. ulmoides* were collected at the Hitachi Zosen Corporation experimental station (Habu 2264-1 Innoshima, Hiroshima, Japan). Preparation of polyprenols from *E. ulmoides* leaf was performed according to the reported procedure of Bamba et al. (13).

Capillary HPLC

The HPLC of polyprenols was performed on an ODS-monolithic silica capillary column (MonoCap) [250 × 0.2 mm inner diameter (ID); macropore size, 2 μm; mesopore size, 13 nm; (GL Sciences Inc.; Tokyo, Japan) and 500 × 0.2 mm ID; macropore size, 2 μm; mesopore size, 13 nm (Kyoto Monotech Co., Kyoto, Japan)] using a dual pump apparatus (MP711; GL Sciences Inc.), and a UV-visible (UV-VIS) detector with a capillary optical fiber flow cell (set at 210 nm; GL Sciences Inc.). A carrier reservoir (CR791; GL Sciences Inc.), and a data station (SIC-480II data station; System Instruments Co., Hachioji, Japan) were also used. For elution purposes, a gradient was applied from an 80% methanol-2-propanol-water mixture (60:40:5; v/v/v) in pump A to 80% N-hexane-2-propanol (70:30; v/v) in pump B. The solvent flow rate was 4 μl/min. The end of the gradient was reached after 40 or 80 min, respectively, and then held at A-B (20:80; v/v) for 10 min. The column was at room temperature.

To investigate the sensitivity of this analysis system for polyprenol, 10 nl of 10 pg/nl chloroform solution of undecaprenol (100 pg) was injected into the capillary HPLC with 250 mm capillary column (N = 3).

RESULTS AND DISCUSSION

First, we tried to separate the polyprenol mixture (16–21 mer) derived from *G. biloba* leaves by using a 250 × 0.2 mm ID ODS-monolith capillary column. For elution purposes, a gradient using a mixed solvent system comprising MeOH, 2-propanol, N-hexane, and H_2O was applied in this experiment. The *Rs* between octadecaprenol (prenol 18) and nonadecaprenol (prenol 19) showed 4.7 and a high value, which exceeded by ≥2-fold the level recorded when using a conventional ODS-packed column (250 × 4.6 mm ID, *Rs* = 2.1) under the same elution conditions (**Fig. 2**). In addition, the detection sensitivity was mark-

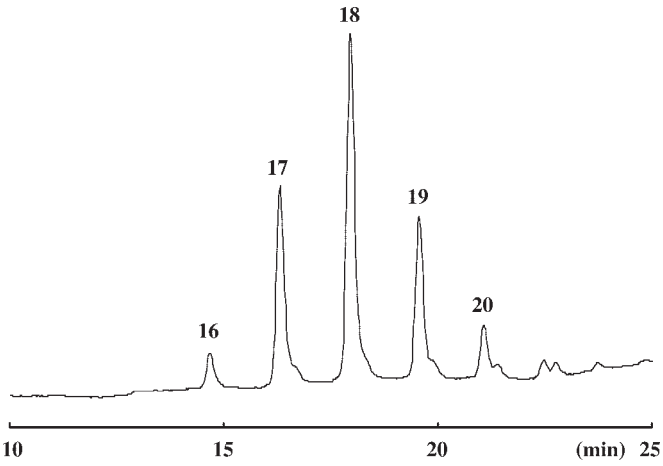

Fig. 2. Chromatogram of authentic polyprenol mixtures separated using a 250 mm monolith capillary column. Conditions: mobile phase, pump A: methanol-2-propanol-water (60:40:5; v/v/v); pump B: N-hexane-2-propanol (70:30; v/v), A-B (80:20; v/v) to A-B (20:80; v/v) over 40 min; flow rate, 4.0 μl/min; UV detection at 210 nm. The numbers represent degrees of polymerization for polyprenol homologs.

edly higher than that of a conventional system. In detail, the UV-VIS detector with the capillary optical fiber flow cell, with a light path length of 4 mm, was adopted for use within this analysis system. Accordingly, the injection amount of the sample was 50 ng (5 mg/ml, 10 nl), and the presence of even a few nanograms per individual component was sufficiently detectable. The sensitivity of this capillary HPLC system for polyprenol was investigated using undecaprenol (C_{55}), and the average of signal to noise was 65 (N = 3) in the injection amount of 100 pg.

Next, the mixture of polyprenols derived from *G. biloba* leaves (prenol 16-21) and dihydropolyprenols derived from bovine serum (dolichol 17-21) was subjected to this capillary HPLC system, and the separation of polyprenols and dolichols was successfully achieved (**Fig. 3a**). It was proven that dolichol polymerized to the same degree was eluted behind the polyprenol under these conditions. There has been no report concerning the separation of polyprenol and dolichol within a liquid chromatography system until now, making this the first example. Additionally, the polyprenol fraction from *E. ulmoides* leaves, containing *trans* and *cis* isomers, was subjected to this analysis system. Consequently, although peaks thought to be dolichol were found (shown as circled peaks in **Fig. 4a**), except for the *trans* and *cis* isomers confirmed previously (13), the separation of *cis*-polyprenols was insufficient.

Under the conditions used, the initial backpressure of this column was low at 5.3 Mpa, so improvement of the *Rs* was attempted by extending the column length. First, through the use of a 500 × 0.2 mm ID ODS-monolith capillary column, the *Rs* between the *cis*-polyprenol and dolichol in the polyprenols-and-dolichols mixture sample was checked. The *Rs* on prenol 18 increased to 1.8, compared with that of the 250 mm column (*Rs* = 0.99) (Fig. 3b). Subsequently, analysis of the polyprenol fraction derived from *E. ulmoides* leaves was similarly carried out, and effective separation of three components, i.e., the *trans*-polyprenols, *cis*-polyprenols, and dolichols, was found to have occurred (Fig. 4b).

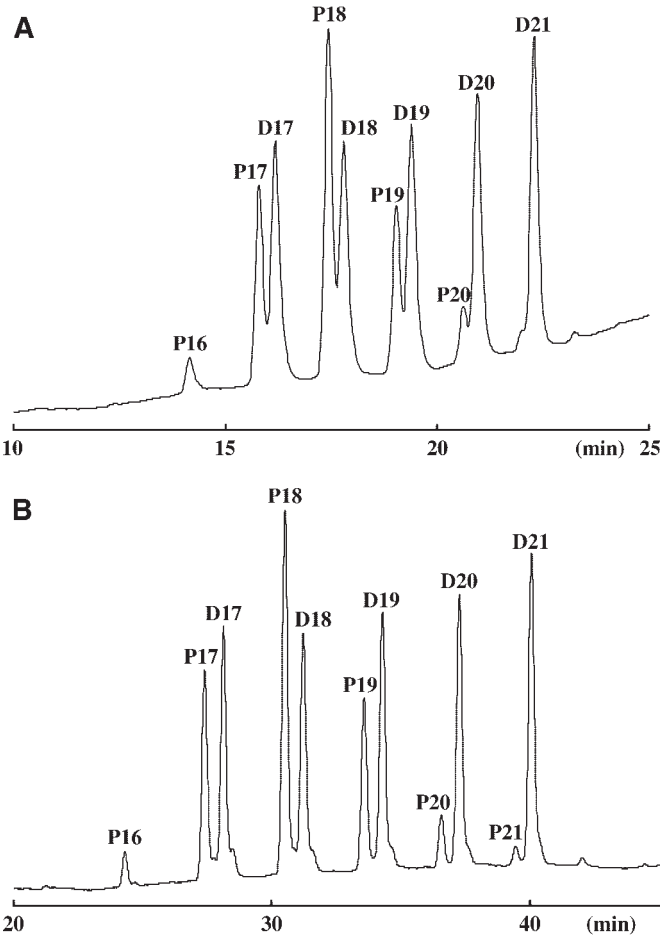

Fig. 3. Chromatogram of authentic polyprenol and dolichol mixtures separated using (A) 250 mm and (B) 500 mm monolith capillary column. Mobile phase, pump A to A-B (20:80; v/v) over (A) 40 min or (B) 80 min; flow rate, 4.0 μl/min; UV detection at 210 nm. The numbers represent degrees of polymerization for polyprenol and dolichol homologs. P, polyprenol; D, dolichol.

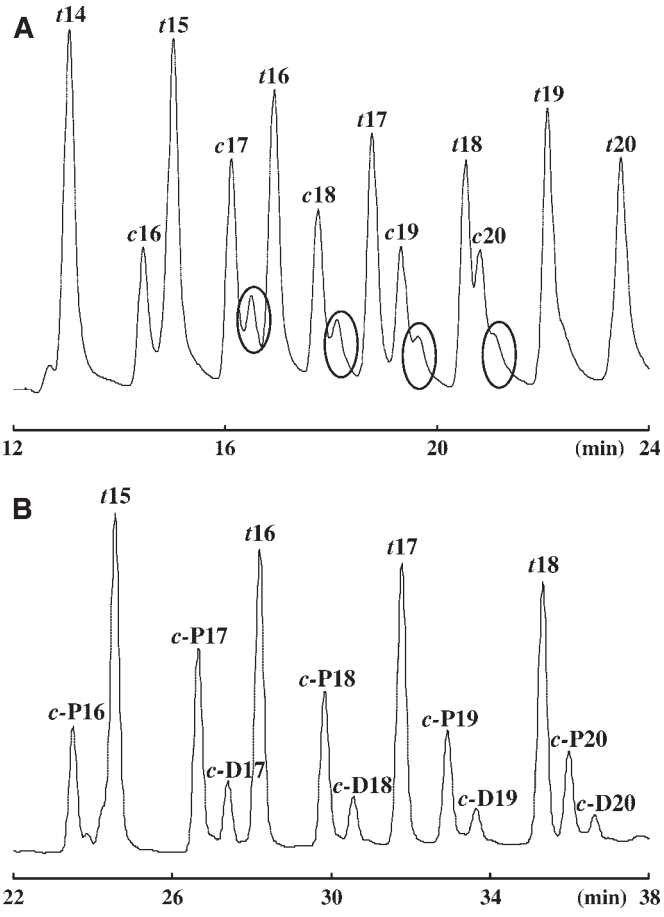

Fig. 4. Chromatogram of polyprenol fraction from *E. ulmoides* leaves separated using (A) 250 mm and (B) 500 mm monolith capillary column. Mobile phase, pump A to A-B (20:80; v/v) over (A) 40 min or (B) 80 min and then held at A-B (20:80; v/v) for 10 min; flow rate, 4.0 μl/min; UV detection at 210 nm. The numbers represent degrees of polymerization for polyprenol and dolichol homologs. *c*, *cis* isomers; *t*, *trans* isomers; P, polyprenol; D, dolichol. The peaks presumed to be dolichol are circled (A). These peaks were identified in an earlier experiment (13).

Thus, this monolith capillary HPLC system was seen to allow the high-resolution analysis of a small sample, an analysis associated with increased and facilitated reproducibility, as compared with two-dimensional TLC. This sensitive high-resolution system is thus considered very useful for the analysis of structurally close and low-content polyprenol. The monolithic capillary column would be ideal as a powerful tool for metabolite analysis in various organisms, inasmuch as the column length can be freely adjusted in proportion to the necessary resolution and includes a widely variable separation mode, enabling various modifications, as well as the particle-packed column.

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