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**Note**

# High-performance liquid chromatography of natural prenylquinones

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Green, yellow and white plant tissues contain several prenylquinones, which are lipophilic benzo- and naphthoquinone derivatives with isoprenoid side-chains[1–3]. The main components are ubiquinone-9 or -10 bound to mitochondria and in plastids plastoquinone-9, α-tocoquinone, α-tocopherol, the chromanol form of α-tocohydroquinone and the naphthoquinone vitamin $K_1$ (for chemical structures, see Fig. 1). The prenylquinones are usually separated from other prenyllipids, *e.g.*, β-carotene, phytol and chlorophylls, by one-dimensional thin-layer chromatography[1,4]. The separation of plastoquinone-9 from phylloquinone, of α-tocopherol from ubiquinone or of phytol from α-tocoquinone is, however, often not satisfactory or requires two-dimensional chromatography with further purification steps. Gas chromatography cannot be applied, as prenylquinones are degraded at higher temperatures. In attempting to devise a faster separation method with better resolution, we tried high-performance liquid chromatography (HPLC)[5], which has been used successfully for the separation of sterols[6], flavonoids[7] and xanthones[8]. It is shown in this paper that HPLC can be applied routinely to the separation of prenylquinones.

## EXPERIMENTAL

### *Apparatus*

The high-pressure liquid chromatograph used was a Siemens S 100 equipped with a pneumatic syringe system (10-μl syringe) and a Zeiss PM2 D spectrophotometer (200–850 nm) as detector, cuvette volume 8 μl. The dual-piston diaphragm pump permits pressures of up to 325 bar with flow-rates of up to 5 ml/min to be used.

### *Column switching valve*

For the analysis of the natural prenylquinones, the application of a high-pressure column-switching valve in connection with two columns was found to be useful[9,10]. The plant lipid fraction was injected and first led over column 1 (length 12.5 cm) directly to column 2 (length 25 cm). After a few seconds, when the apolar lipid components (β-carotene, vitamin $K_1$ and plastoquinone-9) were on column 2, the valve was switched over and the late peaks from column 1 (α-tocopherol, ubiquinone-10, phytol and α-tocoquinone) were fed directly to the detector, as shown in Fig. 2. After elution of the last prenylquinone peak from column 1, the valve was switched again to elute the apolar compounds from column 2.

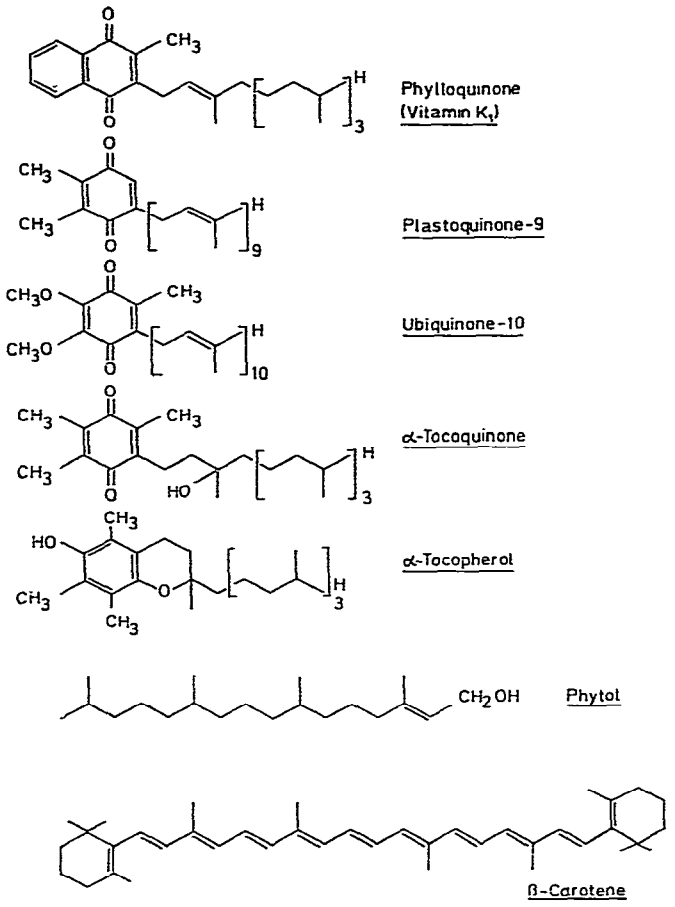


Fig. 1. Structures of prenylquinones, $\beta$-carotene and phytol.

*Columns*

Stainless-steel columns (V 4 A) filled with LiChrosorb SI 60, 5 $\mu$m (Merck, Darmstadt, G.F.R.) were used, column 1 with a length of 12.5 cm and column 2 25 cm. The I.D. of each column was 3 mm. The columns were self-packed with a special filling apparatus purchased from Siemens. The columns were usually run at room temperature at a pressure of 60 bar for column 1 (flow-rate 2.7 ml/min) and 105 bar for column 2 (flow-rate, 1.9 ml/min). When both columns were used in series, a pressure of 120 bar (1.3 ml/min) was applied.

*Solvents*

Mixtures of *n*-hexane and dioxan (both reagent grade from Merck) were used.

*Chemicals*

Phytol was obtained from Merck (7499) and vitamin $K_1$, $\alpha$-tocopherol, ubiquinone-9 and -10 and $\alpha$-tocoquinone were gifts from Hoffmann-La Roche (Basle,

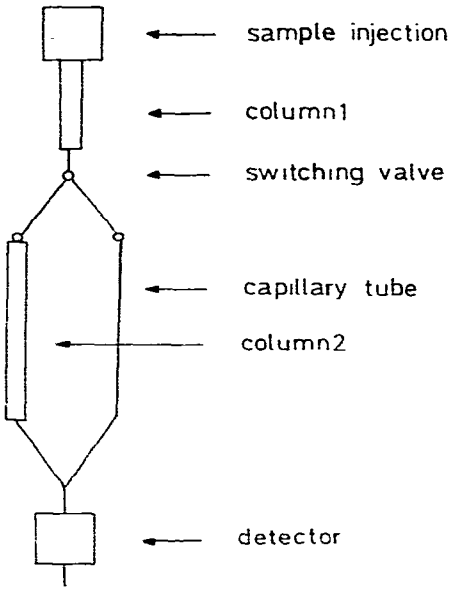


Fig. 2. Schematic diagram of column-switching system.

Switzerland). Plastoquinone-9, β-carotene and also the prenylquinones just mentioned were isolated from green Fagus leaves. Before application of HPLC, all compounds were purified by adsorption and partition thin-layer chromatography[4].

*Detection*

The monochromator was set at a standard wavelength of 260 nm for detection of the eluted compounds by absorption of UV light. In order to obtain better sensitivity, a second run was carried out at particular wavelengths corresponding to the absorption maximum of the individual components: 450 nm for β-carotene, 290 nm for α-tocopherol, 265 nm for α-tocoquinone and 220 nm for phytol. The higher peaks (broken lines in the figures) are those obtained from the second run. The initial wavelength of 260 nm resulted in good detection of vitamin $K_1$, plastoquinone-9 and ubiquinone.

## RESULTS AND DISCUSSION

We compared several solvents for use in the HPLC of prenylquinones and the best results were obtained with mixtures of *n*-hexane and dioxan. β-Carotene appeared from the columns after *ca.* 4 min when pure *n*-hexane was used, while vitamin $K_1$ and plastoquinone-9, which are nearly as lipophilic as β-carotene, were not eluted after 40 min. On the addition of 0.1 % of dioxan to the *n*-hexane, β-carotene appeared after 1.3 min, while the synthetic vitamin $K_1$ gave two peaks after 5.5 min (*cis*-$K_1$) and 6 min (*trans*-$K_1$) and plastoquinone-9 appeared after 12.5 min (Fig. 3). When the pressure was increased from 105 to 200 bar, the compounds were eluted earlier but the sensitivity of detection decreased owing to the higher flow-rates. On increasing the polarity of the solvent by adding more dioxan, the other quinone derivatives,

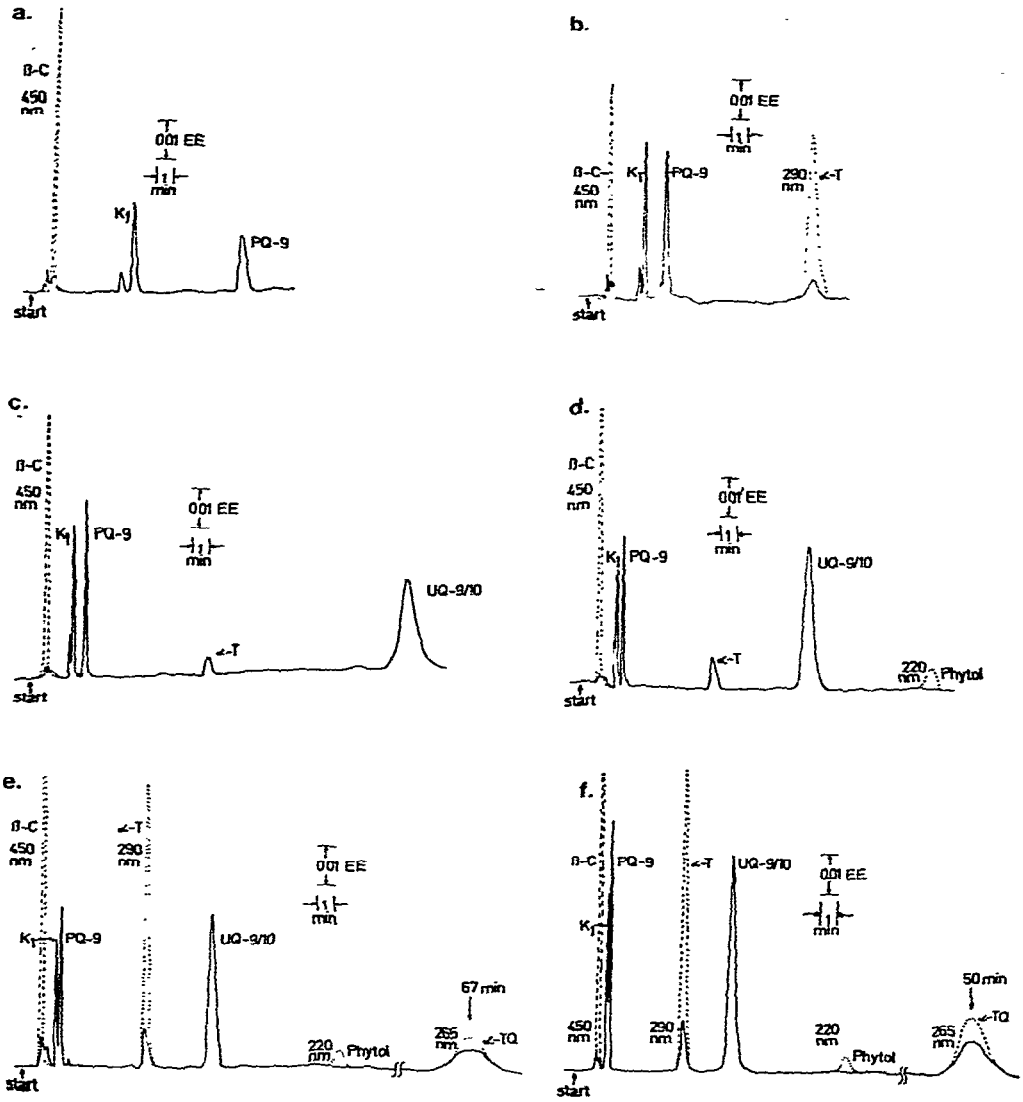


Fig. 3. Separation of β-carotene, vitamin $K_1$ ($K_1$), plastoquinone-9 (PQ-9), α-tocopherol (α-T), ubiquinone-9 and -10 (UQ-9/10), phytol and α-tocoquinone (α-TQ) on LiChrosorb SI 60. Column, 25 cm × 3 mm I.D.; flow-rate, 1.9 ml/min. Mobile phase: $x$% of dioxan in *n*-hexane; $x$ = (a) 0.1; (b) 0.3; (c) 0.5; (d) 0.8; (e) 1; (f) 1.25%. Absorbance units shown as EE.

such as α-tocopherol, ubiquinone and phytol, were eluted successively (Fig. 3), but ubiquinone-9 and -10 were not separated. The resolution of vitamin $K_1$ and plastoquinone decreased with increasing solvent polarity, and α-tocoquinone first appeared after about 1 h when 1% of dioxan in *n*-hexane was used. With 1.25% of dioxan in *n*-hexane, α-tocoquinone was detected earlier (after 50 min), but vitamin $K_1$ and plastoquinone-9 were then poorly resolved (Fig. 3).

To shorten the time of analysis, we developed a chromatographic system with two columns and a column-switching valve (Fig. 2). The injected sample is led on to

columns 1 and 2 in series. After 90 sec, when the non-polar prenyllipids ($\beta$-carotene, vitamin $K_1$ and plastoquinone-9) are on column 2, the valve is switched and the more polar compounds are eluted from column 1. $\alpha$-Tocoquinone appears after 20 min (Fig. 4). The valve is then switched back to elute the sample from column 2. This

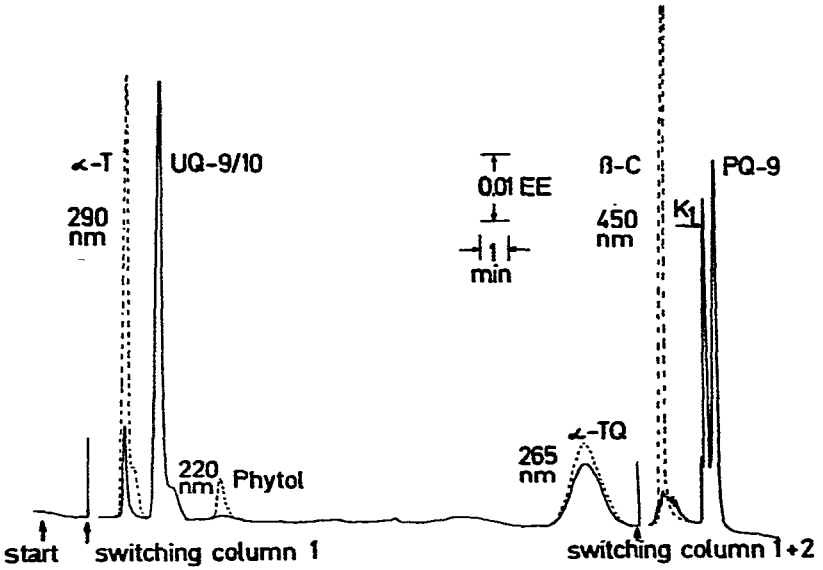


Fig. 4. Separation of prenylquinones on LiChrosorb SI 60 using two columns with a column-switching valve. Column 1, 12.5 cm × 3 mm I.D.; column 2, 25.0 cm × 3 mm I.D. Flow-rate: column 1, 2.7 ml/min; columns 1 + 2, 1.35 ml/min. Mobile phase, 1.25% of dioxan in *n*-hexane.

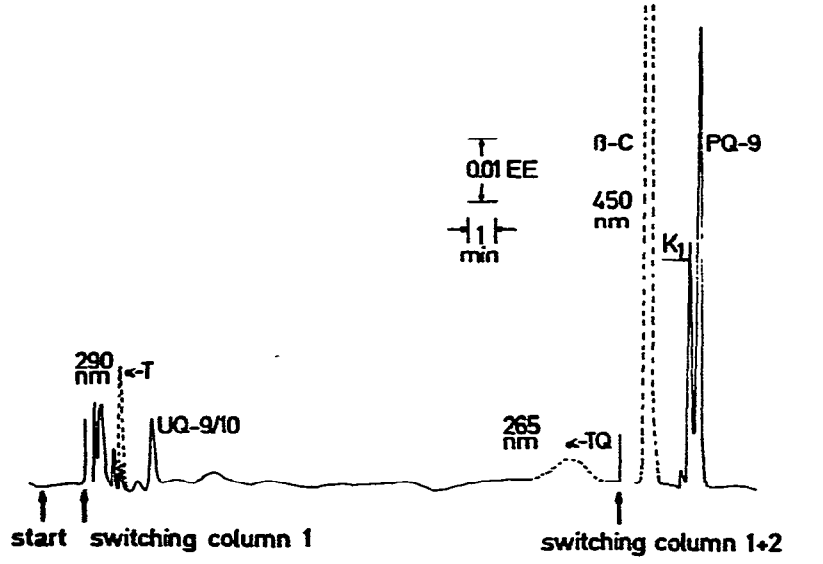


Fig. 5. Separation of natural prenylquinones from a prenyllipid extract from 6-day-old green Raphanus seedlings. HPLC conditions as in Fig. 4.

method has been applied successfully to a prenyllipid fraction from green Raphanus seedlings (Fig. 5) and other plants. Several other peaks of lipid material appear, which have not yet been identified. Depending on the plant species and on the type of plant tissue, the heights of the known and unidentified peaks vary considerably.

The method developed can be applied routinely to the prenylquinone analysis of plant lipid extracts. It is also practicable for quantitative determinations, particularly for the more polar prenylquinones. For the quantitative determination, plastoquinone-9, vitamin $K_1$ and $\beta$-carotene must be well separated from unknown lipids which may interfere with their resolution. In this instance, a mixture of 0.3% of dioxan in *n*-hexane is applied (Fig. 3). By multiple injection and collection of the corresponding peaks, one obtains enough material to identify the prenylquinones by UV spectroscopy.

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