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Note

High-performance liquid chromatographic separation and quantitative determination of 1,8-dihydroxyanthraquinones in plant cell cultures

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High-performance liquid chromatography (HPLC) of some naturally occurring glycosides of 1,8-dihydroxyanthracene derivatives (especially sennosides) has been used in research into plant drugs[1–5]. For the HPLC separation of the anthraquinone aglycones chrysophanol, physcion, emodin, aloe-emodin and rhein, procedures based on isocratic conditions[2] and gradient elution[5–7] have been described. For the quantitative determination of emodin an isocratic HPLC procedure has been reported[8]. Of the described HPLC separations of the anthraquinone aglycones, the isocratic procedure[2] could not be used, because of incomplete experimental data. To perform routine quantitative determinations, HPLC separations based on gradient elution are less useful.

Plant cell cultures can accumulate compositions of anthracene derivatives which are different from those in the intact plants. In the cultures developed and investigated in our laboratory, 1,8-dihydroxyanthracene derivatives occur mainly as anthrones and dianthrones, besides the anthraquinone form (Fig. 1). These oxygenated anthracene derivatives were found to accumulate as such and also as glycosides[9]. Reference samples of such derivatives are rare and sometimes difficult to obtain.

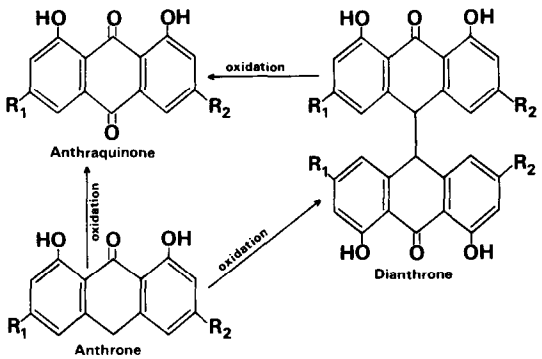

Fig. 1. Structural relationships of the anthrones, dianthrones and anthraquinones under consideration. The anthraquinones were chrysophanol (R_1 = H, R_2 = CH_3), physcion (R_1 = OCH_3, R_2 = CH_3), emodin (R_1 = OH, R_2 = CH_3), aloe-emodin (R_1 = H, R_2 = CH_2OH), rhein (R_1 = H, R_2 = COOH).

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In order to study the potential for oxygenated anthracene production of these cultures an analytical procedure was elaborated. Originally present free and glycosidic-bound anthracene derivatives were converted into the corresponding anthraquinone aglycones. For the separation and quantitative determination of the obtained anthraquinone aglycones, the present communication describes an isocratic HPLC procedure for the good resolution of chrysophanol, physcion, emodin, aloe-emodin and rhein within a relatively short time.

EXPERIMENTAL

Extraction and pretreatment procedures

Freeze-dried plant cells (500 mg) were exhaustively extracted with 100 ml of methanol under reflux. After filtration the methanol was removed from the extract under reduced pressure at 50°C. To the residue, 50 ml of water and 1.2 g of ferric chloride were added and the mixture was refluxed for 30 min. The mixture was cooled, 5 ml of concentrated sulphuric acid were added, and refluxing was performed for 1 h. After cooling the mixture was exhaustively extracted with chloroform. The chloroform solution was washed with water and dried over anhydrous sodium sulphate. The solvent was evaporated under reduced pressure at 50°C. The solution of the residue in 0.5–20.0 ml of methanol–chloroform (1:1), depending on the amount of anthraquinones obtained from each culture, was used for HPLC. (Totally present anthracene derivatives (free or as glycosides) obtained as anthraquinone aglycones.)

When differentiation had to be made for the aglycone and glycoside content of plant cell cultures, the freeze-dried cell material was first exhaustively extracted with 100 ml of diethyl ether. After filtration the marc was subsequently extracted with 100 ml of methanol. The methanol extract was treated according to the above procedure. (Glycosidic-bound anthraquinones, anthrones and dianthrones obtained as anthraquinone aglycones.)

From the ether extract the ether was evaporated and the residue was treated analogously to the residue of the methanol extracts. (Free anthracene derivatives obtained as anthraquinone aglycones.)

HPLC procedure

A Beckman Model 342 gradient liquid chromatograph was used, connected to a C-R1B Chromatopac data processor (Shimadzu). The variable-wavelength detector was operated at 430 nm and the sample-injection valve used in combination with a 20-μl sample loop.

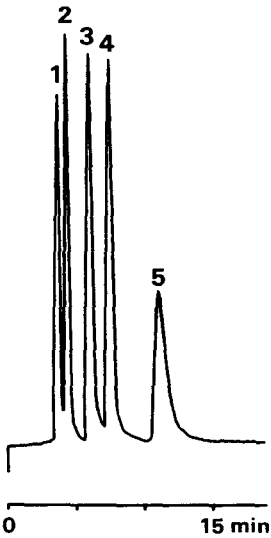
Separations were performed on a prepacked Spherisorb 5 ODS column, 25 cm × 4.6 mm (Chrompack, Cat. No. 26339). The mobile phase consisted of methanol–water containing 2.5% formic acid (81.5:18.5). Distilled water and analytical reagent grade methanol and formic acid (Merck) were used. The solvent flow-rate was 1.2 ml/min. Sample injector and column were operated at room temperature.

Standard solutions in chloroform–methanol (1:1) contained the anthraquinones chrysophanol, physcion, emodin, aloe-emodin and rhein in concentrations of 0–6 mg% (w/v) each.

RESULTS AND DISCUSSION

To study the production capacity of plant cell cultures of *Rhamnus frangula* and *R. purshiana*, the procedure was followed to convert the 1,8-dihydroxyanthrone and 1,8,1′,8′-tetrahydroxydianthrone derivatives into the corresponding anthraquinone aglycones chrysophanol, physcion and emodin. Thin-layer chromatographic (TLC) analysis[9] of the isolated fractions, after oxidation and hydrolysis, showed the absence of anthrones and dianthrones. A similar procedure has been reported previously for some dianthrones[10].

The HPLC procedure described provides a separation of the five anthraquinones with good resolution within 15 min (Fig. 2). The anthraquinones emerged from the column in the sequence: aloe-emodin, rhein, emodin, chrysophanol, physcion. The composition of the mobile phase was rather crucial for a satisfactory resolution of aloe-emodin and rhein. A linear detector response was obtained for each anthraquinone in the range 0–1.2 μg per injection.



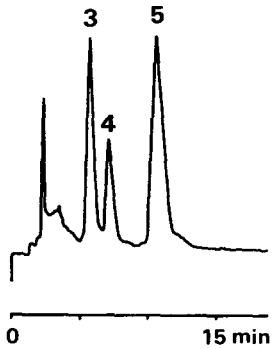


Fig. 2. Chromatogram of anthraquinones; conditions as in Experimental. Peaks: 1 = aloe-emodin; 2 = rhein; 3 = emodin; 4 = chrysophanol; 5 = physcion.

Fig. 3. Chromatogram of a pretreated extract from a *Rhamnus purshiana* plant cell culture; conditions as in Experimental. Peaks: 3 = emodin; 4 = chrysophanol; 5 = physcion.

A chromatogram of an extract of plant cell material of *R. purshiana* is shown in Fig. 3. For a series of quantitative determinations of emodin, chrysophanol and physcion in 28 plant cell cultures, the calculated standard deviations were 0.20, 0.23 and 0.32, respectively.

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