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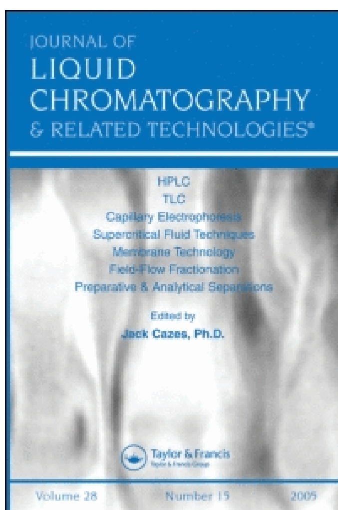

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A High Performance Preparative Procedure for the Isolation of Degradation Products from D-Xylose

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A HIGH PERFORMANCE PREPARATIVE PROCEDURE FOR THE ISOLATION OF DEGRADATION PRODUCTS FROM D-XYLOSE

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ABSTRACT

An improved method for the preparative isolation of 3,8 dihydroxy-2-methylchromone, formed by degradation of D-xylose at 100°C is presented. From the ethyl acetate extractable part of the reaction mixture the chromone was isolated by preparative HPLC in less than one hour using a highly cross-linked dextran gel.

INTRODUCTION

Aromatic compounds are formed by degradation of carbohydrates in aqueous solution at different pH. This has been extensively studied by Theander et al (1). The purpose has been to characterise the products and elucidate their mechanism of formation to throw some light upon caramelisation and non-enzymic browning reaction of importance in food manufacturing and pulping processes.

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The isolation of degradation products and their precursors are often time-consuming due to the complex mixtures and low yields (0,1 - 3% or less), which causes severe extraction and separation problems.

The general method is usually group separation on Sephadex LH-20, followed by purification on silica gel.

This paper describes a simple and reproducible high performance liquid chromatographic procedure for the analysis of aromatic compounds from the degradation of D-xylose and the isolation of the main product (Fig. 1).

MATERIAL AND METHODS

Instrumentation

The liquid chromatograph consisted of the following components: Chromatronics CMP-2 pump (Laboratory Data Control, Riviera Beach, Fla), a SR10/50 J column packed with Sephasorb HP Ultrafine, two SRV-4 valves with teflon loops of different volumes, for injection under pressure, a dual path monitor UV-2 (Pharmacia Fine Chemicals, Uppsala, Sweden), and a Waters differential refractometer R 403 (Waters Associates, Milford MA).

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Degradation and extraction

The method used by Popoff (2) was modified as follows: D-xylose (20,0 g) in sodium acetate buffer (0,5M, 750 ml, pH 4,5) was refluxed for 48 hours. The reaction mixture was cooled and extracted with ethyl acetate (3x100 ml). The dried (sodium sulfate) and evaporated extract was dissolved in the appropriate amount of methanol.

Chromatography

The column was dry packed. The bed height after equilibration with water was 40 cm. The temperature was kept at 65°C. A 100

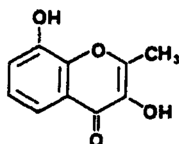

Figure 1. 3,8-Dihydroxy-2-methylchromone.

μl or a 250 μl loop was used for the analytical experiments. For preparative purpose a 2 ml loop was used. With a flow rate of 1 ml/min the pressure was about 8 bar.

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Eluants

Ethanol was purchased from AB Svensk Sprit, Sweden. A Milli-Q system was used to obtain highly purified water. (Millipore, Gothenburg, Sweden). The mobile phase was ethanol/water of different compositions or pure water. A gradient was formed at the low pressure side by adding pure ethanol to a water reservoir (400 ml) at the same rate as the flow rate.
The composition of the gradient is approximately:

$$\frac{P}{100} = 1 - e^{-vt/V}$$

P = percent ethanol (volume)
v = flow rate (ml/min.)
t = time (min.)
V = volume of (water) reservoir (ml)

RESULT AND DISCUSSION

The extraction procedure removed most of the unreacted D-xylose and the highly polar by-products. Due to the high adsorption of the main product, 3,8-dihydroxy-2-methylchromone (Fig. 1) and other aromatic products on this matrix, the chromatographic

procedure is tedious when run in water, although the separation of different compounds is good. By an increasing amount of ethanol the chromatogram in Fig. 2 is obtained. All high molecular weight compounds are excluded from the gel, which has a exclusion limit of about 500, and appear close to the void volume. Fig. 2 shows that an optimal solvent composition for the isolation of the main product should consist of ethanol/water at about 2:3 as confirmed (Fig. 3).

Although the amount separated was considerable higher in the preparative run, (Fig. 4), the resolution is close to that in the previous chromatogram.

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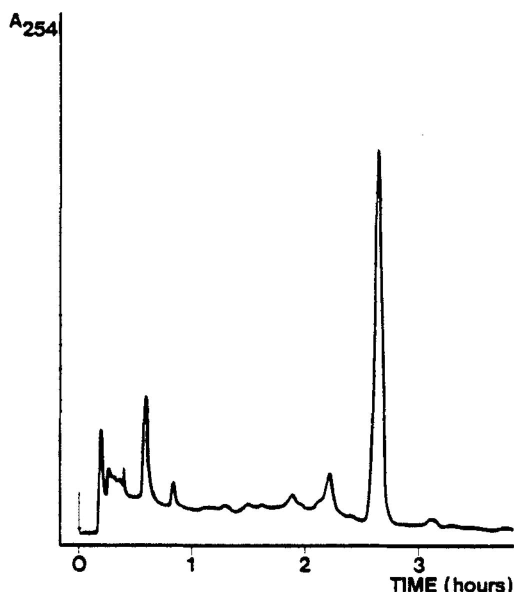

Figure 2. Separation of the ethyl acetate extract.
Column: Sephasorb HP Ultrafine
Solvent: Water-ethanol gradient
Flow rate: 1,0 ml/min.
Temperature: 65°C
Sample: 100 µl, 150 µg

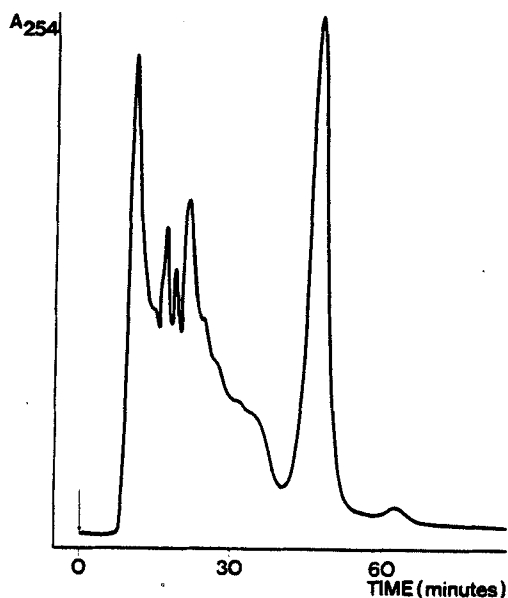

Figure 3. Solvent: Water-ethanol 3:2
Sample: 250 µl, 230 µg

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Sephasorb HP Ultrafine is a highly cross-linked rigid dextran gel, lipofilised with hydroxypropylene oxide, which makes it suitable for use in organic solvents. It has been shown that the affinity for aromatic compounds to dextran gels increases with increasing degree of cross-linking of the carbohydrate back-bone (3). The affinity for phenolic compounds may be due to interaction between phenolic hydroxyls and the ether oxygens of the cross-links (4), but it has also been suggested that a hydrophobic and a special π -electron interaction are involved (5). The hydrophobic effect can be decreased by changing the solvent composition from water to ethanol. In protic solvents like alcohol it is unlikely that hydrogen bonding between the gel and the phenolic hydroxyls predominates. However, the increase in adsorbtion observed in aprotic solvents like acetonitril or chloroform is probably due to this effect (6).

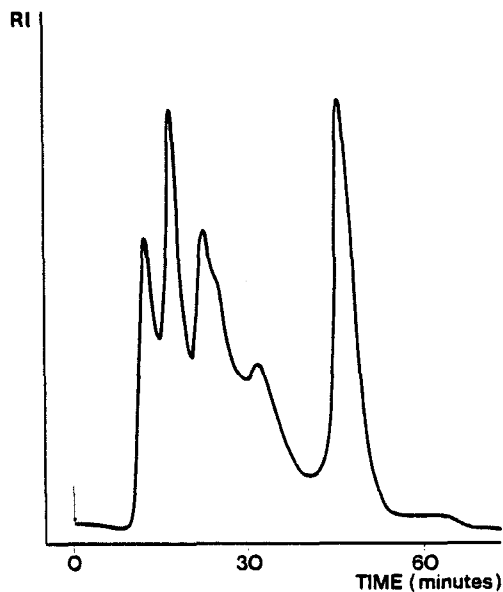

Figure 4. Preparative separation.
Detector: Refractive index
Sample: 2 ml, 60 mg
Yield of chromone 14 mg.

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We think that this method will be of considerable value for identification and preparative scale isolation especially for aromatic compounds such as natural products.

REFERENCES

1. Olsson, K., Pernemalm, P-Å. and Theander, O., Acta Chem. Scand., B 32, 249, 1978, and ref. therein.

2. Popoff, T. and Theander, O., Carbohyd. Res., 22, 135, 1972.

3. Determan, H. and Walter, I., Nature (London), 219, 604, 1968.

4. Brook, C.S., J.W. and Munday, K.C., J. Chromatogr. 47, 1, 1970.

5. Marsden, N.V.B. and Haglund, Å.C. Polymer Sci. in press.

6. Snook, M.E, and Fortson, P.J., Anal. Chem. 51, 1814, 1979.