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Note

Separation of furocoumarins by high-pressure liquid chromatography*

The separation of furocoumarins has been accomplished by thin-layer chromatography (TLC)[1,2] and by column chromatography[3]. In these procedures difficulty has been experienced in obtaining complete separation of the furocoumarin mixture and purification of each compound present in the mixture is often a problem. High-pressure liquid chromatography (HPLC) offers the advantages of high-speed separation and automated operation over other chromatographic methods[4,5]. The aim of the present note is to demonstrate the efficiency possible in applying HPLC to separation of furocoumarin mixtures. A number of natural furocoumarins are phototoxic and the present method will allow quantitation of these toxic materials.

Materials and methods

The liquid chromatographs used were Waters Associates Analytical Model ALC-202 and Prep Model ALC/GPC 301 both with UV detection (254 nm). The recorder used was a Sargent-Welch Model TRG and the fractions were collected as they came off the liquid chromatograph (ALC/GPC 301) on Waters Associates automatic fraction collector. The columns used were 3 ft. × 2.1 mm I.D. and 3 ft. × 6.3 mm I.D. dry packed with Corasil I (single layer), particle size 37–50 μ (Waters Associates No. 27244).

Results

The furocoumarin mixture was obtained from an extraction carried out on spring parsley *(Cymopterus watsonii)*[6]. Fresh plant material** was forced air dried at 60° for 24 h and then ground to 40 mesh. Of the dried plant material, 600 g were extracted in methanol in a Soxhlet for 48 h. The methanol extract was dried to yield 118 g of solid material. This material was then dissolved in a 2:1 water–methanol mixture and extracted on a continuous liquid extractor with Skelly B (*n*-hexane) for 24 h to remove chlorophylls and other fatty materials[7]. The water–methanol mixture was concentrated and back extracted with ether on a continuous liquid extractor for 24 h to remove the furocoumarins. The ether extract was found upon drying to contain 2.7 g of crude furocoumarin mixture.

Of the different solvent systems used, the best separation was obtained using chloroform–cyclohexane mixtures. The above crude furocoumarin mixture was first dissolved in chloroform and then diluted with cyclohexane. The solution was then introduced into the injection port giving peaks shown in Fig. 1. Fractions were col-

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** Plant material obtained from M. C. WILLIAMS, Utah State University, Logan, Utah 85321, U.S.A.

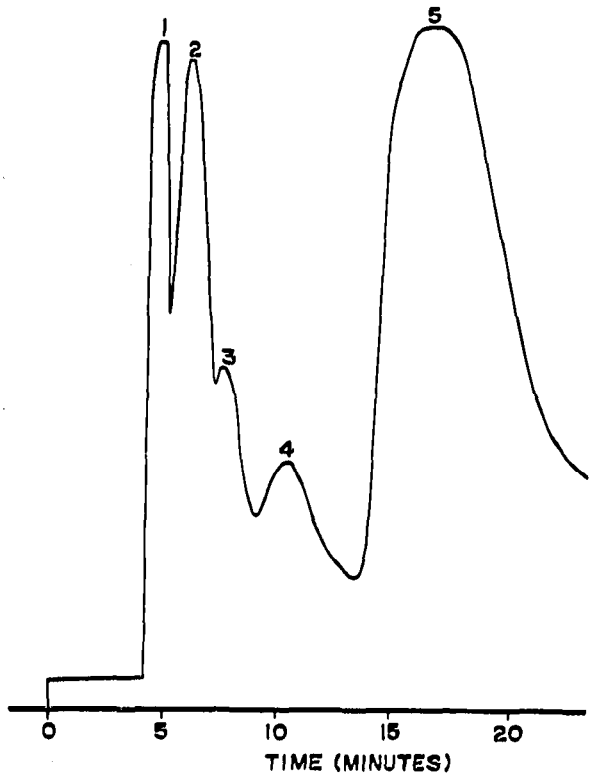

Fig. 1. HPLC analysis of crude furocoumarin mixture (30 μl of a trichloromethane–cyclohexane solution) from *Cymopterus watsonii*. 1 = Bergapten; 2 = xanthotoxin; 3 = isopimpinellin; 4 = unknown coumarin; 5 = unknown furocoumarin. Waters Prep Model ALC/GPC 301; UV detector; 700 p.s.i.; range 08; chart speed 0.2 in./min; mV span 50.

lected for each peak and dried. Each fraction was compared against authentic samples by retention time, UV spectrum, and TLC. Peak No. 1 was shown to be bergapten, peak No. 2 xanthotoxin and peak No. 3 was isopimpinellin. Peak Nos. 4 and 5 were unknown compounds giving UV absorbancies at λ_{max} (ethanol) 210, 237, 244, 260 (shoulder), 276, 290, and 325 (broad) nm, which suggests a coumarin without the furan ring for peak No. 4, and λ_{max} (ethanol) 220, 245, 262 (shoulder), and 300 (broad) nm, which suggests a xanthotoxin derivative for peak No. 5. We are presently isolating (by preparative HPLC) sufficient of peak No. 5 for structural investigation.

The results of this investigation indicate that HPLC can be an important tool in the separation of furocoumarin mixtures.

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