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Note

High-pressure liquid chromatography of naturally occurring anthraquinones

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Anthraquinones are a pharmaceutically important group of plant constituents. Many analytical methods have been reported for the separation of the naturally occurring anthraquinones chrysophanol, physcion, emodin and aloe emodin (for structures see Fig. 1) in plant drugs. The techniques applied include paper chromatography¹⁻⁷, thin-layer chromatography⁸⁻¹³, column chromatography¹⁴ and gas-liquid chromatography¹⁵. This communication describes a high-pressure liquid chromatographic procedure for the separation and identification of chrysophanol, physcion, emodin and aloe emodin by gradient elution. Rai and Turner¹⁶ have recently introduced this method for the detection of anthraquinones in plant material of various kinds.

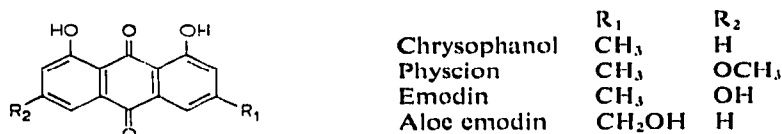


Fig. 1. Structures of anthraquinones involved.

EXPERIMENTAL

Liquid chromatographic separations were performed on a laboratory-assembled instrument equipped with a Model 6000 solvent delivery system from Waters Ass. The chromatograph is fitted with an ultraviolet detector (Cecil Instruments 272) operating at 280 nm. The column used comprised two stainless-steel tubes (3 ft. × $\frac{1}{8}$ in. O.D. each) packed with Corasil II silica gel (Waters). The exponential gradient was cyclohexane to ethyl acetate. 0.025% solutions in chloroform of chrysophanol, physcion, emodin and aloe emodin were prepared; 10- μ l samples were used for injection onto the column.

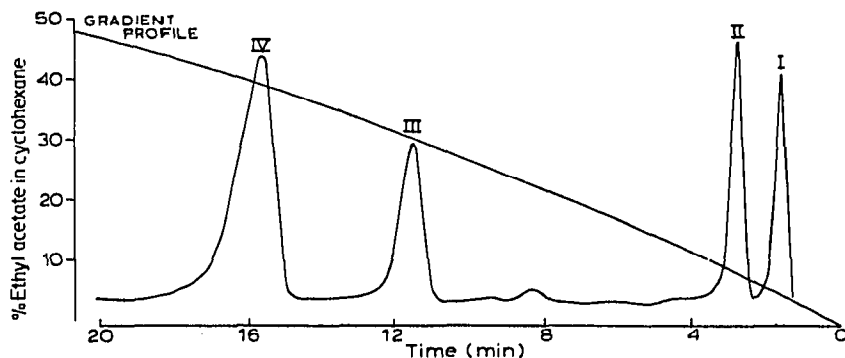


Fig. 2. High-pressure liquid chromatogram of anthraquinones. Conditions as in Experimental; temperature, ambient; flow-rate, 1.0 ml/min. I = Chrysophanol; II = physcion; III = emodin; IV = aloe emodin.

RESULTS AND DISCUSSION

Of several column packings and solvent mixtures investigated, Corasil Type II with gradient cyclohexane to ethyl acetate provided a superior resolution of anthraquinone mixtures. Retention times were directly related to polarity and used to identify the compounds. The retention times were: chrysophanol, 1.7 min; physcion, 3 min; emodin, 11.8 min; aloe emodin, 16 min (see Fig. 2). It is possible to separate all four compounds in less than 25 min. This represents a significant saving of time over previous methods. The technique enables one to monitor the separation of these closely related anthraquinones which generally occur together in purgative plant drugs. It can be applied to preparative separations, as well as to qualitative and quantitative analytical separations.

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