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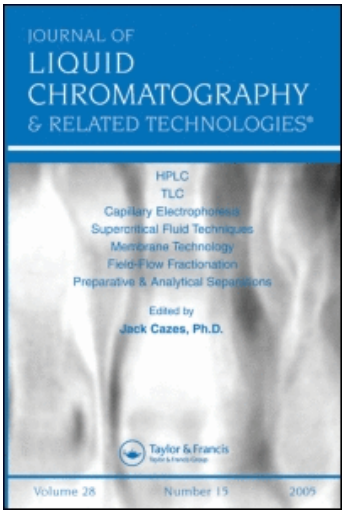

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PREPARATIVE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ISOLATION OF THE TRUE PROAZULENES FROM ARTEMISIA ARBORESCENS L.

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ABSTRACT

The use of the preparative medium-pressure liquid chromatography (prep-MPLC) and the preparative high-pressure liquid chromatography (prep-HPLC) techniques for the separation of the main true proazulenes from a purified extract of *Artemisia arborescens* L. are reported and discussed.

INTRODUCTION

The isolation of unstable substances from natural sources using conventional column chromatography is usually hard and often accompanied by extensive loss of material.The use of preparative medium-pressure liquid chromatography (prep-MPLC) and preparative high-pressure liquid chromatography (prep-HPLC),minimizing the contact between the stationary phase and the substances to be separated,proves very useful with this regard.

We report here an example dealing with the use we made of this techniques during the isolation of the true proazulenes from *Artemisia arborescens* L.

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Although this plant has been known for a long time to yield a dark blue oil (Jona,1914) due to the presence of chamazulene (Meisels and Weizmann,1953),the principles responsible for the formation of this compound during the conditions of hydro-distillation had never been isolated (Šorm and Doleiš,1965).

MATERIALS AND METHODS

Sample preparation

A purified chloroform extract of *A. arborescens* was obtained using a general method previously described (Nano *et al.*,1980).

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Pre-separation of the proazulenic fraction

A sample (10 g) of the purified chloroform extract of the plant was pre-separated by means of prep-MPLC into nine fractions using a Prep-LC/System 500 A Waters,equipped with two Prep PAK-500/Silica columns.As a mobile phase 6 l of a mixture of acetone:n-hexane (1:5) technical grade was used.The refractive index detector was set at x 20 relative response;the flow rate at 300 ml/min;chart speed 1 cm/min.The TLC control of the fractions obtained was performed on 5 x 7.5 cm,layer 0.2 mm,HPTLC-Alufolien Kieselgel 60 F 254 chromatoplates (Merck),using as eluent the system acetone:n-hexane (1:3.5) (1 ascent at 20 °C).The spots on HPTLC plates were detected by spraying with EP reagent (Stahl,1969).The HPLC control of the separated fractions was run on a Perkin-Elmer Series 3B liquid chromatograph;the eluted components were monitored with a LC-75 variable wavelength detector (160-600 nm) equipped with a LC-75 Autocontrol.The column used was a Hibar LiChrosorb RP 18 5 µm (Merck,12.5 x 0.4 cm I.D.).The separations reported were achieved under the following conditions: mobile phase 55% acetonitrile (LiChrosolv,Merck) in water purified by a MILLI-Q-System

(Millipore);flow rate,1.5 ml/min;temperature,28 °C;wavelength,242 and 209 nm;chart speed,0.5 cm/min. Graphs were generally obtained with an attenuation setting corresponding to 0.32 AUFS on a 10 mV Perkin-Elmer 561 recoder.

Lactone A

A crystalline compound (200 mg) was obtained from fraction n°6 by crystallization from EtOAc.

Lactone B

TLC analysis showed that fraction n°5 was a mixture of lactone A and another EP-positive compound.They were separated by prep-MPLC using the same conditions as above,and 60 mg of another crystalline compound were obtained.

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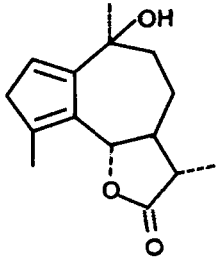
Separation of lactone A into its two constituents

200 mg of lactone A were separated by prep-HPLC using a Perkin-Elmer Series 3B liquid chromatograph,equipped with a LC-75 spectrophotometer detector and recorder unit.A high efficiency preparative liquid chromatography stainless steel column (Perkin-Elmer,25 x 1.6 cm I.D.) packed with LiChrosorb RP 18 10 µm (Merck) was used.The preparative separations were achieved under the following conditions:eluting solvent,55% acetonitrile (LiChrosolv,Merck) in water purified by a MILLI-Q-System (Millipore);flow rate,30 ml/min;temperature,25 °C;chart speed,0.5 cm/min;wavelength,209 nm. Five injections of 100 µl,in a normal volume loop (175 µl) were made.Graphs were obtained with an attenuation setting corresponding to 1.024 AUFS on a 10 mV recorder.

Identification of compounds

Compounds were identified according to their physical and spectral properties;full data will be published elsewhere.



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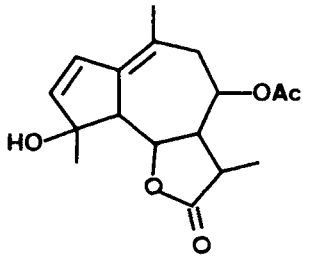

MATRICIN

STRUCTURE 1

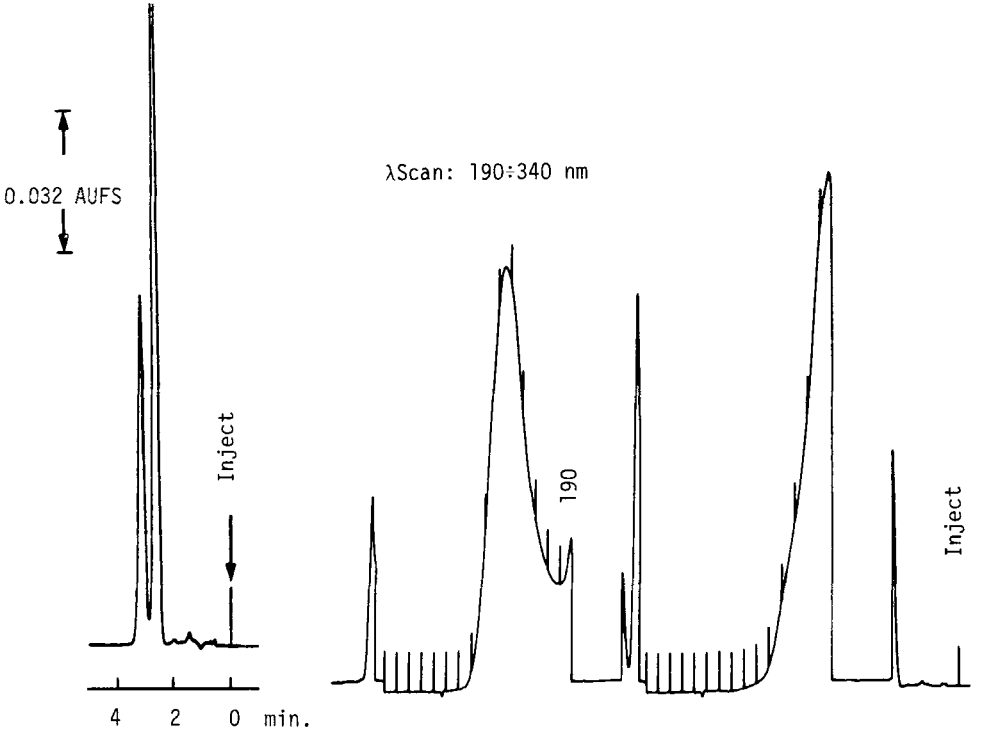

FIGURE 1

HPLC chromatograms illustrating the resolution of lactone A in two sequiterpene lactones and their U.V. spectra.
Column:Hibar LiChrosorb RP 18 5 µm (12.5 x 0.4 cm I.D.);mobile phase: acetonitrile:water (55:45 v/v);flow rate:1.5 ml/min;wavelength:209 nm; temperature:28 C;chart speed:0.5 cm/min.The U.V. spectra were performed in λ-SCAN MODE from 190 to 340 nm.

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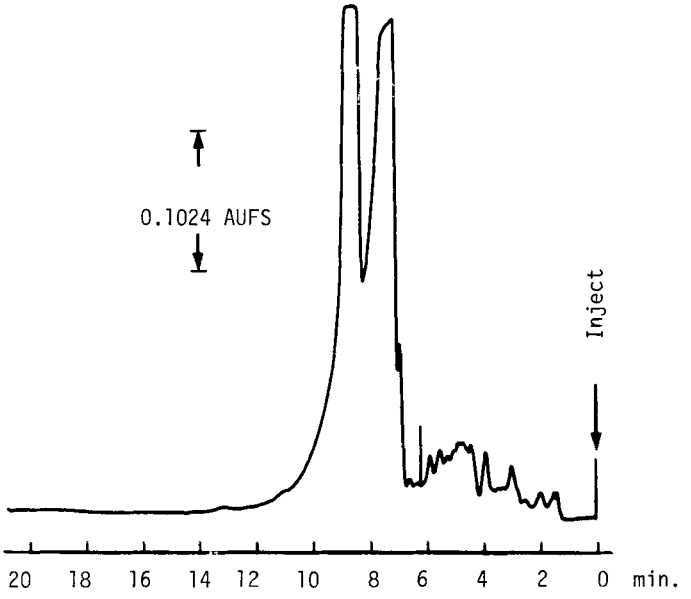

FIGURE 2

Preparative HPLC profile of the separation of lactone A into its two constituents.
Column:Perkin-Elmer (25 x 1.6 cm I.D.) packed with LiChrosorb RP 18 10 µm (Merck);mobile phase:acetonitrile:water (55:45 v/v);flow rate: 30 ml/min;wavelength:209 nm;temperature:25 C;chart speed:0.5 cm/min.

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RESULTS AND DISCUSSION

Using prep-MPLC two crystalline proazulenes were obtained from the plant extract:lactone B was identified as artabsin (Vokáč *et al.*,1969)while lactone A,corresponding to the most EP-positive spot of the extract , was shown by spectral analysis (^{1}NMR,MS) to be a practically equimolecular mixture of two sesquiterpene lactones.Lactone A was apparently unitary,with a definite m.p. and a single spot,turning blue in a few hours due to *in situ* decomposition,in every chromatographic system we tested.The U.V. spectrum of this compound showed,besides a higher absorbition at 242 nm,a lower peak at 209 nm.The HPLC analysis run at

this lower absorbition,gave a slight separation into two peaks;this separation was then optimized allowing us to obtain two baseline resolved peaks having distinctive U.V. spectra (Fig. 1).The analytical conditions of separation were transferred to preparative scale (prep-HPLC). Both components of the crystalline mixture had a relatively high extinction coefficient.As the selected wavelength was optimal for separation, and in order to prevent the U.V. detector overloading,it was decided to inject small aliquots (100 µl) of the sample mixture instead of injecting a higher volume sample and setting the U.V. detector to another wavelength.In this way we were able to obtain in a good purity two sesquiterpene lactones (Fig. 2);one of them was identified as matricin (Čekan *et al.*,1956),while the structure of the second compound as well as the stereochemistry of matricin will be the subject of a separate paper.

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