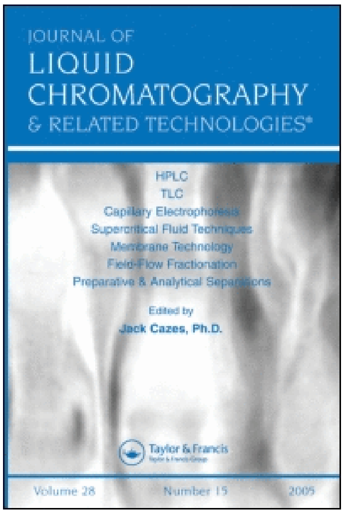

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PREPARATIVE HPLC SEPARATION OF EPIMERIC GUAIANOLIDES

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ABSTRACT

A technique for the preparative HPLC separation of the C-4 epimeric unstable guaianolides matricin and 4-epimatricin,proazulenes isolated from *Artemisia arborescens* L.,is reported.

INTRODUCTION

Mixtures of epimeric sesquiterpene lactones have been reported to occur in some plant (1).They are not separated by TLC under usual conditions (2), and their structural elucidation is generally accomplished by careful ^{1}H-NMR analysis of their crude mixture.

Although the separation of epimeric sesquiterpene lactones has sometimes been accomplished by fractional crystallization (3) and some isomeric sesquiterpene lactones have been separated after suitable derivatization (4,5),to our knowledge only few preparative chromatographic separations of epimeric sesquiterpene lactones have been reported (6).

During our work on the proazulenes from *Artemisia arborescens* L.,we have found that,responsible for the formation of chamazulene during the conditions of hydro-distillation of the plant,was a complex mixture of sesquiterpene

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lactones,whose main constituent was a HPLC-separable 1:1 adduct of matricin with a new sesquiterpene lactone on whose structure work is still in progress (7).

We would like to report here on the HPLC separation of matricin and its C-4 epimer,a new sesquiterpene lactone from this plant.Both compounds are highly unstable,particularly 4-epimatricin and,according to our experience, not separable on a preparative scale by other techniques.

MATERIALS AND METHODS

A crystalline mixture of matricin and 4-epimatricin was obtained from a purified chloroform extract using a technique we have previously described (8).Preliminary analytical HPLC investigation was carried out to establish the best preparative scale high-performance liquid chromatographic conditions. The value of k' and α of the two peaks of interest were determinated from the analytical separation.The maximum injection volume (V_L) and the maximum sample mass (C_{LC}) of the two compounds were calculated using the following chromatographic equations:

$$V_L = V_O\left[(\alpha - 1/R_S)k_1' - 2(2 + k_1' + \alpha k_1')/ N^{\frac{1}{2}}\right]$$

$$C_{LC} = \left[\frac{k' + 1}{k'}\right] \left[\frac{M_C \cdot M_W}{N}\right]$$

where:

M_C = molar capacity number

$N = 4R_S^2\left[2 + K_1'(\alpha + 1)\right]^2 / k'(\alpha - 1)^2$

V_L= injection volume

V_O= void volume

α = separation factor

k'= capacity factor

R_S= resolution between the peaks of interest

M_W= molecular weight of k'_1 solute in g/M

C_{LC}= maximum sample mass

The molar capacity number for the preparative column used was M_C= 0.075 as given from the supplier.A BASIC program for the calculation of scale-up factors for preparative LC columns (Technical Note LC-230,1981 - Perkin-Elmer, Norwalk,U.S.A.) was used.

500 mg of the two C-4 epimeric unstable guaianolides were separated by prep-HPLC using a Perkin-Elmer Series 3B (Perkin-Elmer,Norwalk,U.S.A.) liquid chromatograph equipped with a Rheodyne valve mod.7125 (Rheodyne,Berkeley, Calif.,U.S.A.) ,injection loop:10.3 ml .The eluted components were monitored with a LC-75 variable wavelength detector (190-600 nm) coupled with a LC-75 Autocontrol.A Perkin-Elmer/Preparative C18 10 µm column (25 x 3 cm I.D.) was used.The separations were achieved under the following conditions:mobile phase, 68% MetOH (LiChrosolv,Merck,Darmstadt,F.R.G.) in water purified by a Water I (Gelman-Science,Michigan,U.S.A.);flow rate,12.5 ml/min.;temperature,25 °C,wavelength 215 nm.Six injections of 9 ml of the sample solution in the mobile phase were made.Graphs were obtained with an attenuation setting corresponding to 1.024 AUFS on a Perkin-Elmer mod.561 recorder (10 mV).

RESULTS AND DISCUSSION

Preparative HPLC profile of the separation of the two C-4 epimeric guaianolides is given in Figure 1.Both components of the crystalline mixture had a relatively high extintion coefficient.The U.V. spectra of the two compounds,recorded during the analytical separation,showed a high absorbance at 236 nm (Figure 2) (matricin and 4-epimatricin had λ_{max}^{EtOH} 244 and Log ε 4.28 and 4.22 respectively).In order to prevent U.V. detector overloading it was decided to set the U.V. detector to another wavelength (215 nm) where the

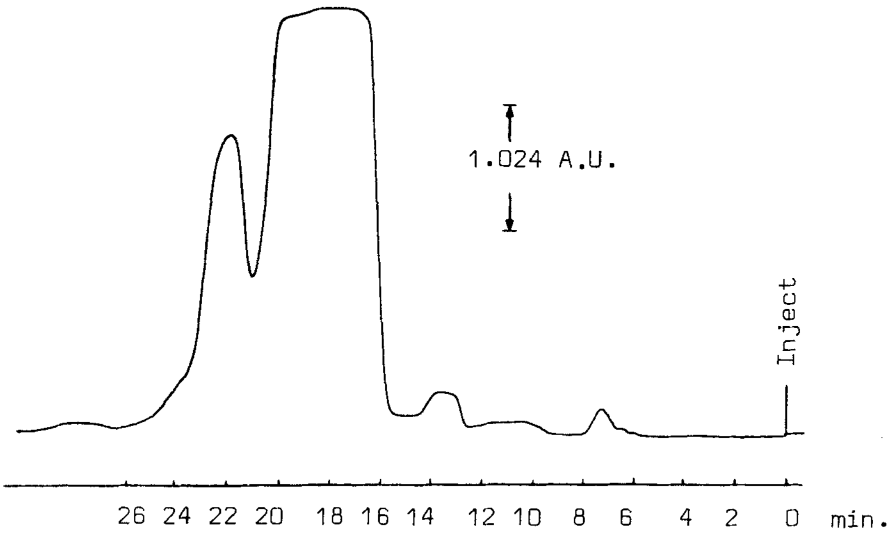


FIGURE 1

Preparative HPLC profile of the separation of matricin and 4-epimatricin Column:Perkin-Elmer/Preparative C18 10 µm (25 x 3 cm I.D.);mobile phase, MetOH:H_2O (68:32);flow rate,12.5 ml/min.;wavelength,215 nm;temperature, 25 C;chart speed,0.5 cm/min.

compounds had lower extinctions.By using the preparative HPLC technique described,we were able to separate,and obtain in good yield (70%),matricin and its C-4 epimer,a new compound for which m.p.,$(\alpha)_D^{21}$ and spectral (MS, U.V.,I.R.,^{1}H-NMR,^{13}C-NMR) data were recorded (9).

Comparision of the spectra of the two substances allowed us to ascertain their stereochemistry at the epimeric carbon C-4,and assign the stereostructures I to matricin and II to 4-epimatricin,substances of hitherto unknown stereochemistry. I.R. spectra of the two epimers are shown in Figure 3.

Both compounds are highly unstable and sensible to traces of acids,turning yellow on simple standing:their separation by conventional column chro-

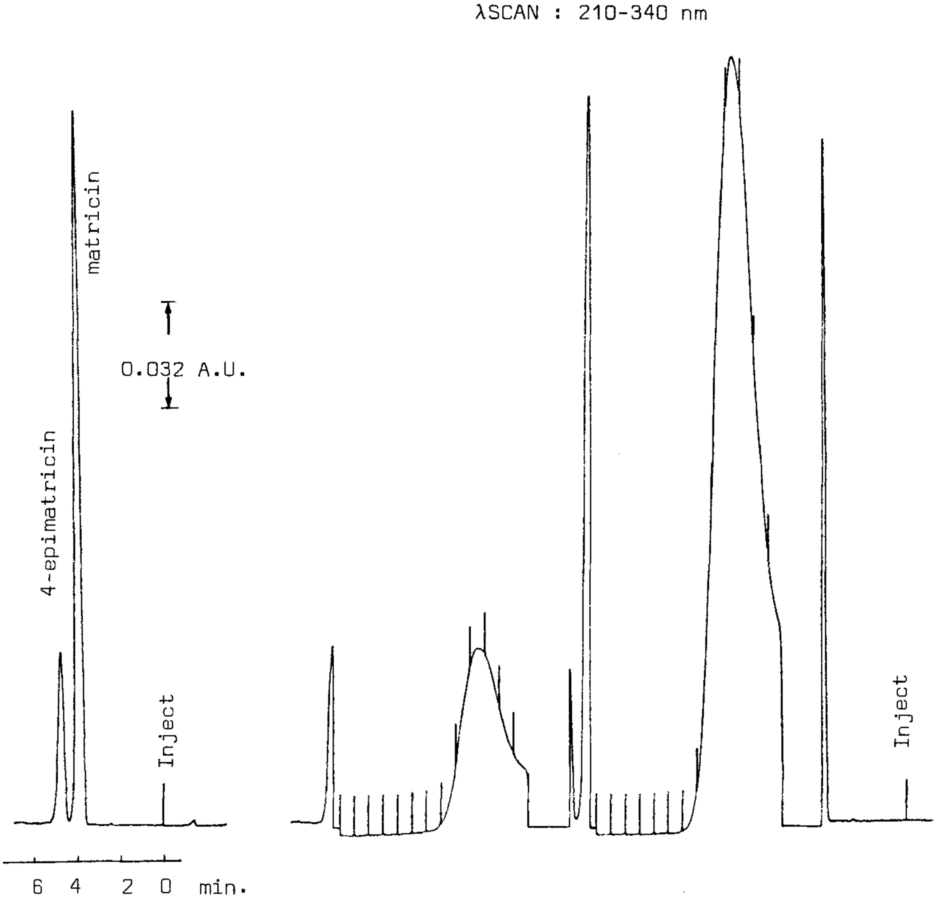
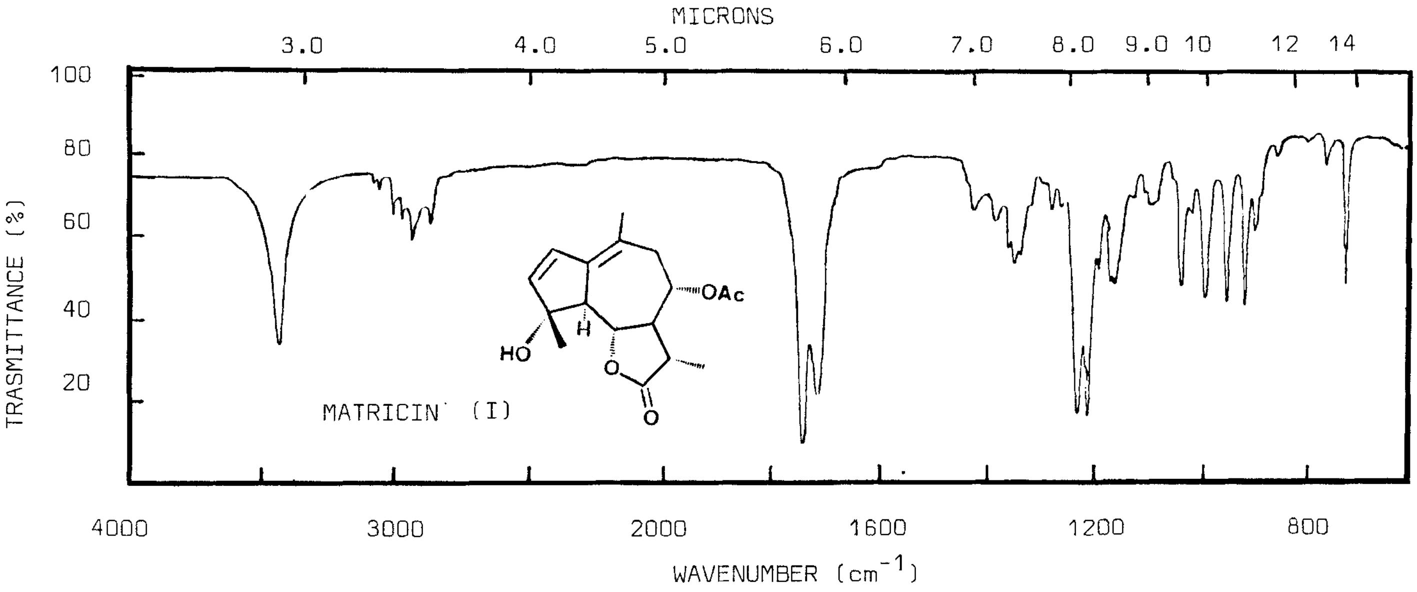


FIGURE 2

HPLC chromatograms illustrating the separation of matricin and 4-epimatricin and their U.V. spectra.
Column:Hibar-LiChrosorb RP 18 10 μm (25 x 0.4 cm I.D.);mobile phase,MetOH:H_2O (68:32);flow rate,1 ml/min;wavelength,244 nm;temperature 25 C;chart speed,0.5 cm/min. The U.V. spectra were recorded in λ-SCAN MODE from 210 to 340 nm.


MICRONS
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10
12
14
100
80
60
40
20
TRASMITTANCE (%)
MATRICIN (I)
HO
H
OAc
O
O
4000
3000
2000
1600
1200
800
WAVENUMBER (cm-1)

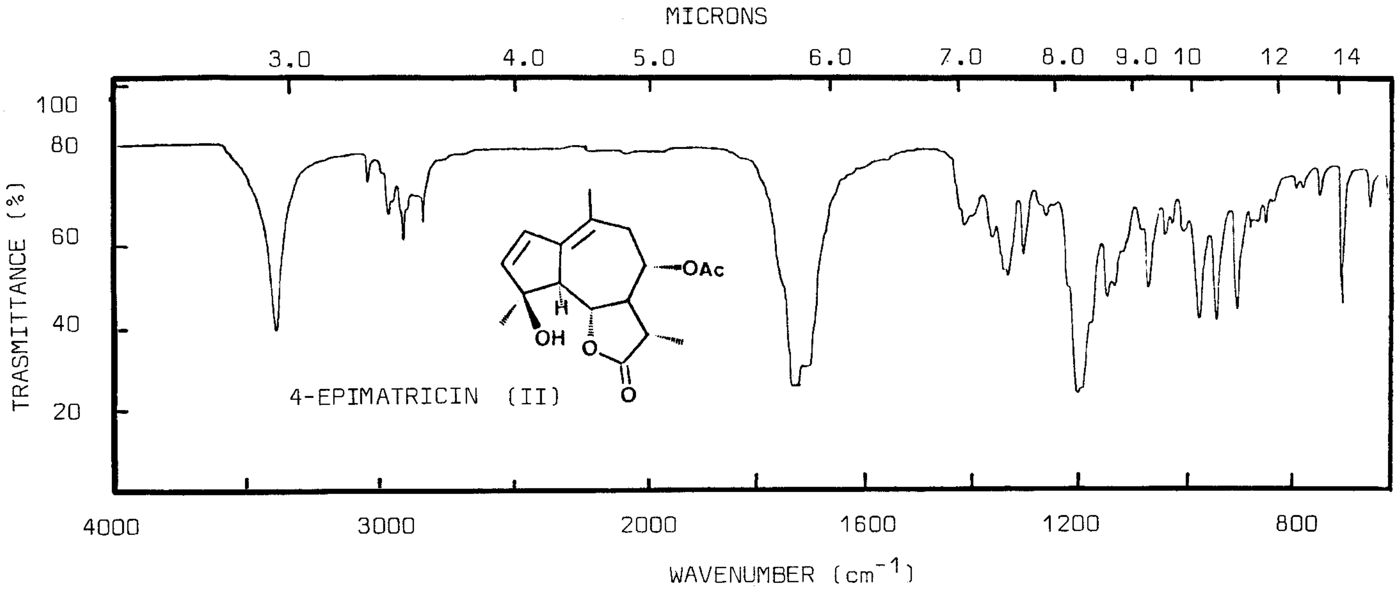


FIGURE 3

I.R. spectra of matricin (I) and 4-epimatricin (II) separated from preparative high-performance liquid chromatography.

matography,even if it had been possible,would have certainly been accompanied by extensive decomposition of them.

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