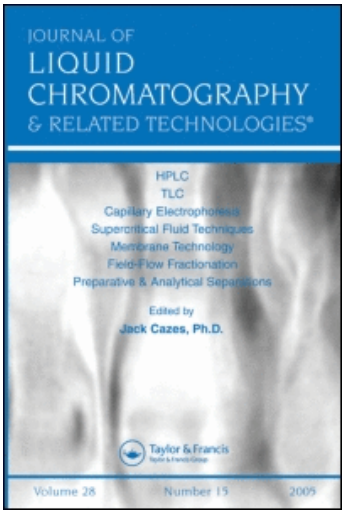

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A Simple Method for the Separation of Triterpene Carboxylic Acids

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A SIMPLE METHOD FOR THE SEPARATION OF TRITERPENE CARBOXYLIC ACIDS

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ABSTRACT

A polybutadiene-coated alumina column has been found to resolve a closely related mixture of naturally occurring triterpene carboxylic acids much more efficiently than conventional octadecylsilyl-bonded (C_{18}) silica columns. Further, this separation could be achieved without ion-pairing, ion-suppression or other buffering techniques.

INTRODUCTION

In connection with our investigation of the HIV-inhibitory and phorbol receptor binding activities of the organic extracts of the tropical plant Maprounea africana (1), we needed a semi-preparative separation strategy for a mixture of closely related triterpene carboxylic acids [**1-3**]. While a number of HPLC methods for separating carboxylic acids exist, most high resolution methods rely on ion-suppression or ion-pairing techniques to reduce tailing and improve resolution. Such techniques work well in qualitative or

quantitative analytical modes, but our need for careful measurement of mass recovery and biological activity, coupled with concerns about hydrolysis or rearrangement (from C-2 to C-3, or vice versa) of the p-hydroxybenzoyl ester substituent, led us to seek a non-buffered, one step separation of these triterpenes.

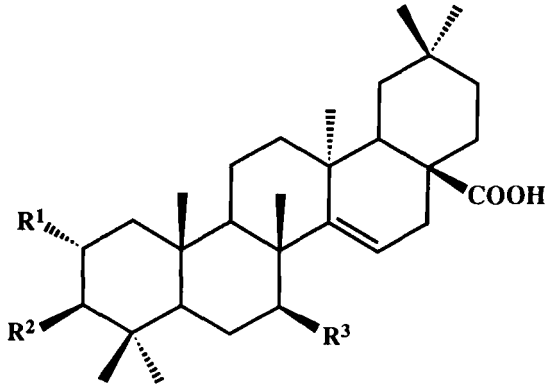
EXPERIMENTAL

General

All HPLC separations were performed on a Waters 990 HPLC system equipped with a diode array detector, using continuous UV monitoring at 210 and 254 nm. Semipreparative analyses utilized a Biotage Unisphere-PBD^R column (1 x 25 cm) at a flow rate of 3.5 mL/min and a C_{18}-bonded silica column (0.4 x 25 cm) at a flow rate of 1.0 mL/min. In either case, 100 μL of a 10 mg/mL solution of the analyte mixture was injected; the elution solvent in either case was CH_3CN-H_2O (11:9).

Triterpene Mixture

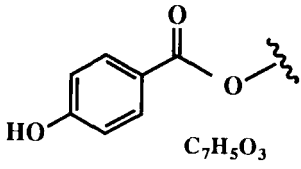
The mixture of **1-3** was obtained from extracts of Maprounea africana by following, with some modification, the procedure described by Wall's group (2). All fractions were assayed for HIV-inhibitory (3) and phorbol receptor binding (4) activity. The structures were determined by careful analysis of the mass spectral and $^1H/^{13}C$ NMR data for the three compounds and comparison to the literature (1,2,5). Full details of the chemical characterization of **1-3** will be more appropriately presented elsewhere (1).



1 $R^1=C_7H_5O_3$, R^2=OH, R^3=H

2 R^1=H, $R^2=C_7H_5O_3$, R^3=OH

3 R^1=OH, $R^2=C_7H_5O_3$, R^3=H



RESULTS AND DISCUSSION

An analysis of the mixture of **1-3** on an analytical C_{18} - reversed phase column is presented in Figure 1A. While compound **3** was reasonably well separated from the rest of the mixture, excessive tailing led to a long run time and minimized prospects for scale-up of the separation with any semblance of resolution. Further, triterpenes **1** and **2** were very poorly resolved and overlap with other impurities eluting at the solvent front.

In contrast, use of the polybutadiene coated alumina column provided good retention and very acceptable resolution for semi-preparative work (Figure 1B). Some tailing was still evident, but it was far less than that observed with the conventional C_{18} approach. The elution volume for **3** on this 1 cm column equaled that observed on the analytical C_{18} column. Fractions obtained by this procedure were characterized as **1-3** without further manipulation.

Triterpenes and, in particular, the triterpene carboxylic acids, are difficult to handle and separate because of poor

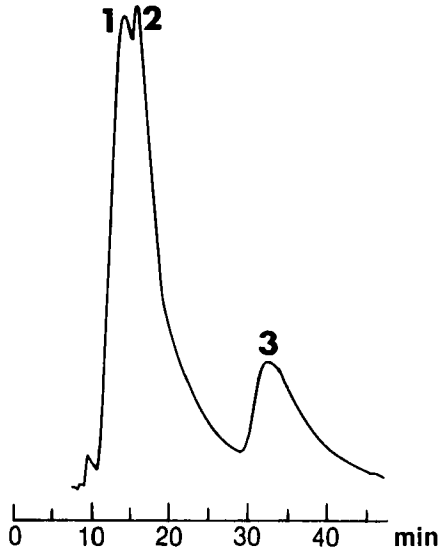


Figure 1A. C_{18}-bonded silica reversed phase HPLC analysis of triterpene mixture from Maprounea africana. Column 0.4 x 25 cm; eluant CH_3CN-H_2O (11:9) at 1.0 mL/min; detection UV @ 254 nm; injection 100 μL of 10 mg/mL solution.

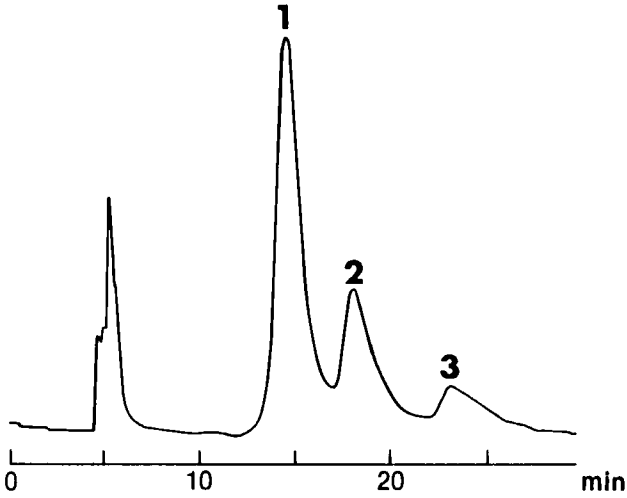


Figure 1B. Semipreparative HPLC analysis of Maprounea africana triterpene mixture. Column: Biotage Unisphere-PBD^R 1 x 25 cm; eluant CH_3CN-H_2O (11:9) at 3.5 mL/min; detection UV @ 254 nm; injection 100 μL of 10 mg/mL solution.

solubility characteristics. These problems are often exacerbated by the concentration of polar functionalities at limited positions in the molecules and the presence of large regions comprised of unfunctionalized hydrocarbon ring segments. The particular case at hand was further complicated by identical numbers and types of functional groups, the potential lability of the benzoyl ester and the presence of a second acidic group in the phenol. This new method for the resolution and semi-preparative separation of very closely related triterpene carboxylic acids should, therefore, provide a useful alternative for separation of this large and important class of compounds. Broader application of this method, to other classes of carboxylic acids, may also be possible.

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