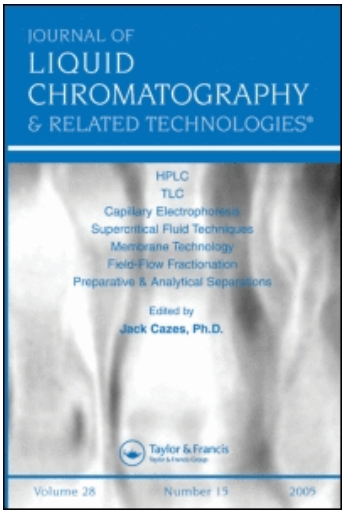

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SEPARATION AND PURIFICATION OF MELIACIN BUTENOLIDES FROM *TRICHILIA ESTIPULATA* BY NORMAL-PHASE HPLC

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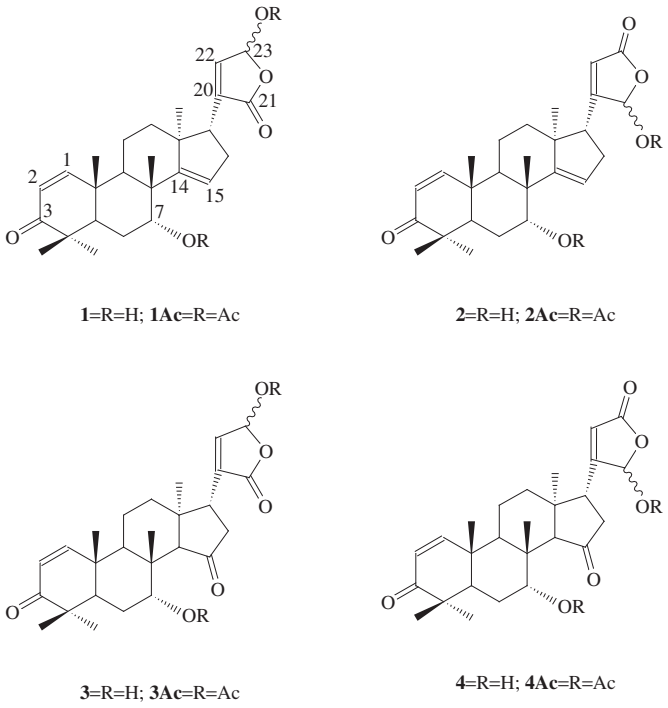
ABSTRACT

Meliacin butenolides mixture was isolated from the dichloromethane extract of the stem bark of *Trichilia estipulata* L. as their acetylated derivatives. The separation and purification of γ-hydroxy butenolides and their epimeric acetylated derivatives have been successfully performed by normal-phase high performance liquid chromatography.

INTRODUCTION

Meliacin butenolides are widely distributed in the families of the order Rutales (Meliaceae, Ruataceae and Cneoreceae) (1). Various limonoids of the tree have been found to have anti-tumor, antipyretic, antibacterial, antifungal, anti-inflammatory, and antilarval activity (2). Many γ-hydroxybutenolides have



1=R=H; **1Ac**=R=Ac

2=R=H; **2Ac**=R=Ac

3=R=H; **3Ac**=R=Ac

4=R=H; **4Ac**=R=Ac

Figure 1. Structures of γ-hydroxybutenolides.

been isolated as epimeric mixtures, which are indicated by the appearance of double signals at C-23 and C-21 (3–5).

This paper presents the results of the application of HPLC to the separation and purification of γ-hydroxy butenolides (Fig. 1) and their epimeric compounds, obtained after acetylation of the mixture isolated from the stem bark of *Trichilia estipulata.*

EXPERIMENTAL

A Waters/Millipore high performance liquid chromatograph was used. The instrument was equipped with a model 510 pump and a UV Lambda-Max model 481 detector set at 254 nm. A semipreparative Hypersil silica column (25 $\times$ 7 mm ID, 5 μm particle size), packed as usual, was used. The mobile phase was delivered at a constant flow rate of 2 mLmin^{-1}.

Dichloromethane-hexane-CH_3CN (45:50:5 and 55:35:10 v/v) was used as mobile-phase. The solvents used were all HPLC grade from Merck (Darmstadt, Germany).

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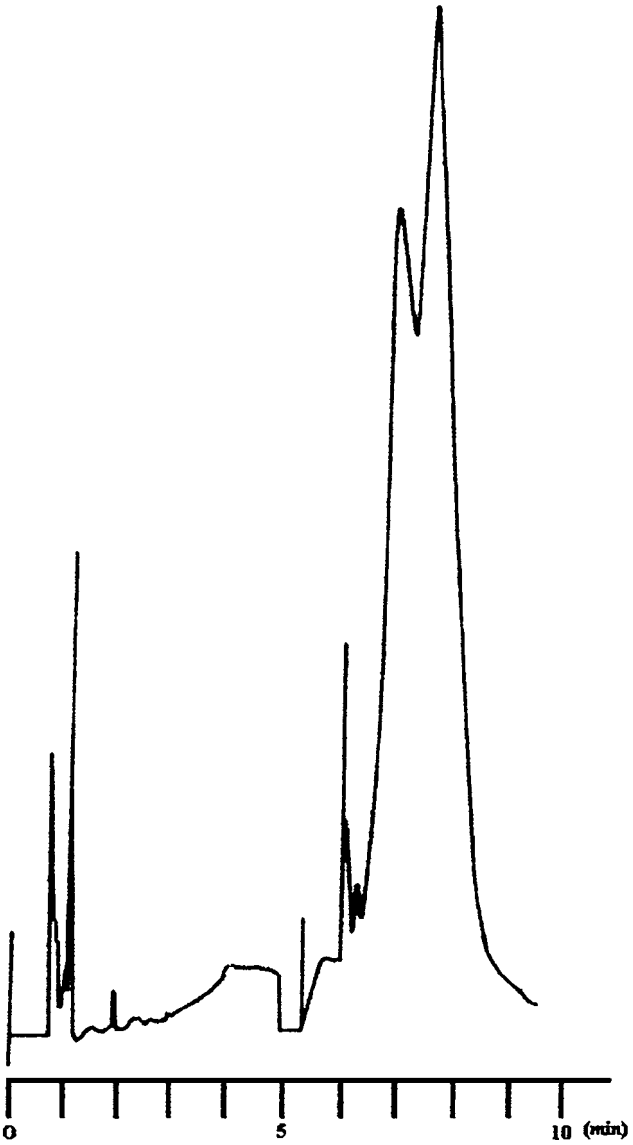

Figure 2. Chromatogram of fraction **A** (compounds **1** and **2**), obtained by HPLC. Column: Hypersil silica (5 μm), 250 $\times$ 7 mm. Eluent: CH_2Cl_2 with a flow rate 2.0 mL min^{-1} at room temperature. Concentration of the fraction **A**: 5 mg/mLx (methanol). Injection: 50 μL.

Two fractions **A** (28 mg) and **B** (3 mg) were obtained from the dichloromethane extract of the stem bark of *Trichilia estipulata* L, as described elsewhere (3).

The following instrumentation was used to record the spectra described here: uv, Hitachi V-3210; ir, Perkin-Elmer735; nmr, Bruker ARX. The nmr spectra were recorded in δ values using TMS as an internal standard. Mass low resolution spectra were obtained on a HP-2576 spectrometer.

RESULTS AND DISCUSSION

The HPLC analysis of fraction **A** and **B** (Fig. 3) showed a mixture of isomeric compounds identified by ^{1}H NMR and ^{13}C NMR spectra as limonoids

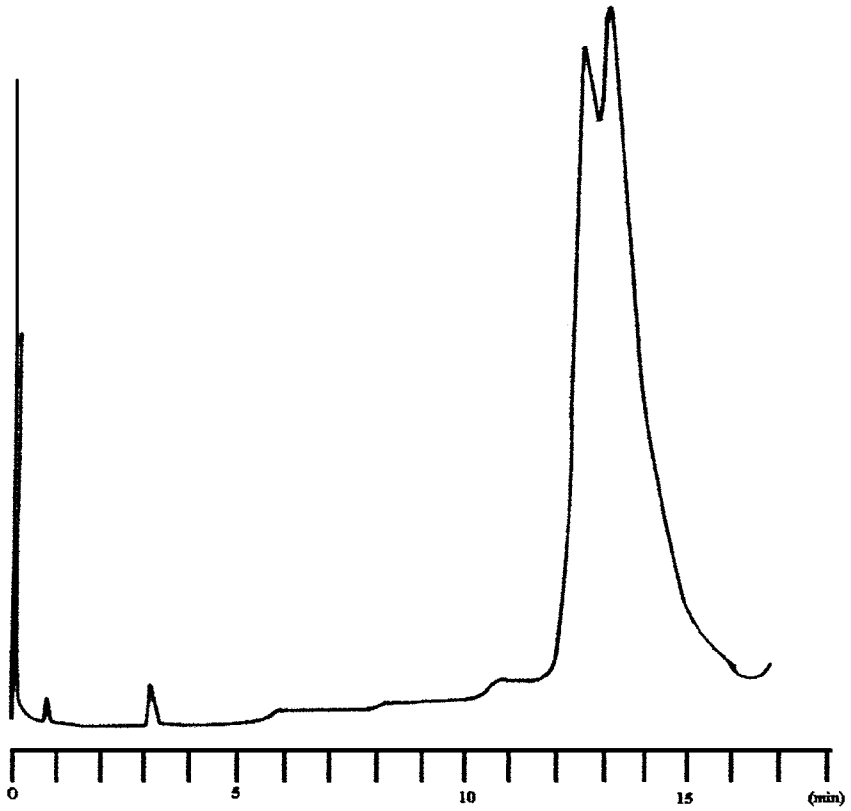

Figure 3. Chromatogram of fraction **B** (compounds **2** and **3**), obtained by HPLC. Column: Hypersil silica (5 μm), 250 $\times$ 7 mm. Eluent: CH_2Cl_2:Hexane:CH_3CN (70:30:10) with a flow rate 2.0 mL min^{-1} at room temperature. Concentration of the fraction **B**: 5 mg/mL (methanol). Injection: 50 μL.

γ-hydroxybutyrolactone. The presence of double signals for the hemiacetal carbons at C-21 and C-23 indicated them to be mixtures of epimeric compounds (6).

Attempts to isolate the epimeric compounds by HPLC were not successful, even when reversed-phase conditions where employed. The fractions **A** and **B** were allowed to react overnight with excess acetic anidride in pyridine.

The acetylated fraction **A** was then submitted to semi-preparative HPLC using dichloromethane:hexane:acetonitrile (45:50:5 v/v) as mobile phase in a Hypersil silica column (5 μ, 120 Å, 250 $\times$ 7 mm). Multiple injections were carried out in order to maintain the best resolution (Fig. 4). Compound **1Ac** (2 mg) was isolated as an epimeric mixture.

This compound was identified as 7α,23-Diacetoxy-3-oxo-24,25,26,27-tetranorapotirucall-1,14,20(22)-trien-21,23-olide (6). Attempts to isolate its epimer were not successful.

The compounds **2Ac(A)** (3.4 mg) and its epimer **2Ac(B)** (0.9 mg) were also isolated and identified as 7α,21-diacetoxy-3-oxo-24,25,26,27-tetranorapotirucall-1, 14,20 (22)-trien-21,23-olide (7).

The above compounds showed similarity (Table 1 and 2) to **1Ac**. The main difference consists in the position of the acetyl group in the acetoxybutyrolactone; in **1Ac** t it is at C-23, while in **2AcA** and **2AcB** is at C21.

The semipreparative Hypersil silica column (5 μ, 120 Å, 250 $\times$ 7 mm) was also used for the isolation of the acetylated fraction **B;** this time dichloromethane:

Table 1. ^{1}H NMR Data of Compounds **1Ac**, **2Ac**(**A** and **B**), **3Ac**(**A** and **B**) and **4Ac**(**A** and **B**) ($CDCl_3$, 200 MHz)

H	**1Ac**	**2Ac(A)**	**2Ac(B)**	**3Ac(A)**	**3Ac(B)**	**4Ac(A)**	**4Ac(B)**
1	7.13 (d 10)	7.09 (d 10)	7.14 (d 10)	7.15 (d 10)	7.13 (d 10)	7.12 (d 10)	7.11 (d 10)
2	5.38 (d 10)	5.84 (d 10)	5.87 (d 10)	5.88 (d 10)	5.88 (d 10)	5.89 (d 10)	5.89 (d 10)
7	5.34 (m)	5.37 (m)	5.36 (m)	4.93 (m)	6.93 (m)	3.88 (m)	4.93 (m)
14				2.46 (s)	2.45 (s)	2.46 (s)	2.46 (s)
15	5.24 (m)	5.23 (m)	5.24 (m)				
17				3.60 (t)	3.64 (m)	3.50 (m)	3.33 (m)
21		6.85 (br s)	6.86 (br s)			6.90 (br s)	7.04 (br s)
22	6.94 (m)	5.99 (br s)	6.05 (br s)	6.95 (br s)	6.99 (br s)	6.13 (br s)	6.03 (br s)
23	6.88 (m)			6.94 (br s)	6.95 (br s)		
Ac(Me)							
7	1.94 (s)	1.94 (s)	1.98 (s)	2.10 (s)	2.09 (s)	2.10 (s)	2.09 (s)
21		2.15 (s)	2.19 (s)			2.20 (s)	2.24 (s)
23	2.14 (s)			2.24 (s)	2.23 (s)		
Me							
				0.89 (s)	0.87 (s)	0.89 (s)	0.87 (s)
	1.18 (s)	1.19 (s)	1.18 (s)	1.14 (s)	1.14 (s)	1.10 (s)	1.11 (s)
	1.17 (s)	1.15 (s)	1.16 (s)	1.05 (s)	1.05 (s)	1.05 (s)	1.05 (s)
	1.05 (s)	1.05 (s)	1.08 (s)	1.07 (s)	1.07 (s)	1.06 (s)	1.06 (s)
	0.90 (s)	0.91 (s)	1.02 (s)	1.17 (s)	1.17 (s)	1.12 (s)	1.12 (s)

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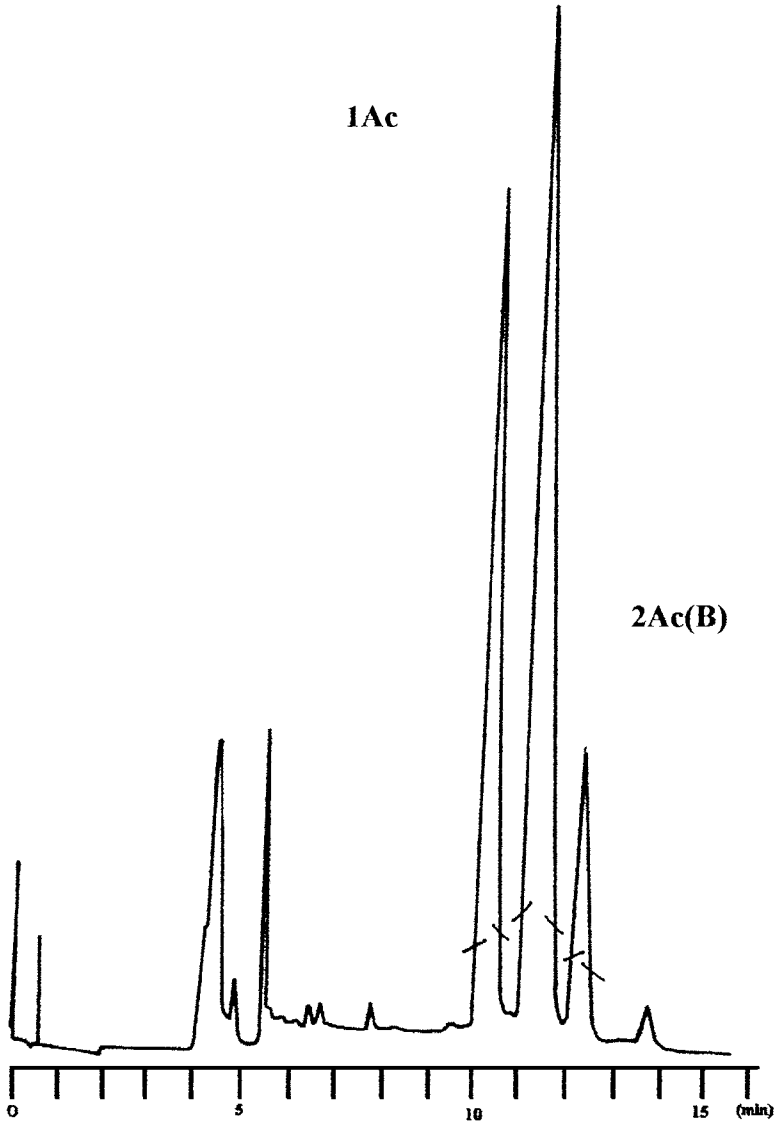

Figure 4. Chromatogram of compounds **1Ac**, **2Ac**(**A**) and **2Ac**(**B**) obtained by HPLC. Column: Hypersil silica (5 μm), 250 $\times$ 7 mm. Eluent: CH_2Cl_2:Hexane:CH_3CN (45:50:5) with a flow rate 2.0 mL min^{-1} at room temperature. Concentration of the acetylated fraction **A**: 5 mg/mL (dichloromethane). Injection: 50 μL.

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Table 2. ^{13}C NMR Data of Compounds **1Ac**, **2Ac**(**A** and **B**) ($CDCl_3$, 50 MHz)

C	**1Ac**	**2Ac**(**A**)	**2Ac**(**B**)
1	158.0	157.5	157.5
2	125.5	125.7	125.7
3	204.5	204.3	204.3
4	44.1	44.1	44.1
5	38.3	38.3	38.3
6	23.4	24.0	24.0
7	74.4	74.2	74.2
8	46.1	46.1	46.1
9	47.5	46.1	46.1
10	39.9	39.8	39.9
11	16.4	16.4	16.5
12	39.2	39.8	39.8
13	47.5	47.5	47.5
14	158.2	58.2	158.3
15	118.3	118.3	118.5
16	33.9	33.9	33.2
17	50.6	52.8	53.5
18	19.0	13.6	13.6
19	20.7	19.0	19.0
20	138.4	166.6	166.0
21	171.0	93.2	94.5
22	143.3	120.1	120.7
23	92.3; 92.4	169.7	169.9
28	21.4	21.2	21.2
29	27.5	27.0	27.0
30	27.0	27.4	27.5
OAc	170.0	170.0	170.0
	169.0	169.0	169.0
Ac(Me)	21.3	21.2	21.3
	21.0		

Multiplicity obtained by DEPT or J-MOD experiments.

hexane:acetonitrile (55:35:10 v/v) was used as mobile phase. Compounds **3Ac**(**A**) (0.6 mg), **3Ac(B)** (0.5 mg), **4Ac(A)** (0.3 mg), and **4Ac(B)** (0.5 mg) were, thus, isolated. Figure 5 is a typical chromatogram obtained during the separation.

The spectral data (Table 1) of compounds **3Ac** (**A** and **B**) showed that they have rings A-C identical with the epimeric compounds **4Ac**(**A** and **B**) also isolated,

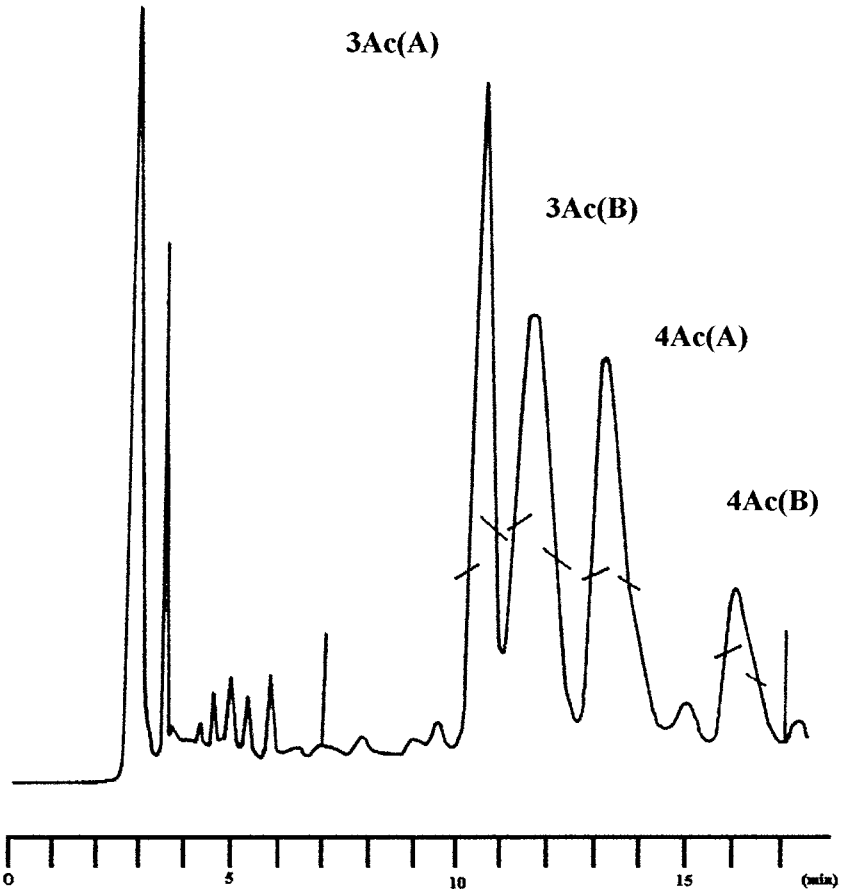

Figure 5. Chromatogram of compounds **3Ac(A), 3Ac(B), 4Ac(A)** and **4Ac(B)** obtained by HPLC. Column: Hypersil silica (5 μm), 250 $\times$ 7 mm. Eluent: CH_2Cl_2:Hexane:CH_3CN (55:35:10) with a flow rate 2.0 mL min^{-1} at room temperature. Concentration of the acetylated fraction **B**: 5 mg/mL (dichloromethane). Injection: 50 μL.

and they differ only in the position of the carbonyl group at C-23, for compounds **3Ac**(**A** and **B**) identified as 23-Acetoxyneotrichilenonelide, and at C-21 for compounds **4Ac**(**A** and **B**) which were identified as 21- Acetoxyneotrichilenonelide (6).

When performing preparative separation there is always a compromise in respect to throughout and purity; since the goal is to isolate very pure compounds, the HPLC separations were carried out under the column capacity for the best resolution. The isolation of the pure compounds was possible by collecting the top of peaks and re-injecting the fractions that were still as a mixture.

CONCLUSION

After acetylation, the appropriate adjustment of the mobile phase composition gave the possibility of optimizing the chromatographic behavior of the compounds studied, and made possible the isolation of these complex mixture of closely related limonoids isomers.

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