Dinorditerpenoids From *Ricinodendron Heudelotii*[*] (Euphorbiaceae)

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ABSTRACT

A new dinorditerpenoid, heudelotenol 1 along with twelve known compounds namely octancosan-1-ol, E-ferulic acid n-octacosylate, stigmasterol, campesterol, â-sitosterol, â-sitosterol glycoside, taraxerone, friedelin, aleuritolic acid, heudelotinone, 1,2-dihydroheudelotinol and labda-8(17),13-dien-3â,15-diol were isolated from *Ricinodendron heudelotii*. The structure of heudelotenol 1 was established by spectral analysis and chemical transformations.

Key words: *Ricinodendrn heudolotii;* Euphorbiaceae; dinorditerpenoids

RÉSUMÉ

Heudelotenol 1, un nouveau dinorditerpène et douze autres composés connus : octancosan-1-ol, E-ferulate de n-octacosyle, stigmasterol, campesterol, â-sitosterol, glucoside de â-sitosterol, taraxerone, friedeline, acide aleuritolique, heudelotinone, 1,2-dihydroheudelotinol et labda-8(17),13-diène-3â,15-diol ont été isolés du *Ricinodendron heudelotii*. La structure de heudelotenol 1 a été établie à partir des données spectroscopiques et des transformations chimiques.

Mots clés : *Ricinodendrn heudolotii*; Euphorbiacée; dinorditerpènes

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[*] Part 2 in the series Studies on *Ricinodendron heudelotii*

INTRODUCTION

Earlier studies on this plant (KIMBU *et al*, 1991) resulted in the isolation of two new dinorditerpenoids heudelotinone **3** and 1,2-dihydroheudelotinol **4** and three known compounds E-ferulic acid octacosylate, 3-methylorselinate and lupeol. In continuation of our research work, an extract of this plant collected from Muyuka, South West Province, Cameroon has yielded the known compounds mentioned above together with a new dinorditerpenoid. Only one of the dinorditerpenoids heudelotinone **3** which was identified earlier in the plant collected from Yaounde was found in the extract of the plant collected from Muyuka (Momeni et al, 2005). This paper describes the results of our investigation.

EXPERIMENTAL

All melting points were determined on a Büchi melting point apparatus model 510 and are uncorrected. UV spectra were measured in methanol solutions using the Anthorpc Version 4.1 H spectrophotometer. Mass spectra were recorded using MAT 312 or MAT 8200 instruments attached to a MSCAN computer. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker AMX 400 instrument (^{1}H, 400 MHZ, ^{13}C 75.5 MHZ) using tetramethylsilane as internal reference in $CDCl_3$ or CD_3OD or C_5D_5N where appropriate. Chemical shifts are given in δ values.
2-D NMR, ^{1}H-^{1}H COSY, HMBC, HMQC and NOESY experiments were performed with usual pulse sequences and data processing was obtained with adequate software.

Plant Material

The stem bark of *Ricinodendron heudelotii* was collected in Muyuka, South West Province, in August 1997. Herbarium specimens documenting the collection are deposited at the National Herbarium, Yaounde, Cameroon.

Extraction and Isolation of Compounds

Dried powdered stem bark of *R. heudelotii* (7.7 kg) was extracted with a mixture methylene chloride/methanol (1:1) at room temperature. The methylene chloride/methanol extract was filtered and evaporated under reduced pressure in a rotatory evaporator to obtain 175 g of a crude extract.
Dried leaves of *R. heudelotii* (290 g) were extracted with a mixture of methylene chloride/methanol (1:1) at room temperature. The methylene chloride/methanol extract was filtered and evaporated under reduced pressure in a rotatory evaporator to yield 30 g of a crude extract. Dried powdered roots of *R. heudelotii* (2.0 kg) were extracted with a mixture of methylene chloride/methanol (1:1) at room temperature. The methylene chloride/methanol extract was filtered and evaporated under reduced pressure in a rotatory evaporator to obtain 20 g of a crude extract. Comparison of this extract by thin layer chromatography (TLC) with one obtained from the stem bark, showed that they were identical and therefore combined.

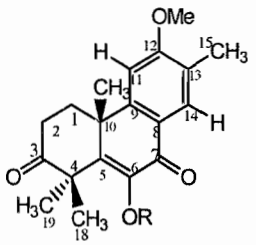
The plant extracts obtained above were separated into different fractions A, B, C, D, E by flash chromatography. These fractions were then chromatographed over silica gel columns and fractions which could not be separated on columns of silica gel were further purified by preparative TLC over Merck kieselgel GF 254. Spots on analytic TLC plates were observed under uv light and by spraying with concentrated sulphuric acid. Eluents for column chromatography were hexane and increasing percentages of ethyl acetate in hexane. TLC plates were developed using appropriate concentrations of ethyl acetate in hexane. Thus the chromatographic separation of the mixture of compounds in fraction C afforded octacosan-1-ol (90 mg), E-ferulic acid octacosylate (450 mg), a mixture of sterols, stigmasterol, campesterol, and β-sitosterol (400 mg). Fraction D afforded heudelotenol (120 mg); fraction E heudelotinone (12 mg); fraction F aleuritolic acid (90 mg) and fraction G β-sitosterol glycoside (400 mg).

Chromatographic separation of the crude extract from the leaves from Muyuka over silica gel and elution with hexane followed by hexane-ethyl acetate afforded friedelin (10 mg) and taraxerone (10 mg). The known compounds isolated were identified by comparison (mp, ^{1}H, ^{13}C NMR) with published data.

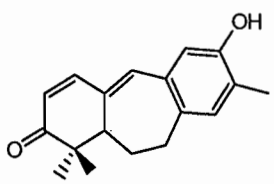
Heudelotenol **1**
White crystalline solid (120 mg); mp: 207-209 °C; $C_{19}H_{22}O_4$; IE m/z (rel. int. %) 314 $[M]^+$ (98), 271 (49); 258 (100); 244 (58); 243 (58); 231 (58); 230 (88); 215 (54); 202 (50); 115 (56); 83 (39); 77 (35); 51 (29). IR (KBr, ν_{max}) cm^{-1}: 3400 (OH); 1712 (α, β- unsaturated C=O); 1691 (simple C=O); 1655, 1601, 1522 (aromatic C=C), 1500; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (75.5 MHz, $CDCl_3$) see Tables 1 and 2

Table 1: ^{1}H NMR (400 MHz, $CDCl_3$) Assignments or compounds (**1**) and (**2**)

Compound **1**	δ_H	J (Hz)	Compound **2**	δ_H	J (Hz)
O–H	7.12 ; s	-	O–CO–C<u>H</u>$_3$	2,36 ; s	-
H–1ax	2.07; dt	13.7; 9.9	H–1ax	2.14	13.9; 9.5
H–1eq	2.46; ddd	13.7; 9.3; 1.5	H–1eq	2.50	13.7; 9.3
H–2ax	2.76; ddd	18.8; 9.8; 8.0	H–2ax	2.80; dd	18.8; 9.8
H–2eq	2.76; ddd	18.8; 9.8; 2.2	H–2eq	2.80; dd	18.8; 9.8
H–11	6.81; s	-	H–11	6.77; s	-
H–14	7.94; s	-	H–14	7.91; s	-
H–15	2.23; s	-	H–15	2.22; s	-
H–18	1.52; s	-	H–18	1.40; s	-
H–19	1.55; s	-	H–19	1.40; s	-
H–20	1.28; s	-	H–20	0.82; s	-
O–C<u>H</u>$_3$	3.90; s	-	O–C<u>H</u>$_3$	3.89; s	-



(**1**) R = H
(**2**) R = Ac



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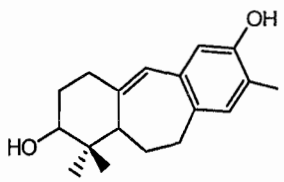
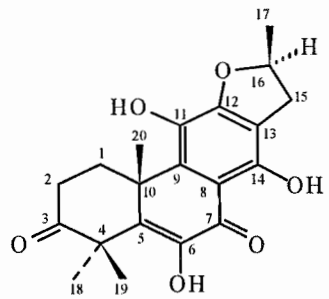


(**4**)



(**5**)

Table 2: ^{13}C NMR (75.5 MHz, $CDCl_3$) Assignments for compounds **1**, **2** and **5**)

Attribution	Compound **1**	Compound **2**	Compound **5**
1	32.7 t	32.9 t	27.05 t
2	33.0 t	37.8 t	33.08 t
3	214.3 s	195.4 s	214.50 s
4	48.5 s	48.4 s	48.7 s
5	142.3 s	142.2 s	140.0 s
6	151.0 s	149.1 s	140.1 s
7	179.5 s	176.8 s	182.9 s
8	120.5 s	122.3 s	107.2 s
9	137.1 s	137.1 s	135.2 s
10	39.1 s	41.0 s	40.6 s
11	106.1 d	105.7 d	131.2 s
12	162.2 s	162.1 s	153.8 s
13	127.0 s	129.2 s	111.5 s
14	128.8 d	150.9 d	154.8 s
15	26.2 q	26.2 q	34.4 t
16	-	-	83.5 d
17	-	-	22.0 q
18	24.4 q	25.7 q	24.4 q
19	20.9 q	21.0 q	21.1 q
20	15.8 q	15.6 q	20.1 q
O–C<u>H</u>$_3$	55.6 q	55.6 q	-
C<u>H</u>$_3$ C =O	-	168.6 s	-

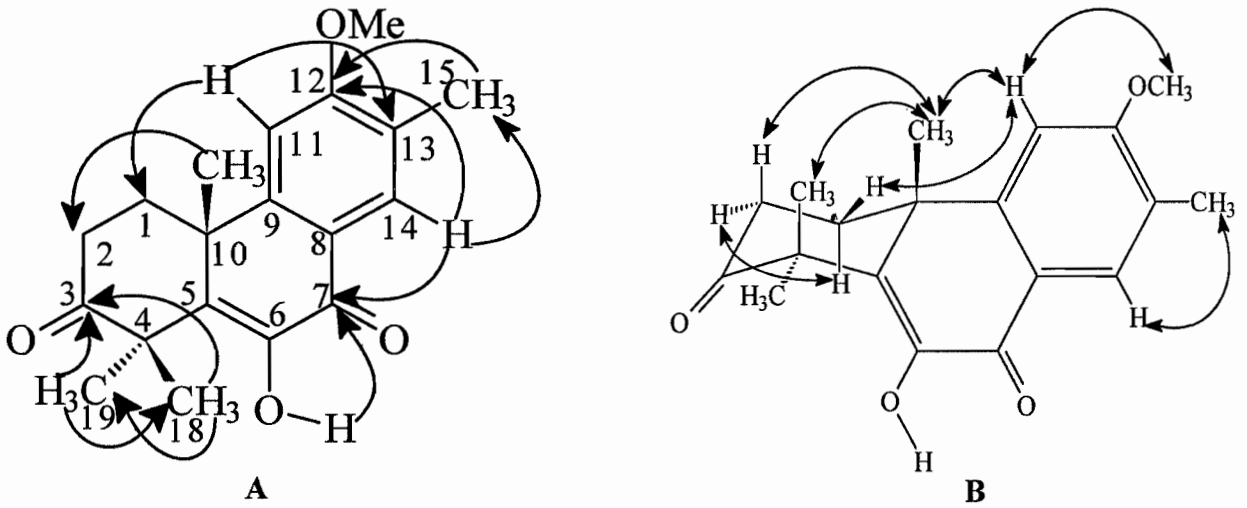


Fig. 1: Pertinent correlations observed with heudelotenol (1) (A); HMBC correlations (Htoc) (B) NOESY correlations.

Heudelotenol Acetate **2**

Compound **1** (25 mg) in pyridine (5 ml) and acetic anhydride were allowed to stand at room temperature for 24 h. Methanol (25 ml) was added to the reaction mixture and the solution obtained passed over activated alumina. The resulting solution was evaporated to dryness under reduced pressure in rotatory evaporator. The acetate (20 mg) was obtained by crystallisation in a mixture of hexane/ ethyl acetate (3:2).

RESULTS AND DISCUSSION

Compound **1** was isolated as a white crystalline solid, mp. 207-209°C, from the methylene chloride/ methanol extract of the dried powdered stem bark/roots as described in section 3. Its molecular formula $C_{19}H_{22}O_4$ was deduced from microanalysis EIMS and NMR data. It showed IR absorption bands for an hydroxyl group at ν_{max} 3400 cm^{-1}; an α, β- unsaturated carbonyl at 1712 and a simple carbonyl function at 1691 cm^{-1}. The ^1H NMR spectrum of compound **1**, revealed the presence of three tertiary methyl groups [δ_H 1.52 (3H, s); 1.55 (3H, s) and 1.28 (3H, s)], two aromatic protons [δ_H 7.94 (1H, s) and 6.81 (1H, s)], one aromatic methyl group [δ_H 2.23 (3H, s)] and one methoxy group [δ_H 3.90 (3H, s)]. The ^{13}C NMR and DEPT spectra of compound **1** showed two carbonyl carbons at δ_C 214.3 and 179.5; eight sp^2 carbons at δ_C 106.1, 120.0, 127.0, 128.8, 137.1, 142.3, 151.0 and 162.2. Other signals observed include two methylenes, two methines and two quaternary carbons (Table 2).

Thus compound **1** is a tricyclic dinorterpenoid including an aromatic ring, two carbonyl groups and an additional tetrasubstituted double bond. The appearance of only two aromatic protons signals in the ^1H NMR spectrum suggest that the benzene ring is tetrasubstituted bearing a methyl at $_H$ 2.23 and one methoxy group at δ_H 3.90 . The location of the different groups on the tricyclic skeleton which is similar to that of teuvincenone A **5** (CARRIERAS *et al*, 1990.) was accomplished by HMBC and NOE correlations. Differences between the two compounds were the absence of two hydroxyl groups on aromatic ring, C-16 and C-17. The ^1H NMR spectrum showed the presence of two adjacent groups [δ_H 2.01 (dt 13.7, 9.9 Hz); 2.46 (ddd 13.7, 9.3; 1.5 Hz); 2.76 (ddd 18.8, 9.0, 8.0 Hz); 2.76 (ddd 18.8, 9.8, 2.2 Hz)] (Table 1) one of which is α to the carbonyl group. Analysis of the HMBC spectrum (summarized in Fig. 1A) showed how various parts were fixed together and pertinent correlations were observed on the spectrum between protons H-14 and C-7, C-12, C-15; H-11 and C-9, C-13, C-1; H-20 and C-2, C-5 and between H-19 and C-18, C-3. These long range couplings in the HMBC spectrum enabled the assignment of the structure of the new compound as **1** and this was further confirmed by correlations observed in the NOE's (summarized in Fig. 1B) and the fragmentations observed in the mass spectrum (Fig. 2).

Acetylation of heudelotenol **1** with acetic anhydride in pyridine gave a white crystalline compound

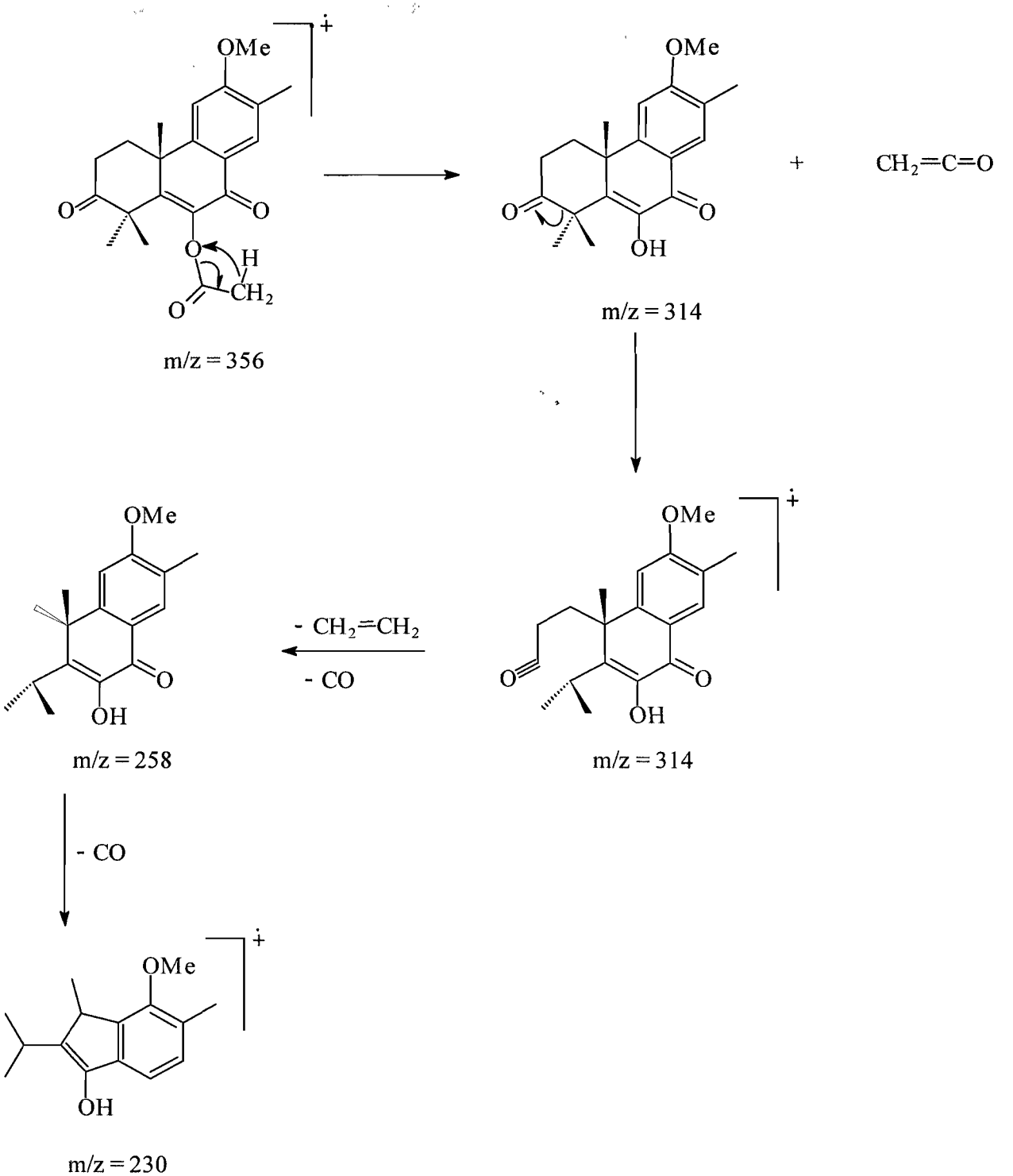


Fig. 2: Principal fragmentations of heudelotenol acetate (2)

which gave M^+ m/z 356 corresponding to the molecular formula $C_{21}H_{24}O_5$. Conspicuously absent in the IR spectrum of compound **2** was the absorption band at 3400 cm^{-1} for the hydroxyl group. Comparison of the ^1H NMR spectroscopic properties of the acetylated product (**2**) and (**1**) (Table 1) showed much similarity the main difference being the absence of the signal at δ_H 7.10 for the hydroxyl group and the presence of the additional signal at δ_H 2.36 for the acetyl group. Thus acetylation transformed the hydroxyl group giving compound **2**.

ACKNOWLEGEMENT
One of us, S. F. K. would like to thank the DAAD Foundation for a research fellowship at the University of Dortmund, Germany, that enabled us to carry out spectroscopic analyses of some of the constituents.

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Received: 16/06/2005
Accepted: 28/08/2006