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Research Article

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Two new compounds from stem barks of *Vepris heterophylla* (Engl.) R. Let. (Rutaceae)

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ABSTRACT

*4-hydroxycinnamate ester namedn-triacontyl-4-hydroxy cinnamate (**1**) and long chain alkanoic ester named n-triacontylpropanoate (**2**), together with 4 known compounds were isolated from the stem bark of Vepris heterophylla. The structures of the two compounds were determined by comprehensive analyses of their 1D and 2D NMR, mass spectral (EI and ESI) data, chemical reactions, and comparison with previously known analogues.*

INTRODUCTION

Vepris heterophylla (Engl.) Letouzey(vernacular names Kinkéliba de kita, Kinkéliba de Boulouli, Kinkéliba des pères, Kinkéliba des roches (French); Kounikoutchoum (Guiziga); Hohoum (Zoulgo); Gougouvetche (Mafa); Kotokolhi (Fulfuldé))[1,2,3] is a tree of 2 – 4 m high, found in Mali, Ghana, Nigeria and Cameroon. This plant is commonly used in traditional medicine in the Far North and North region of Cameroon to treat malaria, rheumatism,cardio-vascular disorders, hypertension and for parasitic diseases [3,4]. The leaves are used traditionally to protect crop, as diuretic, as antipyretic, to treat conjunctivitis, and to reduce high blood pressure [5].Previous phytochemical study of *V. heterophylla* reveals the presence of several furoquinoline alkaloids, maculine, skimmianine, kokusaginine, flindersiamine, evolatine, tecleine and tecleaverdoornine [5,6], kokusaginine and montrifoline[7], flavonoids, coumarines, tritepenoids, steroids and fatty acid esters[8,9].The essential oil obtained from the leaves of *V. heterophylla* contained about 35 compounds, mainly alkaloids, triterpenes and flavonoids. The number and quantity of these compounds differ with the age, freshness and the origin of the plants [10,11,12].The methanol extract of the leaves exhibited high free radical scavenging activities [11]; and the antimicrobial activities [13].Widespread traditional medicinal use and significant biological activities of compounds investigated so far justified continuous investigation of *V. heterophylla*. This paper reports the isolation and structure elucidation of two new esters.

EXPERIMENTAL SECTION

Generals

NMR spectra (1H, ^{13}C, COSYqf45, HSQC, HMBC, and DEPT135) were recorded on a Brùker AV500 spectrometer (500MHz for ^{1}H and 125MHz for ^{13}C) in $CDCl_3$ and TMS as an internal reference. Chemical Shifts were given in parts per million (ppm) and coupling constants in hertz. ESI-MS (ionization voltage 3kV) were recorded with a Q-TOF Ultima spectrometer (Waters). Analytical TLC was performed on aluminium sheets precoated with Si gel F_{254} (20 × 20 cm, 0.2 mm thick; Merck. 1.05735). After development, the dried plates were examined under short-wave (254 nm) or long-wave (365 nm) UV light and sprayed with sulfuric acid 50 % followed by heating at 105°C.

Column Chromatography (CC) was performed on silica gel normal phase 60 (Merck, 63-200 μm) with step gradient of n-hexane-EtOAc as eluent. All solvents used were analytical grade (Merck).

Plant material

The stem barck of *V. heterophylla* was collected in June 2012 in the mountainous massifs of Kaliao (latitude 10°61.508′N, longitude 014°20.1220′E, 437 m altitude) in the Far North Region of Cameroon. The botanical identification was done at the National Herbarium in Yaounde (Cameroon) by referring to the sample number (UICN) EN A1c, B1+2C. The locality of Kaliao is located in the dry savanna where the average annual rainfall reaches 1002 mm. This savannah region has annual average humidity of 73% and an average temperature of 29°C (IRAD, 2007). In this locality, peasants are mostly farmers, and *V. heterophylla* is known there as a very important post harvest botanical insecticide.

Extraction and isolation of compounds

The stem barks of the plant patch were collected, taken to the laboratory and dried at room temperature for about two weeks and powdered to coarse particles.

600 g of powder of stem bark were macerated then percolated with 2.5 L of EtOAc for 4 hours. After decantation and filtration, the solvent was removed under reduced pressure using a rotary evaporator (BÜCHI) (45° C) to yield a paste of 93.5 g (15.58%). The operation was repeated three times with the solvent. The crude EtOAc extract (25 g) of bark was further fractionated by column chromatography on 200 g Merck LichroPrep Si 60 with Hexane-EtOAc mixtures of increasing polarity. Hundred and twenty-two fractions of 150 ml each were collected and pooled to seven major fractions (**A-G**) according to their TLC profile using the mixtures of Hex-EtOAc and Hex/ EtOAc /MeOH as eluent. Compound **1** (4 mg) and **2** (3 mg) crystallized from fraction **D** collected from the column with the mixture 80:20. After washing by hexane, decantation and filtration, we obtained two white powders soluble both in chloroform.

n-tritriacontyl-4-hydroxycinnamate (1)

White powder; ESI-TOF MS *m/z*: 739.5 ([M+3K-4H]$^+$) (calcd. 584.5 for $C_{42}H_{74}O_3$); 711,5; 591,4 ; 381,3. ^{1}H NMR and ^{13}C NMR data see table 1.

triacontylpropanoate (2)

White powder; ESI-TOF MS *m/z* 494.1 [M]$^+$, 495.1 [M+H]$^+$and 496.1 [M+2H]$^+$ (calcd 494.5 for $C_{33}H_{66}O_2$); 354,6; 312,2.^{1}H NMR and ^{13}C NMR data see table 2.

RESULTS AND DISCUSSION

The air-dried, powdered stem barks of *V. heterophylla* were extracted with ethyl acetate. Successive column chromatography of the extracts over silica gel led to the isolation of six constituents including two new esters.

Compound **1**was obtained as a white amorphous powder. It responded positively to the ferric chloride test, indicating its phenolic nature. The molecular formula, $C_{42}H_{74}O_3$ with five degrees of unsaturation, was determined by HR TOF ESIMS, which showed the pseudo molecular ion peak [M+3K-4H]$^+$ at *m/z*739,5. The UV spectrum exhibited several absorption maxima at 312, 291, 225, and at 217 nm. The presence of hydroxyl and ester fonctions was indicated by two IR bands at 3300 and 1670 cm^{-1}respectively.

The ^{1}H NMR spectrum of **1** (Table 1) showed an AB system of two olefinic protons at δ_H 6.30 (*d*, *J* = 18.5 Hz, 1H, H-8) and 7.62 (*d*, *J* = 18.5 Hz, 1H, H-7), an AA'BB' system of four aromatic protons at δ_H 7.43 (*d*, *J*=9.5 Hz, H-3/5) 6.84 (*d*,*J*=9.5Hz, H-2/6), and a free hydroxyl group at δ_H 8.90 (*brs*, 4-OH) exchangeable with D_2O. The large coupling constant (*J* = 18.5 Hz) between H-7 and H-8 indicated a *trans* configuration at the double bond. All these signals suggested the presence of a 4-hydroxycinnamoyl moiety. This inference was supported by the ^{13} C NMR and DEPT data, which showed characteristic signals of a *p*-disubstituted benzene ring at δ= 115.4 (C-3, C-5), 128.2 (C-1), 129.7 (C-2, C-6) and 155.7 (C-4), and the CH2CH2O unit at δ = 65.0 (C-1) and 34.2 (C-2)

Furthermore, the ^{1}H NMR spectrum

A signal at δ_H3.90 as a singlet which integrated for one proton is attributed to an aromatic hydroxyl proton (4-OH). The deshielded nature of the olefinic protons reveals that they are conjugated to an aromatic system.H-7 was significantly more deshielded than the other olefinic H-8; this is probably due to the ring current of the aromatic π electrons. So, **1** is a 1,4-disubstituted benzene with a *trans* olefin conjugated to an ester carbonyl and a hydroxyl group. Compound **1** is therefore a long chain ester of cinnamic acid. The ^{1}H-NMR spectrum also presented a long

chain of fatty alcohol[14]. The most shielded region of the spectrum revealed a signal of three proton as a triplet at δ_H 0.88 which was attributed to a methyl group; methylene protons of long chain fatty alcohol at δ_H1.29-2.35;and theoxymethylene protons at δ_H 4.20 (*t, J*=7.2 Hz). The high chemical shift of these oxymethylene protons suggested that this fatty alcohol was esterified to the 4-hydroxycinnamate.

The ^{13}C and DEPT NMR spectra of compound **1** (Table 1) gave signals following for carbons: a conjugated carbonyl carbon of an ester at *δc* 168.0; conjugated olefinic carbons resonating at *δc* 115.9 and 144.0 attributed to C-2, C-3; protonated aromatic carbons at *δc* 131.0, 115.1, 115.8, 131 respectively for C-5, C-6, C-8, and C-9; non-protonated aromatic carbons appeared at *δc*128.9(C-4) and 158.0 (C-7), this last chemical shift of aromatic carbon suggested that it is bonded to the hydroxyl group; an oxymethylene carbon at *δc* 64.7; methylene carbons at *δc*22.0–31.9; and a methyl carbon at *δc* 14.1. Some of the methylene carbon signals at *δc*22.0–31.9wereoverlapping and supported the presence of fatty alcohols[14,15] in this compound as deduced from the ^{1}H NMR spectrum. The chemical shift of the carbonyl carbon of the ester at *δc* 168.0.confirmed its conjugation to a double bond as suggested in ^{1}H NMR discussion. The complete attribution of the NMR signals (Table 1) was based on the correlations observed in the HMBC, and COSY contour plots.

The COSY spectra revealed five spin systems associated with the cinnamoyl moiety and the side chain. For the cinnamoyl moiety the spectra showed correlations between the aromatic protons at δ_H 7.43 (H-5, H-9) and 6.84 (H-6, H-8); and the olefinicprotons at δ_H 7.62 (H-3) and 6.30(H-2) respectively coupled to each other. In the side chain moiety of compound **1** we have couplage between the Theoxymethylene protons at δ_H 4.20 (2H, *t, J* = 6.8) and the methylene protons at δ_H 1.52, which were in its turn coupled to another set of methylene protons at δ_H 1.30, which were finally coupled to the methyl at δ_H 0.88.Protons directly attached to carbons were assigned (Table 1) from HSQC 2D NMR data and the structure of compound **1** was supported by analysis of the HMBC 2D NMR data. HMBC correlations confirm the cinnamate moiety and the position of the functionality. Thus, Long-range correlations were observed between the protons at δ_H 0.88 (3H-33′) and the carbons at *δc*22.6 (C-31′) and *δc* 34.4 (C-32′)of the long side chain. The oxymethylene protons at δ_H 4.20 (2H, *t, J* = 6.8)correlated with carbons at *δc* 168.0 (C-1), 31.9 (C-2′) and 29.6 (C-3′).This supported the spin systems of the side long chain portion deduced from COSY. On the basis of HMBC correlation between the proton at δ_H 7.62 (H-3) and the carbonyl at *δc* 168 (C-1), the C-1–C-2 bond was identified as the connection point between the 4-hydroxycinnamate moiety and the long chain fatty alcohol. HMBC spectra also displayed correlations between H-3 and a carbon at *δc* 131.0 (probably C-9), protons at δ_H 7.43 (H-3; H-5) and carbons at *δc*128.9, 144.0 and 158.0, protons at δ_H 6.84(H-2; H-6) and 6.30 (H-8) with carbons at *δc* 128.9 (C-3; C-5). Accordingly, compound 1 was assigned as n-tritriacontyl-4-hydroxy cinnamate.

Table 1. NMR Spectroscopic data for compound 1in $CDCl_3$[a]

N°	δ_H (*J* in Hz)	$\delta_C$type	HMBC	COSY
1	/	128.9, C	/	
2	7.43, *d* (*J*=9.5Hz)	131.0, CH	C-4 ;C-1 ; C-7	H-3
3	6.84, *d*(*J*=9.5Hz)	115.1, CH	C-1	H-2
4	/	158.0, C	/	/
5	6.84, *d*(*J*=9.5Hz)	115.8, CH	C-1	H-6
6	7.43, *d*(*J*=9.5Hz)	131.0, CH	C-4 ; C-1 ; C-7	H-5
7	7.62,*d* (*J*=18.5Hz)	144.0, CH	C-9 ;C-2	/
8	6.30,*d* (*J*=18.5Hz)	115.9, CH	C-1	/
9	/	168.0, C	/	/
1′	4.20, *t* (*J*=7.2 Hz)	64.7, CH_2	C-9 ; C-2′ ; C-3′	H-2′
2′- 32′	1.29 – 2.35	22.0 – 31.9, CH_2	/	/
33′	0.88, *t* (*J*=6.8 Hz)	14.2, CH_3	C-31′; C-32′	/

[a]*Spectra were recorder at 500 MHz for ^{1}H and 125 MHz for ^{13}C.*

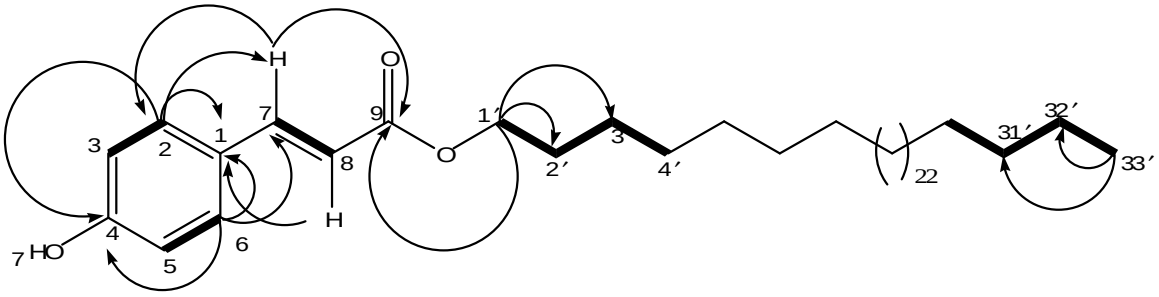

Figure 1:Connectivities deduced by COSY spectra (bold lines) and significant HMBC correlations (solid arrows) of compound (1)

Compound **2**(4 mg) was also obtained as a white powder soluble in chloroform and that crystallized in a Hexane/EtOAc (8:2). The ESI-TOFMSspectrumof this compound showed a molecular ion $[M]^+$at *m/z* 494.1, and

pseudo-molecular ions peaks $[M+H]^+$ at *m/z* 495.1 and $[M+2H]^+$ at *m/z* 496.1 in agreement with the molecular formula $C_{33}H_{66}O_2$. This was supported by ^{13}C-NMR spectrum as 30 carbon resonances were observed.The ^{1}H-NMR spectrum of **2** showed, moreover, the appearance of a broad signal atδ_H 1.30 attributable to the hydrocarbon chain $(CH_2)_n$. The same spectrum revealed a signal atδ_H 4.05 (2H, *t*) due to the 2H-1′deshielded by the ester function fixed on the same carbon C-1′, a signal at δ_H2.30 (2H, *t*, H-2) attributable to the methylene in *α*of the carbonyl group, two triplets at δ_H 0.88 (3H, *t*, H-n') and at δ_H 1.60 (3H, *t*, H-3) corresponding to the terminal methyl group of the hydrocarbon chain for the first, and the second probably deshielded due to the proximity of the ester function.

The ^{13}C NMR spectral data indicated that compound **2** contained 33 carbons, including two methyls, thirty methylene and one quaternary carbon.the spectrum showed a signal at δ_C 173.0 ppm attributable to the carbonyl ester function C-1, a signal at δ_C 64.4ppm due to the resonance of the methylene carbon C-2 and a signal at δ_C 14.2 ppm corresponding to the CH_3-n′ of the hydrocarbon chain.This spectrum showed the appearance of a signal atδ_C34.4ppm (C-2′) and a signal atδ_C 64.4 ppm (C-1′) of the fatty acid chain [15].The COSY spectrum revealed one spin system associated with the long chain. Thus, the methyl at δ_H 0.88 coupled with a proton at δ_H 1.30 which was in its turn coupled to another set of methylene protons at δ_H 1.59. We also had correlation between a methylene at δ_H 2.30 and the methyl at δ_H 1.60, the oxymethylene protons at δ_H 4.05 (2H, *t*) and the methylene protons at about δ_H 1.57. The structure of the side chain was established *inter alia* by the HMBC correlations from 2H-1′ (δ_H 4.05) to C-1 (*δc* 173), C-2′ (*δc*34.4), C-3′ (δ_C25.9) and 2H-2(δ_H 2.30) to C- (173) and C-3 (22.7). Detailed analysis of spectral data led to identification of **2** as triacontylpropanoate.

Table 2. NMR Spectroscopic data for compound 2 in $CDCl_3$[a]

N°	δ_H (*J* in Hz)	δ_C type	HMBC	COSY
1	/	173.0, C	/	/
2	2.30 *q*,	29.7, CH_2	C-1, C-3	H-3
3	1.60*t*,	22.7, CH_3	/	H-2
1′	4.05*t*,	64.4, CH_2	C-1, C-2′, C-3′	H-2′
2′	1.57 *m*	34.4, CH_2	/	H-1′, H-3′
3′-29′	1.29*m*,	25.0-34.4, CH_2	/	/
30′	0.88*t*,	14.1, CH_3	C-28′, C-29′	H-28′, H-29′

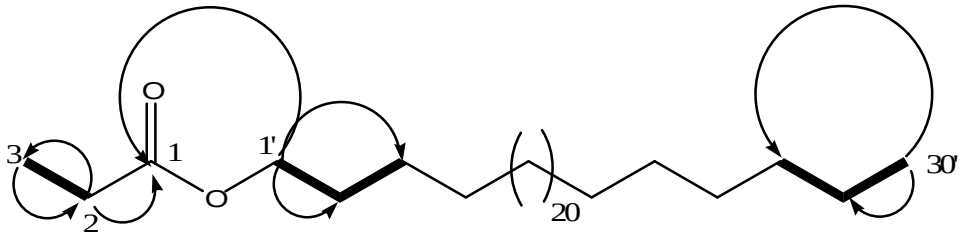

Figure 2:Connectivities deduced by COSY spectra (bold lines) and significant HMBC correlations (solid arrows) of compound (2)

CONCLUSION

A novel long chain 4-hydroxycinnamate ester namedn-tritriacontyl-4-hydroxy cinnamate (**1**) and a new long chain alkanoic ester named triacontylpropanoate (**2**) were obtained from the ethyl acetate extract of stem barks of *Vepris heterophylla*. The structures of 1 and 2have been elucidated by extensive one-dimensional and two-dimensional NMR spectroscopy and mass spectrometry.To the best of our knowledge, this is the first report on the isolation of these two compounds from*V.heterophylla*

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