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Research Article

Campylospermine, an *N*-Hydroxy-alkaloid from the Leaves of *Campylospermum densiflorum* (Ochnaceae)

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ABSTRACT
(*E*)-3-[(*N*-hydroxy)-*N*-methylamino)-*N*-(2-hydroxyethylene)] acrylamide (**1**), an *N*-hydroxy linear alkaloid namely Campylospermine, was isolated from the leaves of *Campylospermum densiflorum*. Four other known compounds were also isolated and identified as: one coumarin, umbelliferone (2) and a mixture of three steroids, campesterol-3-*O*-β-D-glucopyranoside, sitosterol-3-*O*-β-D-glucopyranoside and stigmasterol-3-*O*-β-D-glucopyranoside (**3**). The structures of the new compound and the others ones were determined by spectroscopic methods and by comparison with literature data.

Key words: *Campylospermum densiflorum*; Ochnaceae; steroids; alkaloid; Campylospermine.

INTRODUCTION

Campylospermum densiflorum (De Wild & Th. Durand) Farron of the Ochnaceae family, widely distributed in central Africa area (Cameroon, Gabon, Democratic Republic of Congo, Congo and Nigeria), has been used in traditional medicine by native people of south region of Cameroon to treat malaria, icterus and stomach ache**;** its methanolic extract showed antimicrobial activities[1]. Previous papers have reported the isolation of acylsteryl steroids[2,3], indole alkaloids which are characteristic constituents of the genus[4,5,6], a diterpene derivative[7] and a lignan[8] from this species.

The present investigation focused on secondary metabolites of the leaves of this plant. It led to the isolation of a new linear *N*-hydroxy amide alkaloid, Campylospermine **(1)**, together with a mixture gathering one coumarin known as umbelliferone **(2)**[9] (Fig.1) and three known steroids, campesterol-3-*O*-β-D-glucopyranoside, sitosterol-3-*O*-β-D-glucopyranoside and stigmasterol-3-*O*-β-D-glucopyranoside (**3a**, **3b** and **3c**)[2]. This report focuses on the isolation and structural elucidation of a new amide alkaloid (**1**).

EXPERIMENTAL

General experimental procedure: NMR spectra were recorded in DMSO-d_6 and CD_3OD solutions and were obtained using a Bruker instrument (^1H, 400 MHz; ^{13}C, 100 MHz) spectrometer, with TMS as internal standard. ^1H assignments were made using 2D-COSY and NOESY (mixing time 500 ms) while ^{13}C assignments were made using 2D-HSQC and HMBC experiments. For this latter, the delay was 70 ms. The. EIMS was recorded on a JEOL JMSD-300 instrument. Optical rotations, not corrected, were measured on a Perkin-Elmer 341 polarimeter. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR data were measured on a JASCO FTIR 300E spectrometer with KBr pellets. Chemical shifts (δ) are given in ppm and coupling constants (*J*) are reported in Hz. Column Chromatography was performed using Merck (70-230 mesh) silica gel of an appropriate particle and Sephadex LH-20 (MeOH). Preparative TLC were carried on silica gel plates (Merck silica gel 60 F_{254}). TLC layers were visualized by UV at 254 and 366 nm and exposure to iodine vapour. The solvent systems were the mixture of CH_2Cl_2:MeOH and EtOAc:MeOH of gradually increasing polarity.

Plant material: The leaves of *C. densiflorum* (De Wild & Th. Durand) Farron were collected at Campo in South-Cameroon Region in December 2005, and identified by Mr. Nana Victor, a botanist (National Herbarium of Cameroon). A voucher specimen (N°48270/HNC) was deposited at the National Herbarium in Yaoundé, Cameroon.

Extraction and isolation: Dried and powdered leaves (1.20 kg) of *C. densiflorum* were extracted exhaustively with MeOH at room temperature during 48 h. The solvent was removed under vacuum giving a residue (234 g). The residue was partitioned with hexane and CH_2Cl_2 to remove chlorophyll and non-polar metabolites. The methanolic part was re-extracted with ethyl acetate to yield an EtOAc soluble extract (25.4 g). This latter was analyzed by TLC and submitted to a silica gel CC, eluted successively with

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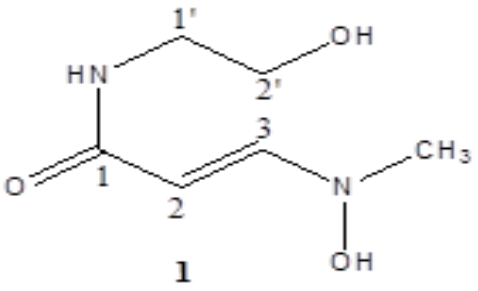
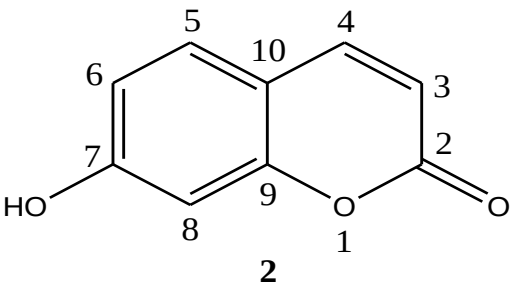


1



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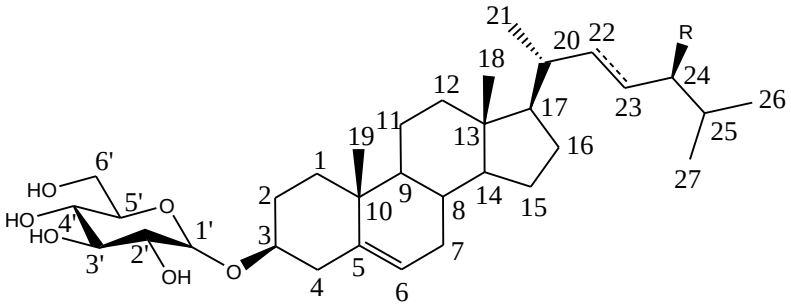


3a *: R=CH_3-, 22,23-dihydro*
3b *: R=CH_3CH_2-, 22,23-dihydro*
3c*: R=CH_3CH_2-, $\Delta^{22,23}$*

Fig. 1*. Structures of compounds* ***1-3****.*

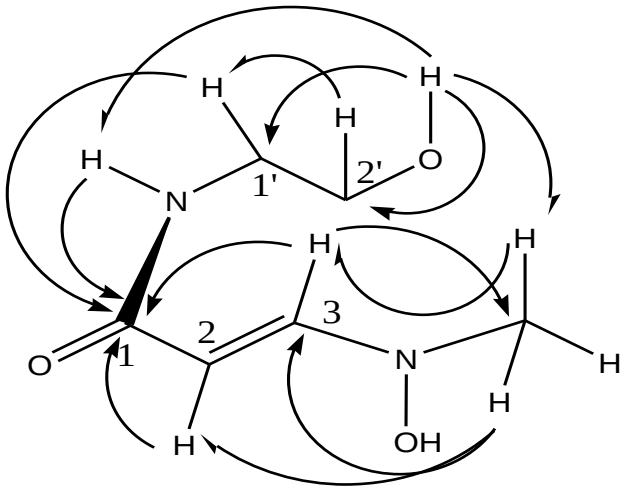


Fig. 2*. Key HMBC (* ⟶ *) and NOESY (* ⟶ *) correlations for compound* ***1****.*

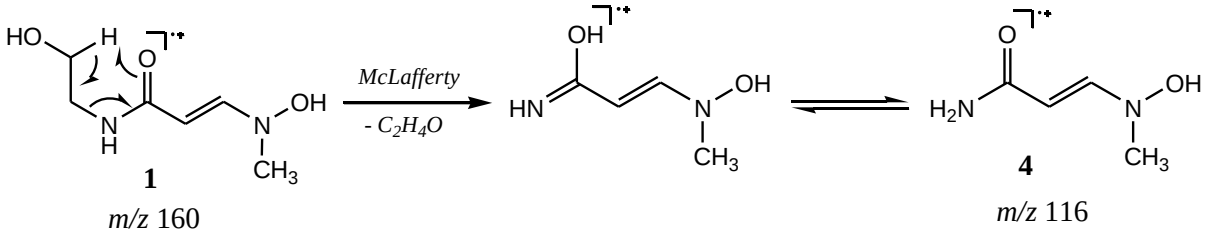
Table 1: ^{1}H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6) data and HMBC correlations of compound 1 (δ in ppm, J in Hz)[a].

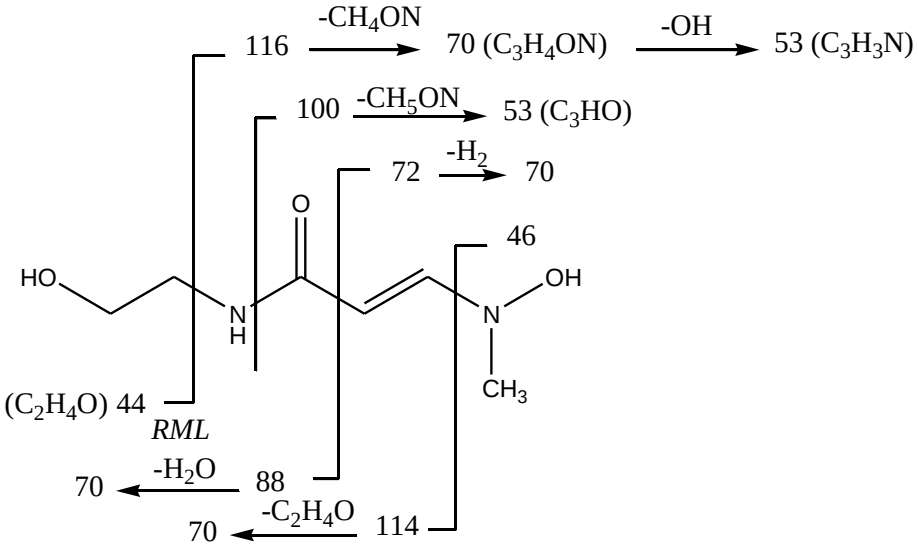
No	1		
	δ_H (mult)	δ_C	Carbon signal correlated
1	-	161.6	-
2	6.97 (d, 15.1)	134.6	C-1; C-3
3	7.46 (d, 15.1)	138.6	C-1; C-2; N-Me
1’	3.23 (q, 9.5; 5.7)	42.0	C-1; C-2’
2’	3.45 (q, 9.6; 5.9)	59.3	C-1’
N-Me	3.13 (s)	41.7	C-3
N-H	8.77 (t, 11.0; 5.5)	-	C-1; C-1’; C-2;
O-H	4.79 (t, 10.5; 5.2)	-	C-1’; C-2’

[a] *Assignments were based on 1D and 2D NMR experiments*

Table 2: ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) of compound 1 (δ in ppm, J in Hz)[a].

No	1	
	δ_H (mult)	δ_C
1	-	164.6
2	7.01 (d, 15.0)	136.3
3	7.44 (d, 15.0)	140.1
1’	3.41 (t, 5.6)	43.5
2’	3.65 (t, 5.6)	61.2
N-Me	3.08 (s)	42.5





Scheme 1. *Fragmentations patterns of MS-EI of compound **1***

CH_2Cl_2, mixtures of CH_2Cl_2: MeOH at increasing polarities, and pure MeOH. Five main fractions were collected [I (5.7 g), II (3.4 g), III (3.8 g), IV (4.2 g) and V (8.3 g)]. Fraction II (3.4 g) was chromatographed in a silica gel (500 g) CC using the solvent system CH_2Cl_2: MeOH (from 30:1 to 15:1) to give three sub-fractions (IIa, IIb and IIc). Sub-fraction IIb (0.91 g) was further purified on a silica gel (180 g) CC using CH_2Cl_2: MeOH (from 10:1 to 6:1) to furnish compound **1** (12 mg). Using the same process, Fraction III (3.8 g) gave three sub-fractions (IIIa, IIIb and IIIc). Fraction sub IIIa (1.08 g) was in turn subjected to a silica gel CC and to Sephadex LH-20 CC one (200 g) to provide three sub-parts (IIIa1, IIIa2 and IIIa3). Sub-fraction IIIa1 (0.6 g) was submitted to a silica gel (100 g) CC eluted with CH_2Cl_2: MeOH (20:1); further chromatographic analyses using repeated preparative TLC (CH_2Cl_2: MeOH 15:1 to 10:1) afforded compound **2** (18 mg). Fraction V (8.3 g) was subjected to CC on silica gel (700 g) and eluted with the solvent system CH_2Cl_2: MeOH (from 10:1 to pure MeOH) to give four sub-fractions (Va, Vb, Vc and Vd). Sub-fraction Va (2,80 g) was chromatographed using a silica gel (200 g) CC with the solvent system EtOAc: MeOH (from 15:1 to 5:1) producing three sub-parts Va1 (0.90 g), Va2 (1.20 g) and Va3 (0.70 g). The first one was submitted to Sephadex LH-20 CC (110 g) with pure MeOH to yield compound **3** (28 mg).

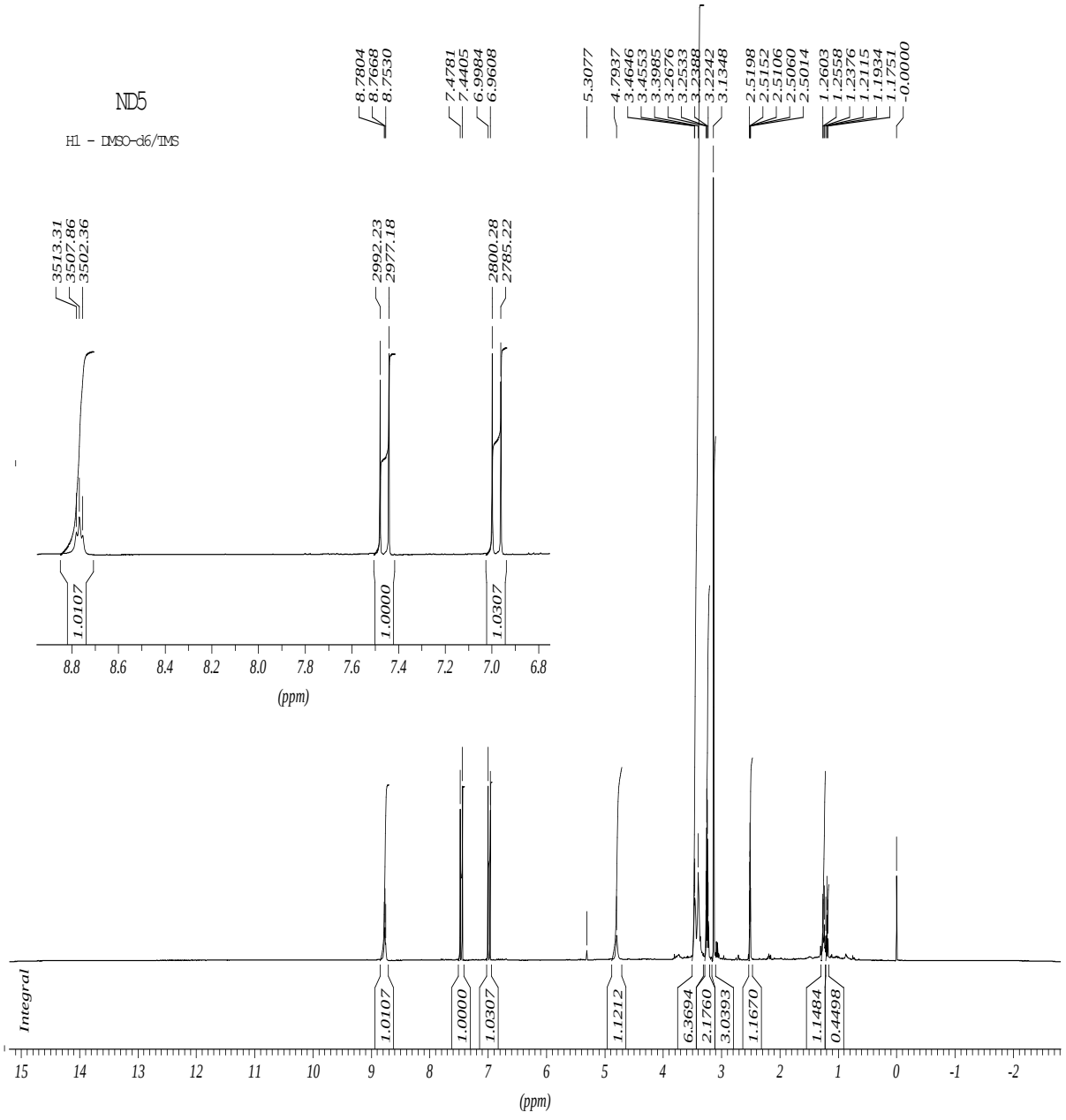
Campylospermine (1): Yellow amorphous powder from CH_2Cl_2: MeOH (6:1), 12 mg : m.p. 209-212 °C, IR υ_{max}^{KBr} cm^{-1}: 3398 (O-H), 3199 (N-H), 3005 (C=C-H), 2988 (C-H), 1678 (N-C=O), 1639 (C=C), 1479 (C-C), 1095 (C-O). TLC: Rf 0.68 (CH_2Cl_2: MeOH 95:5); for ^{1}H and ^{13}C NMR (400 and 100 MHz, DMSO-d_6 and CD_3OD): tables 1 and 2; EI-MS, *m/z* 160 ($M^{+\cdot}$, 69%), 116 (100), 88 (25), 70 (95), 53 (95), 47 (10), 46 (21), 44 (25) Scheme 1.

RMN de ND_5 dans DMSO-d_6

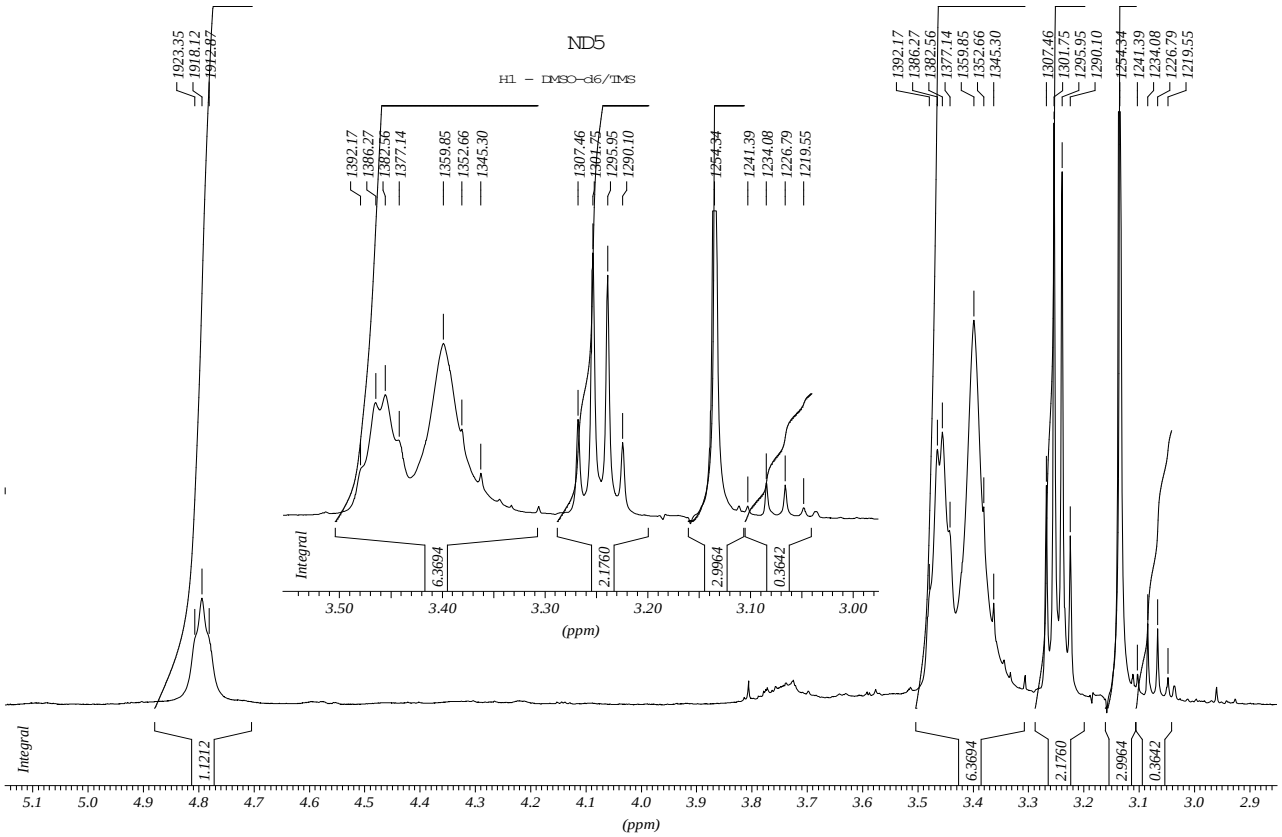
Il y a seulement 6 pics en RMN du C^{13} découplé:

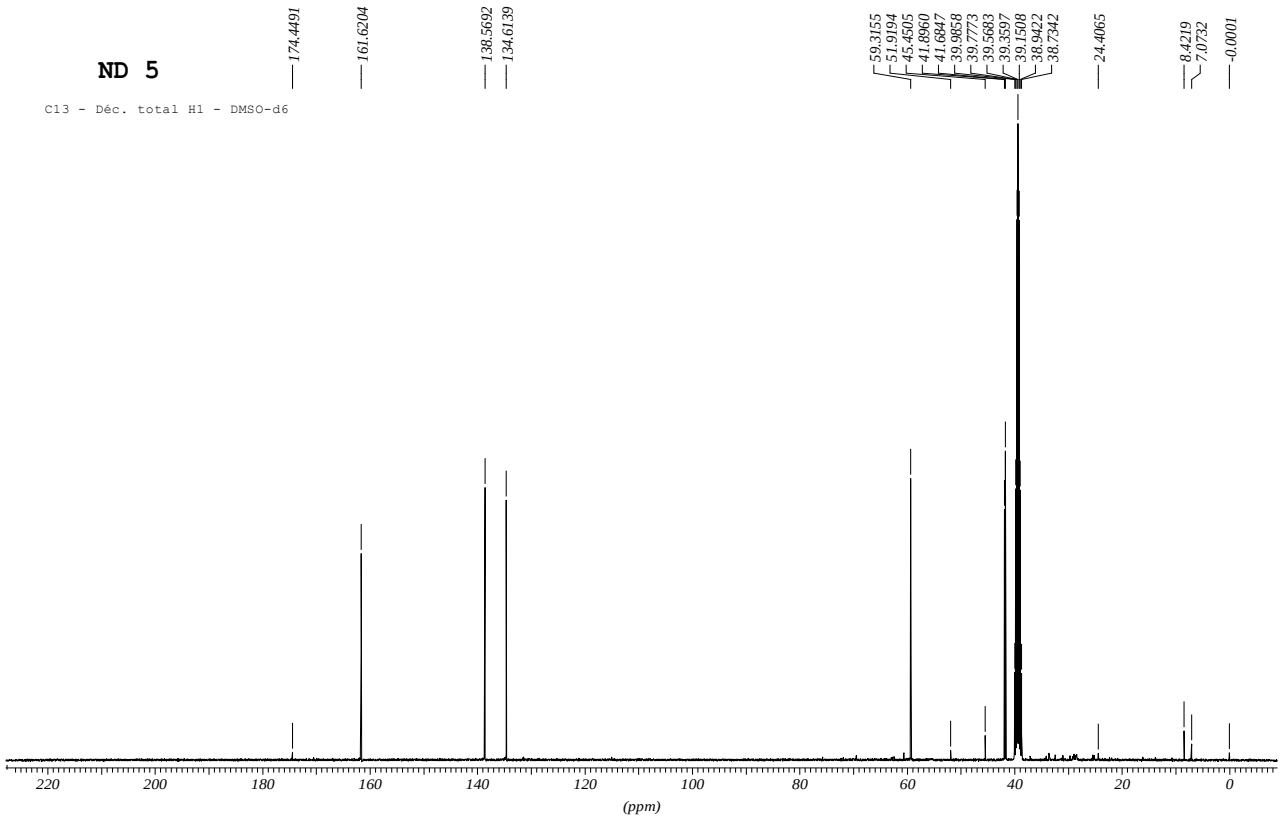
41,6847 ppm	CH_3	corrèle avec H1 à 3,13 ppm
41,8960 ppm	CH_2	corrèle avec H1 à 3,23 ppm
59,3155 ppm	CH_2	corrèle avec H1 à 3,44 ppm
134,6139 ppm	CH	corrèle avec H1 à 6,97 ppm
138,5692 ppm	CH	corrèle avec H1 à 7,46 ppm
161,6204 ppm	C quaternaire	(CO?)

Les protons à 4,79 et 8,77 ppm ne corrèlent pas avec un carbone (OH ou NH?).



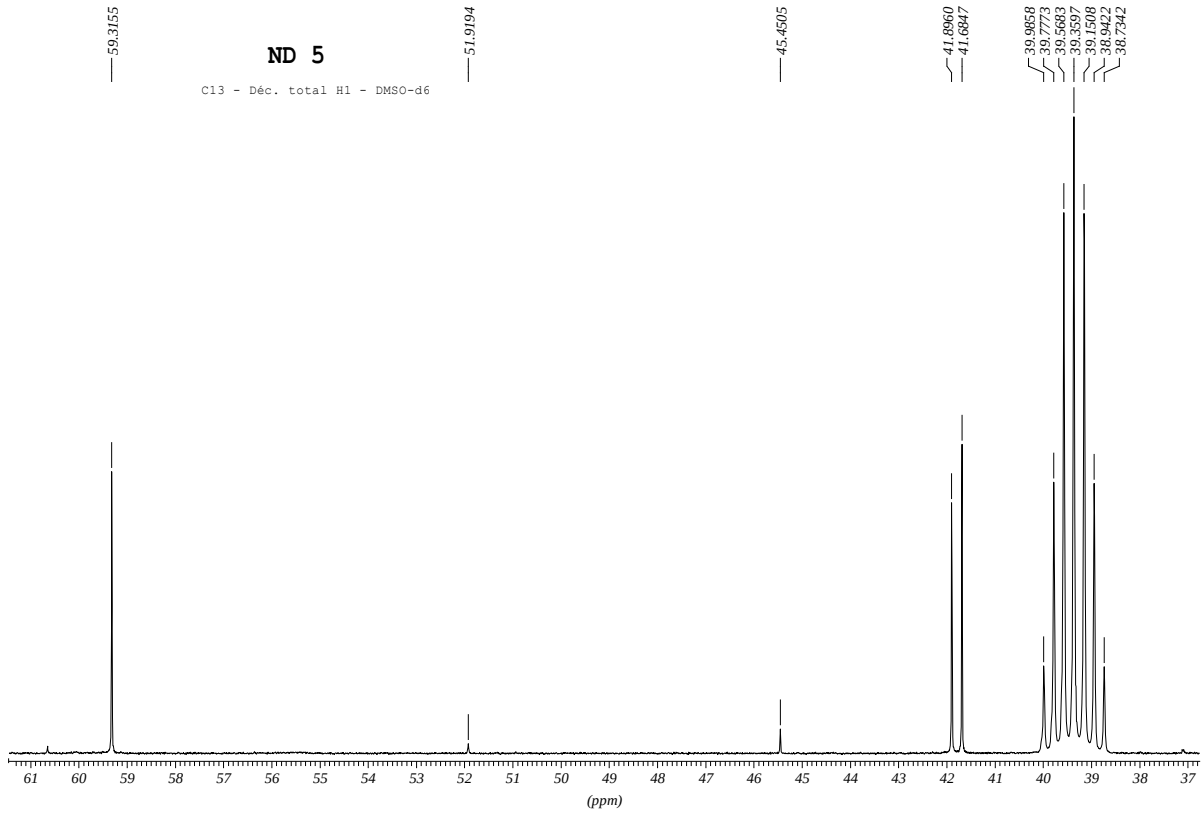
Supplementary data: ^{1}H, ^{13}C spectra for compound 1

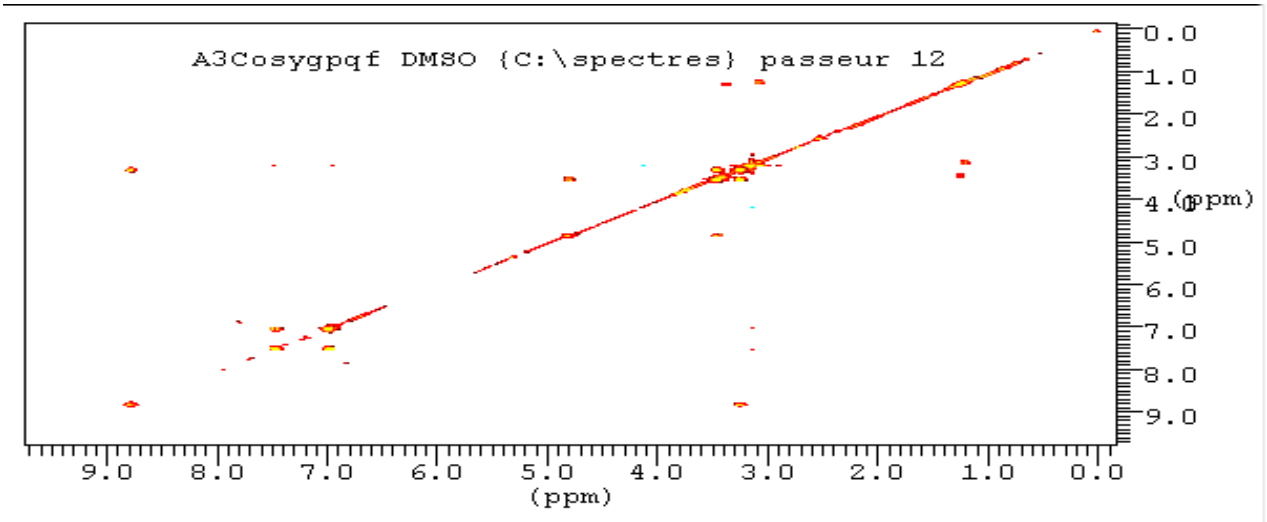


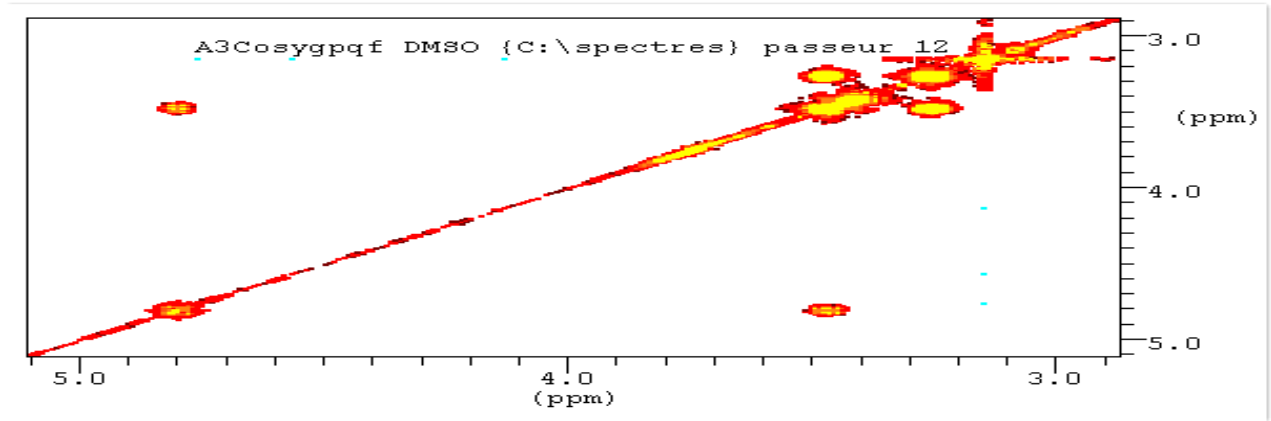


RESULTS AND DISCUSSION

Dried and powdered leaves of *Campylospermum densiflorum* were extracted with MeOH at room temperature during 48 h. The solvent was removed by distillation under vacuum giving a residue (234 g). The

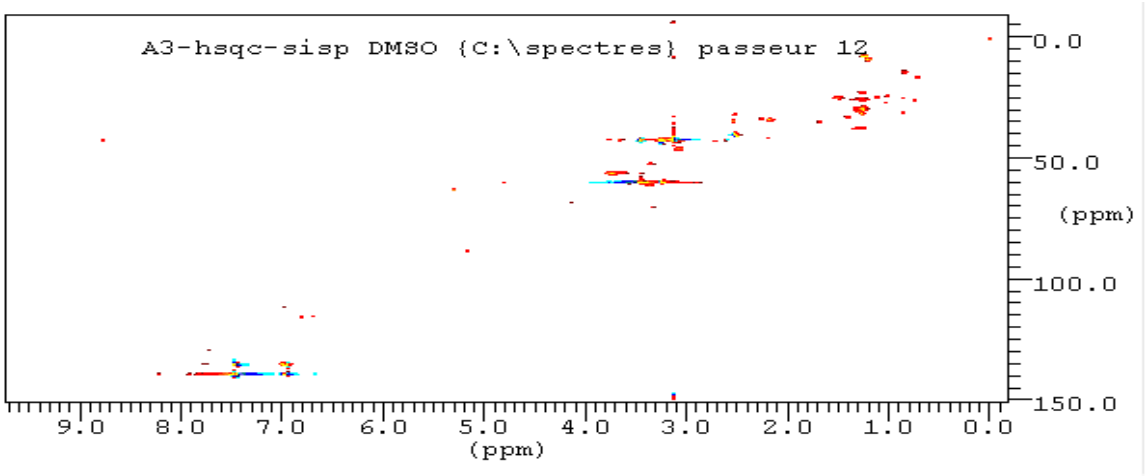

ND 5
C13 - Déc. total H1 - DMSO-d6
59.3155
51.9194
45.4505
41.8960
41.6847
39.9858
39.7773
39.5683
39.3597
39.1508
38.9422
38.7342
(ppm)

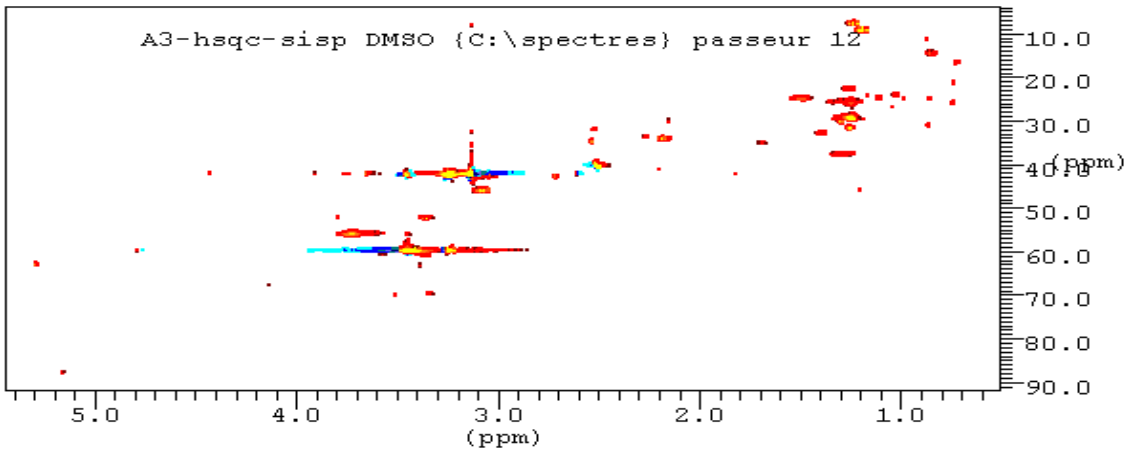

A3Cosygpqf DMSO {C:\spectres} passeur 12
(ppm)
(ppm)

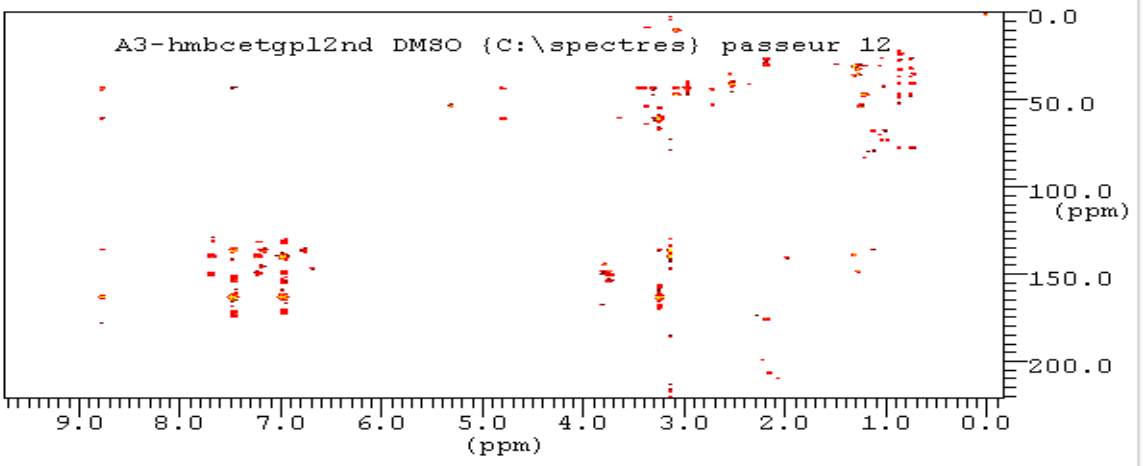

A3Cosygpqf DMSO {C:\spectres} passeur 12
(ppm)
(ppm)

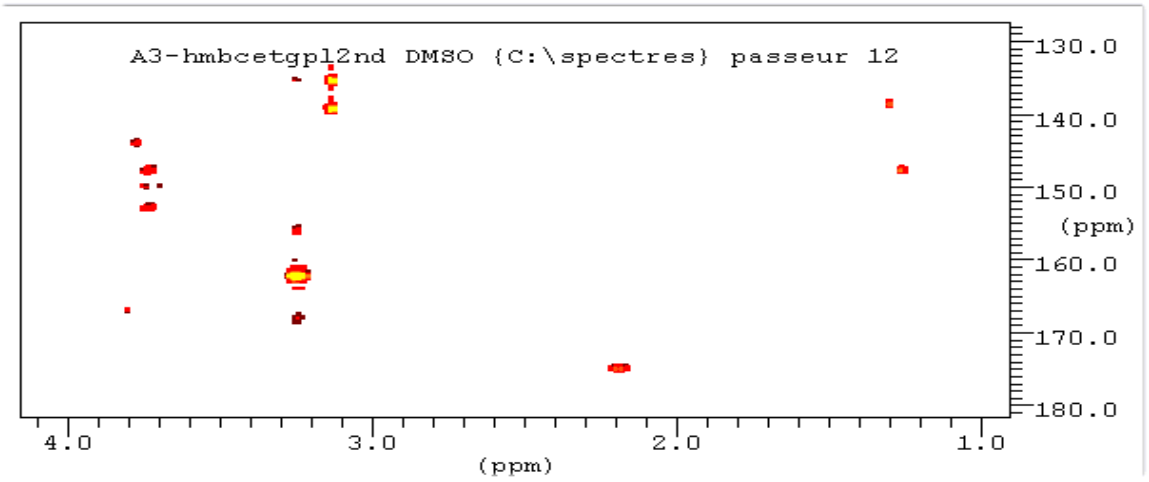
residue was suspended in water and successively extracted with hexane, CH_2Cl_2 and EtOAc, respectively. The EtOAc extract was separated using different chromatographic techniques affording compounds **1** (12 mg), **2** (18 mg) and a mixture of **3a, 3b and 3c** (28 mg). The structure elucidation of the isolated compounds was performed by spectrometric analyses added to comparison with literature data[2,9].

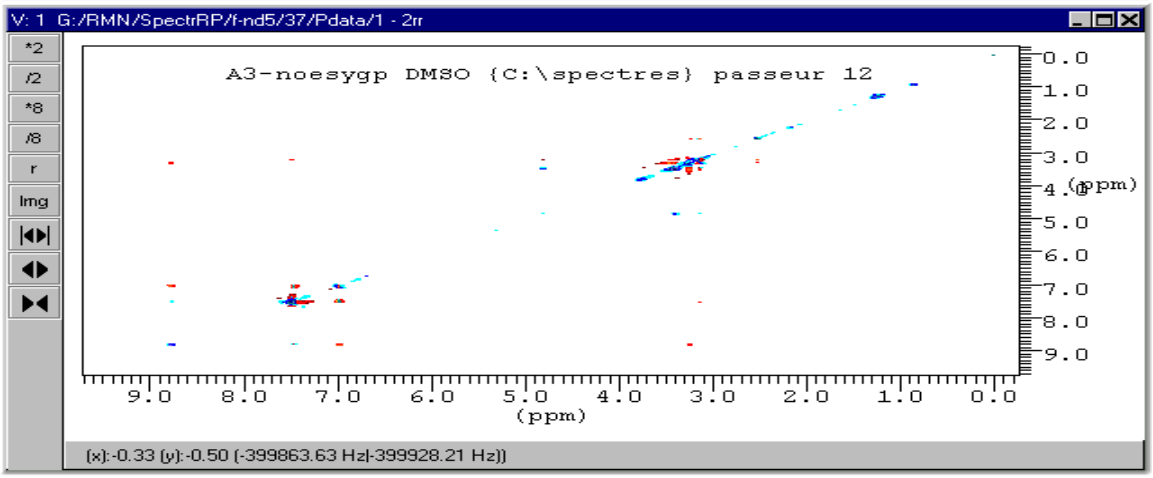
The molecular formula of compound **1** was determined as $C_6H_{12}O_3N_2$ by the value of molecular ion ($M^{+\cdot}$) at m/z 160 detected in the mass spectrum combined to ^1H and ^{13}C NMR analyses. NMR analyses were performed in two solvents (DMSO-d_6 and CD_3OD), but total assignments were established in DMSO-d_6 (Table 1) except for the experience of exchange with D_2O for which CD_3OD was used to avoid interfering signals from OH and NH functions of **1** (Table 2). Its IR spectrum displayed











Demande de résultats

JI 1 (1642)

Passage : 27/04/2011
Source : ESI
Solvant : CH3OH / CH2Cl2 : 90/10
Opérateur : Morgan AUFFRAY

Composé principal

[M+Na]+ (C35 H60 O6 Na)
Masse Théorique : 599.42876
z = 1
m/z Théorique : 599.42821
m/z Trouvé : 599.4282 (0 ppm)

[M+K]+ (C35 H60 O6 K)
Masse Théorique : 615.40270
z = 1
m/z Théorique : 615.40215
m/z Trouvé : 615.4040 (3 ppm)

Autres composés

[M2+Na]+ (C35 H58 O6 Na)
Masse Théorique : 597.41311
z = 1
m/z Théorique : 597.41256
m/z Trouvé : 597.4130 (1 ppm)

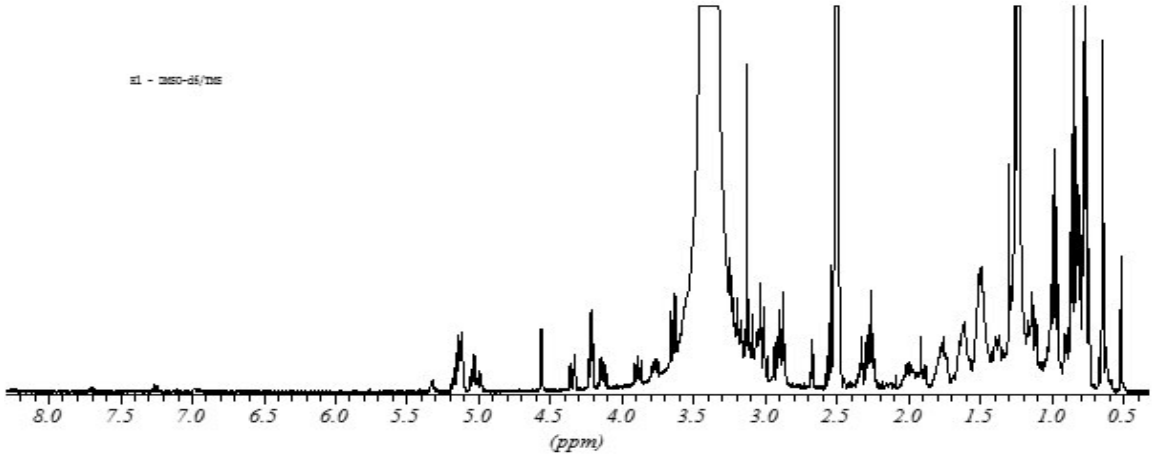
[M3+Na]+ (C34 H58 O6 Na)
Masse Théorique : 585.41311
z = 1
m/z Théorique : 585.41256
m/z Trouvé : 585.4133 (1 ppm)

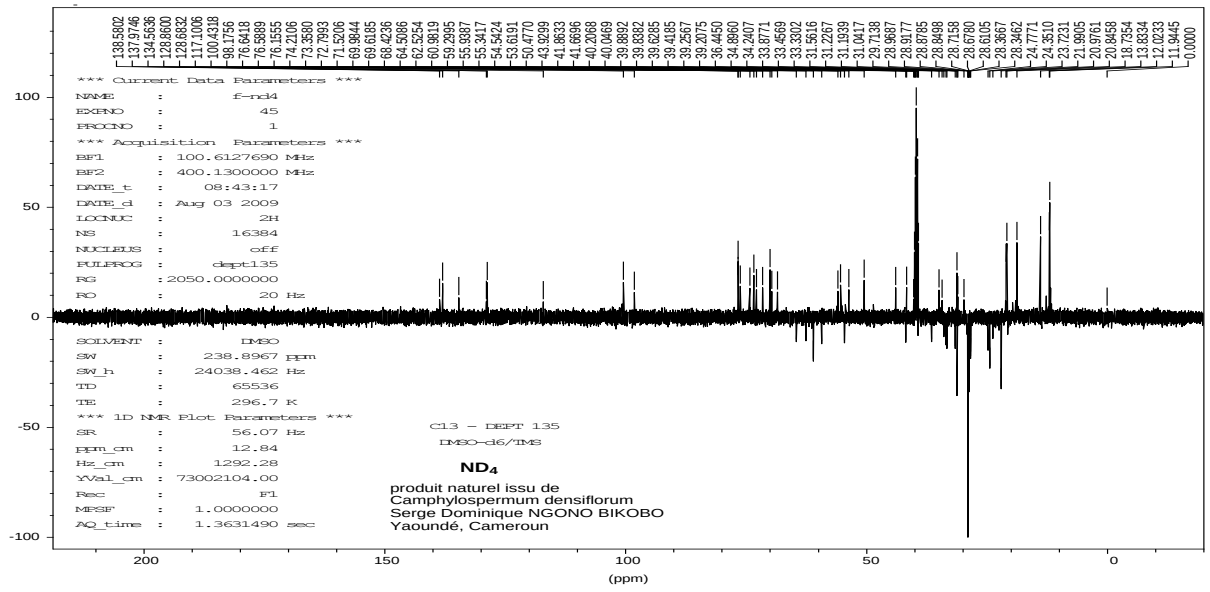
Supplementary data: MS, ^{1}H and ^{13}C spectra for compound 3

characteristic absorptions for hydroxyl groups (3398 cm^{-1}), an *α,β*- unsaturated amide carbonyl (1678 cm^{-1}) and a double bond (1639 cm^{-1}). The ^1H NMR spectrum indicated some remarkable protons: the presence of an olefinic AX system from the signals at δ_H 7.46 (H-**3**, *d*, *J* = 15.1 Hz) and 6.97 ppm (H-**2**, *d*, *J* = 15.1 Hz) showing that the olefin bond is in an *E* form ; an aliphatic A_2X_2 type of methylene protons occurs at δ_H 3.45 (H-**2**', *q*, *J* = 9.6; 5.9 Hz) and 3.23 ppm (H-**1**', *q*, *J* = 9.5; 5.7 Hz), one triplet proton at δ_H 8.77 ppm (*J* = 11.0; 5.5 Hz) which is characteristic of an amide function and a singlet methyl signal appears at δ_H 3.13 ppm, probably linked to a nitrogen atom (Tables 1 and 2). The ^{13}C NMR spectrum showed the presence of 6 carbon signals, one of them was assignable to a carbonyl function at δ_C 161.6 ppm, two olefinic-methines at δ_C 138.6 and 134.6 ppm, two methylenes at δ_C 59.3 and 42.0 ppm and one methyl group at δ_C 41.7 ppm. All protonated carbons were assigned by the results from HSQC spectrum. From these data, it was proposed a shorter branched amide alkaloid for compound **1**.

Further elements from HMBC provided confirmations from two and three-bond connectivities between the methyl protons at δ_H 3.13 ppm and the carbon at position 3 (δ_C 138.6) in one side, the proton at position 3 (δ_H 7.46) and the carbons at position 1 (δ_C 161.6) and the methyl one (δ_{CH3} 41.7) in another side (Fig. 2). This signifies that the single methyl group was attached to olefin carbon C-3 through a nitrogen atom; another indication is consistent with the presence in this structure of an acrylamide moiety as main part of carbon skeleton[10], as supported by the fragment at *m/z* 53 (C_3HO) (*cf.* Scheme 1). The hydroxyethyl group linked to C-1 in the structure of **1** was supported by HMBC experiment (Fig. 2), which showed correlations for the resonances at δ_H 4.79 ppm (terminal hydroxyl) with the signals of methylene carbons at δ_C 59.3 (C-2') and 42.0 ppm (C-1'). In addition, a cross-peak was observed between methylene proton at δ_H 3.23 (H-1') and carbonyl carbon at δ_C 161.6 (C-1) revealing that this remaining unit is attached to the main skeleton of the structure **1**.

The location of other substituents to carbamoylethenyl moiety was confirmed by NOESY experiment (Fig. 2).





For compound 2 , firstly in a mixture, it was obtained after recrystallization from $CHCl_3$ as colorless crystals (melting point at 224-226°C) and identified by TLC with an authentical sample (melting point at 223-225°C) present in our laboratory.

The significant correlations observed for H-2 and H-3 (δ_H 6.97 and 7.46) with CH_3- (δ_{CH3} 3.13), O-H (δ_H 4.79) with N-H (δ_H 8.77), and O-H (δ_H 4.79) with CH_3- (δ_{CH3} 3.13), confirmed the above-discussed data for the structure (**1**).

An interesting feature in the EIMS of **1** was the base peak at *m/z* 116 ($C_4H_8N_2O_2$) corresponding to 3-(*N*-hydroxy-*N*-methylamino) acrylamide (**4**), resulting from *McLafferty* rearrangement[11] (Scheme 1). The others proposed fragment peaks at *m/z* 70 (95 %), *m/z* 53 (95%), detected in the mass spectrum (Scheme 1), were used to confirm the proposed structure (**1)**. Moreover, the presence of *N*-hygroxy group in the structure, induces downfield shifts of the signals for the singlet *N*-CH_3[12,13,14,15,16] (tables 1 and 2) justifying the presence of this unit in **1.**

Based on above evidence, the structure of compound **1** (Fig.1) was assigned as (*E*)-3-[(*N*-hydroxy)-*N*-methylamino)-*N*-(2-hydroxyethylene)] acrylamide, namely Campylospermine (**1**). This is the first report of this class of alkaloid from the Ochnaceae family.

ACKNOWLEDGMENTS

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