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Full Length Research Paper

Structural elucidation of a new furoclerodane from stem barks of *Croton mayumbensis* J. Leonard extracts

Sosthène C. Yamale[1], Jean Koudou[2]*, Abdoulaye Samb[1], Annie Heitz[3] and Jean-Claude Teulade[4]

[1]Laboratoire de chimie et biochimie des produits naturels, Faculté des Sciences et Techniques, UCAD, BP7021 Dakar, Sénégal.
[2]Laboratoire de chimie des substances naturelles, Faculté des Sciences, Université de Bangui, BP908, Bangui, République Centrafricaine.
[3]CCIPE, rue de la Cardonille, F-34094 Montpellier, France.
[4]Laboratoire de chimie organique UMR INSERM 484, Faculté de Pharmacie, Université d'Auvergne, France.

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The stem barks of *Croton mayumbensis* are used by the healers of Centrafricane Republic against many diseases as microbial infections and amoebiasis. The analysis of different extracts by column chromatography, HPLC coupled with mass spectrometry lead to the identification of a new diterpenoïd with a furoclerodane squeleton. Its structure has been elucidated from spectral data: mass spectrometry, ^{1}H NMR, ^{13}C NMR COSY and NOESY.

Key words: *Croton mayumbensis*, euphorbiaceae, stem barks, furoclerodane, Centrafricine I.

INTRODUCTION

Croton mayumbensis (Euphorbiaceae) is a tree up to 34 m growing in the rain forest of the Central African Republic. It is an African medicinal plant that barks and leaves are empirically used to treat microbial infections and human parasitic diseases (Lejolly, 1956). Previously, important studies have been conducted on the genus Teucrium (Labiateae) where the new diterpenoids whose structures have been elucidated, have the clerodane or 19-norclerodane skeleton, and have been characterised by a furan ring with sometimes other additional heterocyclic group (Block et al., 2002; Smith et al., 1976; Roengsumran et al., 1999). However, the genus Croton is well known in a traditional medicine and few previous works have demonstrated the presence of the clerodane structure in the species from South-America (Roengsumran et al., 2002; Piters et al., 1995; Clélia et al., 1999) and Asia (Kittakoop et al., 2001).

Despite the interesting virtues of *C. mayumbensis* no scientific studies have been carried to determine the pharmacological action of this plant and its chemical present work was designed to its phytochemical study and had reported on the isolation from the stem bark of one furoclerodane compound.

*Corresponding author. E-mail: jean_koudou@yahoo.fr. Tel.: +22650368146. Fax: +22650368573.

EXPERIMENTAL RESULTS AND DISCUSSION

General procedure

EI/MS: Hewlett-Packard 5985B and 5989A mass spectrometer; NMR: 400MHz (^{1}H) and 100MHz (^{13}C), Brucker AC 400 spectrometer using $CDCl_3$ and DMSO d6 as solvents, the chemical shifts were measured either from 2D COSY or 2D HMBC spectra with TMS as internal standard.

HPLC analyses were performed on a column (20 cm x 8 mm x 3.5 µm) coated with reversed-phase C-18, equipped with a security guard system. System of solvants CH_3CN-H_2O contained 0.1 % HCOOH with a flow rate: 0.3 ml/min (30 to 50% CH_3CN/15 min, then 10 min to 100 % CH_3CN, from 100 to 30% per 5 min and 5 min to 30% CH_3CN). HPLC/MS analyses were carried out on a Hewlett-Packard 5985B and 5989A mass spectrometer using the same HPLC parameters. The column chromatography was performed with Merck neutral aluminium oxide 90 standardized (63 – 200 µm). The thin- layer chromatography was performed on Merck neutral aluminium oxide $60F_{254}$ plates. The plates were visualized with UV light (254 nm).

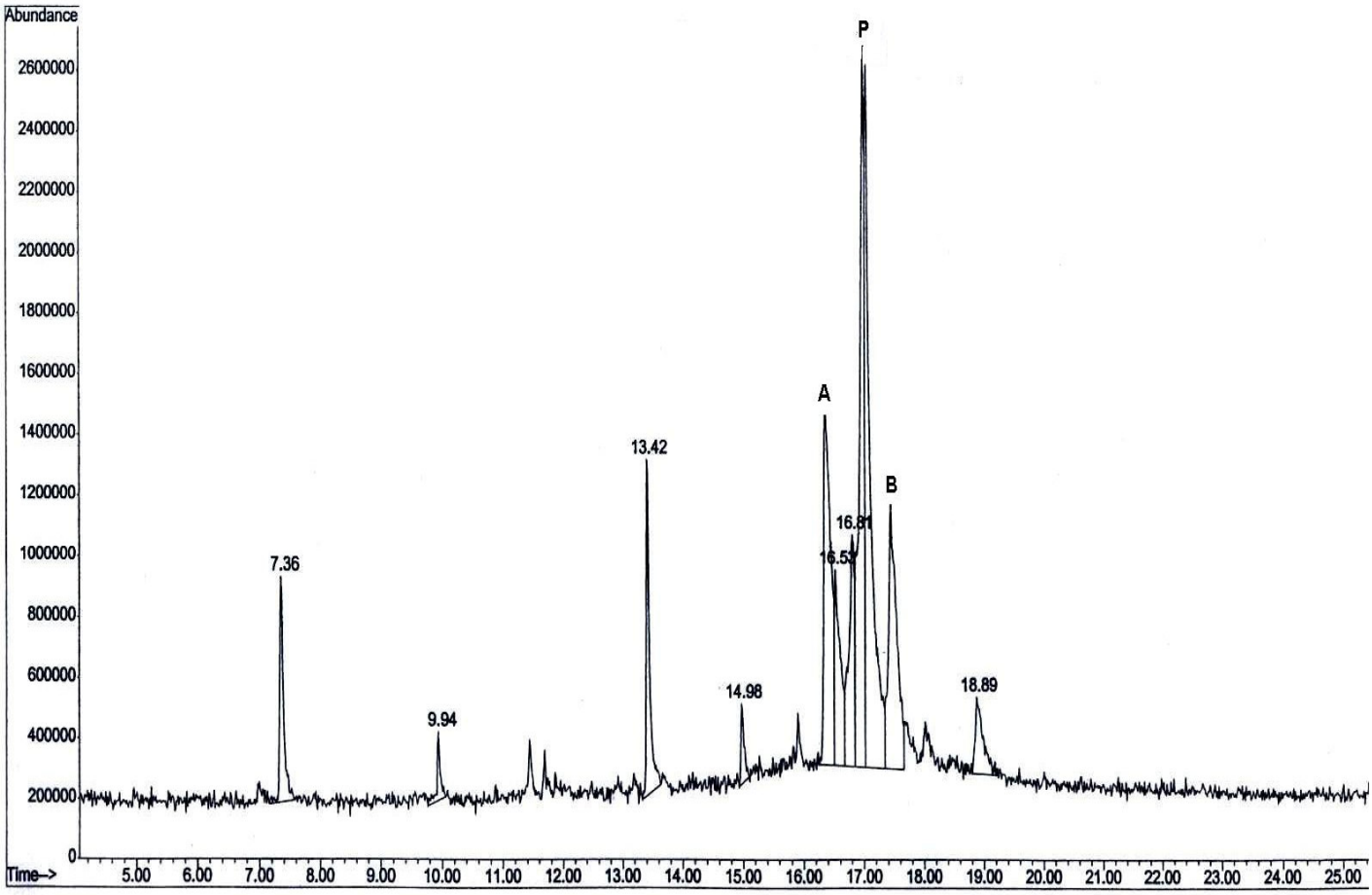


Figure 1. HPLC of the fraction F'

Plant material

The stem bark of *C. mayumbensis* J. Leonard was collected from Boukoko a village (162 km south of Bangui) of Central African Republic in October 2003. A voucher specimen was deposited at Cerphametra, University of Bangui.

Extraction

The air-dried and commutted stem bark of *Croton mayumbensis* (100 g), was extracted at room temperature with the mixture MeOH-CH_2CL_2 (1:1). The extract was concentrated to dryness under reduced pressure to yield a brown crude (16 g). This residue was fractionated by silica gel column according to a standard method and afforded six fractions: F1 (1.8 g): ether (1000 ml), F2 (1.2 g): ethyl acetate (1000 ml), F3 (2.4 g): ethanol (1500 ml), F4 (0.6 g): methanol (500 ml).These fractions were chromatographied on preparative plates and eluted with chloroform/diethylamin (95/5, v/v) and yielded eight fractions SF1 to SF8 which were the complex mixtures; HPLC/MS analyses showed that SF2, SF4 and SF6 contained a major compound P. Fractions SF2, SF4 and SF6 were mixed to give the fraction F' (1.33 g). F' was purified by column chromatography over neutral alumina, eluted with a CH_2Cl_2/EtOH gradient (from 9/1 to 9/4, v/v) starting with CH_2Cl_2. The purification controlled by HPLC yielded the compound P with the percentage of 55% from its peak in the HPLC chromatogram (Figure 1).

The HPLC/MS analysis showed $[M+H]^+$ at m/z 372 corresponding to the molecular formula $C_{21}H_{24}O_6$ with 10 as a number of insaturation (Figure 2). The ^{13}CNMR spectrum displayed signals for 21 carbon atoms: eight quarternary carbon atoms, five methynes, five methylenes, three methyls, one lactone group (δc180.2), one acetyl group (δc21.4), two ethylenic carbon atoms (C=C, δc133.5; 132.5). The signals at δc: 127.5, 102.5, 144.3 and 146.9 indicated a furyl ring (Table 1). The ^{1}H NMR spectrum (Figure 3) showed the presence of furannic ring: [δ = 6.72 (1H, dd), 7.40 (1H, m) and 8.0 (1H, m)], méthyl-ester group [δ = 3.69 (3H, s)], and méthyl group in α position of carbonyl group C19 [δ = 0.97 (3H, d)]. The compound P was a furoclerodane, its structure was confirmed by the COSY ^{1}H-^{13}C correlations (Figure 4). The presence of furyl

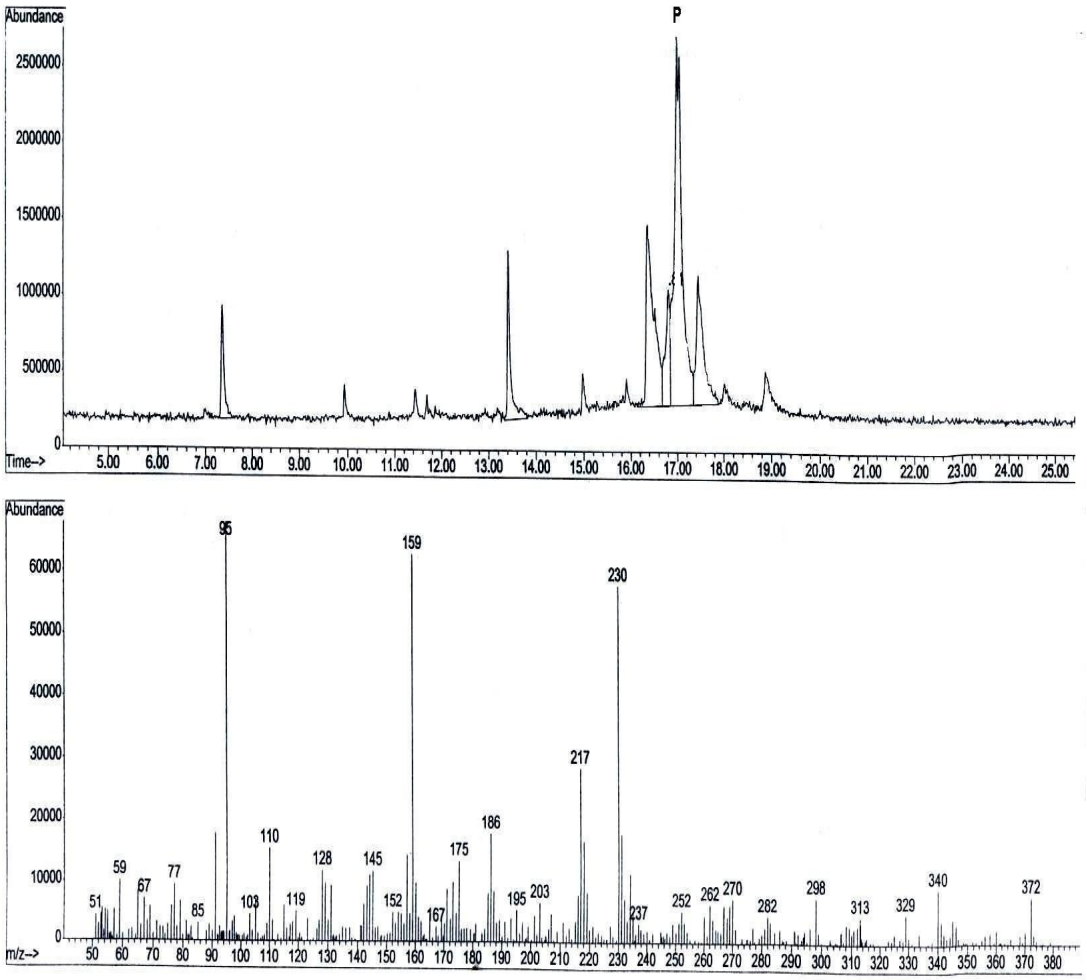


Figure 2. HPLC/MS of Centrafricine I.

group was characterised:

The proton H-14 (δ_H 6.72) was coupled with C-13(δ_c127.5), C-15(δ_c144.3) by ^{2}J coupling and with C-12(δ_c191.4) by ^{3}J coupling. The proton H-16 (δ_H8.0) was coupled with C-14(δ_c102.5) by ^{3}J coupling.

Then, the aliphatic chain was determined:

The methylene H-11(δ_H3.24) was coupled with C-12-(δ_c191.4) by ^{2}J coupling, and with C8(δc18,4) and C-10-(δ_c132.2) by ^{3}J coupling. The methyl H-19 at δ_H0.97 linked with the first hexenic ring was coupled with C-2(δ_c36.8; ^{2}J) and gave ^{3}J coupling with C9(δc26,5) and C7(δc28,9) . H-7 gave ^{3}J coupling with C-9 and C-19(δ_c15.7) and was coupled with C-6(δ_c41.6; ^{2}J). H-6 at (δ_H5.02) gave ^{3}J with C-10.

The second hexenic ring linked with the lactone group was determined: the methyl H-21at δ_H1.36 gave long-range correlation with C-20(δc180.2) and C-3(δ_c31.4) and ^{2}J coupling with C-4(δ_c73.8). The relative configuration of P was confirmed by NOESY correlations: CH_3-21α, CH_3-19α, H-6α (Figure 5). The configuration β for the aliphatic chain was determined by the correlations: H-8 with Hβ-11 (δ_H3.29) and Hβ-7 with Hβ-11. CLHP/SM m/e: 372 $[M]^{\cdot+}$ (11), 340 $[M-32]^+$ (11), 341 $[M-31]^+$, 313 $[M-28]^+$ (5), 312 $[M-28]^+$, 262 $[M-110]^+$ (5), 231 $[M-34]^+$, 230 $[M-110]^+$ (85), 217 $[M-96]^+$ (43), 186 $[M-44]^+$ (22), 173 $[M-44]^+$, 159 $[M-44]^+$ (92), 95 $[M-277]^+$ (100), 67 $[M-28]^+$ (12).

Conclusion

These spectral data in the comparison with literature (Yamalé, 2005) show that this compound is a new furoclerodane and named Centrafricine I. Because of the importance of *C. Mayumbensis* in the traditional medicine the study has to be pursued in the aim to isolate the major components and to evaluate the pharmacological properties of this plant.

Table 1. NMR data of Centrafricine I.

Carbon atoms	^{13}C NMR δ (ppm) (100MHZ, DMSOd6)	^{1}H NMR δ (ppm) (400 MHZ, $CDCl_3$)	HMBC (C→H)	HMBC (C→H)
1	21,4 (CH_2)	1,60 (m), Hα	-	Hα-1, Hβ-1
		2,46 (m), Hβ		-
2	36,8 (CH_2)	1,73 (m)		Hα-2, Hα-1
3	31,4 (CH_2)	1,36 (m),Hα	H-21	Hα-3, Hβ-3
		1,84 (m), Hβ		/
4	73,8 (C)	-	H-21	/
5	133,5 (C)	-	H-21, Hβ-6	/
6	41,6 (CH)	5,02(dd)	Hα-7	/
7	28,9 (CH_2)	1,71 (m),Hα	H-12	Hα-7, Hα-6
		2,24 (m), Hβ		Hβ-7, Hβ-11, Hβ-8
8	18,4 (CH)	2,32 (m)	H-19, Hα-11	Hα-8/, Hα-11
9	26,5 (C)	-	H-19, Hα-7	/
10	132,2 (C)	-	Hα-11, Hβ-6, Hβ-2	/
11	49,2 (CH_2)	3,24 (dd), Hα	-	Hα-11, H-14, H-16
		3,29 (dd), Hβ		/
12	191,4 (C)	-	Hα-11, H-14	/
13	127,5 (C)	-	H-14, H-16	/
14	102,5 (CH)	6,72 (dd)	H-16	/
15	144.3 (CH)	7,44 (m)	H-14	/
16	146,9 (CH)	8.00(m)	-	/
17	170,3 (C)	-	-	/
18	50,8 (CH_3)	3,69 (s)	-	/
19	15,7 (CH_3)	0,97 (d)		H-19, H-18, Hα-7, Hα-6
20	180,2 (C)	-	Hα-7, Hβ-7	/
21	21,5 (CH_3)	1,36 (s)	-	Hα-6

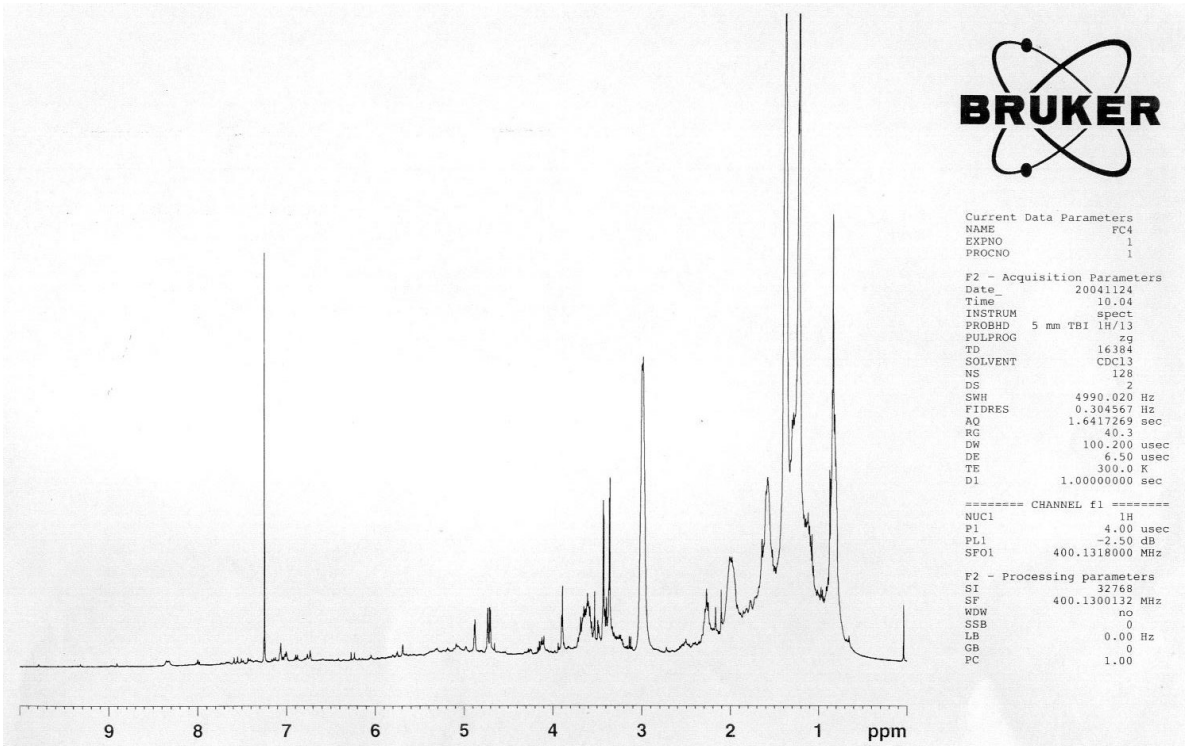


Figure 3. ^{1}H NMR spectrum of the fraction F'.

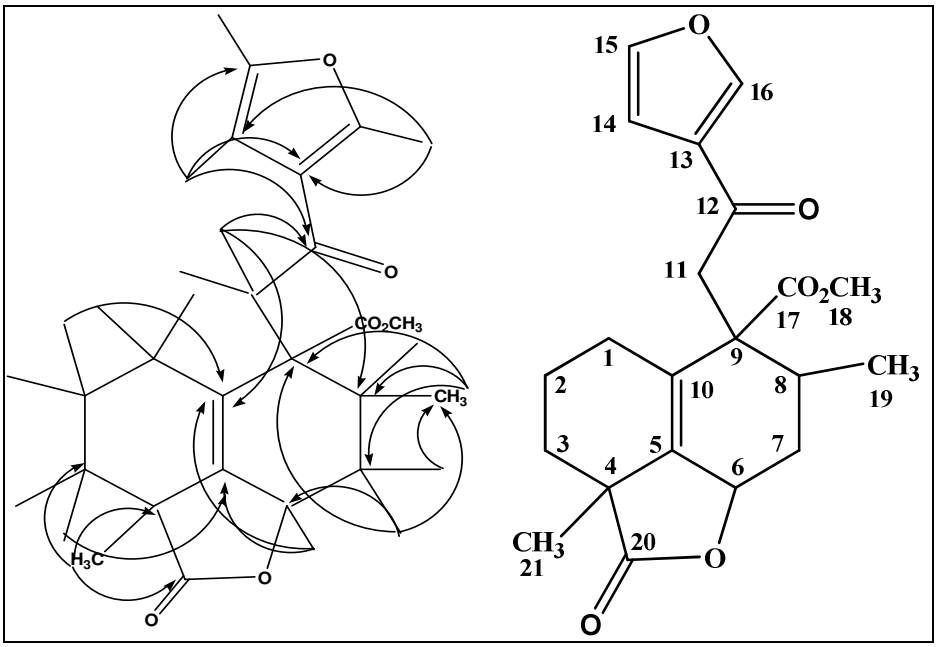
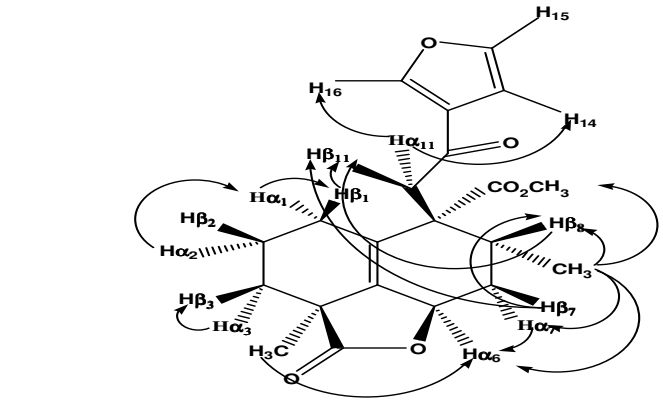
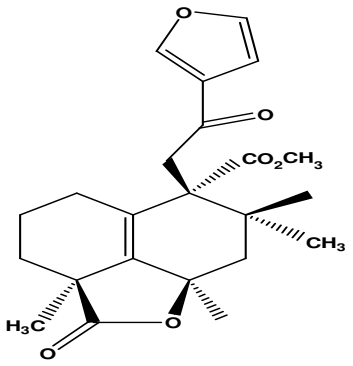


Figure 4. COSY ^{1}H - ^{13}C of Centrafricine I



NOE effects and $^{1}H - ^{1}H$ correlations



Centrafricine I

Figure 5. Structure and configuration.

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