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Triterpenes and Coumaroyltyramide from Ochthocosmus *Africanus*

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Abstract: Chemical investigation of the stem bark of Ochthocosmus *africanus* resulted in the isolation of one new triterpene, ochtofridelane (1) along with four known compounds including stigmasterol (2), N-*p-trans*-coumaroyltyramine (3) and taraxerol (4), the structure of these compounds was established by analysis of their spectra and by comparison of their data with those published in the literature. To the best of our knowledge, it is the first report of chemical constituents of *Ochthocosmus* genus.

Keywords: Ochthocosmus *Africanus*, Ochtofridelane, N-*P-Trans*-Coumaroyltyramine, Triterpene, Ixonanthaceae

1. Introduction

Ochthocosmus or *Phyllocosmus* is an endemic or neotropical genus belonging to Ixonanthaceae family. This genus is distributed mainly in tropical rain forest of South America and Africa. In Africa, 12 to 15 species are common among which, *Ochthocosmus africanus* is well spread [1-4]. *Ochthocosmus africanus* is a tree of 10-15 meter high, and decoction of stem barks of this plant is used in traditional medicine as expectorant and antalgic, and to cure dizziness, diarrhea, stiffness and dysmenorrhea [5-7]. Up to date, few chemical investigations have been reported on Ixonantaceae family; previous researchers indicated the isolation and characterization of triterpene (3-fridelanone, betulenic acid, oleanolic acid, hardwickii acid and ellargic acid derivatives) from *Ivingia gabonensis* [8]. To the best of our knowledge, no previous chemical study has been carried out on the genus *Ochthocosmus*. As part of our continuous effort for phytochemical study of Cameroonian plants, *Ochthocosmus africanus* was selected and investigated. During the investigation, one new triterpene ester named ochtofridelane (1) and three known compounds including stigmasterol (2) [9, 10], N-p-trans-coumaroyltyramine (3) [11] and taraxerol (4) [10, 12] were isolated. It was done using various chromatographic techniques on silica gel and characterized on one hand their spectral data, and the literature on the other hand. In this paper, we report for the first time the isolation and the structure elucidation of secondary metabolites from *ochthocosmus* genus.

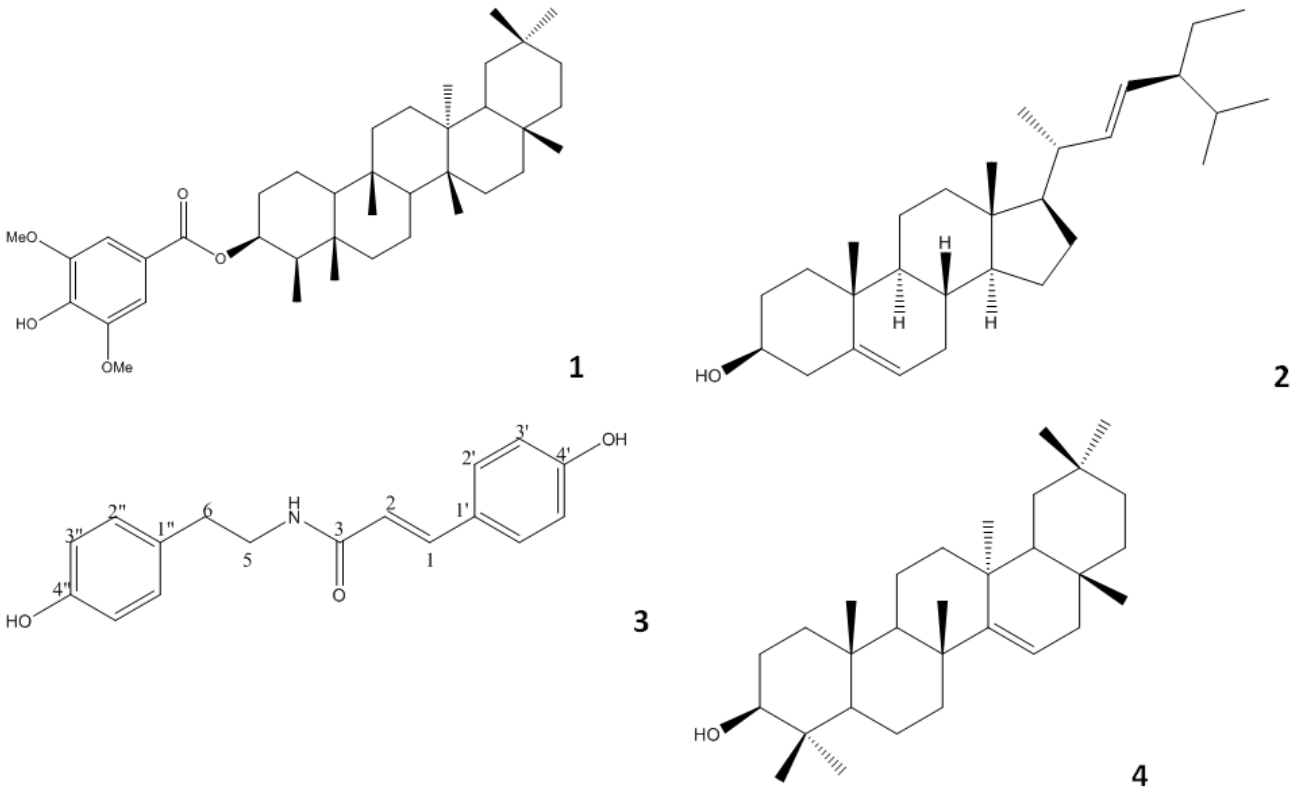

Figure 1. *Structure of compounds 1 – 4.*

2. Results and Discussion

The dried stem bark of *Ochthocosmus africanus* was extracted with a mixture of ethanol and water (7:3) at room temperature. Filtration, vacuum concentration and lyophilization of the resulting solution yielded an oily extract. On part of this extract (50 g) was dissolved in water and extracted with ethyl acetate to give 8.5 g of brown substance. This ethyl acetate extract was subjected to repeated column chromatography and / or preparative TLC to give compounds 1-4 (figure 1) namely ochtofridelane (1) stigmasterol (2), N-p-trans-coumaroyltyramine (3) and taraxerol (4), respectively.

Compound 1 was isolated as colorless needles from *n*-hexane/ethyl acetate (3:7) fraction. It reacted positively both to Lieberman-Burchard and ferric chloride tests suggesting that this compound was a triterpene containing phenolic moiety. Its molecular formula $C_{39}H_{60}O_5$ corresponding to 10 double bond equivalents, was deduced from the analysis of the positive and negative ESI-TOF MS which show pseudo molecular ion pic at *m/z* 609 $[M+H]^+$ and m/z 607 $[M-H]^+$ respectively. The IR spectrum of this compound showed characteristic bands of phenol at 3300 cm^{-1}, ester carbonyl at 1722 cm^{-1}, aromatic ring at 1540 cm^{-1} and ether at 1170 cm^{-1}. The ^{13}C-NMR (Table 1) spectrum of 1 exhibited 36 carbon signals which were assigned using Distorstionless Enhancement by Polarization Transfer (DEPT) and Heteronuclear Single Quantum Correlation (HSQC) to 9 methyl groups among which 7 were angular (δ_C15.0/ δ_H0.89; δ_C18.6/ δ_H0.85; δ_C19.1/ δ_H1.01; δ_C20.6/ δ_H1.00; δ_C32.2/ δ_H1.00; δ_C32.3/ δ_H1.17 and δ_C35.0/ δ_H0.95), one appeared as a doublet (δ_C10.6/ δ_H0.82) and one methoxy (δ_C56.9/ δ_H3.97), 11 sp^3 methylenes, 6 methynes among which one sp^2 (δ_C107.0/ δ_H7,31) and one oxygenated sp^3 methyne δ (δ_C76,4/ δ_H4.86). The 10 remaining signals are attributed to quaternary carbon among which one ester carbonyl of ester at δ_C166.5, two oxygenated sp^2 carbons at δ_C139.4 and δ_C147.0. The presence of 8 methyl signals among which one appeared as doublet in conjunction with the fact that there was no sp^2 carbon on the triterpene moiety indicated that the basic skeleton was fridelane [13]. The integration on ^{1}H NMR spectrum of this compound (Table 1) indicated the presence of two isochrone aromatic protons and methoxy groups which led us to the suggestion of the presence of symmetric aromatic moiety linked to the fridelane skeleton through C_3-O. This aromatic moiety was established to be 4-hydroxy-3,5-dimethoxybenzoyl. In fact, the Heteronuclear Multiple Bond Connectivity (HMBC) of 1 indicated many J^2 and J^3 correlation peaks among which cross peaks between the phenolic proton at δ_H5.87 and the carbon at δ_C139.4 (C4') and δ_C147.0 (C3'), between the methoxy group at δ_H3.97 and the carbon δ_C147.0 (C3') on one hand, and between the aromatic proton at δ_H7,31 and carbon C1'(δ_C122.4), C2' (δ_C107.0), C3' (δ_C147.0), C4' (δ_C139.4) and the carbonyl (δ_C166.5) on other hand. Therefore, the structure of compound 1 was established to be 3-*O*-(4-hydroxy-3,5-dimethoxybenzoyl) fridelane to which the trivial name ochtofridelane was given.

Compound 3 was obtained as colorless needles in *n*–hexane/ethyl acetate (2.5:7.5). Its positive reaction on ferric chloride test showed the phenolic nature of this compound. The molecular formula of 3 was deduced as $C_{17}H_{17}O_3N$ from ESI-TOF MS spectra which showed in positive mode the protonated molecular ion $[M+H]^+$ at *m/z* 284. The ^{13}C NMR spectrum of 3 (Table 1) showed 13 carbon signals which were sorted by DEPT and HSQC techniques as two sp^3 methylenes (δ_C37.2/ δ_H2.68; δ_C44.0/ δ_H3.38) and six sp^2 methynes (δ_C117.7/ δ_H6.64; δ_C118.1/ δ_H6.98; δ_C119.8/ δ_H6.31.0/; δ_C132.0/ δ_H7.32; δ_C132.2/ δ_H6.71; δ_C134.2/ δ_H7.37). The five remaining carbon signals were attributed to sp^2 quaternary carbons among which one amide carbonyl at δ_C168.4 and two oxygenated aromatic carbon at δ_C162.0 and δ_C158.4. The ^1H NMR spectrum of 3 exhibited 8 proton signals which were analyzed using COSY spectrum that showed two AA'BB' system attributed to two *para* substituted aromatic moieties respectively at δ_H7.32 and δ_H6.71 (*J*=7.5 Hz); δ_H6.98 and δ_H6.64 (*J*=8.5 Hz); one pair of doublet of *trans* substituted ethylene moiety protons at δ_H7.37 and δ_H6.31 (*J*=15.5 Hz) and two triplet of two protons each attributed to 1,2-disubstituted ethane moiety at δ_H3.38 and δ_H2.68 (*J*=7.0 Hz). The junction of these different fragments was done using the HMBC spectrum. In fact, this spectrum showed correlation between the ethylene proton at δ_H7.37 and the amide carbonyl (δ_C168.8), aromatic carbon C1' (δ_C132.0); between the aromatic proton at δ_H7.32 and carbon C4' (δ_C162.0), C1 (δ_C143.2). This indicated that one of the *para* substituted aromatic ring was linked to the ethylenic moiety which was also linked to the carbamide in one hand, and between the triplet at δ_H3.38 and the carbonyl of amide (δ_C168.8), carbon C6 (δ_H37.2), C1" (δ_H132.7) and between the aromatic proton at δ_H6.98 and carbon C4" (δ_H158.4), C6 (δ_H37.2) on the second hand, thus the second *para* substituted ring is linked to the ethane moiety which was linked to the carbamide. Therefore, considering the fact that 3 reacted positively with ferric chloride, each aromatic ring should contain one phenolic group. On this basis and by comparison with the literature data, compound 3 was established to be N-*p*-*trans*-coumaroyltyramine [11].

Table 1. *^1H and ^{13}C NMR data of compounds **1** and **3** (δ in ppm and J in Hz); [a] recorded in $CDCl_3$; [b] recorded in CD_3OD (500 MHz for ^1H and 125 MHz for ^{13}C).*

Compound 1[a]			**Compound 3[b]**		
Position	**δ_H**	**δ_C**	**Position**	**δ_H**	**δ_C**
1	1.42; 1.65 (*overlap*)	19.9 t	1	7.37 (d; 15.5)	143.2 d
2	0.93; 1.49 (*overlap*)	39.7 t	2	6.31 (d; 15.5)	119.8 d
3	4.86 (m)	76.4 d	3	-	168.8 s
4	1.49 (*overlap*)	50.2 d	4	-	-
5	-	42.5 s	5	3.38 (t; 7.0)	44.0 t
6	1.10; 1.82 (*overlap*)	41.8 t	6	2.68 (t; 7.0)	37.2 t
7	1.44 (*overlap*)	18.3 t	1'	-	129.1 s
8	1.28(*overlap*)	53.0 d	2'	7.32(d; 7.5)	132.0 d
9	-	39.9 s	3'	6.71(d; 7.5)	118.1 d
10	1.01 (*overlap*)	59.9 d	4'	-	162.0 s
11	1.47; 2.22 (dt)	32.7 t	1"	-	132.7 s
12	1.31 (*overlap*)	31.0 t	2"	6.98(d; 8.5)	132.2 d
13	-	40.1 s	3"	6,64(d; 8.5)	117.7 d
14	-	38.7 s	4"	-	158.4 s
15	1.19; 1.40 (*overlap*)	32.5 t			
16	1.46 (m *overlap*	37.7 t			
17	-	30.4 t			
18	1.55 (*overlap*)	42.7 d			
19	1.52 (*overlap*)	36.1 t			
20	-	28.6 s			
21	1.19; 1.40 (*overlap*)	35.5 t			
22	1.38 (*overlap*)	36.5 t			
23	0.82 (d; 7.0)	10.6 q			
24	0.89 (s)	15.0 q			
25	0.85 (s)	18.6 q			
26	1.01 (s)	19.1 q			
27	1.00 (s)	20.6 q			
28	1.00 (s)	32.2 q			
29	0.95 (s)	35.5 q			
30	1.17 (s)	32.3 q			
1'	-	122.4 s			
2'	7.31 (s)	107.0 d			
3'	-	147.0 s			
4'	-	139.4 s			
C=O	-	166.7 s			
MeO	3.97 (s)	56.9 q			
HO	5,87 (s)	-			

3. Materiel and Methods

General experimental procedures

The chemical constituents of Ochthocosmus africanus were purified and isolated using open column chromatography (CC, Merck Kiesegel 60). A thin layer chromatography (Alu Gram R; SIL G UV 254 Silica gel plates Merck), a gradient of n-hexane and ethyl acetate was use for elution process. Mass spectra were recorded on ESI-MS Shimadzu LC-MS 2020. ^{1}H and ^{13}C NMR spectra as well as 2D NMR experiments were recorded in $CDCl_3$ in a JEOL ECX 500 spectrometer. Chemical shifts are expressed in part per million (δ) relative to TMS as internal standard.

Plant material

Stem bark of (Isonanthaceae) were collected in Yoko, Eastern region of Cameroun in Mars, 2016. This plant was identified by Eric Ngansop a plant taxonomist at National Herbarium of Cameroon (HNC) by comparison with the authentic specimen collected by Letousey and deposited under the number 3431/50B3/SRFK.

Extraction and isolation

The stem bark of this plant was chopped, air dried and crushed to yield 4 kg. This powder was extracted with a mixture of ethanol-water (7:3) by maceration (10 L *2) at room temperature for 48 hours. The filtrate was concentrated and lyophilized to dryness to afford oily material (200 g). 50 g of this crude extract was dissolved in water and extracted with ethyl acetate to yield 8.5 g of brown material. This material was then subjected to repeated column chromatography eluted with gradient of n-hexane – ethyl acetate and monitored by means of TLC to give five fractions F1 (1.0 g; n-hexane), F2 (0.8 g; n–hexane/ethyl acetate; 9:1), F3 (1,1 g; n–hexane/ethyl acetate; 7:3), F4(0.7g; n–hexane ethyl acetate/ethyl acetate 5:5), F5 (1,8 g; ethyl acetate). Less polar fractions F1 and F2, were not studied because they were very rich in fatty acids. F3 (1,1 g), was subjected to column chromatography, followed by preparative TLC eluted with a gradient of n–hexane/ethyl acetate to give stigmasterol 2 (50 mg; n–hexan/ethyl acetate 8:2; Rf=0.75) and N-p-trans-coumaroyltyramine 3 (110 mg; n–hexane/ethyl acetate 7.5:2.5; R_f=0.6), while fraction F4 (0,7 g) was also subjected to successive column chromatography using the same gradient of solvent to yielded compound N-p-trans-coumaroyltyramine 3 (15mg) and (60 mg; n–hexane/ethyl acetate 7:3; R_f=0.7). Finally, in the same manner, F5 (1.8 g) yielded ochtofridelane 1 (30 mg; n–hexane/ethyl acetate 7:3; R_f=0.76).

Ochtofridelane 1: Colorless needles (30 mg), IR ν_{max} (KBr) cm^{-1}: 3300, 1722, 1540, 1170, ESI-TOF MS *m/z* 609 (M+H), ^{1}H and ^{13}C NMR see table 1.

N-*p-trans*-Coumaroyltyramine 3: colorless needles (15mg), IR ν_{max} (KBr) cm^{-1}: 3435, 3100, 1715, 1570, 1080, ESI-TOF MS m/z 284 (M+H), ^{1}H and ^{13}C NMR see Table 1.

Stigmasterol 2: Colorless needles (50 mg), IR ν_{max} (KBr) cm^{-1}: 3435, 3019, 2940, 1636, 1422, 1380, 1215, 1020, 756, 669. ESI-TOF MS m/z 413 (M+H), 13C NMR ($CDCl_3$, 125 MHz): δC 15.6 (C-18), 15.7 (C-29), 17.9 (C-11), 18.9 (C-19), 19.1 (C-26), 19.2 (C-27), 24.8 (C-15), 25.4 (C-21), 25.7 (C-28), 25.9 (C-23), 28.0 (C-16), 28.4 (C-2), 29.1 (C-7), 30.5 (C-8), 34.3 (C-17), 35.3 (C-14), 35.4 (C-4), 35.5 (C-1), 35.9 (C-10), 41.1 (C-20), 44.7 (C-13), 46.2 (C-12), 47.6 (C-25), 49.6 (C-9), 50.0 (C-24), 122.0 (C-6), 125.3 (C-3), 131.1 (C-22), 142.0 (C-5).

Taraxerol 4: colorless needles (60 mg), IR ν_{max} (KBr) cm^{-1}: 3401, 2916, 2849, 1609, 1460, 1441, 1383, 1376, 1037, 762. ESI-TOF MS m/z 427 (M+H), ^{13}C NMR ($CDCl_3$, 125 MHz): δC 16.0 (C-24), 16.1 (C-25), 17.5 (C-11), 19.3 (C-6), 21.5 (C-30), 26.0 (C-27), 27.3 (C-2), 28.1 (C-23), 29.1 (C-20), 29.9 (C-28), 30.2 (C-26), 33.1 (C-22), 33.5 (C-29), 34.0 (C-21), 35.2 (C-7), 35.9 (C-12), 36.8 (C-16), 37.8 (C-13), 37.3 (C-17), 38.3 (C-10), 38.3 (C-1), 38.6 (C-4), 39.6(C-8), 41.9 (C-19), 49.3 (C-9), 49.8 (C-18), 56.1 (C-5), 79.7 (C-3), 117.5 (C-15), 158.7 (C-14).

Conflicts of Interest

The authors declare that there no conflict of interests. All the authors read and approved the final manuscript.

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