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 Ixonanthaceae 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.
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 Distortionless Enhancement by Polarization Transfer (DEPT) and Heteronuclear Single Quantum Correlation (HSQC) to 9 methyl groups among which 7 were angular (δ$_C$ 15.0/ δ$_H$ 0.89; δ$_C$ 18.6/ δ$_H$ 0.85; δ$_C$ 19.1/ δ$_H$ 1.01; δ$_C$ 20.6/ δ$_H$ 1.00; δ$_C$ 32.2/ δ$_H$ 1.00; δ$_C$ 32.3/ δ$_H$ 1.17 and δ$_C$ 35.0/ δ$_H$ 0.95), one appeared as a doublet (δ$_C$ 10.6/ δ$_H$ 0.82) and one methoxy (δ$_C$ 56.9/ δ$_H$ 3.97), 11 sp$^3$ methylenes, 6 methynes among which one sp$^2$ (δ$_C$ 107.0/ δ$_H$ 7.31) and one oxygenated sp$^3$ methyne δ (δ$_C$ 76.4/ δ$_H$ 4.86). The 10 remaining signals are attributed to quaternary carbon among which one ester carbonyl of ester at δ$_C$ 166.5, two oxygenated sp$^2$ carbons at δ$_C$ 139.4 and δ$_C$ 147.0. 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 $^2$J and $^3$J correlation peaks among which cross peaks between the phenolic proton at δ$_H$ 5.87 and the carbon at δ$_C$ 139.4 (C4’) and δ$_C$ 147.0 (C3’), between the methoxy group at δ$_C$ 3.97 and the carbon δ$_C$ 147.0 (C3’) on one hand, and between the aromatic proton at δ$_H$ 7.31 and carbon C1’(δ$_C$ 122.4), C2’ (δ$_C$ 107.0), C3’ (δ$_C$ 147.0), C4’ (δ$_C$ 139.4) and the carbonyl (δ$_C$ 166.5) 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_{22}O_{3}N 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\(_C\)37.2/ δ\(_H\)2.68; δ\(_C\)44.0/ δ\(_H\)3.38) and six sp\(^2\) methylenes (δ\(_C\)117.7/ δ\(_H\)6.64; δ\(_C\)118.1/ δ\(_H\)6.98; δ\(_C\)119.8/ δ\(_H\)6.31.0/; δ\(_C\)132.0/ δ\(_H\)7.32; δ\(_C\)132.2/ δ\(_H\)6.71; δ\(_C\)134.2/ δ\(_H\)7.37). The five remaining carbon signals were attributed to sp\(^3\) quaternary carbons among which one amide carbonyl at δ\(_C\)168.4 and two oxygenated aromatic carbon at δ\(_C\)162.0 and δ\(_C\)158.4. The \(^1\)H 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\(_H\)7.32 and δ\(_H\)6.71 (J=7.5 Hz); δ\(_H\)6.98 and δ\(_H\)6.64 (J=8.5 Hz); one pair of doublet of trans substituted ethylene moiety protons at δ\(_H\)7.37 and δ\(_H\)6.31 (J=15.5 Hz) and two triplet of two protons each attributed to 1,2-disubstituted ethane moiety at δ\(_H\)3.38 and δ\(_H\)2.68 (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\(_H\)7.37 and the amide carbonyl (δ\(_C\)168.8), aromatic carbon C1' (δ\(_C\)132.0); between the aromatic proton at δ\(_H\)7.32 and carbon C4' (δ\(_C\)162.0), C1 (δ\(_C\)143.2). 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\(_H\)3.38 and the carbonyl of amide (δ\(_C\)168.8), carbon C6 (δ\(_C\)37.2), C1'' (δ\(_C\)132.7) and between the aromatic proton at δ\(_H\)6.98 and carbon C4'' (δ\(_H\)158.4), C6 (δ\(_H\)37.2) 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. \(^1\)H 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\(_3\)OD (500 MHz for \(^1\)H and 125 MHz for \(^{13}\)C).

| Position | \(\delta_H\) (\(\delta_C\) t) | Position | \(\delta_H\) (\(\delta_C\) t) |
|----------|-----------------|----------|-----------------|
| 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. Material and Methods

General experimental procedures

The chemical constituents of Ochthocosmus africanus were purified and isolated using open column chromatography (CC, Merck Kieselgel 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-TOF 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 parts per million ($\delta$) relative to TMS as internal standard.

Plant material

Stem bark of (Isonanthaceae) were collected in Yoko, Eastern region of Cameroon 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 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 (0.7 g; 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-hexane/ethyl acetate 8:2; $R_f$=0.6) and N-p-trans-coumaroyltyramine 3 (150 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 $\nu_{\text{max}}$ (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 (150 mg), IR $\nu_{\text{max}}$ (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 $\nu_{\text{max}}$ (KBr) cm$^{-1}$: 3435, 3019, 2940, 1636, 1422, 1380, 1215, 1020, 756, 669, ESI-TOF MS m/z 413 (M+H), $^{13}$C NMR (CDCl$_3$, 125 MHz):

$\delta$C 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 $\nu_{\text{max}}$ (KBr) cm$^{-1}$: 3401, 2916, 2849, 1609, 1440, 1441, 1383, 1376, 1037, 762.

$^{13}$C NMR (CDCl$_3$, 125 MHz):

$\delta$C 34.0 (C-15), 34.3 (C-17), 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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