



Sterols from the fruit barks of *Coelocaryon klainei* Pierre ex Heckel (Myristicaceae)

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ABSTRACT

Two sterols namely β -sitosterol (1) and stigmasterol (2) were isolated from methanolic extract of the fruit barks of *Coelocaryon klainei* Pierre ex Heckel (Myristicaceae). They were isolated for the first time from this species. It is the first isolation of compounds in this genus *Coelocaryon*. The structures were elucidated on the basis of one and two-dimensional NMR, SM, IR and UV.

Key Words: *Coelocaryon klainei*, Myristicaceae, β -sitosterol and stigmasterol.

INTRODUCTION

The genus *Coelocaryon* (Myristicaceae) comprises 4 recognized species (*Coelocaryon botryoides* Vermoesen, *Coelocaryon oxycarpum* Stapf, *Coelocaryon preussii* Warb., *Coelocaryon sphaerocarpum* Fouilloy). The genus *Coelocaryon* is used in traditional medicine to treat various diseases. *Coelocaryon oxycarpum* stems are taken as purgative. The leaves and the seeds of *Coelocaryon sphaerocarpum* are used to treat somnolence. *Coelocaryon klainei* is the most known and used. This species is a tree that grows 30 m in height (Lemmens *et al.*, 2012). You can find it in Africa (Côte d'Ivoire, Bénin, Nigeria, Centrafrique, Gabon and R.D.C) (Fouilloy, 1965; Fouilloy, 1972; Normand, 1993; Foahom, 2002; Christy *et al.*, 2003; Akoègninou *et al.*, 2005; Hawthorne, 2006; Tchouya *et al.*, 2015). The wood is very durable and is commonly harvested, mainly for trade. The tree produces wood, marketed as "ekoune" or "ekun", which is suitable for joinery (Lemmens *et al.*, 2012). In Côte d'Ivoire the seeds are used to treat diabetes and hypertension. Their stem barks are used in the treatment of hemorrhoids, dysentery (Onanga *et al.*, 1999), diarrhea and venereal diseases. This species is poorly studied so far. So it was important to investigate the chemistry of *Coelocaryon*. There is only one chemical study on *Coelocaryon klainei*

Pierre ex Heckel published, reporting the presence of polyphenols, tannins, flavonoids, sterols/triterpenes, coumarins, saponosides and sugars from the leaves and the barks (Tchouya *et al.*, 2015). We report here, the isolation and characterization of two known sterols from the fruit barks of *Coelocaryon klainei*.

MATERIALS AND METHODS

The NMR spectra were recorded on a Brüker Advance-300 operating at 300 MHz, using TMS as internal standard. Chemical shifts were quoted in δ ppm and coupling constant J was measured in Hertz (Hz). One-dimensional ¹H and ¹³C spectra were acquired under standard conditions. Currently, ¹H-¹H homonuclear (COSY, NOESY) and ¹H-¹³C heteronuclear (HSQC, HMBC) correlation techniques were routinely applied in field of constitutional analysis. These techniques were recorded on a Brüker Avance-400 operating at 400 MHz. Column chromatography was performed on silica gel (Kieselgel 60, particle size 0.040–0.063 mm) and Sephadex® LH-20. TLC was run on silica gel pre-coated glass plates (Merck silica gel 60 F₂₅₄). Spots were detected by spraying with phosphomolibdic acid. This operation was followed by a heating. ESIMS Mass spectra were obtained with ITQ 900 spectrometer using an Agilent DB-5HT (30 x 0.32 x 0.1) column. IR spectra were recorded with a PerkinElmer type 257 spectrometer.

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Plant material

The fruit barks of *Coelocaryon klainei* were collected in June 2015 in Abidjan (South of Côte d'Ivoire). They were identified by Pr. Ipou Ipou Joseph (Centre National de Floristique- Université Félix Houphouët-Boigny). A voucher specimen (n° CK-AKOUBET-Abidjan2015-1) is deposited at the Herbarium of the Botanic Laboratory (Université Félix Houphouët-Boigny).

Isolation

Air-dried pulverized fruit barks of *Coelocaryon klainei* (1500 g) were three times defatted with cyclohexane and successively extracted with CH_2Cl_2 , AcOEt and MeOH. The collected extracts were evaporated under reduced pressure to yield 9.8 g of cyclohexane extract, 5.3 g of CH_2Cl_2 extract, 3.8 g of AcOEt extract and 1.9 g of MeOH extract. The cyclohexane extract was chromatographed over silica gel column chromatography, eluting with cyclohexane-dichloromethane gradient systems to give eight fractions (F-1 to F-8). Fraction F-6 was purified using repeated Sephadex[®]LH-20 [$\text{MeOH}/\text{CH}_2\text{Cl}_2$ (1/2)] and column chromatography on silica gel, to yield 4.9 mg of compound 1 and 5.6 mg of compound 2 (Fig. 1). The structures of these compounds (Fig. 1) were established according to their spectral data (NMR, IR and MS).

Identification of compounds 1 and 2

***β*-Sitosterol (1)**: colourless crystalline; ^1H and ^{13}C NMR (400 MHz) data in table 1; IR (CHCl_3): ν_{max} (cm^{-1}) = 3570, 2864, 1585, 1016, 609; UV (CHCl_3): λ_{max} (nm) = 220; ESI-MS (m/z): 415 $[\text{M}+\text{H}]^+$ (molecular formula $\text{C}_{29}\text{H}_{50}\text{O}$).

Stigmasterol (2): white amorphous solid; ^1H and ^{13}C NMR (400 MHz) data in table 1; IR (CHCl_3): ν_{max} (cm^{-1}) = 3428, 1192, 699; UV (CHCl_3): λ_{max} (nm) = 257; ESI-MS (m/z): 413 $[\text{M}+\text{H}]^+$ (molecular formula $\text{C}_{29}\text{H}_{48}\text{O}$).

RESULTS AND DISCUSSION

Compound (1) was isolated as a colourless crystalline. The UV spectrum of this compound showed maximum absorption bands at λ_{max} : 220 nm. Its MS spectrum shown the pseudo-molecular ion fragment $[\text{M}+\text{H}]^+$ at (m/z) = 415. So, its molecular formula was deduced to be $\text{C}_{29}\text{H}_{50}\text{O}$. The IR absorption band at 3570 cm^{-1} was attributed to the hydroxyl group and the broad band (1585 cm^{-1}) indicates the presence of (C=C) stretching. These absorption frequencies resemble the absorption frequencies observed for *β*-sitosterol as resembled data published by (Arjun *et al.*, 2010; Bulama *et al.*, 2015).

The ^1H and ^{13}C NMR spectra (Table 1) allowed the identification of the steroidal skeleton. The typical H-6 of the steroidal skeleton was evident as a multiplet at δ_{H} 5.30 ppm that integrated for one proton. The spectrum further revealed signals at δ_{H} 0.92, 0.84, 0.82 and 0.80 ppm (3H each) assignable to four methyl group at C-19, C-24, C-26, C-27 respectively. This compound is having six methyl, eleven methylene and three quaternary carbons with a hydroxyl group. The above spectral features are in closed agreement to those observed for *β*-sitosterol according to (Escudero *et al.*, 1985; Manoharan *et al.*, 2005).

The ^{13}C -NMR has shown recognizable signals 140.7 and 121.6 ppm, which are assigned C-5 and C-6 double bonds respectively. The carbon peak at δ_{C} 71.9 is in the "alcohol" region (C-O) in the range expected for methine carbon (CH-O), so it was assigned to C-3. HMBC correlation of the proton, H-3 (3.53 ppm), to carbon C-5 (δ_{C} 140.7) and to proton H-3 to the carbon at δ_{C} 37.4 ppm (C-1) confirmed the hydroxyl position as being located on C-3.

Spectra show twenty-nine carbon signal including six methine groups, eleven methylene groups, six methyl groups and three quaternary carbons. The alkene carbons appeared at δ 140.7 and 121.6 ppm. Compound 1 was identified as *β*-sitosterol. Its physical and spectral data are consistent to those reported by literature (Manoharan *et al.*, 2005; Anjoo *et al.*, 2011).

Compound (2) was obtained as white amorphous solid. The UV λ_{max} value of compound 2 was 257 nm. Mass spectrum of isolated compound 2 showed pseudo-molecular ion at m/z $[\text{M}+\text{H}]^+$ peak at m/z 413 which corresponds to the molecular formula $\text{C}_{29}\text{H}_{48}\text{O}$ (fig. 1). IR absorption bands at 3428 and 699 cm^{-1} were due to the presence of hydroxyl and alkenes respectively.

The ^1H NMR spectrum (Table 1) exhibited characteristic signals of protons at δ_{H} 3.50 (H-3) appeared as a triplet of a double doublet (*td*) and at δ_{H} 5.29 (H-6, *m*) for an olefinic proton. Two olefinic protons appeared downfield at δ (4.97, *m*) and (5.13, *m*). Six methyls protons also appeared at δ 0.90, δ 0.83, δ 0.80, δ 0.79, δ 0.72 and δ 1.02 ppm (3H each, *s*, CH_3) (Table 1). These attributions were done on the basis of its HSQC experiment data.

The ^{13}C NMR has shown recognizable signal at δ_{C} 141.1 and 121.6 ppm, which corresponds to double bond at C-5 and C-6 double bonds respectively as well as it also represents signals at 138.4 and 129.4 ppm, which shows one more double bond in between C-20 and C-21. The δ value at 72.0 ppm is due to C-3 β -hydroxyl group. The signal at δ 29.5 and δ 19.7 ppm corresponds to angular carbon atom at C-25 and C-27 respectively (table 1).

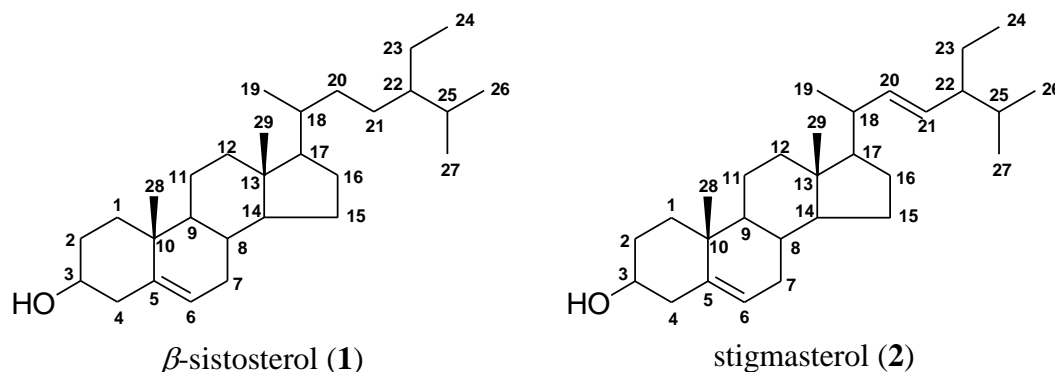


Figure 1: Structures of compounds 1 and 2.

Table 1: ¹H and ¹³C NMR spectral data of β-sitosterol (**1**) and stigmasterol (**2**) in CDCl₃

Position	1		2	
	¹³ C	¹ H	¹³ C	¹ H
1	37.4		37.5	
2	31.8		32.0	
3	71.9	3.53 (<i>tdd</i> ,1H, J = 4.4,4.1,3.9)	72.0	3.50 (<i>tdd</i> ,1H, J = 4.4,4.1,3.9)
4	42.3		42.3	
5	140.7		141.1	
6	121.6	5.30 (<i>m</i> ,1H)	121.6	5.29 (<i>m</i> ,1H)
7	32.0		31.7	
8	32.0		31.7	
9	50.1		50.1	
10	36.4		36.5	
11	21.2		21.4	
12	39.7		39.7	
13	42.4		42.2	
14	56.9		56.4	
15	26.2		42.1	
16	28.4		29.1	
17	56.2		56.0	
18	36.1		40.5	
19	19.0	0.92 (<i>d</i> ,3H, J = 6.5 Hz)	21.6	0.90 (<i>d</i> ,3H, J = 6.1 Hz)
20	34.0		138.4	4.97 (<i>m</i> ,1H)
21	26.1		129.4	5.13 (<i>m</i> ,1H)
22	45.9		45.9	
23	23.1		25.2	
24	12.2	0.84 (<i>d</i> ,3H, J = 7.3 Hz)	12.1	0.83 (<i>d</i> ,3H, J = 7.3 Hz)
25	29.3		29.5	
26	20.0	0.82 (<i>d</i> ,3H, J = 6.3 Hz)	20.1	0.80 (<i>d</i> ,3H, J = 6.7 Hz)
27	19.5	0.80 (<i>d</i> ,3H, J = 6.3 Hz)	19.7	0.79 (<i>d</i> ,3H, J = 6.6 Hz)
28	19.0	0.68 (<i>s</i> ,3H)	18.6	0.72 (<i>s</i> ,3H)
29	11.9	1.00 (<i>s</i> ,3H)	12.1	1.02 (<i>s</i> ,3H)

The ¹H and ¹³C NMR spectroscopy data (Table 1) were similar to those of β-sitosterol (**1**), the only difference was the absence of two methylene groups and the presence of two methine groups for compound **2**. The position of the two methines was established by HMBC correlations of H-24 (δ_H 0.83) to C-21 (δ_C 129.4) and H-20 (δ_H 4.97) to C-22 (δ_C 45.9). From the above observations, isolated compound was found to be stigmasterol (**2**) (Anjoo, *et al.*, 2011; Venkata *et al.*, 2012; Luhata *et al.*, 2015).

CONCLUSION

The phytochemical examination of *Coelocaryon klainei* belonging to the family (Myristicaceae) fruit barks was effectively carried out. From these chemical and spectral evidences compound **1** and compound **2** were confirmed as respectively β-Sitosterol (**1**) and Stigmasterol (**2**) (fig. 1). The complete ¹H and ¹³C NMR spectral assignments of the two isolated compounds were made based on COSY, HSQC, HMBC, and ESI-MS spectroscopic data. Their structures are in agreement with those reported by literature.

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REFERENCES

- Akoègninou, A., Van der Burg, W.J., Van der Maesen, L.J.G. (2006). Flore analytique du Bénin. Backhuys Publishers, Leiden, Netherlands. pp.1034.
- Anjoo Kamboj, Ajay Kumar Saluja. (2011). Isolation of stigmasterol and β-sitosterol from petroleum ether extract of aerial parts of *Ageratum conyzoides* (Asteraceae). *International Journal of Pharmacy and Pharmaceutical Sciences*, 3, 1, 94-96.
- Arjun, P., Jha, S., Murthy, P.N., Manik, M., and Sharone, A. (2010). Isolation and Characterization of β-Sitosterol from the leaves of *Hygrophila spinosa*. *International Journal of Pharma Science & Research*, 1, 2, 95-100.
- Bulama J.S., Dangoggo S.M., Mathias S.N. (2015). Isolation and Characterization of Beta-Sitosterol from ethyl acetate extract of root bark of *Terminalia glaucescens*. *International Journal of Scientific and Research Publications*, 5, 2250-3153.
- Christy, P., Jaffré, R., Ntougou, O., Wilks, C. (2003). La forêt et la filière bois au Gabon. Projet Aménagement Forestier et Environnement, Libreville, Gabon. pp. 389.
- Escudero, J., Lopez, C. Rabanal, R.M., Valverde, S. (1985). Secondary metabolites from *Satureja* species. New triterpenoid from *Satureja acinos*. *Journal of Natural products*, 48, 128 -131. [DOI]
- Foahom, B. (2002). Insect pest incidence on timber tree species in natural forest in South Cameroon. Tropenbos-Cameroon Document 12. Tropenbos Cameroon Programme, Kribi, Cameroon. pp. 54.
- Fouilloy, R. (1965). Myristicacées. Flore du Gabon. Volume 10. Muséum National d'Histoire Naturelle, Paris, France. pp. 83-101.
- Fouilloy, R. (1972). Trois arbres nouveaux d'Afrique tropicale (Laur. – Myrist. – Sapind.). *Adansonia*, ser., 2, 12, 4, 545-555.
- Hawthorne, W., Jongkind, C. (2006). Woody plants of western African forests: a guide to the forest trees, shrubs and lianes from Senegal to Ghana. Kew Publishing, Royal Botanic Gardens, Kew, United Kingdom. pp.1023.

- Lemmens, R.H.M.J., Louppe, D., Oteng-Amoako, A.A. (2012). Ressources végétales de l'Afrique tropicale, 7(2). Bois d'œuvre 2. Fondation PROTA, Wageningen, Pays-Bas/CTA, Wageningen, Pays-Bas. pp. 233-235.
- Luhata, L. P., Munkombwe N. M. (2015). Isolation and Characterisation of Stigmasterol and β -Sitosterol from *Odontonema Strictum* (Acanthaceae). *Journal of Innovations in Pharmaceuticals and Biological Sciences*, 2, 1, 88-95.
- Manoharan, K.P., Haut, B.T.K., Yang, D. (2005). Cycloartane types triterpenoids from the rhizomes of *Polygonum bistorta*. *Phytochemistry*, 66, 1168 - 1173. [\[DOI\]](#)
- Normand D. (1933). Les Bois de Myristicacées du Gabon. In: *Revue de botanique appliquée et d'agriculture coloniale*, 13^e année, bulletin n°143, 471-479. [\[DOI\]](#)
- Onanga, M., Ekouya, U. A., Ouabonzi, A., Itoua G.B. (1999). Ethnobotanical, pharmacological and chemical studies of plants used in the treatment of 'Mwandza' dermatitis. *Fitoterapia*, 70, 579-585. [\[DOI\]](#)
- Tchouya, G. R. F., Souza, A., Tchouankeu, J. C., Yala, J.-F., Boukandou, M., Foundikou, H., Obiang, G. D. N., Boyom, F. F., Mabika, R. M., Menkem, E. Z., Ndinteh, D. T., Lebib J. (2015). Ethnopharmacological surveys and pharmacological studies of plants used in traditional medicine in the treatment of HIV/AIDS opportunistic diseases in Gabon. *Journal of Ethnopharmacology*, 162, 306-316. [\[DOI\]](#)
- Venkata, S. Prakash, C., Indra P. (2012). Isolation of Stigmasterol and β -Sitosterol from the dichloromethane extract of *Rubus suavissimus*. *International Current Pharmaceutical Journal*, 1, 9, 239-242. [\[DOI\]](#)