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Research Article

Isolation and Characterisation of Stigmasterol and B -Sitosterol from *Odontonema Strictum* (Acanthaceae)

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Abstract

Phytochemical screening of the extracts obtained from the leaves of *Odontonemastrictum*, a plant used in folklore medicine in Burkina-faso for its anty-hypertensive properties, indicated the presence of: flavonoids (type of flavones), saponins, glycosides, tannins, steroids and terpenoids. Column chromatography of the crude extracts lead to a number of fractions. TLC fingerprinting and the spraying reagent (Concentrated H_2SO_4 and vanillin in methanol) were used to identify the fraction containing phytosterols. The isolation and purification afforded white crystalline powder which was subjected to physical, chemical and spectral identification by IR, 1H-NMR, 2D-NMR and 13C-NMR. The compound was identified as a mixture of stigmasterol and β-sitosterol.

Key words: *Odontonema strictum, phytosterols, stigmasterol and β-sitosterol*

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1. Introduction

The genus *Odontonema* includes flowering plants of the *Acanthaceae* family and it is mostly found in tropical region and common garden inclusions. The plant belonging to the dicotyledonous angiosperm subclass of *Asteridae*, order of *Scrophulariales*, to the sub family of justiciaceae and gender *Odontonema*.

Three species are used in traditional medicine: the ground leaves and the stem of *Odontonema callistachyum* are applied on open wounds in order to heal them in Sierra Mazateca (Mexico) [1]. The leaves of the species *Odontonema tubiforme (Bertol.) kuntze* is used by Kuna, Ngöbe-Buglé, and Teribe Indians as an anti-inflammatory and for inducing child birth [2]. *Odontonema strictum* is used in Burkina Faso for the treatment of hypertension [3]. The genus *Odontonema* is the likely sources of bioactive secondary metabolites.

Stigmasterol and sitosterol are two phytosterols well spread in plants and animals as well as fungi, and have structural similarity to cholesterol. The most important benefit for these two secondary metabolites is their enrolment amongst the health promoting constituents of natural foods which

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contains them. In fact, the European Foods Safety Authority [4] recommends consuming about 1.5 - 2.4 g/day of phytosterols and/or stanols in order to reduce blood cholesterol. Furthermore, FDA has approved the role of foods containing phytosterol esters inside a low saturated fat and cholesterol diet in reducing the risk of heart disease, especially consumption of at least 1.3 g/day sterols, twice a day [5]. The antibacterial activities of stigmasterol and beta sitosterol have been reported in many reports [6][7][8]. Research has indicated that stigmasterol may be useful in prevention of certain cancers, including ovarian, prostate, breast, and colon cancers. It also possesses potent antioxidant, hypoglycemic and thyroid inhibiting properties [9]. Corfuff and Benedi [10] reported the laxative properties of stigmasterol. β- sitosterol is used as an antioxidant and an antidiabetic agent [11].

The present study is aimed to extract, isolate and characterize by spectroscopic methods the stigmasterol and β- sitosterol from *Odontonema strictum*.

2. Experimental

Collection, Identification and preparation of plant materials

Plant specimens were collected in Lusaka (January-February 2014) and identified by Dr. CHUBA and his team of the Department of Biological Sciences/University of Zambia. Voucher samples were prepared and deposited in the Herbarium of the Department of Biology. The plant samples were shade dried at room temperature and powdered into a fine powder in a blender.

Extraction, fractionation and isolation Procedures

The powdered leaf material (310g) of *Odontonema strictum* (Acanthaceae) were extracted with 800 ml of Methanol (MeOH) and DichloroMethane (DCM) for 24 hours. The supernatant was filtered through Whatman N°1 filter paper. The extract was then left to dry under room temperature. The quantity extracted was 25g.

The crude extract was subjected to the qualitative phytochemical analysis (Table 1). Phytochemical screening of the extracts indicated the presence of flavonoids (type of flavones), saponins, glycosides, tannins, steroids and terpenoids.

Vacuum Liquid chromatography (VLC) was chosen to separate compounds using silica gel as a stationary phase. 90 g of silica gel for Thin Layer Chromatography (TLC)(Merck) was mixed with hexane to form slurry and stirred using a stirring rod. 25g of the crude extracts was mixed with 1 g of silica gel and the mixture was dried in room temperature. A vacuum was created to allow separation of compounds. Initially, hexane was gradually added into the column to remove fats, waxes and some chlorophyll. The polarity was increased by addition of EtOAC (0% -100 %). The total volume used was 200 ml. 26 fractions were collected and left to dry at room temperature. Fractions were mixed according to the results obtained from TLCs. The use of concentrated sulfuric acid and vanillin as a spraying reagent revealed the presence of sterols in fractions 7 and 8 (870 mg).

A normal Column Chromatography (CC) was used to fractionate compounds from fractions 7-8 using Hexane- EtOAc- $CHCl_3$: 5:1:2 as the solvent system. 6 fractions were obtained. A white crystal was formed in one of the eluates. The crystals were named compound 1.

The compound (1) was subjected to TLC using several solvent systems including Ethyl acetate: Hexane (1:5), Ethyl acetate: Hexane: Chloroform (1.5: 2) and it showed

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to be homogenous compound. The white crystalline powder (80mg) with melting point (134-136°C) and R_f value 0.55 (EtAc/Hex: 1/3) was further subjected to IR, Proton NMR (400MHz), Carbon-13 NMR (100 MHz) and 2NMR.

Tests for steroid

Compound (1) gave a positive test to Liebermann Buchard and Salkowski reagents for steroidal nucleus [12][13] [14][15].

Spectroscopic characterization

Different spectroscopic methods were used to elucidate the structure of isolated compound (1), including: IR, 1H NMR and 13C NMR. The infra red spectrum was recorded on FTIR Perkin Elmer, 1H-NMR and 13C-NMR spectra were recorded using $CDCl_3$ as solvent on Bruker Advance II 400 NMR spectrometer at the department of Chemistry, University of Cape Town.

On subjection to IR Spectroscopic analysis, the observed absorption bands are 3547.41cm^{-1} that is characteristic of O-H stretching. Absorption at 3232.75 cm^{-1} is assumed to be due to cyclic olefinic –HC= CH- structure, 3025 cm^{-1} due to =CH structure and 2857.75 cm^{-1} assigned to C-H structure. Other absorption frequencies include 1638.83cm^{-1} as a result of C=C absorption, however, this band is weak (Pretsch et al., 2000). 1462 cm^{-1} is a bending frequency for cyclic (CH2) n and 1382 cm^{-1} for –CH2 (CH3)2γ. The absorption frequency at 1071.28 cm-1 signifies cycloalkane. These absorption frequencies resemble the absorption frequencies observed for Stigmasterol [16].

The 1H NMR spectrum of compound (1) varied between 0.736 to 5.378 ppm, This spectrum showed the presence of 6 high intensity peaks indicating the presence of six methyl groups at δ 0.736 , 0.843, 0.967, 1.037, 1.200 and 1.534 ppm. The proton corresponding to the H-3 of a sterol moiety was appeared as a triplet of doublet at δ 3.529 ppm. At δ 5.197 ppm and at δ 5.378 ppm corresponds to a peak in the form of a single in the region of the ethylene protons suggesting the presence of three protons.

13NMR spectrum of Compound (1) has given signal at 140.943 and 1211.321ppm for C5=C6 double bond respectively, 71.974 for C3 β-hydroxyl group 19.064 and 12.060 for angular methyl carbon atoms for C19 and C18 respectively (table 1).138.404 ppm for C-22 and 129.341ppm for C-23. The C5, C6, C22 and C23 appeared to be alkene carbons.

3. Results and Discussion

From the positive tests for steroids given by compound 1, it is assumed to be a compound containing steroidal nucleus. Compound (1) is white crystalline substance with melting point 134-136°C and Rf value 0.55 (EtAc/Hex: 1/3). On subjection to IR spectroscopic analysis, the observed absorption bands are 3547.41cm^{-1} that is characteristic of O-H stretching. Absorption at 3232.75 cm^{-1} is due to cyclic olefinic –HC= CH- structure, 3025 cm^{-1} due to =CH structure and 2857.75 cm1 assigned to C-H structure. 1462 cm^{-1} is a bending frequency for cyclic (CH2) n and 1382cm^{-1} for –CH2 (CH3)2γ. The absorption frequency at 1071.28cm^{-1} signifies cycloalkane. These absorption frequencies resemble the absorption frequencies observed for Stigmasterol.

The proton NMRshowed the proton of H-3 appeared as a multiplet at δ 3.529 ppm and revealed the existence of signals for Olefinic proton at δ5.067(m), 5.197 (m), 5.378 (m), and 2.323(m). Angular methyl proton at 0.69(s), 0.80(s) and 1.02(s) corresponds to C18 and C19 proton respectively.

The ^{13}C-NMR has shown recognizable signals at 140.943 ppm and 1211.321 ppm which are assigned C5 and C6 double bonds respectively. The value at 19.064ppm corresponds to angular carbon

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atom (C19) 138.404 ppm for C-20 and 129.341ppm for C-21. Spectra show twenty nine carbon signal including six methyls, nine methylenes, eleven methane and three quaternary carbons. The alkene carbons appeared at 140.943, 138.404, 129.341 and 1211.321ppm.

According to the literature β-sitosterol and Stigmasterol are always in a mixture form in which may have maximum portion of stigmasterol. It is very difficult to obtain Stigmasterol in pure state. The only difference between the two compounds is the presence of C22=C23 double bond in Stigmasterol and C22-C23 single bond in β-sitosterol. Furthermore, literatures have shown that sitosterol is difficult to be obtained in pure state [17][18][19]. Stigmasterol and beta-sitosterol have the same Rf value 0.55 (EtAc/Hex: 1/3) despite the use of several solvent systems.

Therefore, compound (1) is a mixture of β-sitosterol and Stigmasterol. β-sitosterol is colorless needle-like solid with a melting point of 147-149°c.

The ^{1}H and ^{13}C NMR values for all the protons and carbons were assigned on the basis of COSY, HMQC and HMBC correlations and were given in Table 2.

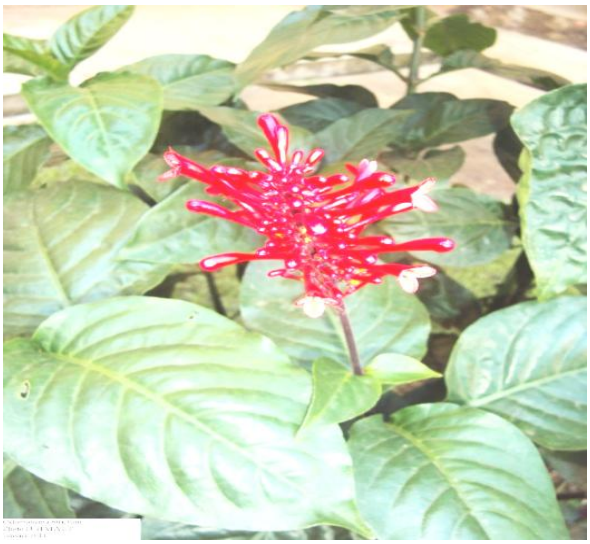

Figure 1: The species *Odontonema strictum*

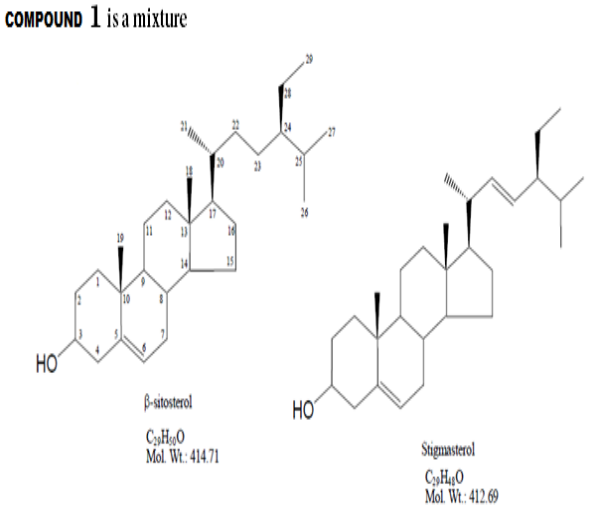

Figure 2: Chemical structure of β-sitosterol and Stigmasterol

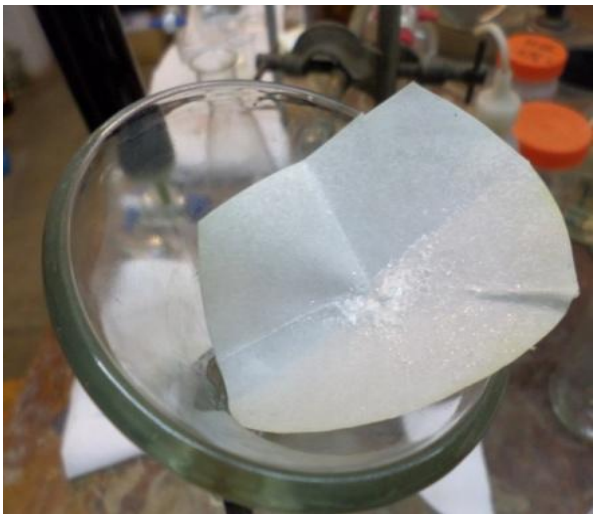

Figure 3: Compound (1) in crystal state

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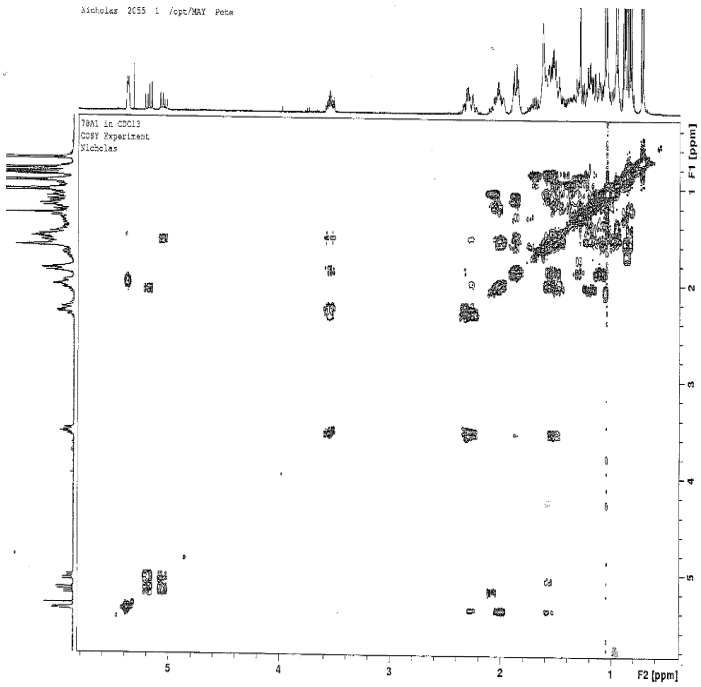

Figure 4: COSY spectrum of compound (1)

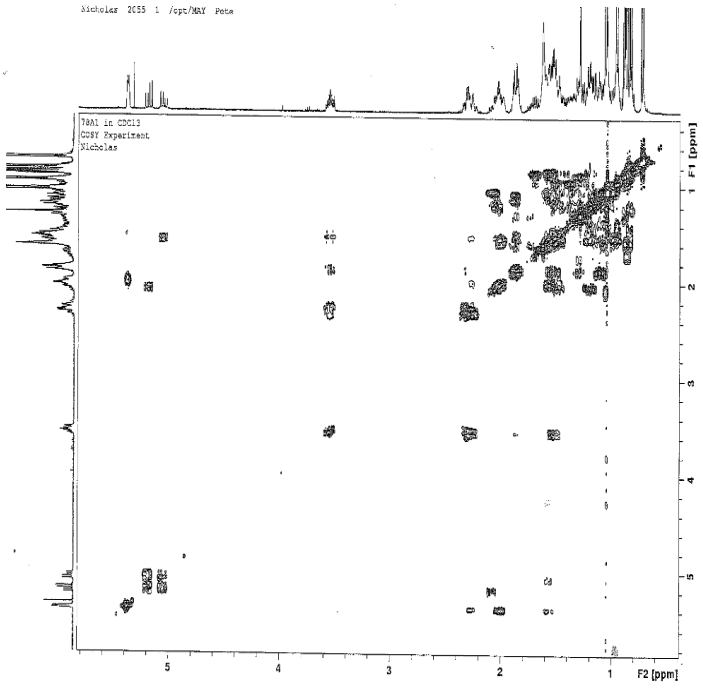

Figure 5: ^{1}H NMR spectrum of compound (1)

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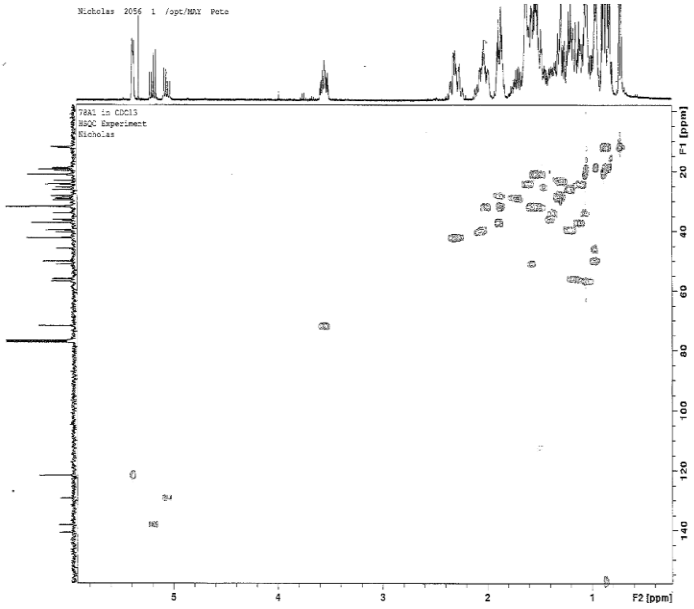

Figure 6: HSQC spectrum of compound (1)

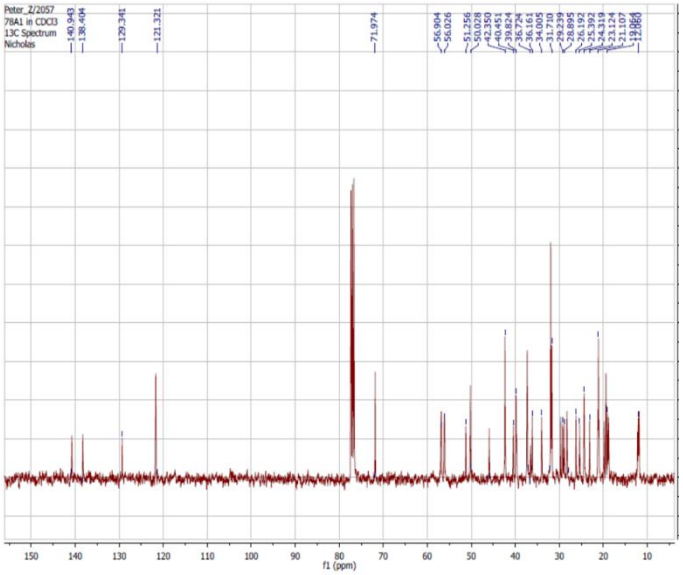

Figure 7: 13C NMR spectrum of compound (1)

Table 1: Phytochemical screening of *O. strictum* extract

Chemical Constituent	Tannins ($FeCl_3$ test)	Saponins (Foam test)	Sterols and Triterpenoids (Libermann-Boucher)	Flavonoids (Shinoda test)	Alkaloids (Mayers test)	Glycosides (Keller-kiliani test)
Leaves	+++	+++	+	+++	±	+
Flowers	++	+++	+	+++	-	+

Legend: - = absent; ± = low present; + = present; ++ = abundant; +++ = very abundant

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Table 2: 1H and 13C NMR chemical shift values for compound 1 recorded in $CDCl_3$ *(400 MHz)* a-b.

Carbon atom	^{13}C NMR Experimental	^{13}C NMR Literature	^{1}H NMR Experimental	^{1}H NMR Literature	Nature of Carbon
C-1	36.72	37.15			CH2
C-2	29.71	31.56			CH2
C-3	71.97	71.71	3.53 (m, 1H)	3.51 (tdd, 1H)	CH
C-4	42.35	42.19			CH2
C-5	140.94	140.81			C=C
C-6	121.32	121.62	5.38 (s, 1H)	5.31 (t, 1H)	C=CH
C-7	31.71	31.56			CH2
C-8	29.24	31.79			CH
C-9	50.03	50.02			CH
C-10	36.16	36.16			C
C-11	24.32	21.12			CH2
C-12	39.82	39.57			CH2
C-13	40.45	42.10			C
C-14	56.90	56.76			CH
C-15	24.32	24.27			CH2
C-16	28.90	28.83			CH2
C-17	56.03	55.84			CH
C-18	12.06	12.15	1.29(d, 3H)	1.03 (s, 3H)	CH3
C-19	19.06	19.88	0.74(d, 3H)	0.71 (s, 3H)	CH3
C-20	39.82	40.40-40.51			CH
C-21	23.12	20.99	1.20(d, 3H)	0.91 (d, 3H)	CH3
C-22	138.40	138.23	5.07(m, 1H)	4.98 (m, 1H)	C=C
C-23	129.34	129.16-129.60	5.20(m, 1H)	5.14 (m, 1H)	C=C
C-24	51.26	51.13-51.30			CH
C-25	34.01	31.94			CH
C-26	21.12	21.23	0.84(d, 3H)	0.80 (d, 3H)	CH3
C-27	22.82	19.01	0.97(d, 3H)	0.82 (d, 3H)	CH3
C-28	25.32	25.40-25.50			CH2
C-29	12.06	12.25-25.30	1.04(t, 3H)	0.83 (t, 3H)	CH3

a- assignments made on the basis of COSY, HMQC and HMBC correlations; b-Chemical shift values are in δ (ppm).

4. Conclusion

According to the results above, compound (1) isolated from the leaves extract of *Odontonema strictum* is a mixture of stigmasterol and beta-sitosterol. Well known phytosterols. The structure of the isolated compounds were identified on the basis of spectroscopic methods and by comparing their physical properties reported in the literature. The complete 1H and 13C NMR spectral assignments of the two isolated compounds were made based on COSY, HSQC and HMBC spectroscopic data.

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