

Original Research Article

Isolation and Characterisation of Stigmasterol and β -Sitosterol from

Anthocleistadjalonensis A. Chev.

ABSTRACT

Aim: *Anthocleistadjalonensis* A. Chev. is a plant with several chemical constituents which accounts for its ethnopharmacological uses. The present study is aimed at identifying and characterizing the active principles from the roots of the plant.

Place and Duration of study: The study was carried out at the Department of Organic Chemistry, Rhodes University, Grahamstown, South Africa between March and July 2016.

Methodology: The root powder was subjected to maceration with methanol to obtain the crude extract. The methanol extract was fractionated using hexane, ethyl acetate and acetone successively. The acetone extract was thereafter subjected to column chromatography to isolate any pure components.

Results: White needle-like crystals were obtained which on spectral analysis (IR, ^1H NMR, ^{13}C NMR, 2D- NMR, and EIMS/HREIMS) were identified as a mixture of stigmasterol and β -sitosterol.

Conclusion: The compounds isolated were identified as stigmasterol and β -sitosterol

Key words: *Anthocleistadjalonensis*, chromatography, maceration, Stigmasterol, β -Sitosterol

1. INTRODUCTION

Anthocleistadjalonensis A. Chev.(Loganiaceae) is a medium-sized tree (30 – 45 m tall) of West tropical Africa [1]. The root bark of the plant is reported to contain irlbacholine [2], which showed very significant antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus fumigatus* and *Trichophyton rubrum*. Other compounds isolated from the plant include triterpenes, a monoterpene-diol djalonenol, a dibenzo-pyrone djalonensone, an iridoid glycosidesweroside (djalonenoside)[3] as well as amplexine and axanthone lichexanthone [4]. The stem bark is also reported to contain the phthalide djalonensin [4]. The stem, roots and leaves of *A. djalonensis* are used traditionally to treat malaria, jaundice, diabetes and abscesses [1, 5]. The Igbos in Nigeria use the seeds, barks and roots as antipyretic, a laxative and remedy for various stomach disorders [6]. It is used to treat epilepsy in Ghana [5,7] and in Southern Nigeria, the leaves and stem bark are used as a remedy for malaria [7, 4, 8]. It is also reported that the plant is useful as febrifuge, abortifacient and relief for pains. The roots of the plant have been reported to contain steroids [9,10] and the present study reports on the isolation of two steroids from the roots of the plant.

2. MATERIAL AND METHODS

2.1 Plant material

The roots of *Anthocleistadjalonensis* were collected from trees growing freely in ZakiBiam, Benue State Nigeria. The plant was identified by Mr Ibe Ndukwe, a taxonomist in the Forestry Department, Michael Okpara University of Agriculture Umudike Nigeria and voucher specimens were deposited in their Herbarium. The roots were dried under a shade for three weeks and milled at the Chemistry Department, University of Agriculture Makurdi using a Thomas model 4 Willey Mill.

2.2 Extraction of plant material

The ground plant material (1200 g) was macerated in methanol for one week and concentrated on a rotary evaporator at 35 °C to yield a viscous light brown residue (93.61 g) which was partitioned successively with hexane (4 x 100 mL), ethyl acetate (4 x 100 mL) and acetone (4 x 100 mL). The extracts were concentrated to give a hexane, ethyl acetate and acetone extracts.

2.4. Isolation and purification of compounds

The acetone extract was subjected to column chromatography on silica gel (Merck 70-30 mesh, (bed surface area 500 m²/g pore volume 0.75 cm²). The column was eluted gradient wise with hexane and dichloromethane and the eluates collected in 50 mL fractions. The fractions were analysed by TLC and similar fractions were combined to obtain 23 fractions including fraction AD-6.

2.5. Purification of fraction AD-6

Fraction AD-6 (11.715 mg) was discovered to have crystals. The crystals were washed separately and repeatedly with Hexane (50 mL). TLC with hexane and dichloromethane (1:2) gave a single spot with R_f 0.62. The purified fraction was labelled AD-03.

2.6. Test for steroid

2.6.1. Salkowski reaction:

A few crystals of AD-03 were dissolved in chloroform and a few drops of concentrated sulfuric acid were added to the solution, A reddish color in the upper chloroform layer [11] was observed by AD-03 indicating presence of steroids.

2.6.2.Liebermann-Burchard reaction:

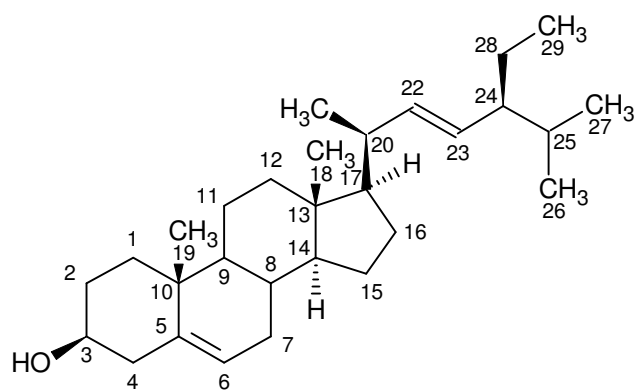
A few crystals of AD-03 were dissolved in chloroform and few drops of acetic anhydride and concentrated sulfuric acid were added to the chloroform solution. Violet blue and finally green color [11] was formed by AD-03 indicating the presence of steroids.

2.7.Spectroscopic characterization

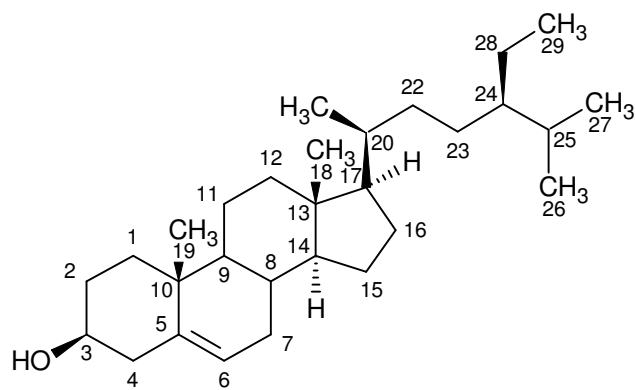
Spectroscopic methods were used to elucidate the structure of the isolated compound. The spectra (^1H , ^{13}C , 2D NMR were recorded using CDCl_3 as solvent on a Bruker Avance II 600 NMR spectrophotometer. EIMS and HREIMS were recorded on a JEOL HX110 MSLC-MS at South African Medical Research Council Drug Discovery and Development Research Unit, University of Cape Town, South Africa.

3. RESULTS AND DISCUSSION

The melting point of AD-03 was 145-147°C. The mass spectrum of AD-03 showed a molecular ion at 415.2117



Stigmasterol



β -Sitosterol

Fig 1: Structures of Stigmasterol and β -Sitosterol

Table 1: ^{13}C NMR and ^1H NMR Spectral data for AD-03 recorded in CDCl_3 (600 MHz)

CARBON ATOMS	¹³ C NMR EXPERIMENT	¹³ C NMR LITERATURE	¹ H NMR EXPERIMENT	¹ H NMR LITERATURE	TYPE OF CARBON
C-1	37.25	36.72			CH ₂
C-2	31.65	29.71			CH ₂
C-3	71.80	71.97	3.54(TDD,1H)	3.53(M,1H)	CH
C-4	42.21	42.35			CH ₂
C-5	141.01	140.94			C=C
C-6	121.65	121.32	5.36(M,1H)	5.38(S,1H)	C=CH
C-7	31.89	31.71			CH ₂
C-8	30.96	29.24			CH
C-9	50.12	50.03			CH
C-10	36.51	36.16			C
C-11	24.30	24.32			CH ₂
C-12	39.76	39.82			CH ₂
C-13	40.52	40.45			C
C-14	56.76	56.90			CH
C-15	23.06	24.32			CH ₂
C-16	28.94	28.90			CH ₂
C-17	56.04	56.03			CH
C-18	12.06	12.06	1.27(D,3H)	1.29(D,3H)	CH ₃
C-19	19.41	19.06	0.71(D,3H)	0.74(D,3H)	CH ₃
C-20	39.67	39.82			CH
C-21	21.07	23.12	1.17(D,3H)	1.20(D,3H)	CH ₃

C-22	138.34	138.40	5.16(M,1H)	5.07(M,1H)	C=C
C-23	129.32	129.34	5.33(M,1H)	5.20(M,1H)	C=C
C-24	51.24	51.26			CH
C-25	36.15	34.01			CH
C-26	18.96	21.12	0.86(D,3H)	0.84(D,3H)	CH ₃
C-27	18.76	22.82	0.94(D,3H)	0.97(D,3H)	CH ₃
C-28	25.42	25.32			CH ₂
C-29	11.96	12.06	1.04(T,3H)	1.04(T,3H)	CH ₃

3.1.Discussion

The result of the positive tests for steroids by AD-03 showed the compound containing a steroidal skeleton with a melting point of 145°C. The ¹H NMR data of AD-03 showed 3 olefinic protons at δ 5.16, δ 5.33 and δ 5.36 (H-22, 23 and 6). A triplet of a doublet of doublet was observed at δ 3.54 (H-3). While carbon 18, 19, 21, 26, 27, and 29 revealed the presence of six methyl protons with their signals at δ 1.27, 0.71, 1.17, 0.86, 0.94, 1.04. These signals and assignments are in accordance with reported values [12, 13, 14, 15, 16]. The ¹³C NMR showed three quaternary carbons, eleven methanes, nine methylenes, and six methyl carbons [14, 17]. C5 and C6 alkene carbons with double bonds revealing distinct signals at 141.01 and 121.65 [18, 19,]. Angular carbon atom signal (C-19, C-18) was also recognized at 19.41 and 12.06. [20]. A signal at 71.80 (C-3) indicated a hydroxyl group [20, 21]. The COSY, HMQC and HMBC correlations aided in the assignments of values of all protons for ¹H NMR and ¹³C NMR.

CONCLUSION

The compound isolated from *Anthocleistadjalonensis* acetone was a mixture of Stigmasterol and β - Sitosterol. The structure identification employed physical and spectroscopic methods. The characterization was made possible comparing the physical properties with that found in literature. Further studies on the pharmacological activities should be undertaken.

CONSENT

It is not applicable.

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