

SHORT COMMUNICATION

Phytosterol from the leaves of *Vernonia conferta* Benth (Asteraceae)

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ABSTRACT

From the dichloromethane soluble part of ethanol extract of the leaves of *Vernonia conferta*, a phytosterol, Stigmast-5-ene-3 β -ol (β -sitosterol) was isolated for the first time from this plant. The structure was elucidated using NMR and MS and was compared with literature.

KEYWORDS: *Vernonia conferta*, Phytosterol,

INTRODUCTION

The genus *Vernonia* is one of the largest genus found predominantly in Africa and South America (Costa, 2008). This species has wide range of ethnopharmacological use (Toyang and Verpoote, 2013). A phytochemical study of the genus has revealed the presence of diterpenes, triterpenes, and steroids (Kos et al, 2006; Liang, QL and Min; 2003; Tchinda et al, 2003). Most frequently isolated constituents are flavonoids and sesquiterpene lactones (Carvalho et al, 1999; Buskohl, 2010). *Vernonia conferta* (Asteraceae) is a plant found in tropical Africa including Nigeria. Ethnomedicinal uses of this plant in Ibibio area of Akwa Ibom state of Nigeria include the following: the young leafy shoots are usually boiled into soup and given to lactating mothers to induce milk, while the crushed leaves are used as dressing to treat cuts and skin infection. In our continuing search for bioactive plant metabolites from the genus *Vernonia*, we report herein the isolation and structure elucidation of a phytosterol from the dichloromethane extract of the leaves of *Vernonia*

conferta. The structure was elucidated using spectroscopic technique.

MATERIALS AND METHODS

IR spectra was recorded on Nicolet 1s 10 FTIR and NMR were recorded on a Bruker DRX 500MHz and 125MHz for ¹H and ¹³C respectively. Mass spectrum (MS) Was recorded on a thermofinnigan mass spectrometry. TLC and column chromatography were performed on silica gel precoated TLC plates 0.2mm(aluminium backed) and silica gel G (200-400mesh)Silicycle, while gel filtration was carried out using sephadex LH20 (Pharmacia).

Plant material

The leaves of *Vernonia conferta* were collected from Ikot Emen in Itam local government area of Akwa Ibom state, Nigeria. It was identified at the herbarium of the Department of Botany and Ecological studies, Faculty of Sciences, University of Uyo. Voucher specimen (UYU 1054) was deposited at the department's herbarium.

Extraction and Isolation

The plant material was air dried and pulverised. The pulverised plant material (300 g) was sequentially extracted to exhaustion at room temperature using n-hexane (2.5L), dichloromethane (2.5L) and 70% methanol (2X2.5L), respectively, and removal of the solvents at reduced pressure afforded greenish mass of n-hexane (37.7 g), dichloromethane (35.7 g) and methanol (47.2 g) extracts. A portion of the methanol extract (30g) was suspended in water and partitioned sequentially with dichloromethane (5x100ml), ethyl acetate (5x100ml) and n-butanol (8x100ml) to give 7.2 g of dichloromethane, 4.3 g of ethyl acetate and 6.12 g of n-butanol soluble fractions. A portion of the dichloromethane soluble fraction(5g) was packed in a column (50cmx3cm) with 100g of silica gel and elution commenced gradiently with n-hexane, followed with increasing amounts of ethyl acetate (95:5; 90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 10:90; 100% ethyl acetate), and finally 5% methanol in ethyl acetate. 50mls aliquots were collected and the progress of elution was monitored on TLC using n-hexane:ethyl acetate (9:1) and dichloromethane:ethyl acetate (2:3).

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Fractions 37-52 eluted from the column (10% ethyl acetate in n-hexane) which showed similar spots on TLC were pooled together and purified over sephadex LH-20 eluting with 100% dichloromethane to give compound 1, a white solid (6 mg). Compound 1 gave positive result to salkowski and Libermann-Burchard tests suggestive of a steroid or triterpene. Structure elucidation: FTIR, ^1H and ^{13}C NMR and MS spectroscopic analysis of compound 1 was undertaken to elucidate the structure.

RESULTS

Compound 1, a white solid (6 mg).
IR(KBr) cm^{-1} : 3431(O-H), 1643(C=C)
 $^1\text{H-NMR}$ (CDCl_3) δ ; (ppm): 0.75 (s), 3H, H-28), 0.80(s), 3H, H-27; 0.82(s), 3H, H-26; 0.83(s), 3H, H-24; 0.91(s), 3H, H-19; 1.03(s), 3H, H-29; 5.31(s), 1H, H-5 and 3.51(m), H-3.
 $^{13}\text{C-NMR}$ δ : (ppm): 37.6(C-1), 32.1(C-2), 72.1(C-3), 42.4(C-4), 141.5(C-5), 121.8(C-6), 31.8(C-7), 31.8(C-8), 50.2(C-9), 36.6(C-10), 21.5(C-11), 39.9(C-12), 42.4(C-13), 56.8(C-14), 24.4(C-15), 29.3(C-16), 56.2(C-17), 40.6(C-18), 21.7(C-19), 46.1(C-22), 25.4(C-23), 12.1(C-24), 29.9(C-25), 20.2(C-26), 18.9(C-27), 19.8(C-28), 12.2(C-29)
MS: m/z, 414(M^+)

DISCUSSION

Compound 1 was isolated as a white solid. The IR spectra revealed the presence of hydroxyl group and double bond with frequencies at 3431 cm^{-1} and 1643 cm^{-1} respectively. The mass spectrum gave a molecular ion peak at m/z 414 consistent with the molecular formula $\text{C}_{29}\text{H}_{50}\text{O}$. The ^1H spectra of compound 1 revealed the presence of six methyl protons which appeared at δ_{H} 0.75, 0.8, 0.82, 0.83, 0.91 and 1.03 ppm, respectively. The vinylic proton appeared at δ_{H} 5.31ppm, while the hydroxymethine proton appeared as a multiplet signal at δ_{H} 3.51ppm. The $^{13}\text{C-NMR}$ spectra of the compound showed the presence of 29 carbons which included six methyl carbons; the vinylic carbons were evident at δ_{C} 141.5 and 121.8ppm respectively typical of Δ^{5-6} sterolenes. The ^1H and ^{13}C spectra of compound 1 are in agreement with the literature data of β -sitosterol (Habib et al, 2007), thus compound 1 was found to be β -sitosterol.

CONCLUSION

From dichloromethane extract, a sterol was isolated for the first time from this plant, and the structure elucidated using spectroscopic techniques.

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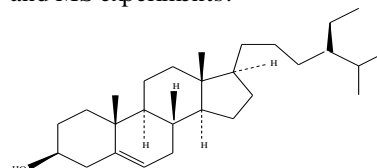


Figure 1: Chemical structure of compound 1 (β -sitosterol).

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