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Vitus A. Nyigo
1: *Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, P.O. Box 3021 Morogoro, Tanzania*
2: *Department of Traditional Medicine Research, National Institute for Medical Research, 3 Barack Obama Drive, P.O. Box 9653, 11101 Dar es salaam, Tanzania*

Xolani Peter
Council for Scientific and Industrial Research (CSIR), DPSS Landward Science, P.O Box 395, Pretoria 0001, South Africa

Faith Mabiki
Department of Physical Sciences, Sokoine University of Agriculture, P.O. Box 3038, Morogoro, Tanzania

Hamisi M. Malebo
Department of Traditional Medicine Research, National Institute for Medical Research, 3 Barack Obama Drive, P.O. Box 9653, 11101 Dar es salaam, Tanzania

Robinson H. Mdegela
Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, P.O. Box 3021 Morogoro, Tanzania

Gerda Fouche
Council for Scientific and Industrial Research (CSIR), Biosciences, P.O Box 395, Pretoria 0001, South Africa

Correspondence:
Mr. Vitus A. Nyigo
Department of Traditional Medicine Research, National Institute for Medical Research, 3 Barack Obama Drive, P.O. Box 9653, 11101 Dar es salaam, Tanzania
Email: nyigoo[at]yahoo.com

Isolation and identification of euphol and β-sitosterol from the dichloromethane extracts of *Synadenium glaucescens*

Vitus A. Nyigo, Xolani Peter, Faith Mabiki, Hamisi M. Malebo, Robinson H. Mdegela, Gerda Fouche

ABSTRACT

Purification of dichloromethane extract from root barks and leaves of *Synadenium glaucescens* respectively resulted into the isolation of two compounds namely Euphol and β-sitosterol. Chemical structures were established mainly by using ^{1}H and ^{13}C NMR data and by comparing current NMR data with those reported in the literature. Both compounds are known and have been isolated from other plant species but are being reported from this plant species for the first time.

Keywords: *Synadenium glaucescens*, euphol, β-sitosterol.

INTRODUCTION

Like many countries around the world, utilization of plants as traditional medicines is a common phenomenon in Tanzania. *Synadenium glaucescens* is a plant species which belongs to a small genus of about 15 species indigenous to eastern Africa [1]. Plants in this genus are shrubs or trees with sub-fleshy cylindrical branches and copious milky latex, monoecious [2]. *S. glaucescens* is among of Tanzanian plants that are traditionally claimed to be used for treatment of various ailments. These include HIV, TB, sores, wounds and worms [3]. Many plants from this genus are known for different biological activities including anti-ulcer activity [4], antinoceptive, anti-inflammatory anti-plasmodial activity [5], anti-asthmatic effect [6], anti-tumoral and anti-angiogenic effects [7]. Albeit few studies have so far been conducted in *S. glaucescens*, the recent laboratory findings have shown that the plant exhibits anti-bacterial activities [8], strong anti-viral activities *in vitro* [9] and against infectious basal disease virus together with fowl pox virus [10]. We could however, hardly find any information regarding secondary metabolites isolated from this plant species.

Phytochemical screening of dichlomethane extracts indicated the presence of lanosterol and cycloartenol sterols as main compounds while ethanolic extracts showed to possess phenolic compounds [11]. The only available information on natural product from this plant is the isolation of one pharmacologically active compound of which the establishment of chemical structure was not accomplished. Preliminary study of the isolated compound indicated it to be β-glycosides attached to alkyl group and was deduced to be an inhibitor of contraction of guinea pig ileum [12]. Earlier Euphol was isolated from *Synadenium grantii* [13] and from some other genera in the family Euphorbiaceae [14, 15]. Different bioassay studies have shown great pharmacological potentials of euphol. Research has indicated the ability of this compound to reduce the severity of colitis in two models of chemically-induced mouse colitis. This suggests that this compound could be a potential molecule in the management of inflammatory bowel diseases [16]. Other pharmacological effects include antinoceptive effects in inflammatory and neuropathic pains [17], inhibition of tumor artificially induced by 12-O-tetradecanoyl phorbol-13-Acetate [18] and selective inhibitions of human gastric cancer cell growth through the induction of ERK1 / 2-mediated apoptosis [19]. β-sitosterol (**2**) is among phytosterols which is found in many plants. Many phytosterols are well known for their anticancer effects against different types of cancer [20]. Although it has been isolated from different species [21, 22, 23], this compound is also being reported for the first time from this plant species. β-sitosterol is known to have many health benefits including control of heart diseases, lowering cholesterol content [24] and inhibition of colon cancer [25].

Research works in this paper describes the isolation and structural elucidation of euphol (**1**) and β-sitosterol (**2**) from *S. glaucescens* for the first time. The two compounds have however, been isolated earlier from other plant species [14, 21].

MATERIALS AND METHODS

General experimental Procedures

Thin layer Chromatography (TLC) and Column Chromatography (CC) were performed on silica gel 60 as stationary phase (particle size 0.04-0.036 mm, 230-400 mesh, ASTM E. Merck, Germany). Melting point measurements of compounds were done using the reichert thermogalen hot stage microscope (NCRL, Austria 1863) which is adapted to the requirements of thermal microscopy and provides optimum conditions to achieve fast and reliable results. Nuclear Magnetic Resonance (NMR) spectroscopy was used to determine the spatial disposition of the molecular frameworks of the isolated compounds within different chemical environments. NMR Spectral data i.e Proton and carbon spectra including the two dimensional spectra were collected in 600MHZ Varian type Nuclear Magnetic Resonance (NMR) spectrophotometer at 30°C temperature in chloroform. Chemical shifts are given in δ (ppm), TMS was used as internal standard material and the coupling constants (J) are given in Hz. A Waters UPLC coupled in tandem to a Waters photodiode array (PDA) detector and a SYNAPT G1 HDMS mass spectrometer was used to generate accurate mass data. [Optimisation of the chromatographic separation was done utilising a Waters BEH C18 column (150 mm x 2.1 mm, 1.7 µm) and the column temperature controlled at 60 °C.

Plant materials

Plant materials were collected from Njombe region, Tanzania in January 2014 and were immediately subjected for drying. Root barks were dried under complete shade in specialised drying room while leaves were dried under half day sun and half day shade [26] to prevent it from rotting due to its high content of latex. Grinding of dried plant materials was effected at the Department of Animal Science and Production (DASP), Sokoine University of Agriculture (SUA), Tanzania.

Extraction and Isolation

Extraction and isolation were carried out using methods described by Harbone [27]. Thus, Dried root barks (1.18 kg) and leaves (1.8 kg) of *Synadenium glaucescens* were successively extracted by maceration at room temperature with n-hexane [2x3000 ml], Chloroform [2x3000 ml], Ethyl acetate [2x3000 ml], methanol [2x3000 ml] and water [2x3000 ml]. The process involved soaking plant materials for 24 hours (x2). Extracts were obtained by removing solvents from the filtrates through rotary evaporation at 40°C water bath temperature. Ten grams of extracts from root barks was dissolved in 500 ml distilled chloroform and pre adsorbed in silica gel. The sample was then partitioned in a vacuum liquid chromatography (VLC) column and eluted with hexane: ethyl acetate respectively starting with ratios of 10:90, 30:70, 40:60, 50:50, 60:40, 80:20 and 100:0 to obtain 7 different fractions. Fraction 2 and 3 were combined and subjected for repeated column chromatography using ethyl acetate: hexane solvent gradient system in increasing polarity from 10:90 ethyl acetate/hexane to 60:40 ethyl acetate/ hexane to obtain 32 fractions. Fractions 10-13 were combined and further purification in preparative TLC using 20: 80 ethyl acetate/Hexane to obtain compound **1**. The dichloromethane leaf extract was partitioned into a silica gel column by first pre adsorbing 2.5g of extract into silica gel and fractionated into a column using hexane/ethyl acetate (5:95 – 0:100) which resulted into 15 fractions. Fraction 2 and 3 were then combined and then partitioned into repeated preparative thin layer chromatography (Prep TLC) using hexane/ethyl acetate (15:85) to obtain compound **2.**

Euphol (1): White powder (5 mg); 1H NMR (CDCl3, 600 MHz): see Table 1; ^{13}C NMR (CDCl3, 600 MHz): see Table 1

β-Sitosterol (2): White powder (4 mg); mp: 128-130°C; 1H NMR (CDCl3, 600 MHz): see Table 2; ^{13}C NMR (CDCl3, 600 MHz): see Table 2; MS (*m/z*): 397 $[M\text{-}OH]^-$

RESULTS AND DISCUSSION

In this paper we report the isolation of two compounds, a triterpene and a sterol namely respectively as **euphol** (**1**) and ***β*-Sitosterol (2)** [Figure 1]. Both compounds have earlier been isolated from other plant species but being reported from *S. glaucescens* for the first time. Isolation of the compounds was effected through chromatographic technique and their structures established based on NMR spectroscopic data together comparison with other existing data (Table 1 & 2). Both compounds are soluble in chloroform and are white crystalline in nature.

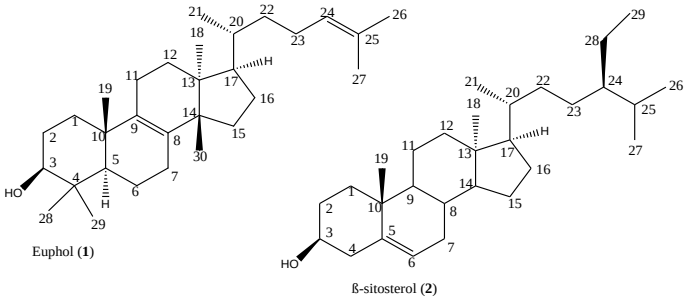

Figure 1: Structures of Euphol (1) and *β* sitosterol (2)

Compound 1 was obtained through Column Chromatography and pre TLC. The ^{13}C NMR spectra showed 30 carbon signals suggesting that the compound could possess a triterpenoid skeleton. The ^{1}H NMR spectra exhibited signals mostly concentrated in the high field region which are typically triterpene signals. The more peculiar signals in the proton NMR included an olefinic proton (δ_H 5.08, 1H, t) and an axial proton on an oxygen-bearing carbon (δ_H 3.21, dd, *J*=11.4, 4.2 HZ). It further exhibited seven singlets characteristic of tertiary methyl groups (δ_H 1.66, 1.58, 0.98, 0.93, 0.85, 0.78, 0.73) and a secondary methyl group (δ_H 0.84, d, J= 6.0) which form the eighth methyl groups in the skeleton. The ^{13}C-NMR spectra exhibited four signals (δ_c 134.27, 133.78, 131.03 and 125.43) characteristic of olefinic carbons indicating that the compound is unsaturated and contains two double bonds. The carbon signal (δ_c 79.29) was characteristic of carbon bearing a hydroxyl group. These data were in agreement with the structure of Euphol (**1**). The NMR data of **1** (Table 1) is in agreement with the published values [14].

Compound 2 was identified using ^{1}H and ^{13}C spectra and confirmed using mass spectral data (*m/z*): 397 $[M\text{-}OH]^-$. Signals in the ^{1}H NMR spectrum were observed mainly in the up field region. The spectra exhibited only two signals with high chemical shifts values; the first one resonated in the olefinic region and the other one was observed a little up field region. The olefinic signal at δ 5.3 (1H, br d, J = 4.8 Hz) appeared to be characteristic of the sterols, and it was assigned to H-6 proton in the *β*–sitosterol (**2**) chemical skeleton. The ^{1}H-NMR spectra of compound 2 also exhibited a signal corresponding to the proton connected to C-3 hydroxyl group which appeared as a multiplet at δ 3.50 (1H, m). Six other proton signals were evident which include four secondary methyl groups (δ_H 0.91, 0.82, 0.81 and 0.79 all doublets with J = 6.6, 7.2, 6.4 and 6.4 Hz respectively) and two tertiary methyl groups (δ_H 0.66 and 0.99. The ^{13}C NMR spectra exhibited 29 carbon signals, characteristic of phytosterols. These data were in agreement with the structure of β-sitosterol (**2**). The NMR data of **2** (Table 2) is in agreement with the published values [21].

Table 1: ^{1}H and ^{13}C NMR data for compound 1 and from the literature

	Experimental		Literature [14]	
Position	**^{13}C**	**^{1}H**	**^{13}C**	**^{1}H**
C-1	35.49		35.4	
C-2	27.9		27.7	
C-3	79.2	3.21 (dd, 1H, J =11.4, 4.2 HZ)	78.9	3.24, (1H, dd, J = 11.6, 4.8 HZ)
C-4	37.5		37.3	
C-5	51.2		50.9	
C-6	19.17		18.9	
C-7	28.16		27.9	
C-8	134.3		134	
C-9	133.8		133.5	
C-10	39.2		38.9	
C-11	21.7		21.5	
C-12	28.3		28.1	
C-13	44.4		44.1	
C-14	50.3		49.9	
C-15	31.1		30.9	
C-16	29.9		29.8	
C-17	49.9		49.6	
C-18	15.74	0.78 (3H, s)	15.6	0.80 (3H, s)
C-19	20.36	0.93 (3H, s)	20.1	0.95 (3H, s)
C-20	36.07		35.8	
C-21	19.12	0.83 (3H, d, J=6.0)	18.9	0.84 (3H, d, J=3.3Hz)
C-22	35.66		35.2	
C-23	24.74		24.7	
C-24	125.43	5.08 (t, 1H)	125.2	5.09 (1H, t, J = 7.5HZ)
C-25	131.03		130.8	
C-26	17.88	1.66 (3H, s)	25.7	1.68 (3H, s)
C-27	25.93	1.58 (3H, s)	17.7	1.60 (3H, s)
C-28	24.68	0.85 (3H, s)	24.4	0.87 (3H, s)
C-29	28.27	0.98 (3H, s)	28	1.00 (3H, s)
C-30	15.85	0.73 (3H, s)	15.5	0.75 (3H, s)

Table 2: ^{1}H and ^{13}C NMR data for compound 2 and from the literature

	Experimental		Literature [21]	
Position	**^{13}C**	**^{1}H**	**^{13}C**	**^{1}H**
1	37.5		37.5	
2	40		31.9	
3	72.1	3.50 (m, 1H)	72	3.53 (tdd, 1H, J=4.5, 4.2, 3.8 HZ)
4	42.6		42.5	
5	141	5.33 (t, 1 H,)	140.9	35.36 (t, 1H, J = 6.4HZ)
6	121.9		121.9	

7	32.2		32.1	
8	31.9		32.1	
9	50.4		50.3	
10	36.8		36.7	
1	21.3		21.3	
12	40.0		39.9	
13	42.6		42.6	
14	57.0		56.9	
15	26.4		26.3	
16	28.5		28.5	
17	56.3		56.3	
18	36.4		36.3	
19	19.3	0.91 (d, 3H, J=6.6)	19.2	0.93 (d, 3H, J = 6.5 HZ)
20	34.2		34.2	
21	24.5		26.3	
22	46.1		46.1	
23	23.3		23.3	
24	12.2	0.82 (t, 3H, J=7.2HZ)	12.2	0.84 (t, 3H, J = 7.2HZ)
25	29.4		29.4	
26	20.0	0.81 (d, 3H, J=6.4HZ	20.1	0.83 (d, 3H, J = 6.4HZ
27	19.6	0.79 (d, 3H, J=6.4)	19.6	0.81 (d, 3H, J = 6.4)
28	19.0	0.66 (s, 3H)	19.0	0.68 (s, 3H)
29	12.1	0.99 (s, 3H)	12.0	1.01 (s, 3H)

Limitation of the study

No biological assays were conducted for these compounds. The intended assays were anti HIV tests but the assay facilities were not available in the country. Samples needed to be sent abroad but time was limiting due to fact that the researcher was a student with a bound registration period

CONCLUSION

Two compounds, a triterpene and a sterol were isolated for the first time from *S. glaucescens*. Respectively, the compounds were isolated from root barks and leaves. The structures of the isolated compounds were identified as euphol (**1**) and β-sitosterol (**2**) on the basis of ^{1}H and ^{13}C NMR spectroscopic data and by comparing them to those reported in the literature.

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