https://dx.doi.org/10.4314/ijs.v21i3.20

ISOLATION AND CHARACTERIZATION OF CAMPEST-5-EN-3-OL FROM THE ROOT BARK OF *UVARIA AFZELII*

Famuyiwa, S. O.
Department of Chemistry, Faculty of Science, Obafemi Awolowo University, Ile Ife, Nigeria.
Corresponding author; Phone: 08166020238; e-mail address: oluwaseyi_f@yahoo.com
(Received: 4th October, 2019; Accepted: 13th November, 2019)

ABSTRACT

This study aimed at the isolation and characterization of a steroidal compound from the root bark of *Uvaria afzelii*. The methanolic crude extract of the root bark was suspended in aqueous methanol and partitioned with *n*-hexane to obtain a fraction rich in volatile oils. The *n*-hexane fraction was subjected to column and preparative thin layer chromatography for isolation and purification of compounds. The *n*-hexane fraction yielded one steroidal compound. The structure of which was determined by ^{1}H-, ^{13}C- and DEPT135-NMR as campest-5-en-3-ol. The spectroscopic data compared very well with published data.

Key words: Extract, *Uvaria afzelii*, chromatography, campest-5-en-3-ol.

INTRODUCTION

Steroids are modified triterpenoid compounds containing tetracyclic ring system but lacking the three methyl groups at C-4 and C-14. Further modifications, especially to the side chain create a wide range of biologically important natural products. They are widely spread in the plants as sterols which are known as phytosterols (Dewick, 2009). They have been reported to possess anti-inflammatory, anti-neoplastic, anti-pyretic, anti-diabetic, anti-cancer, immune-modulating activities and reduction of the risk of cardiovascular diseases (Bouic, 2001; Conforti, *et al*., 2008; Baskar, *et al*., 2010; Othman and Moghadasian, 2011; Genser *et al*., 2012; Pandey, *et al*., 2016; Sanjeewa, *et al*., 2016). The anti-diabetic activity of the phytosterols has been attributed to the ability to lowering the level of cholesterol in the blood (Dumolt and Rideout, 2017). The presence of phytosterol may be responsible for the reported anti-diabetic activity of the root bark of *Uvaria afzelii* (Ayoola *et al*., 2017).

Uvaria afzelii Scot Elliot (Annonaceae), a shrub growing in the West African sub-region, especially in the south and eastern part of Nigeria, is used in the treatment of cough, vaginal tumour, breast aches, swollen hands and feet, diabetes as well as leucorrhea and gonorrhea, infections of liver, kidneys and bladder (Odugbemi, 2008; Kayode *et al.,* 2009; Omoruyi *et. al*., 2014). It has been reported for its antitubercular (Lawal *et al*., 2011), antimicrobial (Lawal *et al.*, 2014) and hepatoprotective (Ofeimun *et al*., 2013) activities. Its bacteriocidal, anti-helminthic and antiparasitic activities have also been investigated (Okoli, 2004; Lawal *et al.,* 2011; Okpekon, 2004). Emorydone, demethoxymatteucinol, 1-indanone and afzeliindanone are some isolated compounds from the plant (Okpekon *et al.,* 2004). The present study isolated and characterized campest-5-en-3-ol from the root bark of the plant.

RESULTS AND DISCUSSION

The light brown solid compound isolated from the hexanes fraction of the root bark of the plant, *Uvaria Afzelii*, was identified as campest-5-en-3-ol according to the ^{1}H-, ^{13}C-, DEPT 135 NMR and comparison with published data (Choi *et al.,* 2007).

The ^{1}H-NMR ($CDCl_3$, 300 MHz) showed up field signals which indicated terpenoid compound with resonance at δ 1.04, 0.94, 0.88, 0.81, 0.80 and 0.72 ppm, each integrated for three protons, indicating the presence of six methyl groups in the compound. A doublet signal at δ 5.38, integrated for one proton with a coupling constant of 4.8 Hz indicated an olefinic proton (H-6). A multiplet signal at δ 3.60, integrated for one proton was assigned to H-3. The hydroxyl proton appeared at δ 2.93 as broad singlet. The analysis of ^{13}C- and DEPT135 NMR spectra was consistent with a molecular formula $C_{28}H_{48}O$. The ^{13}C-NMR

($CDCl_3$, 75 MHz) showed signals for twenty eight carbon atoms including an oxymethine carbon signal at δ 71.8 and two olefinic carbons at δ 140.8 and δ 121.7 ppm. The DEPT135 NMR showed signals for six methyl carbons, ten methylene carbons and eight methine carbons. The assignment of the carbon signals is as shown in Table 2. Thus, the structure of the compound was established as campest-5-en-3-ol, Fig. 1 and the structure was further confirmed by comparing the ^{13}C-NMR data with the published data (Choi *et al.*, 2007).

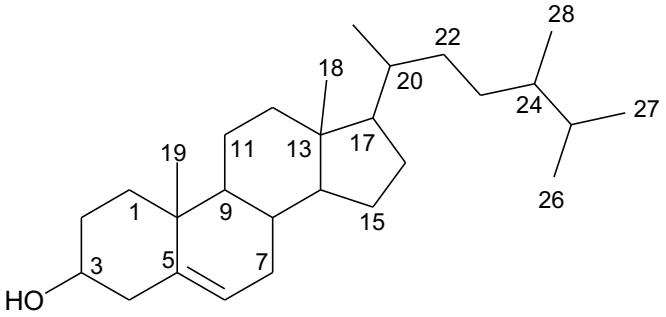

Fig. 1: Structure of campest-5-en-3-ol

Table 1: The comparison of ^{1}H-NMR data between campest-5-en-3-ol and isolated compound

Campest-5-en-3-ol		Isolated compound	
H-atom	ppm (multiplicities)	H-atom	ppm (multiplicities)
H-3	3.51 (brdd, 7.3 Hz)	H-3	3.51 (m)
H-4	2.28 (d, 7.3 Hz)	2H-4	2.29 (dd, 9.3 & 10.5 Hz)
H-6	5.34 (brd, 5.2 Hz)	H-6	5.38 (d, 4.8 Hz)
3H-18	0.68 (s)	3H-18	0.72 (s)
3H-19	1.01 (s)	3H-19	1.04 (s)
3H-21	0.92 (d, 6.6 Hz)	3H-21	0.94 (d, 9.3 Hz)
3H-26	0.81 (d, 7.2 Hz)	3H-26	0.81 (d, 6.9 Hz)
3H-27	0.84 (d, 7.2 Hz)	3H-27	0.88 (d, 6.9 Hz)
3H-28	0.80 (d, 7.0 Hz)	3H-28	0.80 (d)

Table 2: The Comparison of ^{13}C-NMR Data between Campest-5-en-3-ol and isolated compound

Campest-5en-3-ol				Isolated compound			
C-atom	ppm	C-atom	ppm	C-atom	ppm	C-atom	ppm
C-1	37.3	C-15	23.1	C-1	37.3	C-15	23.1
C-2	28.2	C-16	26.1	C-2	28.3	C-16	26.1
C-3	71.8	C-17	56.1	C-3	71.8	C-17	56.1
C-4	42.3	C-18	20.0	C-4	42.2	C-18	12.0
C-5	140.7	C-19	11.8	C-5	140.8	C-19	11.9
C-6	121.7	C-20	31.6	C-6	121.7	C-20	31.6
C-7	31.9	C-21	19.4	C-7	31.9	C-21	19.4
C-8	29.2	C-22	33.9	C-8	29.2	C-22	34.0
C-9	50.1	C-23	21.1	C-9	50.2	C-23	21.1
C-10	36.5	C-24	45.8	C-10	36.5	C-24	45.9
C-11	19.8	C-25	36.1	C-11	19.9	C-25	36.2
C-12	40.0	C-26	18.8	C-12	39.8	C-26	18.8
C-13	42.3	C-27	19.0	C-13	42.4	C-27	19.1
C-14	56.9	C-28	24.4	C-14	56.8	C-28	24.3

CONCLUSION

This study has supported the ethno-medicinal claim of the root bark of *Uvaria afzelii* as an anti-diabetic agent. The hexane fraction was reported to demonstrate lower hyperglycaemia lowering activity compared to a standard drug, glibenclamide (Ayoola *et al*., 2017). This observed activity could be as a result of the presence of campesterol in the hexane fraction. Excess accumulation of cholesterol in the body cells interferes with the secretion of insulin that leads to diabetes (Garber, 2010.). Campesterol controls and balances the accumulation of cholesterol in the body. It plays a number of roles in the human body by keeping the cell membrane healthy, so it allows the right chemicals to pass in and out of the cells but it has to be regulated (Choudhary and Tran, 2011). It is noteworthy that the compound isolated, campest-5-en-3-ol, was reported for the first time from this plant, *Uvaria afzelii*.

MATERIALS AND METHODS

General experimental procedures: All general purpose solvents, *n*-hexane, dichloromethane, ethyl acetate and methanol (JHD, China), used were distilled. Chloroform (analytical grade, Sigma-Aldrich, Germany). Silica gel, 200-400 mesh (Loba chemie, India). Aluminum plate (20 x 20 cm, Merck, Germany) coated with silica gel was used for analytical thin layer chromatography. Spots on the plates were viewed under ultraviolet (UV) light and were further sprayed with vanillin sulphuric acid for the detection of compounds. Nuclear Magnetic Resonance (NMR) was measured on Bruker DMX Advance 300 instrument using chloroform-d as solvent and internal standard.

Plant material: *U. afzelii* roots were collected from the zoological garden of Obafemi Awolowo University, Ile-Ife, Nigeria. The plant was identified and authenticated by Mr. Ademoriyo and a voucher specimen of the plant (IFE16941) was deposited at the Herbarium of the Department of Botany, Obafemi.Awolowo.University, Ile-Ife.

Extraction and isolation of the compound: The roots of the plant were peeled and the barks were oven dried at about 40 °C. These were ground to powder and 1.5 kg of the powder was extracted exhaustively with methanol. The methanolic solution was evaporated at about 40 °C under reduced pressure using rotator evaporator to obtain a residue (142.9 g) and the residue was taken up in 400 ml of methanol/water (1:1). The aqueous methanol solution was partitioned with *n*-hexane (4x400 ml) to obtain *n*-hexane fraction (13.3 g).

The *n*-hexane residue was dissolved in a suitable organic solvent and the solution was adsorbed onto silica gel (13.0 g). The absorbed silica gel was left overnight to dry and it became powdery. The adsorbed silica gel was packed into a glass column and this was gradiently eluted with *n*-hexane, dichloromethane, ethyl acetate and methanol. About 100 ml of eluent were collected into each 250 ml conical flask and a total of 115 conical flasks were used. These were bulked together based on their TLC profile to obtain 23 fractions.

An impure solid (70.4 mg) was collected from the fraction eluted with 80% hexane in dichloromethane. This impure solid contained UV spot when viewed on TLC and it was subjected to preparative TLC using chloroform/methanol (7:3) solvent system to develop the preparative TLC plates. There were three bands and were labeled as **a**, **b** and **c** from the top of the plates (4). These were spotted on analytical TLC and **a** give single spot to yield the isolated compound while others bands did not but contained several spots.

Spectroscopic data of campest-5-en-3-ol

Creamy white powder, IR(KBr): $\tilde{o}_{max}$ (cm^{-1}) 3427, 1643. ^{1}H and ^{13}C-NMR: Tables 1 and 2 respectively.

ACKNOWLEDGEMENTS

The author would like to thank the University of Botswana, Gaborone for the use of her laboratory for the spectroscopic analysis of the isolate and the Network for Analytical and Biological Assay for Southern Africa (NABSA) for funding NMR analysis.

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