The Journal of Phytopharmacology 2022; 11(3):151-154
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2022; 11(3): 151-154
May- June
Received: 09-03-2022
Accepted: 07-05-2022

doi: 10.31254/phyto.2022.11303

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

Hamisi M. Malebo
Mabibo Traditional Medicine Research and Development Center, National Institute for Medical Research, Barack Obama Drive, P.O.Box 9653, 11101 Dar es salaam, Tanzania

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

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

Correspondence:
Dr. Vitus A. Nyigo
Mabibo Traditional Medicine Research and Development Center, National Institute for Medical Research, Barack Obama Drive, P.O. Box 9653, 11101 Dar es salaam, Tanzania
Email: nyigoo@yahoo.com

Isolation and identification of long -chain aliphatic compounds from *Synadenium glaucescens*

Vitus A. Nyigo*, Hamisi M. Malebo, Faith Mabiki, Robinson Mdegela

ABSTRACT

Purification of dichloromethane extract from root barks and leaves of *Synadenium glaucescens* extracts through chromatographic techniques resulted into the isolation of two compounds, namely erythrinacinate C and 1-octacosanol. Chemical structures were established mainly using both one and two dimensional 1H and 13C NMR data and by comparison of the current NMR data with those from literature. Mass spectrophotometry data were used for confirmation through molecular ion peak. Both compounds are known to have been isolated from other plant species but are being reported from this plant species for the first time.

Keywords: *Synadenium glaucescens*, Isolation, Chromatographic technique, Mass spectrophotometry.

INTRODUCTION

Syadenium glaucescent has many recorded traditional utilizations including medicinal uses and others such as construction materials and firewood. Despite the recent biological investigation that has so far been conducted for this plant [1]. very little is known regarding phytochemical investigation for this plant. This study was therefore deliberately conceived to investigate for chemical composition of this plant. Thus, phytochemical investigation of dichloromethane extracts of root barks and leaves respectively resulted into the isolation of erythrinacinate C (1) and 1- Octacosanol (2). erythrinacinate C (1) is an ester of ferulic acid and was first isolated from *Erythrina spp* with very limited bio assay data. The only available information regarding its bioassay information is the anti-microbial assay against various bacteria which indicated the compound to possess low potency as it possessed minimum inhibitory concentrations of above 100 µg/mL [2].

1-Octacosanol (2) is among long chain aliphatic alcohols collectively known as polycosanols. Although it is being reported for the first time from this plant species, this compound has earlier been isolated from other plants of different species, including *Simplices racemose* [3]. *Tinospora cordifolia* [4]. and *Holoptelea integrifolia* [5]. It has also been reported to be isolated from other sources especially waxes [6]. Information shows important uses of long-chain aliphatic alcohols such as treatment of various chronic diseases including diabetes and hypercholesterolemia [6-9]. Octacosanol has specifically been reported to possess interoceptive and anti-inflammatory activities [10]. which signify the relevance of the compound for the pharmacological control of pain and inflammatory processes.

MATERIALS AND METHODS

General methodology

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 recorded on 600 MHz 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 Mullingar village in Njombe district and were immediately subjected to drying process. Root barks were air dried under the shade in special drying room while leaves were dried under half day sun and half day shade. The need for some degree of sun heat for leaf drying was due to the fact that it possesses large amount of latex, thus preventing it from rotting. Grinding of dried plant materials was affected at the Department of Animal Science and Production (DASP), Sokoine University of Agriculture (SUA), Tanzania.

Extraction and Isolation

Respectively, 800 g and 1.2 kg of dried root barks and leaves of *Sy adenium glaucescent* were extracted at room temperature successively with n-hexane [2 x 2000 mL], dichloromethane [2 x 2000 mL], ethyl acetate [2 x 2000 mL], methanol [2 x 2000 mL] and water [2 x 2000 mL]. The filtrates from organic solvents were evaporated using a rotary evaporator with 40°C water bath temperatures and the aqueous extracts were freeze dried. The process involved soaking plant materials for 24 hours (x2) for each extraction in solvents of increasing polarity. After complete drying the extracts were kept in cold room until required for bioassays and phytochemistry.

Thirteen grams of DCM extracts from root bark was dissolved in 300 mL of distilled chloroform and was pre-adsorbed on silica gel. The sample was evaporated through rotary evaporator and then subjected to VLC column and eluting with gradient solvent mixtures of ethyl acetate/hexane (5:95-100:0) followed by chloroform/methanol (5:95-20:80) and a total of 13 VLC fractions were obtained. Fraction 3 and 4 were combined and subjected for repeated column chromatography. Initially gradient elution was applied using ethyl acetate: hexane in increasing polarity from 10: 90 to 20: 80 ethyl acetate/ hexane. Then isocratic elution was applied using 20:80 ethyl acetate/hexane mixture to obtain 25 fractions. Fractions 10-15 were combined and subjected for purification on prep TLC which yielding compound 1. The pre adsorbed dichloromethane leaf extract (5 g) was subjected to VLC and eluted using a gradient ethyl acetate/hexane which resulted into 12 VLC fractions. Fraction 3 (20:80 ethyl acetate/hexane) was subject into column and was eluted using an isocratic (20:80 ethyl acetate/hexane) solvent system. Crystalline compound obtained in fractions 11 through 13 were washed using methanol to obtain compound 2.

RESULTS AND DISCUSSION

Compound 1 was isolated as white crystals. The molecular formula was determined to be $C_{24}H_{38}O_4$ based on both one and two dimensional ^1H and ^{13}C spectra and MS data. Thus, MS analysis displayed a molecular ion peak at m/z 413 [M + Na] $^+$. The proton NMR exhibited signals at δ 7.60 (d, J=15.6 Hz, 1H) and δ 6.28 (d, J=15.6Hz, 1H). The coupling constants of 15.6 Hz implied that the signal belong to olefinic protons in a trans-position and were assigned to carbon 1^{l} and 2^{l} One ^1H doublet of doublet at δ 7.15 (J= 8.4 1.8 Hz) represented the aromatic H-2. The coupling constants are typical of meta- and ortho- splitting patterns between H-3 and H-2 and a long-range coupling in a W pattern through conjugation to H-6. A ^1H doublet at δ 6.90 (J = 8.4 Hz) was ascribed to another aromatic proton H-3. The ortho coupling constant is typically explaining the coupling with H 2. A proton doublet at δ 7.02 (J = 1.8 Hz) was assigned to H-6 while a 3H singlet at δ 3.91 was typical of a methoxy group and the presence of one hydroxyl group was shown by a broad singlet at δ 5.81. The broad singlet at δ 1.10 – 1.38, the presence of two proton triplets at δ 4.17 and the three proton triplets at δ 0.87 indicated the presence of a long chain aliphatic moiety. The signal δ 4.17 and δ 0.87 are typical of the methylene and methyl protons of the aliphatic chain attached respectively at the beginning and at the end of the aliphatic chain. The two-dimensional (2D) NMR spectra, mainly HMBC, HSQC and COSY were key in supporting the proposed structure. COSY revealed correlations between $H2^{l}$-1', H2-3 and H-1"-2". In the HMBC, the methoxy proton at carbon C-5 showed long range correlations to hydroxyl bearing carbon (C-4). Proton at C-6 showed long range correlation to C-4, C-2 and C-1. The carbonyl carbon (C-3^{l}) showed HMBC long range correlations with protons at C-1^{l} and C-1^{ll} These data are also supported by the ^{13}C-NMR data. The signal at δ 169.90 indicated the presence of carbonyl carbon (C-3!) of an ester group. The presence of eight unsaturated carbons in the low field region characteristic of an aromatic carbons and one extra double bond outside the aromatic region were in line with signals in ^1H-NMR. The signals at δ 144.6 and δ 109.3 were due to side chain C-C double bond and the signals were assigned to C-1^{l} and C-2^{l} respectively. Except for carbon $1^{"}$at 67.24 and the terminal methyl group at δ 14.1, the specific assignment of most carbon resonances from the side chain was tricky due to their structural similarity. Thus, the numbers of CH_2 groups between the two signals were determined through integration. The structure was then compared with data from literature [11-13]. All these data together confirmed an ester of ferulic acid. The complete ^1H- and ^{13}C-NMR chemical shift of the compound and those from literature are shown in Table 1.

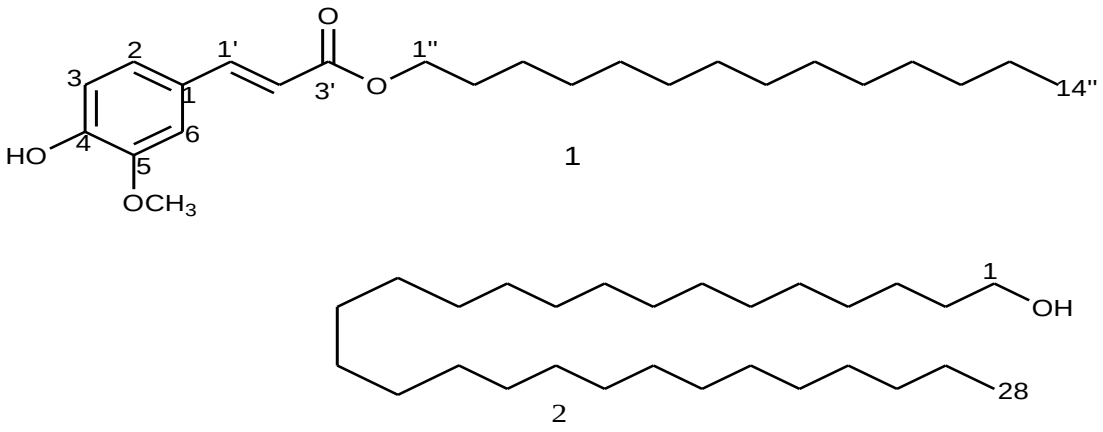

Figure 1: Structures of erythrinacinate C (1) and octacosanol (2)

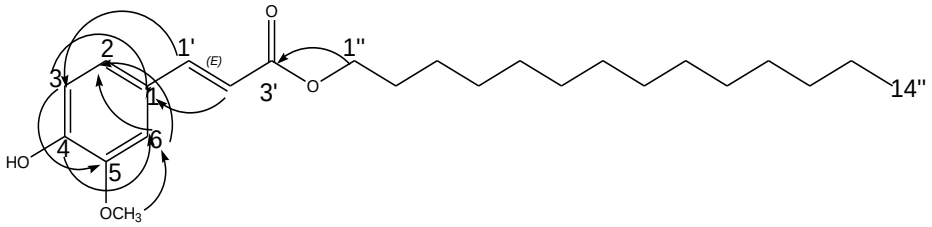

Figure 2: HMBC correlation of erythrinacinate C (1)

Table 1: ^{1}H and ^{13}C NMR Data for compound 1

	Experimental		Literature	
Carbon no	**^{13}C-NMR**	**^{1}H-NMR**	**^{13}C-NMR**	**^{1}H-NMR**
C-3'	167.4		167.3	
C-3	147.9		147.9	
C-4	146.8		147.9	
C-1'	144.6	7.60 (d, 1H, J=15.6Hz)	144.6	7.60 (1H, d, J = 16.0, $=CH_2$, H-2)
C-1	127.1		127.1	
C-6	123.0	7.06 (dd, 1H, J= 1.8Hz, 8.4Hz)	123.0	7.06 (dd, J = 8.6, 2.2 Hz, H-6)
C-2	115.8	7.02 (d, 1H, J= 1.8Hz)	115.7	7.02 (1H, d J=2.2)
C-5	114.7	6.90 (d, 1H, J=8.4Hz)	114.7	6.93 (1H, d J = 8.6, H-5)
C-2'	109.3	6.27 (d, 1H, J=15.6Hz)	109.3	6.26 (1H, J = 16.0 HZ, $=CH_2$),
C-1"	64.6	4.17 (t, 2H, J = 6.6Hz)	64.6	4.15 (2H, t, J = 7.3 HZ, OCH_2R
3-OMe	56.0	3.91 (S, 3H, $-OCH_3$)	55.9	3.8 (3H, s, 3-OCH_3)
C-12"	31.9		31.9	
C-10"	29.7		29.7	
C-6"	29.7		29.7	
C-8"	29.6		29.7	
C-9"	29.6	1.12-1.38 (24H, bs)	29.7	1.10 - 1.39 (24H, bs $(CH_2)12$)
C-7"	29.6		29.7	
C-5"	29.6		29.7	
C-11"	29.4		29.3	
C-4"	29.3		29.3	
C-2"	28.8		28.8	
C-3"	26.0		26.0	
C-13"	22.7		22.7	
C-14"	14.1	0.87 (t, 3H, J = 7.2 Hz)	14.1	0.88 (3H, t, J = 7.3Hz, Me)

Compound 2: Was obtained as white crystals from column fractions (Ethyl acetate/hexane) and the structure were determined based on NMR and MS data. Thus, the Mass spectrum displayed a molecular ion peak at *m/z* values of 409 [M - H] $^{-}$. The proton NMR exhibited four signals, the broad singlet at δ 1.10 – 1.38, the presence of two proton triplets at 3.52, the quintet at δ 1.55 and the three proton triplets at δ 0.86. These signals are characteristic of a long chain aliphatic moiety and the compound was concluded to be a fatty alcohol [12]. The molecular formula was determined as $C_{28}H_{58}O$ which corresponded to *n*-octacosanol. The ^{13}C NMR data of *n*-octacosanol showed only one methyl group (δ_C 14.10), all other signals were methylene groups, one of them (δ_C 63.11) attached to a hydroxyl functional group. These findings indicated the presence of a fatty alcohol. The length of the methylene chain was determined by means of the integrals resulting from the ^1H NMR spectrum. Interpretation of these integrals indicated the presence of 25 methylene groups at δ_H 1.27, leading to the final formula CH_3-$(CH_2)_{27}$-OH and was identified as 1-octacosanol. The melting point (80-82ºC) is typical of 1-octacosanol.

Identification of erythrinacinate C (1) and 1-octacossanol (2)

Erythrinacinate C (1): White crystals (10 mg); MP: 75-76°C ^1H NMR ($CDCl_3$, 600 MHz): see Table 1; ^{13}C NMR ($CDCl_3$, 600 MHz): see Table 1; MS (*m/z*): 413 [M + Na] $^+$

1-Octacosanol (2): White crystals (20 mg), MP: 80-82°C ^1H NMR ($CDCl_3$, 600 MHz): see Table 4; ^{13}C NMR ($CDCl_3$, 600 MHz): MS (*m/z*): 409 [M - H] $^-$

CONCLUSION

Two compounds, including an ester of ferulic acid and a fatty alcohol were isolated from *S. glaucescent.* The first compound was isolated from root bark while the second was isolated from leaves of *S. glaucescent*. The structures of the isolated compounds were identified as erythrinacinate C (1) and 1-Octacosanol (2) on the basis of spectroscopic data and by comparing with those reported in the literature. The complete ^1H and ^{13}C NMR spectral assignments of the

isolated compounds were made based on COSY, HSQC, HMBC and spectroscopic data.

Conflict of Interest

None declared.

Financial Support

None declared.

REFERENCES

1. Mabiki FP, Mdegela RH, Mosha RD, Magadula JJ. In ovo antiviral activity of *Synadenium glaucescens* (pax) crude extracts on Newcastle disease virus. 2013;7(14):863–70.
2. Nkengfack AE, Vouffo TW, Vardamides JC, Kouam J, Fomum ZT, Meyer M, *et al*. Phenolic metabolites from *Erythrina* species. Phytochemistry. 1997;46(3):573-8.
3. Gupta A, Sharma MC. Biologically active long-chain aliphatic alcohols and esters from the bark of *Symplocos racemosa*. International Journal of Pharmacognosy and Phytochemical Research. 2015;7(5):1056-9.
4. Thippeswamy G, Sheela ML, Salimath BP. Octacosanol isolated from *Tinospora cordifolia* downregulates VEGF gene expression by inhibiting nuclear translocation of NF-< kappa> B and its DNA binding activity. European journal of pharmacology. 2008;588(2-3):141-50.
5. Ganie SA, Yadav SS. *Holoptelea integrifolia* (Roxb.) Planch: a review of its ethnobotany, pharmacology, and phytochemistry. BioMed Research International. 2014:3pp.
6. Jackson MA, Eller FJ. Isolation of long-chain aliphatic alcohols from beeswax using lipase-catalyzed methanolysis in supercritical carbon dioxide. The Journal of supercritical fluids. 200637(2):173-7.
7. Damgé C, Hillaire-Buys D, Koenig M, Gross R, Hoeltzel A, Chapal J, *et al*. Effect of n-hexacosanol on insulin secretion in the rat. European journal of pharmacology. 1995;274(1-3):133-9.
8. Yatcilla M. Policosanols: cholesterol solution or controversial problem? Nutraceuticals World. 2002;5: 28.
9. Taylor JC, Rapport L, Lockwood GB. Octacosanol in human health. Nutrition. 2003;19(2):192.
10. Oliveira AM, Conserva LM, de Souza Ferro JN, Brito FD, Lemos RP, Barreto E. Antinociceptive and anti-inflammatory effects of octacosanol from the leaves of *Sabicea grisea* var. grisea in mice. International Journal of Molecular Sciences. 2012;13(2):1598-611.
11. Singh IP, Bharate SB, Bhutani KK. Anti-HIV natural products. Current science. 2005:269-90.
12. Wang X, Zhang M, Zhao Y, Wang H, Liu T, Xin Z, *et al*. Pentadecyl ferulate, a potent antioxidant and antiproliferative agent from the halophyte *Salicornia herbacea*. Food chemistry. 2013;141(3):2066-74.
13. Volken MC. Biological and phytochemical investigations of Euphorbiaceae from Papua New Guinea (Doctoral dissertation, ETH Zurich). 1999:122-25.

HOW TO CITE THIS ARTICLE
Nyigo VA, Malebo HM, Mabiki F, Mdegela R. Isolation and identification of long - chain aliphatic compounds from *Synadenium glaucescens*. J Phytopharmacol 2022; 11(3):151-154. doi: 10.31254/phyto.2021.11303