Taylor & Francis
Taylor & Francis Group

Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: https://www.tandfonline.com/loi/gnpl20

New *n*-nonadecanoyl-β-sitosterol and other constituents from the stem-bark of *Anacardium occidentale*

Abdullahi Shehu, Mangala Gowri Ponnapalli, M. Mahboob, P. V. Prabhakar & Gabriel Ademola Olatunji

To cite this article: Abdullahi Shehu, Mangala Gowri Ponnapalli, M. Mahboob, P. V. Prabhakar & Gabriel Ademola Olatunji (2019): New *n*-nonadecanoyl-β-sitosterol and other constituents from the stem-bark of *Anacardium occidentale*, Natural Product Research, DOI: 10.1080/14786419.2019.1650353

To link to this article: https://doi.org/10.1080/14786419.2019.1650353

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2019.1650353

Taylor & Francis
Taylor & Francis Group

New *n*-nonadecanoyl-β-sitosterol and other constituents from the stem-bark of *Anacardium occidentale*

Abdullahi Shehu[a], Mangala Gowri Ponnapalli[b], M. Mahboob[c], P. V. Prabhakar[c] and Gabriel Ademola Olatunji[d]

[a]Department of Chemistry, Faculty of Science, Federal University Lokoja, Lokoja, Kogi State, Nigeria; [b]Centre for Natural Products and Traditional Knowledge, Indian Institute of Chemical Technology, Hyderabad, India; [c]Toxicology Unit, Biology Division, Indian Institute of Chemical Technology, Hydrabad, India; [d]Department of Industrial Chemistry, Faculty of Physical Science, University of Ilorin, Ilorin, Nigeria

ABSTRACT

A new steroidal ester bearing *n*-nonadecanoyl moiety (**1**) and a mixture of isomeric cerebrosides (**2**) along with two known compounds were isolated from the methanol extract of the stem-bark of *Anacardium occidentale*. The structure of the new steroidal ester was determined as 3-*n*-nonadecanoyl-β-sitosterol on the basis of modern spectroscopic techniques (IR, ESI-MS, HR-ESIMS, 1D and 2D NMR) and chemical degradation studies. The structures of the known compounds were identified as gallic acid and tanacetene by comparison of the spectroscopic data with those of reported data. The mixture of cerebrosides was confirmed based on the analysis of 1D and 2D NMR. These compounds were evaluated for cytotoxicity against human cancer cell lines A549, SCOV3 and rat normal cell line NRK49f.

ARTICLE HISTORY
Received 22 October 2018
Accepted 24 July 2019

KEYWORDS
Anacardium occidentale; 3-n-nonadecanoyl-β-sitosterol; cerebroside; cytotoxicity

1. Introduction

Anacardium occidentale Linn. (Anacardiaceae) is a tropical evergreen tree commonly called cashew. It is native to Brazil and distributed throughout tropical countries like Nigeria, Kenya, Tanzania and India. *A. occidentale* stem-bark extracts are used in some

CONTACT Abdullahi Shehu abdusfa@gmail.com; Mangala Gowri Ponnapalli mangala@iict.res.in; pmgowri@yahoo.com

Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2019.1650353.

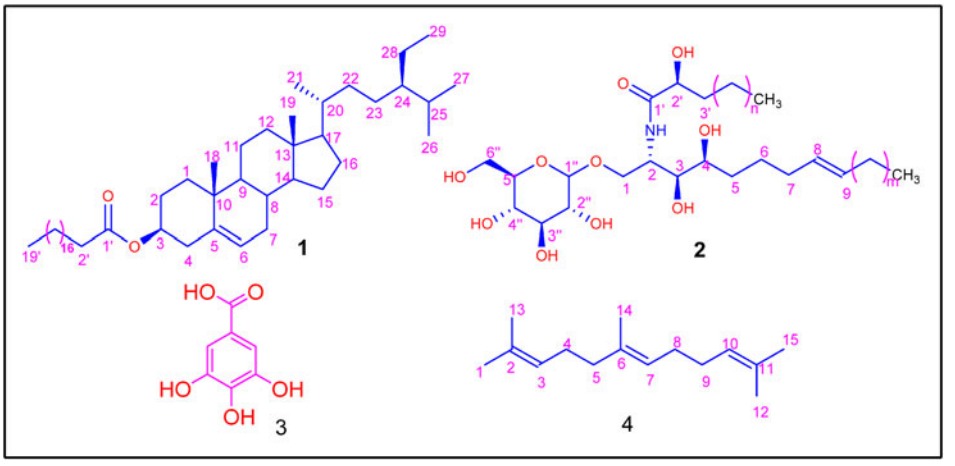

Figure 1. Compounds isolated from *Anacardium occidentale*.

part of Nigeria to treat diabetes and cardiovascular diseases (Eliakim-Ikechukwu et al. 2010; Okonkwo et al. 2010). Crude extracts of leaves, stem-bark, fruits and nut-shell liquid have been reported by various researchers to exhibit wide spectrum of biological activities. The extracts have been shown to possess hypoglycaemic properties (Abdullahi and Olatunji 2010), cytotoxic (Kubo et al. 2011; Santos et al. 2019), genotoxic effects (Barcelos et al. 2007), and tyrosinase inhibitory potential (Kubo et al. 1994). The hydroethanolic extract of the leaves of *A. occidentale* is reported to exhibit pronounced effect on leukaemia cell lines compared to normal T-lymphocytes as well as apoptic effect increased casp3 mRNA level expression in ALL cells (Santos et al. 2019). Further, the prior study of cashew kernel testa, an industrial waste from this plant demonstrated that it is a rich source of industrially significant tannins by HPLC (high performance liquid chromatography), TGA (thermo gravimetric analysis) and FTIR (Fourier-transform Infrared spectroscopy) analysis (Viswanath et al. 2016). Previous phytochemical investigations revealed the presence of polyphenols and tannins (Trevisan et al. 2006; Viswanath et al. 2016), flavonoids, their glycosides (Edy et al. 2007) and sterols (Murthy et al. 1982; Alexander et al. 2004). Herein, we report the isolation and structural elucidation of a new steroidal ester, a mixture of cerebrosides and two known constituents from the stem-bark of *Anacardium occidentale* (Figure 1) and their cytotoxicity evaluation against human cancer cell lines and a rat normal cell line.

2. Results and discussion

Compound **1** was obtained as a white crystalline solid, mp 78.5 °C. The ESIMS of compound **1** displayed a pseudo-molecular ion $[M+Na]^+$ at m/z 717.60127, consistent with the molecular formula of $C_{48}H_{86}O_2Na$, indicating six degrees of unsaturation. The IR spectrum of **1** showed absorption bands for ester carbonyl at 1729 cm^{-1} and olefinic functionality at 1640 cm^{-1}. The analysis of the 1D NMR spectroscopic data indicated that **1** was a steroidal ester structurally related to β-sitosterol. Its ^1H NMR spectrum (Table S1) exhibited characteristic signals for 18-Me at (δ 0.69, s) and 19-Me

Table 1. IC_{50} values (μM) of compounds.

Cell lines			
Compounds	A549	SCOV3	NRK-49f
1	1142.94	502.62	267.90
3	68.29	305.17	199.94
4	634.74	255.63	NA
Doxorubicin	2.5	4.06	6.2

NA: No activity.

at (δ 1.02, s) of sterol nucleus in addition to three secondary methyls at (δ 0.95, d, $J = 6.5$ Hz, 21-Me), (δ 0.92, d, $J = 6.5$ Hz, 26-Me), (0.81, d, $J = 6.5$ Hz, 27-Me) and one primary methyl at (δ 0.84, t, $J = 6.5$ Hz, 29-Me). The diagnostic 6-H of the Δ^5 sterol was noticed at (δ 5.37, br d, $J = 4.7$ Hz) and deshielded oxygenated methine at (δ 4.64, 1 H, m), corresponded to an oxymethine attached to an ester carbonyl. Further, it showed long chain terminal methyl at (δ 0.88, t, $J = 6.9$ Hz). Its ^{13}C NMR spectrum (Supplementary material Table S1) revealed resonances for 48 carbons which were differentiated by DEPT and HSQC spectra into seven methyls, twenty eight methylenes, nine methines and four quaternary carbons. It displayed the signals for an oxygenated methine carbon at (δ 73.7), a trisubstituted olefinic carbons at (δ 122.6, 139.7). Four spin systems involving protons H-19′/H-18′, H-2′/H-3′, H-2/H-3/H-4 and H-6/H-7 were identified by the analysis of COSY spectrum (Supplementary material Table S2 and Figure S1). Its HMBC spectrum (Supplementary material Table S2 and Figure S1) exhibited correlations between H-4 and C-3, C-5 and C-6 confirmed the position of the oxygenated methine at C-3. Other HMBC correlations between H-6/C-7, C-8, 19 Me/C-1, C-5, C-9 were allowed to locate the position of an olefin at C-5. The absence of NOESY (Supplementary material Table S2 and Figure S2) correlations between ring junction protons confirmed that compound **1** possesses *trans* ring fusion. The complete assignment of ^1H and ^{13}C NMR spectra of **1** were done by ^1H-^1H COSY, HSQC and HMBC. Alkaline hydrolysis of **1** afforded β-sitosterol and methyl ester of *n*-nonadecanoic acid, which was further confirmed by IR and GC-MS analysis. Thus, the structure of **1** was established as 3-*n*-nonadecanoyl-β-sitosterol.

The spectroscopic data of compound **2** (Supplementary material Tables S1 and S2) are characteristic of a cerebrosides and similar to the one reported in the literature (Kang et al. 1999). However, the data revealed that compound **2** is a mixture of isomeric cerebrosides as evident from the multiple signals in the olefinic, anomeric and NH regions of the ^1H NMR as well as different signals intensity ratios in the ^{13}C NMR spectrum. Thus, compound **2** is characterised as a mixture of isomeric cerebrosides. Based on the physical and spectroscopic (MS, IR, ^1H and ^{13}C NMR) comparison with literature, the structures of the other known compounds were characterised as gallic acid **3** and tanacetene **4** (Mahmood et al. 2002; Abri and Maleki 2016). Compounds **1, 3** and **4** were tested for their cytotoxicity activity on A 549 (lung adenocarcinoma), SCOV3 (ovarian carcinoma) and NRK-49f (normal rat kidney fibroblast) cell lines using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, compared with doxorubicin as a positive control. The cell growth-inhibitory potencies of the compounds, expressed as IC_{50} values as shown in Table 1. Compound **3** exhibited moderate cytotoxicity activity (IC_{50} 68.29 μM) against human lung adenocarcinoma

(A549). This result lends support to the earlier report by Kubo et al. (2011) and affirmed the role of phenolic compounds from *A. occidentale* as anti-cancer agents. However, compounds **1** and **4** exhibited no significant antiproliferative effect against the cancer cell lines achieving IC_{50} values $>100\,\mu M$. The criteria for cytotoxicity for crude extracts, as established by the U.S. National Cancer Institute (NCI), is an $IC_{50} < 20\,\mu M$ in the preliminary assay (Abdel-Hameed et al. 2012). The cytoxicity of compound **3** was observed in a dose-dependent manner and the cell viability above $10\,\mu M$ was significantly different ($p < 0.05$) from the control (Supplementary material Figures S3 and S4).

3. Experimental

General experimental procedures

The IR spectra were recorded on a Nicolet-740 FT-IR Spectrometer (Thermo Scientific, I.I.C.T., Hyderabad, India). The NMR spectra were recorded with Bruker Avance II (600 MHz), Bruker Avance (500 MHz), Varian Inova (400 MHz) and Bruker Avance (300 MHz) (Bruker, I.I.C.T., Hyderabad, India) for ^{1}HNMR, and 150 MHz,125 MHz, 100 MHz, 75 MHz for ^{13}CNMR in $CDCl_3$,C_5D_5N and $(CD_3)_2CO$ with tetramethylsilane as an internal standard. Coupling constants are given in Hz. The ESI-MS data were recorded on an Agilent 1100 MSD (Agilent Technologies, I.I.C.T., Hyderabad, India) with ESI SL Trap. The HR-ESI-MS data were acquired on an Agilent 6510Q-T.O.F. (Agilent Technologies, I.I.C.T., Hyderabad, India and ESI probe. Thin-layer chromatography (TLC) was performed on precoated silica gel GF_{254} plates (Merck & Co., I.I.C.T., India). The TLC plates were visualised under UV light or by spraying with 5% H_2SO_4 in methanol and heating at 70 °C.

Plant material

The stem-barks of *A. occidentale* were collected at Olorunsogo Area in Ilorin, Kwara State. They were identified and authenticated by Mr. Edward Bolu Ajayi at the Herbarium of the Department of Plant Science, University of Ilorin, Nigeria. Voucher Specimens (UIH 001/970) were deposited at the Department of Plant Science, University of Ilorin, Nigeria.

Extraction and isolation

Air dried stem-bark of *A. occidentale* (1.8 kg) was coarsely powdered and percolated with *n*-hexane, ethyl acetate and ethanol respectively. The ethanol extract (196 g) was subjected to VLC on silica gel (230–400 mesh) and eluted with a mixture of $CHCl_3$-acetone-MeOH in order of increasing polarity yielding 28 fractions of 500 mL each. The identical fractions were pooled based on the TLC profile. A total of eight main fractions (ETF1–ETF8) were obtained (Jone & Kinghorn 2006). Repeated column chromatography of fraction ETF2 (400 mg) afforded sub fractions, which was further subjected to $AgNO_3$-impregnated silica gel column chromatography $CHCl_3$: hexane (1.5:8.5) furnished compound **1** (15 mg). Fraction ETF_6 was suspended in H_2O (5 mL) and then extracted with EtOAc. The EtOAc layers were concentrated under reduced pressure to

yield crude residue (10.4 g). A portion of the fraction (2.5 g) was subjected to further purification on a silica gel (230–400 mesh) CC eluting with a mixture of MeOH/$CHCl_3$ yielded ten fractions. Fraction 2 (5% MeOH/$CHCl_3$) gave compound **2** (6 mg). The ethyl acetate extract (204 g) was also subjected to VLC on silica gel (230–400 mesh) eluting with a mixture of acetone-hexane of increasing polarity to afford a total of six fractions (EAF1–EAF6). The more polar fraction (EAF5) afforded compound **3** (26 mg) while the non-polar fraction (EAF1) gave compound **4** (5 mg).

Hydrolysis of compound 1

Compound degradation study was carried out to separate the steroid moiety from the long chain saturated ester. Compound **1** (5 mg) was dissolved in a mixture of THF/H_2O (5 mL) v/v in a 25 mL round-bottomed flask and KOH (0.273 g) was added to the flask. The reaction solution was refluxed for 12 h at 60 °C. The mixture was extracted with EtOAc (5x 5 mL) and dried over anhydrous Na_2SO_4. The product was purified by recrystallization from MeOH to yield the basic part of the hydrolysed molecule **1a** (2.3 mg). The aqueous layer was acidified with 3% Con. HCl and then extracted with $CHCl_3$ (3x 3 mL) to give the acidic part of the hydrolysed molecule. The $CHCl_3$ extract was then methylated by adding MeOH (3 mL), 3 drops conc. H_2SO_4 and the mixture was refluxed for 12 h. The reaction mixture was then extracted with $CHCl_3$ and the resulting methyl ester **1b** (2.1 mg) was subjected to GC-MS & IR analyses.

Cytotoxicity assay (MTT assay)

Human tumour cell lines, A549 (lung adenocarcinoma), SKOV3 (ovarian carcinoma) and normal cell line NRK-49F (normal rat kidney fibroblast) are seeded onto 96-well micro titre plates at different concentrations and then incubated at 36.5 °C in humidified CO_2 5% incubator for 24 h. The growth medium was removed and maintenance medium added 2 µl containing various concentrations of compounds **1, 3** and **4** whereas positive and negative control as standard drug (doxorubicin) and no standard drug respectively and the plates were incubated for the next 24 h. After incubation, medium was removed from the wells and 100 µl of maintenance medium + 5 µl MTT was added. Plates were incubated for 30–60 minutes, medium/MTT was removed and insoluble product was dissolved in 50 µl DMSO. Finally, the absorbance was measured at 540 nm. The 50% inhibitory concentration (IC_{50}) value of the compounds was calculated (Ata-ur-Rahman et al. 2007).

3-nonadecanoyl-β-sitosterol (**1**)**:** $C_{48}H_{86}O_2$; white crystalline solid from a mixture of 15% EtOAc/n-hexane; R_f 0.5 (15% chloroform/n-hexane); m.p. 78.5 °C; IR (KBr) ν_{max} 2918, 2850, 1736, 1630, 1464, 1376, 1173, 765 cm^{-1}; ^1H, ^{13}C, and 2 D NMR data (see Supplementary material Tables S1 and S2 and Figure S2); HR-ESIMS m/z 717.65200 $[M + Na]^+$ (calcd for $C_{48}H_{86}O_2Na$ 717.60028).

4. Conclusion

The isolation and characterisation of a new steroidal ester are noteworthy. To the best of our knowledge, this is the first time reported a β-sitosterol substituted at a C-3 carbon with a C_{19} long chain saturated aliphatic ester isolated from natural sources and characterised as such. Although similar chain aliphatic ester with longer or shorter carbon chains have been isolated from many plants (Dupont et al. 1997; Dinda et al 2003), compound **1** differ from these owing to its odd number of carbon chains. The existence of the other known compounds in Anacadiaceae family is not well known and furthermore this is the first report of the compounds from *Anacardium occidentale*.

Acknowledgement

The authors acknowledged Tertiary Education Trust Fund (TETFUND), Federal University Lokoja, Nigeria and TWAS-CSIR Postgraduate fellowships given to Abdullahi Shehu and the Director, Dr. S. Chandrasekhar, CSIR-Indian Institute of Chemical Technology, Hyderabad, India for constant support and encouragement.

Disclosure statement

No potential conflict of interest was reported by the authors

Funding

This work was supported by Academy of Science for the Developing World [TWAS-CSIR Postgraduate Fellowship 2014-2015] and Tertiary Education Trust Fund, FU Lokoja, Nigeria.

References

Abdel-Hameed ES, Salih A, Bazaid SA, Shohayeb MW, El-Sayed MM, El-Wakil EA. 2012. Phytochemical studies and evaluation of antioxidant, anticancer and antimicrobial properties of *Conocarpus erectus* L. growing in Taif, Saudi Arabia. Eur J Med Plants. 2(2):93–112.

Abdullahi S, Olatunji GA. 2010. Antidiabetic activity of *Anacardium occidentale* in alloxan-diabetic rats. J Sci Technol. 30:35–41.

Abri A, Maleki M. 2016. Isolation and identification of gallic acid from the *Elaegnus angustifolia* leaves and determination of total phenolic flavonoids contents and investigation of antioxidant activity. Iran. Chem Commun. 4:146–154.

Alexander LRL, Morrison EY, Nair MG. 2004. Hypoglycaemic effect of stigmast-4-en-3-one and its corresponding alcohol from the bark of *Anacardium occidentale* (cashew). Phytother Res. 18(5):403–407.

Ata-Ur-Rahman, Choudhary MI, Thomsen WJ. 2007. Bioassay techniques for drug development. New York (NY): Informa Health care; p. 34–35.

Barcelos GR, Shimabukuro F, Maciel MA, Colus IM. 2007. Genotoxicity and antigenotoxicity of cashew (*Anacardium occidentale* L.) in V79 Cells. Toxicol In Vitro. 21(8):1468–1475.

Dinda B, De UC, Achari B, Arima S, Sato N, Harigaya Y. 2003. Steroid and nitrophenol esters from *Bignonia unguis-cati* root. Indian J Chem Sect B. 42:1514–1518.

Dupont M, Llabrés G, Delaude C, Tchissambou L, Gastmans J. 1997. Sterolic and triterpenoidic constituents of stem bark of *Drypetes gossweileri*. Planta Med. 63(03):282–284.

Edy SDB, Manuela CPDA, Long-Ze L, James H. 2007. Determination of the flavonoid components of cashew apple (*Anacardium occidentale*) by LC-DAD-ESI/MS. Food Chem. 105(3):1112–1118.

Eliakim-Ikechukwu CF, Obri AI, Akpa OA. 2010. Phytochemical and micronutrient composition of *Anacardium occidentale* Linn. (cashew) stem-bark hydroethanolic extract and its effect on the fasting blood glucose levels and body weight of diabetic rats. Int J Nutr Wellness. 10(1): 923–927.

Jone PW, Kinghorn D. 2006. Extraction of plant secondary metabolites. In: Sarker DS, Latif Z, Gray AI, editors. Natural products isolation. 2nd ed. Totowa (NJ): Humana Press; p. 323–351.

Kang SS, Kim JS, Xu YN, Kim YH. 1999. Isolation of a new cerebroside from the root bark of aralia elata. J Nat Prod. 62(7):1059–1060.

Kubo I, Kinst-Hori I, Yokokawa Y. 1994. Tyrosinase inhibitors from *Anacardium occidentale* fruits. J Nat Prod. 57(4):545–551.

Kubo I, Nitoda T, Tocoli FE, Green IR. 2011. Multifunctional cytotoxic agents from *Anacardium occidentale*. Phytother Res. 25(1):38–45.

Mahmood U, Kaul VK, Singh B. 2002. Sesquiterpene and long chain ester from *Tanacetum longifolium*. Phytochemistry 61(8):913–917.

Murthy SS, Anjaneyulu AS, Row LR, Pelter A, Ward RS. 1982. Chemical examination of *Anacardium occidentale*. Planta Med. 45(05):3–10.

Okonkwo TJN, Okorie O, Konta JM, Okonkwo CJ. 2010. Subchronic hepatotoxicity of *Anacardium occidentale* (Anacardiaceae) inner stem bark extract in rats. Indian J Pharm Sci. 72(3):353–357.

Santos JM, Nathalia MC, Jose AY, Jorge AL, Maria LH-M. 2019. Effect of *Anacardium occidentale* leaf extract on human acute lymhoplastic leukaemia cell lines. Nat Prod Res. 33(11): 1633–1636.

Trevisan MTS, Pfundstein B, Haubner R, Wurtele G, Spiegelhalder B, Bartsch H, Owen RW. 2006. Characterization of alkylphenols in cashew (*Anacardium occidentale*) products and assay of their antioxidant capacity. Food Chem Toxicol. 44(2):188–197.

Viswanath V, Leo VV, Sabna Prabha S, Prabhakumari C, Potty VP, Jisha MS. 2016. Thermal properties of tannin extracted from *Anacardium occidentale L*. using TGA and FT-IR spectroscopy. Nat Prod Res. 30(2):223–227.