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Isolation and Characterization of Lanostane Glycoside from the Leaves of *Stachyterpheta jamaicensis* Linn Vahl

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ABSTRACT: Chemical investigation of the bioactive constituents from the leaves of *Stachytarpheta jamaicensis* Linn Vahl (Gervao, Brazilian tea or bastard vervain) resulted in the isolation of a new lanostane triterpenoid glycoside 16β-(β-D-glucopyranosyl-3,8,-dihydroxylanstan-5,22-diene-11-methoxy-1β-yl-6-O-(2,3-dimethoxybenzoyl)-β-D-glucopyranoside. The structure was elucidated using NMR spectroscopy in combination with IR and MS spectral data.
Keywords: Bioactive compound, antioxidant, anti-inflammatory, analgesic, antacid, anti-anaphylactic, natural product.

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INTRODUCTION

Stachytarpheta jamaicensis Linn Vahl (Gervao, Brazilian tea, Verbenaceae) is a Nigerian medicinal plant used in phytomedicine to cure diseases and heal injuries. The plants have various effects on living systems. It exhibits sedative, liver protective, cardio-protective, anti-inflammatory, oxytocic, antispasmodic and immune modulator activity[1]. The plant is widely used in phytomedicine to cure diseases such as asthma, cough, arthritis, bronchitis, bronchial phlegm, constipation, diabetes, inflammation, liver diseases, menstrual disorder, rheumatism and malaria[2,3]. As part of an ongoing search for biologically active secondary metabolites from the Rain Forest Biodiversity of Nigeria, *Stachytarpheta jamaicensis* Linn Vahl (Verbenaceae) was selected for studies because of its application in the treatment of diverse diseases and ailments. In herbal medicine, *S. jamaicensis* is used to treat various ailments such as inflammation, pain, fever, hepatic and renal disorder, helminthiasis, constipation, hypertension, stress and diabetes[4,5] *S. jamaicensis* is an antacid, analgesic, anti-helmithic, anti-inflammatory, diuretic, hypotensive, laxative, lactogogue, progative, sedative, stomach tonic, spasmogenic, vulnerary and vermifuge[6,5]. It is used for allergies and respiratory conditions such as flu, asthma, bronchitis, and cough[5] .It is used for digestive problems such as indigestion, acid reflux, ulcers, constipation, dyspepsia and slow digestion[5]. Pregnant patients and patients with low blood pressure are advised not to use this plant because of its abortive and hypotensive properties[5]. *S. jamaicensis* belongs to the family Verbanaceae. It is an annual weedy herbaceous plant, sometimes perennial that grows 60 – 120cm tall and is reproduced from seeds[7]. It bears small reddish-purple to deep blue flowers. Two common very similar species of *Stachytarpheta* grow in the tropics and are use interchangeably (and share the same common names) in herbal medicine.The species include *Stachytarpheta cayennesis* and *Stachytarpheta jamaicensis*[8]. Phytochemical studies of *S. jamaicensis* revealed that it contains alkaloids ipolamide, β-hydroxyipolamide, verbascoside steroids triterpenes and irridoids[9,10.8,6] .Previous biological studies have documented that secondary metabolites isolated from *S. jamaicensis* exhibited antispasmodic, vasodilator, anti-inflammatory, anti-diarrhea effects, antacid; liver protective, anti-ulcer and laxative properties[8,2,11,12].

We report herein on the isolation and characterization of a new lanostane triterpene glucoside 16β-(β-D-glucopyranosyl-3,8,-dihydroxy lanstan-5,22-diene-11-methoxy 1β-yl-6-O-(2,3-dimethoxybenzoyl)-β-D-glucopyranoside. The structure was elucidated through the combination of spectroscopic analysis and comparison with reported data.

MATERIALS AND METHOD

General Experimental Procedure

IR spectra was determined on a Thermo Nicolet Nexus 470 RT – IR spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 FT NMR

spectrometer using Tetramethylsilane (TMS) as internal standard. Chemical shifts are expressed in parts per million (ppm). LC-ESIMS spectra were determined in the positive ion mode on a PE – Biosystem. API 165 single quadruple instrument. HRESIMS (positive ion mode) spectra were recorded on a Thermo Finniga MAT 95 XL mass spectrometer. Column chromatography was carried out with silica gel (200-300 mesh) and to monitor the analytical thin layer chromatography (TLC) was performed at room temperature on pre-coated 0.25 mm thick silica gel 60 F_{254} aluminum plates (20 x 20 cm) Merck, Darmstadt, Germany. Reagents and solvents like ethanol, chloroform, diethyl ether, hexane, were all of analytic grade and were procured from Merck, Darmstadt, Germany.

Plant Materials

Fresh leaves and stems of *S. jamaicensis* were harvested from Edibe-Edibe, Calabar, Cross River State, Nigeria on 10th January, 2008. Plant samples (flowers, stems and leaves) were identified by Dr. A. Nmeregini of the Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. A voucher specimen No. SJ/3348 has been deposited at the Forestry Department of the University.

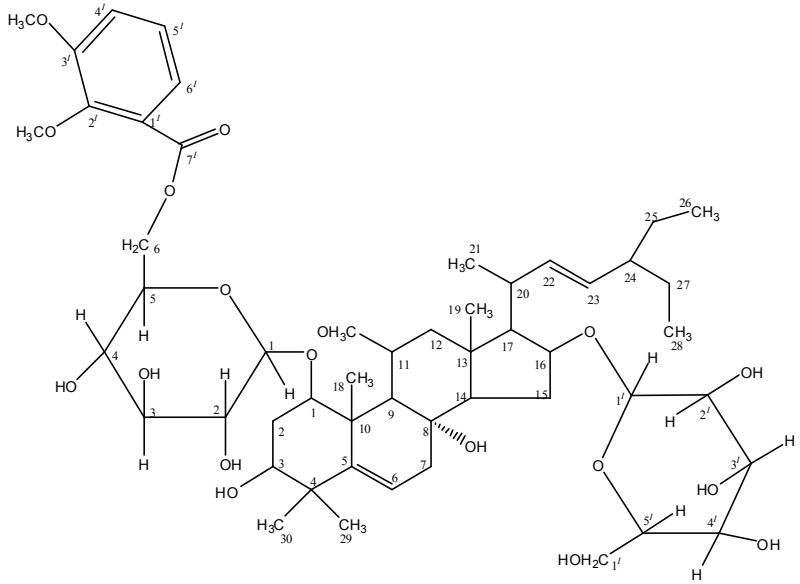
Extraction and Isolation of Plant Materials

Plant materials were treated and analyzed at the Chemistry laboratory, Michael Okpara University of Agricultre, Umudike, Nigeria. The leaves (1Kg) were dried on the laboratory bench for 10 days. The dry sample was milled and ground into powder (850 g) using a Thomas Wiley Machine (Model 5 USA). The powdered plant sample (500 g) was packed into Soxhlet apparatus (2L) and extracted exhaustively with 1000 ml ethanol for 24 hrs. The ethanol extract was concentrated using a rotary evaporator at 45^0C and left on the laboratory bench for 2 days to obtain a dark gummy residues (22.64 g).

The column was parked with silica gel and 20.0 g of the gummy residue placed on top of the silica gel and eluted with chloroform, petroleum ether, methanol (50:30:20) respectively to afford lanostane **1** (1.20 g). Compound **1**: Light yellow oil (1.20 g). Rf 0.43 IR Vmax 3433.24 cm^{-1} (OH), 2930.13 (-CH_2-), 2851.64 (-CH_2-), 1736.50 cm^{-1} (C=O), 1640.21 cm^{-1} (-C=C-), 1052.90 cm^{-1} (C-O), 1022.60 cm^{-1} (C-O). HREIMS 992.41 [m^+] calculated for $C_{52}H_{80}O_{18}$ m/z 992. Base peak m/z 432.37. ^{1}H NMR and ^{13}C NMR were presented in Table **1**.

RESULTS AND DISCUSSION

Lanostanoid triterpenoid glucoside **1** was isolated as light yellow oil and showed a molecular ion peak at m/z 992.41 $[M]^+$ by HREIMS which corresponds to the molecular formula $C_{52}H_{80}O_{18}$ (m/z 992 calculated). The IR spectrum of **1** exhibited absorptions of hydroxyl (3433.24 cm^{-1}), aliphatic (2930.13, 2851.64 cm^{-1}), carbonyl (1736.50 cm^{-1}), aromatic (1640.21 cm^{-1}) and ether (1022.60 cm^{-1}) functional groups. The ^{1}H NMR spectrum of **1** displayed signals due to four tertiary methyls (δH 0.975, 0.9335, 0.8263 and 0.8263), one secondary methyl (δH 0.8740) and two primary methyls (δH 0.8454 and 0.8324) respectively. The compound showed olefinic protons at (δH 5.0235, 5.0455, and 5.0565). This was confirmed by the ^{13}C NMR spectrum which shows the presence of the olefinic carbons at δC 110.2210 (C_6), 107.6520 (C_{22}) and 107.6441 (C_{23}), while the tertiary methyl carbons appeared at δC 20.2410, 20.1001, 23.2012 and 23.5010 respectively.



Compound 1 $C_{52}H_{80}O_{18}$

The benzoyl protons showed a singlet aromatic absorption at δH 7.0389, 7.2599 and 7.6761. The other aromatic protons were fully substituted with methoxy groups. The methoxy protons were observed to absorbed at δH 3.5241 and 3.5859 respectively. The data suggests that **1** is a tetracyclic triterpene with a glucosidic linkage at C_1 and C_{16} and a methoxy group attached to the lanostane skeleton. There are also hydroxyl groups attached to C_3 and C_8 of the lanostane nucleus. The glucosidic moity attached to C_1 have a carbonyl group at $C_7^{\prime}$. This was confirmed by IR peak at 1736.50 cm^{-1} and ^{13}C NMR spectrum which shows the carbonyl carbon at δC 173.3202. Compound **1** was assigned the molecular formula m/z 992.41 calculated for $C_{52}H_{80}O_{18}$ (m/z 992) with base peak at m/z 432.37 calculated for $C_{27}H_{44}O_4$ (m/z 432). The pattern of fragmentation (Figure **1**) showed that compound **1** undergoes cleavage of the methyl group at C_{28} and detachment of the ethyl group at C_{25} followed by the removal of the glucosidic linkage at C_1 and C_{16} of the lanostane nucleus to afford the base peak at m/z 432.37 calculated for $C_{27}H_{44}O_4$ (m/z 432). Other prominent peaks were observed at m/z 429.33, 430.85 and 433.89. These peaks occurred as a result of proton migration and re-arrangement. Detachment of the glucosyl moiety attached to C_1 with the benzoyl group resulted in the formation of the fragment $C_{15}H_{19}O_8$ (m/z 327.36). Combining the MS, NMR and IR spectral data, compound **1** was identified as 16β-(β-D-glucopyranosyl-3,8,-dihydroxy-lanstan-5,22-diene-11-methoxy-1β-yl-6-O-(2,3-dimethoxybenzoyl)β-D-glucopyranoside.

Lanostane triterpenoids have been confirmed to exhibit anti-inflammatory activity[13] and apoptosis induction in myeloid leukemia cells[14]. Lanostane glycosides have been found to exhibit pronounced biological activity such as anti-inflammatory and anticancer activity[14,13]. Clinical trials revealed lanostane to be a potent drug with outstanding ability to block the release of the enzymes responsible for pains and inflammation[15]. It may become a very useful treatment for the symptoms of arthritis and muscular dystrophy. This supported the use of *S. jamaicensis* as a general pain-reliever and anti-inflammatory agent for various internal/external painful inflammatory disorders. The occurrence of lanostane glucoside in *S. jamaicensis* may be the reason behind the use of this herb in phytomedicine for analgesic, antacid, anti-anaphylactic, anti-inflammatory and antispasmodic agent. This also supported the use of *S. jamaicensis* in the treatment of asthma, bronchitis, bile regulation, hormone regulation, lactation stimulation and birth control in phytomedicine in Nigeria. This work therefore shows that lanostane glucoside may be one of the physiologically active compounds in *S. jamaicensis*. The occurrence of lanostane glucoside in *S. jamaicensis* is of significance because this is to the best of our knowledge the first report of its occurrence in any Stachytarpheta species. The plant offer wide-scope for utilization as raw materials by pharmaceutical firms for drug formulation.

Table 1: ^{1}H (400 mHz) and ^{13}C NMR (75 mHz) of Compound 1

Position	δH		δC	
1	1.5422	1Ht	31.5210	CH
2	1.2540	2Ht	20.6900	CH_2
3	1.4830	1Hm	32.82400	HOCH
4			22.5120	C
5			110.2102	C
6	5.0565	2Hm	110.2210	CH_2
7	1.2540	2Hm	25.5208	CH_2
8			34.0802	C
9	1.8241	1Hs	32.8204	CH
10			36.5204	C
11	1.4830	1Hd	25.6750	CH
12	1.2540	2Hm	25.7605	CH_2
13			38.543	C
14	1.0337	1Hd	31.3410	CH
15	1.2540	2Hm	27.9420	CH_2
16	1.4830	1Hm	34.1660	CH
17	1.8241	1Hd	32.0660	CH
18	0.9075	3Hs	20.2410	CH_3

19	0.9335	3Hs	20.1001	CH_3
20	1.8241	1Hm	48.1120	CH
21	0.8740	3Hd	20.6916	CH_3
22	5.0235	1Hd	177.6520	CH
23	5.0455	1Hd	107.6441	CH
24	1.2540	1Hm	25.2010	CH
25	2.2691	2Hm	27.6204	CH_2
26	0.8454	3Ht	14.2510	CH_3
27	2.2691	2Hm	27.61002	CH_3
28	0.8324	3Ht	14.4041	CH_3
29	0.8263	3Hs	23.2012	CH_3
30	0.8263	3Hs	23.5010	CH_3
31	3.4924	3Hs	55.7208	OCH_3
1^{l}			127.5501	C
2^{l}			116.3011	C
3^{l}			116.4022	C
4^{l}	7.0389	1Hs	127.4520	C
5^{l}	7.2599	1Hs	127.6020	C
6^{l}	7.6761	1Hs	109.3024	C
7^{l}			173.3202	C=O
8^{l}	3.5241	3Hs	52.7501	OCH_3
9^{l}	3.5859	3Hs	52.7622	OCH_3
Glu				
1	6.9473	1Hs	107.2021	CH
2	6.3070	1H(bs)	75.6122	HOCH
3	5.9944	1Hd	78.6112	HOC=H
4	5.3612	1Hd	71.7232	HOCH
5	5.3409	1Hs	78.6102	CH
6	5.1696	2Hd	62.9210	CH_2
1^{l}	6.8393	1Hs	107.0022	CH
2^{l}	5.1696	1Hs	75.6022	HOCH
3^{l}	5.1421	1Hm	71.7024	HOCH
4^{l}	5.1094	1Hm	78.7204	HOCH
5^{l}	5.0455	1Hm	72.9102	CH
6^{l}	4.9620	2Hm	62.9122	CH_2

S = singlet, bs = broad singlet, t = triplet, m = multiplet, d = doublet

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