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Isolation and characterization of Steroidal Glycosides from the leaves of *Stachytarpheta Jamaicensis* Linn Vahl

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ABSTRACT

*Steroids represents essential group of natural products which exhibits a broad spectrum of pharmacological profile. They were found to be important hormone regulator which possesses oxytocic, anti-inflammatory, antioxidant, anti-asthmatic, bronchodilator, anti-spasmodic properties; liver detoxifying actions and helps to normalize sticky blood. 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 two new steroidal glucosides 16 β-(β-D glucopyranosyl, 3, 8, 22–trihydroxy) Cholestan–1 β-yl–6–O-(3, 4, 5–trimethoxybenzoyl) β-D, glucopyranoside **1** and 16-β (β-D–glucopyranosyl 3,8,22-trihydroxy-cholest-5,14,16,23 tetraiene 1β-yl, 6-O-(3,4,5-trimethoxybenzonyl) β-D glucopyranoside **2)**. The structures were elucidated using NMR spectroscopy in combination with IR and MS spectral data.*

Keywords: Hormone regulator, antioxidant, anti-inflammatory, bronchodilator, oxytoxic, detoxifying agents, natural products.

INTRODUCTION

Natural extracts derived from plants are proven source of bioactive compounds with therapeutic indications against a wide spectrum of diseases and infections [1-3]. In particular, *Stachytarpheta jamaicensis* has been shown to produce secondary metabolites that display oxytocic, anti-inflammatory, antioxidant, neuroprotective, antiviral, antibacterial, liver protective, cardioactive and antitumorous effects [4-6].

As part of an ongoing search for biologically active secondary metabolites from the Rain Forest Biodiversity of Nigeria, *Stachytarpheta jamaicensis* Linn Vahl (Verbenaceae) commonly known as (Gervao, Brazilian tea, verbena cimarrona, bastard vervain, blue flower or rooter comb) was selected for studies because of its multipurpose utilization as ornamental, vegetable and medicine. *S. jamaicensis* is a weedy annual or perennial herbaceous plant that grows 60 – 120 cm tall [7]. It bears small reddish-purple to deep blue flowers. It is indigenous to most parts of

tropical America and although some consider it a semi-invasive weed to Africa [7]. It is sometimes cultivated as an ornamental plant around homes for its blue flowers and deeply serrated green leaves [8]. *S. jamaicensis* belongs to the verbenaceae family. It is often referred to as bastard vervain or wild verbena. Two very similar species of *Stachytarpheta* grow in the tropics and are used interchangeably (and share the same common names) in many countries' herbal medicine systems. They include *S. cayennensis* and *S. jamaicensis* [8].

The leaves and stem extracts are used extensively in traditional medicine in the preparation of drugs used as a stomach tonic, to stimulate the function of the gastrointestinal tract, for dyspepsia, allergies, asthma and fevers and for chronic liver problems [8-10]. The leaves are used to cure cough, dysentery or chest colds in herbal medicine. Extracts from the leaves prevent colic disorders, cure head or chest colds [11], suppressed cough [12] and is often used in the treatment of cirrhosis and hepatitis (inflammation of the liver) [13]. Externally, it is used to clean ulcers, sores, cuts and wounds [8]. In herbal medicine, the plant is considered to be abortive, laxative, diuretic and sedative and is used to reduce spasms, depress the central nervous system, promote menstruation, and milk production in nursing mother [8,14]).

S. jamaicensis is extensively used by women in southern Nigeria for many types of menstrual disorders and female complaints [15]. The leaves are used to prepare tea for women after childbirth to restore the uterus to its position, regulate hormones and increase the supply of milk in nursing mother [15]. A tea brewed from the aerial parts of the plant is taken for nervousness, heart conditions, stomach, dyspepsia, neuralgia, cough, colds, fever, flu and liver complaints [8]. The mashed leaves are also used in a poultice for boils and infected sores while the leaf juice is taken internally for intestinal parasites [8].

In herbal medicine, *S. jamaicensis* is regarded as a safe, natural remedy when prepared in decoctions or infusions which is normally taken orally or applied externally. The main actions of the plant include antihistamine, bronchodilator, anti-inflammatory, and antacid, anti-parasitic and are mainly used for allergies and respiratory conditions (cold, flu, asthma, bronchitis) [8]. The plant finds application as a remedy to tone, balance, strengthen, protect and detoxify the liver and as a liver bile stimulant and for chronic liver problems [8,16,13]. In some countries it is used for abortions, childbirth, lactation stimulation and treatment of menstrual disorders [8]. These functions are due to the steroids, iridoids or flavonoids compounds available in the plant. *S. jamaicensis* contains flavonoids, terpenes, phenols and steroids. Several of these plant chemicals have been documented [8]. Iridoid glycosides known as verbascoside or acetoside have been isolated from the plant [6]. In clinical research, this powerful antioxidant phytochemical has been documented with neuroprotective, antiviral, antibacterial, liver protective, cardioactive and antitumerous effects [4,5,6,17]. A flavonoid in *S. jamaicensis* called scuttelarein has been documented with cardioprotective, anti-inflammatory and antiviral actions [18]. Another flavonoid found in the plant is known as hopidulin has been reported to have anti-asthmatic, bronchodilator and antispasmodic properties; liver detoxifying actions and helps to normalize sticky blood [16,19]. The main plant chemicals in *S. jamaicensis* include: epigenol–7–glucoronide, alpha–spinasterol, stachytarphine, scutellarein, uroslic acid, scultellarein and verbascoside [8].

Herein we report for the first time the isolation, characterization and structural elucidation of two steroidal glucosides 16β-(β-D- glucopyranosyl-3,8,22–trihydroxy)-Cholestan–1β-yl–6–O-(3,4,5–trimethoxybenzoyl) β-D-glucopyranoside **1** and 16-β(β-D–glucopyranosyl 2) 3,8,22–tri-hydroxy cholest 5, 14, 16,23 tetraiene-1 β-yl–6–O-(3, 4, 5–trimethoxybenzoyl) β-D, glucopyranoside **2** from the leaves of *S. jamaicensis.*

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 preparative separations, 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, and hexane were all of analytical 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 Number 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 Agriculture, Umudike, Nigeria. The leaves (1kg) were dried on the laboratory bench for 10 days. The dry sample was milled and ground into powder (850g) using a Thomas Wiley Machine (Model 5 USA). The powdered plant sample (500g) 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^{0}C and left on the laboratory bench for 2 days to obtain a dark gummy residues (22.64g).

The column was packed with silica gel and 20.0g of the gummy residue placed on top of the silica gel and eluted with chloroform, ethanol, petroleum ether, (50:30:20) respectively to afford steroids **1** (1.53g) and **2** (1.40g).

Compound 1: Dark yellow oil, (1.53 g) Rf 0.20. IR Vmax 3395.41 cm^{-1} (OH), 3009.84 cm^{-1}, 2925.05, 2853.95 cm^{-1} (-CH_2-) respectively, 1739.38, 1710.58 cm^{-1} (C=O) respectively, 1462.89cm^{-1} (C=C aromatic), 1037.46 (C–O) ether. HREIMS m/z 971.87 calculated for $C_{49}H_{78}O_{19}$ m/z 970. Base peak m/z 301.12. ^{1}H NMR and ^{13}C NMR were presented in Table **1**.

Compound 2: Light yellow oil (1.40 g) Rf 0.36; IR Vmax 3376.68cm^{-1} (OH), 2925.15 cm^{-1}, 2853.84 cm^{-1} (-CH_2-) respectively, 1738.93 (=C=O), 1402.97 (C=C) aromatic, 1040.28cm^{-1} (C-O) ether. HREIMS m/z 963.50 calculated for $C_{49}H_{70}O_{19}$ m/z 962. Base peak m/z 432.37. ^{1}H NMR and ^{13}C NMR were presented in Table **1**.

RESULTS AND DISCUSSION

The molecular formula of compound **1** was established as $C_{49}H_{78}O_{19}$ based on its HREIMS and NMR data. The IR spectrum revealed hydroxyl, aliphatic, carbonyl lactone, aromatic and ether

bands at (3395.41, 2925.05, 1739.38, 1462.89 and 1037.46 cm^{-1}) respectively. Compound **1** was identified as 16 β-(β-D glucopyranosyl, 3,8,22–trihydroxy cholestane-1β-yl-6-O- (3,4,5-trimethoxybenzoyl)-β-D-glycopyranoside was assigned the molecular formula m/z 971.87 calculated for $C_{49}H_{78}O_{19}$ (m/z 970) with base peak at m/z 301.12 calculated for $C_{49}H_{78}O_{19}$ (m/z 301). The relative molecular mass of 971.87 with base peak at m/z 301.12 confirmed compound **1** as 16 β-(β-D glucopyranosyl, 3,8,22–trihydroxy cholestane-1β-yl-6-O-(3,4,5 trimethoxybenzoyl) -β-D-glycopyranoside. The pattern of fragmentation (figure **1**) showed that compound **1** undergoes cleavage of the longer aliphatic side chain located at C_{17} and glucoside linkages attached to oxygen at C_1 and C_{16} respectively of the steroid nucleus to produce the base peak $C_{19}H_{25}O_3$ (m/z 301).

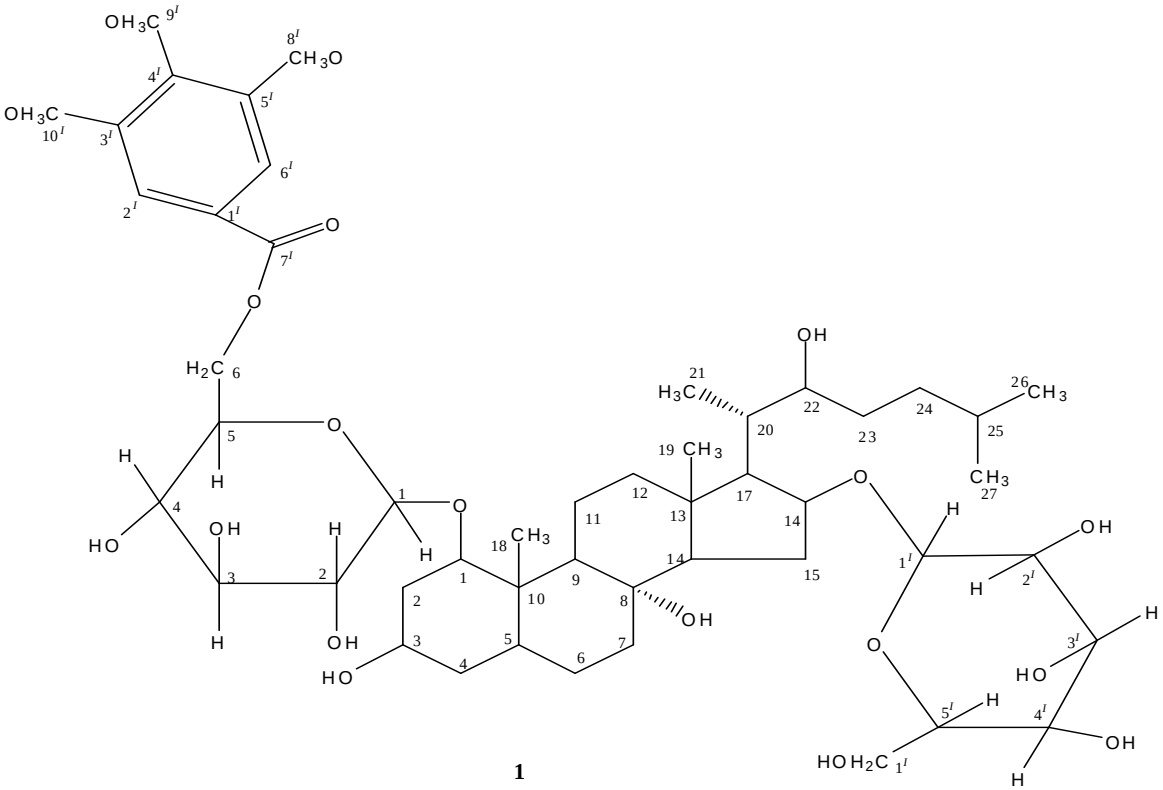
Analysis of the ^1H NMR spectrum is shown in Table **1**. The ^1H NMR spectrum showed the presence of the aromatic proton at δH 7.2633 (1Hs). The other aromatic protons are fully substituted with methoxy groups. These methoxy protons were displayed at δH 3.9056 and 3.8020. The ^1H NMR spectrum also revealed the presence of two angular tertiary methyls (δH 0.9460 and 0.9750) with three secondary methyls observed at δH 0.9750 (3Hd) and δH 1.0064 (6Hd). The ^1H NMR spectrum of the two angular tertiary methyl groups were located at C_{18} and C_{19} and they resonate as singlet at δH 0.9460 and 0.9750 respectively. The C_{18} methyl protons resonate upfield compared to the C_{19} methyl protons which resonate downfield. The C_{26} and C_{27} secondary methyl groups attached to C_{25} methine group give rise to a doublet at δH 1.0064.

Analysis of the ^{13}C NMR spectrum confirmed the presence of a steroid skeleton with 27 carbon atoms and the aromatic carbons were easily established and identified. The spectrum gave the resonance of the aromatic chemical shifts at δC 146.255 (C_1^l), 132.102 (C_2^l), 127.265 (C_3^l), 127.902 (C_4^l), 128.434 (C_5^l), and 132.102 (C_6^l). The presence of the lactone carbonyl carbon was observed at δC 179.277. The methoxy carbons produce the bands at δC 76.652, 77.150 and 77.658 respectively. The compound showed a steroid skeleton with three cyclohexane rings fused in such a way to form an angle with the tertiary methyl groups attached to C_{18} and C_{19}. The fourth ring is a cyclopentane; its addition gives the typical tetracyclic saturated structure with the IR bands of the aliphatic chain with $-CH_2-$ stretching located at 3009.84, 2925.05 and 2853.95 cm^{-1}. The anomeric proton signals were also observed at δH 5.140 and δH 3.420 with their respective ^{13}C NMR chemical shifts observed at δC 90.275 and δC 95.901. The anomeric proton signals indicated β-orientation at the anomeric centers. Compound **1** was identified as 16β-(β-D-glucopyranosyl-3,8,22-trihydroxy-cholestane-1β-yl-6-O-(3,4,5-trimethoxybenzoyl)-β-D-glycopyranoside.

Compound **2** isolated as light yellow oil exhibited an IR absorption band at 3376.68, 2925.15, 1738.93, 1462.97 and 1040.28 cm^{-1} indicating the presence of hydroxyl (OH), aliphatic ($-CH_2-$), carbonyl (C=O), aromatic (C=C) and ether (C–O) functional groups. The elemental composition was defined as $C_{49}H_{70}O_{19}$ by HRESIMS and confirmed by ^{13}C NMR spectrum (Table 1). The ^1H NMR spectrum of **2** contained signals for five steroidal methyl protons at δH 0.9030 (s), 0.9345 (s), 0.8108 (d), 1.0100 (d) and 1.03366 (d) as well as two anomeric protons at δH 5.3420 and 5.0615. Comparism of ^1H NMR and ^{13}C NMR spectra of **2** with those of **1** revealed that the structures of the ring A–D portions and diglucoside moiety attached at C_1 and C_{16} of the anglucone were identical to those of **1**. The benzoyl protons of the two compounds were also similar, each having a singlet aromatic proton at δH 7.26. However, significant differences were recognized in the signals from the rings B and D as well as the C_{23} and C_{24} of the aliphatic long chain of compound **2**. The compound shows olefinic protons at δH 5.200, 5.1433 and 5.3420. This was confirmed by the ^{13}C NMR which shows the presence of the olefinic carbons at δC 133.6 (C_6), 135.11(C_{15}), 134.40 (C_{16}), 135.06 (C_{17}), 109.601 (C_{23}), 109.600 (C_{24}) while the

tertiary methyl carbons were identified at δC 12.20 and 12.05 respectively. Compound **2** has a molecular formula of m/z 963.60 calculated for $C_{49}H_{70}O_{19}$ (m/z 962) with base peak at m/z 433.37 calculated for $C_{23}H_{27}O_8$ (m/z 431). Other prominent peaks at m/z 430.85 and m/z 433.89 were observed and they occurred as a result of proton migration and re-arrangement. The pattern of fragmentation of compound **2** is shown is figure **2**. Combining MS, NMR and IR spectral data, compound **2** is identified as 16-β-(β-D glucopyranosyl, 3,8,22–trihydroxy-cholest-5,14,16,23-tetraiene-1β-yl-6-O-(3,4,5-trimethoxybenzoyl)-β-D -glycopyranoside.

Both compounds **1** and **2** were identified as steroid glucoside with aromatic ring. Steroids are apparently involved in the regulation of large number of biological activities including electrolytic and hormonal balance as well as reaction to allergy. Steroids have anti-inflammatory, oxytocic and antioxidant functions [20-22]. Steroids compounds are used in the treatment of rheumatoid arthritis [21]. Many C_{11} oxygenated steroids are used in the treatment of disorders such as asthma, skin inflammation and for hormonal control [20,22]. The occurrence of steroidal glucoside in *S. jamaicensis* may be the reason behind the use of this herb in phytomedicine for birth control, abortion, treatment of menstrual disorders and as a lactagogue in herbal medicine [14]. The occurrence of steroids in *S. jamaicensis* also confirmed the use of this herb in the treatment of asthma, bronchitis, bile regulation, hormone regulation, lactation stimulation and birth control in phytomedicine in Nigeria. Various phytotherapeuticals with claimed hormonal activity are recommended for prevention of discomforts related to a disturbed hormonal balance [23,24]. There is a renewed interest in naturally occurring phytoestrogens as potential to hormone replacement therapy. It is concluded that these steroids isolated from *S. jamaicensis* may be a contributor to the hormone regulator, liver protector, bronchodilator, antioxidant, anti-inflammatory, oxytocic activity and antacid properties exhibited by *S. jamaicensis*. This plant offer wide-scope for utilization as raw material by pharmaceutical industries for drug formulation.



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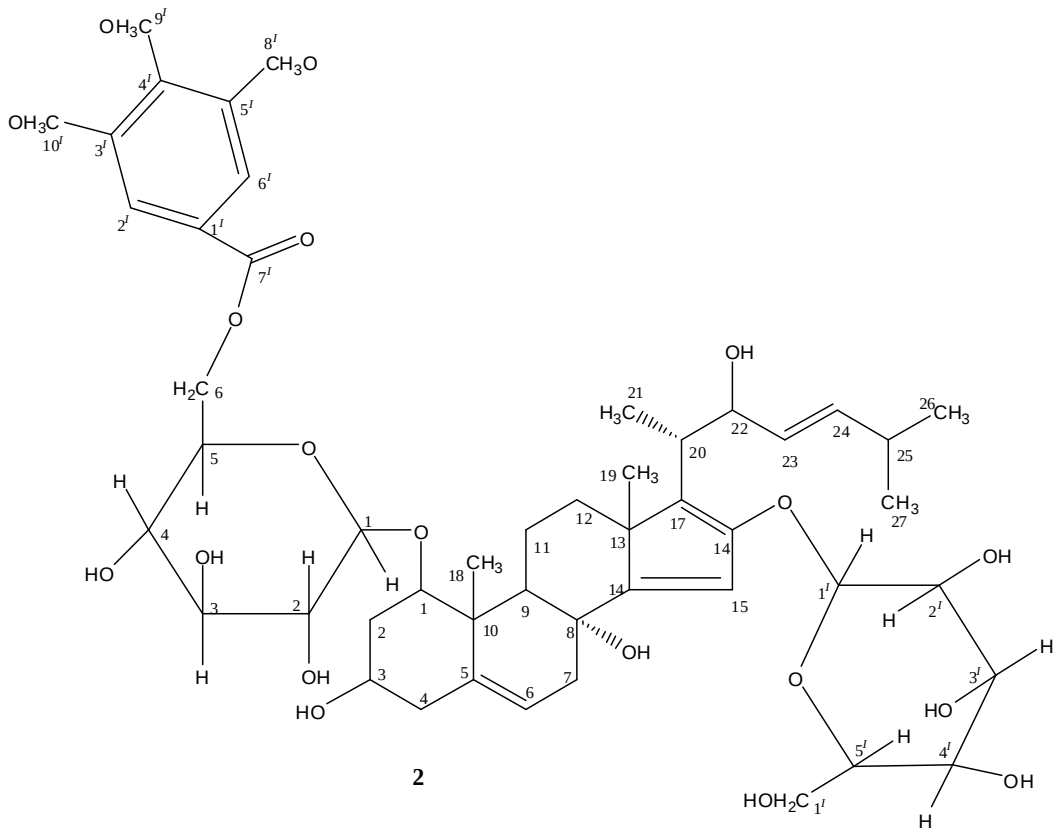

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Table 1: ^{1}H and ^{13}C NMR Chemical Shifts of Compounds 1 and 2

Position	1		2	
	δH	δC	δH	δC
1	1.6012 1Ht	31.543	1.6173 1Ht	31.5434
2	1.2552 2Ht	20.691	1.2509 2Ht	20.6905
3	1.6012 1Hm	32.065	1.6173 1Hm	32.9459
4	1.2843 2Hm	22.829	1.2509 2Hm	22.8289
5	1.6275 1Hm	34.067		34.0671
6	1.3131 2Hm	24.830	5.2035 1Hm	133.600
7	1.2843 2Hm	25.675	1.2509 2Hm	25.6755
8		34.067		34.0671
9	1.6278 1Hs	32.946	1.6173 1Hs	32.9459
10		36.644		34.0671
11	1.2552 2Ht	25.675	1.0366 2Ht	25.7636
12	1.2552 2Ht	25.764	1.0100 2Ht	25.6758
13		38.044		38.0435
14	1.6275 1Hm	31.543		134.10
15	1.3131 2Hm	27.341	5.1433 1Hs	135.11
16	1.6012 1Ht	34.948		134.40
17	1.6275 1Hm	32.066		135.06
18	0.9460 3Hs	12.203	0.9030 3Hs	12.2028

19	0.9750 3Hs	20.631	0.9345 3Hs	12.5060
20	1.6275 1Hm	49.324	1.7677 1Hm	49.0240
21	0.8539 3Hd	20.691	0.8108 3Hd	20.6905
22	1.6012 1Hm	76.652	1.4121 1Hm	75.1650
23	1.2552 2Hm	29.218	5.3420 1Ht	109.601
24	1.2843 2Hm	29.386	5.3420 1Ht	109.601
25	1.6275 1Hm	42.708	1.6173 1Hm	41.2060
26	1.0064 3Hd	14.251	1.0100 3Hs	14.2505
27	1.0064 3Hd	14.404	1.0366 3Hs	14.4044
1[l]		146.255		132.107
2[l]	7.2633 1Hs	132.102	7.2600 1Hs	130.390
3[l]		127.265		127.002
4[l]		127.902		128.434
5[l]		128.434		127.265
6[l]	7.2633 1Hs	133.102	7.2600 1Hs	130.390
7[l]		179.277		180.102
8[l]	3.9056 3Hs	76.652	3.3761 3Hs	51.7102
9[l]	3.8020 3Hs	77.150	3.4898 3Hs	51.8115
10[l]	3.9056 3Hs	77.658	3.5725 3Hs	52.0012
Glu				
1	5.1460 1Hs	90.275	5.3420 1Hs	95.9010
2	5.2895 1Hbs	77.668	5.2035 1Hbs	74.2201
3	5.3113 1Hm	77.150	5.1433 1Hm	79.4102
4	5.3346 1Hm	76.652	5.1096 1Hm	71.5120
5	4.1055 1Hs	71.228	4.1608 1Hs	62.1050
6	4.1658 2Hs	65.187	4.1059 2Hs	62.1055
1[l]	5.4410 1Hs	117.237	5.0615 1Hs	95.9010
2[l]	5.3974 1Hm	113.319	5.1096 1Hm	74.2201
3[l]	5.3974 1Hm	55.083	4.1608 1Hm	79.4102
4[l]	5.3749 1Hm	65.333	4.1354 1Hm	71.5120
5[l]	4.1558 1Hs	51.333	4.1059 1Hs	62.1050
6[l]	5.3113 2Hs	56.053	3.6135 2Hs	62.1055

S = Singlet; d = doublet; t = triplet; m = multiplet; bs = broad singlet

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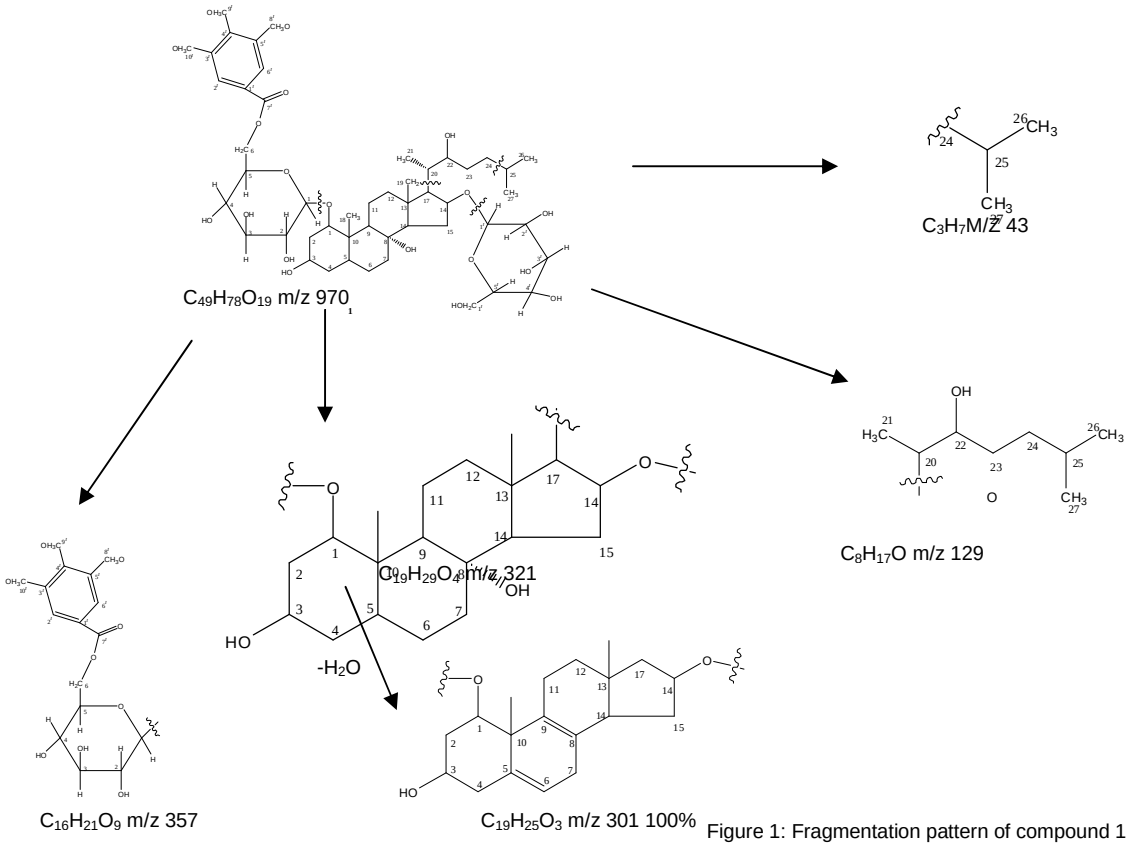

Figure 1: Fragmentation pattern of compound 1

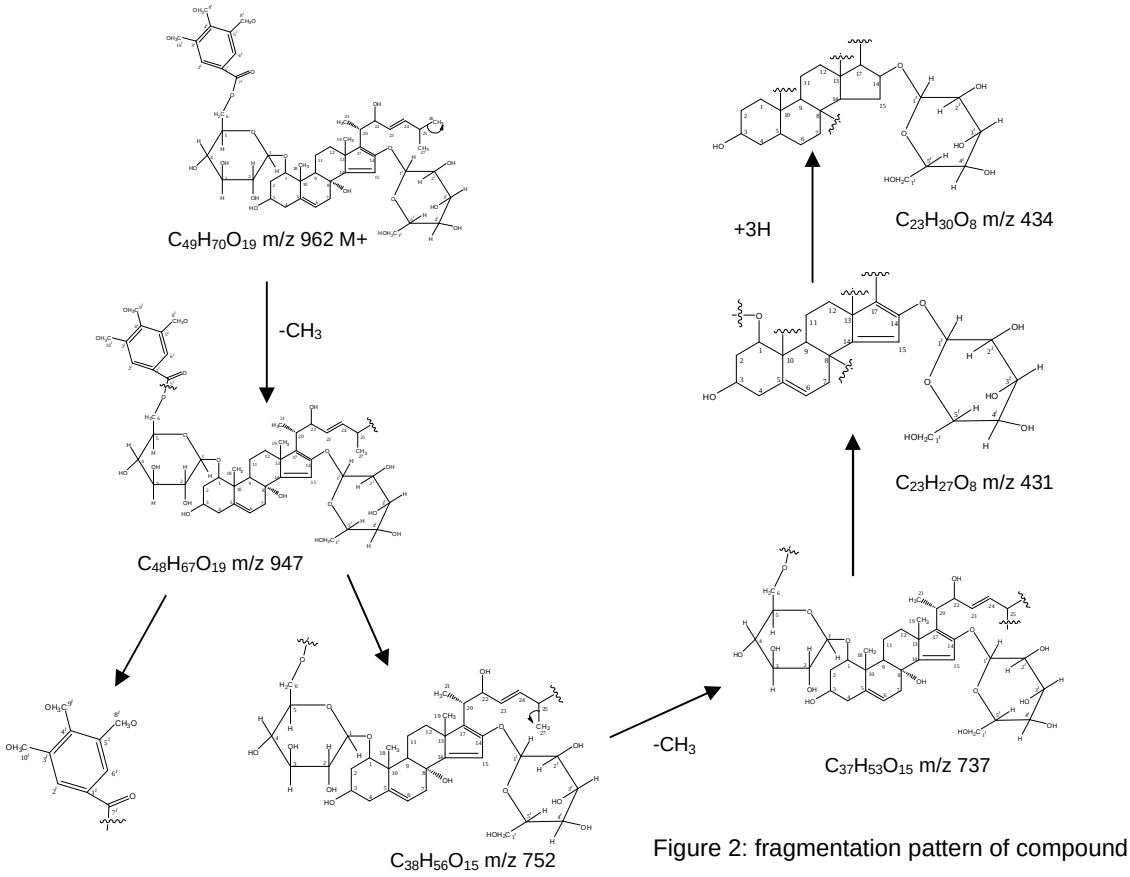

Figure 2: fragmentation pattern of compound

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