Journal of Scientific and Innovative Research 2014; 3 (2): 207-216
Available online at: www.jsirjournal.com

Research Article

ISSN 2320-4818
JSIR 2014; 3(2): 207-216

Received: 14-02-2014
Accepted: 26-04-2014

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Steroidal and triterpenoidal saponins from the stem bark extract of *Stachytarpheta angustifolia* Mill (Vahl) Verbenaceae

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Abstract

Two saponins were isolated from the stem bark extract of *Stachytarpheta angustifolia*. Their structures were established by spectroscopic and chemical analysis as (23S, 25S) - 5α - spirostan – 24 – one - 3β, 23 – diol – 3 – O – {α – L – rhamnopyranosyl – (1→2) – [O – β - D - glucopyranosyl – (1→4)] – β - D – galactopyranoside} (1) and 3β – O –(β- D - Xylopyranosyl – (1-3) – α – L – arabinopyranosyl) - 20β, 23-dihydroxy urs – 12 – en – 28 – O – [- α – L – rhamnopyranosyl – (1-3) – α – L – rhamnopyranosyl – (1-6) – β - D - glucopyranosyl -] ester.

Keywords: *Stachytarpheta angustifolia*, Stem bark, Steroidal and triterpenoidal saponins.

Introduction

Disease prevention is increasingly becoming a public concern of modern health care. Nature manufactures a great deal of diverse structures that may serve as nutraceuticals providing a health benefits including the treatment and prevention of diseases. Edible plants containing nutrients, dietary supplement or secondary metabolites may play an essential role in preventing the incidence of cardio and cerebrovascular diseases, cancer and various chronic diseases.[1]

Stachytarpheta angustifolia is a much branched annual shrub with a tetragonal nearly or quite glabrous stem. The plant is known as devils coach whip, verbena or Bastard Vervain.[2] The leaves are opposite, distinctly petiole, oblong – lanceolate, acute, glabrous, deeply inciso-crenate with tetragonal rachis. The flowers are hollowed out oppositely and calyx nearly as the bract. In Nigeria, the Hausa's called it Tsarkiyar kusu or Wutsiyar Kadangare, while the Yoruba's called it Iru – Alangba or Iru – Amure.[3] The decoction of the whole shrub mixed with natron is taken as a remedy for dysentery and also for similar condition for horses.[4] The cold infusion of the plant mixed with natron is taken as a remedy for, gonorrhea and other forms of venereal diseases. It is also taken as a vermifuge or a purging vehicle for other vermifuge. The whole plant when boiled is taken as a remedy for diabetes.[5] In Asia and America the aerial part of *Stachytarpheta angustifolia* is boiled and taken traditionally as a remedy for diarrhea, intestinal parasites, ulcer and as an abortificient agent.[6] They are mostly found within the tropical part of Africa and other part of the world.

Materials and Methods

General Methods

Optical rotations were measured using a Perkin –Elmer model 341 LC spectrometer at room temperature. IR spectra were recorded on spectrophotometer shimadzu 8400s. Melting Points were determined on XT4A Apparatus and results are uncorrected. ^{1}HNMR and ^{13}CNMR experiments were performed on Bruker spectrometer 600 MHz for ^{1}H and 125 MHz for ^{13}CNMR. NMR spectra were referenced to the CD_3OD solvent signals at ∂3.30 (^{1}H) and 49.00 (^{13}C) with TMS as an internal standard. Chemical shift values (∂) were reported in parts per million (ppm) in relation to the appropriate internal solvent standard (TMS). The coupling constants (J-values) were given in Hertz, HRESI –Ms was measured on a mass Autospec – ultima – TOF spectrometer. TLC was carried out on plates precoated with RP-18 gel (merck) and silica gel F_{254} (Qingdao Marine Chemistry Ltd). Spots on the plates were visualized by spraying with 10% H_2SO_4 and Anisaldehyde – H_2SO_4 (for spirostanol saponins) followed by heating in oven. Column chromatography was performed on silica gel 60 (0.040 - 0.0653 mm), column (40 – 63 µm, 310 mm α 15 mm i.d). GC analysis was performed on a Shimadzu GC-2010 gas chromatographic equipped with an H_2 flame ionization detector and a DB-5 quartz capillary column (30 cm x 0.25 mm x 0.25 µm). Gel filtration technique was carried out on sephadex L_H20 TLC visualization was by UV absorption at 254 mm. All solvents were distilled prior to use.

Plant Material

The plant material *S. angustifolia* was collected from Basawa village out skirt of Zaria, in 2011 and identified by Musa Muhammad of the Herbarium Biological Science Department of Ahmadu Bello University, Zaria, Nigeria. A voucher spectrum (No. 900188) was deposited.

Extraction and Isolation

The stem bark of *S. angustifolia* (1.5 kg) was powdered using pestle and mortar, defatted with n – hexane (3x4L) and subsequently extracted with 75% ethanol (3x6L) at room temperature by maceration. The n – hexane and Ethanolic extract were concentrated using rotary evaporator. The ethanolic extract (175 g) was re suspended in water (2L) and successfully partitioned with chloroform (3x500ml), ethylacetate (2x500ml) and n – butanol (5x500ml). The partition fractions of the ethanolic extract were concentrated using rotary evaporator and then subjected to phytochemical screening using standard protocols (Table 1).[2] The n – butanol fraction rich in Saponin (4 g) was chromatographed on a column of silica gel (500 g, 60 -120 mesh) using isocratic elution with mixture of chloroform: methanol: water (7:2:1). A total of 76 fractions each of 100 ml volume were collected. Fraction 12 -28 (130 mg) with the same TLC pattern was pooled together and subjected to repeated gel filtration techniques using sephadex LH_{20} to obtain compound 1 (amorphous solid 38 mg). Fraction 32 -56 (180 mg) was also subjected to a repeated gel filtration using sephadex LH_{20} and subsequently preparative thin layer chromatography (PTLC) to obtain compound 2, 31 mg.[7]

Acid Hydrolysis for 1

Solution of compound 1 (5mg) in 2M Hcl MeOH (4:1, 5ml) was reflux at 90°C for 6 hrs, after cooling, the reaction mixture was diluted to 20 ml and extracted with CH_2Cl_2 (3x2ml). The aqueous layer was concentrated to an appropriate volume (1 ml) and examined by TLC (Silica gel) with a solvent system $CHCl_3$ /MeOH/H_2O (65:35:10) for sugar analysis. R_f values of D-glucose, D-galactose and D-rhamnose were 0.25, 0.25 and 0.42, respectively. The remaining aqueous layer was concentrated to dryness to give a residue and dissolve in pyridine (1 ml), and then L-cysteine methyl ester hydrochloride (2 mg) was added to the solution. The mixture was heated at 60ºC for 2 hrs; equal volume of acetic anhydride was added, followed by heating at 90ºC for another 2 hrs. The solution was then concentrated to dryness and taken in MeOH (0.5ml), which was analyzed by GC (column: DB-5 quartz capillary column (30m x 0.25mm, 0.25µm), H_2 flame ionization detector column temperature: 160-280ºC programmed increase: 5ºC/min, carrier gas: N_2 (1.5ml/min), injector and detector temperature: 280ºC, injection volume: 1µl, split ratio: 10/1. The derivatives of L-rhamnose, D-glucose and D-galactose were detected. R_f (mm):23.89, 28.07 and 28.70 min respectively. The standard sugars were also subjected to the same reactions and GC analysis under the same conditions as above was observed.[8]

Acid Hydrolysis and GC Analysis for 2

The solution of compound 2 (4.0 mg) in methanol (25 ml) was treated with 3N HCL (15ml) and stirred at 80ºC for 5 hrs. Upon drying with a flow of nitrogen, the residue was dissolved in (-2) -2 – butanol (0.5 mL) and a drop of trifloroacetic acid were added. The solution was transferred to an ampoule which was sealed and heated at 130ºC overnight until complete butanolysis. This was

taken to dryness, the resultant residue was reacted with hex methyl disilazane /chlorotrimethylsilane /pyridine (1:1:5, 0.1 ml) for 35min at room temperature. The solution was centrifuged and the supernatant layer (1 µL) was analyzed by GC using HP-5 column. The injection port and detector temperature was set at 200ºC and 220ºC. A temperature gradient from 140 - 200ºC at 1ºC/min was applied. Four peaks were detected from the hydrolysate at 37.50, 40.13, 42.45 and 43.51. Authentic standards were prepared in a similar manner from commercially available D-and L – galactose which gave rise to peaks at 37.48, 40.18, 40.21, 42.44 and 43.95 min for D – galactose, While 38.42, 40.21, 42.40 and 43.95 min for L- galactose respectively.

Determination of Sugar Compounds in 2

A solution of compound 2 (6 mg) in H_2O (2 ml) and 2N aqueous solution of CF_3COOH (5 ml) were refluxed on a water bath for 3 hrs. After this period, the reaction mixture was diluted with H_2O (20 ml) and extracted with CH_2Cl_2 (4x5 ml). The combined CH_2CL_2 extracts were washed with H_2O and then evaporated to dryness in Vacuo. The sugars were analyzed by silica gel TLC by comparison with standard sugars with those in 2. TLC R_f (rhamnose) 0.50, R_f (glucose) 0.31, R_f (xylose) 0.45 and R_f (arabinose) 0.56. Furthermore, the residue from the sugars were dissolved in anhydrous pyridine (100 µL), and L-cysteine methyl ester hydrochloride (0.06 mol/L) was added. The mixture was stirred at 60ºC for 1hr, and then 150 µL of HMDS –TMCS (Hexamethyldisilazane - trimethylchlorosilane, 3:1) was added. The mixture was stirred at 60ºC for 30 min. The precipitate was centrifuged, and the supernatant layer was concentrated under N_2 stream. The residue was partitioned between n-hexane and H_2O (0.2ml each) and the n-hexane layer (1 µL) was analyzed by GC. L. rhamnose, D-glucose, D-xylose and L-arabinose were detected by co-injection of the hydrolysate with standard silylated samples to give single peaks at (13.48 min) rhamnose, (18.72 min) glucose, (13.18 min) xylose and (12.23 min) arabinose respectively.[9]

Result and Discussion

Compound 1

Compound 1 (Figure 1) was obtained as an amorphous solid with a molecular formula of C45 H72 O19, as determined by the data from the positive – ion HRESI-MS (M/z. 939.4607 (M+ Na)+). mp. at 248-250ºC. The HNMR Spectrum of 1, displayed two oxygenated methylene protons at ∂H 3.67 (IH, t, J=10.9 HZ eq-H-26a) and ∂H 3.96ppm (IH, m, axil H26b) and three oxygenated methine protons at ∂H 3.86ppm (IH, M, H-3), ∂H 4.56ppm (IH, M, - H-16) and ∂H 4.62ppm (IH,M, H-23). The ^{1}HNMR spectrum (Table 2) also displayed four methyl proton signals which could be attributed to steroidal skeleton at ∂H 0.97ppm (3H, S, H-18), ∂H 0.80ppm (3H, S, H-19), ∂H 1.24ppm (3H, d, J=7.0 HZ , H-21) and ∂H 0.94ppm (3H,d, J=6.5 HZ, H-27). The three signals observed at ∂H 4.89ppm (IH, d, J=7.7 HZ, H-I'), ∂H 6.20ppm (IH, brs – H – I") and ∂H 5.16ppm (IH, d, J=7.9 HZ, H-I"') could be attributed to anomeric protons. The signal at ∂H 1.64ppm was due to the methyl group of the 6 – deoxyhexopyranose.[10]

The ^{13}CNMR Spectrum of Compound 1 (Table 2) exhibited 45 carbon signals with 27 attributed to aglycone and 18 carbon atoms to the trisubstituted sugar molecules. The carbon signal at ∂C 208.4ppm/C-24 indicated the presence of a carbonyl group while signals at ∂C 98.6ppm, ∂C 102.9ppm and 106.3ppm corresponding to C-1',C-1'' and C-1"' could be attributed to the anomeric carbons of the tri substituted sugar moieties.[11] The anomeric carbon signal at ∂c 102.9ppm corresponding to ∂c 6.20ppm and the high field signal at ∂c 18.3ppm / C-6" corresponding to ∂H 1.64/H-6" are in conformity with the values of rhamnose moiety.[12]

The Dept. Experiment has exhibited the presence of 4 quaternary carbon signals at ∂c 35.8, ∂c 41.6ppm, ∂c 117.4ppm and ∂c 208.4ppm. The HMBC spectrum has established the correlation of C-7 with C-3'and C-1', C-2' with C-1" while C-4' with C-1"'. So also, the long range correlation of methyl proton at ∂H 0.94 (H-27) with the carbon signals at ∂c 44.5ppm (C-25) ∂c 65.7ppm (C-26) and ∂c 208.4ppm (C-24). The proton at ∂H 2.82ppm (H-25) also showed long-range correlation with the carbon at ∂c 208.4ppm (C-24), indicating the attachment of a Keto group at C-24.[13] The proton at ∂H4.65ppm (H-23) showed long- range correlation with the carbon signals at ∂c 208.4ppm (C-24), indicating the attachment of OH on C-23 and the methyl group (CH3) on (C-25) were regarded to be of α-configuration.[14, 15] The proton at ∂H 4.65ppm(IH,M, H – 23) exhibited a NOESY cross- peak with the proton at ∂H 3.07ppm (IH, M, H- 20β), ∂H 2.82ppm (IH, M, H-25) and ∂H 3.96ppm IH, M, axial, H-26.[16, 17]

The Comparison of the ^{1}H and ^{13}CNMR data for the aglycone moiety of 1 with those of tigogenin 3-O-[-O- α – L – rhamnopyranosyl – (1→2) – O – (β – D – glucopyranosyl – (1→4)] – β- D – galactopyranoside are identical.[18, 8] The orientation of oxygen atom on C-3,

Hydrogen atom at C - 5 and Hydrogen atom on C - 20 justifies (3β, 5α and 20β configuration.[17] Thus, on the basis of this comparison, the aglycone was deduced to be (23S, 25S) – 5α – Spirostane – 24 – one - 3β, 23- diol. The cross peak between the ^{1}HNMR signal at ∂H 4.89(IH, d, J=7HZ H-I') 2, 4 disubstituted galactose), and the carbon signal at ∂C 76.8 (C- 3 aglycone) indicated the glycosidation of aglycone at C – 3 position. The anomeric protons at ∂H 6.20 (IH, brs H – I") and ∂H 5.16 (IH, d, J=7.9 Hz H-I"') exhibit cross –peaks with the carbon signals at ∂c 77.3 (C – 2' of the disubstituted sugars), and also at ∂c 82.3 (C- 4' of the 2, 4 disubstituted galactose). The β anomeric configuration for both the glucose and galactose were judged from the coupling constants (J=>7.0 Hz). The absolute configuration of the sugar moieties were determined to be L – rhamnose, D- glucose and D – galactose on the basis of GC analysis. Acid hydrolysis of compound 1 with 2M HCL in CH_3OH/H_2O (4:1) gave rhamnose, glucose and galactose in the ratio of 1:1:1.

Compound 2

Compound 2 (Figure 2) was obtained as an amorphous white powder; mp. 256-258ºC; UV (MeOH) Δmax: 589nm. IR (KBr) Vmax (cm-1); 3428, 2928, 1436, 1636 and 1048. HREI–MS: M/z 1206.3241 (M) + corresponding to molecular formula of $C_{58}H_{94}O_{26}$. Compound 2 was found to be positive with Lieberman –Burchard and molish reagent.[19] The ^{1}HNMR Spectrum of 2 (Table 3) displayed an oxygenated methine proton on ∂H 3.72ppm, (IH, dd, J=12.2 H – 3), oxygenated methylene proton on ∂H 3.82ppm, dd (12.2 HZ, H – 6"""). The spectrum also showed five anomeric protons on ∂H 4.38ppm d (J=7.3HZ – H – I'), ∂H 4.67ppm d(7.5 H-1"), ∂H 4.78ppm, d (1.92 H – 1"'), ∂H 5.18ppm, d (1.7 H- 1"") and ∂H 5.38ppm, d(7.6 H-1""'). This also showed six numbered of methyl protons on ∂H 0.86ppm (1H, S, H-24), ∂H 0.82ppm (1H, S H-25), ∂H 1.10 (1H, S, H-26), ∂H 1.20 (1H, S, H-27), ∂H 1.0 (1H, d, H-29) and ∂H 1.09, 1H, S, H-30.[10] The ^{13}CNMR spectrum of compound 2 (Table 3 and Table 4) exhibited 58 carbon signals of which 30 were attributed to aglycon moiety and 28 carbon signals to the oligosaccharide unit. The carbon signal at ∂c 178.3ppm indicated the pressure of a carbonyl group while signals at ∂c106.3ppm, ∂c 105.4ppm, ∂c 102.5ppm, ∂c 101.5ppm and ∂c 95.4ppm could be attributed to the anomeric carbons of the sugar unit.[19] The anomeric carbon signals at ∂c102.5ppm/C-1"' and ∂c101.5ppm/C-1"'' corresponding with ∂c 18.3ppm/C-6"' and ∂c18.20ppm/C-6"'' are in conformity with the values of rhamnose moiety.[10] The DEPT experiment exhibited the presence of six quaternary carbon signals at C- 4 , C-8, C -13, C -17, C -20 and C - 28.[15] The HMBC spectrum of compound 2 showed a significant cross – peaks between H -22 and C - 20, H -20, H -18 and C – 20, H -19 with C- 30 and C – 29 with H – 21.[20] The Cosy spectrum also indicated C-18→C-29 connectivies starting from the well resolved signals at ∂H 2.34ppm (IH, d, J=14Hz H – 18) with hydroxyl group at C – 20.[12] The stereochemistry at C – 18, C -19 as well as the orientation of the hydroxyl group at C – 20 were fully determined by NOESY Spectrum which exhibited the prominent correlation peaks between H – 19 and signals of C – 30 as well as between C – 30, H – 21 and H – 22.[21, 9]

The comparison of ^{1}H and ^{13}CNMR data for the aglycon moiety of 2 with those of [Ref. 22] were found to be similar and the aglycon of compound 2 (Table 3) was determined as 3β, 20β, 23 – trihydroyurs – 12 – en – 28 – oic acid.[21] The positions of the sugar moieties were unambiguously ascertain by the HMBC experiment. The glycosidation of the alcoholic function at C – 3 and esterfication at C – 28 (COOH) group were indicated by the down field shift (+ 10ppm) and the high field of (-4ppm) is observed.[23] It was indicated that, the arabinose was the pentose sugar linked to the C – 3 of the aglycon moiety, so also the cross – peak between C- 3' and H – 1 of the terminal xylose (∂H 4.67 d, 7.5Hz) indicated that the xylose was the second sugar unit of the disaccharide chain attached to C – 3 of the arabinose unit.[24, 7] The chemical shifts of the sugar residue at C- 1"'/H -1"' indicated that, the rhamnose sugar was involved in the ester linkage with the C – 28 carboxylic group. The rhamnose has also suggested the point of linkage of the other rhamnose sugar unit. The HMBC experiment, exhibited the long correlation occurrence between H -1' (∂H 4.38ppm) and C – 3 (∂c82.4ppm), H -1"' (∂H 4.78ppm) and C – 28 (∂c178.3ppm). The NOESY spectrum of 2, shows the interaction between the anomeric proton of glucose H -6"'' (∂H 3.82ppm) and H – 6"'' (∂H 1.24ppm) methyl group of the rhamnose moiety.

The chemical shift, multiplicity, absolute values of the coupling constant and magnitude in ^{1}HNMR and ^{13}CNMR spectrum data indicated the β – configuration at the anomeric position for xylose and glucose while α – configuration for arabinose and rhamnose unit respectively. Consequent upon this, Compound 2 was determined to be 3β – O – (- β – D – Xylopyranosyl – (1 – 3) – α – L – arabinopyranosyl) - 20β, 23 – dihydroxy urs – 12 – en – 28 – O – [- α – L – rhamnopyranosyl – (1-3) – α – L – rhamnopyranosyl – (1 -6) – β – D – glucopyranosyl) ester.

Table 1: Preliminary phytochemical screening of the stem bark extract of *Stachytarpheta angustifolia*

Constituents	Test	Ps	Es	CL	EtOAC	n-But	AQ
carbohydrate	Molisch	-	+	-	-	-	++
Fehling's		-	++	-	-	-	+++
Barfoed		-	+	-	-	-	++
Benedict	-		+	-	-	-	++
Alkaloids	Mayer's	-	-	-	-	-	-
Wagner	-		-	-	-	-	-
Dragendorff	-		-	-	-	-	-
Hager's	-		-	-	-	-	-
Flavonoids	Lead Acetate	-	++	+	+	+ +	-
Shinoda	-		++	+	+	++	-
Tetraoxosulphuric acid	-		+	+	-	+ +	-
Glycosides	Borntrager's	-	++	-	+	+	++
Legal	-		+	+	+	+	++
Saponin	Froth test	-	++	-	+	+ +	+++
Cardiac	Keller	-	+	-	++	+	+ +
Glycosides	Killiani	-	++	-	+	+	++
Tannins	Gelatin test	-	+	+	-	+	++
Alkaline reagent test	-		+	-	-	+	++

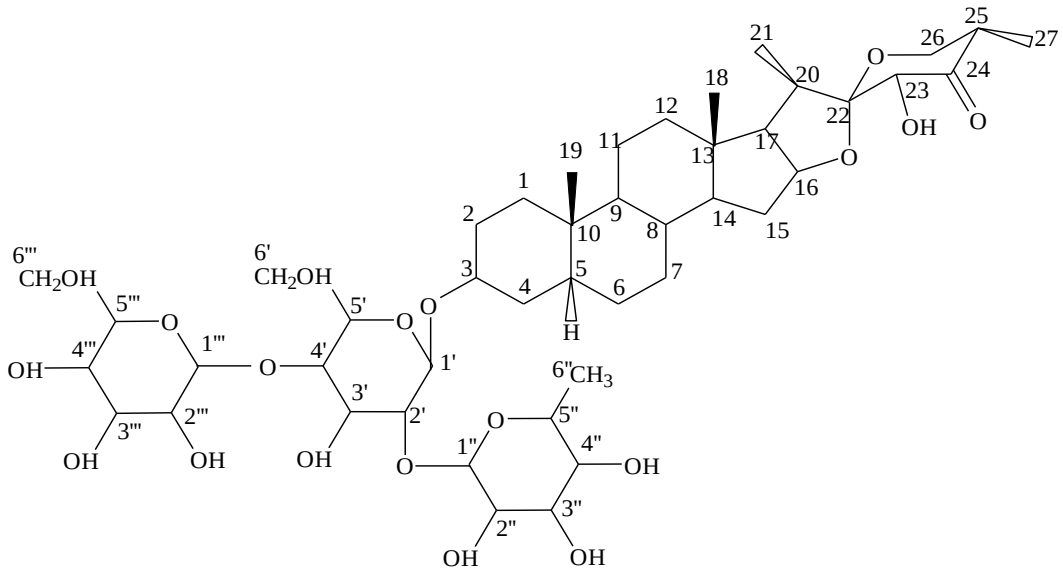

Figure 1: Structure of compound 1

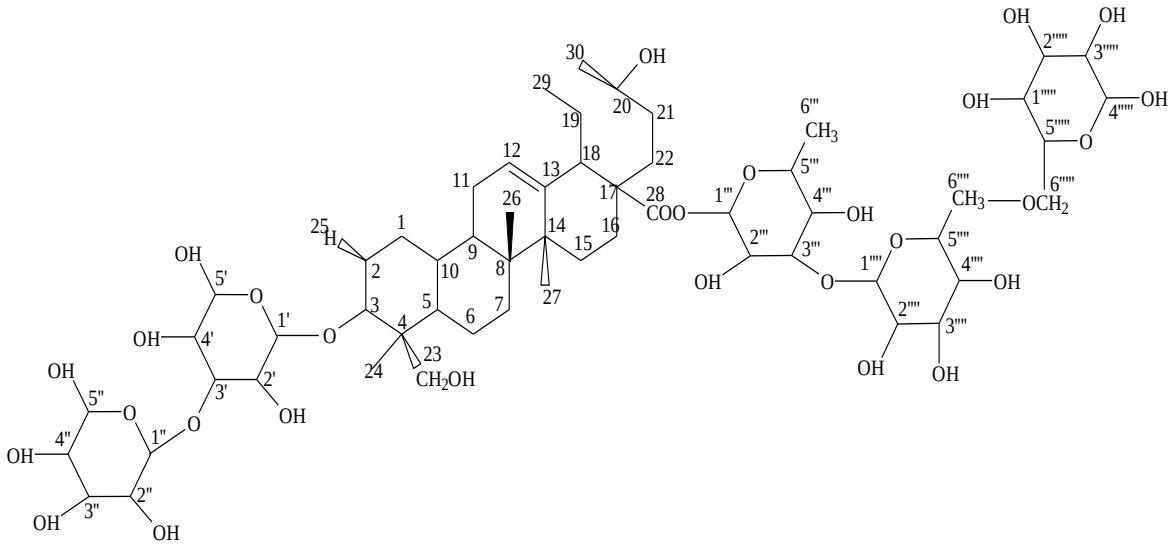

Figure 2: Structure of compound 2

Table 2: ^{1}HNMR and ^{13}CNMR (600 and 125 MH_Z, CD_3OD) for compound 1

S/N	**C** ∂_c	**H** $\partial_{H\ (ppm)}$ **J=HZ**
1.	37.3	1.93
2.	29.7	2.60
3.	76.8	3.86 (1H, M)
4.	34.5	1.54
5.	44.6	
6.	28.3	1.82
7.	32.7	2.10
8.	35.4	
9.	54.4	
10.	35.8	
11.	21.6	2.35
12.	41.2	2.11
13.	41.6	
14.	56.3	
15.	31.6	
16.	82.5	4.56 (1H, M)
17.	61.7	
18.	16.5	0.97 (3H,s)
19.	12.7	0.80 (3H,S)
20.	36.8	3.07 (1H,M)
21.	14.6	1.24 (3H, d, J=7.0H_Z
22.	117.4	
23.	76.3	4.62 (1H,M)

24.	208.4	
25.	44.5	2.82(IH,M)
26.	65.7	3.82 (1H, t J=10H_Z eq.) 3.96(1H, M, axil)
27.	9.4	0.94 (3H, d, J= 6.5 H_Z).
1’	98.6	4.89 (1H, d, J=7.7 H_Z)
2’	77.3	4.51 dd, (7.6)
3’	76.4	4.16 (9.5)
4’	82.3	3.90. d, (6.4)
5’	75.2	3.54
6’	61.3	3.63m
1”	102.9	6.20 (1H, brs)
2”	72.0	5.29 brs
3”	82.6	5.63
4”	78.7	4.66
5”	68.5	4.36
6”	18.3	1.64 d,(6.3)
1”’	106.3	5.16 (1H, D j=7.9 $H_{Z)}$
2”’	75.4	3.90
3”’	78.2	4.32
4”’	72.1	3.83
5”’	78.6	4.39
6”’	62.5	4.04

Table 3: ^{1}HNMR and ^{13}CNMR (600 and 125 MH_Z, CD_3OD) data for Aglycone moiety of compound 2

Position	$\partial_{H\ (ppm)}$ J=HZ	∂_C
1.	1.71 (IH, M)a 1.02 (IH, M)b	39.6
2.	2.0 (IH, M) a 1.58 (IH, M)b	26.8
3.	3.72 (IH, dd, J=12.2)	82.4
4.	----	43.2
5.	1.63 (IH, 3)	49.2
6.	1.50 (IH, M)	19.4
7.	1.84 (IH, m)	32.3
8.	---	40.6
9.	1.67 (IH,s)	49.4
10.	1.28 (IH, m)	39.7
11.	2.06 (IH, m)	23.8
12.	5.41 (IH, s)	128.2
13.	---	139.8
14.	----	44.2
15.	1.21 (IH, m)	29.3

16.	1.18 (IH, m)	25.2
17.	---	48.2
18.	2.34 (IH, d, J =14Hz)	53.4
19.	1.56 (IH, m)	40.1
20.	---	85.7
21.	1.62 (IH, m)	31.3
22.	1.58 (IH, s)	37.2
23.	3.71 (IH, d, J= 12Hz)	63.8
24.	0.86 (IH, S)	13.5
25.	0.82 (IH, s)	16.3
26.	1.10 (IH, s)	16.8
27.	1.20 (IH, s)	24.4
28.	---	178.3
29.	1.0 (IH, d, J=7.1)	21.5
30.	1.09 (IH, s)	23.4

Table 4: 1HNMR and ^{13}CNMR (600 and 125 MH_Z, CD_3OD) for the Oligosaccharide moiety of compound 2

Arabinose	$\partial_{H\ (ppm)}$ J=HZ	∂_C
1’	4.38 d(7.3)	106.3
2’	3.65 dd (7.4)	71.8
3’	3.82 dd (9.2)	80.1
4’	3.57 m	69.2
5’	3.89 d (12.3)	65.8
Xylose		
1’’	4.67 d (7.5)	105.4
2’’	3.24 dd (7.4, 9.3)	74.8
3’’	3.28 m	77.5
4’’	3.42m	72.3
5’’	3.82 dd (5.6, 10.7)	66.5
Rhamnose		
1’’’	4.78 d (1.92)	102.5
2’’’	4.56 dd (1.8, 1.4)	71.8
3’’’	3.98 dd (3.2, 8.6)	81.0
4’’’	3.61	72.6
5’’’	1.34 d (5.8)	18.3
Rhamnose		
1’’’’	5.18 d (1.7)	101.5
2’’’’	3.86 dd (3.2, 1.4)	71.8

3””	3.75 dd (8.1, 4.1)	72.5
4””	3.48 t (8.9)	74.6
5””	3.87 dd (9.2, 6.2)	70.5
6””	1.24 d (7.2)	18.20
Glucose		
1””’	5.38 d (7.6)	95.4
2””’	3.36 dd (97.4, 8.6)	73.2
3””’	3.44 t	77.8
4””’	3.36 d (9.2)	71.0
5””’	3.51 m	76.5
6””	3.82 dd (12.2, 4.9)	62.6

Conclusion

In conclusion, on the basis of spectral data comparison with those of [Ref. 17,8] Compound 1 was elucidated as (23S, 25S) - 5 α – spirostatane-24-One-3β, 23-diol -3 – O - α – L – rhamnopyranosyl – (1 → 2) – O – [- β – D – glucopyranosyl – (1 →4)] – β – D – galactopyranoside. Compound 2 was determined to be 3β – O – (- β – D – Xylopyranosyl – (1 – 3) – α – L – arabinopyranosyl) - 20β, 23 – dihydroxy urs – 12 – en – 28 – O – [- α – L – rhamnopyranosyl – (1-3) – α – L – rhamnopyranosyl – (1 -6) – β – D – glucopyranosyl) ester.

Acknowledgement

We wish to acknowledge the efforts and supports made by Professor Muhammad Idris Bugaje (DG/CEO National Research Institute for Chemical Technology, Zaria – Nigeria) and Professor Abdullahi Mustapha (Vice Chancellor, Ahmadu Bello University Zaria – Nigeria).

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