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Saponins from the Leaves of *Cassia occidentalis* (Caesalpiniaceae)

M. Mohammed, M. M. Adeyemi, M. A. Aboki and S. H. Musa
National Research Institute for Chemical Technology,
P. M. B., 1052, Zaria – Nigeria.

ABSTRACT

Two Saponins 1 and 2 were isolated from the ethanolic extract of the leaves of *Cassia occidentalis*. Their structures were elucidated mainly by using a combination of 400 MH_Z & 100MH_Z 1D and 2D NMR techniques (COSY, NOESY, HSQC, DEPT and HMBC) and Mass spectroscopic method. It has been demonstrated that 1 is 3β, 16β, 23, 28 - tetrahydroxyoleana – 11, 13 - diene - 3 - O - α - L - rhamnopyranosyl - (1 → 4) - β - D - glucopyranosyl - (1 → 3) - [- β - D - glucopyranosyl - (1 → 2)] - β - D - fucopyranoside and 2 as 3 β, 11 β, 16 β, 28 tetrahydroxy - 12 - ene - 3 - O - α - L - rhamnopyranosyl - (1 → 4) - β - D - glucopyranosyl - (1 → 6) - β - D - glucopyranoside.

Keywords: *Cassia occidentalis, Leaves, Saponins, Spectral data.*

I. INTRODUCTION

The genius cassia comprises of 600 species of trees, shrubs, vines and herbs with numerous species widely distributed in the tropics with the greatest diversity in Africa, central and Southern America. *Cassia occidentalis* (Fedegoso) as it's commonly called has been used as natural Medicine in the rainforest and other tropical areas for centuries. The leaves from the plant are used in Brazil for gonorrhea, Typhoid fever, urinary tract disorders, edema and menstrual problems. The decoction of the fresh leaf of the plant is used for general pain, uterine pain and constipitation in Babies. In Panama, the infusion of the leaves are used as an anti-inflammatory agent and as anti-helminthis agent [1, 2].

The wide occurrence of saponins in nature has evoked a lot of interest in their use and considerable data has accumulated concerning their physiological action and other properties. Saponins in general, lowers the surface tension and possess emulsifying properties. They tend to alter the permeability of the cell-wall and therefore, exert a general toxicity on all organized tissue. Their hemolytic, antilipemic activities and capacity to lower the serum cholesterol level can be considered to be their important characteristics [3, 4]. Saponins are constitutive triterpeniod, steroid or steroidal glycoalkaloid molecules having one or more sugar chains and are important in plant defense against microbial infection [5, 6]. Saponins have detergent like properties and are lethal to fungi because of their ability to complex with membrane sterols, resulting in the loss of membrane integrity [7, 8]. Consequent upon this, it was decided to screen this plant for its medicinal important as claim by the folkloric healers.

II. RESULT AND DISCUSSION

COMPOUND 1

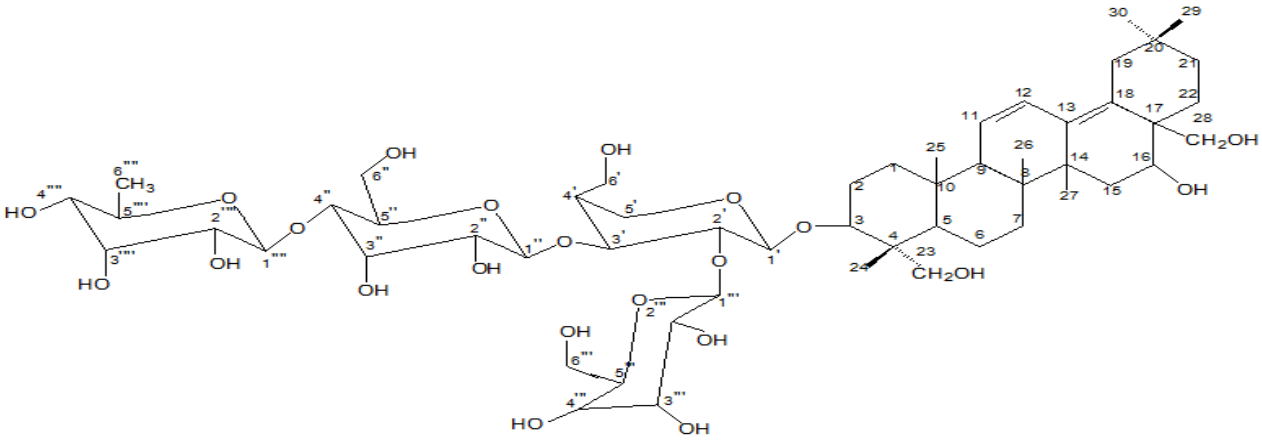
This is an amorphous powder (mp. 286-288) that exhibited the molecular ion at $M/_Z$ 1089 EIMS $[M + H]^+$ corresponding to $C_{54}H_{88}O_{22}$. The IR spectrum showed an absorptions at 3419.90cm^{-1}, 2805.87cm^{-1}, 1385.61cm^{-1}, 1713-1720cm^{-1} and 3080.43cm^{-1} Indicating OH, - CH = CH -, CH_3, Rhamnose unit and - CH_2 - respectively [9]. The HNMR spectrum shows two olefinic signals at δ_H5.6311/δ_C126.4532ppm and δ_H5.4835/δ_C125.7258ppm. This also exhibited the presence of four anomeric sugar proton signals at δ_H4.8654ppm, δ_H4.8346ppm, δ_H4.8642ppm and δ_H5.1045(s). Signals on δ_H0.9501ppm, δ_H0.8421ppm, δ_H0.9322ppm, δ_H 0.8436ppm, δ_H 0.8642ppm and δ_H 0.9463ppm could all be attributed to tertiary Methyl groups of the aglycone. A broad singlet peak of δ_H5.1046(s) indicated the presences of an α - orientation at the anomeric centre of L – rhamnose [10, 11]. In CNMR, Peaks were assigned on the basis of chemical shift consideration and comparism with data for fucose, glucose and rhamnose. This exhibited a total of 54 carbon signals, 30 of it corresponding to aglycone while 24 were attributed to tetra substituted sugar moieties. The J values (>7H_Z) of the tri substituted sugar moieties indicated the β – orientation at the anomeric centre's [12]. The (DEPT) ^{13}CNMR spectrum also showed the presence of eight Methyl groups, 12 Methylene and 26 Methine groups. The presence of 8 quaternary carbon signals were observed on δ_C43.5621ppm, δ_C40.2460ppm, δ_C36.7563ppm, δ_C136.0120ppm, δ_C44.2634ppm, δ_C44.4022ppm, δ_C133.3214ppm and δ_C32.1375ppm respectively [13]. The coupling constants of the olefinic group observed above, suggest their linkage as diene as confirmed from the ^{13}CNMR spectral data δ_C126.4532ppm, δ_C125.7258ppm, δ_C136.0120 and δ_C 133.3214ppm [14]. The HSQC and HMBC spectrum exhibited the correlation of the substituted glucose and fucose on ($1^{II}\rightarrow 4^{I}$, $1^{III} \rightarrow 2^{I}$) for glucose, ($1^{I} \rightarrow 3$) for fucose while ($1^{IIII} \rightarrow 4^{II}$) for rhamnose sugar respectively [15]. However, the Acid hydrolysis of compound 1 yielded – D – fucopyranose, D – glucopyranose and L – rhamnopyranose with 1 : 2 : 1. On the basis of NOESY, COSY, HMBC and HSQC spectra, the linkage of the four sugar moieties were established from the correlation : H – 1^{II} (δ_H4.8346ppm) of glucose with δ_C84.5301ppm of fucose moiety; H – 1^{IIII} (δ_H5.10146ppm) of rhamnose to C-4^{II} (δ_C78.6124ppm) of glucose; H -1^{III} (δ_H4.8642ppm) of glucose to C – 2^{I}

(δ_C71.6342ppm) of fucopyranose moiety [16]. The attachment of the sugar moieties were confirmed between the H – C long – range correlation and H – 1(δ_H4.8654ppm) of fucopyranose with C-3 (δ_C83.6422) observed in the HMBC and NOESY spectrum [17, 18]. The anomeric configurations for the sugar moieties were confirmed as β for glucose and fucose from their coupling constant of 7.8 and 8.3HZ respectively. The ^{13}CNMR spectrum of rhamnose were compared with those of Methyl – α – L and Methyl – β – L – rhamnopyranoside. This conforms well with the values for Methyl α – L – rhamnopyranoside [19]. Thus, the structure of Compound 1 was elucidated as 3β, 16β, 23, 28 – tetrahydroxyoleana – 11, 13 – diene 3 – 0 - α – L – rhamnopyranosyl – (1→ 4) - β – D – glucopyranosyl – (1→ 3) - [-β – D – glucopyranosyl – (1→ 2)] – β – D – fucopyranoside.

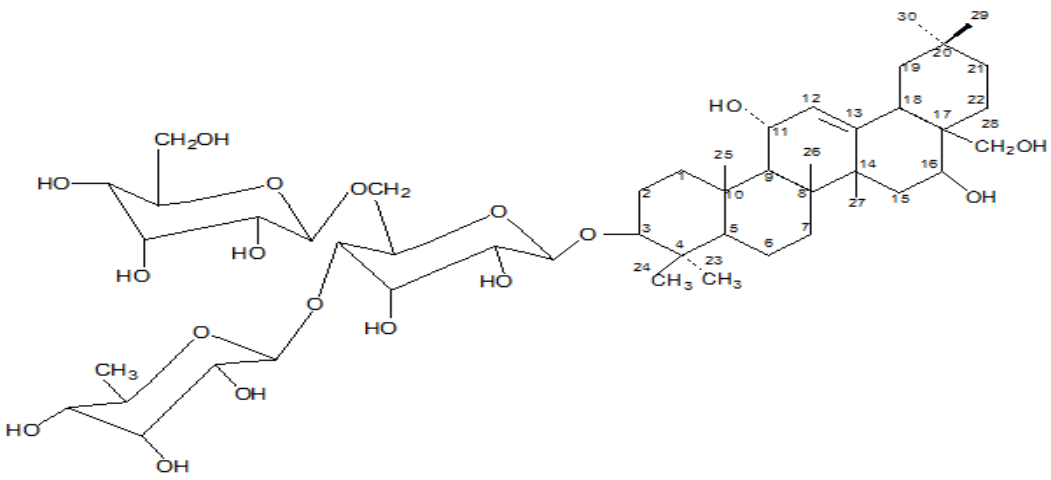
Table 1: ^{13}C NMR(100MHz) and ^{1}H NMR(400MHz) Spectral Data for 1 and 2 (CD_3OD) (δ in ppm, J in Hz).

	COMPOUND 1			COMPOUND 2		
Position	**δ_C(ppm)**	**δ_H(ppm)**	**Hz**	**δ_C(ppm)**	**δ_H(ppm)**	**Hz**
1	38.0143	0.9501		41.2346	1.0648	
2	26.1566	1.432		26.4701	1.3816	
3	83.6422	3.2513		82.7645	3.4631	
4	43.5621	-		44.82	-	
5	47.4825	0.8421		49.0122	0.9643	
6	18.1346	1.3403		19.2163	1.4132	
7	33.3243	1.5638		34.0214	1.6001	
8	40.246	-		41.5642	-	
9	54.8974	1.8624		54.6328	1.6	
10	36.7563	-		30.5521	-	
11	126.4532	5.6311	(1H-) J10.6H_Z	68.321	4.1743	
12	125.7258		(1Hdd J10.6)	128.0142	5.2231	
13	136-0120			147.601	-	
14	44.2634	-		44.8264	-	
15	35.2379	1.9234		36.9321	1.7614	
16	76.7321	5.0631		75.2014	4.2734	
17	44.4022	-		44.9201	-	
18	133.3214			48.5132	2.2314	
19	38.2846	1.3326		38.6654	1.3241	
20	32.1375	-		31.8436	-	
21	34.8324	5.4501		34.52	4.8162	
22	29.4326	2.23113		26.5001	2.4631	
23	64.0761	4.0142		28.6213	1.5639	(s)
24	12.5051	1.0234		18.0132	1.7643	
25	18.3216	0.9322		17.0204	1.6739	
26	16.7532	0.8436		18.7113	1.3246	
27	21.5458	1.0421		27.1526	1.462	
28	63.5236	4.0331		64.764	4.2301	
29	24.6234	0.8642		24.8214	2.321	

30	32.6314	0.9463		32.0112	1.8264	
Fucose				Gluc		
1^{I}	106.2304	4.8654	(8.3)	106.5436	4.8223	(7.5)
2^{I}	71.6342	4.3651		75.4872	3.7201	
3^{I}	84.5301	4.0146		77.1344	4.4821	
4^{I}	72.4253	4.123		81.3462	4.5362	
5^{I}	71.2638	3.6002		75.7011	3.7214	
6^{I}	16.9312	13671	(5.4)	69.52	3.6871	
Gluc				Gluc		
1^{II}	104.7216		(7.5)	104.8634	4.9524	(7.8)
2^{II}	75.0134	3.8512		75.4836	4.0251	
3^{II}	76.0134	4.5642		78.2831	4.1824	
4^{II}	78.6124	4.4613		71.6333	3.6561	
5^{II}	72.1638	3.6721		72.0521	4.3241	
6^{II}	62.3201	4.0615	(4.06)	62.7236	4.0613	
Gluc				Rham		
1^{III}	103.8204		(7.8)	103.0136	5.3115	(s)
2^{III}	76.1432	4.0632		72.432	3.7056	
3^{III}	77.6214	4.1824		72.821	3.3217	
4^{III}	78.4243	3.7249		73.6312	3.4311	
5^{III}	72.2304	4.2316		70.8113	3.6544	
6^{III}	61.5643	4.3301	(4.3)	17.9243	1.6624	
Rham						
1^{III}	102.5451		(S)			
2^{III}	72.4321	4.6273				
3^{III}	72.621	4.5061				
4^{IIII}	73.2012	4.3231				
5^{IIII}	70.602	4.8632				
6^{IIII}	18.3001	1.5736	(6.5)			



Compound (1)



Compound (2)

Compound **2** was obtained as an amorphous powder (mp. 284-286). It gave an accurate positive ion at $M/_Z$ 945 $[M + H]^+$ in the EIMS, corresponding to the molecular formula – C_{48} H_{80} O_{18}. The FTIR spectrum gave characteristic absorption bands at 3426 (hydroxyl groups), 1394cm^{-1} (Methyl groups), 1713- 1725 cm^{-1} (rhamnose unit) and 3087cm^{-1} (-CH2 -) [20]. The ^{1}HNMR showed an olefinic signal at δ_C 128.0142ppm and also exhibited the presence of three anomeric sugar proton signals at δ_H4.8223ppm, δ_H4.9524ppm and δ_H5.3011ppm(s) respectively. Signals on δ_H1.0648ppm, δ_H0.9643ppm, δ_H1.5639ppm, δ_H1.7643ppm, δ_H1.6739ppm, δ_H1.3246ppm, δ_H2.3201ppm and δ_H1.8264ppm could all be attributed to tertiary Methyl groups of the aglycone moiety [21, 22]. In ^{13}CNMR, Peaks were assigned on the basis of chemical shift consideration and comparism with data for glucose and rhamnose. The spectrum exhibited a total of 48 carbon signals, 30 out of it corresponding to aglycone while 18 to trisubstituted sugar moieties. The J values (>7H_Z) of the disubstituted sugars of glucopyranoside moieties indicated the β – orientation at the anomeric regions [23]. The ^{13}CNMR (DEPT) exhibited 7 quaternary carbon signals on C – 4, C – 8, C – 10, C – 13, C – 14, C – 17 and C – 20 respectively. The HSQC and HMBC exhibited the correlation of disubstituted glucopyranosyl moieties unit at ($1^{II}\rightarrow 6^1$), ($1^1\rightarrow 3$) while ($1^{III}\rightarrow 4^1$) is attributed to rhamnose unit respectively [24]. The acid hydrolysis of Compound 2 yielded – D – glucopyranose and L – rhamnopyranose with (2 : 1). The NOESY, COSY, HSQC and HMBC spectra has assisted in establishing the correlations: (δ_H4.9524ppm/δ_C104.8634ppm → δ_H3.6871pm/δ_C69.5200ppm), (δ_H4.8223ppm/ δ_C106.5436ppm → δ_H3.4631ppm/δ_C82.7645ppm) for glucose while (δ_H5.3011ppm/δ_C103.0136 → δ_H4.5362ppm/ δ_C81.3462ppm) for rhamnose unit (25). The anomeric configurations for the sugar moieties were confirmed as β for the glucose and α – for the rhamnose as observed from their coupling constant above. The ^{13}CNMR spectrum was in conformity with the values for Methyl - α – L – rhamnopyranoside. Thus, the structure of compound 2 was elucidated as 3β, 11 β, 16 β, 28 tetraydroxy – 12 – ene – 3 – O – α – L – rhamnopyranosyl – (1→ 4) - β – D – glucopyranosyl – (1→ 6) - β – D – glucopyranoside.

General Experimental procedures: Melting points were determined on Gallenkamp apparatus and were uncorrected. UV (in absolute) ethanol while IR (KBr) absorption spectra were recorded on shimadzu 84003 FTIR spectrophotometer respectively. Proton NMR and ^{13}CNMR spectra both (ID and 2D) were recorded in CD_3OD using spectrometer, with the residual solvent peaks as internal standard. Chemical shift values (δ) were reported in part per million in relations to the appropriate internal solvent standard (TMS). The coupling constant (J-values) were given in Hertz while the HMBC and NOESY were also obtained.

Plant Material: The leaves of *Cassia occidentalis* (Caesalpiniaceae) was collected and identified by Mal. Umar Gallah and Musa Mohammed. A voucher specimen (No 1047) was deposited at the Herbarium Biological Science Department A.B.U Zaria, Nigeria.

Extraction and Isolation: Dried powdered leaves (500g) were exhaustively macerated for 2days with petroleum ether (60^o – 80^oC). The Marc was dried and macerated with 5 liters of 95% ethanol. The petroleum ether and the ethanolic extracts were concentrated using rotary evaporator to afford 25g and 48g respectively. The ethanolic extract (30g) was suspended in water (600ml) and submitted to partition with chloroform, ethylacetate and n-Butanol. The solvent from each portion were concentrated using rotary evaporator to afford chloroform (3g), ethylacetate (2g), N-Butanol (7g) and Aqueous residue (4g) respectively (26). 5g portion of the n-butanol was solubilized in methanol (20ml) and precipitated in diethyl ether (3 x 250ml), yielding 2.96g crude saponin fraction. The resulting mixture was suspended in

water, dialysed for 48hrs and then lyophilized yielding crude saponin mixtures [27]. An aliquot (1.95g) of the mixture above was fractionated by column chromatography over sephadex LH-20. This was subsequently submitted to a repeated PTLC on silica gel precoated glass plates (20 x 20cm) with layer thickness of 0.25mm, using solvent system $CHCl_3$ – MeOH – H_2O (8:5:1). The following solvent system Ethylacetate: pyridine : water (3 : 1 : 3) and n-Butanol : Acetic acid ; water (6 : 1 : 3) were used to monitor the TLC of the isolate [28]. This afford compound 1 (18mg) and 2 (15mg) respectively.

Acid hydrolysis: A solution of (4.6mg) and 4.2mg) of compound 1 and 2 in H_2S0_4 (1ml) were heated at 90^{o}C for 30min under argon atmosphere. After cooling H_2O (5ml) was added to the mixtures each and extracted with $CHCl_3$ (5 x 5ml). The combined $CHCl_3$ layer was washed with brine, dried over Na_2SO_4 and evaporated to afford an aglycone fraction (1.4mg) and 1.2mg). The sugar fraction was dissolved in CH_3OH : H_2O (2 : 8) and after passing through a sep-pa K C_{18} Cartridge, it was analyzed by HPLC using CH_3CN/H_2O (85:15). The sugars were identified as D – glucose and L – rhamnose base on their retention time, t_R, 11.85mm and 13.73mm for glucose and rhamnose (D – glucose t_R = 11.60 mm, 13. 45mm respectively [27].

GC – MS: The dry sugar residue of compound 1 and 2 from the Acid hydrolysis were diluted in 5ml pyridine without water. This was treated with 0.5ml trimethyl chlorosilane (TMCS, Fluka) at room temperature for 30min. The reaction mixture was evaporated to dryness under reduced pressure. The mixture of trimethylsilated derivatives of the monosaccharide were further diluted in 0.5ml CH_3OCH_3 without water. This was then subjected to GC – Mass. GC : AC – 5 Capillary column (30m x 0.25mm); Detector ; Ms; Column temperature 80^{o}c – 220^{o}c, pressure low 108.0KPa, carrier gas He. The sugars in compound 1 and 2 were identified as D-fucose, D – glucose with R_t (s) ; 694, 688 and 430 for L – rhamnose respectively.

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