Research Article

TERPENOID CONSTITUENTS OF *ASPILIA AFRICANA* [PERS] C.D. ADAMS LEAVES

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ABSTRACT

Aspilia africana [Pers] C.D Adams, a medicinal plant used for the treatment of ailments such as cough, gonorrhea, bleeding, wound and sores was investigated for its constituents. Three terpenoids (I-III) were isolated from the leaves of *Aspilia africana*. The structures of the compounds were identified as: 3β-O-[α-rhamnopyranosyl-(1→6)-β-glucopyransyl-(1→3)-ursan-12-ene, 3β-Hydroxyolean-12-ene and 3β-acetoxyolean-12-ene. The molecular structures elucidations of these compounds were carried out using spectroscopic studies (^{1}H NMR and ^{13}C NMR) and comparison with literature. These compounds are reported from this species for the first time.

Keywords: Terpenoids, *Aspilia africana*, Phytoconstituents, Structure elucidation.

INTRODUCTION

Traditional medicine has been a fertile source for revealing novel lead molecules for modern drug discovery. In plants, terpenoids represent a chemical defense against environmental stress and provide a repair mechanism for wounds and injuries[1]. Interestingly, effective ingredients in several plant-derived medicinal extracts are also terpenoid compounds of monoterpenoid, sesquiterpenoid, diterpenoid and carotenoid groups. Inflammatory diseases and cancer are typical therapeutic indications of traditional medicines[1]. According to WHO, herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the vast areas of developing countries[2].

Aspilia africana [PERS] C.D. Adams is a medicinal plant that has been widely used in African folk medicine to stop bleeding, remove corneal opacities, induced delivery and in the treatment of anaemia and various stomach complaints[3,4]. Therefore, *Aspilia africana*, among other African medicinal plants could serve as a source of lead compounds for the development of new drugs. [5]has reported the anti-inflammatory activity of the hexane leaf extract of *Aspilia africana* while the methanolic extract has been reported to possess antiulcer effects in rats[6]. Other studies showed that essential oils from the leaves of *A. africana* were rich in sesquiterpenes and monoterpenes. Also the presence of prococene I was found[7].

Many plant constituents are effective as remedy for some diseases and accounts for large number of pharmaceutical important compounds in Western Pharmacopoeia and a number of important drugs. For example, taxol and artermisinin were reported from plants[8]. In our quest to finding novel anti-inflammatory agents from plants, we have carried out phytochemical study on the leaves extract of *A. africana* to isolate, characterize and identify the terpenoid constituents because, anti-inflammatory activity has been linked to terpenoids compounds[9,1].

MATERIALS AND METHODS

Plant material

The fresh leaves of *A. africana* were obtained from the campus of Obafemi Awolowo University, Ile-Ife, Nigeria in September, 2003, air dried at room temperature for two weeks. The plant was identified and authenticated by Mr. T.K. Odewo of the Forest Research Institute of Nigeria, Ibadan (FRIN) and voucher specimen was deposited at the FRIN Herberium, Ibadan with voucher number; FHI 107695.

Extraction

The air dried leaf powder (2.1 kg) of *A. africana* was exhaustively extracted with 50 % aqueous ethanol at room temperature for 48h. The extract was filtered, concentrated *in vacuo* to dryness to yield 134.2 g of the crude extract of *A. africana*. This was suspended in water and partitioned with *n*-hexane (4 x 400 ml). The combined organic layer was evaporated to dryness *in vacuo* to afford the hexane fraction (9.2 g). The resultant aqueous portion was further partitioned with ethyl acetate (4 x 400 ml). The combined ethyl acetate extracts were concentrated to dryness *in vacuo* at 40^{o}C to afford the ethyl acetate fraction (9.2 g). The resultant aqueous portion was further partitioned with *n*-butanol (3 x 400 ml). The combined butanol fractions were concentrated to dryness *in vacuo* at 40^{o}C to afford the butanol fraction (18.7 g).

Isolation of the compounds

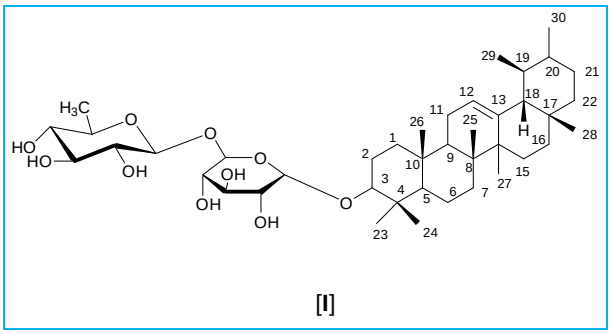
The butanol fraction of the *A. africana* extract (18.1 g) was subjected to column chromatography on silica gel. The column was eluted with a hexane-CH_2Cl_2, CH_2Cl_2-ethylacetate, and ethyl acetate-MeOH gradients

generated by successively eluting with the solvent systems. The fractions collected were monitored by TLC and sprayed with vanillin-H_2SO_4. Fractions that showed similar TLC characteristics were bulked appropriately and concentrated *in vacuo* to dryness to give the following eight major fractions, AABA- AABH.

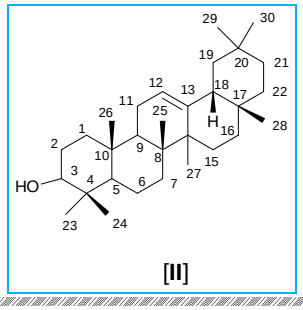
A further fractionation of AABG (2.0 g) on LH-20 Sephadex gel gave fractions AABG1 (553 mg) and AABG2 (620 mg). Fraction AABG2 (620 mg) was further fractionated using Accelerated Gradient Chromatography (AGC) on silica gel to afford fractions AABG2a (120 mg), AABG2b (206 mg) and AABG2c (103 mg). Fractions AABG2b and AABG2c were combined and further fractionated using AGC on silica gel to give fractions AABG2bc1 (196 mg) and AABG2bc2 (50 mg). Fraction AABG2bc1 (190 mg) was purified further on RP-18 lobar column stepwise with MeOH/H_2O mixture with an increasing gradient of methanol up to 100% to afford compound **I** (11 mg).

About 5.9 g of the acetone extract of dried and milled *A. africana* leaf was fractionated on silica gel column chromatography using an increasing gradient of dichloromethane (CH_2Cl_2) in hexane up to 100 %, followed by an increasing gradient of EtOAc up to 100 % and further followed by an increasing gradient of methanol (MeOH) up to 100 %. This gave seven pooled fractions AAAc1-AAAc7. Purification of AAAc3 (550 mg) on silica gel column chromatography using an increasing gradient of CH_2Cl_2 in hexane up to 100 %, followed by an increasing gradient of EtOAc in CH_2Cl_2 up to 100 % afforded compound **II** (17 mg).

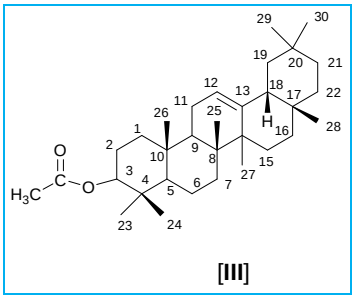
Fresh leaves of *A. africana* were collected and blended with 100 % MeOH using a Philips electric blender, model-BL-902. The green solution was partitioned with Pet. ether 40/60^{0}C range. The Pet. ether fraction was evaporated to dryness *in vacuo* to obtain 920 mg. This was fractionated using an increasing gradient of toluene in hexane up to 100%, followed by an increasing gradient of toluene up to 100 % to afford compound **III** (107 mg).



[I]



[II]



[III]

Spectroscopy Analysis

Spectroscopic data were obtained from the following instruments: NMR-Varian (^{1}H 200 MHz, ^{13}C 50 MHz).

Structure Elucidation

3β-O-[α-rhamnopyranosyl-(1→6)-β-glucopyransyl-(1→3)-ursan-12-ene **(I).** ^{1}H NMR (200 MHz, CD_3OD) δ ppm: 5.30 (1H, br s, H-3), 5.2 (1H,s, H-12), 0.78 (3H, s), 0.84 (3H, s), 0.88 (3H, s), 1.05 (3H, s), 1.08 (3H, s), 1.36 (3H, s), 0.96 (3H, d, J = 3.5Hz), 1.03 (3H, br d, J = 5.3Hz).

^{13}C NMR (50 MHz, CD_3OD) δ ppm: 38.6 (C-1), 33.0 (C-2), 89.4 (C-3), 36.6 (C-4), 55.8 (C-5), 18.1 (C-6), 30.2 (C-7), 41.0 (C-8), 55.8 (C-9), 35.3 (C-10), 23.2 (C-11), 122.2 (C-12), 143.8 (C-13), 39.0 (C-14), 25.7 (C-15), 34.9 (C-16), 38.9 (C-17), 55.8 (C-18), 32.2 (C-19), 40.8 (C-20), 31.5 (C-21), 39.9 (C-22), 26.0 (C-23), 16.7 (C-24), 14.9 (C-25), 15.8 (C-26), 23.7 (C-27), 27.3 (C-28), 16.5 (C-29), 19.9 (C-30), 105.4 (C-1′), 71.0 (C-2′), 74.9 (C-3′), 82.4 (C-4′), 74.1 (C-5′), 68.6 (C-6′), 101.4 (C-1″), 74.1 (C-2″), 71.1 (C-3″), 72.8 (C-4″), 71.0 (C-5″), 18.2 (C-6″). The spectra data for compound **(I)** were in agreement with that of triterpenoids reported in literature[10].

3β-Hydroxyolean-12-ene **(II)**. ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 3.2 (1H, m, H-3), 5.18 (1H, t, H-12), 0.78 (3H, s), 0.84 (3H, s), 0.88 (3H, s), 0.94 (3H, s), 1.0 (3H, s), 1.04 (3H, s), 1.60 (3H, s).

^{13}C NMR (50 MHz, $CDCl_3$) δ ppm: 38.7 (C-1), 27.4 (C-2), 79.2 (C-3), 39.0 (C-4), 55.3 (-5), 18.6 (C-6), 32.8 (C-7), 39.9 (C-8), 47.8 (C-9), 37.1 (C-10), 23.7 (C-11), 121.9 (C-12), 145.4 (C-13), 41.9 (C-14), 26.3 (C-15), 27.1 (C-16), 32.7 (C-17), 47.4 (C-18), 47.0 (C-19), 31.3 (C-20), 34.9 (C-21), 37.3 (C-22), 28.3 (C-23), 15.7 (C-24), 15.8 (-25), 17.0 (C-26), 26.2 (C-27), 28.6 (C-28), 33.5 (C-29), 23.9 (C-30). The spectra data for compound **2** were similar to that of tetra and pentacyclic triterpenoids reported reported in literature[11].

3β-acetoxyolean-12-ene **(III)**. ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 4.5 (1H, m, H-3), 5.18 (1H, t, H-12), 2.3 (2H, t), 0.82 (3H, s), 0.83 (3H, s), 0.85 (6H, s), 0.95 (3H, s), 1.10 (3H, s), 1.25 (3H, s).

^{13}C NMR (50 MHz, $CDCl_3$) δ ppm: 38.4 (C-1), 23.8 (C-2), 80.7 (C-3), 37.9 (C-4), 55.4 (C-5), 18.4 (C-6), 32.7 (C-7), 40.0 (C-8), 47.7 (C-9), 37.0 (C-10), 23.7 (C-11), 121.8 (C-12), 145.4 (C-13), 41.9 (C-14), 26.3 (C-15), 27.1 (C-16), 32.7 (C-17), 47.4 (C-18), 46.9 (C-19), 31.3 (C-20), 39.4 (C-21), 37.3 (C-22), 28.2 (C-23), 15.7 (C-24), 14.3 (C-25), 17.0 (C-26). 26.1 (C-27), 99.3 (C-28), 33.5 (C-29), 23.9 (C-30),

173.9 (C-1′), 17.0 (C-2′). The spectra data for compound **III** were in close agreement with that of 3β-acetoxyolean-12-ene reported in literature as a constituent of *Isodon japonicus* tissue culture[12].

RESULTS AND DISCUSSION

The dried leaves of *A. africana* were extracted successfully with the various solvents as described in the experimental method. Repeated chromatography of the extracts afforded the isolation of compounds **I-III**. Through spectroscopic analysis the compounds were identified as 3β-O-[α-rhamnopyranosyl-(1→6)-β-glucopyransyl-(1→3)-ursan-12-ene I, 3β-Hydroxyolean-12-ene **II** and 3β-acetoxyolean-12-ene **III**.

Table 1 shows the ^{13}C NMR data for compound **I**. These data were extracted from Figures 1a and 1b. ^{13}C NMR of compound **I** showed 30 peaks comprising 8-methyl, 8-methine, 14-methylene and quaternary C signals. This is suggestive of a pentacyclic triterpenoid structure. The fact that not all the methyl proton signals are singlets suggests that the triterpenoid should have an ursane (α-amyrin group) rather than oleanane (β-amyrin) skeleton (Savoir *et al.,* 1967). The down field region of the ^{13}C NMR shows a quaternary carbon signal at δ 143.8 and a CH-carbon signal at δ 105.4 and 122.2. The anomeric methine carbon signals are shown at δ 101.4 and 105.4 while the signals ascribable to sugar carbons were observed at δ 68.6-74.9. For the fact that methylene signals appeared at δ 68.6 and δ 18.2 and for the presence of two anomeric protons suggests that there are two sugars namely; glucose and rhamnose with the rhamnose sugar attached at position 6 of the glucose sugar.

Table 1: ^{13}C NMR spectra data for compound **I**

No	δ_C (ppm)	DEPT	δ_H (ppm)	No	δ_C (ppm)	DEPT	δ_H (ppm)
1	38.6[a]	CH_2		22	39.9[a]	CH_2	
2	33.0[a]	CH_2		23	26.0[d]	CH_3	0.78 (s)
3	89.4	CH	5.30 (br s)	24	16.7[d]	CH_3	0.84 (s)
4	36.6[b]	C		25	14.9[d]	CH_3	0.88 (s)
5	55.8	CH		26	15.8[d]	CH_3	1.05 (s)
6	18.1[a]	CH_2		27	23.7[d]	CH_3	1.08 (s)
7	30.2[a]	CH_2		28	27.3[d]	CH_3	1.36 (s)
8	41[b]	C		29	16.5[d]	CH_3	0.96 (d)[f]
9	55.8	CH		30	19.9[d]	CH_3	1.03 (br d)[f]
10	35.3	C		1′	105.4	CH	
11	23.2	CH_2		2′	71.0	CH	
12	122.2	CH	5.2 (s)	3′	74.9	CH	
13	143.8	C		4′	82.4	CH	
14	39.0[b]	C		5′	74.1	CH	
15	25.7[a]	CH_2		6′	68.6	CH_2	
16	34.9[a]	CH_2		1″	101.4	CH	
17	38.9[a]	C		2″	74.I	CH	
18	55.8	C		3″	71.1	CH	
19	32.2[c]	CH		4″	72.8	CH	
20	40.8[c]	CH		5″	71.0	CH	
21	31.5[a]	CH_2		6″	18.2	CH_3	

N.B : [a,b,c,d,e,f] - assignments carrying a particular letter are interchangeable

Table 2 shows the comparison of ^{13}C NMR spectra data of compounds **II** and **III** with reported data[12]. The data in Table 2 were extracted from Figures 2a to 4b.

Table 2: ^{13}C NMR spectra data for compounds **II** and **III**

No	DEPT	II (δ_C ppm)	III(δ_C ppm)	*δ_C ppm
1	CH_2	38.7	38.4	38.2
2	CH_2	27.4	23.8	23.6
3	CH	79.2	80.7	80.7
4	C	39.0	37.9	37.6
5	CH	55.3	55.4	55.3
6	CH_2	18.6	18.4	18.3
7	CH_2	32.8	32.7	32.6
8	C	39.9	40.0	39.7
9	CH	47.8	47.7	47.6
10	C	37.1	37.0	36.8
11	CH_2	23.7	23.7	23.4
12	CH	121.9	121.8	121.5
13	C	145.4	145.4	144.9
14	C	41.9	41.9	41.7
15	CH_2	26.3	26.3	28.3
16	CH_2	27.1	27.1	26.2
17	C	32.7	32.7	32.5
18	CH	47.4	47.4	47.2
19	CH_2	47.0	46.9	46.8
20	C	31.3	31.3	31.1
21	CH_2	34.9	34.9	34.8
22	CH_2	37.3	37.3	37.1
23	CH_3	28.3	28.2	28.1
24	CH_3	15.7	15.7	16.8
25	CH_3	15.8	14.3	15.7
26	CH_3	17.0	17.0	16.8
27	CH_3	26.2	26.1	26.0
28	CH_3	28.6	28.6	27.0
29	CH_3	33.5	33.5	33.4
30	CH_3	23.9	23.9	23.6
1′	C	-	173.9	170.4
2′	CH_3	-	17.0	21.2

* [12]

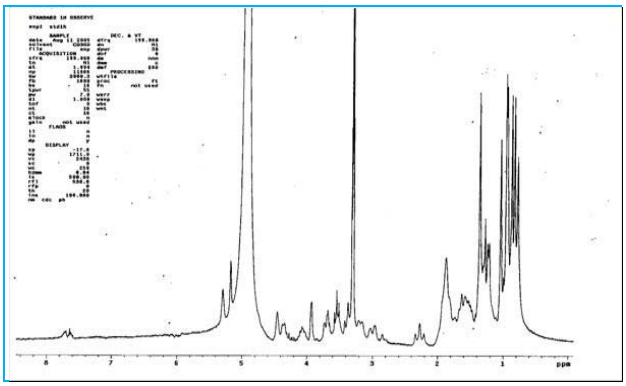


Figure 1a: ^{1}H NMR Spectrum of Compound **I**

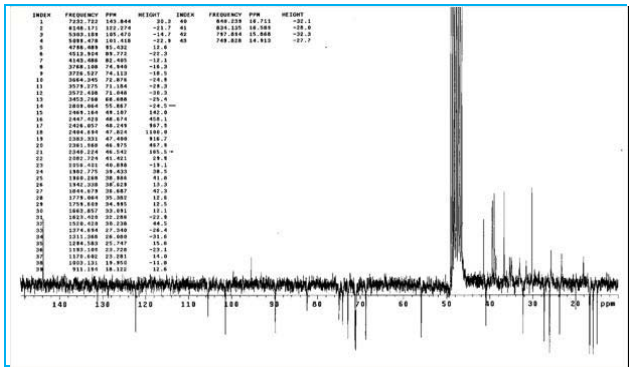


Figure 1b: APT Spectrum of Compound **I**

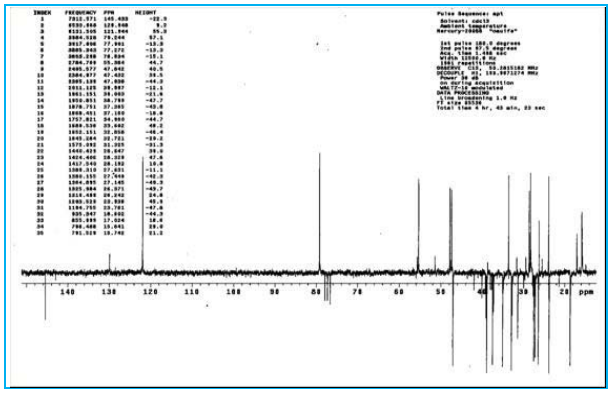


Figure 2a: APT Spectrum of Compound **II**

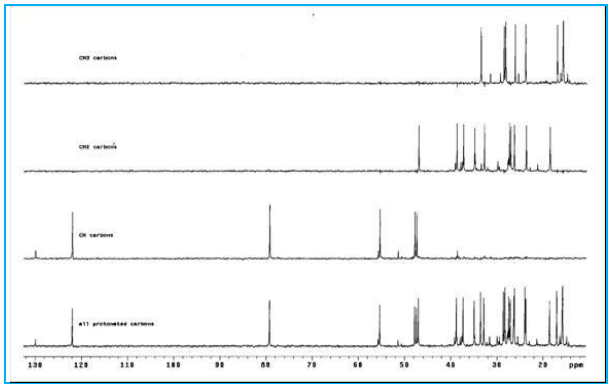


Figure 2b: DEPT Spectrum of Compound **II**

The ^{13}C NMR of compound **II** showed 30 peaks comprising 8-methyl, 10-methylene, 5-methine and 7-quartenary carbon signals, as shown in the Distortionless Enhancement by Polarization Transfer (DEPT) spectrum (Figures 2a & 2b). This is suggestive of a pentacyclic triterpenoid structure. The fact that all the methyl proton signals are singlets suggests that the triterpenoid should have an oleanane (β-amyrin group) rather than ursane (α-amyrin group) skeleton[10]. The downfield region of the ^{13}C NMR shows a quaternary carbon signal at δ 145.4 and a CH-carbon signal at δ 121.9. An oxygenated CH- carbon signal was also located at δ 79.2. All the carbon signals were assigned by comparison with reported data for 3β-Hydroxyolean-12-ene[11].

The ^{1}H- and ^{13}C-NMR spectra data of compound **III** displayed many similarities with those of compound **II**. The ^{13}CNMR of compound **III** showed 32 peaks instead of 30 in compound **II** which comprised of 9-methyl, 10-methylene, 5-methine and 8-quartenary carbon signals (Figures 3a & 3b). The oxygenated CH-carbon signal located at δ 79.2 in compound **II** was located more down field at δ 80.7 suggesting a more electronegative environment at C-3. The down field region of the ^{13}C NMR showed a quaternary carbon signal at δ 145.4 and CH-carbon signal at 121.9 attributed to a double bond in C-12 and C-13. The exceptions concerned mainly signal at δ 173.9 attributed to a quaternary carbonyl carbon (Figure 3a & Table 2). Therefore compound **III** was identified as 3β-acetoxyolean-12-ene as revealed in literature as a constituent of *Isodon japonicus* tissue culture[12].

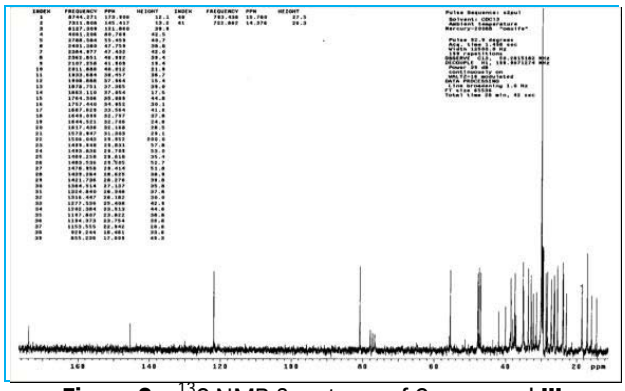


Figure 3a: ^{13}C NMR Spectrum of Compound **III**

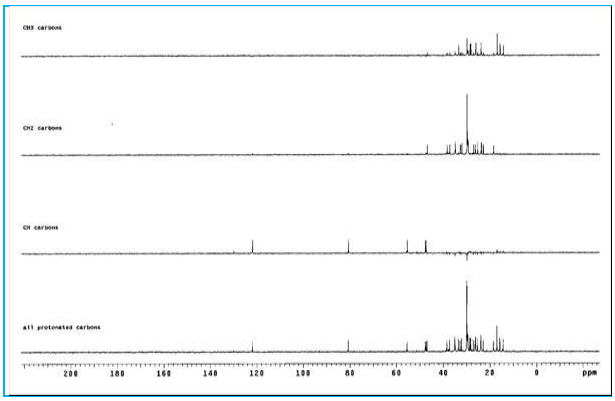


Figure 3b: DEPT Spectrum of Compound **III**

CONCLUSION

In conclusion, many terpenoids have been reported to possess ant-inflammatory activities[9, 1, 13]. The terpenoid constituents isolated and characterized in the present study might have been responsible for the anti-inflammatory and antiulcer activities observed in the leaves extract of *A. africana*[5, 6]. The presence of these compounds in abundance in the leaves extracts of *A. africana* could provide rationale for the use of this plant in folk medicine. However, further study is in progress to investigate biological activities especially anti-inflammatory and antiulcer activities of the isolated terpenoids. These terpenoid constituents are reported from this species for the first time.

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