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Isolation and characterization of three pentacyclic triterpenoids from aerial parts of *Canarium schweinfurthii* (ENGL) (Burseraceae)

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Abstract
General Phytochemical screening of the aerial parts of *Canarium schweinfurthii* revealed the presence of triterpenes. Extensive Phytochemical investigation of the chloroform and ethylaceate extract of the rhizome afforded compounds which were identified by I.R, ^{1}HNMR and ^{13}CNMR. Pentacyclic triterpenoids of three different classes; oleanane-type triterpene, ursane-type triterpene, and lupine-type triterpene were isolated from the methanol extract of the aerial parts of *Canarium schweinfurthii*. On the basis of their spectroscopic data, their structures were elucidated on the basis of physical and spectral techniques, besides comparison with literature data. The structure of the isolated compounds 1, 2 and 3 were established as 3β-hydroxyl olean-12, 18-diene (β-amyrin), 3β-hydroxyllup-20(29)-en-28-oic acid (Betulinic acid) and 3β-hydroxyl ursa-5,11,19-triene (uvaol) respectively. The compounds are reported for the first time from this plant.

Keywords: *Canarium schweinfurthii*; Pentacyclic triterpenoid; TLC; ^{1}HNMR, ^{13}CNMR, HSQC, COSY and HMBC

1. Introduction
Canarium schweinfurthii Engl. (Burseraceae) is a wild tree found mostly in Africa, which produces fruit similar to olives and which is barely used (Weeks *et al*., 2005) [21]. In the past, the resin was exported to Europe for pharmaceutical use. The resin is used against roundworm infections and other intestinal parasites. It is an emollient, stimulant, diuretic and has action on skin-affections and eczema (Kupcham, 1971) [14].
The pounded bark is used against leprosy and ulcers (Jules and Paull, 2006) [12]. Root is used against adenites whereas root scrapings are made into a poultice. The leaves are boiled with other herbs and the decoction used to treat coughs (Cragg and Newman, 2005) [6]. The seeds are roasted and pounded and the resulting powder mixed with skin oil or jelly to treat wounds.
In connection with the biological activities, about 99 compounds have been isolated from 9 species (Zhiyong *et al*., 2008; Zhao *et al.,* 2010; Zhang and Lin, 2008; Zhiyong *et al*., 2009) [24, 23, 22, 25].
Triterpenes have been used extensively in the treatment of many diseases their metabolites play a very important role in a plant's defense mechanism. They protect the plants from both constitutive and induced defensive responses against insects and environmental stress (Keeling and Bohlmann, 2006) [13]. Hence, triterpenoids provide a very good protection shield for plants, indicating their potential for use in the prevention of various inflammatory diseases in humans. Triterpenoids affect multiple signalling pathways, and the clinical properties of triterpenoids, particularly those of pentacyclic triterpenoids, have been shown in various studies. The structure activity relationships indicate that the presence of αβ-unsaturated carbonyl moieties significantly enhance the potency of these pentacyclic triterpenoids tetracyclic C30 compound. The objective of this study is to isolate bioactive pentacyclic triterpenoids of interest from the aerial part of *Canarium schweinfurthii*.

2. Experimental
2.1 Plant material and extraction
The fresh *Canarium schweinfurthii* stem barks were collected from Shendan Local Government Area of Plateau State, around September, 2011. Identified and authenticated by a taxonomist; Mallam Musa Mohammed of the Herbarium unit, Department of Biological

Sciences, Ahmadu Bello University Zaria, Kaduna State, Nigeria. Voucher specimens number 7232 was deposited. The plant materials were air dried at ambient temperature, pulverized, and stored a in air tight containers and abou 1.5 kg were extracted successively with methanol (2L x 3). The filtered extracts were combined, and the solvent evaporated in vacuo to yield the crude extracts.

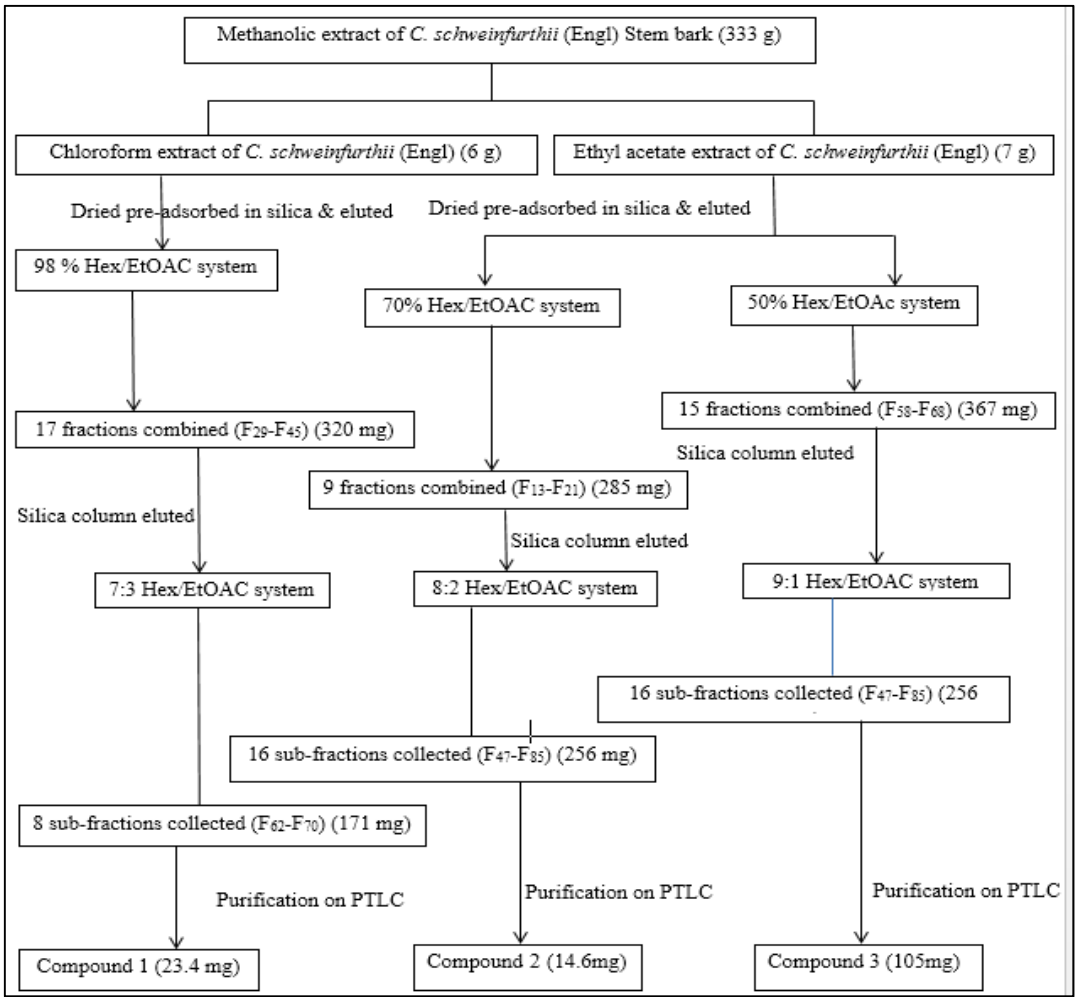
2.2 Column Chromatography

The crude methanol extracts were loaded in a 1 m length open column loaded with silica gel and eluted with neat 1.5 L chloroform and ethyl acetate. About 7 g of eluents were subjected to column chromatography (CC) on silica gel eluted with EtOAc-hexane solvent system. Isolation of Compounds

The crude fractions were subjected to isolation by column chromatography. Few grams of the fractions were dissolved in the appropriate solvent and pre-adsorbed on 5.0 g silica gel (Qualikens 60-120 mesh size). The dried pre-adsorbed extract was transferred to a mortar and ground to give a fine powder and added as a uniform layer on the column. The dry isolates are packed into glass bottles for spectroscopic analysis. The isolation schemes compounds 1-3 are presented in scheme 1.1.

2.3 Spectroscopic Analysis

1D and 2D NMR spectra were recorded on a Bruker Advance 400 MHz spectrometer.



Scheme 1.1: Flow Chart for the Isolation of Compounds 1-3

3. Result

Compound 1 was obtained as a white amorphous solid (23.5 mg). ^{1}H NMR (400 MHz, CD_3Cl) indicates protons resonating at δ 3.40, 5.20, 5.35 and 2.25 (Fig 1.10). The ^{13}C-NMR (400 MHz, CD_3Cl) spectrum showed carbon atoms at δ 64.10, 55.18,121.73, 139.30, 124.42, 38.79, 27.24, 18.38, 34.74, 22.60, 29.71, 39.62, 33.35, 37.15,δ 29.37, 21.41, 15.63, 16.81, 23.70, 26.00, 30.09 and 28.77 (Fig 1.20).

The compound 2 was obtained as white crystalline material (14.7 mg). The ^{1}H NMR (400 MHz, DMSO) with protons at δ 2.79, 4.71, 4.81, 1.56, δ 1.20, δ 1.14, 0.56, 0.37, 1.31 and 4.35 (Fig 1.30). The ^{13}C NMR data (400 MHz, DMSO) spectrum showed carbon atoms at *δ* 110.06, *δ* 150.82, 77.29, 177.70, 38.75, 27.63, 18.44, 19.43, 25.58, 30.51, 32.21, 29.68, 38.76, 55.39, 50.43, 38.09, 47.09 and 49.06, 27.53, 15.22, 16.41, 16.44 and 19.43 (Fig 1.40)

The compound 3 was obtained as colourless crystalline material (105.2 mg). The ^{1}H NMR (400 MHz, DMSO) with protons at δ 5.89, 5.87, 5.23, 3.34, 3.45, 2.10, 1.56, 1.20, 1.14, 0.56 and 0.37 (Fig 1.5). The ^{13}C NMR data (400 MHz, DMSO) spectrum showed carbon atoms at *δ* 143.46, 130.38, 129.39, 122.31, 115.27, 101.00, 77.21, 76.11, 66.04, 61.50, 60.38, 58.93, 49.01, 46.53, 36.25, 33.24, 32.81, 31.91, 30.11, 29.35, 28.06, 27.26, 25.14, 23.40, 22.27, 21.47, 18.24, 16.23, 15.29 and 14.13 (Fig 1.6)

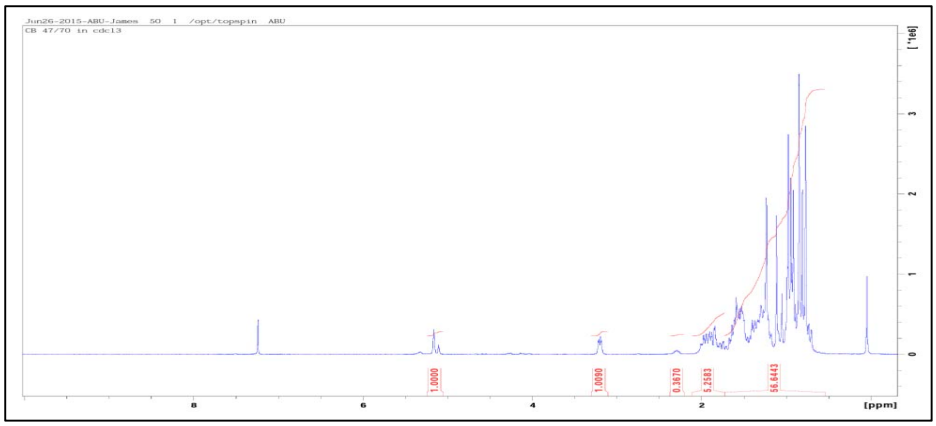


Fig 1.1: Integrated Proton NMR of Compound 1

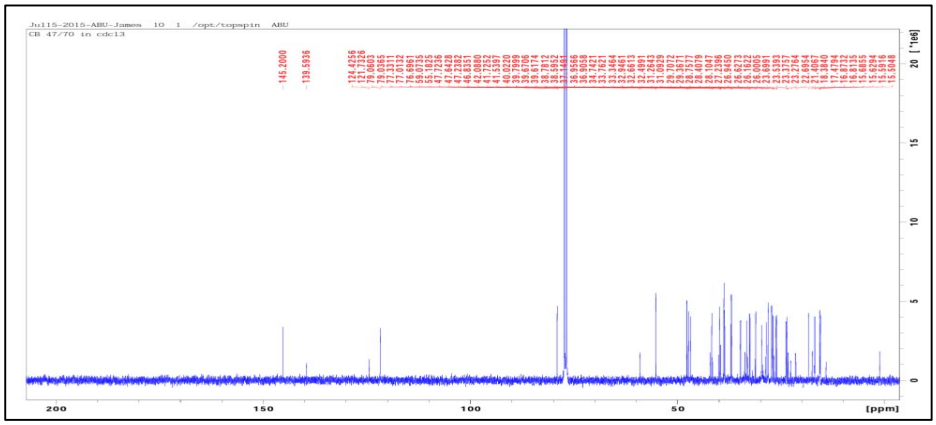


Fig 1.2: ^{13}Carbon NMR Spectrum of Compound 1

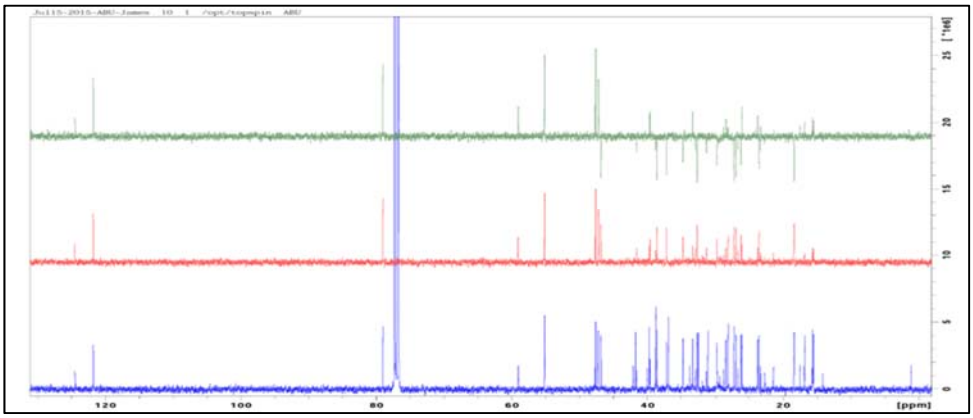


Fig 1. 3: Distortionless Enhancement Polarization Transfer (DEPT) of Compound 1

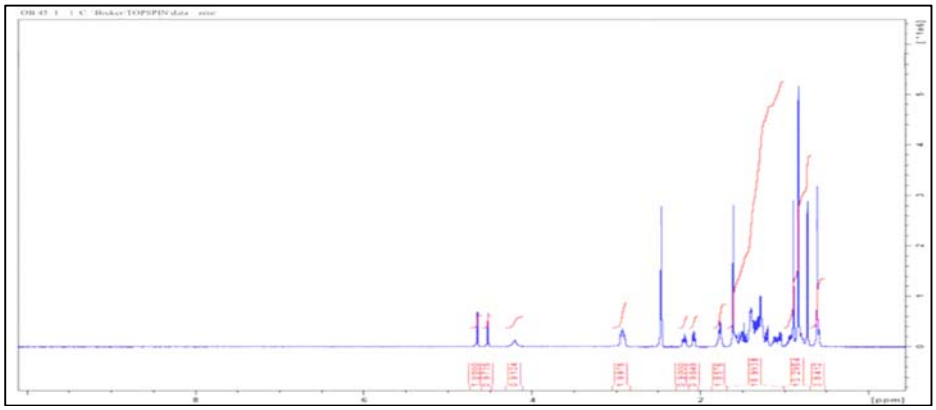


Fig 1.4: Integrated Proton NMR of Compound 2

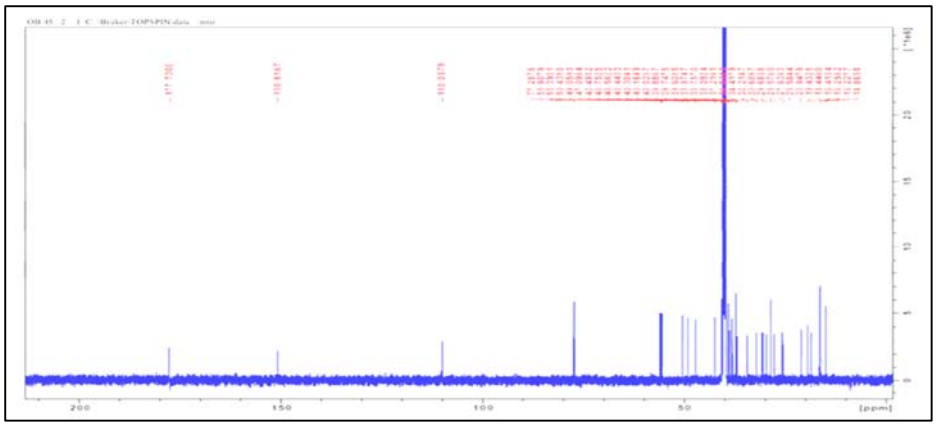


Fig 1.5: [13]Carbon NMR spectrum of Compound 2

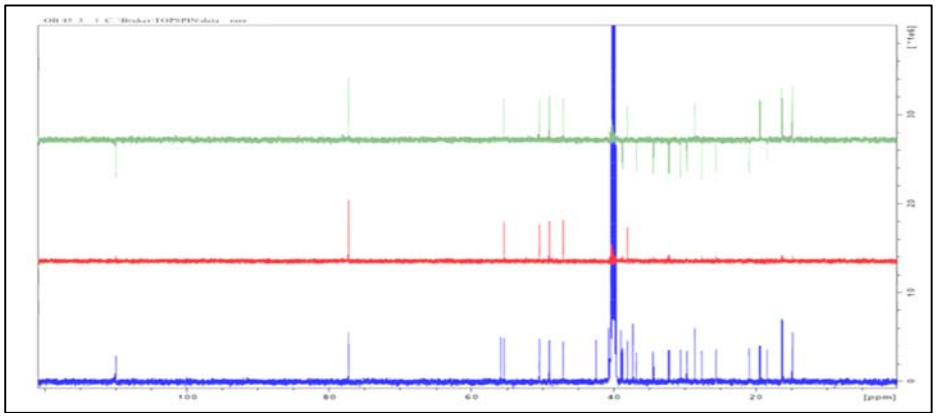


Fig 1.6: Distortionless Enhancement Polarization Transfer (DEPT) of Compound 2

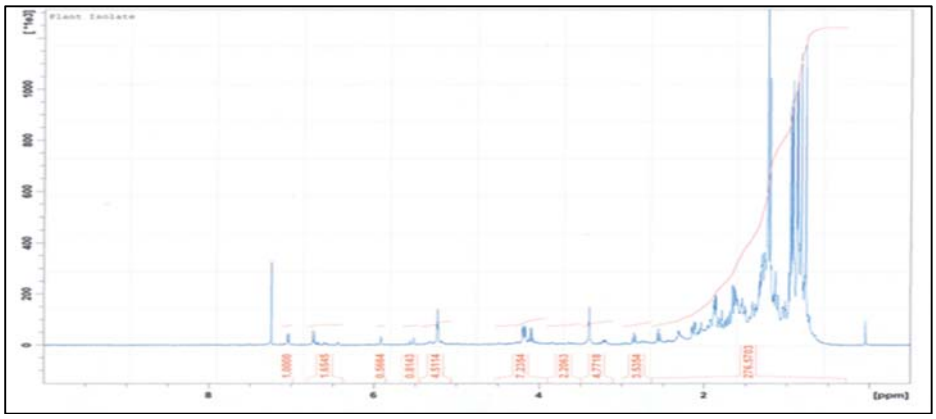


Fig 1.7: Integrated Proton NMR of Compound 3

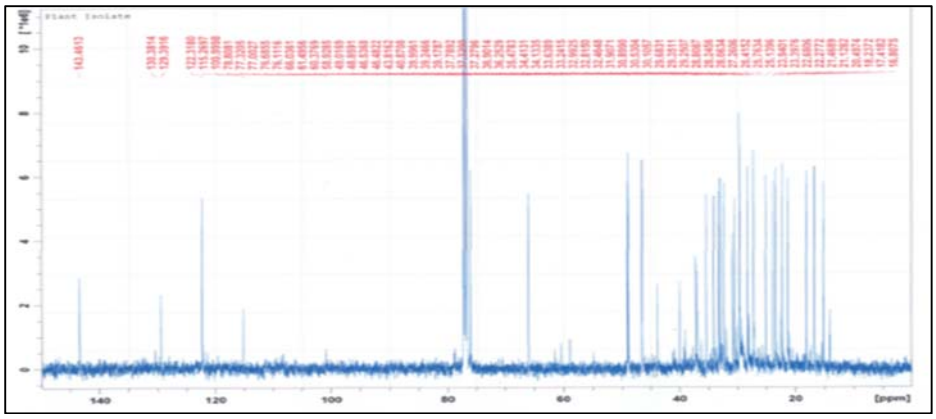


Fig 1.8: [13]Carbon NMR spectrum of Compound 3

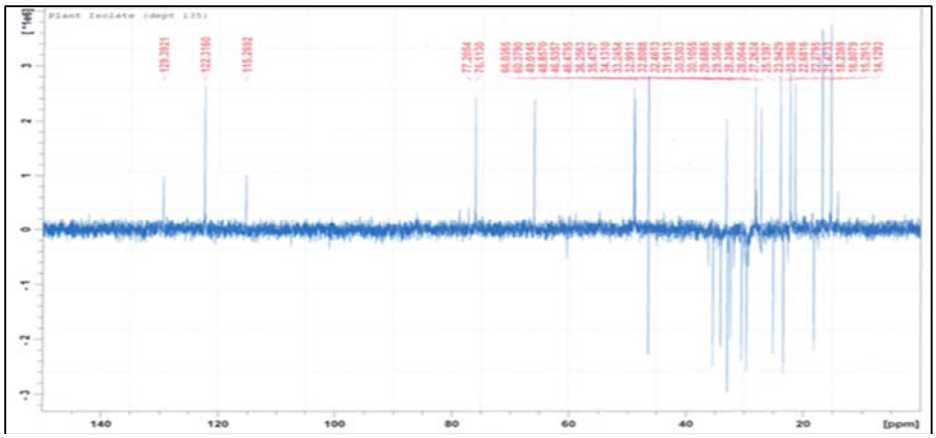


Fig 1.9: Distortionless Enhancement Polarization Transfer (DEPT) of Compound 3

4. Discussion

4.1 Characterization of compound 1

Compound 1 was obtained as a white amorphous solid. The ^{13}C-NMR spectrum showed 30 carbon atoms. Distortionless Enhancement Polarization Transfer (DEPT) sub-spectrum indicated five methine (CH) carbons at δ 64.10, 55.18, 121.73, 139.30, 124.42, nine methylene (CH_2) at δ 38.79, 27.24, 18.38, 34.74, 22.60, 29.71, 39.62, 33.35, 37.15 and eight methyl (CH_3) groups at δ 29.37, 21.41, 15.63, 16.81, 23.70, 26.00, 30.09 and 28.77. Quartenary carbon atoms do not contain attached protons hence do not appear in DEPT sub-spectrum. They may be identified as the signals which are additionally in the proton broadband decoupled ^{13}C-NMR spectra, therefore, eight quaternary carbons were identified (Fig 1.2).

The ^{1}H NMR (400 MHz, CD_3Cl) spectrum showed the presence of an oxymethine proton resonating at δ 3.40 (1H), two olefinic protons at δ 5.20 (1H), δ 5.35 (1H) and hydroxyl proton at δ 2.25 (1H). The signal from δ 0.70-1.98 (multiplet) are due to the presence of overlapping methyl, methylene and methine protons (Fig 1.1).

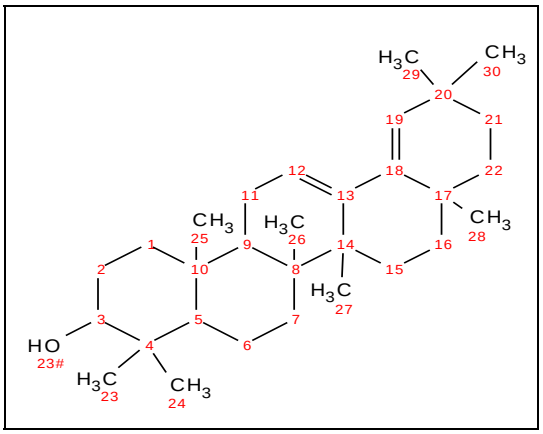
The ^{13}C NMR data (400 MHz, CD_3Cl) spectrum showed the presence of four olefinic carbon atoms resonating at *δ* 124.43, *δ* 139.59, *δ* 121.73 and *δ* 145.20. The oxymethine carbon peaked at *δ* 79.1 while the signals at *δ* 47.72, *δ* 55.18, are methine groups. The signals from δ 37.20, 27.66, 23.70, 28.10, 34.74, 31.26 and 38.60 are probably due to methylene groups, while the methyl groups are indicated by the following signals δ 21.41, 17.48, 18.38, 22.70, 21.41, 23.53, 23.70 and 16.87 respectively (Fig 1.2).

The COSY data indicates that the olefinic protons at δ 5.20 (1H (t)) and δ 5.35 (1H(s)) show correlation with methine protons at δ 1.97and 1.88. The oxymethine proton at δ 3.19 shows correlation with 1.41 methine, 1.51, 1.52 methylene and 0.95, 0.94 methyl protons.

The HSQC was used in assigning the ^{1}H signals to the corresponding ^{13}C signals, the oxymethine proton at δ 3.40 (1H (t)) shows direct correlation to the ^{13}C signals at δ 79.1, olefinic protons at δ 5.20 (1H (d)) and δ 5.35 (1H (d)) show correlation to the ^{13}C signals *δ* 124.43 and *δ* 121.43 respectively. The ^{13}C signals at δ 18.38 - 46.83 show correlation with the methyl protons around *δ* 1.50-1.98.

The oxymethine protons peaked at δ 3.40 shows correlation with 39.62 methine, 37.15 and 27.24 methylene and 29.39 and 21.41 methyl protons. The olefinic protons at δ 5.20 (1H (t)) and δ 5.35 (1H (s)) show multiple bond correlations with 32.92, 33.25, 37.15, 28.77 and 42. 09 while the angular methyl protons at *δ* 1.01 and *δ* 0.99 show correlation to *δ* 40.04 and *δ* 139.8 The COSY data indicates that the olefinic protons at δ 5.20 (1H (t)) and δ 5.35 (1H(s)) show correlation with methine protons at δ 1.97and 1.88. The oxymethine proton at δ 3.19 shows correlation with 1.41 methine, 1.51, 1.52 methylene and 0.95, 0.94 methyl protons.

Based on these information from the the 1 D and on the COSY, HMBC, and NOESY values, Compound 1 was deduced to be a 3β-hydroxyl olean-12,18-diene (β -amyrin) **(1).** The complete ^{13}C NMR spectroscopic assignments are consistent with those reported in literature (Table 1.1) (Mahato and Kundu, 1994; Gonzalez *et al.,* 1981) [15, 9].



3β-hydroxyl olean-12, 18-diene (β-amyrin)(1)

4.2 Characterization of compound 2

The compound 2 was obtained as white crystalline compound. ^{13}C NMR (Fig 1.5) and DEPT (Fig 1.6) showed 30 carbon atoms in the molecule consisting of one carboxyl, seven quaternary, five methine, eleven methylene and six methyl groups. The proton and carbon chemical shifts are characteristic spectral features of triterpenoids (Bross-Walch *et al.*, 2005) [4].

The ^{1}H NMR (400 MHz, DMSO) spectrum showed the presence of an oxymethine proton peaked at δ 2.79 (1H (t)), two olefinic protons at δ 4.71 (1H (s)) and δ 4.81 (1H (s)).The signal at δ 1.56, δ 1.20, δ 1.14, δ 0.56 and δ 0.37 (3H m) are likely due to the presence of overlapping methine and methylene and methyl while the signals at δ 1.31 and δ 4.35 (1H,s) are due to isopropyl protons (Fig 1.4)

The ^{13}C NMR data (400 MHz, DMSO) spectrum showed the presence of two olefinic carbon atoms at *δ* 110.06, *δ* 150.82.

The oxymethine carbon peaked at δ 77.29 and carboxyl carbon at δ 177.70. The signals at δ 38.75, 27.63, 18.44, 19.43, 25.58, 30.51, 32.21, 29.68, 38. 76 are methylene group. The signals from δ 55.39, 50.43, 38.09, 47.09 and 49.06 are due to methine groups, while the CH_3 methyl groups are indicated by the following signals δ 27.53, 15.22, 16.41, 16.44, 19.43 (Fig 1.5).

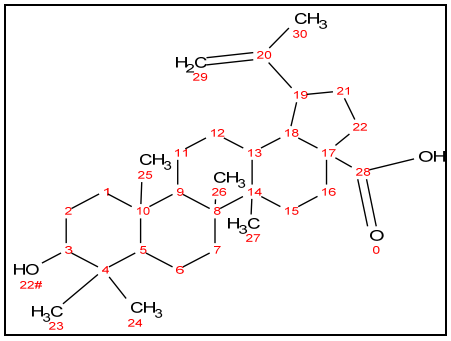
The sp^2 hybridized protons at δ 4.71 and 4.81 show direct correlation to the same carbon at δ 110.06; this implies that the two protons are attached to the same carbon. The oxymethine proton at δ 2.79 is showed a direct correlation to the signal at δ 77.29. The ^{13}C signals at δ 38.75, 30.51, 32.21 and 38.76 showed direct correlation to methylene protons around δ 1.5 – 2.0 ppm.

The olefinic proton at δ 4.71 shows a three bond correlation with the proton signal at δ 1.50. The methyl protons are usually found resonating in this region hence, it is a methyl proton.

Another key cross peak is the oxymethine protons showing correlation with methylene protons around δ 1.4–1.80, this locates the oxymethine proton two to three bonds away from a methylene group.

The olefinic protons showed correlation to the carbon resonating at δ 19.43 and 49.06. The oxymethine proton showed key correlations with the methylene and methyl carbon signals at 38.97 and 15.22 respectively.

Thus, the structure of compound 2 was considered to be a 3β-hydroxyllup-20(29)-en-28-oic acid (betulinic acid) (2). This is consistent with those reported in literature (Table 1.1) (Okwute, and Isyaka, 2014; Habila *et al*., 2013) [19, 9].



3β-hydroxyllup-20(29)-en-28-oic acid (Betulinic acid) (2)

4.3 Characterization of compound 3

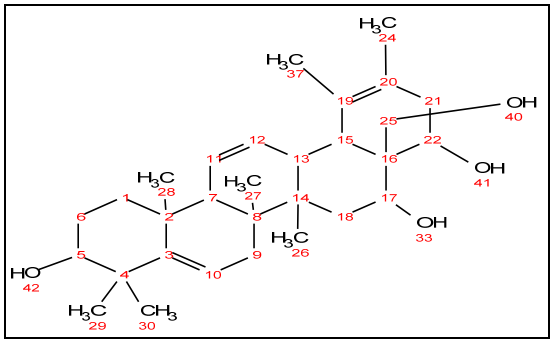
The compound 3 was obtained as colourless crystalline compound. ^{13}C NMR (Fig 1.8) and DEPT 135 (Fig 1.9) showed 30 carbon atoms in the molecule consisting of seven methyl, six methylene, nine methane, eight quaternary groups. The proton and carbon chemical shifts are characteristic spectral features of triterpenoids (Bross-Walch *et al*., 2005) [4].

Table 1. 1: ^{13}C NMR Data of Compounds 1-3 (400 MHz)

	^{13}C NMR Data of Compound 1			**^{13}C NMR Data of Compound 2**			**^{13}C NMR Data of Compound 3**		
Carbon atoms	**Experimental δc(ppm)**	**[1]Literature δc(ppm)**	**DEPT**	**Experimental δc(ppm)**	**[2]Literature δc(ppm)**	**DEPT**	**Experimental δc(ppm)**	**[3]Literature δc(ppm)**	**DEPT**
C1	38.79	38.51	CH_2	38.75	38.7	CH_2	38.79	38.80	CH_2
C2	27.24	27.43	CH_2	27.63	27.4	CH_2	27.24	27.30	CH_2
C3	79.06	79.20	CH	77.29	78.9	CH	79.06	79.00	CH
C4	39.62	39.02	C	38.97	38.8	C	39.62	38.80	C
C5	55.18	55.75	CH	55.39	55.3	CH	143.46*	55.40	CH
C6	18.38	18.31	CH_2	18.44	18.3	CH_2	115.27*	18.40	CH_2
C7	34.74	34.75	CH_2	34.41	34.3	CH_2	34.74	32.90	CH_2
C8	40.02	40.81	C	40.03	40.7	C	40.02	39.40	C
C9	47.72	51.31	C	50.43	50.5	CH	47.72	47.80	C
C10	38.60	37.31	C	37.22	37.2	C	38.60	37.20	C
C11	22.60	22.20	CH_2	19.43	20.8	CH_2	122.31*	23.40	CH_2
C12	121.73	123.88	CH	25.58	25.5	CH_2	129.39*	123.88	CH
C13	139.30	141.50	CH	38.09	38.4	CH	139.30	141.50	CH
C14	42.09	42.84	C	42.49	42.4	C	42.09	42.84	C
C15	29.71	30.85	CH_2	30.51	30.5	CH_2	29.71	30.85	CH_2
C16	39.62	39.01	CH_2	32.21	32.1	CH_2	39.62	39.01	CH_2
C17	34.74	35.65	C	55.91	56.3	C	34.74	35.65	C
C18	145.20	142.25	C	47.09	46.8	CH	145.20	142.25	C
C19	124.42	129.76	CH	49.06	49.2	CH	124.42	129.76	CH
C20	32.95	32.92	C	150.82	150.3	C	32.95	32.92	C
C21	33.35	33.22	CH_2	29.68	29.7	CH_2	33.35	33.22	CH_2
C22	37.15	37.52	CH_2	38.76	37.0	CH_2	37.15	37.52	CH_2
C23	29.37	28.81	CH_3	27.53	27.9	CH_3	29.37	28.81	CH_3
C24	21.41	20.81	CH_3	15.22	15.3	CH_3	21.41	20.81	CH_3
C25	15.63	15.62	CH_3	16.41	16.0	CH_3	15.63	15.62	CH_3
C26	16.81	16.40	CH_3	16.44	16.1	CH_3	16.81	16.40	CH_3
C27	23.70	23.73	CH_3	14.86	14.7	CH_3	23.70	23.73	CH_3
C28	26.00	24.12	CH_3	177.29	180.5	C	26.00	24.12	CH_3
C29	30.09	30.71	CH_3	110.06	109.6	CH_2	30.09	30.71	CH_3
C30	28.77	28.71	CH_3	19.43	19.4	CH_3	28.77	28.71	CH_3

[1&3](Mahato and Kundu, 1994; Gonzalez *et al.,* 1981) [15, 9] and [2] (Okwute, and Isyaka, 2014 and Habila *et al*., 2013) [10, 9].

The ^{1}H NMR (400 MHz, DMSO) spectrum showed the presence of four oxymethine proton peaked at δ 3.24 (1H (s)), 3.39 (1H (t)), 4.29 (1H (t)), 4.30 (1H (t)), three olefinic protons at δ 5.20 (1H (t)), 5.30 (1H (t)), 6.50 (1H (t)) and 5.80 (1H (t)).The signal at δ 1.20 - 0.37 are likely due to the presence of overlapping methine and methylene and methyl.
The ^{13}C NMR data (400 MHz, DMSO) spectrum showed the presence of six olefinic carbons atoms at *δ* 143.46, 130.38, 129.39, 122.31, 115.27 and 101.00. The oxymethine carbons peaked at *δ* 77.21, 76.11, 66.04 and 61.50. The signals at *δ* 60.38, 46.53, 36.25, 33.24, 25.14 and 32.81 are methylene groups. The signals from δ 46.48, 46.54 and 35.48 are due to methine groups, while the CH_3 methyl groups are indicated by the following signals δ 25.14, 23.94, 23.40, 18.24, 16.81, 15.29 and 14.12. The peaks at 48.86, 36.26, 43.81 and 48.88 are quaternary carbons (Fig 1.8).
The sp^2 hybridized protons at δ 5.20, 5.30, 6.50 and 5.80 ppm show direct HSQC correlation to the same carbon at *δ* 122.31, 129.39, 115.27 and 101.00 respectively; this implies that the two protons at δ 5.20 and 5.30 are in the same chemical environment. The oxymethine protons at δ 3.18, 3.39, 4.00 and 4.18, showed direct correlation to the signal at *δ* 77.21, 76.11, 66.04 and 61.50.
Thus, the structure of compound 3 was considered to be a 3β-hydroxyl Ursa-5, 11, 19-triene (uvaol) (3). This is consistent with those reported in literature (Table 1.1) (Mahato and Kundu, 1994) [15].



3β-hydroxyl Ursa-5, 11, 19-triene (uvaol)(3)

5. Conclusion

From the above findings, as 3β-hydroxyl olean-12,18-diene (β-amyrin), 3β-hydroxyllup-20(29)-en-28-oic acid (Betulinic acid) and 3β-hydroxyl ursa-5,11,19-triene (uvaol) were isolated from extract of aerial parts of *Canarium schweinfurthii* (ENGL) (BURSERACEAE). The compounds are reported for the first time from this plant.

6. Acknowledgment

I wish to appreciate the Department of Environmental Water and Earth Sciences, Faculty of Science, Tshwane University of Technology, Pretoria, South Africa and the School of Chemistry University of Kwazulu-Natal Westville campus, Durban, South African, for the use of their nuclear magnetic resonance machine for spectra analyse

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