ISSN: 1858-7658 (Online)
ISSN: 1858-7690 (Print)

Isolation and Characterization of Two Ursane-Skeleton Triterpenoids from Eucalyptus grandis (Myrtaceae)

Ibrahim T. Babalola1 & Esther A. Adelakun

1. Department of Chemistry, Faculty of Sciences, Yobe State University, Damaturu, Nigeria (chemibrabal@gmail.com)
2. Department of Chemistry, Faculty of Natural Sciences, University of Jos, Jos Nigeria (debola2000@yahoo.com)

Abstract

Several naturally occurring ursane or oleane-skeleton pentacyclic triterpenoids occur in a free form as aglycones or in combined forms in medicinal plants. Purification of ethylacetate soluble extract of Eucalyptus grandis leaves through combination of chromatographic techniques led to isolation of two pure compounds. The two isolates were characterized using UV, IR,1H, 13C, DEPT spectroscopic experiments, and in comparison with literature identified as 3β-hydroxyl-urs-12-en-3-ol (α-Amyrin) and as 3β, 28-dihydroxyl-12-ursen (Uvaol) respectively. Triterpenes have been reported in Eucalyptus species, but this is the first report on isolation and characterization of Amyrin and Uvaol in Eucalyptus grandis.

Keywords: Pentacyclic triterpenes, amyrin, uvaol, pharmacological activity.

Introduction

Eucalyptus grandis is a tree belonging to the family Myrtaceae. It is native to the east coast of Australia, and commonly known as Rose gum or Flooded gum. The glossy dark green leaves are stalked, lanceolate, and paler on their undersides, 10 to 16 cm (4-6.4 in) long and 2-3 cm (0.8-1.2 in) wide. They are arranged alternately along the branches. The white flowers appear from mid autumn to late

ISSN: 1858-7658 (Online)
ISSN: 1858-7690 (Print)

winter from April to August, and are arranged in groups of seven to eleven flower heads. The flowers are followed by small pear-or cone-shaped gum nuts which measure 5-8 mm in length and 4-7 mm across [1, 2&3]. Massive planting programmes have been done in South Africa and Brazil [4].

Traditional healers in West Africa use Eucalyptus to treat many illnesses such as infections, colds, flu, sore throats, bronchitis, pneumonia, aching, stiffness, neuralgia and as an antibiotic [5], essential oil extracted from the leaves of Eucalyptus grandis are reported to have antiseptic and disinfectant properties, decoction of the leaves are used in folkloric treatment of flu, bronchitis, pneumonia and respiratory infections [6, 7 & 8]. In addition, its use as an antifungal agent for some skin infections has been reported [9]. The essential oils of Eucalyptus globules and Eucalyptus citriodora, which have 70% of their constituents to be 1,8 cineol (Eucalyptol) have also been reported to stimulate respiration, relieve coughing, helps to expel mucus, relax the respiratory muscles, thus it is used for the management of bronchitis,

ISSN: 1858-7658 (Online)
ISSN: 1858-7690 (Print)

asthma, catarrh, sinusitis and throat infections [10].

In our continous search for active constituents from medicinal plants with therapeutic activity against tuberculosis and other respiratory diseases, this paper reports on the phytochemicals from Eucalyptus grandis (Myrtaceae).

2.0 MATERIAL AND METHODS

2.1 General Experimental Procedure

Melting points of the isolated compounds were determined using Fischer-Johns hot stage melting point apparatus (uncorrected). The 1H and 13C NMR as well as DEPT were carried out using Bruker- Avance 400MHz using residual solvent (CDCl3) peak as internal standard. The Infrared spectra were run on Perkin Elmer Spectrum 100 FTIR Spectrometer. All weight measurements were taken using a Precisa 3100C top loading balance. Column Chromatography was carried out on using silica gel (Merck 7734 , Darmstadt, Germany).Thin-layer chromatography (TLC) was carried out on silica gel precoated plastic plates (Merck 5554) followed by Vanillin-H2SO4

ISSN: 1858-7658 (Online)
ISSN: 1858-7690 (Print)

spray with heating. All commercially available reagents were used without further purification unless otherwise stated.

2.2 Plant Material

The leaves of Eucalyptus grandis were collected in June 2012, at a paper Mill plantation around Empangeni area of Kwazulu – Natal, Republic of South Africa. The plant was identified by Mrs. N.R. Ntuli, of Botany Department, University of Zululand and voucher specimen was prepared and deposited.

2.3 Extraction

500.0 g of dried pulverized leaves of Eucalyptus grandis (collected at a paper plantation Empangeni, Kwazulu Nata Province) was packed in 12L flat bottom flask, soaked in 4L of methanol. The flask was placed on Lab com shaker for 96 hours. The extract was concentrated in vacuo using rotavap to afford 29.8 g (5.96 %). The concentrated extract was transferred into 2L separatory funnel and shaken gently but repeatedly with 500ml portions of hexane and then ethylacetate.

2.4 Fractionation & Isolation of IBF04 & IBF010

10.0g of the ethylacetate

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soluble extract was loaded dry on silica gel eluted with gradient elution of haxane-ethylacetate (5% stepwise increase). 208 fractions were generated, the fractions were monitored with TLC plates, like fractions were pooled together to afford 11 main fractions (GA-GK). A pure compound coded IBF04 was isolated from the fraction GF (fractions 81-82), as a dull-white powder (6 mg). Further purification of fraction GK (350 mg) of the sample (light greenish-yellow solid) obtained from combined fractions 130 – 212 was carried out. The sample was loaded on Silica gel (normal phase) and eluted with gradient solvent system (100%hexane - 75% hexane-EtoAc), 60 fractions were collected and analysed on TLC plates, like fractions were pooled together to afford five main fractions G (KA-KE). Fraction KD gave an intense single purple spot on chromatoplate (Plate 31) which was coded IBF010 (yellow powder; 6.0 mg, Rf value: 0.48). Sample of the compounds were subjected to spectral analysis.

2.5 Spectra Data on IBF 04

Compound IBF 010 was isolated from the ethyl acetate

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soluble extract of E. grandis as a yellow powder with melting point of 184-186oC. UV (MeOH), λmax m: 205, IR (CHCl3)Vmax cm-1: 3409.9(O-H), 2922.7cm-1. 2845.56cm-1 (C-H stretch in CH3 and CH2), 1701.48 cm-1 (C=C stretch), 1445.5 (C-H def. in CH3), 1374.5 cm-1 (C-H), 1050.7 cm-1C-O stretch of secondary alcohol), 729.0 cm-1 (=C-H out of plane bending). 1H NMR δ (CDCl3) : 5.20 (1H, d, J= 6.5, H-12), 3.52 (1H, d, J= 11.2Hz, H-28a), 3.48 (1H, d, J=11.2Hz, H-28b), 3.20 (1H, dd, J= 10.7, 5.5Hz, H-3), 2.15 (1H, d, J= 11.5, H-18), 1.08 (3H, s, H-27), 0.96 (3H, s, H-24), 0.92 (3H, s, H-26), 0.95 (3H, s, H-25), 0.91 (3H, d, J= 6.8Hz, H-30), 0.86 (3H, d, J= 5.5Hz, H-29), 0.77 (3H, s, H-23), 13C-NMR (CDCl3, 125 MHz): Table 1.

2.6 Spectra Data on IBF010

Compound IBF010 was isolated from the crude methanol extract of Eucarlyptus grandis as an orange powdery compound with melting point of 238-240oC. Melting point: 238-240 0C , UV (MeOH), λmax nm: 205, IR (CHCl3) Vmax cm-1:3460(O-H), 2920-2810(C-H), 1630(C=C), 1200(1050(C-O). HREIMS m/z (formula, calculated

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value):442.3801(C30H50O2). 1H NMR δ (CDCl3) : 5.20 (1H, d, J= 6.5, H-12), 3.52 (1H, d, J= 11.2Hz, H-28a), 3.48 (1H, d, J=11.2Hz, H-28b), 3.20 (1H, dd, J= 10.7, 5.5Hz, H-3), 2.15 (1H, d, J= 11.5, H-18), 1.08 (3H, s, H-27), 0.96 (3H, s, H-24), 0.92 (3H, s, H-26), 0.95 (3H, s, H-25), 0.91 (3H, d, J= 6.8Hz, H-30), 0.86 (3H, d, J= 5.5Hz, H-29), 0.77 (3H, s, H-23). 13C-NMR (CDCl3, 125 MHz): Table 2.

3.0 RESULTS AND DISCUSSION

The IR spectrum of the compound IBF04 showed intense absorption bands at 1701, 144.5-1374.5 cm-1 along with broad absorptions in the region around 3409, cm-1 , indicating the presence of olefinic and a hydroxyl functions in the molecule. The presence of end absorption 205nm in the UV spectrum suggested the presence of a unsaturation in the structure. A total of thirty signals were observed in the 13C NMR comprising of six quaternary carbons, seven methines, nine methylenes and eight methyls with the aid of DEPT experiments. The most downfield signals were observed at δ139.3, 123.0 and 78.6 ascribable to C-13,

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C-12 and C-3 respectively (Table 1). The 1H and 13C-chemical shifts of C-12 and C-13 are evidence for the presence of a double bond. The presence of eight methyls at δ16.2 (C-25),), 17.3 (C-26), 23.4 (C-24), 23.4 (C-23), 26.0(C-27), 27.0 (C-28) and two secondary methyls in the ring E, δ21.2(C-29), 21.3 (C-30), suggests an α- amyrin skeleton. The significant features of both the 1H and 13C NMR of the compound were the appearance of two proton doublet ah δ3.52 (1H, d, J = 11.2Hz, H-28a), 3.48 (1H, d, J = 11.2Hz, H-28b) and one methyl at δ 28.0 assigned to C-28.

The IR spectrum of the compound IBF 010 showed intense absorption bands at 1630, 1200-1050 cm-1 along with broad absorptions in the region between 3460, cm-1 , indicating the presence of olefinic and a hydroxyl function in the molecule. The 1H NMR spectrum of the compound displayed a one proton olefinic triplet at δ 5.12 (J= 3.5Hz) indicating the presence of unsaturation in the compound. Five tertiary methyl singlets at δ1.08 (H-27), 0.96 (H-24), 0.92 (H-26), 0.95 (H-25), 0.77 (H-23),

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and two secondary methyl doublets at δ0.91 (d, J = 6.8Hz, H-30) and 0.86 (d, J =5.5Hz, H-29) were observed which further suggested α -type triterpene.

On the basis of literature data, downfield signal that ascribable to C-3 at δ3.20 (1H, m, H-3) must be present. A doublet displayed at δ2.15 (1H, J= 11.2 Hz) was assigned to H -18, indicating that proton at C-18 is in trans position to proton at C-19, two multiplets at δ1.30 and 1.54 of a proton each were assigned to H-19 and H-20 in conformity with literature data of α- amyrin [12,13]. A total of thirty signals were observed in the 13C NMR comprising of six quaternary carbons, seven methines, ten methylenes and seven methyls with the aid of DEPT experiments. The most downfield signals were observed at δ138.7, 125.0 and 79.0 ascribable to C-13, C-12 and C-3 respectively (Table 2). The 1H and 13C-chemical shifts of these carbons(C-12 and C-13) strongly supported the presence of a double bond. The presence of five quaternary methyls at δ15.7 (C-25), 16.8 (C-26), 15.6 (C-24), 23.3 (C-27), 28.1 (C-23) and two secondary methyls in the ring E, δ17.4 (C-

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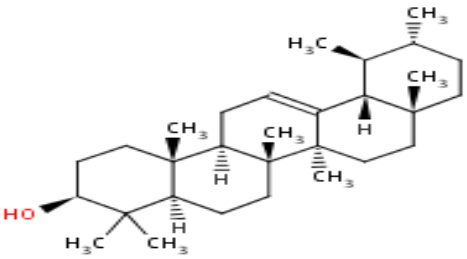
29), 21.3 (C-30), confirmed the α-amyrin type (ursane triterperne). The significant features of both the 1H and 13C NMR of the compound were the appearance of two proton doublet at δ3.52 (1H, d, J = 11.2Hz, H-28a), 3.48 (1H, d, J = 11.2Hz, H-28b) and one methylene at δ 69.9 assigned to C-28.



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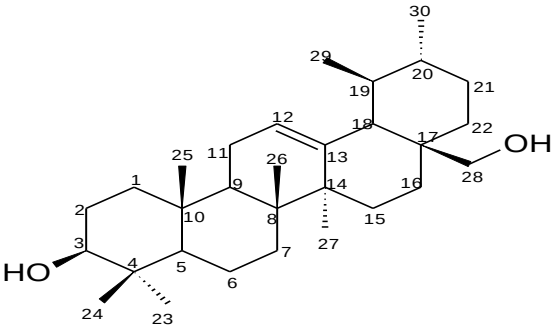

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4.0 CONCLUSION

The natural pentacyclic triterpenoid compounds, ursolic or oleanolic acid and several closely related derivatives, have exhibited biological and pharmacological properties, such as anti-HIV, hepato protective, anti-inflammatory, cytotoxicity or antimicrobial activities [14]. On the basis of the spectral data

ISSN: 1858-7658 (Online)
ISSN: 1858-7690 (Print)

discussed above and in comparison with estimated using Chem draw software and literature values, compound IBF04 was identified as 3β-hydroxyl-urs-12-en-3-ol, α-Amyrin [14]. Similarly, on the basis of the spectral data discussed and by comparative analysis with literature, compound IBF010 was identified as 3β, 28-dihydroxyl-12-ursen (Uvaol) [15, 11 &16]. IBF04 identified as α-amyrin is a pentacyclic triterpenoid synthetized by a number of plants and has been reported [17, 18 &19]. This compound is usually found in plant extracts together with its isomer β-amyrin, which differ in the placement of one methyl group in C-19 and C-20 respectively. There were no previous reports on the isolation of these compounds in E. grandis. It has been demonstrated that a mixture of both isomers produces pronounced antinociception in the capsaicin test (decreased face rubbing behaviour in rats) [20]. The analgesic effect of amyrin was observed only in the case of second phase reaction, corresponding to inflammatory pain rather than to acute neurogenic pain[19]. On the other

ISSN: 1858-7658 (Online)
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hand, the anti-inflammatory activity of these compounds measured in carrageenan-induced mouse paw edema test seemed to be very low[17]. Amyrin esters were also reported to exhibit significant biological activity[20,21]. Alpha amyrin has also been reported to induce proliferation rate of human keratinocytes (HaCaT) by up to 18% [22]. IBF010 identified as Uvaol, is equally a triterpene alcohol found in a number of medicinal plants, which usually occur with its isomer, erythrodiol (an intermediate compound enroute to oleanolic acid). The biological and pharmacological activity of this compound is barely reported. Olive oil obtained from the flowers and fruits of the plant Olea europaea has been reported to posses' therapeutic properties and have been used traditionally for medicinal purposes[23,24]. The major components of the oil are triterpene alcohols and acids, namely uvaol, erythrodio, oleanolic acid and maslinic acid. Uvaol and Erythrodiol were reported to have effectively affect cell proliferation, as well as cell phases and induced 1321N1 cell death. The two triterpene alcohols

successfully modulated the apoptotic response, promoting nuclear condensation and fragmentation. The potential of uvaol and erythrodiol to prevent and effectively treat brain tumours and other cancers were established [25]. The anti-mycobacterial activity of uvaol (isolated from Aspidosperma quebracho-blanco) exhibited MIC of 14µg/ml against M. tuberculosis H37Rv[26].

Table 1: 13C NMR data for compound IBF010 (Chemical Shifts, δ, in ppm)

Position	DEPT	δC (ppm)	δC (Lit.,ppm)	δC (Lit.,ppm)
1	CH2	38.85	38.7	38.5
2	CH2	28.00	28.7	27.4
3	CH	78.94	79.6	78.6
4	C	38.68	38.7	38.8
5	CH	55.19	55.1	55.4
6	CH2	18.23	18.4	18.6
7	CH2	33.00	32.2	33.1
8	C	39.06	40.7	39.9
9	CH	47.7	47.7	47.7
10	C	36.76	36.6	37.9
11	CH2	23.25	23.3	22.8
12	CH	125.53	124.4	123.0
13	C	138.13	139.5	139.3
14	C	42.04	42.0	42.7
15	CH2	26.99	27.2	26.3
16	CH2	26.90	26.6	27.2
17	C	33.00	33.7	32.6

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18	CH	55.19	59.0	59.5
19	CH	39.06	39.6	39.3
20	CH	39.45	39.6	39.2
21	CH2	30.65	31.2	29.0
22	CH2	39.44	41.5	40.4
23	CH3	28.00	28.1	23.4
24	CH3	15.41	15.6	23.4
25	CH3	15.57	15.6	16.2
26	CH3	16.89	16.8	17.3
27	CH3	23.25	23.2	26.0
28	CH3	28.00	28.1	27.0
29	CH3	18.28	17.4	17.0
30	CH3	21.14	21.4	21.2

Table 2: 13C NMR (100.6 MHz) data for compound IBF04 (Chemical shifts, δ, in ppm)

Position	DEPT	δC	δC (Lit.)	C.	DEPT	δC	δC (Lit.)
1	CH2	38.8	38.8	16	CH2	22.3	22.8
2	CH2	27.2	27.3	17	C	36.9	36.8
3	CH	79.0	79.0	18	CH	54.0	54.1
4	C	38.8	38.8	19	CH	39.3	38.9
5	CH	55.2	55.4	20	CH	39.4	39.4
6	CH2	18.3	18.4	21	CH2	30.6	30.7
7	CH2	32.8	32.9	22	CH2	30.6	30.6
8	C	40.0	39.4	23	CH3	28.1	28.1
9	CH	47.6	47.8	24	CH3	15.6	15.4
10	C	36.8	37.2	25	CH3	15.7	15.6
11	CH2	23.4	23.4	26	CH3	16.8	16.9
12	CH	124.01	125.0	27	CH3	23.3	23.4
13	C	138.7	138.0	28	CH2	76.7	69.7
14	C	42.0	42.8	29	CH3	17.4	16.2
15	CH2	29.7	29.2	30	CH3	21.3	21.3

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