TERPENOID COMPOUNDS FROM THE LATEX OF *EUPHORBIA DRUPIFERA*

Famuyiwa, S. O.[a]*, Oladele, A. T.[b], Adeloye, A. O.[c] and Fakunle, C. O.[d]

[a]Department of Chemistry, Obafemi Awolowo University Ile-Ife, Nigeria.
[b]Department of Forestry and Wildlife Management, University of Port Harcourt, Nigeria.
[c]Department of Chemistry, College of Science, Engineering & Tech., Univ. of South Africa.
[d]Department of Chemistry, Joseph Ayo Babalola University, Arakeji, Nigeria.
Corresponding Author: E-mail: oluwaseyi_f@yahoo.com
(Received: 16th Sept., 2013; Accepted: 11th Dec., 2013)

ABSTRACT

This study aimed at the isolation and characterization of terpenoid compounds from the latex of *Euphorbia drupifera*. Methylated spirit extract of the latex was suspended in aqueous methanol and partitioned with petroleum spirit. The aqueous methanol residue was washed with ether. The ethereal fraction was subjected to column and preparative thin layer chromatography for isolation and purification of the compounds. The ethereal fraction of the latex gave two terpenoid compounds. Their structures were determined by 1D-NMR and MS as euphan-8, 24-diene-3-ol (**1**) and 12-deoxyphorbol-20-propanoate (**2**). These compounds could be responsible for the biological properties of the plant.

Keywords: *Euphorbia drupifera*, Euphorbiaceae, Latex, Chromatography, Terpenoids.

INTRODUCTION

Plants are inexhaustible repository of organic molecules most of which possess biological activities of great importance to mankind (Famuyiwa *et al*., 2012). Plants from the family, Euphorbiaceae, are highly reputed for their medicinal properties in the indigenous system of medicine (Abdul *et al*., 1988; Fakunle *et al*., 1989 and Fakunle *et al*., 1992). The medicinal properties of the plants from the genus Euphorbiaceae have been attributed to the presence of diterpenoid compounds (Fakunle *et al*., 1993) and triterpenoid compounds (Lin *et al*., 2000). However, very little information are available in the literature on the medicinal uses of the species *Euphorbia drupifera* Thonn., although it is listed among the plants with medicinal property (Ampofo, 1977). Ingenol and lectins were reported from the latex (Kinghorn and Evans, 1974; Abo, 1990; Lynn and Radford, 1986) and elaeophorbate, a triterpenoid, from the leaves of *Euphorbia drupifera* (Ahiahonu and Goodenowe, 2007).

The fruit is succulent (Kinghorn and Evans, 1974). The latex was reported to have skin irritation effect (Kinghorn and Evans, 1975) and promotes inflammation (Abo, 1994). The leaves are used as filaricide and for the treatment of guinea worm infestation (Comley, 1990). It was reported to contain hypoglycaemic agents (Eno and Itam, 1996) and stimulated autonomic cholinoceptors in the rat uterus (Eno and Itam, 1997). The hexane extract has been found to moderately inhibit HIV-1 and HIV-2 proviral and DNA copying (Ayisi and Nyadedzor, 2003).

The latex and the leaves are used by traditional herbalists in Southern Nigeria for the treatment of hypertension, diabetes, ringworm, tuberculosis, bullet wound and barrenness in women (Eno et al., 2004). In our effort to isolate some of the compounds responsible for these medicinal properties, this paper reports the isolation and characterization of two triterpenoids, euphan-8,24-diene-3-ol (1) and 12-deoxyphorbol-20-propanoate (**2**) from the freshly collected latex of the plant.

MATERIALS AND METHODS

Plant Material

The latex of *Euphorbia drupifera*, 250 ml, was obtained from a standing plant in Ipe/Oka akoko area of Ondo State in Nigeria, by tapping for a period of four months from February to May, 2002. Each time the latex was tapped, it was taken up in distilled methylated spirit and kept in the freezer to prevent deterioration. The plant was authenticated by Mr. Daramola by comparing it

with IFE Herbarium voucher 2588 collected and identified by I. B. Faremi at the Herbarium of the Department of Botany, Obafemi Awolowo University, Ile-Ife.

Extraction and Isolation

A 250 ml of the latex was stirred in the cold between 0 – 5 ºC (Ice chamber) in 500 ml distilled methylated spirit and allowed to stand for 5 minutes. The supernatant layer was decanted and this process was repeated until the extraction was considered completed. The combined methylated spirit extract was evaporated at 35 – 40 °C using rotator evaporator and the residue was taken up in 100 ml of methanol/water (9:1). The aqueous methanol solution was extracted with petroleum spirit (4x100 ml) and was evaporated at 35 – 40 ºC using rotator evaporator. The residue obtained was extracted with ether (4x100 ml). The ether solution was washed with water (2x50 ml), dried over anhydrous sodium sulphate (Na_2SO_4) and filtered. Removal of the ether at 15 °C using rotator evaporator gave a gum (9.1 g).

The extract was dissolved in dichloromethane and the solution was adsorbed onto silica gel (9.0 g) followed by swirling in a water bath until dry and very powdery. The adsorbed silica gel was subjected to column chromatography using accelerated gradient elution of increasing polarity from petroleum spirit through dichloromethane to methanol. The fractions were analyzed on TLC plates using n-hexane/chloroform (3:1) as solvent system. An impure solid (1.0 g) was collected from the fraction eluted with 20% dichloromethane in petroleum spirit. Preparative TLC of the impure solid using n-hexane/chloroform (3.5:0.5) as solvent system was carried out to isolate the prominent component which gave compound **1** (900 mg). At 80% dichloromethane in petroleum spirit gradient, another impure constituent (368 mg) was eluted. There was a UV-active spot in the TLC profile of the constituent which was purified by preparative chromatography to give compound **2** (12.5 mg).

RESULTS AND DISCUSSION

Compound **1** (900 mg) was a white crystalline solid, melting point 126 – 128 ºC. The ^{13}C-NMR ($CDCl_3$, 50 MHz) showed four olefinic carbon atoms at δc 134.2, 133.7, 131.1 and 125.4. One singly bonded oxygen bearing carbon atom at δc 79.2. The ^{1}H-NMR ($CDCl_3$, 200 MHz) showed five tertiary methyl groups at δ_H 0.75 (s), 0.78 (s), 0.89 (s), 0.93 (s) and 0.99 (s). One secondary methyl group at δ_H 0.84 (d, J = 4.0 Hz) and two vinyl methyl groups at δ_H 1.59 (s) and 1.65 (s). The spectrum also showed one vinyl proton at δ_H 5.08 (t, J = 2.1 Hz) and a proton at δ_H 3.24 (dd, 3.5 Hz and 9.3 Hz). The IR spectrum of the compound (Nujol) showed absorption bands at 3429.8 cm^{-1} (O-H str.), 2925 cm^{-1} (C-H str.) and 1646.4 cm^{-1} (C=C str.). The HR-EIMS showed a molecular ion peak at m/z 426.3814 (calc. 426.3862), consistent with the molecular formula $C_{30}H_{50}O$, which was supported by the analyses of ^{13}C- (Table 1) and DEPT-135 NMR spectra.

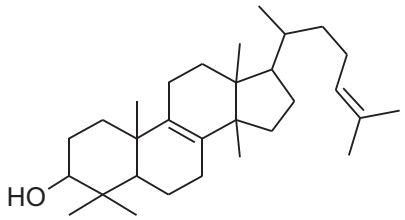

Fig. 1: Structure of Eupha-8,24-diene-3-ol, a Tirucallol.

The ^{1}H-NMR and ^{13}C-NMR data of compound **1** were compared with the ^{1}H-NMR and ^{13}C-NMR data of eupha-8,24-diene-3-ol, a tirucallol (Abdul *et al.,* 1988), and the data agreed well with that of tirucallol (Tables 1 and 2). Thus, compound **1** was identified as eupha-8,24-diene-3-ol.

Table 1: The Comparison of ^{1}H-NMR Data Between Eupha-8,24-diene-3-ol and **1**

Euphan-8,24-diene-3-ol		Compound **1**	
H-atom	ppm (multiplicity)	H-atom	ppm (multiplicity)
H-3	3.24 (dd, J = 3.5 & 9.3 Hz)	H-3	3.22 (dd, J = 3.5 & 9.3 Hz)
3H-18	0.99 (s)	3H-18	0.98 (s)
3H-19	0.93 (s)	3H-19	0.93 (s)
3H-21	0.84 (d, J = 4.0 Hz)	3H-21	0.84 (d, J = 4.0 Hz)
H-24	5.07 (t, 2.1 Hz)	H-24	5.08 (t, 2.1 Hz)
3H-26	1.66 (s)	3H-26	1.65 (s)
3H-27	1.58 (s)	3H-27	1.59 (s)
3H-28	0.86 (s)	3H-28	0.89 (s)
3H-29	0.78 (s)	3H-29	0.78 (s)
3H-30	0.73 (s)	3H-30	0.75 (s)

Table 2: The Comparison of ^{13}C-NMR Data Between Euphan-8,24-diene-3-ol and **1**

Euphan-8,24-diene-3-ol				Compound **1**			
C-atom	ppm	C-atom	ppm	C-atom	ppm	C-atom	ppm
1.	35.2	16.	29.7	1.	35.5	16.	30.0
2.	27.7	17.	49.6	2.	27.9	17.	49.8
3.	79.0	18.	15.5	3.	79.2	18.	15.8
4.	38.9	19.	20.1	4.	39.2	19.	20.4
5.	50.9	20.	35.9	5.	51.2	20.	36.1
6.	24.7	21.	18.9	6.	25.0	21.	19.2
7.	27.9	22.	35.4	7.	28.1	22.	35.6
8.	133.5	23.	24.7	8.	133.7	23.	25.1
9.	134.0	24.	125.2	9.	134.2	24.	125.4
10.	37.3	25.	130.9	10.	37.5	25.	131.1
11.	21.5	26.	17.7	11.	21.8	26.	18.0
12.	28.1	27.	25.7	12.	28.4	27.	26.0
13.	44.1	28.	24.5	13.	44.3	28.	24.7
14.	50.0	29.	28.0	14.	50.2	29.	28.3
15.	30.9	30.	15.6	15.	31.1	30.	15.8

Compound **2** (12.5 mg) was obtained as a white non-crystalline solid. The UV spectrum showed λ_{max} at 238 nm and this was due to α, β-unsaturated carbonyl of phorbol nucleus as reported by Evans and Schmidt, 1976. The IR spectrum (Nujol) showed absorption bands at 3466.3 cm^{-1} (O-H str.), 2961.7 cm^{-1} (C-H str.), 1709.1 cm^{-1} (C=O str.) and 1690.0 cm^{-1} (C=O str. for conjugated carbonyl), 1617.9 cm^{-1} (C=C str.), 1429.6 cm^{-1} and 1368.8 cm^{-1} (C-H bending for methyl, methylene or methine group), 1228.3 cm^{-1} (C-O-C). The ^{1}H-NMR ($CDCl_3$, 200 MHz) showed signals at δ_H 3.05 (q) and 1.30 (t) for methylene and methyl protons of propanoate respectively. Other signals showed at δ_H 6.18 (m) H-1, 5.57 (d, J = 6.0 Hz) H-7, 4.15 (s) 2H-20, 3.85 (m) H-8 and H-10, 2.50 (br.s) 2H-5, 2.08-2.30 (m) 2H-12, 1.95 (t, J = 9.5 Hz) H-11, 1.82 (m) 3H-19, 1.02 (s) 3H-16 and 3H-17, and 0.86 (d, J = 9.0 Hz) 3H-18 and H-14. The ^{13}C-NMR ($CDCl_3$, 50 MHz) of compound **2**, Table 4. The HR-EIMS showed molecular ion peak at m/z 404.2199 (calc. 404.2355), consistent with the molecular formula $C_{23}H_{32}O_6$, which was supported by the analyses of ^{13}C- and DEPT-135 NMR spectra.

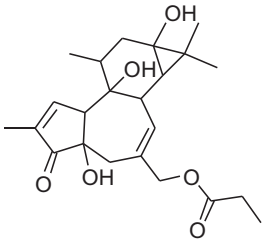

Fig.2: Proposed Structure for Compound 2

The ^{1}H-NMR and ^{13}C-NMR data of the proposed structure for compound **2** were compared with the ^{1}H-NMR data of 12-deoxyphorbol-13-phenyl acetate (Evans and Schmidt, 1976) and ^{13}C-NMR data of 4,20-dideoxy-5-hydroxyphorbol ester (Fakunle et al., 1993) as shown in Tables 3 and 4 respectively. The structure 12-deoxyphorbol-20-propanoate was proposed for compound **2** by this comparison.

Table 3: The Comparison of ^{1}H-NMR Data Between 12-deoxyphorbol-13-phenyl Acetate and **2**

12-deoxyphorbol-13-phenyl Acetate		Compound **2**	
H-atom	ppm (multiplicity)	H-atom	ppm (multiplicity)
H-1	7.60 (m)	H-1	6.18 (m)
2H-5	2.50 (br.s)	2H-5	2.50 (br.s)
H-7	5.62 (d, J = 6.0 Hz)	H-7	5.57 (d, J = 6.0 Hz)
H-8 & H-10	3.0 & 3.22 (m)	H-8 & H-10	3.85 (m)
H-11	-	H-11	1.95 (t, J = 9.5 Hz)
2H-12	1.9-2.18 (m)	2H-12	2.08-2.30 (m)
H-14 & 3H-18	0.86 (d, J = 9.0 Hz)	H-14 & 3H-18	0.86 (d, J = 9.0 Hz)
3H-16 & 3H-17	1.03 (br.s)	3H-16 & 3H-17	1.02 (s)
3H-19	1.76 (m)	3H-19	1.82 (m)
2H-20	3.97	2H-20	4.15 (s)

Table 4: The Comparison of ^{13}C-NMR Data Between 4,20-dideoxy-5-hydroxyphorbol Ester and **2**

4,20-dideoxy-5-hydroxyphorbol Ester				Compound **2**			
C-atom	ppm	C-atom	ppm	C-atom	ppm	C-atom	ppm
1.	154.6	13.	65.2	1.	146.9	13.	67.5
2.	138.3	14.	36.9	2.	139.6	14.	45.4
3.	207.4	15.	26.3	3.	207.2	15.	33.3
4.	56.0	16.	27.3	4.	77.9	16.	29.9
5.	70.9	17.	17.1	5.	43.7	17.	29.9
6.	140.8	18.	15.4	6.	136.2	18.	22.4
7.	127.2	19.	10.1	7.	127.7	19.	24.2
8.	42.4	20.	21.7	8.	35.3	20.	76.6
9.	78.4	1'.	179.3	9.	85.1	1'	168.2
10.	51.5	2'.	34.2	10.	67.5	2'	27.5
11.	43.2	3' & 4'.	18.6	11.	31.4	3'	8.9
12.	77.9			12.	38.6		

CONCLUSION

Compound 1, euphan-8,24-diene-3-ol, a tirucallol was previously isolated from the seeds of *Camellia sinensis*, *Cucumis sativus*, *Cucumis melo* and latex of *Euphorbia tirucalli* and *Euphorbia kansui* but not from *Euphorbia drupifera*. It was reported to possess antiviral properties (Lin et al., 2000). It is noteworthy to report its isolation from this plant, *Euphorbia drupifera*.

Compound 2 identified as 12-deoxyphorbol-20-propanoate may be one of the compounds that are responsible for the biological activities of the latex of the plant.

ACKNOWLEDGEMENTS

The authors are grateful to Mr. Ademoriyo and Mr. Daramola of the IFE Herbarium, Department of Botany, Obafemi Awolowo University, Nigeria for botanical identification and collection of the plant. The authors are also grateful to Pharmacist (Mrs.) Famuyiwa F. Gladys

for proof reading the manuscript. SOF is grateful to Mr. Disang Mosimanethebe of the Department of Chemistry, University of Botswana for his assistance in the acquisition of the MS.

REFERENCES

Abdul Q. K., Rasheed T., Kazmi S. N. H., Ahmed Z. and Malik A. 1988. Cycloeuphordenol, a new triterpene from *Euphorbia tirucalli*. *Phytochemistry* 27 (7), 2279 – 2281.

Abo K. A. 1990. Isolation of ingenol from the lattices of Euphorbia and Elaeophorbia species. *Fitoterapia* 61 (5), 462 – 463.

Abo K. A. 1994. Characterization of ingenol: an inflammatory diterpene from some Nigeria Euphorbia and Elaeophorbia species. *African Journal of Medicine and Medical Science* 23 (2), 161 – 163.

Ahiahonu P. W. and Goodenowe D. B. 2007. Triterpenoids from leaves of *Elaeophorbia drupifera*. *Fitoterapia* 78 (5), 337 – 341.

Ampofo C. 1977. Plants that heal. *World Health Bulletin* 5, 26 – 30.

Ayisi N. K. and Nyadedzor C. 2003. Comparative in-vitro effects of AZT and extracts of *Ocimum gratissimum*, *Ficus palita*, *Clausena anisata*, *Alchornia cordifolia* and *Elaeophorbia drupifera* against HIV-1 and HIV-2 infections. *Antiviral Research* 58 (1), 25 – 33.

Comley J. C. W. 1990. New macrofilaricidal leads from plants. *Tropical Medicine and Parasitology* 41 (1), 1 – 9.

Eno A. E., Azah N., Edet E. E. and Itam E. H. 2004. Induced secretion of pepsin-rich gastric juice in the rat by the crude extract from *Elaeophorbia drupifera* leaves: A dual pathway mechanism. *Nigerian Journal of Physiological Sciences* 19 (1-2), 53 – 59.

Eno A. E. and Itam E. H. 1996. Hypoglycaemic agent(s) in leaves of *Elaophorbia drupifera*. *Phytotherapy Research* 10 (8), 680 – 682.

Eno A. E. and Itam E. H. 1997. Stimulation of autonomic Cholinoceptors in the isolated uterus by the crude extract from *Elaeophorbia drupifera* leaves: *Pharmaceutical Biology* 2, 97 – 102.

Evans F. J. and Schmidt R. J. 1976. Two new toxins from the latex of *Euphorbia poisonii*. *Phytochemistry* 15, 333 – 335.

Fakunle C. O., Connolly J. D. and Rycroft D. S. 1989. A novel penta-ester of 19-hydroxyingol from the latex of *Euphorbia poisonii*. *Fitoterapia* 60 (5), 466 – 467.

Fakunle C. O., Connolly J. D. and Rycroft D. S. 1992. Enukokurin B and C, two other jatrophane diterpenoid esters from the latex of *Euphorbia lateriflora*. *Fitoterapia* 63 (4), 329 – 332.

Fakunle C. O., Connolly J. D. and Rycroft D. S. 1993. A new 4,20-dideoxy-5-hydroxyphorbol ester from the latex of *Euphorbia lateriflora*. *Fitoterapia* 65 (1), 75 – 78.

Famuyiwa S. O., Sichilongo K. F., Yeboah S. O. and Abegaz B. M. 2012. Homoisoflavonoids from the inter-bulb surfaces of *Scilla nervosa* subsp. *rigidifolia*. *Phytochemistry Letters* 5, 591 – 595.

Kinghorn A. D. and Evans F. J. 1974. Occurrence of ingenol in Elaeophorbia species. *Planta Medica* 26, 150 – 154.

Kinghorn A. D. and Evans F. J. 1975. The succulent Euphorbias of Nigeria. *Lloydia* 38, 363 – 365.

Lin J. H., Ku Y. R., Lin Y. T., Teng S. F., Wen K. C. and Liao C. H. (2000): Preparative isolation and gas chromatography-mass spectrometry analysis of triterpenoids in *Kansui radix*. *Journal of Food and Drug Analysis* 8 (4), 278 – 282.

Lynn K. R. and Clevette-Radford N. A. 1986. Lectins from lattices of Euphorbia and Elaeophorbia species. *Phytochemistry* 25, 1553 – 1557.