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Triterpenoids From the Leaves of *Olax mannii* Oliv.

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ABSTRACT: The study of the acetone extract of the leaves of *Olax mannii* Oliv. led to the isolation of two triterpenoids; glutinol and rhoiptelenol. These compounds are reported for the first time in *Olax mannii*. Their structures were elucidated on the basis of one- and two-dimensional NMR spectroscopy, IR and GC-MS.
Keywords: Glutinol, Rhoiptelenol, *Olax mannii*

INTRODUCTION

Olax mannii Oliv. (Family: Olacaceae) is widely distributed in the tropics especially Nigeria, Sierra Leone, and Ghana. It is a shrub that grows up to 2 meters high. The leaves are lanceolate to ovate or elliptic up to 3×4cm with 5-6 pairs at lateral looped nerves. The flowers are greenish white in axillary racemes. The fruits are orange when ripe and about 1/2-3/4cm. The plants natural habitat is close forest (Dalziel, 1956; Hutchinson and Dalziel, 1966). The plant is called in Hausa "Tsada biri". Decoction of the leaves and roots of the plant is used for treatment of fever, yellow fever and snake bite (Burkill, 1997). The twigs are used as chewing sticks in Ghana (Irvine, 1961).

General phytochemical examination of the various crude extracts of the leaves, fruits and root bark of the plant showed the presence of coumarins, steroid/triterpenes, saponins, fatty acids and tannins in all parts of the plant; while volatile oils and flavonoids are present in the fruits and leaves. Alkaloids are absent in all parts of the plant (Sule *et al.*, 2005). The isolation of *(E)* -3-methyl-5-phenyl-2-pentenoic acid from the petroleum ether extract of the leaves has also been reported (Sule *et al.*, 2005). In this paper we report the isolation and structural elucidation of two triterpenoids from the leaves of *O. mannii*.

MATERIALS AND METHODS

Melting points were determined using Electrothermal IA 9300 apparatus and were uncorrected. IR spectroscopy (in KBr) was performed on a Perkin-Elmer Paragon 1000FT-IR spectrophotometer. ^{1}H (600MHz), J mod, ^{13}C-NMR (150.6MHz) and 2D NMR spectra were recorded on a Bruker AMX-400 spectrometer and chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as the internal standard, $CDCl_3$ and C_6D_6 as solvents. The coupling constants J(Hz) were also given. GC-MS (70eV) was performed with a Hewlett Packard HP 5890 gas chromatography that was equipped with a 25m fused silica capillary column with dimethylsiloxane CPsil 5CB coupled to a VG analytical 70-250S mass spectrometer. Thin-layer chromatography (TLC) was performed on precoated silica gel $60F_{254}$ and spots were visualized by spraying with vanillin/sulphuric acid and anisaldehyde followed by heating to 100^oC. Diason HP 20 (Mitsubishi chemical) and silica gel (60-200 mersh size) were used for column chromatography. Preparative TLC was carried out on precoated silica gel glass plates LKD50 (size 20x20 with thickness 250µm) (Kiesegel, Merck).

Collection, identification and preparation of plant materials

The whole plant (aerial and underground parts) materials bearing fruits and leaves growing wild were collected from Samaru village, Zaria, Nigeria in July 2009. The plant was authenticated and deposited (Voucher specimen number 1697) in the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria. Leaves were separated manually, air-dried and powdered using pestle and mortar.

Extraction and isolation

The dried powdered leaves (750g), was defatted with 1L n-hexane. The marc was extracted twice with 2×1L acetone by agitation at room temperature. The acetone extracts were combined and concentrated in vacuo at 40°C to afford 117g of residue. 34.7g of the residue was fractionated on a silica gel column eluted with gradient of increasing polarity of n-hexane to 100% chloroform. The eluents were monitored by TLC and combined to give 14 fractions. The fractions were further subjected to column chromatography and preparative TLC using varied ratio of n-haxane and chloroform as solvent system. This led to the isolation of two compounds (Compound I and Compound II) which were separately purified by recrystallization processes. The compounds were weighed, their melting point determined and subjected to structural analysis.

RESULT AND DISCUSSION

Glutinol (1): m.p. 202-203°C; IR (KBr) Vmax cm^{-1} :3430 (OH), 1680 (C=C), 1040 (C-O).

^{I}H NMR: 0.85, 0.95, 0.99, 1.00, 1.04, 1.09, 1.14, 1.16 (each 3H, s, 8Me), 1.26 (9H, br), 3.5 (lH, m, H- 3), 5.63 (lH. br, d, J=5.5Hz).

ElMS m/z (rel. int.): 426 [M^+] (6.5), 411 [M - Me]$^+$ (20), 274 [fragment a] (75), 260 [a - Me] (50), 245 (3.5), 205 (30), 95 (100).

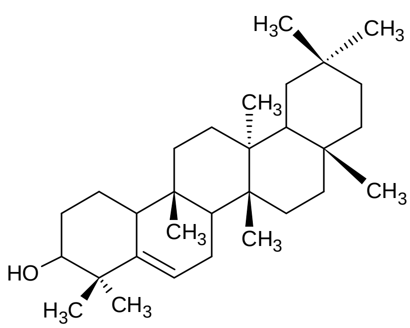

Fig 1: Structure of compound I (Glutinol)

Rhoiptelenol (2): m.p. 211-213°C; IR (KBr) Vmax 3456, 2935, 1445, 1383, 1215, 1035, cm^{-1}.
^{1}H NMR ($CDCl_3$, 500 MHz)δ 0.87 (3H, s, H-25), 0.88 (3H, d, J = 7 Hz, H-30), 0.90 (3H, s, H-27), 0.96 (3H, d, J = 6.5 Hz, H-29), 0.97 (3H, s, H-26), 1.03 (3H, s, H-23), 1.05 (3H, s, H-28), 1.12 (3H, s, H-24), 1.33 (1H, d, J = 2.7 Hz, H-18), 1.50 (1H, dd, J = 10.7 and 2.7 Hz, H-19), 2.02 (1H, m, H-10), 3.46 (1H, br s, H-3) and 5.60 (1H, m, H-6).

EIMS m/z (rel. int): 426 [M^+] (6.7), 274 (75), 245 (3.5), 205 (3.0), 173 (20.1), 152 (35.3), 134 (66.5), 121 (48.5), 109 (57.5), 95 (100).

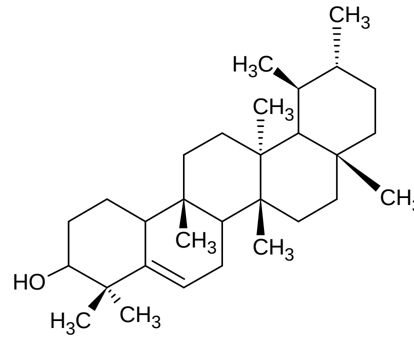

Figure 2: Structure of compound 2 (Rhoiptelenol)

Table 1: ^{13}C-NMR of compounds I and II showing δ values of each carbon atom

C	Glutinol	Rhoiptelenol
1	18.2	18.0
2	27.8	27.7
3	76.3	76.3
4	40.8	40.7
5	141.6	141.7
6	122.1	122.1
7	23.7	23.9
8	47.5	45.2
9	34.9	34.7
10	49.7	49.7
11	34.6	34.2
12	30.4	28.3
13	39.3	39.8
14	37.8	39.8
15	32.1	28.3
16	36.0	34.6
17	30.1	31.3
18	43.1	52.3
19	35.1	35.6
20	28.2	31.8
21	33.1	29.6
22	39.0	37.5
23	25.5	28.4
24	29.0	24.4
25	16.2	16.5
26	19.6	25.1
27	18.4	14.9
28	32.01	38.6
29	34.5	15.0
30	32.4	22.4

From the acetone extract of the leaves of *O. mannii*, two triterpenoids were obtained and purified using silica gel column chromatography and preparative TLC. The two compounds gave positive response to Liebermann-Burchard spray reagent indicating the presence of triterpenoid nucleus. Compound I crystallized as colourless needles from MeOH-$CHCI_3$, with melting point of 202-203°C. Its IR spectrum showed absorption peaks at 3430, 1680 and 1040 cm^{-1}. Thirty carbon atoms were detected on the ^{13}C-NMR of Compound I (Table 1), showing two olefinic carbons signals at δc 141.6 (C-5) and δc 122.1 (C-6) and one hydroxyl group at δc 76.3 (C-3).

The proton ^{1}H-NMR spectrum of Compound I displayed signals due to the eight methyl groups (δ 0.85-1.17), a carbinol proton (δ 3.5) at H-3 and an olefinic proton (δ 5.6) at H-5. A molecular ion at 426 was exhibited in its mass spectrum, thus giving a possible molecular formula of $C_{30}H_{50}O$. Two intense peaks were observed arising from cleavage of the B-ring at m/z 274 (fragment a) and 260 (a-Me) characteristic of a triterpene-5-ene skeleton (Budzikievwicz *et al*., 1965), which is a characteristic fragment due to the retro-Diels-Alder cleavage of ring B. The spectral data suggested that the triterpenoid was glutinol, this was further supported by comparison with literature values (Hui *et al*., 1975; Gaiko *et al*., 1976, Matsunaga *et al*., 1988; Sageer, 2003; and Basar, 2006).

The GC chromatograph showed that compound II has a shorter retention time than compound I. The mass spectrum showed that compound II has a molecular weight of 426, and a possible molecular formula of $C_{30}H_{50}O$ which shows that compound II is possibly an isomer of compound I. The m/z values of nearly all the fragments obtained are very similar to those obtained for compound I and also in agreement with the fragmentation pattern of retro-Diels-Alder cleavage for pentacyclic triterpenoids. The similarity in the mass spectrum of compound II and its shorter retention time when compared with compound I suggested that compound II is β-amyrin. The difference in retention time could be as a result of the shift of the CH_3 group from the axial conformation at C-20 in Oleaneane structures to an equatorial conformation at C-19 in Ursane-type compounds which caused an increased in the polarity of the molecule (Burnough-Radosevich *et al*., 1985).

The ^{1}H-NMR spectra obtained for Compound II further confirm its similarities with compound I. Like Compound I, Compound II has 8 methyl singlet peaks at δ 0.802, 0.841, 0.862, 0.880, 0.947, 0.963, 0.977, and 1.008 (each 3H). However the intensity of the methylene proton at δ1.262 (2H) is higher than the corresponding value for compound 1. This shows that there are more methylene protons in compound 2. The carbinol proton appeared at 3.25 (H-3) and the olefinic proton peaks at δ5.25 (H-6). The peaks at δ1.58 indicate the presence of methine protons in the compound. The absence of methyl doublet peaks in the ^{1}HNMR spectrum of compound II confirms that there is no methyl group at the equatorial conformation of C-19, but an axial conformation at C-20.

CONCLUSION

Analysis of the spectral data, showed compound I to be glutinol an oleaneane-type triterpenoid while compound II was assigned as rhoiptelenol an Ursane-type triterpenoid.

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