Taraxerol and Taraxer-14-en-3-one from *Jatropha tanjorensis* (Ellis and Saroja) leaves.

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Abstract:

Jatropha tanjorensis leaves were collected, air dried and pulverized. The pulverized sample was extracted with solvents of varying polarity (n-hexane, ethylacetate and ethanol) to obtain the crude extracts. Repeated column and thin layer chromatographic separation of the crude extracts afforded two compounds which were characterized by their IR, MS, ^{1}H and ^{13}C-NMR spectra data. Comparison of the data with literature confirmed the compounds to be Taraxerol and Taraxer-14-en-3-one.

Key words: *Jatropha tanjorensis*, Taraxerol, Taraxer-14-en-3-one, TLC, Chromatography

Introduction:

Jatropha tanjorensis is a plant in the *Euphorbiaceae* family. It is a perennial herb which is a hybrid specie with phenotypic character between *Jatropha curcas* and *Jatropha gossypifolia* (Prabakan and Sujatha, 1999). The plant is widely cultivated in Nigeria primarily for fencing, as a source of leafy vegetable and for medicinal purpose (Oboh and Masodje, 2009; O' Hara *et al.*, 1998). The leaves extract of the plant has been shown to have hypoglycaemic properties and are also employed traditionally in the treatment of anaemia, diabetes and cardiovascular diseases

(Iwalewa *et al*, 2005; Olayiwola *et al*., 2004). Phytochemical analysis of the leaf extract revealed the presence of saponins, cardiac glycosides, flavonoids, terpenoids and tannins (Oyewole and Akingbala, 2011). However, to the best of our knowledge, there has been no report in the literature on isolation and characterization of the phytochemical constituents of this plant, hence this study. We hereby report the isolation and characterization of two terpenoid compounds, Taraxerol and Taraxer-14-en-3-one from this plant. Even though these compounds have been reported in related species (Nobuko *et al*, 1987; Claudia *et al*, 2004.), this is the first time they are been reported in this plant.

Materials and Methods:

Plant Material:

Fresh leaves of *Jatropha tanjorensis* were collected in the month of April 2012 beside Ladoke Akintola University of Technology, Ogbomoso, Nigeria. Identification was done in the Department of Pure and Applied Biology, LAUTECH. Harvesting was done with hands properly protected with glove to avoid contact with the milky sap that exudes from the plant which causes irritation and itching on contact with the skin.

Sample Preparation:

The leaves were air dried at room temperature for about two months. Thereafter, the dried leaves were pulverized.

Extraction and Isolation:

755 g of the pulverized sample was successively soaked with three solvents of varying polarity (n-hexane, ethylacetate and methanol), starting with the least polar for one week each. In each

case the mixture was filtered and the filterate concentrated *in vacuo* to obtain the crude hexane extract, crude ethyl acetate extract and crude methanol extract.

The crude n- hexane extract (13 g) was subjected to silica gel column chromatography and the column eluted with either one or a mixture of two of n-hexane, ethylacetate and methanol. Elution was done by gradually increasing the polarity of the solvent system starting with 100% n-hexane. The eluents were collected in fractions of 200 ml each. A total of 50 fractions were collected and analyzed by thin layer chromatography, fractions with similar tlc profile were pooled together and concentrated to dryness *in vacuo*. Rechromatography of fraction 21 gave compound 1(100 mg) as a white crystalline solid.

Fractions 24 to 29 were combined and rechromatographed using the solvent system as stated above and fractions collected at 15ml interval, compound 2 (65 mg) crystallized out of fraction 10 of this column.

Melting points were determined on a Kofler apparatus and are uncorrected. IR spectra were recorded by using a Thermo Nicolet 5700 FT-IR spectrometer, in $CHCl_3$. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on an Agilent DD2 400 NMR spectrometer at 400 MHz and 100 MHz respectively. The chemical shifts as δ- values are reported in parts per million (ppm) relative to teteramethylsilane (TMS, $\delta = 0$) as internal standard.

The positive and negative ion high resolution ESI mass spectra were obtained from a Bruker Apex III Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (Bruker Daltonics, Billerica, USA) equipped with an Infinity™ cell, a 7.0 Tesla superconducting magnet (Bruker, Karlsruhe, Germany), an RF-only hexapole ion guide and an external electrospray ion source (Agilent, off axis spray). Nitrogen was used as drying gas at 150°C. The sample solutions

were introduced continuously via a syringe pump with a flow rate of 120µl h^{-1}. The data were acquired with 512k data points; zero filled to 2048k by averaging 16 scans and evaluated using the Bruker XMASS software (Version 7.0.8).

The electrospray (ESI) mass spectra were performed on a SCIEX API-3200 instrument (Applied Biosystems, Concord, Ontario, Canada) combined with a HTC-XT autosampler (CTC Analytics, Zwingen, Switzerland). The samples were introduced via auto sampler and loop injection. All solvent used for extraction and column chromatography were General Purpose Reagent (GPR), redistilled before use. Column chromatography was carried out on Merck Si gel 60 while thin layer chromatography (TLC) were done with aluminium sheet pre coated with normal phase silica gel 60 F254 (Merck, 0.20 mm thickness). The TLC were run using suitable solvent systems. Spots were located on the developed TLC plates by visualization under ultraviolet light at 254 and 366 nm.

Results and Discussion:

The infra red spectrum of compound 1 (m.p 238-240^{o}C) in Table 1 revealed absorptions at 3048.7 and 3007.7 due to =C – H of alkene, 2956.7, 2913.8 and 2847.9 due to C – H strech of alkane; 1706 due C=O strectching vibration and at 1471, 1461 and 1447 as a result of the bending vibrations of =C – H. The ESI MS of the compound gave the molecular formula of the compound as $C_{30}H_{48}O$. The mass spectrum of the compound showed intense peaks at m/z 300, 285 and 204.

The Infra red spectra of compound 2 (M.pt 277 – 280^{o}C) in Table 2 revealed among others a broad absorption band centred at 3843.0 cm^{-1} due to O-H streching vibration, absorptions at 3052.6cm^{-1} due to =C-H stretching vibration, absorptions at 2914.0 and 2848.0 due to C-H

stretching vibration of alkanes and at 1641 cm^{-1} due to C=C stretching vibrations. The ESI MS of the compound gave a molecular mass of 426 corresponding to a molecular formular of $C_{30}H_{50}O$. The mass spectrum of the compound also showed intense peaks at m/z 302 and 204. The ^{13}C NMR spectrum revealed a total of thirty carbon atoms distributed as follows: 8 methyl, 10 methylene, 5 methine and 7 quartenary carbons as presented in Tables 1 and 2 . The proton nmr also revealed the different proton environments and support the structure of the compounds (Figures 1 and 2). The spectra characteristics of these compounds are in excellent agreement with literature data for these compounds. (Nobuko *et al.*, 1987; Valente *et al.*, 2004.)

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Table 1: ^{1}H and ^{13}C NMR data for compound 1

Position	δC (ppm)	C type	δH(ppm)
1	38.3	CH_2	1.90; 1.60
2	34.1	CH_2	2.60 ; 2.36
3	217.5	C═O	
4	47.6	C	
5	55.8	CH	1.90
6	19.9	CH_2	1.75 ; 1.60
7	35.1	CH_2	1.70 ; 1.60
8	38.9	C	
9	48.7	CH	1.70
10	35.8	C	
11	17.4	CH_2	1.70 ; 1.60
12	37.68	CH_2	1.70; 1.60
13	37.72	C	
14	157.6	C═	
15	117.2	HC═	5.57
16	36.6	CH_2	2.10 ; 1.85
17	37.5	C	
18	48.8	CH	1.70
19	40.6	CH_2	1.70; 1.50
20	28.8	C	

21	33.6	CH_2	1.75; 1.60
22	33.1	CH_2	1.75 ; 1.60
23	26.1	CH_3	0.99
24	21.5	CH_3	0.99
25	14.8	CH_3	1.03
26	29.9	CH_3	1.14
27	25.6	CH_3	1.14
28	29.8	CH_3	1.03
29	33.3	CH_3	0.83
30	21.3	CH_3	0.83

Table 2: ^{1}H and ^{13}C NMR data for Compound 2

Position	δC (ppm)	C type	δH (ppm)
1	37.7	CH_2	1.60; 1.15
2	27.2	CH_2	1.60; 1.40
3	79.1	C—OH	3.2, 3.6
4	39.0s	C	-
5	55.5	CH	1.40
6	18.8	CH_2	1.60; 1.15
7	35.1	CH_2	1.95 ; 1.60
8	38.8	C	-
9	48.8	CH	1.40
10	35.8	C	-
11	17.5	CH_2	1.15
12	37.7	CH_2	1.15
13	37.6	C	-
14	158.1	C=	
15	116.9	HC=	5.5
16	36.7	CH_2	2.01; 1.95
17	38.0	C	-
18	49.3	CH	1.40
19	41.3	CH_2	1.15
20	28.8	C	-

21	33.7	CH_2	1.15
22	33.1	CH_2	1.15
23	28.0	CH_3	0.82
24	15.44	CH_3	0.82
25	15.4	CH_3	0.99
26	29.9	CH_3	1.10
27	25.9	CH_3	1.10
28	29.8	CH_3	0.99
29	33.3	CH_3	0.82
30	21.3	CH_3	0.82

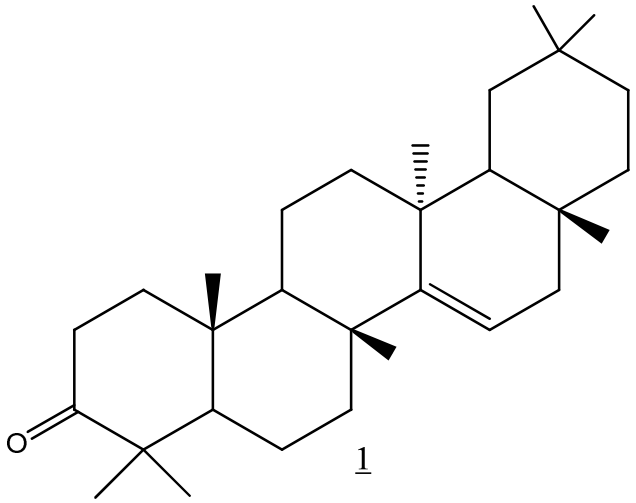

Figure 1: Structure of Taraxer-14-en-3-one

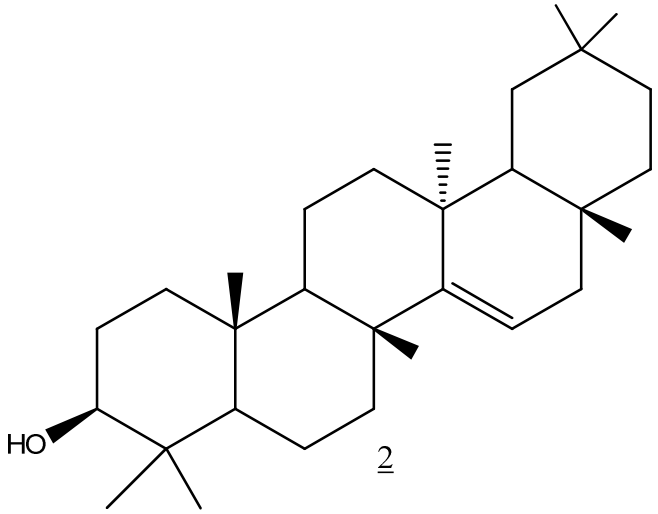

Figure 2: Structure of Taraxerol