Japodic acid, A Novel Aliphatic Acid from *Jatropha podagrica* Hook

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Abstract: A new aliphatic acid named japodic acid (**1**) with a gem-dimethyl cyclopropane ring has been isolated from the roots of *Jatropha podagrica*. Its structure was established by 1D and 2D NMR and mass spectrometric data. Two other known compounds, erythrinasinate (**2**) and fraxidin (**3**) were also isolated from this plant for the first time. Japodic acid showed mild insect growth inhibition activity against *Helicoverpa zea* (37% growth reduction at 100 ppm). Fraxidin and erythrinasinate exhibited antibacterial activity against *Bacillus subtilis* while japodic acid was inactive in the antibacterial assays conducted.

Keywords: *Jatropha podagrica*; aliphatic acid; antibacterial activity; Japodic acid; Insect growth inhibition activity.

1. Introduction

Jatropha podagrica Hook (Euphorbiaceae) is a shrub that is grown in West Africa gardens for its showy red flowers. *Jatropha podagrica* is known locally in south western Nigeria as *lapalapa funfun*. *Jatropha* species are found in Africa, Asia and Latin America where they are used in folk medicine to treat various diseases including skin infections and sexually transmitted diseases [1-3]. Various medicinal and pesticidal properties including antibacterial, antitumour and insect antifeedant have been attributed to this plant [1, 4-6]. Different parts of the plant have been investigated chemically and many compounds including flavonoids, steroids, alkaloids and diterpenoids have been isolated from this plant and related species [7-10].

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In continuation of our studies on *Jatropha* species, the root extracts of *Jatropha podagrica* was reinvestigated for possible new compounds and other biological activities.

2. Materials and Methods

2.1. Plant Materials

Jatropha podagrica plants were collected from the old premises of St. Stephen's Anglican Church, Inalende, Ibadan, Nigeria in June 1995 and were authenticated at the Herbarium of the Forestry Research Institute, Ibadan, Nigeria (Herbarium Voucher Number FHI 93265).

2.2. General

^{1}H and ^{13}C NMR data were recorded on Bruker WM-360 and/or AMX-600 spectrometers. NMR spectra were recorded using $CDCl_3$ solution and were referenced to the corresponding solvent signals. HMQC and HMBC experiments were optimized for $^1J_{CH} = 150$ Hz and $^nJ_{CH} = 8$ Hz, respectively. MS data were recorded on VG Trio1 and VG ZAB-HF mass spectrometers. UV spectra were recorded using the cuvette cell compartment of a Beckman model 168 photodiode array detector. Optical rotations were determined in $CHCl_3$ using a Jasco DIP 1000 digital polarimeter. Melting points were measured on a Fisher-Johns micro melting point apparatus, and are uncorrected.

2.3. Extraction and Isolation

The air-dried and powdered roots of the plant (320g) were extracted successively with hexane, chloroform and methanol (3L, 48h   each) at room temperature. The solvents were removed under vacuum to give the respective extracts: hexane (4.8g), chloroform (6.0g) and methanol (15.0g). The hexane extract (4g) was subjected to vacuum liquid chromatography on silica gel (40-63 µm, 5 x 10 cm). The column elution started with hexane and continued with gradients of ethyl acetate and methanol. Similar fractions were pooled together. The fraction eluted with 30% MeOH in EtOAc showed a very strong antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*. This fraction was subjected to purification by preparative reversed phase HPLC (Rainin Dynamax-60, C18 column, 8µm particles, 21.4mm x 25cm, 10ml/min, UV detection at 215nm) using a gradient of 80-100% MeOH/H_2O for 30 min. This resulted in three fractions labelled JPH1 ($R_t = 6.90$min), JPH2 ($R_t = 9.0$min), and JPH3 ($R_t = 11.60$min). JPH3 which had the most interesting ^{1}H NMR spectrum was further purified by semi preparative RP HPLC using a gradient of 70-100% CH_3CN/H_2O over 30 min (Beckman Ultrasphere C18 column, 5µm, 1.0 x 25 cm, 2.0 ml/min, UV detection at 254 nm) to give compound **1** (3mg) as yellow oil (t_R =9.0min, $R_f = 0.4$, $CHCl_3$/MeOH, 9:1).

Compounds **2** and **3** were isolated from the chloroform and methanol extracts respectively by silica gel vacuum liquid chromatography (VLC) and RP HPLC respectively. 10g of crude extract was subjected to silica gel VLC over a prepacked column bed (silica gel, Type H, 10-40µ, 120g, column of sintered glass funnel (porosity 3)). The column was eluted using a stepwise gradient of CH_2Cl_2 (0-100%) in hexane and MeOH (0-100%) in CH_2Cl_2. Twenty eight 100mL fractions were collected. Similar fractions were pooled together based on their TLC to give six combined fractions. Fraction 4 yielded a white solid which was recrystallized from methanol to give white plates (**2**, 35 mg).
Compound **3** was obtained in a similar way by VLC. It was purified by semipreparative reversed phase HPLC (Beckman Ultrasphere C18 column, 5µm, 10mm x 25cm, 2mL/min, 254nm) using a

gradient of 50% to 100% MeOH in H_2O over 60 minutes. Evaporation of solvent under a stream of nitrogen gas gave a white solid, 13mg. All the compounds were characterized by spectroscopic methods.

2.4. Biological Assays

2.4.1. Antibacterial Assay

The microorganisms employed in the assay are: *Staphylococcus aureus* (ATCC 29213), *Bacillus subtilis* (ATCC 6051), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853). Agar disk diffusion method was employed and filter paper disks (6.25 mm in diameter) were used. 20μl of the test solvents (chloroform and methanol) were applied to each disk using a micropipette to sterilize the paper disks. The inoculated plates (15 x 100 mm) were removed from the refridgerator and labelled accordingly. Once pure solvent has evaporated from the disk, 20μl of the sample solution containing 20μg of the compounds were applied to the disks. Negative and positive controls were also prepared with 20μl of pure solvent and standard antimicrobial agents (streptomycin and gentamycin). The impregnated disks were left to dry and the dry disks were placed on the surface of the bacteria seeded plates. The plates were sealed with parafilm to prevent contamination and incubated overnight (24h). The plates of *B. subtilis* and *S. aureus* were incubated at room temperature, while the plates of *E. coli*, and *P. aeruginosa* were incubated at 37°C. The compound was considered to be active if after 24 hr, a clear zone extended around the impregnated filter paper disk in which no growth of the test organism was observed. The activity was reported as the diameter of the zone of growth inhibition and recorded in mm. The assays were carried out in duplicates [9].

2.4.2. Insect growth Regulatory Assay

The samples were added in 125μL of acetone to test tubes (100 x 16mm) containing 5 mL aliquots of molten diet (60°C). The mixture was then blended vigorously with a vortex mixer for 20 seconds. The diets were dispensed into petri dishes and allowed to cool to room temperature. They were then placed in fume hood for about 20 minutes to remove residual traces of acetone. The diets were then cut into twenty four equal sections. Each section was placed into a single well of a 24-well immunoassay plate. Neonate larvae of *Helicoverpa zea* were used in the assays and a single neonate *H. zea* larva was added to each well. The plate was covered with sheet of paraffin, cardboard and a plastic cover to prevent desiccation. The cover was secured by two rubber bands and the groups of plates were placed in polyethylene bags which were also sealed by rubber bands. The plates were maintained for 7 days at 27°C, 40% relative humidity and a 14:10h (light:dark) photoperiod. The insects were inspected at 2, 4 and 7 days for mortality. Surviving insects were weighed when seven days old. A solvent blank was used as control. Each sample was tested on a total of 40 neonate larvae. The compounds were tested at a concentration of 100 ppm. The activity was measured by comparisons of the tested larval weights relative to those of the controls. The activity was reported as percent reduction in weight gain relative to controls.

3. Results and Discussion

Compound **1** was obtained as a yellow oil. The molecular formula was established as $C_{13}H_{22}O_3$ based on its HR- FABMS which gave a mass of 249.1467 [M + Na$^+$], calculated for 249.1466. The formula indicated 3 sites of unsaturation, the absence of olefinic signals in both the ^{1}H and ^{13}C NMR spectra and the presence of two carbonyl signals suggested that it is monocyclic.
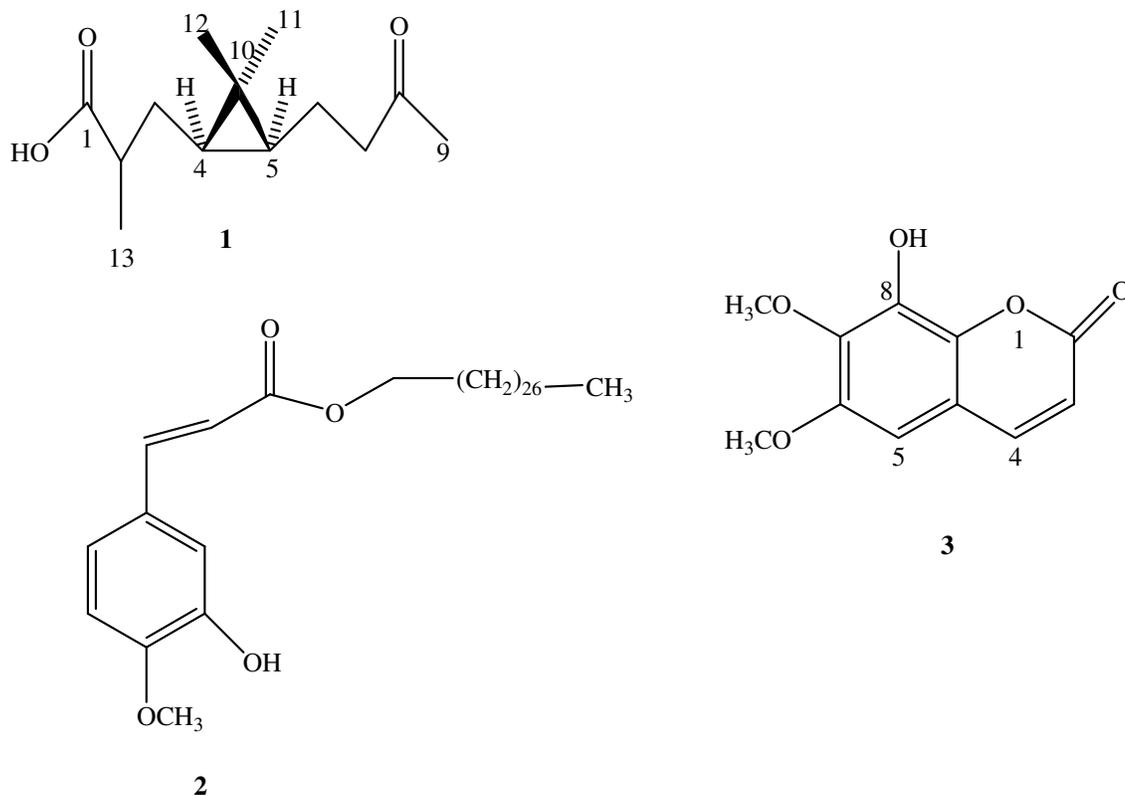
Figure 1. Chemical Structures of japodic acid (**1**), erythrinasinate (**2**) and fraxidin (**3**)

The ^{1}H NMR spectrum revealed the presence of three methyl singlets, one methyl doublet, cyclopropane methine signals and other methines and methylenes. The ^{13}C NMR and APT spectra showed resonances for 13 carbon atoms indicating the presence of 3 quartenary carbons consisting of two carbonyl signals and a tetrasubstituted carbon atom. Other resonances observed were for 3 methylene groups, three methines and four methyl groups (Table 1). These data accounted for twenty one hydrogen atoms indicative of an exchangeable proton in the compound. The structure was resolved with the aid of 2D NMR spectra. The COSY, TOCSY and HMBC spectra of the compound showed the connectivities between the protons and the carbon atoms. The cyclopropane methine signals (H-4 and H-5) showed correlation to the carbon atoms of the gem-dimethyl group on the cyclopropane ring (C-11 and C-12) and the neighbouring carbon atoms (C-2,6,7) (Table 1, Fig.1,).

The gem-dimethyl groups also displayed correlations to the other carbon atoms in the cyclopropane ring (C-4,5,10) and to each other. Further long range HMBC correlations were observed from the methylene protons (H$_2$-6 and H$_2$-7) to the ketone signal (δ 209.0) and C-5 indicating that the two methylenes are in between the ketone and the cyclopropane ring (Table 1). The methyl singlet (δ 2.13) showed correlation to the ketone signal(C-8) and the methylene carbon (C-7) thus establishing the connectivity from the ketone to the cyclopropane ring. The COSY spectrum also confirmed these assignments based on the coupling observed between the protons H-5, H-6 and H-7. The methylene protons H$_2$-3 similarly displayed HMBC correlations to the carbonyl signal (δ 181.5) of the carboxylic acid, the methine (C-2), the cyclopropane methines (C-4 and C-5) and the quartenary carbon C-10. The methine proton (H-2) showed correlations to C-1, 3, 4, 13. The methyl doublet (H$_3$-13) also displayed two and three bond correlations to C-1, C-2 and C-3 establishing the link between the acid group and the cyclopropane ring. The expected COSY correlations were also observed between the

protons. It is interesting to note that the downfield shifts of the methine (H-2) and the methylene protons (H$_2$-7) may be due to the deshielding effect of the adjacent carbonyl groups (Table 1).

The relative stereochemistry proposed for japodic acid was deduced from the analysis of its NOESY spectrum. The cyclopropane methine protons (H-4 and H-5) which were given alpha configuration showed strong NOESY correlations to each other and one of the methyl groups (H$_3$-11) of the gem-dimethyl group. This was indicative of a cis configuration for the cyclopropane ring as shown in the structure. There was also a strong NOESY correlation between H$_3$-12 and H$_3$-13, thus, leading to the proposed structure for japodic acid (**1**).
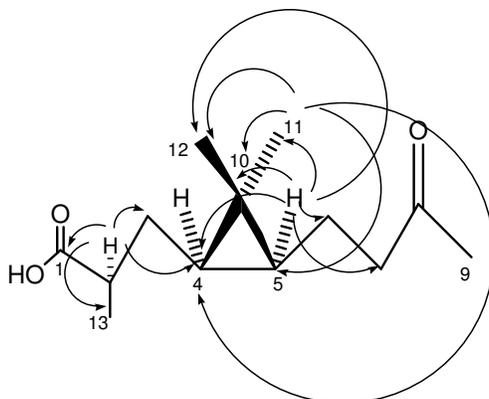
Figure 2. Selected HMBC Correlations of **1**

The structures of compounds **2** and **3** were determined by 1D and 2D NMR data and by comparison with literature data. Compound **2** was found to be erythrinasinate (octacosanyl-3-(3-hydroxy-4-methoxyphenyl)-2-(E)-propenoate), while compound **3** is fraxidin (8-hydroxy-6,7-dimethoxy coumarin) [11-12].

3.1 Spectral Data

Japodic acid *(2-methyl-4,5-dimethylmethylene-8-oxo-nonanoic acid, 1):* yellow oil; [α]$^{22}_D$ -13.14^0 (c 0.0014, CHCl$_3$); UV (MeOH): λ_{max} (log ε) = 223 (3.22) nm; ^{1}H and ^{13}C NMR data: see Table 1; LRFABMS (3-NBA matrix) *m/z* (rel. int) =249 ([M + Na]$^+$, (17), 227 ([M + H]$^+$); HRFABMS (3-NBA matrix) obsd [M + Na]$^+$ at *m/z* 249.1467, (calculated for C$_{13}$H$_{22}$O$_3$Na; 249.1466).

Erythrinasinate *(Octacosanyl-3-(3-hydroxy-4-methoxyphenyl)-2-(E)-propenoate, 2):* White plates from methanol, m.p. = 71-73°C. ^{1}H NMR (600 MHz, CDCl$_3$): δ**:** 7.58 (1H, d, J = 16 Hz, H-2), 7.04 (1H, dd, J = 1.4, 8.0 Hz, H-2', 5'), 7.01 (1H, d, J = 1.4 Hz, H-6'), 6.89 (1H, d, J = 8.0 Hz, H-6'), 6.26 (1H, d, J = 16 Hz, H-3), 5.88 (1H, s), 4.16 (1H, t, J = 6.5, 6.5 Hz), 3.90 (3H, s, OMe-4'), 1.67 (1H, m), 1.35 (1H, m), 0.86 (1H, t). ^{13}C NMR (150 MHz, CDCl$_3$): δ**:** 167.4 (C-1), 115.6 (C-2), 144.6 (C-3), 127.0 (C-1'), 114.7 (C-2'), 147.9 (C-3'), 146.7 (C-4'), 109.2 (C-5'), 123.0 (C-6'), 64.6 (C-1"), 31.9 (C-2"), 29.8 (C-3"), 14.1 (C-28"), 55.9 (C-4' OCH$_3$). HRFABMS 586.4978 [M$^+$], calculated for C$_{38}$H$_{66}$O$_4$, 586.4961.

Fraxidin *(8-hydroxy-6,7-dimethoxy coumarin, 3):* White solid, m.p.= 178 – 180°C.
^{1}H NMR (600 MHz, CDCl$_3$): δ**:** 7.58 (1H, d, J = 9.7 Hz, H-3), 6.49 (1H, s), 6.32 (1H, d, J = 9.4 Hz, H-4), 3.99 (1H, s, OMe-6), 3.88 (3H, s, OMe-7). ^{13}C NMR (150 MHz, CDCl$_3$): δ**:** 160.3 (C-2), 115.2 (C-3), 143.6 (C-4), 100.2 (C-5), 139.5 (C-6), 149.7 (C-7), 137.4 (C-8), 137.9 (C-9), 114.1 (C-10), 61.3 (C-6, OCH$_3$), 56.2 (C-7, OCH$_3$). HRFABMS 223.0593 [M+H]$^+$, calculated for C$_{11}$H$_{11}$O$_5$, 223.0606.

Table 1. NMR data for **1** in CDCl$_3$

Position	δ ^{1}H ^{a} (mult.)	δ ^{13}C^b (mult.)	HMBC^{c}Correlations [H→C]	NOESY^c [H↔H]
1		181.5 (s)	-	-
2	2.46 (m)	39.6 (d)		-
3a	1.44 (m)	28.5 (t)	C - 1, 2, 4, 5, 10	-
3b	1.60 (m)		Ditto	-
4	0.46 (m)	24.2 (d)	C – 2, 5, 10, 11	H-2, 5, 11
5	0.44 (m)	25.9 (d)	C – 4, 6, 7, 10, 11, 12	H-2, 4, 11
6a	1.47 (m)	19.1 (t)	C – 5, 7, 8	-
6b	1.53 (m)		Ditto	-
7a	2.44 (m)	44.2 (t)	C – 5, 6, 8	-
7b	2.45 (m)			-
8	-	209.0 (s)	-	-
9	2.13 (s)	30.0 (q)	C – 7, 8	-
10	-	17.3 (s)	-	-
11	0.99 (s)	29.2 (q)	C – 4, 5, 10, 12	H-4, 5
12	0.89 (s)	14.7 (q)	C – 4, 5, 10, 11	H-13
13	1.18 (d)	16.6 (q)	C – 1, 2, 3	H-12

^{a}600MHz, ^{b}150MHz, ^{c}600MHz (^{1}H Dimension)

3.2. Biological Activity

Compounds **1-3** were tested in agar disk diffusion antibacterial assays (paper disk diameter = 6.25mm) against gram-positive and gram-negative bacteria at a concentration of 20μg/disk. Suprisingly, japodic acid was inactive in all the assays while fraxidin and erythrinasinate displayed moderate activity of 12mm and 15mm zone of inhibition respectively. Furthermore, the compounds were tested in insect growth inhibition assay against *Helicoverpa zea* at 100ppm. (*Helicoverpa zea* is an important pest of maize which causes great damage to the crop, thus resulting in heavy economic losses due to poor yield of the crop). Only japodic acid showed insect growth inhibition activity against this insect at 37.1 % reduction in growth rate while fraxidin and erythrinasinate were inactive.

In conclusion, we isolated a new aliphatic acid named japodic acid **1**; the structure of which was established by spectroscopic analysis as 2-methyl-4,5-dimethylmethylene-8-oxo-nonanoic acid. Two other compounds, fraxidin (**2**), a coumarin and erythrinasinate (**3**), a long chain ester were also isolated from the plant. As far as we know these compounds were isolated from this plant for the first time, and these biological activities are also being reported for the first time.

Acknowledgements

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