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Isolation and Characterization of Constituents from The Extracts of *Momordica angustisepala* Aerial Parts

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Abstract

N-hexane, ethyl acetate and methanol extracts of *Momordica angustisepala* were screened for the presence of metabolites, using column and thin-layer chromatographic techniques. Octadecane (**1**), tetradec-5-enoic acid (**2**), 1,2,3-propanetriyl (8Z,11'Z,14"Z)tris(-8,11,14-eicostrienoate) (**3**), methyl oleana-5,12,15-trienoate-3β-acetate (**4**) and 1,2,3-propanetriyl (9Z,12'Z,15"Z)tris(-9,12,15-uneicostrienooate) (**5**) are the compounds obtained from the extracts of *M. angustisepala* aerial parts using proton and ^{13}C Nuclear Magnetic Resonance Spectroscopy (NMR).

Keywords: *Momordica angustisepala* aerial parts; 1,2,3-propanetriyl (8Z,11'Z,14"Z)tris(-8,11.14-eicostrienoate); 1,2,3-propanetriyl (9Z,12'Z,15"Z)tris(-9,12,15-uneicostrienooate); methyl oleana-5,12,15-trienoate-3β-acetate; Proton and Carbon-13 Nuclear Magnetic Resonance Spectroscopy; Column Chromatography; Thin-Layer Chromatography.

INTRODUCTION

Momordica angustisepala belongs to the family Cucurbitaceae and is a large climbing plant with stout stems attaching themselves into the surrounding vegetation by means of tendrils. The plant, also known as "Ejirin nla" in Yoruba and "bitter gourd" in English, is mostly found in West Tropical Africa (such as Nigeria, Côte D'Ivoire, and Cameroon), mostly in deciduous or semi-deciduous forest. It can also be found at the road side, old plantation, or filthy areas. *M. angustisepala* is used in making sponges due to its fibre-forming polymeric activity (Biswas *et al.*, 2011). This plant is used in herbal medicine to treat tumors, malaria and diabetes, and as abortifacient to guard against unwanted pregnancy (Burkill, 1997; Shehu et al., 2019).

Despite the ethnomedicinal uses of this plant, less attention has been paid to its exploitation. This study was designed to isolate (using chromatography) and to characterize the constituents of *M. angustisepala* extracts using ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) Spectroscopy.

MATERIALS AND METHODS

Plant Materials

Fresh aerial parts of M. angustisepala were collected in Ilorin, Kwara State, Nigeria. The plants were identified and authenticated at the Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria by Mr Bolu Ajayi (a botanist), and voucher specimens of the plants were deposited in the Herbarium of the department.

Extraction and Isolation Procedures

Momordica angustisepala aerial parts were air-dried for three weeks and ground into powdery form with the machine. The weights obtained after grinding were 530.51 g. The ground sample was soaked with n-hexane, ethyl acetate and methanol successively for 10 days each in the order of their polarity, for complete extraction. The extracts were filtered separately with Whatmann No1 filter paper and concentrated at 45 ^{o}C using rotatory evaporator and then freeze-dried to remove trace solvents. The dried extracts were subjected to column chromatography to obtain pure isolates, and thin layer chromatography and recrystallization were carried out on the isolates to affirm their purity.

Extraction of *M. angustisepala* aerial parts with n-hexane, ethyl acetate and methanol gave 22 g, 11.8 g and 5 g extracts respectively. The methanol extract was not reckoned with due to its small quantity. The ethyl acetate-hexane extract (33.8 g) of *Momordica angustisepala* aerial parts (MAEH) was pre-adsorbed on silica gel (60-200 mesh size) (60 g) to form powdered homogeneous slurry. The slurry was then subjected to column chromatography, packed using the slurry method (silica gel 700 g, 60-120 mesh size) and eluted with various solvent systems, starting with 100% of Hexane; then using Hexane: $CHCl_3$ (49:1, 100 mL); (19:1, 100 mL); (23:2, 100 mL); (9:1, 100 mL); (17:3, 100 mL); (4:1, 100 mL); (3:1, 100 mL); (7:3, 100 mL); (3:2, 100 mL); (11:9, 100 mL); and (1:1, 100 mL) separately. A total of 75 fractions (100 mL each) were collected and pooled to five pure fractions, coded MAEH-1–MAEH-5, based on TLC analysis, with masses 20 mg, 15 mg, 24 mg, 15 mg, and 42 mg respectively; and found to be a pure compound each on characterization using ^{1}H NMR and ^{13}C NMR.

***Characterization of Octadecane*,** $C_{18}H_{38}$, white solid (20 mg); **^{1}H NMR ($CDCl_3$, 300 MHz):** δ 0.88 (6H, t, 2 × CH_3), 1.10–1.28 (32H, m, 16 × CH_2); **^{13}C NMR ($CDCl_3$, 75 MHz):** δ 14.25 (C_{-1}), 29.62 ($C_{-2–16}$), 31.8 (C_{-17}), 22.68 (C_{-18}).

***Characterization of Tetradec-5-emoic acid*,** $C_{14}H_{25}O$, yellow viscous oily liquid (15 mg); **^{1}H NMR ($CDCl_3$, 300 MHz):** δ 1.85–2.40 (4H, H_{-2} (t) & H_{-3} (m)), 1.48–1.68 (4H, m, $H_{-4\ \&\ 7}$), 5.11–5.36 (2H, m, $H_{-5\ \&\ 6}$), 1.14–1.41 (12H, m, $H_{-8–13}$), 0.89 (3H, t, H_{-14}); **^{13}C NMR ($CDCl_3$, 75 MHz):** δ 182.05 (C=O, C_{-1}), 29.73 ($C_{-3\ \&\ 4}$), 137.50 (C_{-5}), 128.00 (C_{-6}), 31.95 (C_{-7}), 39.40 (C_{-8}), 37.28 (C_{-9}), 32.83 (C_{-10}), 22.72 (C_{-11}), 19.78 (C_{-12}), 16.04 (C_{-13}), 14.15 (C_{-14}).

***Characterization of 1,2,3-Propanetriyl (8Z,11'Z,14"Z)tris(-8,11,14-eicostrienoate)*,** $C_{63}H_{101}O_6$, creamy solid (24 mg); **^{1}H NMR ($CDCl_3$, 300 MHz):** δ 0.86–0.88 (9H, t, 3 × CH_3), 1.25–1.41 (3 × 6H, m, 3 × 3CH_2), 1.94–2.45 (3 × 18H, m, 3 x 9CH_2), 4.89–6.19 (3 × 6H, m, 3 × 6CH), 3.43–4.28 (4H, d, 2 × O**CH_2** & 1H, m, O**CH**); **^{13}C NMR ($CDCl_3$, 75 MHz):** δ 173.37 (C=O, C_{-1}), 29.29 ($C_{-2–7}$), 139.53 (C_{-8}), 132.56 (C_{-9}), 52.03 (C_{-10}), 131.73 (C_{-11}), 129.72 (C_{-12}), 37.18 (C_{-13}), 127.93 (C_{-14}), 125.30 (C_{-15}), 34.07 (C_{-16}), 31.94 (C_{-17}), 24.87 (C_{-18}), 23.96 (C_{-19}), 14.14 (C_{-20}), 62.12 (OCH_2 of fatty triester, C-1), 68.93 (O**C**H of fatty triester, C-2), 65.06 (OCH_2 of fatty triester, C-3).

***Characterization of Methyl Oleana-5,12,15-trienoate-3β-acetate*,** $C_{33}H_{48}O_4$, yellow solid (15 mg); **^{1}H NMR ($CDCl_3$, 300 MHz):** δ 1.06–1.40 (10H, H_{-1}, $H_{-21\ \&\ 22}$ (t); H_{-2} (m); H_{-19} (d) 5 × R_2**CH_2**,), 1.63–1.79 (4H, H_{-7} (d) & H_{-11} (q), 2 × R_2**CH_2**), 1.48–1.55 (2H, t, H_{-9} & H_{-18}, 2 × R_3**CH**); 5.08–5.25 (1H, t, H_{-6}, R_2C=**CH**R), 5.30–5.35 (1H, t, H_{-12}, R**CH**=CR_2), 5.65–6.50 (2H, d, $H_{-15\ \&16}$, R**CH**=**CH**R), 0.81–0.91 (21H, s, $H_{-23–27,\ 29\ \&\ 30}$, 7 × R**CH_3**), 4.5 (1H, t, H_{-3}, O-**CH**R_2), 2.05–2.37 (3H, s, ROOC**CH_3**), 3.62 (3H, s, OCH_3); **^{13}C NMR ($CDCl_3$, 75 MHz):** δ 39.74 ($C_{-1\ \&\ 2}$), 51.45 (C_{-3}), 29.14 (C_{-4}), 125.03 (C_{-5}), 119.37 (C_{-6}), 29.72 (C_{-7}), 32.22 (C_{-8}), 27.22 (C_{-9}), 29.54 (C_{-10}), 22.71 (C_{-11}), 124.25 (C_{-12}), 142.35 (C_{-13}), 29.54 (C_{-14}), 130.05 (C_{-15}), 135.22 (C_{-16}), 29.37 (C_{-17}), 31.94 (C_{-18}), 29.14 (C_{-19}), 29.54 (C_{-20}), 26.43 (C_{-21}), 25.71 (C_{-22}), 25.71 (C_{-23}), 16.02 (C_{-24}), 14.14 (C_{-25}), 22.65 (C_{-26}), 23.45 (C_{-27}), 174.33 (C=O, C_{-28}), 29.14 (C_{-29}), 24.96 (C_{-30}), 51.45 (ROO**C**CH_3), 60.90 (OCH_3).

***Characterization of 1,2,3-Propanetriyl (9Z,12'Z,15''Z)tris(-9,12,15-uneicostrienoate)*,** $C_{66}H_{107}O_6$, creamy solid (42 mg). **^{1}H NMR ($CDCl_3$, 300 MHz):** δ 1.83–2.42 (42H, H_{-2} (t); H_{-3-8} (m), 3 × 7R_2**CH_2**), 4.56–6.46 (18H, m, $H_{-9,\ 10,\ 12,\ 13,\ 15\ \&\ 16}$, 3 × 3R**CH**=**CH**R), 1.25 – 1.75 (36H, m, $H_{-11,\ 14\ \&\ 17}$, 3 × 3RCH=CH-**CH_2** & $H_{-18–20}$, 3 x 3R_2**CH_2**), 0.88–0.97 (9H, t, 3 × 3R**CH_3**), 3.41–3.94 (4H, d, 2 × O**CH_2** & 1H, m, O**CH**); **^{13}C NMR ($CDCl_3$, 75 MHz):** δ 173.07 (C=O, C_{-1}), 29.70 ($C_{-2–8}$), 135.10 (C_{-9}), 132.81 (C_{-10}), 39.72 (C_{-11}), 130.13 (C_{-12}), 128.77 (C_{-13}), 34.15 (C_{-14}), 125.90 (C_{-15}), 124.24 (C_{-16}), 32.75 (C_{-17}), 27.78 (C_{-18}), 26.41 (C_{-19}), 24.85 (C_{-20}), 14.11 (C_{-21}), 62.07 (2 × O-**C**H_2R of fatty triester C-1 & 3), 68.89 (O-**C**HR_2 of fatty triester, C-2)

RESULTS AND DISCUSSION

Characterization of the Isolated Compounds

The isolates from the plant extracts were characterized using ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) Spectroscopy. The ^{1}H and ^{13}C NMR analyses on the isolates obtained from the ethyl acetate-hexane extracts of *M. angustisepala* aerial parts gave compounds **1–5**. The structures of these compounds are shown in Figure 1.

Compound 1 (20 mg) was isolated as white crystals. The ^{1}H NMR spectrum showed peaks at δ_H 0.88 (6H, t, H_{-1} and H_{-18}) corresponding to the terminal methyl hydrogen atoms. The peak at δ_H 1.10–1.28 was assigned to the cluster of methylene (CH_2) hydrogen atoms at 2–17 positions. The ^{13}C NMR spectrum displayed eighteen carbon resonances and was sorted out to contain sixteen methylene carbons (δ_c 29.62–31.8) and two methyl carbons (δ_c 14.25 (C_{-1}) and 22.68 (C_{-18})). The spectroscopic data of compound **1** were similar to those reported on octadecane in the literature (Lide & Milne, 1994). Hence, the structure of compound **1** was established as octadecane (Figure 1).

Figure 1. Octadecane.

Compound 2 (15 mg) was obtained as a light yellow viscous oily liquid. The ^{1}H NMR spectrum showed signals at δ_H 0.89 (3H, t, H_{-14}) corresponding to the terminal methyl hydrogen atoms. The multiplet peaks at δ_H 1.14–1.41 were attributed to the cluster of methylene hydrogen atoms (CH_2) at 8–13 positions. Allylic hydrogen atoms (-CH=CH-C**H$_2$**) were shown by δ_H 1.48–1.68 at 4 and 7 positions. The signal δ_H 1.85–2.40 corresponds to the α- and β- carbon hydrogen atoms to the carbonyl at positions 2 and 3 respectively. The peaks at δ_H 5.11 – 5.36 represent the olefinic hydrogen atoms (C**H**=C**H**) at positions 5 and 6. The ^{13}C NMR spectrum indicated fourteen carbon resonances and was sorted out as two olefinic carbons (δ_c 128.0, C_{-6}– 137.5, C_{-5}); one carbonyl carbon (δ_c 182.05, C_{-1}); ten methylene carbons (δ_c 19.78–39.40); and one methyl carbon (δ_c 14.15, C_{-14}). The spectroscopic data of compound **2** were similar to those reported on tetradec-5-enoic acid in the literature (Ogunleye et al., 1991; Lie Ken Jie & Lam, 1995). Therefore, compound **2** was established as tetradec-5-enoic acid (Figure 2).

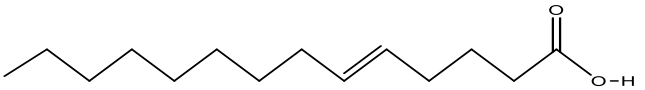

Figure 2. Tetradec-5-enoic acid.

Compound 3 (24 mg) was isolated as a creamy solid. The ^{1}H NMR spectrum signals at δ_H 0.86–0.88 (9H, t, 3 × CH_3) correspond to the cluster of methyl hydrogen atoms. The peaks at δ_H 1.25–1.41 shows the cluster of methylene hydrogen atoms; δ_H 1.94–2.45 corresponds to the cluster of allylic hydrogen atoms and methylene hydrogen atoms immediate to the carbonyl; δ_H 3.43–4.28 indicates oxymethylene hydrogen atoms; and δ_H 4.89–6.19 shows the olefinic hydrogen atoms. The ^{13}C NMR spectrum indicated 23 resonances corresponding to 63 carbons with the carbons assigned positions 1–20 occurring three times. The peak at δ_c 173.37 represents carbonyl carbon; δ_c 62.12–65.06 shows oxymethylene carbons; δ_c 125.30–139.53 indicates olefinic carbons; δ_c 22.71–52.03 represents the methylene carbons; and the peak at δ_c 14.14 corresponds to the methyl carbon. The spectroscopic data of compound **3** were similar to those reported on 1,2,3-Propanetriyl (8Z,11'Z,14"Z)tris(-8,11,14-eicostrienoate) in the literature (Hamid et al., 2017; Lie Ken Jie & Lam, 1995). Hence, the structure of compound **3** was established as 1,2,3-Propanetriyl (8Z,11'Z,14"Z)tris(-8,11,14-eicostrienoate (Figure 3).

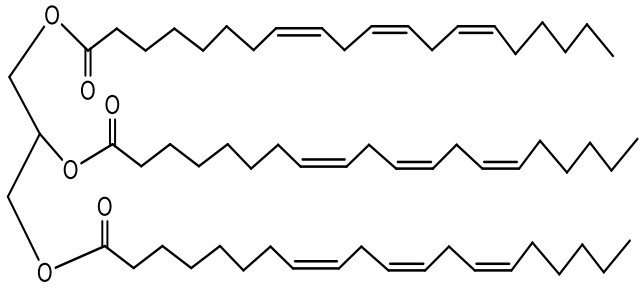

Figure 3. 1,2,3-Propanetriyl (8Z,11'Z,14"Z)tris(-8,11,14-eicostrienoate.

Compound 4 (15 mg) was isolated as a yellow solid. The ^{1}H NMR spectrum signals at δ_H 5.08–6.5 correspond to olefinic hydrogen atoms. The peaks δ_H 0.81–0.91 correspond to the cluster of methyl hydrogen atoms. The peaks at δ_H 3.62 and 4.5 show the oxymethine hydrogen atoms; δ_H 2.05–2.37 corresponds to α- hydrogen atoms to the carbonyl. The peaks at δ_H 1.06–1.55 corresponds to the cluster of methylene hydrogen atoms. The ^{13}C NMR spectrum indicated 31 resonances. The peaks at δ_c 14.14, 16.02, 22.65, 23.45, 24.19, 29.14 correspond to methyl carbons. The signals at δ_c 51.45 and 60.90 represent the oxymethine carbons. δ_c 119.37–142.35 correspond to the olefinic carbons. The spectroscopic data of compound **4** were similar to those reported on oleana-12,15-diene and oleana-5,12-diene in the literature (Bhattacharyya & Cunja, 1992). Therefore, the structure of compound **4** was established as methyl Oleana-5,12,15-trienoate-3β-acetate (Figure 4).

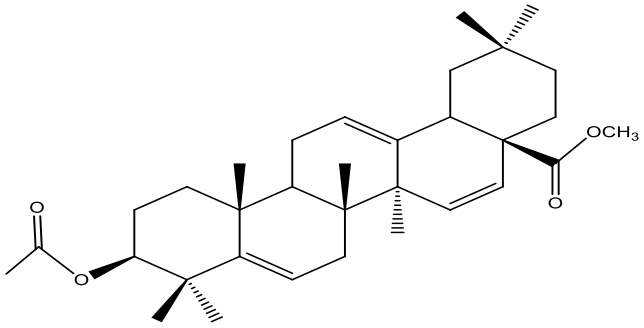

Figure 4. Methyl Oleana-5,12,15-trienoate-3β- acetate.

Compound **5** (42 mg) was obtained as a cream solid. The ^{1}H NMR spectrum signals at δ_H 0.88–0.97 correspond to the methyl hydrogen atoms. The peaks at δ_H 1.25–2.42 correspond to the cluster of methylene hydrogen atoms. The signals at δ_H 4.56–6.46 indicate the olefinic hydrogen atoms and δ_H 3.41–3.94 represent the oxymethylene hydrogen atoms. The ^{13}C NMR spectrum indicated 24 resonances, equivalent to 67 carbons in the compound; with carbon-assignment 1–20 occurring three times. The peak at δ_c 14.11 shows the methyl carbons; δ_c 173.07 indicates the carbonyl carbon. The olefinic carbons are shown by δ_c 124.24–135.10; the oxymethylene carbons are represented by δ_c 62.07–68.89;

and δ_c 24.85–39.72 corresponds to the methylene carbons. The spectroscopic data of compound **5** were similar to those reported on 1,2,3-Propanetriyl (9Z,12'Z,15''Z)tris(-9,12,15-uneicostrienoate) in the literature (Hamid et al., 2017; Chuah et al., 2006; Lie Ken Jie & Lam, 1995). Hence, the structure of compound **5** was established as 1,2,3-Propanetriyl (9Z,12'Z,15''Z)tris(-9,12,15-uneicostrienoate) (Figure 5).

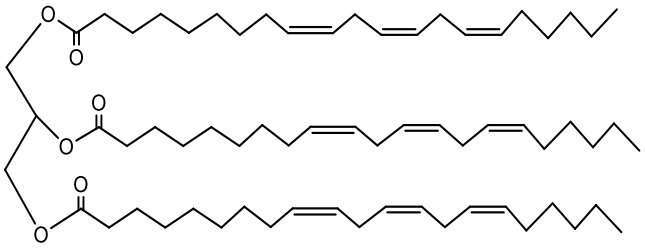

Figure 5. 1,2,3-Propanetriyl (9Z,12'Z,15"Z)tris(-9,12,15-uneicostrienoate)

CONCLUSION

N-hexane, ethyl acetate and methanol extracts of *Momordica angustisepala* indicated the presence of metabolites: triterpenoids, fatty acids and esters. Octadecane (**1**), tetradec-5-enoic acid (**2**), 1,2,3-propanetriyl (8Z,11'Z,14"Z)tris(-8,11.14-eicostrienoate) (**3**), methyl oleana-5,12,15-trienoate-3β-acetate (**4**) and 1,2,3-propanetriyl (9Z,12'Z,15"Z)tris(-9,12,15-uneicostrienooate) (**5**) were isolated from the ethyl acetate-hexane extracts of *M. angustisepala* aerial parts. These compounds are being reported for the first time from this plant.

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Competing Interests: The author declares that there are no competing interests.

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