

SOME FATTY ACID ESTERS OF THE RIPE FRUITS OF *NAUCLEA LATIFOLIA* (FAMILY RUBIACEAE)

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ABSTRACT

Purification of the chloroform extract of the ripe fruits of *N. latifolia* (family: Rubiaceae) using a series of flash chromatography and preparative thin layer chromatography led to the isolation of three fatty acid esters. Characterization and structural elucidation of the esters was based on the use of physical parameters (solubility, melting points), spectroscopic data (UV, IR, ¹H-NMR, ¹³C-NMR, GC-MS) and literature data. The fatty acid esters were identified as ethyl hexadecanoate; methyl, 14-methylpentadecanoate and methyl trans-9-octadecenoate. This is the first report of such esters from the ripe fruits of *N. latifolia*.

Keywords: *N. latifolia*, Ripe Fruits, Chloroform Extract, Spectroscopy, Fatty Acid Esters.

INTRODUCTION

Natural products derived from plants have remained central to traditional medicine and has helped served as sources of new drugs with good therapeutic effect and low toxicity. Unfortunately, "the sleeping giant of pharmaceutical industry"¹ still has several of thousands of plant species yet to be investigated for their phytoconstituents and invariably, their biological potentials. Therefore, the need for continuous search into the phytochemicals of such plants cannot be undermined. *Nauclea latifolia* is a shrub or small tree native to tropical Africa. The leaves are glabrous, opposite, rounded-ovate, glossy green with tufts of hairs. The fruits are usually fleshy, shallow-pitched, with numerous embedded seeds surrounded by a pink edible, sweet- sour pulp. The seeds are usually small, ovoid, numerous and brownish with a pleasant taste but could be emetic if taken in excess.² Traditionally, the plant has been reported as an antimalarial,^{3, 4} antibacterial^{3, 5, 6, 7, 8} and antiviral.^{9, 10, 11} Other potentials of the fruits include, as a laxative¹² and hypocholesterolemic.¹³ Phytochemicals such as alkaloids,^{14, 15} saponins,^{5, 9} tannins, oxalates, phytates^{13, 15} and phthalates^{7, 8, 16} have been detected and isolated from various

parts of the plant. In view of the continuous search for more phytochemicals from the fruits of *N. latifolia*, this paper, therefore, presents the isolation and characterization of some fatty acid esters from the chloroform extract of the ripe fruits of *N. latifolia*.

MATERIALS AND METHODS

Collection of Plant Material

The ripe fruits of *N. latifolia* were collected from a farmland in Maikunkele area of Bosso Local Government area, Minna, Niger State, Nigeria, in the month of September, 2013 and authenticated by Mallam Gallah, of the Herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

Extraction Procedures

Air-dried and pulverized ripe fruits of *N. latifolia* (500g) were extracted with chloroform by continuous extraction until the extractant became colorless. The resulting solution was filtered, concentrated in vacuo and further dried over a water bath to afford a dark brown gummy mass, labeled chloroform extract, C.

Fractionation of Crude Chloroform Extract, C

Three grams of C, was subjected to fractionation by flash column chromatography using silica gel (mesh 60-120, 90g) and hexane as the adsorbent and packing solvent respectively by the slurry method. Elution was carried out with varying proportions of hexane: ethyl acetate and eluents were monitored by TLC, so that identical fractions were pooled. Fractions from solvent system hexane: ethyl acetate (19: 1) and hexane: ethyl acetate (9: 1) yielded promising spots and were labeled fractions C1 and C2 respectively. Both fractions were further purified.

Purification of Fraction C1

TLC of fraction C1 using petroleum ether: chloroform (2:1) as the mobile phase revealed two major and two minor spots which were resolved by further purification. 460mg of C1 was subjected to flash chromatography (silica gel, mesh 230-400, 30g) and elution carried out with varying proportions of petroleum ether and chloroform. Elution with solvent mixture petroleum ether: chloroform (1:1) yielded two major distinct spots on TLC that were resolved using preparative TLC (petroleum ether: ethyl acetate, 9: 1) to yield two different compounds labeled C1-A and C1-B.

Purification of Fraction C2

TLC of fraction C2 using petroleum ether: ethyl acetate (9:1) as the mobile phase revealed one major and three minor spots. 250mg of fraction C2 was subjected to flash chromatography (silica gel, mesh 230-400nm, 30g) and varying proportions of chloroform, ethyl acetate as the mobile phase. Elution with solvent mixture chloroform: ethyl acetate (19:1) yielded one major spot with slight impurities. The obtained sub-fraction was concentrated in vacuo, air-dried and washed severally with methanol in order to remove the impurities. TLC of the sub-fraction revealed a single spot, coded C2-A.

Characterization of Isolates

IR and UV were recorded both in CHCl_3 using FTIR (Spectrolab MB3000) and UV spectra (T60 UV-Visible spectrophotometer) respectively, while GC-MS was recorded using GCMS-QP 2010 plus Shimadzu. $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ (proton-decoupled) and DEPT-135 spectra were taken in CDCl_3 on JEOL LA spectrometer operating at 500MHz. Melting points were uncorrected.

RESULTS AND DISCUSSION

Physical and Spectral Characterization of Isolates.

Compound C1-A: Ethyl hexadecanoate

White waxy-like (29mg); melting point, 25.7-27°C; soluble in chloroform and diethyl ether, slightly soluble in hexane, acetone and ethanol, insoluble in water; TLC, single spotted, R_f 0.50 (no color in sunlight, UV active, golden brown in iodine, blue with vanillin-sulphuric acid + heat and deep brown with 5% alc. KMnO_4). GC-MS revealed its molecular formula and weight as $\text{C}_{18}\text{H}_{36}\text{O}_2$ and 284g/mol respectively. IR (v max, cm^{-1}): 2900-2850 (C-H, aliphatic), 1702.95 (CO) 1487.95 (C-H in CH_3), 1300-1200 (CO, acetate) and 1014.82 (C-H in CH_2). UV (λ max, nm): 274 (CO). $^1\text{H-NMR}$ (δ ppm): 1.26-1.27 (weak triplet, H-1'), 4.04-4.07 (weak quartet, H-2'), 2.18-2.11 (weak quartet, H-2), 1.61-1.63 (weak multiplet, H-3) 1.27 (sharp singlet, H-4 to H-14), 1.30-1.33 (weak multiplet, H-15) and 0.95-1.96 (weak triplet, H-16). $^{13}\text{C-NMR}$ (δ ppm): 13.8 (C-1' and C-16), 60.5 (C-2), 171.6 (C-1), 32.7 (C-2), 24.8 (C-3), 28.2 (C-4), 28.5 (C-5 and C-13), 28.7 (C-6 to C-12), 31.2 (C-14) and 22.3 (C-15). DEPT-135 (δ ppm) one quaternary (C-1), fifteen methylene (C-2', C-2 to C-15), two methyl (C-1' and C-16) and no methine groups. GC-MS (m/z peaks): 284[M⁺, $\text{C}_{18}\text{H}_{36}\text{O}_2$]⁺, 239[M-OC₂H₅]⁺, 157[C₉H₁₇O₂]⁺, 101[C₅H₉O₂]⁺, 88[base peak, C₄H₉O₂]⁺, 73[C₃H₅O₂]⁺, 57[C₄H₉]⁺, 43[C₃H₇]⁺, 41[C₂H₅]⁺, 29 [C₂H₅]⁺, 27 [C₂H₃]⁺. The IR and UV spectra showed characteristic bands indicating the presence of a carboxyl group of an ester. The proton NMR also revealed a single sharp peak at δ 1.27, an indication that several methylene groups were joined together in the same environment, while all other peaks were weak. Carbon-13 revealed that of the ten proton-decoupled peaks, peak at δ 28.7 was the sharpest because of several methylene groups in same environment, while all others were of low intensity, an indication that only one or two carbon atoms were in same environment, while dept 135 showed that the compound was made up of eighteen carbon atoms. GC-MS spectra gave a prominent peak at m/z 239 indicating the loss of OR group from the molecular ion, M⁺. This is typical of an ethyl ester. Characteristic peaks at m/z 86 and 73 are due to Mc-Lafferty rearrangement, while peaks at 239, 157 and 101 and 57, 43, 41, 29 and 27 are due to oxygen- and alkyl-containing fragments respectively. Correlation of obtained data of compound C1-A with literature revealed similar data with ethyl hexadecanoate (Figure 1) a

common fatty acid ester that has been reported and isolated in several medicinal plants.^{18, 19, 20}

Compound C1-B: Methyl, 14-methylpentadecanoate

A golden yellow oil (18.2mg); melting point, 30-34°C; soluble in chloroform, dichloromethane and diethyl ether, slightly soluble in hexane, acetone, ethyl acetate and ethanol, insoluble in water; TLC, single spotted, R_f 0.47 (no color in sunlight; UV active, golden brown in iodine, blue with vanillin-sulphuric acid + heat and deep brown with 5% alc. $KMnO_4$). GC-MS revealed its molecular formula and weight as $C_{17}H_{34}O_2$ and 270gmol^{-1} respectively. IR (ν max, cm^{-1} , CHCl_3): 2905-2800 (C-H, aliphatic), 1701.90 (CO), 1487.95 (C-H in CH_3), 1300-1208 (CO, acetate) and 1012.72 (C-H in CH_2). UV (λ max, nm): 274 (CO). $^1\text{H-NMR}$ (δ ppm, CDCl_3): 3.52-3.54 (sharp singlet, H-1'), 2.08-2.12 (weak triplet, H-2), 1.48-1.51 (weak quintet, H-3), 1.71-1.74 (weak multiplet, H-14), 1.26 (sharp singlet, H-4 to H-13) and 0.99-1.00 (doublet of a doublet, H-14 and H-2'). $^{13}\text{C-NMR}$ (δ ppm): 48.5 (C-1'), 171.9 (C-1), 31.5 (C-2), 25.0 (C-3), 28.7 (C-4), 29.0 (C-5), 29.2 (sharp, C-6 to C-10), 29.8 (C-11), 26.3 (C-12), 36.6 (C-13), 27.6 (C-14), and 22.7 (C-15 and C-2'). DEPT 135 (δ ppm): revealed one quaternary (C-1), twelve methylene (C-2 to C-13), three methyl (C-1' C-2' and C-15) and one methine (C-14) groups. GC-MS (m/z peaks): $270[M^+, C_{17}H_{34}O_2]^+$, $239[M-OCH_3]^+$, $227[M-OC_2H_5]^+$, $101[C_8H_{16}O_2]^+$, $87[C_4H_7O_2]^+$, $74[\text{base peak}, C_3H_6O_2]^+$, $57[C_2H_5]^+$, $43[C_3H_6]^+$, $41[C_3H_5]^+$, $29[C_2H_5]^+$, $27[C_2H_3]^+$. The IR and UV spectra showed characteristic bands indicating the presence of a carboxyl group of an ester. The proton NMR also revealed absence of a peak at carbon-1, an indication that it is a carboxyl group, with no H-atom, while the singlet peak at δ 3.52 is indicative of a methyl group attached to an electron-withdrawing oxygen atom. The weak multiplet peaks at δ 1.71 indicates the proton at H-14 is a methine. A single sharp peak at δ 1.27 is an indication that several methylene groups were joined together in the same environment, while the doublet of a doublet at C-15 and C-2' indicates the protons on both carbons are neighbors to a single proton at C-14 ($n+1$ rule) and therefore in same environment. Carbon-13 NMR revealed twelve proton-decoupled peaks, while dept 135 showed that the compound was made up of seventeen carbon atoms. GC-MS spectra gave prominent peaks at m/z 239, 227 indicating the loss of OR groups from the molecular ion, M^+ . This is typical of a fatty acid ester. Characteristic

peaks at m/z 87 and 74 are due to Mc-Lafferty rearrangement, while peaks at 101 and 57, 43, 41, 29 and 27 are due to oxygen- and alkyl-containing fragments respectively.¹⁷ Correlation of obtained data with literature values revealed that compound C1-B had similar data with methyl, 14-methylpentadecanoate (Figure 2) a fatty acid ester that has been reported and isolated in several medicinal plants.^{21, 22, 23}

Compound C2-A: Methyl trans-9-octadecenoate

Cream-colored oil (15.7mg); melting point, 10-12°C, soluble in hexane, chloroform, diethyl ether, slightly soluble in ethyl acetate, acetone, methanol and ethanol, insoluble water; TLC, single spotted, R_f 0.41 (no color in sunlight, UV active, golden brown in iodine, blue with vanillin-sulphuric acid + heat and deep brown with 5% alc. $KMnO_4$). GC-MS revealed its molecular formula and weight as $C_{19}H_{36}O_2$ and 296gmol^{-1} respectively. IR (ν max, cm^{-1}): 2900-2850 (C-H, aliphatic), 1702.95 (CO), 1612.88 (alkene), 1486.55 (C-H in CH_3), 1302-1200 (CO, acetate) and 1014.77 (C-H in CH_2). UV (λ max, nm): 275 (CO), 180 (alkene). $^1\text{H-NMR}$ (δ ppm): 3.59 (strong doublet, H-1'), 2.18-2.21 (weak multiplet, H-2), 1.59-1.61 (weak triplet, H-3), 1.27 (sharp singlet, H-4, H-5, H-6, H-13, H-14, H-15 and H-16), 1.30 (sharp singlet, H-7, H-12 and H-17), 1.91-1.94 (weak multiplet, H-8 and H-11), 5.29 (weak doublet, H-9 and H-10) and 0.95 (weak triplet, H-18). $^{13}\text{C-NMR}$ (δ ppm): 49.2 (C-1'), 172.8 (C-1), 32.4 (C-2, C-9 and C-11), 24.3 (C-3), 28.2 (C-4), 28.5 (C-5 and C-15), 28.9 (C-6, C-13 and C-14), 29.4 (C-7 and C-12), 128.9 (C-9 and C-10), 30.6 (C-16), 21.8 (C-17) and 13.8 (C-18). DEPT 135 (δ ppm): revealed one quaternary (C-1), fourteen methylene (C-2 to C-8 and C-11 to C-17), two methyl (C-1' and C-18) and two methine (C-9 and C-10) groups. GC-MS (m/z peaks): $296[M^+, C_{19}H_{36}O_2]^+$, $264[M-OCH_3]^+$, $222[M-OC_4H_9]^+$, $180[M-C_6H_{13}O]^+$, $137[M-C_9H_{18}O_2]^+$, $123[M-C_{10}H_{21}O_2]^+$, $101[C_6H_{13}O]^+$, $97[C_6H_9O]^+$, $87[C_4H_7O_2]^+$, $74[C_3H_5O_2]^+$, $69[C_3HO_2]^+$, $56[C_4H_9]^+$, $55[\text{base peak}, C_4H_7]^+$, $43[C_3H_6]^+$ and $41[C_3H_5]^+$. IR suggests it is an ester, while UV shows the compound possesses sp^2 hybridized carbon atoms. Its Proton NMR suggests the presence of deshielded protons of an allylic group (H-9 and H-10) which occurred at δ 5.29, downfield δ 3.59 of a methyl group attached to an electron withdrawing oxygen atom. Generally, π bonds are effective in influencing the chemical shift of nearby atoms, so that allylic H's are shifted downfield/higher δ values.²⁴ Sharp singlets at δ 1.27 and δ 1.30 indicate several protons in same environment.

Carbon-13 revealed twelve proton-decoupled peaks, of which peaks at 832.4, 28.5, 28.9 and 29.4 were of strong intensities. The weak peak at 5172.8 is attributable to the presence of a carbonyl group. Generally, a carbonyl group exhibits anisotropic effect on adjacent atoms. This causes the carbonyl carbon and the hydrogen atoms bonded to them to resonate at the lowest field position because of combined effects of the induced anisotropic field and a nearby electronegative element.²⁴ DEPT 135 revealed the compound is made up of nineteen carbon atoms. Its GC-MS spectrerevealed prominent peaks at m/z 284, 222 and 180 due to the loss of OR groups from the parent, M. Other peaks are as a result of oxygen-containing fragment (137, 123, 101, 97, 84, 74 and 69) and alkyl-containing fragment (56, 55, 43 and 41).²⁵ Correlation of obtained data with literature values revealed similar data with methyl trans-9-octadecenoate (Figure 3), it has been

reported and isolated from some medicinal plants.^{15, 26}

CONCLUSION

Purification of the crude chloroform extract of the ripe fruits of *N. latifolia* led to the isolation of three fatty acid esters. The esters were characterized based on spectroscopic data and identified as palmitic acid, ethyl ester, iso-palmitic acid, methyl ester and elaidic acid, methyl ester. Further work will be directed towards isolating and characterizing more bio-active principles from the ripe fruits of *N. latifolia*.

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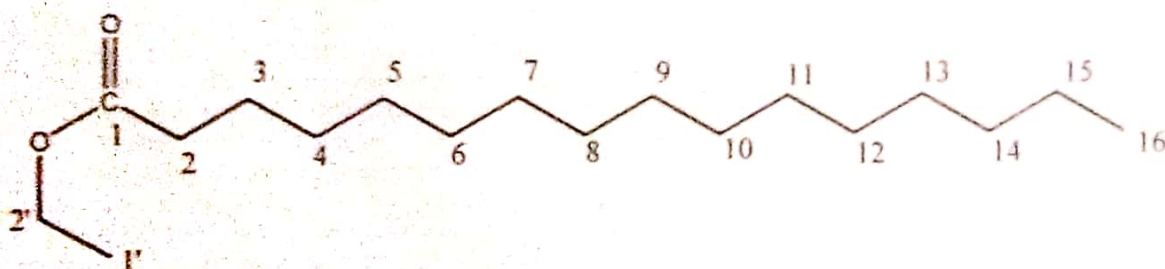


Fig. 1: Ethyl palmitate/Palmitic acid, ethyl ester/Hexadecanoic acid, ethyl ester

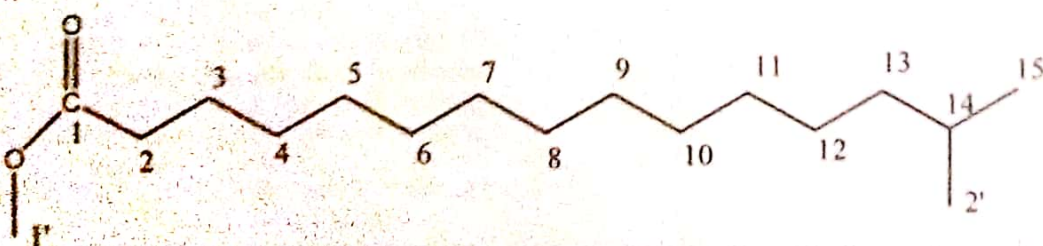


Fig. 2: Pentadecanoic acid, 14-methyl, methyl ester/Methyl, 14-methylpentadecanoate/ Iso-Palmitic methyl ester

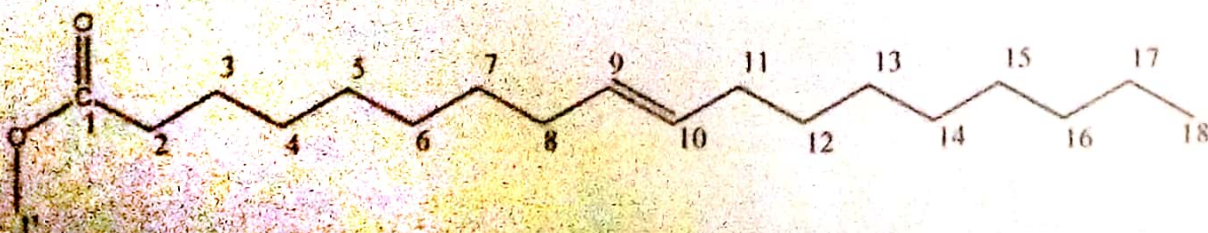


Fig. 3: Methyl trans-9-octadecenoate/9-Octadecenoic acid, methyl ester (E) /Elaidic acid, methyl ester/Methyl elaidate

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