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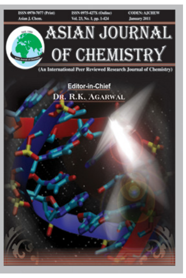

Fatty Acid Esters from Roots of *Amaranthus hybridus* Linn.

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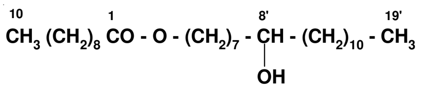
New fatty acid esters were isolated from ethanolic extract of roots of *Amaranthus hybridus* Linn. (Amaranthaceae). The structures of these compounds were established on the basis of the detailed chemical reaction and spectral analysis as 8'-hydroxynonadecanyl-*n*-decanoate (**1**), *n*-docos-11-enoic acid (**2**), *n*-pentacosonyl-*n*-octadec-9-enoate (**3**), *n*-tricosanyl-*n*-octadec-9,12-dienoate (**4**).

Key Words: *Amaranthus hybridus* Linn., Amaranthaceae, Fatty acid ester.

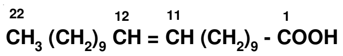
INTRODUCTION

Amaranthus hybridus Linn. (Amaranthaceae) commonly known as smooth pigweed is an erect branched annual herb found throughout tropical and temperate regions of India as a common weed in fields and wastelands[1-5]. The plant has been used in treating dysentery ulcers and hemorrhage of the bowel due to its astringent property[6]. Leaves have a cleansing effect and also help to reduce tissue swelling[1]. The plant has been found to be non-toxic and posses antibacterial potency[7]. In the present paper, the isolation and structure elucidation of 8'-hydroxynonadecanyl-*n*-decanoate (**1**), *n*-docos-11-enoic acid (**2**), *n*-pentacosonyl-*n*-octadec-9-enoate (**3**), *n*-tricosanyl-*n*-octadec-9,12-dienoate (**4**) from root of the plant are described.



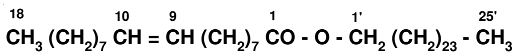
$\overset{10}{CH_3}(CH_2)_8\overset{1}{CO}-O-(CH_2)_7-\underset{OH}{\overset{8'}{CH}}-(CH_2)_{10}-\overset{19'}{CH_3}$

1



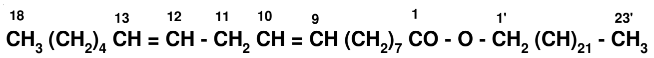
$\overset{22}{CH_3}(CH_2)_9\overset{12}{CH}=\overset{11}{CH}(CH_2)_9-\overset{1}{COOH}$

2



$\overset{18}{CH_3}(CH_2)_7\overset{10}{CH}=\overset{9}{CH}(CH_2)_7\overset{1}{CO}-O-\overset{1'}{CH_2}(CH_2)_{23}-\overset{25'}{CH_3}$

3



$\overset{18}{CH_3}(CH_2)_4\overset{13}{CH}=\overset{12}{CH}-\overset{11}{CH_2}\overset{10}{CH}=\overset{9}{CH}(CH_2)_7\overset{1}{CO}-O-\overset{1'}{CH_2}(CH)_{21}-\overset{23'}{CH_3}$

4

EXPERIMENTAL

Melting points, uncorrected are taken on a Complab melting point apparatus. Optical rotations were measured on JASCO-1020 polarimeter, the UV spectra were measured with UV-visible-NIR spectrophotometer (Carry 5000 Varian Australia Pvt. Ltd.). IR spectra were obtained using a Shimadzu (FTIR 8000 using KBR pellets). ^1H and ^{13}C NMR spectra were recorded on Bruker Avance II (400 MHz, USA). Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. EIMS were recorded on Jeol SX-102 mass spectrometer. Column chromatography was carried out using neutral alumina (Qualigens). Merk precoated silica gel 60 F_{254} plates were used for TLC.

The roots of *Amaranthus hybridus* Linn. were collected from the campus of Guru Jambeshwar University of Science and Technology, Hisar, Haryana in July 2008. Identity and authentification of plant was carried out at National Institute of Science Communication and Information Resources, New Delhi by Dr. H. B. Singh where a voucher specimen was lodged vide reference number, NISCAIR/RHMD/CONSULT/2008-09/1044/75. A voucher specimen of the same has been retained in the Department for the future reference.

Extraction and isolation: The air dried powdered roots of *Amaranthus hybridus* Linn. (3 kg) were soxhlet extracted with 95 % ethanol for 72 h and the combined ethanolic extracts were collected and evaporated under reduced pressure and kept in desiccators. The dried ethanolic extract was dissolved in minimum amount of methanol and adsorbed on silica gel (for column 80-120) to form slurry. The air-dried slurry was chromatographed on neutral alumina column loaded in petroleum ether (b.p. 60-80 ºC). The column was eluted with gradient solvent system of petroleum ether, petroleum ether-chloroform, chloroform, chloroform-ethyl acetate, ethyl acetate, ethyl acetate-methanol and methanol in increment of polarity to afford the following compounds[8,9]:

8'-Hydroxynonadecanyl-*n*-decanoate (1): Elution of the column with petroleum ether furnished colourless crystals of compound **1**, recrystallized from acetone: MeOH (1:1), 265 mg (yield (%) 0.19), R_f: 0.96 (pet. ether), $[\alpha]_D^{30}$ -89º [$CHCl_3$], m.p. 72-73 ºC; UV λ_{max} (MeOH): 268, 272, 284 nm; IR (KBr, ν_{max}, cm^{-1}): 3414, 2923, 2856, 1735, 1649, 1461, 1381, 1229, 1023, 953, 717; ^1H NMR ($CDCl_3$): δ 3.84 (1H, m, H-8'), 3.22 (2H, brs, H_2-1'), 2.05 (1H, d, *J* = 6.6 Hz, H_2-2a), 2.01 (1H, d, *J* = 6.2 Hz, H_2-2b), 1.53 (2H, m, CH_2), 1.25 (44H, brs, 22 × CH_2), 0.88 (3H, t, *J* = 8.1Hz, Me-10), 0.82 (3H, t, *J* = 7.6 Hz, Me-19'); ^{13}C NMR ($CDCl_3$): δ 177.3 (C-1), 63.2 (C-1'), 29.50 (CH_2), 28.96 (CH_2), 28.90 (CH_2), 28.12 (CH_2), 27.21 (CH_2), 25.32 (CH_2), 25.01 (CH_2), 24.04 (CH_2), 22.08 (CH_2), 21.92 (CH_2), 14.42 (C-19), 14.11 (C-10); +ve FAB MS m/z (rel. int.): 454 $[M]^+$ ($C_{29}H_{58}O_3$) (21.5), 185 (100), 171 (35.6), 155 (85.3).

Acetylation of compound 1: Compound **1** (20 mg) was taken in a round bottom flask and (0.5 mL) pyridine was mixed to it and the solution was cooled to 10 ºC. Then acetic anhydride (0.5 mL) was mixed and the solution was kept at room temperature for over night. The reaction mixture was poured on to ice water. A white crystalline solid obtained was extracted with ethyl acetate (4 × 10 mL). The combined organic layer was washed with 5 % aqueous $NaHCO_3$ solution. The organic layer was concentrated to dryness. The white solid was crystallized in $CHCl_3$:MeOH mixture, which afforded white crystalline solid.

***n*-Docos-11-enoic acid (2):** Elution of the column with petroleum ether-chloroform (3:1) afforded colourless crystalline mass of compound **2**, recrystallized from acetone MeOH (1:1), 295 mg (yield (%) 0.21), R_f: 0.48 (petroleum ether:$CHCl_3$, 9:1), $[\alpha]_D^{30}$ + 26º [$CHCl_3$], m.p. 98 -99 ºC; UV λ_{max} (MeOH): 272, 275, 286 nm; IR (KBr, ν_{max}, cm^{-1}): 3408, 2927, 2857, 1667, 1640, 1458, 1359, 1261, 1120, 725; ^1H NMR ($CDCl_3$): δ 5.48 (2H, m, H-11, H-12), 2.62 (1H, m, H_2- 2a), 2.58 (1H, m, H_2-H_2-2b), 2.01 (4H, m, H_2-13, H_2-10), 1.51 (4H, brs, 2 × CH_2), 1.25 (26H, brs 13 × CH_2), 0.88 (3H, t, *J* = 7.8Hz, Me-22); ^{13}C NMR ($CDCl_3$): δ 174.76 (C-1), 129.42 (C-11), 128.63 (C-12), 62.02 (C-1'), 28.42 (20 × CH_2), 28.10 (CH_2), 27.94 (CH_2), 13.69 (C-22); +ve FAB MS m/z (rel. int.): 338 $[M]^+$ ($C_{22}H_{42}O_2$) (9.8).

***n*-Pentacosonyl-*n*-octadec-9-enoate (3):** Compound **3** was obtained a colourless crystalline mass from petroleum-ether $CHCl_3$ (1:1), recrysallized from acetone MeOH (1:1), 298 mg (yield (%) 0.22), R_f: 0.27 (petroleum ether:$CHCl_3$, 75:25), $[\alpha]_D^{30}$ + 12.6º [$CHCl_3$], m.p. 86-87 ºC; UV λ_{max} (MeOH): 272, 275, 286 nm; IR (KBr, ν_{max}, cm^{-1}): 2926, 2857, 1738, 1632, 1462, 1378, 1251, 1179, 1036, 724; ^1H NMR ($CDCl_3$): δ 5.36 (1H, m, H-9), 5.34 (1H, m, H-10), 4.13 (1H, d, *J* = 6.9 Hz, H_2-1'a) 4.11 (1H, d, *J* = 7.2 Hz, H_2-1'b), 2.34 (1H, d, *J* = 8.1 Hz, H_2-2a), 2.27 (1H, d, *J* = 7.5 Hz, H_2-2b), 2.05 (2H, m, H_2-8), 1.85 (2H, m, H_2-11), 1.51 (6H, m, 3 × CH_2), 1.25 (62H, brs, 11 × CH_2), 0.90 (3H, t, *J* = 6.3 Hz), Me-18), 0.88 (3H, t, *J* = 6.2 Hz, Me-25'); ^{13}C NMR ($CDCl_3$): δ 173.12 (C-1), 131.4 (C-11), 129.60 (C-10), 129.02 (C-9), 128.92 (C-10), 60.24 (C-1'), 35.02 (C-2), 29.24 (11 × CH_2), 28.92 (5 × CH_2), 28.0 (4 × CH_2), 26.94 (CH_2), 13.68 (C-18), 14.0 (C-25'), 24.04 (CH_2), 22.08 (CH_2), 21.92 (CH_2), 14.42 (C-19), 14.11 (C-10) ; +ve FAB MS m/z (rel. int.): 632 $[M]^+$ ($C_{43}H_{84}O_2$) (1.5), 367 (29.8), 265 (31.5).

***n*-Tricosanyl-*n*-octadec-9,12-dienoate (4):** Elution of the column with $CHCl_3$ yielded colourless crystals of compound **4**, recrystallized from acetone MeOH (1:1), 385 mg (yield (%) 0.28), R_f: 0.51 ($CHCl_3$), $[\alpha]_D^{30}$ + 4.5º [$CHCl_3$], m.p. 88-89 ºC; UV λ_{max} (MeOH): 275, 279, 286 nm; IR (KBr, ν_{max}, cm^{-1}): 2926, 2857, 1742, 1655, 1461, 1377, 1169, 1045, 722; ^1H NMR ($CDCl_3$): δ 5.37 (1H, m, H-10), 5.34 (1H, m, H-12), 5.14 (1H, m, H-9), 5.06 (1H, m, H-13), 3.61 (1H, m, H_2-1'a), 3.59 (1H, m, H_2-1'b), 2.77 (2H, m, H_2-2), 2.36 (2H, m, H_2-11), 2.31 (2H, m, H_2-8), 2.27 (2H, m, H_2-14), 2.03 (2H, m, CH_2), 1.77 (2H, m, CH_2), 1.62 (2H, m, CH_2), 1.25 (4OH, brs, 24 × CH_2), 0.89 (3H, t, *J* = 6.5 Hz, Me-18), 0.85 (3H, t, *J* = 6.3 Hz, Me-23); ^{13}C NMR ($CDCl_3$): δ 176.0 (C-1), 130.04 (C-13), 129.6 (C-12), 62.08 (C-1'), 35.05 (C-2), 29.88 (22 × CH_2), 28.92(CH_2), 29.01(CH_2), 28.24 (CH_2), 27.98 (CH_2), 27.05 (CH_2), 26.02 (CH_2), 24.76 (CH_2), 21.35 (CH_2), 14.26 (CH_2), 14.15 (CH_2); +ve FAB MS m/z (rel. int.): 602 $[M]^+$ ($C_{41}H_{78}O_2$) (33.6), 339 (100), 263 (25.2).

RESULTS AND DISCUSSION

8'-Hydroxynonadecanyl-*n*-decanoate was obtained as a colourles crystalline mass from petroleum ether eluants. Its IR spectrum showed characteristic absorption bands for hydroxyl group (3414 cm^{-1}), ester group (1735 cm^{-1}) and long aliphatic chain (717 cm^{-1}). The mass spectrum of compound **1** exhibited a molecular ion peak at m/z 454 consistent with the molecular formula of a hydroxyl fatty acid ester $C_{29}H_{58}O_3$. It indicated one double bond equivalent which was adjusted in the ester group. The important ion peaks arising at m/z 155 $[CH_3(CH_2)_8CO]$ and 171 $[CH_3(CH_2)_8COO]^+$ suggested that capric acid was esterfied with C_{19} aliphatic dihydroxy alcohol. The ion peak at m/z 185 $[CHOH\ (CH_2)_{10}CH_3^+]$ indicated the location of the hydroxyl group at C-8'. The ^1H NMR spectrum exhibited a one proton multiplets at δ 3.84 assigned to hydroxy methine H-8' proton. A two proton broad signal at δ 3.22 was ascribed to oxygenated methylene H_2-1' proton. Two one-proton doublets at δ 2.05 (*J* = 6.6 Hz) and 2.01 (*J* = 6.2 Hz) were attributed to methylene H_2-2 protons adjacent to the ester group. A two-proton multiplet and a broad signal at δ 1.25 (44 H) were due to others methylene protons. Two three proton triplets at δ 0.89 (*J* = 8.1 Hz) and 0.82 (*J* = 7.6 Hz) were accounted to primary C-10 and C-19' methyl protons. The absence of any signal beyond δ 3.84 indicated saturated nature of the molecule. The ^{13}C NMR spectrum of the compound fully supported the structure above, it shows 29 carbons and revealed the presence of two terminal methyl and one OH on the CH of 8'. Acid show a peak at δ 177.3. On the basis of the foregoing account the structure of compound **1** has been formulated as 8'-hydroxynonadecanyl-*n*-decanoate. This is a new fatty acid ester isolated from a natural source for the first time.

Compound **2**, *n*-docos-11-enoic acid, was obtained as a colourless crystalline mass from petroleum ether-chloroform (3:1) eluants. It produces effervescences with sodium bicarbonate solution. Its IR spectrum showed distinctive absorption bands for carboxylic group (3408, 1667 cm^{-1}), unsaturation (1640 cm^{-1}) and long aliphatic chain (725 cm^{-1}). The mass spectrum of compound **2** showed a molecular ion peak at m/z 338 corresponding to a formula of a fatty acid, $C_{22}H_{42}O_2$. It had two double bond equivalents which were adjusted one each in the vinaylic linkage and carboxylic function. The ^1H NMR spectrum of compound **2** displayed a two proton multiplet at δ 5.48 assigned to vinylic H-11, H-12 protons. Two-one proton multiplets at δ 2.62 amd 2.58 were ascribed to methylene H_2- 2 protons adjacent to carboxylic group. The remaining methylene protons appeared as a multiplet at δ 2.02 (4H) and as broad signals at δ 1.51 (4H) and 1.25 (26H). A three-proton triplet at δ 0.88 (J = 7.8 Hz) was accounted to C-22 primary methyl protons. Two vinylic carbon showed the peak at δ 128.92 and δ 131.4, acid show at δ 173.12, one methyl which is adjacent to the acid showed the peak at δ 60.24. On the basis of the foregoing account, the structure of compound **2** has been elucidated as *n*-docos-11-enoic acid.

Compound **3**, named *n*-pentacosonyl-*n*-octadec-9-enoate, was obtained as a colourless crystalline mass from petroleum ether chloroform (1:1) eluants. Its IR spectrum showed characteristic absorption bands for ester group (1738 cm^{-1}), unsaturation (1632 cm^{-1}) and long aliphatic chain (724 cm^{-1}). On the basis of mass spectrum, the molecular weight of compound **3** has been established at m/z 632 corresponding to a fatty acid ester, $C_{43}H_{84}O_2$. It had two degrees of unsaturation, one each of them were adjusted in the vinylic linkage and ester group. The formation of the ion peaks at m/z 265 $[CH_3(CH_2)_7CH=CH(CH_2)_7CO]^+$ and 367 $[M-265, O(CH_2)_{24}CH_3]^+$ indicated that oleic acid was esterified with a C_{25} aliphatic alcohol. The ^1H NMR spectrum of compound **3** showed two one-proton multiplet at δ 5.36 and 5.34 assigned to vinylic H-9 and H-10 proton. Two one proton doublets at δ 4.13 (J = 6.9 Hz) and 4.11 (J = 7.2 Hz) were ascribed to oxygenated methylene H_2-1 protons. Two one proton doublets at δ 2.34 (J = 8.1 Hz) and 2.27 (J = 7.5 Hz) were attributed to methylene H_2-2 protons adjacent to the ester group. The remaining methylene protons appeared as multiplets at δ 2.05 (2H), 1.85 (2H) and 1.51 (6H) and as a broad signal at δ 1.25 (62H). Two three proton triplets at δ 0.90 (J = 6.3 Hz) and 0.88 (J = 6.2 Hz were accounted to C-18 and C- 25' primary methyl protons. Two vinylic carbon showed the peak at δ 128.92 and δ 131.4, acid show at δ 173.12, one methyl which is adjacent to the acid showed the peak at δ 60.24. Alkaline hydrolysis of compound **3** yielded oleic acid, Co-TLC comparable. On the basis of spectral data analysis and chemical reactions, the structure of compound **3** has been identified as *n*-pentacosonyl-*n*-octadec-9-enoate.

Compound **4** designated as *n*-tricosanyl-*n*-octadec-9,12-dienoate, was obtained as a colourless crystalline mass from the chloroform eluants. Its IR spectrum showed characteristics absorption bands for ester group (1742 cm^{-1}), unsaturation (1655 cm^{-1}) and long aliphatic chain (722 cm^{-1}). The mass spectrum of compound **4** exhibited a molecular ion peak at m/z 602 consistent with molecular formula of a fatty acid ester, $C_{41}H_{78}O_2$. It had three double bond equivalents; two of them were adjusted in the vinylic linkage and the remaining one in the ester group. The formation of the prominent ion peaks at m/z 263 $[CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7CO]^+$ and 339 $[M-263, O(CH_2)_{22}CH_3]^+$ suggested that linoleic acid was esterified with C23 aliphatic alcohol. The ^1H NMR spectrum of compound **4** showed four one proton multiplets at δ 5.37, 5.34, 5.14 and 5.06 assigned to vinylic H-10, H-12, H-9 and H-13 protons, respectively. Two one-proton multiplets at δ 3.61 and 3.59 were ascribed to oxygenated methylene H_2-1' protons. The remaining methylene protons resonated between δ 2.77-1.25. Two three-proton triplets at δ 0.89 (J = 6.5 Hz) and 0.85 (J = 6.3 Hz) were accounted to terminal C-18 and C-23' primary methyl protons. The ^{13}C NMR spectrum of the compound fully supported the structure suggested above, it showed the presence of two terminal methyl, long chain of methylene in an agreement with the proposed molecular mass (ESMS). Out of these signals at δ 177.02 was for acid. Alkaline hydrolysis of compound **4** yielded linoleic acid, Co-TLC comparable. On the basis of above discussion the structure of compound **4** has been elucidated as *n*-tricosanyl-*n*-octadec-9,12-dienoate.

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