Original Article

Isolation and Characterization of Fatty Acid Esters from the Seeds of *Cichorium intybus*

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ABSTRACT

Phytochemical investigation of the methanolic extract of the seeds of *Cichorium intybus* L. (Asteraceae) yielded five fatty acid esters characterized as *n*-hexadecanyl hexadecanoate (**1**); *n*-pentadecanyl octadec-9-enoate (**2**); *n*-hexadecanyl octadec-9-enoate (**3**); *n*-hexadecanyl octadecan oate (**4**) and *n*-octadecanyl octadecanoate (**5**). The structures of these phytoconstituents were elucidated on the basis of spectral data analysis and chemical reactions.

Keywords: *Cichorium intybus*, Asteraceae, Fatty acid ester.

INTRODUCTION

Cichorium intybus L. (Asteraceae), popularly known as Kasini or Chicory, is an erect perennial herb, 1-3 feet high with a fleshy tap root[1]. Historically, chicory was grown by the ancient Egyptians as a medicinal plant, coffee substitute, and vegetable crop and was occasionally used for animal forage. Despite its long tradition of use, the plant is not described in any official Pharmacopoeia of a country[2]. The plant exhibited antidiabetic[3], antihepatotoxic[4], antimicrobial[5], antioxidant[6], anticancer[7] and antimalarial activities[8]. Chicoric acid has been identified as the major compound in methanolic extracts of chicory. Aliphatic compounds and their derivatives comprise the main fraction while terpenoids comprise minor constituents of the plant. The flowers of chicory contain saccharides, methoxycoumarin, cichorine, flavonoids and essential oils. The principal volatile components of chicory include: octane, *n*-nonadecane, pentadecanone and hexa-

decane[9-12]. As the part of our ongoing research on the phytochemical screening of plants, the present paper describes the isolation and characterization of five fatty acid esters (see figure **1-5**) from the seeds of *C. intybus.*

MATERIALS AND METHODS

General experimental procedures

MPs were determined on a Perfit apparatus and are uncorrected. The IR spectra were recorded in KBr pellet on FTIR spectrometer 135 instruments (Biorad, USA). ^{1}H (300 MHz) and ^{13}C (75 MHz) spectra were recorded on a Bruker spectrospin NMR instrument in deuterated $CDCl_3$ using TMS as internal standard. Column chromatography was performed on silica gel (Merck, 60–120 mesh) and thin layer chromatography on silica gel G-coated TLC plates (Merck).

Plant material

The seeds of *Cichorium intybus* (3 kg) were procured from local crude drug market, Delhi. A voucher specimen (PRL/JH/2010/02) was deposited in the Phytopharmaceuticals Research Laboratory, Faculty of Pharmacy, Hamdard University, New Delhi, India.

Extraction and isolation

The dried seeds of *Cichorium intybus* were coarsely powdered and extracted with methanol in a Soxhlet apparatus for 72 h. The extract was concentrated under reduced pressure to obtain a dark viscous mass. It was chromatographed over silica gel (60-120 mesh) packed in petroleum ether (60-80°C). Elution with chloroform yielded 26 fractions (250 ml each), from these fractions compounds **1-5** were isolated.

Compound 1

Fraction 4-8 gave colourless semi-solid mass of compound **1**, from $CHCl_3$: MeOH (1:1). UV λ_{max} (MeOH): 214, 268 nm, IR ν_{max} (KBr): 2925, 2856, 1740, 1458, 1379, 1257, 1164, 1097, 725 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 4.21 (1H, d, J=5.3 Hz, H_2-1′a), 4.08 (1H, d, J=5.6 Hz, H_2-1′b), 2.24 (2H, t, J=7.2 Hz, H_2-2), 1.54 (2H, m, CH_2), 1.23 (8H, brs, 4xCH_2), 1.18 (44H, brs, 22xCH_2), 0.81 (3H, t, J= 6.2Hz, Me-16), 0.78 (3H, t, J=6.5 Hz, Me-16′). ^{13}C NMR ($CDCl_3$): δ 171.27 (C-1), 67.34 (C-1′), 32.57 (CH_2), 32.27 (CH_2), 30.26 (CH_2), 28.02 (14xCH_2), 27.83 (CH_2), 27.68 (CH_2), 27.33 (CH_2), 27.23 (CH_2), 25.55 (CH_2) 23.39 (CH_2), 23.24 (CH_2), 23.18 (CH_2), 23.05 (CH_2), 21.01 (CH_2), 12.39 (Me-16), 12.33 (Me-16′). ESIMS *m/z (rel. int.):* 480 $[M]^+$ ($C_{32}H_{64}O_2$), (7.2), 239 (5.3), 225 (100).

Compound 2

Fraction 11-15 yielded pale yellow sticky mass of compound **2**, from $CHCl_3$: MeOH (1:1). UV λ_{max} (MeOH): 212, 270 nm. IR ν_{max} (KBr): 2928, 2736, 1729, 1651, 1452, 1273, 1110, 1045, 750 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 5.35 (1H, m, H-9), 5.33 (1H, m, H-10) 4.13 (2H, t, J=6.6 Hz, H_2-1′), 2.27 (2H, t, J=7.5 Hz, H_2-2), 2.03 (1H, m, H_2-8), 1.84 (2H, m, H_2-11), 1.64 (2H, m, 4×CH_2), 1.59 (2H, m, CH_2), 1.29 (8H, brs, 4×CH_2), 1.25 (36H, brs, 18×CH_2), 0.87 (3H, t, J=6.3 Hz, Me-18), 0.84 (3H, t, J=6.5 Hz, Me-15′). ^{13}C NMR ($CDCl_3$): δ 173.06 (C-1), 130.96 (C-9), 128.85 (C-10), 71.82 (C-1′), 31.94 (CH_2), 31.51 (CH_2), 29.71 (20×CH_2), 29.38 (CH_2), 29.12 (CH_2), 29.07 (CH_2), 27.73 (CH_2), 22.71 (CH_2), 19.81 (Me-15), 14.16 (Me-18). ESIMS *m/z (rel. int.):* 492 $[M]^+$ ($C_{33}H_{64}O_2$) (24.3).

Compound 3

Fraction 16-19 afforded colourless amorphous powder of compound **3**, from $CHCl_3$: MeOH (1:1). IR ν_{max} (KBr): 2927,

2839, 1736, 1645, 1402, 1115, 803, 725 cm^{-1}. ^1H NMR ($CDCl_3$) 5.21 (2H, m, H-9, H-10), 4.24 (2H, t, J=6.5 Hz, H_2-1′), 2.52 (2H, t, J=7.2 Hz, H_2-2), 2.24 (2H, m, H_2-8), 2.20 (2H, m, H_2-11), 1.52 (2H, m, CH_2), 1.23 (28H, brs, 24×CH_2), 0.85 (3H, t, J=6.1 Hz, Me-18), 1.82 (3H, t, J=6.3 Hz, Me-16′). ESIMS *m/z (rel. int.):* 506 $[M]^+$ $C_{34}H_{66}O_2$ (11.5).

Compound 4

Fraction 20-24 yielded colourless semi solid of compound **4**, from $CHCl_3$: MeOH (1:1). IR ν_{max} (KBr): 2927, 2855, 1737, 1445, 1382, 1262, 725 cm^{-1}. ^1H NMR ($CDCl_3$) : δ 4.26 (2H, t, J=10.5 Hz, H_2-1′), 2.31 (2H, t, J=7.5 Hz, H_2-2), 1.82 (2H, m, CH_2), 1.64 (2H, m, CH_2), 1.59 (2H, m, CH_2), 1.32 (8H, brs, 4xCH_2), 1.23 (44H, brs, 22xCH_2), 0.87 (3H, t, J=6.3 Hz, Me-18), 0.84 (3H, t, J=6.1 Hz Me-16′). ESIMS *m/z (ret. int.):* 508$[M]^+$ ($C_{34}H_{68}O_2$) (23.7), 283(14.1.)

Compound 5

Fraction 25-26 furnished pale yellow sticky mass of compound **5**, from $CHCl_3$: MeOH (1:1). IR ν_{max} (KBr): 2926, 2855, 1735, 1459, 1370, 1251, 1196, 726 cm^{-1}. ^1H NMR ($CDCl_3$): δ 4.06 (2H, t, J=6.0 Hz, H_2-1′), 2.30 (2H, t, J=7.2 Hz, H_2-2), 1.64 (2H, m, CH_2), 1.59 (2H, m, CH_2), 1.30 (6H, brs, 3xCH_2), 1.28 (6H, brs, 3xCH_2), 1.25 (46H, brs, 23xCH_2) , 0.90 (3H, t, J=6.5 Hz, Me-18), 0.88 (3H, t, J=6.3 Hz, Me-18′). ^{13}CMNR ($CDCl_3$): δ 173.80 (C-1), 63.79 (C-1′), 51.59 (C-2), 34.07 (CH_2), 33.69 (CH_2), 31.78 (CH_2), 31.57 (CH_2), 31.23 (CH_2), 29.49 (13xCH_2), 29.49 (CH_2), 29.44 (CH_2), 29.34 (CH_2), 29.19 (CH_2), 29.15 (CH_2), 29.06 (CH_2), 29.01 (CH_2), 28.92 (CH_2), 28.33 (CH_2), 28.77 (CH_2), 27.85 (CH_2), 27.02 (CH_2), 25.65 (CH2), 24.87 (CH_2), 24.83 (CH_2), 24.64 (CH_2), 22.57 (CH_2), 14.38 (C-18), 14.27 (C-18′). ESIMS *m/z (rel. int.)*: 536 $[M]^+$ ($C_{36}H_{72}O_2$) (18.2), 283 (4.6).

RESULT AND DISCUSSION

Compound 1, a fatly acid ester, was obtained as semisolid mass from chloroform eluants. Its FTIR displayed characteristic absorption bands for ester group (1740 cm^{-1}) and aliphatic moiety (1097, 725 cm^{-1}). Its ESI-MS exhibited a molecular ion peak at *m/z* 480 corresponding to the molecular formula $C_{32}H_{64}O_2$ of a fatty acid ester. It indicated the presence of one double bond equivalent that was adjusted in an ester linkage. The base peak at *m/z* 225 $[C_{16}H_{33}]^+$ and other ion peak at *m/z* 239 $[C_{16}H_{31}O]^+$ arising due to ester linkage fission supported the presence of two C_{16} units in the compound. The ^1H NMR of compound **1** displayed two one-proton doublets at δ 4.21 (J=5.3 Hz) and 4.08 (J=5.6 Hz) assigned to H-l′a and H-l′b oxygenated methine protons, respectively. A two-proton triplet at δ 2.24 (J=7.2 Hz) was attributed to H-2 methylene protons adjacent to ester linkage. The remaining methylene protons resonated between δ 1.54-1.18. Two three-proton triplets at δ 0.81 (J=6.2 Hz) and 0.78 (J=6.5 Hz) were correspondingly assigned to H-16 and H-16′ primary methyl protons. The ^{13}C NMR of compound **1** exhibited signals for thirty two carbons. Diagnostic peaks appeared for ester carbon at δ 171.27 (C-1), oxygenated methylene carbon at δ 67.34 and primary methyl carbons at δ 12.39 (C-16) and 12.33 (C-16′). On the basis of above discussion, the structure of compound **1** was elucidated as *n*-hexadecanyl hexadecanoate.

Compound 2, designated as pentadecanyl oleate, was obtained as pale yellow sticky mass. It responded positive to tests of unsaturation. Its FTIR spectrum exhibited important absorption bands for ester (1729 cm^{-1}), unsaturation (1651 cm^{-1}) and aliphatic moiety (1045, 750 cm^{-1}). On the

basis of ESI-MS and ^{13}C NMR spectra, its molecular mass was found to be 492, consistent with the molecular formula $C_{33}H_{64}O_2$ of a fatty acid ester. The ^{1}H NMR of compound **2** exhibited two downfield one-proton multiplets at δ 5.35 and 5.33 ascribed to H-9 and H-10 vinylic protons, respectively. Two two-proton multiplets at δ 2.03 and 1.84 were attributed to H-8 and H-11 methylene protons adjacent to vinylic linkage, respectively. Two two-proton triplets at δ 4.13 and 2.27 were correspondingly ascribed to H-1′ oxygenated methylene protons and H-2 methylene protons adjacent to ester linkage. The remaining methylene protons resonated from δ 1.64 to 1.25. Two three-proton triplets at δ 0.87 (J=6.3 Hz) and 0.84 (J=6.5 Hz) were assigned to primary methyl protons Me-18 and Me-15′, respectively. The ^{13}C NMR of compound **2** displayed important signals for ester carbon at δ 173.06 (C-1); vinylic carbons at δ 130.96 (C-9), 128.85 (C-10); oxygenated methylenic carbon at δ 71.82 (C-1′) and methyl carbons at δ 19.18 (C-15′) and 14.16 (C-18). The remaining methylene carbons appeared from δ 31.94 to 22.71. On the basis of above discussion the structure of compound **2** was elucidated as *n*-pentadecanyl octadec-9-enoate.

Compound 3, designated as hexadecanyl oleate, was obtained as colorless amorphous powder from acetone-methanol (1:1). It responded positive to tests for unsaturation. Its FTIR spectrum exhibited characteristic absorption bands for ester (1736 cm^{-1}), unsaturation (1645 cm^{-1}) and aliphatic chain (1115, 803 cm^{-1}). On the basis of ESI-MS, its molecular mass was found to be 506, consistent with the molecular formula $C_{34}H_{66}O_2$ of fatty acid ester. Its formula indicated the presence of two double bond equivalents, one of which was adjusted in an ester group and other in a vinylic linkage. The ^{1}H NMR of compound **3**, displayed a downfield multiplets at δ 5.21, integrating for two protons, ascribed to H-9 and H-10 vinylic protons. Two two-proton triplets at δ 4.24 (J=6.5 Hz) and 2.52 (J=7.2 Hz) were attributed to H-1′ oxygenated methylene proton and H-2 methylene protons adjacent to ester linkages respectively. Two multiplets at δ 2.24 and 2.20, each integrating for two protons, were correspondingly attributed to H-8 and H-11 methylene protons adjacent to vinylic linkage. The remaining methylene protons resonated at δ 1.52 (m, 2H) and 1.23 (brs, 28H).Two three-proton triplets at δ 0.85 (J=6.1 Hz) and 0.82 (J=6.3 Hz) were assigned to H-18 and H-16′ primary methyl protons, respectively. On the basis of above spectral data and chemical tests, the structure of compound **3** was elucidated as *n*-hexadecanyl octadec-9-enoate.

Compound 4, designated as hexdecanyl stearate, was obtained as colorless semi-solid mass from chloroform eluants. Its FTIR spectrum exhibited characteristic absorption bands for ester group (1737 cm^{-1}) and aliphatic moiety (725 cm^{-1}). On the basis of ESI-MS and ^{13}C NMR spectrum, its molecular mass was found to be 508, consistent with molecular formula $C_{34}H_{64}O_2$. It indicated the presence of one-double bond equivalent that was adjusted in the ester group. An important ion peak at *m/z* 283 $[C_{18}H_{64}O_2]^+$ arouse due to ester linkage fission further supported the presence of stearic acid moiety. The ^{1}H NMR spectrum of compound **4** displayed a downfield triplet at δ 4.26 (J=10.5 Hz), integrating for two protons, assigned to H-1′ oxygenated methylene protons. Another two-proton triplet at δ 2.31 (J=7.5 Hz) was ascribed to H-2 methylene protons adjacent to ester linkage. The remaining methylene protons resonated from δ 1.82 to 1.23. Two triplet signals at δ 0.87 (J=6.3 Hz) and 0.84 (J=6.1 Hz), each integrating for three protons, were attributed to H-18 and H-16′ primary methyl protons, respectively. On the basis of above discussion the structure of compound **4** was elucidated as *n*-hexadecanyl octadecanoate.

Compounds 5, designated as octadecanyl stearate, was obtained as pale yellow sticky mass from chloroform eluants. Its FTIR spectrum exhibited characteristic absorption bands for ester group (1735 cm^{-1}) and aliphatic moiety (1196, 726 cm^{-1}). On the basis of ESI-MS and ^{13}C NMR data, its molecular mass was established at 536, consistent with the formula $C_{36}H_{72}O_2$. It indicated the presence of one double bond equivalent that was adjusted in an ester linkage. The other significant ion peak at *m/z* 283 $[C_{18}H_{35}O_2]^+$ arising due to ester linkage fission supported the compound 5 to be a stearic acid ester. The ^1H NMR of compound **5**, exhibited two two-proton triplets at δ 4.06 (J=6.0 Hz) and 2.30 (J=7.2 Hz) ascribed correspondingly to H-1′ oxygenated methylene protons and H-2 methylene protons adjacent to ester group. The remaining methylene protons resonated between δ 1.64-1.25. Two three-proton triplets at δ 0.90 (J=6.5 Hz) and 0.88 (J=6.3 Hz) were attributed to H-18 and H-18′ primary methyl protons, respectively. The ^{13}C NMR spectrum of compound **5** displayed signals for ester carbon at δ 173.80 (C-1), oxygenated methylene carbon at δ 63.76 (C-1′) and primary methyl carbons at δ 14.38 (C-18) and 14.27 (C-18′). On the basis of above discussion, the structure of compound **5** was elucidated as *n*-octadecanyl octadecanoate.

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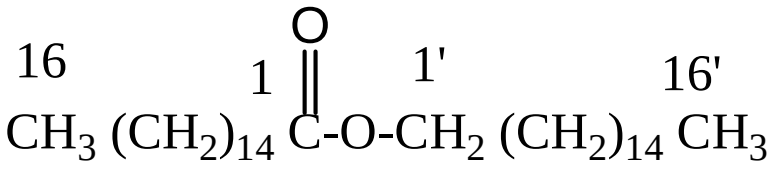

Figure 1. Chemical structure of *n*-hexadecanyl hexadecanoate (1)

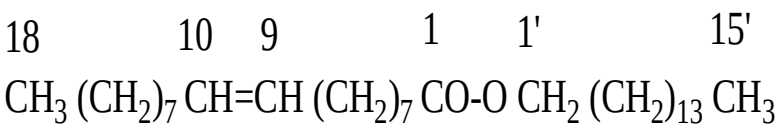

Figure 2. Chemical structure of pentadecanyl oleate (2)

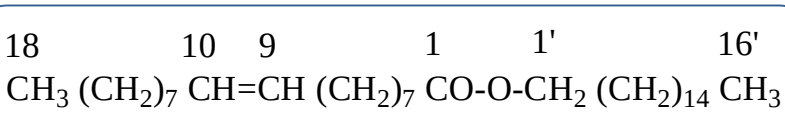

Figure 3. Chemical structure of hexadecanyl oleate **(3)**

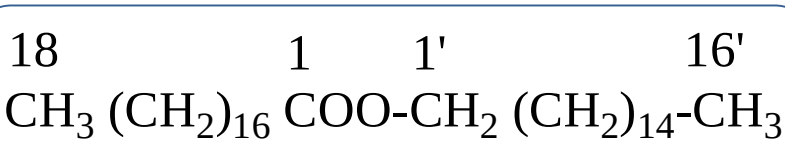

Figure 4. Chemical structure of hexadecanyl stearate (4)

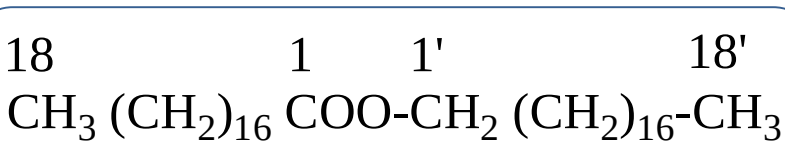

Figure 5. Chemical structure of octadecanyl stearate (5)