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PHYTOCHEMICAL INVESTIGATION OF THE STEM BARK OF *MORINGA OLEIFERA* LAM.

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ABSTRACT
Phytochemical investigation of the stem bark of *Moringa oleifera* Lam. (Moringaceae) furnished two new phytoconstituents identified as *n*-heptacosanyl *n*-octadec-9,12,15 trieneoate (moringyl linoleneate) and *n*- docas- 4-en-11-one-1-yl *n*-decanoate (oleiferyl capriate) along with the known compounds β-sitosterol, epilupeol, glyceropalmityl phosphate and glycerol-oleiostearyl phosphate. The structures of all the phytoconstituents have been elucidated on the basis of spectral data analyses and chemical reactions.
KEYWORDS : *Moringa oleifera*, stem bark, moringyl linoleneate, oleiferyl capriate, Phytoconstituents

INTRODUCTION

Moringa oleifera Lam. (Moringaceae) is a small or medium-sized, about 10 m. high tree, distributed in many tropical and subtropical countries. It is found wild in the sub-Himalayan tract from Chenab eastwards and cultivated all over the plains of India[1]. *M. oleifera* is an exceptionally nutritious vegetable plant with a variety of potential uses. It is considered one of the world's most useful tree, as almost every part of the Moringa tree can be used for food or has some other beneficial property. The plant parts act as cardiac stimulant and possess various bioactivities[2]. The stem barks of *M. oleifera* are thick, soft, corky and deeply fissured. All parts of the tree are used in the treatment of ascites, rheumatism, venomous bites and as cardiac and circulatory stimulants. The root and root bark of the young tree are rubefacient and vesicant. The leaves are rich in vitamins A and C and beneficial to cure scurvy, catarrhal affection and as emetic. A paste of the leaves is applied to heal wounds. The flowers are used as tonic, diuretic and cholagogue. The seeds are considered antipyretic, acrid and bitter. The seed oil is applied to cure rheumatism and gout[1-3]. The plant parts contained carotenoids[4], benzyl carbamate derivative[5,6], niazimicin, niazirin, steroids[5], flavonoids[7,8], caffeoyl quinic acid[2,8], triacylglycerols[9] and monoterpenes[10]. The present paper describes the isolation and characterization of two new phytoconstituents along with four known compounds from the tem bark of *M. oleifera.*

MATERIALS AND METHODS

General Precedure

Melting points were determined on Perfit melting point apparatus and are uncorrected. FTIR: Jasco FT/IR-5000; UV: Lambda Bio 20 Spectrophotometer, MeOH; ^{1}H-NMR (400 MHz): Advance DRY 400, Bruker Spectrospin, $CDCl_3$; ^{13}C NMR (75 MHz): Advance DRY 100, Bruker Spectrospin, $CDCl_3$ with TMS as an internal standard; MS: FAB ionization on JEOL-JMS-DX 303; CC: Silica gel (Qualigens), 60-120 mesh; TLC: Silica gel G (Qualigens). Spots were visualized by exposure to iodine vapours, UV radiation and by spraying reagents.

Plant Material

The bark of *M. oleifera* was collected from the local market of Khari Baoli, Delhi and identified by Dr. M. P. Sharma, taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard (Hamdard University). A voucher specimen No. PRL/JH/09/13 is deposited in the herbarium of the Faculty of Pharmacy, Jamia Hamdard, New Delhi.

Extraction and isolation

The air-dried bark (1.3 kg) of of *M. oleifera* was coarsely powdered, defatted with petroleum ether and then exhaustively extracted in a Soxhlet apparatus with methanol for 72 hours. The methanolic extract was concentrated under reduced pressure to obtain dark brown viscous mass. Small portion of the extract was analyzed chemically to determine the presence of different chemical constituents. The viscous dark brown mass was adsorbed on silica gel (60-120 mesh) for column after being dissolved in little quality of methanol for preparation of slurry. The slurry (110 g) was air dried and chromatographed over silica gel column packed in petroleum ether. The column was eluted successively with petroleum ether, mixture of petroleum ether and chloroform (9:1, 3:1, 1:1 and 1:3), pure chloroform and finally the mixture of chloroform and methanol (99:1, 49:1, 24:1, 95:5, 97:3, 9:1). Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions (having same R_f values) were combined and crystallized. The isolated compounds were recrystalized to get the pure compounds.

Moringyl linoleneate (1)

Elution of the column with petroleum ether: chloroform (3:1) mixture furnished colourless crystals of **1**, recrystallized from acetone, (450 mg, 0.035 % yield). R_f value: 0.9 (petroleum ether: chloroform) (3:2); m.p.: 50-52 ^oC; UV λ_{max} (MeOH): 213, 236 nm (log ε 1.3, 2.7); IR ν_{max} (KBr): 2911, 2845, 1721, 1645, 1601, 1488, 1444, 1377, 1020, 792, 719 cm^{-1}; ^{1}H NMR ($CDCl_3$) : δ 5.82 (1H, m, H-12), 5.37 (2H, m, H-13, H-15, 4.96 (2H, m, H-10, H-16), 4.61 (1H, m, H-9), 4.07 (1H, d, J= 6.0 Hz, H_2-1'a), 4.03 (1H, d, J= 6.0 Hz, H_2-1'b), 2.30 (2H, m, H_2-11), 2.28 (1H, d, J= 8.0 Hz, H_2-2a), 2.24 (1H, d, J= 8.0 Hz, H_2-2b), 2.02 (2H, m, H_2-14), 1.99 (2H, m, H_2-17), 1.86 (2H, m, H_2-3), 1.52 (2H, m, CH_2), 1.27 (50H, brs, 25 × CH_2), 1.02 (6H, m, 3 × CH_2), 0.89 (3H, t, J= 6.1 Hz, Me-18). 0.84 (3H, t, J= 6.2 Hz, CH_3-27'). +ve ion FAB MS*m/z* (rel. int.): 656 $[M]^+$ (C_{45} H_{84} O_2) (1.1), 395 (26.1), 261 (11.3), 135 (70.6), 109 (18.5), 95 (22.7).

Hydrolysis of 1

Compound **1** (35 mg) was heated with alkaline ethanol (5 ml) on a steam bath for 1 hr. The reaction mixture was dried under reduced pressure and the residue dissolved in $CHCl_3$ (5 ml). It was washed with H_2O (2 × 5 ml), dried over anhydrous Na_2SO_4 and evaporated to get heptacosanol. The residue after seperating alcohol was acidified to pH 3 and chromatograph on TLC with a standard solution of linolenic acid, R_f value was comparable.

β-sitosterol (2)

Elution of the column with chloroform: methanol (1:1) furnished colourless amorphous powder of **2**, recrystallized from methanol, (115 mg, and 0.008 % yield). R_f value: 0.43 (chloroform: methanol) (2:1); m.p.: 136-138 ^oC; +ive FAB MS *m/z* (rel. int.): 414

$[M]^+(C_{29}H_{50}O)$, (3.9).

Epilupeol (3)

Elution of the column with petroleum ether: chloroform (1:1) mixture afforded colourless crystals of **3**, recrystallized from acetone, (280 mg, 0.021 % yield). R_f value: 0.71 (Pet. Ether : $CHCl_3$) (3:2); m.p.: 162-164 ^oC; +ve ion FAB MS *m/z* (rel. int.): 426 $[M]^+$ (C_{30} H_{50} O) (21.9).

Oleiferyl capriate (4)

Elution of the column with chloroform: methanol (99:1) mixture yielded colourless semisolid mass of **4**, recrystallized from methanol, (500 mg, 0.038 % yield). R_f value: 0.72 ($CHCl_3$); m.p.: 70^{o}C; UV λ_{max} (MeOH): 213 nm (log ε 5.1); IR ν_{max} (KBr): 2922, 2852, 1721, 1709, 1640, 1461, 1282, 943, 723 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.35 (2H, m, H-4, H-5), 4.13 (2H, m, H_2-1) 2.78 (1H, d, J= 6.4 Hz, H_2-2'a), 2.75 (1H, d, J = 6.4 Hz, H_2-2'b), 2.36 (1H, d, J= 7.6 Hz, H_2-10a), 2.32 (1H, d, J= 7.6 Hz, H_2-10b), 2.05 (1H, d, J= 7.2 Hz, H_2-12a), 2.02 (1H, d, J= 7.2 Hz, H_2-12b), 1.64 (2H, m, H_2-3), 1.61 (2H, m, H_2-6), 1.31 (14H, brs, 7 × CH_2), 1.25 (26H, brs, 13 × CH_2), 0.88 (3H, t, J= 6.8 Hz, Me-22), 0.82 (3H, t, J=7.6 Hz, Me-10'). +ve ion FAB MS *m/z* (rel. int.): 492 $[M]^+$ (C_{32} H_{60} O_3) (3.1), 337 (18.3), 279 (32.6), 213 (12.8), 183 (11.7), 171 (22.5), 155 (147.1). ^{13}C NMR ($CdCl_3$): δ 202.16 (C-11), 171.69 (C-1'), 129.42 (C-4), 128.18 (C-5), 61.33 (C-1), 51.37 (C-2'), 33.89 (CH_2), 31.49 (CH_2), 28.52 (CH_2), 25.41 (21 x CH_2), 22.52 (CH_2), 14.18 (Me-22), 14.09 (Me-10').

Alkaline Hydrolysis of 4

Compound **4** (30 mg) was heated with alkaline ethanol (5 ml) on a steam bath for 1 hr. The reaction mixture was extracted with $CHCl_3$ (2 × 5 ml) to separate alcohol, acidified with dil. HCl to pH 3 and chromatographed on TLC plate along with a standard solution of capric acid, R_f value was comparable.

Glyceropalmityl phosphate (5)

Elution of the column with chloroform: methanol (49:1) mixture gave colourless crystals of **5**, recrystallized from methanol, (520 mg, 0.04 % yield). R_f value: 0.82 ($CHCl_3$: CH_3OH) (99:1); m.p.: 215 ^{o}C; IR ν_{max} (KBr): 3444, 1735, 722 cm^{-1}. ^1H NMR (DMSO-d_6): δ 4.09 (1H, m, H-2), 3.80 (2H, m, H_2-3), 3.59 (2H, m, H_2-1), 2.73 (2H, brs, H_2-2'), 2.27 (2H, brs, H_2-2''), 1.48 (12H, brs, 6× CH_2), 1.24 (40H, brs, 20× CH_2), 0.85 (6H, brs, CH_3-16', CH_3-16'').

Glycero-oleiostearyl phosphate (6)

Elution of the column with chloroform: methanol (9:1) mixture furnished colourless crystals of **6**, recrystallized from methanol, (240 mg, 0.018 % yield). R_f value: 0.8 ($CHCl_3$: CH_3OH) (19:1); m.p.: 52 ^{o}C; UV λ_{max} (MeOH): 207 nm (log ε 5.6); IR ν_{max} (KBr): 3412, 1736, 1630, 1462, 719 cm^{-1}. +ve ion FAB MS *m/z* (rel. int.): 702 $[M]^+$ (C_{39} H_{75} O_8 P) (1.2).

RESULT AND DISCUSSION

Compound **1,** named moringyl linoleneate, was obtained as a colourless crystalline mass from petroleum ether : chloroform (3:1) eluants. It decolourized bromine water indicating unsaturated nature of the molecule. Its IR spectrum exhibited characteristic absorption bands for ester group (1721cm^{-1}), unsaturation (1645, 1601 cm^{-1}) and long aliphatic chain (792, 719 cm^{-1}). Its mass spectrum displayed a molecular ion peak at*m/z* 656 corresponding to a molecular formula of an aliphatic ester, $C_{45}H_{84}O_2$. It indicated four double bond equivalents; three of them were adjusted in the vinylic linkages and the remaining one in the ester group. The ion fragments generated at*m/z* 395 [O-CO fission, $CH_3(CH_2)_{26}O]^+$ and 261 [M-395, CO $(CH_2)_7C_9H_{12}CH_3]^+$ indicated that linolenic acid was esterified with a C_{27} - fatty alcohol. The ion fragments arising at*m/z* 135 $[C_9H_{12}CH_3]^+$, 109 $[C_7H_{10}CH_3]$ and 95 $[C_6H_8CH_3]^+$ suggested the location of the vinylic linkages at C-9 and C-12 positions [4-6]. The absence of $[M\text{-}Me]^+$ ion suggested its straight chain nature [7], where as the presence of $[M+1]^+$ ion arose due to its unsymmetrical nature [11-15]. The ^1H NMR spectrum of **1** showed two one-proton multiplets at δ 5.82 and 4.61 assigned to vinylic H-12 and H-9, respectively. Two multiplets at δ 5.37 and 4.96, both integrated for two protons each, were ascribed to vinylic H-13, H-15 and H-10, H-16, respectivly. Two one-proton doublets at δ 4.07 (J= 6.0 Hz) and 4.03 (J= 6.0 Hz) were attributed to oxygenated H_2-1' methylene protons. Four two-proton multiplet at δ 2.30, 2.02, 1.99 and 1.86 were associated with the methylene protons adjacent to the vinylic carbons. Two one-proton doublets at δ 2.28 (J= 8.0 Hz) and 2.24 (J= 8.0 Hz) were due to methylene H_2-2 protons adjacent to ester group. Two three-proton triplets at δ 0.89 (J= 6.1 Hz) and 0.84 (J= 6.2 Hz) were accounted to primary C-18 and C-27' methyl protons. The remaining methylene protons resonated at δ 1.52 (2H), 1.27 (50H) and 1.02 (6H). Alkaline hydrolysis of **1** yeilded linolenic acid. On the basis of foregoing discussion the structure of **1** has been established as *n*-heptacosanyl *n*-octadec-9,12,15-trieneoate. It is a new fatty ester isolated from a herbal drug.

Compound **4**, designated as oleiferyl capriate, was obtained as a colourless semisolid mass obtained from chloroform: methanol (99:1) eluants. It decolorized bromine water indicating unsaturated nature of the molecule. Its IR spectrum showed distinctive absorption bands for ester group (1721 cm^{-1}), carbonyl group (1709 cm^{-1}), unsaturation (1640 cm^{-1}) and long aliphatic chain (723 cm^{-1}). Its mass spectrum displayed a molecular ion peak at *m/z* 492 corresponding to a fatty acid ester with an unsaturated ketoalcohol $C_{32}H_{60}O_3$. It indicated three degrees of unsaturation, one each of them were adjusted to the carbonyl and ester groups and vinylic linkage. The prominent ion fragments generated at *m/z* 155 [C_{11} – C_{12} fission, $CH_3(CH_2)_{10}]^+$, 183 [C_{10}-C_{11} fission, $CH_3(CH_2)_{10}CO]^+$, 279 [C_3- C_4 fission, $CH_3(CH_2)_{10}CO\text{-}C_7H_{12}]^+$, and 213 [M-279, $(CH_2)_3$ O-$CO(CH_2)_8$ $CH_3]^+$ indicated the location of the carbonyl group at C-11 and vinylic linkage at C-4. The ion fragments arising at *m/z* 171 $[OCO(CH_2)_8CH_3]^+$, 155 [$CO(CH_2)_8CH_3]^+$ and 337 [M-155]$^+$ supported the esterification of capric acid with C_{22}-ketoalcohol[5-9]. The ^1H NMR spectrum of **4** exhibited two multiplets at δ 5.35 and 4.13, both integrated for two protons each, assigned correspondingly to vinylic H-4 and H-5 and to oxygenated methylene H_2-1 protons. Six one-proton doublets at δ 2.78 (6.4 Hz) and 2.75 (J=6.4 Hz), at δ 2.36 (J=7.6 Hz) and 2.32 (J=7.6 Hz) and at 2.05 (J=7.2 Hz) and 2.02 (J=7.2 Hz) were assigned to methylene H_2-1' adjacent to the ester group and to H_2-10 and H_2-12 nearby to the carbonyl group, respectively. Two three-proton triplets at δ 0.88 (J=6.8 Hz) and 0.82 (J=7.6 Hz) were attributed to primary methyl H_3-22 and H_3-10', respectively. The remaining methylene protons resonated between δ 1.64-1.25. The ^{13}C MNR spectrum of **4** displayed signals for carbonyl carbon at δ 202.16 (C-11), vinylic carbons at δ 129.42 (C-4) and 128.18 (C-5), ester carbon at δ 171.69 (C-1), oxygenated methylene carbon at δ 61.33 (C-1), methyl carbons at δ 14.18 (C-22) and 14.09 (C-10') and the remaining methylene carbons between δ 51.37-22.52. Alkaline hydrolysis of **4** yielded capric acid. On the basis of the above mentioned discussion the structure of **4** was characterized as *n*- docas- 4-en-11-one-1-yl *n*-decanoate. This is a new aliphatic ester isolated from a natural or synthetic source for the first time.

The compounds **2**, **3**, **5** and **6** are the known compounds identified as β-sitosterol, epilupeol, glyceropalmityl phosphate and glycerol-oleiostearyl phaosohate respectively, on the basis of spectral data analyses and chemical reactions.

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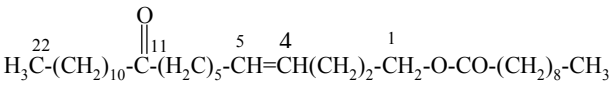
$$CH_3(CH_2)_{25}CH_2\text{-}O\text{-}CO(CH_2)_7(CH{=}CHCH_2)_3\text{-}CH_3$$

1



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