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Der Pharmacia Lettre, 2011, 3 (6):74-81
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Isolation of four new phytoconstituents from the roots of *Albizzia lebbeck* Benth

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ABSTRACT

Phytochemical investigation of the methanolic extract of the roots of Albizzia lebbeck Benth. (Fabaceae) led to the isolation of one new fatty acid ester 2' α- hydroxy octyl hexadecanoate (hydroxyoctyl palmitate) and two new phenolic acid glucosidic ester characterized as salicylic acid-2-O–β-D-glucofuranosyl-6'-octadec-9''-enoate (salicylic acid arabinosyl oleate) along with a fatty acid phytoconstituent, docos-3-en-1-oic acid (docosenoic acid), and two esters tricosanyl hexadecanoate and hexacosanyl octadec-9-en-1-oate (hexacosanyl oleate). The structures of all these phytoconstituents have been elucidated on the basis of spectral data analysis and chemical reactions.

Keywords: *Albizzia lebbeck*, Fabeceae; roots, salicylic acid glucosyl esters, fatty acid esters.

INTRODUCTION

A. lebbeck Benth (family: Fabaceae), commonly known as siris or East India walnut, is a native to tropical southern Asia, and widely cultivated and naturalized in other tropical and subtropical regions. It is a tree growing to a height of 18-30 [1]. Its root bark is used to cure inflammation; blood related diseases, leucoderma, itching, skin diseases, piles, bronchitis [2]. The root bark powder is prescribed to strengthen the gums, when they are spongy and ulcerative [3]. The bark contained saponins [4], seed yielded alkaloid like Budmunchiamine L_1, L_2, L_3 [5]; L_4, L_5, L_6 [6]. Two phytoconstituents (-)-2,3-cis-3, 4-cis-3-O-methylmelaccacidin and 3'-O-methylmelanoxetin were isolated from heart wood [7]. The roots possessed echinocystic acid (saponin) glycoside [8]. The present paper desgribes the isolation and characterization of four new constituents and two known fatty acid esters from the roots of *A. lebbeck*.

MATERIALS AND METHODS

General experimental procedure

The melting points were determined on a Perfit apparatus and are uncorrected. The IR spectra were recorded in KBr pellet on Win IR FTS 135 instrument (Biorad, USA). ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were run by Bruker spectrospin NMR instrument in $CDCl_3$, using TMS as internal standard. FAB MS were scanned at 70 eV on a Jeol D-300 instrument. Column chromatography was performed on silica gel (Merck, 60-120 mesh) and thin-layer chromatography on silica gel G coated TLC plates (Merck). Spots were visualized by exposure to iodine vapours, UV radiation and by spraying reagent.

Plant Material

The roots of *A. lebbeck* were collected from West Champaran, Bihar, (India) and identified by Dr. H. B. Singh, Scientist F and Head, Raw Materials, Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India. A voucher specimen (No. NISCAIR/RHMD/Consult/-2008-09/1114/145) was deposited in The Herbarium of NISCAIR, New Delhi.

Extraction and isolation

The roots (1.6 kg) were shade dried, coarsely powdered and extracted exhaustively with methanol. The methanolic extract was concentrated under reduced pressure in a Buchi rotavapor to obtain a dark green viscous mass (85.5 g). The viscous mass was dissolved in little amount of methanol and adsorbed on silica gel (60-120 mesh) for column for preparation of slurry. The slurry 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, 98:2, 96:4, 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 following compounds:

Hydroxyoctyl palmitate (1)

Elution of the column with petroleum ether-chloroform mixture (1:1) furnished colourless crystals of **1**, recrystallized from acetone; 200 mg (0.24%) yield; R_f: 0.48 ($CHCl_3$-MeOH, 4:1); m.p: 114-116 °C; UV λ_{max} (MeOH): 222 nm (log ε 3.8); IR ν_{max} (KBr): 3418, 2921, 2853, 1743, 1460, 1375, 1240, 1164, 973, 724 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.98 (1H, dd, *J*= 6.9, 6.3 Hz, H-2' β), 3.59 (2H, dd, *J*= 7.5, 6.0 Hz, H_2-1'), 2.22 (2H, t, *J*= 7.2 Hz, H_2-2), 1.92 (2 H, m, CH_2), 1.54 (4H, m, 2 × CH_2), 1.18 (30 H, brs, 15 × CH_2), 0.81 (3 H, t, *J*= 6.3 Hz, Me-16), 0.76 (3 H, t, *J*= 6.2 Hz, Me-8'); ^{13}C NMR ($CDCl_3$): δ 171.23 (C-1), 69.06 (C-2'), 62.13 (C-1'), 34.42 (CH_2), 32.53 (CH_2), 30.39 (CH_2), 30.26 (CH_2), 30.09 (CH_2), 30.05 (CH_2), 29.82 (CH_2), 29.63 (CH_2), 25.24 (CH_2), 23.36 (CH_2), 14.75 (Me-16, Me-8), 14.73 (C-8'); +ve ion FAB MS *m/z* (*rel. int.*): 384 $[M]^+$ ($C_{24}H_{48}O_3$) (19.8), 269 (21.9), 255 (31.6), 239 (28.3), 211 (21.8), 173 (42.1), 145 (69.0), 129 (47.1), 115 (37.2), 85 (73.1).

Acid hydrolysis of **1**: Compound **1** (25 mg) was refluxed with 1N NaOH solution in 80% MeOH (1:1, 15 ml) for one hour. After cooling, the reaction mixture was extracted with EtOAc to

separate diol. It was then acidified with dil. HCl to pH 3 and extracted with chloroform to separate palmitic acid, m.p 63-64 ^{o}C, Co-TLC comparable.

Salicylic acid glucosyl oleate (2)

Elution of the column with chloroform-methanol (97:3) gave pale yellow coloured crystals of **2**, recrystallized from methanol, 1240 mg (1.46% yield); R_f: 0.78 ($CHCl_3$-MeOH, 5:1); m.p: 202-204°C; UV λ_{max} (MeOH): 225 nm; IR ν_{max} (KBr): 3427, 3350, 2919, 2850, 1736, 1632, 1516, 1464, 1273, 1167, 1053, 721 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.54 (1H, dd, *J*= 7.8, 2.7 Hz, H-3), 6.92 (1H, dd, *J*= 7.8, 2.9 Hz, H-6), 6.83 (1H, m, H-4), 6.22 (1H, m, H-5), 5.27 (1H, m, H-9''), 5.24 (1H, m, H-10''), 4.94 (1H, d, *J*= 7.2 Hz, H-1'), 4.19 (1H, dd, *J*= 7.2, 8.4 Hz, H-2'), 3.96 (1H, m, H-3'), 3.82 (1H, m, H-3'), 3.62 (2H, brs, H_2-6'), 3.41 (1H, brs, H_2-5'a), 3.38(1H, brs, H2-5'b), 2.70 (1H, d, *J*= 6.1 Hz, H_2-2''a), 2.66 (1H, d, *J*= 6.1 Hz, H_2-2''b), 2.24 (2H, m, H_2-8''), 2.22 (2H, m, H_2-11''), 1.96 (2H, m,CH_2), 1.53 (4H, brs, 2 × CH_2), 1.33 (2H, m, CH_2), 1.31 (2H, m, CH_2), 1.16 (12H, brs, 6 × CH_2), 0.79 (3H, t, *J*= 6.6 Hz, Me-18''); ^{13}C NMR ($CDCl_3$): δ 178.37 (C-7), 173.11 (C-1''), 146.80 (C-2), 144.74 (C-1), 129.95 (C-3), 129.91 (C-6), 126.93 (C-4), 122.99 (C-5), 115.47 (C-9''), 114.77 (C-10''), 109.41 (C-1'), 83.52 (C-4'), 72.02 (C-2'), 69.18 (C-3'), 64.99 (C-6'), 64.64 (C-5'), 55.88 (C-2''), 33.92 (CH_2), 31.87 (CH_2), 29.64 (5 × CH_2), 29.59 (CH_2), 29.41 (CH_2), 27.14 (CH_2), 25.93 (CH_2), 24.82 (CH_2), 22.63 (CH_2), 14.06 (Me-18''). +ve ion FAB MS *m/z* (*rel. int.*): 564 $[M]^+$ ($C_{31}H_{48}O_9$) (9.8), 442 (8.3), 281 (21.3), 265 (18.2), 237 (11.5), 223 (15.6), 209 (19.5), 195 (5.6),137 (32.5), 121 (43.8).

Acid hydrolysis of 2: Compound **2** (15 mg) was refluxed with 2N HCl in 80% MeOH (1:1, 15 ml) for one hour. After cooling, the reaction mixture was poured into crushed ice, and the hydrolysate was then extracted with EtOAc to give the salicylic acid, (m.p. 157-158°C) and oleic acid (m.p. 13°C). The concentrated aqueous hydrolysate showed the presence of D-glucose on comparison with authentic sample on silica gel TLC, R_f 0.18 (EtOAc-AcOH-H_2 O-MeOH, 6:1:1:2).

Salicylic acid glucosyl palmitate (3)

Elution of the column with chloroform-methanol (19:1) yielded pale yellow coloured crystals of **3**, recrystallized from acetone, 740 mg (0.85% yield); R_f: 0.62 ($CHCl_3$-MeOH, 4:1); m.p: 258-260 °C; UV λ_{max} (MeOH): 224 nm (log ε 6.1); IR ν_{max} (KBr): 3422, 3375, 3280, 2920, 2851, 1734, 1685, 1633, 1515, 1463, 1376, 1271, 1169, 1085, 720 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.57 (1H, dd, *J*= 8.1, 2.9 Hz, H-3), 7.03 (1H, dd, *J*= 7.8, 2.7 Hz, H-6), 6.91 (1H, m, H-4), 6.26 (1H, m, H-5), 5.11 (1H, d, *J*= 7.1 Hz, H-1'), 4.30 (1H, m, H-5'), 4.16 (1H, m, H-2'), 4.05 (1H, m, H-3'), 3.76 (1H, m, H-4'), 3.66 (1H, d, *J*= 7.5 Hz, H_2-6'a), 3.63 (1H, d, *J*= 7.5 Hz, H_2-6'b), 2.33 (1H, d, *J*= 7.8 Hz, H_2-2''a), 2.28 (1H, d, *J*= 7.8 Hz, H_2-2''b), 2.01 (2H, m, CH_2), 1.60 (4H, brs, 2 × CH_2), 1.24 (18H, brs, 9 × CH_2), 1.03 (2H, m, CH_2), 0.85 (3H, t, *J*= 6.6 Hz, Me-16''); ^{13}C NMR ($CDCl_3$): δ 178.56 (C-7), 174.11 (C-1''), 144.70 (C-2), 144.26 (C-1), 129.63 (C-6), 129.36 (C-3), 127.01 (C-4), 123.12 (C-5), 101.58 (C-1'), 86.53 (C-5'), 73.42 (C-2'), 65.01 (C-3'), 64.62 (C-4'), 62.07 (C-6'), 55.89 (C-2''), 34.08 (CH_2), 31.89 (CH_2), 29.65 (6 × CH_2), 29.48 (CH_2), 27.15 (CH_2), 25.92 (CH_2), 24.85 (CH_2), 22.65 (CH_2), 14.08 (Me-16''); +ve ion FAB MS *m/z* (*rel. int.*): 538 $[M]^+$ ($C_{29}H_{46}O_9$) (6.3), 401 (26.8), 299 (19.5), 255 (42.7), 239 (40.8), 211 (33.4), 197 (29.8), 183 (28.4), 169 (23.5), 155 (27.1), 141 (19.2), 137 (18.3), 127 (21.6), 121 (20.9).

Acid hydrolysis of **3**: Compound **3** (15 mg) was refluxed with 2N HCl in 80% MeOH (1:1, 15 ml) for one hour. After cooling, the reaction mixture was poured into crushed ice, and the hydrolysate was then extracted with EtOAc to give the salicylic acid (m.p. 157-158 °C) and palmitic acid (m.p. 63-64 °C). The concentrated aqueous hydrolysate showed the presence of glucose on comparison with authentic sample on silica gel TLC, R_f 0.18 (EtOAc-AcOH-H_2 O-MeOH, 6:1:1:2).

Docosenoic acid (**4**)

Elution of the column with chloroform-methanol (99:1) (fraction no. 125-146) afforded colourless crystals of **4**, recrystallized from acetone, 1130 mg (1.32% yield); R_f: 0.72 ($CHCl_3$-MeOH, 4:1); m.p: 86-87°C; UV λ_{max} (MeOH): 225 nm (log ε 3.7); IR ν_{max} (KBr): 3340, 2920, 2852, 1708, 1630, 1462, 1374, 1168, 724 cm^{-1}; ^{1}H NMR ($CDCl_3$): 5.27 (2H, m, H-3, H-4), 2.29 (1H, d, *J*= 7.5 Hz, H_2-2'a), 2.24 (1H, d, *J*= 7.5 Hz, H_2-2'b), 1.96 (2H, m, H_2-5), 1.55 (2H, m, CH_2), 1.53 (2H, m, CH_2), 1.18 (32H, brs, 16 × CH_2), 0.80 (3H, t, *J*= 6.9 Hz, Me-22).^{13}C NMR ($CDCl_3$)**:** δ 179.65 (C-1), 129.97 (C-3), 128.03 (C-4), 34.01 (CH_2), 30.90 (CH_2), 30.87 (CH_2), 29.67 (9 × CH_2), 29.33 (2 × CH_2), 27.17 (CH_2), 25.60 (CH_2), 24.66 (CH_2), 22.66 (CH_2), 14.08 (CH_3-22). +ve ion FAB MS *m/z* (*rel. int.*): 338 $[M]^+$ ($C_{22}H_{42}O_2$) (32.9).

Tricosanyl palmitate (**5**)

Elution of the column with chloroform-methanol (93:7) afforded colourless crystalline mass of **5**, recrystallized from acetone, 250 mg (0.03% yield); R_f: 0.54 ($CHCl_3$); m.p: 120-122°C; UV λ_{max} (MeOH): 224 nm (log ε 4.1); IR ν_{max} (KBr): 2919, 2850, 1732, 1642, 1463, 1270, 1064, 717 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 3.70 (2H, brs, H_2-1'), 2.47 (2H, brs, H_2-2), 1.94 (2H, m, CH_2), 1.72 (2H, m, CH_2), 1.54 (4H, m, 2 × CH_2), 1.20 (58H, brs, 29 × CH_2), 1.01 (2H, brs, CH_2), 0.80 (3H, t, *J*= 6.1 Hz, Me-16), 0.76 (3H, t, *J*= 6.0 Hz, Me-23'); ^{13}C NMR (DMSO-d_6): δ 171.53 (C-1), 62.81 (C-1'), 31.81 (CH_2), 29.56 (32 × CH_2), 29.24 (CH_2), 22.61 (CH_2), 14.31 (C-16, C-23'); +ve ion FAB MS *m/z* (*rel. int.*): 578 $[M]^+$ ($C_{39}H_{78}O_2$) (23.6), 339 (25.2), 323 (18.5), 255 (23.6), 239 (28.1).

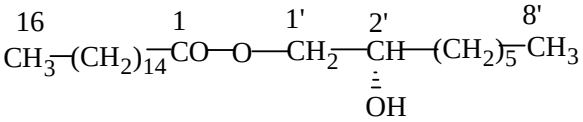
Hexacosanyl oleate (**6**)

Elution of the column with chloroform-methanol (91:9) furnished colourless mass of **6,** recrystallized from acetone, 670 mg (0.78% yield); R_f: 0.82 ($CHCl_3$-MeOH, 3:2); m.p: 84-85 °C; UV λ_{max} (MeOH): 218 nm (log ε 2.8); IR ν_{max} (KBr): 2920, 2851, 1732, 1636, 1462, 1271, 1061, 717 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.22 (1H, m, H-9), 5.19 (1H, m, H-10), 3.82 (1H, d, *J*= 6.3 Hz, H_2-1'a), 3.78 (1H, d, *J*= 6.0 Hz, H_2-1'b), 2.17 (1H, d, *J*= 7.5 Hz, H_2-2a), 2.12 (1H, d, *J*= 7.5 Hz, H_2-2b), 1.95 (2H, m, H_2-8), 1.67 (2H, m, H_2-11), 1.51 (2H, m, CH_2), 1.41 (2H, m, CH_2), 1.11 (66H, brs, 33 × CH_2), 0.73 (3H, t, *J*= 6.3 Hz, Me-18), 0.65 (3H, t, *J*= 7.2 Hz, Me-26'); ^{13}C NMR ($CDCl_3$): δ 173.16 (C-1), 130.96 (C-9), 129.57 (C-10), 63.25 (C-1'), 34.13 (CH_2), 31.69 (CH_2), 29.07 (CH_2), 28.96 (2 × CH_2), 26.53 (CH_2), 25.33 (CH_2), 25.05 (CH_2), 24.94 (CH_2), 22.06 (CH_2), 21.24 (CH_2), 18.92 (CH_2), 14.08 (Me-26'), 12.11 (Me-18); +ve ion FAB MS *m/z* (*rel. int.*): 646 $[M]^+$ ($C_{44}H_{86}O_2$) (19.8), 281 (25.1), 265 (14.2).

RESULTS AND DISCUSSION

Compound **1**, designated as hydroxyoctyl palmitate, was obtained as colourless crystalline mass from petroleum ether-chloroform (1:1) eluants. It did not decolourise bromine water indicating

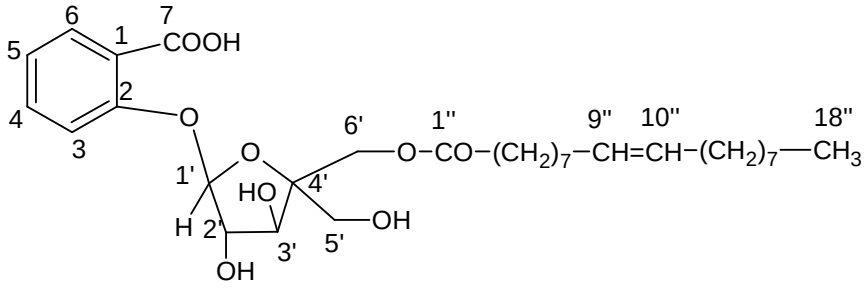
saturated nature of the molecule. Its IR spectrum exhibited distinctive absorption bands for the hydroxyl group (3418 cm^{-1}), ester group (1743 cm^{-1}) and long aliphatic chain (724 cm^{-1}). The mass spectrum of **1** showed a molecular ion peak at *m/z* 384 corresponding a molecular formula of a hydroxyoctyl palmitate, C_{24} $H_{48}O_3$. It indicated the presence of one degree of double bond equivalent, which was adjusted to an ester function. The prominent ion fragments arising at *m/z* 211 $[CH_3(CH_2)_{14}]^+$, 173 [M-211,$COOCH_2CHOH(CH_2)_5CH_3]^+$, 239 $[CH_3(CH_2)_{14}CO]^+$, 145 [M-239, $OCH_2CHOH(CH_2)_5CH_3]^+$, 255 $[CH_3(CH_2)_{14}COO]^+$ and 129 [M-255, $CH_2CHOH(CH_2)_5CH_3]^+$ suggested that palmitic acid was esterified with dihydroxy octane. The ion peaks appearing at *m/z* 269 [$C_{1'}$-$C_{2'}$ fission, CH_3 $(CH_2)_{14}CO$-$OCH_2]^+$, 115 [M-269, CHOH $(CH_2)_5CH_3]^+$ and 85 $[(CH_2)_5CH_3]^+$ indicated the existence of the hydroxyl group at C-2' . The ^1H NMR spectrum of **1** displayed an one-proton double- doublet at δ 3.98 (*J*= 6.9, 6.3 Hz) assigned to H-2' carbonyl proton while as a two-proton double- doublet at δ 3.59 (*J*= 7.5, 6.0 Hz) was ascribed to oxygenated methylene H_2-1' protons. A two-proton triplet at δ 2.22 (*J*= 7.2 Hz) was attributed to methylene H_2-2 proton adjacent to ester linkage. Two multiplets at δ 1.92 (2H) and 1.54 (4H) and a broad signal at δ 1.18 (30H) were associated with the remaining methylene protons. Two three-proton triplets at δ 0.81 (*J*= 6.3 Hz) and 0.76 (*J*= 6.2 Hz), were accounted to C-16 and C-8' primary methyl protons, respectively. The ^{13}C NMR spectrum of **1** showed signals for ester carbon at δ 171.23 (C-1); carbinol carbon at δ 69.06 (C-2'); oxygenated methylene carbon at δ 62.13 (C-1'); methylene carbons between δ 32.42-23.36 and methyl carbons at δ 14.75 (C-16, C-8') and 14.73 (C-8'). The absence of any proton signal beyond δ 3.98 in the ^1H NMR spectrum and between δ 171.23-69.06 in the ^{13}C NMR spectrum supported saturated nature of the molecule. The ^1H-^1H COSY spectrum of **1** showed interactions of methyl protons with methylene protons; and H-2' with H_2-1' and H_2-3'. Alkaline hydrolysis of **1** yielded palmitic acid. On the basis of the foregoing account, the structure of **1** has been elucidated as 2'α-hydroxy octyl hexadecanoate. This is a new fatty acid ester isolated from natural or synthetic source for the first time.



1

Compound **2**, designated as salicylic acid arabinosyl oleate, was obtained as pale yellow coloured crystals from chloroform-methanol (97:3) eluants. It gave effervescence with sodium bicarbonate. Its IR spectrum displayed important absorption peaks for hydroxyl group (3427 cm^{-1}), carboxylic group (3350,1736 cm^{-1}), aromatic moiety (1632, 1516, 1053 cm^{-1}) and long aliphatic chain (721 cm^{-1}). Its +ve FAB mass spectrum exhibited a molecular ion peak at *m/z* 564 consistent with the molecular formula of a salicylic acid glycosidic ester, $C_{31}H_{48}O_9$. It displayed important fragment ion peaks at *m/z* 121 $[C_6H_5COO]^+$**,** 137 $[(C_6H_4O)COO]^+$ and 442 $[M-121]^+$ due to glycosidic linkage fission and at *m/z* 281 $[C_{18}H_{33}O_2]^+$ and 265 $[C_{18}H_{33}O]^+$ due to ester linkage fission. The fragment ion peaks at *m/z* 237 $[265-CO]^+$, 223 $[237-CH_2]^+$, 209 $[223-CH_2]^+$ and 195 $[209-CH_2]^+$ supported the presence of a long chain fatty acid. The ^1H NMR spectrum of **2** exhibited two downfield ortho-, meta-coupled double-doublets at δ 7.54 (*J*= 7.8, 2.7 Hz), 6.92 (*J*= 7.8, 2.9 Hz) and two multiplets at δ 6.83 and 6.22, each integrating for one-proton, assigned correspondingly to H-3, H-6, H-4, H-5 aromatic protons. Two one-proton multiplets at δ 5.27 and 5.24 were ascribed to H-9" and H-10" vinylic protons, respectively. A one-proton doublet at

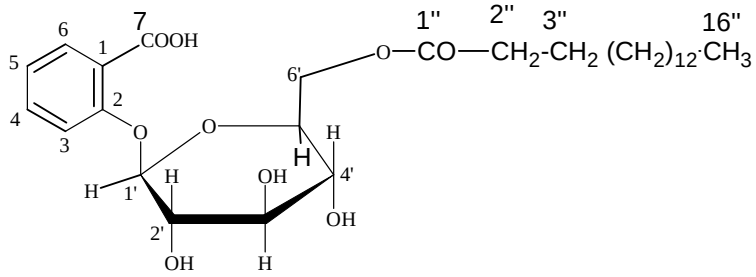
δ 4.94 (*J*= 7.2 Hz) and a one-proton double-doublet at δ 4.19 (*J*= 7.2, 8.4 Hz) were attributed correspondingly to anomeric H-1' and H-2' glycone protons. A two-proton broad signals at δ 3.62 and two one-proton broad signals at δ 3.41 and 3.38 were accounted to oxygenated methylene H_2-6 and H_2-5 protons, respectively. A one proton multiplet at δ 3.82 was associated with carbinol H-3' proton. The shifting of H_2-6' signal in the desheilded region at δ 3.62 suggested the attachment of ester function at C-6'. Two one-proton doublets at δ 2.70 and 2.66 (*J*= 6.1 Hz, each) were assigned to H_2-2" methylene protons adjacent to ester linkage while as two two-proton multiplets at δ 2.24 and 2.22 were attributed to H_2-8" and H_2-11" methylene protons adjacent to vinylic linkage, respectively. The remaining methylene protons resonated between δ 1.96 to 1.16. A three-proton triplet at δ 0.79 (*J*= 6.6 Hz) was assigned to Me-18" primary methyl protons. The ^{13}C NMR spectrum of **2** displayed signals for carboxylic carbon at δ 178.37 (C-7); ester carbon at δ 173.11 (C-1"); vinylic carbons at δ 115.47 (C-9"), 114.77 (C-10"); anomeric carbon at δ 109.41 (C-1') and primary methyl carbon at δ 14.06 (C-18"). The other sugar carbons appeared from δ 72.02 to 64.64. The appearance of the anomeric carbon in the deshielded region at δ 109.41 and C-4' carbon at δ 83.52 suggested that furonic form of the sugar. The shifting of C-6' signal in the downfield region at δ 64.99 supported attachment of the ester function at C-6'. The HMBC spectrum of **2** showed correlations of C-7 with H-6; C-2 with H-3, H-1' and H-2'; C-4' with H-3'; H_2-5' and H_2-6'; C-1" with H_2-6' and H_2-2"; C-9" with H_2-8" and H-10"; and C-18" with H_2-17". The hydrolysis products of **2** confirmed the presence of salicylic acid, D-glucose and oleic acid (m.p., co-TLC comparable). On the basis of above discussion the structure of **2** was characterized as salicylic acid–2-O-β-D-glucofuranosyl-6'-octadec-9"-enoate. This is a new phenolic acid glucosidic ester isolated from a plant source.



2

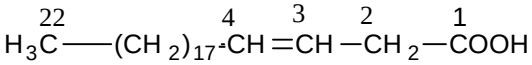
Compound **3**, designated as salicylic acid glucosyl palmitate was obtained as pale yellow crystals from chloroform-methanol (19:1) eluants. It gave effervescences with sodium bicarbonate. Its IR spectrum exhibited important absorption peaks for hydroxyl group (3422, 3375 cm^{-1}), ester group (1734 cm^{-1}), carboxylic group (3280, 1685 cm^{-1}), aromatic moiety (1633, 1515, 1085 cm^{-1}) and long aliphatic chain (720 cm^{-1}). Its +ve FAB mass spectrum displayed a molecular ion peak at *m/z* 538 corresponding to the molecular formula $C_{29}H_{46}O_9$. It showed important fragment ion peaks at *m/z* 239 $[CH_3\ (CH_2)_{14}CO]^+$ and 299 [M-239] due to ester linkage fission and at *m/z* 121 $[C_7H_5O_2]^+$, 137 $[C_7H_4O_3]^+$, 211 $[CH_3(CH_2)_{14}]^+$ and 401 $[M\text{-}137]^+$ due to glycosidic linkage fission. The fragment ion peaks at *m/z* 197 $[211\text{-}CH_2]^+$, 183 $[197\text{-}CH_2]^+$, 169 $[183\text{-}CH_2]^+$, 155 $[169\text{-}CH_2]^+$, 141 $[155\text{-}CH_2]^+$ and 127 $[141\text{-}CH_2]^+$, supported the presence of a long chain fatty acid. The ^{1}H NMR spectrum of **3** exhibited two downfield one-proton multiplet signals at 6.91, 6.26 and two one-proton ortho-, meta-coupled double-doublets at δ 7.57 (*J*= 8.1, 2.9 Hz) and δ 7.03 (*J*= 7.8, 2.7 Hz) assigned correspondingly to H-4, H-5, H-3 and H-6 aromatic protons. A

doublet at δ 5.11 (*J*= 7.1 Hz), integrating for one-proton, was ascribed to H-1' anomeric proton, while other sugar protons appeared as four one-proton multiplets at δ 4.30 (H-5'), 4.16 (H-2'), 4.05 (H-3') and 3.76 (H-4'). Two two-proton doublets at δ 3.66 and 3.63 (*J*= 7.5 Hz, each) were attributed to hydroxyl methylene H_2-6'a and H_2-6'b protons, respectively. Two one-proton doublets at δ 2.33 and 2.28 (*J*= 7.8 Hz, each) were accounted to H_2-2"a and H_2-2"b methylene protons adjacent to the ester group. The other methylene protons appeared between δ 2.01-1.03. A three-proton triplet at δ 0.85 (*J*= 6.6 Hz) was associated with Me-16" primary methyl protons. The shifting of the oxygenated methylene H_2-6' proton signals in the desheilded region at δ 3.66 and 3.63 indicated the attachment of the ester group at C-6'. The ^{13}C NMR spectrum of **3** displayed signals for carboxylic carbon at δ 178.56 (C-7); ester carbon at δ 174.11 (C-1"); aromatic carbons between δ 144.70-122.32; anomeric carbon at δ 101.58 (C-1') and primary methyl carbon at 14.08 (Me-16"). Acid hydrolysis of **3** yielded salicylic acid, palmitic acid and D-glucose. On the basis of above discussion the structure of **3** was characterized as salicylic acid-2-O-ß-D-glucopyranosyl-6'-hexadecanoate. This is a new phenolic acid glucosidic ester isolated from a plant source.



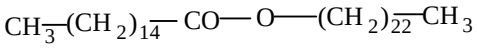
3

Compound **4,** docosenoic acid, was obtained as a colourless crystalline powder from chloroform-methanol (99:1) eluents. It gave effervescence with sodium bicarbonate indicating carboxylic nature of the molecule. Its IR spectrum exhibited characteristic absorption bands for carboxylic group (3340, 1708 cm^{-1}), unsaturation (1630 cm^{-1}) and a long aliphatic chain (724 cm^{-1}). The mass spectrum of **4** showed a molecular ion peak at *m/z* 338 corresponding to a molecular formula $C_{22}H_{42}O_2$ of a fatty acid. It indicated the presence of two double bond equivalent that were adjusted in a vinylic linkage and a carboxylic group. The ^{1}H NMR spectrum of **4** displayed a two-proton multiplet at δ 5.27 assigned to vinylic H-3 and H-4 protons, respectively. Two one-proton doublets at 2.29 and 2.24 (*J*= 7.5, each) were ascribed correspondingly to H_2-2'a and H_2-2'$_b$ methylene protons adjacent to the carboxylic group. The other methylene protons appeared at δ 1.96 (2H), 1.55 (2H), 1.53 (2H) and 1.18 (32H). A triplet at δ 0.80 (*J*= 6.9 Hz), integrating for three-protons was accounted to the terminal H-2 primary methyl protons. The ^{13}CNMR spectrum of **4** exhibited important signals for carboxylic carbon at δ 179.65 (C-1); vinylic carbons at δ 129.97 (C-3), 128.03 (C-4) and methyl carbons at δ 14.08 (C-22). The methylene carbons resonated in the range between δ 34.01 to 22.66. On the basis of the above mentioned discussion the structure of **4** has been established as docos-3-en-1-oic acid or 3-docosenoic acid. This is a new fatty acid.

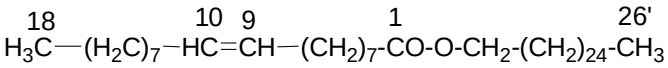


4

Compounds **5** and **6** were the known fatty acid esters characterized as tricosanyl hexadecanoate and hexacosanyl octadec-9-en-1-oate, respectively.



5



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