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Isolation of New Aliphatic Ester Linked with δ-lactone cos-11-enyl pentan-1-oic-1, 5-olide from the Roots of *Streblus asper* Lour.

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ABSTRACT: Phytochemical investigation of the methanolic extract of the roots of *Streblus asper* Lour. (Moraceae) led to the isolation of three new esters characterized as hexacosa-11′-enoyl pentan-1,5-olide(hexcosenyl lactone), β-D-glucopyranosyl hexacosa-1-oate (cerotic acid glucoside) and *n*-nonadecanyl-2-O-β-D- glucopyranosyloxy benzoate (nonadecanyl salicylate glucoside) along with *n*-octacosonoic acid, nonadecanyl -1-*n*- cosanoate and ursolic acid. The structures of all these phytoconstituents have been elucidated on the basis of spectral data analysis and chemical reactions.

KEYWORDS: *Streblus asper*; Roots; Fatty Acids; Aliphatic Ester; Salicyl Glucoside Ester.

INTRODUCTION

Streblus asper Lour. (Moraceae), commonly known as Sihora is a rigid shrub or medium sized tree distributed in tropical countries including India, Sri Lanka, Malaysia, Philippines and Thailand[1][2]. Its roots are used to treat inflammation, sinuses, filariasis, nervous disorders, leprosy, piles, dysentery, diarrhoea, elephantiasis, ulcers and fevers[1,3,4]. The reported phytoconstituents in the roots and stem bark are cardiac glycosides[9][7]. ,steroidal glycosides[8,10], triterpenoids and steroids [11,13,6,12]. The present paper describes the isolation and characterization of three new esters of δ-lactone, cerotic acid and salicylic acid glucoside from the roots of *S asper*.

EXPERIMENTAL SECTION

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 ceric sulphate solution.

Plant Material

The roots of *S. asper* 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 (2 kg) were shade dried, coarsely powdered and extracted exhaustively in Soxhlet apparatus with methanol. The methanolic extract was concentrated under reduced pressure to obtain a dark green viscous mass (94 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 re crystallized to get the following compounds:

Hexacosenyl lactone **(1)**

Elution of the column with chloroform-methanol (99:1) mixture afforded pale yellow crystals of **1**, recrystyallised from $CHCl_3$-MeOH (1:1), 500 mg (0.56 % yield); R_f: 0.72 ($CHCl_3$); m.p: 65-68°C; UV λ_{max} (MeOH): 265 (log ε 3.6); IR ν_{max} (KBr): 2920, 2851, 1737, 1645, 1465, 1376, 1265, 1170, 723 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.29 (1H, m, H-11'), 5.27 (1H, m, H-12'), 4.09 (1H, dd, *J*= 5.7,5.5 Hz , H-5β), 2.29 (1H, d, *J*= 7.5 Hz, H_2-2'a), 2.24 (1H, d, *J*= 7.5 Hz, H_2-2'b), 2.02 (2H, m, H_2-2), 1.78 (2H, m, H_2-10'), 1.73 (2H, m, H_2-13'), 1.55 (4H, m, 2× CH_2), 1.18 (26H, brs, 13 × CH_2), 0.80 (3H, t, *J*= 6.6 Hz, Me-20'); ^{13}C NMR ($CDCl_3$): δ 177.16 (C-1), 174.55 (C-1'), 129.81 (C-11'), 127.27 (C-12'), 99.96 (C-5), 34.09 (CH_2), 33.88 (CH_2), 31.91 (CH_2), 29.67 (13 × CH_2), 27.18 (CH_2), 24.85 (CH_2), 22.67 (CH_2), 14.10 (CH_3-20'); +ve ion FAB MS *m/z* (*rel. int.*): 408 $[M]^+$ ($C_{25}H_{44}O_4$) (23.1), 309 (11.8), 295 (4.5), 293 (2.1), 269 (3.3), 265 (7.1), 143 (32.6), 139 (20.2), 115 (21.1), 113 (10.1), 99 (11.2).

Nonadecanyl arachidonate **(2)**

Elution of the column with chloroform-methanol (99:1) mixture furnished colourless crystals of **2**, recrystyallised from methanol, 820 mg (0.91% yield); R_f: 0.76 ($CHCl_3$); m.p: 55-56°C; IR ν_{max} (KBr): 1737, 1641, 722 cm^{-1}; ^{13}C NMR (DMSO-d_6): δ 171.53 (C-1), 63.47 (C-1'), 34.90 (CH_2), 31.89 (CH_2), 29.68 (31× CH_2), 24.87 (CH_2), 22.26 (CH_2), 14.09 (2 × CH_3); +ve ion FAB MS *m/z* (*rel. int.*): 578 $[M]^+$ ($C_{39}H_{78}O_2$) (14.3), 311 (11.6), 295 (10.1).

Cerotic acid **(3)**

Elution of the column with chloroform-methanol (19:1) gave colourless crystals of **3**, recrystyallized from methanol, 420 mg (0.47% yield); R_f: 0.69 ($CHCl_3$-MeOH, 5:1); m.p: 77-78°C; IR ν_{max} (KBr): 3436, 1708, 1645, 720 cm^{-1}; ^{13}C NMR ($CDCl_3$): δ 181.13 (C-1), 31.84 (CH_2), 31.64 (CH_2), 31.64 (CH_2), 31.44 (CH_2), 30.10 (CH_2), 29.92 (CH_2), 29.39 (CH_2), 29.21 (CH_2), 22.13 (CH_2), 14.16 (CH_3-26); +ve ion FAB MS *m/z* (*rel. int.*): 396 $[M]^+$ ($C_{26}H_{52}O_2$) (22.3).

n-octacosanoic acid **(4)**

Elution of the column with chloroform-methanol (19:1) mixture yielded colourless amorphous powder of **4**, recrystyallised from $CHCl_3$-MeOH (1:1), 160 mg (0.18% yield); R_f: 0.48 ($CHCl_3$); m.p: 81-82°C; IR ν_{max} (KBr): 3416, 1708, 724 cm^{-1}; ^{13}C NMR ($CDCl_3$): δ 179.16 (C-1), 33.75 (CH_2), 31.92 (CH_2), 29.69 (CH_2), 24.69 (22×CH_2), 22.69 (CH_2), 14.11 (CH_2); +ve ion FAB MS *m/z* (*rel. int.*): 424 $[M]^+$ ($C_{28}H_{56}O_2$) (12.3).

Cerotic acid glucoside **(5)**

Elution of the column with chloroform-methanol (93:7) yielded colourless crystals of **5**, recrystyallised from acetone, 920 mg (1.02% yield); R_f: 0.62 ($CHCl_3$-MeOH, 4:1); m.p: 264-266°C; UV λ_{max} (MeOH): 221 (log ε 3.9); IR ν_{max} (KBr): 3376, 3295, 2921, 2852, 1737, 1647, 1462, 1374, 1272, 1122, 1073, 718 cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.31 (1H, d, *J*= 7.1 Hz, H-1'), 4.92 (1H, m, H-5'), 4.26 (1H, dd, *J*= 7.5, 7.1 Hz, H-2'), 3.59 (1H, m, H-3'), 3.48 (1H, m, H-4'), 3.21 (1H, d, *J*= 6.5 Hz, H_2-6'a), 3.19 (1H, d, *J*= 6.5 Hz, H_2-6'b), 2.49 (2 H, t, *J*=7.2 Hz, H_2-2), 1.98 (2H, m, CH_2), 1.78 (2H, m, CH_2), 1.54 (4 H, m, 2 × CH_2), 1.23 (38 H, brs, 19 × CH_2), 0.82 (3 H, t, *J*= 6.9 Hz, Me-26); ^{13}C NMR (DMSO-d_6): δ 173.56 (C-1), 101.39 (C-1'), 83.56 (C-5'), 73.48 (C-2'), 71.57 (C-3'), 69.81 (C-4'), 60.45 (C-6'), 55.98 (CH_2-2), 35.89 (CH_2), 30.92 (CH_2), 28.64 (14 × CH_2), 28.30 (CH_2), 26.24 (CH_2), 24.19 (CH_2), 23.53 (CH_2), 21.65 (CH_2), 20.34 (CH_2), 18.63 (CH_2), 13.33 (Me-26); +ve ion FAB MS *m/z* (*rel. int.*): 558 $[M]^+$ ($C_{32}H_{62}O_7$) (11.3), 395 (26.2), 379 (22.6).
Acid hydrolysis of **5**: Compound **5** (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 cerotic acid. The neutralized and concentrated aqueous hydrolysate showed the presence of glucose on comparison with authentic sample on silica gel TLC, R_f 0.4 (EtOAc-HOAc-H_2O-MeOH, 6:1:1:2).

Nonadecanyl salicylate glucoside **(6)**

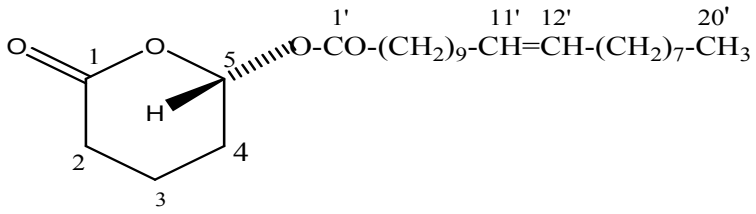
Elution of the column with chloroform-methanol (9:1) mixture yielded pale yellow crystals of **6**, recrystyallised from methanol, 980 mg (1.08 % yield); R_f: 0.8 ($CHCl_3$-MeOH, 3:2); m.p: 208-212°C; UV λ_{max} (MeOH): 225 (log ε 5.7); IR ν_{max} (KBr): 3397, 2922, 2852,

1737, 1646, 1512, 1462, 1374, 1270, 1122, 1077 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.51 (1H, dd, *J* =9.1, 2.8 Hz , H-3), 6.91 (1H, dd, *J* =8.9, 2.5 Hz, H-6), 6.74 (1H, m, H-5), 6.62 (1H, m, H-4), 5.29 (1H, d, *J*= 7.1 Hz, H-1"), 4.88 (1H, m, H-5"), 4.64 (1H, m, H-2"), 4.29 (1H, m, H- 3"), 4.14 (1H, m, H-4"), 3.31 (2H, t, *J*=8.8 Hz, H_2-1'), 3.15 (1H, d, *J*= 6.5 Hz, H_2-6"a), 3.06 (1H, d, *J*= 6.5 Hz, H_2-6"b), 1.93 (2H, m,CH_2), 1.68 (2H, m,CH_2), 1.48 (2H, m,CH_2), 1.19 (28H, brs,14 × CH_2), 0.80 (3H, t, *J*= 6.5 Hz, Me-19'); ^{13}C NMR (DMSO-d_6): δ 171.52 (C-7), 145.11 (C-2), 133.01 (C-1), 131.03 (C-3), 129.68 (C-6), 114.26 (C-5), 111.46 (C-4), 101.37 (C-1"), 86.59 (C- 5"), 76.54 (C-2"), 72.55 (C-3"), 69.58 (C-4"), 63.15 (C-1'), 60.01 (C-6"), 55.96 (C-2'), 41.90 (CH_2), 34.89 (CH_2), 32.60 (CH_2), 31.46 (CH_2), 29.32 (8 × CH_2), 26.70 (CH_2), 24.64 (CH_2), 22.21 (CH_2), 21.21 (CH_2), 13.95 (CH_3-19'); +ve ion FAB MS *m/z* (*rel. int.*): 562 $[M]^+$ ($C_{32}H_{50}O_8$) (5.3), 283 (70.5), 179 (19.6), 163 (25.2), 120 (61.7), 104 (73.8).

Acid hydrolysis of **6**: Compound **6** (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 get salicylic acid (m.p.159 °C). The neutralized and concentrated aqueous hydrolysate showed the presence of glucose on comparison with authentic sample on silica gel TLC, R_f. 0.4 (EtOAc-AcOH-H_2O-MeOH, 6:1:1:2).

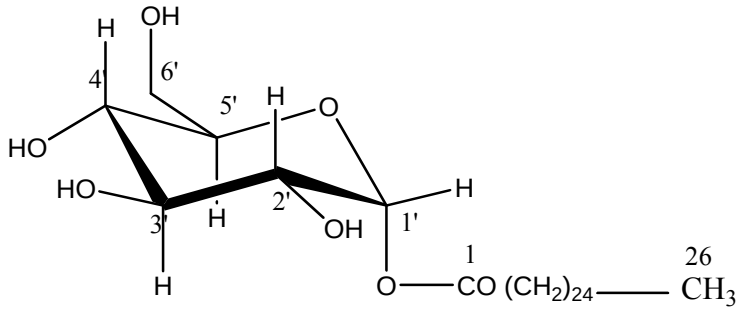
RESULTS & DISCUSSION

Compound **1,** hexcosenyl lactone, was obtained as pale yellow crystals from chloroform- methanol (99:1) eluants. Its IR spectrum exhibited absorption bands for δ lactone group (1737 cm^{-1}), unsaturation (1645 cm^{-1}) and long aliphatic chain (723 cm^{-1}). Its mass spectrum showed a molecular ion peak at *m/z* 408 corresponding to the molecular formula, $C_{25}H_{44}O_4$. It indicated the presence of four double bond equivalents; two of them were adjusted in the δ lactone, and one each in the ester group and a vinylic linkage. The prominent ion peaks arising at *m/z* 309 $[CH_3 (CH_2)_7 CH{=}CH (CH)_9 COO]^+$ and 99 $[M - 309, C_5H_70_2]^+$ indicated the presence of a δ-lactone esterified with gadoleic acid. The ^1H NMR spectrum of **1** exhibited two one-proton multiplets at δ 5.29 and 5.27 ascribed correspondingly to H-11' and H-12' vinylic protons. A double doublet integrating for one proton at δ 4.09 was assigned to H-5 oxygenated methine proton placed in β-orientation on the basis of its coupling constant (*J*= 5.7, 5.5 Hz). Two one-proton doublets at δ 2.29 and 2.24 (*J*= 7.5 Hz each) were ascribed to H_2-2'a and H_2-2'b methylene protons adjacent to ester group of the aliphatic chain. A two-proton multiplet at δ 2.02 was attributed to H_2-2′ methylene protons adjacent to carbonyl group in the lactone ring. Two two-proton multiplets at δ 1.78 and 1.73 were assigned to H_2-10' and H_2-13' methylene protons adjacent to vinylic linkage, respectively. The remaining methylene protons appeared as broad singlets at δ 1.55 (4H) and 1.18 (26H). Terminal primary methyl protons resonated as a three proton triplet at δ 0.80 (*J*= 6.6 Hz) C-20'. The ^{13}C NMR spectrum of **1** displayed important signal for lactone carbonyl carbon at δ 177.16 (C-1), ester carbon at δ 174.55 (C-1'), vinylic carbons at δ 129.81 (C-11') and 127.27 (C-12'), dioxygenarated methine carbon at δ 99.96 (C-5), methylene carbons resonated in the range of δ 34.09-22.67 and primary methyl carbon Me-20' at δ 14.10. The HMBC spectrum of **1** showed correlations of C-1 with H_2-2; C-1' with H-5 and H_2-2'; C-11' with H_2-10' and H-12'; and C-20' with H_2-19'. On the basis of above discussion the structure of **1** was elucidated as hexacos-11'-enoyl pentan- 1, 5-olide. This is a new aliphatic ester linked with δ-lactone.



1

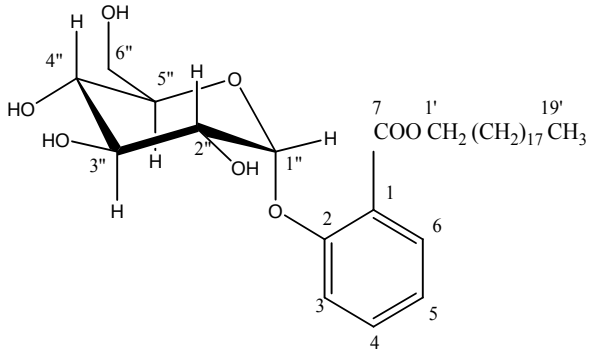
Compound **5,** designated as cerotic acid glucoside, were obtained as colourless crystals from chloroform –methanol (93:7) eluants. Its IR spectrum exhibited important absorption bands for hydroxyl groups (3376, 3295 cm^{-1}), ester group (1737 cm^{-1}) and long aliphatic chain (718 cm^{-1}). Its +ve FAB mass spectroscopy displayed a molecular ion peak at *m/z* 558 corresponding to the molecular formula of a fatty acid glucoside, $C_{32}H_{62}O_7$. The fragment ion peaks at *m/z* 379 $[M\text{-}C_6H_{11}O_6]^+$and 395 $[M\text{-}C_6H_{11}O_5]^+$ suggested that a long chain fatty acid was esterified to a hexose sugar. The ^1H NMR spectrum of **5** displayed a doublet, integrating for one-proton, at δ 5.31 (*J*= 7.1 Hz) assigned to H-1' anomeric proton. A one-proton double-doublet at δ 4.26 (*J*= 7.5, 7.1 Hz) was ascribed to H-2' carbinol proton. The remaining protons of the sugar unit resonated at δ 4.92 (H-5'), 3.59 (H-3') and 3.48 (H-4') as one-proton multiplets. Two one-proton doublets at δ 3.21 and 3.19 (*J*= 6.5 Hz, each) were attributed correspondingly to hydroxymethylene H_2-6'a and H_2-6'b protons of the sugar moiety. A two-proton triplet at δ 2.49 (*J*=7.2 Hz) was assigned to H_2-2 methylene protons adjacent to ester group. The remaining methylene protons resonated between δ 1.98 - 1.23. A three-proton triplet at δ 0.82 (*J*= 6.9 Hz) was accounted to Me-26 primary methyl protons. The ^{13}C NMR spectrum of **5** displayed important signals at δ 173.56 for C-1 ester carbon, δ 101.39 for C-1' anomeric carbon and δ 13.33 for C-26 methyl carbon. The remaining carbon signals of glucose unit resonated between δ 83.56-60.45. The methylene carbons appeared between δ 55.98-18.63. The HMBC spectrum showed interations of C-1 with H_2-2 and H-1'; C-2' with H-1' and H-4'; and C-26 with H_2-25. The acid hydrolysis of **5** yielded cerotic acid and glucose. On the basis of above discussion the structure of **5** was elucidated as D-glucopyranosyl-hexacosan-1-oate.



5

Compound **6,** designated as nonadecanyl salicylate glucoside, was obtained as pale yellow crystals from chloroform-methanol (91:9) eluants. It gave positive tests for glycosides. Its IR spectrum exhibited important absorption bands for hydroxyl groups (3397 cm^{-1}), ester group (1737 cm^{-1}) and aromatic moiety (1649,1512, 1077cm^{-1}). Its +ve FAB mass spectroscopy displayed a molecular ion peak at *m/z* 562 corresponding to the molecular formula of a benzoyl glucosyl ester, $C_{32}H_{50}O_8$. A prominent fragment ion peak at *m/z* 283 $[C_6H_4CO\ (OC_6H_{11}O_5)]^+$ arouse due to ester link cleavage suggested the presence of a glycoside of hydroxybenzoic acid esterified to C_{19} alcohol. The presence of hexose as sugar moiety was supported by fragment ions at *m/z* 120 $[283\text{-}C_6H_{11}O_5]^+$, 104 $[283\text{-}\ C_6H_{11}O_6]^+$, 163 $[C_6H_{11}O_5]^+$ and 179 $[C_6H_{11}O_6]^+$. The ^1H NMR spectrum of **6** displayed two one-proton

doublets at δ 7.51 (*J*= 9.1, 2.8Hz) and 6.91 (*J*= 8.9, 2.5Hz) assigned to orthos, meta-coupled H-3 and H-6, respectively. Two one proton multiplets at δ 6.74 and 6.62 were ascribed to aromatic H-5 and H-4, respectively. A single-proton doublet at δ 5.29 (*J*= 7.1 Hz) was accounted to H-1" anomeric proton. Four one-proton multiplets at δ 4.88, 4.64, 4.29 and 4.14 were due to sugar H-5", H-2", H-3" and H-4" protons, respectively. Two one-proton doublets at δ 3.15 and 3.06 (*J*= 6.5 Hz each), were attributed to H_2-6"a and H_2-6"b hydroxymethylene protons. A two-proton triplet at δ 3.31 (*J*= 7.2 Hz) was ascribed to H_2-1' oxygenated methylene proton. The remaining methylene protons appeared at 1.93 (2H), 1.68 (2H), 1.48 (4H) and 1.19 (28 H). The primary methyl protons Me-19' resonated as a three-proton triplet at δ 0.80 (*J*= 6.5 Hz). The ^{13}C NMR spectrum of **6** displayed important signals for ester carbon at δ 171.53 (C-7); aromatic carbons between δ 145.11-111.46; anomeric carbon at δ 101.37 (C-1") and primary methyl carbon at δ 13.95 (Me-19'). The ^{1}H-^{1}H COSY spectrum of compound **6** showed correlation of H-3 with H-5 and H-4; H_2-1'with H_2-2'; and H_2-1'' with H_2-2"; and H-3" with H-2" and H-4". The HMBC spectrum exhibited interactions of C-7 with H-6 and H_2-1'; C-2 with H-3, H-6 and H-1" ; H-4" with H_2-6''.



6

Acid hydrolysis of **6** yielded salicylic acid and D-glucose. On the basis of above discussion the structure of **6** was elucidated as n- nonadecanyl-2-*O*-*β*-*D* –glucopyranosyloxybenzoate. Compounds **2, 3** and **4** are the known phytoconstituents characterized as nonadecanyl arachidonate, cerotic acid and n-octacosanoic acid[14,15].

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