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Lupene-type triterpenic and steroidal constituents from the roots of *Streblus asper* Lour

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Abstract

Streblus asper Lour. (Moraceae)is a small tree found in tropical regions of the world and used for the treatment of fever, diarrhea, dysentery, elephantiasis, filariasis, gingivitis, leprosy, piles, skin diseases, toothache and wounds.Phytochemical investigation of a methanol extract of the roots led to the isolation of two new compounds characterized as lup-20(29)-en-3β-olyl octadec-9′-enoate (1) and stigmast-5-en-3β-olyl-26-oic acid-3β-hexadecanoate (4) together with the four known constituents identified as lupeol linoleate (2), stigmasterol palmitate (3), cerotic acid (5) and octacosanoic acid (6). The structures of all these compounds were elucidated on the basis spectral data analysis and chemical reactions.

Keywords: Streblus asper, Roots, Lupenic esters, Steroidal esters, Fatty acids.

Introduction

Streblus asper Lour. (Moraceae) is a small tree indigenous to tropical countries such as India, Sri Lanka, Malaysia, the Philippines and Thailand. It is used traditionally to treat leprosy, piles, dysentery, elephantiasis, skin diseases, filariasis, leprosy, toothache, wounds, fever, diarrhoea, dysentery and is effective against the oral cavity infections. The bark extract is used to relieve fever, dysentery, toothache, gingivitis, heart diseases and hypertension; the branch is used as a toothbrush for strengthening the teeth and gums [1-3]. *S. asper* is a rich source of cardiac glycosides [4,5]. The plant parts yielded triterpenoids, phytosterols,strebloside,mansonin, a pregnane glycoside named sioraside, aliphatic constituents, cerotic acid glucoside, nonadecanyl salicylateglucoside and cos-11-enyl pentan-1-oic-1,5-olide [6-13]. The present paper describes isolation and characterization of triterpenic, steroidal and fatty acid constituents from the roots of *S. asper*.

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 with ceric sulphate solution.

Plant Material

The roots of *S. asper* were collected from western 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/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 a 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 a 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 the same R_f values were combined and crystallized. The isolated compounds were re-crystallized to get the following compounds:

Lupeololeate (1)

Elution of the column with petroleum ether-chloroform (3:1) gave colourless crystals of **1**, recrystallized from acetone, 748 mg (0.79% yield); R_f: 0.52 ($CHCl_3$-MeOH, 4:1); m.p. 166-168 ^oC;UV λ_{max} (MeOH): 226 (log ε 4.9); IR ν_{max} (KBr): 2925, 2854, 1736, 1647, 1459, 1371, 1245, 1024, 896, 724 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.08 (1H, m, H-9′), 5.06 (1H, m, H-10′), 4.88 (2H, brs, H_2-29), 4.25 (1H, dd, J=5.1, 8.9 Hz, H-3α), 1.77 (3H, brs, Me-30), 1.11 (3H, brs, Me-23), 0.82 (3H, t, J=6.1 Hz, Me-18′), 0.77 (3H, brs, Me-24), 0.73 (3H, brs, Me-25), 0.65 (3H, brs, Me-26), 0.60 (3H, brs, Me-28), 0.55 (3H, brs, Me-27), 2.51-1.21 (31H, m, 13 × CH_2;7 × CH); ^{13}C NMR ($CDCl_3$): δ 39.55 (C-1), 26.85 (C-2), 80.81 (C-3), 38.39 (C-4), 55.19 (C-5), 18.17 (C-6), 34.68 (C-7), 40.78 (C-8), 51.27 (C-9), 37.62 (C-10), 21.31 (C-11), 26.06 (C-12), 39.94 (C-13), 42.74 (C-14), 28.67 (C-15), 28.01 (C-16), 58.98 (C-17), 47.15 (C-18), 47.56 (C-19), 150.75 (C-20), 29.08 (C-21), 33.65 (C-22), 27.11 (C-23), 17.43(C-24), 16.69 (C-25), 16.67 (C-26), 15.65(C-27), 17.41 (C-28), 109.33 (C-29), 22.61 (C-30), 170.77 (C-1′), 46.70 (C-2′), 37.01 to29.27 (C-3′ to C-8′), 139.53 (C-9′), 124.23 (C-10′), 33.99 to 23.16 (C-11′ to C-17′), 14.43 (C-18′); +ve ion FAB MS *m/z (rel. int.)*: 690 $[M]^+$ ($C_{48}H_{82}O_2$) (1.5), 425 (15.2), 410 (100), 408 (39.8).

Lupeollinoleate (2)

Elution of the column with petroleum ether-chloroform (1:1)furnished colourless crystals of **2,** recrystallized from acetone, 813 mg (0.86% yield); R_f: 0.58 ($CHCl_3$-MeOH, 7:3); m.p. 152-154 ^oC; IR ν_{max} (KBr): 2934, 2852, 1735, 1643, 1458, 1371, 1245, 1025, 977, 881 cm^{-1} ; ^1H NMR ($CDCl_3$): δ 5.10 (2H, m, H-9′, H-12′), 5.08 (2H, m, H-10′, H-13′), 4.86 (2H, brs, H_2-29), 4.24 (1H, dd, J=5.2, 8.7 Hz, H-3α), 1.78 (3H, brs, Me-30), 1.10 (3H, brs, Me-23), 0.81 (3H, t, J=6.3 Hz, Me-18′), 0.75 (3H, brs, Me-24), 0.72 (3H, brs, Me-25), 0.68 (3H, brs. Me-26), 0.61 (3H, brs, Me-27), 0.59 (3H, brs, Me-28), 2.51-1.21 (31H, m, 13 × CH_2,7 × CH); ^{13}C NMR ($CDCl_3$): δ 39.57 (C-1), 27.15 (C-2), 80.85 (C-3), 38.36 (C-4), 55.70 (C-5), 18.57 (C-6), 34.15 (C-7), 40.79 (C-8), 50.69 (C-9), 37.73 (C-10), 21.83 (C-11), 26.69 (C-12), 39.95 (C-13), 42.77 (C-14), 29.07(C-15), 28.70 (C-16), 58.99 (C-17), 47.93 (C-18), 47.58 (C-19), 150.80 (C-20), 29.66 (C-21), 33.69 (C-22), 27.38 (C-23), 16.13(C-24), 16.46 (C-25), 16.70 (C-26), 15.10(C-27), 17.45 (C-28), 109.35 (C-29), 19.24 (C-30), 170.86 (C-1′), 47.12 to 24.15 (C-2′ C-8′), 139.56 (C-9′), 124.26 (C-10′), 130.12 (C-12′), 130.12 (C-13′), 42.91 to 22.53 (C-11′ to C-17′), 14.45 (C-18′); +ve ion FAB MS *m/z (rel. int.)*: 688 $[M]^+$ ($C_{48}H_{80}O_2$) (1.3), 425 (24.9), 410 (100), 408 (61.2).

Stigmasterolpalmitate (3)

Elution of the column with petroleum ether- chloroform (1:3)yielded colourless amorphous powder of **3**,recrystallized from chloroform, 837 mg (0.89% yield); R_f: 0.42 (chloroform); m.p. 137-138 ^oC;UV λ_{max} (MeOH): 229 (log ε 3.1); IR ν_{max} (KBr): 2927, 2857, 1737, 1640, 1461, 1375, 1244, 1181, 1106, 1049, 960, 727 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.35 (1H, m, H-22), 5.32 (1H, m, H-23), 5.11 (1H, m, H-6), 4.01 (1H, brm, $w_{1/2}$ =16.5 Hz, H-3α), 1.04 (3H, brs, Me-19), 0.93 (3H, d, J= 6.2 Hz, Me-21), 0.87 (3H, d, J= 6.4 Hz, Me-26), 0.84 (3H, d, J= 6.1 Hz, Me-27), 0.82 (3H, t, J= 6.1 Hz, Me-16′), 0.80 (3H, t, J=5.6 Hz, Me-29), 0.67 (3H, brs, Me-18), 2.48 (2H, t, J=7.2 Hz, H_2-2′), 2.27 to 2.03 (4H, m, H_2-3′, H_2-4′), 1.25 (22H, brs, 11 × CH_2); ^{13}C NMR ($CDCl_3$): δ 69.76 (C-3), 140.71 (C-5), 121.66 (C-6), 1.37 (C-18), 19.25 (C-19), 18.31 (C-21), 138.29 (C-22), 129.97 (C-23), 45.83 (C-24), 31.92 (C-25), 20.36 (C-26), 22.50 (C-27), 23.07 (C-28), 11.58 (C-29), 173.12 (C-1′), 55.35to 23.56 (C-1, C-2, C-4, C-7 to C-17, C-20, C-2′ to C-15′), 17.26 (C-16′) ;+ve ion FAB MS *m/z (rel. int.)*: 650 $[M]^+$ ($C_{45}H_{78}O_2$) (7.5), 411 (8.5), 394 (11.5).

Sterblusterylpalmitate (4)

Elution of the column with chloroform afforded colourless crystals of **4**,recrystallized from acetone, 758 mg (0.80% yield); R_f: 0.36 ($CHCl_3$); m.p. 190-194 ^oC; IR ν_{max} (KBr): 3405,2922, 2852, 1725, 1707, 1645, 1462, 1376, 1239, 1180, 1059, 962, 725 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.35 (1H, m, H-6), 4.12 (1H, brm, $w_{1/2}$ =18.6 Hz, H-3α), 1.01 (3H, brs, Me-19), 0.93 (3H, d, J= 6.3 Hz, Me-21), 0.87 (3H, d, J=7.5 Hz, Me-27), 0.84 (3H, t, J= 6.6 Hz, Me-16′), 0.82 (3H, t,J= 6.3 Hz, Me-29),0.67 (3H, brs, Me-18), 2.78 – 1.18 (31H, m, 12 × CH_2, 7 × CH), 1.26 (20H, brs, 10 × CH_2), 1.12 (6H, brs, 3 × CH_2) ; ^{13}C NMR ($CDCl_3$): δ 37.21 (C-1), 31.87 (C-2), 71.77 (C-3), 42.21 (C-4), 140.70 (C-5), 121.67 (C-6), 31.55 (C-7), 32.93 (C-8), 50.10 (C-9), 38.57 (C-10), 23.02 (C-11), 39.72 (C-12), 40.46 (C-13), 56.73 (C-14), 24.27 (C-15), 28.20 (C-16), 55.98 (C-17), 111.93 (C-18), 19.35 (C-19), 36.46 (C-20), 18.73(C-21), 33.90 (C-22), 25.38 (C-23), 45.83 (C-24), 31.92 (C-25), 178.49 (C-26), 21.04 (C-27), 22.66 (C-28), 11.91 (C-29), 171.13 (C-1′),55.73 (C-2′), 35.59 (C-3′), 29.66 (C-4′ to C-10′), 26.02 (C-11′), 26.02 (C-12′), 24.72 (C-13′), 24.69 (C-14′), 22.68 (C-15′), 14.08 (C-16′); +ve ion FAB MS *m/z (rel. int.)*: 682 $[M]^+$ ($C_{45}H_{78}O_4$) (1.7), 638 (2.1), 441 (12.6), 427 (5.6), 412 (95.3), 397 (42.1), 383 (12.7), 381 (5.6), 368 (5.1), 270 (18.6), 256 (6.6), 255 (21.5), 240 (8.8), 239 (7.8), 213 (20.3), 198 (17.8).

Cerotic acid (5)

Elution of the column with chloroform-methanol (99:1) gave colourless crystals of **5**, recrystallized from methanol 423 mg (0.47% yield); R_f: 0.69 ($CHCl_3$-MeOH, 5:1); m.p. 88-89 ^oC; IR ν_{max} (KBr): 3436, 1708, 1645, 720 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.21 (2H, brs, H_2-2), 1.93 (2H, brs, CH_2), 1.50 (4H, brs, 2 × CH_2), 1.16 (40H, brs, 20 × CH_2), 0.79(3H, t, J=6.9 Hz, Me-26);^{13}C NMR ($CDCl_3$): δ 181.13 (C-1), 31.84 to 29.21 (23 × 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).

Octacosanoic acid (6)

Elution of the column with chloroform-methanol(19:1) mixture yieldedcolourless amorphous powder of **6,** recrystallized from $CHCl_3$-MeOH (1:1), 158 mg (0.18% yield); R_f: 0.48 ($CHCl_3$); m.p. 55-57 ^oC; IR ν_{max} (KBr): 3416, 1708, 724 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.37 (1H, t, J=5.7, H_2-2), 2.02 (2H, m, H_2-3), 1.63 (2H, m,CH_2), 1.25 (46H, brs, 23 × CH_2), 0.90 (3H, t, J=6.0 Hz, Me-28); ^{13}C NMR ($CDCl_3$): δ 176.16 (C-1), 33.75 to 24.69 (25 × 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), 379 (25.9), 129 (26.6), 115 (23.8).

Results and Discussion

Compound **1**, designated as lupeol palmitate, showed IR absorption bands for ester group (1736 cm^{-1}), unsaturation (1647 cm^{-1}) and long aliphatic chain (724 cm^{-1}). Its +ve FAB mass spectrum of exhibited a

molecular ion peak at *m/z* 690 consistent with the molecular formula of a triterpenic ester, $C_{48}H_{82}O_2$. It indicated seven double bond equivalents; five of them were adjusted in the pentacyclic carbon skeleton of the triterpenoid, one each in the vinylic linkage and ester group. The prominent ion peaks generating at *m/z* 425 [M - $CH_3(CH_2)_7CH$=CH- $(CH_2)_7CO]^+$, 410 [425 - $CH_3]^+$ and 408[425 - OH]$^+$ suggested thatit was esterified with oleic acid. The ^{1}H NMR spectrum of **1** displayed two one-proton multiplets at δ 5.08 and 5.06 assigned to vinylic H-9′ and H-10′ proton, respectively. A two-proton broad singlet at δ 4.88 was due to exocyclic methylene protons of a lupene-type triterpene. A one-proton double doublet at δ 4.25 with coupling interactions of 5.1 and 8.9 Hz was accounted to α-oriented oxygenated H-3 methine proton. A three-proton broad singlet at δ 1.77 was due to C-30 methyl protons located on C-20 vinylic carbon of the lupene molecule. The other tertiary methyl protons appeared as three-proton broad singlets at δ 1.11, 0.77, 0.73, 0.65,0.60 and 0.55 associated with tertiaryC-23, C-24, C-25, C-26, C-28 and C-27 methyl protons, respectively.A three-proton triplet at δ 0.82 (*J*= 6.1 Hz) was accounted to C-18′ primary methyl protons. The ^{13}C NMR spectrum of **1** showed signal for ester carbon at δ 170.77 (C-1′), vinylic carbons at δ 150.75 (C-20), 109.33 (C-29), 139.53 (C-9′) and 124.23 (C-10′), oxygenated methine carbon at δ 80.81 (C-3) and methyl carbons from δ 27.11 to 14.43.The ^{1}H and ^{13}C NMR signals of **1** were compared with the related lupene-type triterpenoids [14,15]. On the basis of the foregoing discussion the structure of **1** has been established as lup-20(29)-en-3β-olyl octadec-9′-enoate (Figure 1).

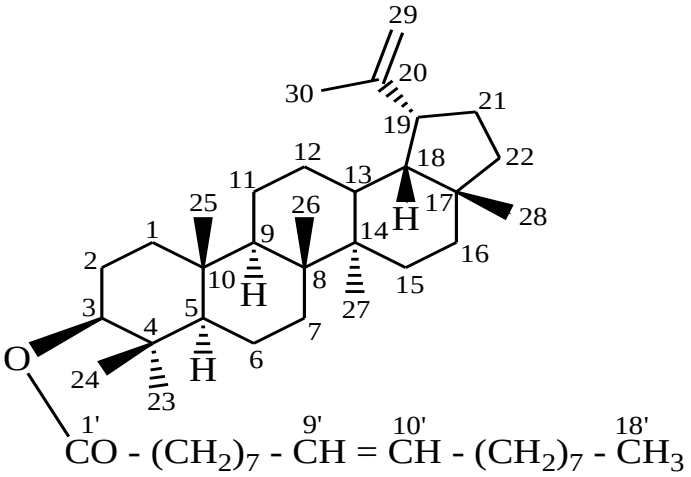

Figure 1: Structure of lupeol oleate (Compound **1**)

Compound **4**, named streblusteryl palmitate, produced effervescences with sodium bicarbonate solution suggesting carboxylic nature of the molecule. Its IR spectrum showed characteristic absorption bands for carboxylic group (1707 cm^{-1}), ester function (1725 cm^{-1}), unsaturation (1645 cm^{-1}) and long aliphatic chain (725 cm^{-1}). On the basis of mass and ^{13}C NMR spectra the molecular ion peak of **4** was established at *m/z* 682 consistent with the molecular formula of a steroidal acid ester, $C_{45}H_{78}O_4$. The prominent ion peaks generating at *m/z* 427 [M-$CH_3(CH_2)_{14}COOH]^+$, 412 [427- $CH_3]^+$, 397 [412- $CH_3]^+$, 381 [397-$CH_3]^+$, 383 [427- $CO_2]^+$, 368 [383- $CH_3]^+$, 256 [427- $C_{10}H_{19}O_2$, side chain]$^+$, 240[256-Me]$^+$, 213 [256-ring D]$^+$, 198 [213-Me]$^+$, 441 [M-$C_{16}H_{31}O]^+$, 270 [441-side chain]$^+$, 255 [$C_{16}H_{32}O_2$] and239 [$C_{16}H_{31}O$]suggested the steroid was esterified to C_{16} fatty acid and possessed a C_{10}-saturated side chain with the carboxylic group. The ^{1}H NMR spectrum of **4** displayed a one-proton doublet at δ 5.35 (*J*=5.3 Hz) assigned to vinylic H-6 proton. A one-proton broad multiplet at δ 4.12 with half width of 18.6 Hz was ascribed to α-oriented H-3 carbinol proton. A two-proton triplet at δ 2.33 (*J*=7.2 Hz) was accounted to H_2-2′ methylene protons adjacent to the ester linkage. Two three-proton broad singlets at δ 0.67 and 1.01 were due to C-18 and C-19 tertiary methyl protons, respectively. Two three-proton doublets at δ 0.93 (*J*= 6.3 Hz) and 0.87 (*J*= 7.5 Hz) were associated correspondingly to C-21 and C-27 secondary methyl protons. Two three-proton triplets at δ 0.82 (*J*= 6.3 Hz) and 0.84 (*J*= 6.6 Hz) were accounted to H_3-29 and H_3-16′ primary methyl protons, respectively. The presence of methyl signals in the range δ 1.01-0.67 indicated the location of these functionalities on the saturated carbons. The ^{13}C NMR spectrum of **4** showed signal for carboxyl carbon at δ 178.49 (C-26), ester carbon at δ 171.13 (C-1′), vinylic carbons at δ 140.70 (C-5) and 121.67 (C-6), oxygenated methine carbon at δ 71.77 (C-3) and methyl carbons from δ 21.04 to 11.93.The ^{1}H and ^{13}C NMR signals of **4** were compared with the related steroids [16-17]. Alkaline hydrolysis of **4** yielded a steroid acid and palmitic acid. On the basis of the foregoing discussion the structure of **4** has been established as stigmast-5-en-3β-olyl-26-oic acid-3β-hexadecanoate (Figure 2). This is the new steryl ester isolated from a plant source for the first time.

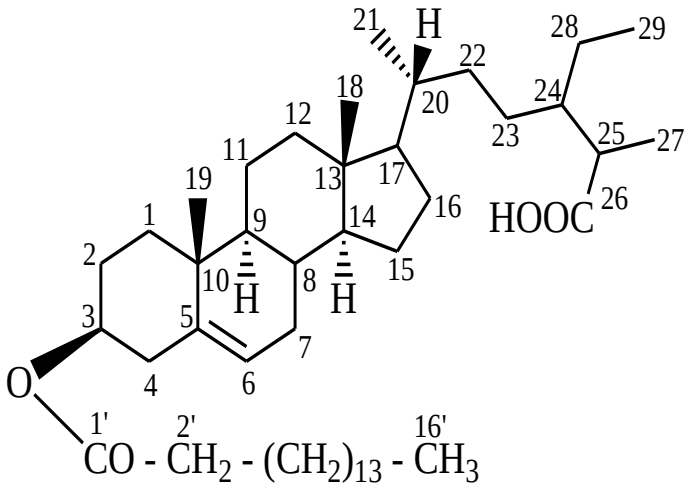

Figure 2: Structure ofsterblusteryl palmitate (Compound **4**)

The compounds **2, 3, 5** and **6** were known phytoconstituents characterized as lupeol linoleate [18], stigmasterol palmitate [19], cerotic acid and octacosanoic acid [13], respectively.

Conclusion

Phytochemical investigation of the roots of *Streblus asper* led to the isolation and characterization of lupene-type triterpenic esters, steroidal esters and fatty acids. This study has enhanced the phytochemical nature of the plant. These compounds may be used as chromatographic markers for standardization, as it is a drug of controversial identity in the traditional systems of medicine.

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