Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2016; 5(4): 01-05
Received: 01-05-2016
Accepted: 02-06-2016

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Phytochemical investigation of the roots of *Panax ginseng* C.A. Meyer

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Abstract
Panax ginseng C. A. Meyer (Araliaceae) is an oriental medicinal plant distributed in eastern Asia. It is used as an aphrodisiac, sexual rejuvenator, panacea for longevity and to treat headache, fatigue, diabetes, dizziness, nausea, asthma, Alzheimer's disease, hemorrhage and impotence. Phytochemical investigation of a methanolic extract of the roots of *P. ginseng* resulted in the isolation of new chemical constituents characterized as *cis*-,*cis*-*n*-tetratriacont-20,23-dienoic acid (1), (E,E)-5α-hydroxylabda-1,6-dien-20-oic acid 12β-olyl-O-β-D-xylopyranoside (2), stigmast-5-en-3β,4α,12β, 21-tetraol-21-octadec-9′,12′-dienoate (3) and lanost-24-en-3β-ol-3-O-β-D-arabinopyranosyl-(2′→1″)-O-β-D-arabinoside (5) along with a known steroidal constituent characterized as β-sitosterol 3-β-D-glucopyranoside (4). The structures of all the isolated phytoconstituents have been established on the basis of spectral data analysis and chemical reactions.

Keywords: *Panax ginseng*, roots, fatty acid, diterpenic xyloside, sterol ester, triterpenic diarabinoside

1. Introduction
Panax ginseng C. A. Meyer (Araliaceae) is a slow growing perennial umbel plant with fleshy roots. It is one of the most important oriental medicinal plants distributed in eastern Asia including north-eastern China, Korea, Bhutan and eastern Siberia typically in cool and shady climates [1, 2]. Ginseng is used to relieve headache, fatigue, diabetes, dizziness, nausea, asthma, Alzheimer's disease, hemorrhage and impotence, to strengthen the viscera, physical stamina and mental capacity, to improve resistance against diseases and work efficiency and to prevent muscle damage from exercise and athletic endurance [3-7]. Modern scientific data is substantiating many of these claims. It is imported into India and is used as an aphrodisiac, sexual rejuvenator and panacea for longevity. In Ayurvedic medicine, it is considered as a powerful adaptogen [8]. Ginseng species contain ginsenosides as active components which are triterpene saponins, essential oil, polyacetylenes, polysaccharides, peptidoglycans, nitrogenous compounds and various ubiquitous constituents such as fatty acids, carbohydrates and phenolic compounds [9-19]. This paper describes isolation and characterization of one each of a fatty acid, diterpenic glycoside, steroidal ester and lanostene diglycoside from the roots of *P. ginseng* procured from Delhi.

2. Materials and Methods
2.1. General procedure
Melting points were determined on a Perfit melting apparatus (Ambala, Haryana, India) and are uncorrected. UV spectra were measured with a Lambda Bio 20 spectrophotometer (Perkin-Elmer-Rotkreuz, Switzerland) in methanol. Infrared spectra were recorded on a Bio-Rad FTIR 5000 (FTS 135, Kawloon, Hong Hong) spectrophotometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were scanned on Advance DRX Bruker spectrospin 400 and 100 MHz, respectively, instruments (Karlsruhe, Germany) using TMS as an internal standard. Mass spectra were obtained by effecting FAB ionization at a 70 eV on a JEOL-JMS DX 303 spectrometer (Japan) equipped with direct inlet probe system. Column chromatography was performed on a silica gel (60-120 mesh; Qualigen, Mumbai, India) column. TLC was run on silica gel G (Qualigen) coated plates. Spots were visualized by exposing to iodine vapors, UV radiation and spraying with ceric sulfate solution.

2.2. Plant material

The roots of *P. ginseng* were procured from the Khari Baoli market of Delhi and identified by Prof. M.P. Sharma, Department of Botany, Jamia Hamdard, New Delhi. A voucher specimen is deposited in the herbarium of the Phytochemical Research Laboratory, Faculty of Pharmacy.

2.3. Extraction and isolation

The air-dried roots (2.0 kg) were coarsely powdered, defatted with petroleum ether and extracted with methanol exhaustively in a Soxhlet apparatus. The combined extracts were filtered and concentrated under reduced pressure to get a dark brown viscous mass (125 g, 6.25%). The dried extract was dissolved in minimum quantity of methanol and adsorbed on silica gel (60-120 mesh) for preparation of a slurry. It was dried in air and chromatographed over silica gel column (1.6 m x 16 mm x 2 mm) packed in petroleum ether. The column was eluted successively in increasing order of polarity in various combinations with chloroform, chloroform-methanol (19.9 : 0.1; 99 : 1; 97 : 3; 19 : 1; 93 : 7; 9 : 1; 17 : 3; 3 : 1; 3 : 2; 2 : 3, v/v) and methanol. The 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 recrystallized to get pure compounds. The following compounds were isolated from the methanolic extract of the roots of *P. ginseng:*

2.4. *n*-Tetratriacont-20, 23-dienoic acid (1)

Elution of the column with chloroform furnished colorless crystals of **1,** recrystallized from chloroform-methanol (1:1), m.p. 108-109 °C; UV λ_{max} (MeOH): 223 nm (log Ɛ 2.9); IR ν_{max} (KBr): 3335, 2924, 2854, 1708, 1651, 1458, 1413, 1376, 1248, 1073, 1034, 991, 722 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.38 (1H, m, $w_{1/2}$ = 8.6 Hz, H-23), 5.36 (1H, m, $w_{1/2}$ = 8.4 Hz, H-10), 5.34 (1H, m, $w_{1/2}$ = 9.1 Hz, H-23), 5.32 (1H, m, $w_{1/2}$ = 7.9 Hz, H-24), 2.78 (2H, dd, *J* = 7.5, 7.2 Hz, H_2-22), 2.34 (2H, t, *J* = 7.2 Hz, H_2-2), 2.06 (2H, m, H_2-19), 2.03 (2H, m, H_2-25), 1.64 (2H, m, CH_2), 1.54 (2H, m, CH_2), 1.36 (14H, brs, 7 x CH_2), 1.32 (20H, brs, 10 x CH_2), 1.30 (8H, brs, 4 x CH_2), 1.26 (4H, m, 2 x CH_2), 0.87 (3H, t, *J* = 7.1 Hz, Me-34); ^{13}C NMR ($CDCl_3$): δ 180.37 (C-1), 130.13 (C-21), 129.94 (C-23), 127.85 (C-20), 127.70 (C-24), 34.06 (C-22), 31.89 (C-19), 31.49 (C-25), 29.63 (CH_2), 29.54 (CH_2), 29.40 (CH_2), 29.31 (CH_2), 29.31 (CH_2), 29.20 (CH_2), 29.11 (CH_2), 29.03 (7 x CH_2), 28.99 (6 x CH_2), 27.16 (CH_2), 27.14 (CH_2), 25.58 (CH_2), 24.61 (CH_2), 22.65 (CH_2), 22.53 (CH_2), 14.01 (Me-34); +ve ion FAB MS *m/z (rel. int.)*: 505 $[M]^+$ ($C_{34}H_{65}O_2$) (11.2), 459 (23.3), 363 (7.1), 337 (8.6), 337 (8.7), 323 (20.5), 297 (12.8), 207 (48.9).

2.5. 5α-Hydroxylabd-1,6-dien-20-oic acid 12β-olyl O-β-D-xylopyranoside (2)

Elution of the column with chloroform-methanol (49 : 1) furnished colourless crystals of 2, recrystallized from methanol-methanol (1:1), m.p : 165-167 ° C, IR ν_{max} (KBr) : 3405, 3326, 3262, 2927, 2842, 1695, 1637, 1462, 1260, 1083, 913 cm^{-1}; ^1H NMR ($CDCl_3$) : δ 5.94 (1H, m, $w_{1/2}$ = 18.3 Hz, H-2), 5.45 (1H, d, J = 16.8 Hz, H-6), 5.25 (1H, d, J = 16.6 Hz, H-1), 4.98 (1H, m, $w_{1/2}$ = 16.2 Hz, H-7), 3.68 (1H, $w_{1/2}$ = 15.8 Hz, H-12α), 2.58 (2H, brs, H_2-3), 2.29 (1H, m, H-8), 2.04 (1H, m, H-13), 1.78 (1H, m, H-9), 1.63 (2H, m, H_2-11), 1.49 (2H, m, H_2-14), 1.29 (3H, brs, Me-17), 1.26 (3H, brs, Me-16), 1.11 (3H, brs, Me-19), 0.93 (3H, J = 6.8 Hz, Me-18.6), 0.87 (3H, t, J = 6.5 Hz, Me-15), 5.01 (1H, d, J = 7.5 Hz, H-1′), 4.24 (2H, d, J = 8.5 Hz, H-5′), 3.67 (1H, m, H-2′), 3.64 (1H, m, H-3′), 3.60 (1H, m, H-4′); ^{13}C NMR ($CDCl_3$): δ 116.94 (C-1), 138.92 (C-2), 28.54 (C-3), 51.48 (C-4), 74.88 (C-5), 136.10 (C-6), 114.28 (C-7), 31.78 (C-8), 48.75 (C-9), 33.65 (C-10), 29.64 (C-11), 70.44 (C-12), 33.43 (C-13), 22.60 (C-14), 14.05 (C-15), 29.01 (C-16), 28.76 (C-17), 24.06 (C-18), 25.18 (C-19), 177.20 (C-20), 101.25 (C-1'), 73.07 (C-2'), 72.19 (C-3'), 66.46 (C-4'), 63.20 (C-5'); +ve FAB MS *m/z* (rel.int) : 469 $[M+H]^+$ ($C_{25}H_{41}O_8$) (7.1), 149 (12.5), 133 (22.1).

2.6. 21-Linolenyloxy stigmast-5-en-3β,4α,12β-triol (3)

Elution of the column with chloroform-methanol (19:1) gave pale yellow crystals of 3, m.p. 167-168° C, UV λmax (MeOH): 217 nm (log ξ 4.9); IR ν_{max} (KBr): 3381, 3276, 2928, 2847, 1733, 1645, 1454, 1371, 1250, 1068, 1032, 721 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.35 (1H, d, J = 6.7 Hz, H-6), 5.32 (1H, m, H-9′), 5.29 (1H, m, H-10′), 5.27 (1H, m, H-12′), 5.25 (1H, m, H-13′), 4.12 (1H, d, J = 11.2 Hz, H_2-21α), 4.06 (1H, d, J = 11.2 Hz, H_2-21β), 3.99 (1H, d, J = 4.6 Hz, H- 4β), 3.54 (1H, dd, J = 3.9, 9.1 Hz, H-12α), 3.41 (1H, ddd, J = 4.6, 3.8, 9.2 Hz, H-3α), 2.73 (2H, m, H_2-11″), 2.25 (2H, t, J = 7.5 Hz, H_2-2′), 2.21- 1.04 (31H, m, 12 x CH_2, 7 x CH), 1.23 (8H, brs, 4 x CH_2), 1.20 (6H, brs, 3 x CH_2), 0.98 (3H, brs, Me-19), 0.87 (3H, d, J = 6.5 Hz, Me-26), 0.83 (3H, d, J = 6.8 Hz, Me- 27), 0.80 (3H, t, J = 6.3 Hz, Me- 18′), 0.77 (3H, t, J = 6.2 Hz, Me-29), 0.65 (3H, brs, Me- 18); ^{13}C NMR ($CDCl_3$): δ 39.51 (C-1), 31.72 (C-2), 71.82 (C-3), 75.56 (C-4), 139.41 (C-5), 122.47 (C-6), 32.42 (C-7), 34.19 (C-8), 51.53 (C-9), 37.89 (C-10), 22.37 (C-11), 72.11 (C-12), 45.64 (C-13), 56.57 (C-14), 25.08 (C-15), 27.07 (C-16), 55.91 (C-17), 11.69 (C-18), 19.52 (C-19), 35.94 (C-20), 60.98 (C-21), 33.72 (C-22), 25.71 (C-23), 42.08 (C-24), 29.01 (C-25), 18.78 (C-26), 18.52 (C-27), 24.09 (C-28), 11.61 (C-29), 174.86 (C-1′), 33.86 (C-2′), 33.48 (C-3′), 32.77 (C-4′), 29.95 (C-5′), 29.49 (C-6′), 29.41 (C-7′), 32.44 (C-8′), 130.58 (C-9′), 129.81 (C-10′), 36.77 (C-11′), 129.61 (C-12′), 127.75 (C-13′), 31.32 (C-14′), 29.35 (C-15′), 29.08 (C-16′), 22.68 (C-17′), 14.06 (C-18′); +ve FAB MS *m/z* (rel. int.): 725 $[M+H]^+$ ($C_{47}H_{81}O_5$) (2.1), 445 (6.8), 427 (5.2), 409 (11.4), 279 (8.1).

2.7. β-Sitosterol 3-β-D-glucopyranoside (4)

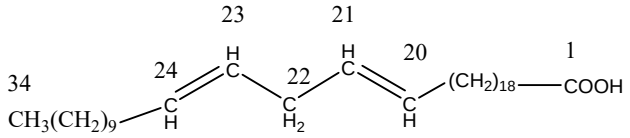
Further elution of the column with chloroform-methanol (19:1) yielded colourless amorphous powder of 4, recrystallized from methanol, yield: 200 mg; R_f 0.35 (chloroform: methanol: 9: 1); m.p. 265- 267°C, UV λ_{max} (MeOH): 241 nm (log ε 2.9); IR υ_{max} (KBr): 3401, 2918, 2849, 1654, 1377, 1261, 1172, 1082 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.34 (1H, m, H-6), 5.11(1H, d, J= 7.2 Hz, H-1′), 4.37 (1H, m, H-5′), 4.31 (1H, m, H-4′), 4.16 (1H, m, H-3′), 3.91 (1H, m, H-2′), 3.54 (1H, brs, $w_{½}$= 18.5 Hz, H-3), 3.37 (2H, brs, H_2-6′), 0.99 (3H, brs, M-19), 0.92 (3H, d, J= 6.2 Hz, Me-21), 0.87 (3H, brs, Me-27), 0.84 (3H, brs, Me-26), 0.82 (3H, brs, Me-29), 0.67 (3H, brs, Me-18); ^{13}C NMR ($CDCl_3$): δ 37.28 (C-1), 31.93 (C-2), 70.62 (C-3), 42.33 (C-4), 140.31 (C-5), 122.10 (C-6), 29.33 (C-7), 34.23 (C-8), 50.21 (C-9), 36.14 (C-10), 22.66 (C-11), 38.89 (C-12), 39.78 (C-13), 56.18 (C-14), 27.21 (C 15), 28.22 (C-16), 56.12 (C-17), 11.85 (C-18), 19.33 (C-19), 36.73 (C-20), 19.03 (C-21), 33.98 (C-22), 26.18 (C-23), 45.68 (C-24), 29.68 (C-25), 21.07 (C-26), 19.78 (C-27), 24.94 (C-28), 14.07 (C-29), 101.20 (C-1′), 73.92 (C-2′), 68.61 (C-3′), 73.62 (C-4′), 78.58 (C-5′), 62.11 (C-6′); +ve ion FABMS *m/z* (rel.int.): 576 $[M]^+$ ($C_{35}H_{60}O_6$) (11.3), 413 $[M - C_6H_{11}O_5]^+$ (10.1), 398 (100).

2.8. Lanosten- 3β-olyl diarabinoside (5)

Elution of the column with chloroform-methanol (9:1) yielded colorless crystals of 5, m.p. 201-203° C, IR ν_{max} (KBr): 3415, 3355, 3263, 2925, 2842, 1635, 1454, 1374, 1260, 1071, 1045, 906 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.11 (1H, t, J = 6.4 Hz, H-24), 3.38 (1H, dd, J = 5.6, 9.2 Hz, H-3α), 1.62 (3H, brs, Me-26), 1.57 (3H, brs, Me-27), 1.12 (3H, brs, Me-19), 0.97 (3H, brs, Me-29), 0.93 (3H, brs, Me-28), 0.84 (3H, d, J = 6.6 Hz, Me - 21), 0.78 (3H, brs, Me-30), 0.73 (3H, brs, Me-18), 2.28-1.01 (25H, m, 10 x CH_2, 5 x CH), 4.99 (1H, d, J = 7.5 Hz, H-1′), 3.98 (1H, m, H-2′), 3.48 (1H, m, H-3′), 3.39 (1H, m, H-4′), 3.77 (2H, dd, J = 3.1, 3.2 Hz, H_2-5′), 4.71 (1H, d, J=7.3 Hz, H-1″), 3.46 (1H, m, H-2″), 3.36 (1H, m, H-3″), 3.26 (1H, m, H-4″), 3.70 (2H, d, J = 4.2, 4.3 Hz, H_2-5″); ^{13}C NMR ($CDCl_3$): δ 34.71 (C-1), 27.69 (C-2), 81.38 (C-3), 38.75 (C-4), 53.51 (C-5), 17.98 (C-6), 27.72 (C-7), 48.19 (C-8), 49.81 (C-9), 36.71 (C-10), 22.13 (C-11), 34.06 (C-12), 47.72 (C-13), 51.45 (C-14), 30.96 (C-15), 34.55 (C-16), 51.38 (C-17), 15.56 (C-18), 17.46 (C-19), 32.36 (C-20), 16.82 (C-21), 39.13 (C-22), 25.11 (C-23), 124.91 (C-24), 131.27 (C-25), 29.48 (C-26), 26.03 (C-27), 28.57 (C-28), 26.82 (C-29), 16.11 (C-30), 105.04 (C-1′), 76.18 (C-2′), 73.94 (C-3′), 70.66 (C-4′), 62.02 (C-5′), 92.16 (C-1″), 75.36 (C-2″), 73.21 (C-3″), 69.96 (C-4″), 61.85 (C-5″); +ve FAB MS *m/z* (ret.int.): 693 $[M + H]^+$ ($C_{40}H_{69}O_9$) (1.8), 559 (4.2), 427(16.4), 133 (25.3).

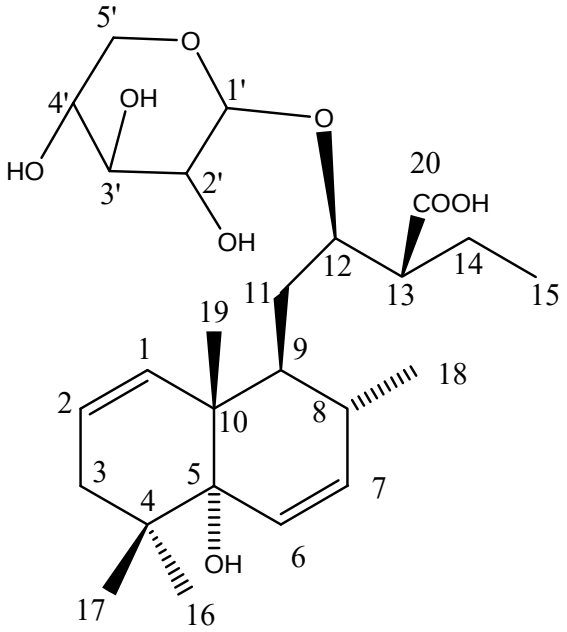
3. Results and Discussion

Compound 1 produced effervescences with sodium bicarbonate solution suggesting carboxylic nature of the molecule. Its IR spectrum showed absorption bands for carboxylic group (3335, 1708 cm^{-1}), unsaturation (1651 cm^{-1}) and long aliphatic chain (722 cm^{-1}). Its +ve FAB mass spectrum displayed a molecular ion peak at *m/z* 505 $[M+H]^+$ corresponding to a molecular formula of an aliphatic unsaturated acid, $C_{34}H_{65}O_2$. The ion peaks arising at *m/z* 459 $[M - COOH]^+$, 207 [C_{19}–C_{20} fission, $CH_3(CH_2)_8(CH_2CH{=}CH)_2]^+$ and 297 [M– 207; $(CH_2)_{18}COOH]^+$ indicated the existence of the carboxylic function at one of the terminal carbon of the molecule. The ion fragments generated at *m/z* 323 [C_{21}–C_{22} fission, $CH{=}CH(CH_2)_{18}COOH]^+$, 337 [$C_{22}$–$C_{23}$ fission, $CH_2CH{=}CH(CH_2)_{18}COOH]^+$ and 363 [C_{24}–C_{25} fission, $(CH{=}CH\text{-}CH_2)_2\ (CH_2)_{17}COOH]^+$ suggested the presence of the two vinylic linkages at C_{20} and C_{23} positions. The ^1H NMR spectrum of 1 exhibited four one-proton multiplets between δ 5.38 - 5.32 with half-width from 9.1 to 7.9 Hz assigned to *cis*-oriented H-23, H-21, H-20 and H-24 vinylic protons. A two-proton double doublet at δ 2.78 (J = 7.5, 7.2 Hz) and a two-proton triplet at δ 2.34 (J = 7.2 Hz) were ascribed to methylene H_2-22 protons between the vinylic carbons and methylene H_2-2 protons adjacent to the carboxylic group. The remaining methylene protons resonated between δ 2.06 to 1.26. A three-proton triplet at δ 0.87 (*J* = 7.1 Hz) was accounted to terminal C-34 primary methyl protons. The ^{13}C NMR spectrum of 1 displayed important signals for carboxylic carbon at δ 180.37 (C-1), vinylic carbons between δ 130.13–127.70, methylene carbons from δ 34.06 to 22.53 and primary methyl carbon at δ 14.01 (Me-34). The ^1H-^1H COSY spectrum of 1 showed correlations of H-21 with H_2-19, H-20 and H_2-22; H-24 with H_2-22, H-23 and H_2-25; and Me-34 with H_2-32 and H_2-33. On the basis of these evidences the structure of 1 has been characterized as *cis*-, *cis*-*n*-tetratriacont-20,23-dienoic acid. The is a new unsaturated fatty acid.



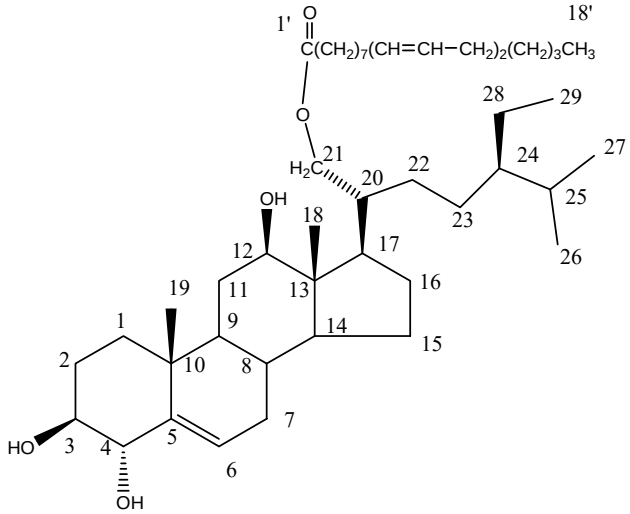
cis-cis- n-Tetratriacont-20,23-dienoic acid (1)

Compound 2 gave effervescences with sodium bicarbonate solution, responded glycoside tests positively and showed IR absorption bands for hydroxyl groups (3405, 3326 cm^{-1}), carboxylic function (3262, 1695 cm^{-1}) and unsaturation (1637 cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectra, its molecular weight was established at *m/z* 469 $[M+H]^+$ consistent with a molecular formula of a bicyclic diterpenic glycoside, $C_{25}H_{41}O_8$. The ion peaks arising at *m/z* 149 $[C_5H_9O_5]^+$ and 133 $[C_5H_9O_4]^+$ suggested the attachment of a C_5 - sugar with the diterpenic residue. The ^1H NMR spectrum of 2 showed two one-proton doublets at δ 5.45 (J = 16.8 Hz) and 5.25 (J = 16.6 Hz) and two one-proton multiplets at δ 5.94 ($w_{1/2}$ = 18.3 Hz) and 4.98 ($w_{1/2}$ =16.2 Hz) assigned to *trans*-oriented vinylic H-6, H-1, H-2 and H-7 protons, respectively. A one-proton multiplet at δ 3.68 with half-width of 15.8 Hz was ascribed to oxygenated H-12α methine proton. Three broad singlets at δ 1.29, 1.26 and 1.11, a doublet at δ 0.93 (J = 6.8 Hz) and a triplet at δ 0.87 (J = 6.5 Hz), all integrating for three protons each, were associated with tertiary C-17, C-16 and C-19, secondary C-18 and primary C-15 methyl protons, respectively. A one-proton doublet at δ 5.01 (J = 7.5 Hz) was accounted to anomeric H-1′ proton. The other sugar protons appeared as a two-proton doublet at δ 4.24 (J = 8.4 Hz, H_2-5′) and as one-proton multiplets between δ 3.67 – 3.60. The ^{13}C NMR spectrum of 2 displayed signals for the carboxylic carbon at δ 177.20 (C-20), vinylic carbons from δ 138.94 to 114.28, anomeric carbon at δ 101.25 (C-1'), oxygenated methine carbon at δ 70.44 (C-12), hydroxyl quaternary carbon at δ 74.88 (C-5), other sugar carbons in the range of δ 73.07-63.20 and methyl carbons from δ 29.01 to 14.05. The ^1H- ^1H COSY spectrum of 2 showed correlations of H-2 with H-1 and H_2-3; H-6 with H-7 and H-8; H-12 with H_2-11 and H-13; and H-1′ with H-12, H-2′ and H-3′. The HMBC spectrum of 2 exhibited interactions of H-1 and H_2-3 with C-2; H-6, H-7 and Me-19 with C-5; H-12, H-13 and H_2-14 with C-20; H-2′, H-3′ and H-12 with C-1′; Me-16, Me-17 and H_2-3 with C-4; H-13 and H_2-14 with C-15. The HSQC spectrum of 2 displayed correlations of proton signals at δ 5.94 (H-2), 5.45 (H-6), 5.25 (H-1) and 4.98 (H-7) with their respective carbons at δ 138.94 (C-2), 136.10 (C-6), 116.94 (C-1) and 114.28 (C-7); anomeric proton at δ 5.01 with C-1′ at δ 101.25 and oxygenated methine proton at δ 3.68 (H-12) with δ 70.44 (C-12). The ^1H and ^{13}C NMR spectral values of 2 were compared with the reported labdanyl-type diterpenoids [20-22]. Acid hydrolysis of 2 yielded D-xylose, co-TLC comparable. On the basis of the foregoing account, the structure of 2 has been elucidated as (E, E)-5α-hydroxylabda-1,6-dien-20-oic acid 12β-olyl-O-β-D-xylopyranoside. This is a new labdane-type xyloside.



(E, E)-5α-Hydroxylabda-1,6-dien-20-oic acid 12β-olyl-O-β-D-xylopyranoside (2)

Compound 3 had characteristic IR absorption bands for hydroxyl groups (3381, 3276 cm^{-1}), ester function (1733 cm^{-1}), unsaturation (1645 cm^{-1}) and long aliphatic chain (721 cm^{-1}). Its molecular ion peak was determined at *m/z* 725 $[M+ H]^+$ on the basis of FAB mass and ^{13}C NMR spectra corresponding to the molecular formula of a steroidal ester $C_{47}H_{81}O_5$. The ion peaks arising at *m/z* 445 [C_{21}-O fission, $C_{30}H_{49}O_3]^+$, 427 $[445\text{-}H_2O]^+$, 409 $[427\text{-}H_2O]^+$ and 279 $[CH_3(CH_2)_3(CH_2\text{-}CH{=}CH)_2(CH_2)_7CO]^+$ indicated that linoleic acid was esterified with a steryl tetraol. The ^1H NMR spectrum of 3 showed five deshielded signals as a doublet at δ 5.35 (J = 6.7 Hz) and as multiplets at δ 5.32, 5.29, 5.27 and 5.25 assigned to vinylic H-6, H-9′, H-10′, H-12′ and H-13′ protons, respectively. Three one-proton signals as a triple doublet at δ 3.41 (J = 4.6, 9.2, 3.8 Hz), as a doublet at δ 3.99 (J = 4.6 Hz) and as a double doublet at δ 3.54 (J = 3.9, 9.1 Hz) were ascribed to carbinol H-3α, to H-4β adjacent to the vinylic carbon C-5 and to H-12α protons, respectively. Two one-proton doublets at δ 4.12 and 4.06 with coupling constant of 11.2 Hz each were attributed to oxygenated methylene H_2-21 protons linked to the ester function. Six three-proton signals as broad singlets at δ 0.65 and 0.95, as doublets at δ 0.87 (J= 6.5 Hz) and 0.83 (J = 6.8 Hz) and as triplets at δ 0.77 (J = 6.2 Hz) and 0.80 (J = 6.3 Hz) were associated with the tertiary C-18 and C-19, secondary C-26, and C-27 and primary C-29 and C-18′ methyl protons, all attached to saturated carbons. The other methine and methylene protons resonated from δ 2.73 to δ 1.04. The ^{13}C NMR spectrum of 3 exhibited signals for ester carbon at δ 174.86 (C-1′), vinylic carbons between δ 139.41- 122.50, carbinol carbons at δ 71.82 (C-3), 75.56 (C-4) and 72.11 (C-12), oxygenated methylene carbon at δ 60.98 (C-21) and methyl carbons from δ 19.52-11.69. The ^1H-^1H COSY spectrum of 3 showed correlations of H-3 with H_2-2 and H-4; H-12 with H_2-11, H-9 and Me-18; H_2-21 with H-20, H-17 and H_2-22; H-10′ with H-9′, H_2-11′ and H-12′; and H-24 with H_2-23, H_2-28, Me-29, H-25, Me-26 and Me-27. The HMBC spectrum of 3 exhibited interactions of H-3, H-4, H-6 and Me-19 with C-5; H_2-11, H-12, H-17 and Me-18 with C-13; H_2-21 and H_2-2′ with C-1′; and H-9′, H-10′, H-12′ and H-13′ with C-11. Its HSQC spectrum showed correlation of H-3 at δ 3.41 with C-3 at 71.82, H-4 at δ 3.99 with C-4 at δ 75.56; H-12 at δ 3.54 with C-12 at 72.11; H_2-21 at δ 4.12 and 4.06 with C-21 at δ 60.98; H-6 at δ 5.35 with C- 6 at δ 122.47; and other vinylic and methyl protons with the respective carbons. The ^1H and ^{13}C NMR spectral data of the steroidal nucleus were compared with the reported spectral values of the related compounds [23-25]. Alkaline hydrolysis of 3 yielded linoleic acid, co-TLC comparable. On the basis of these evidences, the structure of 3 has been established as stigmast-5-en-3β,4α,12β,21-tetraol-21-octadec-9′,12′-dienoate. This is a new steroidal ester.

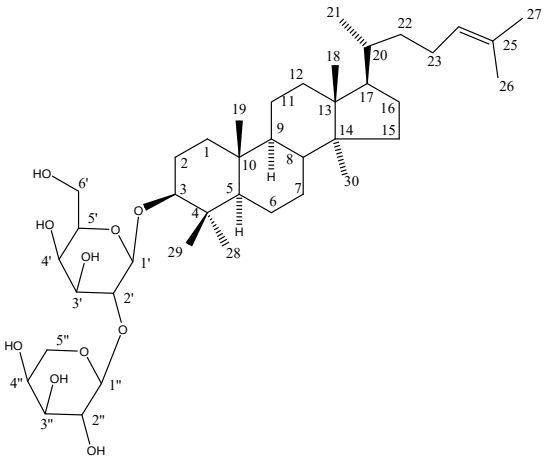


Stigmast-5-en-3β,4α,12β, 21-tetraol-21-octadec-9′,12′-dienoate (3)

Compound 4 was the known steroidal constituent characterized as β-sitosterol 3-β-D- glucopyranoside.

Compound 5 showed IR absorption bands for hydroxyl groups (3415, 3355, 3263 cm^{-1}) and unsaturation (1635 cm^{-1}). Its molecular ion peak was determined at *m/z* 693 $[M+H]^+$ on the basis of FAB mass and ^{13}C NMR spectra relating to a triterpenic diglycoside, $C_{40}H_{69}O_9$. The ion fragments generating at *m/z* 559 $[M\text{-}C_5H_9O_4]^+$, 427 $[M\text{-}C_5H_9O_4\text{-}C_5H_8O_4]^+$ and 133 $[C_5H_9O_4]^+$ suggested that the triterpenyl moiety was linked to a dipentose unit. The ^1H NMR spectrum of 5 exhibited four one-proton signals as a triplet at δ 5.11 (J= 6.4 Hz), as a double doublet at δ 3.38 (J = 5.6, 9.2 Hz) and as doublets at δ 4.99 (J = 7.5 Hz) and 4.71 (J = 7.3 Hz) assigned correspondingly to vinylic H-24, oxygenated methine H-3α and anomeric H-1′α and H-1″α protons. The other sugar protons appeared from δ 3.98 to 3.26. Eight three-proton signals as broad singlets at δ 1.62, 1.57, 1.12, 0.97, 0.93, 0.78 and 0.73 and as a doublet at δ 0.84 (J = 6.6 Hz) were associated with C-26 and C-27 methyl protons attached to vinylic C-25 carbon, tertiary C-19, C-29, C-28, C-30 and C-18 methyl and secondary C-21 methyl protons, respectively. The ^{13}C NMR spectrum of 5 displayed signals for vinylic carbons at δ 124.91 (C-24) and 131.27 (C-25), oxygenated C-3 methine carbon at δ 81.73, anomeric carbons at δ 105.04 (C-1′) and 92.16 (C-1″) and other sugar carbons between δ 76.18-61.85. The presence of H-2′ at δ 3.98 in the ^1H NMR spectrum and C- 2′ at δ 76.18 in the deshielded region suggested attachment of the second sugar at C- 2′. The ^1H and ^{13}C NMR spectral data of the triterpenic unit of 5 were compared with the reported spectral data of lanostene-type triterpenoids [21, 26, 27]. The ^1H-^1H COSY spectrum of 5

showed correlations of H-3 with H_2-2, H_2-1, Me-28 and H-1′; H- 1″ with H- 2″, H- 3″, H_2- 5″ and H- 2′; and H-24 with H_2-23, Me-26 and Me-27. The HMBC spectrum of 5 exhibited interactions of H-3, H_2-2′ and H_2-5′ with C-1′; H- 2′, H-2″ and H_2-5″ with C-1″; and H_2- 23, H- 24, Me- 26 and Me- 27 with C-25. The ^1H and ^{13}C NMR values were correlated in the HSQC spectrum of 5. Acid hydrolysis of 5 yielded D-arabinose, co-TLC comparable. On the basis of these evidences the structure of 5 was formulated as lanost-24-en-3β-ol-3-O-β-D-arabinopyranosyl-(2′→1″)-O-β-D-arabinoside. This is a new lanostenyl diarabinoside.



Lanost-24-en-3β-ol-3-O-β-D-arabinopyranosyl-(2′→1″)-O-β-D-arabinoside (5)

4. Conclusion

Phytochemical investigation of a methanolic extract of the roots of *P. ginseng* yielded one each of a new fatty acid, diterpenic glycoside, steroidal ester and triterpenic diglycoside. These compounds may be used as chromatographic markers for standardization of the ginseng roots.

5. Acknowledgements

The authors are thankful to the instrumentation centers, Central Drug Research Institute, Lucknow and Jawaharlal Nehru University, New Delhi for recording spectral data of the compounds.

6. References

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