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New Steroidal Constituents from the Roots of *Artemisia annua* L. Cultivar *Jwarharti*

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ABSTRACT

Artemisia annua L. (Asteraceae) is an aromatic annual herb up to 2 m in height found in temperate Asia, especially in China and naturalized throughout the world. The plant is prescribed against fever, malaria, skin diseases, jaundice, malignant ulcers and hemorrhoids. Phytochemical investigation of a methanolic extract of the roots of *A. annua* cultivar *jwarhati* led to the isolation of eudesm-3-en-5*α* H- 6*α*-ol-15-al (annuaeudesmanal, 3), 2-methoxybenzyl *n*- eicos- 9′-enoate (gadolyl methoxysalicylate, 5), 9, 12-octadecadienoyl-β-D-glucopyranosyl-(6′→1″)-β-D-glucopyranoside (linolyl diglucoside, 6), stigmast-5-en-3β,21-diol-3β-D-glucopyranosyl-4′-(2″,3″,4″-trihydroxybenzoate)- 6′-*n*-octadecanoate (21-hydroxy-β-sitosteryl glycosyl dioate, 7) and stigmast-5,22-dien-3β-ol-3-O-β D-xylopyranoside (stigmasterol xyloside, 8) along with the known compounds β-amyrin acetate (1), stigmasterol (2), stigmasteryl palmitate (4), glucuronic acid (9) and D-galacturonic acid (10). The structures of all these phytoconstituents have been identified on the basis of spectral data analysis and chemical reactions.

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INTRODUCTION

Artemisia annua L. (Asteraceae), known as sweet wormwood, sweet sagewort or annual wormwood (Chinese: qinghao), is an aromatic annual herb up to 2 m in height with yellow flowers. It is a native to temperate Asia, especially China but naturalized throughout the world including Argentina, Bulgaria, France, Hungary, India, Italy, Romania, Spain and USA. Currently, it is the source for the production of artemisinin and semi-synthetic artemisinin derivatives (including dihydroartemisinin, artesunate, artemether and arteether) which are cadinane-type sesquiterpenic lactones used for the production of combination therapies to treat malaria[1,2]. It is cultivated in Kashmir valley to produce artemisinin. The plant is highly pollinated and the seeds exhibited a great variation in maturity, biomass and the quantity of artemisinin (Qinghaosu). Artemisinin is the most potent and efficacious compound against chloroquine and quinine-resistant *Plasmodium falciparum* and other malaria-causing parasites. Beside antimalarial effects, *A. annua* exhibited biological activities such as antibacterial, anti-inflammatory, angiotensin converting enzyme inhibitory, cytokinin-like and antitumor effects[3]. In China, an aqueous preparation of the dried herb is prescribed against fever, malaria, skin diseases, jaundice, malignant ulcers and hemorrhoids. It is effective against pathogenic 'shu', a summer heat syndrome characterized by headache, fever, dry mouth, irritability, profuse sweating and full pulse. *A. annua* is one of the important ingredients in several Ayurvedic formulations. World Health Organization shows high interest with the active constituent artemisinin and its chemical derivatives. A. annua is included in the official Pharmacopoeia of China as qinghao and in the drug directories of India, Japan and Vietnam.

The prominent constituents reported from *A. annua* were the coumarins aesculetin, iso-fraxidin, scopoletin, scopolin and tomentin. The phenolic components of *A. annua* were quercetin glucoside, flaviolin, rhamnetin, chrysoplenol D, pillion and chlorogenic acid. In addition, other phenolic compounds such as 2,4-dihydroxy-6-methoxy-acetophenone, 5-nonadecyl-3-O-methyletherresorcinol, 2,2,6-trihydroxychromene, and 2,2-dihydroxy-6-methoxychromene have also been isolated from *A. Annua*[4 - 11]. The plant also contained phytols, flavones, chrysoplenetin, chrysosplenol-D, friedelin, casticin, terpenoid lactones sterols and anthraquinones[12- 15]. The major constituents of the plant essential oils reported camphor were (- 48%), germacrene D (-18.9%), artemisia ketone (- 68%) and 1, 8 cineole (- 51.5%)[16]\. The root volatile oil consists mainly of cis-arteannuic alcohol (25.9%), β-farnesene

(6.7%), β-maaliene, β-caryophyllene, its oxide and 2-phenylbenzaldehyde[17]. The shoot volatile oil is rich in sesquiterpenes. The manuscript describes isolation and characterization of an eudesmanal, acyl esters, steroids, β-amyrin ester and monosaccharides from the roots of *A. annua* cultivar *jwarharti*.

MATERIALS AND METHODS

General

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 Bio-Rad FTIR 5000 (FTS 135, Kawloon, Hong Hong) spectrophotometer using KBr pellets; γ_{max} values are given in cm^{-1}. ^{1}H and ^{13}C NMR spectra were screened on advance DRX 400, Bruker spectrospin 400 and 100 MHz instrument in 5 mm spinning tubes at 27 ºC, respectively (Karlsruhe, Germany) using TMS as an internal standard. Mass spectra were scanned by affecting FAB ionization at 70 eV on a JEOL-JMS-DX 303 spectrometer (Japan) equipped with direct inlet probe system. Column chromatography was performed on silica gel (60-120 mesh; Qualigen, Mumbai, India). TLC was run on silica gel G (Qualigen). Spots were visualized by exposing to iodine vapors, UV radiation, and spraying with ceric sulfate solution.

Collection of plant material

The roots of *A. annua* cultivar Jwarharti were collected from the experimental field of National Institute of Plant Genome Research (NIPGR), Aruna Asif Ali Road, New Delhi – 110067 and identified by Dr. Sushil Kumar, Scientist Emeritus, NIPGR.

Extraction and isolation

The dried root powder (1.0 kg) was exhaustively extracted with methanol in a Soxhlet apparatus. The methanolic extract was evaporated under reduced pressure to get a brown viscous mass (50 g, 5.0% yield). The dried extract was dissolved in minimum quantity of methanol and added to silica gel (60-120 mesh) to prepare slurry. It was air-dried, powdered and loaded on a silica gel column prepared in petroleum ether. The column was run with petroleum ether (b.p. 60- 80^{0}C), petroleum ether-chloroform (9:1, 3:1, 1:1, 1:3, v/v), chloroform, chloroform- methanol (99:1, 49:1, 19:5, 9:1, 17:3,4:1 7:3 and 1:1, v/v) and

methanol. 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 recrystallized to get pure compounds. The following compounds were isolated:

β-Amyrin acetate (1)

Elution of the column with petroleum ether-chloroform (3:1) furnished colourless crystals of **1**; recrystallized from petroleum ether; 103 mg (0.20% yield); R_f : 0.73 (petroleum ether: chloroform ; 1:9); m.p.: 240°-241°C; IR γ_{max} (KBr): 1725, 1640, 1471 cm^{-1}; +ve ion FAB MS *m/z* (rel. int.): 468 $[M]^+$ ($C_{32}H_{52}O_2$) (21.3), 408 (45.3), 218 (3.8), 203 (12.5).

Stigmasterol (2)

Elution of the column with petroleum ether–chloroform (1:1) yielded colourless crystals of **2**; 731 mg (1.46% yield); R_f : 0.31 (methanol-chloroform, 1:99); m.p.: 166 - 168 ^oC; IR γ_{max} (KBr): 3416, 1640, 1465, 1376, 1223, 1171, 805 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.36 (1H, m, H-6), 5.16 (1H, m, H-22), 5.01 (1H, m, H-23), 3.65 (1H, brm, $w_{1/2}$= 16.5 Hz, H-3α), 2.23 to 1.23 (25 H, m, 9 x CH_2. 7 x CH), 1.05 (3H, brs, Me-19), 0.96 (3H, d, J = 6.3 Hz, Me-21), 0.87 (3H, d, J = 6.6 Hz, Me-26), 0.84 (3H, d, J = 6.0 Hz, Me-27), 0.80 (3H, t, J = 6.6 Hz, Me-29), 0.71 (3H, brs, Me-18); ^{13}C NMR ($CDCl_3$): δ 36.52 (C-1), 31.90 (C-2), 71.81 (C-3), 41.90 (C-4), 140.76 (C-5), 121.69 (C-6), 31.66 (C-7), 33.94 (C-8), 51.24 (C-9), 37.26 (C-10), 21.07 (C-11), 39.76 (C-12), 42.30 (C-13), 56.87 (C-14), 24.17 (C-15), 28.67 (C-16), 55.96 (C-17), 12.24 (C-18), 19.41 (C-19), 36.68 (C-20), 18.79 (C-21), 138.30 (C-22), 129.28 (C-23), 45.83 (C-24), 27.28 (C-25), 19.83 (C-26), 18.99 (C-27), 23.11 (C-28), 12.05 (C-29); +ve ion FAB MS *m/z* (rel. int.): 412 $[M]^+$ ($C_{29}H_{48}O$) (32.1), 396 (51.3), 394 (43.8), 384 (13.6), 381 (12.1), 273 (8.9), 271 (19.8), 256 (25.6), 253 (16.2), 240 (12.6), 213 (19.7) 198 (21.2).

Annuaeudesmanal (3)

Further elution of the column with petroleum ether–chloroform (1:1) mixture afforded colourless crystals of 3; recrystallized from chloroform; 66 mg (0.12% yield); R_f : 0.36 (petroleum ether: chloroform; 1:9); m.p.: 240^0-242^0C; UV λ_{max} (MeOH): 205 nm (log ε 3.1). IR γ_{max} (KBr): 3484, 2932, 2859, 1699, 1638, 1467, 1375, 1132, 1033, 998, 813 cm^{-1}; ^1H NMR ($CDCl_3$): δ 9.21 (1H, brs, H-15), 5.54 (1H, m, H-5), 3.99 (1H, dd, J = 4.9, 12.5 Hz, H-6α), 2.55 (1H, d, J = 12.5 Hz, H-5α), 2.03 (2H, m, H_2-2), 1.97 (3H, brs, Me-11), 1.50 (1H, m,

H-12), 1.40 (1H, m, H-7), 1.36 (2H, m, H_2-8), 1.31 (2H, m, H_2-1), 1.12 (2H, m, H_2-9), 0.98 (3H, d, J = 6.1 Hz, Me-14), 0.95 (3H, d, J = 6.2 Hz, Me-14); ^{13}C NMR ($CDCl_3$): δ 31.82 (C-1), 24.53 (C-2), 121.65 (C-3), 139.46 (C-4), 48.51 (C-5), 71.28 (C-6), 53.92 (C-7), 23.88 (C-8), 38.43 (C-9), 36.81 (C-10), 25.16 (C-11), 22.75 (C-12), 21.17 (C-13), 20.82 (C-14), 198.21 (C-15); +ve ion FAB MS *m/z* (rel. int.): 236 $[M]^+$ ($C_{15}H_{24}O_2$) (3.6), 218 (11.6), 203 (26.7), 189 (16.8), 188 (18.6), 175 (15.5), 160 (19.2), 146 (23.4), 131 (21.3).

Stigmasteryl palmitate (4)

Elution of the column with chloroform produced colourless crystals of **4**; recrystallized from methanol; 429 mg (0.86% yield); R_f : 0.17 (chloroform); m.p.: 125^0-126^{0}C; UV λ_{max} (MeOH): 205, 235 nm (log ε 5.8, 3.5); IR γ_{max} (KBr): 1721, 1640, 1462, 1375, 1243, 1114, 1043, 963, 725 cm^{-1}; +ve ion FAB MS *m/z* (rel. int.): 650 $[M]^+$ ($C_{45}H_{78}O_2$) (1.1), 411 (85.2), 397 (41.3), 394 (42.8), 382 (13.6), 271 (38.1), 255 (38.3), 253 (28.5), 239 (31.3).

Gadolyl methoxysalicylate (5)

Elution of the column with chloroform–methanol (99:1) gave colourless crystals of **5**; recrystallized from methanol; 89 mg (0.18 % yield); R_f : 0.74 (chloroform -methanol, 1:9); m. p.: 82^0 – 84^{0}C; UV λ_{max} (MeOH): 206, 228, 290 nm (log ε 6.3, 3.1, 2.5); IR γ_{max} (KBr): 2923, 2855, 1721, 1508, 1458, 1375, 1264, 1175, 1031 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.62 (1H, dd, J = 2.5, 9.2 Hz, H-3), 6.92 (1H, m, H-4), 6.85 (1H, m, H-5), 6.67 (1H, dd, J = 2.8, 9.2 Hz, H-6), 5.34 (1H, m, H-9′), 5.31 (1H, m, H-10′), 4.18 (1H, brs, H_2-7α), 4.08 (1H, brs, H_2-7β), 3.95 (3H, brs, OMe), 2.34 (2H, t, J = 7.1 Hz, H_2-2′), 2.19 (2H, m, H_2-8′), 2.03 (2H, m, H_2-11′), 1.62 (4H, m, 2 x CH_2), 1.25 (22H, brs, 11 x CH_2), 0.88 (3H, t, J = 6.3 Hz, Me-20′); ^{13}C NMR ($CDCl_3$): δ 147.21 (C-1), 150.12 (C-2), 143.81 (C-3), 103.64 (C-4), 107.96 (C-5), 113.72 (C-6), 65.01 (C-7), 55.98 (OMe), 172.80 (C-1′), 36.09 (C-2′), 129.97 (C-9′), 127.98 (C-10′), 33.90 (2 x CH_2), 31.88 (CH_2), 31.83 (CH_2), 29.68 ((CH_2), 29.66 (7 x CH_2), 27.16 (CH_2), 24.68 (CH_2), 22.65 (CH_2), 14.08 (C-20′); +ve ion FAB MS *m/z* (rel. int.): 430 $[M]^+$ ($C_{28}H_{46}O_3$) (22.3), 309 (6.8), 293 (11.6), 289 (15.3), 265 (18.5), 263 (13.2), 167 (23.1), 165 (47.8), 141 (21.3), 137 (87.3), 135 (25.2), 120 (39.8), 104 (52.3).

Linolyl diglucoside (6)

Elution of the column with chloroform–methanol (19:1) mixture provided colourless mass of **6**; recrystallized from methanol; 250 mg (0.50% yield); R_f **:** 0.91 (chloroform–methanol; 1:1);

m.p.: 60^0- 61^0C; UV λ_{max} (MeOH): 208 nm (log ε 2.9); IR γ_{max} (KBr): 3450, 3379, 3285, 2924, 2853, 1740, 1640, 1462, 1374, 1150, 1080 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.34 (1H, m, H-9), 5.30 (1H, m, H-13), 5.25 (1H, m, H-10), 5.22 (1H, m, H-12), 5.01 (1H, d, *J*=7.5 Hz, H-1′), 4.90 (1H, d, J = 7.3 Hz, H-1"), 4.55 (2H, m, H-5′, H-5″), 4.13 (1H, d, J = 6.5 Hz, H-2′), 4.10 (1H, d, *J*=6.5 Hz, H-2"), 3.85 (1H, m, H-4′), 3.82 (1H, m, H-4"), 3.76 (2H, m, H-3', H-3"), 3.17 (2H, brs, H_2-6'), 3.09 (2H, brs, H_2-6"), 2.50 (2H, brs, H_2-11), 2.03 (2H, t, J = 7.2 Hz, H_2-2), 1.91 (4H, brs, H_2-8, H_2-14), 1.48 (6H, brs, 3xCH_2), 1.22 (10H, brs, 5 x CH_2), 0.82 (3H, t, J = 6.1 Hz, Me-18); ^{13}C NMR (DMSO- d_6): δ 173.91 (C-1), 55.89 (C-2), 31.48 (C-3), 29.30 (C-4), 29.30 (C-5), 29.30 (C-6), 29.30 (C-7), 31.48 (C-8), 127.49 (C-9), 130.31 (C-10), 50.01 (C-11), 129.41 (C-12), 118.26 (C-13), 33.48 (C-14), 29.28 (C-15), 26.72 (C-16), 22.24 (C-17), 13.93 (C-18), 103.53 (C-1′), 73.51 (C-2′), 70.65 (C-3′), 70,62 (C-4′), 76.88 (C-5′), 63.13 (C-6′), 100.17 (C-1″), 72.55 (C-2″), 69.87 (C-3″), 70.04 (C-4″), 76.54 (C-5″), 61.09 (C-6″); +ve ion FAB MS *m/z* (rel. int.): 604 $[M]^+$ ($C_{30}H_{52}O_{12}$) (1.1), 341 (3.2), 263 (11.5), 137 (38.2), 97 (18.5).

21-Hydroxy-β-sitosteryl glycosyl dioate (**7**)

Elution of the column with chloroform–methanol (93:7) furnished colourless crystals of **7**; recrystallized from methanol; 73 mg (0.14% yield); R_f : 0.65 (methanol-chloroform, 3:17); m.p.: 78-79^0 C; UV λ_{max} (MeOH): 205 nm (log ε 4.9); IR γ_{max} (KBr): 3442, 3350, 2919, 2849, 1735, 1721, 1640, 1608, 1510, 1466, 1378, 1246, 1181, 1090, 830, 721 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.13 (1H, d, J = 7.5 Hz, H-5″), 6.81 (1H, d, J = 7.5 Hz, H-6″), 5.36 (1H, m, H-6), 5.02 (1H, d, J = 7.5 Hz, H-1′), 4.36 (1H, m, H-4′), 4.31 (1H, m, H-5′), 4.10 (1H, dd, J = 7.5, 7.3 Hz, H-2′), 3.89 (1H, brm, $w_{1/2=}$ 17.1 Hz, H-3α), 3.78 (1H, m, H-3′), 3.48 (2H, d, J = 8.5 Hz, H_2-6′), 3.39 (2H, d, J = 9.5 Hz, H_2-21), 2.34 (2H, t, J = 7.5 Hz, H_2-2‴), 2.32 – 1.27 (41H, m, 17 x CH_2, 7 x CH), 1.25 (18 H, brs, 9 x CH_2), 1..01 (3H, brs, Me-19), 0.87 (3H, d, J = 6.3 Hz, Me-26), 0.85 (3H, d, J = 6.2 Hz, Me-27), 0.83 (3H, t, J = 6.5 Hz, Me-18‴), 0.80 (3H, d, J = 6.1 Hz, Me-29), 0.67 (3H, brs, Me-18); ^{13}C NMR (DMSO-d_6) δ 38.81 (C-1), 31.87 (C-2), 73.51 (C-3), 41.67 (C-4), 140.21 (C-5), 122.12 (C-6), 32.67 (C-7), 30.95 (C-8), 50.10 (C-9), 37.21 (C-10), 21.03 (C-11), 40.47 (C-12), 42.25 (C-13), 56.71 (C-14), 24.90 25.67 (C-15), 25.67 (C-16), 55.96 (C-17), 11.96 (C- 18), 19.31 (C-19), 36.67 (C-20), 63.35 (C-21), 24.20 (C-22), 27.18 (C- 23), 45.76 (C-24), 29.85 (C-25), 19.25 (C-26), 19.16 (C-27), 24.87 (C-28), 12.03 (C-29), 101.10 (C-1′), 72.77 (C-2′), 70.10 (C-3′), 73.82 (C-4′),79.50 (C-5′), 63.29 (C-6′), 143.67 (C-1″), 156.13 (C-2″), 152.62 (C-3″), 150.25 (C-4″), 127.79 (C-5″),

116.12 (C-6″), 170.69 (C-7″), 172.93 (C-1‴), 39.66 (C-2‴), 37.23 (CH_2), 30.17 (CH_2), 29.71 (7 x CH_2), 29.69 (3 x CH_2), 29.53 (CH_2), 25.70 (CH_2), 22.64 (CH_2), 14,09 (C-18‴); +ve ion FAB MS *m/z* (rel. int.): 994 $[M]^+$ ($C_{60}H_{98}O_{11}$) (1.1), 565 (9.2), 429 (5.6), 411 (18.6), 283 (9.7), 267 (25.3), 273 (23.6), 255 (27.8), 240 (30.2), 231 (26.5), 213 (26.6), 170 (22.1), 157 (12.5), 153 (27.6).

Stigmasterol xyloside (8)

Further elution of the column with chloroform–methanol (93:7) mixture afforded colourless crystals of **8**; recrystallized from methanol; 467 mg (0.92 % yield); R_f : 0.4 (methanol-chloroform, 1:5); m. p.: 254^0-255^0C; UV λ_{max} (MeOH): 205 nm (log ε 5.3); IR γ_{max} (KBr): 3455, 3396, 3290, 2923, 2853, 1643, 1462, 1374, 1070, 1023 cm^{-1}; ^{1}H NMR (DMSO- d_6): δ 5.32 (1H, m, H-6), 5.13 (1H, dd, J = 8.1, 7.8 Hz, H-22) , 5.01 (1H, dd, J = 7.8, 8.7 Hz, H-23), 4.88 (1H, d, J = 7.3 Hz, H-1′), 4.42 (1H, m, H-2′), 4.22 (1H, dd, J = 7.3, 7.3 Hz, H-3′), 3.63 (1H, m, H-4′), 3.23 (1H, brm, $w_{1/2}$ = 18.5 Hz, H-3α), 3.09 (1H, d, J = 12.6 Hz, H_2-5α), 3.05 (1H, d, J = 12.6 Hz, H_2-5β), 1.01 (3H, brs, Me-19), 0.95 (3H, d, J = 6.2 Hz, Me-21), 0.89 (3H, d, J = 6.3 Hz, Me-26), 0.81 (3H, d, J = 6.1 Hz, Me-27), 0.77 (3H, d, J = 6.6 Hz, Me-29), 0.66 (3H, brs, Me-18); ^{13}C NMR (DMSO- d_6): δ 36.85 (C-1), 27.80 (C-2), 73.45 (C-3), 38.32 (C-4), 140.41 (C-5), 121.15 (C-6), 35.53 (C-7), 31.38 (C-8), 49.62 (C-9), 36.21 (C-10), 22.60 (C-11), 38.65 (C-12), 45.13 (C-13), 56.20 (C-14), 24.89 (C-15), 28.70 (C-16), 55.45 (C-17), 12.10 (C-18), 19.06 (C-19), 41.85 (C-20), 20.62 (C-21), 138.03 (C-22), 128.85 (C-23), 50.63 (C-24), 29.13 (C-25), 20.88 (C-26), 21.87 (C-27), 25.47 (C-28), 11.88 (C-29), 100.84 (C-1′), 76.73 (C-2′), 76.97 (C-3′),70.03 (C-4′), 61.05 (C-5′); +ve ion FAB MS *m/z* (rel. int.): 544 $[M]^+$ ($C_{34}H_{56}O_5$) (2.1), 411 (18.2), 396 (71.3), 394 (63.7), 381 (17.6), 379 (12.1), 255 (22.8), 213 (24.3), 198 (17.9), 174 (23.5), 146 (62.1), 131 (28.3), 109 (63.8).

Glucuronic acid (9)

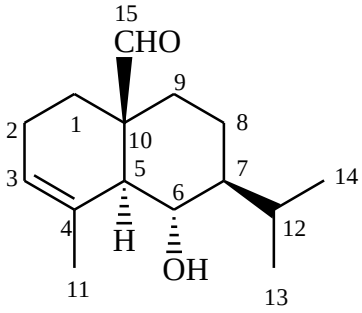
Elution of the column with chloroform–methanol (9:1) mixture gave colourless crystals of **9**; recrystallized from ethanol; 396 mg (0.80% yield); R_f : 0.69 (MeOH:$CHCl_3$, 1:1); m.p.: 164^0-165^0C, IR γ_{max} γ_{max} (KBr): 3410, 3398, 3265, 2931, 1690, 1637, 1386, 1066 cm^{-1}, ^{1}H NMR (DMSO- d_6): δ 4.90 (1H, d, *J*=7.4 Hz, H-1), 4.69 (1H, d, *J*=6.9 Hz, H-5), 4.59 (1H, m, H-2), 4.40 (1H, m, H-3), 3.83 (1H, m, H-4); +ve ion FAB MS *m/z* (rel. int): 194 $[M]^+$ ($C_6H_{10}O_7$) (11.6).

D-Galacturonic acid (10)

Further elution of the column with chloroform–methanol (9:1) mixture yielded colourless crystals of **10**; recrystallized from methanol; 1.6 mg (3.2% yield), R_f : 0.67 (chloroform–methanol, 3:1), m. p.: 157^0 -159^0C, IR γ_{max} (KBr): 3450, 3408, 3325, 2925, 1705, 1391, 1115, 831 cm^{-1}, ^{1}H NMR (DMSO- d_6): δ 4.71 (1H, d, J = 7.3 Hz, H-1), 3.40 (1H, brs, H-5), 3.36 (2H, m, H-3, H-4), 3.15 (1H, dd, J = 7.3, 4.5 Hz, H-2), +ve ion FAB MS *m/z* (rel. int.): 194 $[M]^+$ ($C_6H_{10}O_7$) (21.2); co-TLC comparable.

RESULTS AND DISCUSSION

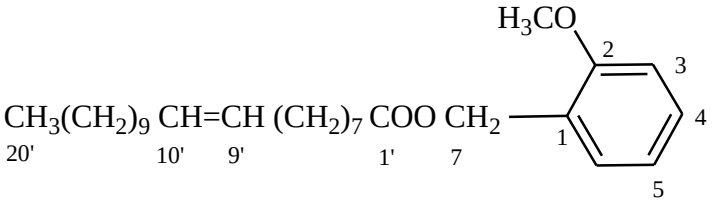
Compounds **1**, **2**, **4, 9** and **10** are the known phytoconstituents identified as β-amyrin acetate, stigmasterol, stigmasterol palmitate, glucuronic acid and D-galacturonic acid, respectively[19 - 21]. Compound **3**, named annuaeudesmanal, displayed characteristic IR absorption bands for hydroxyl group (3484 cm^{-1}), aldehydic group (1699 cm^{-1}) and unsaturation (1638 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, the molecular ion peak of **3** was determined at *m/z* 236 consistent with the molecular formula of a hydroxy aldehydic sesquiterpene, $C_{15}H_{24}O_2$. The prominent ion peaks generated at *m/z* 218 $[M - H_2O]^+$ and 189 $[218 - CHO]^+$ supported the presence of the hydroxyl and aldehydic groups in the molecule. The ^{1}H NMR spectrum of **3** showed a one-proton broad singlet at δ 9.21 assigned to the aldehydic H-15 proton. A one-proton broad multiplet at *δ* 5.54 and a one-proton double doublet at δ 3.39 (J = 4.9, 12.5 Hz) were attributed to vinylic H-3 and β-oriented H-6 carbinol protons, respectively. A three-proton broad singlet in the deshielded region at δ 1.97 was ascribed to C-11 methyl protons located on the C-4 vinylic carbon. A one-proton doublet at δ 2.55 (J = 12.5 Hz) was assigned to H- 5*α* and a one-proton multiplet at δ 1.40 to H-7 methine protons. Two three-proton doublets at δ 0.98 (J = 6.1 Hz) and 0.95 (J = 6.2 Hz) were attributed to secondary C-13 and C-14 methyl protons, respectively. The ^{13}C NMR spectrum of **3** showed 15 carbon signals including aldehydic carbon at δ 198.21 (C-15), vinylic carbons at δ 121.65 (C-3) and 139.46 (C-4), carbinol carbon at δ 71.28 (C-6) and methyl carbons at δ 25.11 (C-11), 21.17 (C-13) and 20.82 (C-14). The HMBC spectrum of **3** exhibited correlations of H_2-2, H-3, H-5 and Me-11 with C-4; H-5, H-7 and H_2-8 with C-6; and H_2-1 and H_2-9 with C-15. On the basis of the above discussion the structure of **3** was designated as eudesm-3-en-5*α* H- 6*α*-ol-15-al. This is a new sesquiterpene aldehyde.



3. Annuaeudesmanal

Compound **5,** designated as gadolyl methoxysalicylate, showed an IR absorption band for ester group (1721 cm^{-1}). On the basis of +ve FAB mass and ^{13}C NMR spectra the molecular ion peak of **5** determined at *m/z* 430 consistent with the molecular formula of a fatty ester $C_{28}H_{46}O_3$. The generation of prominent ion fragments at *m/z* 137 $[OCH_2–C_6H_4OCH_3]^+$, 165 $[COOCH_2–C_6H_4OCH_3]^+$, 263 $[(CH_2)_7COOCH_2–C_6H_4OCH_3]^+$ and 289 $[CH=CH(CH_2)_7COOCH_2–C_6H_4OCH_3]^+$ indicated the esterification of methoxysalicyl alcohol with the C_{20} fatty acid. The ion peaks arising at *m/z* 309 $[CH_3(CH_2)_9CH=CH(CH_2)_7COO]^+$, 293 $[CH_3(CH_2)_9CH=CH(CH_2)_7CO]^+$, 265 $[CH_3(CH_2)_9CH=CH- (CH_2)_7]^+$, 167 $[CH_3(CH_2)_9CH=CH]^+$ and 141$[CH_3(CH_2)_9]^+$ indicated that gadoleic acid was esterified with the aromatic alcohol. The ^1H NMR spectrum of **5** exhibited two one- proton double doublets at δ 7.62 (*J* = 2.5, 9.2 Hz) and 6.67 (*J* = 2.8, 9.2 Hz) assigned to meta-, ortho-coupled aromatic H-3 and H-6 protons, respectively. Two one-proton multiplets at δ 6.92 and 6.85 were attributed correspondingly to aromatic H-4 and H-5 protons. Two one-proton multiplets at δ 5.34 and 5.31 were ascribed to vinylic H-9′ and H-10′, respectively. Two one- proton broad singlets at δ 4.18 and 4.08 were associated with the oxygenated methylene H_2-7 protons. A two-proton triplet at δ 2.34 (*J* = 7.1 Hz) was accommodated to methylene H_2-2′ protons nearby the ester group. Two multiplets at *δ* 2.19 and 2.03, both integrated for two protons each, were accounted to methylene H_2-8′ and H_2-11′ protons, respectively, adjacent to the vinylic carbons. A three - proton broad singlet at δ 3.95 and a three-proton triplet at δ 0.88 (J = 6.3 Hz) were accommodated to methoxy and C- 20′ primary methyl protons, respectively. A four-proton multiplet at δ 1.62 and a broad signal at δ 1.25 (22 H) were accounted to the remaining methylene protons. The ^{13}C NMR spectrum of **5** showed signals for ester carbon at δ 172.80 (C-1′), aromatic and vinylic carbons between δ 150.12 - 107.96, oxymethylene carbon at δ 65.01 (C-7), methoxy carbon at δ 55.98, methyl carbon at δ 14.08 (C-20′) and methylene carbons between δ 36.09- 22.65. The HMBC

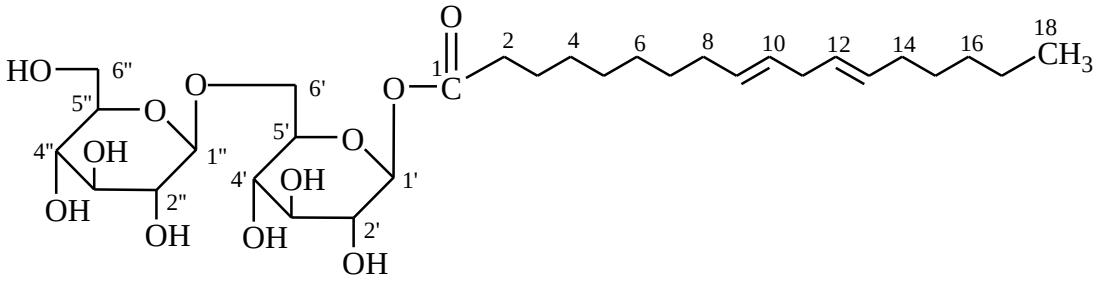
spectrum of **5** indicated interactions of H_2-7 and H_2-2′ with C-1′; H-3, H-6, OMe and H_2-7 with C-1; and H_2-8′, H-9′ and H_2-11′ with C-10′. Alkaline hydrolysis of **5** yielded gadoleic acid, m. p. 23 to 24 ^{0}C, co-TLC comparable; and 2- methoxysalicylic alcohol. On the basis of the foregoing discussion, the structure of the new fatty acid ester **5** has been elucidated as 2-methoxybenzyl *n*- eicos- 9′-enoate.



5. Gadolyl methoxysalicylate

Compound **6,** named linolyl diglucoside, gave positive test for glycosides and exhibited IR absorption bands for hydroxyl group (3450, 3379, 3285 cm^{-1}), ester function (1740 cm^{-1}) and unsaturation (1640 cm^{-1}). On the basis of its FAB mass and ^{13}C NMR spectra, its molecular weight was established at *m/z* 604 consistent with the molecular formula of a fatty acid diglycoside, $C_{30}H_{52}O_{12}$. The important ion peak generated at *m/z* 263 $[C_{18}H_{31}O]^+$ and 341 [M -263]$^+$ suggested fission of the glycoside moiety from the fatty acid chain and the presence of diglucosidic nature of the glycone moiety. The ion fragments arising at *m/z* 137 $[CH=CH–CH_2–CH=CH–(CH_2)_4–CH_3]^+$ and 97 $[CH=CH–(CH_2)_4–CH_3]^+$ supported the location of the vinylic linkage at C-9 and C-12. The ^{1}H NMR spectrum of **6** displayed four one-proton multiplets at δ 5.34 5.30, 5.25 and 5.22 assigned to vinylic H-9, H-13 and H-10 and H-12 protons, respectively. Two one-proton doublets at δ 5.01 (J = 7.5 Hz) and 4.90 (J = 7.3 Hz) were accounted for anomeric H-1′ and H-1″ protons, respectively. The other sugar protons appeared between δ 4.55- 3.09. The methylene protons resonated from δ 2.50 to 1.22 . A three-proton triplet at δ 0.82 (J = 6.1 Hz) was ascribed to the terminal C-18 primary methyl protons. The ^{13}C NMR spectrum of **6** showed important signals for ester carbon at δ 173.91 (C-1), vinylic carbons between δ 130.31 - 118.26, anomeric carbon at δ 103.53 (C-1$^{'}$) and 100.17 (C-1$^{''}$), other sugar carbons between δ 76.88 - 61.09, methylene carbons from δ 55.89 to 22.24 and methyl carbon at δ 13.93 (C-18). The presence of the oxymethylene protons signal in the deshielded region at δ 3.17 (H_2-6') and the respective carbon signal at δ 63.13 (C-6′) suggested (1→6) linkage of the sugar units. The HMBC spectrum of **6** showed interactions of H-1′ and H_2-2 with C-1; H-1″ and H-5′ with C-6′; and H_2-8, H-9, H_2-11 and H-12 with C-10. Alkaline hydrolysis of **6** yielded linoleic acid and D- glucose, co-TLC

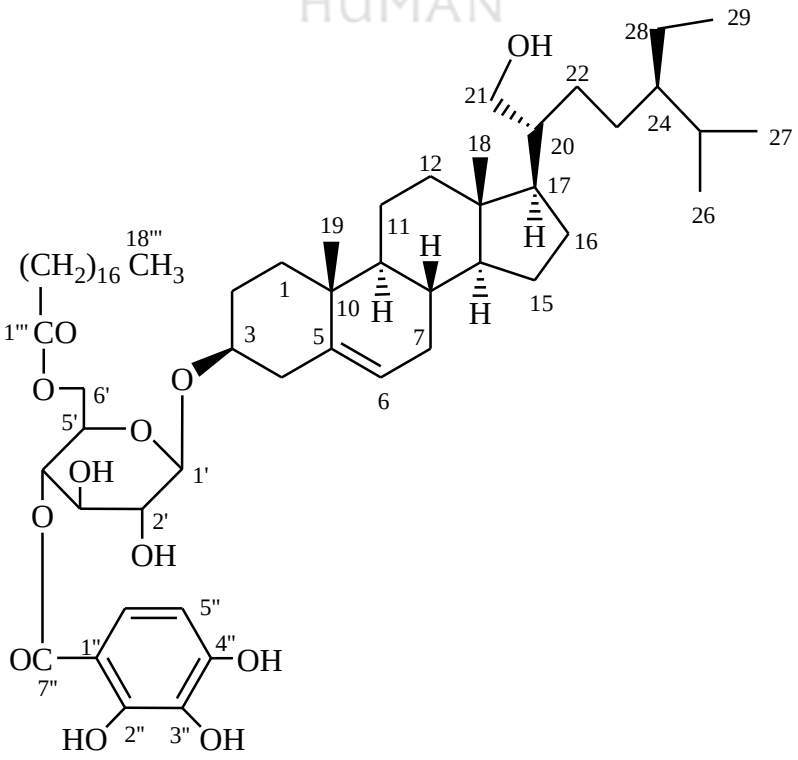
comparable. On the basis of these findings, the structure of **6** has been established as 9, 12-octadecadienoyl-β-D-glucopyranosyl-(6′→1″)-β-D-glucopyranoside. This is a new fatty acid diglucoside.



6 . Linolyl diglucoside

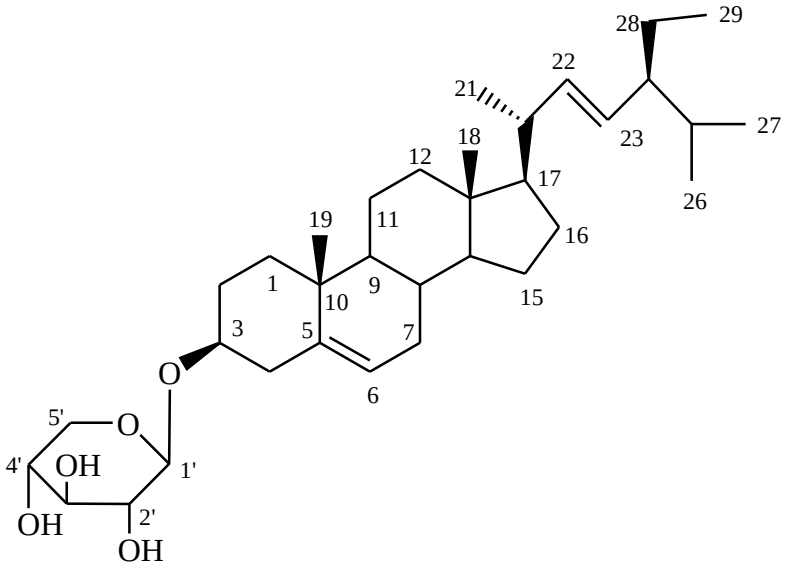
Compound **7,** designated as 21-hydroxy-β-sitosteryl glycosyl dioate, responded positively to tests for sterol glycoside and phenol and exhibited IR absorption bands for hydroxyl groups (3442, 3350 cm^{-1}), ester functions (1735, 1721 cm^{-1}), unsaturation (1640 cm^{-1}) and aromatic ring (1608, 1510, 1090 cm^{-1}) and long aliphatic chain (721 cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectra, its molecular weight was established at m/z 994 consistent with a molecular formula of a diesterified glycosidic steroid, $C_{60}H_{98}O_{11}$. The prominent ion fragments generated at *m/z* 429 [M - glycone]$^+$, 412 [429 - H_2O]$^+$, 157 [$C_{10}H_{21}O$, side chain]$^+$ and 565 [$C_6H_9O_5$-$CH_3(CH_2)_{16}CO)(C_6H_2CO(OH)_3]^+$ suggested that the steroidal moiety was a β- sitosterol type molecule possessing one hydroxyl group in the side chain. The ion peaks arising at *m/z* 267 [$OC-(CH_2)_{16}–CH_3]^+$, 283 [$OOC–(CH_2)_{16}CH_3]^+$, 170 [$OOC–C_6H_2(OH)_3]^+$ and 153 [$CO–C_6H_2(OH)_3]^+$ indicated that stearic and trihydroxybenzoic acids were esterified with the sugar residue. The ^1H NMR spectrum of **7** displayed a one-proton multiplet at δ 5.36 ascribed to vinylic H-6 proton. A one-proton doublet at δ 5.02 (J = 7.5 Hz) was accounted to anomeric H-1′ proton. A one - proton broad multiplet at δ 3.89 with half - width of 17.1 Hz as attributed to *α*-oriented H-3 oxymethine proton. A two-proton doublet at δ 3.48 (J = 8.5 Hz, H_2-6′), was assigned to oxymethylene H_2-6′ protons and it's shifting in the deshielded region indicated location of one of the ester group at this function. The appearance of H-4′ in the downfield region as a one-proton multiplet at δ 4.36 suggested the presence of another ester group at this function. The other sugar protons resonated between δ 4.36- 3.78. A two-proton doublet at δ 3.39 (J = 9.5Hz) was accounted to hydroxymethylene H_2-21 protons. Two three-proton broad singlets at *δ* 1.01 and 0.67 were due to C-19 and C-18 tertiary methyl protons, respectively. Three three-proton doublets at δ 0.87 (J = 6.3 Hz), 0.85 (J = 6.2 Hz) and 0.80 (J = 6.1 Hz) and a three- proton triplet at *δ* 0.83 (J = 6.5 Hz) were

accommodated correspondingly to the secondary C-27 and C-26 and primary C-29 and C-18″ methyl protons. A two-proton triplet δ 2.34 (J = 7.5 Hz), was associated with H_2-2‴′ methylene protons adjacent to the ester group. Two one-proton deshielded doublets at δ 7.13 (J = 7.5 Hz) and 6.81 (J = 7.5 Hz) were due to *ortho*-coupled H-5″ and H-6″ protons, respectively. The remaining methylene and methine protons appeared in the range of δ 2.36 - 1.23. The ^{13}C NMR spectrum of **7** showed signals for vinylic carbons at δ 140.21 (C-5) and 122.12 (C-6), anomeric carbon at δ 101.10 (C-1′), ester carbons at δ 170.69 (C-7″′) and 172.93 (C-1‴′), oxygenated methine carbon at δ 73.51 (C-3), hydroxymethylene and sugar carbons between *δ* 79.50 - 63.29, aromatic carbons between δ 156.13- 116.12 and methyl carbons in the range of δ 19.31 – 11.96. The ^{13}C NMR signals of the steroidal moiety were compared with other stigmastene type molecules[22,23]. The HMBC spectrum of **7** showed interactions of H-3 and H-2′ with C-1′; H_2-4, H-6 and H_2-7 with C-5; H-17 and H-20 with C-21; H_2-6′ and H_2-2‴ with C-1‴; and H-4′ and H-2″ with C-1″. Acid hydrolysis of **7** yielded stearic acid, m. p. 69°– 70°C, co-TLC comparable, isogallic acid, D-glucose, D- glucose, R_f = 0.41 (EtOAc: HOAc: H_2O: MeOH ; 6:1:1:2) and 21- hydroxy-*β*-sitosterol. On the basis of the foregoing account, the structure of **7** has been established as stigmast-5-en-3β,21-diol-3β-D-glucopyranosyl-4′-(2″,3″,4″-trihydroxybenzoate)- 6′-*n*-octadecanoate. This is a new phytosterol glycosidic diester.



7. 21-Hydroxy-β-sitosteryl glycosyl dioate

Compound **8**, named stigmasterol xyloside, responded positively to test for steroidal glycosides. Its IR spectrum exhibited characteristic absorption band for hydroxyl group (3455, 3396, 3290 cm^{-1}) and unsaturation (1643 cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectra the molecular ion peak of **8** was determined at *m/z* 544 consistent with the molecular formula of a steroidal glycoside, $C_{34}H_{56}O_5$. The prominent ion peaks generated at *m/z* 411 [M - $C_5H_9O_4$]$^+$, 396 [411- Me]$^+$, 381 [396 - Me]$^+$, 394 [M - $C_5H_{10}O_5$]$^+$, 255 [394 - $C_{10}H_{19}$, side chain]$^+$, 213 [255 - ring D fission]$^+$ and 198 [213 - Me]$^+$ indicated the attachment of C_5- sugar to the stigmasterol. The ^1H NMR spectrum of **8** displayed three one-proton signals as a multiplet at δ 5.32 and as double doublets at δ 5.13 (J = 8.1, 7.8 Hz) and 5.01 (J = 7.8, 8.7 Hz) assigned correspondingly to *cis*- oriented vinylic H-6, H-22 and H-23 protons. A one - proton doublet δ 4.88 (J = 7.3 Hz) was attributed to anomeric H-1′ proton. A one-proton broad multiplet at δ 3.23 with half width of 18.5 Hz was accounted to H-3*α* oxymethine proton. The remaining sugar protons resonated between *δ* 4.42 - 3.05. Two three-proton broad signals at *δ* 1.01 and 0.66 were attributed to C-19 and C-18 tertiary methyl protons, respectively. Four three- proton doublets at *δ* 0.95 (J = 6.2 Hz), 0.89 (J = 6.3 Hz), 0.81 (J = 6.1 Hz) and 0.77 (J = 6.6 Hz) were assigned correspondingly to secondary C-21, C-26, C-27, and primary C-29 methyl protons, all of them were located on the saturated carbons. The ^{13}C NMR spectrum of **8** exhibited signal for 34 carbons. The important signals appeared for oxymethine carbon at δ 73.45 (C-3), vinylic carbons between δ 140.41 - 121.15, anomeric carbon at δ 100.84 (C-1′), other sugar carbons from δ 76.97 to 61.05. The ^{13}C NMR data of the steroidal skeleton were compared with other stigmastene-type molecules[22,23]. The HMBC spectrum of **8** showed correlations of H-1′, H_2-2 and H_2-4 with C-3; H_2-4 and H-6 with C-5; H-17, H-20, Me-21, H-23 and H-24 with C-22. Acid hydrolysis of **8** yielded D-xylose, R_f = 0.26 (*n*- butanol: ethanol: water; 4:1:2.2) and stigmasterol, m. p.167–168 ^0C, TLC comparable. On the basis of the spectral data analyses and chemical reactions, the structure of **8** has been elucidated as stigmast-5,22-dien- 3β-ol-3-O-β D-xylopyranoside. A related steroidal xyloside, β-sitosterol xyloside, has been reported from *Butea monosperma* seeds[24].



8. Stigmasterol xyloside

CONCLUSION

Phytochemical investigation of a methanolic extract of the roots of *A. annua* cultivar *jwarhati* led to the isolation of a variety of natural products including a sesquiterpenoid, acyl esters, steroids, β-amyrin ester and monosaccharides. This work has enhanced understanding about the phytoconstituents of the plant. These secondary metabolites can provide necessary information for the further researchers as an effective analytical marker, identity, purity and quality control of this plant in future.

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