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ALIPHATIC AND STEROIDAL CONSTITUENTS FROM THE SEEDS OF *CAESALPINIA BONDUCELLA* L.

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ABSTRACT
Caesalpinia bonducella L. (Caesalpiniaceae) is found in India, Myanmar and Sri Lanka. Its seeds are used to treat colic, convulsions, edema, abdominal pain, fever, leprosy, malaria, menstrual complaints, pneumonia, skin diseases, pimples, hydrocele, orchitis, pulmonary tuberculosis and palsy. A methanol extract of the seeds of *C. bonducella* L. was concentrated and adsorbed on silica gel (60-120 mesh) for the preparation of a slurry. The dried slurry was chromatographed over silica gel column packed in petroleum ether. The column was eluted with petroleum ether, chloroform and methanol successively in order of increasing polarity to isolate four new phytoconstituents characterized as *n*-triacontan-7,13α-diol (**1**, triacontanyl diol), *n*-pentatetracontan-23β-ol (**2**, pentatetracontanyl alcohol), *n*-hexacos-15-en-1,5-olide (**5**, bonducelloide) and 21-hydroxystigmast-5-en-3β-ol- 3-O-β-D-glucopyronosyl-6'-octadec-9"-enoate (**6**, bunducsteroid) together with three known compounds stigmasterol (**3**), β-sitosterol (**4**) and β-sitosterol glucoside (**7**). The structures of all these phytoconstituents were elucidated on the basis of the spectral data analysis and chemical reactions.

KEYWORDS: *Caesalpinia bonducella*, seeds, fatty acids, steroids, aliphatic lactone.

INTRODUCTION
Caesalpinia bonducella L., syn. *C. crista*, *C. bonduc* (L.) Roxb, *C. paniculata* (Lam.) Roxb. (Caesalpiniaceae), commonly known as Kantkarej, fever nut and bunduc hindi, is a large straggling, thorny shrub with armed branches, hooks and straight, hard yellow prickles; leaves compound; flowers pale yellow in supra-axillary racemes at the top; fruits inflated pods, covered with prickles; seeds globular, hard, bluish grey with a smooth shiny surface resembling eyeballs.[1] It is distributed in tropical and subtropical regions of south-eastern Asia including India, Myanmar and Sri Lanka.[2] The seeds are considered as an adaptogenic, antimicrobial, antiproliferatve, antidiabetic, anti-filarial, uterine stimulant, hepatoprotective, antitumor, antioxidant, febrifugal, periodic, tonic and vesicant; used to treat colic, convulsions, edema, abdominal pain, fever, leprosy, malaria, menstrual complaints, pneumonia, skin diseases, pulmonary tuberculosis and palsy. The seed oil is lapped to soften the skin and to remove pimples. The seed powder mixed with castor oil is applied to cure hydrocele and orchitis.[3,4] The bark is rubefacient and utilized to alleviate toothache.[2,4-6] In Siddha, *C. bonducella* is recommended to relieve psoriasis.[1]

The seeds contained bonducin, proteins, saponin, starch, sucrose, enzymes, phytosterols, phenolic compounds, alkenes, amino acids, 4-O-methyl myoinositol, fatty acids, furano-cassane-diterpenes[7-10], triterpenoids[11], quercetin and tocopherols.[12] The leaves contained pinitol, glucose, bonducin, waxy material composed of an ester of myristic acid and an amorphous bitter principle.[13,14] The stem and root afforded peltogynoids, pulcherrimin, 6-methoxy pulcherrimin, homoisoflavonoid, 8-methoxybonducellin, bonducellin, 2,6-dimethoxybenzoquinone, 2',4',4- trihydroxychalcone and 2',4-dihydroxy-4'-methoxychalcone.[15] The paper describes isolation and characterization of aliphatic and steroidal constituents from the seeds of *C. bonducella* L. procured from Delhi.

MATERIAL AND METHODS

General procedure

Melting points were determined on a Perfit apparatus without correction. The infrared (IR) spectra were measured in KBr pellet on a Bio-Rad Fourier transform-IR spectrometer (Spectra Lab Scientific Inc., Ontario, Canada). Ultraviolet (UV) spectra were obtained in methanol with a Lambda Bio 20 spectrometer (Perkin-Elmer, Rotkreuz, Switzerland). ^{1}H (400 MHz) and ^{13}C (100 MHz) nuclear magnetic resonance (NMR) spectra were recorded on Bruker spectrospin spectrometer (Bruker AXS, Karlsruhe, Germany). Deutrated chloroform and dimethyl suphoxide (Sigma-Aldrich, Bengaluru, India) were used as solvents and TMS as an internal standard. Mass spectra were scanned at 70 eV on a Jeol D-300 instrument (Jeol, USA). Column chromatographic separations were carried out on silica gel (Merck, 60–120 mesh, Mumbai, India). Precoated silica gel plates (Merck, Silica gel 60 F_{254}) were used for analytical thin layer chromatography. The spots were visualized by exposure to iodine vapors, UV radiations and spraying with ceric sulphate solution.

Plant material

The seeds of *C. bonducella* were procured from a local market of Khari Baoli, Delhi and authenticated by Prof. M. P. Sharma, Department of Botany, Jamia Hamdard, New Delhi. A voucher specimen is deposited in the herbarium of the Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard.

Extraction and isolation

The seeds (2 kg) were coarsely powdered and extracted exhaustively with methanol in a Soxhlet apparatus. The methanolic extract was then concentrated on a steam water bath and dried under reduced pressure to get a dark brown mass (162 g, 8.1% yields). It was dissolved in a small 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 a 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, 1:3 v/v), pure chloroform and finally the mixture of chloroform and methanol (99:1, 98:2, 96:4, 95:5, 97:3, 9:1 v/v). 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 recrystallized to get the following compounds:

Triacontanyl diol (1)

Elution of the column with petroleum ether- chloroform (9:1) mixture yielded colorless crystals of **1**, recrystallized from chloroform-methanol (1:1), 215 mg (0.014 % yield); R_f: 0.48 (petroleum ether: chloroform, 9:1); m. p.: 64-65 °C; UV λ_{max} (methanol): 210 nm (log ε 4.3); IR ν_{max} (KBr): 3510, 3450, 2921, 2855, 1405, 1026, 1002, 725 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.16 (1H, brm, $w_{1/2}$ = 15.3 Hz, H-7α), 3.13 (1H, brm, $w_{1/2}$ = 6.1 Hz, H-7β), 2.54 (2H, brs, CH_2), 2.50 (4H, brs, 2 x CH_2), 1.22 (46H, brs, 23 x CH_2), 0.84 (3H, t, *J* = 6.1 Hz, Me-1), 0.81 (3H, t, *J* = 6.2 Hz, Me-30); ^{13}C NMR ($CDCl_3$): δ 79.15 (C-7), 78.49 (C-13), 29.03 (2 x CH_2), 28.89 (2 x CH_2), 22.89 (20 x CH_2), 21.12 (CH_2), 19.67 (CH_2), 14.15 (Me-1), 13.87 (Me-30); EIMS *m/z* (*rel. int.*): 454 $[M]^+$ ($C_{30}H_{62}O_2$) (12.2), 369 (14.1), 339 (11.8), 239 (22.5), 225 (19.8), 215 (18.5), 183 (21.2), 155 (33.6), 141 (32.8), 127 (31.3), 115 (41.9).

Pentatetracontanyl alcohol (2)

Elution of the column with petroleum ether-chloroform (3:1) mixture afforded colorless crystals of **2**, recrystallized from chloroform, 435 mg (0.029 % yield); R_f: 0.64 (petroleum ether: chloroform, 3:1); m.p.: 91-92 °C; UV λ_{max} (methanol): 204 nm (log ε 4.8); IR ν_{max} (KBr): 3450, 2919, 2852, 1467, 1361, 993, 909, 733, 715 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.21 (1H, brm, $w_{1/2}$ = 14.8 Hz, H-23α), 2.50 (4H, brs, 2 x CH_2), 1.97 (2H, m, CH_2), 1.48 (2H, m, CH_2), 1.22 (76H, brs, 38 x CH_2), 0.83 (3H, t, *J* = 6.0 Hz, Me-1), 0.81 (3H, t, *J* = 6.1 Hz, Me-45); EIMS *m/z* (*rel. int.*): 648 $[M]^+$ ($C_{45}H_{92}O$) (42.8), 339 (12.6).

Stigmasterol (3)

Elution of the column with petroleum ether-chloroform (1:1) furnished colourless amorphous powder of **3**, recrystallized from chloroform-methanol (1:1), 515 mg (0.034 % yield); R_f: 0.66 (petroleum ether: chloroform: methanol, 5:2:1); m.p.: 168-170 °C; IR ν_{max} (KBr): 3419, 2922, 2852, 1636, 1463, 1378, 1164, 965 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.16 (1H, m, H-6), 5.14 (1H, m, H-22), 5.07 (1H, m, H-23), 1.12 (3H, brs, Me-19), 0.97 (3H, d, *J* =7.8 Hz, Me-21), 0.88 (3H, d, *J* = 6.5 Hz, Me-26), 0.85 (3H, d, *J* = 6.3 Hz, Me-27), 0.81 (3H, d, *J* = 6.0 Hz, Me-29), 0.68 (3H, brs, Me-18); ^{13}C NMR ($CDCl_3$): δ 37.33 (C-1), 31.09 (C-2), 69.74 (C-3), 41.92 (C-4), 141.08 (C-5), 119.83 (C-6), 33.23 (C-7), 34.97 (C-8), 50.18 (C-9), 36.68 (C-10), 21.09 (C-11), 39.78 (C-12), 41.92 (C-13), 55.98 (C-14), 24.34 (C-15), 27.29 (C-16), 55.33 (C-17), 11.48 (C-18), 20.31 (C-19), 35.09 (C-20), 18.65 (C-21), 137.38 (C-22), 128.69 (C-23), 45.08 (C-24), 28.52 (C-25), 25.73 (C-26), 18.69 (C-27), 23.50 (C-28), 18.71 (C-29); +ve ion EIMS *m/z* (*rel. int.*): 412 $[M]^+$ ($C_{29}H_{48}O$) (83.1), 397 (83.6), 394 (62.7), 382 (51.6), 379 (31.6), 271 (21.3), 255 (24.1), 253 (12.3), 229 (10.1), 213 (19.8).

β-Sitosterol (4)

Further elution of the column with petroleum ether-chloroform (1:1) gave colorless amorphous powder of **4**, recrystallized from methanol, 260 mg (0.013 % yield); R_f: 0.62 (petroleum ether-chloroform-methanol, 5:2:2); m.p.: 137-138 °C; IR υ_{max} (KBr): 3465, 2955, 2845, 1640, 1475, 1365, 1210, 1105 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.30 (1H, d, *J* = 5.5 Hz, H-6), 3.51 (1H, brs, $w_{½}$ = 16.5 Hz, H-3α), 1.01 (3H, brs, Me-19), 0.97 (3 H, d, *J* = 6.5 Hz, Me-21), 0.86 (3 H, d, *J* = 6.0 Hz, Me-29), 0.85 (3 H, d, *J* = 6.0 Hz, Me-27), 0.83 (3 H, t, *J* = 6.2 Hz, Me-26), 0.67 (3H, brs, Me-18); ^{13}C NMR ($CDCl_3$): δ 37.33 (C-1),

31.63 (C-2), 69.51 (C-3), 41.98 (C-4), 141.17 (C-5), 119.94 (C-6), 31.15 (C-7), 31.81 (C-8), 49.57 (C-9), 36.74 (C-10), 21.66 (C-11), 39.80 (C-12), 41.98 (C-13), 56.04 (C-14), 24.19 (C-15), 28.60 (C-16), 55.41 (C-17), 11.36 (C-18), 19.30 (C-19), 36.74 (C-20), 18.75 (C-21), 33.30 (C-22), 25.73 (C-23), 45.14 (C-24), 29.15 (C-25), 20.37 (C-26), 19.30 (C-27), 23.56 (C-28), 11.03 (C-29); EIMS *m/z* (*rel. int.*): 414 $[M]^+$ ($C_{29}H_{50}O$) (22.3), 399 (21.6), 397 (33.1), 395 (35.2), 381 (16.5), 365 (11.3), 339 (12.6), 371 (17.8), 273 (12.5), 255 (13.2), 239 (9.6), 213 (15.6), 198 (17.9), 159 (39.7), 145 (52.6), 119 (62.6), 105 (100).

Bonducelloide (5)

Elution of the column with chloroform produced colorless crystals of **5**, recrystallized from methanol, 240 mg (0.012 % yield); R_f: 0.74 (chloroform-methanol, 2:1); m.p.: 184-185 °C; UV λ_{max} (methanol): 204 nm (log ε 4.3); IR ν_{max} (KBr): 2916, 2847, 1739, 1645, 1464, 1392, 1215, 1084, 724 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.32 (1H, m, H-15), 4.99 (1H, m, H-16) 4.09 (1H, d, *J* = 6.0 Hz, H_2-5a), 4.07 (1H, d, *J* = 7.2 Hz, H_2-5b), 2.70 (1H, m, $w_{1/2}$ = 6.0 Hz, H-4β), 2.30 (2H, t, *J* = 7.6 Hz, H_2-2), 2.06 (2H, m, H_2-14), 1.56 (2H, m, H_2-17), 1.27 (2H, brs, H_2-3), 1.22 1.22 (16H, brs, 8 x CH_2), 1.18 (16H, brs, 8 x CH_2), 0.82 (3H, t, *J* = 6.4 Hz, Me-26); ^{13}C NMR ($CDCl_3$): δ 172.81 (C-1), 128.75 (C-15), 113.92 (C-16), 33.86 (CH_2), 31.95 (CH_2), 29.73 (CH_2), 29.40 (CH_2), 29.27 (CH_2), 29.19 (CH_2), 28.97 (CH_2), 22.73 (CH_2), 14.17 (Me-26); EIMS *m/z* (*rel. int.*): 392 $[M]^+$ ($C_{26}H_{48}O_2$) (22.3), 293 (11.5), 251 (12.6), 225 (13.1), 167 (28.6), 141 (23.8), 99 (22.7).

Bunducsteroid (6)

Elution of the column with chloroform-methanol (19:1) provided colorless crystals of **6,** recrystallized from methanol, 215 mg (0.014 % yield); R_f: 0.74 (chloroform-methanol, 9.5: 0.5); m.p.: 230-232 °C; UV λ_{max} (methanol): 210 nm (log ε 5.6); IR ν_{max} (KBr): 3410, 3250, 3133, 2912, 2855, 1735, 1645, 1465, 1391, 1260, 1175, 1080, 759, 726 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.33 (1H, m, H-6), 3.47 (1H, brm, $w_{1/2}$ = 18.5 Hz, H-3α), 3.40 (2H, d, *J* = 6.3 Hz, H_2-21), 0.98 (3H, brs, Me-19), 0.90 (3H, d, *J* = 6.1 Hz, Me-26), 0.88 (3H, d, *J* = 6.0 Hz, Me-27), 0.80 (3H, t, *J* = 6.3 Hz, Me-29), 0.67 (3H, brs, Me-18), 5.01 (1H, d, *J* = 7.2 Hz, H-1′), 4.34 (1H, m, H-5′), 4.26 (1H, m, H-2′), 4.15 (1H, m, H-4′), 4.06 (1H, m, H-3′), 3.50 (2H, d, *J* = 6.5 Hz, H_2 -6′), 5.13 (1H, m, H-9″), 5.09 (1H, m, H-10″), 2.38 (2H, t, *J* = 7.2 Hz, H_2-2″), 1.25 (10 H, brs, 5 x CH_2), 1.22 (8 H, brs, 4 x CH_2), 0.85 (3H, t, *J* = 6.3 Hz, Me-18″), 1.22 (8 H, brs, 4 x CH_2), 2.33-1.11 (39 H, m, 16 x CH_2, 7 x CH); ^{13}C NMR ($CDCl_3$): δ 37.29 (C-1), 34.93 (C-2), 73.31 (C-3), 42.29 (C-4), 140.34 (C-5), 122.10 (C-6), 31.97 (C-7), 31.85 (C-8), 50.13 (C- 9), 36.67 (C-10), 21.13 (C-11), 39.78 (C-12), 42.33 (C-13), 56.77 (C-14), 24.34 (C-15), 28.26 (C-16), 56.19 (C-17), 11.97 (C-18), 19.84 (C-19), 36.21 (C-20), 62.81 (C-21), 33.93 (C-22), 26.15 (C-23), 45.77 (C-24), 29.09 (C-25), 19.41 (C-26), 19.02 (C-27), 23.04 (C-28), 11.88 (C-29), 101.28 (C-1′), 72.87 (C-2′), 70.89 (C-3′), 69.66 (C-4′), 76.73 (C-5′), 63.03 (C-6′), 174.31 (C-1″), 38.92 (C-2″), 29.86 (C-3″), 29.81 (C-4″), 29.75 (C-5″), 26.15 (C-6″), 29.58 (C-7″), 29.58 (C-8″), 130.02 (C-9″), 129.95 (C-10″), 29.86 (C-11″), 29.43 (C-12″), 29.36 (C-13″), 25.03 (C-14″), 22.73 (C-15″), 21.29 (C-16″), 18.80 (C-17″), 14.17 (C-18″); +ve ion FAB MS *m/z* (*rel. int.*): 857 $[M+H]^+$ ($C_{53}H_{93}O_8$) (10.1), 429 (38.5), 414 (26.2), 412 (100), 282 (14.6), 270 (29.8), 255 (24.3), 240 (22.6), 162 (21.7).

β-Sitosterol glucoside (7)

Elution of the column with chloroform-methanol (9:1) gave colorless amorphous powder of **7**, recrystallized from methanol, 275 mg (0.183 % yield); R_f: 0.72 (chloroform-methanol, 9:1); m.p.: 275-277 °C; UV λ_{max} (methanol): 241 nm (log ε 2.9); IR ν_{max} (KBr): 3401, 2918, 2849, 1654, 1465, 1377, 1261, 1172, 1082 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.34 (1H, brs, H-6), 5.11 (1H, brs, 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, $w_{1/2}$= 18.5 Hz, H-3), 3.37 (2H, brs, H_2- 6'), 0.99 (3H, brs, Me-19), 0.92 (3H, d, *J* = 6.2 Hz, Me-21), 0.87 (3H, d, *J* = 6.0 Hz, Me-27), 0.84 (3H, d, *J* = 6.1 Hz, Me-26), 0.82 (3H, d, *J* = 6.3 Hz, Me-29), 0.67 (3H, brs, Me-18); ^{13}C NMR ($CDCl_3$): δ 140.31 (C-5), 122.10 (C-6), 101.20 (C-1'), 78.58 (C-5'), 73.92 (C-2') 73.62 (C-3), 70.62 (C-3), 68.68 (C-4'), 62.11 (C- 6'), 56.18 (C-14), 56.12 (C-17), 150.21 (C- 9), 45.87 (C-25), 42.33 (C-4), 39.78 (C-13), 38.89 (C-12), 37.28 (C-1), 36.73 (C-20), 36.14 (C-10), 34.23 (C-8), 33.98 (C-22), 31.9 (C-2), 29.68 (C-25), 29.33 (C-7), 28.22 (C-16), 27.20 (C-15), 26.18 (C-23), 24.94 (C-28), 22.66 (C-11), 21.07 (C-26), 19.78 (C-27), 19.33 (C-19), 19.03 (C-21), 14.07 (C-29), 11.85 (C-18); +ve ion FAB MS *m/z* (*rel. int.*): 576 $[M]^+$ (C_{35} H_{60} O_6) (11.3), 413 $[M- C_6 H_{11}O_5]^+$ (10.1), 397 (100).

RESULTS AND DISCUSSION

Compound **1**, named triacontanyl diol, displayed characteristic IR absorption bands for hydroxyl groups (3510, 3450 cm^{-1}) and long chain aliphatic moiety (725 cm^{-1}). Its mass spectrum showed a molecular ion peak at *m/z* 454 corresponding to the molecular formula of a saturated alcohol, $C_{30}H_{62}0_2$. The ion fragments arising at *m/z* 215 [C_{13}-C_{14} fission, $C_{13}H_{27}O_2]^+$, 239 $[M-215]^+$, 115 [C_7-C_8 fission, $C_7H_{15}O]^+$, 339 $[M-115]^+$ and 369 $[M-C_6H_{13}]^+$ supported the location of the hydroxyl groups at C-13 and C-7. Other ion peaks generating at *m/z* 225 $[239-CH_2]^+$, 183 $[239-4 \times CH_2]^+$, 155 $[183-2 \times CH_2]^+$, 141 $[155-CH_2]^+$, and 127 $[141-CH_2]^+$ suggested the aliphatic nature of **1**. The ^1H NMR spectrum of **1** displayed a one-proton broad multiplet cantered at δ 4.16 assigned to H-7 carbinol proton placed in α-orientation on the basis of its $w_{1/2}$ = 15.3 Hz. Other one-proton broad multiplet centered at δ 3.18 was attributed to H-13 carbinol proton placed in β-orientation on the basis of its $w_{1/2}$ = 6.1 Hz. The remaining methylene protons appeared at δ 2.54 (1 x CH_2), 2.50 (2 x CH_2) and 1.22 (23 x CH_2). Two terminal C-1 and C-30 primary methyl protons resonated as three-proton triplets at δ 0.84 (*J* =

6.1 Hz) and δ 0.81 (*J* = 6.2 Hz), respectively. The ^{13}C NMR spectrum of **1** displayed signals for carbinol carbons at δ 79.15 (C-7) and δ 78.49 (C-13), methyl carbons at δ 14.15 (CH_3-1) and 13.87 (CH_3-30) and methylene carbons from δ 29.03 to 19.67. The absence of any signal beyond δ 4.16 in ^1H NMR spectrum and δ 79.15 in the ^{13}C NMR spectrum supported saturated nature of the molecule. On the basis of above discussion the structure of **1** was elucidated as *n*-triacontan-7β,13α-diol, a new long chain aliphatic alcohol.

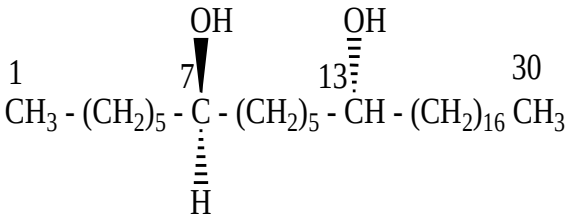
Compound **2**, named pentatetracontanyl alcohol, exhibited characteristic IR absorption bands for hydroxyl group (3450 cm^{-1}) and long aliphatic chain (733, 715 cm^{-1}). Its mass spectrum showed a molecular ion peak at *m/z* 648 corresponding to a molecular formula of a long chain saturated alcohol, $C_{45}H_{92}O$. An important fragment ion at *m/z* 339 $[C_{23}H_{47}O]^+$ arising due to fission of C_{22}-C_{23} linkage indicated the existence of the hydroxyl group at C_{23}. Its ^1H NMR spectrum displayed a one-proton broad multiplet centered at δ 4.21 assigned to carbinol H-23 proton placed in α-orientation on the basis of its $w_{1/2}$ = 14.8 Hz. The remaining methylene protons appeared as two-proton multiplets at δ 1.97 (CH_2) and 1.48 (CH_2) and as broad singlets at δ 2.50 (2 x CH_2) and 1.22 (38 x CH_2). Two three-proton triplets at δ 0.83 (*J* = 6.0 Hz) and δ 0.81 (*J* = 6.1 Hz) were accounted correspondingly to terminal C-1 and C-45 primary methyl protons. The absence of any signal beyond δ 4.21 in the ^1H NMR spectrum supported saturated nature of the molecule. On the basis of above discussion the structure of **2** was elucidated as *n*-pentatetracontan-23β-ol. This is a new long chain aliphatic alcohol.

Compound **5**, named bonducelloide, exhibited IR absorption bands for lactone ring (1739 cm^{-1}), unsaturation (1645 cm^{-1}) and long chain aliphatic moiety (724 cm^{-1}). Its mass spectrum showed a molecular ion peak at *m/z* 392 corresponding to a molecular formula of an unsaturated aliphatic δ-lactone, $C_{26}H_{48}O_2$. It indicated three double bond equivalents, one each of which was adjusted in the carbonyl function, cyclic ring and vinylic linkage. The prominent ion peaks arising at *m/z* 141, 251 $[C_{16}$-C_{17} fission$]^+$ and 225, 167 $[C_{14}$-C_{15} fission$]^+$ indicated location of the vinylic linkage at C-15. The ion peaks generating at *m/z* 99, 293 $[C_4$-C_6 fission$]^+$ supported the existence of the saturated δ-lactone ring. The ^1H NMR spectra of **5** displayed two one-proton downfield multiplets at δ 5.32 and 4.99 assigned correspondingly to H-15 and H-16 vinylic carbons. Two one-proton doublets at δ 4.09 (*J* = 6.0 Hz) and 4.07 (*J* = 7.2 Hz) were ascribed to oxygenated H_2-5a and H_2-5b methylene protons of the lactone ring. A one-proton multiplet at δ 2.70 was ascribed to methine H-4 proton of the lactone ring placed in β-orientation on the basis of its $w_{1/2}$ = 6.0 Hz. A two - proton triplet at δ 2.30 (*J* = 7.6 Hz) was accounted to methylene H_2-2 protons adjacent to the C-1 carbonyl group. Other two-proton multiplets at δ 2.06 and 1.56 were assigned to H_2-14 and H_2-17 methylene protons adjacent to vinylic linkage. The methylene protons of aliphatic chain appeared as broad signals at δ 1.27 (CH_2-3), 1.22 (8 x CH_2) and 1.18 (8 x CH_2). Terminal C-26 primary methyl protons resonated as a three-proton triplet at δ 0.82 (*J* = 6.4 Hz). The ^{13}C NMR spectrum of **5** displayed important signals for carbonyl carbon at δ 172.81 (C-1), vinylic carbons at δ 128.75 (C-15) and 113.92 (C-16), methylene carbons in the range δ 33.86 - 22.73 and primary methyl carbon at δ 14.17 (C-26). On the basis of above discussion the structure of **5** was elucidated as *n*-hexacos-15-en-1,5-olide, a new aliphatic δ-lactone derivative.

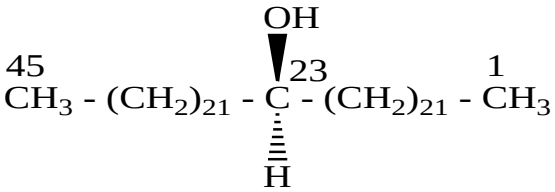
Compound **6**, named bunducsteroid, gave positive test for steroidal glycosides and exhibited characteristic IR absorption bands for hydroxyl groups (3410, 3250, 3133 cm^{-1}), unsaturation (1645 cm^{-1}), ester linkage (1735 cm^{-1}) and long aliphatic chain (759, 726 cm^{-1}). The +ve FAB mass spectrum of **6** displayed a molecular ion peak at *m/z* 857 $[M+H]^+$ consistent with molecular formula of a phytosterol glycosyl ester $C_{53}H_{93}O_8$. The prominent fragment ion peaks generating at *m/z* 429 $[C_{29}H_{49}O_2]^+$, 282 $[C_{18}H_{34}O_2]^+$ and 162 $[C_6H_{10}O_5]^+$ indicated the presence of a sterol glucoside esterified with a C_{18} carboxylic acid moiety. Other fragment ions appearing at *m/z* 414 $[429\text{-Me}]^+$, 412 $[429\text{-OH}]^+$, 270 [429 – side chain, $C_{10}H_{21}O$ - 2H$]^+$, 255 $[270\text{-Me}]^+$ and 240 $[255\text{-Me}]^+$ suggested β-sitosterol-type carbon framework containing a saturated side chain with one hydroxyl group. The ^1H-NMR spectrum of **6** displayed three one-proton deshielded multiplets at δ 5.33, 5.13 and 5.09 ascribed correspondingly to vinylic H-6, H-9" and H-10" protons. A one-proton deshielded doublet at δ 5.01 (*J* = 7.2 Hz) was attributed to anomeric H-1' proton. Other sugar protons appeared as one-proton multiplets at δ 4.34 (H-5'), 4.15 (H-4'), 4.26 (H-2'), 4.06 (H-3') and as a two-proton doublet at δ 3.50 (*J* = 6.5 Hz, H_2-6'). A one-proton broad signal centered at δ 3.47 with half width of 18.5 Hz was accounted to oxygenated methine H-3α proton. A two-proton doublet at δ 3.40 (*J* = 6.3 Hz) was ascribed to hydroxymethylene H_2-21 protons. The presence of oxygenated H_2-6' methylene protons in deshielded region at δ 3.50 indicated the attachment of the fatty acid at this carbon. Two singlets at δ 0.98 and 0.67, two doublets at δ 0.90 (*J* = 6.1 Hz) and 0.88 (*J* = 6.0 Hz) and two triplets at δ 0.80 (*J* = 6.3 Hz) and 0.85 (*J* = 6.3 Hz), integrating for three protons each, were associated with tertiary C-19 and C-18, secondary C-26 and C-27 and primary C-29 and C-18″ methyl protons, respectively, all attached to the saturated carbons. The remaining methylene and methine protons resonated between δ 2.38 - 1.11. The ^{13}C NMR spectrum of **6** displayed signals for 53 carbons. The important signals appeared for ester carbon (δ 174.31; C-1"), vinylic carbons (δ 140.34, C-5; 130.02, C-9"; 129.95, C-10"; 122.10, C-6), anomeric carbon (δ 101.28, C-1'), other sugar carbons between δ 76.73 – 63.03, oxygenated methine carbon (δ 70.31; C-3) and hydoxymethylene carbon (δ 62.81, C-21). The shifting of C-6' oxygenated

methylene carbon signal in the deshielded region at δ 63.03 indicated the location of the fatty acid moiety at this carbon. The ^{1}H and ^{13}C NMR data of steroidal nucleus of **6** were compared with the reported values of other steroidal compounds.[16-18] On the basis of the forgoing discussion the structure of **6** was established as 21-hydroxystigmast-5-en-3β-ol- 3-O-β-D-glucopyronosyl-6'-octadec-9''-enoate. This is a new phytosterol isolated from a natural source for the first time.

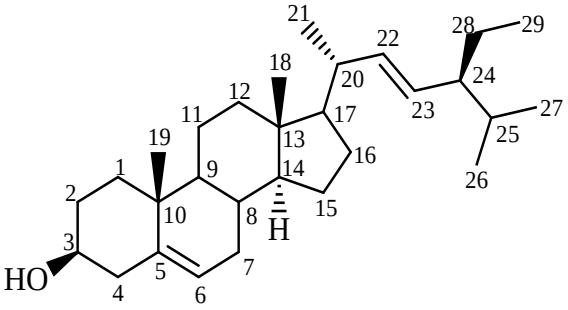
Compound **3**, **4** and **7** were the known chemical constituents characterized as stigmasterol[19,20], β-sitosterol[21,22] and β-sitosterol 3-O-glucoside.[23]



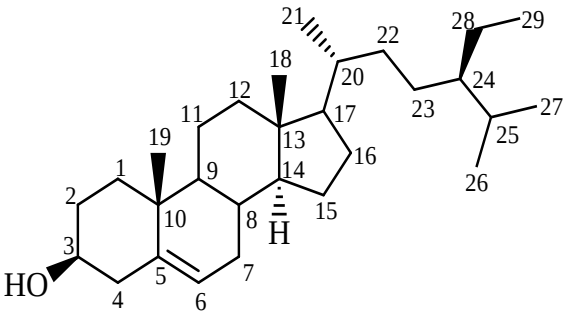
Triacontan-7,13-diol **(1)**



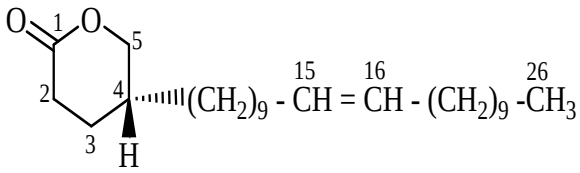
Pentatetracontanyl alcohol (2)



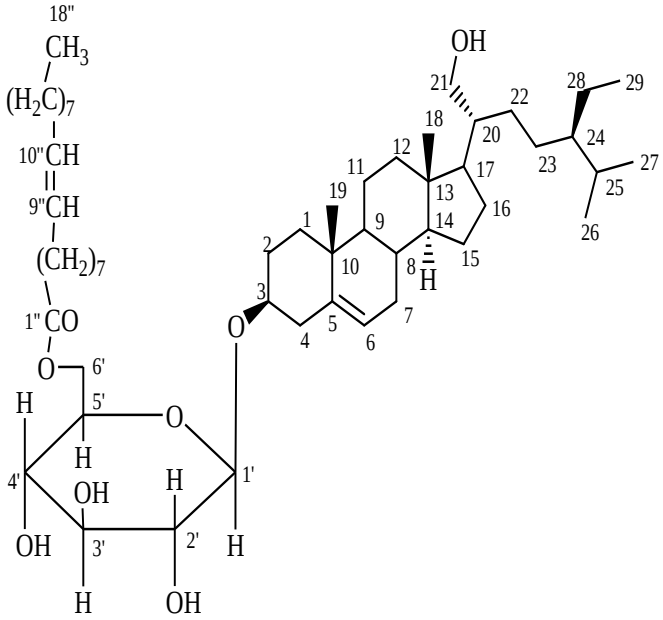
Stigmasterol (3)



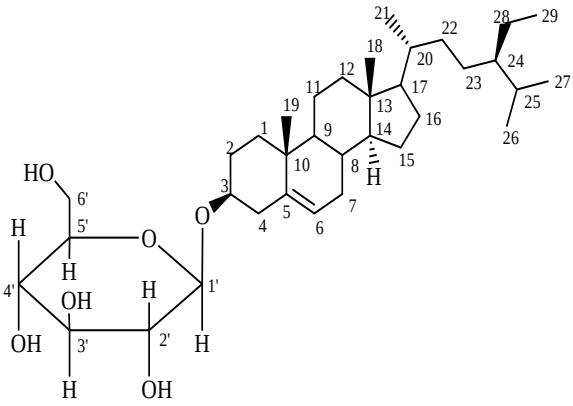
β-Sitosterol (4)



Bonducelloide (5)



Bunducsteroid (6)



β-Sitosterol glucoside (7)

Figure: 1 Structural formulae of compounds 1-7.

CONCLUSION

Phytochemical investigation of a methanolic extract of the seeds of *C. bonducella* yielded two new aliphatic alcohols, an alkyl lactone and four steroids. This work has enhanced understanding about the phytoconstituents of the plant. These compounds may be used as chromatographic markers for standardization of the seeds.

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