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CHEMICAL CONSTITUENTS FROM THE ROOTS OF *TRICHODESMA INDICUM* (L.) R. BR.

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ABSTRACT

Trichodesma indicum (L.) R. Br. (family Boraginaceae), found throughout India, is an erect, annual, branched, scabrous, and spreading herb. Its roots are useful to treat diarrhea, children's dysentery, fever, joint swellings, skin injuries, snake bites and for expulsion of dead foetus. Our study was planned to isolate chemical constituents from the methanolic extracts of the roots of *T. indicum* and to characterize their structures on the basis spectral data analysis. Phytochemical investigation of the methanolic extract led to isolate two known fatty acid esters identified as *n*-decanyl dodecanoate (decyl laurate, **1**), and *n*-tetradecyl dodecanoate (myristyl laurate, **2**), a new saturated aliphatic ketones characterized as *n*-pentacosan-12-one (**3**), an unknown fatty acid ester formulated as nonacosan-1-oyl *n*-dodecanoate (nonacosanyl myristate, **4**), an unsaturated carbonyl compound identified as (*Z*)-*n*-dotriacont-13-en-9-one (**5**), a rare steroidal ketone and its structure was determined as stigmat-5-en-3β -ol-23-one (β-sitosterol 23-one, **6**), an unfamiliar steroidal lactone and its structure was established as stigmast-5-en-3 β-ol-21(24)-olide (**7**) and a triterpenic lactonic glucoside with the structure elucidated as lanast-5,20(22)-dien-3 β-olyl-3-O-D-glucopyranosyl-21(24)-olide (**8**).

KEYWORDS: *Trichodesma indicum*, roots, phytoconstituents, isolation, characterization.

INTRODUCTION

Trichodesma indicum (L.) R. Br., syn. *Borago indicus* L., *Trichodesma amplexicaule* Roth, and *T. hirsutum* Edgew. (family Boraginaceae), known as Chhota kalpa, and Indian borage, is found throughout India, in the Himalayas and western Ghats up to 1500 m elevation, Afghanistan, Mauritius, Pakistan, and Philippines. It is an erect, annual, branched, scabrous, spreading plant; it grows about 50 cm tall; leaves alternate, opposite, linear-oblong, apex obtuse, base auricled; flowers solitary, axillary or in lax racemes, light blue or violet; fruits ellipsoid, smooth, white or blue nutlets, enclosed by the calyx.[1, 2] The plant is acrid, alexiteric, anodyne, anti-inflammatory, bitter, carminative, constipating, depurative, diuretic, emollient, febrifuge, ophthalmic, pectoral and refrigerant; used to treat arthritis, bone fractures, coughs, dyspepsia, diarrhoea, dysentery, dysmenorrhoea, ear and eye diseases, inflammations, influenza, skin diseases and strangury.[1-3] The leaf infusion is depurative. The leaves are used as remedy for ear pain, eczema, joint pain, snake bites and wounds.[4,5] The flowers are diuretic, pectoral and sudorific, used to heal wounds. The roots are useful to relieve diarrhea, children's dysentery, fever, joint swellings, skin injuries, snake bites and for expulsion of dead foetus.[1-6]

The plant contained oleanolic acid, lupeol and linoleic acid,[7] rutin, salicylic acid, benzoic acid. 9,19-cyclolanosta-3-ol 24,24-epoxymethano acetate, piperidine, cyclohexanol, 1.2-dimethoxy-4-(1-methyl-1-propenyl)-benzene, diisoctyl phthalate, and 1,10-phenanthroline 2,9-dimethyl 2-propenoic acid,[8] tannins and saponins,[9] 1,2-benzenedicarboxylic acid diisooctyl ester,[10] ß-sitosterol, catechin and gallic acid, tannins.[11,12] The leaves possessed phytol, tetradecamethyl-cyclo-heptasiloxane and hexadecanoic acid methyl ester.[13] The seeds contained an oil, phenolic compounds, pyrrolizidine alkaloids, rosmarinic acid, isomers of salvianolic acid, melitric acid A and sebestenoid C/D, linoleic, γ-linolenic, α-linolenic, and stearidonic acids, oleic and palmitic acids, γ-tocopherol and β-sitosterol.[14] The presence of herbal chemical constituents vary due to many factors such as geographic regions, soils, seasonal changes, plant species and application of fertilizers. Keeping in views the various therapeutic values and variation aspects of chemical constituents of the plants and development of ecofriendly, biodegradable and safer herbal preparations, it has been aimed to establish chemical structures of phytoconstituents isolated from the roots *Trichodesma indicum*.

MATERIALS AND METHODS

The protocols of all methodologies (procedures, experimental designs and spectral data analysis) were adopted from the earlier published work.[17, 18, 20]

General Procedures

Melting points were measured using one end open capillary tubes on a thermoelectrically heated melting point apparatus (Perfit, India) without correction. UV spectra were determined with Lambda Bio 20 spectrophotometer (Perkin Elmer, Schwerzenbach, Switzerland) in methanol. The IR spectra were obtained by using KBr pellets with Jasco FT/IR-5000 Spectrometer (FTS 135, Hong Kong). The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on Bruker DRX Spectrometer (Rheinstetten, 2 Germany) using $CDCl_3$ and DMSO-d_6 as solvents. TMS (Fluka analytical, Sigma-Aldrich, Netherland) was taken as an internal standard and the coupling constants (*J* values) are expressed in Hertz (Hz). Mass spectra were recorded by affecting electron impact ionization at 70 eV on a Jeol SX-102 mass spectrometer (Waters Corp., UK) instrument equipped with direct inlet prob system. The *m/*z values of the more intense peaks are mentioned and the figures in bracket attached to each *m/*z values indicated relative intensities with respect to the base peak. Column chromatography was performed on silica gel (Qualigens, Mumbai, India) with 60-120 mesh particle size. The purity of the isolated compounds was checked on precoated TLC plates with silica gel 60 F_{254} (0.25 mm, Merck, Mumbai, India). The spots were visualized by exposure to iodine vapours and under UV radiations at 254 and 366 nm and spraying with ceric sulphate solution.

Collection and Authentication of Plant Material

The roots of *Trichodesma indicum* were collected from Dehradun, Uttarakhand. The plant material was identified and authenticated by Prof. M. P. Sharma, Taxonomist, Department of Botany, Jamia Hamdard, New Delhi. A Voucher specimen of the plant materials was preserved in the herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

Extraction and Isolation

The roots of *Trichodesma indicum* (1 kg) were dried in air, coarsely powdered and extracted exhaustively with methanol in a Soxhlet apparatus. The extracts were concentrated under reduced pressure to get a dark brown mass, 121 g. The dried residue (100 g) was dissolved in minimum amount of methanol and adsorbed on silica gel column grade (60-120 mesh) to obtain a slurry. It was air-dried and chromatographed over a silica gel column loaded in petroleum ether (b. p. 60 – 80 °C). The column was eluted with petroleum ether, petroleum ether - chloroform (9:1, 3:1, 1:1, 1:3, *v/v*), chloroform and chloroform - methanol (99:1, 49:1 *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 with solvents. The isolated compounds were recrystallized to get pure compounds.

Decyl laurate (1)

Elution of the column with petroleum ether produced a colourless amorphous powder of **1**, yield 117 mg, recrystallized from chloroform-methanol (1:1), m. p. 28 - 30 °C; IR υ_{max} (KBr) : 2921, 2852, 1730, 1464, 1295, 1169, 724 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.12 (2H, m, H_2 -1′), 2.43 (2H, t, J = 7.2 Hz, H_2 -2), 1.55 (2H, m, H_2-3), 1.33 (4H, m, H_2 -2′, H_2-3), 1.25 (24H, brs, 12 x CH_2), 1.19 (4H, m, H_2 -9′, H_2-11), 0.81 (3H, t, J = 6.3 Hz, Me-12), 0.73 (3H, t, J = 6.2 Hz, Me-10′); EIMS *m/z* (rel.int.): 340 $[M]^+$ ($C_{22}H_{44}O_2$) (11.6), 313 (23.1), 239 (20.8), 183 (100), 155 (50.7), 127 (32.5), 113 (22.6).

Myristyl laurate (2)

Further elution of the column with petroleum ether gave a colourless amorphous powder of **2**, yield 132 mg, recrystallized from chloroform-methanol (1:1), m. p. 35 - 37 °C; UV λmax (MeOH): 210 nm (log ε 3.6); IR υ_{max} (KBr): 2919, 2850, 1734, 1466, 1375, 1179, 1015, 725 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.15 (2H, m, H_2 -1′), 2.46 (2H, t, J = 7.3 Hz, H_2 -2), 1.57 (2H, m, H_2-3), 1.35 (4H, m, H_2 -2′, H_2-3), 1.25 (38H, brs, 19 x CH_2), 0.81 (3H, t, J = 6.1 Hz, Me-12), 0.78 (3H, t, J = 6.3 Hz, Me-14′); EIMS *m/z* (rel.int.): 396 $[M]^+$ ($C_{26}H_{52}O_2$) (38.1), 339 (23.6), 267 (19.5), 239 (19.6), 183 (100), 141 (90.1).

n-Pentacosan-12-one (3)

Elution of the column with petroleum ether – chloroform (3:1) furnished colorless powder of **3**, 168 mg, m. p. 72 - 74 °C, UV λmax (MeOH): 216 nm (log ε 3.8); IR υ_{max} (KBr): 2922, 2850, 1710, 1635, 1467, 1244, 721 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.55 (4H, m, H_2-11, H_2-13), 1.51 (2H, m, H_2-10), 1.28 (38 H, brs, 19 x CH_2), 0.81 (3 H, t, J = 6.3 Hz, Me-1), 0.78 (3 H, t, J = 6.5 Hz, Me-25); ^{13}C NMR ($CDCl_3$): δ 195.73 (C-12), 31.97 (C-11), 29.72 (18 x CH_2), 29.31 (C-23), 22.72 (C-2, C-24), 14.28 (Me-1), 14.17 (Me-25); EI MS *m/*z (rel. int.): 366 $[M]^+$ ($C_{25}H_{50}O$) (33.6), 268 (31.7), 225 (15.3), 183 (100), 155 (7.5), 140 (88.1), 113 (17.6).

n-Nonacosanyl myristate (4)

Elution of the column with chloroform yielded a colourless amorphous powder of **4**, yield 136 mg, recrystallized from chloroform-methanol (1:1), m. p. 84 - 86 °C; UV λmax (MeOH): 218 nm (log ε 3.8); IR υ_{max} (KBr) : 2921, 2852, 1735, 1463, 1174, 1104, 726 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.21 (2H, t, J = 6.7 Hz, H_2 -1′), 2.16 (2H, t, J = 7.3 Hz, H_2 -2), 1.55 (2H, m, H_2-3), 1.34 (2H, brs, H_2-2′), 1.28 (72H, brs, 36 x CH_2), 0.86 (3H, t, J = 6.3 Hz, Me-14), 0.83 (3H, t, J = 6.2 Hz, Me-29′); ^{13}C NMR ($CDCl_3$): δ 171.25 (C-1), 34.87 (C-2), 32.73 (C-3), 30.64 (C-4), 29.61 (C-5 to C-11), 27.06 (C-12), 25.82 (C-13), 16.23 (C-14), 64.73 (C-1′), 29.27 (C-2′ to C-26′), 25.81 (C-27′), 22.74 (C-28′), 14.76 (C-29′); EI MS *m/z* (rel. int.): 634 $[M]^+$ ($C_{43}H_{86}O_2$) (19.8), 561 (12.3), 437 (11.6), 423 (12.3), 337 (19.7), 267 (22.5), 253 (37.1), 211

(73.8), 197 (64.9), 169 (32.7), 155 (25.2), 127 (36.2), 113 (56.1), 99 (48.9).

(*Z*)-*n*-Dotriacont-13-en-9-one (5)

Further elution of the column with chloroform afforded a colorless powder of **5**, yield 157 mg, m. p. 96 - 98 °C; UV λmax (MeOH): 243 nm (log ε 2.9); IR υ_{max} (KBr): 2922, 2853, 1705, 1636, 1464, 1370, 1057, 956, 725 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.35 (1H, m, $w_{1/2}$ = 9.1 Hz, H-13), 5.31 (1H, m, $w_{1/2}$ = 8.8 Hz, H-14), 2.73 (2H, t, J = 7.1 Hz, H_2-8), 2.51 (2H, m, H_2-10), 2.18 (2 H, m, H_2-12), 2.03 (2 H, m, H_2-15), 1.42 (2 H, m, H_2-7), 1.27 (44 H, brs, 22 x CH_2), 0.78 (3 H, t, J = 6.1 Hz, Me-1), 0.75 (3 H, t, J = 6.3 Hz, Me-32); ^{13}C NMR ($CDCl_3$): δ 207.21 (C-9), 133.89 (C-13), 129.18 (C-14), 50.47 (C-8), 37.26 (C-10), 33.85 (C-12), 31.96 (C-15), 31.62 (C-7), 30.97 (C-11), 30.19 (C-16), 30.06 (C-12), 29.72 (8 x CH_2), 29.51 (C-4), 29.38 (C-3), 29.18 (C-2), 28.96 (C-29), 27.41 (C-30), 22.68 (C-31), 14.15 (C-1), 14.11 (C-32); EI-MS *m/z* (rel. int.): 462 $[M]^+$ ($C_{32}H_{62}O$) (6.7), 439 (21.9), 393 (17.8), 359 (65.1), 349 (8.7), 341 (40.6), 321 (10.5), 279 (100), 263 (37.1), 191 (29.8), 183 (55.3), 141 (49.2), 127 (36.5), 113 (12.6).

β- Sitosterol 23-one (6)

Further elution of the column with chloroform produced a colourless crystalline mass of **6**, recrystallized by (chloroform-methanol, 1:1), 143 mg, UV λmax (MeOH): 213 nm (log ε 5.7); m. p. 112 - 114 °C; IR υ_{max} (KBr): 3413, 2921, 2849, 1711, 1636, 1463, 1377, 1274, 1168, 1031 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.33 (1H, d, J = 5.5 Hz, H-6), 3.37 (1H, brm, $w_{1/2}$ = 16.5 Hz, H-3α), 2.43 (2H, m, H_2-22), 2.25 (1H, m, H-24), 1.98 (2H, m, H_2-4), 1.74 (2H, m, H_2-7), 1.71 - 1.19 (20H, m, 7 x CH_2, 8 x CH), 1.15 (3H, brs, Me-19), 1.06 (3H, d, J = 6.5 Hz, Me-21), 0.82 (3H, d, J = 6.3 Hz, Me-26), 0.79 (3H, d, J= 6.4 Hz, Me-27), 0.77 (3H, d, J = 6.3 Hz, Me-29), 0.63 (3H, brs, Me-18); ^{13}C NMR ($CDCl_3$): δ 36.87 (C-1), 32.72 (C-2), 73.16 (C-3), 41.89 (C-4), 140.12 (C-5), 122.36 (C-6), 31.27 (C-7), 31.12 (C-8), 49.57 (C-9), 37.64 (C-10), 23.17 (C-11), 39.81 (C-12), 41.91 (C-13), 55.78 (C-14), 24.15 (C-15), 28.93 (C-16), 55.46 (C-17), 11.51 (C-18), 19.21 (C-19), 36.83 (C-20), 18.45 (C-21), 38.39 (C-22), 207.25 (C-23), 45.12 (C-24), 27.87 (C-25), 21.03 (C-26), 22.48 (C-27), 23.71 (C-28), 11.64 (C-29); EI MS *m/z* (rel. int.): 428 $[M]^+$ ($C_{29}H_{48}O_2$) (6.7), 413 (17.5), 410 (14.1), 398 (11.2), 395 (9.5), 393 (22.6), 359 (38.7), 341 (64.9), 315 (27.8), 304 (11.3), 264 (27.6), 239 (41.3), 180 (100), 164 (15.2), 155 (26.1), 141 (87.3), 138 (47.9), 124 (52.3), 113 (20.1).

Stigmat-5-en-3β -ol-21(24)-olide (7)

Elution of the column with chloroform – methanol (19:1) afforded an amorphous powder of **7**, recrystallized from acetone, yield 136 mg, m. p. 118 - 120 °C; UV λmax (MeOH): 249 nm (log ε 3.4); IR υ_{max} (KBr): 3404, 2926, 2855, 1738, 1636, 1461, 1376, 1255, 1103, 1065 cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.35 (1H, m, H-6), 3.28 (1H, brs, $w_{1/2}$ = 18.5 Hz, H-3α), 2.55 (1H, m, H-20), 2.42 (2H, m, H_2-4), 2.26 (2H, m, H_2-7), 1.07 (3H, brs, Me-19), 0.87 (3H, d, J = 6.7 Hz, Me-26), 0.84 (3H, d, J = 6.4 Hz, Me-27), 0.82 (3H, t, J = 6.1 Hz, Me-29), 0.65 (3H, brs, Me-18), 2.21 - 1.12 (23H, m, 9 × CH_2, 5 × CH); ^{13}C NMR (DMSO-d_6): δ 38.91 (C-1), 31.36 (C-2), 73.38 (C-3), 41.89 (C-4), 141.05 (C-5), 121.19 (C-6), 34.38 (C-7), 35.51 (C-8), 49.56 (C-9), 36.87 (C-10), 23.25 (C-11), 39.71 (C-12), 39.87 (C-13), 56.41 (C-14), 28.47 (C-15), 29.08 (C-16), 55.72 (C-17), 11.69 (C-18), 19.03 (C-19), 41.26 (C-20), 173.82 (C-21), 33.94 (C-22), 26.21 (C-23), 78.451 (C-24), 29.28 (C-25), 18.53 (C-26), 19.67 (C-27), 20.49 (C-28), 11.63 (C-29); EI-MS *m/z* (rel. int.): 442 $[M]^+$ ($C_{29}H_{46}O_3$) (2.5), 413 (5.1), 399 (2.9), 397 (13.4), 380 (11.6), 370 (9.6), 278 (23.5), 276 (9.2), 250 (6.1), 234 (23.5), 192 (11.3), 188 (100), 166 (12.7), 164 (15.1), 141 (76.5), 138 (8.3),124 (43.8).

Lanast-5,20(22)-dien-3 β-olyl-3-O-D-glucopyranosyl-21(24)-olide (8)

Elution of the column with chloroform – methanol (9:1) gave an amorphous powder of **8**, recrystallized from chloroform – methanol (1:1), yield 181 mg, m. p. 129 - 131 °C; UV λmax (MeOH): 210, 235 nm (log ε 4.8, 3.7); IR υ_{max} (KBr): 3405, 3353, 2923, 2854, 1737, 1643, 1462, 1375, 1251, 1169, 1075 cm^{-1}; ^1H NMR (DMSO-d_6): δ 6.33 (1H, m, H-22), 5.37 (1H, m, H-6), 4.96 (1H, m, $w_{1/2}$ = 16.3 H, H-24 α), 3.89 (J = 5.5, 8.9 Hz, H-3α), 2.91 (1H, m, H-17), 2.47 (2H, m, H_2-2), 2.31 (2H, m, H_2-23), 2.19 (2H, m, H_2-7), 2.16 (2H, m, H_2-1), 1.98 (1H, m, H-9), 1.95 (1H, m, H-8), 1.81 (1H, m, H-25), 1.63 (2H, m, H_2-12), 1.54 (2H, m, H_2-11), 1.46 (2H, m, H_2-15), 1.40 (2H, m, H_2-16), 1.25 (3H, brs, Me-28), 1.21 (3H, brs, Me-19), 0.86 (3H, brs, Me-30), 0.80 (3H, brs, Me-29), 0.78 (3H, d, J = 6.1 Hz, Me-26), 0.75 (3H, d, J = 6.2 Hz, Me-27), 0.63 (3H, brs, Me-18), 5.03 (1H, d, J = 7.1 Hz, H-1′), 4.43 (1H, m, H-5′), 4.21 (1H, m, H-2′), 357 (1H, m, H-3′), 3.45 (1H, m, H-4′), 3.16 (2H, d, J = 9.1 Hz, H_2- 6′); ^{13}C NMR (MeOD): δ 34.53 (C-1), 27.29 (C-2), 78.31 (C-3), 42.56 (C-4), 138.84 (C-5), 119.76 (C-6), 31.296 (C-7), 41.92 (C-8), 49.25 (C-9), 38.16 (C-10), 21.81 (C-11), 26.52 (C-12), 48.29 (C-13), 55.11 (C-14), 35.97 (C-15), 47.86 (C-16), 53.34 (C-17), 13.71 (C-18), 20.26 (C-19), 142.74 (C-20), 173.91 (C-21), 123.352 (C-22), 34.95 (C-23), 76.46 (C-24), 29.43 (C-25), 21.79 (C-26), 22.16 (C-27), 24.51 (C-28), 28.14 (C-29), 14.24 (C-30); 101.38 (C-1′), 71.69 (C-2′), 64.39 (C-3′), 67.81 (C-4′), 79.68 (C-5′), 62.31 (C-6′); EI-MS *m/z* (rel. int.): 616 $[M]^+$ ($C_{36}H_{56}O_8$) (1.2), 436 (6.6), 421 (11.3), 406 (20.6), 379 (58.3), 354 (11.6), 288 (12.3), 277 (18.6), 195 (35.7), 179 (12.3), 174 (23.2), 163 (14.1), 157 (100), 148 (25.8), 139 (9.7), 134 (74.1).

RESULTS AND DISCUSSION

Compound **1** was a known fatty acid ester identified as *n*-decanyl dodecanoate (decyl laurate) (Fig.1).[15] Compound **2** was a familiar fatty acid ester characterized as *n*-tetradecyl dodecanoate (myristyl laurate) (Fig.1).[15, 16]

Compound **3** showed IR absorption bands for carbonyl group (1710 cm^{-1}) and long aliphatic chain (721 cm^{-1}). Its

mass spectrum displayed a molecular ion peak at *m/z* 366 corresponding to a molecular formula of a saturated aliphatic ketone, $C_{25}H_{50}O$. The ion peaks generating at *m/z* 183 [C_{12} – C_{13} fission, CH_3 $(CH_2)_{10}CO]^+$, 155 [C_{11} – C_{12} fission, CH_3-$(CH_2)_{10}]^+$ and 140 [155 – Me] + suggested the presence of the carbonyl function at C_{12} carbon. The ^{1}H NMR spectrum of **3** exhibited a four-proton multiplet at δ 2.55 assigned methylene H_2-11 and H_2-12 protons adjacent to the carbonyl carbon, other methylene protons as a two-proton multiplet at δ 1.51 and as a broad singlet at δ 1.28 (38H). Two three-proton triplets at δ 0.81 (J = 6.3 Hz) and 0.78 (J = 6.5 Hz) were accounted to terminal C-1 and C-25 primary methyl protons, respectively. The ^{13}C NMR spectrum of **3** showed signals for the carbonyl carbon at δ 195.73 (C-9), methylene carbons between δ 31.97 - 22.72 and methyl carbons at δ 14.28 (C-1) and 14.17 (C-25). The absence of any signal beyond δ 2.55 in the ^{1}H NMR spectrum and between δ 195.73 - 31.97 in the ^{13}C NMR spectrum ruled out the unsaturated nature of the molecule. On the basis of foregoing spectral data analysis, the structure of **3** has been elucidated as *n*-pentacosan-12-one, a new aliphatic ketone (Fig. 1).

Compound **4** exhibited IR absorption bands for an ester group (1735 cm^{-1}) and long aliphatic chain (726 cm^{-1}). Its mass spectrum showed a molecular ion peak at *m/z* 634 $[M]^+$ consistent with a molecular formula of a fatty acid ester, $C_{43}H_{86}O_2$. A prominent ion peak formed due to removal of the acyl group at *m/z* 211 [C_1 - O fission, $CH_3(CH_2)_{12}$-$CO]^+$ and at *m/z* 423 [M – 211, O-CH_2-$(CH_2)_{27}$-$CH_3]^+$ suggested that myristic acid was esterified with nonacosan-1-ol.

The ^{1}H NMR spectrum of **4** exhibited two two-proton triplets at δ 4.21 (J = 6.7 Hz) and 2.16 (J = 7.3 Hz) attributed correspondingly to oxymethylene H_2-1′ and methylene H_2 -2 protons adjacent to the ester function. The remaining methylene protons appeared as two-proton multiplets from δ 1.55 and 1.34 and as a broad singlet at δ 1.28 (72H). Two three-proton triplets at δ 0.86 (J = 6.3 Hz) and 0.83 (J = 6.2 Hz) were ascribed to primary C-14 and C-29′ methyl protons, respectively. The ^{13}C NMR spectrum of **4** displayed signals for the ester carbon at δ 171.25 (C-1), oxymethylene carbon at δ 64.73 (C-1′), other methylene carbons between δ 34.87 – 22.74 and methyl carbons at δ 16.23 (C-14) and 14.76 (C-29′). On the basis of above discussion the structure of **4** has been characterized as nonacosan-1-oyl *n*-dodecanoate (nonacosanyl myristate), a new fatty acid ester (Fig. 1).

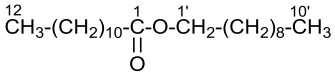
Compound **5** showed its IR absorption bands for carbonyl group (1705 cm^{-1}), unsaturation (1636 cm^{-1}) and long aliphatic chain (725 cm^{-1}). Its mass spectrum displayed a molecular ion peak at *m/z* 462 corresponding to a molecular formula of an unsaturated aliphatic ketone, $C_{32}H_{62}O$. The ion fragments arising at *m/z* 113 [C_8 – C_9 fission, CH_3 $(CH_2)_7]^+$, 349 $[M – 113]^+$, 141 [C_9 – C_{10} fission, CH_3 $(CH_2)_7$-$CO]^+$ and 321 $[M – 141]^+$ suggested the existence of the carbonyl group at C_9 carbon position. The ion peaks generating at *m/z* 183 [C_{12} – C_{13} fission, CH_3 $(CH_2)_7CO$-$(CH_2)_3]^+$ and 279 [M - 183, CH_3 $(CH_2)_{17}$-CH=CH]$^+$ indicated the presence of the vinylic linkage at C_{13} carbon. The ^{1}H NMR spectrum of **5** exhibited two one -proton multiplets at δ 5.35 and 5.31 with half-widths of 9.1 Hz and 8.8 Hz, respectively, assigned correspondingly to *cis*-oriented vinylic H-13 and H-14 protons and methylene protons as a two -proton triplet at δ 2.73 (J = 7.1 Hz) and a two-proton multiplet at δ 2.51 ascribed to methylene protons adjacent to the carbonyl group, other methylene protons as two-proton multiplets at δ 2.18, 2.03, and 1.42 as a broad singlet at δ 1.27 (44 H). Two three-proton triplets at δ 0.78 (J = 6.1 Hz) and 0.75 (J = 6.3 Hz) were accounted to terminal C-1 and C-32 primary methyl protons, respectively. The ^{13}C NMR spectrum of **5** showed signals for the keto carbon at δ 207.21 (C-9), vinylic carbons at δ 133.89 (C-13) and 129 (C-20), methylene carbons between δ 50.47 - 22.68 and methyl carbons at δ 14.15 (C-1) and 14.11 (C-25). The absence of any signal between δ 5.31 - 2.73 in the ^{1}H NMR spectrum and from δ 129.18 to 50.47 in the ^{13}C NMR spectrum ruled out the presence of any carbinol function in the molecule. On the basis of foregoing spectral data analysis, the structure of **5** has been elucidated as (*Z*)-*n*-dotriacont-13-en-9-one, a new aliphatic ketone (Fig. 1).

Compound **6** showed characteristics IR absorption bands for a carbinol function (3413 cm^{-1}), carbonyl group (1711 cm^{-1}), and unsaturation (1636 cm^{-1}). Its molecular ion peak was established at *m/z* 428 on the basis of mass and ^{13}C NMR spectra consistent with a molecular formula of a steroid, $C_{29}H_{48}O_2$. The prominent ion peaks produced at *m/z* 413 [M - Me, $C_{28}H_{45}O_2]^+$, 398 [M – Me]$^+$, 410 $[M – H_2O]^+$ and 395 [410 – Me]$^+$ indicated the presence of the carbinol group in the molecule. The ion peaks generated at *m/z* 124 [$C_{6,7}$ – $C_{9,10}$ fission, $C_8H_9O]^+$, 304 $[M – 124]^+$, 138 [$C_{7,8}$ – $C_{9,10}$ fission, $C_9H_{11}O]^+$, 164 [$C_{8,14}$ – $C_{9,11}$ fission, $C_{11}H_{16}O]^+$ and 264 $[M – 164]^+$ suggested the existence of the hydroxyl group in ring A which was placed at C-3 on the basis biological analogy. The ion peaks formed at *m/z* 113 [C_{22} -C_{23} fission, $C_7H_{12}O]^+$, 315 $[M – 113]^+$ and 155 [C_{20} -C_{21} fission, $C_{10}H_{19}O]^+$ supported the location of the carbonyl group at C-23 position. The ^{1}H NMR spectrum of **6** displayed a one-proton doublet at δ 5.33 (J = 5.5 Hz) and a one-proton multiplet at δ 3.37 with half-with of 16.5 Hz attributed to vinylic H-6 and alpha-oriented carbinol H-3 protons, respectively. Two three-proton singlets at δ 1.15 and 0.63, and four three-proton doublets at δ 1.06 (J = 6.5 Hz), 0.82 (J = 6.3 Hz), 0.79 (J = 6.4 Hz) and 0.77 (J = 6.3 Hz) were associated correspondingly with the tertiary C-19 and C-18, secondary C-21, C-26 and C-27 and primary C-29 methyl protons. The remaining methine and methylene protons resonated from δ 2.43 to 1.19. The ^{13}C NMR spectrum of **6** displayed 29 carbon signals including important signals for vinylic carbons at δ 140.12 (C-5) and 122.36 (C-6), carbonyl carbon at δ 207.25 (C-23), carbinol carbon at δ 73.16 (C-3), methyl

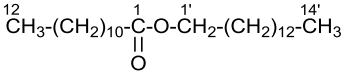
carbons at δ 11.51 (C-18), 19.21 (C-19), 18.45 (C-21), 21.03 (C-26), 22.48 (C-27) and 11.64 (C-29). The ^{1}H NMR and ^{13}C NMR spectral data of the steroidal nucleus were compared with other stigmastene-type molecules.[17, 18] On the basis of spectral data analysis, the structure of **6** has been determined as stigmat-5-en-3β -ol-23-one (β- sitosterol 23-one), a new keto steroid (Fig. 1).

Compound **7** gave positive tests of steroids and showed IR absorption bands for a hydroxyl group (3404 cm^{-1}), δ-lactone (1738 cm^{-1}) and unsaturation (1636 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, its molecular ion peak was established at *m/z* 442 consistent with a molecular ion peak of a steroidal lactone, $C_{29}H_{46}O_3$. The mass ion peaks generated at *m/z* 370 [$C_{1,10}$ – $C_{4,5}$ fission]$^+$, 124 [$C_{6,7}$ – $C_{9,10}$ fission]$^+$, 138 [$C_{7,8}$ – $C_{9,10}$ fission]$^+$, 164 [$C_{8,14}$ – $C_{9,11}$ fission]$^+$, 178 [$C_{8,14}$ – $C_{11,12}$ fission]$^+$, 192 [$C_{8,14}$ – $C_{12,13}$ fission]$^+$, 278 [M – 164]$^+$, 250 [M -192]$^+$, 413 [M – CH_2CH_3]$^+$, 398 [413 – Me]$^+$ and 380 [398 – H_2O]$^+$ indicated the presence of the hydroxy group in ring A kept at C-3 on the basis of biogenetic analogy, vinylic bond in ring B at $C_{5,6}$ – position, and saturated nature of rings A and C. The ion peaks arising at *m/z* 166 and 276 [C_{17} – C_{20} fission]$^+$ supported the existence of the lactone ring at C_{20}- C_{24} position. The ^{1}H NMR spectrum of **7** displayed a one-proton doublet at δ 5.35 and a one-proton multiplet at δ 3.28 ($w_{1/2}$ = 18.5 Hz) assigned to vinylic H-6 and α-oriented carbinol H-3 protons, respectively. Two three-proton broad singlets at δ 0.65 and 1.07 were ascribed to tertiary C-18 and C-19 methyl protons, respectively. Two doublets at δ 0.87 (J = 6.7 Hz) and 0.84 (J = 6.4 Hz) and a triplet at δ 0.82 (J = 6.1 Hz), all integrating for three protons each, were accounted to secondary C-26 and C-27 methyl and primary C-29 methyl protons, respectively, all attached to the saturated carbons. Three multiplets at δ 2.55 (1H), 2.42 (2H) and 2.26 (2H) were due to correspondingly methine H-20 linked to lactone carbonyl carbon and methylene H_2-4 and H_2-7 protons adjacent to the vinylic linkage. The remaining methylene and methine protons resonated as multiplets between δ 2.21 - 1.12. The ^{13}C NMR spectrum of **7** showed signals for 29 carbons including vinylic carbons at δ 141.05 (C-5) and 121.19 (C-6), carbinol carbon at δ 73.38 (C-3), lactone carbonyl carbon at δ 173.82 (C-21), tertiary oxygenated carbon at δ 78.451 (C-24), methyl carbons at δ 11.69 (C-18), 19.03 (C-19), 18.53 (C-26), 19.67 (C-27), and 11.63 (C-29) and the remaining methylene and methine carbons between δ 56.41 -20.49. The ^{1}H and ^{13}C NMR spectral data of the steroidal nucleus of **7** were compared with related stigmasterol-type molecules.[17, 18] On the basis of spectral data analysis, the structure of **7** has been formulated as stigmast-5-en-3 β-ol-21(24)-olide, a new steroidal lactone (Fig. 1).

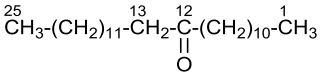
Compound **8** gave positive tests for glycosides and showed characteristics IR absorption bands for hydroxyl groups (3405, 3353 cm^{-1}), lactone group (1737 cm^{-1}) and unsaturation (1643 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, the molecular ion peak of 8 was determined at *m/z* 616 corresponding to the molecular formula of a tetracyclic triterpenic monoglycoside, $C_{36}H_{66}O_8$. The ion fragments arising at *m/z* 179 [C_3 – O fission, $C_6H_{11}O_6]^+$, 163 [$C_6H_{11}O_5]^+$ and 436 [M – 179]$^+$ indicated the presence of the hexose unit linked with the triperpenic unit. The ion peaks formed at *m/z* 354 [$C_{1,10}$ - $C_{4,5}$ fission, $C_{24}H_{34}O_2]^+$, 134 [$C_{6,7}$ -$C_{9,510}$ fission, $C_{10}H_{14}]^+$, 148 [$C_{7,8}$ -$C_{9,10}$ fission, $C_{11}H_{16}]^+$, 288 [436 – 148]$^+$, 174 [$C_{8,14}$ -$C_{9,11}$ fission, $C_{13}H_{18}]^+$ suggested the existence of the oxy group in ring A which was placed at C-3 on the basis of biological analogy and vinylic linkage at C-5 position. The ion peak produced at *m/z* 139 [C_{17} – C_{20} fission, $C_8H_{11}O_2]^+$ supported the presence of the lactone ring in the side chain at C_{21} – C_{24} position. The ^{1}H NMR spectrum of **8** showed two one-proton multiplets at δ 6.33 (J = 10.3 Hz) and 5.37 assigned to vinylic H-22 and H-6 protons, respectively. A one-proton double doublet at δ 3.89 (J = 5.5, 8.9 Hz) and a one-proton multiplet at δ 4.96 ($w_{1/2}$ = 16.3 H) were ascribed correspondingly to α-oriented oxymethine H-3 and H-23 protons. Five three-proton singlets at δ 1.25, 1.21, 0.86, 0.80 and 0.63 were attributed to C-28, C-19, C-30, C-29 and C-18 tertiary methyl protons, respectively. Two three-proton doublets at δ 0.78 (J = 6.1 Hz) and 0.75 (J = 6.2 Hz) were ascribed to secondary C-26 and C-27 methyl protons, respectively. A one-proton doublet at δ 5.03 (J = 7.1 Hz), four one-proton multiplets at δ 4.43, 4.21, 357, 3.45, and a two -proton doublet at δ 3.16 (J = 9.1 Hz) were accounted to anomeric H-1′, and other sugar methine protons H-5′, H-2′, H-3′ and H-4′ and hydroxymethylene H_2- 6′ protons, respectively. The remaining methine and methylene protons appeared between δ 2.91 – 1.40. The ^{13}C NMR spectrum of **8** showed important signals for oxymethine carbons at δ 78.31 (C-3) and 76.46 (C-24), lactone carbonyl group at δ 173.91 (C-21), vinylic carbons at δ 138.84 (C-5), 119.76 (C-6), 142.74 (C-20) and 123.352 (C-22), methyl carbons between δ 13.71 – 28.14, anomeric carbons at δ 101.38 (C-1′), other sugar carbons from δ 79.68 to 62.31 and the remaining methylene and methine carbons between δ 55.11 – 21.81. The ^{1}H NMR and ^{13}C NMR spectral data of the triterpenic nucleus were compared with other lanostene –type molecules.[19,20] Acid hydrolysis of **8** yielded D-glucose, R_f 0.26 (*n*-butonal- acetic acid-water, 4:1:5), specific rotation, $[\alpha]^{D}_{25^{o}}$ + 52.5 ° (water). On the basis of spectral data analysis and chemical reactions, the structure of **8** has been characterized as lanast-5,20(22)-dien-3 β-olyl-3-O-D-glucopyranosyl-21(24)-olide, a new lanostane lactonic glucoside (Fig. 1).



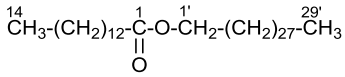
Decyl laurate **(1)**



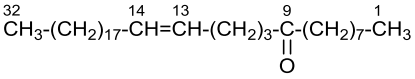
Myristyl laurate **(2)**



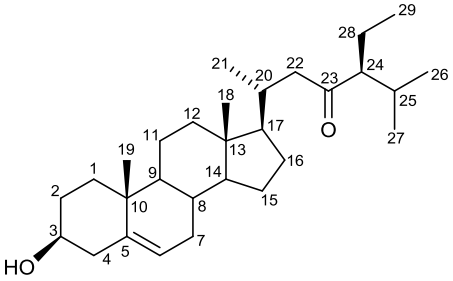
n-Pentcosan-12-one **(3)**



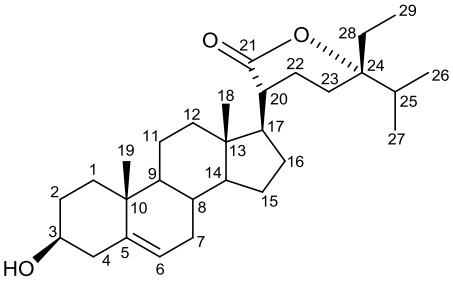
n-Nonacosanyl myristate **(4)**



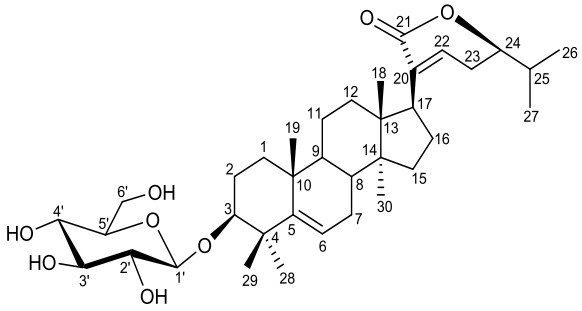
(*Z*)-*n*-Dotriacont-13-en-9-one **(5)**



Stigmast-5-en-3β-ol-23-one **(6)**



Stigmast-5-en-3β-ol-21(24)-olide **(7)**



Lanost-5, 20(22)-dien-3β-olyl-3-*O*-β-D-glucopuranosyl-21(24)-olide **(8)**

Fig 1: Chemical constituents 1 to 8 isolated from the roots of *Trichodesma indicum* (L.) R. Br.

CONCLUSION

Phytochemical investigation of the roots of *Trichodesma indicum* (L.) R. Br. led to isolate two known fatty acid esters identified as *n*-decanyl dodecanoate (decyl laurate, **1**), and *n*-tetradecyl dodecanoate (myristyl laurate, **2**), a new saturated aliphatic ketones characterized as *n*-pentacosan-12-one (**3**), an unknown fatty acid ester formulated as nonacosan-1-oyl *n*-dodecanoate (nonacosanyl myristate, **4**), an unsaturated carbonyl compound identified as (*Z*)-*n*-dotriacont-13-en-9-one (**5**), a rare steroidal ketone and its structure was determined as stigmat-5-en-3β -ol-23-one (β- sitosterol 23-one, **6**), an unfamiliar steroidal lactone and its structure was established as stigmast-5-en-3 β-ol-21(24)-olide (**7**) and a triterpenic lactonic glucoside with the structure elucidated as lanast-5,20(22)-dien-3 β-olyl-3-O-D-glucopyranosyl-21(24)-olide (**8**). This work has enhanced understanding about the chemical constituents of the undertaken plant. Further research is recommended to screen bioactivities of the isolated phytoconstituents with a view for supplementing conventional drug development especially in developing countries.

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