Chemical examination of *Citrullus colocynthis* roots

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Chemical investigation of *Citrullus colocynthis* roots yields three new compounds besides four known compounds characterised as tridecyl 2-methyl-19-ene-triacontanoate 1, heptadecyl trieicosanoate 2, nonaeicosanol 3, dotriacontanol 4, 1-hentriacontanol 5, dotriacontanoic acid 6 and tetraeicosanoic acid 7 using IR, $^1$H NMR, $^{13}$C NMR and mass spectral data.

*Citrullus colocynthis* (Hindi: Indrayan) belongs to the family cucurbitaceae. The roots have beneficiary effects in inflammation of breast, pain in joints, in ophthalmia and in uterine pains. In rheumatism equal parts of root and long pepper is prescribed. A paste of the root is applied to enlarged abdomen of the children. Oral administration of root powder and muscular injection is used for testicular enlargement and in Hernia. The ethanol extract of roots, shoot and fruits have exhibited antimicrobial activities. Literature survey revealed the presence of steroidal sapogenins, diosgenin, kryptogenin, lanosterol and $\beta$-sitosterol in various parts of *C. colocynthis*. Many medicinal properties of various parts particularly fruit, pulp and roots have motivated us, for the chemical investigation of its roots. Herein we report the isolation and characterisation of three new and four known aliphatic acids, esters and alcohols from acetone extract of the roots.

The shade dried roots were extracted with acetone and separated by column chromatography using silica gel and alumina grade III to yield seven compounds characterised as tridecyl 2-methyl-19-ene-triacontanoate, heptadecyl trieicosanoate, nonaeicosanol, dotriacontanol, 1-hentriacontanol, dotriacontanoic acid and tetraeicosanoic acid.

**Tridecyl 2-methyl-19-ene-triacontanoate 1.** (20 mg, $C_{44}H_{86}O_2$, $M^+$ 646), mp 67 °C; its IR spectrum showed the presence of ester and olefinic group (1740, 1170 and 1680 cm$^{-1}$) and aliphatic nature of the molecule. Its $^1$H NMR spectrum (300 MHz, TMS, CDCl$_3$) showed a triplet at δ 0.8 for six protons of the two terminal methyl groups. A multiplet at δ 4.08 for four protons, two for the methylene group attached to the ester and two olefinic protons, and a triplet at δ 2.25 for one proton was due to the methylene group attached to the carbonyl group indicated the presence of an ester group in the molecule.

Mass spectrum showed a major fragment at m/z 256 (100) formed by McLafferty rearrangement. The other fragment at m/z 419 resulted from the $\alpha$-cleavage next to carbonyl group confirmed the structure of compound 1 as tridecyl 2-methyl-19-ene-triacontanoate, being reported first time in the literature.

**Heptadecyl trieicosanoate 2.** (28 mg, $C_{40}H_{82}O_2$, $M^+$ 592), (methanol), mp 108 °C; its IR spectrum showed it to be a long chain saturated acyclic ester. The position of ester group was determined from its mass fragmentation pattern. In the mass spectrum the separation of most of the peaks by 14 mass units and appearance of [C$_n$H$_{2n}$] ions series confirmed its long chain aliphatic nature.

Major fragments at m/z 353, 309 and 239 were obtained. The peak at m/z 298 resulted due to McLafferty rearrangement. The $^1$H NMR spectrum showed a triplet at δ 4.09 for two protons of methylene group attached to the ester group. Another triplet at δ 2.25 was observed for two protons of methylene group attached to carbonyl group of the ester. Rest of the methylenes merged into a single peak at δ 1.24. Thus, the compound was identified as heptadecyl trieicosanoate. The above structure was further supported by $^{13}$C NMR spectrum which was in consistent with the above structure. A peak at 180.0 ppm corresponded to the carbon of the ester group.
The \( \alpha \)- and \( \beta \)-methylene carbons attached to ester group resonated at \( \delta \) 34.5 and 32.0 ppm, respectively. The peak at 14.2 ppm corresponded to the end methyl groups. Thus, compound 2 was characterised as heptadecyl tricosanoic acid, being reported for the first time in the literature.

Nonaeicosanol 3. \( (32\, \text{mg}, \text{C}_{29}\text{H}_{60}\text{O}, \text{M}^+: 424) \) (methanol), mp 83 \(^o\text{C}\), in its IR spectrum showed the presence of hydroxyl group (3440 and 1060 cm\(^{-1}\)) and aliphatic nature of the molecule. The \(^1\text{H} \) NMR spectrum showed a triplet at \( \delta \) 0.9 for three protons of terminal methyl group. A triplet at \( \delta \) 3.64 for two protons was due to methylene attached to hydroxyl group. The remaining methylenes merged into a single peak at \( \delta \) 1.28. Mass spectrum showed it to be an aliphatic alcohol. Thus, the compound was identified as nonaeicosanol which is being reported for the first time in the literature.

Compounds 4 (25 mg), \( \text{C}_{32}\text{H}_{64}\text{O}, \text{M}^+: 466 \) and 5 (25 mg) \( \text{C}_{33}\text{H}_{60}\text{O}, \text{M}^+: 452 \) were identified as dotriacontanol and 1-hentriacontanol respectively by comparison of IR, \(^1\text{H} \) NMR and mass spectral data with those of compounds isolated from \textit{Arbutus unedo} and \textit{Phyllanthus commaricine} respectively.

Compounds 6 (32 mg) \( \text{C}_{34}\text{H}_{66}\text{O}, \text{M}^+: 480 \) and 7 (34 mg) \( \text{C}_{29}\text{H}_{54}\text{O}, \text{M}^+: 368 \) were identified as dotriacontanoic acid and tetra eicosanoic acid by comparison of spectral data with those of compounds isolated from leaves waxes.

Experimental Section

General. All mp's are uncorrected. IR spectra were recorded in KBr on a Perkin-Elmer-377 infrared spectrophotometer, \(^1\text{H} \) NMR spectra (300 MHz) on a Brucker AM-300 NMR spectrometer using TMS as internal standard, \(^13\text{C} \) NMR spectra on a Brucker AM-75 MHz, FT-NMR spectrometer and Mass spectra on a Jeol D 300. Silica gel-G was used for TLC and spots were visualised by exposure to iodine vapours or by charring with 10% H\(_2\)SO\(_4\). Roots of \textit{C. colocynthis} were collected from the near by area of Ujjain city and identified, washed, air-dried and ground to a coarse powder.

Extraction and isolation. Powdered roots (4 kg) were exhaustively extracted in a soxhlet with acetone and solvent removed by rotary vacuum evaporator to yield a thick dark brown mass. The extract was separated by column chromatography using silica gel. The column was eluted by increasing order of polarity of the solvent, starting with \( n \)-hexane. The fractions were checked by TLC and those of similar compositions were combined and solvent was recovered.

Compound 1 was obtained from rechromatography of benzene eluate using hexane as solvent, recrystallised from methanol, yield 20 mg, mp 67 \(^o\text{C}\); IR: 2920, 2840, 1740, 1680, 1470, 1460, 1170, 730 and 720 cm\(^{-1}\); \(^1\text{H} \) NMR: \( \delta \) 4.08 (m, 4H, -CH\(_2\)-O-C=O, -CH=CH), 2.25 (m, 1H, OC-O-CH\(_2\)), 1.68 (m, 4H, H\(_2\)-HC=CH-CH\(_2\), \( J = 5 \) Hz), 1.28 (bs, 68H, 34-CH\(_2\)), 1.0 (d, 3H, CH\(_3\)-CH\(_2\), \( J = 7.0 \) Hz, 0.8 (t, 6H, 2-CH\(_2\), \( J = 7.0 \) Hz); MS (rel. int. %): m/z 646 (M\(^+\); 1), 602 (3.1), 546 (2.4), 490 (5.4), 462 (6.8), 451 (7.9), 447 (4.5), 424 (7.1), 423 (5.3), 419 (4.0), 418 (9.5), 390 (8.3), 361 (2.2), 348 (3.1), 333 (3.4), 305 (4.2), 295 (3.6), 278 (4.7), 264 (3.1), 256 (100.0), 250 (4.0), 249 (3.4), 239 (6.0), 227 (5.2), 223 (3.6), 197 (3.3), 183 (3.3), 167 (4.5), 153 (5.7), 139 (8.7), 127 (9.0), 111 (10.2), 99 (11.0), 75 (11.5), 71 (11.0), 57 (11.8), 55 (7.5), 44 (7.9) and 42 (4.3).

Compound 2 was obtained from 2.5% methanol-benzene eluate, recrystallised from methanol, yield 28 mg, mp 108 \(^o\text{C}\); IR: 2960, 2840, 1730, 1470, 1460, 1170, 730 and 720 cm\(^{-1}\); \(^1\text{H} \) NMR: \( \delta \) 4.09 (t, 2H, -CH\(_2\)-O-C=O, \( J = 7.5 \) Hz), 2.25 (t, 2H, OC-O-CH\(_2\), \( J = 7.5 \) Hz), 1.6 (m, 2H, -CH\(_2\)), 1.24 (bs, 68H, 32-CH\(_2\)), 0.88 (t, 6H, 2 \times CH\(_3\), \( J = 7.0 \) Hz); MS (rel. int. %): m/z 592 (M\(^+\); 2.5), 353 (2.0), 309 (2.5), 302 (2.5), 298 (2.5), 294 (3.0), 287 (2.6), 286 (9.7), 283 (5.6), 281 (4.4), 279 (3.1), 273 (3.4), 271 (4.4), 264 (4.4), 262 (2.5), 253 (8.8), 248 (3.4), 242 (3.4), 239 (2.5), 234 (2.5), 231 (2.5), 230 (2.8), 227 (2.8), 226 (7.7), 224 (2.8), 219 (4.4), 218 (2.5), 217 (4.4), 211 (4.4), 210 (3.3), 208 (2.5), 207 (2.8), 204 (4.4), 203 (6.9), 202 (3.3), 201 (2.5), 200 (2.5), 199 (2.8), 196 (2.0), 193 (4.4), 193 (4.4), 191 (5.0), 185 (4.3), 183 (9.4), 182 (4.8), 181 (8.0), 178 (22.1), 167 (9.1), 165 (11.3), 159 (9.9), 153 (7.7), 151 (9.9), 150 (9.0), 149 (9.5), 147 (9.6), 135 (7.5), 134 (7.3), 121 (7.3), 120 (7.2), 119 (7.0), 111 (7.2), 97 (7.0), 91 (8.9), 85 (10.9), 83 (11.9), 57 (14.1), 55 (14.0) and 43 (10.1); \(^13\text{C} \) NMR: (75 MHz CDCl\(_3\), 180.0, 64.51, 34.5, 32.0, 29.7, 29.4, 29.3, 29.2, 28.7, 26.0, 25.1, 22.7 and 14.2 ppm.

Compound 3 was obtained from rechromatography of 5% methanol-benzene eluate using 1% ether-benzene as solvent, recrystallised from methanol, yield 32 mg, mp 83 \(^o\text{C}\); IR: 3440, 2940, 2900, 1480, 1460, 1060, 730, 720 cm\(^{-1}\); \(^1\text{H} \) NMR: \( \delta \) 0.9 (t, 3H, -CH\(_3\), \( J = 7.0 \) Hz), 1.28 (bs, 54H, 27x CH\(_2\)), 1.55 (s, 1H, -OH, D\(_2\)O exchangeable), 3.64 (t, 2H, -CH\(_2\)OH, \( J = 7.0 \) Hz); MS (rel. int., %) m/z: 424 (M\(^+\); 1.0), 409 (1.4), 381 (1.8), 364 (1.4), 337 (1.2), 324 (1.4), 306 (1.1), 295 (1.1), 280 (1.7), 279 (1.1), 267 (2.1), 252
Compound 4 was obtained from rechromatography of benzene eluate using 10% benzene-hexane solvent, recrystallised from methanol, yield 25 mg, mp 87 °C; IR: 3400-3150, 2950, 2850, 1480, 1460, 1080, 1050, 730-720, 710 cm⁻¹; IH NMR: \( \delta \) 0.9 (t, 3H, -CH₃, \( J = 7.5 \) Hz), 1.25 (bs, 58H, -CH₂), 1.6 (s, 1H, -OH), 3.65 (t, 2H, -CH₂OH, \( J = 7.5 \) Hz); MS (rel. int. %): m/z 452 (M⁺; 0.12), 434 (0.15), 420 (4.8), 392 (6.2), 364 (0.2), 335 (0.1), 307 (2.8), 279 (2), 251 (3), 223 (4), 195 (8), 167 (8), 139 (14.1), 111 (34), 97 (64.2), 57 (100), 55 (61.9); \( ^{13} \)C NMR: 13.99, 22.63, 25.73, 29.65, 31.89, 32.83, 63.07 ppm.

Compound 5 was obtained from rechromatography of 5% methanol-benzene eluate using hexane as solvent, recrystallised from methanol, yield 25 mg, mp 87 °C; IR: 3400-3150, 2950, 2850, 1480, 1460, 1080, 1050, 730-720, 710 cm⁻¹; IH NMR: \( \delta \) 0.89 (t, 3H, -CH₃, \( J = 7.5 \) Hz), 1.25 (bs, 58H, -CH₂), 1.6 (s, 1H, -OH), 3.65 (t, 2H, -CH₂OH, \( J = 7.5 \) Hz); MS (rel. int. %): m/z 452 (M⁺; 0.12), 434 (0.15), 420 (4.8), 392 (6.2), 364 (0.2), 335 (0.1), 307 (2.8), 279 (2), 251 (3), 223 (4), 195 (8), 167 (8), 139 (14.1), 111 (34), 97 (64.2), 57 (100), 55 (61.9); \( ^{13} \)C NMR: 13.99, 22.63, 25.73, 29.65, 31.89, 32.83, 63.07 ppm.

Compound 6 was obtained from rechromatography of 5% benzene-methanol eluate using acetone-benzene eluate as solvent, recrystallised from methanol, yield 32 mg, mp 95 °C; IR: 3400, 2910, 2825, 1705, 1480, 1440, 1420, 1300, 1180, 735 and 725 cm⁻¹; IH NMR: \( \delta \) 2.35 (t, 2H, -CH₂COOH, \( J = 7.0 \) Hz), 1.62 (bm, 2H, -CH₂), 1.25 (bs, 56H, -CH₂), 0.89 (t, 3H, -CH₃, \( J = 7.5 \) Hz); MS (rel. int. %): m/z 480 (M⁺; 3.6), 451 (5.8), 423 (5.3), 367 (7.4), 340 (16.1), 321 (4.0), 312 (4.5), 296 (17.5), 268 (4.4), 252 (3.6), 250 (3.9), 247 (6.3), 241 (4.3), 236 (6.2), 221 (7.5), 217 (6.6), 213 (14.3), 197 (4.6), 195 (10.2), 190 (4.0), 185 (4.1), 178 (4.2), 168 (4.5), 160 (4.2), 155 (7.8), 152 (4.8), 143 (13.5), 123 (14.2), 115 (14.6), 111 (13.9), 102 (10.1), 97 (10.1), 83 (7.8), 73 (5.1), 60 (4.1), 57 (7.8), 55 (6.0), 44 (3.5) and 43 (3.0); \( ^{13} \)C NMR: 179.00, 31.89, 29.60-29.9, 24.69, 22.63, 14.0 ppm.

Compound 7 was obtained from rechromatography of 5% methanol-benzene eluate using 75% benzene-hexane as solvent, recrystallised from methanol, yield 34 mg, mp 50 °C; IR: 3400, 2910, 2825, 1705, 1480, 730 and 725 cm⁻¹; IH NMR: \( \delta \) 2.4 (t, 2H, -CH₂OH, \( J = 7.0 \) Hz), 1.6 (bm, 2H, -CH₂), 1.25 (bs, 40H, -CH₂), 0.88 (t, 3H, -CH₃, \( J = 7.5 \) Hz) MS (rel. int. %): m/z 367 (M⁺; 4.5), 327 (4.0), 305 (4.1), 286 (4.1), 279 (3.6), 254 (3.0), 250 (2.6), 235 (2.6), 223 (4.5), 216 (2.4), 212 (2.0), 208 (3.4), 196 (5.1), 194 (3.8), 193 (3.9), 184 (3.3), 182 (3.4), 169 (4.8), 167 (4.5), 155 (5.1), 153 (5.7), 139 (20.3), 125 (34.1), 112 (14.9), 111 (15.3), 97 (10.2), 85 (7.4), 83 (6.2), 71 (8.1), 69 (8.5), 57 (8.5), 44 (8.4) and 42 (5.0).

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References