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THREE NEW COMPOUNDS FROM THE ROOTS OF *ALTHAE OFFICINALIS* L.

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ABSTRACT
Two new phenolic compound and one new acid ester characterized as 3,4 –dihydroxy benzyl octadecane, 24β, 28β -dihydroxy octa tetracont – 36 – en – 1- oic – acid and 5 β, 13 β -dihydroxynonacosanyl godoleate have been isolated from the roots of *Althaea officinalis* L. (Malvaceae) along with the known compounds n –triacontanoic acid, n-tetracosane, n –pentatriacontane and althealanostenoic acid glucoside. The structures of all the phytoconstituents have been elucidated on the basis of spectral data analysis and chemical reactions.
KEYWORDS: *Althaea officinalis,* Malvaceae, altheaoctatetracontenoic acid, godoleic acid ester.

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INTRODUCTION

Althaea officinalis L. (Malvaceae), commonly known as gukhairo or marshmallow, is a downy, perennial herb, distributed in the Himalayan region from Kashmir to Himachal Pradesh[1]. The seeds of this plant are demulcent, diuretic and febrifuge[2].In Unani system, *Althaea officinalis is* prescribed as an expectorant and is known to relieve bronchitis and bronchial catarrh[3] .The roots counteract excess of stomach acid, and are prescribed to treat peptic ulcer, gastritis and intestinal problems including ileitis, colitis, diverticulitis and irritable bowel syndrome. An ointment prepared from the roots is applied to cure inflammatory tumors, burns, boils and abscesses. A root decoction is utilized as mouth wash to relieve inflammation[4], bruises, sprain; as an expectorant in cough, hoarseness of voice, bronchitis and whooping cough[5]. The phytochemical studies carried out so far have revealed herniarin and umbelliferone. Tiliroside, diosetin-8- hydroxy-8-o- β-D- glucoside[6], scopoletin, quercetin, kaempferol, chlorogenic acid, caffeic acid, and p-coumaric acid were isolated from the dried roots of *A. officinalis* var. Russalka[2].

From the roots of *A. officinalis* two flavonoid glycosides were separated. Phenolic acids and coumarins were identified as hypolaetin 8-glucoside and the new flavonoid sulphate isoscutellarein 4-Me ether 8-Glucoside. The present manuscript describes isolation and characterization of three new phytoconsisituents from the roots of *A. officinalis.*

MATERIALS AND METHODS

Plant material

The dried roots of *Althaea officinalis* were purchased from Khari Baoli market of Delhi. The authenticity of the material was established by Prof.M.P.Sharma, Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi. A voucher specimen No-PRL/ JH/08/38 in deposited in the herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

Extraction and fractionation

The roots were chopped into small pieces. The chopped material (3 kg) was extracted exhaustively with ethyl alcohol in a Soxhlet apparatus. Recovery of the solvent left a semi – solid brown mass (170 g) which was treated with petroleum ether at room temperature several times till the last extract became colourless. The combined extracts were concentrated to a small volume. It was coded as fraction A (17 g). The insoluble residue left after extraction with petroleum ether at room temperature was successively extracted by refluxing with petroleum ether and chloroform to give the respective fraction B (5 g) and C (6 g).

Fraction of A

The fraction A was diluted to 200 ml with petroleum ether and poured over silica gel column (60-120 mesh, 100g; diameter of the column 2.5 cm). It was exhaustively eluted with petroleum ether (60-80^{o}C, 5 lit) and then after with ethyl acetate (2 lit). Recovery of

solvents from these eluates gave respective subfractions A-1 (4.9 g) and A-2 (5.5 g).

Isolation

n-Triacontanoic acid (1)

The subfraction A_1 was dissolved in minimum quantity of chloroform and to it methanol (50 ml) was added. It was left at room temperature for crystallization. A colourless crystalline compound **1** was obtained, R_f 0.4, petroleum ether: benzene (4:1), 18 mg, m.p 89- 90 ^oC;UV λmax (MeOH): 208 nm (log ε 4.9) ; IR υmax (KBr) : 3490, 2919, 2851, 1690, 1655, 1461, 1335, 1263, 1101, 803, 721 cm^{-1} ^1H NMR ($CDCl_3$): δ 2.30 (1H, d, J = 7.2 Hz, H_2–2a), 2.25 (1H, d, J = 7.2 Hz, H_2–2b), 1.56 (2H, m, H_2–3), 1.18 (54H, brs, 27×CH_2), 0.81 (3H, t, J = 7.08 Hz, Me-29); ^{13}C NMR ($CDCl_3$): δ 176.19 (C-1), 34.10 (C – 2), 32.15 (C – 3), 29.93 (22 × CH_2), 29.60 (2 × CH_2), 29.27 (CH_2), 24.89 (CH_2), 22.92 (CH_2), 14.37 (CH_3 – 29); +ve ESI.MS m/z (rel. int): 452 $[M]^+$ ($C_{30}H_{60}O_2$) (2. 5).

p-n-Octadecanyl-o-catechol (2)

The subfraction A-2 was dissolved in ethyl acetate for crystallization. A dark brown amorphous powder of **2** was obtained. It was filtered and washed with small volume of ethyl acetate. On TLC examination in $CHCl_3$: MeOH (9:1), it showed only one spot, R_f 0.9, 20 mg, mp 170-171 ^oC; UV λmax (MeOH): 261 nm (log ε 5.3); IR υmax (KBr): 3410, 3360, 2922, 2845, 1655, 1597, 1046, 795 cm^{-1} ^1H NMR ($CDCl_3$): δ 6.98 (1H, d, J = 3.0 Hz, H- 2), 6.89 (1 H, m, H-6), 6.70 (1 H, d, J = 7.8 Hz,, H – 5), 2.79 (2 H, brs, H_2 – 7), 2.58 (2 H, brs, H_2 -7b), 1.57 (2 H, brs, CH_2), 1.37 (30 H, brs, 15 x CH_2,) 0.84 (3 H, t, J = 6.1 Hz, Me – 24); +ve ESI.MS m/z (rel. int): 362 $[M]^+$ ($C_{24}H_{42}O_2$) (22. 6).

Altheaoctatetracontenoic acid (3)

The mother liquor obtained after removal of **2** was evaporated to dryness. It was dissolved in methanol-chloroform (9:1) and left for crystallization. A colourless amorphous powder **3** was obtained. It was filtered and washed with little volume of methanol. R_f 0.7 (petroleum ether- $CHCl_3$, 2:3), 30 mg, mp 199-200^oC; UV λmax (MeOH): 214 nm (log ε 4.7).; IR υmax (KBr): 3430, 3295, 2922, 2853, 1687, 1656, 1460, 1263, 1263, 1077, 1025, 797, 774, 720 cm^{-1} ;^1H NMR ($CDCl_3$) : δ 5.30 (2H, m, H – 36), 5.28 (1H, m, H – 37), 4.08 (1H, m, $w_{½}$ 16.7 Hz, H – 28β), 3.59 (1H, m, $w_{½}$ 15.3 Hz, H – 24β), 2.24 (2H, m, H_2 – 2), 1.94 (2H, m, H_2 = 35), 1.54 (2H, m, H_2 – 38), 1.18 (80H, brs, 40 × CH_2) 0.80 (3H, t, J = 6.1 Hz, Me – 48);^{13}C NMR ($CDCl_3$) : δ 182.50 (C – 1), 128.33 (C – 36), 122.1 (C – 37), 65.07 (C – 28), 63.08 (C – 24), 34.3 (CH_2), 32.6 (CH_2), 32.1 (CH_2), 29.9 (25 × CH_2), 29.6 (10 ×CH_2), 28.4 (CH_2), 24.8 (CH_2), 22.9 (CH_2), 14.35 (Me – 48) ;+ve ESI MS m/z (rel. int) : 734 $[M]^+$ ($C_{48}H_{94}O_4$), (11.6), 455 (12.3), 383 (12.7), 181 (22.5).

n-Tetracosane (4)

Mother liquor obtained after filtration of **3** was concentrated to small volume and left at room temperature to obtain **4** R_f 0.71, benzene: chloroform, 7:3) 13.2 mg, mp 79-80 ^oC; UV λmax (MeOH): 207 nm (log ε 4.3); IR υmax (KBr): 2934, 2845, 1462, 1440, 1370, 1261, 1165, 1074, 1025, 797, 773 cm^{-1} ; ^1H NMR ($CDCl_3$): δ 1.54 (8H, brs, 4 × CH_2), 1.25 (36H, brs, 18 × CH_2), 0.84 (3H, t, J = 6.1 Hz, Me – 1), 0.82 (3H,t,J=6.0 Hz, Me-24); +ve ESI MS m/z (rel. int): 338 $[M]^+$ ($C_{24}H_{50}$) (2.1).

β –Sitosterol (5)

Fraction B, obtained by dissolving the alcoholic extract in petroleum ether on crystallization with methanol containing few drops of $CHCl_3$ gave colourless crystals of **5,** R_f 0.43 , (petroleum ether : chloroform, 1:3), 38 mg (0.0038%), m.p 138-140^oC; ^1H NMR (DMSO-d_6) : δ 5.36 (1H, d, J = 4.64 Hz, H–6), 3.52 (1H, brs, $w_{1/2}$ 18.5 Hz, H–3 β), 1.01 (3H, brs, Me-10), 0.93 (3H, d, J = 6.5, Hz, Me– 21), 0.86 (3H, t, J = 6.03 Hz, Me–29), 0.84 (3H, d, J = 6.0, Hz, Me–26 , 0.80 (3H, d, J= 6.0 Hz, Me–27), 0.68 (3H, brs, Me–18); +veESI MS m/z (rel. int.) : 414$[M]^+$ ($C_{29}H_{50}O$) (54.4), 399(16.2), 396 (58.3), 381 (31.6), 273 (21.5), 255 (40.0), 231 (23.4), 213 (40.5), 198 (13.6), 173 (17.4), 163 (33.7), 161 (33.6), 159 (33.2), 145 (45.1), 133 (28.1), 121 (38.9), 107 (59.2), 105 (23.1), 95 (60.8), 93 (61.2), 83 (67.2), 81 (82.1), 71 (80.3), 69 (93.1), 57 (100), 55 (41.2).

Dihydroxy –n- nonacosanyl godoleate (6)

The chloroform soluble fraction C of the ethanolic extract was dissolved in ethyl acetate and left at room temperature. A colourless amorphous compound **(6)** was obtained, R_f 0.51 ($CHCl_3$: MeOH, 19: 1), 17 mg, (0.00177%yield), mp 135-137 ^oC;UV λmax (MeOH): 215 nm (log ε 3.7);IR υmax (KBr): 3450, 3365, 2923, 2855, 1721, 1640, 1476, 1440, 1335, 1075, 773 cm^{-1}.; ^1H NMR ($CDCl_3$) : δ 5.39 (2H, m, H – 9′, H – 10′), 4.16 (1H, brm, $w_{½}$ = 18.5 Hz, H – 5α) 3.60 (1H, brm, $w_{½}$ = 16.2 Hz, H – 13α), 3.11 (2H, brm, H_2 – 1), 2.36 (2H, m, H_2 - 2′), 1.99 (2H, m, H_2 – 8′), 1.84 (2H, m,H-11′), 1.56 (20 H, brs, 10 × CH_2), 1.25 (32 H, brs, 16× CH_2), 1.01 (4H, brs, 2 × CH_2), 0.87 (3H, t, J = 6.3 Hz, Me – 29), 0.82 (3H, t, J = 6.1Hz, Me - 20′); ^{13}C NMR ($CDCl_3$): δ 173.1 (C–1′), 123.6 (C–9′), 122.5 (C–10′), 73.8 (C–5), 67.9 (C–13). 60.3 (C–1), 45.7 (C-8′), 44.2 (C-11′), 37.3 (CH_2), 33.6 (CH_2), 29.94 (36 × CH_2), 24.9 (CH_2), 17.6 (2 ×CH_3); +veESI MS m/z (rel.

int): 748 $[M]^+$ ($C_{49}H_{96}O_4$), (13.6), 455 (12.7), 383 (25.9), 293 (18.5), 255(38.3).

n- Pentatriacontane (7)

The mother liquor obtained after removal of **6** was concentrated to dryness and dissolved in methanol containing few drops of chloroform. It was left at room temperature for crystallization to obtain a colourless compound **7**; R_f 0.8 ($CHCl_3$: acetone, 3:2), 21 mg (0.0021% yield); mp 89-90° C; UV λmax (MeOH): 207 nm. (log ε 4.2); IR υmax (KBr) :2925, 2855, 1440, 1260, 1075, 721 cm^{-1}.^{1}H NMR ($CDCl_3$):δ 1.61 (2H, brs, CH_2), 1.25 (64H, brs, 32 × CH_2), 0.87 (3H, t, J=6.3 Hz, Me-1), 0.84 (3H, t, J=6.0 Hz, Me – 35); ^{13}C NMR ($CDCl_3$):δ 32.14 (CH_2), 29.24 (28 × CH_2), 29.59(3 × CH_2), 22.91 (CH_2), 14.37 (Me-1, Me-35);+veESI MS m/z (rel. int) : 492 $[M]^+$ ($C_{35}H_{72}$), (3.5).

Diglucosyl oleate (8)

Elution of the column with chloroform - methanol (87:13) gave colourless crystalline mass of **8**, recrystallized from methanol, 231 mg (0.28 % yield). R_f value 0.51 (chloroform - methanol, 43:7), m.p. 102-103 °C;UV λ_{max} (MeOH) : 256 nm (log ε 3.9); IR υ_{max} (KBr) : 3420, 2926, 2857, 1726, 1637, 1450, 1375, 1063 cm^{-1}.^{1}H NMR (DMSO-d_6): δ 5.28 (2H, m, H-9, H-10), 4.71 (2H, m, H-1', H-1''), 4.50 (2H, m, H-5', H-5''), 3.72 (2H, m, H-2, H-2''), 3.62 (1H, m,H-4'), 3.47 (1H, m, H-4''), 3.40 (1H, m, H-3'), 3.38 (2H, m, H_2-3''), 3.19 (2H, brs, H_2-6''), 3.05 (2H, brs, H_2-6''), 2.72 (2H, brs, H_2-2), 2.20 (4H, brs, H_2-8, H_2-11), 2.03 (2H, m, CH_2), 1.97 (2H, m, CH_2), 1.88 (2H, m, CH_2), 1.80 (2H, m, CH_2), 1.65 (2H, m, CH_2), 1.50 (2H, m, CH_2), 1.26 (2H, m, CH_2), 1.21 (8H, brs, 4 x CH_2), 0.87 (3H, t, *J* = 7.2 Hz, CH_3-18);^{13}C NMR (DMSO-d_6) : δ 172.68 (C-1), 128.13 (C-9), 127.18 (C-10), 103.16 (C-1'), 100.01 (C-1''), 77.04 (C-5', C-5''), 73.89 (C-2'), 73.86 (C- 2''), 72.81 (C-2'), 72.27 (C-4'), 70.36 (C-4''), 68.75 (C-3'), 68.91 (C-3''), 63.43 (C-6'), 61.28 (C-6''), 55.98 (C-2), 49.10 (CH_2), 34.01 (CH_2), 31.91 (CH_2), 29.74 (6 x CH_2), 27.13 (CH_2), 25.53 (CH_2), 24.92 (CH_2), 22.66 (CH_2), 21.35 (CH_2), 4.10 (CH_3-18); +ve ES MS *m/z* (*rel. int.*): 606 $[M]^+$ ($C_{30}H_{54}O_{12}$) (5.1), 443 (16.2), 427 (18.1), 265 (15.8), 281 (14.9).

RESULTS AND DISCUSSION

Compound **1,4,5,7,8** are the known phytoconstituents identified as n –triacontanoic acid, n-tetracosane, stigmast -5 –en -3β – ol, n –pentatriacontane, lanost-7-en-3β-ol-26-oic acid-3β-D-glucopyranoside, β-D-glucopyranosyl (4→1)-β-D-glucopyranosyl octadec-9-enoate respectively, on the basis of spectral data analysis. The subfraction A-2 was dissolved in ethyl acetate for crystallization. A dark brown amorphous powder was obtained for compound 2. Its UV spectrum displayed absorption band maxima at λ 216 nm. Its IR spectrum exhibited characteristic absorption bands for hydroxylic group 3360 cm^{-1} and long aliphatic chain (795 cm^{-1}). Its +ve ESI mass spectrum displayed a molecular ion peak at m/z 362 corresponding to the molecular formula of an octadecane substituted catechol $C_{24}H_{42}O_2$.The ^{1}H NMR spectrum of **2** displayed two one-proton doublets at δ 6.98 (J = 3.0 Hz) and δ 6.70 (J = 7.8 Hz) assigned correspondingly meta-coupled H-2 and ortho coupled H – 5. A one-proton multiplet at δ 6.89 was ascribed to ortho -, meta coupled H – 6. Two one proton broad signals at δ 2.79 and 2.50 attributed to methylene protons adjacent to aromatic ring. Two broad signals at δ 2.58 (2 H) and 1.57 (2 H) and a broad signal at δ 1.37 were assigned to the remaining methylene protons. A three proton triplet at δ 0.84 (J = 6.1 Hz) was accounted to C – 24 primary methyl protons. The compound formed a diacetate with acetic anhydride and pyridine. On the basis of the spectral data analysis and chemical reactions, the structure of **2** has been formulated as 3, 4-dihydroxybenzyl octadecane. (p-n-octadecanyl-o-catechol). This is a new phenolic compound isolated from a natural or synthetic source for the first time.

The mother liquor obtained after removal of **2** was evaporated to dryness. It was left at room in methanol containing few drops of chloroform to give compound **3**. It produced effervescences with sodium bicarbonate solution and decolourized bromine water indicating unsaturated nature of the fatty acid. Its IR spectrum showed characteristic absorption bands for hydroxyl groups at 3430 cm^{-1}, carboxylic group at 3295 and 1687 cm^{-1}, unsaturation at 1656 cm^{-1} and long aliphatic chain 797, 774, 720 cm^{-1}. Its +ve ESI mass spectrum exhibited a molecular ion peak m/z 734 corresponding to a molecular formula $C_{48}H_{94}O_4$. It indicated two double bond equivalents which were adjusted to the vinylic linkage and the carboxlic group. The ^{1}H NMR of **3** displayed two one-proton deshielded multiplets at δ 5.30 and 5.28 assigned to vinylic H – 36 and H – 37 protons, respectively. Two one-proton broad multiplets at δ 4.08 ($w_{½}$ = 16.7 Hz) and 3.59 ($w_{½}$ = 15.3 Hz) were ascribed correspondingly to hydroxymethine proton, H – 28β and H – 24β. Three two- proton multiplets at δ 2.24 and δ 1.94 and 1.54 were associated correspondingly to methylene H_2 – 2, H_2 – 35 and H_2 – 38 protons adjacent to the carboxylic group and vinylic linkage. A broad signal at δ 1.18, integrated for 80 protons was accounted to the remaining methylene proton. One three-proton triplet at δ 0.80 (J = 6-1Hz) was attributed to C–48 primary methyl protons. The ^{13}C NMR spectrum of **3** displayed important signals for carboxylic carbon (δ

182.581, C - 1), carbinol carbon at δ 63.08 (C-24) and 65.07 (C-25), vinylic carbons at δ 128.33 (C-36) and 122.1 (C- 37) and methyl carbon at δ 14.35 (C-48) The ^{1}H-^{1}H COSY spectrum of **3** showed correlation of H-24 with H_2-23, H_2-22, H_2-25 and H_2-26; H-28 with H_2-27, H_2-26, H_2-29 and H_2-30; H-36 with H_2-35, H_2-34 and H-37 with H-36, H_2-35 and H_2-38. On the basis of above discussion the structure of **3** was elucidated as 24 β, 28 β -dihydroxy octatetraconta–36 –en–1- oic–acid. This is a new altheaoctatetracontenoic acid isolated from a natural or synthetic source for the first time.

The chloroform soluble fraction was dissolved in ethyl acetate and left at room temperature to obtain colourless amorphous compound **6**. It decolourised bromine water indicating unsaturated nature of the molecule. Its IR spectrum. displayed characteristic absorption bands for hydroxyl groups at 3450, 3365 cm^{-1}, unsaturation 1640 cm^{-1}, ester group at 1721 cm^{-1} and long aliphatic chain at 773 cm^{-1}. Its +ve ESI mass spectrum displayed a molecular ion peak m/z 748 corresponding to the molecular formula of a fatty acid ester $C_{49}H_{96}O_4$. It indicated two double bond equivalents; one each of them was adjusted to the vinylic linkage and ester group. The ion peaks arising at m/z 293 [O-CO fission, CO $(CH_2)_7$ CH=CH $(CH_2)_9$ CH_3] and 455 [M-293] $^{+}$ suggested that godoleic acid was estrified with C_{29} trihydroxy alcohol. The ion peaks arising at m/z 255 [CH_3 $(CH_2)_{15}$CHOH] and 383 [CH_3 $(CH_2)_{15}$CHOH $(CH_2)_7$CHOH indicated the existence of the hydroxyl groups at C-5 and C-13.The ^{1}H NMR spectrum of **6** exhibited a two-proton multiplet at δ 5.39 assigned to vinylic H-9′ H-10′ protons . Two one-proton multiplets at δ 4.16 ($w_{½}$ = 18.5 Hz) and 3.60 ($w_{½}$ = 16.2 Hz) were ascribed to carbinol protons H-5α and H-13α, respectively. Three two - proton multiplets δ 2.36 (H_2 - 2′), 1.99 (H_2-8′) and 1.84 (H_2 - 11′) were accounted to methylene protons adjacent to the ester group and vinylic carbons. A 20-proton broad signalet at δ 1.56, 32-proton broad signal at 1.25 and a 4-proton broad signal δ 1.01 were attributed to the remaining methylene protons in the compound. Two three-proton triplets at δ 0.87 (J = 6.3 Hz) and 0.82 (J = 6.1 Hz) were associated correspondingly with Me-29 and Me - 20′ terminal primary methyl protons. The ^{13}C NMR spectrum of **6** displayed important signals for ester carbon (δ 173.1, C-1′), vinylic carbons (δ 123.6, C - 9′, 122.5, C - 10′) and carbinol carbons (δ 73.8, C – 5) and 67.9 (C-13). The methylene carbons resonated between δ 45.7 – 24.9 whereas the C – 29 and C-20′ terminal methyl carbons appeared at δ 17.6. On the basis of above discussion the structure of **6** has been elucidated as 5β, 13β, dihydroxynonacosanyl godoleate. This is a new godoleic acid ester isolated a natural or synthetic source for the first time.

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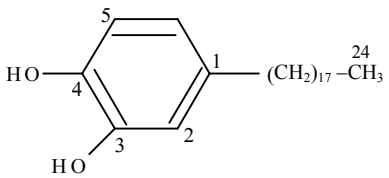
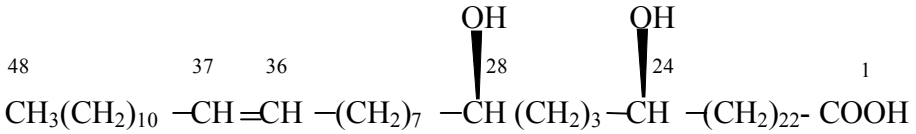
REFERENCES

1. The wealth of India, Raw materials. National institute of science communication and information Resource New Delhi: CSIR 2003; 1: 207 -208.
2. Mhaskar KS, Blatter E,Caius JF. Kirtikar and Basu's illustrated Indian medicinal plants. Delhi: Sri Satguru Publication 2000; 2: 405-407.
3. Anonymous, Reviews on Indian medicinal plants. Indian council of medical Research, New Delhi 2004; 2: 159-161.
4. Prajapati ND, Purohit SS, Sharma AK, and Kumar T. A handbook of Medicinal Plants Agrobios India 2003; 37.
5. Anonymous. The Wealth of India A dictionary of Indian Raw Material and Industrial Product. NISCAIR (CSIR) New Delhi 2003; 208.
6. Gudej J. Flavonoids phenolic acids and coumarins from the roots of *Athaea officinalis*. Planta Med 1991; 57(3): 284-285.

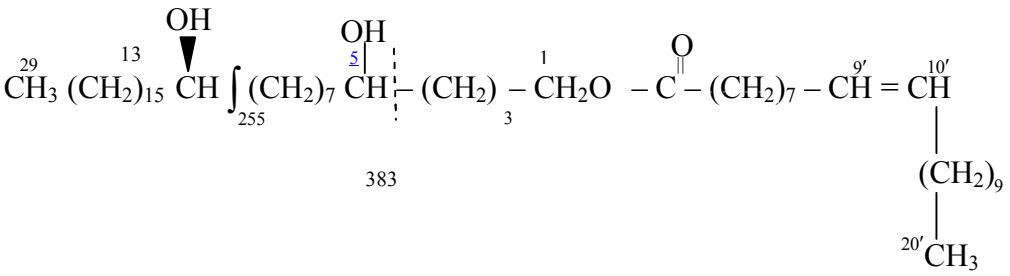


3, 4 –Dihydroxybenzyl octadecane **(2)**



24 β, 28 β -Dihydroxyoctatetracont – 36 – en – 1- oic – acid

(3)



Godoleic acid ester

5 β, 13 β - Dihydroxynonacosanyl godoleate **(6)**

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