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NEW FATTY ACID DERIVATIVES FROM *OCIMUM SANCTUM* L. LEAVES

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ABSTRACT

Two new fatty acid derivatives characterized as dotriacont-20-en-14-ol-1-oic acid (sanctumoic acid) and 4′-benzoylglucopyranosyl octadec-9-enoate (benzoyl gluco-oleate) were isolated from the leaves of *Ocimum sanctum* L. (Lamiaceae) together with five known compounds, vanillic acid, ocimumnapthanoic acid, β-sitosterol glucoside, oleiyl glucoside and diglucosyl oleate. The structures of isolated compounds have been established on the basis of spectral data analysis and chemical reactions.

Keywords: *Ocimum sanctum*, Lamiaceae, Fatty acid derivatives, Sanctumoic acid, Benzoyl gluco-oleate.

INTRODUCTION

The genus *Ocimum* (Lamiaceae) comprises number of important culinary, economic and medicinal herbs in which the most prominent and well known species is *Ocimum sanctum* L. It is commonly known as tulsi or holy basil, described as sacred by Hindus all over India and grown in courtyards and temples [1,2]. Tulsi is an annual, upto 60 cm high, much branched, erect, soft hairy aromatic herb with elliptic oblong, obtuse or acute, entire or serrate and minutely gland dotted leaves [3]. The plant is grown throughout India from Andaman and Nicobar islands to the Himalayas up to 1800 meters above the sea level. It is also found in Nepal, Malaysia, Australia, Brazil, West Africa and some of the Arab countries [4,5]. Its leaves have long been the part of Indian Ayurvedic system of medicine and used to treat various health problems including skin diseases, gastric and hepatic disorders. Various preparations of leaves are also used as diaphoretic, antiperiodic, antihyperlipedimic, antioxidant, antimicrobial, abortifacient, antifertility, hypoglycemic, hypotensive, cardiac depressant, smooth muscle relaxant, sedative, antiinflammatory, analgesic, antipyretic and adaptogenic [6,7,8,9,10]. Previous chemical investigations of the leaves indicated the presence of volatile oil with eugenol, methyl eugenol and β-caryophyllene as the main constituents [11,12,13,14]. The leaves are also enriched with flavonoids, vanillin, rosmarinic acid, ursolic acid, gallic acid, vanillic acid, 4-allyl-1-O-β-D-glucopyranosyl-2-hydroxy benzene, 4-allyl-1-O-β-D-glucopyranosyl-2-methoxybenzene and 1,3-dilinoleneoyl-2-palmitin [15,16,17,18,19]. The present manuscript describes isolation and identification of two new fatty acid derivatives from *O. sanctum* leaves collected from Delhi region.

MATERIALS AND METHODS

General

Melting points were determined on a Perfit apparatus without correction. The IR spectra were measured in KBr pellet on a Bio-Red FT-IR spectrometer. UV spectra were obtained in methanol with a Lambda Bio 20 spectrometer. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on Bruker spectrospin spectrometer. DMSO-d_6 (Sigma-aldrich) was used as a solvent and TMS as an internal standard. ES MS analyses were performed

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on a Micromass Quattro II triple quadrupole mass spectrometer. Column chromatography separations were carried out on silica gel (Merck, 60-120 mesh). Precoated silica gel plates (Merck, Silica gel 60 F_{254}) were used for analytical thin layer chromatography (TLC) visualised by exposure to iodine and UV radiations.

Plant Material

The leaves of *O. sanctum* L. were collected from the herbal garden of Jamia Hamdard, New Delhi. Drug sample was identified by Dr. H.B. Singh, Scientist F and Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. Voucher specimen of drug was deposited in the Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, with reference number NISCAIR/RHMD/Consult/-2008-09/1059/90.

Extraction and Isolation of Compounds

Fresh leaves of *O. sanctum* were shade dried for one week and in oven at 45 °C for 48 h. The dried leaves (2 kg) were coarsely powdered and extracted exhaustively with methanol using Soxhlet apparatus. The extract was dried under reduced pressure to get a residue (183 g) of dark brown mass. The extract was redissolved in methanol and treated with equal volume of aqueous lead acetate (10%) to precipitate tannins and other impurities and filtered. The filtrate was partitioned with petroleum ether (500 x 3 ml) and re-dried. The residue (80 g) was dissolved in minimum amount of methanol and adsorbed on silica gel column grade (60-120 mesh) to obtain slurry. The slurry was dried in air and chromatographed over silica gel column loaded in chloroform. The column was eluted with chloroform-methanol (97:3, 19:3, 93:7, 91:9, 89:11, 87:13) mixtures.

Vanillic Acid (1)

Elution of the column with chloroform-methanol (97:3) afforded colourless fine powder of 1, recrystallized from methanol, 129 mg (0.16 % yield). R_f 0.28 (chloroform-methanol, 24:1); m.p. 194-195 °C; UV λ_{max} (MeOH): 216 nm (log ε 6.2); IR υ_{max} (KBr): 3484, 2925, 2853, 1682, 1597, 1523, 1434, 1298, 1239, 1205, 1112, 1028, 917 cm^{-1}; +ve ES MS *m/z* (*rel. int.*): 168 $[M]^+$ ($C_8H_8O_4$) (9.3), 153 (9.8).

Sanctumoic Acid (2)

Elution of the column with chloroform-methanol (19:1) afforded pale yellow mass of 2, recrystallized from methanol, 412 mg (0.51 % yield). R_f 0.59 (chloroform-methanol, 47:3); m.p. 71:72 °C; UV λ_{max} (MeOH): 258 nm; IR υ_{max} (KBr): 3422, 2928, 3360, 1695, 1631, 1455, 1269, 1040, 859 cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.32 (2H, m, H-20, H-21), 3.75 (1H, brs, $w_{1/2}$ 18.0 Hz, H-14β), 2.52 (2H, m, H_2-2), 2.26 (2H, m, H_2-19), 2.18 (2H, m, H_2-22), 2.03 (2H, m, CH_2), 1.90 (2H, m, CH_2), 1.81 (2H, m, CH_2), 1.68 (2H, m, CH_2), 1.54 (6H, m, 3 x CH_2), 1.27 (12H, brs, 6 x CH_2), 1.24 (40H, brs, 20 x CH_2), 0.83 (3H, t, *J* = 6.3 Hz, CH_3-32); ^{13}C NMR (DMSO-d_6): δ 184.16 (C-1), 130.13 (C-20), 116.31 (C-21), 78.74 (C-14), 55.76 (C-2), 39.78 (C-19), 39.51 (C-22), 39.23 (CH_2), 38.95 (CH_2), 32.03 (CH_2), 31.01 (CH_2), 28.72 (10 x CH_2), 28.27 (5 x CH_2), 28.25 (5 x CH_2), 26.06 (CH_2), 25.01 (CH_2), 24.28 (CH_2), 21.72 (CH_2), 20.42 (CH_2), 14.27 (CH_3-32); +ve ESMS *m/z* (*rel. int.*): 495 $[M + H]^+$ ($C_{32}H_{63}O_3$) (16.2), 281 (18.3), 251 (32.6), 213 (6.9), 181 (10.0), 155 (27.3).

Ocimumnaphthanoic Acid (3)

Elution of the column with chloroform-methanol (19:3) yielded colourless amorphous powder of 3, recrystallized from chloroform-methanol (1:1), 156 mg (0.19 % yield). R_f 0.4 (chloroform-methanol, 47:3); m.p. 240-241 °C; UV λ_{max} (MeOH): 268, 335 nm (log ε 4.9, 2.1); IR υ_{max} (KBr): 3287, 3094, 2924, 1685, 1588, 1557, 1502, 1354, 1244, 1181, 829 cm^{-1}; +ve ES MS *m/z* (*rel. int.*): 220 $[M]^+$ ($C_{11}H_8O_5$) (2.5).

β-Sitosterol Glucoside (4)

Elution of the column with chloroform-methanol (93:7) yielded colourless amorphous powder of 4, recrystallized from methanol, 90 mg (0.11 % yield). R_f 0.72 (Chloroform : methanol, 9.3:0.7); m.p. 275-277

°C; UV λ_{max} (MeOH): 241 nm (log ε 2.9); IR ν_{max} (KBr): 3401, 2918, 2849, 1654, 1465, 1377, 1261, 1172, 1082 cm^{-1}; +ve ES MS *m/z* (*rel. int.*): 576 $[M]^+$ ($C_{35}H_{60}O_6$) (11.3), 413 $[M-C_6H_{11}O_5]^+$ (10.1), 397 (100).

Benzoylgluco-oleate (5)

Elution of the column with chloroform-methanol (91:9) gave light brown mass of 5, recrystallized from methanol, 426 mg (0.53 % yield). R_f 0.27 (chloroform-methanol, 9:10); m.p. 99-100 °C; UV λ_{max} (MeOH): 259, 278 nm (log ε 4.2, 1.3); IR υ_{max} (KBr): 3421, 3265, 2924, 2852, 1722, 1625, 1525, 1448, 1366, 1272, 1209, 1074, 950 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.54 (1H, m, H-2″), 7.37 (1H, m, H-6″), 7.29 (1H, m, H-4″), 6.69 (1H, m, H-3″), 6.58 (1H, m, H-5″), 5.31 (2H, m, H-9, H-10), 4.71 (1H, d, *J* = 7.1 Hz, H-1′), 4.50 (1H, m, H-5), 4.26 (1H, m, H-2′), 4.11 (1H, m, H-4′), 3.74 (1H, m, H-3′), 3.05 (2H, d, *J* = 9.1 Hz H_2-6′), 2.21 (1H, d, *J* = 7.2 Hz, H_2-2a), 2.19 (1H, d, *J* = 7.2 Hz, H_2-2b), 1.98 (2H, m, H_2-8), 1.79 (2H, m, H_2-11), 1.65 (2H, m, H_2-3), 1.24 (20H, brs, 10 x CH_2), 0.85 (3H, t, *J* = 6.3 Hz, CH_3-18); ^{13}C NMR (DMSO-d_6): δ 174.37 (C-7″), 169.43 (C-1), 147.54 (C-1″), 145.35 (C-4″), 128.23 (C-9), 127.75 (C-10), 125.26 (C-2″), 121.97 (C-6″), 115.47 (C-3″), 113.72 (C-5″), 102.23 (C-1′), 76.90 (C-5′), 75.40 (C-2′), 70.20 (C-4′), 67.19 (C-3′), 61.24 (C-6′), 55.65 (C-2), 44.36 (C-8), 38.08 (5 x CH_2), 29.28 (CH_2), 24.70 (CH_2), 22.65 (CH_2), 22.29 (CH_2), 13.93 (CH_3-18); +ve ES MS *m/z* (*rel. int.*): 549 $[M+H]^+$ ($C_{31}H_{49}O_8$) (2.5), 424 (22.1), 281 (23.5), 267 (18.7), 265 (13.1).

Hydrolysis of 5**:** Compound benzoylgluco-oleate (25 mg) was dissolved in ethanol (5ml), dilute HCl (2 ml) added and heated for 1 hour on a steam bath. The solvent was evaporated under reduced pressure and the residue was dissolved in chloroform to separate oleic and benzoic acids, co-TLC comparable. The insoluble residue was dissolved in water and chromatographed along with a standard sample of D-glucose, R_f comparable.

Oleiyl glucoside (6)

Elution of the column with chloroform-methanol (89:11) furnished colourless crystals of 6, recrystallized from methanol, 125 mg (0.15 % yield). R_f 0.89 (chloroform-methanol, 22:3)**;** m.p. 113-114 °C; UV λ_{max} (MeOH): 257 nm (log ε 2.1); IR υ_{max} (KBr): 3372, 3265, 2927, 1722, 1645, 1435, 1373, 1270, 1044, 862 cm^{-1}; +ve ES MS *m/z* (*rel. int.*): 444 $[M]^+$ ($C_{24}H_{44}O_7$) (5.1), 281 (27.9), 265 (21.6), 180 (19.2).

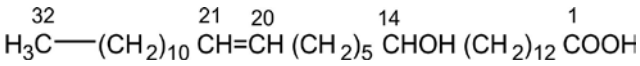
Diglucosyl oleate (7)

Elution of the column with chloroform-methanol (87:13) gave colourless crystalline mass of 7, recrystallized from methanol, 231 mg (0.28 % yield). R_f 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}; +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

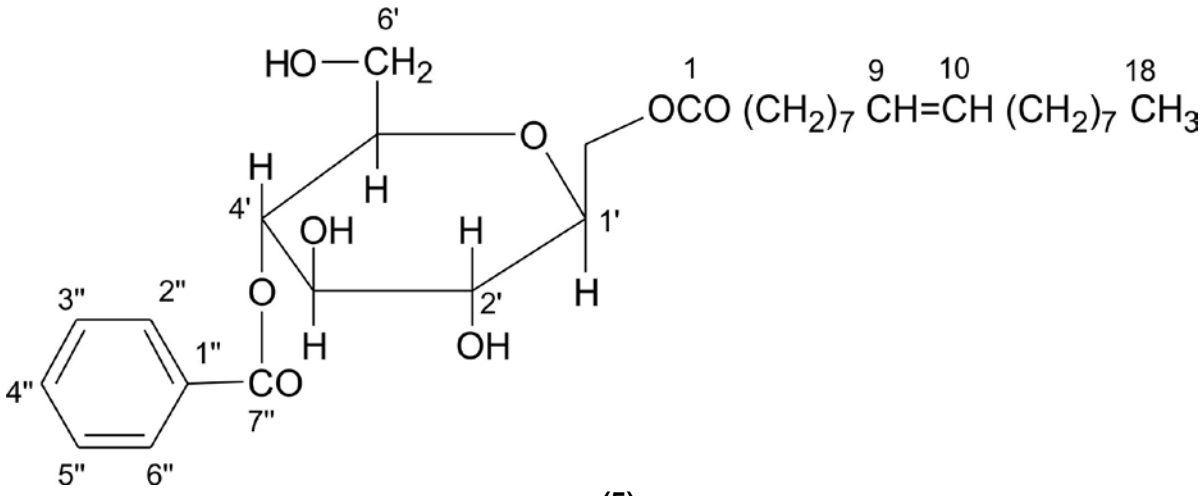
Compound 2, designated as sanctumoic acid, was obtained as a pale yellow mass from chloroform-methanol (19:1) eluents. It produced effervescence with sodium bicarbonate solution. Its IR spectrum exhibited characteristic absorption bands for carboxylic group (3422, 1695 cm^{-1}) and unsaturation (1631 cm^{-1}). The mass spectrum of 2 showed a molecular ion peak at m/z 495 $[M+H]^+$ corresponding to a molecular formula of a fatty acid, $C_{32}H_{63}O_3$. It indicated two double bond equivalents, one of each of them was adjusted in the carboxylic function and vinylic linkage. The prominent ion peak arising at m/z 155 [C_{21}-C_{22} fission, $CH_3(CH_2)_{10}]^+$, and 181 [C_{19}-C_{20} fission, CH_3 $(CH_2)_{10}CH=CH]^+$ indicated the presence of the vinylic linkage at C-20. The ion fragments generating at *m/z* 251 [C_{14}-C_{15} fission, $CH_3(CH_2)_{10}CH=CH(CH_2)_5]^+$, 281 [$C_{13}$-$C_{14}$ fission, $[CH_3(CH_2)_{10}CH=CH(CH_2)_5$ $CHOH]^+$ and 213 [M-281, $(CH_2)_{12}COOH]^+$ supported the existence of the hydroxyl group at C-14. The ^1H NMR spectrum of 2 showed a two-proton multiplet at δ 5.32 assigned to vinylic H-20 and H-21 proton. A one-proton broad multiplet at δ 3.75 with half-width of 18.0 Hz was ascribed to β-oriented H-14 carbinol proton. The methylene protons resonated between δ 2.52-1.24.

A three-proton triplet at δ 0.83 (*J* = 6.3 Hz) was accounted to C-32 primary methyl protons. The ^{13}C NMR spectrum of 2 displayed signals for carboxylic carbon at δ 184.16 (C-1), vinylic carbons at δ 130.13 (C-20) and 116.31 (C-21), hydroxymethine carbon at δ 78.74 (C-14), methylene carbons between δ 55.76-20.42 and methyl carbon at δ 14.27 (C-32). On the basis of the forgoing account the structure of 2 has been elucidated as dotriacont-20-en-14-ol-1-oic acid. It is a new hydroxyfatty acid.



(2)

Compound 5, named benzoylgluco-oleate, was obtained as a light brown mass from chloroform-methanol (91:9) eluents. It decolourized bromine water and gave positive tests for glycosides. Its IR spectrum displayed characteristic absorption bands for hydroxyl groups (3421, 3265 cm^{-1}), ester group (1722 cm^{-1}), unsaturation (1625 cm^{-1}) and aromatic ring (1525, 930 cm^{-1}). On the basis of mass and ^{13}C NMR spectra the molecular ion peak of 5 was determined at *m/z* 549 $[M+H]^+$ consistent to a glycoside of a fatty acid, $C_{31}H_{49}O_8$. The important ion peak arising at *m/z* 265 $[CH_3(CH_2)_7CH=CH(CH_2)_7\ CO]^+$, 281 $[CH_3(CH_2)_7CH=CH(CH_2)_7\ COO]^+$, 267 [M-281, $C_6H_5CO\text{-}C_6H_{10}O_5]^+$ and 424 $[M\text{-}C_6H_5CO]^+$ indicated that benzoylglycosyl unit was linked to oleic acid. The ^1H NMR spectrum of 5 exhibited aromatic proton signals as multiplets between δ 7.54-6.58, vinylic proton signal as a two-proton multiplet at δ 5.31 and anomeric proton signal as a one proton doublet at 4.71 (*J* = 7.1 Hz). Four one-proton multiplets between δ 4.50-3.74 and a two-proton doublet at δ 3.05 (*J* = 9.1 Hz) were attributed to other glucose protons. Two one-proton doublets at δ 2.21 (*J* = 7.2 Hz), and 2.19 (*J* = 7.2 Hz) were accounted to C-2 methylene protons adjacent to the ester group. Three two-proton multiplets at δ 1.98, 1.79 and 1.65 and a broad singlet at δ 1.24 (20H) were associated with the remaining methylene protons. A three-proton triplet at δ 0.85 (*J* = 6.3 Hz) was ascribed to C-18 primary methyl protons. The ^{13}C NMR spectrum of 5 showed signals for ester carbons at δ 174.37 (C-7″) and 169.43 (C-1), aromatic and vinylic carbons between δ 147.54-113.72, anomeric carbons at δ 102.23 (C-1′), other sugar carbons in the range of δ 76.90-61.24, methylene carbons in the range of δ 55.65-22.29 and methyl carbon at δ 13.93. The appearance of the ^1H NMR signal for H-4′ in the deshielded region at δ 4.11 and C-4′ carbon signal at δ 70.20 suggested the location of the benzoyl group at C-4′. Acid hydrolysis of 5 yielded D-glucose, oleic acid and benzoic acid (TLC comparable). On the basis of spectral data analysis and chemical reactions, the structure of 5 has been established as 4′-benzoylglucopyranosyl octadec-9-enoate. This is an unreported fatty acid glycoside.



(5)

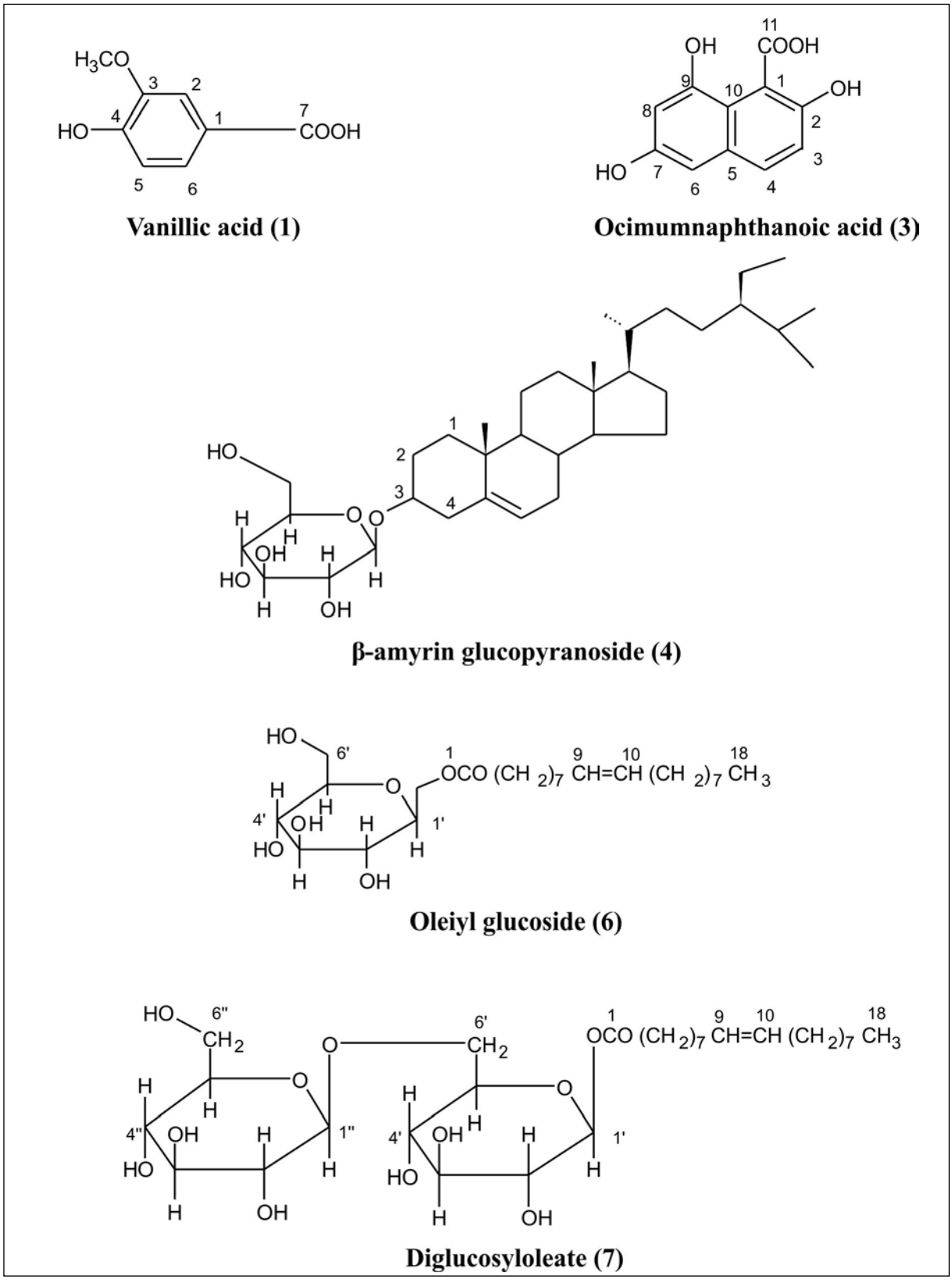


Fig.1: Structure of isolated known compounds

CONCLUSION

The phytochemical investigation of the methanolic extract of the leaves of *O. sanctum* furnished two new fatty acid derivatives sanctumoic acid and benzoyl gluco-oleate specific to the species grown in Delhi. These compounds may be responsible for medicinal properties of the drug.

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