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CHEMICAL CONSTITUENTS FROM THE FRUIT PEELS OF *CITRUS RETICULATA* BLANCO VAR. *KINNOW*

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ABSTRACT

Citrus reticulata Blanco var. *kinnow* (family Rutaceae), is a small, evergreen, spinous, tree, up to 8 m tall cultivated around the world in the warm temperate and tropical areas. Its fruit rind is used as a spice, to improve digestion, to reduce cough with profuse phlegm and to relieve abdominal and gastric distension, dyspepsia, hiccup and vomiting. Our study was planned to isolate chemical constituents from a methanolic extract of the fruit peels of this plant and to characterize their structures on the basis spectral data analysis. Phytochemical investigation of the fruit peels of *Citrus reticulata* Blanco var. *kinnow* led to isolate two fatty esters identified as *n*-hexadecanyl butyrate (cetyl butyrate, **1**) and methyl arachidate (methyl eicosanoate, **2**), a new acyclic diterpenic acid formulated as 3,7,11,15-tetramethyl hexadecane-14α-ol-1-oic acid (**3**), a known pentacyclic triterpenic methyl ester characterized as oleanolic acid methyl ester (methyl oleanolate, **4**) and a new steroidal glycosidic flavone and its structure was elucidated as β-sitosterol 3β-D-glucuronosyl –(6'→ 7″)-apigenin (**5**).

KEYWORDS: *Citrus reticulata* Blanco var. *kinnow*, fruit peels, phytoconstituents, isolation, spectral data analysis, structure characterization.

INTRODUCTION

Citrus reticulata Blanco var. *kinnow* (family Rutaceae), known as Mandarin, Tangerine, Satsuma orange and Temple orange, is indigenous to the subtropical and tropical zones of Asia including China, India, Japan and Vietnam. It is cultivated around the world in the warm temperate and tropical areas. It is a small, evergreen, spinous tree, up to 8 m tall; leaves are dark green, long, narrow and shiny with small petioles; flowers star shaped, white, oblate to subglobose, in the leaf-axils; fruits reddish-orange in colour, taste less sour, sweeter and stronger; peel is very thin with little mesocarp; seeds 10-15 per fruit, ovoid, base rounded, apex narrow and acute. The mandarin fruits possess antiemetic, aphrodisiac, astringent, laxative, refrigerant and tonic properties. The fruit rind is analgesic, antiasthmatic, anti-inflammatory, antiscorbutic, antiseptic, aromatic, astringent, carminative, expectorant, stomachic and tonic, used as a spice, to improve digestion, to reduce cough with profuse phlegm and to relieve abdominal and gastric distension, dyspepsia, hiccup and vomiting.[1,2] The unripe green exocarp is used to treat chest pains, hypochondrium, gastro-intestinal distension, swelling of the liver and spleen and cirrhosis of the liver. The fruit peel regulates skin moisture, softens hard and rough skin and cleanses oily skin. The peel essential oil is useful as an anodyne, antispasmodic, flavouring for candy, in gelatines, ice cream, chewing gum, liquers, baked goods, perfumery, beverages, medical formulations, toiletries and other cosmetic products.[1, 2] The seed is analgesic and carminative, beneficial to manage hernia, lumbago, mastitis and pain or swellings of the testes.

The mandarin peels contained ascorbic acid, carotenoids, and polyphenols, [3] essential oil composed of beta-myrcene, 3-carene, alpha-pinene, p-cymene, beta-pinene, sabinene, terpinolene and alpha-thujene, [4] limonene, γ-terpinene and α-pinene, linalool, myrcene and sabinene,[5-9] decenals, decadienals, decatrienals, dodecenals, dodecadienals, (1,3Z,5Z)-undecatriene, undecatetraenes, 3-butylpyridine, indole, alkylpyrazines, diethyl disulfide and alcohols[10], linalool, (E,E)- deca-2,4-dienal, winelactone, α-pinene, myrcene and octanal, [11] *n*-hexacosonoic acid, reticulataursenoside, citrusteryl arachidate, and lanost-5-en-3beta-ol-3beta-D-glucopyranosyl-4'-eicosanoate (citruslanosteroside).[12] The fruits possessed beta-cryptoxanthin, zeaxanthin and lutein,[13] essential oil,[14] flavanone glycosides (narirutin, naringin, hesperidin, and neohesperidin), polymethoxylated flavones (nobiletin and tangeretin), limonoid, and synephrine,[15] hesperidin and ferulic acid.[16] The leaves yielded methyl-N-methylanthranilate,[17] essential oil composed of sabinene, linalool, β-myrcene, limonene, γ-terpinene,

terpinen-4-ol, thymol, methyl ether, α-selinene, germacrene B, β-sinensal and α-sinensal.[18,19] 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 fruit peels of *Citrus reticulata* Blanco var. *kinnow*.

MATERIALS AND METHODS

The protocols of all methodologies (procedures, experimental designs and spectral data analysis) were adopted from the earlier published work.[12,26,27]

General procedures: The melting points were determined in one end open capillary tubes on a melting point M-560 apparatus (Perfit, India) heated thermoelectrically. UV spectra were determined with Lambda Bio 20 spectrophotometer (Perkin Elmer, Schwerzenbach, Switzerland) in methanol. The IR spectra were recorded 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 $CDCl_3$ as a solvent and TMS (Fluka analytical, Sigma-Aldrich, Netherland) as an internal standard. Mass spectra were recorded on a Jeol JMS-D 300 instrument using Argon/Xenon gas as the FAB. Petroleum ether, chloroform, methanol and other solvents of analytical grade were purchased from E. Merck(India) Ltd , New Delhi. Silica gel with 60-120 mesh particle size was procured from Qualigens, Mumbai, India) and used for column chromatography. The purity of the isolated compounds was checked on precoated TLC plates with Silica gel 60 F_{254} (Merck, 0.25 mm) and the spots were visualized by exposure to iodine vapors or under UV radiations and spraying with ceric sulfate solution.

Collection and authentication of plant material

The fruit peels of *C. reticulata* Blanco var. *kinnow* were collected from a local market of Delhi. 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 material was preserved in the herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

Extraction and isolation

The fruit peels of *C. reticulata* Blanco var. *kinnow* (1 kg) were dried in air, coarsely powdered and extracted exhaustively with methanol in a Soxhlet apparatus. The extract was concentrated under reduced pressure to get a dark brown mass, 123.6 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 silica gel columns 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, 19:1, 9:1, *v/v*) mixtures. 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.

n-Hexadecanyl butyrate (1)

Elution of the column with petroleum ether-chloroform (1:1) afforded colourless crystals of **1**, yield 195 mg, recrystallized from chloroform-methanol (1:1), m. p. 58 – 60 °C; IR υ_{max} (KBr) : 2924, 2851, 1737, 1464, 1377, 1265, 1221, 1196, 1179, 1014, 801, 725 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.03 (2H, t, J = 7.1 Hz, H_2 -1′), 2.21 (2H, t, J = 7.2 Hz, H_2 -2), 1.54 (2H, m, H_2-3), 1.39 (2H, m, H_2-2′), 1.25 (26H, brs, 13 x CH_2), 0.98 (3H, t, J = 6.2 Hz, Me-4), 0.95 (3H, t, J = 6.1 Hz, Me-16′); ^{13}C NMR ($CDCl_3$): δ 173.21 (C-1), 62.10 (C-1′), 30.95 (C-2), 29.70 (11 x CH_2), 29.31 (C-21′), 28.49 (C-13′), 25.01 (C-14′), 22.67 (C-15′), 14.77 (C-4), 14.17 (C-16′); +ve FAB MS *m/z* (rel. int.): 312 $[M]^+$ ($C_{20}H_{40}O_2$) (3.1), 241 (1.5), 87 (100).

Methyl arachidate (2)

Further elution of the column with petroleum ether - chloroform (1:1) yielded a colourless amorphous powder of **2**, yield 153 mg, purified by preparative TLC using chloroform-methanol (1:1), m. p. 53 - 54 °C; UV λmax (MeOH): 209 nm (log ε 5.1); IR υ_{max} (KBr) : 2925, 2853, 1722, 1464, 1377, 1284, 1180, 723 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.46 (3H, brs, OMe), 2.32 (2H, t, J = 4.8 Hz, H_2 -2), 1.61 (2H, m, H_2-3), 1.29 (20H, brs, 10 x CH_2), 1.24 (12H, brs, 6 x CH_2), 0.90 (3H, t, J = 6.8 Hz, Me-20); ^{13}C NMR ($CDCl_3$): δ 176.12 (C-1), 34.05 (C-2), 31.91 (C-3 to C-11), 29.64 (C-12), 29.60 (C-13), 29.45 (C-14), 29.35 (C-15), 29.27 (C-16), 29.11 (C-17), 24.83 (C-18), 22.68 (C-19), 14.10 (C-20), 50.49 (OMe); +ve FAB MS *m/z* (rel. int.): 326 $[M]^+$ ($C_{21}H_{42}O_2$) (35.6), 255 (100), 239 (54.1), 229 (68.5), 213 (69.2), 185 (98.3), 171 (97.1), 85 (96.7), 71 (99.8).

Tetramethyl hexadecane-14*α*-ol-1-oic acid (3)

Elution of the column with chloroform furnished a colourless amorphous powder of **3**, recrystallized from chloroform – methanol (9: 1), m. p. 146 -147 ^{o}C; IR υ_{max} (KBr): 3287, 3165, 2950, 2845, 1695, 1640, 1315, 1201, 1005, 725 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.13 (1H, m, $w_{1/2}$ = 6.9 Hz, H-14α), 2.33 (2H, d, J = 7.5 Hz, H_2-2), 2.30 (1H, m, H-3 α), 1.76 (1H, m, H-7 α), 1.65 (1H, m, H-11α), 1.63 (1H, m, H-15α), 1.61 (2H, m, H_2-4), 1.59 (2H, m, H_2-5), 1.25 (10H, brs, H_2-6, H_2-8, H_2-9, H_2-10, H_2-12, H_2-13), 1.11 (2H, m, H_2-5), 0.87 (3H, d, J = 6.9 Hz, Me-17), 0.85 (3H, d, J = 6.9 Hz, Me-18), 0.80 (3H, d, J = 6.5 Hz, Me-16), 0.78 (3H, d, J = 6.5 Hz, Me-19), 0.69 (3H, d, J = 6.3 Hz, Me-20); ^{13}C NMR ($CDCl_3$): δ 179.13 (C-1), 39.68 (C-2), 56.01 (C-3), 37.24 (C- 4), 34.31 (C-5),

33.91 (C-6), 51.21 (C-7), 29.64 (C-8), 29.64 (C-9), 29.64 (C-10), 45.81 (C-11), 24.68 (C- 2), 31.87 (C-13), 60.14 (C-14), 42.19 (C-15), 22.64 (C-16), 21.03 (C-17), 18.97 (C-18), 14.06 (C-19), 22.73 (C-20); +ve FAB MS *m/z* (rel. int.): 328 $[M]^+$ ($C_{20}H_{40}O_3$) (3.2), 285 (4.3), 255 (4.8), 225 (9.1), 199 (10.4), 157 (6.3), 129 (3.6), 101 (13.6), 87 (11.7), 59 (21.1).

Oleanolic acid methyl ester (4)

Elution of the column with chloroform- methanol (49:1) produced colourless crystalline powder of **4**, recrystallized from acetone, yield 219 mg, m. p. 201 - 203 °C; IR υ_{max} (KBr): 3468, 2927, 2856, 1735, 1639, 1456, 1375, 1236, 1193, 1032, 882 cm^{-1} ; ^1H NMR ($CDCl_3$): δ 5.32 (1H, m, H-12), 3.51(3H, brs, OMe), 3.24 (1H, dd, J = 5.5, 9.3 Hz, H-3α), 2.31 – 1.37 (23H, m, 10 x CH_2, 3 x CH), 1.30 (3H, brs, Me-23), 1.19 (3H, brs, Me-25), 0.86 (3H, brs, Me-30), 0.82 (3H, brs, Me-29), 0.81 (3H, brs, Me-24), 0.74 (3H, brs, Me-27), 0.68 (3H, brs, Me-26); ^{13}C NMR ($CDCl_3$): δ 39.08 (C-1), 28.18 (C-2), 78.23 (C-3), 39.42 (C-4), 55.93 (C-5), 18.82 (C-6), 33.46 (C-7), 39.86 (C-8), 48.27 (C-9), 37.46 (C-10), 23.81 (C-11), 122.61 (C-12), 144.85 (C-13), 42.26 (C-14), 28.06 (C-15), 23.87 (C-16), 46.73 (C-17), 42.17 (C-18), 46.62 (C-19), 31.07 (C-20), 34.34 (C-21), 33.24 (C-22), 28.84 (C-23), 16.51 (C-24), 15.67 (C-25), 17.58 (C-26), 26.27 (C-27), 173.28 (C-28), 23.43 (C-29), 24.65 (C-30); +ve FAB ESI *m/z* (rel.int.): 470 [M]+ ($C_{31}H_{50}O_3$) (1.8), 455 (11.6), 410 (9.3), 247 (28.8), 203 (36.5).

β-Sitosterol 3β-D-glucuronosyl –(6'→7″)-apigenin (5)

Elution of the column with chloroform – methanol (9:1) gave a light yellow crystalline mass of **5**, recrystallized from methanol, yield 348 mg, m. p. 104 - 106 °C; UV λmax (MeOH): 224, 275, 330 nm (log ε 5.2, 4.9, 4.1); IR υ_{max} (KBr): 3446, 3311, 2926, 2854, 1732, 1690, 1619, 1561, 1462, 1380, 1273, 1207, 1133, 1012, 922, 824 cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.33 (1H, m, H-6), 3.83 (1H, brs, $w_{1/2}$ = 18.3 Hz, H-3), 1.19 (3H, brs, Me-19), 0.83 (3H, d, J = 6.1 Hz, Me-21), 0.81 (3H, d, J = 6.3 Hz, Me-26), 0.79 (3H, d, J = 6.2 Hz, Me-27), 0.77 (3H, t, J = 6.1 Hz, Me-29), 0.63 (3H, brs, Me-18), 2.55 - 1.03 (29H, m, 11 × CH_2, 7 × CH), 5.23 (1H, d, J = 7.2 Hz, H-1′), 4.24 (1H, d, J = 6.4 Hz, H-5′), 4.01 (1H, m, H-2′), 3.91 (1H, m, H-3′), 3.79 (1H, m, H-4′), 6.71 (1H, d, J = 2.7 Hz, H-8″), 6.65 (1H, s, H-3″), 6.16 (1H, d, J = 2.7 Hz, H-6″), 7.57 (1H, m, H-2‴), 7.54 (1H, m, H-6‴), 7.19 (1H, m, H-5‴), 7.16 (1H, m, H-3‴); ^{13}C NMR (DMSO-d_6): δ 38.89 (C-1), 31.41 (C-2), 73.38 (C-3), 41.86 (C-4), 141.32 (C-5), 122.11 (C-6), 39.42 (C-7), 35.49 (C-8), 49.53 (C-9), 36.21 (C-10), 22.60 (C-11), 39.48 (C-12), 39.78 (C-13), 56.14 (C-14), 28.59 (C-15), 29.03 (C-16), 55.71 (C-17), 11.97 (C-18), 19.17 (C-19), 34.36 (C-20), 18.79 (C-21), 33.95 (C-22), 26.21 (C-23), 45.17 (C-24), 29.24 (C-25), 18.56 (C-26), 19.69 (C-27), 20.57 (C-28), 11.65 (C-29), 101.64 (C-1′), 69.87 (C-2′), 62.39 (C-3′), 67.72 (C-4′), 79.84 (C-5′), 173.61 (C-6′), 163.21 (C-2″), 102.94 (C-3″), 182.57 (C-4″), 161.63 (C-5″), 106.38 (C-6″), 164.39 (C-7″), 98.63 (C-8″), 156.42 (C-9″), 104.56 (C-10″), 122.18 (C-1‴), 128.96 (C-2‴), 116.34 (C-3‴), 160.89 (C-4‴), 114.31 (C-5‴), 129.47 (C-6‴); FAB MS *m/z* (rel. int.): 842 $[M]^+$ ($C_{50}H_{66}O_{11}$) (3.2), 573 (6.4), 413 (5.6), 396 (3.7), 381 (6.9), 273 (11.7), 269 (7.1), 255 (10.8), 213 (98.6).

RESULTS AND DISCUSSION

The IR spectrum of compound **1** showed absorption bands for an ester function (1737 cm^{-1}) and long aliphatic chain (725 cm^{-1}). Its mass spectrum exhibited a molecular ion peak at *m/z* 312 consistent with a fatty acid ester, $C_{20}H_{40}O_2$. The ion fragments arising at *m/z* 87 [$C_{1'}$ – O fission, $CH_3(CH_2)_{14}$-$CH_2]^+$ and 241 [M – 87]$^+$ indicated that butyric acid was esterified with 1-hexadecanol (cetyl alcohol). The ^1H NMR spectrum of **1** displayed two two-proton triplets at δ 4.03 (J = 7.1 Hz) and 2.21 (J = 7.2 Hz) assigned to oxymethylene H_2 -1′ and methylene H_2 -2 respectively, adjacent to the ester group. Two two-proton multiplets at δ 1.54 and 1.39 and a broad singlet at δ 1.25 (26 H) were attributed to other methylene protons. Two three- proton triplets at δ 0.98 (J = 6.2 Hz) and 0.95 (J = 6.1 Hz) were accounted to terminal C-4 and C-16′ primary methyl protons. The ^{13}C NMR spectrum of **1** exhibited signals for the ester carbon at δ 173.21 (C-1), oxymethylene carbon at δ 62.10 (C-1′), methylene carbons from δ 30.95 to 22.67 and methyl carbons at δ 14.77 (C-4) and 14.17 (C-16′). The absence of any signal beyond δ 4.03 in the ^1H NMR spectrum and carbon signals between δ 173.21 - 62.10 in the ^{13}C NMR spectrum ruled out the existence of any vinylic linkage in the molecule. On the basis of these spectral data evidences, the structure of **1** has been established as *n*-hexadecanyl butyrate (cetyl butyrate), a new alkyl ester (Fig. 1).

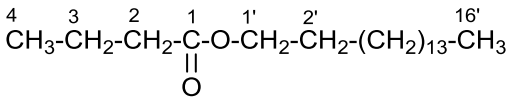
Compound **2** was a fatty acid ester identified as methyl arachidate (methyl eicosanoate).[20,21]

Compound **3** produced effervescences with sodium bicarbonate solution indicating the existence of a carboxylic group and exhibited characteristic IR absorption bands for a hydroxyl group (3287 cm^{-1}) and carboxylic function (1695 cm^{-1}). Its molecular ion peak was determined at *m/z* 328 on the basis of mass and ^{13}C NMR spectra corresponding to a molecular formula of an acyclic diterpenic acid, $C_{20}H_{40}O_3$. The ion peaks arising at *m/z* 59 [C_2 – C_3 fission, CH_2-$COOH]^+$, 87 [C_3 – C_4 fission, CH_3-CH- CH_2-$COOH]^+$, 129 [C_6 – C_7 fission, $(CH_2)_3$-(CH_3)-CH-CH_2-$COOH]^+$, 157 [C_7 – C_8 fission,CH_3-CH-$(CH_2)_3$-(CH_3)-CH-CH_2-$COOH]^+$, 199 [C_{10} – C_{11} fission, $(CH_2)_3$-CH_3-CH-$(CH_2)_3$-(CH_3)-CH-CH_2-$COOH]^+$, 227 [C_{11} – C_{12} fission, $[CH_3$-CH-$(CH_2)_3]_2$-(CH_3)-CH-CH_2-$COOH]^+$ and 255 [C_{13} – C_{14} fission, $C_{16}H_{31}O_2]^+$ suggested the presence of the carboxylic group at the terminal C-1 position. The ion fragments produced at *m/z* 129 [M – 199, $(CH_3)_2$-CH-CH(OH)-$(CH_2)_2$-(CH_3)-$CH]^+$,101 [M – 227, $(CH_3)_2$-CH-CH(OH)-$(CH_2)_2]^+$, and 285 [C_{14} - C_{15} fission, $C_{17}H_{33}O_3]^+$ supported the location of the hydroxyl group at C-14 carbon. The ^1H NMR spectrum of **3** showed a one-proton multiplet at δ 4.13 with half-width of 6.9 Hz assigned to alpha-

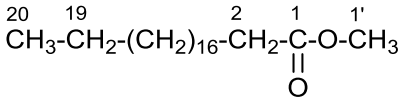
oriented carbinol H-14 proton. Four methine protons appeared as one-proton multiplets at δ 2.30 (H-3 α), 1.76 (H-7 α), 1.65 (H-11α) and 1.63 (H-15α). A two-proton doublet at δ 2.33 (J = 7.5 Hz) was ascribed to methylene H_2-2 protons adjacent to the carboxylic function. The other methylene protons resonated as two-proton multiplets at δ 1.61 (H_2-13), 1.59 (H_2-12) and 1.11 (H_2-10) and as a broad singlet at δ 1.25 (10H). Five three-proton doublets at δ 0.87 (J = 6.9 Hz), 0.85 (J = 6.9 Hz), 0.80 (J = 6.5 Hz), 0.78 (J = 6.5 Hz) and 0.69 (J = 6.3 Hz) were associated with the secondary C-16 to C-20 secondary methyl protons. The ^{13}C NMR spectrum of **3** displayed signals for the carboxylic carbon at δ 179.13 (C-1), carbinol carbon at δ 60.14 (C-14), methine carbons at δ 56.01 (C- 3), 51.21 (C- 7), 45.81 (C- 11) and 42.19 (C- 15), methylene carbons from δ 39.68 to 24.68 and methyl carbons between δ 22.73 -14.06. The absence of any signal beyond δ 4.13 in the ^{1}H NMR spectrum and carbon signals between δ 179.13 - 60.14 in the ^{13}C NMR spectrum supported saturated nature of the molecule. On the basis of spectral data analysis and chemical reactions, the structure of **3** was formulated as 3,7,11,15-tetramethyl hexadecane-14α-ol-1-oic acid, a new acyclic diterpenic acid (Fig. 1).

Compound **4** was a known pentacyclic triterpenic methyl ester identified as oleanolic acid methyl ester (methyl oleanolate).[22]

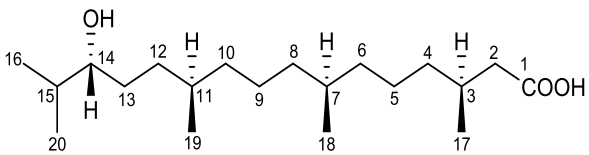
Compound **5**, gave positive tests of glycosides and exhibited UV absorption maxima at 275 and 330 nm for a flavone molecule and IR absorption bands for hydroxyl groups (3446, 3311 cm^{-1}), carbonyl group (1690 cm^{-1}), ester function (1732 cm^{-1}), unsaturation (1619 cm^{-1}) and aromaticity (1561, 1012 cm^{-1}). There was a shift of UV band I with sodium methoxide suggesting the presence of free hydroxy groups. There was no shift of UV bands with sodium acetate solution indicating bound nature of 7-hydroxyl group. A shift of band I with aluminum chloride supported the presence of free 5-hydroxyl group. There was no shift of band I with aluminum chloride and hydrochloric acid excluding the existence of B-ring *o*-dihydroxy functions. [23,24] On the basis of mass and ^{13}C NMR spectra , the molecular ion peak of **5** was determined at *m/z* 842 corresponding to the molecular formula of a steroidal glycosidic ester, $C_{50}H_{66}O_{11}$. The ion fragments produced at *m/z* 413 [$C_{1'}$ - O fission, $C_{29}H_{49}O]^+$, 396 [$C_{29}H_{49}$ O - OH]$^+$, 381 [396 – Me]$^+$, 273 [M – side chain]$^+$, 255 [273 – $H_2O]^+$ and 213 [255 – ring C]$^+$ supported the presence of the steroidal unit attached to the sugar moiety. An ion fragment arising at *m/z* 573 [$C_{6'}$ - O fission, $C_6H_8O_5$-O-$C_{29}H_{49}]^+$ indicated that glucuronosyl unit was linked to the steroidal unit. An ion peak generated at *m/z* 269 [M – 573]$^+$ suggested the attachment of a flavone unit to the sugar moiety. The ^{1}H NMR spectrum of **5** showed steroidal protons as one-proton multiplets at δ 5.33 and 3.83 ($w_{1/2}$ = 18.3 Hz) assigned to vinylic H-6 and oxymethine H-3α protons, respectively. Two three-proton singlets at δ 1.19 and 0.63, three three-proton doublets at δ 0.83 (J = 6.1 Hz), 0.81 (J = 6.3 Hz), 0.79 (J = 6.2 Hz) and a three-proton triplet at 0.77 (J = 6.1 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 glycosidal protons resonated as a one- proton doublet at δ 5.23 (J = 7.2 Hz) ascribed to β-oriented anomeric H-1′ proton and other sugar protons as one-proton signals from δ 4.24 to 3.79. The flavone signals appeared as one-proton doublets at δ 6.71 (J = 2.7 Hz) and 6.16 (J = 2.7 Hz), respectively, attributed to A-ring H-8″ and H-6″ protons, as a one-proton singlet at δ 6.65 due to flavone H-3 proton, and ring B protons as one-proton multiplets at δ 7.57 (H-2‴), 7.54 (H-6‴), 7.19 (H-5‴) and 7.16 (H-3‴). The ^{13}C NMR spectrum of **5** showed important signals for steroidal vinylic carbons at δ 141.32 (C-5) and 122.11 (C-6), oxymethine carbon at δ 73.38 (C-3), methyl carbons at δ 11.97 (C-18), 19.17 (C-19), 18.73 (C-21), 18.56 (C-26), 19.69 (C-27) and 11.65 (C-29), sugar anomeric carbon at δ 101.64 (C-1′), ester carbon at δ 173.53 (C-6′) and other sugar carbons from δ 79.84 to 62.39, and flavone signals for the carbonyl carbon at δ 182.57 (C-4″), vinylic carbons at δ 163.21 (C-2″) and 102.94 (C-3″) and aromatic carbons between δ 164.39 – 98.61. The ^{1}H NMR and ^{13}C NMR spectral data of the steroidal nucleus were compared with other stigmastene-type molecules. [24,25] The ^{13}C NMR spectral data of **5** were compared with the values of the reported flavones.[26] Acid hydrolysis of **5** yielded β-sitosterol, m. p. 136 – 138 °, D-glucuronic acid, m. p. 159 – 161 °C, $[\alpha]_D$ 35 – 37 °, conc. 6 % w/v in water, R_f = 0.26 (*n*-butanol – pyridine – water, 6: 4: 3, v/v) and apigenin , m. p. 345 - 348 °C; R_f = 0.83 (benzene-acetic acid- water , 125:72:3). On the basis of spectral data analysis and chemical reactions, the structure of **5** has been formulated as β-sitosterol 3β-D-glucuronosyl –(6'→ 7″)-apigenin, a new steroidal glycosidic flavone (Fig. 1).



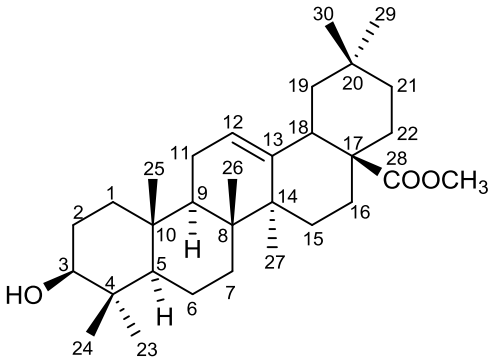
n-Hexadecanyl butyrate **(1)**



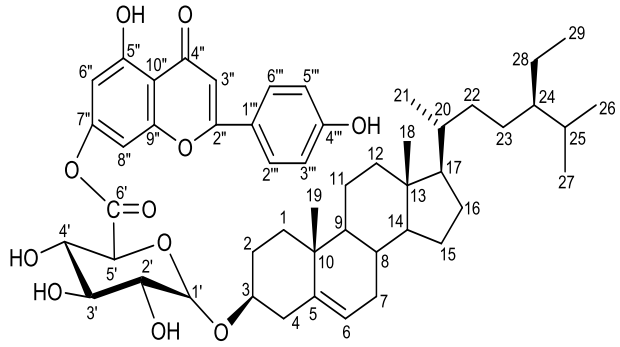
Methyl arachidate **(2)**



Tetramethyl hexadecanyl-14α-ol-1-oic acid **(3)**



Oleanolic acid methyl ester **(4)**



β-Sitosterol-3β-D-glucuronosyl-(6′→7″)-apigenin **(5)**

Fig. 1: Chemical constituents 1 to 5 isolated from the fruit peels of *Citrus reticulata* Blanco var. *kinnow*.

CONCLUSION

Phytochemical investigation of the fruit peels of *Citrus reticulata* Blanco var. *kinnow* led to isolate two fatty esters identified as *n*-hexadecanyl butyrate (cetyl butyrate, **1**) and methyl arachidate (methyl eicosanoate, **2**), a new acyclic diterpenic acid formulated as 3,7,11,15-tetramethyl hexadecane-14α-ol-1-oic acid (**3**), a known pentacyclic triterpenic methyl ester characterized as oleanolic acid methyl ester (methyl oleanolate, **4**) and a new steroidal glycosidic flavone and its structure was elucidated as β-sitosterol 3β-D-glucuronosyl –(6'→ 7″)-apigenin (**5**). This work has enhanced understanding about the chemical constituents of the undertaken plants. 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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