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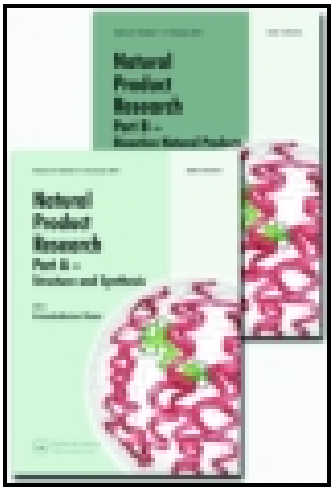

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Phytochemical investigation of the fruit peels of Citrus reticulata Blanco

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Phytochemical investigation of the fruit peels of *Citrus reticulata* Blanco

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Phytochemical investigation of the fruit peels of *Citrus reticulata* Blanco (Rutaceae) resulted in the isolation of three new phytoconstituents along with *n*-hexacosonoic acid. Their structures have been established as 18βH-urs-5,11-dien-3β-ol-11-one-3β-D-glucopyranosyl-(4′→1″)-D-glucopyranosyl-6″-(3‴,4‴-dihydroxyl)-benzoate (reticulataursenoside), stigmast-5-en-3β-ol-3β-D-glucopyranosyl-4′-eicosanoate (citrusteryl arachidate), and lanost-5-en-3β-ol-3β-D-glucopyranosyl-4′-eicosanoate (citruslanosteroside) on the basis of spectral data analysis and chemical reactions.

Keywords: *Citrus reticulata*; Rutaceae; hexacosanoic acid; reticulataursenoside; citrusteryl arachidate; citruslanosteroside

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1. Introduction

Citrus reticulata Blanco (Rutaceae) is commonly known as narangi or santra (orange). Mandarin is a group name for this class of orange with thin, loose peel, which have been dubbed 'kid-glove' oranges. These are treated as members of a distinct species, *C. reticulata* Blanco. The name 'tangerine' could be applied as an alternate name to the whole group, but in trade, it is usually confined to the types with red-orange skin. In the Philippines all mandarin oranges are called naranjita. Spanish-speaking people in the American tropics call them mandarina. The fruit peel regulates skin moisture, softens hard and rough skin and has a cleansing effect on oily skin. It also helps skin tone and removes skin blemishes (Mahalwal & Ali, 2001). 5-Hydroxy-7,8,4′ trimethoxy flavone, 4′-hydroxy-5,6,7,8-tetramethoxy-flavone, substituted triterpene-flavone methyl ethers, (Rastogi & Mehrotra, 1993), elemol, 5-hydroxy-3,7,8,3′, 4′-pentamethoxy flavone, 3,5,6,7,3′,4′-hexamethoxy flavone, 5,7,8,4′-tetramethoxy flavone and 3,5,7,8,3′,4′-hexamethoxy flavone, β-sitosterol, β-cymerin and naringenin have all been reported from the fruit peel (Rastogi & Mehrotra, 1995; Saxena & Shrivastava, 1995). This article describes the isolation and characterisation of three new triterpenic and sterol glycosides, along with *n*-hexacosanoic acid, from the fruit of *C. reticulata*.

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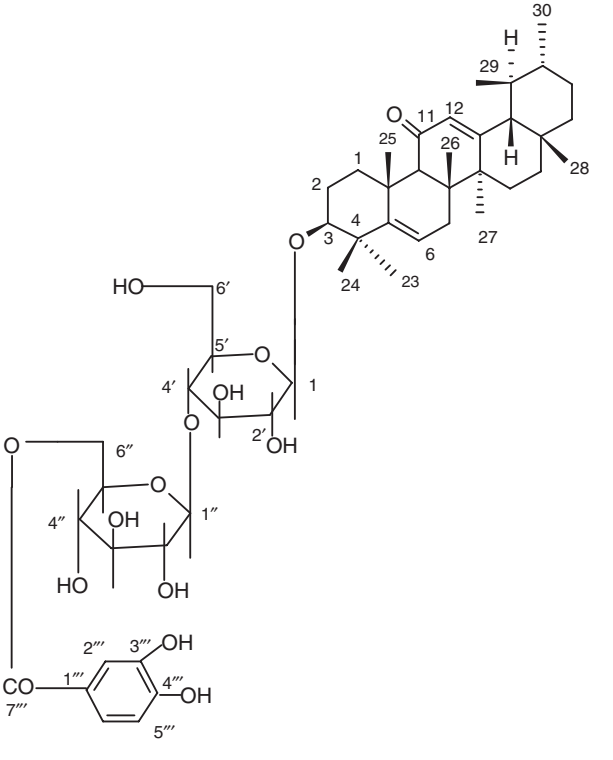
2. Results and discussion

Compound **1** was identified as *n*-hexacosanoic acid on the basis of spectral data analyses and chemical reactions.

$CH_3(CH_2)_{24}COOH$

(1)



(2)

Compound **2**, named reticulataursenoside, was obtained as a colourless crystal from chloroform–methanol (97 : 3) eluents. It gave positive tests of triterpenic glycosides. Its IR spectrum showed the characteristic absorption band for hydroxyl groups (3510, 3459, 3390 cm^{-1}), an ester group (1746 cm^{-1}), a keto group (1710 cm^{-1}), and unsaturation (1635 cm^{-1}). The fast atom bombardment (FAB) mass spectrum of **2** displayed a molecular peak at *m*/*z* 898 corresponding to a pentacyclic diglycoside esterified with an aromatic acid, $C_{49}H_{70}O_{15}$. Removal of the glycosidic unit yielded an ion fragment at *m*/*z* 438 related to $C_{30}H_{46}O_2$. It indicated eight double-bond equivalents. Five of them were adjusted in the pentacyclic triterpenic carbon framework, two in the vinylic linkages and one in the carbonyl function. The retro-Diels–Alder fragmentation of the triterpenic moiety yielded ion fragments at *m*/*z* 205 and 232, suggesting the presence of one vinylic linkage and a keto group in ring C (Ali, 2001; Budzikiewicz, Wilson, & Djerassi, 1963). The ion fragments generated at *m*/*z* 217 $[232 - Me]^+$, 202 $[217 - Me]^+$, 190 $[205 - Me]^+$, 175 $[190 - Me]^+$, 160 $[175 - Me]^+$, 145 $[160 - Me]^+$, 187 $[205 - H_2O]^+$, 172 $[187 - Me]^+$,

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152 $[C_{6,7}–C_{9,10}$ fission$]^+$and 166 $[C_{7,8}–C_{9,10}$ fission$]^+$ suggested the location of another vinylic linkage at C-5 and a hydroxyl group in ring A, which was placed at C-3 on the basis of biogenetic considerations. The ion fragments arising at m/z 343 $[C_{12}H_{24}O_{11}]^+$, 179 $[C_6H_{11}O_6]^+$, 146 $[C_6H_{10}O_4]^+$, 154 $[C_7H_6O_4]^+$ and 137 $[C_7H_5O_3]^+$ indicated the presence of two glucosidic moieties esterified with a dihydroxybenzoic acid in the molecule.

The ^{1}H NMR spectrum of **2** exhibited a one-proton broad signal at δ 6.49 and a one-proton doublet at δ 5.48 ($J = 5.3$ Hz) assigned to vinylic H-12 and H-6, respectively. A one-proton double doublet at δ 3.80 with coupling interactions of 7.2 and 5.7 Hz was ascribed to an α-oriented H-3 carbinol proton. The existence of H-12 in the downfield region at δ 6.49 as a broad signal indicated the location of the carbonyl group at C-11. Two one-proton doublets at δ 5.26 ($J = 7.1$ Hz) and 4.92 ($J = 7.2$ Hz) were attributed to anomeric H-1′ and H-1′ protons, respectively. Four one-proton doublets at δ 3.11 ($J = 11.3$ Hz), 3.06 ($J = 11.3$ Hz) and at δ 3.89 ($J = 10.2$ Hz) and 3.86 ($J = 10.2$ Hz) were accounted to oxygenated methylene H_2-6′ and H_2-6″ protons, respectively. The remaining carbinol protons of the sugar moieties appeared between δ 4.87 and 3.96. The presence of H-4′ signal at δ 4.87 and H_2-6″ signals at 3.89 and 3.86 in deshielded regions supported the attachment of one of the glucosidic moieties at C-4′ and an aromatic acid group at C-6″. Two one-proton doublets at δ 6.74 ($J = 2.5$ Hz) and 7.64 ($J = 10.5$ Hz) and a one-proton double doublet at δ 7.19 ($J = 10.5$, 2.5 Hz) were accounted to meta-coupled H-2‴, ortho-coupled H-5‴ and ortho-meta coupled H-6‴, respectively, suggesting a 3″,4″-dioxygenated pattern of the aromatic ring. Six three-proton broad signals at δ 0.94, 0.83, 1.13, 1.20, 1.24 and 1.06 were assigned to tertiary C-23, C-24, C -25, C-26, C-27 and C-28 methyl protons, respectively, all attached to the saturated carbons. Two doublets at δ 1.04 ($J = 6.3$ Hz) and 1.02 ($J = 6.3$ Hz), integrated for three protons each, were associated with the secondary C-29 and C-30 methyl proton of an ursane type triterpene, respectively. A one-proton doublet at δ 2.80 with a coupling interaction of 16.2 Hz was accounted to an H-18 β-methine proton. The remaining methine and methylene protons resonated between δ 2.66 and 1.28 (Table 1). The ^{13}C NMR spectrum of **2** showed carbon signals for a carbonyl carbon at δ 207.67 (C-11), an ester carbon at δ 169.81 (C-7‴), vinylic carbons at δ 141.48 (C-5), 119.37 (C-6), 120.10 (C-12) and 143.11 (C-13), aromatic carbons between δ 166.96 and 109.97, glycosidic carbons between δ 106.31 and 60.77 and methyl carbons in the range δ 27.53–16.93. The ^{1}H and ^{13}C NMR spectral values of the triterpenic moiety were compared with the related ursene-type triterpenes (Ali, Gupta, Neguerulea, & Perez-Alonso, 1998; Ali, Ravinder, & Ramachandram, 2000; Mahato & Kundu, 1994). The ^{1}H–^{1}H COSY spectrum of **2** showed correlations of H-3 with H_2-2 and H-1′; H-12 with H-18; H-4′ with H-5′, H-4′ and H-1″; and H-2‴with H-6‴. The ^{1}H–^{13}C HETCOR spectrum of **2** exhibited interactions of C-3 with H-2 and H-1′; C-11 with H-12 and H-9; C-4′ with H-3′ and H-1″; and C-7‴ with H_2-6″ and H-2‴. Alkaline hydrolysis of **2** yielded 3,4-dihydroxybenzoic acid, β-D-glucose and an ursene-type triterpene. On the basis of spectral data analysis and chemical reactions, the structure of **2** has been formulated as 18βH-urs-5, 11-dien-3β-ol-11-one-3β-D-glucopyranosyl-(4′→1″)-D-glucopyranosyl-6″-(3‴,4‴-dihydroxyl)-benzoate. This is a new ursene-type diglycoside.

Compound **3**, designated as citrusteryl arachidate, was obtained as a colourless crystalline mass from chloroform–methanol (23 : 2) eluents. It gave a positive test for steroidal glycosides. Its IR spectrum exhibited characteristic absorption bands for hydroxyl groups (3490, 3421, 3365 cm^{-1}), an ester group (1735 cm^{-1}) and unsaturation (1637 cm^{-1}). Its mass spectrum displayed a molecular ion peak at m/z 870 corresponding to a steroidal glycoside esterified with a C_{10} fatty acid, $C_{55}H_{98}O_7$. It indicated seven

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Table 1. ^{1}H and ^{13}C NMR spectral data of reticulataursenoside (**2**), citrustery arachidate (**3**) and citruslanosteroside (**4**).

	^{1}H NMR						^{13}C NMR		
	2		**3**		**4**				
Position	*α*	*β*	*α*	*β*	*α*	*β*	**2**	**3**	**4**
1	2.31 m	2.42 m	2.14 m	2.50 m	1.87 ddd (6, 15.6, 8.5)	2.29 ddd (10.2, 8.5, 6.0)	38.95	36.71	35.73
2	1.98 m	2.26 m	1.82 m	1.96 m	2.16 m	2.09 m	28.72	29.14	27.52
3	3.80 dd (7.2, 5.7)	–	3.45 br m (w ½ = 18.5 Hz)	–	3.69 dd (5.5, 9.5)	–	79.33	73.39	78.16
4	–	–	2.50 m	2.65 m	–	–	37.56	40.33	38.62
5	–	–	–	–	–	–	141.48	140.41	142.08
6	5.48 d (5.3)	–	5.33 d (5.5)	–	5.22 d (5.3)	–	119.37	120.91	121.82
7	2.66 m	2.60 m	1.93 m	2.48 m	2.47 br s	2.47 br s	35.47	31.33	25.35
8	–	–	–	1.48 m	–	1.73 m	45.19	31.23	42.33
9	2.56 s	–	1.51 m	–	1.92 d (6.0)	–	53.64	49.56	49.97
10	–	–	–	–	–	–	36.06	36.09	37.26
11	–	–	1.27 m	1.29 m	1.47 m	1.73 m	207.67	22.56	21.15
12	6.49 br s	–	2.14 m	1.96 m	2.84 br s	2.15 m	120.10	38.26	36.81
13	–	–	–	–	–	–	143.11	41.74	46.83
14	–	–	1.51 m	–	–	–	46.42	56.07	51.80
15	1.76 m	1.28 m	1.27 m	1.39 m	1.37 dd (13.2, 6.50	1.33 dd (12.8, 6.5)	29.06	25.61	34.22
16	1.52 m	1.28 m	1.15 m	1.49 m	1.61 m	1.55 m	23.66	28.49	31.78
17	–	–	1.46 m	–	1.47 m	–	50.22	55.40	51.01
18	–	2.80 d (16.2)	0.67 m	–	0.55 br s	–	58.05	11.61	16.36
19	2.50 m	–	1.01 br s	–	1.13 br s	–	38.68	20.46	18.59
20	–	1.76 m	–	1.96 m	2.16 m	–	39.22	35.29	36.36
21	2.50 m	1.77 m	0.96 d (6.5)	–	0.89 d (8.8)	–	29.59	18.83	19.52
22	1.58 m	1.29 m	2.14 m	1.95 m	2.19 m	2.19 m	31.24	39.24	38.25
23	0.94 br s	–	1.28 m	1.51 m	1.47 m	1.47 m	21.23	33.31	24.78
24	0.83 br s	–	1.53 m	–	1.45 m	1.55 m	16.93	45.13	39.63
25	1.13 br s	–	1.56 m	–	1.73 m	–	17.44	27.55	37.33
26	1.20 br s	–	0.91 d (6.2)	–	0.87 d (8.8)	–	18.45	19.47	17.71
27	1.24 br s	–	0.85 d (6.3)	–	0.76 D (5.6)	–	20.44	18.89	20.28
28	1.06 br s	–	1.51 m	1.49 m	0.72 br s	–	27.53	23.68	24.16
29	1.04 d (6.3)	–	0.83 br s	–	0.70 br s	–	18.92	11.49	22.15
30	1.02 d (6.3)	–	–	–	0.68 br s	–	19.48	–	18.79
1′	5.26 d (7.1)	–	5.22 d (7.1)	–	4.97 d (7.1)	–	106.31	100.76	101.43

(*Continued*)

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Table 1. Continued.

	^{1}H NMR						^{13}C NMR		
	2		**3**		**4**				
Position	α	β	α	β	α	β	**2**	**3**	**4**
2′	4.24 brs	–	4.68 m	–	4.28 d (6.8)	–	77.32	76.72	76.18
3′	4.03 m	–	3.66 m	–	3.95 m	–	64.68	70.15	70.45
4′	4.87 m	–	4.25 m	–	4.52 m	–	66.13	76.55	76.59
5′	4.47 brs	–	4.70 m	–	4.58 m	–	78.34	76.97	76.99
6′	3.11 d (11.3)	3.06 d (11.3)	3.08 brs	3.06 brs	3.48 d (9.5)	3.31 d (9.5)	60.77	61.10	61.23
1″	4.92 d (7.2)	–	–	–	–	–	97.13	173.11	171.68
2″	4.12 brs	–	2.94 brs	2.94 brs	2.65 d (6.4)	2.63 d (6.4)	77.32	28.75	28.67
3″	3.96 m	–	1.96 brs	1.96 brs	1.47 m	1.47 m	64.67	28.75	28.67
4″	4.51 m	–	1.51 brs	1.51 brs	1.17 brs	1.17 brs	64.66	28.75	28.67
5″	4.47 brs	–	1.51 brs	1.51 brs	1.17 brs	1.17 brs	78.34	28.75	28.67
6″	3,89 d (10.2)	3.86 d (10.2)	1.24 brs	1.24 brs	1.17 brs	1.17 brs	61.61	28.75	28.67
7″	–	–	1.24 brs	1.24 brs	1.13 brs	1.13 brs	–	28.75	26.43
8″	–	–	1.24 brs	1.24 brs	1.13 brs	1.13 brs	–	28.75	26.43
9″	–	–	1.24 brs	1.24 brs	1.13 brs	1.13 brs	–	28.75	26.43
10″	–	–	1.24 brs	1.24 brs	1.13 brs	1.13 brs	–	28.75	25.67
11″	–	–	1.24 brs	1.24 brs	1.13 brs	1.13 brs	–	28.75	25.67
12″	–	–	1.24 brs	1.24 brs	1.13 brs	1.13 brs	–	28.75	25.67
13″	–	–	1.24 brs	1.24 brs	1.13 brs	1.13 brs	–	28.75	25.67
14″	–	–	1.24 brs	1.24 brs	1.13 brs	1.13 brs	–	28.75	25.67
15″	–	–	1.24 brs	1.24 brs	1.13 brs	1.13 brs	–	26.43	23.84
16″	–	–	1.24 brs	1.24 brs	1.13 brs	1.13 brs	–	25.60	23.84
17″	–	–	1.24 brs	1.24 brs	1.13 brs	1.13 brs	–	24.90	22.56
18″	–	–	1.24 brs	1.24 brs	1.13 brs	1.13 brs	–	24.34	22.56
19″	–	–	1.24 brs	1.24 brs	1.13 brs	1.13 brs	–	21.83	22.19
20″	–	–	0.83 brs	–	0.83 brs	0.75 t (6.0)	–	18.46	17.43
1‴	–	–	–	–	–	–	129.65	–	–
2‴	6.74 d(2.5)	–	–	–	–	–	109.40	–	–
3‴	–	–	–	–	–	–	160.13	–	–
4‴	–	–	–	–	–	–	166.96	–	–
5‴	7.64 d (10.5)	–	–	–	–	–	111.83	–	–
6‴	7.19 dd (10.5, 2.5)	–	–	–	–	–	109.97	–	–
7‴	–	–	–	–	–	–	169.81	–	–

Note: Coupling constants (in hertz) are provided in parentheses.

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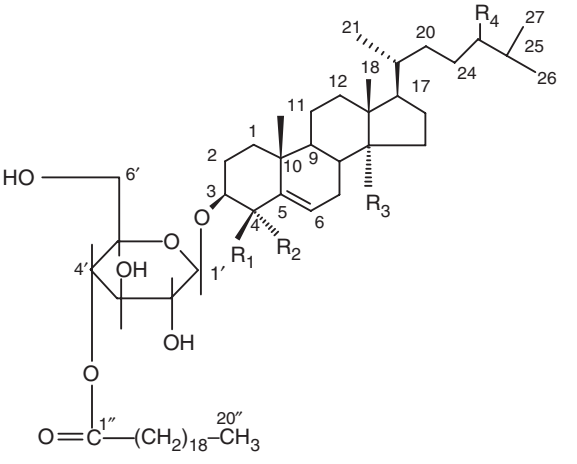
double-bond equivalents. Four of them were adjusted in the tetracyclic carbon framework of the steroidal nucleus and one each in the vinylic glycosidic and ester linkages. Removal of the glycosidic moiety from the molecular ion peak generated an ion fragment at *m/z* 396 related to dehydroxylated β-sitosterol, $C_{29}H_{48}$. The ion peaks arising at *m/z* 381 $[396 - Me]^+$, 255 $[396 - C_{10}H_{21}$, side chain$]^+$, 240 $[255 - Me]^+$, 225 $[240 - Me]^+$, 213 [255-ring C]$^+$, 198 $[213 - Me]^+$, and 183 $[198 - Me]^+$ supported that the steroidal moiety was β-sitosterol possessing a C_{10} saturated side chain (Alam, Chopra, Ali, Niwa, & Sakaet, 1994; Das, Venkateswarlu, Srinivas, & Rama Rao, 1992; Gupta, Ali, Alam, Niwa, & Sakai, 1992). Elimination of the $C_6H_{10}O_5CO(CH_2)_{18}CH_3$ moiety from the molecular ion peak yielded an ion fragment at *m/z* 413, related to a β-sitosterol ion. The ion peaks formed at *m/z* 123 $[C_{6,7}–C_{9,10}$ fission$]^+$, 137 $[C_{7,8}–C_{9,10}$ fission$]^+$, 163 $[C_{8,14}–C_{9,11}$ fission$]^+$, 177 $[C_{11,12}–C_{13,14}$ fission$]^+$, 191 $[C_{8,14}–C_{12,13}$ fission$]^+$, 106 $[123 - OH]^+$, 120 $[137 - OH]^+$, 146 $[163 - OH]^+$, 160 $[177 - OH]^+$, and 174 $[191 - OH]^+$ suggested the saturated nature of the rings C and D and the presence of the vinylic linkage at C-6 and a hydroxyl group in ring A, which was placed at C-3 on the basis of biogenetic considerations. The ion peaks produced at *m/z* 457 $[C_6H_{10}O_5 - CO(CH_2)_{18}CH_3]^+$, 576 $[C_{29}H_{49} - O–C_6H_{10}O_5]^+$, 295 $[CO\ (CH_2)_{18}CH_3]^+$, 267 [295-CO]$^+$ and the ion peaks generated at *m/z* 253, 239, 225, 211, 197, 183, 169, 155 and 141 on subsequent removal of methylene groups from the mass unit 267 indicated that an arachidic acid was esterified with the glycosidic unit.



(3) $R_1 = R_2 = R_3 = H$, $R_4 = CH_2CH_3$

(4) $R_1 = R_2 = R_3 = CH_3$, $R_4 = H$

The ^{1}H NMR spectrum of **3** showed a one-proton doublet at *m/z* 5.33 ($J = 5.5$ Hz) assigned to a vinylic H-6 proton. A one-proton broad multiplet at *m/z* 3.46 with half-width of 18.5 Hz was attributed to carbinol H-3 α-proton. Two three-proton broad signals at δ 0.67 and 1.01 were attributed to a tertiary C-18 and C-19 methyl protons. A six-proton broad signal at δ 0.83 was associated with C-29 and C-20″ methyl protons. Two three-proton doublets at δ 0.91 ($J = 6.2$ Hz) and 0.85 ($J = 6.3$ Hz) were due to secondary C-26 and C-27 methyl protons. The remaining methylene and methine protons appeared between δ 2.50 and 1.27. The presence of all the methyl signals in the range δ 1.01–0.67 suggested that all these functionalities were located on the saturated carbons. A one-proton doublet at δ 5.22 ($J = 7.1$ Hz) was assigned to an anomeric H-1′ proton. The other

protons of the sugar moiety appeared between δ 4.70 and 3.06. The appearance of a H-4′ carbinol proton in the deshielded region at δ 4.23 supported the location of the ester linkage at C-4′. The methylene protons of the fatty acid chain resonated at δ 2.94 (2H), 1.96 (2H), 1.51 (4H) and 1.24 (28H). The ^{13}C NMR spectrum of **3** exhibited signals for vinylic carbons at δ 140.41 (C-5) and 120. 91 (C-6), an ester carbon at 173.11 (C-1″), an anomeric carbon at δ 100.76 (C-1′), and other sugar carbons at δ 76.72 (C- 2′), 70.15 (C-3′), 76.55 (C-4′), 76.97 (C-5′) and 61.10 (C-6′). The appearance of C-4′ in the deshielded region at δ 76.55 indicated the attachment of a fatty acid moiety at this carbon. The C-3 carbinol signal appeared at δ 73.39. The carbon signals in the upfield region at δ 11.61, 20.46, 18.83, 19.47, 18.89, 11.49 and 18.46 were associated with the methyl functionalities. The remaining methylene and methine carbon resonated between δ 56.07 and 20.46. The ^{1}H–^{1}H COSY spectrum of **3** showed correlations of H-6 with H_2-7 and H-8; H-3 with H_2-2, H_2-4 and H-1′; and H-4′ with H-5′ and H_2-6′. The ^{1}H–^{13}C NMR spectrum of **3** exhibited interactions of C-3 with H_2-4 and H-1′; C-6 with H_2-7, H_2-4 and H-8; and C-1″ with H_2-2″ and H-4′. The ^{1}H and ^{13}C NMR spectral data of steroidal nucleus of **3** were compared with related steroidal constituents (Alam et al., 1994; Greca, Manaco, & Previtera, 1990; Gupta et al., 1992). Alkaline hydrolysis of **3** yielded β-sitosterol, D-glucose and arachidic acid. On the basis of the foregoing discussion, the structure of **3** has been elucidated as stigmast-5-en-3β-ol-3β-D-glucopyranosyl-4′-eicosanoate. This is an unknown steroidal glycoside.

Compound **4**, designated as citruslanosteroside, was obtained as a colourless crystalline mass from chloroform–methanol (9 : 1) eluents. It responded positively to triterpenic glycoside tests and exhibited characteristic absorption bands for hydroxyl groups (3510, 3433, 3315 cm^{-1}), an ester group (1737 cm^{-1}) and unsaturation (1631 cm^{-1}). Its mass spectrum exhibited a molecular ion peak at m/z 884, corresponding to a triterpenic glycoside esterified with a fatty acid, $C_{56}H_{100}O_7$. It indicated seven double-bond equivalents. Four of them were adjusted in the tetracyclic triterpenic carbon skeleton and one each in the vinylic, glycosidic and ester linkages. The prominent ion peaks generating at m/z 410 $[M - C_6H_{11}O_6CO(CH_2)_{18}CH_3]^+$, 297 [410 − C_8H_{17}, side chain]$^+$, 255 [297 – ring D]$^+$, and 240 [25 − Me]$^+$ indicated that the aglycone moiety was a lanosterol-type triterpene. The ion peaks arising at m/z 134 [$C_{6,7}$ − $C_{9,10}$ fission]$^+$, 148 [$C_{7,8} - C_{9,10}$ fission]$^+$, 174 [$C_{8,14} - C_{9,11}$ fission]$^+$, 188 [$C_{8,14} - C_{12,13}$ fission]$^+$ and 202 [$C_{8,14} - C_{12,13}$ fission]$^+$ indicated the presence of the vinylic linkage in the ring B at C-5, the saturated nature of the ring C and a hydroxyl group in ring A placed at C-3 on the biogenetic analogy (Ansari, Ali, & Quadri, 1994; Sharma & Ali, 1996). The ion peaks arising at m/z 162[$C_6H_{10}O_5$]$^+$, 312[$CH_3(CH_2)_{18}COOH$]$^+$ and 295[$CH_3(CH_2)_{18}CO$]$^+$ supported the location of the glucosidic moiety esterified with a C_{20} fatty acid.

The ^{1}H NMR spectrum of **4** displayed a one-proton doublet at δ 5.22 (J = 5.3 Hz) assigned to vinylic H-6 proton. A one-proton double doublet at δ 3.69 with coupling interaction of 5.5 and 9.5 Hz was ascribed to an α-oriented H-3 carbinol proton. Three doublets at δ 0.89 (J = 8.8 Hz), 0.87 (J = 8.8 Hz) and 0.76 (J = 5.6 Hz), all integrated for three-protons each, were attributed correspondingly to C-21, C-26 and C-27 secondary methyl protons. Five three-proton broad signals at δ 0.55, 1.13, 0.72, 0.70 and 0.68 were associated with the C-18, C-19, C-28, C-29 and C-30 tertiary methyl protons, respectively. The remaining methylene and methine protons resonated between δ 2.47 and 1.33. The presence of all methyl signals in the range δ 0.55–1.13 indicated their location on the saturated carbons. A one-proton doublet at δ 4.97 (J = 7.1 Hz) was accounted to an anomeric H-1′ proton. Two one-proton doublets at δ 3.38 (J = 9.5 Hz) and 3.31

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($J = 9.5$ Hz) were ascribed to oxygenated H_2-6′ methylene protons. The remaining sugar protons resonated between δ 4.58 and 3.95. The presence of an H-4′ carbinol proton in the deshielded region δ 4.52 as a multiplet supported esterification of the fatty acid at this carbon. Two one-proton doublets at δ 2.65 ($J = 6.4$ Hz) and 2.63 ($J = 6.4$ Hz) were assigned to H_2-2″ methylene protons adjacent to the ester group. A three-proton triplet at δ 0.75 ($J = 6.0$ Hz) was associated with C-18″ primary methyl protons. The remaining methylene protons appeared as a multiplet at δ 1.47 (2 H) and as broad signals at δ 1.17 (6 H) and 1.13 (22 H). The ^{1}H and ^{13}C NMR values of the triterpenic skeleton were compared with the similar lanostone-type molecules (Ali, 2001; Shiao, Lin, & Yeh, 1988; Tanaka & Matsunaga, 1990). ^{1}H–^{1}H COSY spectrum of **4** showed correlations of H-3 with H_2-2, H_3-28 and H-1′; H-6 with H_2-7 and H-8; and H-4′ with H-3′, H-5′ and H_2-6′. The ^{1}H–^{13}C HETCOR spectrum of **4** exhibited interactions of C-3 with H_2-2, H_3-28 and H-1′; C-5 with H-6, H_3-29 and H_2-7; and C-1″ with H_2-2″ and H-4′. Alkaline hydrolysis of **4** yielded lanosterol, D-glucose and arachidic acid. On the basis of the above discussion, the structure of **4** has been formulated as lanost-5-en-3β-ol-3β-D-glucopyranosyl-4′-eicosanoate. This is a new lanosterol-type glycoside.

3. Experimental

3.1. *General experimental procedure*

The melting points were determined on a Perfit apparatus and are uncorrected. The IR spectra were recorded in KBr pellet on a Win IR FTS 135 instrument (Bio-Rad, USA). ^{1}H (300 MHz), ^{13}C (75 MHz), and 2D NMR spectra were recorded by a Bruker spectrospin NMR instrument in $CDCl_3$, using TMS as the internal standard. FAB MS spectra were scanned on a JEOL-JMS-DX 303. Column chromatography was performed on silica gel (Merck, 60–120 mesh) and thin layer chromatography (TLC) on silica gel G coated TLC plates (Merck).

3.2. *Plant material*

The fruit peel of *C. reticulata* was collected from the local market of Khari Baoli, Delhi, and identified by Dr M.P. Sharma, Taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard (Hamdard University). A voucher specimen (No. PRL/JH/06/13) was deposited in the Herbarium of the Phytochemistry Research Laboratory, Jamia Hamdard.

3.3. *Extraction and isolation*

The air-dried peel (2 kg) of *C. reticulata* was coarsely powdered, defatted with petroleum ether and then exhaustively extracted in a Soxhlet apparatus with methanol for 72 h. The methanolic extract was concentrated under reduced pressure to obtain a dark brown viscous mass. A small portion of the extract was analysed chemically to determine the presence of different chemical constituents. The viscous dark brown mass was adsorbed on silica gel (60–120 mesh) for column after being dissolved in a little quantity of methanol for the preparation of slurry. The slurry (110 g) was air dried and chromatographed over silica gel column packed in petroleum ether. The column was eluted successively with petroleum ether, a mixture of petroleum ether and chloroform

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(9 : 1, 3 : 1, 1 : 1 and 1 : 3), pure chloroform, and finally a mixture of chloroform and methanol (99 : 1, 49 : 1, 23 : 2, 19 : 1, 97 : 3, 9 : 1). Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions (having the same R_f values) were combined and crystallised. The isolated compounds were recrystallised to obtain the pure compounds.

***n*-Hexacosanoic acid (1).** Elution of the column with chloroform afforded colourless crystals of **1**, recrystallised from chloroform–methanol (1 : 1), 160 mg (0.01% yield) R_f 0.81 (chloroform), m.p.: 80–81°C; IR υ_{max} (KBr): 3430, 2921, 2853, 1712, 1454, 1376, 1271, 1074, 722 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.28 (2H, m, H_2-2), 1.72 (2H, m, H_2 -3), 1.61 (2H, m, H_2 -4), 1.57 (2H′, m, CH_2), 1.39 (2H, m, CH_2), 1.34 (2H, m, CH_2), 1.22 (36 H′ br s, 18CH_2), 0.85 (3H, t, $J = 6.8$ Hz, Me-26). +ve ion FAB MS: m/z (rel. int.): 396 $[M]^+$ ($C_{26}H_{52}O_2$) (100), 381 (12.6), 339 (3.2), 255 (10.6), 213 (11.6), 199 (11.7), 185 (12.3), 171 (15.8), 157 (16.9), 143 (20.7), 129 (21.1).

Reticulataursenoside (2). Elution of the column with chloroform–methanol (97 : 3) furnished colourless crystals of **2**, recrystallised from methanol, 275 mg, (0.183% yield). R_f 0.73 (chloroform–methanol, 97 : 3), m.p. 210–211°C. UV λ_{max} (MeOH): 223, 266 (log ε 5.6, 2.1). IR υ_{max} (KBr): 3510, 3459, 3390, 2962, 2850, 1746, 1710, 1635, 1511, 1460, 1359, 1124, 1027 cm^{-1}. ^1H NMR (DMSO-d_6): Table 1, ^{13}C NMR (DMSO-d_6): Table 1. +ve ion FAB MS m/z (rel. int.): 898 $[M]^+$ (C_{49} H_{70} O_{15}) (1.1), 438 (11.5), 343 (5.6), 232 (1.1), 217 (11.3), 205 (16.3), 202 (17.6), 190 (14.8), 187 (19.5), 179 (12.3), 175 (26.8), 172 (9.8), 166 (31.9), 160 (34.1), 154 (98.6), 152 (26.3), 146 (26.5), 145 (53.2), 137 (71.6).

Alkaline hydrolysis of 2. Compound **2** (45 mg) was dissolved in ethanol (5 mL), 1 N NaOH solution (1 mL) was added, and the reaction mixture was heated on a steam bath for 1 h. It was dried under reduced pressure and the residue was dissolved in $CHCl_3$ (5 mL). The $CHCl_3$ layer was washed with H_2O (2 × 5 mL), dried over anhydrous Na_2SO_4 and evaporated to get triterpene (IR υ_{max} 3450, 1710 cm^{-1}). The residue was dissolved in H_2O (5 mL), acidified with diluted HCl and re-extracted with $CHCl_3$, which, on drying, afforded protocatechuic acid, m.p. 197–199°C. The aqueous layer was concentrated and chromatographed over paper with a standard sample of D-glucose. R_f 0.12 (*n*-butenol–acetic acid–water, 4 : 1 : 5, top layer).

Citrusteryl arachidate (3). Elution of the column with chloroform–methanol (23 : 2), gave colourless amorphous powder of **3**, recrystallised from methanol, 310 mg (0.26% yield) R_f 0.82 $CHCl_3$–MeOH (8%) m.p.: 239–240°C, IR υ_{max} (KBr): 3490, 3421, 3365, 2854, 1735, 1637, 1463, 1374, 1166, 1073, 1020 cm^{-1}. ^1H NMR (DMSO-d_6): Table 1, ^{13}C NMR (DMSO-d_6): Table 1. +ve ion FAB MS m/z (rel. int.): 870 $[M]^+$ ($C_{55}H_{98}O_7$) (1.1), 576 (11.6), 457 (2.3), 413 (13.2), 396 (22.5), 381 (16.1), 295 (31.9), 278 (11.3), 267 (11.7), 255 (31.6), 253 (22.1), 240 (19.8), 239 (17.6), 225 (20.3), 213 (26.5), 211 (22.6), 198 (37.5), 197 (31.0), 191 (20.4), 183 (35.6), 177 (32.5), 174 (79.1), 169 (41.5), 163 (52.6), 162 (61.8), 160 (78.2), 155 (49.1), 146 (98.3), 141 (43.5), 137 (91.5), 123 (65.2), 120 (95.6), 106 (97.2), 95 (99.1).

Alkaline hydrolysis of 3. Compound **3** (45 mg) was dissolved in ethanol (5 mL), 1N NaOH solution (1 mL) added and the reaction mixture heated on the steam bath for 1 h. It was dried under reduced pressure and the residue was dissolved in $CHCl_3$ (5 mL). The $CHCl_3$ layer was washed with water (2 × 5 mL), dried over anhydrous Na_2SO_4 and evaporated to get β-sitosterol, m.p. 136–137°C. The residue dissolved in H_2O (5 mL),

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acidified with diluted HCl and re-extracted with $CHCl_3$. Evaporation of the organic layer gave arachidic acid, m.p. 75°C, TLC comparable. The aqueous phase was chromatographed on a TLC plate with a standard solution of D-glucose; R_f values were comparable.

Citruslanosteroside (4). Elution of column with chloroform–methanol (9 : 1) mixture (fractions 170–200), furnished a colourless crystalline mass of **4**, recrystallised from methanol, 110 mg, (0.073% yield) R_f 0.74 (chloroform–methanol; 9 : 1), m.p.: 249–250°C, IR υ_{max} (KBr): 3510, 3433, 3315, 2924, 2855, 1737, 1631, 1462, 1373, 1167, 1080, 1022 cm^{-1}. ^1H NMR: Table 1. +ve ion FAB MS *m/z* (rel. int.): 884 $[M]^+$ ($C_{56}H_{100}O_7$) (1.1), 410 (26.3), 397 (100), 382 (16.9), 314 (6.9), 312 (10.1), 297 (11.8), 295 (10.9), 240 (5.3), 202 (11.3), 188 (10.1), 174 (18.3), 162 (22.3), 148 (24.7), 134 (21.2).

Hydrolysis of 4. Compound **4** (35 mg) was heated with alkaline ethanolic solution (5 mL) on a steam bath for 1 h. The solvent was evaporated under reduced pressure and the residue was dissolved with chloroform to get lanosta-5-en-3β-ol. Water (5 mL) was added to the residue, acidified with dilute HCl and re-extracted to secure arachidic acid, m.p. 75°C, Co-TLC comparable. The aqueous solution was chromatographed over paper with standard D-glucose, R_f 0.12 (*n*-butenol–acetic acid–water, 4 : 1 : 5, top layer).

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