

A New Acylglycosyl Sterol from *Quisqualis Fructus*

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(Received January 22, 2003)

A new acylglycosyl sterol (**4**) was isolated from the MeOH extract of *Quisqualis Fructus* together with four known compounds. On the basis of spectroscopic data, their structures were elucidated as clerosterol (**1**), betulinic acid (**2**), methylursolate (**3**), 3-O-[6'-O-(8Z-octadecenoyl)- β -D-glucopyranosyl]-clerosterol (**4**) and α -xylofuranosyluracil (**5**).

Key words: *Quisqualis indica*, *Quisqualis Fructus*, Acylglycosyl sterol

INTRODUCTION

The fruits of *Quisqualis indica* (Combretaceae), *Quisqualis Fructus*, is a Korean traditional medicine to treat ascariasis (Sho-ugakukan, 1985). Tannins (Lin *et al.*, 1997) and non-peptide amino acids (Takemoto *et al.*, 1975) were reported from *Quisqualis Fructus*. Our research on this source led to the isolation of a new acylglycosyl sterol, 3-O-[6'-O-(8Z-octadecenoyl)- β -D-glucopyranosyl]-stigmasta-5, 25(27)-diene (**4**), together with four known compounds **1-3**, **5**. The present paper describes the isolation and structural characterization of clerosterol (**1**), betulinic acid (**2**), methylursolate (**3**), 3-O-[6'-O-(8Z-octadecenoyl)- β -D-glucopyranosyl]-clerosterol (**4**) and α -xylofuranosyluracil (**5**).

MATERIALS AND METHODS

General

Melting points were determined on Gallenkamp melting point apparatus and uncorrected. Optical rotations were measured on a Jasco P-1020 Polarimeter. NMR spectra were recorded on either a Bruker AMX or a Varian UNITY INOVA 500 NMR spectrometer. MS data were obtained on a JEOL JMS700 mass spectrometer and GC-MS data were taken on a Hewlett-Packard 6890 GC (column: HP-5MS 30 m 0.25 mm)/Hewlett-Packard 5973 MSD system. Open column chromatography was carried out over silica

gel (Merck, 70-230) or Sephadex LH-20 (Pharmacia). Low pressure liquid chromatography was carried out over Merck Lichroprep Lobar-A Si 60 (240 \times 10 mm) or Lichroprep Lobar-B RP-18 (240 \times 10 mm) column with FMI QSY-0 pump (ISCO).

Material

Quisqualis Fructus was purchased at KyungDong market in March, 2001, Seoul, Korea. The voucher specimen (SKK-161) is deposited at the College of Pharmacy in SungKyunKwan University.

Extraction and isolation

The dried and ground *Quisqualis Fructus* (1.0 kg) was extracted with MeOH five times at room temperature and three times at 60°C. The resultant methanol extract (300 g) was suspended in H₂O and then successively partitioned to give *n*-hexane (100 g), chloroform (90 g) and *n*-butanol (16 g) soluble fractions. The hexane extract (100 g) was chromatographed over silica gel using gradient solvent system of *n*-hexane-EtOAc (5:1-0:1) to give six main fractions (H1-H6). Fr. H3 (5 g) was chromatographed over Sephadex LH-20 eluted with CH₂Cl₂-MeOH (1:1) to give two fractions (H31 and H32). Fr. H32 (1.7 g) was subjected to Lobar[®] RP-18 chromatography eluted with methanol to give four subfractions (H321-H324). Fr. H324 (800 mg) was purified with silica gel Lobar[®]-A column (*n*-hexane-EtOAc, 5:1) to yield **1** (120 mg). Fr. H4 (5.0 g) was chromatographed over Sephadex LH-20 eluted with CH₂Cl₂-MeOH (1:1) to give three fractions (H41 and H43). Fr. H42 (230 mg) was purified with Lobar[®]-A Si-60 column (*n*-hexane-EtOAc, 5:1) to afford **2** (100 mg). Fr. H5 (3.0 g)

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was chromatographed over Sephadex LH-20 eluted with CH_2Cl_2 -MeOH (1:1) to give two fractions (H51 and H52). Fr. H52 (600 mg) was subjected to Lobar[®] RP-18 chromatography eluted with 95% methanol to give three subfractions (H521-H523) and Fr. H522 (70 mg) was purified with Lobar[®]-A Si-60 column (*n*-hexane-EtOAc, 5:1) to yield **3** (30 mg). The chloroform extract (90 g) was chromatographed on silica gel using gradient solvent system of *n*-hexane-EtOAc (3:1 0:1) to give six subfractions (C1-C6). Fr. C5 was chromatographed on silica gel eluting with *n*-hexane:EtOAc (1:1) and Sephadex LH-20 with CH_2Cl_2 -MeOH (1:1) to give the major subfraction C52. Fr. C52 was purified with silica gel Lobar[®]-A column (*n*-hexane-EtOAc, 2:1) to yield **4** (17 mg). The BuOH extract (16 g) was subjected to silica gel column chromatography using EtOAc-MeOH-H₂O (9:3:1) to give five subfractions (B1-B5). Fr. B2 (622 mg) was chromatographed over Sephadex LH-20 (MeOH) to give two subfractions (B21-B23). Fr. B23 (120 mg) was subjected to silica gel chromatography eluted with EtOAc-MeOH-H₂O (9:2:0.5) to give two subfractions (B231 and B232). Fr. B232 was purified with Lobar[®]-A Si-60 column (EtOAc-MeOH-H₂O, 9:2:0.5) to afford **5** (10 mg).

Clerosterol (1)

White powder, mp. 145°C; $[\alpha]_D -45.2$ (c 1.00, CHCl_3); EI-MS *m/z* (rel. int.): 412 (*M*⁺, 100), 394 (65), 379 (32), 314 (35), 299 (28), 273 (24), 255 (38), 229 (33), 213 (59); ¹H-NMR (500 MHz, CD_3OD) δ : 0.69 (3H, s, H-18), 0.82 (3H, t, *J* = 7.4 Hz, H-29), 0.92 (3H, d, *J* = 6.5 Hz, H-21), 1.02 (3H, s, H-19), 1.58 (3H, s, H-27), 3.53 (1H, m, H-3), 4.66 (1H, br.d, *J* = 2.3 Hz, H-26), 4.74 (1H, br.dd, *J* = 2.3, 1.4 Hz, H-26), 5.36 (1H, br.d, *J* = 5.2 Hz, H-6); ¹³C-NMR (125 MHz, CD_3OD): Table I.

Betulinic acid (2)

White powder, mp. 250°C; $[\alpha]_D +3.7$ (c 0.15, MeOH); ¹H-NMR (500 MHz, CD_3OD) δ : 0.77 (3H, s), 0.84 (3H, s), 0.96 (3H, s), 0.98 (3H, s), 0.99 (3H, s), 1.71 (3H, s, H-30), 3.03 (1H, m, H-19), 3.20 (1H, m, H-3), 4.63 (1H, br.s, H-29), 4.75 (1H, br.s, H-29); ¹³C-NMR (125 MHz, CD_3OD): Table I.

Methylursolate (3)

White powder, mp 285°C; ¹H-NMR (500 MHz, CDCl_3) δ : 0.75 (3H, s), 0.78 (3H, s), 0.86 (3H, d, *J* = 6.5 Hz), 0.92 (3H, s), 0.94 (3H, d, *J* = 6.5 Hz), 0.99 (3H, s), 1.08 (3H, s), 3.21 (1H, m, H-3), 3.73 (3H, s), 5.24 (1H, m, H-12); ¹³C-NMR (125 MHz, CDCl_3): Table I.

3-O-[6'-O-(8Z-Octadecenoyl)-β-D-glucopyranosyl]-clerosterol (4)

Colorless gum, $[\alpha]_D -40.7$ (c 0.15, CHCl_3); EI-MS *m/z*

Table I. ¹³C-NMR Data of Compounds **1-4** (125 MHz, CDCl_3 , δ ppm)

	1	2	3	4
1	37.5	38.7	39.9	37.5
2	31.9	27.4	29.0	29.4
3	72.0	79.0	80.0	74.1
4	42.5	38.9	40.2	39.1
5	141.0	55.3	56.7	140.5
6	121.9	18.3	19.6	122.4
7	32.1	34.3	34.4	32.2
8	32.1	40.7	40.8	32.2
9	50.4	50.5	48.9	50.4
10	36.7	37.2	38.3	37.0
11	21.3	20.9	24.5	21.3
12	40.0	25.5	126.4	40.0
13	42.6	38.4	140.1	42.6
14	57.0	42.4	43.3	57.0
15	24.5	30.6	29.5	24.5
16	28.4	32.2	25.8	28.4
17	56.3	56.3	48.9	56.3
18	12.3	46.9	54.4	12.1
19	19.6	49.3	40.3	19.6
20	35.8	150.4	40.2	35.8
21	18.9	29.7	31.9	18.9
22	33.9	37.0	38.1	33.9
23	29.6	28.0	29.6	29.5
24	49.8	15.4	16.5	49.8
25	147.8	16.0	17.4	147.8
26	18.0	16.1	18.3	18.0
27	111.6	14.7	24.7	111.7
28	26.8	180.4	179.0	26.8
29	12.1	109.7	18.3	12.3
30		19.4	22.2	
			OMe 52.6	
1'				101.5
2'				73.7
3'				76.3
4'				70.4
5'				76.3
6'				63.6
1''				174.9
2''				32.1
8'', 9''				130.0, 130.3
18''				14.4
3''~7'', 10''~17''				23.0, 25.2, 27.5, 29.5, 29.6, 29.8, 29.9, 30.0, 34.5

(rel. int.): 574 [M-fatty acid chain]⁺ (5), 412 [M-fatty acid-glucose]⁺ (13), 394 (100); ¹H-NMR (500 MHz, CDCl_3) δ : 0.67 (3H, s, H-18), 0.78 (3H, t, *J* = 7.5 Hz, H-29), 0.88 (3H, t, *J* = 7.0 Hz, H-18''), 0.91 (3H, d, *J* = 6.5 Hz, H-21), 1.00 (3H, s, H-19), 1.25 (22H, br.s, -CH₂-), 1.59 (3H, s, H-27), 2.00 (4H, m, H-7'', H-10''), 2.34 (2H, t, *J* = 7.0 Hz, H-2''), 3.37 and 3.54 (each 2H, m, H-2', 3', 4', 5'), 3.45 (1H, m, H-3), 4.25 (1H, br.d, *J* = 12.0 Hz, H-6'a), 4.37 (1H, d, *J* = 7.5 Hz, H-1'), 4.42 (1H, dd, *J* = 12.0, 5.0 Hz, H-6'b), 4.64 (1H, br.d, *J* = 2.5 Hz, H-26), 4.72 (1H, m, H-26), 5.35 (3H, m, H-5, H-8'' and H-9''); ¹³C-NMR (125 MHz, CDCl_3): Table I.

Alkaline hydrolysis of 4

A solution of **4** (1 mg) with 2.3 g dry NaOMe in MeOH

(1 mL) was stirred at room temperature for 12 hours. The reaction mixture was neutralized with 1N HCl and partitioned between MeOH and *n*-hexane. The *n*-hexane layer was evaporated in vacuo to yield fatty acid methyl ester. The fatty acid methyl ester was identified as 8Z-octadecenoic acid methyl ester by GC-MS analysis.

α -Xylcfuranosyl-uracil (5)

Colorless gum, $^1\text{H-NMR}$ (500 MHz, CD_3OD) δ : 3.72 (1H, dd, $J = 12.2, 3.1$, H-5'), 3.83 (1H, dd, $J = 12.2, 2.8$, H-5'), 3.99 (1H, br. dt like, $J = 4.5, 2.8$, H-4'), 4.14 (1H, dd, $J = 5.1, 4.5$ Hz, H-3'), 4.17 (1H, dd, $J = 5.1, 4.6$ Hz, H-2'), 5.68 (1H, d, $J = 8.1$ Hz, H-5), 5.89 (1H, d, $J = 4.6$ Hz, H-1'), 8.00 (1H, d, $J = 8.1$ Hz, H-6); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD) δ : 62.2 (C-5'), 71.3 (C-4'), 75.7 (C-3'), 86.3 (C-2'), 91.7 (C-1'), 102.6 (C-5), 142.7 (C-6), 152.4 (C-2), 166.2 (C-4)

RESULTS AND DISCUSSION

Compound **1** was obtained as white powder and its molecular ion peak appeared at m/z 412 in EI-MS spectrum. The molecular formula was assigned as $\text{C}_{29}\text{H}_{48}\text{O}$ based on $^{13}\text{C-NMR}$ data ($\text{C}\times 29$) and the molecular ion peak in EI-MS. The $^1\text{H-NMR}$ spectrum showed signals for two tertiary methyl groups at δ 0.69 (3H, s) and 1.02 (3H, s), an allylic methyl group at δ 1.58 (3H, s), a secondary methyl group at δ 0.92 (3H, d, $J = 6.5$ Hz), a primary methyl group at δ 0.82 (3H, t, $J = 7.4$ Hz), an oxygenated

proton at δ 3.53 (1H, m), terminal methylene protons at δ 4.66 (1H, br.d, $J = 2.3$ Hz) and 4.74 (1H, br.dd, $J = 2.3, 1.4$ Hz), and an olefinic proton at δ 5.36 (1H, br.d, $J = 5.2$ Hz). In the $^{13}\text{C-NMR}$ spectrum, 29 carbon signals appeared, which included an oxygenated carbon at δ 72.0 and four olefinic carbons at δ 111.6, 121.9, 141.0 and 147.8. The NMR data of **1** were very similar to those of β -sitosterol but major differences were the presence of terminal methylene and the downfield shift of H-27 signal (δ 1.58) in the $^1\text{H-NMR}$ spectrum of **1**, indicated the structure of **1** include $\Delta_{25,26}$ terminal methylene. On the basis of the above evidences, the structure of **1** was suggested to clerosterol. The NMR data and optical rotation (-45.2°) of **1** were in good agreement with the previous data of clerosterol (Gaspar *et al.*, 1996).

Compound **2** was obtained as white powder. $^1\text{H-}$ and $^{13}\text{C-NMR}$ spectra showed the typical pattern of pentacyclic triterpene (Mahato and Kundu, 1994). Especially, the $^1\text{H-NMR}$ spectrum of **2** was characteristic of the presence of an allylic methyl group at δ 1.71 (3H, s) and terminal methylene protons at δ 4.63 (1H, br.s) and 4.75 (1H, br.s). On the basis of the above evidences, the structure of **2** was suggested to betulinic acid. The NMR and physical data of **2** were in good agreement with the previous data of betulinic acid (Mahato and Kundu, 1994).

Compound **3** was obtained as white powder. $^1\text{H-}$ and $^{13}\text{C-NMR}$ spectra of **3** also showed the typical pattern of pentacyclic triterpene (Mahato and Kundu, 1994). The $^1\text{H-NMR}$ spectrum showed five methyl singlets at δ 0.75 (3H,

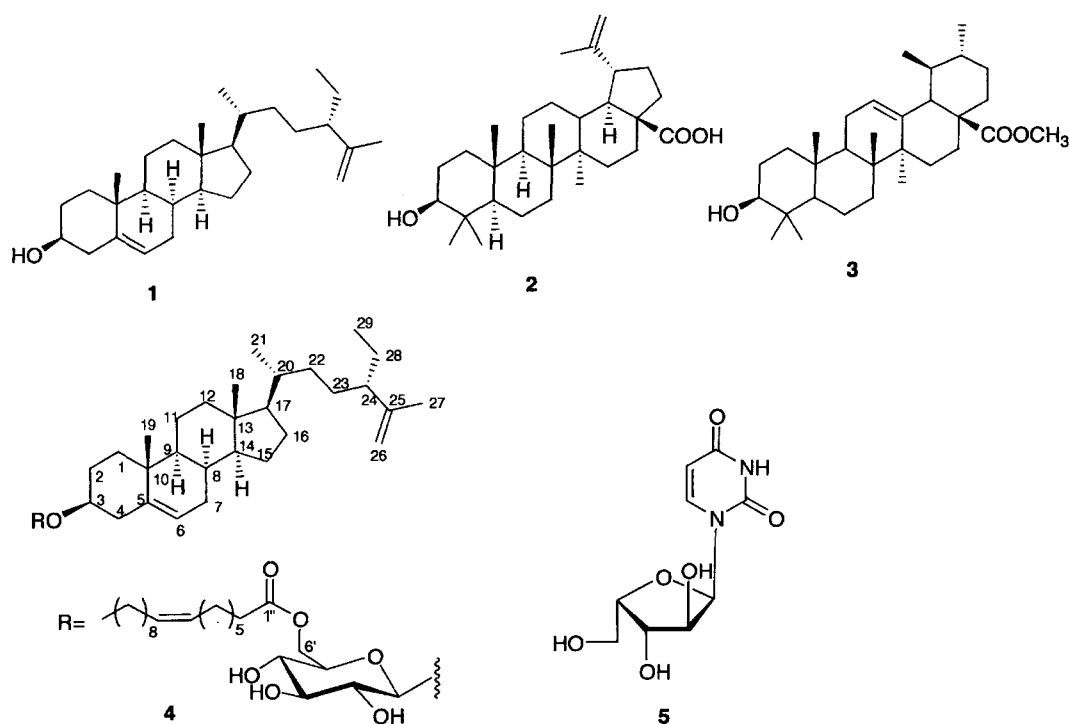


Fig. 1. Structures of Compounds 1-5

s), 0.78 (3H, s), 0.92 (3H, s), 0.99 (3H, s) and 1.08 (3H, s), two methyl doublets at δ 0.86 (3H, d, $J = 6.5$ Hz) and 0.94 (3H, d, $J = 6.5$ Hz), an oxygenated proton signal at δ 3.21 (1H, m), a methoxy signal at δ 3.73 (3H, s), and an olefinic proton at δ 5.24 (1H, m). In the ^{13}C -NMR spectrum, 31 carbon signals appeared, which included an carbinol carbon at δ 80.0, methoxy group at δ 52.6, two olefinic carbons at δ 126.4 and 140.1, and carbonyl group at δ 179.0. The above evidences suggested the structure of **3** was methylursolate. The ^{13}C -NMR spectrum of **3** was in good agreement with the literature data (Mahato and Kundu, 1994).

Compound **4** was obtained as colorless gum. The characteristic signals of a fatty acid chain were observed at δ 0.78 (3H, t, $J = 7.5$ Hz), 1.25 (22H, br.s), 2.00 (4H, m), 2.34 (2H, t, $J = 7.0$) and 5.35 (2H, m) in the ^1H -NMR spectrum. The ^1H -NMR spectrum also showed the signals corresponding to a sugar moiety at δ 3.37 and 3.54 (each 2H, m), 4.25 (1H, br.d, $J = 12.0$ Hz), 4.37 (1H, d, $J = 7.5$ Hz) and 4.42 (1H, dd, $J = 12.0, 5.0$ Hz, H-6). The ^{13}C -NMR spectrum showed the signals for a sugar moiety at δ 63.6, 70.4, 73.7, 76.3 ($\times 2$) and 101.5. The NMR data of an aglycon unit in **4** were very similar to those of **1** but major differences were the downfield shift of C-3 (74.1) and the upfield shift of C-2 (δ 29.4) and C-4 (δ 39.1) in the ^{13}C -NMR spectrum of **4**, indicated the sugar unit was bonded at C-3 of clerosterol. The alkaline hydrolysis with NaOMe in MeOH of **4** and the solvent fractionation of reaction mixture with *n*-hexane/ H_2O yielded 8Z-octadecenoic acid methyl ester in *n*-hexane soluble portion, which was identified by GC-MS analysis. The aqueous portion treated with 1N-HCl yielded glucose, which was identified by cellulose TLC with authentic glucose. The downfield shift of H-6' in glucose unit was suggested that fatty acid unit was bonded at C-6 of glucose. On the basis of above evidences, the structure of **4** was determined as 3-O-[6'-O-(8Z-octadecenoyl)- β -D-glucopyranosyl]-clerosterol. Recently, several acylglucopyranosylclerosterols were isolated from natural sources (Yang *et al.*, 2000; Guevara *et al.*, 1989) but 8Z-octadecenoylglucopyranosylclerosterol derivatives have not been reported.

Compound **5** was obtained as colorless gum. The ^1H -NMR spectrum showed the signals corresponding to a sugar moiety at δ 3.72 (1H, dd $J = 12.2, 3.1$ Hz), 3.83 (1H, dd, $J = 12.2, 2.8$ Hz), 3.99 (1H, br. dt like, $J = 4.5, 2.8$ Hz), 4.14 (1H, dd, $J = 5.1, 4.5$ Hz), 4.17 (1H, dd, $J = 5.1, 4.6$ Hz), 5.68 (1H, d, $J = 8.1$ Hz). The ^{13}C -NMR spectrum showed

the signals for a sugar moiety at δ 62.2, 71.3, 75.7, 86.3 and 90.7. The coupling constants of proton signals and the chemical shift of five carbon signals in sugar unit suggested the sugar is α -xylofuranose (Pouchert and Behnke, 1993). The ^1H - and ^{13}C -NMR spectra also showed the signals for uracil unit at δ 5.68 (1H, d, $J = 8.1$ Hz) and 8.00 (1H, d, $J = 8.1$ Hz), and at δ 102.6, 142.7, 152.4 and 166.2, respectively (Pouchert and Behnke, 1993). Thus, the structure of **5** was suggested to 3-amino-2Z-propenamide and the NMR data of **5** were in good agreement with the previous values of α -L-xylofuranosyl-uracil (Holy, 1973).

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