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A New Acylglycosyl Sterol from Quisqualis Fructus

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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*-(8*Z*-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 (Shougakukan, 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*-(8*Z*-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*-(8*Z*-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 (240×10 mm) or Lichroprep Lobar-B RP-18 (240×10 mm) column with FMI QSY-0 pump (ISCO).

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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 Qusqualis 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 H_2O 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 CH_2Cl_2-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 CH_2Cl_2-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)

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-H_2O (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-H_2O (9:2:0.5) to give two subfractions (B231 and B232). Fr. B232 was purified with Lobar®-A Si-60 column (EtOAc-MeOH-H_2O, 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); ^{1}H-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); ^{13}C-NMR (125 MHz, CD_3OD) : Table I.

Betulinic acid (2)

White powder, mp. 250°C; $[\alpha]_D$ +3.7 (c 0.15, MeOH); ^{1}H-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); ^{13}C-NMR (125 MHz, CD_3OD) : Table I.

Methylursolate (3)

White powder, mp 285°C; ^{1}H-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); ^{13}C-NMR (125 MHz, $CDCl_3$) : Table I.

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

Colorless gum, $[\alpha]_D$ -40.7 (c 0.15, $CHCl_3$); EI-MS *m/z* (rel. int.): 574 [M-fatty acid chain]$^+$ (5), 412 [M-fatty acid-glucose]$^+$ (13), 394 (100); ^{1}H-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, -CH_2-), 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″); ^{13}C-NMR (125 MHz, $CDCl_3$) : Table I.

Table I. ^{13}C-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

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 8*Z*-octadecenoic acid methyl ester by GC-MS analysis.

α-Xylofuranosyl-uracil (5)

Colorless gum, ^{1}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}C-NMR (125 MHz, CD_3OD) δ : 62.2 (C-5′), 71.3 (C-4′), 75.7 (C-3′), 86.3 (C-2′), 90.7 (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 $C_{29}H_{48}O$ based on ^{13}C-NMR data (C×29) and the molecular ion peak in EI-MS. The ^{1}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}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}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}H- and ^{13}C-NMR spectra showed the typical pattern of pentacyclic triterpene (Mahato and Kundu, 1994). Especially, the ^{1}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}H- and ^{13}C-NMR spectra of **3** also showed the typical pattern of pentacyclic triterpene (Mahato and Kundu, 1994). The ^{1}H-NMR spectrum showed five methyl singlets at δ 0.75 (3H,

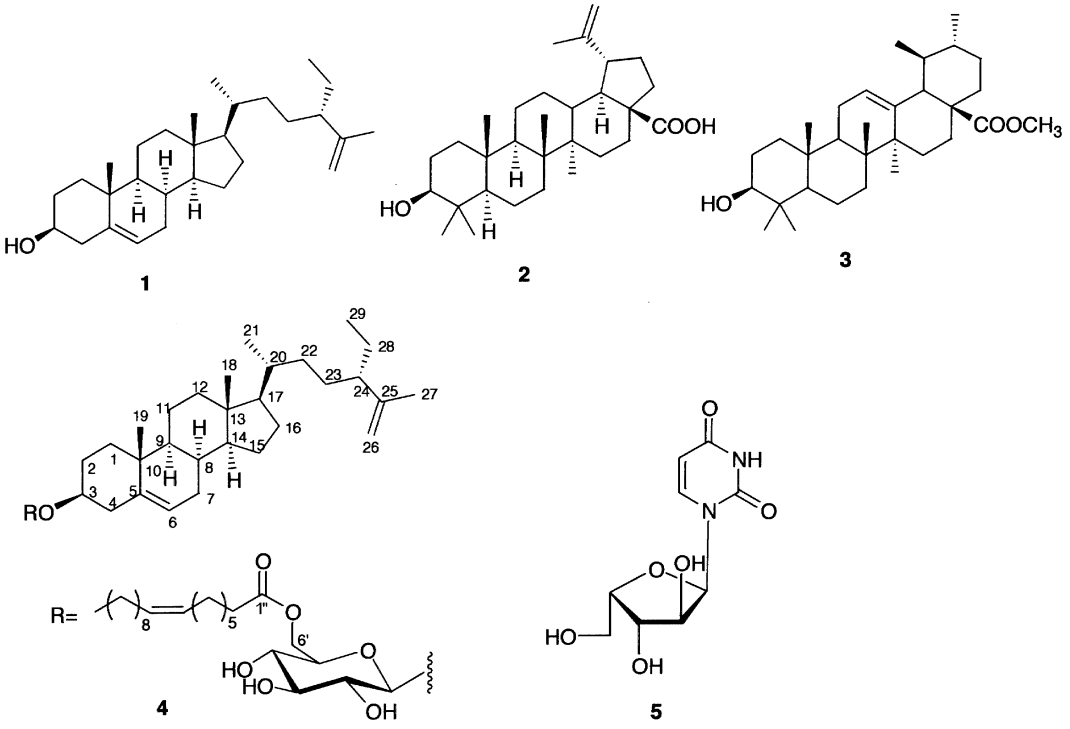

Fig. 1. Structures of Compounds **1**-**5**

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 ^{1}H-NMR spectrum. The ^{1}H-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 (×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 8*Z*-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*-(8*Z*-octadecenoyl)-β-D-glucopyranosyl]-clerosterol. Recently, several acylglucopyranosylclerosterols were isolated from natural sources (Yang *et al.,* 2000; Guevara *et al.,* 1989) but 8*Z*-octadecenoylglucopyranosylclerosterol derivatives have not been reported.

Compound **5** was obtained as colorless gum. The ^{1}H-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 ^{1}H- 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-2*Z*-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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