A New Pentacyclic Cucurbitane Glucoside and a New Triterpene from the Fruits of *Gymnopetalum integrifolium*

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A new pentacyclic cucurbitane glucoside, named aoibaclyin (1) and a new triterpene (2) have been isolated from the EtOH extract of the fruits of *Gymnopetalum integrifolium* KURZ (Cucurbitaceae), together with three known compounds, bryoamaride (3), 25-*O*-acetylbryoamaride (4) and β-sitosterol 3-*O*-β-D-glucopyranoside (5). The structures of these compounds were elucidated by spectroscopic analyses.

Key words *Gymnopetalum integrifolium*; Cucurbitaceae; pentacyclic cucurbitane glucoside; aoibaclyin; bitter principle; 3,29-*O*-dibenzoyloxykarounidiol

Gymnopetalum integrifolium KURZ (Cucurbitaceae) is a medicinal plant which has been used as a purgative folk medicine in Thailand. A number of cucurbitanes were previously reported from the family Cucurbitaceae,[1,2] however no phytochemical study has been undertaken on this species or the genus *Gymnopetalum*. As a part of our phytochemical studies on Asian medicinal plants and crude drugs,[3—5] the nonpolar fraction of the EtOH extract of the fruits of *G. integrifolium*, collected in Thailand, was investigated.

This paper describes the isolation and structure determination of five constituents (**1**—**5**), including two new compounds (**1**, **2**), from the fruits of this plant.

Results and Discussion

The 75% EtOH extract of the fruits was partitioned with *n*-hexane, benzene, EtOAc and *n*-BuOH successively. Each extract was concentrated and the *n*-hexane and EtOAc extracts were purified by chromatography with silica gel, octadecyl silica (ODS) and Sephadex LH-20 to yield bitter-tasting compounds (**1**, **3**, **4**) from the EtOAc extract and non-tasting compounds (**2**, **5**) from the *n*-hexane extract. Spraying **1**—**5** on TLC plates with 50% H_2SO_4 suggested a triterpene structure. Compound **5** was identified as β-sitosterol 3-*O*-β-D-glucopyranoside by comparison of its physicochemical data with those of an authentic sample.

Inspection of the ^{1}H- and ^{13}C-NMR spectra revealed that **1**, **3** and **4** possess a monosaccharide unit, despite being relatively nonpolar. The UV and IR spectra were typical for the diosphenol structure of ring A in cucurbitacin.[6,7]

Compound **3** showed the molecular formula $C_{36}H_{54}O_{12}$ by the presence of an $[M+H]^+$ ion at *m/z* 701 in the FAB-MS and by ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) analysis. The ^{13}C-NMR spectrum of **3** displayed 36 carbon signals, of which 30 were attributed to the triterpenoid moiety and six to the sugar moiety (Table 1). Thus compound was identified as bryoamaride (Fig. 1) by comparison of its NMR data with values reported in the literature.[8]

The FAB-MS of **4** exhibited an $[M+Na]^+$ ion at *m/z* 743, which exceeded that of **3** by 42 mass units. The ^{1}H- and ^{13}C-NMR spectra of **4** showed similar patterns to **3**, including the region of sugar signals (Table 1). The remarkable low field shift of the C-25 signal (+8.1 ppm) and the high field shifts of the C-24 (−3.4 ppm), C-26 (−3.0 ppm) signals of **4** are apparently due to the presence of the acetyl group at the C-25 position in **4**. Therefore **4** was concluded to be 25-*O*-acetylbryoamaride (Fig. 1).[6]

Aoibaclyin (**1**) was isolated as a pale yellow amorphous

Table 1. ^{13}C-NMR Data of Compounds **1**, **3** and **4**

Carbon	**1**	**3**	**4**
1	128.4	123.6	124.2
2	144.6	147.1	145.5
3	199.6	199.8	198.6
4	49.2	50.1[a)]	48.2
5	135.3	137.5	135.6
6	121.6	122.3	121.2
7	23.8	24.6	23.7
8	41.2	43.1	41.3
9	49.8	50.3[a)]	49.0
10	35.4	36.4	35.3
11	215.3	216.5	213.9
12	48.5	50.2	49.0
13	48.2[a)]	49.0[b)]	49.1
14	48.0[a)]	51.6[b)]	50.5
15	41.0	46.8	45.6
16	76.0	71.4	70.9
17	55.3	59.4	57.8
18	19.7	20.6	19.9
19	20.2	20.6	18.1
20	72.1	80.8	81.3
21	29.0	25.5	24.5
22	48.8	217.2	214.3
23	73.0	33.1	30.7
24	124.9	38.1	34.7
25	136.6	70.8	78.9
26	25.8	29.1[c)]	26.1
27	18.4	29.4[c)]	27.8
28	20.3[b)]	18.7	20.2
29	27.7	28.3	25.8
30	20.2[b)]	20.8	20.1
$\underline{C}OCH_3$	—	—	170.4
$CO\underline{C}H_3$	—	—	22.4
1′	101.2	101.1	100.4
2′	71.9	74.3	72.3
3′	75.5	77.5	76.7
4′	69.0	70.6	69.4
5′	77.0	78.1	75.8
6′	61.5	61.9	61.7

a—*c*) Assignments may be interchangeable in each vertical column.

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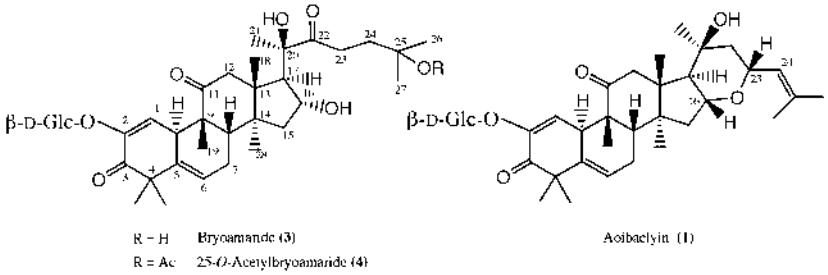

Fig. 1. Structures of Cucurbitane Glucosides (**1**, **3**, **4**)

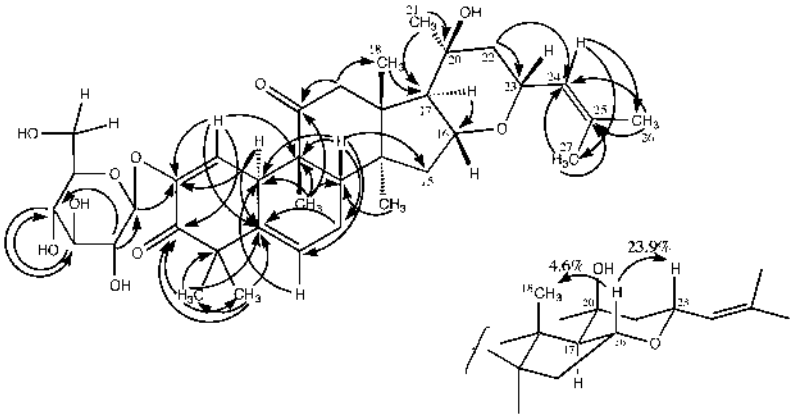

Fig. 2. ^{13}C–^{1}H Long-Range Correlations and NOE Experiments of **1**

solid with positive optical rotation, showing a violet spot on TLC when sprayed with a 50% H_2SO_4 solution followed by heating on a hot plate. In the positive ion FAB-MS spectrum, an $[M+Na]^+$ ion at *m/z* 667 as well as a fragment ion $[M-162+H]^+$ at *m/z* 483 due to the loss of a hexose unit without the glycosidic oxygen, and an $[M+Li]^+$ ion at *m/z* 651 due to addition of LiI, indicated its molecular weight to be 644. The high resolution (HR)-FAB-MS of **1** displayed an $[M+Na]^+$ ion at *m/z* 667.3422 corresponding to the molecular formula $C_{36}H_{52}O_{10}$. The ratio of carbons to hydrogen in the molecule indicates eleven degrees of unsaturation. The general features of the NMR, as described bellow, indicated two carbonyls, three C=C double bonds, and one hexose group. Because no other unsaturated function was indicated by the spectral data, the five remaining unsaturations were accounted for by five rings, suggestive of a pentacyclic triterpene derivative.

The ^{1}H-NMR spectrum of **1** showed the presence of eight tertiary methyl groups [δ 0.90, 0.93, 1.30, 1.67, 1.69, 1.27, 1.21, 1.27], three olefinic hydrogens [δ 6.27 (H-1, d, J=2.4 Hz), 5.77 (H-6, br s), 5.12 (H-24, d, J=8.2 Hz)] and an anomeric hydrogen [δ 4.68 (H-1′, d, J=7.9 Hz)] in the sugar part. The ^{13}C-NMR spectrum exhibited 36 carbons (Table 1), including two carbonyls at δ 199.6 (C-4) and 215.3 (C-11), an olefinic quaternary carbon at δ 135.3 (C-5) and an olefinic carbon at δ 121.6 (C-6) which are characteristic for the cucurbitane skeleton.[9]

The presence of an olefinic carbon signal at δ 128.4 and an olefinic quaternary carbon signal at δ 144.6 suggested that the 1, 2 position was unsaturated. This assignment was supported by the strong UV absorbance at 254 nm, and also by the high field position of the C-3 signal in the ^{13}C-NMR spectrum. A comparison of the ^{1}H- and ^{13}C-NMR spectra of **1** with those of **3** revealed that the sugar moiety and the A, B and C ring part of the aglycone of both compounds share the same structure, the difference being the side chain part (C-22—C-27) in **1**. Namely, the carbonyl signal for C-22, the methylene signals for C-23 and C-24, and the quaternary carbon signal for C-25 seen in the spectrum of **3** were absent in **1**. Instead, a methylene carbon signal at δ 48.8 due to C-22, an oxymethine carbon signal at δ 73.0 due to C-23 and a set of olefinic carbon signals due to C-24 and C-25 at δ 124.9, 136.6, respectively, were observed in **1**. Tracing out the ^{1}H–^{1}H coupling systems from the H-22 signals by ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY) combined with the ^{1}H-detected multiple quantum coherence (HMQC) spectrum, allowed the sequential assignments of the signals of the side chain, giving rise to the partial structure, –CH_2–CH(O–)–CH=$C(CH_3)_2$. This partial structure explains the three bond correlations for H-22/C-24, H-24/C-26, C-24/H-27, H-26/C-24, H-27/C-24 observed in the ^{1}H-detected heteronuclear multiple bond correlation (HMBC) spectrum of **1** (Fig. 2). Despite the lack of effective information on the ether linkage between C-16 and C-23 in the HMBC spectra, the linkage was apparent from the chemical shift values of C-16 (δ 76.0) and C-23 (δ 73.0), and also from the remaining degrees of unsaturation.

Confirmation that the glucose unit was situated at C-2 was obtained by the ^{3}J correlations between H-1′ (δ 4.68) and C-2 (δ 144.6) in the HMBC spectrum. The β-configuration at the anomeric center of glucopyranose was suggested by the large coupling constant (J=7.9 Hz) of the anomeric hydrogen at δ 4.68 in the ^{1}H-NMR spectrum and by the resonances of C-3′ (δ 75.5) and C-5′ (δ 77.0) which appear at lower field

Table 2. ^{13}C-NMR Data of Compounds **2** and **2a**

Carbon	**2**	**2a**
C-1	23.0	23.1
C-2	30.6	30.0
C-3	79.0	79.1
C-4	37.1	37.2
C-5	43.4	43.5
C-6	23.8	23.8
C-7	118.2	117.9
C-8	142.1	142.2
C-9	144.5	144.3
C-10	36.2	36.3
C-11	114.5	114.3
C-12	39.3	39.2
C-13	37.3	37.4
C-14	40.0	40.1
C-15	27.4	27.1
C-16	34.2	34.2
C-17	31.5	31.0
C-18	44.6	44.5
C-19	28.3	29.6
C-20	31.7	31.7
C-21	30.0	29.7
C-22	36.6	36.7
C-23	22.0	22.0
C-24	19.4	19.1
C-25	20.5	20.6
C-26	22.0	22.1
C-27	27.5	27.5
C-28	31.0	30.6
C-29	72.7	71.1
C-30	30.4	29.9
C-1′	130.8	130.9
C-2′, 6′	129.4	129.5
C-3′, 5′	128.4	128.4
C-4′	132.8	132.6
C-7′	166.7	Not reported
C-1″	130.6	—
C-2″, 6″	129.4	—
C-3″, 5″	128.3	—
C-4″	132.6	—
C-7″	165.9	—

than those of the corresponding α anomers.

The relative stereostructure of **1** was characterized by careful comparison of the ^{13}C-NMR data with those of **3**, and by the difference nuclear overhauser effect (NOE) experiment, which showed NOE enhancement of β-oriented H-18 (4.6%) and H-23 (23.9%) when irradiated at H-16, as shown in Fig. 2. The large coupling constant (J=9.8 Hz) between H-16β and H-17α supported the D/E *trans* ring junction. The absolute configuration at C-16 (*R*) and C-20 (*S*) of **1** are assumed on biogenetic grounds since cucurbitane derivatives isolated from Cucurbitaceae plants have (*R*) and (*R*)-configurations at C-16 and C-20.[1,10] From these lines of evidences, the structure of **1** was elucidated as 16α,23α-epoxy-2,20β-dihydroxycucurbita-1,5,24-triene-2-*O*-β-D-glucopyranoside (Fig. 1). The co-occurrence of these three cucurbitacines (**1**, **3**, **4**) in *G. integrifolium* is of biosyhthetic significance. These compounds were the first cucurbitanes isolated from the genus *Gymnopetalum*.

Compound **2** was obtained as colorless needles and its molecular formula was calculated as $C_{44}H_{56}O_4$ based on HR-FAB-MS analysis. The IR spectrum had an absorption band at 1714 cm^{-1} indicating the presence of an ester function.

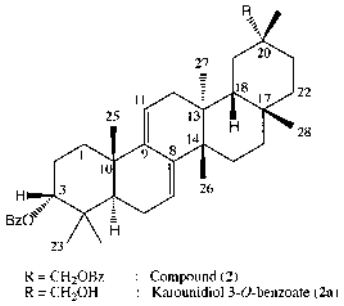

Fig. 3. Structure of **2**

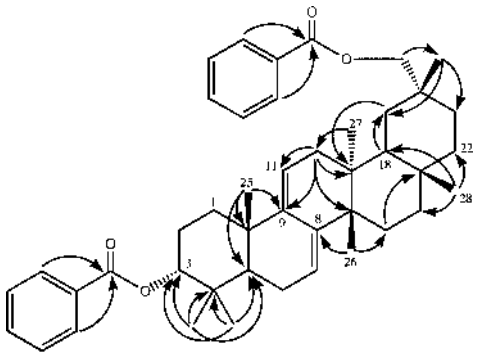

Fig. 4. ^{13}C–^{1}H Long-Range Correlations of **2**

The ^{1}H-NMR of **2** displayed signals corresponding to seven tertiary methyls (δ 1.04, 0.90, 0.96, 0.93, 0.90, 1.10, 1.09), two olefinic hydrogen [δ 5.50 (H-7), 5.21 (H-11)], an oxygenated methylene [δ 4.11, 4.14, each d, J=16.0 Hz (H-29)] and an oxygenated methine [δ 4.89, br t (H-3)] together with two sets of benzoyl group signals. The ^{13}C-NMR (Table 2), on the other hand, showed 40 signals including four intense signals due to two benzoyl moieties, and seven primary, ten secondary, three tertiary and six quaternary sp^3 carbons, and two tri-substituted olefins with typical signals (δ 118.2, 142.1, 144.5, 144.0) for double bonds at C-7/C-8 and C-9/C-11 of multiflora-7,9(11)-dien-3β-ol (D: C-friedo-oleana-7,9(11)-dien-3β-ol) type triterpenes. Thus **2** was considered to be a multiflorenol-type triterpene dibenzoate. Additional support for this proposal was obtained from the diagnostic fragment ion at *m*/*z* 253 in the FAB-MS spectrum.[11,12]

The ^{13}C-NMR spectrum of **2** was almost superimposable on that of karounidiol 3-*O*-benzoate (**2a**) (Fig. 3)[11] except for the carbon signals of an additional benzoyl group. The molecular weight of **2** was 122 mass units higher than that of **2a** suggesting that **2** contained an additional benzoyl group at C-29. This assumption was also supported by homo- and heteronuclear two-dimensional (2D) NMR analysis (Fig. 4). We therefore assigned the structure of **2** as 3,29-*O*-dibenzoyloxykarounidiol (Fig. 3).

Experimental

^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM α-500 spectrometer. Chemical shifts are shown as δ values, using tetramethylsilane (TMS) as an internal standard. COSY, HMQC, HMBC and differential NOE experiments were obtained with the usual pulse sequences and data processing were performed with the standard JEOL software. The *J* values in the HMBC experiments were 4, 8, and 12 Hz. FAB-MS was measured on a JEOL HX-110A mass spectrometer. UV spectra were recorded on a Hitachi U3200 spectrometer, and IR spectra on a JASCO FT-IR-230 spectrometer.

Optical rotations were determined with a JASCO DIP-181 polarimeter. Silica gel 60 (70—230 mesh, Merck), Sephadex LH-20 (Pharmacia) and Cosmosil 75C_{18}-OPN (Nacalai tesque) were used for column chromatography. Analytical TLC was performed with Silica gel 60 F_{254} (Merck) and ODS RP-18F_{254}S (Merck) plates, and detection of the spots was accomplished by UV absorption and spraying with 50% H_2SO_4 followed by heating.

Plant Material Fruits of *G. integrifolium* were collected at Nakhon Ratchasima Province, Thailand. A voucher specimen is deposited at the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Extraction and Isolation Dried fruits of *G. integrifolium* (450 g) were extracted with 75% EtOH. After evaporation of the solvent, the extract (90 g) was suspended in H_2O and extracted successively with *n*-hexane, benzene, EtOAc and *n*-BuOH. The EtOAc-soluble fraction was concentrated and a portion of the resulting residue (2.0 g) was chromatographed on a silica gel column. Elution with a gradient EtOAc–MeOH mixture gave fourteen fractions. A portion (106 mg) of fraction 9 (15% MeOH eluent, 276 mg) was further purified by ODS (H_2O–MeOH), Sephadex LH-20 (H_2O–MeOH), and silica gel ($CHCl_3$–MeOH) column chromatographies to give bryoamaride (**3**) (69.4 mg). Fractions 7 and 8 (10% MeOH eluent, total 301 mg) were also purified in the same manner to afford 25-*O*-acetylbryoamaride (**4**) (25.7 mg) and aoibaclyin (**1**) (9.7 mg). The *n*-hexane-soluble fraction was re-extracted with *n*-hexane. Evaporation of the solvents gave an *n*-hexane-soluble residue (9.1 g) and a water-soluble residue (0.4 g). A portion (2.5 g) of the *n*-hexane-soluble fraction (2.8 g) was repeatedly chromatographed on silica gel columns, eluting with a gradient *n*-hexane–$CHCl_3$, *n*-hexane–EtOAc mixtures to give a compound **2**-rich fraction (7.7 mg). Compound **2** (5.7 mg) was purified by recrystallization of the fraction with *n*-hexane. The water-soluble fraction (0.4 g) was subjected to silica gel, Sephadex LH-20, ODS column chromatographies to afford compound **5** (3.4 mg).

Aoibaclyin (**1**): Pale yellow amorphous solid, UV λ_{max} (MeOH) nm [log ε]: 255 [3.75], IR ν_{max} (KBr) cm^{-1}: 3427, 2970, 2929, 1688, 1651, 1460, 1379, 1217, 1074, 1030, 673, $[\alpha]_D$: +3.5° (c=0.78, $CHCl_3$), HR-FAB-MS *m/z*: 667.3422 $[M+Na]^+$ (Calcd for $C_{36}H_{52}O_{10}$, 667.3458), FAB-MS *m/z*: 667 $[M+Na]^+$, 483 $[M-162+H]^+$, ^{1}H-NMR ($CDCl_3$, 500 MHz): 6.27 (1H, d, J=2.4 Hz, H-1), 5.77 (1H, br s, H-6), 2.01 (1H, m, H-7), 2.34 (1H, br d, J=11.6 Hz, H-7), 2.03 (1H, m, H-8), 3.43 (1H, br s, H-10), 2.57 (1H, d, J=15.0 Hz, H-12), 3.04 (1H, d, J=15.0 Hz, H-12), 1.53 (1H, unresolved dd, H-15), 1.83 (1H, overlapped, H-15), 4.38 (1H, unresolved dt, H-16), 1.90 (1H, d, J=9.8 Hz, H-17), 0.90 (3H, s, H-18), 0.93 (3H, s, H-19), 1.30 (3H, s, H-21), 1.40 (2H, m, H-22), 4.52 (1H, m, H-23), 5.12 (1H, d, J=8.2 Hz, H-24), 1.67 (3H, s, H-26), 1.69 (3H, s, H-27), 1.27 (3H, s, H-28), 1.21 (3H, s, H-29), 1.27 (3H, s, H-30), 4.68 (1H, d, J=7.9 Hz, H-1′), 3.31 (1H, unresolved dd, H-2′), 3.60 (1H, overlapped, H-3′), 3.56 (1H, overlapped, H-4′), 3.40 (1H, overlapped, H-5′), 3.92 (1H, br d, J=10.4 Hz, H-6′), 4.07 (1H, br d, J=11.0 Hz, H-6′), ^{13}C-NMR ($CDCl_3$, 125 MHz). See Table 1.

3,29-*O*-Dibenzoyloxykarounidiol (**2**): Colorless needles, mp 149—152° (*n*-hexane), UV λ_{max} (*n*-hexane) nm [log ε]: 228 [4.48], IR ν_{max} (KBr) cm^{-1}: 2941, 1714, 1645, 1549, 1525, 1462, 1271, 677, $[\alpha]_D$: +9.1° (c=0.17, $CHCl_3$), HR-FAB-MS *m/z*: 648.4203 $[M]^+$ (Calcd for $C_{44}H_{56}O_4$, 648.4179), FAB-MS *m/z*: 649 $[M+H]^+$, 648 $[M]^+$, 527 $[M-PhCOOH+H]^+$, 405 $[M-2\times PhCOOH+H]^+$, 253 $[C_{19}H_{25}]^+$, 105 $[PhCO]^+$, ^{1}H-NMR ($CDCl_3$, 500 MHz): 8.05 (2H, dd, J=8.2, 1.2 Hz, H-3′, 7′), 7.92 (2H, dd, J=8.2, 1.2 Hz, H-3″, 7″), 7.50 (1H, dddd, J=7.6, 7.6, 1.2, 1.2 Hz, H-5′), 7.41 (3H, overlapped, H-4′, 6′, 5″), 7.27 (2H, dd, J=8.2, 1.2 Hz, H-4″, 6″), 5.50 (1H, m, H-7), 5.21 (1H, br d, H-11), 4.89 (1H, br t, H-3), 4.12 (2H, d, J=5.2 Hz, H-29), 2.18 (1H, ddd, J=18.0, 4.9, 4.9 Hz, H-6), 2.10 (1H, overlapped, H-6), 2.10 (1H, overlapped, H-12), 1.99 (1H, m, H-1), 1.87 (1H, ddd, J=15.0, 3.4, 3.4 Hz, H-1), 1.80 (1H, overlapped, H-2), 1.80 (1H, overlapped, H-5), 1.80 (1H, overlapped, H-19), 1.77 (1H, overlapped, H-22), 1.73 (1H, overlapped, H-12), 1.73 (1H, overlapped, H-16), 1.70 (1H, overlapped, H-15), 1.64 (1H, m, H-18), 1.55 (1H, overlapped, H-19), 1.51 (1H, overlapped, H-2), 1.50 (1H, overlapped, H-16), 1.45 (1H, overlapped, H-21), 1.39 (1H, m, H-15), 1.23 (1H, m, H-21), 1.10 (3H, s, H-28), 1.09 (3H, s, H-30), 1.04 (3H, s, H-23), 0.96 (3H, s, H-25), 0.95 (1H, overlapped, H-22), 0.93 (3H, s, H-26), 0.90 (3H, s, H-24), 0.90 (3H, s, H-27), ^{13}C-NMR ($CDCl_3$, 125 MHz). See Table 2.

Bryoamaride (**3**): Yellow amorphous solid, UV λ_{max} (MeOH) nm [log ε]: 255 [4.89], IR ν_{max} (KBr) cm^{-1}: 3410, 2979, 2926, 1687, 1472, 1387, 1230, 1077, 1031, $[\alpha]_D$: −47.8° (c=1.2, EtOH), FAB-MS *m/z*: 701 $[M+Na]^+$, 679 $[M+H]^+$, 499 $[M-162-H_2O+H]^+$, ^{1}H-NMR (CD_3OD, 500 MHz): 6.11 (1H, d, J=2.4 Hz, H-1), 5.83 (1H, br t, H-6), 2.09 (1H, unresolved d, H-7), 2.38 (1H, m, H-7), 2.06 (1H, d, J=8.2 Hz, H-8), 3.69 (1H, br s, H-10), 2.64 (1H, d, J=15.0 Hz, H-12), 3.39 (1H, overlapped, H-12), 1.43 (1H, m, H-15), 1.86 (1H, unresolved dd, H-15), 4.45 (1H, dd, J=7.6, 7.6 Hz, H-16), 2.57 (1H, d, J=7.6 Hz, H-17), 0.94 (3H, s, H-18), 1.01 (3H, s, H-19), 1.39 (3H, s, H-21), 2.73 (1H, m, H-23), 2.85 (1H, m, H-23), 1.72 (1H, m, H-24), 1.18 (3H, s, H-26), 1.19 (3H, s, H-27), 1.40 (3H, s, H-28), 1.26 (3H, s, H-29), 1.29 (3H, s, H-30), 4.64 (1H, d, J=7.3 Hz, H-1′), 3.39 (1H, overlapped, H-2′), 3.39 (1H, overlapped, H-3′), 3.52 (1H, dd, J=9.7, 9.2 Hz, H-4′), 3.35 (1H, ddd, J=9.7, 3.4, 2.4 Hz, H-5′), 3.86 (1H, dd, J=12.2, 3.4 Hz, H-6′), 4.04 (1H, dd, J=12.2, 2.4 Hz, H-6′), ^{13}C-NMR (CD_3OD, 125 MHz). See Table 1.

25-*O*-Acetylbryoamaride (**4**): Pale yellow amorphous solid, UV λ_{max} (MeOH) nm [log ε]: 256 [4.78], IR ν_{max} (KBr) cm^{-1}: 3417, 2975, 2935, 1688, 1455, 1371, 1262, 1222, 1076, 1027, 685, $[\alpha]_D$: −61.0° (c=0.94, $CHCl_3$), FAB-MS *m/z*: 743 $[M+Na]^+$, 661 $[M-OCOCH_3]^+$, 499 $[M-162-OCOCH_3]^+$, ^{1}H-NMR ($CDCl_3$, 500 MHz): 6.19 (1H, d, J=2.4 Hz, H-1), 5.76 (1H, br s, H-6), 2.00 (1H, overlapped, H-7), 2.34 (1H, m, H-7), 2.00 (1H, d, J=9.5 Hz, H-8), 3.46 (1H, overlapped, H-10), 2.73 (1H, d, J=14.7 Hz, H-12), 3.22 (1H, d, J=14.7 Hz, H-12), 1.41 (1H, overlapped, H-15), 2.00 (1H, overlapped, H-15), 4.31 (1H, m, H-16), 2.49 (1H, d, J=6.7 Hz, H-17), 0.94 (3H, s, H-18), 0.96 (3H, s, H-19), 1.40 (3H, s, H-21), 2.78 (1H, m, H-23), 2.50 (1H, m, H-23), 1.83 (1H, br t, H-24), 1.37 (3H, s, H-26), 1.38 (3H, s, H-27), 1.43 (3H, s, H-28), 1.21 (3H, s, H-29), 1.27 (3H, s, H-30), 1.94 (3H, s, C_{25}-OCOMe), 4.66 (1H, d, J=7.6 Hz, H-1′), 3.46 (1H, overlapped, H-2′), 3.46 (1H, overlapped, H-3′), 3.61 (1H, m, H-4′), 3.61 (1H, m, H-5′), 3.91 (1H, br d, J=9.3 Hz, H-6′), 4.07 (1H, br d, 11.3 Hz, H-6′), ^{13}C-NMR ($CDCl_3$, 125 MHz). See Table 1.

β-Sitosterol-3-*O*-β-D-glucoside (**5**)[13]: White amorphous solid, FAB-MS *m/z*: 599 $[M+Na]^+$, 615 $[M+K]^+$, 395.

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References

1) Lavie D., Glotter E., Israel R., "Progress in the Chemistry of Organic Natural Products," ed. by Zechmeeister L. *et al.*, Springer-Verlag, New York, 1971, **29**, pp. 307—362.
2) Miro M., *Phytotherapy Res.*, **9**, 159—168 (1995).
3) Sekine T., Ikegami F., Fukasawa N., Kashiwagi Y., Aizawa T., Fujii Y., Ruangrungsi N., Murakoshi I., *J. Chem. Soc. Perkin Trans. 1*, **1995**, 391—393.
4) Sekine T., Inagaki M., Ikegami F., Fujii Y., Ruangrungsi N., *Phytochemistry*, **52**, 87—94 (1999).
5) Sekine T., Fukasawa N., Ikegami F., Saito K., Fujii Y., Murakoshi I., *Chem. Pharm. Bull.*, **45**, 148—151 (1997).
6) Andersen J. F., Plattner R. D., Weisleder D., *Insect Biochemistry*, **18**, 71—77 (1988).
7) Hylands P. J., Mansour E. S., *Phytochemistry*, **21**, 2703—2707 (1982).
8) Ripperger H., *Tetrahedron*, **32**, 1567—1569 (1976).
9) Velde V. V., Lavie D., *Tetrahedron*, **39**, 317—321 (1983).
10) Kupchan S. M., Sigel C. W., Guttman J. L., Restivo R. J., Bryan R. F., *J. Am. Chem. Soc.*, **94**, 1353—1354 (1972).
11) Akihisa T., Tamura T., Matsumoto T., Eggleston D. S., Kokke W. C. M. C., Shimizu N., *J. Chem. Soc. Perkin Trans. 1*, **1988**, 439—443.
12) Hylands P. J., Oskoui M. T., *Phytochemistry*, **18**, 1843—1845 (1979).
13) Kojima H., Sato N., Hatano A., Ogura H., *Phytochemistry*, **29**, 2351—2355 (1990).