Compositions of Royal Jelly II. Organic Acid Glycosides and Sterols of the Royal Jelly of Honeybees (*Apis mellifera*)

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Two organic acid glycosides (1, 2) and 16 sterols were isolated from the royal jelly of honeybees (*Apis mellifera*). The former two were monoglucosides of 10-hydroxy-2*E*-decenoic and 10-hydroxydecanoic acids. They are the first examples of glycosides isolated from royal jelly. The latter 16 were sterols mainly composed of 28 or 29 carbons. Among them, four compounds were new isofucosterol derivatives, and their structures were characterized as (24*Z*)-stigmasta-5,24(28)-dien-3β-ol-7-one (3), (24*Z*)-stigmasta-5,24(28)-diene-3β,7β-diol (4), (24*Z*)-stigmasta-5,24(28)-diene-3β,7α-diol (5), and (24*Z*)-stigmast-24(28)-ene-3β,5α,6β-triol (6) on the basis of various NMR spectroscopic data.

Key words royal jelly; organic acid glycoside; isofucosterol derivative; honeybee; *Apis mellifera*

Royal jelly is a secretion from the hypopharyngeal and mandibular glands of worker bees, and it is the exclusive food of the queen bee for the entire span of both her larval and adult lives. With regard to the constituents of this material, it contains remarkably high amounts of organic acids in the total lipid fraction.[1)] Many organic acids mainly consisting of 8 to 11 carbon atoms such as 10-hydroxy-2-decenoic[2,3)] and 10-hydroxydecanoic acids have been isolated. Among them, a minor component, 9-hydroxy-2-decenoic acid,[4)] is known to be a queen honeybee pheromone with swarm-stabilizing activity,[5)] and it is also regarded as a precursor of the so-called queen substance, 9-keto-2-decenoic acid, which controls the caste of honeybee colonies.[6)] Recently, we have isolated mono- or diesters of 10-hydroxy-2-decenoic acid in which the hydroxyl group was esterified by another organic acid residue from the total lipid fraction of the royal jelly of the honeybees (*Apis mellifera*).[7)] In view of these findings, it seems that other unknown compounds including pheromones or their precursors exist in royal jelly. The present study was undertaken to examine the constituents of royal jelly in the hope of discovering the biologically active compounds that control the hierarchy of honeybee colonies. By application of the recycling HPLC technique for the isolation of minor constituents from a complex mixture, two glycosides (**1**, **2**) together with 16 compounds (**3**—**18**) were obtained in the pure state. The former two were fatty acid monoglucosides and the latter were sterols mainly composed of 28 or 29 carbons. This paper deals with the isolation and structural elucidation of these compounds.

Results and Discussion

The total lipid fraction obtained from lyophilized royal jelly powder (6.0 kg) was separated repeatedly on silica gel column chromatography with a mixture of $CHCl_3$–MeOH to yield crude glycoside and sterol fractions (see Experimental). Both fractions were further separated with HPLC including a recycling mode, and two (**1**, **2**) from the former and 16 (**3**—**18**) compounds from the latter were isolated in pure form. Among them, compounds **7**—**18** were identified as the known sterols 24-methylenecholesterol (**7**),[8)] isofucosterol (**8**),[9)] cholesta-5,24(24′)-dien-3β-ol-7-one (**9**),[10,11)] cholesta-5,24(24′)-diene-3β,7β-diol (**10**),[10,12)] cholesta-5,24(24′)-diene-3β,7α-diol (**11**),[10,12)] β-sitosterol (**12**),[13)] stigmast-5-en-3β-ol-7-one (**13**),[13)] stigmast-5-ene-3β,7β-diol (**14**),[13)] stigmast-5-ene-3β,7α-diol (**15**),[13)] cholest-24(24′)-ene-3β,5α,6β-triol (**16**),[14)] stigmastane-3β,5α,6β-triol (**17**),[15)] and desmosterol (**18**)[16)] by comparison of their ^{1}H- and ^{13}C-NMR spectroscopic data with those described in the literature.

Structures of Compounds 1 and 2 Negative-ion FAB-MS of **1** gave an $[M-H]^-$ ion peak at *m*/*z* 347, and HR-FAB-MS revealed the molecular formula of **1** to be $C_{16}H_{28}O_8$. The ^{1}H-NMR spectrum showed one anomeric proton signal at δ 4.87 ppm, in addition to typical signals ascribable to a 10-hydroxy-2-decenoyl group.[7)] Based on the coupling constants of the signals arising from the sugar moiety together with a cross-peak between H-1 of the sugar moiety and C-10 of the acid group observed in the heteronuclear multiple-bond connectivity (HMBC) spectrum, compound **1** was considered to be a 10-hydroxy-2-decenoic acid 10-*O*-β-glucopyranoside. Acid hydrolysis of **1** gave products, which were identified as 10-hydroxy-2*E*-decenoic acid and D-glucose by GC analysis. From the information obtained above, compound **1** was assigned to be 10-hydroxy-2*E*-decenoic acid 10-*O*-β-D-glucopyranoside (Fig. 1).

Compound **2** exhibited an $[M-H]^-$ ion peak at *m*/*z* 349, which was two mass units greater than that of **1**. The ^{1}H-NMR spectrum of **2** was similar to that of **1** except for the lack of two olefinic proton signals in **1**, indicating that **2** is another monoglucoside. Acid hydrolysis of **2** gave 10-hydroxydecanoic acid and D-glucose. From these results together with the HMBC correlation (H-1 of glc/C-10 of decanoyl group) of **2**, the structure of **2** was determined to be 10-hydroxydecanoic acid 10-*O*-β-D-glucopyranoside (Fig. 1).

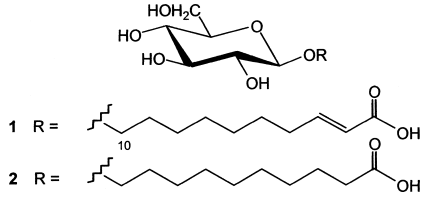

Fig. 1. Structures of Compounds **1** and **2**

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Structures of Compounds 3—6 EI-MS of compound **3** exhibited a molecular ion peak at *m/z* 426. The ^{13}C-NMR spectrum of **3** gave 29 carbon signals, and its ^{1}H-NMR spectrum was quite similar to that of isofucosterol (**8**). The secondary methyl proton at δ_H 1.59 (d, J=7.2 Hz) was coupled with the olefinic proton at δ_H 5.11 (q, J=7.2 Hz), and they were assigned as H_3-29 and H-28, respectively, by analyses of the ^{1}H–^{1}H and ^{1}H–^{13}C COSY spectra. The geometry of the side-chain double bond could be determined from the chemical shift of the characteristic septet signal due to H-25, namely, the signal observed at *ca*. δ_H 2.8 ppm in the *Z* type and *ca*. δ_H 2.2 ppm in the *E* type.[17] The septet signal of H-25 appeared at δ_H 2.83 ppm, and hence the geometry of the double bond was the same *Z* as that of **8**. This conclusion was further supported by the observation of a nuclear Overhauser effect (NOE) correlation between H_3-29 and H-25 in its NOESY spectrum. From these findings, **3** was confirmed to be a monoketo derivative of **8**. In the HMBC spectrum, correlations of the carbonyl carbon (δ_C 202.3) with H-6, H-9, and H-14 were observed, and therefore the C-7 position of the carbonyl group was confirmed. On the basis of the above information, the structure of **3** was characterized as (24*Z*)-stigmasta-5,24(28)-dien-3β-ol-7-one (Fig. 2).

The EI-MS of compounds **4** and **5** exhibited the same molecular ion peak at *m/z* 428, which was 16 mass units greater than that of **8**. Based on the assignment of proton and carbon signals arising from the side chain portion by the same method as described for **3**, they were considered to be other analogues with a (24*Z*)-stigmast-24(28)-ene-type side chain. The ^{1}H- and ^{13}C-NMR spectra of **4** and **5** were similar to each other, but differed markedly in the splitting pattern of a hydroxymethine assignable to H-7, suggesting that they are 7-epimers with each other. The H-7 signal (δ_H 3.85) of **4** was coupled with H-8 (J=7.8 Hz), while that (δ_H 3.86) of **5** appeared as a broad singlet. These observations indicate that the former has a 7β-OH and the latter has a 7α one.[13] The chemical shifts and coupling constants of diagnostically important signals (H-7, H-8) of **4** were in good agreement with those of **10** and **14**, which have a 7β-hydroxyl group, whereas those of **5** were consistent with the corresponding values of **11** and **15** with a 7α-hydroxyl group. Based on the results obtained above, the structures of **4** and **5** were determined to be (24*Z*)-stigmasta-5,24(28)-diene-3β,7β-diol and (24*Z*)-stigmasta-5,24(28)-diene-3β,7α-diol, respectively.

Compound **6** also has a (24*Z*)-stigmast-24(28)-ene-type side chain. The ^{1}H-NMR spectrum of **6** showed two hydroxymethine at δ_H 4.02 and 3.47 ascribable to H-3 and H-6, respectively, while the ^{13}C-NMR spectrum gave one new quaternary carbon signal at δ_C 75.9, together with two oxymethine signals at δ_C 67.4 (C-3) and 76.3 (C-6). In the HMBC spectrum, this quaternary carbon was correlated with Hα-3 and H_3-19. Further, the signals of Hα-3 (+0.85 ppm), Hβ-4 (+0.96 ppm), and H_3-19 (+0.50 ppm) showed the typical pyridine-induced deshielding effect[14,15] due to 1,3-diaxial interactions ($\delta_{\text{pyridine-}d_5} - \delta_{CDCl_3:CD_3OD(9:1)}$ with the C-5 and C-6 hydroxyl groups, confirming a 3β,5α,6β-trihydroxyl configuration. From these findings, together with comparison of ^{1}H-NMR spectroscopic data with the corresponding signals of compounds (**16**, **17**) with the same steroid ring skeleton, the structure of **6** was characterized as (24*Z*)-stigmast-24(28)-ene-3β,5α,6β-triol (Fig. 2).

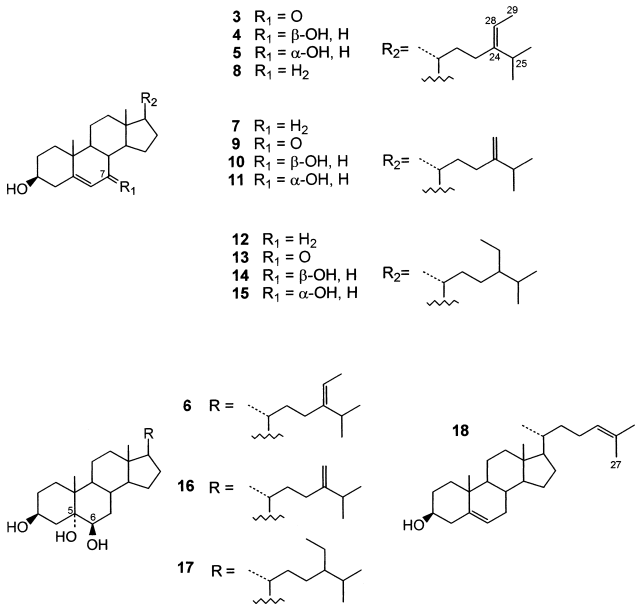

Fig. 2. Structures of Compounds **3**—**18**

Table 1. ^{13}C-NMR Data for **8**, **3**, **4**, **5** and **6** in $CDCl_3$ (100 MHz)

Position	8	3	4	5	6[a]
1	37.2	36.4	37.0	36.9	33.4
2	31.6	31.2	31.6	31.3	32.6
3	71.7	70.6	71.5	71.2	67.4
4	42.3	41.8	41.8	41.9	42.9
5	140.5	165.0	143.5	145.5	75.9
6	121.5	126.2	125.5	123.6	76.3
7	31.6	202.3	73.4	65.2	35.8
8	31.8	45.4	41.0	37.4	31.3
9	50.0	50.0	48.3	42.2	46.0
10	36.4	38.3	36.5	37.4	39.2
11	21.0	21.2	21.1	20.6	21.8
12	39.7	38.7	39.6	39.1	40.7
13	42.2	43.1	43.0	42.1	43.1
14	56.6	50.0	55.4	49.3	56.5
15	24.3	26.3	26.4	24.3	24.7
16	28.2	28.6	28.6	28.2	28.5
17	55.9	54.7	56.0	55.5	56.6
18	11.8	12.0	11.8	11.6	12.4
19	19.4	17.3	19.2	18.2	17.3
20	36.1	36.1	36.1	36.0	36.5
21	18.8	19.0	18.9	18.8	19.0
22	35.9	36.0	36.0	35.8	36.5
23	27.8	27.9	27.9	27.6	28.7
24	145.6	145.8	145.8	145.9	145.9
25	28.6	28.6	28.6	28.5	29.0
26	21.0	21.0	21.0	21.0	21.2
27	21.0	21.0	21.0	21.0	21.2
28	116.2	116.6	116.5	116.2	117.1
29	12.7	12.0	12.8	12.7	13.0

a) **6** was dissolved in C_5D_5N.

In conclusion, two organic acid glucosides (**1**, **2**) and 16 sterols (**3**—**18**) were isolated in pure form. Both of the formers are the first glycosides isolated from royal jelly, although **1** has already been synthesized by Takimoto and Motoyoshi.[18] It is noteworthy that the main organic acids, 10-hydroxy-2*E*-decenoic and 10-hydroxydecanoic acids, also exist as glucosides in royal jelly. Concerning sterols, only eight compounds have been identified so far.[19] This study

showed that royal jelly contains a very complex steroidal composition. A major sterol is 24-methylenecholesterol (**7**), followed by sitosterol (**12**) and isofucosterol (**8**). These compounds comprised as much as 80% of the total sterol fraction. Except for desmosterol, all other sterols consist of 28 or 29 carbons. At present, it remains unclear whether all sterols obtained originate from pollen. To the best of our knowledge, compounds **3**—**6** are the first isofucosterol derivatives isolated from natural products. Further investigation will be made with the objective of finding more of the compounds that control the caste of honeybee colonies.

Experimental

General Experimental Procedures Optical rotations were measured at 25 °C with a JASCO P-1020 polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM-GX400 or ECA-600SN spectrometer, using tetramethylsilane as an internal reference. Samples were measured at a probe temperature of 25 °C. The NOESY spectrum was obtained using a mixing time of 500 ms. The HMBC spectrum was recorded at 600 MHz with 64 scans ($^{2,3}J_{CH}$=7 Hz). EI-MS and FAB-MS including high-resolution MS were recorded on a JEOL JMS-700T spectrometer. (EI-MS: ionization voltage, 30 eV; accelerating voltage, 10 kV; FAB-MS: accelerating voltage, 10 kV; matrix, triethanolamine; collision gas, He). TLC was carried out on silica gel precoated Al sheets (Merck). Spots were visualized with 5% H_2SO_4 in MeOH (by heating). Column chromatography was carried out on Sephadex LH-20 (Amersham Pharmacia Biotech AB), silica gel (Kieselgel 60, Merck), and Cosmosil 75C_{18}-OPN (Nacalai Tesque) columns. Preparative HPLC was conducted over Inertsil ODS-3 (4 μm, 4.6 mm×100 mm), Inertsil ODS-3 (5 μm, 10 mm×250 mm), Cosmosil 5SL-II (5 μm, 10 mm×250 mm), and L-column ODS (5 μm, 10 mm×250 mm) columns with a JASCO 980-PU unit. The elution profile was monitored with a refractive index detector, RI504R. Recycling HPLC was carried out on a JASCO 880-PU equipped with a JASCO preparative recycling valve.

Material Lyophilized royal jelly powder was supplied by Yamada Apiculture Center Inc. (no. L030411B).

Extraction and Isolation Lyophilized royal jelly powder (6.0 kg) was extracted successively with *n*-hexane, $CHCl_3$, $CHCl_3$–MeOH (1 : 1), and MeOH successively. Part of the $CHCl_3$–MeOH extract (36.0 g) was chromatographed on silica gel and eluted successively with $CHCl_3$–MeOH–H_2O (7 : 2 : 0.2)→(7 : 3 : 0.5)→(6 : 4 : 1) to give a fraction (907.6 mg). This was chromatographed on a Sephadex LH-20 column using MeOH to give another fraction (743.9 mg). This was chromatographed on a Cosmosil 75C_{18}-OPN column using MeOH–H_2O gradient (from 1 : 9 to 10 : 0) to give a glycoside fraction (42.6 mg). This was subjected to preparative HPLC (Inertsil ODS-3) using CH_3CN–H_2O–CF_3CO_2H (25 : 75 : 0.5) to give **1** (2.8 mg) and **2** (0.4 mg). The remaining $CHCl_3$–MeOH extract (277.8 g) was treated with $CHCl_3$–MeOH–H_2O (1 : 1 : 1, 900 ml), and the lower layer was collected and concentrated to give a total lipid fraction (185.2 g). Part of this fraction (35.0 g) was chromatographed on a Cosmosil 75C_{18}-OPN column and eluted successively with 25% MeOH→50% MeOH→MeOH. The 50% MeOH eluent was concentrated to give a fraction (7.0 g). This was repeatedly column chromatographed on silica gel using an *n*-hexane–EtOAc gradient (from 1 : 0 to 0 : 1) to give fr. 1 (1.6 g) and a sterol fraction, fr. 2 (2.9 g). Fr. 1 was subjected to preparative HPLC on a Cosmosil 5SL-II column using *n*-hexane–EtOAc (1 : 1) to give four sterol factions, fr. 3 (66 mg), fr. 4 (106 mg), fr. 5 (44 mg), and fr. 6 (1.3 g). Part of fr. 2 (147.4 mg) was separated by conventional HPLC (Inertsil ODS-3) using 90% MeOH to give **7** (80.0 mg), **8** (20.4 mg), **12** (30.2 mg), and **18** (0.2 mg). Each of fr. 3—fr. 5 was separated by HPLC (Inertsil ODS-3+L-column ODS) in a recycling mode using 95% MeOH to give **3** (4.4 mg), **9** (38.4 mg), **13** (6.8 mg) from fr. 3, **4** (0.2 mg), **5** (10.0 mg), **10** (5.6 mg), **11** (25.6 mg), **14** (12.0 mg), **15** (6.8 mg) from fr. 4, and **6** (4.5 mg), **16** (6.0 mg), **17** (1.6 mg) from fr. 5.

Acid Hydrolysis of 1 and 2 Separate solutions of **1** and **2** in aqueous-1,4-dioxane (1 : 1, 1 ml) were acidified with H_2SO_4 (pH 2) and heated at 96 °C for 2 h. After cooling, the reaction mixture was neutralized with $Ba(OH)_2$ and shaken with $CHCl_3$–H_2O (1 : 1, 1 ml) to give an organic acid (lower layer) and sugar fractions (upper layer). The former was methylated with diazomethane and the reaction mixture was analyzed with gas chromatography on a Hitachi G-3000 equipped with a 30 : 1 splitter and a flame ionization detector [(fused silica capillary column Bonded MPS-50, Quadrex, 0.25 mm×50 m, column temperature 230 °C, carrier gas He: 33.4 (ml/min)]. t_R (min): 5.8 (methyl 10-hydroxy-2*E*-decenoate), 5.4 (methyl 10-hydroxydecanoate). According to Hara *et al.*,[20] conversion of the sugar fraction with L-cysteine methyl ester hydrochloride leads to a methyl thiazolidine 4(*R*)-carboxylate derivative, followed by *N*-(trimethylsilyl)imidazole. The product was examined using gas chromatography under the same analytical conditions as described above. t_R (min): 18.2, which was identical to that of the authentic D-glucose derivative.

10-Hydroxy-2*E*-decenoic Acid 10-*O*-β-D-Glucopyranoside (**1**): $[\alpha]_D$ −18.8° (*c*=0.29, MeOH). ^{1}H-NMR (600 MHz, C_5D_5N) δ: 1.14—1.15 (4H, m, H_2-6 and H_2-7), 1.28—1.30 (4H, m, H_2-5 and H_2-8), 1.64 (2H, m, *J*= 6.6 Hz, H_2-9), 2.09 (2H, dt, *J*=6.6, 6.6 Hz, H_2-4), 3.67 (1H, dt, *J*=6.6, 9.6 Hz, H_2-10), 3.99 (1H, m, H-5 of Glc), 4.07 (1H, dd, *J*=7.8, 7.8 Hz, H-2 of Glc), 4.11 (1H, dt, *J*=6.6, 9.6 Hz, H_2-10), 4.24—4.29 (2H, m, H-3 and H-4 of Glc), 4.41 (1H, dd, *J*=4.8, 11.4 Hz, H_2-6 of Glc), 4.59 (1H, dd, *J*=2.4, 11.4 Hz, H_2-6 of Glc), 4.87 (1H, d, *J*=7.8 Hz, H-1 of Glc), 6.18 (1H, d, *J*= 15.6 Hz, H-2), 7.23 (1H, dt, *J*=6.6, 15.6 Hz, H-3). ^{13}C-NMR (150 MHz, C_5D_5N): δ 26.3 (C-8), 28.3 (C-5), 29.3, 29.5, 30.2 (C-9), 32.2 (C-4), 62.9 (C-6 of Glc), 69.8 (C-10), 71.8 (C-4 of Glc), 75.3 (C-2 of Glc), 78.6 (C-3 of Glc), 78.6 (C-5 of Glc), 104.8 (C-1 of Glc), 123.2 (C-2), 148.8 (C-3), 169.0 (C-1). HR-FAB-MS *m/z*: 347.1706 $[M-H]^-$ (Calcd for $C_{16}H_{27}O_8$: 347.1706).

10-Hydroxy-decanoic Acids 10-*O*-β-D-Glucopyranoside (**2**): $[\alpha]_D$ −0.2° (*c*=0.06, MeOH). ^{1}H-NMR (600 MHz, C_5D_5N) δ: 1.07—1.67 (14H, m, C$\underline{H}_2$×7), 2.32 (2H, t, *J*=7.2 Hz, H_2-2), 3.68 (1H, m, H_2-10), 3.99 (1H, m, H-5 of Glc), 4.07 (1H, dd, *J*=7.8, 7.8 Hz, H-2 of Glc), 4.11 (1H, m, H_2-10), 4.24—4.29 (2H, m, H-3 and H-4 of Glc), 4.41 (1H, dd, *J*=4.8, 11.4 Hz, H_2-6 of Glc), 4.59 (1H, dd, *J*=2.4, 11.4 Hz, H_2-6 of Glc), 4.87 (1H, d, *J*=7.8 Hz, H-1 of Glc). ^{13}C-NMR (150 MHz, C_5D_5N): δ 25.2, 26.4, 29.3, 29.6, 29.6, 29.6, 34.1, 34.9, 62.9 (C-6 of Glc), 69.8 (C-10), 71.8 (C-4 of Glc), 75.3 (C-2 of Glc), 78.6 (C-3 of Glc), 78.6 (C-5 of Glc), 104.8 (C-1 of Glc), 174.0 (C-1). HR-FAB-MS *m/z*: 349.1869 $[M-H]^-$ (Calcd for $C_{16}H_{29}O_8$: 349.1862).

(24*Z*)-Stigmasta-5,24(28)-dien-3β-ol-7-one (**3**): White powder, mp 72—76 °C (MeOH). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.69 (3H, s, H_3-18), 0.95 (3H, d, *J*=6.6 Hz, H_3-21), 0.98 (6H, d, *J*=7.2 Hz, H_3-26 and H_3-27), 1.20 (3H, s, H_3-19), 1.34 (overlapped, H-9), 1.51 (overlapped, H-14), 1.59 (3H, d, *J*=7.2 Hz, H_3-29), 2.24 (1H, dd, *J*=10.8, 12.8 Hz, H-8), 2.40 (1H, ddd, *J*= 1.8, 11.4, 13.8 Hz, Hβ-4), 2.51 (1H, ddd, *J*=1.8, 7.2, 13.8 Hz, Hα-4), 2.83 (1H, septet, *J*=7.2 Hz, 1H, H-25), 3.68 (1H, m, H-3), 5.11 (1H, q, *J*=7.2 Hz, H-28), 5.69 (1H, d, *J*=1.8 Hz, H-6). HR-EI-MS *m/z*: 426.3497 $[M^+]$ (Calcd for $C_{29}H_{46}O_2$: 426.3498).

(24*Z*)-Stigmasta-5,24(28)-diene-3β,7β-diol (**4**): White powder, mp 75—78 °C (MeOH). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.70 (3H, s, H_3-18), 0.95 (3H, d, *J*=6.6 Hz, H_3-21), 0.98 (6H, d, *J*=7.2 Hz, H_3-26 and H_3-27), 1.05 (3H, s, H_3-19), 1.59 (3H, d, *J*=6.6 Hz, H_3-29), 2.26 (1H, m, Hβ-4), 2.34 (1H, ddd, *J*=1.8, 7.2, 13.8 Hz, Hα-4), 2.83 (1H, septet, *J*=7.2 Hz, H-25), 3.55 (1H, m, H-3), 3.85 (1H, ddd, *J*=1.8, 1.8, 7.8 Hz, Hα-H), 5.11 (1H, q, *J*=7.2 Hz, H-28), 5.29 (1H, dd, *J*=1.8, 1.8 Hz, H-6). HR-EI-MS *m/z*: 428.3651 $[M^+]$ (Calcd for $C_{29}H_{48}O_2$: 428.3654).

(24*Z*)-Stigmasta-5,24(28)-diene-3β,7α-diol (**5**): White powder, mp 91—94 °C (MeOH). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.69 (3H, s, 3H, H_3-18), 0.96 (3H, d, *J*=6.8 Hz, H_3-21), 0.97 (3H, s, H_3-19), 0.99 (6H, d, *J*=5.2 Hz, H_3-26 and H_3-27), 1.59 (3H, d, *J*=6.8 Hz, H_3-29), 2.29 (1H, m, Hβ-4), 2.35 (1H, ddd, *J*=1.8, 7.2, 13.8 Hz, Hα-4), 2.83 (1H, septet, *J*=7.2 Hz, H-25), 3.59 (1H, m, H-3), 3.86 (1H, br s, Hb-7), 5.11 (1H, q, *J*=6.8 Hz, H-28), 5.61 (1H, dd, *J*=1.6, 5.2 Hz, H-6). HR-EI-MS *m/z*: 428.3650 $[M^+]$ (Calcd for $C_{29}H_{48}O_2$: 428.3654).

(24*Z*)-Stigmast-24(28)-ene-3β,5α,6β-triol (**6**): White powder, mp 213—215 °C (MeOH). ^{1}H-NMR (600 MHz, CD_3OD–$CDCl_3$, 1 : 9) δ: 0.68 (3H, s, H_3-18), 0.94 (3H, d, *J*=6.8 Hz, H_3-21), 0.98 (6H, d, *J*=5.2 Hz, H_3-26 and H_3-27), 1.16 (3H, s, H_3-19), 2.00 (1H, dd, *J*=11.4, 12.6 Hz, Hβ-4), 1.59 (3H, d, *J*=7.2 Hz, H_3-29), 2.04 (1H, dd, *J*=5.4, 12.6 Hz, Hα-4), 2.83 (1H, septet, *J*=7.2 Hz, H-25), 3.47 (1H, dd, *J*=3.0, 3.0 Hz, H-6), 3.86 (1H, br s, H-7), 4.02 (1H, m, H-3), 5.10 (1H, q, *J*=7.2 Hz, H-28). (600 MHz, C_5D_5N) δ: 0.75 (3H, s, H_3-18), 1.02 (3H, d, *J*=6.6 Hz, H_3-21), 1.04 (6H, d, *J*= 6.6 Hz, H_3-26 and H_3-27), 1.64 (3H, d, *J*=6.6 Hz, H_3-29), 1.66 (3H, s, H_3-19), 2.34 (1H, dd, *J*=5.4, 12.6 Hz, Hα-4), 2.87 (1H, septet, *J*=7.0 Hz, H-25), 2.96 (1H, dd, *J*=11.4, 12.6 Hz, Hβ-4), 4.17 (1H, br s, H-6), 4.87 (1H, m, H-3), 5.24 (1H, q, *J*=6.6 Hz, H-28). HR-EI-MS *m/z*: 446.3770 $[M^+]$ (Calcd for $C_{29}H_{50}O_3$: 446.3760).

^{13}C-NMR (100 MHz, $CDCl_3$) spectroscopic data of **3**—**6** are shown in Table 1.

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