Structure Elucidation of Glycosidic Antibiotics, Glykenins, from *Basidiomycetes* sp. IV. Structure of Glykenin III

Fumiko NISHIDA,^a Yuji MORI,^a Makoto SUZUKI,^{*,a} Vithaya MEEVOOTISOM,^b Timothy W. FLEGEL,^b Yodhathai THEBTARANONTH,^b and Suthum INTARARUANGSORN^b

Faculty of Pharmacy, Meijo University,^a Tempaku-ku, Nagoya 468, Japan and Faculty of Science, Mahidol University,^b Rama VI Road, Bangkok 10400, Thailand. Received May 1, 1991

Three new glycosidic antibiotics, glykenin (GK)-III A, B, and C (2a–c), were isolated as a mixture from a strain of *Basidiomycetes* sp. and identified as glycosides of C₂₆-fatty acids and a diacetylated trisaccharide composed of glucose and two xyloses. The locations of the two acetyl groups were elucidated by secondary ion mass spectrometry (SIMS), double quantum filter (DQF), and relayed chemical shift correlation spectroscopy (COSY) spectral analysis.

Keywords *Basidiomycetes* sp.; glykenin III; glycosidic antibiotic; SIMS; double quantum filter ¹H COSY; relayed COSY

Glycosidic antibiotics known as glykenins (GK), which are produced by a *Basidiomycetes* sp., exhibit inhibitory activity against gram-positive bacteria. GK is composed of two major components (GK-III and GK-IV) and five minor components (GK-I, GK-II, and GK-V–VII), as determined by silica gel thin layer chromatography (TLC). In the previous papers,^{1,2)} we reported the structures of deacetyl GK (DG-A–C, 1a–c), and the basic structures of GK, which are composed of unusual tetrahydroxylated long chain (C₂₆) fatty acids as aglycones, and trisaccharides. We describe here the precise structure elucidation of the major component GK-III (2a–c) by the use of two-dimensional nuclear magnetic resonance (NMR) methods, and the structural relationship of 1a–c and 2a–c (Chart 1).

The major component GK-III was isolated from the ethyl acetate extract of the culture broth of a strain of *Basidiomycetes* sp. in glucose–glutamine medium by Sephadex LH-20 chromatography and repeated chromatography on silica gel (CHCl₃:MeOH:50% AcOH = 65:15:5). Although TLC, mass spectrometry and ¹H-NMR examinations suggested that GK-III was pure,

analysis by high-performance liquid chromatography (HPLC) of the peracetyl phenacyl ester derivative (3a–c) of GK-III using CH₃CN:MeOH:H₂O = 9.0:0.5:0.5 as the mobile phase indicated the presence of three peaks, which were identical with those obtained from the peracetyl phenacyl esters of 1a–c as shown in Fig. 1. The retention times of 3a–c were 11.1, 11.5, and 10.0 min, respectively. These results revealed that GK-III is a mixture of three components derived from DG-A–C, which represent the basic structures of GK. The three components of GK-III differ in the aglycone parts, and they were proved to be regio- and stereoisomers of hydroxy groups by chemical transformation.^{1,2)}

GK-III is an amorphous powder and showed molecular ions at *m/z* 969 (M–H)[–] and *m/z* 993 (M+Na)⁺ in the negative and positive secondary ion mass spectrometry (SIMS) using glycerol as a liquid matrix, respectively. GK-III showed an absorption at 1730 cm^{–1} in the infrared (IR) spectrum, and showed ¹H-NMR signals at 2.07 and 2.08 ppm, indicating that it possesses two acetyl groups. Separation of the three components by normal and reversed-phase chromatographies was not successful due

Chart 1. Structures of DG, GK-III and Peracetyl Phenacyl Esters

Fig. 1. HPLC Analyses of the Peracetyl Phenacyl Esters of GK-III (2) and DG-A—C (1a, 1b, and 1c)

to the structural similarity of the aglycone parts. The NMR spectrum of GK-III, however, appeared to be that of a single compound and therefore we decided to analyze GK-III as it was. We employed the double quantum filter (DQF) and relayed proton chemical shift correlation spectroscopy (^1H COSY) techniques^{3,4)} to determine the positions of the two acetyl groups. Using these techniques, we could elucidate the connectivities of many overlapped methine proton signals of the sugar moiety as follows.

In the DQF ^1H COSY spectrum, the anomeric proton (H-1) of glucose (Glc) was connected with Glc-H-2 by cross peaks, and Glc-H-2 was correlated with Glc-H-3. Glc-H-4, the lower-field-shifted signal (4.85 ppm), was easily assigned from Glc-H-3 based on the cross peaks and was correlated with Glc-H-5. The chemical shifts of Glc-H-6 and Glc-H-6' assigned from Glc-H-5 were 4.14 and 4.22 ppm, which were lower-field signals. In the relayed COSY, Glc-H-1 was connected with Glc-H-2 and Glc-H-3 and in the 2-relayed COSY, with Glc-H-4, as shown in Fig. 2. In addition, starting from Glc-H-4, connections were observed between Glc-H-4 and Glc-H-1, H-2, H-3, H-5, H-6, and H-6' in 2-relayed COSY. As mentioned above, the lower-field-shifted signals connected with acetyl groups, 4.85, 4.14 and 4.22 ppm, were assigned to Glc-H-4, Glc-H-6 and Glc-H-6', respectively. The proton signals of xylose-1 (Xyl^1) were upfield signals, and the xylose-2 (Xyl^2) moiety showed similar signals to Xyl^1 . It was confirmed by DQF, relayed and 2-relayed COSY spectra that no acetyl group was present on the two xylose moieties.

The fragmentations in negative SIMS also suggested the positions of the two acetyl groups. The fragment ions of GK-III include m/z 927, 723, 591 and 459 (aglycone-H)⁻, indicating the sequential elimination of the three sugar moieties. The mass differences between of the fragment ions, corresponded to the loss of ketene (Δ 42), glucose and

keten (Δ 42 + 162), xylose-2 (Δ 132), and xylose-1 (Δ 132), respectively. The fragment ions of DG-C (1c) at m/z 723, 591 and 459 indicated only the sequential elimination of the three sugars. These data support the conclusion that the two acetyl groups are present on the glucose moiety in the structures of GK-III (Fig. 3).

Based on the analyses described above, the structures of GK-III A—C were determined to be as shown in Chart 1.

Experimental

IR spectra were recorded on a Hitachi 215 spectrometer, and ultraviolet (UV) spectra were measured on a Hitachi 200-10 spectrophotometer. NMR spectra were recorded on JEOL JNM-GX270 (270 MHz for ^1H -NMR), and JNM-GX400 (400 MHz for ^1H -NMR and 100 MHz for ^{13}C -NMR) spectrometers, and chemical shifts are given on the ppm (δ) scale with tetramethylsilane as an internal standard. Mass spectra (MS) were taken on a Hitachi M-80 spectrometer and a JEOL HX-110. Column chromatographic separations were carried out using Sephadex LH-20 (Pharmacia) or Silica gel 60 (Nacalai). Analytical TLC was performed on precoated silica gel 60 plates (Merck, Art. 5715) and RP-18F_{254S} (Merck, Art. 15685). HPLC was performed on a JASCO Trirotar V using a Develosil ODS column.

Isolation and Purification The fermentation broth of GK was extracted 3 or 4 times with AcOEt. The organic layer was concentrated and the residue was chromatographed on the Sephadex LH-20 column with MeOH to give GK complex (9.88 g). GK complex (9.88 g) was chromatographed on a silica gel column using CHCl_3 :MeOH:50% AcOH (65:15:5), and repeated chromatographies using CHCl_3 :MeOH:50% AcOH (65:10:5) gave GK-III (12.5 mg), GK-IV (51.6 mg), and a mixture of minor components (90.8 mg).

GK-III: Amorphous powder. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: end absorption. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3600—3200, 1730. SIMS (positive) m/z : 993 ($\text{M} + \text{Na}$)⁺. SIMS (negative) m/z : 969 ($\text{M} - \text{H}$)⁻. Positive high-resolution fast atom bombardment mass spectrum (FAB-MS): Found 1015.5050. Calcd for $\text{C}_{46}\text{H}_{81}\text{O}_{21}\text{Na}_2$ ($\text{M} + 2\text{Na} - \text{H}$)⁺ 1015.5065. ^1H -NMR (400 MHz, CD_3OD) δ : 4.85 (1H, t, $J=9.8$ Hz, G-3), 4.72 (1H, d, $J=8.1$ Hz, G-1), 4.61 (1H, d, $J=7.1$ Hz, X²-1), 4.41 (1H, d, $J=7.1$ Hz, X¹-1), 4.22 (1H, dd, $J=4.9$, 11.7 Hz, G-6'), 4.19 (1H, m, aglycone-2), 4.14 (1H, dd, $J=1.9$, 11.7 Hz, G-6), 3.86 (2H, dd, $J=4.9$, 10.7 Hz, X²-5', X¹-5'), 3.78 (1H, m, aglycone-22), 3.72 (1H, m, G-5), 3.58 (1H, t, $J=9.8$ Hz, G-3), 3.56 (1H, t, $J=9.5$ Hz, X²-3), 3.51 (2H, t, $J=9.8$ Hz, X¹-3 and m, X²-4), 3.50 (1H, m, X¹-5), 3.43 (1H, t, $J=9.5$ Hz, X²-2), 3.35 (1H, t, $J=9.8$ Hz, G-2), 3.27 (1H, t, $J=9.8$ Hz, X¹-2), 3.26 (2H, m, aglycone-17, 18), 3.18 (2H, dd, $J=3.9$, 9.8 Hz, X¹-4, X²-5), 2.08 (3H, s, COCH_3), 2.07 (3H, s, COCH_3), 1.53, 1.29 (CH_2), 0.92 (3H, t, $J=6.5$ Hz, CH_3). ^{13}C -NMR (100 MHz, CD_3OD) δ : 172.8, 171.9, 105.5, 104.2, 102.5, 84.2, 83.6, 80.2, 77.3, 77.1, 76.0, 75.9, 75.7, 75.4, 75.3, 75.2, 73.6, 71.9, 70.9, 70.7, 66.8, 66.4, 63.5, 37.4, 35.9, 35.5, 34.6, 34.0, 33.5, 30.9, 30.8, 30.7, 30.5, 28.4, 27.1, 26.2, 24.2, 23.8, 22.9, 22.6, 20.9, 14.6.

The Peracetyl Phenacyl Esterifications A methanolic solution (0.5 ml) of the mixture of GK-III A—C (2.8 mg) was treated with 1% NaOH in MeOH (23 μl) and stirred for 1 h at room temperature. The residue obtained by evaporation *in vacuo* was dissolved in *N,N*-dimethylformamide. Dicyclohexyl 18-crown-6 (2.2 mg) and phenacyl bromide (1.7 mg) were added to the solution, and the whole was stirred for 4 h. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel using CHCl_3 -MeOH- H_2O (65:15:5) to give the phenacyl esters (2.2 mg). The phenacyl esters (2.2 mg) were acetylated with acetic anhydride (0.1 ml) and pyridine (0.1 ml) and the products obtained was chromatographed on Sephadex LH-20 (MeOH) to give a mixture of peracetyl phenacyl esters (3.1 mg).

Peracetyl Phenacyl Ester of GK-III: Oil. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 242. IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 1750, 1365, 1240. SIMS (positive) m/z : 1489 ($\text{M} + \text{Na}$)⁺. ^1H -NMR (400 MHz, CD_3OD) δ : 7.90 (2H, d, $J=2.4$ Hz, aromatic proton), 7.62 (1H, t, $J=7.5$ Hz, aromatic proton), 7.49 (2H, t, $J=7.4$ Hz, aromatic proton), 5.50 (1H, d, $J=16.2$ Hz, CH_2), 5.28 (1H, d, $J=16.2$ Hz, CH_2), 5.18 (1H, t, $J=9.5$ Hz, G-4), 5.16 (1H, t, $J=9.0$ Hz, X¹-3), 5.11 (1H, t, $J=9.5$ Hz, G-3), 5.09 (1H, m, aglycone-2), 5.07 (1H, t, $J=8.8$ Hz, X²-3), 5.01 (1H, m, aglycone-18), 4.98 (1H, m, aglycone-17), 4.95 (1H, dd, $J=9.5$, 8.1 Hz, G-2), 4.87 (1H, m, X¹-4), 4.84 (1H, m, X²-4), 4.64 (1H, d, $J=6.4$ Hz, X²-1), 4.54 (1H, d, $J=8.1$ Hz, G-1), 4.43 (1H, d, $J=7.0$ Hz, X¹-1), 4.35 (1H, dd, $J=12.2$, 2.2 Hz, G-6'), 4.23 (1H, dd, $J=12.2$, 3.9 Hz, G-6), 4.05 (1H, dd, $J=12.5$, 5.1 Hz, X²-5'), 3.96 (1H, dd,

7.49 (2H, t, $J=7.3$ Hz, aromatic proton), 5.50 (1H, d, $J=16.2$ Hz, CH_2), 5.28 (1H, d, $J=16.2$ Hz, CH_2), 5.19 (1H, t, $J=9.5$ Hz, G-4), 5.16 (1H, t, $J=9.2$ Hz, X^1 -3), 5.11 (1H, t, $J=9.5$ Hz, G-3), 5.10 (1H, dd, $J=8.1$, 4.6 Hz, aglycone-2), 5.07 (1H, t, $J=8.9$ Hz, X^2 -3), 4.99 (1H, m, aglycone-18), 4.96 (1H, m, aglycone-17), 4.95 (1H, dd, $J=9.5$, 8.1 Hz, G-2), 4.87 (1H, m, X^1 -4), 4.83 (1H, m, X^2 -4), 4.63 (1H, d, $J=6.4$ Hz, X^2 -1), 4.55 (1H, d, $J=8.1$ Hz, G-1), 4.44 (1H, d, $J=6.8$ Hz, X^1 -1), 4.37 (1H, dd, $J=12.2$, 1.9 Hz, G-6'), 4.22 (1H, dd, $J=12.2$, 3.7 Hz, G-6), 4.05 (1H, dd, $J=12.8$, 5.0 Hz, X^2 -5'), 3.95 (1H, dd, $J=11.6$, 5.3 Hz, X^1 -5'), 3.62 (1H, ddd, $J=9.8$, 3.8, 2.3 Hz, G-5), 3.59 (1H, dd, $J=9.2$, 6.8 Hz, X^1 -2), 3.55 (1H, m, aglycone-22), 3.51 (1H, dd, $J=8.9$, 6.4 Hz, X^2 -2), 3.38 (1H, dd, $J=11.6$, 9.0 Hz, X^1 -5), 3.33 (1H, dd, $J=12.8$, 7.6 Hz, X^2 -5), 2.17, 2.15, 2.10, 2.06, 2.05, 2.04, 2.04, 2.02, 2.01, 2.00, 1.99 (3H $\times$ 11, s, COCH_3), 0.92 (3H, t, $J=7.1$ Hz, CH_3). ^{13}C -NMR (100 MHz, CD_3OD) δ : 193.4, 172.3, 172.2, 172.0, 171.6, 171.3, 170.9, 170.0, 135.4, 135.0, 129.9, 128.9, 126.1, 101.2, 101.1, 100.9, 79.6, 77.9, 76.9, 74.3, 74.2, 74.2, 73.4, 72.4, 72.2, 71.9, 70.9, 70.0, 69.8, 68.2, 67.7, 62.3, 61.9, 35.2, 34.9, 32.2, 30.9, 30.7, 30.4, 30.2, 30.0, 28.0, 26.5, 25.9, 24.0, 22.2, 21.4, 21.2, 21.0, 20.5, 14.7.

Peracetyl Phenacyl Ester of **1c**: Oil. $[\alpha]_{\text{D}}^{20} -1.49^\circ$ ($c=0.4$, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 242. IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 1750, 1365, 1240. SIMS (positive) m/z : 1489 ($\text{M}+\text{Na}$) $^+$. ^1H -NMR (400 MHz, CD_3OD) δ : 7.90 (2H, d, $J=7.4$ Hz, aromatic proton), 7.62 (1H, t, $J=7.5$ Hz, aromatic proton), 7.49 (2H, t, $J=7.4$ Hz, aromatic proton), 5.50 (1H, d, $J=16.2$ Hz, CH_2), 5.28 (1H, d, $J=16.2$ Hz, CH_2), 5.18 (1H, t, $J=9.5$ Hz, G-4), 5.16 (1H, t, $J=9.0$ Hz, X^1 -3), 5.11 (1H, t, $J=9.5$ Hz, G-3), 5.09 (1H, m, aglycone-2), 5.07 (1H, t, $J=8.8$ Hz, X^2 -3), 5.01 (1H, m, aglycone-18), 4.98 (1H, m, aglycone-17), 4.95 (1H, dd, $J=9.5$, 8.1 Hz, G-2), 4.88 (1H, m, X^1 -4), 4.84 (1H, m, X^2 -4), 4.64 (1H, d, $J=6.4$ Hz, X^2 -1), 4.55 (1H, d,

$J=8.1$ Hz, G-1), 4.44 (1H, d, $J=6.8$ Hz, X^1 -1), 4.35 (1H, dd, $J=12.2$, 2.2 Hz, G-6'), 4.23 (1H, dd, $J=12.2$, 3.9 Hz, G-6), 4.05 (1H, dd, $J=12.5$, 5.1 Hz, X^2 -5'), 3.97 (1H, dd, $J=11.7$, 5.4 Hz, X^1 -5'), 3.64 (1H, ddd, $J=9.5$, 3.9, 2.2 Hz, G-5), 3.59 (1H, dd, $J=9.0$, 6.8 Hz, X^1 -2), 3.55 (1H, m, aglycone-22), 3.52 (1H, dd, $J=8.8$, 6.4 Hz, X^2 -2), 3.39 (1H, dd, $J=11.7$, 9.0 Hz, X^1 -5), 3.33 (1H, dd, $J=12.5$, 7.6 Hz, X^2 -5), 2.17, 2.15, 2.10, 2.08, 2.07, 2.06, 2.03, 2.02, 2.01, 2.00, 1.99 (3H $\times$ 11, s, COCH_3), 0.91 (3H, t, $J=7.9$ Hz, CH_3). ^{13}C -NMR (100 MHz, CD_3OD) δ : 193.6, 172.3, 171.8, 171.6, 171.5, 171.1, 170.0, 135.5, 135.1, 130.1, 128.9, 126.1, 101.2, 101.1, 100.8, 79.3, 77.9, 76.9, 74.2, 74.0, 73.7, 73.4, 73.2, 72.4, 71.9, 70.9, 70.1, 69.9, 68.3, 67.6, 62.3, 61.9, 61.4, 34.9, 32.3, 31.7, 30.9, 30.7, 30.2, 28.2, 26.3, 25.9, 24.0, 21.5, 21.2, 21.1, 21.0, 20.7, 14.7.

The HPLC Analyses The HPLC analyses were performed using a column of Develosil ODS-5 (260 $\times$ 4.6 mm, Nomura Kagaku). The solvent system was CH_3CN - MeOH - H_2O (9.0:0.5:0.5) and the flow rate was 1 ml/min. The HPLC analysis of the peracetyl phenacyl ester of GK-III showed three peaks at $t_{\text{R}}=11.1$, 11.5, and 10.0 min, which coincided with the respective peaks of **1a**—**c**.

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