Notes

STRUCTURE ELUCIDATION OF GLYCOSIDIC ANTIBIOTICS GLYKENINS FROM *Basidiomycetes* sp.

III. STRUCTURE OF GLYKENIN IV

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(Received for publication December 3, 1990)

A strain of *Basidiomycetes* sp. has been found to produce glycosidic antibiotics glykenins (GK), which exhibit inhibitory activity against Gram-positive bacteria. GK complex is composed of two major components (GK-III and IV) and five minor components (GK-I, II, V ~ VII) as revealed by silica gel TLC. In a previous paper,[1] we reported the structures of deacetyl compounds of GK (DG-A ~ C, **1a** ~ **1c**), basic structures of GK, consisting of unusual tetrahydroxylated long chain (C_{26}) fatty acids as aglycones and trisaccharides. We describe here the structures of GK-IV (**2a** ~ **2c**) by the use of the 2D NMR methods (Fig. 1).

GK-IV, amorphous powder, was separated from GK complex by repeated chromatography on silica gel ($CHCl_3$-MeOH-50% AcOH, 65:15:5) and seemed to be pure judging from MS and ^1H NMR spectra. HPLC analysis of a peracetyl phenacyl ester of GK-IV showed three peaks, which coincided with those of the peracetyl phenacyl esters (**3a** ~ **3c**) of DG-A ~ C.[1] This result revealed that structural differences between the three components of GK-IV lay in the aglycone parts which proved to be regio

Fig. 1. Structures of DG, GK-IV and peracetyl phenacyl esters.

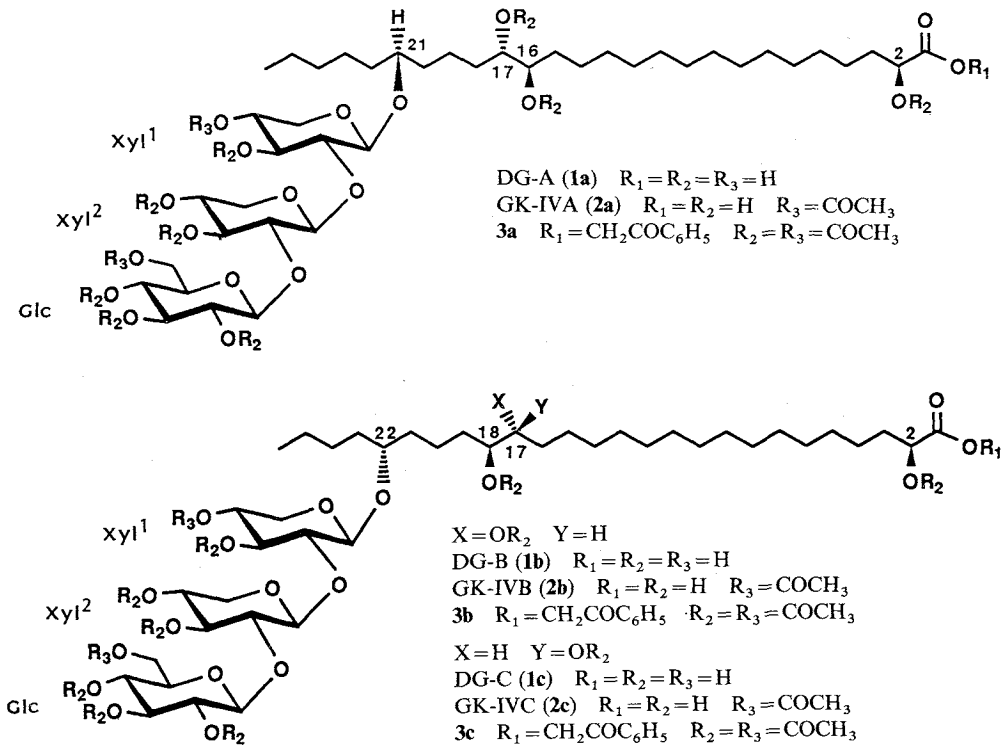
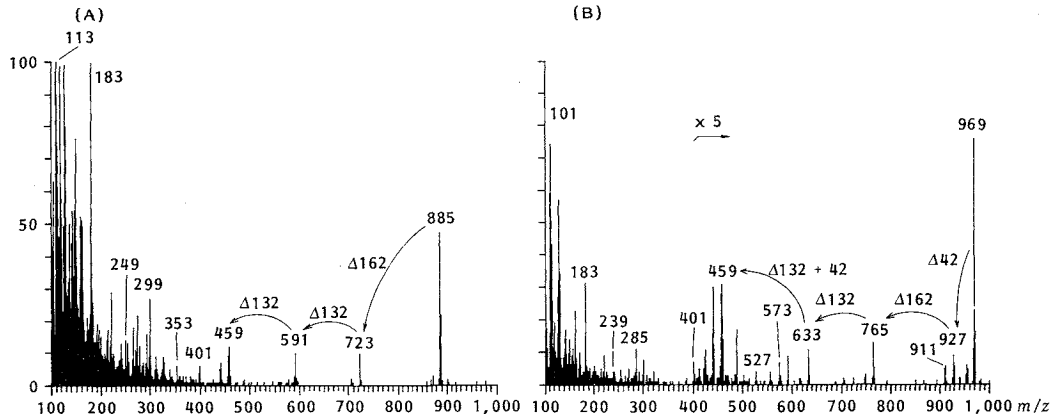


Fig. 2. Liquid SI-MS (glycerol) spectra of DG-C and GK-IV.

(A) DG-C, (B) GK-IV.



and stereoisomers of hydroxy groups by chemical transformations.[2] GK-IV showed an IR absorption at 1730 cm^{-1} and ^1H NMR signals at 2.05 and 2.09 ppm, indicating that GK-IV have two acetyl groups. In the negative and positive SI-MS spectra, GK-IV showed molecular ions at *m/z* 969 $(M-H)^-$ and *m/z* 993 $(M+Na)^+$, respectively, and were established to be diacetylated compounds of DG-A ~ C that showed the strong peaks at *m/z* 909 $(M+Na)^+$ and 931 $(M+2Na-H)^+$ in the positive SI-MS spectra. The fragment ions of DG-A ~ C observed in negative mode are *m/z* 723, 591 and 459 $(aglycone-H)^-$ due to the sequential elimination of the three sugar moieties (Fig. 2). The same fragmentation pattern was observed with GK-IV. The mass differences of the fragment ions between *m/z* 969, 765, 633 and 459 correspond to the loss of ketene and glucose (Glc), xylose-2 (Xyl^2), and ketene and xylose-1 (Xyl^1), respectively. These data suggested the presence of two acetyl groups on Glc and Xyl^1 in the structure of GK-IV.

HPLC analysis of a peracetyl phenacyl ester of GK-IV showed three peaks, indicating that GK-IV is a mixture of three compounds. Separation of these three compounds by silica gel chromatography was not successful because of the structural similarity of the aglycone parts. GK-IV, however, showed the NMR spectrum as a single compound, enabling us to analyze as it was. The positions of two acetyl groups were determined by the use of the 2D ^1H NMR methods. Because many methine proton signals of the sugar moieties overlapped in the ^1H NMR spectrum (CD_3OD), we employed DQF and relayed ^1H COSY techniques[3,4] to disclose the connectivities of the sugar protons (Fig. 3). Starting form the anomeric proton (1-H) of Xyl^1, the Xyl^1-2-H was easily assigned by the cross peaks in the DQF ^1H COSY spectrum. Xyl^1-2-H was correlated with Xyl^1-3-H, where the 3-H signal was connected with the 4-position. Assignment of the lowerfield shifted signal (4.71 ppm) to Xyl^1-4-H was also discernible from cross peaks in the DQF ^1H COSY spectrum and confirmed by cross peaks in the single and double-relayed COSY spectra. Therefore, one of the acetyl groups was found to locate on C-4 position of Xyl^1. The signal of 5-H of Xyl^1 was easily assigned by corroborated analysis of DQF ^1H COSY, single, and double-relayed COSY spectra.

In a similar manner, the anomeric proton of Glc was connected with Glc-2-H, while Glc-2-H was related to Glc-4-H, *via* a cross peak of Glc-3-H in the DQF ^1H COSY spectrum. Glc-4-H was further connected with Glc-5-H and Glc-6-H by the single and double-relayed COSY spectral analyses. The signals assigned to Glc-6-H were observed in the lowerfield than the other signals of glucose.

Based on the analysis described above, the signals at 4.71 ppm, and 4.18 and 4.41 ppm showing lowerfield shifts due to acetylation were assigned to Xyl^1-4-H and Glc-6-H, respectively.

Therefore, the structures of GK-IV A ~ C are as shown in Fig. 1. Structure elucidation of other components is in progress.

Experimental

General Procedure

The IR spectra were recorded on a Hitachi 215 spectrometer, and UV spectra were measured on a

Fig. 3. Multiple-relayed COSY spectra of GK-IV (CD_3OD, 400 MHz).

The arrows in the structure show the correlated protons from 1-H and 4-H in xylose and from 1-H and 6-H in glucose. 2-Relayed-COSY (A, C and E), relayed-COSY (B, D and F). DQF ^1H COSY (G).

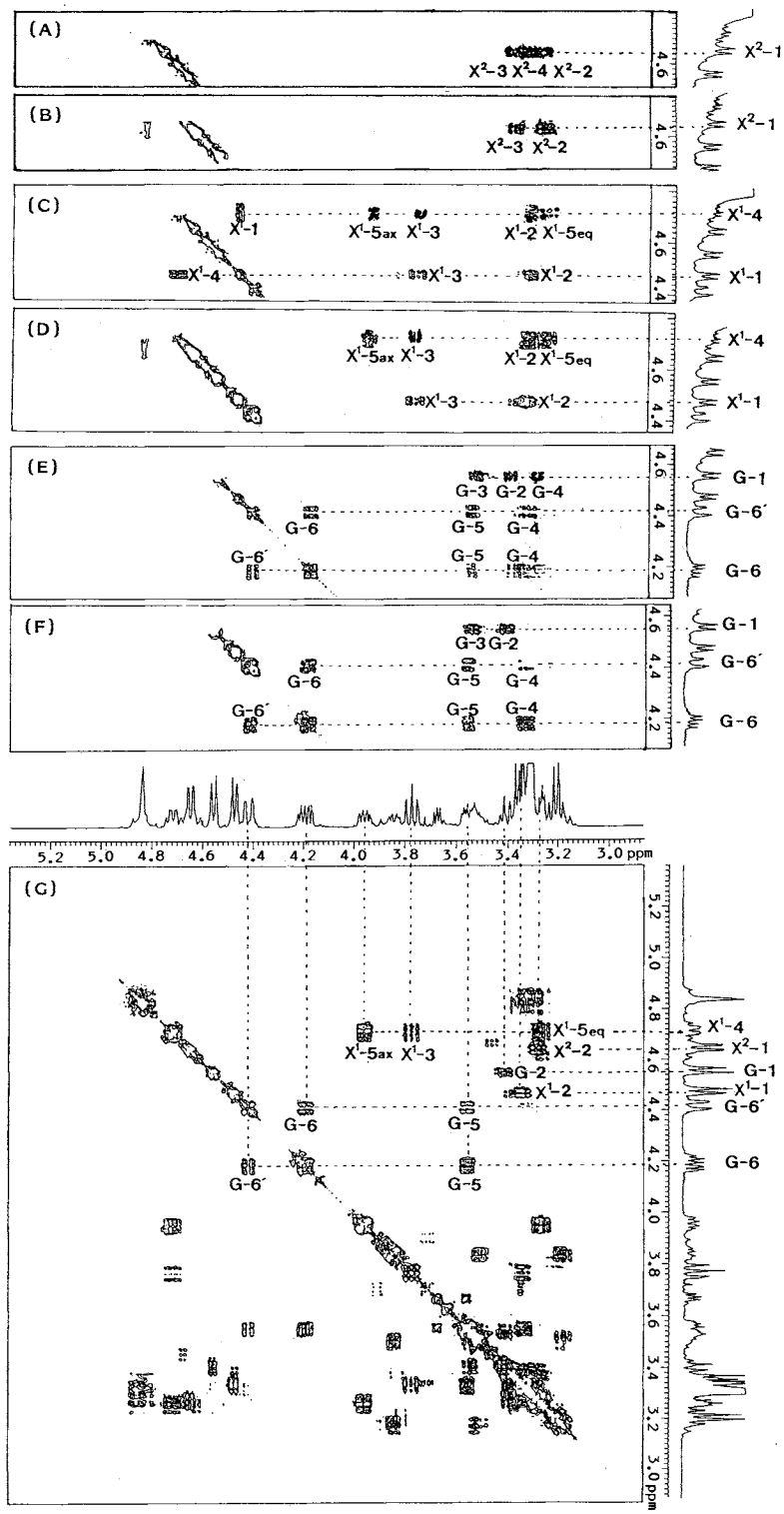
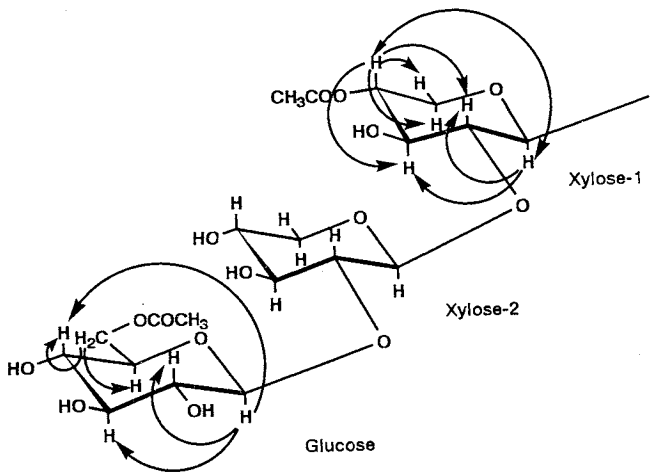


Fig. 3. (Continued)



Hitachi 200-10 spectrophotometer. NMR spectra were recorded on Jeol JNM-GX 400 (400 MHz for ^1H and 100 MHz for ^{13}C) and 270 (270 MHz for ^1H) spectrometers, and chemical shifts are given in ppm (δ) scale with TMS as an internal standard. MS were taken on a Hitachi M-80 spectrometer. 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. No. 5715). HPLC was performed on a Jasco Trirotar-V using a Develosil ODS column.

Isolation and Purification

The fermentation broth[2] was extracted with EtOAc (3~4 times) and the organic layer was concentrated to give a brown oil. Sephadex LH-20 column chromatography with MeOH of the oil (10.17 g) gave GK complex (9.88 g). It (9.88 g) was chromatographed on a silica gel column using $CHCl_3$-MeOH-50% AcOH (65:15:5) to give eight fractions. Fraction 7 (1.95 g) was rechromatographed and seven fractions were collected. Silica gel chromatography of fraction 4 (343.3 mg) using the same solvent system gave five fractions. GK-IV (51.6 mg) was isolated from fraction 3 (86.9 mg) of the silica gel chromatography using $CHCl_3$-MeOH-50% AcOH (65:10:5).

GK-IV: Amorphous powder; UV λ_{max}^{MeOH} nm end absorption; IR (KBr) cm^{-1} 3600~3200, 1730; SI-MS (positive) *m/z* 993 $(M+Na)^+$; SI-MS (negative) *m/z* 969 $(M-H)^-$; ^1H NMR (400 MHz, CD_3OD) δ 4.71 (1H, dd, J=9.2 and 13.9 Hz, X^1-4), 4.64 (1H, d, J=7.8 Hz, X^2-1), 4.55 (1H, d, J=7.3 Hz, G-1), 4.47 (1H, d, J=6.8 Hz, X^1-1), 4.41 (1H, dd, J=2.2 and 10.0 Hz, G-6), 4.18 (1H, dd, J=5.5 and 10.0 Hz, G-6), 4.17 (1H, m, aglycone 2-H), 3.95 (1H, dd, J=4.2 and 11.7 Hz, X^1-5_{eq}), 3.85 (1H, dd, J=3.2 and 11.7 Hz, X^2-5_{eq}), 3.78 (1H, t, J=9.1 Hz, X^1-3), 3.67 (1H, m, aglycone 22-H), 3.55 (1H, m, G-5), 3.54 (1H, t, J=9.1 Hz, G-3), 3.52 (1H, m, X^2-4), 3.40 (1H, t, J=9.1 Hz, G-2), 3.37 (1H, t, J=8.8 Hz, X^2-3), 3.35 (1H, t, J=9.1 Hz, G-4), 3.34 (1H, t, J=9.1 Hz, X^1-2), 3.27 (1H, m, X^2-2), 3.25 (1H, dd, J=5.1 and 11.7 Hz, X^1-5_{ax}), 3.19 (1H, dd, J=7.7 and 11.7 Hz, X^2-5_{ax}), 3.26 (2H, m, aglycone 17, 18-H), 2.09 (3H, s, $COCH_3$), 2.05 (3H, s, $COCH_3$), 1.76~1.29 (CH_2), 0.92 (3H, t, J=6.5 Hz, CH_3). ^{13}C NMR (100 MHz, CD_3OD) δ 172.7, 172.3, 106.1, 104.7, 102.5, 85.3, 84.7, 80.1, 77.6, 77.5, 75.9, 75.8, 75.6, 75.3, 75.2, 74.2, 73.0, 71.1, 70.9, 70.7, 66.9, 64.7, 63.6, 35.9, 35.7, 34.8, 34.5, 34.0, 33.5, 30.9, 30.8, 30.4, 30.2, 28.4, 27.1, 26.8, 25.9, 24.2, 23.8, 22.9, 22.6, 22.5, 21.0, 20.9, 14.6.

Peracetyl Phenacyl Ester of GK-IV

A solution of 1% NaOH in MeOH (33 μl) was added to a methanolic solution (0.5 ml) of GK-IV A~C (4.2 mg). The reaction mixture was stirred for 3 hours at room temperature and then the solvent was removed *in vacuo*. The residue was dissolved in DMF (0.5 ml), and dicyclohexyl 18-crown-6 (3.09 mg) and phenacyl bromide (2.48 mg) were added to the solution. The mixture was stirred for 5 hours and the solvent was removed *in vacuo*. The residue was chromatographed on silica gel using $CHCl_3$-MeOH-H_2O (65:15:5) to give the esters (2.8 mg). The esters (2.8 mg) were acetylated with acetic

anhydride (0.1 ml) and pyridine (0.1 ml) and the products obtained by removal of the solvents were chromatographed on Sephadex LH-20 (MeOH) to give peracetyl phenacyl esters (2.8 mg).

HPLC analysis of the peracetyl phenacyl esters of GK-IV A~C showed three peaks, which coincided with those of the peracetyl phenacyl esters prepared from DG-A~C. HPLC analysis was performed using a column of Develosil ODS-5 (260×4.6 mm, Nomura Kagaku). The solvent system is CH_3CN-MeOH-H_2O (9.0:0.5:0.5) and the flow late was 1 ml/minute. The Rt's of peracetyl phenacyl ester-A, -B, and -C were 11.1, 11.5, and 10.0 minutes, respectively.

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