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STRUCTURES OF DEACETYL GLYKENINS-A, B, AND C, GLYCOSIDIC ANTIBIOTICS FROM BASIDIOMYCETES SP.

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Abstract: The structures of deacetyl Glykenins (DG)-A, B, and C, basic structures of Glykenins, were elucidated by chemical degradation and interpretation of spectral data.

In the course of study on antibiotics a strain of Basidiomycetes sp. was found to produce antibiotics Glykenins (GK) which exhibit inhibitory activity against Gram-positive bacteria. The antibiotics contain unusual tetrahydroxylated long-chain (C_{26}) fatty acids as aglycones and trisaccharides. The straight chain nature of the aglycones and the presence of many hydroxyl groups produced major obstacles in stereochemical elucidation. But degradations to simpler fragments and exquisite chemical transformations enabled to determine the absolute configurations of the aglycones. We wish to report the structures of the deacetyl compounds of GK, DG-A (**1a**), B (**1b**), and C (**1c**), which are basic structures of GK (Fig. 1).

The ethyl acetate extract of the cultured broth of a strain of Basidiomycetes sp. was chromatographed on Sephadex LH-20 (MeOH) to give a mixture of GK, from which two major components GK-III and GK-IV were separated by silica gel chromatography ($CHCl_3$:MeOH:50%AcOH = 65:15:5). Spectral analyses showed that GK-III and IV had same molecular weight 970

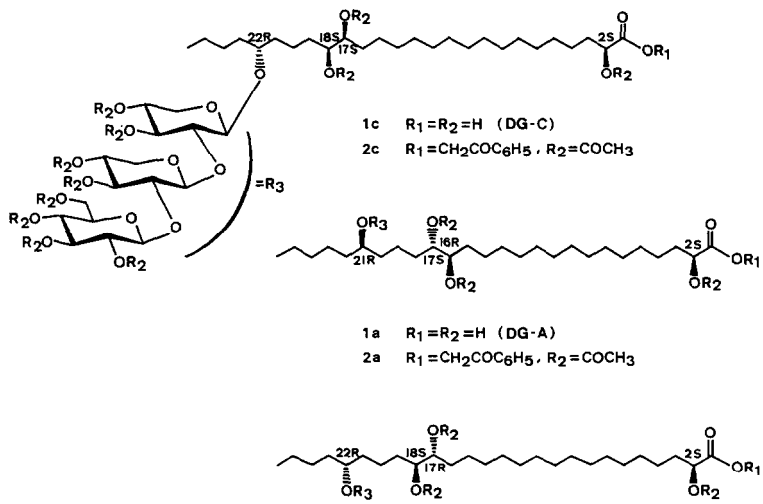


Fig. 1

[negative SIMS, m/z 969 $(M-H)^-$] and had two acetyl, one carboxyl and many hydroxyl groups. But the HPLC analyses of the peracetyl phenacyl esters of GK-III and IV showed same chromatograms with three major peaks, indicating that both components contained three compounds **2a-c**, respectively. The compounds **2a-c** were also obtained directly by preparative ODS-HPLC (CH_3CN:MeOH:H_2O = 9.0:0.5:0.5) separation of the peracetyl phenacyl esters of GK. Treatment of **2a-c** with NaOMe in MeOH yielded DG-A (**1a**), B (**1b**), and C (**1c**), respectively.

Compounds **2a-c**, colorless oil, had same molecular formula $C_{72}H_{106}O_{31}$ (MW 1466) and were closely related one another, containing three molecules of sugars with β-glycosidic linkages judging from their ^1H and ^{13}C-NMR spectra. Hydrolysis of **2a-c** with 5%HCl-MeOH followed by silica gel chromatography using stepwise elution with $CHCl_3$:MeOH:H_2O [(i) 65:5:5, (ii) 65:15:5] gave methyl esters of C_{26}-fatty acids (**3a-c**), methyl D-glucoside, and methyl D-xyloside. Analysis of the ^1H-^1H and ^{13}C-^1H COSY spectra of **2a-c** allowed to assign the signals of one glucose and two xylose moieties (Table 1). The chemical shifts of H-2 protones in xylose-1 (Xyl^1) and xylose-2 (Xyl^2) indicated that the sugar moieties of **2a-c** formed trisaccharides with 1,2-glycosidic linkages, respectively.

The sequence of the trisaccharide and the complete assignments of proton and carbon signals of **2c** were established by the ^{13}C-^1H and long-range ^{13}C-^1H COSY spectral analyses. Three long range cross peaks diagnostic of sequence were observed between Glc• H-1 and Xyl^2• C-2, Glc C-1 and Xyl^2• H-2, and Xyl^2• H-1 and Xyl^1• C-2. Therefore, **2c** had a peracetylated O-β-D-glucopyranosyl-(1-2)-O-β-D-xylopyranosyl-(1-2)-O-β-D-xylopyranosyl structure. Comparisons of ^1H and ^{13}C NMR and ^1H-^1H COSY spectra of **2c** with those of **2a** and **2b** indicated that **2a-c** had the same trisaccharide chain in common.

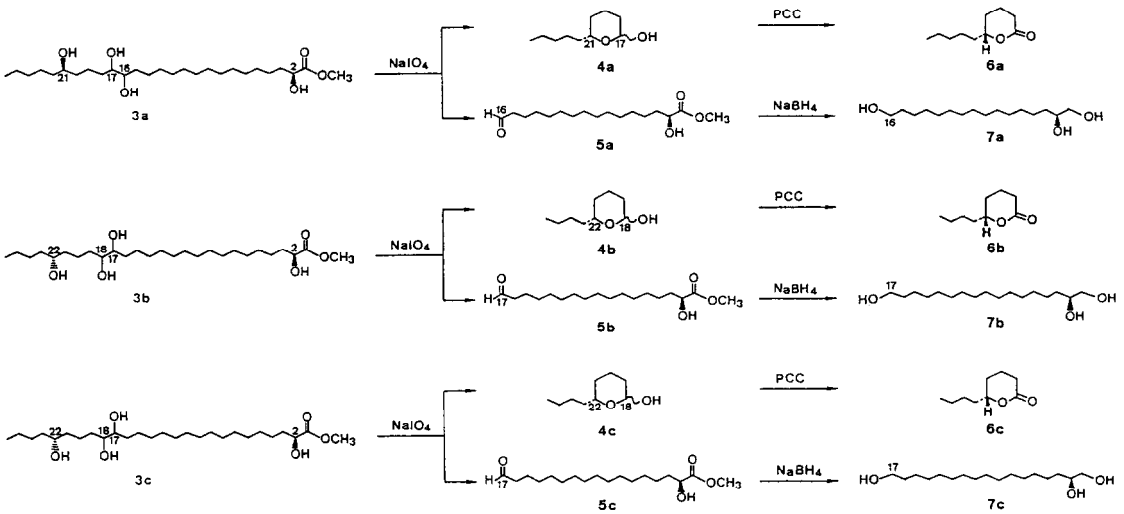
The methyl esters of fatty acids (**3a-c**) had same molecular weight ($C_{27}H_{54}O_4$, 474) and

Table 1

Position		^{13}C-NMR (100MHz, $CDCl_3$) 2a	2b	2c	^1H-NMR (400MHz, $CDCl_3$) (Hz) 2a	2b	2c
Aglycone	1	170.0	170.0	170.0			
	2	72.3	72.4	72.4	5.12 (m)	5.10 (dd, 8.1, 4.6)	5.09 (m)
	16	74.2	-	-	•4.99 (m)		
	17	74.2	74.2	•73.7	•5.01 (m)	•4.96 (m)	•4.98 (m)
	18	-	74.2	•74.0		•4.99 (m)	•5.01 (m)
	21	79.6	-	-	3.55 (m)		
	22	-	79.6	79.3		3.55 (m)	3.55 (m)
Xylose-1	1	100.9	100.9	100.8	4.43 (d, 7.1)	4.44 (d, 6.8)	4.44 (d, 6.8)
	2	76.9	76.9	76.9	3.58 (dd, 9.0, 6.8)	3.59 (dd, 9.2, 6.8)	3.59 (dd, 9.0, 6.8)
	3	74.3	74.3	74.2	5.15 (t, 9.0)	5.16 (t, 9.2)	5.16 (t, 9.0)
	4	70.0	70.0	70.1	4.86 (m)	4.87 (m)	4.88 (m)
	5	62.3	62.3	62.3	3.38 (dd, 11.7, 9.3)	3.38 (dd, 11.6, 9.0)	3.39 (dd, 11.7, 9.0)
					3.95 (dd, 11.7, 5.6)	3.95 (dd, 11.6, 5.3)	3.97 (dd, 11.7, 5.4)
Xylose-2	1	101.2	101.2	101.2	4.63 (d, 6.4)	4.63 (d, 6.4)	4.64 (d, 6.4)
	2	77.9	77.9	77.9	3.51 (dd, 8.8, 6.4)	3.51 (dd, 8.9, 6.4)	3.52 (dd, 8.8, 6.4)
	3	73.5	73.4	73.4	5.07 (t, 8.8)	5.07 (t, 8.9)	5.07 (t, 8.8)
	4	69.8	69.8	69.9	4.83 (m)	4.83 (m)	4.84 (m)
	5	61.9	61.9	61.9	3.32 (dd, 12.7, 7.8)	3.33 (dd, 12.8, 7.6)	3.33 (dd, 12.5, 7.6)
					4.05 (dd, 12.7, 5.1)	4.05 (dd, 12.8, 5.0)	4.05 (dd, 12.5, 5.1)
Glucose	1	101.1	101.1	101.1	4.54 (d, 8.1)	4.55 (d, 8.1)	4.55 (d, 8.1)
	2	70.9	70.9	70.9	4.95 (dd, 9.3, 8.1)	4.95 (dd, 9.5, 8.1)	4.95 (dd, 9.5, 8.1)
	3	73.2	72.2	73.2	5.11 (t, 9.3)	5.11 (t, 9.5)	5.11 (t, 9.5)
	4	68.2	68.2	68.3	5.18 (t, 9.3)	5.19 (t, 9.5)	5.18 (t, 9.5)
	5	71.9	71.9	71.9	3.62 (m)	3.62 (ddd, 9.8, 3.8, 2.3)	3.64 (ddd, 9.5, 3.9, 2.2)
	6	61.4	61.9	61.4	4.22 (dd, 12.2, 3.7)	4.22 (dd, 12.2, 3.7)	4.23 (dd, 12.2, 3.9)
					4.36 (dd, 12.2, 1.9)	4.37 (dd, 12.2, 1.9)	4.35 (dd, 12.2, 2.2)

• may be interchanged.

gave tetraacetates on acetylation. Since the trisaccharide moieties in **2a-c** were identical, **3a-c** should be regio and/or stereoisomers of four hydroxyl groups. Periodate oxidation of **3a-c** gave lactols (**4a-c**) and aldehydes (**5a-c**), respectively (Scheme 1). PCC oxidation of **4a-c** gave (R)-lactones[1], **6a** :$[\alpha]_D^{23}$ +50.8° (c 0.32, $CHCl_3$); **6b** :$[\alpha]_D^{25}$ +51.4° (c 0.58, $CHCl_3$); **6c** :$[\alpha]_D^{22}$ +53.9° (c 0.65, $CHCl_3$). On the other hand, reduction of **5a-c** with $NaBH_4$ afforded (S)-triols[1], **7a** :$[\alpha]_D^{21}$ -6.39° (c 0.27, MeOH); **7b** :$[\alpha]_D^{23}$ -6.87° (c 0.75, MeOH); **7c** :$[\alpha]_D^{21}$ -6.41° (c 0.27, MeOH). These results revealed that the position of hydroxyl groups were C-2 (S), 16, 17 and 21 (R) in **3a**, and C-2(S), 17, 18 and 22 (R) in **3b** and **3c**.

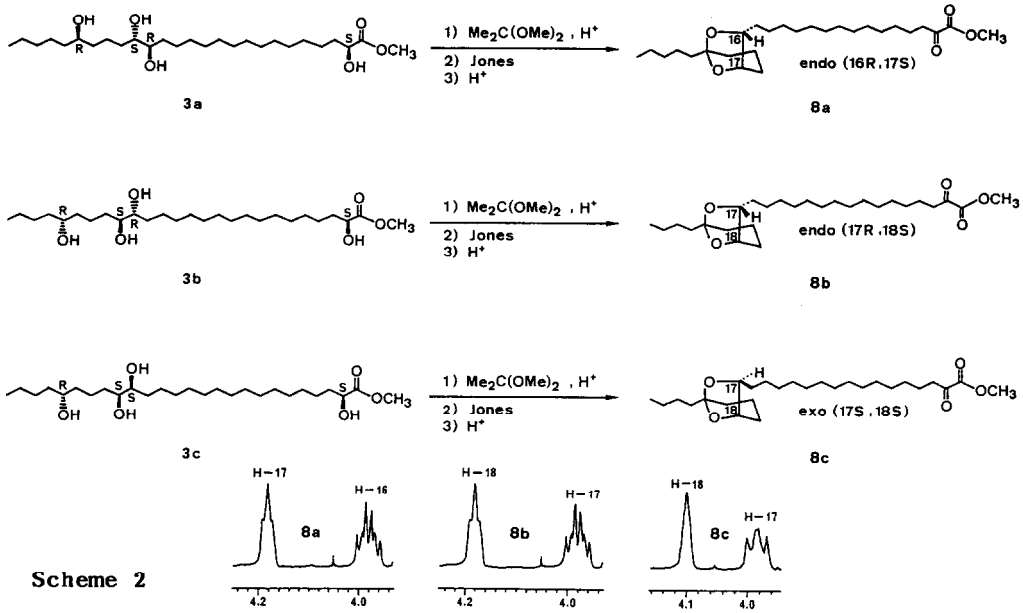


Scheme 1

In order to determine the relative configurations of 1,2-diols, **3a-c** were converted to the corresponding acetonides. Analysis of their ^1H NMR spectra were found to be unfruitful because of overlap of methine protons in the 1,3-dioxolane rings. But the arrays of three hydroxyl groups at C-16, 17, and 21 in **3a** and C-17, 18, and 22 in **3b** and **3c** were suggestive that **3a-c** could be transformed into 6,8-dioxabicyclo[3.2.1]octane derivatives. Treatments of the acetonides of **3a-c** with Jones reagent followed by TsOH in MeOH yielded endo-ketals **8a** and **8b** and exo-ketal **8c** (Scheme 2). In the ^1H NMR spectrum of **8c** the coupling constant ($J_{17,18}$) was 0Hz[2] and the bridgehead proton H-18 appeared at higher field by 0.09 ppm than those of endo **8a** and **8b**. These NMR data led to the relative configurations of the 1,2-diols erythro for **3a** and **3b** and threo for **3c**.

The spacial disposition of the threo-diol in **3c** is suited for stereochemical analysis by the dibenzoate chirality method[3], and in fact the positive split CD spectrum [273nm ($\Delta\varepsilon$ +1.77), 249nm ($\Delta\varepsilon$ -7.39)] of the 2,22-diacetyl-17,18-di-p-methoxybenzoate derivative of **3c** established the absolute configurations of the threo-diol to be 17S and 18S. The chirality method was not applicable to the erythro-diols **3a** and **3c**. By the way, the 6,8-dioxabicyclo[3.2.1]octane structure of **8a-c** reminded us brevicomin[4], a pheromone of western pine beetle, which have the same skeleton. Comparison of the molecular rotations of **8a-c** with those of the synthetic brevicomins[5] revealed that the absolute configurations of the 1,2-diol moieties were 16R and 17S in **8a**, 17R and 18S in **8b**, and 17S and 18S in **8c** (Table 2).

The results stated above established that the absolute configurations of the aglycones of



Scheme 2

DG-A, B, and C were **3a** (2S, 16R, 17S, 21R), **3b** (2S, 17R, 18S, 22R), and **3c** (2S, 17S, 18S, 22R).

Table 2

Brevicomin	
(+) - endo (1S,7R) $[\alpha]_D^{25}$ = +96.6° (c 0.98, Et_2O) $[\phi]$ = +150.7°	**8a** endo (16R,17S) $[\alpha]_D^{25}$ = +36.9° (c 0.17, Et_2O) $[\phi]$ = +166.8°
(−) - endo (1R,7S) $[\alpha]_D^{25}$ = −93.1° (c 1.01, Et_2O) $[\phi]$ = −145.2°	**8b** endo (17R,18S) $[\alpha]_D^{23}$ = +35.4° (c 0.17, Et_2O) $[\phi]$ = +160.0°
(−) - exo (1S,7S) $[\alpha]_D^{25}$ = −80.3° (c 2.23, Et_2O) $[\phi]$ = −125.3°	**8c** exo (17S,18S) $[\alpha]_D^{23}$ = −36.1° (c 0.38, Et_2O) $[\phi]$ = −163.2°
(+) - exo (1R,7R) $[\alpha]_D^{25}$ = +80.9° (c 2.18, Et_2O) $[\phi]$ = +126.2°	

The location of the trisaccharides in **3a-c** were determined as follows. Permethylation of **2a-c** followed by methanolysis and PCC oxidation of the aglycones yielded trimethoxy keto-esters, whose ^1H NMR spectra showed two methylene signals adjacent to the ketone carbonyl group about at 2.0 (t) and 2.10 (t)ppm. Therefore, the trisaccharide linked to C-21-OH in **4a** and C-22-OH in **4b** and **4c**.

References

1) The stereostructures were determined by comparison with authentic synthetic samples.
2) B. P. Mundy, K. B. Lipkowitz, and G. M. Dirks, Heterocycles, **6**, 51 (1977).
3) N. Harada, and K. Nakanishi, "Circular Dichroic Spectroscopy-Exiton Coupling in Organic Chemistry", University Science Books, Mill Valley, CA, 1983.
4) R. M. Silverstain, R. G. Brownee, T. E. Bellas, D. L. Wood, and L. E. Browne, Science, **159**, 889 (1968).
5) F. Sato, O. Takahashi, T. Kato, and Y. Kobayashi, J. Chem. Soc., Chem. Commun., 1638 (1985).

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