J. Antibiot. 59(10): 640–645, 2006

ORIGINAL ARTICLE

THE JOURNAL OF ANTIBIOTICS

Talosins A and B: New Isoflavonol Glycosides with Potent Antifungal Activity from *Kitasatospora kifunensis* MJM341

II. Physicochemical Properties and Structure Determination

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Received: June 9, 2006 / Accepted: October 3, 2006
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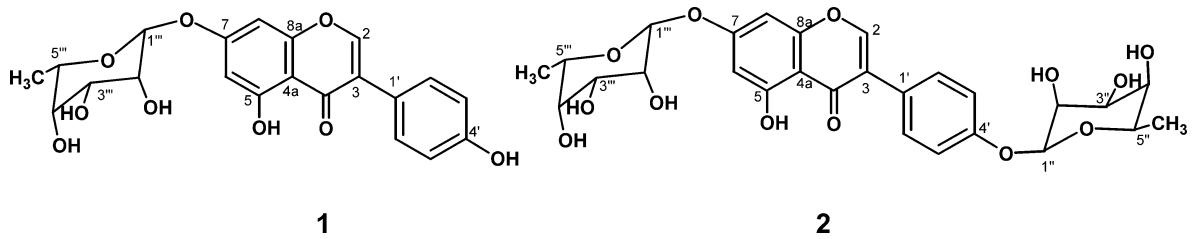
Abstract In our screening program for new antifungal agents from microbial secondary metabolites, two new isoflavonol glycosides with potent antifungal activity, talosins A and B, were isolated from the culture broth of *Kitasatospora kifunensis* MJM341. Talosins A and B were determined to be genistein 7-α-L-6-deoxy-talopyranoside and genistein 4′,7-di-α-L-6-deoxy-talopyranoside, respectively, by spectroscopic studies. They are the first flavonoid glycosides incorporating 6-deoxy-talose as a sugar component.

Keywords *Kitasatospora kifunensis*, antifungal agent, isoflavonol glycoside, genistein, 6-deoxy-talose, talosin A, talosin B

Introduction

In our screening program for new antifungal agents from microbial secondary metabolites, we isolated two new isoflavonol glycosides named talosin A (**1**) and talosin B (**2**) from the culture broth of a rare actinomycetes, *Kitasatospora kifunensis* MJM341 (Fig. 1). To our knowledge, **1** and **2** are the first flavonoid glycosides with 6-deoxy-talose as a sugar component. The compounds exhibited strong antifungal activity against *Candida albicans*, *Aspergillus niger*, and *Cryptococcus neoformans* with low toxicity because there is no trace of cytotoxicity against the human hepatic HepG2 cell. In the proceeding paper [1], we described the taxonomy of the producing



1 **2**

Fig. 1 The absolute structures of talosins A (**1**) and B (**2**).

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Table 1 Physico-chemical property of **1** and **2**

	1	**2**
Appearance	Pale yellow powder	Pale yellow powder
$[\alpha]_D^{25}$	−16.8 (*c* 0.1, MeOH)	−41.0 (*c* 0.1, MeOH)
FAB-MS (*m*/*z*)	439 $(M+Na)^+$	585 $(M+Na)^+$
HRFAB-MS (*m*/*z*)		
found	439.1017 $(M+Na)^+$	585.1547 $(M+Na)^+$
calcd.	439.1005	585.1584
Molecular formula	$C_{21}H_{20}O_9$	$C_{27}H_{30}O_{13}$
UV λ_{max} nm (ε) (MeOH)	203 (11416), 261 (12583)	203 (19269), 260 (15505)
IR (KBr) ν_{max} cm^{-1}	3415, 2120, 1647, 1050	3404, 2135, 1654, 1050

strain, fermentation, isolation, and biological activities of these new compounds. In this paper, we report on the physicochemical properties and structure determination of **1** and **2**.

Physicochemical Properties of 1 and 2

Physicochemical properties of compounds **1** and **2** were shown in Table 1. They were soluble in methanol and dimethylsulfoxide, and insoluble in water, $CHCl_3$, ether, and *n*-hexane. The optical rotation values [−16.8° (*c*, 0.1, MeOH) and −41.0° (*c*, 0.1, MeOH)] of **1** and **2** were different from those of the genistein 4′,7-α-L-rhamnopyranoside [−169° (*c*, 1, MeOH)] and genistein 7-α-L-rhamnopyranoside [−130° (*c*, 1, MeOH)], respectively [2].

Structure of 1

The molecular formula of **1** was determined to be $C_{21}H_{20}O_9$ on the basis of a high resolution FAB-MS [$(M+Na)^+$, 439.1017 *m*/*z* (+1.2 mmu error)] in combination with the ^1H and ^{13}C NMR data. The ^1H and ^{13}C NMR spectral data (Table 2) of **1**, together with the ^1H-^1H COSY, HMQC, and DEPT spectral data, revealed the presence of a 1,4-disubstituted benzene ring (δ_H 6.83; δ_C 115.0 and δ_H 7.38; δ_C 131.1), three isolated olefinic methines (δ_H 8.38; δ_C 154.3: δ_H 6.72; δ_C 94.4: δ_H 6.46; δ_C 99.9), an anomeric signal (δ_H 5.69; δ_C 98.8), four oxygenated methanes (δ_H 3.56; δ_C 71.9: δ_H 3.77; δ_C 69.8: δ_H 3.79; δ_C 65.0: δ_H 3.82; δ_C 68.4), one carbonyl carbon (δ_C 180.2), and seven sp^2 quaternary carbons (δ_C 161.7, δ_C 160.8, δ_C 157.6, δ_C 157.1, δ_C 122.6, δ_C 120.9, δ_C 106.4). These ^1H and ^{13}C spectral data suggested that **1** was composed of an isoflavone and a sugar moiety. In the HMBC spectrum, the olefinic proton at δ_H 8.38 (H-2) was coupled to the carbonyl carbon at δ_C 180.2 (C-4) and three sp^2 quaternary carbons at δ_C 157.1 (C-8a), δ_C 122.6 (C-3), and δ_C 120.9 (C-1′). The olefinic proton at δ_H 6.72 (H-8) was coupled to three sp^2 quaternary carbons at δ_C 157.1 (C-8a), δ_C 106.4 (C-4a), and δ_C 99.9 (C-6). Also, coupling was observed between the proton at δ_H 7.38 (H-2′) of the 1,4-disubstituted benzene ring and the carbon at C-3. These spectral data indicated the presence of genistein.

The planar structure of the sugar was determined using ^1H-^1H COSY and HMBC experiments. In the ^1H-^1H COSY spectrum, the methyl protons at δ_H 1.11 (H_3-6‴) and the anomeric proton at δ_H 5.69 (H-1‴) were correlated with the oxygenated methine protons at δ_H 3.82 (H-5‴) and at δ_H 3.77 (H-2‴), respectively. In the HMBC spectrum, the anomeric proton at δ_H 5.69 (H-1‴) was coupled to the oxygenated methine carbons at δ_C 65.0 (C-3‴) and δ_C 68.4 (C-5‴). In addition, couplings were observed from the methyl of H-6‴ to the carbons at C-4‴ and C-5‴. These spectral data showed the presence of a rhamnose-type planar structure. The linking position of the sugar moiety and the 1,4-disubstituted benzene ring was determined using the HMBC data, which showed the cross peaks between H-1‴ and C-7 and between H-2′ and C-3. This linkage was confirmed by the NOESY spectral data (Fig. 2). Thus, the planar structure of **1** was determined as shown in Fig. 1.

The NMR data of the sugar moiety suggested that the sugar could be 6-deoxy-talose rather than rhamnose, since the ^{13}C-NMR chemical shifts of the sugar moiety were almost identical to those of 6-deoxy-talose [3], while they were different from those of rhamnose [4] in terms of C-3, as shown in Table 3. To confirm the presence of 6-deoxy-talose in **1**, the sugar moiety was analyzed using a gas chromatography after an acid methanolysis, and followed by silylation. The retention time (16.85 minutes) of the silylated sugar component of **1** was different from the authentic silylated L-rhamnose (16.09 minutes), L-fucose (17.54 minutes), and D-fucose (17.56 minutes). In addition,

Table 2 The ^{1}H and ^{13}C NMR data of **1** and **2**

Atom	**1**			**2**		
	δ_C		δ_H (*J*, Hz)	δ_C		δ_H (*J*, Hz)
2	154.3	CH	8.38 (1H, s)	154.7	CH	8.46 (1H, s)
3	122.6	C		121.9	C	
4	180.2	C		179.9	C	
4a	106.4	C		105.8	C	
5	161.7	C		161.0	C	
6	99.9	CH	6.46 (1H, s)	99.6	CH	6.50 (1H, s)
7	160.8	C		161.0	C	
8	94.4	CH	6.72 (1H, s)	94.5	CH	6.77 (1H, s)
8a	157.1	C		156.9	C	
1′	120.9	C		123.8	C	
2′,6′	131.1	CH	7.38 (2H, d, 10.2)	129.9	CH	7.50 (2H, d, 10.2)
3′,5′	115.0	CH	6.83 (2H, d, 10.2)	116.0	CH	7.11 (2H, d, 10.2)
4′	157.6	C		155.7	C	
1″				98.4	CH	5.54 (1H, br s)
2″				69.7	CH	3.75 (1H, m)
3″				64.8	CH	3.77 (1H, m)
4″				71.7	CH	3.53 (1H, m)
5″				67.6	CH	3.83 (1H, m)
6″				16.4	CH_3	1.05 (3H, d, 6.6)
1‴	98.8	CH	5.69 (1H, br s)	98.5	CH	5.70 (1H, br s)
2‴	69.8	CH	3.77 (1H, m)	69.3	CH	3.75 (1H, m)
3‴	65.0	CH	3.79 (1H, m)	64.6	CH	3.77 (1H, m)
4‴	71.9	CH	3.56 (1H, m)	71.5	CH	3.53 (1H, m)
5‴	68.4	CH	3.82 (1H, m)	68.1	CH	3.83 (1H, m)
6‴	16.4	CH_3	1.11 (3H, d, 6.6)	16.3	CH_3	1.05 (3H, d, 6.6)

The ^{1}H and ^{13}C NMR spectra of **1** and **2** were recorded at 500 and 125 MHz, respectively, in DMSO-d_6. The assignments were aided by ^{1}H-^{1}H COSY, DEPT, NOESY, HMQC, and HMBC.

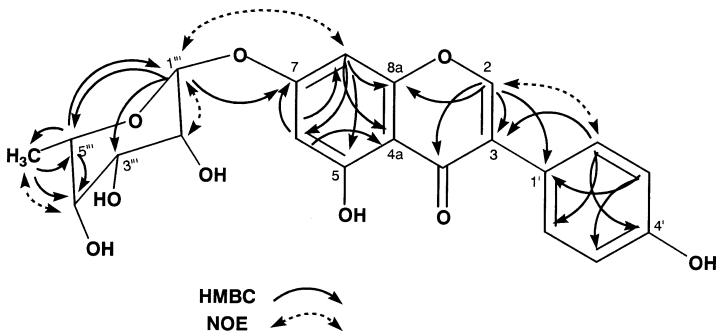

Fig. 2 HMBC and NOE data of talosin A (**1**).

comparing the ^{1}H-NMR data assigned by the ^{1}H-^{1}H COSY spectrum, the peak of the H-2 of the silylated sugar component of **1** shifted upfield in the authentic silylated L-rhamnose, wherase the peak of H-5 shifted downfield in the authentic silylated L-rhamnose (data not shown). Together with the ^{13}C-NMR data, this indicated that the sugar moiety of **1** was not L-rhamnose but 6-deoxy-talose. The absolute configuration of the sugar component was shown to be L from its negative $[\alpha]_D^{25}$ value (−16.8° (*c*, 0.1, MeOH); reported value [5], −13.7° (*c*. 0.15)). The sugar's

anomeric configuration was determined to be α based on the small $J_{H\text{-}1,H\text{-}2}$ value (3.2 Hz) of the silylated sugar component (Fig. 3). The small $J_{H\text{-}2,H\text{-}3}$ and $J_{H\text{-}4,H\text{-}5}$ values of the silylated sugar component correlated well with those of α-L-6-deoxy-talopyranoside [6]. From these above data, the sugar component of **1** was determined as α-L-6-deoxy-talopyranoside. The conformation of the sugar was examined by the NOE spectroscopic data of the silylated sugar measured at 800 MHz in acetone-d_6. An NOE between H-3‴ and H-5‴ was observed, while there was no NOE between H-2‴ and H-4‴ (Fig. 3). These data suggested that the sugar could be $^1C_4$ conformation. Thus, the structure of **1** was determined to be genistein-7-α-L-6-deoxy-talopyranoside, as shown in Fig. 1.

Structure of 2

The molecular formula of **2** was determined to be $C_{27}H_{30}O_{13}$ on a basis of high resolution FAB-MS [$(M+Na)^+$, 585.1547 m/z (−3.7 mmu error)] in combination with the ^1H and ^{13}C NMR data. The ^1H and ^{13}C NMR spectral data (Table 2) of **2** were similar to those of **1**. The major differences between **1** and **2** in the ^1H and ^{13}C NMR spectra with regards to the HMQC data were that an additional set of one anomeric methine, four oxygenated methines, and one methyl signal were observed in **2**. These spectral data suggested that one more 6-deoxy-talose could be present in **2**. The presence of one more 6-deoxy-talose was confirmed by HMBC experiments (Fig. 4). One anomeric proton at δ_H 5.54 (H-1″) was coupled to the oxygenated methine carbons at δ_C 64.8 (C-3″) and δ_C 67.6 (C-5″), while the other anomeric proton at δ_H 5.70 (H-1‴) was correlated to the oxygenated methine carbons at δ_C 64.6 (C-3‴) and δ_C 68.1 (C-5‴). Also, couplings were observed from the methyl protons (δ_H 1.05, H-6″ and H-6‴) to four oxygenated carbons at δ_C 67.6 (C-5″), δ_C 71.7 (C-4″), δ_C 68.1 (C-5‴), and δ_C 71.5 (C-4‴). These spectral data indicated that there were two 6-deoxy-taloses in **2**. The linkage position of the two 6-deoxy-taloses was determined using the NOESY and HMBC data. The anomeric proton of H-1″ was coupled to the carbon at δ_C 155.7 (C-4′) while the other anomeric proton of H-1‴ was coupled to the carbon at δ_C 161.0 (C-7). In addition, there were NOE effects from the anomeric proton of H-1‴ to the aromatic

Table 3 Comparison of ^{13}C chemical shifts of the sugar component of **1** with those of the known α-6-deoxy-L-talose and α-L-rhamnose

	The sugar of **1** (DMSO-d_6)	α-6d-L-talose[a] (D_2O)	α-L-rhamnose[b] (DMSO-d_6)
1	98.8	98.0	98.9
2	69.8	71.1	70.9
3	65.0	66.6	71.2
4	71.9	73.6	72.6
5	68.4	68.5	70.3
6	16.4	16.7	18.5

[a] 6-deoxy-talose residue of *O*-deacetylated polysaccharide [3].

[b] 7-*O*-α-L-rhamnopyranoside residue of astrasikokioside I (kaempferol 3-*O*-α-rhamnopyranosyl-(1→6)-[α-L-rhamopyranosyl-(1→2)]-β-D-galactopyranosyl-7-*O*-α-L-rhamnopyranoside) [4].

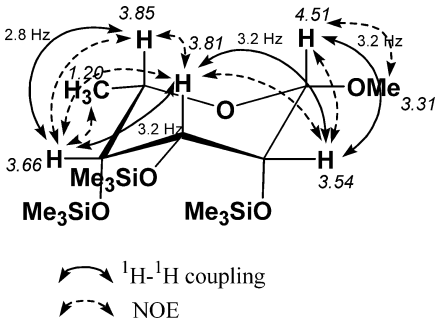

Fig. 3 ^1H-NMR chemical shifts, coupling constants ($^1J_{H,H}$), and NOE effects of the silylated sugar component of talosin A (**1**) at 800 MHz in acetone-d_6.

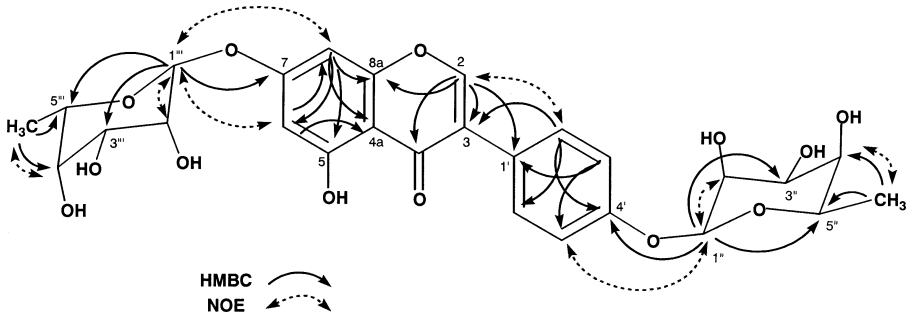

Fig. 4 HMBC and NOE data of talosin B (**2**).

protons at δ_H 6.50 (H-6) and δ_H 6.77 (H-8). From these spectral data, one 6-deoxy-talose with the anomeric proton at δ_H 5.54 should be connected to C-4′, while the other 6-deoxy-talose with the anomeric proton at δ_H 5.70 should be connected to C-7. The other remaining structure was also confirmed using the HMBC spectral data (Fig. 4). The absolute and anomeric configurations of the sugar components should be L and α respectively, since the $[\alpha]_D^{25}$ value (−41.0° (*c*, 0.1, MeOH)) and the coupling constants of the anomeric protons of **2** were similar to those of **1** Thus, structure of **2** was determined to be genistein-4′,7-di-α-L-6-deoxy-talopyranoside, as shown in Fig. 1.

Discussion

Talosins A and B are new isoflavonol glycosides with 6-deoxy-L-talose as a sugar moiety. The 6-deoxy-L-talose is a stereoisomer at C-4 of L-rhamnose. Even though many of flavonoid glycosides with L-rhamnose as a sugar component have been known, a flavonoid glycoside with a 6-deoxy-L-talose has not been reported yet, to our knowledge. The 6-deoxy-L-talose has been known as a residue of the outer-membrane lipopolysaccharide in some Gram (−) bacteria such as *E. coli* [6], *Aeromonas hydrophila* [3], and *Actinobacillus actinomycetemcomitans* [5]. To the best of our knowledge, 6-deoxy-L-talose is reported as a component of a small molecule metabolite in this study for the first time.

Both talosins A and B showed potent antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus niger* which cause systemic mycosis. Very interestingly, genistein 4′,7-di-rhamnopyranoside [7], genistein 7-rhamnopyranoside [7], and genistein 7-glucopyranoside, however, did not inhibit the growth of yeasts and fungi at 100 μg/ml as described in the proceeding paper [1]. These results indicate that the 6-deoxy-talose moiety of talosins A and B could be crucial for antifungal activity. So, talosin A and talosin B would be very useful for studying the mode of action of their antifungal activity.

Experimental

Instrumental Analysis

The NMR spectra were recorded in DMSO-d_6 solutions on a Varian Unity 500 spectrometer. The NOE NMR spectra of the silylated sugar were measured on a Bruker Avance 800. The proton and carbon NMR spectra were measured at 500 and 125 MHz, respectively. All of the chemical shifts were recorded in δ (ppm) using TMS as the internal standard. Mass spectra were obtained by a Jeol JMS-HX 110 high-resolution mass spectrometer, provided by Korea Basic Science Institute, Korea.

Acid Hydrolysis and GC Analysis

Methanol containing 0.5 M HCl was prepared by adding acetyl chloride (140μl) to anhydrous methanol (1 ml). 1 (2 mg) was suspended in MeOH/HCl (0.5 ml) and kept for 16 hours at 80°C. Then, the cooled solution was concentrated to dryness at 40°C under a stream of nitrogen gas. An excess (0.3 ml) of TriSil® reagent (Trimethylsilylation reagent (Hexamethyldisilazane : Trimethylchlorosilane : Pyridine (2 : 1 : 10)), Pierce (Rockford, USA)) was added, and the solution was kept for 20 minutes at 80°C. The reagent was removed at 40°C with a stream of nitrogen gas. The residue was then extracted with hexane (1 ml). The hexane was concentrated to 50 μl, and 2 μl was used for the GC-MS analysis. All analyses were performed in triplicate. The GC-MS was performed with a Shimadzu QP2010 GC-MS using a DB-5 ms column (0.32 mm×30 m, 0.25 μm thickness). Both temperatures of the injector and detector were 250°C. The transfer line to the MSD was set at 280°C. The GC was operated using temperature programming (120∼145°C at 1°C/minute, 145∼180°C at 0.9°C/minute, and 180∼230°C at 50°C/minute).

Acknowledgment This study was supported by a grant (grant no. MG05-0303-1-0) from the 21C Frontier Microbial Genomics and Application Center program, the Korean Ministry of Science and Technology. The authors appreciate for this support. We also thank to ECUM (Extract Collection of Useful Microorganism), a member of the Korean National Research Resource Center (KNRRC) supported by the Korean Ministry of Science and Technology, for providing talosins and the producing strain.

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