

New Aldose Reductase Inhibitors N99-596 A and B from *Streptomyces*

Yuesheng Dong, Junshan Yang, Xiao Ren, Hua Zhang, Jiangong He

Received: September 26, 2005 / Accepted: October 26, 2005

© Japan Antibiotics Research Association

Abstract The actinomycete *Streptomyces diannanensis*, isolated from a soil sample collected in Yunnan province, China, was found to produce two novel isoflavone rhamnopyranosides, namely, 7,4'-dihydroxyisoflavone 3'-*O*- α -L-rhamnopyranoside (**1**) and 5,7,4'-trihydroxyisoflavone 3'-*O*- α -L-rhamnopyranoside (**2**). The structures of **1** and **2** were elucidated on the basis of extensive 1D and 2D NMR and MS spectral analyses. Compounds **1** and **2** exhibited *in vitro* activities against aldose reductase with an IC₅₀ of 170 and 165 μ M, respectively.

Keywords aldose reductase, inhibitor, isoflavone rhamnopyranosides

The sorbitol pathway appears to be involved in metabolism of the excess glucose in diabetic tissues. This may lead to development of some of these complications. Aldose reductase (AR), an enzyme in the polyol pathway, catalyzes the reduction of glucose to sorbitol. Many AR inhibitors have been isolated from microorganisms [1–3]. The clinical efficacy of certain AR inhibitors such as epalrestat has been reported [4,5]. In the course of our screening program for aldose reductase inhibitors, two new isoflavonoids N99-596A (**1**) and B (**2**) were found in the fermentation broth of an actinomycete strain N99-596,

which was identified as a new actinomycete species named *Streptomyces diannanensis*. This paper reports the basic taxonomy and fermentation of the strain N99-596 and the isolation, biological properties and structure elucidation of **1** and **2**.

Strain N99-596 was isolated from a soil sample collected at Diannan in Yunnan Province, China. The culture of N99-596 was characterized by the gray aerial mycelium and the spiral spore chains with whole cell hydrolysate containing LL-diaminopimelic acid, which is of Type I. Strain N99-596 was concluded to be a new actinomycete species based on chemotaxonomy and molecular biology (The result will be reported elsewhere). The strain was deposited in China General Microbiological Culture Collection, Beijing, China, under the name of *Streptomyces diannanensis* and the accession number is CGMCC No. 0834.

Strain N99-596 was cultivated at 27°C on a rotary shaker at 220 rpm in the medium consisting of soluble starch 4.0%, soybean meal 2.0%, Na₂S₂O₃ solution (0.1 mol/liter) 32 μ l/liter, FeSO₄·7H₂O 0.05%, K₂HPO₄ 0.05%, KCl 0.03% in water (pH 6.5). After fermentation for 6 days, the cultured broth (5.6 liters) was centrifuged to separate mycelial cake and supernatant. The mycelial cake was extracted with acetone (2.0 liters). The acetone solution was filtered and evaporated to remove acetone, and then extracted with ethyl acetate (2.5 liters). The ethyl acetate layer was evaporated to give 2.2 g of crude extract. This extract was subjected to a silica gel column and eluted with chloroform-methanol. The active fractions showing

J. Yang (Corresponding author), **Y. Dong**: Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100094, People's Republic of China, E-mail: jsyang2005@yahoo.com.cn

Y. Dong, X. Ren, H. Zhang, J. He: New Drug Research and Development Center, North China Pharmaceutical Group Corporation and National Microbial Medicine Engineering and Research Center, Shijiazhuang 050015, People's Republic of China

inhibitory activity against AR were collected and evaporated to give 85.2 mg of a residue. The residue was separated by HPLC using a reverse phase column (Phenomenex PRODIGY 10 μ -Prep, 250 \times 20 mm i.d.), and a solvent of 30% acetonitrile/water with a flow rate of 6 ml/minute at a room temperature. The eluates were monitored with a photodiode array detector at 254 nm.

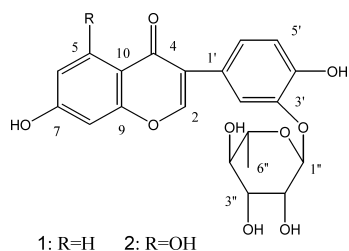


Fig. 1 Structures of compounds **1** and **2** from *Streptomyces diannanensis*.

Compounds **1** and **2** were eluted at 13.2 and 15.2 minutes, respectively, to give pure **1** (12.1 mg) and **2** (9.6 mg).

AR was prepared from pig lenses. Pig lenses were homogenized in 60 mM Na, K-phosphate buffer (pH 6.2) containing 0.5 mM phenylmethylsulfonyl fluoride (PMSF) and 0.5 mM 2-mercaptoethanol, and centrifuged at 100,000 $\times g$ for 30 minutes. The supernatants were further purified by ammonium sulfate precipitation, and saved as enzyme fractions. All the procedures for enzyme preparation were carried out at 4 $^{\circ}$ C.

AR activity was assayed according to the method previously described by Nishikawa *et al.* [1] with slight modifications. The reaction mixture contained 30 mM Na, K-phosphate buffer (pH 6.2), 0.4 M LiSO₄, 0.125 mM NADPH, 3 mM DL-glyceraldehyde and proper enzyme fraction, with or without 10 μ l of various concentrations of **1** and **2** (dissolved in DMSO) in a total volume of 200 μ l. The reaction was initiated by the addition of NADPH, the concentration change in NADPH was monitored

Table 1 ¹H (500 MHz) and ¹³C (125 MHz) NMR spectral data of **1** and **2** in DMSO-*d*₆ at 25 $^{\circ}$ C

Position	1			2	
	δ_{H}	δ_{C} (mult)	HMBC (H to C)	δ_{H}	δ_{C} (mult)
2	8.29 (1H, s)	154.5 d	C-4, 9	8.34 (1H, s)	153.7 d
3		122.9 s			121.9 s
4		174.5 s			179.9 s
5	7.95 (1H, d, 9)	127.3 d	C-4, 7, 9		164.0 s
6	6.93 (1H, dd, <i>J</i> =9, 2.5)	115.1 d	C-8, 10	6.22 (1H, d, <i>J</i> =2.5)	99.9 d
7		162.5 s			161.8 s
8	6.86 (1H, d, <i>J</i> =2.5)	102.0 d	C-7, 9	6.38 (1H, d, <i>J</i> =2.5)	93.4 d
9		157.3 s			157.3 s
10		116.3 s			106.7 s
5-OH	—			12.58 (1H, s)	
7-OH	10.23 (1H, s)			10.32 (1H, s)	
1'		123.2 s			123.2 s
2'	7.28 (1H, d, <i>J</i> =2)	119.4 d	C-1', 3', 4', 6'	7.28 (1H, d, <i>J</i> =2)	121.5 d
3'		143.6 s			143.6 s
4'		147.8 s			147.9 s
5'	6.87 (1H, d, <i>J</i> =8.5)	116.6 d	C-1', 3', 4', 6'	6.88 (1H, d, <i>J</i> =8.5)	115.9 d
6'	7.05 (1H, dd, <i>J</i> =8.5, 2)	123.4 d	C-1', 2', 4', 5'	7.07 (1H, dd, <i>J</i> =8.5, 2)	120.9 d
4'-OH	9.15 (1H, s)			9.09 (1H, s)	
Sugar					
1''	5.27 (1H, br s)	99.9 d	C-3'', 2'', 3'', 5''	5.27 (1H, s)	98.8 d
2''	3.70 (1H, m)	70.1 d		3.64 (1H, m)	69.9 d
3''	3.90 (1H, m)	70.3 d		3.90 (1H, m)	70.2 d
4''	3.16 (1H, m)	71.9 d		3.14 (1H, m)	71.8 d
5''	3.72 (1H, m)	69.4 d		3.75 (1H, m)	69.2 d
6''	1.13 (3H, d, <i>J</i> =6.5)	17.8 q	C-3'', 4'', 5''	1.05 (3H, d, <i>J</i> =6.5)	17.4 q

spectrophotometrically for 1 minute at 340 nm using a spectrophotometer at 37°C. As a result, **1** and **2** inhibited AR at the IC₅₀ of 170 μM and 165 μM, respectively.

Compound **1** was obtained as a pale yellow solid with UV (MeOH) λ_{max} 249 and 320 (sh) nm, and its formula was established as C₂₁H₂₀O₉ by HRFAB-MS *m/z* 417.1194 [M+H]⁺, Calculated for 417.1185. The ¹H and ¹³C NMR spectra revealed that **1** was an isoflavonoid with glycosidic functionalities present (Table 1). The ABX spin system in ¹H NMR δ 7.95 (1H, d, *J*=9 Hz), 6.93 (1H, dd, *J*=9, 2.5 Hz) and 6.86 (1H, d, *J*=2.5 Hz), which correlated to carbons at δ 127.3, 115.1, 102.0 in the HMQC, were characteristic of H-5, H-6, H-8 protons of the A ring and the other three signals in ¹H NMR δ 7.28 (1H, d, *J*=2 Hz), 6.87 (1H, d, *J*=8.5 Hz) and 7.05 (1H, dd, *J*=8.5, 2 Hz), which correlated to carbons at δ 119.4, 116.6, 123.4 in the HMQC, were assigned to H-2', H-5' and H-6' protons of the B ring of **1**, respectively. Moreover, one proton broad singlet at δ 5.27 with very small *J* values and a set of three-proton doublets (*J*=6.5 Hz) at δ 1.13 were presented. These facts suggested the presence of a deoxysugar with a α-configuration because of the very low *J* values of its anomeric protons due to trans-diequatorial interactions. Analysis of the ¹³C NMR spectrum confirmed the identity of the sugar as rhamnose. In HMBC spectra, strong correlations from H-1" (δ 5.27) to δ 143.6 (C-3') (three bonds correlation) and relative weak correlations from H-2' (δ 7.28) to C-3' (two bonds correlation) suggested that the rhamnose group was located at C-3'. Acid hydrolysis of **1** liberated L-rhamnose, identified by TLC with authentic samples. Therefore **1** is 7,4'-dihydroxyisoflavone 3'-*O*-α-L-rhamnopyranoside (daidzein 3'-*O*-α-L-rhamnopyranoside).

Compound **2** was obtained as a pale yellow solid with UV (MeOH) λ_{max} 260 and 320 (sh) nm, and its molecular formula was confirmed as C₂₁H₂₀O₁₀ from HRFAB-MS *m/z* 433.1156 [M+H]⁺, Calculated for 433.1134. NMR and other spectroscopic data were nearly identical with those of **1** (Table 1). The only difference was that two *meta*-coupled doublets at δ 6.22 (1H, d, *J*=2.5 Hz) and 6.38 (1H, d, *J*=2.5 Hz), which correlated to carbons at δ 99.9 and 93.4 ppm in the HMQC, were characteristic of H-6, and H-8 protons of A ring. In conclusion, the structure of **2** was elucidated as 5,7,4'-trihydroxyisoflavone 3'-*O*-α-L-rhamnopyranoside (genistein 3'-*O*-α-L-rhamnopyranoside).

Compounds **1** and **2** were isolated only from the fermentation broth of *Streptomyces diannanensis* which was cultured with medium containing soybean meal (data not shown), but the two compounds could not be obtained from the uninoculated soybean meal. This

suggested that both soybean meal and *Streptomyces diannanensis* are necessary for the production of these two compounds.

Although some novel isoflavones and isoflavone glucosides have been isolated from actinomycetes, especially from *Streptomyces* [6–9], only Hu *et al.* [6] and Hazato *et al.* [7] reported the production of isoflavone rhamnopyranosides from microorganisms. Novel compounds N99-596 A and B which belong to isoflavone rhamnopyranosides are rare microbial metabolites as far as we know.

References

1. Nishikawa M, Tsurumi Y, Namiki T, Yoshida K, Okuhara M. Studies on WF-3681, a novel aldose reductase inhibitor I. Taxonomy, fermentation, isolation and characterization. *J Antibiot* 40: 1394–1399 (1987)
2. Rao KCS, Divakar S, Srinivas M, Babu KN, Karanth NG, Sattur AP. Asperaldin, a new aldose reductase inhibitor from *Aspergillus niger* CFR-1046. I. Fermentation, isolation and characterization. *J Antibiot* 56: 173–176 (2003)
3. Rao KCS, Divakar S, Babu KN, Rao AGA, Karanth NG, Sattur AP. Nigerloxin, a novel inhibitor of aldose reductase and lipoxxygenase with free radical scavenging activity from *Aspergillus niger* CFR-W-105. *J Antibiot* 55: 789–793 (2002)
4. Dvornik E, Simard-Duquesne N, Krami M, Sestanj K, Gabbay KH, Kinoshita JH, Varma SD, Merola LO. Polyol accumulation in galactosemic and diabetic rats: control by an aldose reductase inhibitor. *Science* 182: 1146–1148 (1973)
5. Nakamura N, Yamazaki K, Satoh A, Urakaze M, Kobayashi M, Yamabe H, Osawa H, Shirato K, Sugawara T, Nakamura M, Tamura M, Okumura K. Effects of eparlestat on plasma levels of advanced glycation end products in patients with type 2 diabetes. *In Vivo* 17: 177–180 (2003)
6. Hu JF, Wunderlich D, Sattler I, Thiericke R, Grabley S, Feng XZ. New 2-*O*-methylrhamno-isoflavones from *Streptomyces* sp. *Nat Prod Res* 17: 451–458 (2003)
7. Hazato T, Naganawa H, Kumagai M, Aoyagi T, Umezawa H. β-Galactosidase-inhibiting new isoflavonoids produced by actinomycetes. *J Antibiot* 32: 217–222 (1979)
8. Umezawa H, Tobe H, Shibamoto N, Nakamura F, Nakamura K, Matsuzaki M, Takeuchi T. Isolation of isoflavones inhibiting DOPA decarboxylase from fungi and *Streptomyces*. *J Antibiot* 28: 947–952 (1975)
9. Komiyama K, Funayama S, Anraku Y, Mita A, Takahashi Y, Omura S, Shimasaki H. Isolation of isoflavonoids possessing antioxidant activity from the fermentation broth of *Streptomyces* sp. *J Antibiot* 42: 1344–1349 (1989)