Chemistry of Natural Compounds, Vol. 44, No. 5, 2008

FIVE UNUSUAL NATURAL CARBOHYDRATES FROM *Actinosynnema pretiosum*

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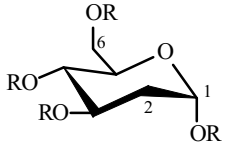
UDC 547.752

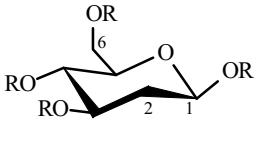
Five unusual hexose derivatives were isolated from the carbohydrate portion of the solid-state fermentation extract of Actinosynnema pretiosum *ssp.* auranticum *ATCC 31565, which is a producing strain of maytansinoids that are a family of 19-membered macrocyclic lactams having extraordinary cytotoxic and antineoplastic activities. Their structures were determined to be 2-deoxy-α-D-*arabino-*hexopyranose (**1**), 2-deoxy-β-D-*arabino-*hexopyranose (**2**), 3,6-anhydro-2-deoxy-α-D-*arabino-*hexcofuranose (**3**), 3,6-anhydro-2-deoxy-β-D-*arabino-*hexofuranose (**4**), and 2-(D-glycerol-1,2-dihydroxyethyl)furan (**5**) by NMR spectroscopic experiments.*

Key words: *Actinosynnema pretiosum* ssp. *auranticum* ATCC 31565, 2-deoxyglucose (2-DG), 3,6-anhydro-2-deoxyglucose, 2-(D-glycerol-1,2-dihydroxyethyl)furan.

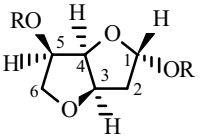
Actinosynnema pretiosum ssp. *auranticum* ATCC 31565 is a producing strain of maytansinoids that are a family of 19-membered macrocyclic lactams having extraordinary cytotoxic and antineoplastic activities [1, 2]. Recently, Floss and coworkers have reported the cloning, sequencing and characterization of the maytansinoid, ansamitocin, biosynthetic gene cluster (*asm*) from a cosmid library of *A. pretiosum* ssp. *auranticum* ATCC 31565 [3]. However, the biological functions of many loci are unknown, indicating that this strain has the potential to generate a diverse array of secondary metabolites. Recently, solid-state fermentation (SSF) has become more and more attractive for the production of antibiotics because it produces a high product concentration with a relatively low energy requirement in a shorter time period [4]. To explore the potential productivity of secondary metabolites of *A. pretiosum* ssp. *auranticum* ATCC 31565, this strain was cultivated by SSF on YMG media to afford five unusual carbohydrates (**1**–**5**) through isolation by repeated column chromatography.



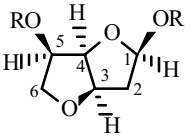
1, 1a



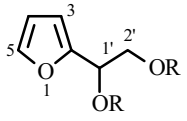
2, 2a



3, 3a



4, 4a



5, 5a

1 - 5: R = H; **1a - 5a:** R = Ac

Inspection of the NMR data of **1**/**2** (proton, carbon, DEPT, HMQC, HMBC, ^{1}H–^{1}H COSY) revealed 12 carbon signals for 4 methylene and 8 methine groups, respectively, indicating the existence of two 2-deoxyhexoses, and that one was in the α-form (**1**) and the other in the β-form (**2**) as indicated by the coupling constants of the two anomeric protons (Table 1). The complete NMR assignments were carried out on the basis of HMQC, HMBC, and ^{1}H–^{1}H COSY experiments. Moreover, the HMBC experiment indicated no linkage between **1** and **2**, revealing that **1** and **2** were anomeric mixtures, which was further supported by the proton signal integrations (**1:2** ≈ 7:5) and the ESIMS data (*m/z* 354.9 $[M+Na]^+$) and HRFABMS data (*m/z* 333.1191 $[M+H]^+$, $C_{14}H_{21}O_9$, calcd.: 333.1186) of the tetra-*O*-acetyl products **1a**/**2a** ($[\alpha]_D^{28}$ +34.7 (*c* 2.19, CH_3OH)).

1) Key Laboratory of the Ministry of Education for Cell Biology and Tumor Cell Engineering; Xiamen Engineering Research Center of Marine Microbial Drug Discovery; Fujian Laboratory of Pharmaceutical Engineering; School of Life Science, Xiamen University, Xiamen, Fujian 361005, P. R. China, Fax: 86 592 2181722, e-mail: yshen@xmu.edu.cn; 2) Bio-X Life Science Research Center, Shanghai Jiaotong University, Shanghai 200030, China. Published in Khimiya Prirodnykh Soedinenii, No. 5, pp. 481-483, September-October, 2008. Original article submitted June 6, 2007.

 0009-3130/08/4405-0594

TABLE 1. The NMR Assignments for **1**-4 and **1a-4a**

C atom	**1**[a]		**1a**[b]		**2**[a]		**2a**[b]	
	δ_C	δ_H (J/Hz)	δ_C	δ_H (J/Hz)	δ_C	δ_H (J/Hz)	δ_C	δ_H (J/Hz)
1	92.7 d	5.26 (d, J = 2.6)	91.3 d	6.47 (br.d, J = 2.0)	95.0 d	4.81 (d, J = 9.5)	91.6 d	6.18 (dd, J = 2.2, 10.0)
2a	41.7 t	2.05 (br.dd, J = 5.0, 12.8)	34.2 t	2.42 (dd, J = 1.3, 5.1)	39.4 t	2.15 (ddd, J = 1.7, 4.5, 12.4)	35.2 t	2.49 (ddd, J = 2.2, 5.1, 12.3)
2b		1.62 (dt, J = 2.6, 12.8)		2.00 m		1.50 (dt, J = 9.5, 12.4)		2.00 (dt, J = 10.0, 12.3)
3	73.4 d	3.80 m[e]	70.8	5.36 m[e]	72.5 d	3.68 m[e]	70.6	4.29 m[e]
4	69.6 d	3.90 m[e]	69.0	5.40 m[e]	72.9 d	3.18 m[e]	71.3	5.36 m[e]
5	73.4 d	3.26 m[e]	69.9	4.33 m[e]	77.9 d	3.24 m[e]	74.5	4.55 m[e]
6a	62.8 t	3.68 m[e]	62.6 t	5.34 (m[e], 2H)	62.9 t	3.86 m[e], 3.78 m[e]	62.5 t	5.34 (m[e], 2H)
6b		3.64 m[e]						
Ac	-	-	20.5	2.01 (s, 3H)	-	-	20.5	2.01 (s, 3H)
			20.7	2.02 (s, 3H)			20.7	2.02 (s, 3H)
			20.7	2.02 (s, 9H)			20.7	2.02 (s, 9H)
			169.9	2.02 (s, 3H)			169.9	2.02 (s, 3H)
			170.0	2.05 (s, 3H)			170.0	2.05 (s, 3H)
			170.1	2.08 (s, 3H)			170.1	2.08 (s, 3H)
			170.2				170.2	
			170.4				170.4	

C atom	**3**[a]		**3a**[b]		**4**[a]		**4a**[b]	
	δ_C	δ_H[c] (J/Hz)	δ_C	δ_H[c] (J/Hz)	δ_C	δ_H[c] (J/Hz)	δ_C	δ_H[c] (J/Hz)
1	101.5	5.61 (dd, J = 2.9, 4.2)	100.7	6.66 (dd, J = 2.6, 5.5)	101.0	5.57 (d, J = 4.5)	99.5	6.45 (d, J = 5.5)
2a	43.1	2.07 m	40.2	2.30 (ddd, J = 2.6, 6.9, 14.5)	42.0	2.04 m	40.2	2.00 m
2b		2.16 m		2.36 (ddd, J = 2.9, 5.5, 14.5)		2.13 m	83.4e	2.24 m
3	83.3	4.73 m	81.8[d]	4.86 m	85.5	4.63 m	82.2e	4.73 (t, J = 5.5)
4	82.2	4.57 (t, J = 5.9)	81.6[d]	5.01 (t, J = 6.0)	83.8	4.59 m	73.9	4.98 (t, J = 5.5)
5	73.2	4.19 m	73.7	5.19 (dt, J = 6.0, 6.9)	72.6	4.16 m	69.1	5.15 (dt, J = 5.5, 7.2)
6a	71.7	3.51 (dd, J = 6.4, 8.6)	68.7	3.89 (dd, J = 6.9, 9.1)	73.7	3.72 (dd, J = 5.5, 8.9)		4.10 (dd, J = 7.2, 8.9)
6b		3.80 (dd, J = 6.2, 8.6)		4.03 (dd, J = 6.9, 9.1)		3.89 (dd, J = 5.5, 8.9)		4.19 (dd, J = 7.2, 8.9)
Ac	-	-	20.9,	1.91 (s, 3H)	-	-	21.2,	1.99 (s, 3H)
			20.4,	2.01 (s, 3H)			20.4,	2.00 (s, 3H)
			170.3,				170.2,	
			169.7				170.0	

[a]^{1}H, ^{13}C NMR, and HMBC spectra were obtained at 400 MHz, 100 MHz, and 500 MHz, respectively, and recorded in CD_3OD at room temperature.
[b]^{1}H, ^{13}C NMR, ^{1}H–^{1}H COSY, and ROESY spectra were obtained at 500 MHz, 125 MHz, and 500 MHz, respectively, and recorded in C_5D_5N at room temperature.
[c]Coupling constants are presented in Hertz. Unless otherwise indicated, all proton signals integrate to 1H.
[d,e]The asignments are interchangeable.

The ROESY experiments on **1**/**2** and their tetra-*O*-acetyl products **1a**/**2a** further supported the configuration assignments for the anomeric carbons. The ratio of **1a** to **2a** was reversed to 5:7 from 7:5 of the starting materials as revealed by the proton signal integrations, indicating that **1** and **2** were anomers. Therefore, compounds **1** and **2** were determined to be 2-deoxy-α-D-*arabino*-hexopyranose and 2-deoxy-β-D-*arabino*-hexopyranose, respectively [5–7].

TABLE 2. The NMR Assignments for **5**[a]

C atom	δ_C	δ_H[b]	HMBC	C atom	δ_C	δ_H[b]	HMBC
234	155.0 s	-	-	5	142.3 d	7.47 (d, J = 1.8)	C-2, C-3, C-4
	107.7 d	6.34 (d, J = 3.2)	C-2, C-4, C-5	1′	69.5 d	4.70 (t, J = 6.4)	C-2, C-3, C-2′
	111.2 d	6.39 (dd, J = 1.8, 3.2)	C-3	2′	65.8 t	3.81 (dd, J = 6.4, 9.5, 2H)	C-2, C-1′

[a]^{1}H, ^{13}C NMR, and HMBC spectra were obtained at 500 MHz, 100 MHz, and 500 MHz, respectively, and recorded in CD_3OD at room temperature.

[b]Coupling constants are presented in Hertz. Unless otherwise indicated, all proton signals integrate to 1H.

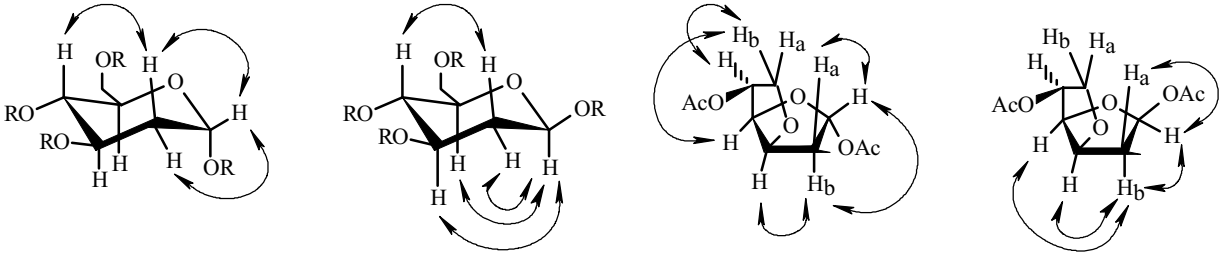


Fig. 1. The selected ROESY correlation of **1**, **2** , **3a** and **4a**.

TLC developed by several different solvent combinations gave only one spot for **3**/**4**, but the NMR data, particularly the proton signal integrations, indicated that **3** and **4** were in a mixture ratio of 2:1. The acetylation of this mixture in pyridine and acetic anhyride produced two compounds **3a** and **4a** in 2:1 ratio after isolation with column chromatography over Si gel eluted with a chloroform–methanol gradient as well. Inspection of the NMR data of **3**/**4** (proton, carbon, DEPT, HMQC, HMBC, ^{1}H–^{1}H COSY) indicated that they were 2-deoxyhexoses. The complete NMR assignments based on HMQC, HMBC, and ^{1}H–^{1}H COSY experiments revealed that **3** and **4** were furanoses (Table 2, Fig. 1).

The ^{1}H–^{13}C long-range correlations between H-6 and C-3 revealed the linkage between C-3 and C-6 via oxygen. The structure elucidation was further supported by the HRFABMS data of **3a** (*m/z* 231.0860 $[M+H]^+$, $C_{10}H_{15}O_6$, calc.: 231.0869) and **4a** (*m/z* 231.0868 $[M+H]^+$, $C_{10}H_{15}O_6$, calc.: 231.0867). The relative stereochemistry of **3**, **4**, **3a**, and **4a** was determined with the aid of ROESY experiments (Fig. 1), showing that **3** and **4** were α- and β-anomers; therefore, **3** and **4** were determined to be 3,6-anhydro-2-deoxy-β-D-glucofuranose and 3,6-anhydro-2-deoxy-β-D-*arabino*-hexofuranose with the aid of optical rotation data (**3a** +65 (*c* 0.2, CH_3OH), **4a** +122 (*c* 0.4, CH_3OH)) [8].

The ^{13}C-NMR spectra, including DEPT of compound **5**, showed six carbon signals for one methylene, four methanes and one quaternary carbon (Table 2). The complete NMR assignments were carried out based on the HMQC, HMBC, and ^{1}H–^{1}H COSY experiments, to gether with the optical rotation data $[\alpha]_D^{28}$ +15.1° (*c* 0.06, CH_3OH), proving compound **5** is 2-(D-glycerol-1,2-dihydro-xyethyl)furan [9, 10].

EXPERIMENTAL

Optical rotations were measured with a JASCO DIP-370 digital polarimeter in MeOH solution. Mass spectra were measured on a VG Auto Spec-3000 spectrometer. NMR spectra were obtained using Bruker AM-400 or DRX-500 NMR spectrometers with TMS as internal standard. Silica gel (200–300 mesh) for column chromatography and precoated TLC plates (Si gel G) were purchased from the Qindao Marine Chemical Factory, Qingdao, P. R. China. Reversed-phase C18 silica gel for column chromatography and C18-RP-TLC plates were obtained from Merck. Sephadex LH-20 for column chromatography was purchased from Amersham Biosciences.

Culture Conditions and Extraction. *A. pretiosum* ssp. *aurantium* ATCC31565, stored in glycerol, was used to inoculate on a slope of YMG media in a test tube at 28°C for 5 days to afford seed cultures. The YMG media had the following composition (g/liter): glucose 4.0, malt extract 10.0, yeast extract 4.0, pH 7.2. Solid-state fermentation was performed with the

YMG media (3.0 L) at 28°C for 7 days, and the seed culture was inoculated with the inoculum loop. The cultured agar was chopped, diced, and extracted with EtOAc–MeOH–AcOH (80:15:5, 3.0 L) at room temperature overnight. The organic solution was collected through filtration, and the remaining agar residue was extracted several times more as described above until the filtrate was colorless. The combined filtrates were concentrated under vacuum to remove organic solvents. The aqueous solution was extracted five times with chloroform. Removal of the solvents under vacuum afforded a methanol-soluble extract (12.2 g). This extract was subjected to MPLC over reversed-phase C18 Si gel (130 g) eluted with water. The water eluents were detected by TLC and combined and concentrated under vacuum to produce a syrupyfraction (6.7 g), which contained three major carbohydrate components indicated by TLC. This syrup was subjected to column chromatography over Si gel (150 g) and eluted with EtOAc, EtOAc–MeOH (10:1, 8:1, v/v), and MeOH to afford four fractions 1–4, and each fraction was subjected to column chromatography over Sephadex LH-20 (130 g) and eluted with methanol to produce mixtures of **1**/**2** (100 mg, from fraction 4), **3**/**4** (40 mg, from fraction 2), and the pure compound **5** (3 mg, from fraction 1) (Fig. 1).

Acetylation of 1/2. The mixture of **1**/**2** (20 mg, syrup) was dissolved in 0.5 mL pyridine and 0.5 mL acetic anhydride. This reaction solution was kept at 42°C overnight. This reaction was quenched by adding 15 mL distilled water. The aqueous solution was extracted four times with an equal volume of chloroform. This chloroform extract was subjected to column chromatography over Si gel (5.5 g) and eluted with chloroform–acetone (20:1, 10:1, v/v) to produce the mixture of acetylated **1**/**2** (**1a**/**2a**, 50 mg), which gave only one spot on normal phase TLC with various developing solvent combinations (chloroform–acetone 20:1, chloroform–methanol 100:1, petroleum ether–acetone 1:1), and RP-8 TLC (acetone–water 6:4).

Acetylation of 3/4. The mixture of **3**/**4** (36 mg, syrup) was dissolved in 0.6 mL pyridine and 0.6 mL acetic anhydride. This reaction solution was kept at 42°C for 6 h. This reaction was quenched by adding 20 mL distilled water. The aqueous solution was extracted four times with an equal volume of chloroform. This chloroform extract was subjected to column chromatography over Si gel (7 g) and eluted with chloroform–acetone (100:2, 100:3, v/v) to produce **3a** (more polar than **4a**; **3a** showed the same R_f value as **1a**/**2a** on TLC developed with chloroform–acetone 4:1, v/v).

Acetylation of 5. Compound **5** (3 mg) was dissolved in 0.2 mL pyridine and 0.2 mL acetic anhydride. The reaction solution was kept at 42°C overnight. This reaction was quenched by adding 5 mL distilled water. The aqueous solution was extracted four times with an equal volume of ethyl acetate. This EtOAc extract was subjected to column chromatography over Si gel (1.5 g) and eluted with petroleum ether–acetone (10:1, v/v) to produce **5a** (2 mg).

ACKNOWLEDGMENT

This work was partially supported by the National Science Fund for Distinguished Young Scholars to Y.-M. Shen (30325044), and the National Natural Science Foundation of China (30430020).

REFERENCES

1. E. Higashide, M. Asai, K. Ootsu, S. Tanida, Y. Kozai, T. Hasegawa, T. Kishi, Y. Sugino, and M. Yoneda, *Nature*, **270**, 721 (1977).
2. M. Asai, E. Mizuta, M. Izawa, K. Haibara, and T. Kishi, *Tetrahedron,* **35**, 1079 (1979).
3. T.-W. Yu, L.-Q. Bai, D. Clade, D. Hoffmann, S. Toelzer, K. Q Trinh, J. Xu, S. J.Moss, E. Leistner, and H. G. Floss, *Proc. Natl. Acad. Sci. USA,* **99**, 7968 (2002).
4. T. Robinson, D. Singh, and P. Nigam, *Appl. Microbiol. Biotechnol.*, **55**, 284 (2001).
5. K. Bock and, C. Pedersen, *J. Chem. Soc. Perkin Trans II*, 293 (1974).
6. W. A. Bonner, *J. Org. Chem.*, **26**, 908 (1960).
7. H. Maluszynska, J. R. Ruble, and G. A. Jeffrey, *Carbohydr. Res.*, **97**, 199 (1981).
8. A. B. Foster, W. G. Overend, M. Stacey, and G. Vaughan, *J. Chem. Soc.*, 3367 (1954).
9. M. Hayashi, H. Kawabata, and K. Yamada, *Chem. Commun.*, 965 (1999).
10. B. S. Babu and K. K. Balasubramanian, *J. Org. Chem.*, **65**, 4198 (2000).