Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Research 343 (2008) 470–476
Carbohydrate RESEARCH

Two novel aminooligosaccharides isolated from the culture of *Streptomyces coelicoflavus* ZG0656 as potent inhibitors of α-amylase

Peng Geng[a] and Gang Bai[a,b,*]

[a]*Department of Microbiology, College of Life Sciences, Nankai University, 94 Weijin Road, Tianjin 300071, People's Republic of China*
[b]*College of Pharmaceutical Sciences, Nankai University, 94 Weijin Road, Tianjin 300071, People's Republic of China*

Received 12 September 2007; received in revised form 8 November 2007; accepted 10 November 2007
Available online 19 November 2007

Abstract—Two novel aminooligosaccharides were separated from the culture filtrate of *Streptomyces coelicoflavus* ZG0656. Their chemical structures were determined by electrospray ionization tandem mass spectrometry (ESI-MS/MS) and 2D nuclear magnetic resonance (NMR) spectroscopy. Because of their acarviosine core structures, the names acarviostatins II23 and II13 were given to the novel compounds. The two acarviostatins were both mixed noncompetitive inhibitors of porcine pancreatic α-amylase (PPA), with inhibition constants (K_i) of 0.009 μM (acarviostatin II23) and 0.010 μM (acarviostatin II13). Therefore, acarviostatin II23 and acarviostatin II13 are, respectively, 231 and 208 times more potent than acarbose.

Keywords: *Streptomyces coelicoflavus*; Aminooligosaccharides; Acarviosine; Acarviostatin II23; Acarviostatin II13

1. Introduction

α-Amylases are among the most important saccharide-cleaving enzymes, and act by catalyzing the hydrolysis of α-glucosidic linkages. It is well known that inhibitors of saccharide hydrolases, such as amylase and glucodase, may be used in the treatment or prevention of diabetes, hyperlipoproteinemia, hyperlipidemia, obesity, or other secondary symptoms caused by these conditions.[1] In this regard, several aminooligosaccharide derivatives, for example, acarbose,[2] adiposins,[3] amylostatins,[4] oligostatins,[5] trestatins,[6,7] (glucose)$_n$-deoxynojirimycin,[8] salbostatin,[9] CK-4416,[10] isovalertatins,[11] and butytatins[12] have been reported as potent inhibitors of the saccharide hydrolases. Among these, acarbose was the first reported α-amylase inhibitor. Acarbose analogues containing more glucose residues, such as trestatins, were found to display increased inhibitory potencies in subsequent researches.

In the course of our screening program to find new α-amylase inhibitors, we previously discovered a complex, AIB656, in the culture filtrate of a strain of *Streptomyces coelicoflavus* ZG0656. The complex was composed of aminooligosaccharide derivatives that showed remarkable inhibitory activity against porcine pancreatic α-amylase (PPA).[13] This paper describes the isolation, structural elucidation, and inhibitory activities against α-amylase of the acarviosine-containing oligosaccharides identified from *S. coelicoflavus* ZG0656.

2. Experimental

2.1. General methods

Optical rotations were measured on a Perkin–Elmer 241MC polarimeter. Infrared (IR) spectra were taken on a Bruker IFS 55 FT-IR spectrometer and recorded using the KBr pellet method. Nuclear magnetic resonance (NMR) experiments were conducted on a Bruker UltraShieid-300 instrument at 25 °C. The D_2O solutions

* Corresponding author. Tel./fax: +86 22 23508371; e-mail: gangbai@nankai.edu.cn

0008-6215/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2007.11.012

(0.7 M, pH ~ 7) were used with sodium 4,4-dimethyl-4-silapentane-5-sulfonate (DSS) as the external standard, and the NMR spectra were recorded in 5 mm tubes at 300.07 MHz for ^{1}H and 75.45 MHz for ^{13}C spectra. Electrospray ionization (ESI) multistage mass spectrometry (MS) experiments were run on a Thermofinnigan LCQ Advantage mass spectrometer, and electrospray ionization-Fourier transformed mass spectrometry (ESI-FTMS) data were acquired on an IonSpec QFT mass spectrometer with PEG1450 as the internal standard. Semi-preparative high-pressure liquid chromatography (HPLC) was carried out on a Shimadzu series instrument.

2.2. Microorganism

S. coelicoflavus strain ZG0656, which was collected from soil at the Nankai University campus, Tianjin, China, in 2005, was identified by the Department of Microbiology, Nankai University. A voucher specimen (CGMCC 2097) was deposited in China General Microbiological Culture Collection Center, Institute of Microbiology, Academia Sinica.

2.3. Preparation of the AIB656 complex

The culture (5 L) of *S. coelicoflavus* ZG0656 was filtered and concentrated in vacuo. About a onefold volume of EtOH was added to the concentrated aqueous solution, and the residue was discarded after centrifugation. The supernatant was concentrated to 200 mL in vacuo, and passed through a D301R macroporous resin column (300 × 40 mm) to partly remove pigments, followed by a column of X-5 macroporous resin (400 × 35 mm, washed with water, eluted with 40% aqueous EtOH), and a column of 001 × 7 cation-exchange resin (200 × 20 mm, washed with water, eluted with 1 M aqueous NH_3). A Sephadex G-25 column (eluted with water) was used at the end to obtain the 500–2000 Da fractions, which were lyophilized to give the AIB656 complex (1.3 g).

2.4. Purification of acarviostatins

The aminooligosaccharide-containing complex, AIB656 (1.3 g), was dissolved in water; filtered through a 0.45-μm membrane; separated by semi-preparative reversed-phase HPLC, using a stainless-steel column filled with Kromasil C_{18} (250 × 10 mm, i.d., 10 μm), at 25 °C. The mobile phase was 7:93 (v/v) acetonitrile–1.5 mM aqueous NH_3 at a flow rate of 3.0 mL/min with UV detection at 210 nm. A combined fraction was collected from the 9.0–10.3 min eluate. This eluate was further separated on the same column at 25 °C with 5:95 (v/v) acetonitrile–1.5 mM aqueous NH_3 as the mobile phase to give **1** (5.3 mg) at 23.6 min and **2** (1.8 mg) at 25.1 min, respectively. The purities of these oligomers were determined by positive-ion mode ESI-MS analysis, which showed the compounds to be contaminated with only very small traces of other products.

2.5. Kinetics of PPA inhibition by acarviostatins

Soluble amylose (0.05–0.5% w/v) in 25 mM imidazolium–HCl buffer (pH 6.5) containing 1.0 mM $CaCl_2$ was used as the substrate solution for the PPA inhibition studies. The substrate solution (450 μL) containing various concentrations of inhibitors (acarbose, acarviostatins II23 and II13) was preincubated at 37 °C for 10 min. The reactions were started by adding 50 μL of PPA (Sigma, USA) enzyme solution (5 U/mL). One unit (U) of α-amylase was defined as the hydrolysis of 1 μmol of α-(1→4) glycosidic linkages per minute. A 50 μL sample was taken every 5 min, and the reaction was stopped by adding 50 μL of 3 M NaOH. The reducing value indicating the amount of products was measured by adding 75 μL of 3,5-dinitrosalicylic acid (DNS) and boiling to acquire an absorbance at 490 nm, using maltose as a standard. Curves of product amount, in terms of μg maltose equivalents, versus time in minutes, were obtained. The initial velocities (v_i) were then determined from the slope of the linear portion (within 30 min) of the curve. Dixon plots of $1/v_i$ versus inhibitor concentration for four concentrations of soluble amylose were used to determine the type of inhibition and the inhibition constants, K_i and K'_i.[14]

2.6. Acarviostatin II23 (1)

White amorphous powder; $[\alpha]_D^{18}$ +162 (*c* 0.1, water); UV (water): end absorption; IR ν_{max} (KBr): 3393, 2927, 1661, 1385, 1035, 577 cm^{-1}; ESI-FTMS (pos.): *m/z* 1759.6518 $[M+H]^+$ ($C_{68}H_{114}N_2O_{50}$ requires 1759.6512); for ESI-MS/MS (pos.), see Figure 1; for ^{1}H and ^{13}C NMR data, see Table 1.

2.7. Acarviostatin II13 (2)

White amorphous powder; $[\alpha]_D^{18}$ +153 (*c* 0.1, water); UV (water): end absorption; IR ν_{max} (KBr): 3398, 2926, 1660, 1383, 1040, 579 cm^{-1}; ESI-FTMS (pos.): *m/z* 1597.5991 $[M+H]^+$ ($C_{62}H_{104}N_2O_{45}$ requires 1597.5984); for ESI-MS/MS (pos.), see Figure 2; ^{1}H NMR (300 MHz, D_2O): δ 1.31 (d, 6H, $J_{5,6} = 6.3$ Hz, E-6, H-6), 2.44 (t, 2H, $J_{vic} = 9.0$ Hz, E-4, H-4), 3.24 (t, 0.6H, $J_{vic} = 8.4$ Hz, A-2β), 3.40 (t, 1H, $J_{vic} = 9.0$ Hz, J-4), 3.51 (m, 2H, F-1, I-1), 3.53–4.09 (overlapping), 4.13 (m, 4H, F-3, F-6a, I-3, I-6a), 4.16 (m, 2H, F-6b, I-6b), 4.20 (m, 2H, F-4, I-4), 4.63 (d, 0.6H, $J_{1,2} = 8.4$ Hz, A-1β), 5.21 (d, 0.4H, $J_{1,2} = 4.2$ Hz, A-1α), 5.30 (m, 2H, E-1, H-1), 5.34–5.39 (overlapping,

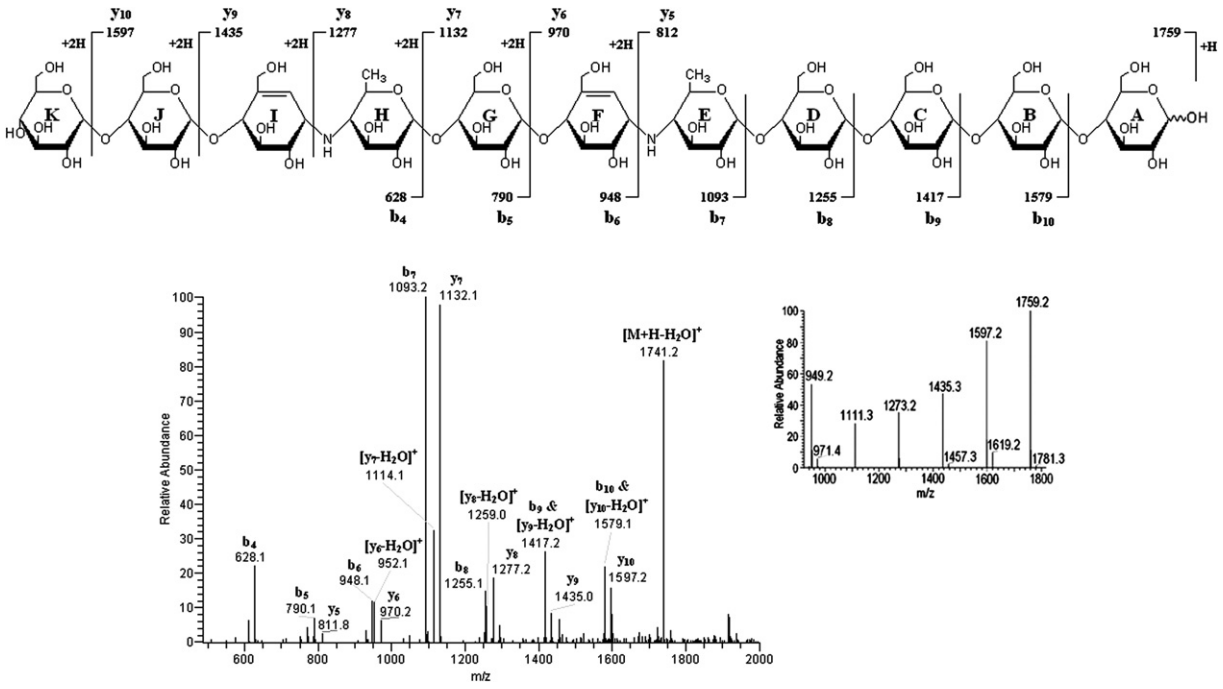


Figure 1. Positive ESI-MS/MS fragmentation and spectrum of $[M+H]^+$ of **1** at *m*/*z* 1759. (inset: full scan MS of **1**).

Table 1. ^1H and ^{13}C NMR data of **1** in D_2O[a]

Position	δ_C	δ_H	Position	δ_C	δ_H	Position	δ_C	δ_H	Position	δ_C	δ_H
A1α	93.0	5.16	B1,C1,D1	~100.7	~5.34	F1,I1	56.1	3.46	G1,J1	98.5	5.31
A2α	72.6	3.49	B2,C2,D2	~72.6	~3.53	F2,I2	72.6	3.71	G2,J2	72.6	3.53
A3α	74.4	4.04	B3,C3,D3	~74.4	~3.90	F3,I3	71.8	4.08	G3,J3	74.4	3.87
A4α	79.3	3.57	B4,C4,D4	~77.9	~3.57	F4,I4	77.0	4.15	G4,J4	77.9	3.57
A5α	71.0	3.91	B5,C5,D5	~72.2	~3.77	F5,I5	137.5	—	G5,J5	72.2	3.84
A6α	61.5	~3.68	B6,C6,D6	~61.5	~3.77	F6,I6	63.1	4.08/4.12	G6,J6	61.5	~3.80
A1β	96.8	4.58	E1,H1	100.7	5.27	F7,I7	127.4	5.90	K1	100.7	5.34
A2β	75.0	3.20	E2,H2	70.6	3.67				K2	72.6	3.53
A3β	77.3	3.62	E3,H3	73.7	3.53				K3	74.4	3.90
A4β	77.9	3.57	E4,H4	65.2	2.40				K4	70.4	3.35
A5β	75.6	3.50	E5,H5	70.4	3.73				K5	72.2	3.77
A6β	61.5	~3.71	E6,H6	18.4	1.26				K6	61.5	3.77

[a] Assignments are supported by TOCSY, HMQC, and HMBC experiments.

5H, B-1, C-1, D-1, G-1, J-1), 5.95 (d, 2H, $J_{1,7} = 3.6$ Hz, F-7, I-7).

3. Results and discussion

The complex, AIB656, was isolated from the culture filtrate of *S. coelicoflavus* ZG0656. It was then separated by semi-preparative HPLC to afford two aminooligosaccharide derivatives **1** and **2**. Complete acidic hydrolysis followed by monosaccharide analysis with the 1-phenyl-3-methyl-5-pyrazolone (PMP) pre-column derivatization HPLC method[15] revealed that **1** and **2** were composed of two structural units, D-glucose and acarviosine. Acarviosine[16] is composed of a cyclohexitol unit (hydroxymethylconduritol residue) and a 4-amino-4,6-dideoxy-D-glucopyranose (4-amino-4-deoxy-D-quinovopyranose) unit. The cyclohexitol–nitrogen bond is defined as a pseudoglycosidic bond. Acarviosine cannot further be hydrolyzed under these acidic conditions.[17]

The structural elucidations (in detail below) showed that **1** and **2** have a core, pseudotrisaccharide, formed by an acarviosine unit and a D-glucopyranose group attached through an α-(1→4) quinovosidic bond. Aminooligosaccharides are therefore named acarviostatins followed by a Roman numeral and two numbers, that is, acarviostatins II23 and II13. *Acarvios* originates from the acarviosine core; the Roman numeral II represents two pseudotrisaccharide residues; the middle digit represents the number of glucose units at the non-reducing end; the last digit represents the number of glucose units at the reducing end.

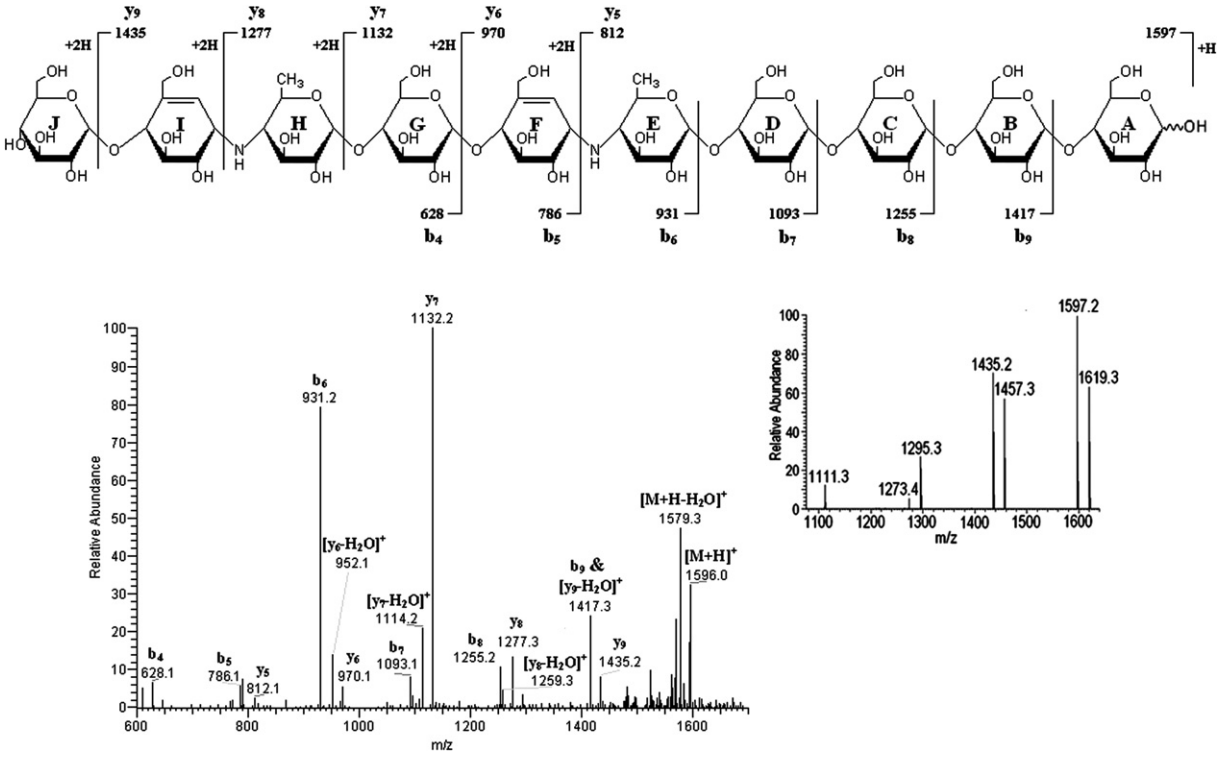
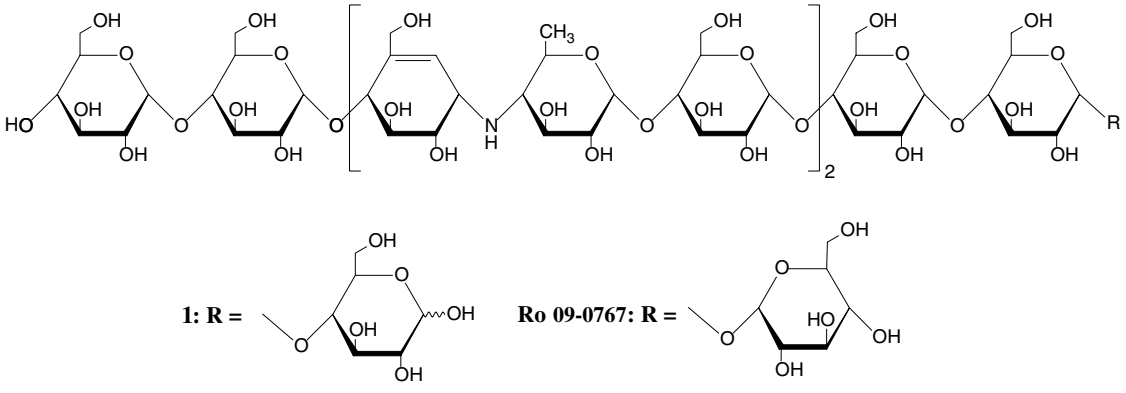


Figure 2. Positive ESI-MS/MS fragmentation and spectrum of $[M+H]^+$ of **2** at *m/z* 1597 (inset: full scan MS of **2**).

3.1. Structural determination of 1

Acarviostatin II23 (**1**) was a white amorphous powder. The molecular formula was deduced as $C_{68}H_{114}N_2O_{50}$ by combined positive ESI-FTMS (Anal. Found $[M+H]^+$ 1759.6518, Calcd 1759.6512) and NMR data. The IR spectrum of **1** displayed absorption at 1661 cm^{-1} for the C=C double bond and broad bands at 3393 and 1035 cm^{-1} for the oligosaccharide.

The positive full-scan ESI-MS of **1** showed a strong $[M+H]^+$ signal at *m/z* 1759 (Fig. 1). The signals at *m/z* 1597, 1435, 1273, 1111, and 949 were due to the loss of one to five glucose units from the molecule. The ESI-MS/MS spectrum from $[M+H]^+$ is shown in Figure 1. The daughter ion at *m/z* 1741 corresponded to the neutral loss of one water molecule. The product ions at *m/z* 1579 (b_{10}), 1417 (b_9), and 1255 (b_8) matched with the ordinal loss of one to three glucose units attached to the reducing end. The ions at *m/z* 1597 (y_{10}) and 1435 (y_9) agreed with the ordinal loss of one and two glucose units attached to the non-reducing end. The cleavages occurred on every C-1-oxygen bond, which could differentiate a monosaccharide unit on the 'left side' from one on the 'right side'. The most abundant fragment ions at *m/z* 1132 (y_7), 1093 (b_7), and 628 (b_4) were produced by the cleavages of the α-(1→4) quinovosidic bond between the quinovopyranose unit and glucose unit, which indicated the relatively weaker



Scheme 1.

intensity compared to the other ordinary glycosidic bonds in the molecule. The ions at m/z 1277 (y_8), 812 (y_5), and 948 (b_6) resulted from the dissociations of the pseudoglycosidic bond within the acarviosine moiety. The ions at m/z 970 (y_6) and 790 (b_5) were due to the cleavage of the glycosidic bond between two pseudotrisaccharide residues. Generally, every abundant fragment possessed at least one nitrogen-containing group; the ions y_j could further form a water-loss fragment due to the existence of a hydroxyl group at the reducing terminus, whereas b_i could not because of the absence of a reducing hydroxyl group. These features of the MS/MS spectrum indicated the structure of **1** outlined in Figure 1.

The ^1H, ^{13}C, total correlation spectroscopy (TOCSY), and heteronuclear multiple quantum correlation (HMQC) NMR spectra of **1** showed the presence of two inner units of acarviosine in the molecular,[6,7,11,12] including δ_C 100.7/δ_H 5.27 (m, 2 H), δ_C 65.2/δ_H 2.40 (t, 2H, $J_{vic} = 9.6$ Hz), δ_C 18.4/δ_H 1.26 (d, 6H, $J_{5,6} = 5.7$ Hz), δ_C 56.1/δ_H 3.46 (m, 2H), δ_C 77.0/δ_H 4.15 (d, 2H, $J_{3,4} = 4.8$ Hz), δ_C 63.1/δ_H 4.08 (m)/4.12 (m), δ_C 127.4/δ_H 5.90 (d, 2 H, $J_{1,7} = 3.0$ Hz), and δ_C 137.5. The NMR data confirmed that there were glucose units attached to the non-reducing end of acarviosine moieties. The HMQC spectrum of **1** showed typical characteristic signals for the reducing terminal glucose unit[11,12] at δ_C 93.0/δ_H 5.16 (d, 0.4H, $J_{1,2} = 3.6$ Hz), δ_C 96.8/δ_H 4.58 (d, 0.6H, $J_{1,2} = 9.9$ Hz), and δ_C 75.0/δ_H 3.20 (t, 0.6H, $J_{vic} = 8.1$ Hz).

The NMR signals for the methine group at C-4 in a glucose unit normally resonate at δ_C 70.4/δ_H 3.42.[11,12] In the HMQC spectrum of **1**, besides one methine signal at δ_C 70.4/δ_H 3.35 (t, $J_{vic} = 9.0$ Hz) for K-4 in the non-reducing terminus, the other six C-4 methine signals of six glucose units appeared at about δ_C 77.9–79.3/δ_H 3.55–3.59 (overlapped), revealing that the six C-4 hydroxyl groups of the six glucose units were glycosylated. Meanwhile, the two methine signals at δ_C 77.0/δ_H 4.15 (d, 2H, $J_{3,4} = 4.8$ Hz) for F-4 and I-4 indicated their glycosylations. As the chemical shifts for eight anomeric C-1 methines on hexoses occurred at δ_C 98.5–100.7/δ_H 5.27–5.35 (m, 8H, ~3.0 Hz), the configuration of the glycosidic bonds was determined to be α-(1→4).[11,12]

The complete proton and carbon signal assignments of **1**, aided by TOCSY, HMQC, and heteronuclear multiple bond correlation (HMBC) experiments, and comparisons with reported data for trestatins,[6,7] isovalertatins,[11] and butytatins[12] are shown in Table 1.

Compound **1** had the same molecular formula as the known compound trestatin Ro 09-0767.[7] However, the spectral characteristics of **1** showed that it contained a reducing glucose terminus and lacked α-(1→1) glycosidic bonds, which differs from trestatin Ro 09-0767 (Scheme 1). Therefore, **1** is a novel compound and was named acarviostatin II23.

3.2. Structural determination of 2

Acarviostatin II13 (**2**), obtained as a white amorphous powder, was assigned the molecular formula of $C_{62}H_{104}N_2O_{45}$ by positive ESI-FTMS (Anal. Found $[M+H]^+$ 1597.5991, Calcd 1597.5984) and NMR data. The spectroscopic characteristics and liquid chromatographic behavior of **2** closely resembled those of **1**, suggesting that it was an analog with a repeating moiety appended.

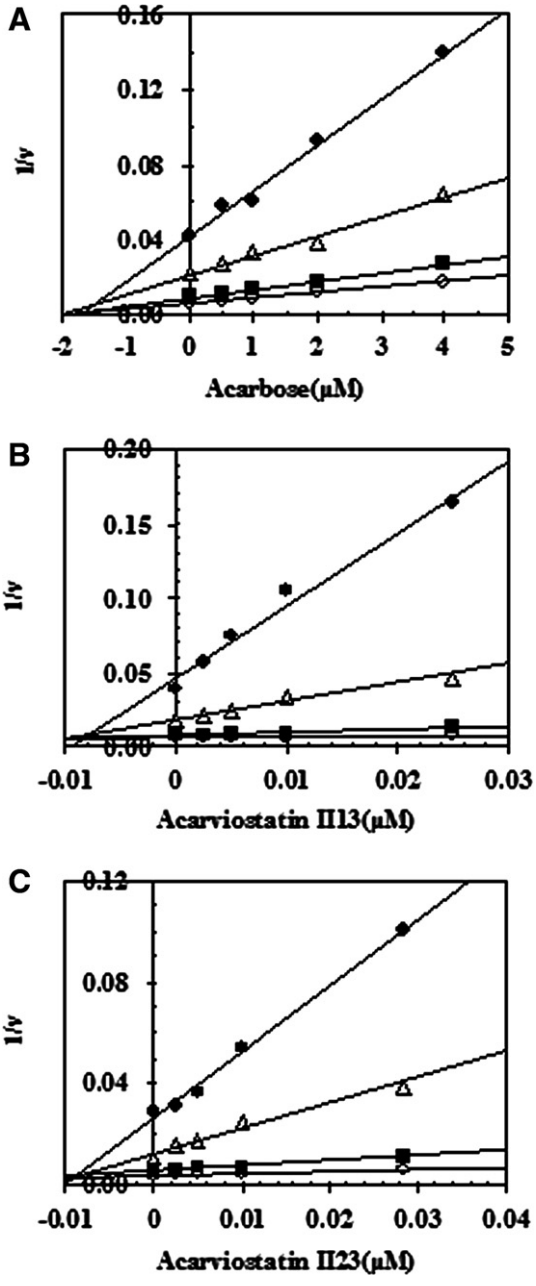


Figure 3. Dixon plots showing the velocity of amylose hydrolysis by PPA in the presence of (A)acarbose, (B) acarviostatin II13, and (C) acarviostatin II23. Initial concentrations of amylose for lines in each plot are (○) 0.5%, (■) 0.25%, (△) 0.1%, and (●) 0.05%; v is defined as μg maltose equivalent/mL/min.

Table 2. Inhibition constants of acarbose and acarviostatins for PPA

Inhibitor	Inhibition type	K_i (μM)[a]	K_i' (μM)[b]	Inhibition potency[c]
Acarbose	Mixed	2.077 ± 0.463	2.066 ± 0.681	1
Acarviostatin II13	Mixed	0.010 ± 0.006	0.011 ± 0.002	208
Acarviostatin II23	Mixed	0.009 ± 0.002	0.009 ± 0.003	231

[a] K_i is the inhibition constant, defined as [E][I]/[EI].
[b] K_i' is the inhibition constant, defined as [ES][I]/[ESI].
[c] Inhibition potency was obtained by dividing the K_i of acarbose by the K_i of acarviostatins.

The $[M+H]^+$ ion at *m/z* 1597 and $[M+Na]^+$ ion at *m/z* 1619 of **2** were found in the full-scan ESI-MS (Fig. 2). The signals at *m/z* 1435, 1273, and 1111 corresponded to the loss of one to three glucose units from the molecule. The ESI-MS/MS spectrum from $[M+H]^+$ (Fig. 2) displayed a quite similar fragmentation pathway compared to **1**. The fragment ion at *m/z* 1579 indicated the loss of one water molecule. The ordinal loss of one to three glucose units attached to the reducing end produced ions at *m/z* 1417 (b_9), 1255 (b_8), and 1093 (b_7). The daughter ion at *m/z* 1435 (y_9) was due to the loss of one glucose unit at the non-reducing end. Similar to **1**, the α-(1→4) quinovosidic bond cleavages yielded the most abundant daughter ions at *m/z* 1132 (y_7) and 931 (b_6). The dissociations of the pseudoglycosidic bond resulted in ions at *m/z* 1277 (y_8), 812 (y_5), and 786 (b_5). The cleavage of the glycosidic bond between two pseudotrisaccharide residues led to the ions at *m/z* 970 (y_6) and 628 (b_4). A significant number of water-loss fragments produced by the ions y_j were easily observed. The cleavage pathway suggested the structure of **2** outlined in Figure 2.

Because the yield of **2** was low, we recorded only its ^{1}H NMR spectrum. The ^{1}H NMR spectrum of **2** appeared almost the same as that of **1** besides the relative abundance of different signals. It showed the diagnostic signals for two inner acarviosine moieties at δ 1.31 (d, 6H, $J_{5,6} = 6.3$ Hz), 2.44 (t, 2H, $J_{vic} = 9.0$ Hz), 3.51 (m, 2H), 4.13 (m, 4H), 4.16 (m, 2H), 4.20 (m, 2H), 5.30 (m, 2H), and 5.95 (d, 2H, $J_{1,7} = 3.6$ Hz); for a reducing glucose terminus at δ 3.24 (t, 0.6H, $J_{vic} = 8.4$ Hz), 4.63 (d, 0.6H, $J_{1,2} = 8.4$ Hz), and 5.21 (d, 0.4H, $J_{1,2} = 4.2$ Hz); for a free hydroxyl group on position J-4 at the non-reducing terminus at δ 3.40 (t, $J_{vic} = 9.0$ Hz); the typical anomeric proton signals at δ 5.30 (m, 2H) and 5.34–5.39 (overlapping, 5H) having coupling constants of ~3.0 Hz for the α-(1→4) glycosidic bonds. All of the foregoing evidence confirmed the chemical structure of **2** as shown in Figure 2. Compound **2** is a novel oligomer and was named acarviostatin II13.

3.3. Kinetics of PPA inhibition by acarviostatins

Lineweaver-Burk plots of $1/v_i$ versus 1/[S] (data not shown) and Dixon plots of $1/v_i$ versus [I] (Fig. 3) were made to determine the type of inhibition each of the two acarviostatins had against PPA. Both acarviostatins were mixed noncompetitive inhibitors in which both the enzyme-inhibitor complex (EI) and enzyme-substrate-inhibitor complex (ESI) were formed. The kinetic parameters, V_m, K_m, K_i, and K_i' were determined from the Michaelis–Menten equation for mixed noncompetitive inhibition, using nonlinear regression analysis:[14]

$$v_i = V_m[S]/(K_m(1 + ([I]/K_i)) + [S](1 + ([I]/K_i')))$$

where, v_i is the initial velocity; [S] and [I] are the concentrations of substrate and inhibitor, respectively; V_m is the maximum velocity; K_m is the Michaelis–Menten constant; K_i is the dissociation constant of EI; K_i' is the dissociation constant of ESI. The inhibition constants, K_i and K_i' , for each compound are given in Table 2. The relative inhibition potencies of the acarviostatins, compared to acarbose, were calculated from the ratio of K_i.

Acarviostatins II13 and II23 were both potent PPA inhibitors with K_i values of 0.010 and 0.009 μM, respectively. These compounds are thus 208 and 231 times stronger inhibitors than acarbose. The results show that one additional acarviosine–glucose moiety at the non-reducing terminus than acarbose could improve the inhibitory potencies with two orders of magnitude. Furthermore, acarviostatin II23 inhibited PPA a little more effectively compared to acarviostatin II13 due to its one more glucose unit at the non-reducing terminus.

4. Conclusions

In conclusion, acarviostatins II13 and II23 are produced by *S. coelicoflavus* ZG0656. They are both novel acarviosine-containing oligosaccharides and are mixed noncompetitive inhibitors of PPA. These compounds inhibit PPA with K_i values two orders of magnitude more potent than acarbose.

Acknowledgments

This work was supported in part by The National High Technology Research and Development Program of China (863 Program) (No. 2006AA020502).

References

1. Si, D.; Zhong, D.; Chen, X. *Anal. Chem.* **2001**, *73*, 3808–3815.
2. Schmidt, D. D.; Frommer, W.; Junge, B.; Müller, L.; Wingender, W.; Truscheit, E.; Schafer, D. *Naturwissenschaften* **1977**, *64*, 535–536.
3. Namiki, S.; Kangouri, K.; Nagate, T.; Hara, H.; Sugita, K.; Omura, S. *J. Antibiot.* **1982**, *35*, 1234–1236.
4. Fukuhara, K.; Murai, H.; Murao, S. *Agric. Biol. Chem.* **1982**, *42*, 1941–1945.
5. Omoto, S.; Itoh, J.; Ogino, H.; Iwamatsu, K.; Nishizawa, N.; Inouye, S. *J. Antibiot.* **1981**, *34*, 1429–1433.
6. Yokose, K.; Ogawa, K.; Suzuki, Y.; Umeda, I.; Suhara, Y. *J. Antibiot.* **1983**, *36*, 1116–1175.
7. Yokose, K.; Ogawa, M.; Ogawa, K. *J. Antibiot.* **1984**, *37*, 182–186.
8. Arai, M.; Sumida, M.; Fukuhara, K.; Kainosho, M.; Murao, S. *Agric. Biol. Chem.* **1986**, *50*, 639–644.
9. Vertesy, L.; Fehlhaber, H. W.; Schulz, A. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1844–1846.
10. Kim, J. W.; Lee, K. M.; Chun, H. S.; Kim, J. G.; Chang, H. B.; Kim, S. H.; Min, K. B.; Moon, K. S. PCT Int. Appl. WO 9620145, 1996; *Chem. Abstr*. **1996**, *125*, 193577z.
11. Zhong, D.; Si, D.; He, W.; Zhao, L.; Xu, Q. *Carbohydr. Res.* **2001**, *331*, 69–75.
12. Si, D.; Zhong, D.; Xu, Q. . *Carbohydr. Res.* **2001**, *335*, 127–132.
13. Bai, G.; Geng, P.; Zhang, L.; Gao, Z.; Zhang, Q. CN Patent 200710058410.1, 2007.
14. Yoon, S. H.; Robyt, J. F. *Carbohydr. Res.* **2003**, *338*, 1969–1980.
15. Honda, S.; Akao, E.; Suzuki, S.; Okuda, M.; Kakehi, K.; Nakamura, J. *Anal. Biochem.* **1989**, *180*, 351–357.
16. Salvatore, T.; Giugliano, D. *Clin. Pharmacokinet.* **1996**, *30*, 94–106.
17. Qian, M.; Nahoum, V.; Bonicel, J.; Bischoff, H.; Henrissat, B.; Payan, F. *Biochem.* **2001**, *40*, 7700–7709.