ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 4:2 (2010) 131-140

records of natural products

Structures of the Aspartocin Antibiotics[§] - A Consideration of Requirements for Cyclopeptide Structures

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(Received January 25, 2010; Revised May 2, 2010, Accepted May 5, 2010)

Abstract: Three lipocyclopeptides, aspartocins A (**1**), B (**2**), and C (**3**), were obtained from the aspartocin complex by reversed-phase HPLC separation. Their structures were elucidated by spectroscopic studies coupled with the previously published chemical degradation results. All three compounds, **1, 2**, and **3**, share the same cyclic decapeptide core of cyclo(Dab2-Pip3-MeAsp4-Asp5-Gly6-Asp7-Gly8-Dab9-Val10-Pro11-). They differ only in the side chain moiety corresponding to Asp1-isotetradecenoic acid, Asp1-anteisotetradecenoic acid, and Asp1-isotridecenoic acid for aspartocins A, B, and C, respectively. The cyclic substructure of aspartocins contains two D-amino acid residues, and the geometry of the peptide linkages appears to be all *trans* including the two tertiary amide bonds. The result is consistent with the hypothesis that a normal peptide to be cyclic requires D-configured residues or *cis* amide bond(s) incorporated.

Keywords: Lipopeptide antibiotics; cyclopeptide requirement; *trans* and *cis* amide bonds; L- and D-amino acid residues; pipecolic acid conformation; NMR spectroscopy

1. Introduction

The lipopeptide antibiotics as a class include a large number of cyclic peptides [1] including amphomycin [2] and recently marketed Cubicin® (daptomycin) [3]. Amphomycin, a highly surface-active lipopeptide mixture, was introduced in the United States in the 1960s as a calcium salt in ointments for topical use [4], and was later patented as a feed additive [5]. Amphomycin was described as the first member of a series of closely related peptide antibiotics including glumamycin, aspartocin, laspartomycin, zaomycin, crystallomycin, and tsushimycin [6]. Although the amphomycin

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[§] This material was presented as a poster at the International Union of Microbiological Societies annual meeting in 2005 in San Francisco, USA

[‡] Wyeth was acquired by Pfizer on October 16th, 2009

complex was first isolated from a soil actinomycete in 1953 [2], details of its structure were unknown until 1972 when Bodanszky proposed a linear lipopeptide structure for amphomycin containing a C-terminal diketopiperazine moiety [6]. The linear structure was later found to be incorrect by Vertesy *et al*. in 2000 who proposed a cyclic structure for amphomycin [7]. Our degradation study of the glycinocin antibiotics, structurally related to amphomycin, showed that the diketopiperazine-containing fragments were hydrolytic artifacts [8]. This result provides additional evidence that this antibiotic family, typified by amphomycin, does contain a cyclic peptide core. Very recently, laspartomycin C has been described as the major component of the original laspartomycin complex [9]. Laspartomycin C is identical to glycinocin A [9].

The aspartocin complex, an acidic lipopeptide antibiotic mixture, was produced by two soil isolates *Streptomyces griseus* var. spiralis and *Streptomyces violaceus* [10]. As reported previously, eight amino acids, L-aspartic acid, L-proline, L-valine, glycine [10], D-pipecolic acid, β-methyl-L-aspartic acid, α,β-diaminobutyric acid [11], D-*erythro* and L-*threo* α,β-diaminobutyric acids [12], and two fatty acids, 13-methyl-3-tetradecenoic acid and (+)-12-methyl-3-tetradecenoic acid [13], were identified from the acid hydrolysate of the antibiotic complex. However, the complete structure of aspartocin has never been reported, although several articles suggested that it was identical to amphomycin in the peptide part of the molecule [6] and its principal constituent was identical to A-1437 G [7]. Adding to the confusion, the term aspartocin was also used to describe an oxytocin-like hormone in articles published in 1972 [14-15]. Since we had archived a sample of the original aspartocin complex, we reinvestigated its composition by HPLC separation and spectroscopic structural elucidation for each separated component. The results of our analyses are presented herein (Figure 1).

2. Materials and Methods

2.1 General

Electrospray mass spectra and MS-MS data were measured on a Micromass Q-TOF I mass spectrometer. High resolution mass spectra were recorded on a 9.4 Tesla FT-ICR mass spectrometer. NMR spectra were recorded on a Bruker DRX 500 or DPX 400 spectrometer in deuterated solvents. ^{1}H-NMR and ^{13}C-NMR chemical shifts were measured in parts per million relative to partially deuterated solvent peaks of DMSO-d_6 at δ 2.49 and δ 39.5, and MeOH-d_4 at δ 3.30 and δ 49.0 for ^{1}H-NMR and ^{13}C-NMR signals, respectively. All 2D experiments were run non-spinning.

2.2 HPLC Systems

A Hewlett-Packard 1100M LC system with diode array detection and a Finnigan LCQ LC-MS system employing a YMC-ODS-A reversed-phase column (5μ, 4.6 x 150 mm) were used for analysis of preparative HPLC fractions. Preparative HPLC separations were accomplished on an YMC-Pack ODS-A (C18, 250 x 10 mm) column. Fractions from preparative column were generally collected using an ISCO Foxy fractional collector and monitored by analytical HPLC or LC-MS. All solvents used were obtained from EM Science or J. T. Baker, Inc., and were of the highest commercially available purity.

2.3 Separation

Aspartocin complex (0.11 gram, Batch 3146C-165) was dissolved in a solution of MeOH:H_2O (4:1, 3 ml) and centrifuged. The supernatant was repeatedly injected into a Hewlett-Packard 1100M LC system equipped with a reversed-phase HPLC column (YMC-Pack, ODS-A, 250 x 10 mm). The column was washed with a gradient of 45-52% acetonitrile in 0.05% trifluoroacetic acid buffer over 25 minutes at a flow rate of 4 ml/min. The peaks were detected by UV absorbance at 215 nm and collected by a fractional collector. Fractions corresponding aspartocins A to C from each HPLC run

were combined and concentrated to dryness by Speed-Vac and lyophilization to yield white powders (**1**, 15.2 mg; **2**, 14.7 mg; **3**, 3.5 mg). A typical chromatogram of the aspartocin complex is shown in Figure 2. Aspartocin A eluted out at 23.6 minutes, aspartocin B at 22.7 minutes, and aspartocin C at 17.5 minutes.

3. Results and Discussion

The aspartocin antibiotic complex was isolated from the fermentation broth as a calcium salt [10]. The complex consisted of three main analogs, aspartocin A (**1**), aspartocin B (**2**), and aspartocin C (**3**). These three analogs were separated using reversed-phase HPLC with detection by UV absorbance at 215 nm. Aspartocins A and B are the major components occurring in a nearly 1:1 ratio and aspartocin C is a minor component (Figure 2). Based on their respective retention times, the major components have similar polarities whereas the minor one is more polar. Semi-preparative HPLC separation of the aspartocin complex led to the isolation of these three components, aspartocins A, B, and C, in milligram quantities. All compounds were finally obtained as white amorphous powders after lyophilization.

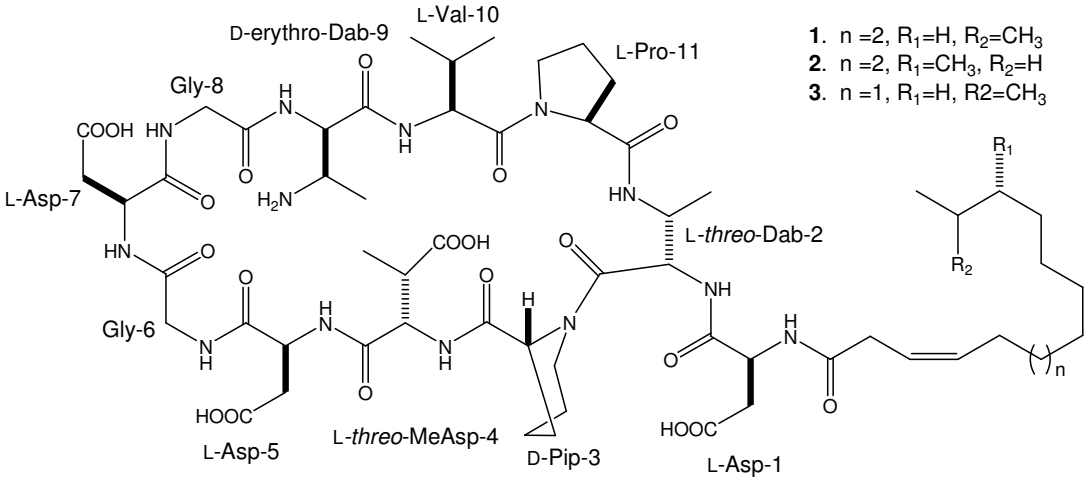

Figure 1. Structures of Aspartocins A (**1**), B (**2**), and C (**3**)

Aspartocin A (**1**) was obtained from the HPLC mobile phase as the free acid, whereas the original aspartocin complex was a calcium salt. Aspartocin A had a nondescript UV absorption spectrum showing only end absorption (Supporting Information). High resolution FT-ICR mass spectrometry yielded a quasi molecular ion at *m/z* 1318.6905 ([M + H]$^+$, calcd: 1318.6889), consistent with a molecular formula of $C_{60}H_{95}N_{13}O_{20}$. Consideration of the molecular formula in conjunction with the previously published amino acid and fatty acid composition indicated that at least a portion of the molecule was cyclic.

Visual inspection of the NMR data acquired on **1** suggested that the molecule was a lipopeptide. Identification of the fatty acid chain with one double bond present in the molecule was straightforward. The proton NMR spectrum of **1** showed two olefinic proton resonances both at δ 5.56 (H-3 and H-4) with a complex splitting pattern. The enhanced resolution afforded by 1D NMR analysis at higher field (600 MHz) was inadequate to define the coupling constants and thereby assign olefinic geometry. However, a ^{13}C coupled HSQC experiment with CW homonuclear ^{1}H decoupling at ~3ppm clearly showed the double bond to be in the *Z*-configuration as H-3 has a coupling constant of

11.3 Hz with H-4 (Figure 3). In the HSQC spectrum, H-2 at δ 2.94 was attached to a carbon resonance at δ 29.0 (C-2) and H-5 was correlated to a carbon signal at δ 26.9 (C-5), typical chemical shifts for *cis* allylic carbons. A methyl doublet at δ 0.82, integrating for six protons, was evidence that the fatty acid chain terminated in an isopropyl group. The length of the fatty chain was revealed by the MS data (*vide infra*). Detailed analyses of the 1D and 2D NMR data confirmed the lipid chain as a 13-methyl-3-tetradecenoyl moiety, which had previously been reported from the acid hydrolysates of the complex [13].

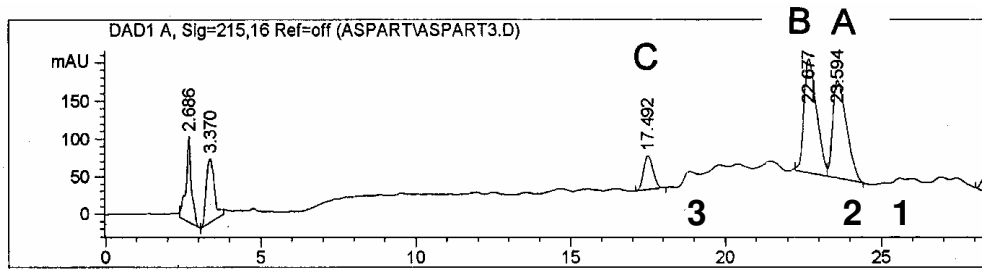

Figure 2. The aspartocin complex is composed of three analogs, aspatocin A (**1**), aspartocin B (**2**), and aspartocin C (**3**)

The peptidic nature of aspartocin A was apparent from the observation in the ^{1}H NMR spectrum of a series of exchangeable amide proton signals between δ 7.0 and δ 10.0 depending on which NMR solvents were used. These amide signals were coupled to the amino acid residue's α-protons between δ 3.0 and 5.0. Full assignments of the proton and carbon signals for all residues and determination of the sequence of the residues proved to be very challenging. This was due to the poor quality of spectra caused by limited solubility and existence of multiple conformers giving rise to significant line broadening even with mixtures of acetonitrile and water [7]. Ultimately, given the reported composition of amino acids and detailed analyses of the NMR data acquired in deuterated methanol, deuterated DMSO, and mixture of water and deuterated acetonitrile, all the NMR assignments for aspartocin A were accomplished.

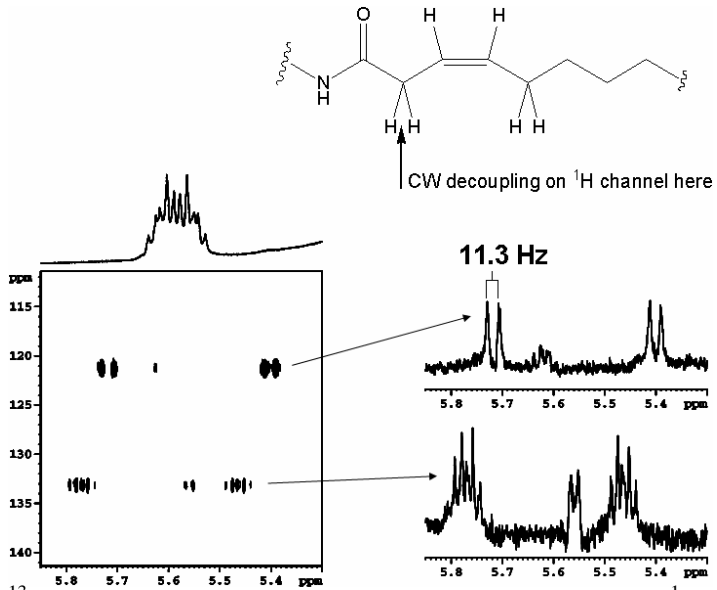

Figure 3. ^{13}C coupled HSQC experiment with CW homonuclear ^{1}H decoupling

Table 1. ^{1}H and ^{13}C NMR assignments for aspartocin A (**1**) in DMSO-d_6 and aspartocin B (**2**) in MeOH-d_4

		Aspartocin A (1)		Aspartocin B (2)	
Residue	**Position**	δ_H/500 MHz	δ_C [a]	δ_H/400 MHz	δ_C
Asp1	NH	8.23		-	
	α	4.65	49.0	4.79	51.6
	β	2.50/ 2.60	36.0	2.89/ 2.73	36.3
Dab2	NH	7.37		-	
	α	4.88	53.0	5.01	55.1
	β	4.42	50.0	4.47	47.6
	γ	0.99	18.9	1.14	18.1
	β-NH	7.95		-	
Pip3	α	4.90	53.3	4.72	56.8
	β	2.22/ 1.39	27.2	2.06/ 1.79	27.0
	γ	1.47/ 1.38	20.5	1.63/ 1.51	20.8
	δ	1.50	24.5	1.77	25.1
	ε	3.87/ 3.68	42.6	3.88/ 3.51	44.8
Me-Asp4	NH	8.85		-	
	α	5.05	52.8	4.50	56.5
	β	2.80	42.3	3.17	40.6
	γ	0.88	14.1	1.19	14.6
Asp5	NH	8.77		-	
	α	4.24	51.2	4.59	52.5
	β	2.51	36.0	3.05/ 2.88	36.3
Gly6	NH	8.45		-	
	α	3.94/ 3.46	43.0	4.06/ 3.92	44.3
Asp7	NH	7.55		-	
	α	4.28	50.8	4.50	52.3
	β	3.00/ 2.20	36.3	3.04 2.75	36.0
Gly8	NH	8.21		-	
	α	3.98/ 3.44	43.1	3.92/ 3.89	44.3
Dab9	NH	9.66		-	
	α	4.30	56.0	4.71	55.3
	β	3.20	47.4	3.82	49.5
	γ	1.21	17.4	1.33	15.9
Val10	NH	7.00		-	
	α	3.83	61.2	4.39	58.7
	β	2.19	29.4	2.16	31.3
	γ	0.98	18.9	1.05	19.9
	γ'	0.94	19.7	0.96	18.9
Pro11	α	4.15	59.6	4.27	61.9
1	β	2.10/ 1.71	29.4	2.22/ 1.88	31.0
3	γ	1.89/ 1.80	24.9	2.05/ 1.94	26.1
	δ	3.77/ 3.56	47.3	3.90/ 3.66	49.2
FA	1				174.3
	2	2.94	34.0	3.06	35.6
	3	5.56	121.2	5.53	122.8
	4	5.62	133.1	5.56	134.8
	5	1.99	27.0	2.08	28.4
	6	1.29	29.0	1.36	30.4
	7-9	1.23	29.0-29.5	1.30	30.6-31.1
	10	1.23	29.0-29.5	1.28	28.2
	11	1.23	26.8	1.09/ 1.30	37.8
	12	1.13	38.3	1.29	35.8
	13	1.49	27.3	1.14/ 1.32	30.6
	14	0.84	22.4	0.87	11.7
	12-Me			0.85	19.7
	13-Me	0.84	22.4		

[a] ^{13}C chemical shifts were extracted from the 2D spectra.

The data set from NMR experiments in DMSO was chosen to report here as the amide proton signals from the major conformer showed the important sequence information. Detailed analyses of the COSY, TOCSY, HSQC-TOCSY, and HSQC spectra allowed assignment of the proton and carbon resonances for each spin system through their ^{1}H-^{1}H and ^{1}H-^{13}C correlations. As a result, all eleven amino acid residues, three Asp, two Gly, two Dab, one Pip, one MeAsp, one Val, and one Pro, were characterized. The proton and carbon chemical shifts are listed in Table 1. The identity of these individual residues was consistent with the literature information reported previously from the acid hydrolysate of the antibiotic complex [10-13].

The sequence for aspartocin A was mainly determined by analysis of the NOESY and ROESY data. This was achieved by observing the NOESY correlations between the amide protons of adjacent residues. In the amide proton region between δ 7.0 and δ 10.0 two coupling systems were observed. One involved the most upfield signal at δ 7.00 that exhibited a cross-peak at δ 9.66 and the latter was also correlated to a resonance at δ 8.20; the δ 8.20 signal was further correlated to δ 7.55, and continued from δ 7.55 to δ 8.45, from δ 8.45 to δ 8.75, and finally from δ 8.75 to δ 8.85. These signals were assigned to the amide protons of Val10, Dab9, Gly8, Asp7, Gly6, Asp5, and MeAsp4, respectively, establishing the partial sequence Val-Dab-Gly-Asp-Gly-Asp-MeAsp. The other system involved correlations observed from the signal at δ 8.22 to the signal at δ 7.37 and from the signal at δ 7.37 to the signal at δ 7.95. These signals were assigned to the amide protons of the side chain Asp1 and Dab2's α and β amino groups, respectively (Supporting Information).These NOESY correlations were observed using a 400-ms mixing period and were caused by spin diffusion. Moreover, the NOESY and ROESY correlations observed between the NH and the α-proton of the preceding residue at every amide bond supported the above assignment. The lack of correlations between α-protons of neighboring residues further supported the all-*trans* nature of the amide bonds. The remaining portion of the molecule's linear sequence was established by correlations between the NH (δ 8.85) of MeAs4 and the α-proton (δ 4.90) of Pip3, and between the β-NH (δ 7.95) of Dab2 and the α-proton (δ 4.15) of Pro11, and between the NH (δ 8.23) of Asp1 and the α-protons (δ 2.94) of the fatty chain.

Finally, the connectivity between Val10 and Pro11 within the cyclic peptide core was determined by analysis of the MS-MS data. The sequence determination of cyclic peptides is generally not practical by MS-MS, because a very complex spectrum is generated due to many combinations of two bond cleavages [16]. However, in the case of aspartocin A, the tertiary peptide linkage of Dab2/Pip3 was favored for protonation, resulting in initial cleavage between the diaminobutyric acid and protonated pipecolic acid residues. Subsequent MS-MS fragmentation generated a series of fragments at *m/z* 1207, 1078, 963, 535, corresponding to sequential losses of Pip3, MeAsp4, Asp5, and Gly6-Asp-Gly-Dab-Val10, respectively, supporting the Val10 and Pro11 linkage for the molecule (Figure 4). Other key fragment at *m/z* 981 represented the cyclic peptide core. The length of the fatty acid chain was revealed by the fragments at *m/z* 338 and 310, corresponding to the Asp1-FA side chain and loss of a carbonyl group, respectively, as shown. All MS-MS information was consistent with the cyclic peptide structure of **1** as shown in Figure 1 [17]. It should be noted that for the glycinocin peptides that are closely related to aspartocins, the preferred protonation site is the tertiary peptide linkage of the proline residue rather than the pipecolic acid one [8].

The information of absolute configurations of the amino acids comes from the previously published data determined from the hydrolysate of the aspartocin complex. Thus, they are L-aspartic acid, L-proline, L-valine [10], D-pipecolic acid, β-methyl-L-aspartic acid, D-*erythro* and L-*threo* α,β-diaminobutyric acids [11, 12]. By analogy to the absolute stereochemistry of amphomycin [6] and glycinocins [8], which both contain a cyclodecapeptide core and two D-amino acid residues, it is assumed that the D-*erythro* α,β-diaminobutyric acid is for residue-9 (Dab9) and the L-*threo* one is for residue-2 (Dab2) to meet the spatial arrangement requirement. Therefore, the structure of aspartocin A with stereochemistry, as depicted in Figure 1, is a cyclodecapeptide with an acyl-Asp side chain. It is important to note that the cyclic core contains two D-amino acid residues (Pip3 and Dab9) and two tertiary amide bonds (Pip3 and Pro11).

Interestingly, many cyclopeptides and cyclodepsipeptides contain two D-amino acid residues such as mannopeptimycins [18], gramicidin S [1], and daptomycin (Cubicin) [3]. It is well known that a peptide consisting of all L-amino acid residues adapts the α-helical backbone conformation and therefore cannot form a cyclic structure. It has been long speculated that a minimum of two D-configured amino acid residues is required for a peptide to be cyclic. Unless a peptide contains residues with no side chain (glycine) or small side chains like alanine and serine, and approaches a flat-sheet structure, it is unlikely to form a cyclic structure without the incorporation of D-configured residues. Cyclopeptides with a combination of glycine residues and one D-configured amino acid residue fit into this group [19]. However, if there is a *cis* amide bond present, which usually occurs at an N-alkyl peptide site, the peptide backbone of the α-helix (all L-amino acid residues) is disrupted to make a turn to form the cyclic structure. Examples include many proline rich cyclic peptides with at least one *cis* amide bond such as pseudoaxinellin [20], hymenamides [21], and cycloleonurinin [22]. The role a *cis* peptide linkage plays is more apparent in the simplest cyclopeptide diketopiperazine that contains two *cis* amide bonds.

Tertiary amide bonds in a molecule can exist either in *trans* or *cis* conformation in solution due to the partial double bond character of the carbon-nitrogen bond and comparable steric effects between the two rotamers. The NMR data indicated that the geometry of the two tertiary amide bonds in **1** appeared to be all *trans*. The chemical shifts of Cβ (δ 29.4) and Cγ (δ 24.9) of the proline residue supported the *trans* conformation of the Pro11 amide bond [20-22]. The assignment of *trans* conformation for the Pip3's tertiary amide bond was derived by analogy to the NMR investigation of *trans* and *cis* rotamers of rapamycin [23-24]. The proton and carbon chemical shifts of the α and ε positions of the pipecolic acid residue have unique patterns for the *trans* and *cis* isomers. The pattern of NMR signals at the α (δ_H 4.90/ δ_C 53.3) and ε (δ_H 3.68, 3.87/ δ_C 42.6) positions of Pip3 revealed that it was the *trans* rotamer. This result is apparently different from the Pip3's tertiary amide bond conformation of glycinocins that appears to be the *cis* rotamer based on the characteristic chemical shifts at positions α (δ_H 4.79/ δ_C 56.2) and ε (δ_H 2.86, 4.33/ δ_C 39.9) [8]. The magnitude of the α-positioned chemical shifts indicates that for the *trans* rotamer, the pipecolinyl's carboxylic group appears to be in axial orientation and its α-proton equatorial as shown in Figure 1. This is consistent with the pipecolinyl ring conformation of the major isomer of rapamycin that has been determined to be *trans* both by NMR and X-ray analyses. For the case of glycinocin A, the pipecolinyl amide bond is in *cis* geometry as evidenced by a strong ROESY correlation observed between the α-proton of Pip3 and α-proton of Dap2. Therefore, it is possible to predict the correct conformation by a comparison of characteristic chemical shifts with the corresponding values for the pipecolinyl residue. The assignment of the carbon chemical shift of αC-Pip3 at δ 53.3 (aspartocin A) for the *trans* amide bond and δ 56.2 (glycinocin A) for the *cis* one is further supported by the literature data. It is well known that the carbon resonance of an amide N-carbon with the *cis* geometry is shifted about 3ppm downfield in comparison with the corresponding *trans* one [25].

Aspartocin B (**2**) was isolated as a white powder. Its molecular formula was established by high resolution FT-ICR mass spectrometry as $C_{60}H_{95}N_{13}O_{20}$ (measured 1340.6675, calculated 1340.6708 for $[M + Na]^+$), identical to compound **1**. The ^{1}H NMR spectrum of **2** was nearly identical to that recorded for **1** in MeOH-d_4, indicating that the two compounds were closely related (Supporting Information). In fact, the quality of the NMR data for aspartocins in deuterated methanol was better than in deuterated DMSO, however, this resulted in the loss of the amide proton information. Detailed analyses of the ^{1}H-NMR, ^{13}C-NMR, COSY, HSQC, and HMBC spectra allowed us to assign the proton and carbon resonances for aspartocin B, listed in Table 1. Another commonly accepted approach to predict the amide bond geometry of a proline residue is to compare the difference of the Cβ and Cγ's chemical shifts [21,22]. The difference ($\Delta_{\beta\text{-}\gamma}$ value of 5 ppm) of chemical shifts of Cβ (δ 31.0) and Cγ (δ 26.1) of Pro11 and the difference ($\Delta_{\alpha\text{-}\varepsilon}$ value of 12 ppm) of Cα (δ 56.8) and Cε (δ 44.8) of Pip3 indicated that both tertiary amide bonds adopted *trans* conformation in methanol as well. The MS-MS fragmentation of **2** showed that it had the same cyclic core at *m/z* 981

and same size of the side chain at *m/z* 338 and 310. Therefore, aspartocin B was identified to contain the same cyclic core of cyclo-(Dab2-Pip3-MeAsp4-Asp5-Gly6-Asp7-Gly8-Dab9-Val10-Pro11-) with Asp1-anteisotetradecenoyl acid side chain as depicted in Figure 1. The absolute stereochemistry for the fatty moiety was determined previously as (+)-12-methyl-3-tetradecenoic acid [13]. Aspartocin B is identical to A-1437 G [7].

Aspartocin C (**3**) is a white powder whose molecular formula was determined to be $C_{59}H_{93}N_{13}O_{20}$ by high resolution FT-ICR mass spectrometry (measured 1304.6734, calculated 1304.6733 for $[M + H]^+$). It only differed from the molecular formula of aspartocin A by the loss of a CH_2 unit. The MS-MS spectrum of **3** showed the identical fragmentation pattern as that of **1**, revealing that it differed from **1** only in the fatty chain. The ^1H NMR spectrum of **3** was almost identical to that recorded for **1** in MeOH-d_4, (Supporting Information). Therefore, aspartocin C was identified as cyclo-(Dab2-Pip3-MeAsp4-Asp5-Gly6-Asp7-Gly8-Dab9-Val10-Pro11-) with Asp1-isotridecenoyl side chain as shown in Figure 1. Aspartocin C (**3**) is identical to A-1437 B [7].

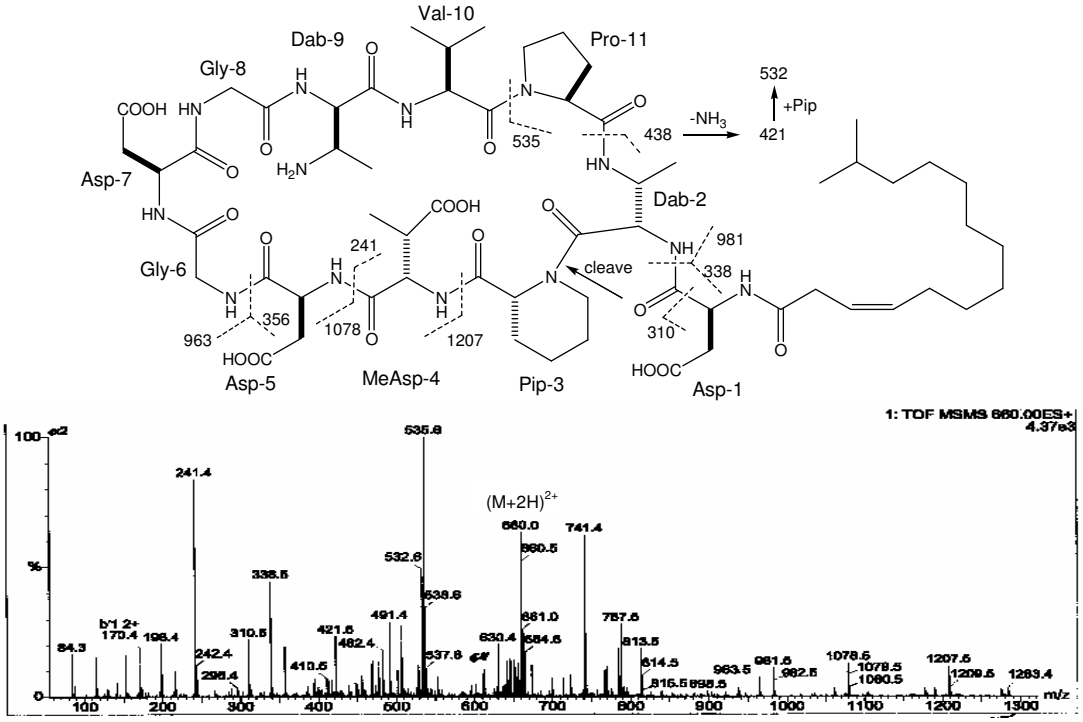

Figure 4. MS/MS fragmentation pattern of aspartocin A (**1**)

4. Conclusion

The aspartocin complex is composed of three main components, aspartocins A, B, and C. Their structures are determined as a cyclic peptide core of cyclo-(Dab2-Pip3-MeAsp4-Asp5-Gly6-Asp7-Gly8-Dab9-Val10-Pro11-) with an Asp1-FA side chain attached at Dab2's α-amino group. They differ only in the fatty acid part of side chain as 13-methyl-3-tetradecenoyl, 12-methyl-3-tetradecenoyl, and 12-methyl-3-tridecenoyl for aspartocins A, B, and C, respectively. Aspartocins are structurally related to the revised structure of amphomycin with the same cyclic peptide core but differing in the fatty chain. Ironically, the revised structure of amphomycin is identical to one of the two possible structures originally proposed by Fujino in 1965 for glumamycin [26]. Additionally, the

term aspartocin used for an oxytocin-type hormone with molecular formula $C_{42}H_{64}N_{12}O_{12}S_2$ should be avoided [14-15].

The cyclic substructure of aspartocins contains two D-amino acid residues, supporting the hypothesis that a minimum of two D-configured residues or *cis* amide bond(s) is required for a peptide to be cyclic. The geometry of the two tertiary amide bonds (Pip3 and Pro11) in the molecule appears to be all *trans*. It has been well accepted that the characteristic chemical shifts of Cβ and Cγ of a proline residue are consistent with the geometry of the prolinyl amide bond. Likewise, it is possible to predict the amide bond geometry as well as the pipecolinyl ring conformation only based on the unique chemical shifts of Cα and Cε of a pipecolic acid residue.

Acknowledgments

The authors would like to thank Xidong Feng for HRMS measurement and R. Thomas Williamson for providing ^{13}C coupled HSQC with CW homonuclear ^{1}H decoupling data. The authors also wish to thank Gerhard Schlingmann, Marshall M. Siegel, and Thomas G. Pagano for insightful discussions.

Supporting Information

Supporting information data associated with this article can be found at the http://www.acgpubs.org/RNP/2010/Volume%204/Issue%201/SupportingInformation-RNP1001-182.pdf.

References

[1] E.M. Jr. Wise (1992). In *Kirk-Othmer Encycl. Chem. Technol*., Kroschwitz, J.I. & Howe-Grant, M., 4th edn., John Wiley & Sons, New York, Vol. **3**, pp 266-305.

[2] B. Heinemann, M.A. Kaplan, R.D. Muir and I.R. Hooper (1953). Amphomycin, a new antibiotic. *Antibiotics and Chemotherapy* (Washington, D. C.). **3**, 1239-1242.

[3] R.H. Baltz, V. Miao and S.K. Wrigley (2005). Natural products to drugs: daptomycin and related lipopeptide antibiotics. *Nat. Prod. Rep.* **22(6),** 717-741.

[4] B. Heinemann (1964). Amphomycin and its production and recovery. U.S. Pat. **3126317**. Bristol-Myers Co.

[5] M. Gordon and G.J. Christie (1983). Animal feeds. U.S. Pat. **4414206**. Bristol-Myers Co.

[6] M. Bodanszky, G.F. Sigler and A. Bodansky (1973). Structure of the peptide antibiotic amphomycin. *J. Am. Chem. Soc.* **95**, 2352-2357.

[7] L. Vertesy, *et al.* (2000). Friulimicins: novel lipopeptide antibiotics with peptidoglycan synthesis inhibiting activity from *Actinoplanes friuliensis* sp. nov., II. Isolation and structural characterization. *J. Antibiot.* **53,** 816-827.

[8] F. Kong and G.T. Carter (2003). Structure determination of glycinocins A to D, further evidence for the cyclic structure of the amphomycin antibiotics. *J. Antibiot*. **56**(6), 557-564.

[9] D.B. Borders, R.A. Leese, H.Jarolmen, N.D. Francis, A.A. Fantini, T.Falla, J.C. Fiddes and A.Aumelas (2007). Laspartomycin, an Acidic Lipopeptide Antibiotic with a Unique Peptide Core. *J. Nat. Prod.* **70(3),** 443-446**.**

[10] A.J. Shay and J. Adam *l.* (1960). Aspartocin. I. Production, isolation, and characteristics. *Antibiotics Annual.* 194-198.

[11] J.H. Martin and W.K. Hausmann (1960). Isolation and identification of D-α-pipecolic acid, α [L]-β-methylaspartic acid, and α,β-diaminobutyric acid from the polypeptide antibiotic aspartocin. *J. Am. Chem. Soc.* **82,** 2079-2079.

[12] W.K. Hausmann, D.B. Borders and J.E. Lancaster (1969). α ,β -Diaminobutyric acid obtained from aspartocin. *J. Antibiot.* **22**, 207-210.

[13] W.K. Hausmann, A.H. Struck, J.H. Martin, R.H. Barritt and N. Bohonos (1963). Structure determination of fatty acids from the antibiotic aspartocin. *Antimicr. Agents & Chemoth.* (1961-70). 352-359.

[14] R. Acher, J. Chauvet and M.T. Chauvet (1972). Identification of two new neurohypophysial hormones, valitocin (8-valine oxytocin) and aspartocin (4-asparagine oxytocin) in a selachian shark (Squalus ocanthias). *Comptes Rendus des Seances de l'Academie des Sciences, Serie D: Sciences Naturelles.* **274**(2), 313-316;

[15] R. Acher, J. Chauvet and M.T. Chauvet (1972). Phylogeny of the neurohypophysial hormones. Two new active peptides isolated from a cartilaginous fish, Squalus acanthias. *Eur. J. Biochem.* **29(1)**, 12-19.

[16] K. Eckart (1994). Mass spectrometry of cyclic peptides. *Mass Spectrometry Reviews* **13(1)**, 23-55.

[17] M.M. Siegel, F. Kong, X. Feng and G.T. Carter (2009). Structure characterization of lipocyclopeptide antibiotics, aspartocins A, B & C, by ESI-MSMS and ESI-nozzle-skimmer-MSMS. *J. Mass Spectrometry*, **44(12)**, 1684-1697.

[18] H. He, *et al*. (2002). Mannopeptimycins, novel antibacterial glycopeptides from Streptomyces hygroscopicus, LL-AC98. *J. Am. Chem. Soc.* **124(33)**, 9729-9736.

[19] H. Huang, Z. She, Y. Lin, L.L.P. Vrijimoed and W. Lin (2007). Cyclic peptides from an endophytic fungus obtained from a mangrove leaf (Kandelia candel). *J. Nat. Prod.* **70(11)**, 1696-1699.

[20] F. Kong, D.L. Burgoyne, R.J. Andersen and T.M. Allen (1992). Pseudoaxinellin, a cyclic heptapeptide isolated from the Papua New Guinea sponge Pseudoaxinella massa. *Tetra. Lett.* **33(23)**, 3269-3272.

[21] J. Kobayashi, M. Tsuda, T. Nakamura, Y. Mikami and H .Shigemori (1993). Hymenamides A and B, new proline-rich cyclic heptapeptides from the Okinawan marine sponge Hymeniacidon sp.*Tetrahedron*. **49(12)**, 2391-2402.

[22] K. Kinoshita, *et al*. (1991). Cycloleonurinin, a cyclic peptide from Leonuri Fructus. *Chem. Pharm. Bull.* **39(3)**, 712-715.

[23] H. Kessler, R. Haessner and W.Schueler (1993). Structure of rapamycin: an NMR and molecular-dynamics investigation. *Helv. Chim. Acta.* **76(1)**, 117-30;

[24] C.C. Zhou, K.D. Stewart and M.K. Dhaon (2005). An intramolecular ionic hydrogen bond stabilizes a *cis* amide bond rotamer of a ring-opened rapamycin-degradation product. *Magn. Reson. Chem.* **43(1)**, 41-46.

[25] E. Pretsch, P. Bühlmann and C. Affolter (2000). Structure determination of organic compounds, Tables of spectral data. 3rd ed., pp 140-141, Springer Press, Germany.

[26] M. Fujino (1965). Glumamycin, a new antibiotic. VI. Approach to the amino acid and sequence. *Bull. Chem. Soc. Jap.* **38**, 517-522.