Notes

WS-7338, NEW ENDOTHELIN RECEPTOR ANTAGONISTS ISOLATED FROM *Streptomyces* sp. No. 7338

III. STRUCTURES OF WS-7338 A, B, C AND D AND TOTAL SYNTHESIS OF WS-7338 B

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In the course of our screening program for endothelin (ET)-1 receptor antagonists we found new cyclic pentapeptides, WS-7338 A~D (**1**~**4**), in the culture broth of a *Streptomyces* sp. No. 7338. The taxonomy, fermentation, isolation and physicochemical properties of **1**~**4** have been described in the preceding paper[1]. Herein we report on the structure elucidation of **1**~**4** and a total synthesis of WS-7338 B (**2**).

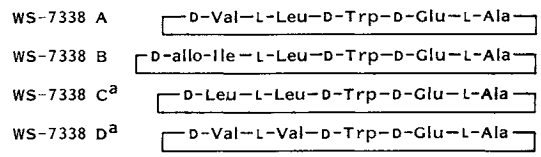
HRFAB-MS measurement yielded a molecular formula of $C_{30}H_{42}N_6O_7$ for WS-7338 A (**1**), which was consistent with elementary analysis[1] and ^{13}C NMR data (Table 1). Total acid hydrosis (6 N HCl, 110°C, 15 hours) of **1** detected Glu, Ala, Val, Leu and Trp in a 1:1:1:1:0.3 molar ratio. The individual amino acid residues were characterized in the ^1H NMR spectra of **1** with the aid of ^1H-^1H COSY, and the complete ^1H NMR assignment is presented in Table 1. The absolute configurations of the amino acids were established by chiral GC-MS analysis[2] of *N*-trifluoroacetyl *n*-butyl ester derivatives of the WS-7338 A acid hydrolysate: Glu (D), Ala (L), Val (D), Leu (L), Trp (D). The sum of the formulae for the amino acid residues account for the molecular formula of **1** and twelve degrees of unsaturation. The remaining one unsaturation required by the molecular formula of **1** indicated that **1** is a cyclic pentapeptide.

Addition of 1 eq of NaOD to a DMSO-d_6 solution of **1** induced a up-field shift of Glu-γ-CH_2 signal by 0.12 ppm and thus the γ-COOH function of the Glu residue in intact WS-7338 A must be free. In agreement with this assumption, the regiochemistry of the Glu linkage was assigned as normal linkage because of the NOE observation between $C_\alpha H$ of the Glu residue and NH of Ala. The order of linkage of the five residues was determined by interpretation of NOE obtained from NOESY experiment (phase-sensitive mode)[3]. The NH of D-Trp showed a strong cross peak with $C_\alpha H$ of L-Leu, the NH of L-Leu was correlated with $C_\alpha H$ of D-Val, the NH of D-Val gave cross peak with $C_\alpha H$ of L-Ala, and the NH of L-Ala was correlated with $C_\alpha H$ of D-Glu. These NOE correlations indicated the following amino acid sequence: –D-Glu–L-Ala–D-Val–L-Leu–D-Trp–. There is only one reasonable combination of the D-Glu and the D-Trp and hence the structure of WS-7338 A was concluded to be **1**.

A combination of HRFAB-MS, elementary analysis[1] and ^{13}C NMR (Table 1) established the molecular formula of WS-7338 B as $C_{31}H_{44}N_6O_7$. Conventional amino acid analysis and inspection of ^1H NMR spectra of WS-7338 B (**2**) confirmed the presence of one residue each of Glu, Ala, allo-Ile, Leu and Trp. The chiral assignment of the individual amino acid was performed by chiral GC-MS analysis[2], which permitted us to assign the D-configuration for Glu, allo-Ile and Trp and the L-configuration for Ala and Leu. The displacement of Val in **1** with allo-Ile in **2** can account for the difference of the molecular formulae between **1** and **2**. Therefore, structure, cyclo(–D-allo-Ile–L-Leu–D-Trp–D-Glu–L-Ala–), (**2**), was proposed for WS-7338 B.

In an effort to confirm the structure of **2**, the total synthesis of WS-7338 B (**2**) was carried out, as shown in Scheme 1. Alanine phenacyl ester (**5**) was coupled with Boc–D-Glu(OBzl)–OH (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (WSCD) and 1-hydroxybenzotriazole (HOBt) in CH_2Cl_2 at 0°C for 3 hours) to give Boc–D-Glu(OBzl)–Ala–O Phenacyl (Pac) (80.4%). Because of the hygroscopic nature

Fig. 1. Structures of WS-7338 A, B, C and D.



[a] Structures of WS-7338 C and D are the tentative structures.

Table 1. ^{1}H and ^{13}C NMR data for WS-7338 A (**1**) and B (**2**).

		WS-7338 A (**1**)			WS-7338 B (**2**)		
		$^{1}H^{a}$ δ_H	$^{13}C^{b}$ δ_C	Mult.	^{1}H δ_H	^{13}C δ_C	Mult.
D-Val	NH	7.53			7.45		
[D-allo-Ile][c]	α	4.15	57.4	d	4.31	55.2	d
	β	1.78	30.9	d	1.58	37.4	d
	γ	0.81	18.3	q	0.78	14.7	q
		0.83	19.2	q	1.30	26.0	t
					1.08		
	δ				0.87	11.5	q
L-Leu	NH	8.54			8.59		
	α	4.11	52.2	d	4.07	52.7	d
	β	1.24	38.8	t	1.20	38.7	t
		~1.18					
	γ	1.02	24.0	d	0.96	23.9	d
	δ	0.63	22.2	q	0.63	22.1	q
		0.74	22.4	q	0.73	22.5	q
D-Trp	NH	8.76			8.77		
	α	4.30	55.3	d	4.28	55.6	d
	β	2.92	27.0	t	2.90	26.9	t
		3.26			3.28		
	1-NH	10.80			10.79		
	2	7.12	123.8	d	7.12	123.8	d
	3		110.7	s		110.7	s
	3_a		127.1	d		127.0	s
	4	7.51	118.4	d	7.52	118.4	d
	5	6.96	118.2	d	6.96	118.1	d
	6	7.04	120.9	d	7.05	120.9	d
	7	7.32	111.4	d	7.31	111.4	d
	7_a		136.3	s		136.3	s
D-Glu	NH	7.40			7.50		
	α	4.25	52.4	d	4.25	52.5	d
	β	1.87	27.4	t	1.89	27.4	t
	γ	2.15	30.3	t	2.16	30.3	t
L-Ala	NH	8.70			8.74		
	α	4.45	47.4	d	4.46	47.4	d
	β	1.13	14.5	q	1.13	14.4	q
CO-X			174.0	s		173.9	s
			172.0	s		172.4	s
			171.9	s		172.0	s
			171.9	s		172.0	s
			171.6	s		171.6	s
			170.4	s		170.3	s

[a] 400 MHz in DMSO-d_6.
[b] 100 MHz in DMSO-d_6, assignment was made by C-H COSY.
[c] D-allo-Ile for WS-7338 B (**2**).

of the TFA salt, the protected dipeptide was treated with TFA followed by 4 N HCl in dioxane to afford **6** as the HCl salt (99.2%). Compound **6** was coupled with Boc–D-Trp(For)–OH (WSCD - HOBt in DMF at 0°C for 3 hours) to give Boc–D-Trp(For)–D-Glu(OBzl)–Ala–OPac (85.6%) which was treated with TFA followed by 4 N HCl in dioxane to afford **7** (99.7%). In substantially the same manner, **8** was prepared from **7** (94%). Compound **8** was coupled with Boc–D-allo-Ile–OH (WSCD - HOBt in DMF at 0°C overnight) to give protected linear pentapeptide (**9**) (93.5%). The action of Zn on **9** (Zn - AcOH, room temperature for 1 hour) resulted in removal of the Pac ester, and treatment with TFA followed by 4 N HCl in dioxane gave **10** (84.6%). Cyclization of **10** (WSCD - HOBt in DMF at 10°C overnight)

Scheme 1.

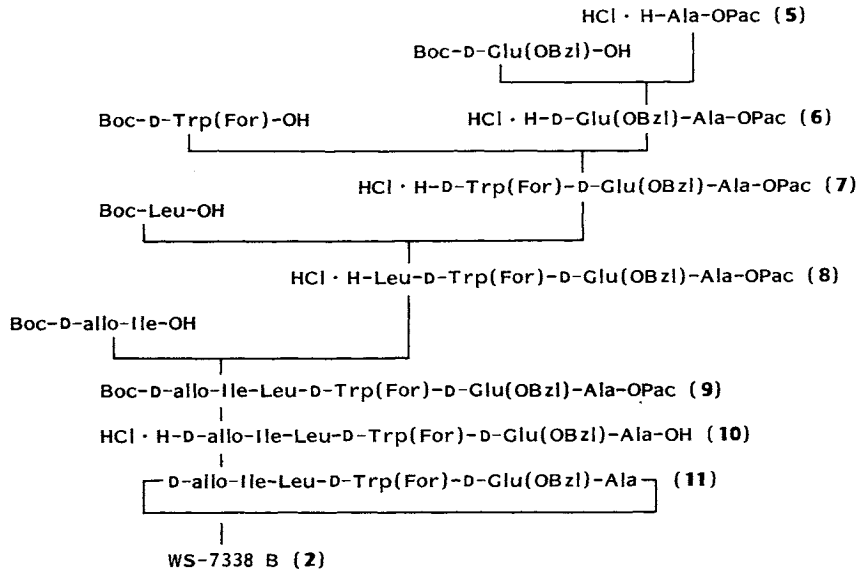


Table 2. ^{1}H NMR signal assignment for WS-7338 C (3) and D (4).

		WS-7338 C (3)	WS-7338 D (4)			WS-7338 C (3)	WS-7338 D (4)
		^{1}H[a] δ_H	^{1}H δ_H			^{1}H[a] δ_H	^{1}H δ_H
Leu[1]	NH	7.55	7.80	D-Trp	1-NH	10.79	10.78
[Val[1]][c]	α	4.38[b]	4.08		2	7.13	7.12
	β	1.36	1.82		3		
	γ	1.53	0.82		3_a		
			0.83		4	7.53	7.53
	δ	0.86			5	6.96	6.96
		0.87			6	7.03	7.03
Leu[2]	NH	8.47	8.38		7	7.32	7.30
[Val[2]][c]	α	4.10	3.78		7_a		
	β	1.18	1.70	D-Glu	NH	7.48	7.58
		1.24			α	4.24	4.18
	γ	0.99	0.36		β	1.87	1.80
			0.78		γ	2.15	1.90
	δ	0.63		L-Ala	NH	8.56	8.60
		0.73			α	4.38[b]	4.38
D-Trp	NH	8.68	8.80		β	1.13	1.13
	α	4.29	4.26				
	β	2.92	2.96				
		3.24	3.18				

[a] 400 MHz in DMSO-d_6.
[b] Overlapping.
[c] Val for WS-7338 D (4).

led to protected cyclic pentapeptide (**11**) (89.7%). Base treatment of **11** (1 N NaOH, room temperature for 15 minutes) gave cyclic pentapeptide (83.3%) which was identical with natural WS-7338 B (**2**) in all respects.

The structure elucidation of the minor congeners, **3** and **4**, was achieved as follows. The molecular formula of **4** was deduced as $C_{29}H_{40}N_6O_7$ from HRFAB-MS[1]. On the basis of conventional amino acid analysis, ^{1}H NMR data (Table 2), and chiral GC-MS analysis, one residue each of D-Glu, L-Ala, D-Val, L-Val and D-Trp is present in the intact molecule of **4**. The amino acid sequence analysis was performed by interpreting the NOE between NH

Table 3. Vicinal coupling constants ($J_{NH,C_\alpha H}$) of corresponding amino acid residue in WS-7338 A (**1**), B (**2**), C (**3**) and D (**4**).

Position[a]	1	2	3	4
1	9[b]	9.5	9	9
2	7	6.5	6.5	7
3	8.5	8.5	8	8
4	7.5	7.5	7.5	7.5
5	8	8	8	7.5

[a] For convenience, L-Ala was located at position 5.
[b] Expressed in Hz.

and $C_\alpha H$ pairs from neighboring amino acid residues and showed the following sequence: $-Val^1-Val^2-$D-Trp^3-D-Glu^4-L-Ala^5-.

The close similarity of vicinal coupling constants ($J_{NH,C_\alpha H}$) between relevant amino acid residues in **1** and **2** (Table 3) implied that the conformation of the peptide back bone of **1** closely matched that of **2**. These conformations could be stabilized by the two intramolecular hydrogen bonds as judged from the presence of two amide protons showing small temperature coefficients ($\Delta\delta/\Delta T$ [ppb/deg] **1**: D-Val (2.1), D-Glu (1.0), **2**: D-allo-Ile (1.7), D-Glu (1.9)). The striking resemblance of vicinal coupling constants of **4** (Table 3) to **1** and **2** suggested that the conformation of the peptide core of **4** might resemble those of **1** and **2**. From this assumption, we placed the D-Val residue at the 1 position (Val^1) and L-Val at 2 (Val^2), thus deriving structure **4** for WS-7338 D.

The structure of WS-7338 C was tentatively assigned as **3** in the same way.

Recently, it came to our attention from a CAS registry search that WS-7338 A is identical with BE-18257A[4] and WS-7338 B with BE-18257B[4].

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