WS75624 A and B, New Endothelin Converting Enzyme Inhibitors Isolated from *Saccharothrix* sp. No. 75624

II. Structure Elucidation of WS75624 A and B

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The structures of WS75624 A and B, novel endothelin converting enzyme inhibitors, were determined to be **1** and **2A**, respectively, by a combination of chemical evidence and a series of 2D NMR spectral analyses.

Endothelin 1 (ET-1) is a well-known potent vasoconstrictor peptide. It is generated from big endothelin-1 (precursor of ET-1) by the action of endothelin converting enzyme (ECE). We therefore expected that an endothelin converting enzyme inhibitor would have a possibility of a therapy for treatment of hypertension. In the course of our screening program for new ECE inhibitors, WS75624 A (**1**) and B (**2A**) were discovered from the fermentation of *Saccharothrix* sp. No. 75624. The taxonomy, fermentation, isolation, physical-chemical properties and biological activities are the subjects of the preceding paper[1]. In this paper we report structure elucidation of WS75624 A and B on the basis of chemical and physical evidence.

Structure Elucidation of WS75624 B

Initial structural efforts were focused on the most abundant WS75624 B (**2A**). The major metabolite **2A** analyzed for $C_{18}H_{24}N_2O_5S$ by interpretation of elemental analysis[1] and high resolution fast atom bombardment MS (HRFAB-MS)[1] and ^{13}C NMR spectral data (Table 1). All 18 carbon signals were seen in the ^{13}C NMR spectrum, consisting of one methyl, 5 CH_2, 2 methoxy, one oxymethine, 2 =CH and 7 sp^2 quaternary carbons. These groups accounted 22 non-exchangeable protons and therefore **2A** possesses 2 exchangeable protons. It reacted quickly with trimethylsilyldiazomethane ($TMSCHN_2$) at room temperature to give a methyl ester (**3**) (FAB-MS *m/z* 395 $(M+H)^+$, δ 3.91 (3H, s)). Treatment of **3** with acetic anhydride-pyridine gave an acetyl product (**4**) (FAB-MS *m/z* 437 $(M+H)^+$, δ 2.03 (3H, s)) in which oxymethine had shifted from 3.82 to 4.91 ppm. These results proved the presence of a carboxylic acid and a secondary alcohol functional groups in **2A**. At first we assigned a carbon signal at 173.5 ppm to the carboxylic acid carbonyl carbon but comparison of ^{13}C NMR data (Table 1) of **2A** with that of a sodium salt (**2B**), prepared by neutralization followed by evaporation (see Experimental), gave the indication of the misassignment. The carbon signal at 173.5 ppm showed a negligible change while a signal at 166.5 ppm exhibited a significant change and thus the signal at 166.5 ppm reassigned to the COOH. Eventually the ^{13}C assignment was unambiguously made by 2D INADEQUATE (natural abundant ^{13}C-^{13}C NMR)[2] experiments *vide infra*.

NOE correlations (3-H⇔4-OMe and 4-OMe⇔5-OMe) observed in NOESY spectra in combination with long-range C-H correlations as shown in Fig. 1 led to fragment **a**. No long-range C-H correlation was observed between C-2 and H-3 because C-2 carbon signal appeared as singlet in ^1H-coupled ^{13}C NMR spectrum. Although severe ^1H signal overlapping due to methylene protons on C-3″, C-4″ and C-5″ (δ 1.51～1.37 (6H, m)) failed to reveal alkyl chain of **2A**, the corresponding ^1H signals in **2B** were well separated (Table 1) and thus a combined spectral analysis of ^1H-^1H COSY and ^{13}C-^1H COSY revealed the alkyl chain $CH_3CH(OH)CH_2CH_2CH_2$-CH_2CH_2–.

Extensive analyses of 2D INADEQUATE experiments of **2A** showed all C-C connections: C7″-C6″-C5″-C4″-C3″-C2″-C1″-C2′ and C5′-C4′-C6-C5-C4-C3-C2-C7 which extend the above alkyl chain to substructure **b** and the fragment **a** to substructure **c** as shown in Fig. 2. The carbon chemical shift of C-2′ (δ 173.5) showed the carbon situated between two heteroatoms. The long-range C-H

Table 1. ^{1}H and ^{13}C NMR data for **2A**, **2B** and **1**.

Position	2A			2B		1	
	^{1}H[a]	^{13}C[b]	J_{C-H}[c]	^{1}H[a]	^{13}C[b]	^{1}H[a]	^{13}C[b]
2		144.6 s			146.6		144.6 s
3	7.79 (1H,s)	109.1 d	168	7.82 (1H,s)	109.0	7.81 (1H,s)	109.1 d
4		163.2 s			162.1		163.3 s
5		147.3 s			149.3		147.3 s
6		142.7 s			144.1		142.8 s
7		166.5 s			169.8		166.5 s
OMe on 4	4.09 (3H,s)	57.4 q	147	4.08 (3H,s)	56.9	4.09 (3H,s)	57.4 q
OMe on 5	3.99 (3H,s)	61.0 q	147	3.99 (3H,s)	60.7	4.00 (3H,s)	61.0 q
2'		173.5 s			173.0		173.5 s
4'		148.6 s			151.0		148.6 s
5'	8.30 (1H,s)	123.8 d	195	8.29 (1H,s)	122.2	8.32 (1H,s)	123.8 d
1"	3.11 (2H,m)	34.0 t	130	2.89 (2H,m)	34.1	3.13 (2H,m)	34.0 t
2"	1.85 (2H,m)	31.3 t	125	1.63 (2H,m)	31.5	1.85 (2H,m)	31.9 t
3"	1.51	30.2 t	125	1.30 (2H,m)	30.3	1.54 ~	24.9 t
4"	~	26.5 t	125	1.20 (2H,m)	26.4	1.50 (4H,m)	44.3 t
5"	1.37 (6H,m)	40.0 t	126	1.35 (1H,m)	40.0		71.3 s
				1.28 (1H,m)			
6"	3.71 (1H,m)	68.4 d	142	3.64 (1H,m)	68.4	1.18 (6H,s)	29.2 q
7"	1.13 (3H,d,6)	23.5 q	125	1.11 (3H,d,6)	23.5		29.2 q

[a] 400 MHz in CD_3OD, [b] 100 MHz in CD_3OD, [c] One-bond coupling constant in Hz

Fig. 1. Long range C-H coupling (COLOC) and NOE of substructure **a**.

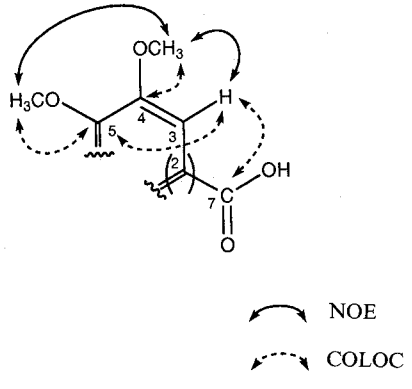
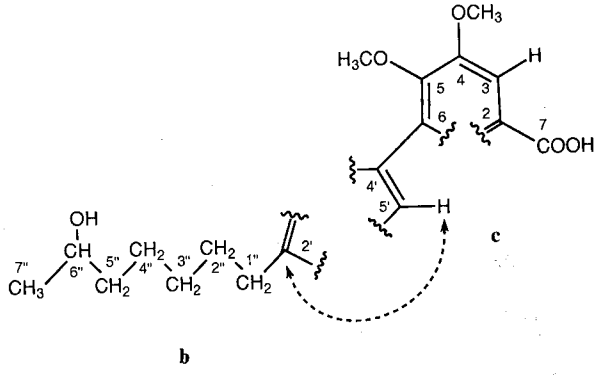


Fig. 2. Substractures **b** and **c**.

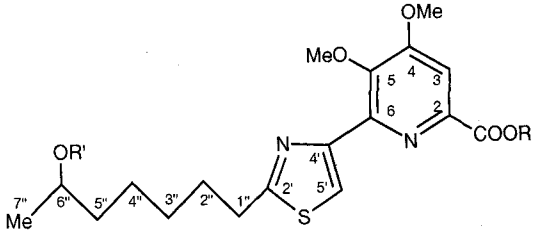


coupling between C-2′ and 5′-H indicated the presence of a five-membered heterocycle which was ascertained by a large one-bond C-H coupling ($J_{C-H}=195$ Hz)[3]. Inspection of carbon chemical shift of C-2 (δ 144.6) and C-6 (δ 142.7) revealed the presence of a pyridine nucleus and thus identified the five-membered heterocycle as a thiazole. The regiochemistry of the thiazole ring was assigned on the basis of the carbon chemical shift comparison of C-4′ and C-5′ to the relevant carbon in 2,4-disubstituted-thiazole-containing marine natural product, mycothiazole[4]. From the above information, the structure of WS75624 B (**2A**) was concluded to be 4,5-dimethoxy-6-(2-(6-hydroxyheptyl)-4-thiazolyl)-2-pyridinecarboxylic acid as depicted in Fig. 3. The stereochemistry of the WS75624 B side chain remains to be established.

Structure of WS75624 A

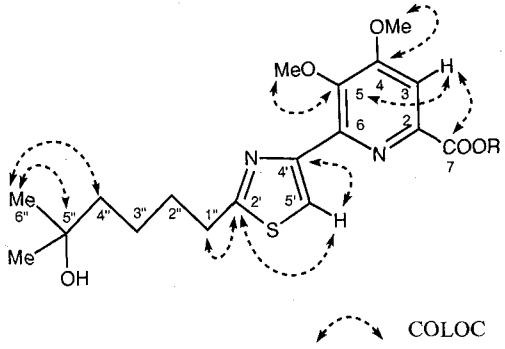
The molecular formula of WS75624 A (**1**) was determined to be $C_{18}H_{24}N_2O_5S$ on the basis of elementary analysis[1], HRFAB-MS[1] and ^{13}C NMR data (Table 1). WS75624 A (**1**) has the same molecular formula and the difference between **1** and **2A** lies in the alkyl chains. The chromophore portion of **1** is the same as in **2A** was indicated by that the UV spectrum of **1** was superimposable on that of **2A** and that the carbon chemical shift due to **1** heterocylce was in excellent agreement with that of the relevant carbon in **2A** (Table 1). The assignment was also confirmed by the long-range

Fig. 3. Structures of **2A**, its sodium salt **2B** and derivatives **3** and **4**.



2A R=H R'=H
2B R=Na R'=H
3 R=Me R'=H
4 R=Me R'=Ac

Fig. 4. Structures of **1** and its derivative **5**.



1 R=H
5 R=Me

C-H couplings as shown in Fig. 4. As in the case of **2A**, **1** reacted with $TMSCHN_2$ to give the methyl ester (**5**). Failure of acetylation of **5** demonstrated the presence of a tertiary alcohol instead of a secondary alcohol. A combination of ^1H-^1H COSY, ^{13}C-^1H COSY and COLOC revealed the substructures: $(CH_3)_2C(OH)CH_2-$ and $-CH_2CH_2CH_2-$. The connectivity of C3″ and C4″ was not clearly obtained because of the ^1H signal overlapping (δ 1.54~1.50 (4H, m)). The assumption of a bond between the carbons 3″ and 4″ completed the structure, giving 4,5-dimethoxy-6-(2-(5-hydroxy-5-methylhexyl)-4-thiazolyl)-2-pyridinecarboxylic acid, shown in Fig. 4.

Experimental

General Procedures have been previously described[1,5].

Preparation of **2B** (**2A** Sodium Salt)

To a solution of **2A** (100 mg) in MeOH (3 ml) was added 0.1 N NaOH (3 ml). After removal of MeOH, the solution was applied to a column of Diaion HP-20 and the column was washed with deionized water and then eluted with MeOH. The eluate was evaporated to dryness under reduced pressure to give 72 mg of sodium salt **2B** as a colorless powder: FAB-MS m/z 425 $(M+Na)^+$, *Anal* found: C 51.84, H 6,15, N 6.63, calcd for $C_{18}H_{23}N_2O_5SNa \cdot H_2O$: C 51.42, H 5.99, N 6.66, ^1H NMR and ^{13}C NMR see Table 1.

Preparation of **2A** Methyl Ester (**3**)

A solution of **2A** (10 mg) in MeOH (1 ml) was treated with 0.2 ml of $TMSCHN_2$ (10% by wt in hexane). After 5 minutes at room temperature, the solution was evaporated to dryness. The residue obtained was purified by PTLC ($CHCl_3$ - MeOH (95 : 5)) to give 8 mg of methyl ester (**3**) as an oil: FAB-MS m/z 395 $(M+H)^+$, ^1H NMR (400 MHz, $CDCl_3$) δ 7.93 (1H, s), 7.75 (1H, s), 4.05 (3H, s), 4.00 (3H, s), 3.91 (3H, s), 3.82 (1H, m), 3.12 (2H, m), 1.88 (2H, m), 1.49 (6H, m), 1.20 (3H, d, J=6 Hz).

Acetylation of **3**

Acetic anhydride (0.2 ml) was added to a solution of **3** (5 mg) in prydine (0.5 ml) and the solution was allowed to stand at room temperature overnight. The solution was evaporated to dryness under reduced pressure to give an oil which was separated by PTCL ($CHCl_3$-MeOH (95 : 5)) to afford 6 mg of acetyl product (**4**): FAB-MS m/z 437 $(M+H)^+$, ^1H NMR (400 MHz, $CDCl_3$) δ 7.92 (1H, s), 7.74 (1H, s), 4.91 (1H, m), 4.05 (3H, s), 4.00 (3H, s), 3.90 (3H, s), 3.12 (2H, m), 1.88 (2H, m), 1.55~1.34 (6H, m), 1.21 (3H, d, J=6 Hz).

Preparation of **1** Methyl Ester (**5**)

Methyl ester of **1** was prepared in a similar manner. **5**: FAB-MS m/z 395 $(M+H)^+$, ^1H NMR (400 MHz, $CDCl_3$) δ 7.92 (1H, s), 7.72 (1H, s), 4.03 (3H, s), 3.99 (3H, s), 3.90 (3H, s), 3.12 (2H, m), 1.88 (2H, m), 1.56~1.50 (4H, m), 1.20 (6H, s).

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