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Transvalencin A, a Thiazolidine Zinc Complex Antibiotic Produced by a Clinical Isolate of *Nocardia transvalensis*

II. Structure Elucidation

YASUTAKA HOSHINO[a,d], AKIRA MUKAI[a], KATSUKIYO YAZAWA[a], JUN UNO[a], AKIKAZU ANDO[b], YUZURU MIKAMI[a,*], TOSHIO FUKAI[c], JUN ISHIKAWA[d] and KENTARO YAMAGUCHI[e,†]

[a] Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba, Chiba 260-8673, Japan
[b] Department of Biotechnology, Graduate School of Science and Technology, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba, Chiba 263-8522, Japan
[c] School of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan
[d] Department of Bioactive Molecules, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan
[e] Chemical Analytical Center, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba, Chiba 263-8522, Japan

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A novel antifungal antibiotic, transvalencin A, is produced by *Nocardia transvalensis* IFM 10065 isolated from a patient with actinomycotic mycetoma in Japan. The antibiotic structure was elucidated using NMR, mass spectrometric investigations, and X-ray crystallographic analysis. Transvalencin A is a 1 : 1 complex of a zinc and an organic acid with a phenolic substituent. Transvalencin A is comprised of *o*-substituted *p*-chlorophenol, tetrasubstituted oxazoline, disubstituted thiazolyl-*N*-methylthiazolidine and monosubstituted *N*-methylthiazolidine.

Our continuing studies on antifungal agents from pathogenic *Nocardia*, have revealed a novel compound designated as transvalencin A (**1**) from cultured mycelia of *Nocardia transvalensis* IFM 10065, which was isolated from a male Japanese patient with actinomycotic mycetoma[1]. In a preceding paper[1], we reported the taxonomy of the producing strain, fermentation, isolation, and biological activities of the novel compound (**1**). The antibiotic is a thiazole-class compound that is related structurally to a known family of antibiotic whose members include thiocillins[2], micrococcins[3,4], YM-266183 and YM-266184[5], radamycin[6], nocathiacins[7,8], nosiheptide[9], glycothiohexides[10], cystothiazoles[11], and melithiazols[12]. This paper describes the structural elucidation of the new antibiotic using spectroscopic studies including two-dimensional NMR experimentation and X-ray crystallographic analysis.

Results and Discussion

The FAB-MS of transvalencin A (**1**) gave positive pseudomolecular ions at *m/z* 655 $[M+H]^+$, 653 $[M+H]^+$, and 651 $[M+H]^+$, and potassium adduct ions $[M+K]^+$ at *m/z* 693, 691, and 689 with the addition of KI (Table 1). Furthermore, positive pseudomolecular ions $[M+Na+CH_3CN]^+$ were observed at *m/z* 718, 716, and 714 in the ESI-MS spectrum of **1**. These data suggest that

* Corresponding author: mikami@faculty.chiba-u.jp
† Present address: Faculty of Pharmaceutical Sciences, Tokushima Bunri University at Kagawa Campus, 1314-1 Shido, Sanuki, Kagawa 769-2193, Japan.

Table 1. Physico-chemical properties of transvalencin A (**1**).

Appearance	colorless prisms
	mp. 280°C (dec, shattered at 258°C)
Molecular formula	$C_{23}H_{27}ClN_4O_6S_3Zn$
FAB-MS *m/z*	655 [M (^{37}Cl, ^{66}Zn) + H]$^+$, 653 [M (^{37}Cl, ^{64}Zn / ^{35}Cl, ^{66}Zn) + H]$^+$, 651 [M (^{35}Cl, ^{64}Zn) + H]$^+$, 637, 635, 633 (M – 17), 307, 289, 176, 154, 136.
FAB-MS (+ KI) *m/z*	693 [M (^{37}Cl, ^{66}Zn) + K]$^+$, 691 [M (^{37}Cl, ^{64}Zn / ^{35}Cl, ^{66}Zn) + K]$^+$, 689 [M (^{35}Cl, ^{64}Zn) + K]$^+$, 637, 635, 633 (M – 17), 580, 578, 576, 344.5, 306, 288, 191, 154, 136
ESI-MS* *m/z*	720 (24.6%), 719 (24.6%), 718 (72.2%), 717 (38.7%), 716 (100%), 715 (29.8%), 714 (91.9%)
IR ν_{max} (KBr) cm^{-1}	3400 (sh), 3190, 2835, 1660, 1625, 1595 (infl.), 1520, 1460, 1365, 1340, 1235, 1150, 1085, 980, 965, 595.
CD (c 1.2 × 10^{-4} mol/L, MeOH) [θ] (nm)	+ 24000 (217), + 9900 (238), 0 (244), – 11000 (254), – 16000 (276), 0 (317), + 1900 (347), 0 (387)
UV (MeOH) λ_{max} (log ε) nm	225 (4.47), 251 (4.05), 358 (3.65)

* Theoretical ion distribution of the pseudomolecular ions [M + Na + CH_3CN]$^+$ of $C_{23}H_{27}ClN_4O_6S_3Zn$ (M): *m/z* 720 (25.3%), 719 (25.6%), 718 (72.4%), 717 (39.3%), 716 (100%), 715 (30.0%) and 714 (91.7%).

this compound includes halogen (X) and/or metal (M) atoms, 2×X or 2×M or X+M, whose stable isotopes have relatively high natural abundance. ^{1}H and ^{13}C NMR spectra of **1** (Table 2) indicated that the antibiotic comprises 27 hydrogen and 23 carbon atoms and more than 12 heteroatoms such as oxygen, nitrogen, and sulfur atoms. Simulation of the MS spectrum indicated that the molecular formula of **1** is $C_{23}H_{27}ClN_4O_6S_3Zn$ because the calculated relative abundance of the pseudomolecular ions at *m/z* 718 [M(^{37}Cl,^{66}Zn)+Na+CH_3CN]$^+$ (72.4%), 716 [M(^{37}Cl,^{64}Zn/^{35}Cl,^{66}Zn)+Na+CH_3CN]$^+$ (100%), and 714 [M(^{35}Cl,^{64}Zn)+Na+CH_3CN]$^+$ (91.7%) was appropriate for the intensity of the (M+Na+CH_3CN) ions observed in the ESI-MS of **1** (Table 1). The UV spectrum of **1** exhibited maxima at 225, 251, and 358 nm, indicating the presence of an *ortho*-oxygenated phenyl chromophore. The IR spectrum also suggested the presence of a benzene ring (1595 and 1520 cm^{-1}) in the molecule as well as the presence of a hydrogen-bonded hydroxyl group (3400 cm^{-1}), >C=O and >C=N– bonds (1660 and 1625 cm^{-1}).

Table 2 summarizes the ^{1}H and ^{13}C NMR data of **1**, which were assigned with COSY, NOESY, HMQC, and HMBC spectra. The ^{1}H NMR spectrum showed ABC-type aromatic protons, 7 methine and 4 non-equivalent methylene protons, 12 protons of methyl groups (two *N*-CH_3, an *O*-CH_3 and a doublet methyl), and a hydroxyl proton. The ^{13}C NMR spectrum revealed 23 carbons, of which there were 4 methyl, 2 methylene, 7 methine, and 7 quarternary resonances, and 3 aromatic carbons bearing hydrogen atoms. The ^{13}C-^{1}H long-range couplings of ^2J and ^3J that were observed in the HMBC experiments (Table 2) indicated the following spin-spin networks. Cross-peaks between H-3 and C-1, C-2 (oxygen-bearing carbon, δ 169.7), C-5, between H-4 and C-2, C-5, C-6 and between H-6 and C-2, C-5 supported the presence of a benzene ring (*A*-ring). The H-6 was also correlated to the sp^2 carbon at δ 168.5 (C-7, heteroatoms and a sp^2 carbon bearing carbon[13]), which was further correlated with H-9. The quartet proton (H-9) was coupled with doublet methyl protons (H_3-8), which were correlated (^2J and ^3J) with C-9 and C-10. The hydroxy proton [H-24 (O<u>H</u>-10), δ 7.08] correlated with C-9 and C-10 (δ 100.6) suggesting the presence of the hydroxyl group at the C-10 position. These data indicated the presence of a five-member ring (*B*-ring) with a methyl group, a hydroxyl group, two heteroatoms (nitrogen and oxygen/sulfur) and a double bond. The C-10 signal was correlated with H-11 on a five-member ring (*C*-ring) with a *N*-CH_3 group and an unidentified heteroatom (oxygen or sulfur). From these data, a partial structure A

Table 2. NMR data for transvalencin A (**1**) in $CDCl_3$ (δ [ppm], J [Hz]).

Position	δ_H, multiplicity, coupling constant(s)*	δ_C	HMBC; ^3J (^2J) correlations** (position number of protons)
1	---	109.7	3
2	---	169.7	(3), 4, 6
3	6.65, d, 9.3	124.5	---
4	7.15, dd, 3, 9.3	134.9	6
5	---	117.5	3, (4, 6)
6	7.61, d, 3	129.1	4
7	---	168.5	6, 9
8	1.42 (3H), d, 6.6	14.8	---
9	4.82, q, 6.6	82.3	(8), 24
10	---	100.6	8, (11, 24)
11	4.30, s	78.2	9, (H-12, δ 2.96), 21
12	3.02, dd, 10.3, 11.5 / 2.96, dd, 5.8, 11.5	37.2	11, (13)
13	3.96, dd, 5.8, 10.3	72.6	11, (H_2-12), 21
14	---	186.7	H_2-12, H_2-15, 16
15	3.70, t, 12 / 3.31, dd, 6.3, 12	34.4	---
16	5.43, m (br ddd-like)	71.8	(H_2-15, 17)
17	4.61, d, 4	79.0	H_2-15, (16), 18, 19, 22
18	5.52, br s	93.4	(19), 23
19	3.91, s	81.4	22
20	---	171.4	18, (19)
21	2.78 (3H), s	44.4	11, 13
22	2.92 (3H), s	45.4	17, 19
23	3.38 (3H), s	56.4	18
24	7.08 (O<u>H</u>-10), s	---	

* All HMQC correlations were observed on the protons except H-24 (O<u>H</u>-10).
** Correlation with 4 or 8 Hz.

Fig. 1. Structure of transvalencin A (**1**).

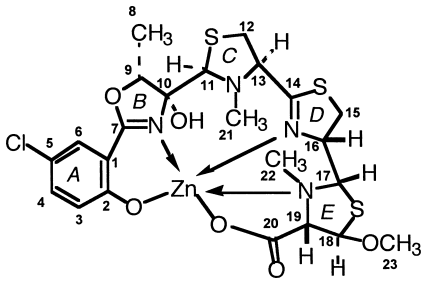
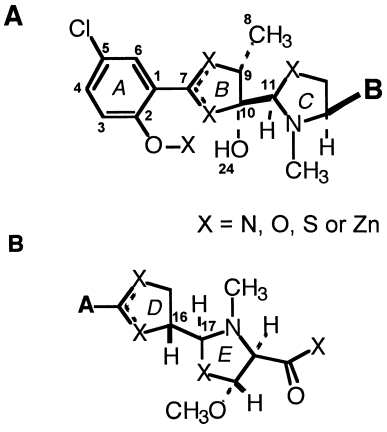
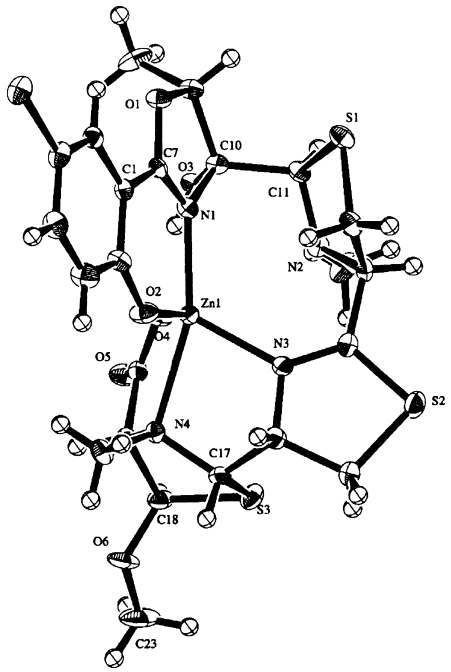


Fig. 2. Partial structures A and B of transvalencin A (**1**).



(Fig. 2) was elucidated along with the following partial structure B (*D*- and *E*-rings). Similar NMR experiments indicated a five-member ring (*E*-ring) with a carbonyl group, a methoxyl group, a *N*-methyl group and an unidentified heteroatom (oxygen or sulfur), which all existed in the partial structure B. The H-17 of the heterocyclic ring correlated with the proton (H-16) of the other five-member ring (*D*-ring) with two unidentified heteroatoms (nitrogen, oxygen, or sulfur). Further effective data for structural elucidation were not obtained from NMR

Fig. 3. ORTEP picture of transvalencin A (**1**).*



* Inter atomic distances: C(2)-O-Zn, 1.955 Å; C(20)-O-Zn, 1.99 Å, C(7)-N-Zn, 2.048 Å; C(14)-N-Zn, 2.03 Å; C(17)-N-Zn, 2.355 Å, C(11)-N-Zn, 3.00 Å, C(10)-OH-O-C(20)=O, 1.79 Å. Bond angles: C(2)-O-Zn-N-C(7), 89.3°; C(2)-O-Zn-N-C(14), 104.6°; C(2)-O-Zn-N-C(17), 90.2°; C(2)-O-Zn-O-C(20)=O, 136.5°; C(10)-O-H-O-C(20)=O, 165.3°, C(2)-O-Zn-N-C(11), 134.8°.

experiments.

Collectively, the molecular formula, UV, IR, and NMR spectral data suggested that transvalencin A (**1**) is a thiazolidine/oxazoline/thiazoline type antibiotic. However, a complete structure was not determined because of the presence of N, O, S, and Zn atoms in the molecule. Several repeated attempts to obtain a suitable crystal for X-ray analysis were performed and eventually we obtained a crystal of **1** from methanol. This crystallized sample was analyzed using X-ray diffraction to give the structure of **1** as shown in Figure 3.

Although many thiazole type antibiotics have been reported, thiazolidine, or thiazoline type antibiotics are very rare. Most thiazole antibiotics are produced by *Streptomyces*[6,9], *Bacillus*[2,3], or myxobacteria[11,12]. Recently, new thiazolyl peptide antibiotics were isolated from a *Nocardia* sp. strain isolated from soil[6,7]. We believe that the thiazolidine-class antibiotic reported here is the first member of this class from a clinical isolate of pathogenic *Nocardia*. The transvalencin A structure is unique because it contains a zinc atom in the molecule. The active role of the zinc atom in exerting antifungal activity is unclear, but it is known that a zinc atom plays an important role in protein structure conformation[14]. For that reason, this antibiotic may be a useful tool to elucidate the inhibitory role of the zinc atom in enzymes such as adenylyl cyclase.

Experimental

General

Melting points were determined using a Yanaco MP-500V apparatus (GTR TEC Corp., Kyoto, Japan); they are uncorrected. The UV spectrum was measured with a spectrophotometer (UV-265; Shimadzu Corp., Kyoto, Japan). The ^{1}H and ^{13}C NMR spectra were recorded on an NMR spectrometer (JEOL JNM EXP-500; JEOL, Akishima, Japan). Chemical shifts were reported with respect to $CDCl_3$; δ_H 7.24 ($CHCl_3$) and δ_C 77.0. Electrospray ionization (ESI) MS and FAB-MS were measured with JEOL JMS-700 and JMS-AX500 instruments, respectively. The sample for ESI-MS analysis was dissolved in CH_3OH-CH_3CN-H_2O (70 : 25 : 5). Nitrobenzyl alcohol was used as a FAB-MS matrix. We obtained IR and CD spectra using a Jasco FT/IR-300E spectrometer and a Jasco J-720W CD spectropolarimeter, respectively (Jasco Inc., Hachioji, Japan).

X-Ray Crystallographic Analysis of Transvalencin A (**1**)

A clear prism crystal of $C_{23}H_{27}ClN_4O_6S_3Zn$ with approximate dimensions of 0.49×0.25×0.08 mm was mounted on a glass fiber. All measurements were made using a diffractometer (Bruker Smart 1000 CCD; Bruker Analytik, GmbH, Germany) with graphite monochromated Mo-Kα radiation. Cell constants and an orientation matrix for data collection corresponded to a primitive monoclinic cell with the following dimensions: a=10.056(2) Å, b=7.919(2) Å, c=17.678(3) Å, β=105.799(3)°, V=1354.7(4) Å^3. The calculated density is 1.60 g/cm^3 for Z=2 and F.W.=652.50. Based on the systematic absences of: 0k0: k±2 n packing considerations, a statistical analysis of intensity distribution, and the successful solution and refinement of the structure, the space group was determined to be $P2_1$. Data were collected at 173±1 K to a maximum 2θ value of 57.0°. Final R and weighted R values were 0.029 and 0.034, respectively.

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References

1) Hoshino, Y.; A. Mukai, K. Yazawa, J. Uno, J. Ishikawa, A. Ando, T. Fukai & Y. Mikami: Transvalencin A, a thiazolidine zinc complex antibiotic produced by a clinical isolate of *Nocardia transvalensis*. I. Taxonomy, fermentation, isolation and biological activities. J. Antibiotics 57: 798～802, 2004
2) Shoji, J.; T. Kato, Y. Yoshimura & K. Tori: Studies on antibiotics from the genus *Bacillus*. XXIX. Structural studies on thiocillins I, II and III. J. Antibiotics 34: 1126～1136, 1998
3) Shoji, J.; H. Hinoo, Y. Wakisaka, K. Koizumi, M. Mayama, S. Matsuura & K. Matsumoto: Studies on antibiotics from the genus *Bacillus*. VIII. Isolation of three new antibiotics, thiocillins I, II, III, related to micrococcin P. J. Antibiotics 29: 366～374, 1976
4) Walker, J.: Total structure of the polythiazole-containing antibiotic micrococcin P. A carbon-13 nuclear magnetic resonance study. J. Chem. Soc., Chem. Commun. 1977: 706～708, 1997
5) Nagai, K.; K. Kamigiri, N. Arao, K. Suzumura, Y. Kawano, M. Yamaoka, H. Zhang, M. Watanabe & K. Suzuki: YM-266183 and YM-266184, novel thiopeptide antibiotics produced by *Bacillus cereus* isolated from a marine sponge. I. Taxonomy, fermentation, isolation, physicochemical properties and biological properties. J. Antibiotics 56: 123～128, 2003
6) Rodriguez, J. C.; G. G. Holgado, R. I. S. Sanchez & L. M. Canedo: Radamycin, a novel thiopeptide produced by *Streptomyces* sp. RSP9. II. Physico-chemical properties and structure determination. J. Antibiotics 55: 391～395, 2002
7) Li, W.; J. E. Leet, H. A. Ax, D. R. Gustavson, D. M. Brown, L. Turner, K. Brown, J. Clark, H. Yang, J. Fung-Tomc & K. S. Lam: Nocathiacins, new thiazolyl peptide antibiotics from *Nocardia* sp. I. Taxonomy, fermentation and biological activities. J. Antibiotics 56: 226～231, 2003
8) Leet, J. E.; W. Li, H. A. Ax, J. A. Matson, S. Huang, R. Huang, J. L. Cantone, D. Drexler, R. A. Dalterio & K. S. Lam: Nocathiacins, new thiazolyl peptide antibiotics from *Nocardia* sp. II. Isolation, characterization, and structure determination. J. Antibiotics 56: 232～242, 2003
9) Benazet, F.; M. Cartier, J. Florent, C. Godard, G. Jung, J. Lunel, D. Mancy, C. Pascal, J. Renaut, P. Tarridec, J. Theilleux, R. Tissier, M. Dubost & L. Minet: Nosiheptide, a sulfur-containing peptide antibiotic isolated from *Streptomyces actuosus* 400037. Experientia 36: 414～416, 1980
10) Steinberg, D. A.; V. S. Bernan, D. A. Motenegro, D. R. Abbanat, C. J. Pearce, J. D. Korshalla, N. V. Jacobus, P. J. Petersen, M. J. Mroczenski-Wildey, W. M. Maiese & M. Greenstein: Glycothiohexides, novel antibiotics produced by "*Sebekia*" sp., LL-14E605. I. Taxonomy, fermentation, and biological evaluation. J. Antibiotics 47: 887～893, 1994
11) Suzuki, Y.; M. Ojika, Y. Sakagami, R. Fudou & S. Yamanaka: Cystothiazoles C～F, new bithiazole-type antibiotics from the myxobacterium *Cystobacter fuscus*. Tetrahedron 54: 11399～11404, 1998
12) Bohlendorf, B.; M. Herrmann, H.-J. Hecht, F. Sasse, D. Forche, B. Kunze, H. Reichenbach & G. Hofle: Antibiotics from gliding bacteria. Part 85. Melithiazoles A～N, new antifungal β-methoxyacrylates from myxobacteria. Eu. J. Org. Chem. 2601～2608, 1999
13) Carnio, M. C.; A. H. Ltzel, M. Rudolf, T. Henle, G. Jung & S. Scherer: The macrocyclic peptide antibiotic micrococcin P_1 is secreted by the food-borne bacterium *Staphylococcus equorum* WS 2733 and inhibits *Listeria monocytogenes* on soft cheese. Appl. Environ. Microbiol. 66: 2378～2384, 2000
14) Klein, C.; T. Heyduk & R. K. Sunahara: Zinc inhibition of adenylyl cyclase correlates with conformational changes in the enzymes. Cell Signal 16: 1177～1185, 2004