Revised Structure and Stereochemistry of Hypothemycin

Tsutomu AGATSUMA,[a] Akira TAKAHASHI,[a] Chizuko KABUTO,[b] and Shigeo NOZOE*,[a]

Faculty of Pharmaceutical Sciences,[a] and Instrumental Analysis Center for Chemistry, Faculty of Sciences,[b] Tohoku University, Aoba-ku, Sendai 980, Japan. Received June 25, 1992

A cytotoxic 14-membered resorcylic macrolide, isolated from *Coriolus versicolor* (L.: FR.) QUÉL., was found to be identical with hypothemycin, previously isolated from *Hypomyces trichothecoides* TSUBAKI. The structure of hypothemycin has been elucidated to be 1 rather than the originally proposed 2.

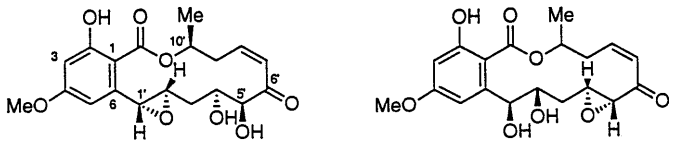
Keywords hypothemycin; revised structure; 2D-NMR: X-ray crystallographic analysis; *Coriolus versicolor*; *Hypomyces trichothecoides*

During the course of our search for new cytotoxic agents from Basidiomycetes, a crude extract of *Coriolus versicolor* (L.: FR.) QUÉL. was found to demonstrate potent activity *in vitro* against P388 leukemia. A cytotoxic substance (**1**)[1] was isolated by chromatography over silica gel, and its physico-chemical properties were found to be identical with those of hypothemycin (**2**),[2] a 14-membered resorcylic macrolide[3] isolated from the fermentation broth of *Hypomyces trichothecoides* TSUBAKI (*mycoparasitic fungus*) by Nair *et al.* Reinvestigation of the chemical structure of hypothemycin by means of two dimensional (2D)-NMR spectroscopies, including correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC), led to conclude that the structure of hypothemycin should be represented by **1** rather than the previously assigned **2**. This paper deals with the structural revision of hypothemycin.

The ethyl acetate-soluble material from the methanolic extract of fruiting bodies of *C. versicolor* was chromatographed on silica gel (hexane–ethyl acetate) to obtain a crude crystalline compound, which was further purified by recrystallization from ethyl acetate to give hypothemycin (**1**) as colorless needles.

^{1}H- and ^{13}C-NMR analyses (Table I) of **1**, including COSY and HMQC, revealed the partial structures from C1′ to C5′ (I) and C7′ to C10′ (II) in **1** (Fig. 1). Although a phenolic proton signal was not observed in the ^{1}H-NMR spectrum of **1** (lit.[2] δ 12.1), an nuclear Overhauser effect (NOE) difference experiment on the corresponding triacetyl derivative indicated a 4-methyl resorcylic structure for **1**.

In order to clarify the connectivities of the partial structures I and II attached to the resorcylic chromophore, an HMBC experiment was carried out. The HMBC spectrum revealed that one of the aromatic proton signals



1 **2** (relative)

Fig. 1

TABLE I. ^{1}H- and ^{13}C-Chemical Shifts Assignments of **1** in $CDCl_3$

Carbon	δ (ppm)[a]	Proton	δ (ppm)[a]	J (Hz)
1	104.0 s			
2	166.2 s[b]			
3	101.1 d[c]	3	6.39 d[d]	2.8
4	165.2 s[b]			
5	103.6 d[c]	5	6.38 d[d]	2.8
6	142.3 s			
1′	56.0 d	1′	4.37 d	1.7
2′	62.6 d	2′	2.88 dd	1.7, 9.3
3′	34.6 t	3′a	1.10 dd	15.0, 9.3
		3′b	1.96 dd	15.0, 9.3
4′	70.7 d	4′	3.99 br ddd	9.3, 9.3, 1.5
5′	81.0 d	5′	4.58 br dd	5.7, 1.5
6′	199.5 s			
7′	126.4 d	7′	6.37 dd	11.4, 2.8
8′	145.4 d	8′	6.17 ddd	11.4, 11.4, 2.2
9′	37.0 t	9′a	2.56 dddd	16.4, 2.8, 2.2, 2.2
		9′b	3.09 ddd	16.4, 11.4, 11.4
10′	73.2 d	10′	5.49 ddq	11.4, 2.2, 5.7
–*C*OO–	171.2 s			
10′-CH_3	21.1 q	10′-CH_3	1.41 d	5.7
4-OCH_3	55.5 q	4-OCH_3	3.77 s	
		2-*OH*	—[e]	
		4′-*OH*	2.21 br d	9.3
		5′-*OH*	3.48 br d	5.7

a) ^{1}H- and ^{13}C-NMR chemical shifts were recorded at 500 MHz and 125 MHz, respectively, relative to the tetramethylsilane peak. *b*—*d*) Assignments are interchangeable. *e*) Obscured.

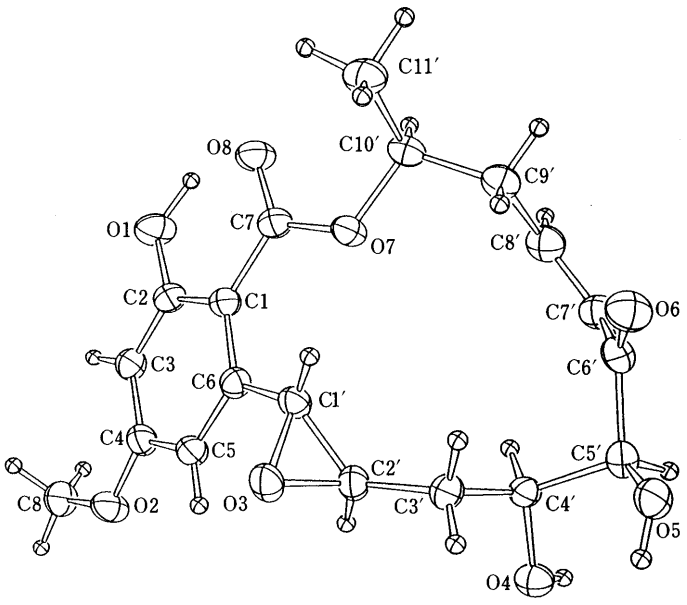


Fig. 2. ORTEP Drawing of **1**

at δ 6.38, 6.39 (ABq, 2H, J=2.8 Hz) was coupled to the epoxy carbon signal at δ 56.0 (d), one of the epoxy proton signals at δ 4.37 (d, 1H, J=1.7 Hz) was coupled to the aromatic carbon signal at δ 142.3 (s), and one of the carbinyl proton signals at δ 4.58 (br dd, 1H, J=5.7, 1.5 Hz) was coupled to the carbonyl carbon signal at δ 199.5 (s). Thus, the total planar struture of hypothemycin has been determined as 7′-dehydro-4′,5′-dihydroxy-1′ : 2′-epoxyzearalenone 4-methyl ether (**1**).

The partial relative stereochemistry of hypothemycin was previously reported as 1′*S**, 2′*R**, 4′*R**, 5*S** on the basis of the 2D-*J* resolved method. However, because of the presence of many possible conformations due to the 14-membered ring in **1**, it was difficult to determine the relative stereostructure of **1** from ^{1}H–^{1}H coupling constants. We therefore chose to perform an X-ray crystallographic analysis.

Colorless prisms of **1** were grown in an ethanol–chloroform medium. The X-ray structure determination of **1** is described in the experimental section. An ORTEP drawing of the molecule is shown in Fig. 2. Some structural features are noteworthy. As can be seen in Fig. 2, the enone function (C6′ to C8′) has *cisoid* form in the solid state. The dihedral angle between the 2′-proton and one of the 3′-protons is about 90°. This is consistent with ^{1}H-NMR data which showed that the ^{1}H–^{1}H coupling constant of these vicinal protons is 0 Hz. Thus, the five asymmetric centers in hypothemycin have been established as having the 1′*R*, 2′*R*, 4′*S*, 5′*S* and 10′*S* configurations.

Experimental

Melting points were determined on a Yanagimoto micro hot plate and are uncorrected. Optical rotation value was measured on a JASCO DIP-370 polarimeter. The spectroscopic data were measured by using the following instruments; IR spectra: JASCO A-100S IR spectrometer; UV spectrum: Hitachi U-3200 spectrophotometer; mass spectra: JEOL DX-303 spectrometer; ^{1}H- and ^{13}C-NMR spectra: JEOL GX-500 (500 MHz and 125 MHz, respectively). Chemical shifts are shown in δ(ppm) and multiplicities are given as follows: singlet = s, doublet = d, triplet = t, multiplet = m, and broad = br. Coupling constants (J) are shown in hertz (Hz). TLC analyses were performed on Kieselgel 60 F_{254} (Merck) and spots were detected under UV irradiation and by heating on a hot plate after spraying 50% surfuric acid reagent.

Isolation Procedure The fruiting bodies (25 g) of *C. versicolor*, collected in Miyagi prefecture in Oct. 1991, were extracted twice with MeOH (150, 100 ml). The combined extracts were concentrated under reduced pressure and then the residue was partitioned between EtOAc (50 ml × 3) and water. The EtOAc-soluble fraction (98 mg) was chromatographed on silica gel, then active fractions (fr. 4) eluted with EtOAc–hexane (2 : 1) were further purified by recrystallization from EtOAc to give hypothemycin (**1**), 4.4 mg as colorless needles. Skyrin[4] was obtained as an orange crystalline compound from the fractions (fr. 2) eluted with EtOAc–hexane (1 : 1), after chromatography over Sephadex LH-20 with $CHCl_3$–MeOH (1 : 1) as the eluent.

Properties of 1 Colorless needles; mp: 169—173 °C; $[\alpha]_{24}^{D}$: −17.3° (c = 0.50, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 308 (4.34), 267 (4.38), 220 (4.73). IR ν_{max}^{KBr} cm^{-1}: 3350, 1690, 1645, 1620, 1580, 1250. These values were in good agreement with the reported data. Electron impact mass spectrum (EI-MS) m/z: 378.1293 (M^+, Calcd for $C_{19}H_{22}O_8$, 378.1315), 360, 180, 179 (base peak) Rf: 0.16 (hexane : EtOAc = 1 : 1), 0.25 ($CHCl_3$: EtOAc = 1 : 1).

Acetylation of Hypothemycin A solution of hypothemycin and dihydrohypothemycin mixture (8.7 mg) in pyridine (100 μl) was treated with Ac_2O (100 μl) and then the mixture was left to stand at room temperature overnight. After concentration of the mixture, the residue was subjected to preparative TLC (TLC plate: Kieselgel 60 F_{254}, 10 cm × 15 cm × 0.5 mm; solvent system: $CHCl_3$: EtOAc = 3 : 1) to give hypothemycin triacetate (**3**, 5.6 mg) and dihydrohypothemycin triacetate (**3′**, 2.3 mg).

Properties of 3 EI-MS m/z: 504 (M^+), 486, 462, 444, 427, 179. IR ν_{max} ($CHCl_3$) cm^{-1}: 1770, 1750, 1720, 1690, 1620, 1370, 1220, 1155. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_{ppm}: 7.24 (m, H-8′, overlapped with solvent peak), 6.76 (d, J=2.4 H-3), 6.57 (d, J=2.4, H-5), 6.37 (dd, J=16.1, 1.0, H-7′), 5.96 (d, J=2.4, H-5′), 5.69 (ddd, J=8.8, 2.4, 2.2, H-4′), 5.61 (m, H-10′), 3.78 (s, 4-OCH_3), 3.52 (d, J=2.0, H-1′), 3.01 (ddd, J=7.3, 4.0, 2.0, H-2′), 2.75 (dddd, J=16.1, 4.9, 2.0, 2.0, H-9′a), 2.58 (ddd, J=16.1, 8.8, 8.8, H-9′b), 2.46 (ddd, J=15.1, 8.8, 4.0, H-3′a), 2.27, 2.20, 2.09 (each s, $OCOCH_3$), 1.44 (d, J=6.3, 10′-CH_3), 1.18 (ddd, J=15.1, 9.3, 1.0, H-3′b).

Properties of 3′ EI-MS m/z: 506 (M^+), 464, 429, 355, 225, 180; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_{ppm}: 6.84 (d, J=2.6, H-3), 6.55 (d, J=2.6, H-5), 5.72 (dd, J=10.0, 1.3, H-4′), 5.44 (d, J=1.3, H-5), 5.28 (m, H-10′), 3.80 (s, 4-OCH_3), 3.67 (d, J=2.0, H-1′), 3.43 (m, H-7′a), 2.96 (ddd, J=10.4, 2.0, 2.0, H-2′), 2.53 (m, H-3′a), 2.35 (m, H-7′b), 2.30, 2.22 (m, H-8′a, H-8′b), 2.18 (m, H-3′b), 2.26, 2.17, 2.10 (each s, $OCOCH_3$), 1.60, 1.65 (m, H-9′a, H-9′b), 1.37 (d, J=6.2, 10′-CH_3).

X-Ray Structure Determination of 1 Crystal data for **1** are as follows: Molecular formula = $C_{19}H_{22}O_8$, Molecular weight = 378.13, orthorhombic space group $P2_12_12_1$, a=8.729(2), b=39.954(5), c=5.255(1) Å, V=1832.7 Å^3, Z=4, D_{calc}=1371 g/cm^3. In total, 2585 independent reflections within 2θ=124° were measured on a Rigaku AFC5R diffractometer using monochromated Cu$K\alpha$ radiation (λ=1.5409 Å). Lorenz and polarization corrections, and an empirical absorption correction were applied. The structure was solved by the direct method and refined by full-matrix least-squares method anisotropically for non-hydrogen atoms and isotripically for hydrogen atoms which were located on the D-map. The final R factor based on the absolute structure shown in Fig. 2 was 0.039 (R_W=0.047) for 2207 reflections with $I_0 > 3\sigma(I_0)$. The corresponding R factor for the enantiomer was 0.040 (R_W=0.048), showing no significant difference. We remeasured the intensities of Bijvoet pairs of reflections to examine the absolute configuration using anomalous dispersion of oxygen atoms. All eight equivalent pairs of 256 reflections with a large dispersion effect ($F_O > 5\sigma(F_O)$ and $\Delta F_C/F_C > 0.3\%$) were carefully measured. The 11 reflections with $F_O > 30.0$ all gave correct signs of equality. However, futher tests for the lower intensities gave consistency of only 78%, 71% and 68% for 59, 170 and 210 reflections with $F_O > 20.0$, 15.0, 10.0, respectively. Therefore, we concluded that the absolute configuration of the five asymmetric centers of **1** is probably, though not certainly, was the 1′*R*, 2′*R*,4′*S*, 5′*S* and 10′*S*, as shown in Fig. 2.

Acknowledgement We thank Mr. K. Kawamura and Miss M. Inada for the MS measurements and Miss K. Mushiake for the NMR spectral measurements. We are grateful to Fuji Chemical Industry Co. Ltd. for the biological assays. This work was supported by a Grant-in-Aid (01470135) for Scientific Research from the Ministry of Education, Science and Culture, Japan.

References and Notes

1) Cytotoxic activities (IC_{50}) of **1** *in vitro* are as follows: P388, 1.20 μg/ml; L1210, 0.634 μg/ml; Colon 26, 0.252 μg/ml; A549, 1.50 μg/ml; DLD-1, 0.826 μg/ml. For previously reported antibiotic activity of hypothemycin, see references 2.

2) *a*) M. S. R. Nair and S. T. Carey, *Tetrahedron Lett.*, **21**, 2011 (1980); *b*) M. S. R. Nair, S. T. Carey, and J. C. James, *Tetrahedron*, **37**, 2445 (1981).

3) For a review: S. Omura, "Macrolide Antibiotics; Chemistry, Biology, and Practice," Academic Press, New York, 1984, Chapter 13. See also the following references for the (10′*S*)-6′-oxofamily of this class: *a*) Zearalenone, W. H. Urry, H. L. Wehrmeister, E. B. Hodge, and P. H. Hidy, *Tetrahedron Lett.*, **1966**, 3109; *b*) Co-metabolites of zearalenone, G. Bolliger and C. Tamm, *Helv. Chim. Acta*, **55**, 3030 (1972); *c*) Antibiotic LL-Z1640-1, 2, G. A. Ellestad, F. M. Lovell, Perkinson, R. T. Hargreaves, and W. J. McGahren, *J. Org. Chem.*, **43**, 2339 (1978); *d*) Recently, LL-Z1640-1 has been isolated from the plant pathogenic fungus, *Drechslara Portulacae*. F. Sugawara, K.-w. Kim. K. Kobayashi, J. Uzawa, S. Yoshida, N. Murofushi, N. Takahashi, and G. A. Strobel, *Phytochemistry*, **31**, 1978 (1992); (10′*S*)-6′-Hydroxy-family: *e*) Zeaenol, M. Nukina, T. Sassa, and S. Marumo, 21st Tennen Yuki Kagoubutsu Toronkai Koen Yoshishu, 1978, p. 144; *f*) Antibiotic LL-Z1640-3, 4, ref. 3*c*; (10′*R*)-2′-Oxofamily: *g*) Radicicol R. N. Mirrington, E. Ritchie, C. W. Shoppee, W. C. Taylor, and S. Sternhell, *Tetrahedron Lett.*, **1964**, 365; *h*) Radicicol was found to be identical with monorden. F. McCapra, A. I. Scott, P. Delmotte, J. Delmotte-Plaquee, and N. S.

Bhacca, *ibid.*, **1964**, 869; *i*) X-Ray analysis of monorden H. G. Culter, R. F. Arrendale, J. P. Springer, P. D. Cole, R. G. Roberts, and R. T. Hanlin, *Agric. Biol. Chem.*, **51**, 3331 (1987); *j*) Monocillins I-V, W. A. Ayer, S. P. Lee, A. Tsuneda, and Y. Hiratsuka, *Can. J. Microbiol.*, **26**, 766 (1980); *k*) Nordinone and nordinonediol, W. A. Ayer and L. Pena-Rodriguez, *Phytochemistry*, **26**, 1353 (1987).

4) This macrolide might be produced by *H. trichothecoides* parasitical on *C. versicolor*, since we also isolated skyrin from the same source. S. T. Carey and M. S. R. Nair, *Lloydia*, **38**, 357 (1975).