Isolation, Structure Determination, and Biological Properties of Cyclothiazomycins B1 and B2

Takanori Murakami, Takatsugu Kimura Toshikatsu Okuno, Hyouta Himeno, Masaru Hashimoto  (Hirosaki University)

Cyclothiazymycin B1:
DHHA1 = (Z)-isomer

Cyclothiazymycin B2:
DHHA1 = (E)-isomer
Isolation of Cyclothiazomycin B1 and B2

Streptomyces sp. A307 strain culture broth (4.8 L)

1) centrifuge
2) MeOH extracts residue

1) remove MeOH
2) AcOEt extracts residue

Myceal cake MeOH supernatant residue

MeOH-CHCl₃ (1:1) supernatant (150 mg) residue

Sep-Pak ODS (CH₃CN:H₂O) active fraction (eluted with 80:20) ODS HPLC

Cyclothiazomycin B2 (3.0 mg)
Cyclothiazomycin B1 (20 mg)

¹H-NMR spectra (pyridine-d₅ + D₂O)
Cyclothiazomycin B2
Cyclothiazomycin B1
Properties of Cyclothiazomycin B1 ($C_{61}H_{69}N_{21}S_{7}O_{13}$)

**ESIMS**
(aq.CH$_3$CN-NaCl) $m/z = 1528$ [M+H]$^+$, 1550 [M+Na]$^+$

**ESIMS**
(CH$_3$CN-D$_2$O) $m/z = 1545$ [M'+D]$^+$
(suggesting 16 exchangeable protons)

**HRESIMS** $m/z = 1528.3448$ (many candidates)

**COSY and HSQC** 53 non-exchangeable protons

**$^{13}$C-NMR and HSQC** 61 carbons (60 by 1D-NMR)

**UV (PDA)** 271, 300, 315 nm

**IR (KBr)** 3400, 1675, 1516 cm$^{-1}$

**Amino acid analysis**
\{ Gly$\times$1, Arg$\times$1, Pro$\times$1, Asx$\times$1, Cys, (not quantitative) \}

**Suggested hetero atom**
\{ 21 nitrogens (due to nitrogen rule), 7 sulfurs \}

**Hydrolysates**

![Saramycetic acid](image)
FGHSQC (pyridine-\textit{d}_5-D_2O)

Chemical Shift Alteration in $^{13}$C-NMR by D$_2$O

pyridine-\textit{d}_5/D$_2$O (3:1)
pyridine-\textit{d}_5/H$_2$O (3:1)
overlay
$^1$H, $^{13}$C Chemical Shifts

ROESY and HMBC

HMBC

ROE (strong)

ROE (weak)
MALDI-TOF/TOF Analysis of Cyclothiazomycin B1

The last nitrogen should be carboxylic acid.
Stereochemistry of Amino Acids

Marfey's regent (FDAA)

acidic hydrolysate

aq. NaHCO₃
40°C, 2 hr

UV (330 nm)

Ion chromatography (LC-ESIMS)

L-Cystine
LL : DL = 5:1
(L-Cys : D-Cys = 10 : 1)

meso-Cystine

Racemate!
Cyclothiazomycin B1
Structure of Cyclothiazomycins B2

HPLC analysis in Pyridine-$d_5$
- 0 hr
- 80 hr
- 200 hr

Slowly isomerized into cyclothiazomycin B1

$^1$H-NMR spectra of Cyclothiaomycins (in DMSO-$d_6$)

cyclothiazomycin B2

cyclothiazomycin B1
Distribution of the chemical shift alterations

Possibilities

1. Conformational isomer?
   δ alteration should be observed over the molecule

2. Diastereomer due to the hemithioacetal?
   It hardly occurs by entropic factor.

3. Diastereomer about the DHHA moieties?
   Most plausible. But DHHA2 in both are Z-configuration on the basis of ROEs.

Diastereomer of DHHA1!!

Chemical shift alteration localizes around DHHA1.

Consideration by Molecular Orbital Calculation (HF631G*)

(Z)-isomer 5.93 ppm  
(E)-isomer 6.56 ppm

Cyclothiazomycin B1  
Cyclothiazomycin B2
Cyclothiazomycin B1  
Cyclothiazomycin B2
Inhibition of DNA-Dependent RNA Synthesis

RNA polymerase
DNA
mRNA

Inhibition of DNA-dependent RNA synthesis is employed using thiostrepton and cyclothiazomycin B1.

Transcription of DNA for dihydrofolate reductase

Transcription

RNA polymerase
DNA
mRNA

Cyclothiazomycin B1

Translation on ribosome

thiostrepton

protein
(dihydrofolate reductase)