Thiazohalostatin is a new cytoprotective substance produced by Actinomadura sp. HQ24. Its structure was elucidated as shown in Fig. 1 by NMR spectral analyses and chemical modifications. Thiazohalostatin was found to possess a novel skeleton containing trichloropyrrole and thiazoline ring moieties.

In the screening for new cytoprotective substances, Actinomadura sp. HQ24 was found to produce novel substances named thiazohalostatin. In the preceding paper\(^1\), we described the fermentation, isolation and biological properties of thiazohalostatin. This paper describes the physico-chemical properties and structural studies of thiazohalostatin.

Thiazohalostatin (I) is a colorless powder with mp 67~69°C. The molecular formula of I was determined as C\(_{20}\)H\(_{25}\)N\(_2\)O\(_4\)SCl\(_3\) by HRFAB-MS ((M+H)+ m/z calcd: 495.0747, found: 495.0713) and elemental analyses (Table 1). The IR spectrum of I had broad absorption bands at 1720 (sh), 1640 (sh)

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Fig. 1. The structures of thiazohalostatin and its derivatives.
and 1590 cm\(^{-1}\), indicating the presence of a carbonyl group and an enolized carbonyl function.

The \(^1\)H NMR spectrum showed extremely broad lines in CDCl\(_3\) due to the tautomeration of 1, but the spectrum of 1 in pyridine-\(d_5\) (Fig. 2) showed 14 signals clearly, which could be attributed to one tertiary methyl group, three doublet methyl groups, three methylene groups, three \(sp^3\) methine groups and one olefinic methine group. The \(^13\)C NMR spectrum of 1 gave 20 carbon signals, which were assigned to four methyl, three methylene, four methine, and 9 quaternary carbons by a DEPT experiment. The \(^13\)C and \(^1\)H NMR spectral data of 1 are summarized as shown in Table 2.

The following units A, B, C and D (Fig. 3) as partial structures of 1 were elucidated by the analysis of \(^1\)H and \(^13\)C NMR data including 2D NMR.

A \(^1\)H-\(^1\)H COSY experiment showed alkyl proton spin networks representing unit A as shown in Fig. 3.

Methylation of 1 with CH\(_3\)I in the presence of NaH gave a trimethyl derivative (2) (Fig. 1). In the \(^1\)H NMR spectrum of 2, a methoxy signal due to a methoxycarbonyl group was observed at \(\delta_H 3.78\) (OCH\(_3\)). \(^1\)H-\(^13\)C long range coupling from the methoxy protons to the carbonyl carbon (C-18, \(\delta_C 173.9\)) in the HMBC spectrum of 2\(^2\)) indicated the presence of a carboxylic acid residue in 1. In the HMBC spectrum of 1, \(^1\)H-\(^13\)C long range correlations were observed from 16-H (\(\delta_H 3.36\) and 4.02) to C-14 (\(\delta_C 182.5\)), C-17 (\(\delta_C 186.5\)), C-22 (\(\delta_C 23.1\)) and C-18 (\(\delta_C 178.1\)) and from 22-H (\(\delta_H 1.68\)) to C-16, C-17 and C-18 (Fig. 3), thereby showing that the 4-carboxy-4-methyl-2-thiazoline ring was comprised of C-14 to C-17, C-18 and C-22. The \(^13\)C NMR chemical shifts of this moiety were in good agreement with those of ferrithiocin\(^3\). Based on these results, the structure of unit B was established.

The \(^1\)H NMR spectrum of 2 showed a methine signal 7-H (\(\delta_H 4.90\)) as a quartet which was coupled with a newly observed doublet methyl signal 7-CH\(_3\) (\(\delta_H 1.39\)), and comparison of the \(^13\)C NMR data for 1 and 2 revealed downfield shifts of C-6 (\(\delta_C 174.3\) v.s. \(\delta_C 188.0\)) and C-8 (\(\delta_C 199.6\) v.s. \(\delta_C 210.1\)). In addition, the long range coupling of 2 from 7-CH\(_3\) to C-6 and C-8 and from 7-H to C-6 and C-8 indicated that 2 contains a 2-methyl-1,3-propanedione moiety consisting of C-6 to C-8 and 7-CH\(_3\). Therefore, the existence of the enol form of the 1,3-propanedione moiety in 1 was confirmed (Fig. 3, unit C).

### Table 1. Physico-chemical properties of thiazohalostatin.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Colorless powder</td>
</tr>
<tr>
<td>MP (dec)</td>
<td>67 ~ 69°C</td>
</tr>
<tr>
<td>([\alpha]_D^2)</td>
<td>(-122^\circ) (c 1.0, MeOH)</td>
</tr>
<tr>
<td>Molecular formula</td>
<td>C(<em>{20})H(</em>{25})N(_2)O(_4)SCl(_3)</td>
</tr>
<tr>
<td>HRFAB-MS</td>
<td>Calcd: 495.0747</td>
</tr>
<tr>
<td></td>
<td>Found: 495.0713 ((M + H)^+)</td>
</tr>
<tr>
<td>Analysis (%)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>48.58 48.95</td>
</tr>
<tr>
<td>H</td>
<td>5.09 5.22</td>
</tr>
<tr>
<td>N</td>
<td>5.67 5.38</td>
</tr>
<tr>
<td>O</td>
<td>12.95 13.17</td>
</tr>
<tr>
<td>S</td>
<td>6.47 6.32</td>
</tr>
<tr>
<td>Cl</td>
<td>21.23 20.91</td>
</tr>
<tr>
<td>UV (\lambda_{max}) nm ((\varepsilon))</td>
<td>252 (7,650), 275 (5,110), 287 (4,690), 345 (33,470), 360 (28,920)</td>
</tr>
<tr>
<td>(in MeOH)</td>
<td></td>
</tr>
<tr>
<td>IR v (KBr) cm(^{-1})</td>
<td>3421, 2960, 2930, 1720 (sh), 1640 (sh), 1590, 1540, 1500, 1477, 1454, 1439, 1417, 1012</td>
</tr>
</tbody>
</table>

Fig. 2. 500 MHz \(^1\)H NMR spectrum of thiazohalostatin in pyridine-\(d_5\).
Table 2. 125 MHz $^{13}$C NMR and 500 MHz $^1$H NMR spectral data of thiazohalostatin (1)a, trimethylthiazohalostatin (2)b and tribromo analog of thiazohalostatin (3)c.

<table>
<thead>
<tr>
<th>Position</th>
<th>$\delta_C$ (ppm)</th>
<th>$\delta_H$ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>116.2 (s)</td>
<td>124.1 (s)$^a$</td>
</tr>
<tr>
<td>3</td>
<td>112.0 (s)$^c$</td>
<td>110.8 (s)$^e$</td>
</tr>
<tr>
<td>4</td>
<td>110.0 (s)$^e$</td>
<td>117.3 (s)$^e$</td>
</tr>
<tr>
<td>5</td>
<td>128.3 (s)</td>
<td>125.9 (s)$^e$</td>
</tr>
<tr>
<td>6</td>
<td>174.3 (s)</td>
<td>188.0 (s)</td>
</tr>
<tr>
<td>7</td>
<td>97.8 (d) 6.79 (br s)</td>
<td>55.1 (d) 4.90 (q, 7.0)</td>
</tr>
<tr>
<td>8</td>
<td>199.6 (s)</td>
<td>210.1 (s)</td>
</tr>
<tr>
<td>9</td>
<td>40.1 (d) 2.75 (m)</td>
<td>43.1 (d) 2.66 (m)</td>
</tr>
<tr>
<td>10</td>
<td>35.6 (t) 1.22$^a$, 1.92 (ddd, 1.5, 13.0, 13.5)</td>
<td>39.5 (t) 1.30$^a$</td>
</tr>
<tr>
<td>11</td>
<td>29.3 (d) 1.75 (m)</td>
<td>27.9 (d) 1.50$^b$</td>
</tr>
<tr>
<td>12</td>
<td>42.1 (t) 1.20$^a$, 2.11 (ddd, 1.8, 12.0, 12.0)</td>
<td>43.7 (t) 1.28$^a$, 1.53$^a$</td>
</tr>
<tr>
<td>13</td>
<td>35.6 (d) 3.16 (m)</td>
<td>37.0 (d) 2.89 (m)</td>
</tr>
<tr>
<td>14</td>
<td>182.5 (s)</td>
<td>177.2 (s)</td>
</tr>
<tr>
<td>15</td>
<td>41.9 (t) 3.36 (d, 11.5), 4.02 (d, 11.5)</td>
<td>40.9 (t) 3.10 (d, 11.2), 3.69 (d, 11.2)</td>
</tr>
<tr>
<td>16</td>
<td>86.5 (s)</td>
<td>83.7 (s)</td>
</tr>
<tr>
<td>17</td>
<td>178.1 (s)</td>
<td>173.9 (s)</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>21.8 (q) 1.29 (d, 6.7)</td>
<td>15.9 (q) 1.03 (d, 6.7)</td>
</tr>
<tr>
<td>20</td>
<td>20.0 (q) 1.10 (d, 6.7)</td>
<td>18.6 (q) 0.84 (d, 6.5)</td>
</tr>
<tr>
<td>21</td>
<td>16.2 (q) 0.67 (d, 6.8)</td>
<td>20.2 (q) 1.17 (d, 6.7)</td>
</tr>
<tr>
<td>22</td>
<td>23.1 (q) 1.68 (s)</td>
<td>23.8 (q) 1.49 (s)</td>
</tr>
</tbody>
</table>

- \( ^1\text{NCH}_3 \)
- \( 7\text{-CH}_3 \)
- \( 18\text{-OCH}_3 \)

a Taken in pyridine-$d_5$.
b 'Taken in CDCl$_3$.
c,d,e,f The assignments may be interchanged.
g,h,i Resonances in one-dimensional spectra obscured by overlapping signals.
Fig. 3. Partial structures of thiazohalostatin and methyl derivative.

The solid-line arrows indicate $^1$H-$^{13}$C long range couplings detected by HMBC experiment.

A

\[ \begin{array}{c}
\text{CH}_3 \\
19 \\
\text{CH}_3 \\
20 \\
\text{CH}_3 \\
21 \\
\text{CH}_3 \\
10 \\
9 \\
\end{array} \]

B

\[ \begin{array}{c}
\text{CH}_3 \\
14 \\
\text{CH}_3 \\
16 \\
\text{CH}_3 \\
18 \\
\text{COOH} \\
1 \\
22 \\
\end{array} \]

The solid-line arrows indicate $^1$H-$^{13}$C long range couplings.

Fig. 4. The connectivities of partial structures.

D

\[ \begin{array}{c}
\text{Cl} \\
7 \\
\text{Cl} \\
8 \\
\text{Cl} \\
3 \\
\text{Cl} \\
2 \\
\text{Cl} \\
1 \\
\text{N} \\
4 \\
\text{H} \\
5 \\
\text{OH} \\
6 \\
\text{O} \\
9 \\
\text{O} \\
10 \\
\text{O} \\
11 \\
\text{O} \\
12 \\
\text{O} \\
13 \\
\text{O} \\
14 \\
\text{O} \\
15 \\
\text{O} \\
16 \\
\text{O} \\
17 \\
\text{O} \\
18 \\
\text{O} \\
19 \\
\text{O} \\
20 \\
\text{O} \\
21 \\
\text{O} \\
22 \\
\end{array} \]

C

\[ \begin{array}{c}
\text{Cl} \\
2 \\
\text{Cl} \\
3 \\
\text{Cl} \\
4 \\
\text{N} \\
5 \\
\text{H} \\
6 \\
\text{O} \\
7 \\
\text{C} \\
8 \\
\text{H} \\
9 \\
\text{C} \\
10 \\
\text{H} \\
11 \\
\text{C} \\
12 \\
\text{H} \\
13 \\
\text{C} \\
14 \\
\text{H} \\
15 \\
\text{C} \\
16 \\
\text{H} \\
17 \\
\text{C} \\
18 \\
\text{H} \\
19 \\
\text{C} \\
20 \\
\text{H} \\
21 \\
\text{C} \\
22 \\
\end{array} \]

D

\[ \begin{array}{c}
\text{Cl} \\
2 \\
\text{Cl} \\
3 \\
\text{Cl} \\
1 \\
\text{N} \\
4 \\
\text{H} \\
5 \\
\text{O} \\
6 \\
\text{C} \\
7 \\
\text{H} \\
8 \\
\text{C} \\
9 \\
\text{H} \\
10 \\
\text{C} \\
11 \\
\text{H} \\
12 \\
\text{C} \\
13 \\
\text{H} \\
14 \\
\text{C} \\
15 \\
\text{H} \\
16 \\
\text{C} \\
17 \\
\text{H} \\
18 \\
\text{C} \\
19 \\
\text{H} \\
20 \\
\text{C} \\
21 \\
\text{H} \\
22 \\
\end{array} \]

The solid-line arrows indicate $^1$H-$^{13}$C long range couplings.

The remaining elements of 1 (four $sp^2$ quaternary carbons, one proton, one nitrogen and three chlorine atoms) suggested the presence of a trichloropyrrole ring, which was substantiated by the characteristic $^{13}$C chemical shifts ($\delta_c$ 110.0, $\delta_c$ 112.0, $\delta_c$ 116.2 and $\delta_c$ 128.3). In order to determine the locations of the chlorine atoms, a tribromo analog (3) was prepared by the addition of KBr$^{+4}$ to the culturing medium of Actinomadura sp. HQ24. The carbon signals of C-2 ($\delta_c$ 116.2), C-3 ($\delta_c$ 112.0) and C-4 ($\delta_c$ 110.0) were assigned to chlorinated $sp^2$ carbons, because the corresponding signals in the $^{13}$C NMR spectrum of 3 showed upfield shifts by 7.7~10.5 ppm$^4$ compared with those in the spectrum of 1 (Table 2) due to the substitution of chlorine atoms with bromine atoms. Furthermore, $^1$H-$^{13}$C long range couplings of 2 were observed from N-CH$_3$ to C-2 ($\delta_c$ 124.1) and C-5 ($\delta_c$ 125.9). These results established a 2,3,4-trichloropyrrole moiety$^{5}$ as represented by unit D in Fig. 3.

The HMBC experiment on 1 also showed the long range couplings of 19-H (CH$_3$) to C-8 and 21-H (CH$_3$) to C-14. Thus, the connectivities of unit B, unit A and unit C were established (Fig. 4). The linkage of unit B and unit D was revealed by the long range coupling relationship between 7-H and C-5 (pyrrole carbon) in the long range selective proton decoupling (LSPD) experiment (Fig. 4). From the results above, the structure of 1 was determined to be 2-[6,8-dioxo-1,3,5-trimethyl-8-(2,3,4-trichloropyrrol-5-yl)-1-octyl]-4-methyl-2-thiazoline-4-carboxylic acid as shown in Fig. 1. Further studies on the stereochemistry and biosynthesis are in progress.

Experimental

General

Optical rotation was obtained on a Jasco DIP-140 spectropolarimeter at 589.6 nm and 22°C. Mass spectra were measured on a VG Analytical ZAB-HF. UV and IR spectra were measured on a VG Analytical ZAB-HF. UV and IR spectra were recorded on a Hitachi U-3200 spectrophotometer and a Jasco A-3 spectrophotometer, respectively. NMR spectra were obtained on a JEOL JNM-GX500 spectrophotometer with $^1$H NMR recorded at 500 MHz and $^{13}$C NMR at 125 MHz. Chemical shifts are given in ppm using TMS as internal standard.
Methylation of Thiazohalostatin

To a stirred solution of 1 (50 mg) and NaH (20 mg) in 3 ml of DMF was added CH$_3$I (70 mg). The mixture was stirred for 1 hour at room temperature. The resulting solution was evaporated in vacuo and chromatographed on a silica gel column (1.5 x 20 cm) eluted with hexane-EtOAc (6:1) to yield 25 mg of 2. FD-MS $m/z$ 534 (M$^+$); $^1$H NMR (CDCl$_3$): see Table 2; $^{13}$C NMR (CDCl$_3$): see Table 2.

Tribromo Analog of Thiazohalostatin (3)

The strain HQ24 was inoculated into 100 ml of seed medium consisting of soluble strach 0.8%, glycerol 0.8%, soy bean meal 0.3%, fish meal 0.8%, CaCO$_3$ 2% and KBr 2% in a 500-ml Erlenmeyer flask, and cultured at 28°C for 5 days on a rotary shaker (180 rpm). The isolation procedures of 3 were essentially the same as described in the preceding paper$^1$. Six mg of 3 was obtained from 1 liter cultured broth: FAB-MS $m/z$ 627 (M+H)$^+$ and 649 (M+Na)$^+$; $^1$H NMR (pyridine-d$_5$): see Table 2; $^{13}$C NMR (pyridine-d$_5$): see Table 2.

References