THIAZOHALOSTATIN, A NEW CYTOPROTECTIVE SUBSTANCE PRODUCED BY *Actinomadura*

II. PHYSICO-CHEMICAL PROPERTIES AND STRUCTURE DETERMINATION

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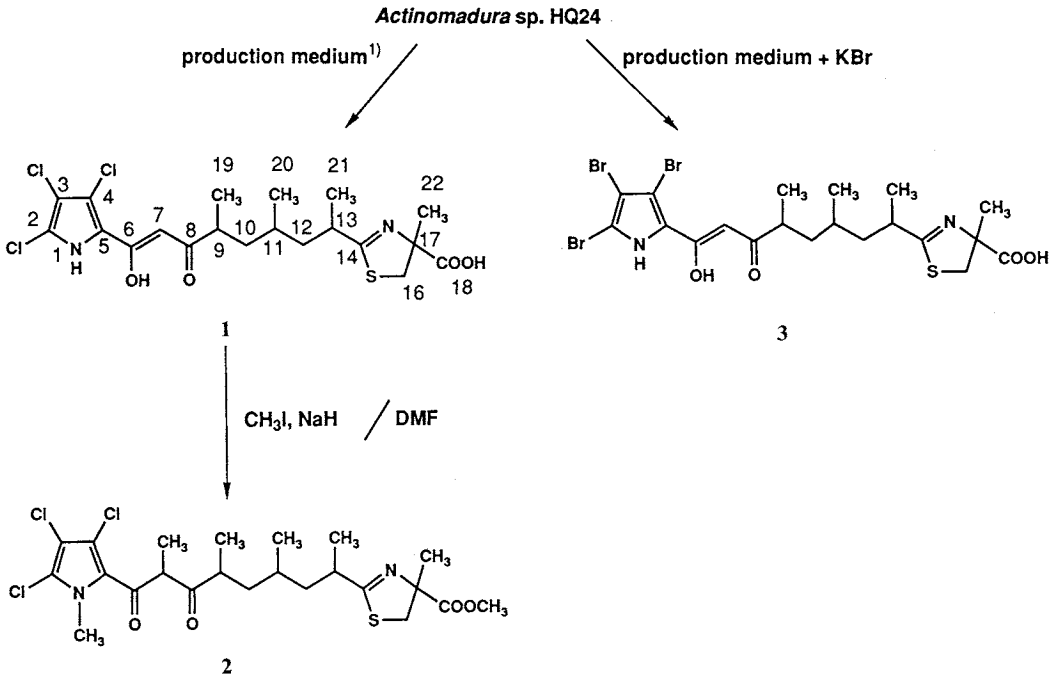
Thiazohalostatin is a new cytoprotective substance produced by *Actinomadura* sp. HQ24. Its structure was elucidated as shown in Fig. 1 by NMR spectral analyses and chemical modifications. Thiazohalostatin was found to possess a novel skeleton containing trichloropyrrole and thiazoline ring moieties.

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 (**1**) is a colorless powder with mp 67~69°C. The molecular formula of **1** was determined as $C_{20}H_{25}N_2O_4SCl_3$ by HRFAB-MS ($(M+H)^+$ *m/z* calcd: 495.0747, found: 495.0713) and elemental analyses (Table 1). The IR spectrum of **1** had broad absorption bands at 1720 (sh), 1640 (sh)

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 ^1H NMR spectrum showed extremely broad lines in $CDCl_3$ due to the tautomerizm 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 ^1H 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 ^1H and ^{13}C NMR data including 2D NMR.

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

Methylation of **1** with CH_3I in the presence of NaH gave a trimethyl derivative (**2**) (Fig. 1). In the ^1H NMR spectrum of **2**, a methoxy signal due to a methoxycarbonyl group was observed at δ_H 3.78 (OCH_3). ^1H-^{13}C long range coupling from the methoxy protons to the carbonyl carbon (C-18, δ_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**, ^1H-^{13}C long range correlations were observed fom 16-H (δ_H 3.36 and 4.02) to C-14 (δ_C 182.5), C-17 (δ_C 86.5), C-22 (δ_C 23.1) and C-18 (δ_C 178.1) and from 22-H (δ_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.

Table 1. Physico-chemical properties of thiazohalostatin.

Appearance	Colorless powder
MP (dec)	67~69°C
$[\alpha]_D^{22}$	−122° (*c* 1.0, MeOH)
Molecular formula	$C_{20}H_{25}N_2O_4SCl_3$
HRFAB-MS Calcd:	495.0747
Found:	495.0713 $(M+H)^+$
Analysis (%)	Calcd: Found:
C	48.58 48.95
H	5.09 5.22
N	5.67 5.38
O	12.95 13.17
S	6.47 6.32
Cl	21.23 20.91
UV λ_{max} nm (ε) (in MeOH)	252 (7,650), 275 (5,110), 287 (4,690), 345 (33,470), 360 (28,920)
IR ν (KBr) cm^{-1}	3421, 2960, 2930, 1720 (sh), 1640 (sh), 1590, 1540, 1500, 1477, 1454, 1439, 1417, 1012

The ^1H NMR spectrum of **2** showed a methine signal 7-H (δ_H 4.90) as a quartet which was coupled with a newly observed doublet methyl signal 7-CH_3 (δ_H 1.39), and comparison of the ^{13}C NMR data for **1** and **2** revealed downfield shifts of C-6 (δ_C 174.3 *v.s.* δ_C 188.0) and C-8 (δ_C 199.6 *v.s.* δ_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).

Fig. 2. 500 MHz ^1H NMR spectrum of thiazohalostatin in pyridine-d_5.

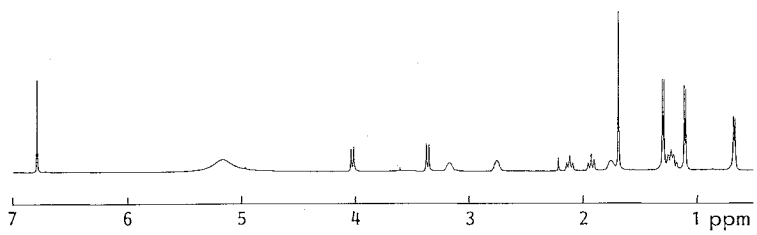


Table 2. 125 MHz ^{13}C NMR and 500 MHz ^{1}H NMR spectral data of thiazohalostatin (**1**)[a], trimethylthiazohalostatin (**2**)[b] and tribromo analog of thiazohalostatin (**3**)[a].

Position	**1**		**2**		**3**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	116.2 (s)		124.1 (s)[d]		105.7 (s)[f]	
3	112.0 (s)[c]		110.8 (s)[e]		104.3 (s)[f]	
4	110.0 (s)[c]		117.3 (s)[e]		101.1 (s)	
5	128.3 (s)		125.9 (s)[d]		133.0 (s)	
6	174.3 (s)		188.0 (s)		174.6 (s)	
7	97.8 (d)	6.79 (br s)	55.1 (d)	4.90 (q, 7.0)	97.5 (d)	6.92 (br s)
8	199.6 (s)		210.1 (s)		199.6 (s)	
9	40.1 (d)	2.75 (m)	43.1 (d)	2.66 (m)	40.1 (d)	2.75 (m)
10	35.6 (t)	1.22[g],	39.5 (t)	1.30[h],	35.6 (t)	1.22[i],
		1.92 (ddd, 1.5, 13.0, 13.5)		1.40[h]		1.94 (ddd, 1.5, 13.0, 13.5)
11	29.3 (d)	1.75 (m)	27.9 (d)	1.50[h]	29.3 (d)	1.75 (m)
12	42.1 (t)	1.20[g],	43.7 (t)	1.28[h],	42.2 (t)	1.20[i],
		2.11 (ddd, 1.8, 12.0, 12.0)		1.53[h]		2.10 (ddd, 1.8, 12.0, 12.0)
13	35.6 (d)	3.16 (m)	37.0 (d)	2.89 (m)	35.7 (t)	3.14 (m)
14	182.5 (s)		177.2 (s)		182.5 (s)	
16	41.9 (t)	3.36 (d, 11.5), 4.02 (d, 11.5)	40.9 (t)	3.10 (d, 11.2), 3.69 (d, 11.2)	42.0 (t)	3.36 (d, 11.5), 4.02 (d, 11.5)
17	86.5 (s)		83.7 (s)		86.5 (s)	
18	178.1 (s)		173.9 (s)		178.1 (s)	
19	21.8 (q)	1.29 (d, 6.7)	15.9 (q)	1.03 (d, 6.7)	21.8 (q)	1.29 (d, 6.7)
20	20.0 (q)	1.10 (d, 6.7)	18.6 (q)	0.84 (d, 6.5)	20.1 (q)	1.12 (d, 6.7)
21	16.2 (q)	0.67 (d, 6.8)	20.2 (q)	1.17 (d, 6.7)	16.2 (q)	0.66 (d, 6.7)
22	23.1 (q)	1.68 (s)	23.8 (q)	1.49 (s)	23.1 (q)	1.68 (s)
1-NCH_3			35.3 (q)	3.86 (s)		
7-CH_3			13.5 (q)	1.39 (d, 7.0)		
18-OCH_3			52.8 (q)	3.78 (s)		

[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.

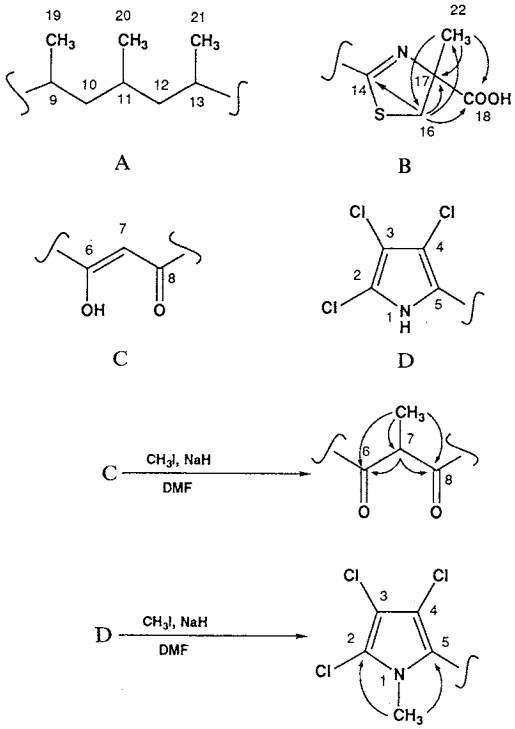
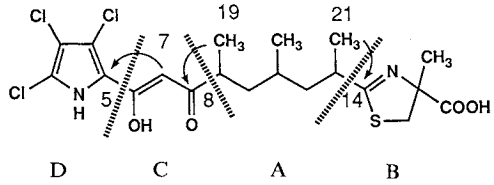


Fig. 4. The connectivities of partial structures.



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 (δ_C 110.0, δ_C 112.0, δ_C 116.2 and δ_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 (δ_C 116.2), C-3 (δ_C 112.0) and C-4 (δ_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 (δ_C 124.1) and C-5 (δ_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_3I (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 × 20 cm) eluted with hexane - EtOAc (6 : 1) to yield 25 mg of **2**. FD-MS *m/z* 534 (M^+); ^1H 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 satrch 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 $(M+Na)^+$; ^1H NMR (pyridine-d_5): see Table 2; ^{13}C NMR (pyridine-d_5): see Table 2.

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