BISUCABERIN, A NEW SIDEROPHORE, SENSITIZING TUMOR CELLS TO MACROPHAGE-MEDIATED CYTOLYSIS

II. PHYSICO-CHEMICAL PROPERTIES AND STRUCTURE DETERMINATION

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The structure of bisucaberin, a new siderophore, was determined to be 1,12-dihydroxy-1,6,12,17-tetraazacyclodocosane-2,5,13,16-tetrone by spectroscopic analysis and X-ray crystallographic analysis. The molecule of bisucaberin consists of a cyclic dimer of 1-hydroxy-1,6-diazaundecane-2,5-dione moiety and is closely related to nocardamine, the trimer of the same moiety.

Bisucaberin, isolated from a culture broth of a marine bacterial strain identified as *Alteromonas haloplanktis* SB-1123, is a new siderophore which sensitizes tumor cells to macrophage-mediated cytolysis. We have reported the fermentation, isolation and biological properties of bisucaberin in the preceding paper[1].

In this report, we describe here the physico-chemical properties and structure determination of bisucaberin.

Results and Discussion

Physico-chemical Properties of Bisucaberin

Bisucaberin (Fig. 1) was obtained as colorless crystals, mp 180°C (dec). It was soluble in dimethyl sulfoxide, slightly soluble in methanol and hardly soluble or insoluble in other organic solvents and water. The UV spectrum of bisucaberin is as follows; UV λ_{max}^{MeOH} nm (ε) 215 (5,740). Bisucaberin was positive to $FeCl_3$ and chlorine - tolidine reactions. On silica gel thin-layer chromatogram, it gave a single spot at Rf 0.52 (chloroform - methanol, 9:1). The molecular formula of bisucaberin was established as $C_{18}H_{32}N_4O_6$ (MW 400) by elemental analysis and secondary ion mass spectrometry (SI-MS), *anal* calcd for $C_{18}H_{32}N_4O_6$: C 54.00, H 8.00, N 14.00; found: C

Fig. 1. Structure of bisucaberin.

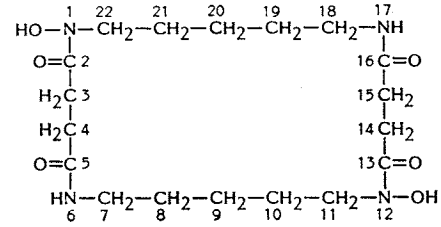
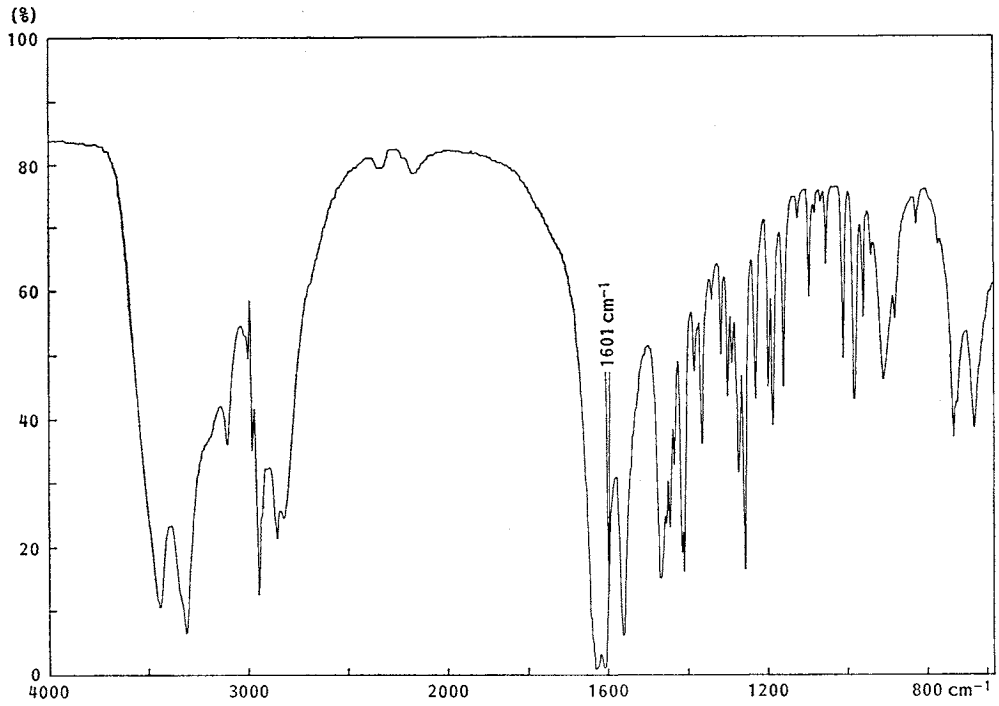


Fig. 2. IR spectrum of bisucaberin (KBr).



53.69, H 7.97, N 12.20; SI-MS *m/z* 401 (MH^+). As shown in Fig. 2, the IR spectrum indicated the presence of the amide bond in the structure.

The ^{13}C NMR spectrum (DMSO-d_6, 100 MHz) of the compound showed only 9 carbon signals at δ 171.8 (C=O), 171.5 (C=O), 46.3 (CH_2), 37.9 (CH_2), 30.5 (CH_2), 28.2 (CH_2), 27.8 (CH_2), 25.5 (CH_2) and 22.5 (CH_2). As shown in Table 1, the ^1H NMR spectral data (400 MHz, DMSO-d_6) indicated the presence of seven methylene groups, one NH group and one OH group. Spin decoupling experiment indicated the presence of two kinds of carbon chain, cadaverine moiety ($NHCH_2CH_2CH_2CH_2CH_2N$) and succinyl moiety ($COCH_2CH_2CO$).

Table 1. ^1H NMR data of bisucaberin in DMSO-d_6 (400 MHz).

Proton	Chemical shift (δ value in ppm) and coupling constants (Hz)
3, 14 or 4, 15	2.27 (t, J=7.4)
4, 15 or 3, 14	2.57 (t, J=7.4)
6, 17 (NH)	7.61 (br t)
7, 18	3.01 (br m)
8, 19	~1.35 (m)
9, 20	~1.15 (m)
10, 21	1.47 (m)
11, 22	3.48 (t, J=6.3)
1, 12 (OH)	9.53 (s)

The positive reaction to $FeCl_3$ indicated the presence of hydroxamate moiety (N(OH)CO) in the structure.

These data suggest that bisucaberin consists of a cyclic dimer of succinyl-(*N*-hydroxycadaverine).

Structure Determination of Bisucaberin by X-Ray Diffraction

The structure of bisucaberin was determined by X-ray crystallographic analysis to be 1,12-dihydroxy-1,6,12,17-tetraazacyclodocosane-2,5,13,16-tetrone (Figs. 1, 3 and 4).

Colorless crystals with thick plate form were grown in a methanol solution. The X-ray specimen

of approximate dimensions $0.25\times0.25\times0.1$ mm was chosen and mounted on a Philips PW-1100 diffractometer. All diffraction measurements were made with CuKα radiation monochromated by a graphite monochrometor. Crystal data are: Bisucaberin $C_{18}H_{32}N_4O_6\cdot2H_2O$. FW=436.5 Monoclinic, space group $P2_1/C$, $Z=2$. Lattice constants: $a=9.321(6)$, $b=13.002(9)$, $c=9.560(6)$ Å, $\beta=111.77(6)°$, $U=1111$ Å^3. $D_{calc}=1.305$ gcm^{-3}, μ for CuK$\alpha=8.19$ cm^{-1}.

Intensities of 1887 reflections out of 2416 possible ones were observed above the 2σ(I) level, within the 2θ range of 6° through 156°. These were measured by the ω-2θ scan method with the scan speed 6° θ sec^{-1}. Scan was repeated twice when the net counts during the first scan were less than twice the standard deviation of the background counts measured at both ends of the scan.

The space group and the volume of the unit cell indicated that the cell contains half the molecule of bisucaberin and this half must be linked to the other half by a center of symmetry. This view was confirmed by the structure determination by the direct method which also revealed the presence of the molecule of hydration water. The structural parameters were refined by the block-diagonal least-squares method to an R value of 0.047. The atomic parameters for 15 heavier atoms in an asymmetric unit are listed in Table 2 along with 18 hydrogen atoms which were located on the difference electron-density map and refined with isotropic temperature factors.

The molecule consists of a cyclic dimer of 1-hydroxy-1,6-diazaundecane-2,5-dione moiety (hereafter abbreviated as diazaundecane moiety) and is closely related to nocardamine[2], the trimer of the same moiety. The molecular structure of bisucaberin is shown in Fig. 3 which was drawn by PLUTO program[3].

Table 3 lists the bond length and valency angles found in the diazaundecane moiety. The values are normal for the types of chemical bonds. The two amide groups involved in a diazaundecane

Fig. 3. Molecular structure viewed nearly perpendicular to the 22 membered ring.
Numbering of the atoms and torsion angles along the main chain are given.

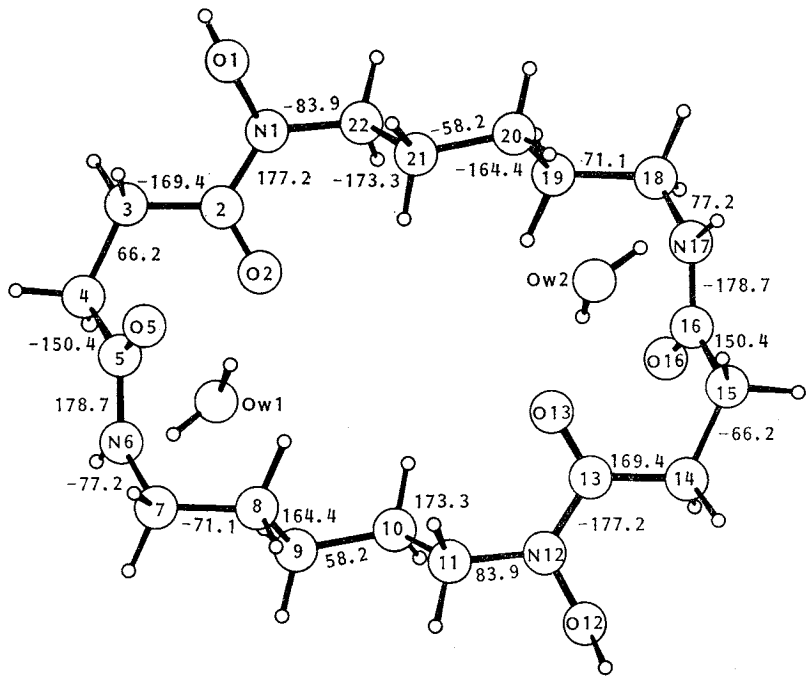


Fig. 4. Molecular structure showing intermolecular hydrogen bond.
Fig. 4 is a birds-eye view of Fig. 3.

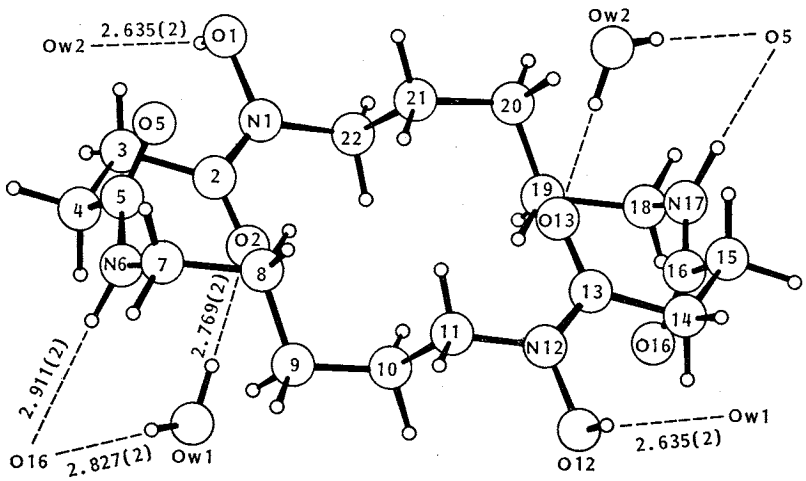


Table 2. The positional parameters and equivalent isotropic thermal parameters with estimated standard deviations in parentheses.

Atom	X (×10⁴)	Y (×10⁴)	Z (×10⁴)	B_{eq} (Å²)	Atom	X (×10³)	Y (×10³)	Z (×10³)	B_{eq} (Å²)
N1	9284 (2)	156 (1)	7058 (2)	3.04 (3)	H (O1)	1134 (3)	31 (2)	844 (3)	6 (1)
C2	8808 (2)	652 (1)	5740 (2)	2.51 (3)	H (C3)	979 (2)	202 (2)	654 (2)	3 (0)
C3	9633 (2)	1624 (1)	5662 (2)	2.73 (3)	H′(C3)	1070 (3)	144 (2)	576 (3)	4 (1)
C4	8847 (2)	2237 (1)	4228 (2)	2.56 (3)	H (C4)	873 (2)	182 (2)	330 (3)	3 (0)
C5	7365 (2)	2661 (1)	4160 (2)	2.36 (3)	H′(C4)	949 (3)	291 (2)	422 (3)	4 (0)
N6	6312 (2)	2786 (1)	2798 (2)	2.66 (2)	H (N6)	657 (3)	263 (2)	192 (3)	4 (0)
C7	4818 (2)	3172 (2)	2590 (2)	3.15 (3)	H (C7)	432 (3)	349 (2)	147 (3)	5 (1)
C8	3841 (2)	2341 (2)	2904 (2)	3.41 (3)	H′(C7)	492 (3)	375 (2)	321 (3)	3 (0)
C9	3350 (3)	1505 (2)	1694 (2)	3.73 (4)	H (C8)	443 (3)	193 (2)	391 (3)	4 (1)
C10	2748 (3)	535 (2)	2188 (3)	3.60 (4)	H′(C8)	284 (3)	277 (2)	300 (3)	4 (1)
C11	1417 (2)	762 (2)	2636 (2)	3.25 (3)	H (C9)	423 (3)	132 (2)	138 (3)	5 (1)
O1	10324 (2)	636 (1)	8329 (1)	3.46 (2)	H′(C9)	267 (3)	181 (2)	73 (3)	5 (1)
O2	7771 (2)	293 (1)	4644 (1)	3.42 (2)	H (C10)	244 (3)	−1 (2)	135 (3)	4 (1)
O5	7162 (2)	2905 (1)	5330 (1)	3.28 (2)	H′(C10)	349 (3)	20 (2)	310 (3)	5 (1)
O(w)	7106 (2)	130 (1)	1576 (2)	3.73 (3)	H (C11)	180 (3)	118 (2)	357 (2)	4 (0)
					H′(C11)	65 (3)	119 (2)	180 (3)	4 (1)
					H (Ow)	720 (3)	26 (2)	255 (3)	4 (1)
					H′(Ow)	700 (3)	71 (2)	110 (3)	5 (1)

moiety are *trans* amide configuration and both have similar dimensions although the imino hydrogen atom of the one amide group, N6H, is replaced in the other amide group by a hydroxyl group O1H, forming a hydroxamic acid residue ($C22H_2$–N1(–O1H)–C2(=O2)–$C3H_2$–). If the bond lengths and angles are compared between the two amide groups, only the largest difference is found in the bond angles subtended at the imino nitrogen atoms. While the angle C5-N6-C7 of the usual amide group is 121.8(2)°, C22-N1-C2 of the hydroxamic acid residue is 124.3(2)°. Substitution of imino hydrogen by hydroxyl group causes the widening of this angle.

Table 3. Bond distances (Å) and angles (°).

Distances		Angles	
N1-C2	1.336 (2)	C2-N1-O1	119.2 (2)
N1-O1	1.402 (2)	C2-N1-C22	124.3 (2)
C2-C3	1.509 (3)	O1-N1-C22	115.3 (2)
C2-O2	1.240 (2)	C3-C2-N1	116.6 (2)
C3-C4	1.523 (2)	C3-C2-O2	123.2 (2)
C4-C5	1.507 (3)	N1-C2-O2	120.2 (2)
C5-N6	1.330 (2)	C4-C3-C2	112.9 (2)
C5-O5	1.246 (2)	C5-C4-C3	112.1 (2)
N6-C7	1.465 (3)	N6-C5-C4	116.8 (2)
C7-C8	1.533 (3)	N6-C5-O5	122.1 (2)
C8-C9	1.529 (3)	C4-C5-O5	121.0 (2)
C9-C10	1.534 (3)	C7-N6-C5	121.8 (2)
C10-C11	1.524 (4)	C8-C7-N6	112.0 (2)
N1-C22	1.460 (3)	C9-C8-C7	113.1 (2)
		C10-C9-C8	113.4 (2)
		C11-C10-C9	112.0 (2)
		N12-C11-C10	112.9 (2)

Table 4. Hydrogen bond distances.

X (i*)-H (i)·····Y	X·····Y	H·····Y	<Y-X-H
Ow1-H ·····O2 (i)	2.769 (2) Å	1.87 (3) Å	9.3°
Ow1-H′·····O16 (ii)	2.827 (2)	1.97 (3)	8.2
O1-H ·····Ow2 (iii)	2.635 (2)	1.61 (3)	4.2
N6-H ·····O16 (ii)	2.911 (2)	1.94 (3)	6.0

* (i) x, y, z. (ii) $1-x, (1/2)+y, (1/2)-z$. (iii) $1+x, y, z$.

Though the amide groups take a *trans* planar configuration, the torsional angles about the NCH_2 bonds immediately linked to the amide nitrogen atoms adopt a gauche conformation. Some methylene-methylene bonds also adopt a gauche form resulting in the formation of a macrocyclic 22 membered ring.

The conformation along the main chain bonds can be described roughly as, $\bar{g}ttgtt\bar{g}\bar{g}tgt\ gtt\bar{g}ttggt\bar{g}t$ starting at N1-C22 (Fig. 3), where g, $\bar{g}$ and t denote gauche, minus gauche and *trans* conformation, respectively.

Fig. 4 shows the role of the hydration water molecules. This molecule is situated near at the hydrophilic corner of the ring and is separated by some distance from the plane. Thus it forms three hydrogen bonds to the carbonyl and hydroxyl oxygen atoms of the neighboring rings. Table 4 lists the hydrogen bond distances found in the present crystal structure. The molecules are linked together through these intermolecular hydrogen bonds.

Experimental

Melting point was determined with a Yazawa melting point apparatus and was uncorrected. UV spectrum and IR spectrum were recorded with a Hitachi 220S spectrophotometer and a Hitachi 260-10 IR spectrophotometer, respectively. The ^1H and ^{13}C NMR spectra were measured with a JEOL JNM-GX400 spectrometer. TLC was performed on a silica gel (Kieselgel 60 F_{254}, Merck) developed with a mixture of $CHCl_3$ - MeOH (9 : 1).

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