STRUCTURE OF APLASMOMYCIN

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A new antibiotic, aplasmomycin, was isolated from a broth cultivated with a marine isolate of actinomycete, and inhibits Gram-positive bacteria *in vitro* and *Plasmodium berghei in vivo*. It is a natural ionophore and the structure of the Ag-salt was solved by an X-ray crystallographic analysis. It has a symmetric structure having boron in the centre of the molecule.

OKAMI *et al.*[1] have directed a search for biologically active substances from marine microorganisms. In the course of their screening, they isolated from a marine habitat of an actinomycete, *Chainia*, a new substance named SS-228Y[1,2] which has antibacterial activity and anti-malignant activity as well as inhibitory activity against dopamine-β-hydroxylase. This active principle was produced only in a medium containing Kobu-cha (powdered tangle sea weed, *Laminarium*). Another marine isolate belonging to *Streptomyces griseus* was found to produce a new active principle[3] only in diluted medium or Kobu-cha medium. The strain was named SS-20 and the active principle was extracted from the culture broth by organic solvents such as *n*-butyl acetate. It had inhibitory activity against Gram-positive bacteria *in vitro* and plasmodium *in vivo* when it was administered orally to mice infected with *Plasmodium berghei*. Because of its anti-plasmodium activity, it was named aplasmomycin. Further studies [flame reaction with alcohol (green color), color reaction with carminic acid-sulfuric acid (red color) and colorimetric determination with BF_4^- - methylene blue[4]] indicated that the antibiotic contained boron. The presence of boron was further confirmed by comparison with the derivative of triethanolamine-boric acid complex and the authentic derivative from boric acid.[5] The molecular formula of aplasmomycin is $C_{40}H_{60}O_{14}BNa$ instead of the formula presented formerly, $C_{41}H_{60}O_{14}Na$[3]. The result of elemental analysis is as follows: calcd. for $C_{40}H_{60}O_{14}BNa$ (mw 798.7); C 60.15, H 7.52, O 28.07, B 1.38, Na 2.88, found; C 59.87, H 7.79, O 28.14 (by diff.), B 1.44, Na 2.76. The mass spectrometric ion peak at *m/e* 798 shows a molecular ion for aplasmomycin. The silver salt of aplasmomycin, thus, is $C_{40}H_{60}O_{14}BAg$ (mw 882.9): calcd. C 54.42, H 6.85, B 1.23, found; C 54.15, H 7.06, B 1.30.

Table 1. Crystal data

$C_{40}H_{60}O_{14}BAg \cdot 2H_2O$	
F.W.	919.6
$P2_12_12_1$	
a	=18.572 (8) Å
b	=21.246 (9)
c	=11.595 (5)
U	=4575.2 Å^3
Z	=4
D_x	=1.336 gcm^{-3}

Crystals of silver salt of aplasmomycin were grown from an aqueous methanol solution as colorless hexagonal thick plates. Lattice constants and intensity data were obtained by a Phillips four-circle X-ray diffractometer with graphite-monochromated CuKα radiation

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Table 2. Final atomic parameters ($\times 10^4$).

The temperature factors are of the form: $T=\exp[-(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+2\beta_{12}hk+2\beta_{13}hl+2\beta_{23}kl)]$.

	X	Y	Z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
Ag	4053 (2)	1434 (2)	1775 (3)	54 (1)	50 (1)	152 (3)	28 (1)	−8 (2)	−20 (2)
B	4630 (13)	1699 (12)	4392 (28)	9 (8)	26 (7)	91 (30)	−7 (6)	−17 (14)	−22 (14)
C (1)	3934 (11)	73 (10)	4119 (21)	9 (7)	15 (6)	66 (24)	−7 (5)	3 (13)	1 (11)
C (2)	4187 (13)	637 (9)	4702 (24)	24 (8)	5 (5)	80 (29)	−4 (5)	11 (15)	4 (11)
C (3)	5012 (12)	676 (9)	4852 (26)	17 (8)	9 (5)	119 (33)	6 (5)	−31 (16)	−12 (12)
C (4)	5342 (13)	259 (11)	5889 (22)	25 (9)	14 (6)	50 (25)	−7 (6)	−1 (14)	6 (11)
C (5)	6148 (14)	332 (13)	5804 (25)	24 (10)	32 (8)	78 (29)	0 (7)	−22 (16)	24 (14)
C (6)	6403 (15)	170 (14)	4551 (33)	24 (10)	38 (9)	152 (44)	−5 (9)	−12 (20)	−30 (19)
C (7)	6085 (14)	591 (11)	3710 (26)	11 (9)	16 (7)	121 (29)	6 (6)	−9 (15)	25 (12)
C (8)	6268 (13)	505 (10)	2391 (24)	23 (9)	13 (6)	77 (26)	8 (6)	−6 (15)	−6 (11)
C (9)	5795 (13)	910 (12)	1664 (24)	26 (9)	19 (7)	60 (26)	−8 (6)	18 (15)	2 (13)
C (10)	6018 (17)	982 (15)	420 (29)	41 (12)	35 (9)	115 (37)	−9 (9)	−15 (22)	1 (17)
C (11)	5552 (22)	1471 (16)	−293 (33)	82 (19)	38 (10)	126 (42)	−25 (12)	24 (25)	−14 (18)
C (12)	5516 (23)	2091 (18)	61 (31)	82 (21)	78 (14)	106 (31)	19 (15)	−32 (23)	10 (19)
C (13)	5192 (16)	2631 (17)	−717 (27)	30 (11)	56 (12)	51 (29)	16 (10)	7 (18)	26 (17)
C (14)	5242 (18)	3243 (12)	38 (25)	51 (13)	24 (8)	53 (25)	−1 (9)	20 (18)	−3 (13)
C (15)	4566 (16)	3215 (11)	690 (23)	44 (11)	20 (7)	29 (23)	5 (7)	8 (15)	2 (12)
C (16)	3976 (16)	2933 (12)	−58 (23)	47 (12)	22 (7)	48 (23)	−7 (8)	8 (17)	−6 (12)
C (17)	3577 (19)	3346 (19)	−796 (27)	53 (16)	72 (15)	53 (28)	22 (14)	−42 (20)	0 (19)
C (18)	5059 (19)	425 (14)	7036 (26)	67 (15)	33 (9)	65 (32)	2 (10)	−36 (20)	27 (16)
C (19)	6147 (14)	−202 (12)	1975 (29)	35 (10)	16 (7)	146 (38)	−6 (7)	37 (19)	−17 (15)
C (20)	7066 (12)	647 (13)	2251 (27)	12 (8)	37 (9)	110 (36)	−9 (7)	26 (15)	1 (16)
O (1)	3902 (9)	−439 (7)	4616 (16)	30 (6)	14 (4)	107 (20)	−8 (4)	18 (11)	−5 (8)
O (2)	4035 (7)	1207 (6)	4096 (13)	20 (5)	19 (4)	78 (15)	4 (4)	25 (9)	−4 (7)
O (3)	5128 (7)	1305 (6)	5053 (12)	23 (5)	17 (3)	37 (12)	6 (3)	−12 (7)	2 (6)
O (4)	5287 (8)	477 (6)	3771 (14)	18 (5)	11 (4)	58 (15)	4 (4)	9 (8)	−14 (6)
O (5)	5039 (9)	709 (7)	1656 (16)	32 (6)	14 (4)	77 (18)	−4 (4)	0 (11)	0 (7)
O (6)	4413 (11)	2493 (10)	−855 (19)	41 (8)	37 (6)	87 (22)	−2 (6)	−10 (13)	−10 (11)
O (7)	4652 (8)	2823 (7)	1738 (15)	27 (6)	19 (4)	42 (15)	−2 (4)	13 (10)	0 (8)
C (1′)	4955 (14)	3037 (11)	2651 (20)	30 (9)	21 (7)	13 (19)	−7 (7)	−15 (13)	−4 (10)
C (2′)	4981 (12)	2635 (12)	3674 (22)	12 (8)	27 (8)	55 (25)	12 (6)	14 (13)	−2 (12)
C (3′)	4337 (12)	2768 (10)	4565 (23)	21 (8)	10 (6)	74 (27)	−3 (6)	16 (14)	−6 (12)
C (4′)	4443 (14)	3346 (10)	5317 (22)	31 (10)	17 (6)	44 (25)	−1 (6)	5 (14)	−6 (11)
C (5′)	3693 (15)	3468 (13)	5946 (25)	37 (10)	26 (7)	76 (30)	13 (8)	20 (17)	0 (14)
C (6′)	3046 (14)	3463 (11)	5148 (24)	26 (9)	21 (7)	82 (27)	−1 (8)	9 (15)	−6 (12)
C (7′)	3058 (12)	2884 (10)	4445 (23)	8 (7)	16 (6)	78 (28)	4 (6)	18 (14)	4 (12)
C (8′)	2435 (12)	2789 (11)	3512 (21)	12 (7)	27 (7)	36 (23)	8 (6)	6 (12)	21 (11)
C (9′)	2549 (12)	2162 (10)	2951 (20)	13 (7)	12 (6)	56 (23)	0 (5)	9 (13)	−17 (10)
C (10′)	1955 (13)	2005 (11)	2179 (26)	23 (8)	15 (6)	101 (32)	14 (6)	6 (16)	−2 (13)
C (11′)	1965 (16)	1302 (19)	1740 (39)	24 (10)	67 (17)	178 (54)	7 (11)	−33 (24)	51 (30)
C (12′)	2406 (15)	840 (12)	2207 (24)	34 (10)	14 (7)	63 (28)	−11 (7)	8 (15)	−6 (12)
C (13′)	2379 (16)	159 (16)	1756 (29)	35 (11)	52 (12)	89 (35)	−1 (9)	−19 (21)	−8 (19)
C (14′)	2601 (14)	−369 (11)	2617 (27)	22 (9)	19 (7)	120 (34)	1 (7)	−30 (17)	−4 (14)
C (15′)	3416 (14)	−412 (13)	2423 (27)	19 (9)	29 (8)	84 (30)	−1 (7)	−18 (15)	−1 (14)
C (16′)	3552 (18)	−272 (14)	1121 (27)	63 (15)	26 (8)	73 (30)	8 (10)	−30 (20)	−21 (14)
C (17′)	3455 (20)	−886 (13)	416 (31)	67 (17)	28 (8)	119 (39)	25 (10)	−22 (23)	−42 (16)
C (18′)	5081 (16)	3275 (12)	6206 (26)	43 (12)	25 (7)	78 (27)	3 (8)	−35 (18)	−14 (13)
C (19′)	2461 (15)	3345 (11)	2660 (27)	32 (10)	14 (7)	115 (33)	7 (7)	−2 (17)	−10 (13)
C (20′)	1687 (14)	2776 (13)	4202 (29)	14 (9)	35 (9)	112 (35)	2 (7)	24 (17)	0 (17)
O (1′)	5127 (9)	3578 (9)	2701 (15)	26 (6)	30 (5)	61 (17)	−6 (5)	−2 (9)	15 (9)
O (2′)	4872 (7)	1987 (5)	3395 (12)	23 (5)	8 (3)	57 (14)	−1 (3)	14 (8)	−8 (6)
O (3′)	4323 (7)	2193 (6)	5156 (13)	22 (5)	12 (3)	70 (14)	−1 (3)	1 (8)	−3 (7)
O (4′)	3714 (7)	2860 (6)	3824 (13)	9 (4)	12 (3)	49 (14)	−2 (3)	0 (8)	−3 (6)
O (5′)	3162 (9)	2200 (8)	2196 (15)	24 (6)	28 (5)	69 (17)	5 (5)	1 (10)	−16 (8)
O (6′)	2951 (11)	155 (9)	791 (18)	58 (9)	26 (5)	96 (22)	14 (6)	−12 (13)	−4 (10)
O (7′)	3769 (8)	135 (7)	2985 (13)	23 (5)	16 (4)	39 (15)	−2 (4)	5 (9)	3 (7)
O (W1)	3512 (19)	1261 (10)	−60 (37)	110 (18)	31 (7)	389 (60)	−23 (9)	−72 (30)	28 (19)
O (W2)	3694 (15)	1848 (10)	−2723 (24)	81 (14)	36 (7)	154 (31)	−5 (8)	35 (19)	−16 (13)

Fig. 1. Chemical structure of aplasmomycin

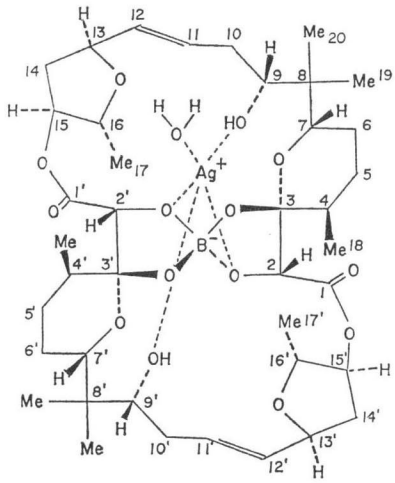
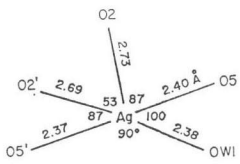


Fig. 3. Coordination geometry around the silver ion



O2'-Ag-O5	84°
O2-Ag-O5'	85°
O2-Ag-OWI	148°
O5'-Ag-O5	170°
O2'-Ag-OWI	159°

Fig. 2. Bond lengths (Å) and bond angles(°) of aplasmomycin molecule in its silver salt

The values found in the other part of the molecule related by the pseudo diad axis are given in parentheses.

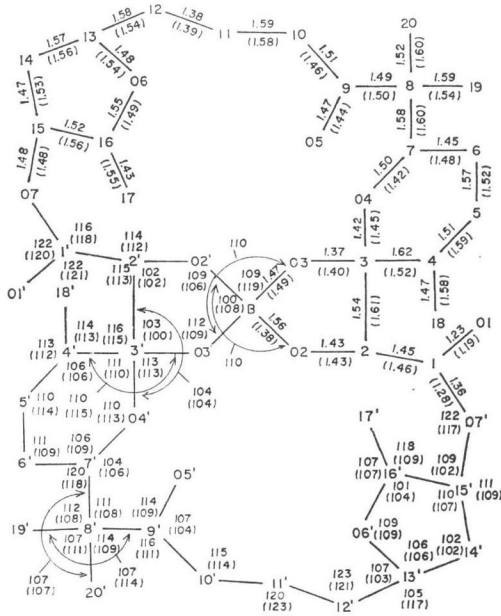
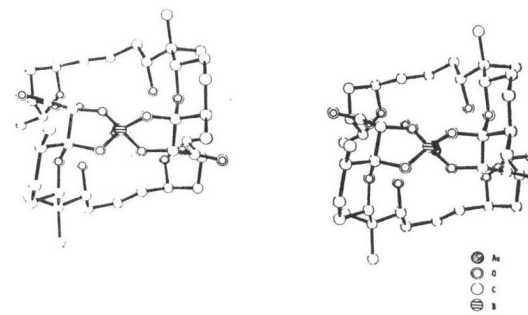


Fig. 4. Stereoscopic drawing of the molecule of the silver salt viewed along the molecular pseudo diad axis

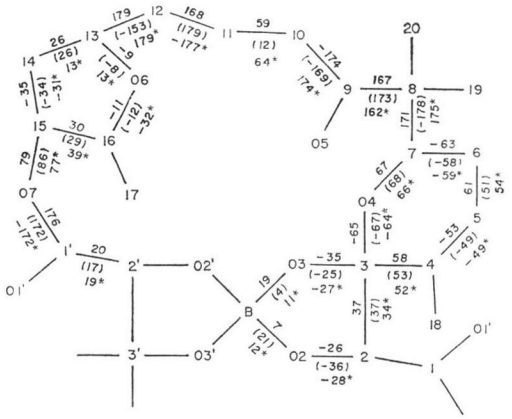


using a crystal of $0.12\times0.10\times0.25$ mm in size. Intensities were measured by a $\theta-2\theta$ scan method with the scan speed of $\theta=4°\text{min}^{-1}$. For weak reflexions with total counts less than 2×10^3, scans were repeated twice. The background was measured as each end of the scan for half the total scan time. The three rather strong reflexions, 800, 060, 014 were chosen as intensity standards and the integrated intensities of these reference reflexions were measured every 2 hours. During the data collection which took about 100 hours, the intensities of the reference reflexions varied about 20%, those of 800 and 014 decreased gradually while that of 060 increased, and at the same time the crystal became slightly brownish in color. This is undoubtedly due to crystal deterioration resulting from X-ray irradiation but a correction to the intensity data was not applied. The intensities of 1513 hk$l$ reflexions out of 2561 theoretically possible ones within $2\theta=100°$ were measured along with 370 $\bar{h}k\bar{l}$ and $\bar{h}k2$ reflexions. Those FRIEDEL reflexions were measured in pairs so that their intensity ratios would not be seriously affected by the deterioration. The crystal data are shown in Table 1.

The structure was solved by the heavy atom method. Successive use of FOURIER, difference FOURIER and least-squares methods yielded the locations of all atoms. The refinement of the structure was carried out by the block-matrix least-squares method using the HBLS program[6] in which anisotropic temperature factors were applied for all atoms but no hydrogen atom contributions were taken into account. The absolute configuration was determined at the stage when the R-factor was 0.11. The dispersion corrections for the atomic scattering factor of Ag for CuKα radiation were taken as $\Delta f' = -0.060$ and $\Delta f'' = 4.282$ (International Tables for X-ray Crystallography Vol. IV)[7]. Of the total of 190 FRIEDEL pairs* 185 showed clearly that the right handed coordinate system is to be taken for the parameters listed in Table 2. The final least-squares refinement was carried out in which the dispersion corrections for Ag atom were taken into accout and the following weighting system was adopted, $\sqrt{w} = 0$ when $Fo < 10$, $\sqrt{w} = 10/Fo$ when $Fo \geqq 10$. The R-factor decreased to 0.084. The final atomic parameters are listed in Table 2.

Fig. 5. Endocyclic torsion angles (°) in the five- and six-membered rings

The torsion angles along the bonds: 6-7-8-9-10-11-12-13-14 and 14-15-07-1′-2′-02′ are also shown. The values formed in the other part of the molecule related by the pseudo diad axis are given in parentheses. The values marked with asterisks are those formed in boromycin.



The chemical structure of the molecule is shown in Fig. 1. As is clear from Fig. 1, the chemical structure possesses a twofold rotation axis of symmetry (diad axis) passing through the B and Ag atoms. Bond lengths and bond angles are shown in Fig. 2. They also show the approximate twofold symmetry and the corresponding values agree with each other with the mean deviations of 0.05 Å and 4°. Because of the unstable nature of the specimen crystal towards X-ray irradiation it was not possible to obtain high precision data for the atomic parameters. The average standard deviations of the bond lengths and angles are estimated to be σ(Ag–O) = 0.02 Å, σ(C–C) = 0.04 Å, σ(B–O) = 0.04 Å, σ(O–Ag–O) = 1°, σ (C–C–C) = 2° and σ (O–B–O) = 2° but some of them, especially those involving C(10), C (11), C (12), C (13), C(11′) and C (13′) are as much as 0.05 Å and 3° owing to the apparently large anisotropic thermal vibrations. The average bond length and angle for the particular type of bond are; C–C = 1.53, C=C = 1.39, C–O = 1.44, B–O = 1.48, C=O = 1.21 and C–OH = 1.46 Å and C–C–C and

Table 3. Deviations of atoms from the least-squares planes in the five-membered rings

C (13)	0	C (13′)	0	B	0.040Å	B	−0.023Å
O (6)	0	O (6′)	0	O (2)	−0.042	O (2′)	0.014
C (16)	0	C (16′)	0	O (3)	−0.027	O (3′)	0.023
C (14)*	−0.232Å	C (14′)*	−0.220Å	C (2)	0.029	C (2′)*	0.578
C (15)*	0.294	C (15)*	0.323	C (3)*	−0.529	C (3′)	−0.014

* These atoms were not included in the least-squares calculations.

* Among the 370 observed FRIEDEL pairs, 190 pairs were selected because they satisfied the following three conditions. $|\Delta Fo| > 2\sigma(Fo)$; $|\Delta Fo| > 0.03|Fo|$; $|\Delta Fc| > 0.03|Fc|$; where $\Delta F = |F(hkl)| - |F(\bar{h}\bar{k}\bar{l})|$.

C–C–O=110, C–C=C=121, C–O–C=111, O–C–O=113, O–B–O=109 and C–C=O and O–C=O=121°, which agree with the usual values.

The silver ion is coordinated to two oxygen atoms of boric acid, two hydroxyl oxygen atoms and a water oxygen atom completing a five coordinated system. The geometry of the coordination is depicted in Fig. 3. The Ag-O lengths range from 2.37 Å to 2.73 Å. The conformation of the molecule is illustrated in Fig. 4 by a stereoscopic perspective drawing (plotted by the ORTEP program[8]) viewed nearly parallel to the molecular diad axis. The conformations of the five- and six-membered heterocyclic rings can be seen in Fig. 5 in which the endocyclic torsion angles are written along with some other torsion angles. These angles differ only about several degrees between the two halves of the molecule related by the pseudo diad axis. Therefore, the following discussions will be made only for one half of the molecule unless otherwise stated. It is seen that the tetrahydropyran ring, consisting of C (3)~C (7) and O (4), adopts a regular chair form. The tetrahydrofuran ring (C (13)~C (16) and O (6)) has an approximate diad axis through O (6) and the midpoint of C (14)-C (15) indicating that the ring adopts a puckered form. The five-membered ring formed by the complex of boric acid with diolide (B, O (2), C (2), C (3) and O (3)) has an approximate mirror plane through C (3) and the midpoint of B-O (2). Hence the ring adopts an envelope form with a flagpole at C (3). The other complex ring consisting of B, O (2′), C (2′), C (3′) and O (3′) atoms, exhibits the same conformation but the approximate mirror plane passes through C (2′) and the midpoint of B-O (3′). Therefore the flagpole atom is moved from C (3′) to C (2′) and the symmetry of the molecule is violated in this part. The puckering of the five-membered rings are shown in Table 3. The conformations along the carbon chain C (6)–C (7)–C (8)–C (9)-C (10)–C (11)=C (12)–C (13)–C (14) are all trans except the gauche conformation about the C (10)–C (11) bond. The ester linkage C (15)–O (7)–C (1′)–C (2′) takes the trans conformation as usual. As a whole, there seems to be no particularly unstable conformation found in the present molecule except for the gauche conformation mentioned above. The silver ion is trapped in the molecule without causing much distorsion. The structure of aplasmomycin resembles that of boromycin. Especially, the structure of one of the two halves of the des-valine boromycin[9] molecule containing a tetrahydrofuran ring is very similar. In Fig. 5, the corresponding torsion angles formed in this part of the molecule are denoted with asterisks. It is seen that although the C11=C12 double bond in aplasmomycin is hydrogenated in boromycin, they have very similar conformations and identical configurations at the asymmetric carbon atoms.

As shown in Fig. 6, the ^{13}C-nmr spectrum of aplasmomycin (dioxane-d_8) showed 20 peaks. Twenty of them indicated two carbon atoms by each signal. These data agree with the symmetric structure determined by X-ray crystallography. As shown in the structure, the boron atom is placed at the center of the symmetric molecule and forms a BÖESEKEN complex of boric acid as in the boromycin[9]. In contrast to boromycin, aplasmomycin does not contain D-valine. In the ^{13}C-nmr spectrum of the preceding paper[3], the small signal at δ 131.4 was thought to be one carbon atom, but this appears to be a noise signal.

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