BIOSYNTHESIS OF THE BORON-CONTAINING ANTIBIOTIC APLASMOMYCIN[1]

NUCLEAR MAGNETIC RESONANCE ANALYSIS OF APLASMOMYCIN AND DESBOROAPLASMOMYCIN

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The 360 MHz ^1H NMR spectra of the boron-containing macrolide antibiotic aplasmomycin and of desboroaplasmomycin were analyzed to extract most of the parameters revealing the conformations of these compounds in $CDCl_3$ solution. It was found that the conformation of aplasmomycin in $CDCl_3$ solution is identical to that in the solid state and that removal of the boron atom from aplasmomycin results only in a slight conformational change of the molecule in $CDCl_3$. All resonances observed in the ^{13}C NMR spectrum of the antibiotic have been assigned on the basis of chemical shift theory, multiplicity analyses, single frequency proton decoupling experiments, comparison with several derivatives and model compounds, specific deuteration experiments, and analysis of one-bond carbon-carbon couplings of pairs of carbon atoms.

Aplasmomycin is a novel ionophoric macrolide antibiotic which was isolated from strain SS-20 of *Streptomyces griseus*[2]. Its structure has been determined by a single-crystal X-ray analysis[3] as a symmetric dimer built around a boron atom. In this paper we discuss the solution conformation as well as the ^{13}C NMR assignments of aplasmomycin.

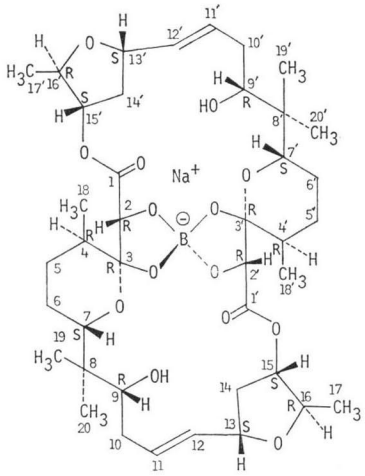
Results and Discussion

The well-resolved 360 MHz ^1H NMR spectra of aplasmomycin and desboroaplasmomycin were analyzed primarily by extensive spin decoupling experiments as shown in Fig. 2 and by protium-deuterium exchange experiments (Table 1). The distinction of the two geminal methyl singlets is based on nuclear OVERHAUSER effect measurements[4]. Irradiation of the H-9 signal caused a 13% area increase of the singlet at 0.73 ppm, and irradiation of the H-7 signal caused a 10% area increase of the same singlet. The singlet at 0.73 ppm therefore must be due to the H-19 resonance signal which is *cis* to H-7 and H-9.

The dihedral angles were calculated from the measured coupling constants using the following modified KARPLUS equations: (A) $^3J_{H,H}=10.5\cos^2\phi-1.2\cos\phi$ for the dihedral angles in the tetrahydrofuran ring[5]; (B) $^3J_{H,H}=6.6\cos^2\phi+2.6\sin^2\phi\,(0°\leqq\phi\leqq90°)$ and $^3J_{H,H}=11.6\cos^2\phi+2.6\sin^2\phi\,(90°\leqq\phi\leqq180°)$ for H_{13}–C_{13}–C_{12}–H_{12} and H_{11}–C_{11}–C_{10}–H_{10} fragments in which one of the intervening carbon atoms is sp^2 hybridized[6]; (C) $^3J_{H,H}=9.3\cos^2\phi+0.3(0°\leqq\phi\leqq90°)$ and $^3J_{H,H}=10.4\cos\phi^2+0.3(90°\leqq\phi\leqq180°)$ which takes into consideration the influence of substituent electronegativity on the H_{10}–C_{10}–C_9–H_9 and H_7–C_7–C_6–H_6 fragments[7].

These dihedral angles can then be utilized to portray the conformational features of each half of aplasmomycin in chloroform solution. However, the conformation around the bonds C_1–C_2, C_1–$O_{15'}$

Fig. 1. Structure of aplasmomycin.



and the five-membered ring formed by the complex of boric acid with the geminal diol functions (B, O–2, C–2, C–3 and O–3) cannot be assessed by this approach.

The large coupling $J_{13,14R}$ (9.3 Hz), and the small coupling $J_{14R,15}$ (4.9 Hz) and the absence of couplings $J_{13,14S}$, $J_{14S,15}$ and $J_{15,16}$ suggest that the tetrahydrofuran moiety occurs in the *S*-conformation rather than the *N*-conformation[4].

S *N*

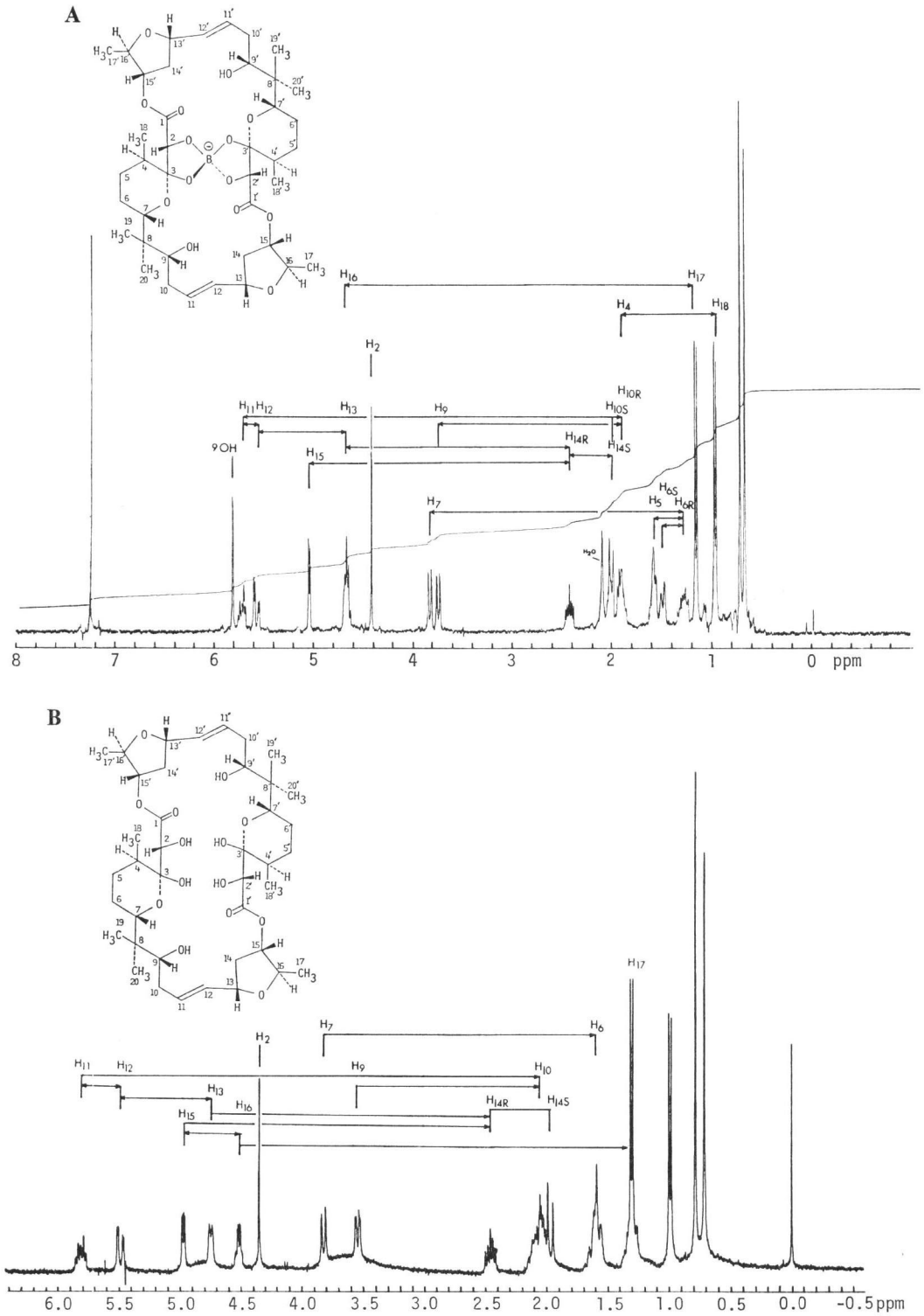
This conformation also accounts for the unusual shielding of H-14S relative to H-14R because of the orientation of the carbonyl group.

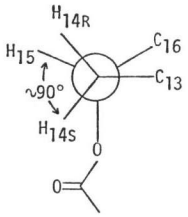
Two possible arrangements around C_{12}–C_{13} (A and A′) are consonant with the coupling constant $J_{12,13}$ (4.5 Hz). The conformer A′ is thermodynamically less favorable than the conformer A because the

Table 1. The ^1H chemical shifts (ppm) and ^1H-^1H spin coupling constant (Hz) for aplasmomycin and desboroaplasmomycin.

Assignment	Aplasmomycin		Assignment	Desboroaplasmomycin	
	Chemical shifts	Coupling constant (coupled proton)		Chemical shifts	Coupling constant (coupled proton)
H_2	4.44 (s)		H_2	4.42 (s)	
H_4	2.04 (m)		H_4	2.05 (m)	
H_5	1.60 (m)		H_5	1.60 (m)	
H_{6S}	1.51 (bd)	12.5 (H_{6R})	H_6	1.60 (m)	
H_{6R}	1.32 (dq)	5.5 (H_5), 10.7 (H_7), 11 (H_{5S}), 12.5 (H_{6S})	H_7	3.82 (d)	11.3 (H_6)
			H_9	3.54 (dd)	10.9 (H_{10R}), 3.0 (H_{10S})
H_7	3.84 (d)	10.7 (H_{6R})	H_{10}	2.05 (m)	
H_9	3.75 (d)	11.0 (H_{10R})	H_{11}	5.80 (td)	7.3 (H_{10}), 15.5 (H_{12})
H_{10R}	1.92 (ddd)	5.9 (H_{11}), 11.0 (H_9), 13.6 (H_{10S})	H_{12}	5.48 (dd)	2.3 (H_{13}), 15.5 (H_{11})
			H_{13}	4.75 (bd)	7.9 (H_{14R})
H_{10S}	2.04 (dd)	5.9 (H_{11}), 13.6 (H_{10R})	H_{14R}	2.45 (ddd)	6.5 (H_{15}), 7.9 (H_{13}), 14.5 (H_{14S})
H_{11}	5.74 (td)	5.9 (H_{10}), 15.8 (H_{12})			
H_{12}	5.58 (dd)	4.5 (H_{13}), 15.8 (H_{11})	H_{14S}	1.96 (d)	14.5 (H_{14R})
H_{13}	4.69 (dd)	4.5 (H_{12}), 9.3 (H_{14R})	H_{15}	4.96 (dd)	2.1 (H_{16}), 6.5 (H_{14S})
H_{14R}	2.45 (ddd)	4.9 (H_{15}), 9.3 (H_{13}), 14.3 (H_{14S})	H_{16}	4.51 (dq)	2.1 (H_{16}), 6.5 (H_{17})
			H_{17}	1.29 (d)	6.5 (H_{16})
H_{14S}	2.03 (d)	14.3 (H_{14R})	H_{18}	0.98 (d)	6.7 (H_4)
H_{15}	5.06 (d)	4.9 (H_{14R})	H_{19}	0.78 (s)	
H_{16}	4.68 (q)	6.7 (H_{17})	H_{20}	0.70 (s)	
H_{17}	1.18 (d)	6.7 (H_{16})			
H_{18}	0.98 (d)	6.6 (H_4)			
H_{19}	0.73 (s)				
H_{20}	0.68 (s)				

Fig. 2. 360 MHz ^1H NMR spectra of (A) aplasmomycin and (B) desboroaplasmomycin in $CDCl_3$ solution. The arrow x→y indicates that proton y is observed while proton x is irradiated.

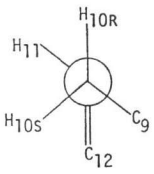




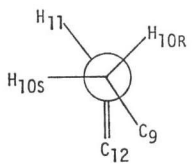
S

bulky groups C_{11} and O_6 are eclipsed in **A'**.

The equivalence of the coupling constants $J_{10R,11}$ and $J_{10S,11}$ (5.9 Hz) reflects two possible arrangements around C_{10}–C_{11}. The conformer **C** is the more favorable one. The conformer **C'** suffers severe steric hindrance due to the eclipsed relationship of C_9 and C_{12}.

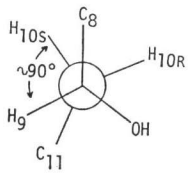


C



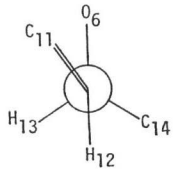
C'

The coupling constant $J_{9,10R}$ (11.0 Hz) and $J_{9,10S}$ (0 Hz) reveal the *anti* relationship of H-9 and H-10R and the orthogonal disposition of H-9 and H-10S.

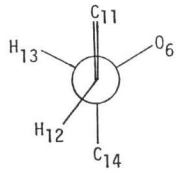


D

As discussed above, the *cis* relationship between H-19 and H-7(H-9) has been shown by the NOE results. Inspection of models and consideration of steric hindrance, suggest that the more favorable conformers around C_8–C_9 and C_7–C_8 would be the ones shown below (**E** and **F**), in which the bonds C_9–C_{10} and C_7–C_8, C_6–C_7 and C_8–C_9, stay antiperiplanar.



A'



A

Table 2. Torsional angles of aplasmomycin along the carbon chain in solution and in solid state.

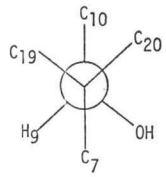
Condition	Carbon-carbon bond				
	6–7	7–8	8–9	9–10	10–11
Solution	60	165	175	175	50
Solid state	63	171	167	174	59
Condition	11–12	12–13	13–14	14–15	15–16
Solution	180	170	25	30	20
Solid state	177	179	26	34	30

Table 3. ^{13}C NMR spectral data for aplasmomycin.

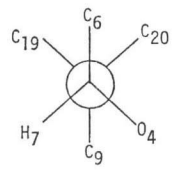
Carbon No.	δc*	Multiplicity**	$^1J_{c-c}$, Hz
1	170.4	s	64.7
2	78.2	d	65.2
3	106.0	s	47.6
4	32.9	d	47.6
5	28.6	t	31.7
6	25.0	t	31.7
7	79.5	d	39.1
8	39.0	s	39.1
9	79.3	d	39.1
10	32.1	t	39.1
11	128.0	d	72.0
12	131.8	d	72.0
13	76.4	d	34.7
14	36.0	t	34.7
15	80.4	d	
16	78.2	d	
17	19.4	q	
18	16.5	q	
19	12.9	q	
20	21.6	q	

* Chemical shifts are given in parts per million downfield from internal Me_4Si in $CDCl_3$.

** Multiplicities in the off-resonance decoupled spectrum.



E



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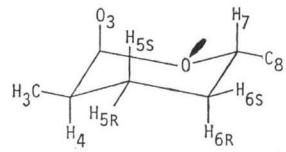

The chair conformation of the tetrahydropyran ring is corroborated by the findings that the coupling constants of $J_{6R,7}$, $J_{5S,6R}$, $J_{6R,5R}$ and $J_{6S,7}$ are 10.7, 11, 6 and 0 Hz respectively. The unusually small coupling between H-6S and H-7 is probably due to the presence of a free oxygen orbital in a W-conformation (a planar zig-zag path) with respect to a coupling C-H bond (O_4–C_7–C_6–H_{6S})[8].

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* with the exception of the gauche conformation about the C(10)-C(11) bond. The solid state conformation of the silver salt of aplasmomycin has been determined by X-ray crystallographic analysis.[3] The present results show that the solution conformation is very similar to that in the crystalline state. The torsional angles of aplasmomycin in solution and in the solid state are shown in Table 2.

Desboroaplasmomycin was easily obtained by acid hydrolysis of aplasmomycin[9]. The 360MHz ^{1}H NMR spectrum of desboroaplasmomycin (Fig. 2) is much less well resolved in the methylene proton region than that of aplasmomycin. The assignments of the proton resonances of desboroaplasmomycin in $CDCl_3$ were made by spin decoupling experiments. A conformational change is seen in the tetrahydrofuran ring: in aplasmomycin $J_{12,13}$=4.4 Hz, $J_{13,14R}$=9.3 Hz, $J_{14R,15}$=4.9 Hz, $J_{15,16}$=0 Hz but in desboroaplasmomycin $J_{12,13}$=2.3 Hz, $J_{13,14R}$=8.0 Hz, $J_{14R,15}$=6.5 Hz, $J_{15,16}$=2.1 Hz. A second change is found in the torsional angle around the C_9-C_{10} bond. In aplasmomycin the coupling between H-10S and H-9 is small (~0 Hz), but in desboroaplasmomycin $J_{9,10S}$=3.0 Hz, reflecting a slight change from their original orthogonal disposition. These results indicate that, unlike in boromycin[10], removal of the boron atom from aplasmomycin has led to slight conformational changes of the molecule in $CDCl_3$ solution*. Yet, the boron could easily be reinserted into desboroaplasmomycin by treatment with boric acid at pH 6 and 8, respectively.

Chemical shifts for the carbon atoms in aplasmomycin were determined on proton-noise-decoupled spectra. Because of the symmetry of the molecule the natural abundance proton-noise-decoupled ^{13}C NMR spectrum of aplasmomycin (Table 3) shows only 20 signals corresponding to 40 carbon atoms of the symmetrical macrocyclic dilactone ring[9]. Each signal represents two identical carbon atoms. The proton-coupled spectrum shows 3 singlets, 9 doublets, 4 triplets and 4 quartets. The singlets at 170.4 ppm, 106.0 ppm and 39.0 ppm, and the doublet at 32.9 ppm were assigned to the carbonyl carbon C-1, the quaternary carbon C-3 bearing two oxygens, the aliphatic quaternary carbon C-8 and the aliphatic methine carbon C-4 not carrying oxygen, respectively, on the basis of their characteristic chemical shifts and multiplicities. Assignments of the remaining carbons were made through more detailed analysis.

The signals at 12.9, 16.5, 19.4, 21.6, 25.0, 36.0, 78.2, 79.3, 80.4, 128.0 and 131.8 ppm were assigned to C-19, C-18, C-17, C-20, C-6, C-14, C-2, C-9, C-15, C-11 and C-12, respectively, by selective proton single frequency decoupling experiments. The assignment for the signal of C-9 was confirmed by the β-deuterium isotope-effect on the ^{13}C NMR signal[11]. Deuteration of the hydroxy groups caused an up-

* A comparison of the 360 MHz ^{1}H NMR spectra of desvalinoboromycin and desboro-desvalinoboromycin shows them to be virtually identical. Thus the solution conformation of desvalinoboromycin also does not change significantly upon removal of the boron (CHEN, CHANG and FLOSS, unpublished observation).

field displacement of the signal at 79.3 ppm by 0.15 ppm. The carbon chemical shift difference between the two heterotopic methyl carbons C-19 and C-20 was 8.7 ppm. The sterically induced polarization of perturbed C_{19}-H bonds through their *cis* relationship to H-7 and H-9 tends to increase the charge density at C-19, thereby increasing its shielding.

Of the remaining two aliphatic methylene carbon resonances at 28.6 and 32.1 ppm, the former was assigned to C-5 by measuring the ^{13}C-^{13}C one-bond coupling constants of pairs of methylene signals in aplasmomycin derived biosynthetically from $^{13}CH_3$-$^{13}COONa$. The signal at 28.6 ppm was found to have the same coupling constant of 31.7 Hz as C-6 at 25.0 ppm. The assignment for the signals of C-5 and C-6 was further confirmed by chemical shift correlation.[12,13] Chemical shift increments, determined for 1-butyl, ethyl, hydroxy and methyl groups in mono and disubstituted cyclohexanes were added to the appropriate chemical shifts for the carbon atoms in tetrahydropyran to give 28.6 ppm for C-5 and 25.0 ppm for C-6. The last methylene carbon signal at 32.1 ppm, by difference, must be C-10.

The three remaining oxygen-bearing methine carbon atoms C-7, C-13 and C-16 could be distinguished as follows: Selective single frequency irradiation of the multiplet at 4.7 ppm decoupled both protons H-13 and H-16 simultaneously, and let to collapse of both carbon signals at 76.4 and 78.2 ppm. Since in aplasmomycin obtained from [1,2-^{13}C]acetate only the signals at 76.4 ppm and that for C-14 had one-bond carbon-carbon coupling constants of 34.7 Hz, the carbon atom which resonated at 76.4 ppm must be coupled to C-14.

The signals at 76.4 and 78.2 ppm were thus assigned to C-13 and C-16, respectively. The remaining signal at 79.5 ppm was thus assigned to C-7. These complete ^{13}C resonance assignments allowed us to determine the biosynthetic origin of the carbon skeleton of aplasmomycin using ^{13}C NMR spectroscopy[1].

Experimental

High-field ^1H NMR spectra were determined at 360 Hz on a Nicolet NTC-360 super-conducting nuclear magnetic resonance spectrometer. ^{13}C NMR spectra were recorded on Jeol PFT-100, Varian FT-80, Nicolet NTC-150 and Nicolet NTC-360 nuclear magnetic resonance spectrometers operating at 25.2, 20.16, 37.8 and 90.72 MHz, respectively. Chemical shifts are given in parts per million (ppm) relative to Me_4Si (TMS) as internal standard, or adjusted to the TMS scale by reference to the $CHCl_3$ resonance at δ_H 7.26 or δ_C 76.9. Coupling constants are given in Hertz (Hz). Splitting patterns are designated s, singlet; d, doublet; t, triplet; q, quartert; m, multiplet; b, broad.

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