SOLUTION CONFORMATION OF SEPTAMYCIN AND ITS SODIUM SALT

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The interpretation of the 300 MHz ^{1}H-nmr spectra of septamycin (**1**) and its sodium salt (**1**$^+$) allow one to extract most of the parameters revealing their resemblant conformations in solution. The backbone forms a pseudocyclic structure closed by head-to-tail hydrogen bonding between the carboxylate fragment and the OH-14. In **1**$^+$, the sodium ion is trapped in a central hole by coordinating around it six to seven oxygen atoms (including COO^-). The external lipophilic zone of the molecule keeps the sodium ion away from the surroundings. The dangling sugar-like fragment does not participate in the metal binding. It was not possible to detect any water molecule participating in the cyclization of septamycin-free acid, nor in septamycin-Na$^+$, as was found in an X-ray study for the *p*-bromophenacyl ester.

Septamycin, $C_{48}H_{82}O_{16}$ (**1**) is a relatively large polyether antibiotic[1,2], that can be isolated[3] as its sodium salt, $C_{48}H_{81}O_{16}$-Na$^+$ from *Streptomyces hygroscopicus*. Only its *p*-bromophenacyl ester has yet been studied[4] by X-ray measurements, from which the covalent structure and absolute configuration has been revealed.

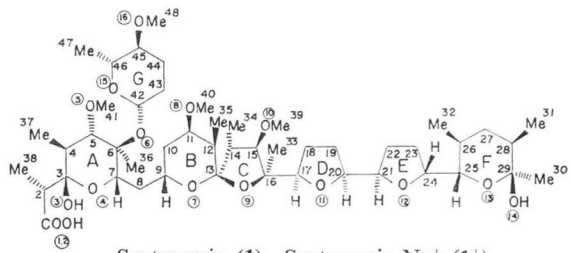
Continuing our high-resolution ^{1}H-nmr spectroscopic studies of polyether antibiotics[5~12] we report here on the results obtained for septamycin (**1**) and its sodium complex (**1**$^+$). The covalent structure of **1** is almost identical to that of the antibiotic A-204 A[8,13] except that it lacks a methoxy group at C-27 in ring F (for coding see **1**), and that it differs in ring A, where the methoxy group at C-5 and the pyranose ring at C-6 are positionally exchanged. Furthermore the pyranose ring has a β-configuration in **1** where it is α in A-204 A.

We have identified by consecutive double irradiation experiments (nmdr) most of the absorption patterns in the spectra of **1** and **1**$^+$ taken in different solvents ($CDCl_3$, CS_2 and C_6D_6; See Figs. 1 and 2 for some representatives). The extracted parameters are given in Tables 1 and 2.

The apparent values of coupling constants (Table 2) are believed to be close to the true ones, if they remain unaffected by changing the solvent system.

Eighteen to twenty patterns (depending on the solvent system used) at low field ($\delta > 2.0$) in both **1** and **1**$^+$ are more or less separated from each other. This gives direct access to nmdr experiments, allowing inter-proton patterns to be followed and to reveal the different structural fragments corresponding to structure **1**.

Scheme **1**.



Septamycin (**1**) Septamycin-Na$^+$ (**1**$^+$)

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In the spectrum of **1** there is a doublet at $\delta=5.88$ ($CDCl_3$) belonging to the mobile hydroxylic proton OH-3, while it couples with H-4 ($^4J(4, OH\text{-}3)=1.6$ Hz; Table 2). In monitoring the latter proton absorptions we were able to assign the doublet of H-5 (*i.e.* OH-3⇄H-4⇄H-5)* and so to distinguish between H-5 and H-15, which also displays a doublet in the same region and between H-4 and H-14, which show almost the same pattern in the overcrowded region $\delta\sim1.5\sim2.0$.

The four hexanoid rings occur basically in chair conformations ($^3J(4a, 5a)>10.8$ Hz; $^3J(9a, 10a)\sim12.0$ Hz; $^3J(25a, 26a)=9\sim10$ Hz; $^3J(45a, 46a)=8.7\sim9.0$ Hz). It was not possible to determine the position of the substituents on C-3, C-6, C-13 and C-29, as these carbons do not bear hydrogens. We took the configurations on these carbons from solid state data. Thus OH-3 and Me-36 in ring A, C_{13}-O_9 in ring B and OH-14 in ring F are axial. All remaining ring-substituents stay equatorial

Fig. 1. 300 MHz ^{1}H-nmr spectrum of septamycin free acid in C_6D_6 (TMS internal).
The assignments are made by consecutive double irradiation experiments[9,11] starting from the low field patterns and the methyl doublets.

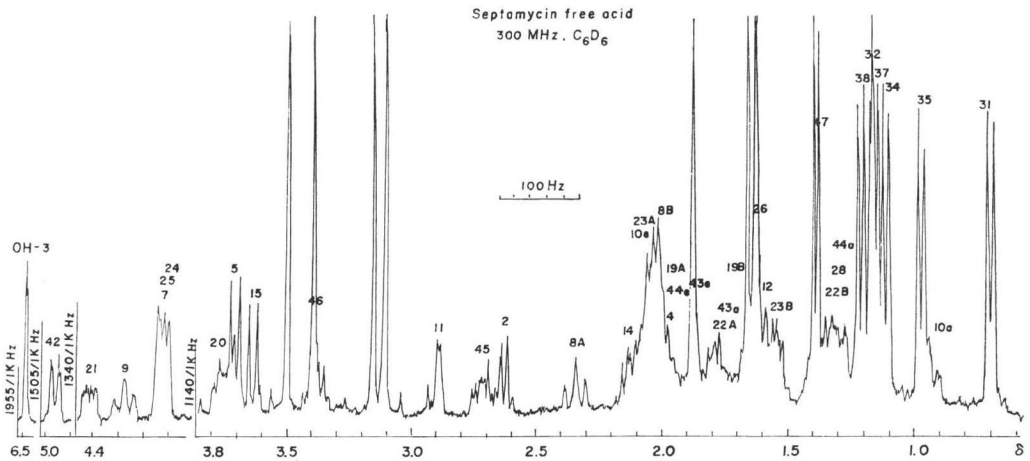
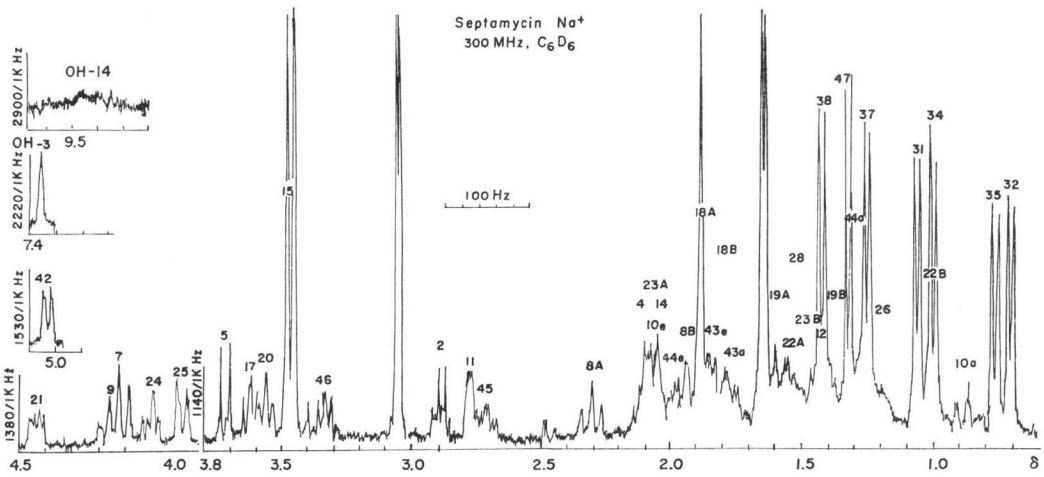


Fig. 2. 300 MHz ^{1}H-nmr spectrum of septamycin-Na^+ in C_6D_6 (TMS internal).
The assignments are made as in Fig. 1.



* The arrow A→B indicates that A has been irradiated while observing B.

Table 1. Proton shift values (in p.p.m. from TMS-internal) observed for septamycin free acid (**1**) and septamycin-Na^+ ($\mathbf{1}^+$) in various solvents

Compound (Solvent)	Proton															
	2	4	5	7	8A	8B	9	10e	10a	11	12	14	15	17	18A	18B
1 ($CDCl_3$)	2.623	1.73	3.35	3.762	1.77	1.54	3.983	2.05	1.084	3.35	1.73	1.98	3.53	3.57	~1.78	~1.78
1 (CS_2)	2.415	1.57	3.140	3.529	1.65	1.36	3.82	1.94	0.98	3.273	1.67	1.86	3.428	~3.38	~1.67	~1.67
1 (C_6D_6)	2.563	1.90	3.642	4.11	2.279	1.93	4.273	1.93	0.867	2.824	1.48	2.08	3.570			
Δ(Asis)[a]·10^2	−6.0	17.0	29.2	34.8	50.9	39.0	29.0	−12.0	−21.7	−52.6	−25.0	10.0	4.0			
$\mathbf{1}^+$ ($CDCl_3$)	2.462	1.47[c]	3.792[c]	3.42	1.78	1.50	4.020	2.15	1.15	3.407	1.77	2.117[c]	3.549[c]	3.683	1.93	1.77
$\mathbf{1}^+$ (CS_2)	2.218	1.545[c]	3.092[c]	3.524	1.65	1.293	3.883	2.07	1.02	3.31	1.725	1.997[c]	3.451[c]	3.620	1.83	1.68
$\mathbf{1}^+$ (C_6D_6)	2.89	2.10[c]	3.71[c]	4.21	2.30	1.95	4.273	2.083	0.87	2.771	1.43	2.07[c]	3.47[c]	3.62	1.90	~1.77
Δ(Asis)[a]·10^2	42.8	63.0	−8.2	79.0	52.0	45.0	25.3	−6.7	−28.0	−63.6	−34.0	−4.7	−7.9	−6.3	−3.0	0.0
$\Delta(CDCl_3)$[b]·10^2	16.1	26	−44.2	34.2	−1.0	4.0	−3.7	−10.0	−6.6	−5.7	−4.0	−13.7	−1.9	−11.3	−1.5	1.0

Compound (Solvent)	Proton															
	19A	19B	20	21	22A	22B	23A	23B	24	25	26	28	42	43e	43a	44A (e)	44B (a)
1 ($CDCl_3$)	1.73	1.22	3.78	4.247	1.97	1.4	2.03	1.78	4.195	3.847	1.35	1.53	4.837	1.84	1.58	2.21	1.32
1 (CS_2)	1.72	1.58	3.717	4.05	1.87	1.35	2.02	1.72	4.115	3.78	1.23	1.43	4.671	1.70	1.43	2.11	1.23
1 (C_6D_6)	1.92	1.62	3.703	4.157	1.70	1.2	2.06	1.5(?)	4.12	~4.12	1.22[d] or 1.55	1.55[d] or 1.22	4.959	1.80	1.71	1.93	1.20
Δ(Asis)[a]·10^2	19.0	40	−7.7	−9.0	−27.0	−20.0	3.0	−28.0	−7.5	27.3			12.2	−4.0	13.0	−28.0	−12.0
$\mathbf{1}^+$ ($CDCl_3$)	~1.72	~1.72	3.923	4.563	1.93	1.38	2.17	1.80	4.304	3.817	1.32	1.80	4.862	1.82	1.55	2.217	1.32
$\mathbf{1}^+$ (CS_2)	~1.72		3.867	4.441	1.95	1.38	2.18	1.77	4.227	3.70	1.24	1.38	4.686	1.72	1.43	2.217	1.28
$\mathbf{1}^+$ (C_6D_6)	1.62	1.38	3.55	4.55	1.55	1.02	2.08	1.45	4.12	3.995	1.18	1.50	5.035	1.87	1.75	2.027	1.32
Δ (Asis)[a]·10^2	−10.0	−34.0	−37.3	−1.3	−38.0	−36.0	−9.0	−35.0	−18.4	17.8	−14.0	−30.0	17.3	5.0	20.0	−19.0	0.0
$\Delta(CDCl_3)$[b]·10^2	1.0	−50	−14.3	−31.6	4.0	2.0	−14.0	−2.0	−10.9	3.0	3.0	−27.0	−2.5	2.0	3.0	−0.7	0.0

(to be continued)

Table 1. (Continued)

Compound (Solvent)	Proton														
	45	46	Me-30[e]	Me-31	Me-32	Me-33[e]	Me-34	Me-35	Me-36[e]	Me-37	Me-38	Me-47	OMe	OH-3	OH-14
1 ($CDCl_3$)	2.82	3.31	1.488	0.926	0.839	1.314	0.962	0.999	1.222	0.987	1.098	1.263	3.536/3.422 3.352/3.343	5.881	
1 (CS_2)	2.633	3.139	1.337	0.833	0.800	1.171	0.911	0.941	1.097	0.88	0.996	1.223	3.396/3.345 3.244(2MeO-6-H)	5.776	
1 (C_6D_6)	2.652	3.283	1.810	1.102[d] or 0.635	0.645[d] or 1.102	1.591	1.047	0.908	1.559	1.088	1.145	1.320	3.430/3.324 3.065/3.041	6.488	
Δ(Asis)[a]·10^2	−16.8	−2.7	32.2			27.7	8.5	9.1	33.7	10.1	4.7	5.7		60.7	
1^+ ($CDCl_3$)	2.810	3.327	1.624	1.002	0.817	1.277	0.997	0.982	1.218	0.899	1.059	1.262	3.539/3.477 3.410/3.349	6.220	~8.86
1^+ (CS_2)	2.642	3.145	1.496	0.835	0.789	1.165	0.963	0.932	1.078	0.861	0.871	1.137	3.413/3.389 3.358/3.158	6.480	~8.45
1^+ (C_6D_6)	2.702	3.348	1.88	1.062	0.71	1.647	1.002	0.777	1.633	1.520	1.422	1.322	3.476/3.450 3.052/3.040	7.35	~9.61
Δ(Asis)[a]·10^2	−10.8	2.1	25.6	6.0	−10.7	37.0	0.5	−20.5	41.5	62.1	36.3	6.0		113.0	75.0
$\Delta(CDCl_3)$[b]·10^2	1.0	−1.7		−7.6	2.2		−3.5	1.7		8.8	3.9	0.1		−33.9	

[a] $\Delta(\text{Asis}) = \delta(C_6D_6) - \delta(CDCl_3)$;

[b] $\Delta(CDCl_3) = \delta(CDCl_3)\text{-free-acid} - \delta(CDCl_3)\text{-}Na^+$;

[c] Assignment made by comparison of coupling constants in free and complexed species;

[d] First row represents the values of our choice, if solvent effects would be comparable to the behaviour of the complexed ionophore;

[e] Me-30, Me-33 and Me-36 may be interchanged.

Table 2. Apparent coupling constants in Hz of septamycin free acid (**1**) and septamycin-Na^+ ($\mathbf{1}^+$)

Compound (Solvent)	Couplings																	
	2, 38	4, 5	4, 37	7, 8A	7, 8B	8A, 8B	8A, 9	8B, 9	9, 10e	9, 10a	10a,10e	10e,11e	10a,11e	11e, 12a	12, 35	14, 15	14, 34	17, 18A	17, 18B
1 ($CDCl_3$)	7.0	11.0	6.8	2.0	~12.0	~12.5	~12.0	<2.0	~1.0	~12.0	>12.5	~3.0	3.0	~3.5	7.0	9.5	6.8		
1 (CS_2)	7.0	10.8	6.7	2.0	12.2	~12.5	~12.0	$\sum$J(9,10e,8B)	~4.5[a]	~12.0	~12.0	>2.0	~3.0	~3.5	7.0	9.5	6.7		
1 (C_6D_6)	7.0	11.0	6.6	2.0	~12.0	12.5	~12.0	$\sum$J(9,10e,8B) ~4.5[b]	~1.0	~12.0	>12.5	~2.0	~3.0	~3.5	7.2	9.6	6.7		
$\mathbf{1}^+$ ($CDCl_3$)	7.0	10.8	6.4	small	>11.0	~14.0	~12.0	small	<1.5	~12.0	12.5	small		>3.5	7.2	9.2	6.6	$\sum$(17,18)	>14.5
$\mathbf{1}^+$ (CS_2)	7.0	10.8	6.7	1.5	12.2	~14.0	11.3	<1	<1.5	large	13.0	<1.0	2.5	3.8	7.2	9.3	6.7		
$\mathbf{1}^+$ (C_6D_6)	7.0	10.8	6.7	<2	~12.0	13.5	~12.0	<1	~2.0	~12.5	~14.0	small	~3.0	~3.6	7.1	~9	6.7	7.0	9.0

Compound (Solvent)	Couplings																		
	19A,20	19B,20	20, 21	21, 22A	21, 22B	23A, 24	23B, 24	24, 25	25, 26	26, 32	28, 31	42, 43e	42, 43a	43e, 44a	44e, 45	44a, 45	45, 46	46, 47	4, OH-3
1 ($CDCl_3$)	$\sum$J(20)≃16		~2.0	5.0	10.0	8.0	8.0	3.0	9.0	~5.8	6.7	~2.0	9.2	large	4.2	~10.0	~9.0	6.1	1.6
1 (CS_2)	~7	~7	2.2	5.0	~11.0	~8.0	~7.5	~2.8	~9.0	5.8	6.6	2.2	9.0	~12.5	4.5	10.5	8.6	6.1	1.6
1 (C_6D_6)	~6	~8	2.2	5.0	10.5				>8.0	6.2 or 6.0	6.0 or 6.2	2.4	~9.0		4.4	10.5	8.8	6.1	1.6
$\mathbf{1}^+$ ($CDCl_3$)	$\sum^3$J(20)≳16.5		~2.0	~5.0	~11.0	~7.5	~7.5	~2.6	<10.0	5.6	6.6	~2.0	9.0		4.5	10.4	8.7	6.3	
$\mathbf{1}^+$ (CS_2)			~2.5	~5.5	~11.0	6.6 or 8	8 or 6.6	~2.8	9.5	5.8	6.3	~2.5	9.0		4.5	10.2	8.7	6.0	
$\mathbf{1}^+$ (C_6D_6)	~8	~8	~2.0	~5.0	~11.0	8.0	~6.5	~2.6	10.0	6.3	6.5	~2.0	9.0	>13.5	4.4	10.8	8.7	6.1	

[a] Only approximate value because of strong overlap of H-9 pattern with H-25 and H-20. The decoupling phenomena could only be interpreted inspecting an outside low-field peak of small intensity of H-9.

[b] Only approximate sum-value because H-8B and H-10e are isochronous.

except for OMe-40 in ring B, as revealed from inspection of the coupling constant data and in agreement with the X-ray findings.

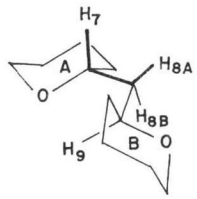
Ring B seems to be somewhat distorted, presumably by a slight outwards bending of the syn-diaxial substituents C_{11}-O_8 and C_{13}-O_9.

(Note how small $^3J(10e, 11e) \simeq 1 \sim 2$ Hz is compared to $^3J(11e, 10a) \simeq {}^3J(11e, 12a) = 3.7 \sim 3.8$ Hz, a relatively large value in view of the fact that H-10a suffers from the antiperiplanar position of O-8 and H-12a from antiperiplanar of both O-8 and O-9).[14]

The β-configuration of ring G is clearly revealed by the fact that H-42 is an axial proton ($^3J(42a, 43a) = 9.0$ Hz and $^3J(42a, 43e) = 2.0 \sim 2.5$ Hz), at variance to the α-sugar fragment in A-204 A.[8]

The assignments concerning the conformation of the tetrahydrofurane rings are speculative. $^3J(14, 15)$ is large ($9 \sim 9.5$ Hz); hence protons H-14 and H-15 must be quasi-antiperiplanar while Me-34 and OMe-39 are trans. We may tentatively propose a time-averaged conformation of a predominant half chair form for the rings D and E, as could be indicated by the equality of coupling constants near 7 Hz, *e.g.* $^3J(17, 18A) \simeq {}^3J(17, 18B)$ and $^3J(19A, 20) \simeq {}^3J(19B, 20)$ as well as $^3J(23A, 24) \simeq {}^3J(23B, 24)$. (Note that $^3J(21, 22A) = 5 \sim 5.5$ Hz $\neq {}^3J(21, 22B) = 10 \sim 11$ Hz).

Fig. 3. Conformation along the fragment C_7-C_8-C_9 in septamycin.



The relevant parameters concerning some rotors around acyclic fragments are of special interest. The large coupling between H-7 and one of the vicinal methylene protons ($^3J(7, 8B) \sim 12$ Hz) is accompanied by a much smaller coupling with the other geminal partner ($^3J(7, 8A) \leq 2$ Hz). An analogous situation is found for H-9 in respect to the same methylene protons, but with an inversed partnership ($^3J(8A, 9) = 11.3 \sim 12$ Hz and $^3J(8B, 9) < 2$ Hz). This points to rigid rotors in the C_7-C_8-C_9 fragment, nl. H-8A vicinal to H-7 but antiperiplanar to H-9 and H-8B quasi-antiperiplanar to H-7 but vicinal to H-9 (and presumably with $\tau > 60°$) (See Fig. 3).

The coupling values between H-20/H-21 and H-24/H-25, *e.g.* $^3J(20, 21) = 2 \sim 2.5$ Hz and $^3J(24, 25) = 2.6 \sim 3.0$ Hz, indicate that these protons are vicinal (and presumably with $\tau < 60°$, because the observed values are lowered by the presence of a quasi-axial oxygen[14]).

For the remaining rotors around C_{16}-C_{17} and C_2-C_3 we accepted the results extracted from X-ray data, as it was impossible to extract any information about them (no interproton connection between C-16 and C-17, as well as between C-2 and C-3).

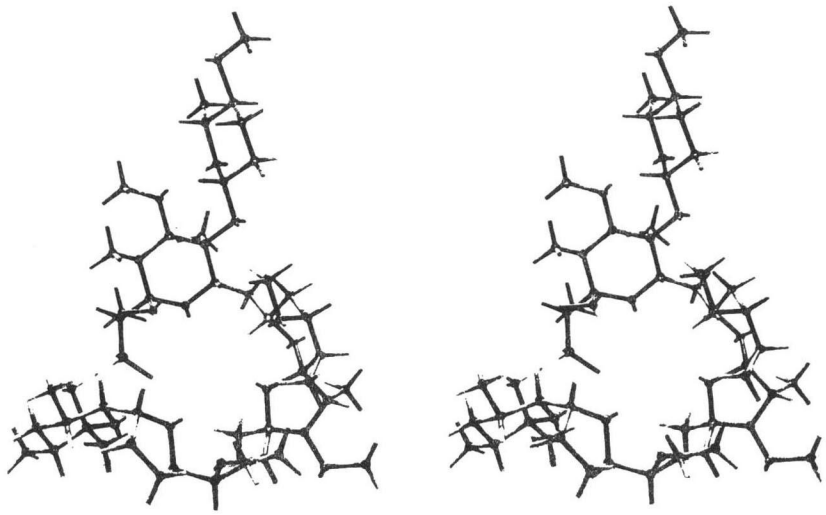
The peaks due to the hydroxyls are of particular interest. We have already mentioned the peak at $\delta \simeq 5.9$ in free acid ($CDCl_3$), corresponding to OH-3. The other hydroxyl protons in the free acid, *e.g.* COOH and OH-14, give rise to a very broad common signal at $\delta \sim 6.4$. Both peaks collapse in a broad band at $\delta \sim 6.2$ after addition of small amounts of Et_3N. It was not possible to have accurate integration data, but we incline to the conclusion that only three protons in total are involved (COOH, OH-3 and OH-14) and no extra water is present. It is clear that OH-3 is not involved in a rapid exchange with the remaining two mobile protons (COOH and OH-14). The observed coupling with H-4 ($^4J(4, \underline{OH}\text{-}3) = 1.6$ Hz) discloses an essential planar zig-zag H_4-C_4-C_3-O_3-H fragment, hence OH-3 is orientated towards the inner cavity into the direction of the OMe-40. Although the distance OH-3/OMe-40 is at least 3 Å (from model inspection) a residual hydrogen bonding mechanism may remain. The other two mobile protons are then involved in a more rapid exchange as would follow

from mutual hydrogen bridging.

Two distinct signals are displayed in the spectrum of septamycin-Na^+, each representing one proton. We assigned the peak at $\delta = 6.2$ to the OH-3, by analogy to the free acid form. The peak at $\delta \simeq 10$ must then belong to the OH-14, which is involved in H-bridging with the carboxylate head of the molecule. In contrast to the free acid, OH-3 is not coupled apparently with the H-4, although displaying a distinct signal, indicative of the absence of a rapid chemical exchange *e.g.* with COO^-. This might mean that the inward orientation of OH-3 as found in the free acid, is no longer present, as *e.g.* the mere result of some slight conformational relaxation of the backbone in the sodium complex, while OMe-40 has now become a ligand to the central cation.

The main conclusions from this study are that the free and complexed form have essential identical conformations, whereby from considerations extracted from the present ^{1}H-NMR data and model building, a quasi cyclic structure occurs as represented in stereo drawing in Fig. 4 for the Na^+-salt (see also legend to Fig. 4 for main characteristics).

Fig. 4. Stereoscopic view of septamycin-Na^+ with the typical circular backbone wrapped around the hydrophilic cavity and characterized by head-to-tail hydrogen bond binding (COO^- . . . OH-14).
In this model the O_6-C_{42} bond of the sugar fragment stays in quasi-synaxial fashion to C_5-OMe-41. Model inspection and consideration of steric constraint reveal that other orientations are competing and perhaps even more probable, *e.g.* three rotamers (around O_6-C_{42}) with the same bond in quasi-synaxial position to H-7. Anyway, ring G does not participate to ligand formation with the central Na^+: Probable coordinators are then COO^-, O-4, O_8Me, O-9, O-11, O-12 (and O-13?).



Experimental

The spectra were obtained at room temperature on a ^{1}H-nmr Varian HR-300 apparatus, equipped with homo-INDOR and decoupling facilities (SC8252–2). Septamycin free acid and septamycin-Na^+ were gifts of Sandoz Ltd., Basel. More details of the way extracting the spectral parameters and interpreting of the spectra are found in our previous papers[9,11].

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