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THE MOLECULAR STRUCTURES OF TETRANACTIN AND ITS ALKALI METAL ION COMPLEXES(1)

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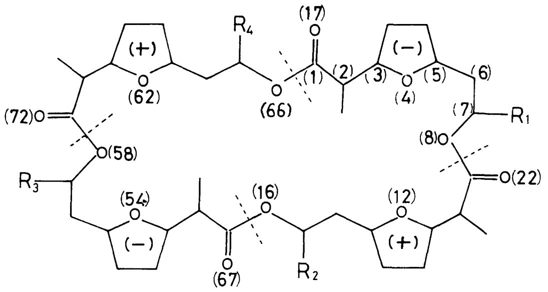
Molecular structures of tetranactin and its alkali metal ion complexes were determined by the X-ray crystal structure analysis. Molecular shape of free tetranactin is fairly flat, elongated and twisted, and outline of the molecule resembles that of propeller. Molecular conformation of the complexes is similar to that of K^+-nonactin complex. A complex formation mechanism is proposed.

Tetranactin($C_{44}H_{72}O_{12}$)(2) is a macrotetrolide antibiotic produced by Streptomyces, and is composed of four units of homononactic acids; this substance is one of the homologous compounds of nonactin(Fig. 1). Biological property of nonactins, which is similar to that of valinomycin, is well known; that is to transport alkali ions through biological membranes in the form of complexes.(3) The crystal and molecular structures of K^+-nonactin complex were reported previously(4); but only the crystals of disordered structure were obtained for nonactin.(5)



$R_1, R_2, R_3, R_4 = -CH_2CH_3$; TETRANACTIN
$R_1, R_2, R_3, R_4 = -CH_3$; NONACTIN
$R_1, R_2, R_3 = -CH_3$, $R_4 = -CH_2CH_3$; MONACTIN
$R_1, R_3 = -CH_3$, $R_2, R_4 = -CH_2CH_3$; DINACTIN
$R_1 = -CH_3$, $R_2, R_3, R_4 = -CH_2CH_3$; TRINACTIN
R_j : $-CH_2CH_3$ ---- HOMONONACTIC ACID
R_j : $-CH_3$ --------- NONACTIC ACID

Fig. 1. Structures of macrotetrolide antibiotics.
The signs discriminate between the antipodes of the constituent acids.

Accordingly, we tried to elucidate the molecular structure of uncomplexed tetranactin and to compare with those of its alkali ion complexes, in order to solve the complex formation mechanism of nonactin homologues.

The crystals of tetranactin and its metal ion complexes were obtained by the method described in the previous paper.6) The crystal of K^+-complex(Form I), which belongs to the space group $P2_1/n$, is isomorphous with that of Rb^+-complex. Another form of K^+-complex(Form II) belongs to the space group C2/c and is isomorphous with Na^+-complex. The refinement of the structures was carried out on the reflexion data collected by an automatic 4-circle diffractometer with Cu Kα radiation. The methods of the crystal structure analysis and the results of the refinement are shown in Table 1.

The molecular structures of the metal ion complexes are very similar to each other and also to the structure of K^+-nonactin complex, although a strict crystallographic diad axis of symmetry exists in the molecule of nonactin complex. However an approximate twofold symmetry axis, which passes through the metal ion and is roughly parallel to the b-axis, is found for the complex molecules in the crystals of Form I. Stereoscopic drawing of the molecular structure of Rb^+-complex is shown in Fig. 2. On the other hand, this axis of symmetry makes an angle of about 34° to [010] in the crystals of Form II. Consequently, in the complexes of tetranactin, distorsions from the twofold symmetry around the metal ion are obvious as shown in Table 2. The totally averaged ion to oxygen distances are longer than the sum of the van der Waals and the ionic radii, indicating that the bond strengths are not so strong. It is very interesting to note that in Na^+-complex, carbonyl oxygen-ion distances are about 2.44Å which are close to the value expected assuming the ionic radius of Na^+ is about 0.4Å smaller than that of K^+, while ether oxygen-ion distances are much longer. The estimated standard deviations of metal ion to oxygen distances do not exceed 0.012Å in all cases. The conformations of the tetrahydrofuran ring in the complexes are all of the envelope type.

In the case of uncomplexed tetranactin, crystallographic twofold symmetry exists at the center of the molecule, and the conformation is markedly different from those of the complexes; namely four keto oxygen atoms of ester groups are projected outside the molecule, and a pair of ethyl groups and also two carbon atoms of tetrahydrofuran rings approach each other to keep the distances of van der Waals contacts(Fig. 3). These intramolecular interactions, which suggest

Table 1. Methods and results of structure analyses.

SAMPLE	SPACE GROUP	CELL CONST.	Z	METHOD	RESULT
RbSCN-TETRANACTIN (Form I)	$P2_1/n$	a=15.927Å b=20.555 c=15.495 β=90° 23'	4	PATTERSON	R=8.5 % β_{ij}---Rb,S N,O,C B---H
KSCN-TETRANACTIN (Form I)	$P2_1/n$	a=15.796 b=20.630 c=15.613 β=90° 18'	4	(ISO-MORPHOUS)	R= 9.9% β_{ij}---K,S,N O,C B---H
KSCN-NONACTIN	Pnna	a=20.17 b=15.554 c=15.325	4	M.Dobler, et al.[4)] DIRECT METHOD	R=12.5 % β_{ij}----K,O B---S,C,H,N
KSCN-TETRANACTIN (Form II)	C2/c	a=27.455Å b=12.420 c=30.211 β=98° 09'	8	DIRECT METHOD	R=11.9 % β_{ij}---K,S O,N,C
NaSCN-TETRANACTIN (Form II)	C2/c	a=27.484 b=12.364 c=30.050 β=97° 18'	8	(ISO-MORPHOUS)	R=12.1 % β_{ij}---S,Na O,N,C
UNCOMPLEXED TETRANACTIN	C2/c	a=25.437 b= 9.455 c=24.515 β = 129° 50'	4	DIRECT METHOD	R=5.9 % β_{ij}---C,O B---H

R is defined as $100\times\Sigma\big|\,|Fo|-|Fc|\,\big|/\Sigma|Fo|$. β ij and B denote that the anisotropic and isotropic thermal vibrations were taken into account respectively for the atoms indicated.

Table 2. Metal ion to oxygen distances (Å)

Sample	Rb^+-complex	K^+-complexes		Na^+-complex
Form	Form I		Form II	
Space group	$P2_1/n$	$P2_1/n$	C2/c	C2/c
M^+---O< (4),(54)	2.953 ; 2.981	2.896 ; 2.932	2.924 ; 2.877	2.941 ; 2.838
M^+---O< (12),(62)	2.899 ; 2.925	2.855 ; 2.888	2.829 ; 2.864	2.704 ; 2.798
MEAN	2.940	2.893	2.874	2.820
M^+---O= (17),(67)	2.912 ; 2.884	2.795 ; 2.775	2.776 ; 2.760	2.426 ; 2.433
M^+---O= (22),(72)	2.934 ; 2.907	2.795 ; 2.789	2.748 ; 2.812	2.429 ; 2.452
MEAN	2.909	2.789	2.774	2.435
SUM of van der Waals' & ionic radii	2.88	2.73		2.35

The ionic radii of Rb^+, K^+ and Na^+ are taken to be 1.48, 1.33 and 0.95Å, respectively and the van der Waals radius of oxygen to be 1.40Å.

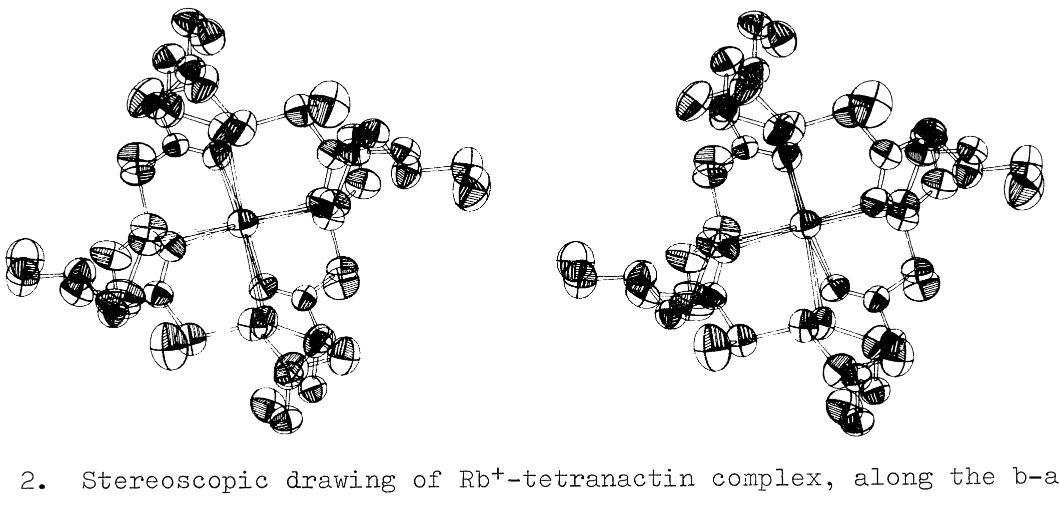


Fig. 2. Stereoscopic drawing of Rb^+-tetranactin complex, along the b-axis.

the existence of hydrophobic bonds, may contribute to stabilize the conformation of the uncomplexed tetranactin molecule. In the molecules of the complexes, metal ion-oxygen interactions are predominating. Conformation of the two of the four tetrahydrofuran rings in the uncomplexed tetranactin is envelope type and that of the remaining two is half-chair type. The molecular shape of the uncomplexed tetranactin is fairly flat, elongated and twisted, and the outline of the molecule resembles that of propeller. This is clear in Fig. 4. Difference in the molecular conformations of the complexed and uncomplexed molecules is surprising. However the internal rotation angles about the bonds of bold lines or black lines in Figs. 4 and 5 do not differ more than 10°. Accordingly, one can imagine how the free molecule of tetranactin changes its shape by the complex formation. In Figs. 4 and 5, we shall depict several modes of intramolecular motions of tetranactin during the complex formation. When metal ion approaches under the molecule of tetranactin, the rotations around the axes ① cause the carbonyl oxygen atoms(A) to come close to it and push it up through the cyclic backbone of tetranactin, while the ethyl groups turn outside of the molecule; the carbonyl oxygen atoms(B) turn inside by the rotations around the axes ② and prevent the metal ion from escaping away; the oxygen atoms(C) of tetrahydrofuran rings turn inside by the rotations around the axes ③ and hold the metal ion in the molecule; the other tetrahydrofuran rings stand up by the rotations around the axes ④ and ⑤. By these intramolecular motions, tetranactin may be able to curl up to take the ion into the molecule and form the complex.

Proton magnetic resonance(PMR) spectra of tetranactin exhibited the magnetic equivalence of the four constituent acids, indicating that the molecule of tetranactin is very flexible in the solution. This is in agreement with the results reported by J.H.Prestegard and S.I.Chan,7) and even at lower temperatures no substantial change was observed in the spectra, although coalescences of the proton signals occurred which were probably due to the decrease of speed of molecular motions and also due partly to the increase of viscosity of the solution. Furthermore PMR spectra of the complex indicated that the life time of the ions, staying at the complexed state, was fairly short in the CD_3OD solution.8) All these physicochemical properties may facilitate the ion-pump action of nonactins in the biological environments.

After completing this manuscript, we received the paper9) describing the result of the structure determination of uncomplexed nonactin, which was remarkably

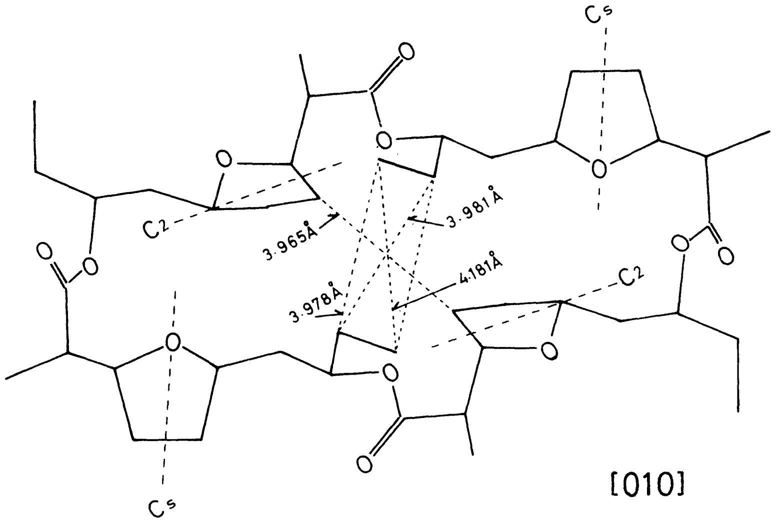


Fig. 3. Intramolecular short contacts in tetranactin.

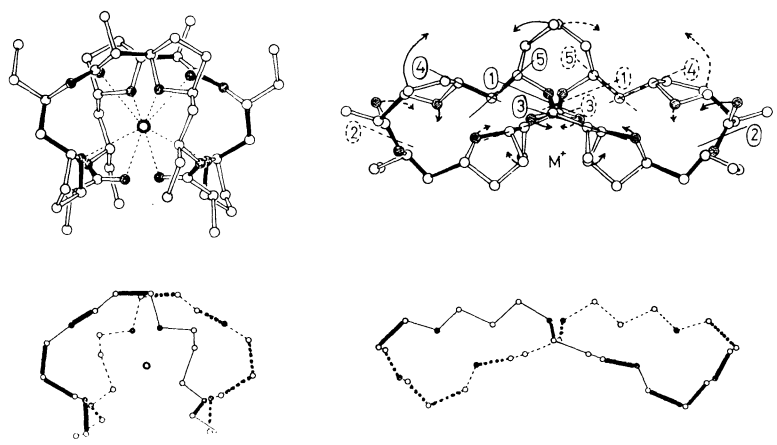


Fig. 4. Molecular conformations of tetranactins. Upper left figure is the projection of Rb^+-tetranactin complex along [100] and upper right one is that of uncomplexed tetranactin along [101]. Lower figures are the projections of the corresponding 32 membered rings showing the outlines of the molecules.

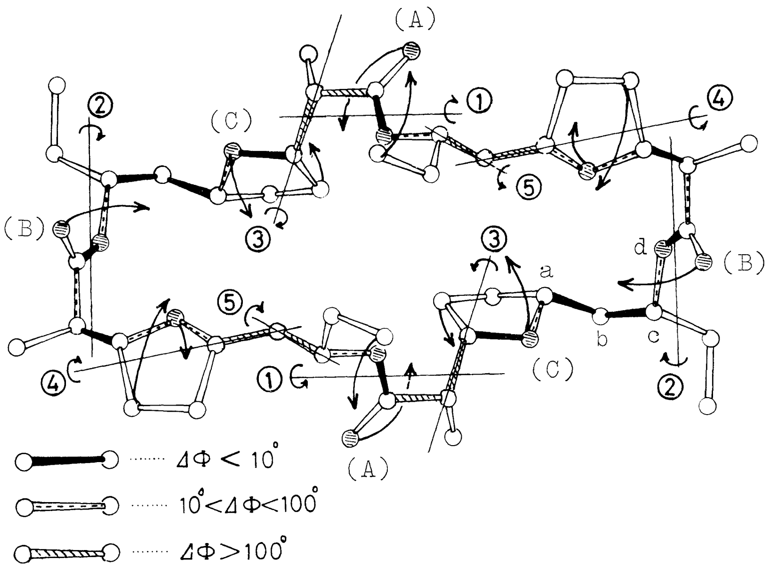


Fig. 5. Intramolecular motions of tetranactin. Internal rotation angle, Φ, around the b-c bond of a-b-c-d along the 32 membered ring is defined as the angle formed by the projection of the a-b bond with that of c-d when the projection is taken along the b-c bond. $\Delta\Phi$ denotes the difference of Φ between the uncomplexed and complexed tetranactin molecules at the corresponding bonds.

different from that of uncomplexed tetranactin. The difference of the molecular conformations may be caused by the presence of ethyl groups in tetranactin.

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