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The Crystal Structures of Rb+, K− and Na+-Tetranactin Complexes

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The molecular structures of several tetranactin complexes have been determined with X-ray reflexion data collected on a four-circle diffractometer. The structures of the form I crystals of the Rb^+ complex [space group $P2_1/n$, $Z=4$, $a=15{\cdot}927$ (9), $b=20{\cdot}555$ (14), $c=15{\cdot}495$ (6) Å, $\beta=90{\cdot}38°$ (3)] and K^+ complex were solved by the heavy-atom method, and refined by the block-diagonal matrix least-squares method: the R indices are 8·4 and 9·9%, respectively, including H atoms. The structures of the form II crystals of the K^+ complex [space group $C2/c$, $Z=8$, $a=27{\cdot}455$ (6), $b=12{\cdot}420$ (1), $c=30{\cdot}211$ (6) Å, $\beta=97{\cdot}15°$ (2)] and the Na^+ complex were determined by the symbolic addition procedure; the R indices were 11·1 and 11·9%, respectively, excluding hydrogen atoms. The molecular structures are approximately identical with those of the nonactin complexes in which all ethyl groups are replaced by methyl. Conformational energies of the complexes were calculated and the dependence of the cation selectivity on intra-ligand steric interactions was estimated. Strong attractive ion–dipole interactions were observed in an inverse proportion to ionic radii of the central cations. However, it was concluded that the ion selective behavior of the macrotetrolide must be closely related to the free energy of the hydration of cations in aqueous solutions.

Introduction

Macrotetrolide antibiotics (nonactin and its homologues) have been investigated physico-chemically as one of the electrically neutral ionophores which exhibit high K^+ selectivity in the complex formation with alkali metal ions (Morf & Simon, 1971) as well as in ion transport through biological and artificial membranes (Szabo, Eisenman & Ciani, 1969).

One of the purposes of this study was to clarify the relationship between the ion selectivity and the molecular structures of the complexed macrotetrolides in the crystal. A part of this work was presented in a preliminary report (Iitaka, Sakamaki & Nawata, 1972). In the present paper a detailed description of the crystal structure analyses of Rb^+, K^+, and Na^+-tetranactin complexes is given, in comparison with those of K^+ and Na^+-nonactin complexes (Dobler, Dunitz & Kilbourn, 1969; Dobler & Phizackerley, 1974), and their conformational energies are estimated.

Experimental

Tetranactin complexes were crystallized from a solution containing approximately equal molar concentrations of tetranactin and alkali thiocyanate by slow evaporation of the solvent. The form I crystals (prisms with square cross section, developed along [010]) of the Rb^+, and K^+ complexes, and the form II crystal (rhombic plate, with well developed faces of {101}) of the Na^+ complex were prepared from ethyl acetate–acetone (1:1, v/v); the form II crystal of the K^+ complex was prepared from acetone solutions. These crystals were colourless, transparent and brittle. Intensity data were collected on a Rigaku four-circle X-ray diffractometer by the θ–2θ scanning method (scan speed 4° 2θ min^{-1}) with Ni-filtered Cu radiation (Cu $K\alpha$, $\lambda=1{\cdot}54052$ Å). Data for the intensity measurements are summarized in Table 1. Lattice constants were determined by the least-squares refinement of the angular settings for 15 reflexions carefully centred on a diffractometer at 21 °C. The densities were measured by flotation in aqueous KI solution at 21 °C.

Crystal structure analyses

(*a*) The heavy-atom method was applied to the crystal structure analysis of the Rb^+-tetranactin complex. The rubidium ion lies close to the glide plane ($y=0{\cdot}2553$) and a pseudo-mirror symmetry of the molecule appeared on the subsequent electron density map. However, 48 out of 57 non-hydrogen atoms were located by assuming a molecular structure similar to that of the K^+-nonactin complex. In the final stage of the refinement, the three highest peaks on the difference electron density map were assigned to the SCN^- group. That the peak heights did not correspond to the atomic species and that they were not arranged linearly suggested that the thiocyanate ion is disordered in the crystal. 72 H atoms were also found on the map and they were included in the calculation of the structure factors but their parameters were not refined. Refinement of the structure was carried out by the least-squares method (block-diagonal matrix approximation) with unit weights. The atomic scattering factors for C and O were those given in *International Tables for X-ray Crys-*

tallography (1962) as SX-6 and 8 respectively and for H those given by Stewart, Davidson & Simpson (1965). The final R index was 8·4% including anisotropic temperature factors for the non-hydrogen atoms. Estimated standard deviations (e.s.d.'s) for C–C bonds and C–C–C angles were 0·022 Å and 1·6°, respectively.

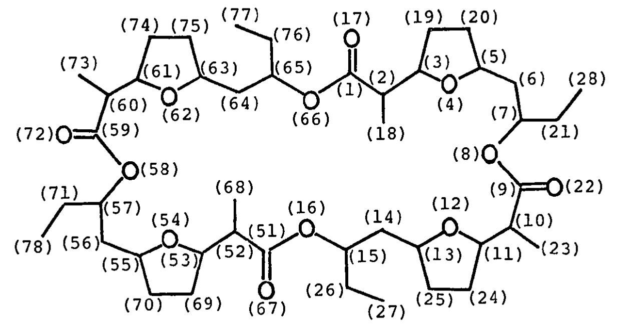
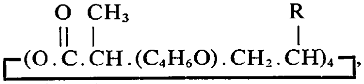


Fig. 1. Structure of tetranactin. The chemical structures of tetranactin (I) and nonactin (II) are



$$\text{(O.}\overset{\overset{O}{\|}}{C}\text{.}\overset{\overset{CH_3}{|}}{CH}\text{.(C}_4\text{H}_6\text{O).CH}_2\text{.}\overset{\overset{R}{|}}{CH}\text{)}_4$$

where R = Et in (I) and Me in (II).

(*b*) The crystal structure of K$^+$–tetranactin complex (form I crystal) was determined by assuming the same structure as the Rb$^+$ complex with Rb$^+$ replaced by K$^+$. The refinement was carried out with the observed structure factors of the K$^+$ complex. The final R index was 9·9%. The e.s.d.'s for C–C and C–C–C were 0·016 Å and 0·9°, respectively.

(*c*) The symbolic addition method (Karle & Karle, 1966) was applied to the crystal structure analysis of the K$^+$ complex (form II crystal). Statistical values for the normalized structure factors ($|E_o|$) indicated the centrosymmetric structure. The phases of 471 reflexions with $|E_o| > 1{\cdot}8$ were determined starting with two origin-specifying reflexions plus four symbols. The phase refinement by the tangent formula was successfully carried out; the R index for the normalized structure factors was 26%. The molecular structure of the complex was recognized on the E map and the positions of 53 non-hydrogen atoms including K$^+$ were easily determined. The thiocyanate ion was also disordered in the form II crystal, and the three highest peaks on the difference electron density map were assigned to the SCN$^-$ group. The structure was refined as in (*a*). The final R index was 11·1% excluding H

Table 1. *Crystal data and intensity-measurement data*

M$^+$(I) and M$^+$(II) represent the form I and form II crystals of tetranactin complexes respectively.

Sample	Rb$^+$(I)	K$^+$(I)	K$^+$(II)	Na$^+$(II)
Formula	$C_{44}H_{72}O_{12}RbSCN$	$C_{44}H_{72}O_{12}KSCN$	$C_{44}H_{72}O_{12}KSCN$	$C_{44}H_{72}O_{12}NaSCN$
F.W.	936·0	889·6	889·6	873·5
Crystal form	Form I	Form I	Form II	Form II
Crystal system	Monoclinic	Monoclinic	Monoclinic	Monoclinic
a (Å)	15·927 (9)	15·796 (3)	27·455 (6)	27·484 (7)
b (Å)	20·555 (14)	20·630 (15)	12·420 (1)	12·364 (2)
c (Å)	15·495 (5)	15·613 (11)	30·211 (6)	30·050 (5)
β (°)	90·38 (3)	90·30 (3)	97·15 (2)	97·30 (3)
U (Å^3)	5072·3	5078·7	10195·1	10129·1
Space group	$P2_1/n$	$P2_1/n$	$C2/c$	$C2/c$
Z	4	4	8	8
D_x (g cm^{-3})	1·225	1·161	1·159	1·145
D_m (g cm^{-3})	—	1·14	1·19	—
μ(Cu $K\alpha$) (cm^{-1})	23·0	17·3	17·3	11·0
Crystal size (mm)	0·4 × 0·4 × 0·4	0·8 × 0·3 × 0·3	0·6 × 0·5 × 0·2	0·6 × 0·5 × 0·2
2θ max (°)	110	110	100	110
Number of observed reflexions	3694	3441	2838	3694

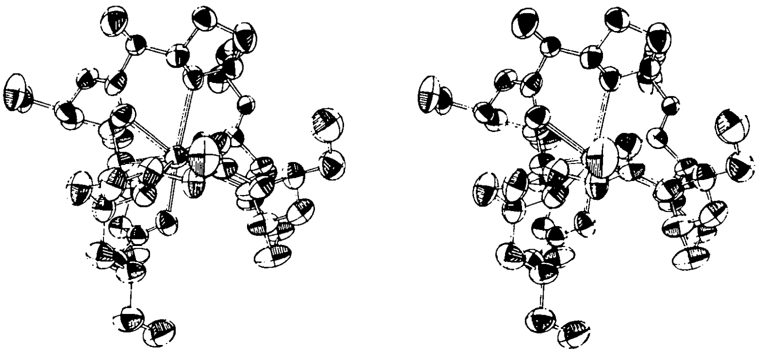


Fig. 2. Stereoscopic drawing of the Na$^+$–tetranactin complex along [010] drawn by *ORTEP* (Johnson, 1965).

atoms. The e.s.d.'s for C–C and C–C–C were 0·022 Å and 1·2° respectively.

(*d*) The crystal structure of the Na$^+$–tetranactin complex (form II crystal) was determined by refining the structure of the isomorphous K$^+$ complex, and replacing K$^+$ by Na$^+$. The final R index was 11·9% excluding H atoms. The e.s.d.'s for C–C and C–C–C were 0·020 Å and 1·1° respectively.

Results and discussions

Molecular structure

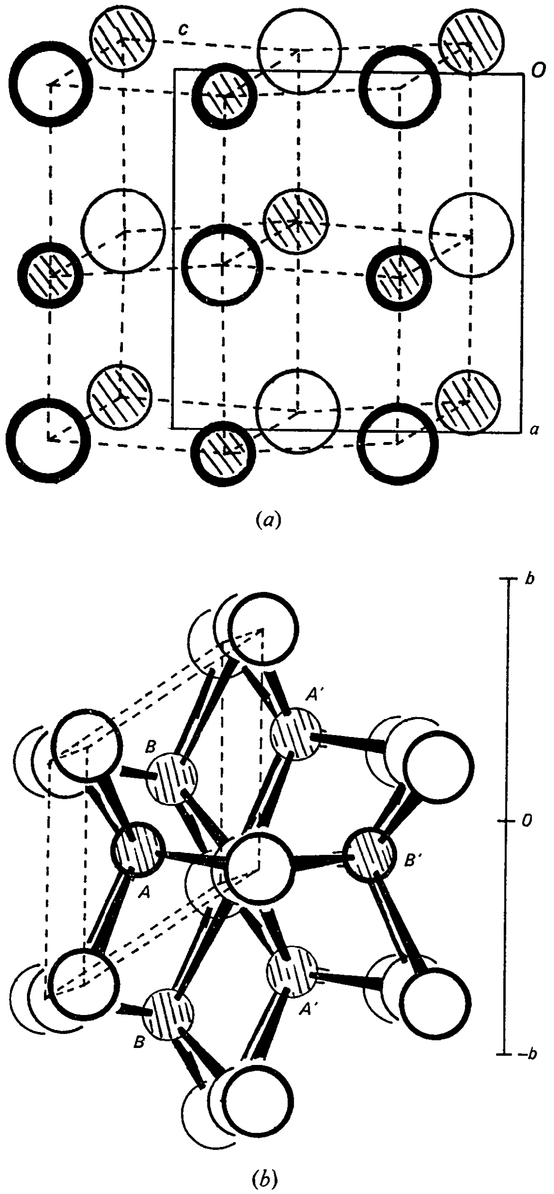
The atom numbering is shown in Fig. 1. The final atomic parameters are listed in Table 2(*a*), (*b*), (*c*), and (*d*).* The interatomic distances and angles, and the internal rotation angles are given in Table 3(*a*) and (*b*) respectively.*

The molecular structures of the alkali metal ion–tetranactin complexes, Fig. 2, are approximately identical with those of the nonactin complexes, which possess an approximate $S_4(\bar{4})$ symmetry. The 32-membered ring of the complex is rounded up to form a saddle or seam of a tennis ball, and adopts a spherical molecular shape in which a distorted cubic coordination of the O atoms to the central metal ion (M$^+$) is completed. The four O atoms of the tetrahydrofuran (THF) rings are arranged tetrahedrally around M$^+$, while the methylene parts of the THF's turn to the outside of the complex. Although the four O atoms of the ester carbonyl groups are located in the vicinity of the surface of the molecule, they are sheltered by the four ethyl groups

Table 5. *Coordination geometry around the central cation*

The bond distances (Å) between the central cation and the ligand O atoms, and the angles (°) between the directions of the bond and the lone-pair orbitals of the O atoms, are listed.

	Rb$^+$(I)	K$^+$(I)	K$^+$(II)	Na$^+$(II)
Ether				
O(4)	2·98	2·91	2·86	2·80
	7;102	10;99	11;98	11;98
O(12)	2·93	2·88	2·92	2·94
	8;101	9;100	5;104	6;103
O(54)	2·95	2·89	2·83	2·70
	9;100	10;99	9;99	13;96
O(62)	2·90	2·85	2·88	2·84
	10;100	10;100	4;104	6;104
av.	2·94	2·88	2·87	2·82
	8;101	10;99	7;101	9;101
Carbonyl				
O(17)	2·88	2·78	2·81	2·45
	78;81	71;84	69;93	62;83
O(22)	2·91	2·77	2·77	2·43
	76;83	73;83	63;98	59;81
O(67)	2·91	2·79	2·75	2·43
	76;83	68;85	63;100	58;87
O(72)	2·93	2·78	2·76	2·43
	73;86	70;84	65;97	65;82
av.	2·91	2·78	2·77	2·44
	76;83	71;84	65;97	61;83



(*a*)

(*b*)

Fig. 3. (*a*) Arrangement of ions in the form I crystals viewed along [010] showing a distorted NaCl type structure. The tetranactin complex cations and thiocyanate anions are shown by large circles and small shaded circles respectively. The circles drawn with heavy lines represent the ions at $y \simeq 0{\cdot}7$ and those with thin lines at $y \simeq 0{\cdot}2$. For clarity, nearest neighbour ions are connected by broken lines. (*b*) Arrangement of ions in the form II crystals projected on $(10\bar{1})$ showing a modified NiAs type structure. The heights of A and B thiocyanate ions are about $\frac{1}{4}$ and $\frac{3}{4}$, respectively and those of A' and B' $\frac{3}{4}$ and $\frac{1}{4}$, instead of $\frac{1}{4}$ and $\frac{3}{4}$ in NiAs. For clarity, nearest neighbour ions are connected by dark bonds, and the hexagonal unit cell is shown by broken lines.

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* F_o and F_c tables and Tables 2 (positional parameters and temperature factors), 3 and 4 have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 31287 (67 pp., 2 microfiches). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

from the exterior. These structural features may enhance the lipophilic nature of the molecular surface, and contribute to stabilization of the complexes (Ueno, Akutsu, Kyogoku & Nawata, 1974).

There are no appreciable differences in conformation among the complexes for the 32-membered ring, as is indicated by the Φ* values given in Table 3(*b*). The Φ's at the ester groups in the Na^+ complex are slightly different from those in the others, causing the torsional energy around these groups to be increased. The conformation of the THF rings of the complexes is of the envelope type, as can be seen from the Φ's in the THF rings. In the THF rings C(19), C(24), C(69), and C(74) deviate by about 0·6 Å from the respective least-squares planes formed by the rest of the atoms (Table 4).*

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* Internal rotation angle.

* See footnote on p. 770.

Table 6. *Short contacts* (Å) (*less than* 3·7 Å)

	Rb^+(I)	K^+(I)
$O(8^{i})\cdots C(70^{ii})$	3·66	3·67
$O(16^{i})\cdots C(75^{iii})$	3·58	3·63
$O(16^{i})\cdots C(64^{iii})$	3·62	3·64
$C(25^{i})\cdots O(66^{iii})$	3·59	–
$C(28^{i})\cdots C(77^{ii})$	–	3·61

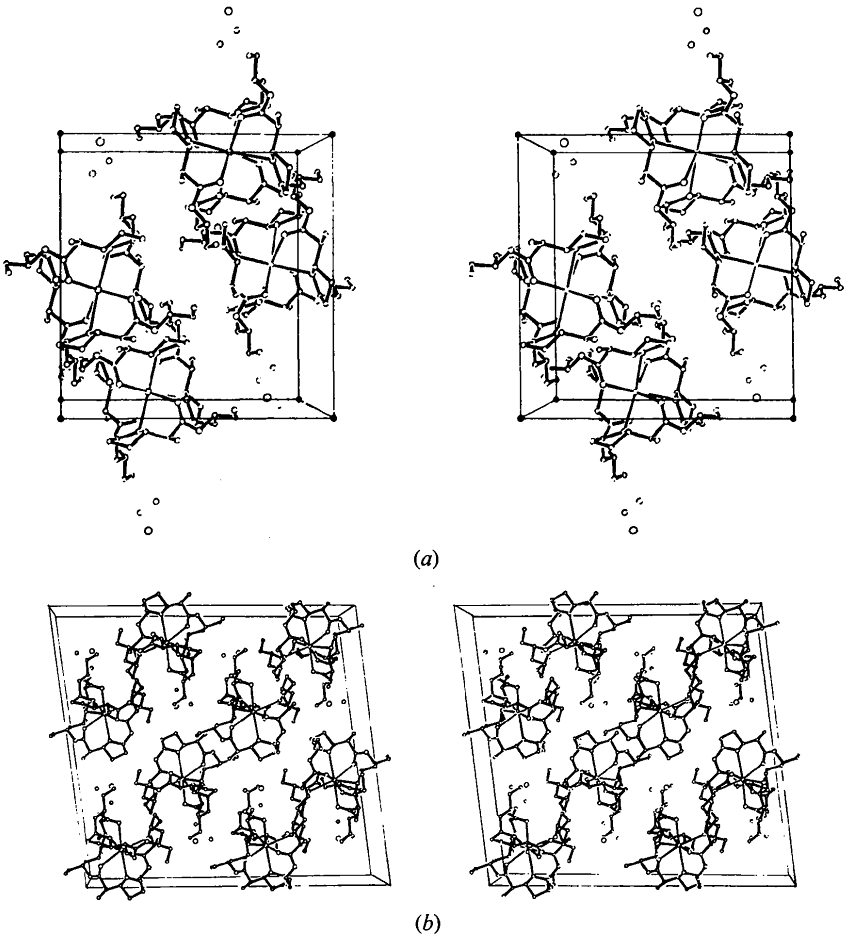
Symmetry code

(i) $x, \quad y, \quad z$
(ii) $-\frac{1}{2}+x, \frac{1}{2}-y, \frac{1}{2}+z$
(iii) $-\frac{1}{2}+x, \frac{1}{2}-y, -\frac{1}{2}+z$

	K^+(II)	Na^+(II)
$C(6^{i})\cdots O(8^{ii})$	3·56	3·58
$O(8^{i})\cdots C(21^{ii})$	3·59	3·60
$O(16^{i})\cdots C(24^{iii})$	3·42	3·50
$O(16^{i})\cdots C(25^{iii})$	3·57	3·63
$C(64^{i})\cdots O(66^{iv})$	3·67	3·67

Symmetry code

(i) $x, \quad y, \quad z$
(ii) $-x, \quad y, \frac{1}{2}-z$
(iii) $\frac{1}{2}-x, \frac{1}{2}+y, \frac{1}{2}-z$
(iv) $-x, 1-y, -z$


(*a*)

(*b*)

Fig. 4. (*a*) Stereoscopic view of the crystal structure of the K^+-tetranactin complex (form I) along [010]. (*b*) Stereoscopic view of the crystal structure of the Na^+-tetranactin complex (form II) along [010].

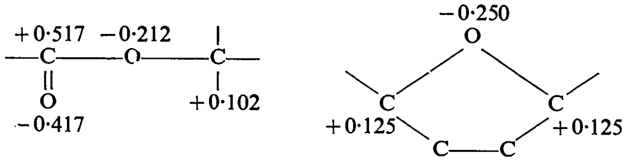
Table 5 shows the geometry of the coordination around M^+. The directions of lone-pair orbitals of the O atoms were calculated by assuming a tetrahedral arrangement for the ether O atoms and a trigonal arrangement for the carbonyl O atoms. It can be seen that for each ether O atom, the direction of a lone-pair orbital nearly coincides with that of the bond connecting the O atom to M^+, while for the carbonyl O atoms, none of the orbital directions coincide with the bond directions. It is therefore concluded that the $M^+\cdots O$ (ether) interactions may be more strengthened by the influence of lone-pair electrons than would be expected for usual electrostatic interactions involving ether O atoms. In the ammonium–tetranactin complex (Nawata, Sakamaki & Iitaka, 1975) the ether O atoms form stronger hydrogen bonds than the carbonyl O atoms to the central ammonium ion.

The distances between M^+ and the carbonyl O atoms are close to the sum of the ionic radius of M^+ and the van der Waals radius of O atoms (Pauling, 1960), which shows that the electrostatic interactions involving the carbonyl O atoms will be increased as the ionic radius of the central ion becomes smaller. The distances between M^+ and the ether O atoms, on the other hand, do not vary greatly with the radius of M^+, as is apparent from Table 5. In particular, if one compares the difference in the average $M^+\cdots O$ (ether) distances between K^+ and Na^+ complexes, it can be seen that the difference is only about $\frac{1}{6}$ of that found in $M^+\cdots O$ (carbonyl) distances. In this case, a close approach of the ether O atoms to the central ion may be obstructed by repulsions between the coordinated O atoms and/or by the deformations of the ligand molecule which compel it to take an unstable conformation.

Table 7. *Conformational energy* (kcal $mole^{-1}$)

U_{NB} = non-bonded interaction energy by Lennard-Jones 6–12 potential function. Cut-off distance was 7·0 Å. H atoms, for which positions were stereochemically assumed as follows, were included in the calculation. (i) Methyl hydrogen atoms: staggered conformation with C–H bond length 1·10 Å, and C–C–H bond angles 109·5°. (ii) Methylene hydrogen atoms: C–H bond length 1·08 Å, and C–C–H bond angles 109·5°. (iii) Methine hydrogen atoms: C–H bond length 1·07 Å, and main chain C or O–C–H bond angles 109·5°. $U_{TOR}=\sum(U_o/2)\,(1\pm\cos n\Phi)$. $U_o=U_{TOR}$ when $\Phi=0°$, Φ = torsional angle, n = n-rotational symmetry around the bonds. Negative and positive signs indicate that at $\Phi=0°$ the bonds possess the minimum and maximum U_{TOR} respectively.

The following values were used: $U_o=2{\cdot}8$ kcal $mole^{-1}$ and $+\cos 3\Phi$ around $-\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}-$ bond, $U_o=0{\cdot}2$ kcal $mole^{-1}$ and $-\cos 3\Phi$ around $-\overset{|}{\underset{|}{C}}-\underset{\overset{\|}{O}}{C}-$ bond, $U_o=8{\cdot}75$ kcal $mole^{-1}$ and $-\cos 2\Phi$ around $-\overset{|}{\underset{|}{C}}-\underset{\overset{\|}{O}}{C}-O-\overset{|}{\underset{|}{C}}-$ bond, $U_o=1{\cdot}07$ kcal $mole^{-1}$ and $+\cos 3\Phi$ around $-\overset{|}{\underset{|}{C}}-O-\overset{|}{\underset{|}{C}}-$ and $-\overset{|}{\underset{|}{C}}-O-\underset{\overset{\|}{O}}{C}-\overset{|}{\underset{|}{C}}-$ bonds. $U_{EL}=332{\cdot}0\ \sum(q_iq_j/Dr_{ij})$ = Coulomb's force. U_{ID} = ion–dipole interaction = Coulomb's force. r_{ij} = distance (Å) between the partial charge q_i and q_j; D = apparent dielectric constant. $D=1$ was used in the calculation. No contribution to the U_{EL} and U_{ID} was included for interaction between atoms within a group of atoms constituting a dipole. Electronic partial charges used are as follows:



${}^{0}_{H}\Delta G$ = hydration energy (kcal $mole^{-1}$) of the cations (*Chemical Handbook*, 1956). K = complex formation constant (in methanol).

Sample	U_{NB}	U_{TOR}	U_{EL}	$U_{NB}+U_{EL}+U_{TOR}$	U_{ID}	$-\Delta G^0_H$	K (l $mole^{-1}$)
Na^+(II)	−83·07	26·00	10·45	−46·62	−79·42	96·5	5×10^2 [f]
K^+(I)	−80·54	25·72	6·22	−48·60	−63·85	76·5	3×10^4 [f]
Rb^+(I)	−78·35	25·55	4·24	−48·56	−53·09	69	2×10^4 [f]
Na^+-nonactin[a]	−76·52	24·73	10·63	−41·16	−80·41	96·5	$2{\cdot}4\times10^2$ [g]
K^+-nonactin[b]	(−57·64)[e]	27·58	4·23	–	−56·85	76·5	$3{\cdot}8\times10^3$ [g]
Tetranactin[c]	−73·19	31·31	0·53	−41·35			
Tetranactin (nonactin-form conformation)	−65·20	28·73	2·55	−33·92			
Nonactin[d]	−57·85	28·73	2·55	−26·57			
Nonactin (tetranactin-form conformation)	−64·55	31·31	0·53	−32·71			

(*a*) Dobler & Phizackerley (1974). (*b*) Dobler, Dunitz & Kilbourn (1969). (*c*) Nawata, Sakamaki & Iitaka (1974). (*d*) Dobler (1972). (*e*) The distance between C(14) and H2(C26) in the nonactin molecule is extraordinarily short (2·05 Å), and its contribution to U_{NB} (+5·03 kcal $mole^{-1}$) is unreasonably larger than the others. (*f*) Ueno *et al.* (1974). (*g*) Morf & Simon (1971), at 30°C.

Crystal structures

The crystal structures of K^+ and Na^+–tetranactin complexes are shown in Figs. 3 and 4, respectively, which represent the structures of the form I and form II crystals.

The form I crystals of the tetranactin complexes possess unit-cell dimensions approximately identical with those of the nonactin complexes. The crystal structure of this form can be described as a distorted rock-salt type structure, if the complex and SCN^- ions are regarded as simple spherical ions [Fig. 3(*a*)]. In the tetranactin complexes, the distortion is more remarkable than in the nonactin complexes and for smaller ions such as Na^+ only the crystals of form II are found.

In the form II crystals of the tetranactin complexes, the axis of approximate symmetry [$S_4(\bar{4})$] of the molecule makes an angle of about 34° with [010], whereas this axis is roughly parallel to [010] in the form I crystals. The crystal structure of this form can be described as a modification of the NiAs type structure [Fig. 3(*b*)], although no particular interaction corresponding to that observed in the latter structure between the Ni atoms is found in this case.

In both crystal structures, each ionic group is surrounded by six counter ionic groups. The packing of the molecules in the form II crystals is slightly more dense than in form I. The volumes for one structural unit calculated for the two forms of K^+ complex are 1271·9 Å^3 (form I) and 1274·4 Å^3 (form II), and some shorter intermolecular distances are found in K^+ (form II) as compared with K^+ (form I) (Table 6). This is not surprising, since in the NiAs structure, a close contact is permissible not only between unlike atoms but also between like atoms.

Conformational energy

In order to estimate the stability of various conformations of tetranactin and nonactin molecules, the conformational energy was calculated by summing up non-bonded interaction energies (U_{NB}), torsional energies (U_{TOR}), and electrostatic interaction energies (U_{EL} and U_{ID}) (Ooi, Scott, Vanderkooi & Scheraga, 1967; Yan, Vanderkooi & Scheraga, 1968). The result is shown in Table 7.

The free molecule of tetranactin whose atomic coordinates were assumed to be those given by the X-ray analysis (Nawata, Sakamaki & Iitaka, 1974) possesses considerably larger attractive U_{NB} than that of nonactin for which the conformation was found to be like a torus (Dobler, 1972). With formation of the complex, a more negative (stable) U_{NB} was estimated for either molecule, in inverse proportion to the ionic radii of M^+.

U_{TOR}'s of the complexed molecules are, in general, lower than those of the uncomplexed molecules. Although the Na^+–nonactin complex has a lower U_{TOR} than K^+–nonactin, there is no appreciable difference in U_{TOR} among the tetranactin complexes. On the other hand, the repulsive U_{EL} of the ligand molecule in the complexed state makes the complex less stable, in inverse proportion to the ionic radii of M^+, but this is completely compensated by the strong attractive ion–dipole interactions [U_{ID}, calculated following the procedure of Ooi *et al.* (1967)] between M^+ and the ester and ether groups, in inverse proportion to the ionic radii.

It is interesting that a certain dependence of the cation selectivity (represented by K) on intra-ligand steric interaction ($U_{NB}+U_{TOR}+U_{EL}$) is observed for the complexes, although more reasonable values may be estimated by using larger partial charges on ester and ether groups induced by the central ion. The fact that the tetranactin complexes possess higher stabilities than those of the corresponding nonactin complexes may be caused by the more negative U_{NB} energies of the former, and by the more negative U_{ID} energy found in the K^+–tetranactin complex.

As pointed out by Prestegard & Chan (1970), the free energy of hydration plays an important role in ion selectivity as far as the selectivity for the alkali metal ions in aqueous solvents is concerned.

In the case of uncomplexed molecules, U_{TOR} of tetranactin is higher and less stable [owing to the molecule's elongated conformation (Nawata, Sakamaki & Iitaka, 1974)] than that of nonactin, whereas U_{NB} of tetranactin is considerably lower, as stated before; hence the intra-ligand steric interaction of tetranactin becomes appreciably lower than that of nonactin. The same also holds for the two structures of tetranactin, one of which is that of free tetranactin and the other the hypothetical nonactin-type conformation.* Furthermore it can be shown that even for two structures of nonactin where one is the hypothetical tetranactin-type structure† and the other that found in free nonactin, U_{NB} is lower in the tetranactin-type structure. The present conformational energy calculation indicates that the tetranactin-type conformation is more stable than the nonactin-type conformation. Although the calculated conformational energy of the tetranactin molecule is lower than that of nonactin, the corresponding protons and carbons of the four structural subunits in each of these molecules were observed to be magnetically equivalent in the n.m.r. spectra (Prestegard & Chan, 1969; Pretsch, Vasák & Simon, 1972; Kyogoku, Ueno, Akutsu & Nawata, 1975), probably because of their rapid intramolecular motions. On the other hand, the broad Raman band of the ester carbonyl groups of uncomplexed nonactin in chloroform solution suggested the simultaneous presence of several related conformers (Asher, Phillies & Stanley, 1974); it is therefore probable that the tetranactin-type conformer is present in solution.

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* In the hypothetical structure, four methyl groups are added to the nonactin molecule (Dobler, 1972) at the sites where the internal rotation angles at C–C–C–C bonds are consistent with those observed in the tetranactin molecule.

† In the hypothetical structure, four ethyl groups observed in the tetranactin molecule are replaced by four methyl groups.

The calculations were carried out on the HITAC 5020 E and 8800/8700 computers at the Computer Centre of the University of Tokyo, with the programs *SYMMAIN* (Matsuzaki & Iitaka, 1971) for the symbolic addition procedure and application of the tangent formula, *HBLS* IV (Okaya & Ashida, 1967) for the least-squares refinement and *ORTEP* (Johnson, 1965) for the stereoscopic drawing.

The purified samples of tetranactin were isolated by Dr K. Ando of Chugai Pharmaceutical Co. Ltd to whom the authors' thanks are due.

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