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MOLECULAR STRUCTURE OF DINACTIN[1)]

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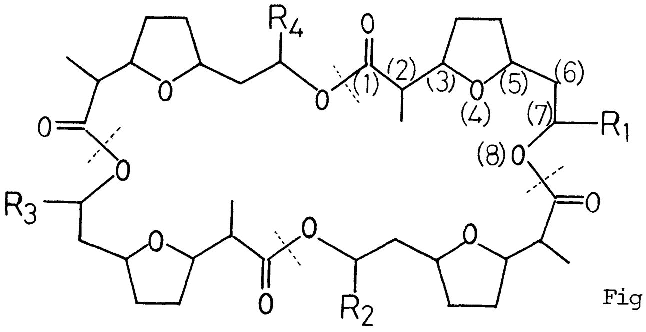
The molecular conformation of dinactin, one of the homologues of macrotetrolide antibiotics nonactin, was determined by the X-ray diffraction method using a mixed crystal of dinactin and monactin (7:3). The conformation of the 32-membered ring looks a deformed cradle with an asymmetric shape, and can approximately be described as a hybrid of the nonactin and tetranactin type conformations. Dinactin type conformer has the lowest conformation energy among the three.

Macrotetrolide antibiotics, nactins (nonactin and its homologues), are widely known as ionophoric substances which can carry K^+ ions selectively across biological and artificial membranes.[2)] The structures of various alkaline ion complexes as well as the free molecules of nonactin and tetranactin have been extensively studied in both crystalline states[3-7)] and in solutions.[8,9)] Dinactin ($C_{42}H_{68}O_{12}$), one of the nactins, consists of an alternating sequence of homononactic and nonactic acid residues (Fig. 1). The IR spectra of mixed crystals of dinactin and monactin (7:3) exhibit broader and simpler absorption bands than those observed for crystalline nonactin and tetranactin and resemble closely the spectra observed for the nactins in solution.[10)]



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_1, R_2, R_3, R_4 = $-CH_2CH_3$; TETRANACTIN
R_i: $-CH_2CH_3$ ···· HOMONONACTIC ACID
R_i: $-CH_3$ ············ NONACTIC ACID

Fig. 1. Chemical structure of nactins

Dinactin is barely crystallized in usual organic solvents and the crystals suitable for the analysis are not obtained. But a mixture of dinactin and monactin ($C_{41}H_{66}O_{12}$) (approximate molar ratio = 7:3, determined by MS analysis) gives a triclinic crystal in a benzene solution having the cell dimensions a=10.531(5), b=25.230(10), c=9.422(4) Å, α=91.18(4), β=116.07(5), γ=96.84(5)°, space group $P\bar{1}$, Z=2, ρ_{calc}=1.13 g cm^{-3}, mol wt = 760.8 (calculated for the formula $C_{41.7}H_{67.4}O_{12}$). Intensity data were collected on a four circle diffractometer (Philips PW 1100) using Cu $K\alpha$ radiation monochromated by a graphite plate. 5174 independent reflexions ($I_o > 2\sigma(I_o)$) were used for the structure analysis. The crystal structure was solved by the direct method (MULTAN)[11)] using 386 reflexions. The terminal carbon atoms of ethyl groups in dinactin and monactin were found to be randomly distributed over the four positions. Thus no distinctions were made for ethyl and methyl groups at the four C7 positions of the molecule and a half weight carbon atom was assumed for the four terminal ethyl groups. Least-squares calculations with block-diagonal approximations yielded the final R index of 8.2% including hydrogen atoms. The e.s.d.'s of bond lengths and angles for the non-hydrogen atoms (excluding those of the ethyl groups) were 0.008 Å and 0.4°, respectively.

As is shown by an ORTEP drawing (Fig. 2), the shape of the dinactin molecule is rather elongated but curled up on one end and looks like a lobster. The overall conformation of the 32-membered ring (deformed cradle shape with C_1 symmetry) is markedly different from either nonactin (torus shape with C_2 symmetry but approximate S_4 symmetry)[6] or tetranactin (propeller shape with C_2 symmetry)[7] and constitutes a new conformation which has not been previously observed. However, a comparison of the torsional angles (Φ) along the 32-membered rings of dinactin and other nactins revealed that the conformations of the four subunits in dinactin may be regarded as the hybrid of those found in the tetranactin and nonactin molecule (Fig. 3). The largest differences in Φ exceed 90° which correspond to the change from trans to gauche or vice versa. In the dinactin molecule, the intramolecular short contacts are observed between the alkyl groups and the oxygen atoms (Fig. 4). These van der Waals contacts may stabilize the molecular conformation of dinactin. In the case of tetranactin molecule, two ethyl groups and two methylene groups approach each other in pairs to give

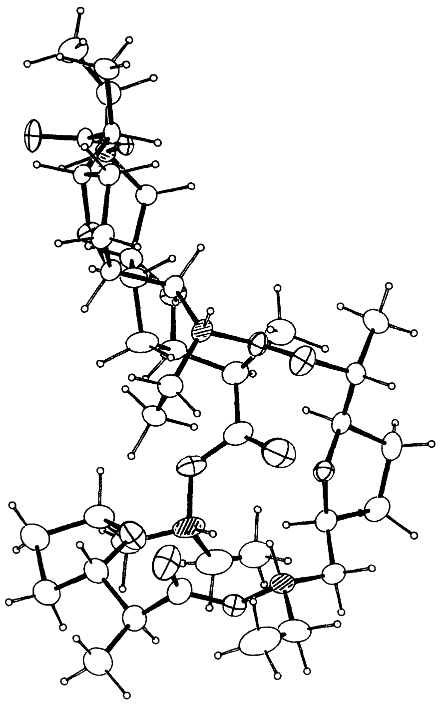


Fig. 2. Molecular structure of dinactin viewed along [100].[12] The two ethyl groups are distributed at the four C7 sites (shaded atoms). Non-hydrogen atoms are represented by the thermal ellipsoids of 20% probability. The principal ellipses are added to the oxygen atoms.

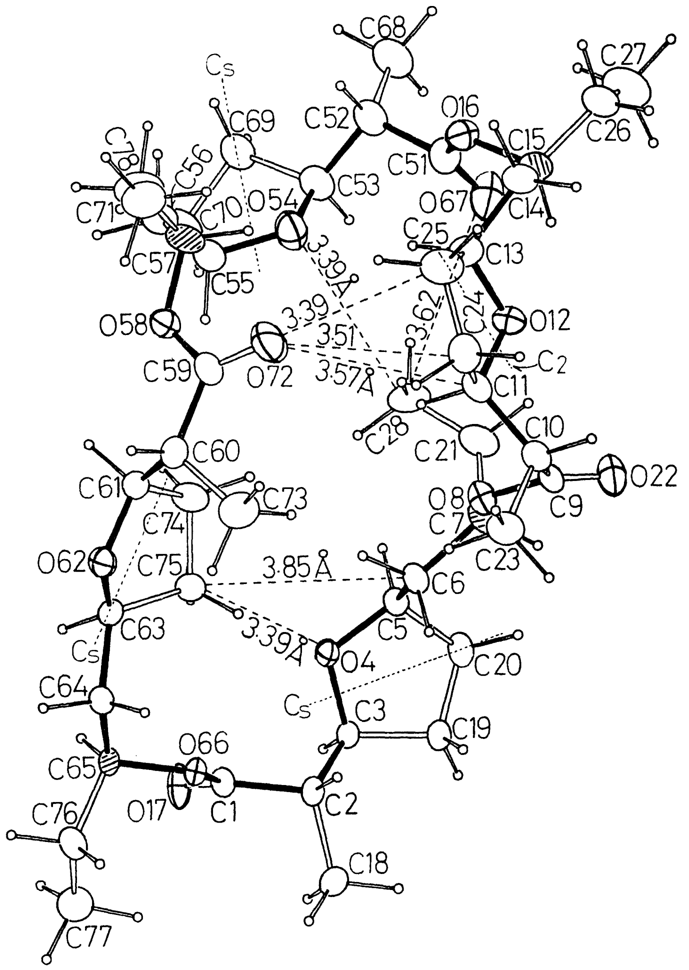


Fig. 4. Intramolecular short contacts in dinactin viewed along [001].[12] A 32-membered ring is shown by thick bonds, and the approximate symmetry of each tetrahydrofuran ring is indicated.

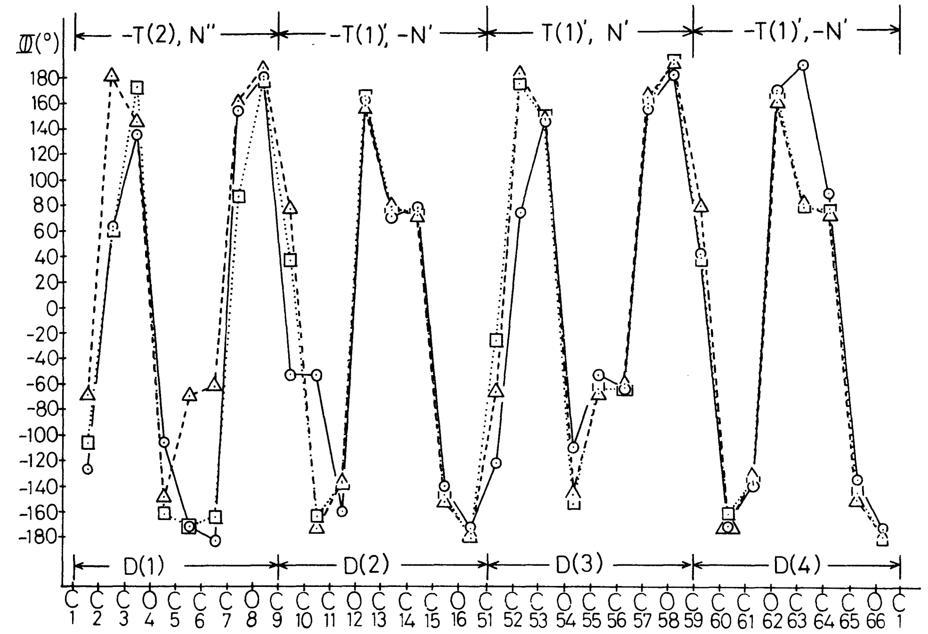


Fig. 3. A comparison of the backbone torsional angles along the 32-membered ring: dinactin (⊙), nonactin (△) and tetranactin (⊡). The conformations of the subunits in dinactin are grouped in the four types, D(1), D(2), D(3) and D(4) and they are compared with those of tetranactin (T(1), T(2)) and of nonactin (N, corresponding Φ values in the four subunits are averaged assuming the approximate S_4 symmetry), where a minus sign indicates the enantiomer of the subunit and the primes denote that one or two Φ values differ from dinactin more than 90° (double primes denote more differences). Note that in dinactin the two ethyl groups are distributed over four C7 positions and no distinctions have been made for the nonactic and homononactic acid residues.

Table 1. Spin-spin coupling constants of ^{1}H-NMR spectra (220 MHz) of nactins

	dinactin (calc.)	tetranactin (calc.)	nonactin (calc.)	nonactin (obs.†)[8] (in $(CD_3)_2CO$)	tetranactin (obs.)[9] (in $CDCl_3$)
$J_{H2,H3}$	8.4	7.4	2.6	7.6±0.4	7.8
$J_{H5,H6}$ $J_{H5,H6'}$	6.2††	6.2	5.4	6.2±0.4	7.0
$J_{H7,H6}$ $J_{H7,H6'}$	5.6	6.4	6.0	6.4±0.4	6.0

J(Hz) = Jo $\cos^2\Phi - 0.28$ (Jo = 10.9 Hz for $90° \leq \Phi \leq 180°$, Jo = 9.9 Hz for $0° \leq \Phi \leq 90°$)[9]

Spin-spin coupling constants are evaluated using the observed dihedral angles by the Karplus relation described above. The averaged J values of the four subunits are listed in the Table. The dihedral angles of tetranactin and nonactin are calculated by the atomic coordinates given by the papers, refs. 7 and 6, respectively.

†There is no observation for the J values of dinactin at 220 MHz, but 60 MHz spectra of nactins show very similar line profiles of the main chain proton signals to each other.

††The mean value $(J_{H5,H6}+J_{H5,H6'})/2$. The same holds for $J_{H7,H6'}$ and $J_{H7,H6}$.

short intramolecular contacts. It would be interesting to know which is the most stable conformation of the molecule in these three types of structures. Conformation energy of the dinactin type conformer is estimated according to the method described in the previous papers.[5)] The result shows that non-bonded interactions = -320.9 kJ mol^{-1}, electrostatic interactions = 0.4 kJ mol^{-1} and torsional energies = 118.4 kJ mol^{-1}. These values are all lower than the corresponding ones found in the other two conformers. Especially, the non-bonded energies are lower by about 48.1 and 14.6 kJ mol^{-1} than those of the nonactin type and tetranactin type conformers, respectively.[5)] In the dinactin molecule, the conformations of the four subunits are somewhat different from each other. Three of the four tetrahydrofuran rings are of the envelope type in which chemically non-equivalent C20, C63 and C69 atoms are out of the least-squares planes, formed by the rest of the atoms in each ring. The fourth ring takes a half-chair conformation which has not been observed in the crystal structure of nactins. These structural features may be responsible for the broadening of the IR spectra of dinactin in crystalline state. ^{1}H-NMR spectra of nactins indicated that the proton signals of the four subunits in the 32-membered ring are magnetically equivalent and the nactin molecules in solution are in a dynamic equilibrium of numerous conformers.[8,9)] This was considered to be in accord with the approximate S_4 symmetry observed for the crystal structure of nonactin. However, the vicinal spin coupling constant $J_{H2,H3}$ for the nonactin molecule, calculated by the Karplus relation using the observed dihedral angles (2.6 Hz), is too small compairing with the observed values for nonactin (7.6 Hz) and tetranactin (7.8 Hz) (Table 1) while the calculated J values of dinactin and tetranactin molecules are larger and close to the observed values. This suggests that the conformations of the H2-C2-C3-H3 in nactins may be trans at least in two subunits (the nonactin type molecule of S_4 symmetry has all gauche H2-C2-C3-H3 conformations while the tetranactin type has two and the dinactin type has three trans conformations). It is therefore most probable that this type of structure of dinactin molecule, reported here having an asymmetric conformation, may be one of the predominant intermediate structures of nactins in solution. The similarities of IR spectra of nactin solutions and dinactin crystals support the view. Calculations were carried out on an ACOS 500 computer at the Computer Center of Chugai Pharmaceutical Co. Ltd. The authors thank to Professor Yoshimasa Kyogoku of Osaka University for valuable discussions.

References and Note

1) A part of this work was presented at the Annual Meeting of the Crystallographic Society of Japan, Nagoya (Nov. 15, 1979).
2) G. Eisenman, S. Krasne, and S. Ciani, Ann. N.Y. Acad. Sci., 264, 34 (1975).
3) M. Dobler, J.D. Dunitz, and B.T. Kilbourn, Helv. Chim. Acta, 52, 2573 (1969).
4) M. Dobler and R.P. Phizackerley, Helv. Chim. Acta, 57, 664 (1974).
5) T. Sakamaki, Y. Iitaka, and Y. Nawata, Acta Crystallogr., Sect. B, 32, 768 (1976); ibid, 33, 52 (1977).
6) M. Dobler, Helv. Chim. Acta, 55, 1371 (1972).
7) Y. Nawata, T. Sakamaki, and Y. Iitaka, Acta Crystallogr., Sect. B, 30, 1047 (1974).
8) J.H. Prestegard and S.I. Chan, Biochemistry, 8, 3921 (1969).
9) Y. Kyogoku, M. Ueno, H. Akutsu, and Y. Nawata, Biopolymers, 14, 1049 (1975).
10) It was first pointed out by Y. Kyogoku that the conformations of the four subunits in dinactin are slightly different from each other, even in crystals, and the molecular conformation is closely related to that of the nactins observed in solution (private communication, 1976).
11) P. Main, M.M. Woolfson, and G. Germain, Acta Crystallogr., Sect. A, 27, 368 (1971).
12) C.K. Johnson, ORTEP II. Report ORNL-3794, 2nd revision. Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA (1970).

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