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Pseudomerohedrally twinned monoclinic structure of unfolded 'free' nonactin: comparative analysis of its large conformational change upon encapsulation of alkali metal ions

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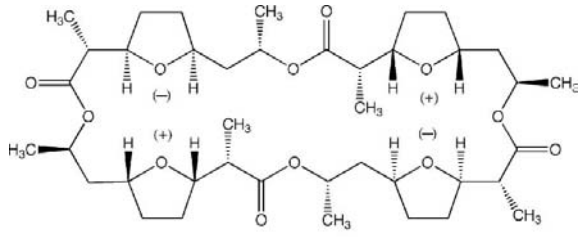
The title compound, $C_{40}H_{64}O_{12}$, crystallizes in a pseudomerohedrally twinned primitive monoclinic cell with similar contributions of the two twin components. There are two symmetry-independent half-molecules of nonactin in the asymmetric unit. Each molecule has a pseudo-S_4 symmetry and resides on a crystallographic twofold axis; the axes pass through the molecular center of mass and are perpendicular to the plane of the macrocycle. The literature description of the room-temperature structure of nonactin as an order–disorder structure in an orthorhombic unit cell is corrected. We report a low-temperature high-precision ordered structure of 'free' nonactin that allowed for the first time precise determination of its bond distances and angles. It possesses an unfolded and more planar geometry than its complexes with encapsulated Na^+, K^+, Cs^+, Ca^{2+} or NH_4^+ cations that exhibit more isometric overall conformations.

Comment

Nonactin, (I), is a naturally occurring optically inactive macrotetrolide antibiotic whose activity includes the ability to transport cations across biological and artificial membranes. New antibiotics are urgently needed to combat persistent, emerging and re-emerging infectious diseases, hospital-acquired resistance, and bioterrorism agents. Currently, there is a renaissance of drug discovery from natural products. Exploration of microbial diversity in underexplored environments offers renewed hope for the discovery of novel antibiotics, anticancer agents, and other drugs from nature. In a pilot effort to explore the Great Lakes as an untapped freshwater environment for microbial resources from which new chemical entities may be obtained as antibiotics, dozens of actinomycete isolates were obtained from Lake Michigan sediment samples and found to produce chemical extracts with antimicrobial activities. In particular, a *Streptomyces* sp. isolate was found to produce a significant amount of what was proven later to be nonactin anhydride.



(I)

Nonactin has been isolated previously from numerous bacterial sources as an antibiotic and anticancer agent (Corbaz *et al.*, 1955; Solov'eva, 1973; Wallhaeusser *et al.*, 1964). This macrotetrolide ionophore consists of the (+)(−)(+)(−)-ester linkage of the enantiomeric nonactic acid building blocks. However, the elucidation of the solid-state structure of nonactin has proven difficult. Dunitz (1964) determined that impure crystals (described as 'not highly purified') of nonactin gave diffraction patterns with *para*-orthorhombic symmetry. Dornberger-Schiff (1966) noted that the structure may be of the type 'order–disorder'. The orthorhombic unit-cell dimensions were determined by Dominguez *et al.* (1962) as a = 47.6 Å, b = 31.5 Å and c = 5.70 Å, and Dobler (1972) reported the room-temperature order–disorder structure of nonactin in this cell, but noted that the superposition structure corresponds to the orthorhombic space group *Pbam* with a unit cell of $a/2$, $b/2$, c. Although Dobler reported the atomic parameters, he commented that the large magnitude of the standard deviations on bond distances and angles did not allow for a detailed discussion of the molecular geometry of nonactin. Dobler observed diffuse streaks in the diffraction pattern of crystals of nonactin; we also recorded streaking in the diffraction pattern of our crystal.

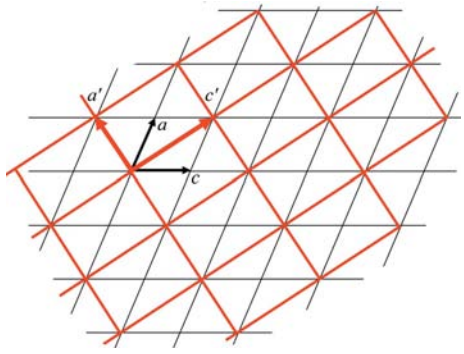


Figure 1
Relationship between the correct monoclinic unit cell under $P2/n$ symmetry and the apparent C-centered orthorhombic unit cell. The unique twofold symmetry axis is perpendicular to the plane of the page.

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Herein we report molecular parameters of nonactin established with high precision. Our low-temperature (100 K) structural investigation of a colorless unknown (subsequently proven to be nonactin) proceeded as follows. The initial indexing of the unit cell suggested a *C*-centered orthorhombic lattice consistent with the reported dimensions (a = 31.147 Å, b = 47.166 Å, c = 5.569 Å and V = 8180.55 Å^3), which is frequently a sign of trouble. The program *CELL_NOW* (Sheldrick, 2009) was used to index the reflections and the crystal appeared to be single. A full sphere of data was collected. The program *XPREP* (Sheldrick, 2008) suggested the space group *Cmma*, which is encountered in the Cambridge Structural Database (CSD; Allen, 2002) only 36 times. Not surprisingly, the structure could not be solved in this space group and a monoclinic unit cell was selected instead. Systematic absences were consistent with the space groups *P*2/*n* and *Pn* and the *E* statistics were indiscriminate as to the centrosymmetry. Although the structure was successfully solved in *P*2/*n*, the refinement stalled, with an *R* factor of ∼22%. Scrutiny of the data revealed many F_{obs} values much higher than the corresponding F_{calc} values, a likely indication of twinning. The program *PLATON* (Spek, 2009) was then used to analyze the data, and indeed pseudomerohedral twinning was detected. The suggested transformation matrix ($00\bar{1}$, $0\bar{1}0$, $\bar{1}00$) corresponds to interchange of the *a* and *c* axes that have very similar lengths. The suggested twin law was incorporated into the instruction file with a TWIN/BASF combination for the program *XL* (Sheldrick, 2009) and the batch scale factor refined to indicate a 45.08 (11)% contribution of the minor twin component. The resulting refinement converged to an *R* factor of 4.30%. Fig. 1 shows the relationship between the primitive monoclinic unit cell and the apparent incorrect *C*-centered orthorhombic unit cell. The monoclinic unit cell can be converted into the *C*-centered orthorhombic cell with a transformation matrix (101, $\bar{1}$01, $0\bar{1}0$). A recent related example of pseudomerohedral twinning of cyclopentadecanone was reported by Noe *et al.* (2008).

Nonactin (Fig. 2) crystallizes in a centrosymmetric lattice of *P*2/*n* symmetry, with two symmetry-independent half-molecules in the asymmetric unit (four molecules in the unit cell, Z = 4). Each molecule occupies a crystallographic twofold axis. The two independent molecules have virtually identical conformations with an approximate S_4 symmetry. The twofold and pseudo-S_4 axes pass through the molecular center of mass and are perpendicular to the plane of the macrocycle. The molecules form stacks in the [010] direction. There is a solvent-accessible void in the center of the macrocycle with an approximate volume of 69 Å^3 (*PLATON*). The dimensions of

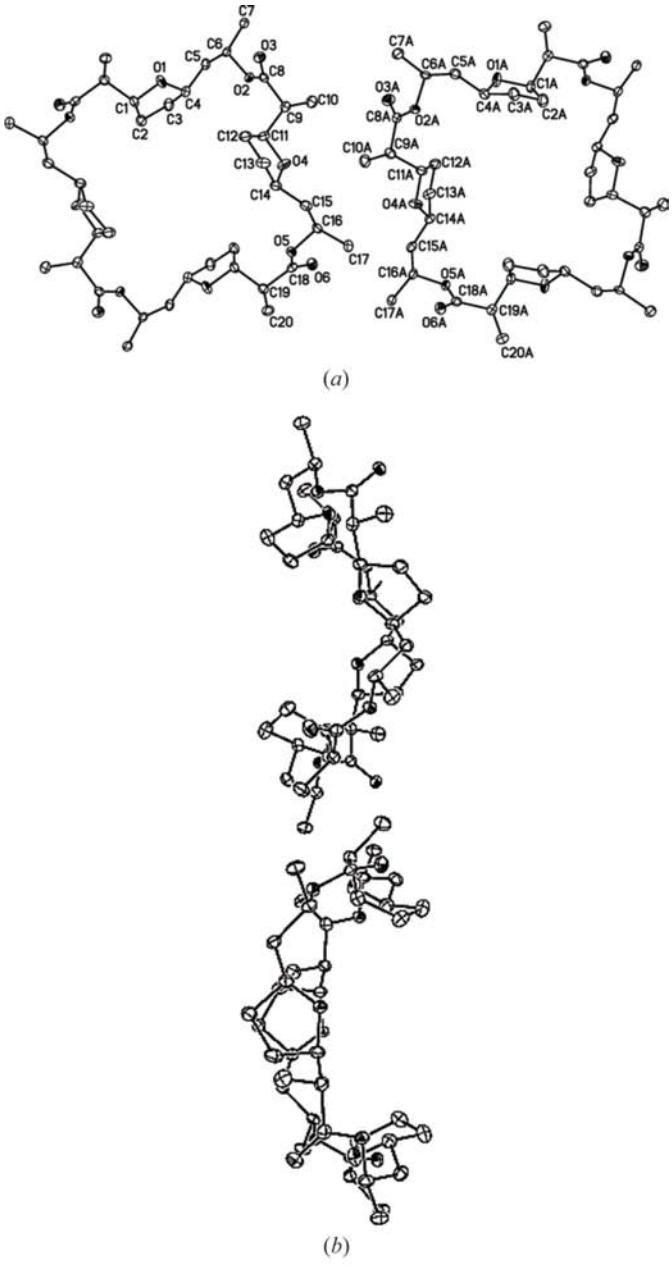

Figure 2
(*a*) The molecular structure of nonactin, with displacement ellipsoids shown at the 40% probability level and all H atoms omitted. (*b*) Sideways view of the two molecules along the crystallographic *a* axis. All atoms are shown with 40% probability ellipsoids, but the O-atom ellipsoids are drawn with octant shading.

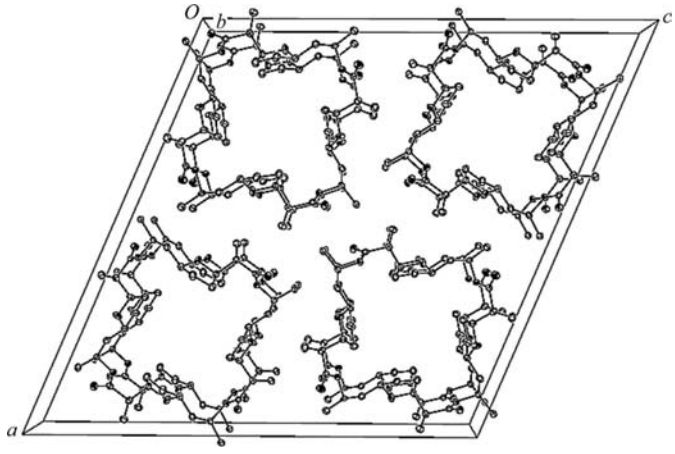

Figure 3
A packing diagram of nonactin, viewed along the *b* axis. The voids in the center of the macrocycle form continuous channels in the lattice. All atoms are shown with 40% probability ellipsoids, but the O-atom ellipsoids are drawn with octant shading.

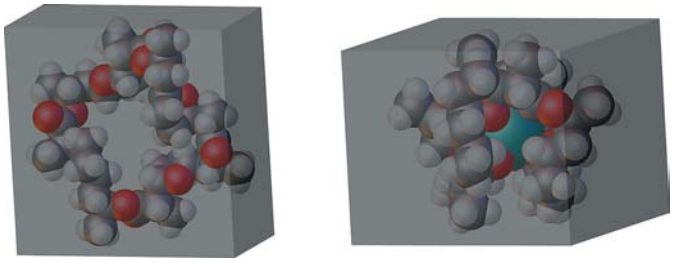

Figure 4
Illustration of the smallest parallelepiped containing free nonactin (left) and nonactin coordinating a Cs^+ cation. The box on the right is noticeably more isometric. All atoms are shown with their van der Waals spheres (see Table 1 for the radii). C atoms are gray, O atoms are red, and H atoms are white.

this molecular cavity can be described with the centroid–centroid distances between the tetrahydrofuran rings related by the twofold axes. These dimensions are 7.688 (2) × 7.544 (2) Å in the O1 molecule and 7.563 (3) × 7.593 (2) Å in the O1*A* molecule. These continuous voids form channels in the crystal along the *b* axis (Fig. 3).

The conformation of 'free' nonactin was compared with that observed in the previously reported nonactin complexes with Na^+ (Dobler & Phizackerley, 1974), K^+ (Kilbourn *et al.*, 1967), Cs^+ (Sakamaki *et al.*, 1977), Ca^{2+} (Vishwanath *et al.*, 1983) and NH_4^+ (Neupert-Laves & Dobler, 1976) using a new subroutine WBOX developed specifically for this project in the program *OLEX2* (Dolomanov *et al.*, 2009) to compute the dimensions of the smallest parallelepiped superscribing each of the nonactin complexes (Fig. 4). The obtained dimensions (Table 1) clearly show that 'free' nonactin *per se* is unfolded and relatively square-planar, but becomes globular when encapsulating a cation, a fact noted by others and now supported quantitatively. For example, the dimensions of the parallelepiped superscribing nonactin are ∼9 × 17 × 17 Å, whereas those for the parallelepiped encompassing nonactin coordinated to a Ca^{2+} cation are ∼12 × 14 × 14 Å. In the four metal cationic complexes of nonactin, the macrocycle coordinates to the metal with four carbonyl and four tetrahydrofuran O atoms in a distorted cubic arrangement. In the cases of the smaller Na^+ and Ca^{2+} cations (hard Lewis bases), the metal–oxygen distances to the carbonyl O atoms are shorter, whereas in the cases of the larger Cs^+ and K^+ cations (softer Lewis bases), the distances to the tetrahydrofuran O atoms are shorter. It is noteworthy that encapsulation of larger Cs^+ and K^+ cations produces more spheroidal and compact structures – the 'encompassing' box sizes for the corresponding complexes are smaller than those of the complexes involving Na^+ and Ca^{2+} (Table 1). This is likely due to the better fit between the sizes of the larger cations and the internal nonactin cavity. In the case of the ammonium salt, the four H atoms of the cation form hydrogen-bonding interactions with four tetrahydrofuran O atoms. Thus, the conformational changes of nonactin are, as expected, dependent on the size and nature of the encapsulated cation.

The elusive structure of anhydrous 'free' nonactin has finally been unambiguously established. Nonactin crystallizes in a pseudomerohedrally twinned primitive monoclinic cell with two twin components of similar sizes.

Experimental

Nonactin anhydride was isolated as an amorphous powder from the culture broth of *Streptomyces* sp. by ethyl acetate extraction, followed by silica-gel chromatography and reverse phase preparation high-pressure liquid chromatography. Its antimicrobial activities were monitored by agar diffusion assays during purification steps. A crystalline sample was isolated after being recrystallized three times from acetone.

Crystal data

$C_{40}H_{64}O_{12}$
M_r = 736.91
Monoclinic, $P2/n$
a = 28.252 (10) Å
b = 5.569 (2) Å
c = 28.270 (11) Å
β = 113.120 (19)°
V = 4090 (3) Å^3
Z = 4
Cu $K\alpha$ radiation
μ = 0.71 mm^{-1}
T = 100 K
0.55 × 0.20 × 0.12 mm

Data collection

Bruker SMART APEXII area-detector diffractometer
Absorption correction: multi-scan (*SADABS*; Bruker, 2009)
T_{min} = 0.694, T_{max} = 0.918
51667 measured reflections
7460 independent reflections
7285 reflections with $I > 2\sigma(I)$
R_{int} = 0.024

Refinement

$R[F^2 > 2\sigma(F^2)]$ = 0.043
$wR(F^2)$ = 0.110
S = 1.05
7460 reflections
478 parameters
H-atom parameters constrained
$\Delta\rho_{max}$ = 0.55 e Å^{-3}
$\Delta\rho_{min}$ = −0.20 e Å^{-3}

Table 1
Symmetry and size of selected nonactin complexes.

The box is the smallest rectangular box encompassing the nonactin molecule.

	Space group	Symmetry	Box dimensions (Å)	Box volume (Å^3)
Nonactin – molecule O1	$P2/n$	$C2$	9.09 × 17.21 × 17.24	2695.4
Nonactin – molecule O1*A*	$P2/n$	$C2$	9.02 × 17.74 × 17.95	2871.6
Na(nonactin)-(NCS)	$C2/c$	$C2$	12.43 × 15.16 × 15.25	2873.9
Ca(nonactin)-$(ClO_4)_2$	$Pnna$	$C2$	12.21 × 13.83 × 14.00	2362.1
K(nonactin)-(NCS)	$Pnna$	$C2$	12.27 × 13.12 × 13.04	2099.1
Cs(nonactin)-(NCS)	$P2/n$	$C2$	12.47 × 12.15 × 12.70	1922.8
(NH_4)(nonactin)-(NCS)	$P\bar{1}$		12.57 × 13.84 × 13.40	2329.5

Note: the important van der Waals radii used for these computations were H = 1.20 Å, C = 1.70 Å and O = 1.52 Å.

All H atoms were placed in geometrically idealized locations, with primary, secondary and tertiary C—H distances of 0.98, 0.99 and 1.00 Å, respectively. The H atoms were refined as riding, with isotropic displacement coefficients of U_{iso}(H) = 1.5U_{eq}(C) for methyl groups or 1.2U_{eq}(C) otherwise.

Data collection: *APEX2* (Bruker, 2009); cell refinement: *SAINT-Plus* (Bruker, 2009); data reduction: *SAINT-Plus*; program(s) used to solve structure: *SHELXTL* (Sheldrick, 2008); program(s) used to

refine structure: *SHELXTL*; molecular graphics: *SHELXTL* and *OLEX2* (Dolomanov *et al.*, 2009); software used to prepare material for publication: *SHELXTL*.

The manuscript was prepared with beta test version 1.9.3 of the program *publCIF* (Westrip, 2009) and the programs *FCF_filter*, *INSerter*, and *modiCIFer* (Guzei, 2007). This work was supported in part by a grant from the US National Institute of Health (grant No. R03 AI073498 to YQC). We thank Lihua Jiang (UW–Milwaukee) for technical assistance in the assay-guided purification process. We are grateful to Professor Lawrence F. Dahl (UW–Madison) for fruitful discussions and to Dr A. I. Yanovsky (Pfizer) for insightful suggestions.

Supplementary data for this paper are available from the IUCr electronic archives (Reference: SQ3212). Services for accessing these data are described at the back of the journal.

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