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Structure of a Model Compound for the Lysozyme–Substrate Complex

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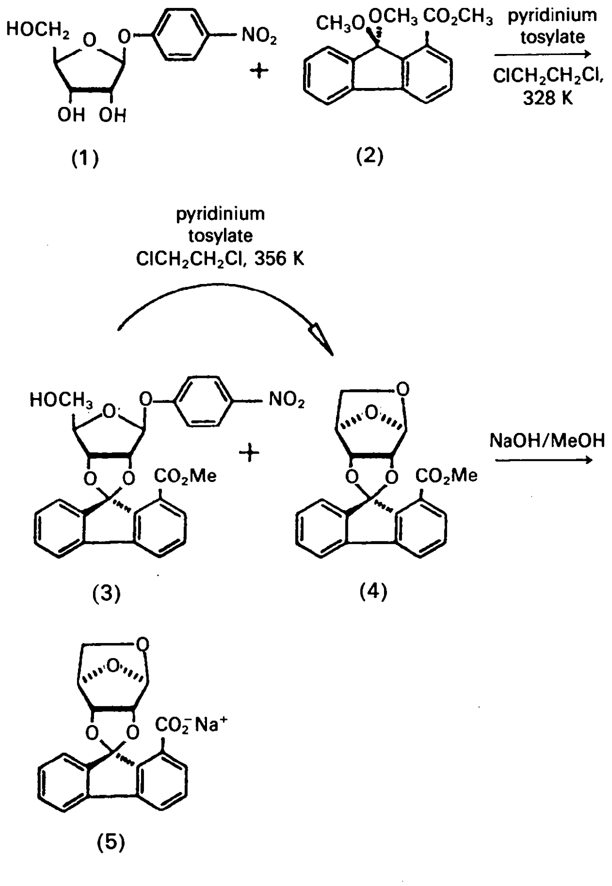
Abstract. Sodium spiro[fluorene-9,8′-(3,7,9,10-tetraoxatricyclo[4.3.0.$1^{2,5}$]decane)]-1-carboxylate methanol solvate, $Na^+.C_{19}H_{13}O_6^-.CH_3OH$, $M_r = 392{\cdot}34$, orthorhombic, $P2_12_12_1$, $a = 11{\cdot}771\,(2)$, $b = 17{\cdot}751\,(2)$, $c = 8{\cdot}275\,(2)$ Å, $V = 1728{\cdot}9\,(6)$ Å^3, $Z = 4$, $D_x = 1{\cdot}51$ g cm^{-3}, $\lambda(\text{Mo } K\bar{\alpha}) = 0{\cdot}71069$ Å, $\mu = 1{\cdot}45$ cm^{-1}, $F(000) = 816$, $T = 295$ K, $R = 0{\cdot}051$ for the 1430 indepedendent reflections with $I > 1\sigma(I)$. The structure consists of Na^+ and $C_{19}H_{13}O_6^-$ ions and a disordered methanol solvent molecule. There is a sixfold coordination geometry of O atoms about the sodium ion such that Na—O distances range from 2·28 (1) to 2·714 (4) Å. The compound serves as a potential model compound for the lysozyme–substrate complex.

Introduction. The role of lysozyme's Asp-52 residue in its mechanism of action remains one of the most long-lived enigmas in enzyme mechanism chemistry (Sinnott, 1984). We have proposed that the lone-pair exchange repulsion (LPER) effect may be important in the enzyme mechanism (Bakthavachalam & Czarnik, 1987) and have been engaged in nucleoside chemistry aimed at testing our idea experimentally (Bakthavachalam, Lin, Cherian & Czarnik, 1987; Lin, Bakthavachalam, Cherian & Czarnik, 1987; Cherian, Van Arman & Czarnik, 1988). In order to model accurately the spatial relationship between the Asp-52 carboxylate group and lysozyme's bound substrate, two criteria must be met: (1) the carboxylate should be *syn*-oriented with respect to the acetal structure, (2) the distance between the carboxylate and carbohydrate ring O atoms should be rigidly fixed at about 2·5–3 Å (Ford, Johnson, Machin, Phillips & Tijan, 1974). Compounds approximating these criteria have not been reported in the literature previously, nor have their structures. Recently, this group has described the synthesis of a compound with approximately the requisite spatial arrangement of funtional groups (Cherian *et al.*, 1988). The structure of our model compound for the lysozyme–substrate complex was determined in an effort to detect structural deformations that might occur as a result of LPER.

Experimental. The synthesis of the title compound (5) was carried out as detailed in the scheme (Cherian *et al.*, 1988). The *endo* ester of 1,5-anhydroribose (4), obtained as a minor product, was hydrolyzed to the corresponding sodium salt of carboxylic acid (5) in methanolic sodium hydroxide, from which the product crystallized as clear, colorless crystals with octahedral morphology.



Examination of the diffraction pattern with a Rigaku AFC5 diffractometer indicated an orthorhombic crystal system with systematic absences $h00$, $h = 2n + 1$, $0k0$, $k = 2n + 1$, and $00l$, $l = 2n + 1$, which uniquely determine the space group as $P2_12_12_1$. At room temperature the cell constants were

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determined by a least-squares fit of the diffractometer setting angles for 25 reflections with 2θ values in the range 20 to 30° and with graphite-monochromated Mo $K\bar{\alpha}$ radiation.

Intensity data were measured by the θ–2θ scan method. Six standard reflections were measured after every 150 reflections and indicated that the crystal was stable throughout data collection. Data reduction, which included corrections for Lorentz and polarization effects, and all subsequent calculations were performed with the *TEXSAN* package of crystallographic programs (Molecular Structure Corportion, 1987).

The structure was solved using the direct-methods program *MITHRIL* (Gilmore, 1983). The fluorene fragment of the molecule was used as a random group in the calculation of the E values and the resulting electron density map contained a planar five-membered ring with one of the atoms tetrahedrally bonded to two other atoms not in the ring (presumably O atoms). This fragment was then used as a phasing model in *DIRDIF* (Beurskens, Bosman, Doesburg, Gould, Van den Hark, Prick, Noordik, Beurskens, Parthasarathi, Haltiwanger & Bruins Slot, 1984), and the whole molecule, including the sodium ion, was then located on the electron density map. The correct enantiomer was chosen based on the known chemistry which produced this molecule.

During the course of isotropic least-squares refinement of the model it became evident that a solvent molecule of methanol was present and disordered in the lattice. There appear to be two positions for the O atom, labeled as O(7*A*) and O(7*B*). The occupancy factor, χ, for O(7*A*) was refined to a value of 0·62 (2) and that for O(7*B*) was then set to $(1 - \chi)$. Because of the disorder, the thermal parameters for the methanol molecule were kept at the isotropic stage, and no H atoms were added to this molecule.

After a cycle of anisotropic refinement, most of the H atoms were located on a difference electron density map. The H atoms were then included in the model as fixed contributions at their calculated positions with the assumptions C—H = 0·98 Å and $B(\mathrm{H}) = 1{\cdot}2 \times B_{\mathrm{eq}}$(attached C atom). All full-matrix least-squares refinements were based on F so that the function minimized in least squares was $\sum w(|F_o| - |F_c|)^2$ with $w = 1/\sigma^2(F_o)$. The final refinement cycle for the 1430 intensities with $F_o^2 > 1\sigma(F_o^2)$ and the 247 variables (anisotropic non-H atoms, except for isotropic methanol molecule, and fixed H atoms) yielded agreement indices of $R = 0{\cdot}051$ and $wR = 0{\cdot}047$. The final difference electron density map contained maximum and minimum peak heights of 0·26 and $-0{\cdot}19$ e Å^{-3}. The maximum shift-to-e.s.d. ratio in the final refinement cycle was 0·05. Scattering factors for the non-H atoms were obtained from the usual source (*International Tables for X-ray Crystallography*, 1974); those for the H atoms are from Stewart, Davidson & Simpson (1965). The real and imaginary anomalous-scattering components were also included for C, O and Na (*International Tables for X-ray Crystallography*, 1974). Table 1 contains additional crystallographic details. Final positional parameters and equivalent isotropic temperature factors are listed in Table 2.*

Table 1. *Additional crystallographic details*

Crystal size (mm)	0·23 × 0·31 × 0·38
2θ limits (°)	$4 \le 2\theta \le 55$
Data collected	h 0→14, k 0→22, l 0→10
Unique data	2313
Unique data used in refinement*	1430 with $I > 1\sigma(I)$
$R(F)$	0·051
$wR(F)$	0·047
S, error in observation of unit weight	1·83

* $\sigma(I) = [\sigma^2_{\mathrm{CS}}(I) + (0{\cdot}02I)^2]^{1/2}$ where σ_{CS} is based on counting statistics.

Table 2. *Final positional and isotropic thermal parameters for* (5)

$$B_{\mathrm{eq}} = (8\pi^2/3)\textstyle\sum_i\sum_j U_{ij}a_i^*a_j^*\mathbf{a}_i.\mathbf{a}_j.$$

	x	y	z	B_{eq} or B(Å^2)
Na	0·2835 (2)	0·0407 (1)	0·0025 (2)	5·4 (1)
O(1)	0·1947 (3)	0·1582 (2)	0·1702 (4)	3·2 (2)
O(2)	0·2199 (3)	0·1980 (2)	0·4265 (4)	3·3 (2)
O(3)	0·0945 (3)	0·0583 (2)	0·4122 (4)	4·1 (2)
O(4)	−0·0790 (3)	0·0913 (2)	0·3196 (4)	4·6 (2)
O(5)	0·3367 (4)	0·0101 (2)	0·2645 (5)	5·3 (2)
O(6)	0·3792 (4)	0·0411 (2)	0·5154 (4)	5·7 (2)
O(7*A*)	0·3851 (6)	0·1141 (4)	−0·1990 (8)	5·3 (1)*
O(7*B*)	0·3064 (9)	0·1226 (6)	−0·208 (1)	4·4 (2)*
C(1)	0·4410 (5)	0·1219 (3)	0·3040 (6)	3·6 (3)
C(2)	0·5577 (6)	0·1187 (4)	0·2836 (7)	5·0 (3)
C(3)	0·6181 (6)	0·1776 (4)	0·2208 (8)	5·5 (4)
C(4)	0·5640 (5)	0·2437 (3)	0·1737 (7)	4·8 (3)
C(5)	0·3892 (6)	0·3816 (3)	0·0993 (7)	4·6 (3)
C(6)	0·2974 (7)	0·4305 (3)	0·0866 (8)	5·5 (3)
C(7)	0·1918 (6)	0·4099 (3)	0·1375 (8)	5·5 (4)
C(8)	0·1714 (5)	0·3377 (3)	0·1966 (7)	4·7 (3)
C(9)	0·2601 (5)	0·2879 (3)	0·2076 (6)	3·5 (2)
C(10)	0·2629 (4)	0·2065 (3)	0·2682 (5)	2·9 (2)
C(11)	0·3861 (5)	0·1872 (3)	0·2578 (6)	3·1 (2)
C(12)	0·4467 (5)	0·2469 (3)	0·1941 (6)	3·5 (2)
C(13)	0·3682 (5)	0·3093 (3)	0·1609 (6)	3·5 (2)
C(14)	0·3807 (5)	0·0528 (3)	0·3672 (7)	4·1 (3)
C(15)	0·3524 (6)	0·1882 (4)	−0·2192 (8)	6·3 (2)*
C(1′)	0·0353 (5)	0·0815 (3)	0·2721 (6)	3·9 (3)
C(2′)	0·0829 (5)	0·1600 (3)	0·2383 (6)	3·5 (2)
C(3′)	0·1001 (5)	0·1882 (3)	0·4131 (6)	3·4 (2)
C(4′)	0·0572 (5)	0·1214 (3)	0·5090 (6)	4·0 (3)
C(5′)	−0·0713 (6)	0·1164 (3)	0·4854 (6)	5·0 (3)

* Atoms refined isotropically.

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* Tables of final anisotropic thermal parameters, bond angles, calculated H-atom positions, least-squares planes, and observed and calculated structure-factor amplitudes have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 52492 (17 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 3. *Intramolecular distances for* (5)

Distances are in Å. E.s.d.'s in the least-significant figure(s) are given in parentheses. Code for symmetry-related atoms: (i) $\frac{1}{2}-x,-y,z-\frac{1}{2}$.

Na	O(7*B*)	2·282 (10)	C(1)	C(11)	1·381 (7)
Na	O(5)	2·322 (5)	C(1)	C(2)	1·385 (7)
Na	O(3$^{\mathrm{i}}$)	2·391 (4)	C(1)	C(14)	1·511 (7)
Na	O(6$^{\mathrm{i}}$)	2·406 (5)	C(2)	C(3)	1·367 (8)
Na	O(7*A*)	2·431 (7)	C(3)	C(4)	1·391 (8)
Na	O(5$^{\mathrm{i}}$)	2·587 (4)	C(4)	C(12)	1·393 (7)
Na	O(1)	2·714 (4)	C(5)	C(6)	1·390 (8)
O(1)	C(10)	1·428 (5)	C(5)	C(13)	1·402 (7)
O(1)	C(2′)	1·432 (6)	C(6)	C(7)	1·363 (9)
O(2)	C(10)	1·412 (5)	C(7)	C(8)	1·392 (7)
O(2)	C(3′)	1·425 (6)	C(8)	C(9)	1·372 (7)
O(3)	C(1′)	1·413 (6)	C(9)	C(13)	1·383 (7)
O(3)	C(4′)	1·444 (6)	C(9)	C(10)	1·529 (6)
O(4)	C(1′)	1·412 (6)	C(1′)	C(2′)	1·529 (7)
O(4)	C(5′)	1·446 (6)	C(10)	C(11)	1·493 (7)
O(5)	C(14)	1·251 (6)	C(11)	C(12)	1·382 (7)
O(6)	C(14)	1·243 (6)	C(12)	C(13)	1·467 (7)
O(7*B*)	O(7*A*)	0·94 (1)	C(2′)	C(3′)	1·544 (6)
O(7*B*)	C(15)	1·288 (11)	C(3′)	C(4′)	1·515 (7)
O(7*A*)	C(15)	1·381 (9)	C(4′)	C(5′)	1·529 (8)

Discussion. Bond distances for (5) are listed in Table 3. The *ORTEP* drawings (Johnson, 1976) in Fig. 1 show two views of the carboxylate anion, one of which displays the labeling scheme. Fig. 2 contains a stereoview of the unit-cell packing diagram.

The structure contains Na^+ and $\mathrm{C_{19}H_{13}O_6^-}$ ions along with a disordered $\mathrm{CH_3OH}$ solvent molecule. Two of the anions and one solvent molecule are arranged about the sodium ion to form a sixfold coordination geometry consisting of various types of O atoms (ether, carboxylate and alcohol O atoms) with Na—O distances ranging from 2·28 (1) to 2·714 (4) Å. As seen in Fig. 2, two of these O atoms, O(1) and O(5), are from one anion, three O atoms, O(3), O(5), and O(6) with the carboxylate group as bidentate ligand, are from a second anion, while the sixth O atom is from the disordered methanol molecule, either O(7*A*) or O(7*B*). The geometry about the sodium ion is best descibed as a distorted octahedron. Adjacent octahedra share the O(5) atom as a common vertex, so that infinite chains of linked octahedra lie approximately along the *c*-axis direction.

The anion consists of a fluorene group with a carboxylate substituent, where atom C(10) is a spiro C atom bonded to the 2′,3′-*O*-acetal of 1′,5′-anhydroribose. The fluorene portion of the anion contains an approximate non-crystallographic mirror plane passing through atom C(10) and bisecting the C(12)—C(13) bond. The one pair of bond lengths which breaks this symmetry is the C(9)—C(10) and C(11)—C(10) pair. All the pairs of bond angles related by this pseudo-mirror plane are not significantly different. A least-squares plane through this fluorene group indicates that this part of the molecule is non-planar, with the maximum deviation from the plane for atom C(7) at 0·091 (7) Å. The orientation of the carboxylate group with respect to the fluorene group is governed by steric interactions with the anhydroribose portion of the molecule. As a result, the dihedral angle between the least-squares planes through the carboxylate group, consisting of atoms C(1), C(14), O(5), and O(6), and the fluorene group is 97°. The distances between the carboxylate and carbohydrate ring O atoms are O(5)···O(3) = 3·22 Å and O(6)···O(3) = 3·47 Å. Both distances are larger than the desired distance (2·5–3·0 Å) between the Asp-52 carboxylate group and lysozyme's bound substrate (Ford *et al.*, 1974). It should be noted that the metrical parameters of the fluorene group are in good agreement with those reported for the low-temperature structure of fluorene (Gerkin, Lundstedt & Reppart, 1984) and for the room-temperature

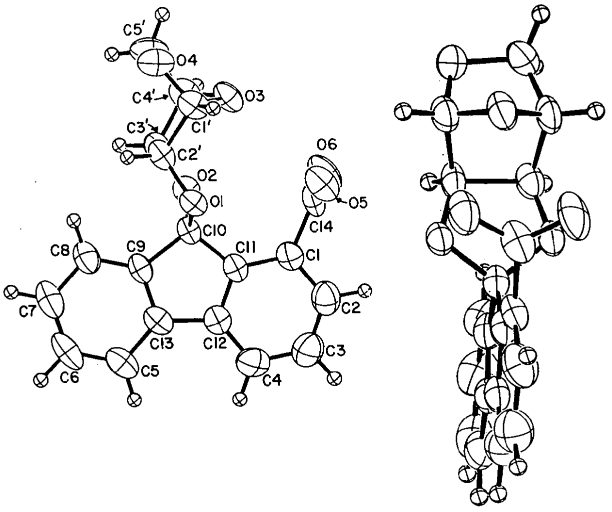

Fig. 1. Two views of the $\mathrm{C_{19}H_{13}O_6^-}$ ion of (5). The non-H atoms are represented by 50% probability thermal ellipsoids. The H atoms are drawn with an artificial radius.

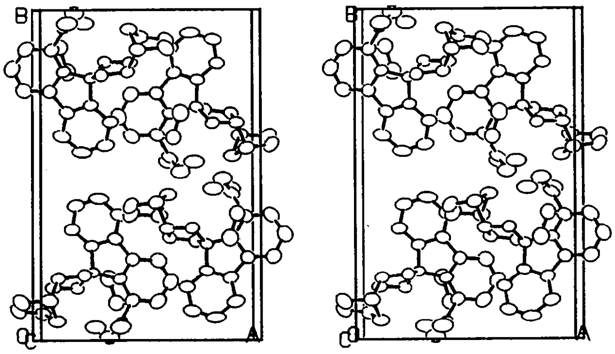

Fig. 2. Stereoview drawing of the unit cell. H atoms have been omitted for clarity. Only one of the disordered O atoms of the methanol molecule has been included.

structure (Belsky, Zavodnik & Vozzhennikov, 1984). The only significant differences are again in the C(9)—C(10) and C(10)—C(11) bond lengths, which are 1·504 Å in both the low-temperature and room-temperature structures. Also the C(12)—C(13) bond length in this study is 1·467 (7) Å, which agrees well with the low-temperature structure [1·472 (3) Å] and not very well with the room-temperature structure (1·491 Å).

The remainder of the anion, consisting of the 2′,3′-*O*-acetal of 1′,5′-anhydroribose, contains three five-membered rings each in an envelope conformation. In the 1′,5′-anhydroribose segment the two five-membered rings share three atoms which causes the six-membered ring of C(1′), C(2′), C(3′), C(4′), C(5′) and O(4) to be forced into a boat conformation. The conformation, bond lengths, and bond angles of this 1′,5′-anhydroribose fragment agree well with the structural results for this same fragment within the 2,6-anhydro-β-D-fructofuranose molecule (Dreissig & Luger, 1973).

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Structure of Tetramethylammonium Tetrabromomanganate in its Low-Temperature Phase

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Abstract. $[N(CH_3)_4]_2MnBr_4$, $M_r = 522{\cdot}8$, monoclinic, $P2_1/c$, $a = 9{\cdot}236\,(4)$, $b = 15{\cdot}983\,(9)$, $c = 12{\cdot}641\,(7)$ Å, $\beta = 90{\cdot}26\,(4)^\circ$, $V = 1866$ Å^3, $Z = 4$, $D_x = 1{\cdot}861$ g cm^{-3}, $\lambda(\text{Mo}\ K\alpha) = 0{\cdot}7107$ Å, $\mu = 91{\cdot}49$ cm^{-1}, $F(000) = 1004$, $T = 223$ K, final $R = 0{\cdot}052$ for 1639 independent reflections with $F > 3\sigma(F)$. Compared with the high-temperature phase, a large decrease in the thermal parameters and in the degree of the distortion of NC_4 tetrahedra are found. The structure is characterized by two kinds of chains composed of $MnBr_4$ and $N(1)(CH_3)_4$; one is along [001] and the other is along [100].

Introduction. It is well known that among insulators of the A_2MX_4 family the compounds $[N(CH_3)_4]_2MX_4$, with M = Zn, Co, Ni, Fe, Mn, Cu, Hg and X = Cl, Br, I, exhibit a wide variety

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