# THREE-DIMENSIONAL STRUCTURE OF OLIGOMYCIN B

Marianne von GLEHN, Rolf NORRESTAM, Peder KIERKEGAARD
and Lajos MARON
*Institute of Inorganic and Physical Chemistry*
and
Lars ERNSTER
*Institute of Biochemistry, University of Stockholm, Stockholm, Sweden*

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## 1. Introduction

The antibiotic oligomycin has been demonstrated by Lardy et al. [1] to act as a potent inhibitor of oxidative phosphorylation. As the first specific inhibitor acting at the site of coupling between respiration and phosphorylation, oligomycin has become a most valuable tool for investigating the mechanism of this process, and has as such been widely used over the past years in studies with mitochondria [2], submitochondrial particles [3] and purified enzymes [4]. Its precise mode of action, however, has not yet been understood, and progress to this end has been seriously limited by the lack of knowledge of the structure of oligomycin.

Preparations of oligomycin contain 3 molecular species, denoted A, B and C, which all inhibit oxidative phosphorylation with equal efficiencies [5]. By chemical studies of degradation products of purified oligomycin B, parts of its structure have been determined by Prouty et al. [6]. These studies have indicated that the molecule, with an observed chemical composition $C_{45}H_{72}O_{12}$, consists of a large, 25- or 26-membered, heterocyclic ring containing 3 double-bonds. The ring also contains keto, hydroxy and methyl substituents, and is linked to 2 saturated, hetero-atomic, 6-membered rings.

The present paper reports the three-dimensional structure of oligomycin B, as determined from single-crystal X-ray diffraction data.

## 2. Materials and methods

Oligomycin B was separated from a commercially available preparation of oligomycins A and B (Sigma Chemical Company, St. Louis, Mo., USA) by thin layer chromatography. Crystals were obtained by slow evaporation of methanolic solutions of the compound. Single-crystal X-ray diffraction data were collected on a diffractometer (Siemens) with monochromatized Cu$K\alpha$ radiation.

## 3. Results and discussion

The dimensions of the unit cell agree reasonably well with those reported by Masamune et al. [7] for single crystals of oligomycin B prepared from methanolic solutions as shown in table 1. Because of the lack of atoms heavier than oxygen in the molecule, and also of the lack of knowledge of the total chemical structure, direct methods for phase-determination (cf. [8]) were applied, with the assumption that the molecule consisted of 57 nonhydrogen atoms. Some difficulties were encountered during the initial applications of direct methods, due to comparatively bad choices of the basis sets. These problems were satisfactorily solved when applying a more systematic choice of basis set as described by Norrestam [9]. The phases obtained were refined by using variance-weighted [9] $\Sigma_2$ formulas [8]. The 11 highest, chemically meaningful peaks in the calculated electron-density map ($E$ map) were accepted and used to

Table 1
Comparison of unit cell parameters of oligomycin B (space-group $P2_12_12_1$) obtained in the present study with those given by Masamune et al. [7].

| Parameter | Present study (Å) | Masamume et al. (Å) |
|---|---|---|
| *a* | 10.33 | 10.25 |
| *b* | 17.38 | 16.92 |
| *c* | 26.99 | 26.34 |

obtain estimates of phases to be included in a new basis set. After 2 such phase refinements using partial structure information, the subsequent *E* map showed 24 reasonable atomic positions. The positions of the remaining nonhydrogen atoms were determined by conventional analysis of difference electron densities and by least-squares refinements. In all, 60 nonhydrogen positions were found per asymmetric unit. Three of these seem to originate from water and/or methanol molecules, linked by hydrogen-bonding to the remaining 57 nonhydrogen atoms, which constitute one molecule of oligomycin B. Least-squares refinement of the structural parameters for the 60 nonhydrogen atoms (assuming isotropical thermal vibrations) yielded a conventional crystallographic *R* value of 0.13 for the 3160 reflections used. A detailed description of the structure determination and a more elaborate discussion of the crystal structure will be published elsewhere [10].

A comparison of the intramolecular distances and angles with standard values [11] indicate the schematic formula shown in fig. 1. The assignment of the oxygens was also reasonably consistent with the thermal parameters obtained in a least-squares refinement where all atoms were treated as carbons. As seen in fig. 1, the oligomycin B molecule consists of a large, 26-membered ring. Attached to this ring are a number of ethyl, methyl, keto and hydroxyl groups, and condensed to it is a substituted tetrahydropyran ring which in turn is linked to another tetrahydropyran ring by a spiro-junction. The conformation of the molecule, which is shown in fig. 2, indicates, *inter alia*, that the 2 tetrahydropyran rings both occur in the chair conformation.

The large number of proper hydrogen-bond donors and acceptors within the molecule gives rise to an extensive hydrogen-bond scheme, the importance of

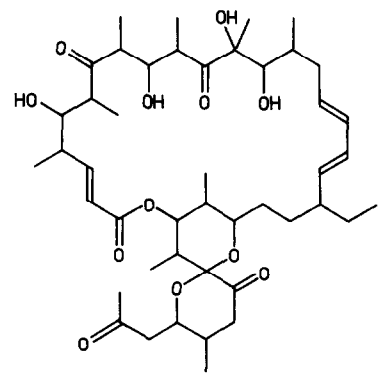


Fig. 1. Schematic structural formula of oligomycin B, as indicated by the present X-ray study.

which for biochemical implications is quite obvious. However, a thorough investigation of the hydrogen bonds requires knowledge of all the 76 hydrogen positions. Probably the quality of the data collected will permit a future location of most of the hydrogens. Details of the hydrogen-bond scheme will be given

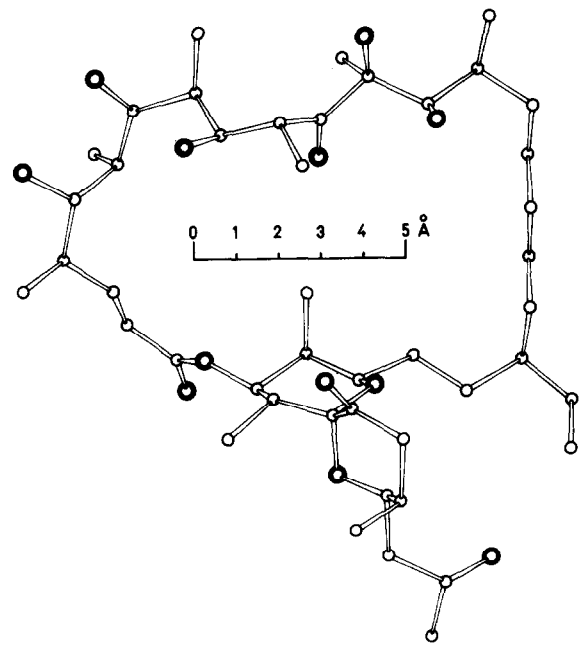


Fig. 2. Molecular conformation in the crystal structure of oligomycin B. The thicker large circles denote oxygen atoms and the smaller circles carbon atoms.

elsewhere [10].

## Acknowledgements

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## References

[1] H.A. Lardy, D. Johnson and W.C. McMurray, Arch. Biochem. Biophys. 78 (1958) 587.
[2] F. Huijing and E.C. Slater, J. Biochem. (Japan) 49 (1961) 493.
[3] C.P. Lee and L. Ernster, European J. Biochem. 3 (1968) 391.
[4] A. Tzagoloff, K.H. Byington and D.H. MacLennan, J. Biol. Chem. 243 (1968) 2405.
[5] H.A. Lardy, P. Witonsky and D. Johnson, Biochemistry 4 (1965) 552.
[6] W.F. Prouty, R.M. Thompson, H.K. Schnoes and F.M. Strong, Biochem. Biophys. Res. Commun. 44 (1971) 619; W.F. Prouty, Thesis, University of Wisconsin, 1971.
[7] S. Masamune, J.M. Sehgal, E.E. Van Tamelen, F.M. Strong and W.H. Peterson, J.Am. Chem. Soc. 80 (1958) 6092.
[8] J. Karle and I.L. Karle, Acta Cryst. 21 (1966) 849.
[9] R. Norrestam, Acta Cryst. A 28 (1972) in press.
[10] R. Norrestam and M. von Glehn, to be published.
[11] L.E. Sutton, Tables of Interatomic Distances and Configuration of Molecules and Ions (The Chemical Society, London, 1958).