Crystal Structure of 3,6-Spiro-dicyclohexyliden-1,2,4,5-tetra-oxa-cyclohexan ("Dimeric Cyclohexanone Peroxide")

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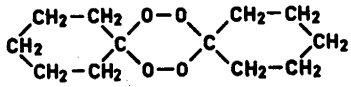
The reaction of cyclohexanone with hydrogen peroxide has been described by several investigators.[1-3] Crystals of "dimeric cyclohexanone peroxide", prepared by



T. Ledaal, belong to the triclinic system and the Dirichlets reduced unit cell, containing one molecule, has the following parameters:

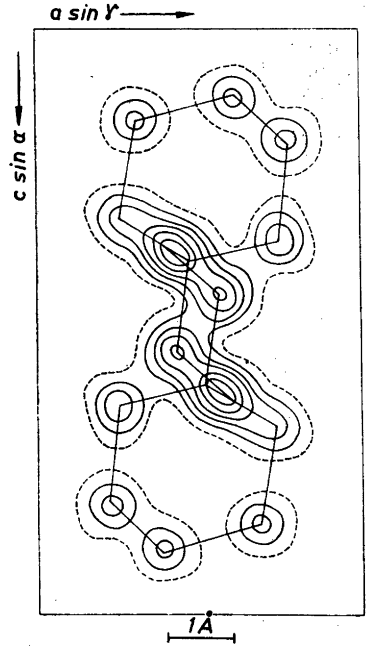


Fig. 1. Fourier projecticon along the *b* axis.

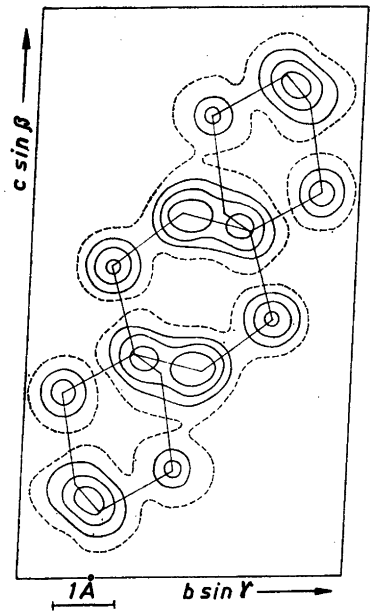


Fig. 2. Fourier projection along the *a* axis.

$a = 5.76$ Å, $b = 6.05$ Å, $c = 9.44$ Å
$\alpha = 86.0°$, $\beta = 88.8°$, $\gamma = 62.4°$

The Wilson ratios and $N(Z)$-curves for the $h0l$- and $0kl$-projections indicate the space group $P\bar{1}$.

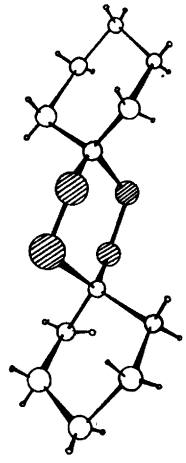


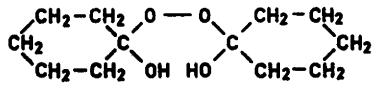
Fig. 3. Schematical drawing of the molecule.

Signs for about 30 % of the structure factors were determined by a computer procedure based on the Cochran—Douglas method[4] (programmed in SPS for IBM-1620 by the author). The corresponding Fourier maps could easily be interpreted, and the final R-values arrived at after least squares refinements were about 10 % for each projection. The final electron density maps (Figs. 1 and 2) show considerable overlapping and one cannot expect the final atomic positions to be very accurate. A three-dimensional analysis is therefore now being undertaken.

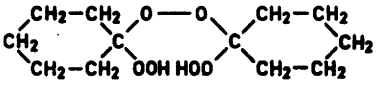
Fig. 3 shows the three chair formed rings of the molecule.

The corresponding compounds obtained from cycloheptanone (space group $P2_1/c$, $Z = 2$), cyclooctanone and cyclododecanone are also being studied, and the investigation will be extended to some other organic peroxides namely

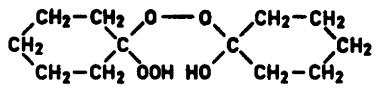
a) 1,1′-dihydroxycyclohexanylperoxide-1,1′



b) 1,1′-dihydroperoxycyclohexanylperoxide-1,1′



c) 1-hydroperoxycyclohexanyl-1′-hydroxycyclohexanyl-peroxide-1,1′



(space group $P2_1/c$, $Z = 4$)

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Crystalline β-Glucuronidase

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Several papers have appeared on the preparation of β-glucuronidase. Bernfeld *et al.*[1] in 1953 claimed that the pure enzyme had an activity of about 1×10^5 Units per mg. Recently Fialkow *et al.*[2] reported an activity of 1.4×10^5 while Plapp *et al.*[3] reported a value of about 2.2×10^5 Units per mg for a fraction obtained by zone electrophoresis. In all cases only small amounts of enzyme were available. The protein determination was made according to the method of Lowry *et al.*[4]

As starting material beef liver was chosen. One kg of fresh liver contains about 6×10^6 Units. The liver was ground in a blendor and extracted overnight in the cold with 3 times its weight of either water or acetate buffer pH 5, 0.1 M. From this crude extract there are many ways to arrive at a product containing about 500—1000 Units per mg protein. Repeated ammonium sulphate fractionation, treatment according to Tsuchihashi [5] as in the preparation of ADH [6] or fractionation with alcohol at a low temperature. In the present paper the first method was used.

When the above mentioned purity is reached two or three more ammonium sulphate fractionations at pH 5 give a product of about 4000—5000 Units per mg. The solution is now dialyzed against 0.1 M acetate buffer, pH 5, until practically all sulfate is removed. Some loss of enzyme usually occurs. The more concentrated the solution is, the smaller is the loss. A heavy precipitate of inactive protein formed during the dialysis is removed by centrifugation.

Next step is an alcohol fractionation to get rid of the sticky brown colored material that usually is found in liver extracts. At pH 4.2 all enzyme is precipitated with about 8—10 % alcohol (v/v) at 0 to +2°C, while at pH 7.8 the enzyme is not precipitated until 30—50 % alcohol is reached.

This behaviour gives a wide latitude for chosing conditions during the alcohol fractionation. More than two successive fractionations should not be made as this gives rise to losses of enzyme.