with acetylcholine (14×10^{-3} M) as substrate. ChE was completely phosphorylated by incubation with 10^{-5} M Sarin, Soman, or Tabun and excess inhibitor removed by dialysis. After dialysis a check was made that no phosphorus compound was left.[8] The reactivation experiments were performed at 25° for 90 min in a Michels buffer[7] at pH 8.14. Reactivation is given as a percentage of the ChE activity in an enzyme control containing the reactivator but no phosphorus compound.

Synthesis. 4(5)-Chloromethylimidazole hydrochloride was prepared according to Turner *et al.*[9] 4(5)-Bromoethylimidazole hydrobromide was prepared from histamine.[10] The pyridinealdoximes used have the following m.p.: 2-aldoxime 109–111° (lit.[11] 114°), 3-aldoxime 148–150° (lit.[11] 150–151°), 4-aldoxime 130–132° (lit.[11] 132°). The quaternizations were performed in dimethylformamide at 50° for about 12 h using two equivalents of aldoxime. After cooling and chloroform addition, the precipitate was filtered, washed with chloroform and recrystallized.

The reactions could be followed by TLC on silica gel with pentanol-acetic acid-acetone-water (56:6:24:14) as eluent. Compounds containing the imidazole ring were detected by spraying with a diazonium salt (Echtblausalz B). For the other compounds, Dragendorffs reagent was used.

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X-Ray Investigation of *N,N*-Dimethyl-*p*-nitrosoaniline, a Disordered Structure

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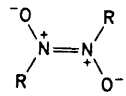
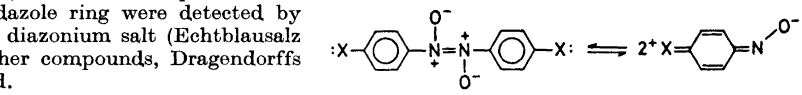
Recent investigations have shown the dimers of organic nitroso compounds to be azodioxy-derivatives:[1-3]



The correlation between the electron donor ability of R and the stability of these compounds has been demonstrated by Lüttke.[4-6] In *para*-substituted nitrosobenzenes a shift to the right in the equilibrium



was found when X is a strong electron donor.

In view of the powerful electron donating property of the dimethylamino group, *N,N*-dimethyl-*p*-nitrosoaniline was selected as the subject of the present investigation. A comparison of the bonds in this molecule and in those with X = H, Br, I [2,3,7] and X = OH (now being investigated in this laboratory) should be of importance for the understanding of the dimerisation process.

Owing to the disorder observed in crystals of the title compound two structure refinements were carried out with crystals grown in different ways; both gave the same results with exception of small differences in the thermal parameters. The crystals were formed (I) by slow evaporation of a diethyl ether solution and (II) by cooling a similar solution in liquid nitrogen. A third attempt was carried out by a zone melting technique; X-ray photographs proved also these crystals to be disordered. The crystal data are as follows: *N,N*-Dimethyl-*p*-nitrosoaniline, triclinic, space group $P\bar{1}$. Cell dimensions (II): $a =$

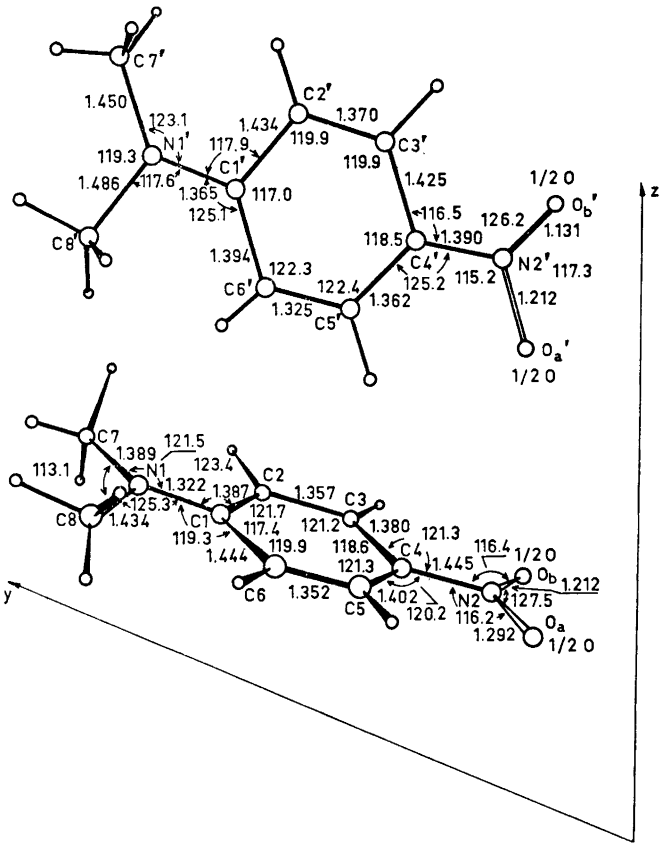


Fig. 1. *N,N*-Dimethyl-*p*-nitrosoaniline. Bond lengths (Å) and angles (°), $R = 8.8$ %.

9.617(2) Å, $b = 10.137(2)$ Å, $c = 9.817(2)$ Å, $\alpha = 68.21(1)°$, $\beta = 68.51(1)°$, $\gamma = 76.61(1)°$. $D_{obs} = 1.19$ g cm^{-1}, $Z = 4$, $D_{calc} = 1.21$ g cm^{-1}.

The intensity data were collected using an automatic Picker diffractometer with graphite crystal monochromated Mo$K\alpha$-radiation. Reflections with sin θ/λ up to 0.6 Å^{-1} were measured; data set I comprised 1036 observed reflections ($I > 1.5\ \sigma(I)$) and data set II 1702 observed reflections ($I > 2\sigma(I)$).

Cell dimensions in crystal I did not deviate significantly from those of crystal II. A conventional agreement factor between the two data sets of 5 % was determined after scaling the data set I with a factor $C \exp(\Delta B \sin^2\theta/\lambda^2)$. ΔB was found to be 0.58 Å^2 by a least-squares procedure.

The transformation $\mathbf{a}' = \mathbf{b}$, $\mathbf{b}' = \mathbf{a} - \mathbf{c}$, $\mathbf{c}' = \mathbf{a} + \mathbf{c}$ gives a cell with dimensions $a' = 10.1$ Å, $b' = 5.5$ Å, $c' = 8.2$ Å, $\alpha' = 91°$, $\beta' = 68°$, $\gamma = 84°$. This is a pseudo monoclinic cell with an intensity distribution resembling that of the space group $P2_1/c$.

With data set I a three-dimensional Patterson function and subsequent Fourier refinements revealed the positions of the two independent molecules. Both showed the same kind of disorder resulting in a pseudo two-fold axis of symmetry passing through the nitrogen atoms of each molecule. The disorder was maintained when refining the model against the second data set. Least-squares refinements were terminated (parameter shifts less than 10 % of the e.s.d.) with $R = 14.6$ % using data set I and $R = 8.8$ % with data set II.

All hydrogen atoms were located. The results of the structure analysis for data set II are given in Fig. 1. The estimated standard deviations are 0.008 – 0.015 Å in bond lengths and 0.5 – 1.0° in angles. Apparently significant differences in the bond lengths and large thermal parameters of the oxygen atoms are probably due to the disorder in the structure.

Both of the independent molecules appear to be almost planar with respect to the heavy atoms; this is even the case for the "half" oxygen atoms. At the present stage of the refinement there are indications of a weak quinonoid character in the molecule.

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Antigenic Relationship between Lysozymes and its Use in Isolation and Purification of this Type of Enzyme

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Lysozymes or endo-β-*N*-acetylmuramidases (E.C. 3.2.1) have been isolated from a variety of sources including mammalian tissues, birds, insects, plants, and microorganisms.[1-3] These enzymes hydrolyze the glycan of the bacterial cell walls to oligosaccharides of the *N*-acetylglucosaminyl-*N*-acetylmuramic acid structure.

Immunological cross-reaction is a widespread phenomenon, and several cases of serological cross-reactions between related enzymes have been reported in recent years, *e.g.* between trypsin and chymotrypsin [4,5] and between papain and chymopapain.[6]

The many immunological studies carried out on hen egg-white lysozyme (E.C. 3.2.1.17), of which the primary as well as tertiary structures have been fully characterized, have given us a considerable knowledge of the antigenic determinants of this enzyme.[7-14] Comparative immunological studies of lysozyme from different sources have, in spite of the striking similarity in amino acid sequences and three-dimensional structures, detected few or no cross-reactions.[15-19] This is the case also for human lysozyme and hen egg-white lysozyme, which have also been found to differ in amino acid sequences at a considerable number of points near the catalytic site.[20,21] However, cross-reactions between human and chicken lysozymes have been detected by methods necessitating only one antigenic determinant in common.[17,19] Immunoadsorbents, therefore, are expected to be a useful tool in isolation and purification of related enzymes. In the present study rabbit anti-hen egg-white lysozyme has been used to isolate lysozymes from human milk and saliva.

Materials and methods. Antiserum to hen egg-white lysozyme (EL) (Sigma; Grade I, 3 × crystalline) was raised in rabbits given injections of a saline solution of the enzyme emulsified in an equal volume of Freund's complete adjuvant (Difco). Three injections (5 mg of antigen/dose) were given 3 weeks apart, the first one in a foot pad (0.5 ml), the two other intramuscularly (1 ml into 2 sites). IgG was precipitated from rabbit anti-EL sera at 1.33 M $(NH_4)_2SO_4$ concentration, chromatographed on a column of DEAE-cellulose (Eastman),[22] and coupled to Sepharose 4B (Pharmacia) and Sephadex G-15 (Sigma) activated with cyanogen bromide following the procedure described by Porath *et al.*[23] Casein-free milk whey was prepared by acidifying (to pH 4.6 with 1 N HCl) skimmed human milk (kindly provided by Dr. B. Haneberg, The University Hospital) which was subsequently centrifuged to sediment the precipitated casein. The whey