An Electron Diffraction Investigation of

2,2,6,6-Tetramethyl-4-piperidone-1-hydroxyl

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The molecular structure of 2,2,6,6-tetramethyl-4-piperidone-1-hydroxyl has been determined in the gas phase by electron diffraction. The molecule has a chair conformation which is considerably distorted. The $N-O$ bond is in an equatorial position and the bond length is 1.46 Å. The dihedral angles in the ring are: $N-C(2)-C(3)-C(4) = 53°$, $C(2)-C(3)-C(4)-C(5) = 66°$, and $C(5)-C(6)-N-C(2) = 47°$. The molecular C, N, O skeleton has a plane of symmetry through the nitrogen and $C(4)$ carbon atom.

At the present time organic nitroxyl free radicals are of great interest in radiobiology due to their ability to sensitize bacteria and mammalian cells to ionizing radiation when present during irradiation in absence of oxygen.[1] It has been demonstrated that these compounds may exert their effect, as other oxygen mimics, through interaction with DNA radicals, giving either rise to covalent binding [2–4] or to red-ox reactions with such DNA radicals.[5]

In the present paper the structure of 2,2,6,6-tetramethyl-4-piperidone-1-hydroxyl (TAN-H) has been studied by the electron diffraction method in order to get insight into the possible sterical configuration of the TAN-DNA complexes, in which TAN mainly is bound through its 1-oxyl group to the 5 (or 6) position of the pyrimidin bases within DNA.[6]

For the subsequent structure determination of the organic nitroxyl free radical 2,2,6,6-tetramethyl-4-piperidone-1-oxyl (TAN) it will be of great importance to know the structure of its reduction product TAN-H. The difference in the two structures could be expected to be mainly associated with the difference between the

$\diagdown N-O \cdot$ and the $\diagdown N-O \diagup^H$ arrangement.
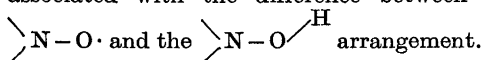
EXPERIMENTAL

2,2,6,6-Tetramethyl-4-piperidone-1-hydroxyl (TAN-H) was obtained by reduction of 2,2,6,6-tetramethyl-4-piperidone-1-oxyl (TAN) with hydrogen in absolute alcohol, with platinum as a catalyst. The product could be recrystallized as TAN-H.HCl from absolute alcohol – ether – HCl (dry) (m.p. 179 °C). Since TAN-H is easily autoxidized in presence of O_2, it was found necessary to synthesize this compound immediately before use. When the theoretical amount of H_2 had reacted with TAN, the reaction was interrupted, the solution rapidly filtered in N_2-atm., and the solvent removed by evaporation in vacuum at room temperature. The residuum was dissolved in alcohol and once more evaporated in vacuum to dryness. The compound was kept at low temperature in vacuum until used for the diffraction experiment. In various runs TAN-H was formed in nearly quantitative yield. A recrystallized sample of TAN-H.HCl had a $pK_1 = 4.2$.
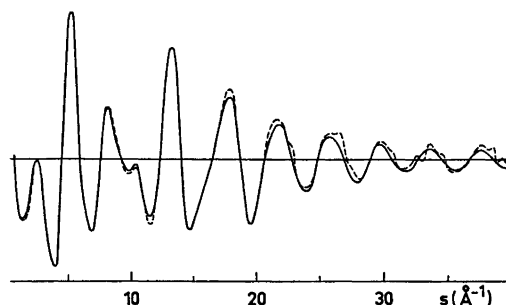

Fig. 1. 2,2,6,6-Tetramethyl-4-piperidone-1-hydroxyl (TAN-H). Calculated (solid line) and experimental (dotted line) molecular intensity curve.

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The diffraction data were recorded on photographic plates at two nozzle-plate distances of 202 mm and 481 mm. The electron wave length was 0.06454 Å and the temperature of the nozzle 120 °C. The microphotometer curves of four long-distance plates covering an s range from 1.375 Å^{-1} to 19.25 Å^{-1} and five short-distance plates covering an s range from 7.00 Å^{-1} to 42.75 Å^{-1} were used. The resulting modified molecular intensity curve has an s range from 1.625 Å^{-1} to 39.75 Å^{-1} and is reproduced in Fig. 1. All calculations were carried out using the programs available in Oslo.[9]

STRUCTURE ANALYSIS

Of the 29 atoms in TAN-H 17 are hydrogen atoms which are difficult to detect. The carbon, oxygen, and nitrogen skeleton of the molecule was initially assumed to have a plane of symmetry through O(7), N(1), C(4), and O(8) (Fig. 2). The experimental radial distribution (RD) curve is reproduced in Fig. 3 and the starting parameters for the bond distances could
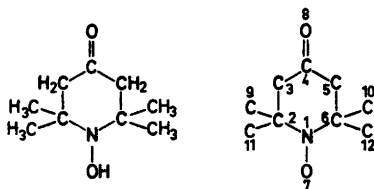

Fig. 2. 2,2,6,6-Tetramethyl-4-piperidone-1-hydroxyl (TAN-H) and the numbering system of the carbon, nitrogen, and oxygen atoms.
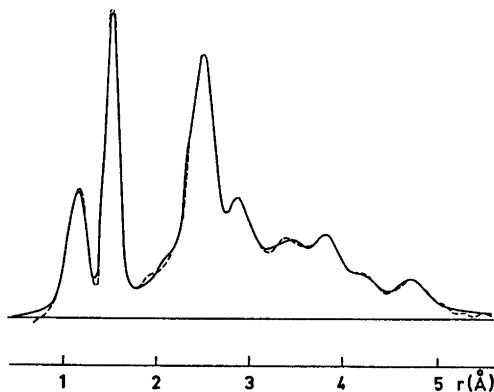

Fig. 3. 2,2,6,6-Tetramethyl-4-piperidone-1-hydroxyl (TAN-H). Calculated (solid line) and experimental (dotted line) radial distribution curve. Artificial damping constant $k = 0.0009$ Å.

be deduced from the two first peaks on the RD curve. The part of the curve outside 2 Å contains information about the longer distances, which also determine the conformation. A preliminary analysis of the RD curve indicated that the assumed chair form of the ring could be present. However, one could not neglect to investigate the possibility of a twisted boat conformation of the ring.

The crystal structure of cyclohexane-1,4-dione [10] shows that a twisted boat form is preferred in the crystalline state, and Rozantsev [8] has proposed on basis of measured and calculated dipole moments of TAN that this molecule should have a twisted boat conformation. Crystal structure determinations of similar molecules [11-13] show that the chair conformation is present in the solid state. That these molecules should have another conformation in the gas phase is possible but probably not likely. In the case of TAN-H the two CH_3 groups in the axial positions on the C(2) and C(6) atoms would come too close together if an ideal sixmembered ring with a chair conformation is present, and considerable deviations in the ring and methyl group positions could be expected.

Preliminary sets of molecular parameters assuming both a chair and a twisted boat conformation, were derived from the RD curve. Independent successive refinements of the parameters of the two forms using the least squares procedure, showed that only the chair conformation parameters could be successfully refined. The experimental and calculated RD curves and the difference between the two is shown in Fig. 3 and the experimental and calculated molecular intensity curves in Fig. 1. The molecular parameters used in calculating the curves are those which gave the best fit of the intensity curves without having unacceptable interatomic distances.

RESULTS AND DISCUSSION

The final molecular parameters, the corresponding u-values (vibrational amplitudes), and standard deviations are listed in Table 1. The numbering of atoms is shown in Fig. 2. All bond lengths and angles are reasonable except for the C(2) − C(3) bond which is 1.56 Å. Similar lengthening of this type of bond has been found in other structures. In, for instance, cyclo-

Table 1. Structure parameters for 2,2,6,6-tetra-methyl-4-piperidone-1-hydroxyl (TAN-H) obtained by least squares refinement on the intensity data. Distances (r_a-values) and mean amplitudes of vibration (u-values) are given in Å, angles in degrees. The standard deviations given in parentheses have been corrected to take into account data correlation. The uncertainty arising from error in the electron wave length is included. (For numbering system of the atoms see Fig. 2).

Distances	r	u
N(1) — O(7)	1.465 (18)	0.046
N(1) — C(2)	1.500 (20)	0.048
C(2) — C(3)	1.560 (38)	0.047
C(3) — C(4)	1.510 (39)	0.047
C(4) — O(8)	1.212 (9)	0.036
C(2) — C(9)	1.530 (20)	0.050
C(2) — C(11)	1.530 (20)	0.050
C — H	1.108 (7)	0.070

Angles	deg.
C(2) — N(1) — C(6)	118 (3.5)
C(2) — N(1) — O(7)	114 (1.8)
N(1) — C(2) — C(3)	111 (1.8)
C(3) — C(2) — C(9)	103 (1.8)
C(3) — C(2) — C(11)	107 (1.8)
C(2) — C(3) — C(4)	106 (1.8)
C(3) — C(4) — C(5)	113 (1.8)
C(3) — C(4) — O(8)	122 (1.7)
N(1) — C(2) — C(9)	117 (1.8)
N(1) — C(2) — C(11)	104 (1.9)
C(2) — C(9) — H	107 (1.8)

hexane-1,4-dione [10] is the corresponding bond 1.56 Å. In the ring the angle at the nitrogen atom is 118° whereas the angle at C(4) is 113°, this makes the C(3)···C(5) distance 2.52 Å and the C(2)···C(6) distance 2.57 Å. This is a favourable increase for the methyl groups in axial positions on C(2) and C(6), in addition are these methyl groups bent out to make the C(9)··· C(10) distance 3.35 Å. The dihedral angles in a regular sixmembered ring should be 60°, the following angles have been found in this ring: C(5) — C(6) — N — C(2) = 47°,   N — C(2) — C(3) — C(4) = 53°,   C(2) — C(3) — C(4) — C(5) = 66°.   An INDO closed shell calculation gave a dipole moment of 2.87 D while the measured value is 2.86 D.

In model experiments it has been found that radiation induced binding of TAN to deoxynucleotides takes place at the 5 — 6 position of the pyrimidin nucleus.[5,6] The data indicate that

nearly all radiation-induced radicals located in the 5 — 6 position of the thymine residue bind TAN covalently, and that a high fraction of radicals located in the 5 — 6 position of cytosine induced by OH bind TAN.

The very high yield of binding of TAN to thymine radicals is surprising in view of the sterical hindrance around the organic nitroxyl radical and the presence of the methyl group in 5-position of thymine. It has been calculated that the free valence of the pyrimidine nucleotides to a large extent is located at the 6-position and that thus OH may add to position 6 of the pyrimidine nucleus.[7] The resulting nucleotide radicals may exist in at least two stereoisomers. The 5-methyl group of the thymine radicals may cause less sterical hindrance than in the parent residue.

In TAN approximately 70 % of the free electrons are located on the oxygen atom of the nitroxyl group.[8] On a model of double stranded DNA it can be shown that TAN, with a conformation as in TAN-H, may be bound through its nitroxyl oxygen atom to the 5 or 6 position of the pyrimidines being located in the minor groove of the double helix without sterical hindrance from neighbouring groups in DNA.

REFERENCES

1. Emmerson, P. T. and Howard-Flanders, P. *Nature (London)* 204 (1964) 1005.
2. Nakken, K. F., Sikkeland, T. and Brustad, T. *FEBS Lett. 8* (1970) 33.
3. Nakken, K. F. and Pihl, A. In Teply, J., Ed., *International Discussion on Progress and Problems in Contemporary Radiation Chemistry*, Prague 1971, p. 678.
4. Brustad, T., Jones, W. B. G. and Nakken, K. F. *Int. J. Radiat. Phys. Chem. 3* (1971) 55.
5. Nakken, K. F., Tomas, E. and Brustad, T. *Ninth Annu. Meeting of the European Society for Radiation Biology, Book of Abstr.* (1972) 122.
6. Nakken, K. F., Tomas, E. and Brustad, T. *I. A. E. A. Panel on Modification of Radiosensitivity in Biological Systems*, Stockholm June 1973. *To be published.*
7. Nofre, C. and Cier, A. In Pullman, B., Ed., *Electronic Aspects of Biochemistry*, Academic, New York 1964.

8. Rozantsev, E. G. In Ulrich, H., Ed., *Free Nitroxyl Radicals*, Plenum, New York 1970.
9. Andersen, B., Seip, H. M., Strand, T. G. and Stølevik, R. *Acta Chem. Scand. 23* (1969) 3224.
10. Groth, P. *Acta Chem. Scand. 18* (1964) 923.
11. Lajzerowicz-Bonneteau, P. J. *Acta Crystallogr. B 24* (1968) 196.
12. Berliner, L. J. *Acta Crystallogr. B 26* (1970) 1198.
13. Hawley, D. M., Ferguson, G. and Robertson, J. M. *J. Chem. Soc. B* (1968) 1255.