

The Molecular Structure of 2,2,6,6-Tetramethyl-4-piperidone-1-oxyl Free Radical Determined by Electron Diffraction in the Gas Phase

PER ANDERSEN,^a E. E. ASTRUP,^a P. S. FREDERICHSEN^a and K. F. NAKKEN^{b,*}

^aDepartment of Chemistry, University of Oslo, Oslo 3, Norway and ^bNorsk Hydro's Institute for Cancer Research, Montebello, Oslo 3, Norway

The structure determination of 2,2,6,6-tetramethyl-4-piperidone-1-oxyl free radical has been carried out in the gas phase using the electron diffraction method. The N—O bond length has been found to be 1.247 (11) Å and the angle of this bond with the C(2)—N—C(6) plane is 14°. All bond lengths and angles have acceptable values, only the C(2)—C(3) bond (Fig. 2) of 1.570 (36) Å is longer than what is usually found. The ring has a chair conformation. Attempts to refine a twisted boat conformation resulted in unacceptable non-bonded distances.

2,2,6,6-Tetramethyl-4-piperidone-1-oxyl (TAN) is a stable free radical of relatively low toxicity, high solubility, and strong radiosensitizing effect in a number of biological systems.^{1,2} Organic nitroxyl free radicals may be stabilized by a low ground state energy of the three-electron bond and by sterical hindrance of the nitroxyl group from alkyl substituents in the α positions.³ Such sterical hindrance may affect the radiosensitizing effect of the different nitroxyl free radicals when these are used in low molar concentration.²

TAN has previously been shown to interact with approximately 50 % of radiation induced DNA radicals through covalent binding^{4,5} or through red-ox reactions.⁵ Due to low reactivity or sterical hindrance or both, a significant fraction of DNA radicals are unable to interact with TAN.

The advantage of having solved the structure of 2,2,6,6-tetramethyl-4-piperidone-1-hydroxyl

(TAN-H)⁶ using the electron diffraction method in the gas phase has been utilized in the structure determination of TAN. By comparing the radial distribution (RD) curves and interpreting the difference between the two curves, which on beforehand was assumed mainly to be associated with the difference between the two

groups $\text{>N}-\text{O}-\text{H}$ and $\text{>N}-\text{O}\cdot$, one could more easily arrive at reliable molecular parameters. A number of structure determinations of different nitroxyl radicals⁷⁻¹⁵ have been carried out using X-ray crystallographic and electron diffraction methods. There is still some doubt as

to the planarity of the $\text{>N}-\text{O}\cdot$ group and we

felt that the structure determination in the gas phase could be of some value while the molecules are removed from the effect of their surroundings in the crystal. The comparison of TAN with TAN-H should make it possible to get a good estimate of the N—O bond angle

with the >N plane.

EXPERIMENTAL

2,2,6,6-Tetramethyl-4-piperidone-1-oxyl (TAN) was synthesized from 2,2,6,6-tetramethyl-4-piperidone-HCl (Fluka AG) according to published procedures^{3,8} with some minor modifications. The sample, after recrystallization from light petroleum, had a m.p. of 36.7 °C.

The intensity of the scattered electrons were recovered on photographic plates in the usual

* Present address: Radiotherapy and Isotope Department, Ullevål Hospital, Oslo, Norway

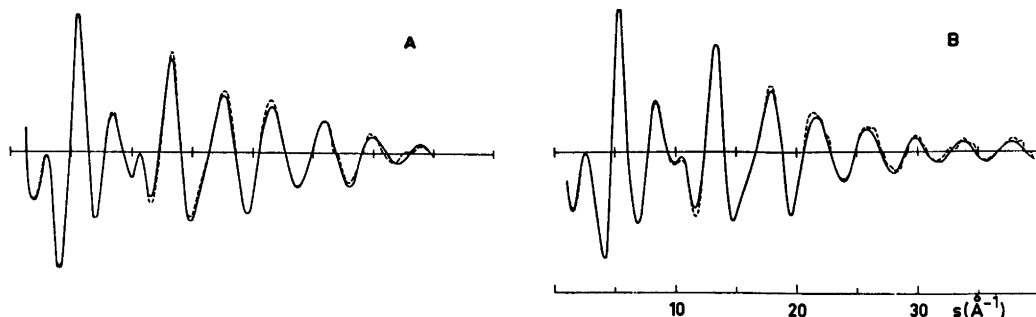


Fig. 1. Calculated (solid line) and experimental (dotted line) molecular intensity curves. A: 2,2,6,6-tetramethyl-4-piperidone-1-oxyl (TAN), B: 2,2,6,6-tetramethyl-4-piperidone-1-hydroxyl (TAN-H).

way at two nozzle-plate distances. One set of six plates at 480 mm and a nozzle temperature of 125 °C and a second set of eight plates at 200 mm and a nozzle temperature of 125 °C were used. The electron wave length was for both sets 0.06457 Å. The first set covered an s range of 1.625–19.875 Å⁻¹ and the second an s range of 7.50–43.00 Å⁻¹. The total modified molecular intensity curve is reproduced in Fig. 1 and extends over an s range from 1.625 Å⁻¹ to 35.00 Å⁻¹.

The RD curve of TAN was compared with the RD curve of TAN-H and analyzed to determine approximate molecular parameters for the structure. The parameters were refined by a least squares method in the usual way. The computer programs available in Oslo¹⁷ were used throughout this investigation.

STRUCTURE ANALYSIS

It was initially assumed that TAN and TAN-H would both have a chair conformation and that there should be a plane of symmetry through N and C(4) (Fig. 2). In Fig. 1 are shown the molecular intensity curves of TAN and TAN-H and in Fig. 3 are shown the RD curves of the two compounds. The curves showed that not only the parameters for the free radical part of the molecule had to be changed but also other molecular parameters

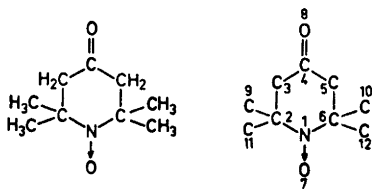


Fig. 2. 2,2,6,6-Tetramethyl-4-piperidone-1-oxyl (TAN) and the numbering system of the carbon, nitrogen and oxygen atoms.

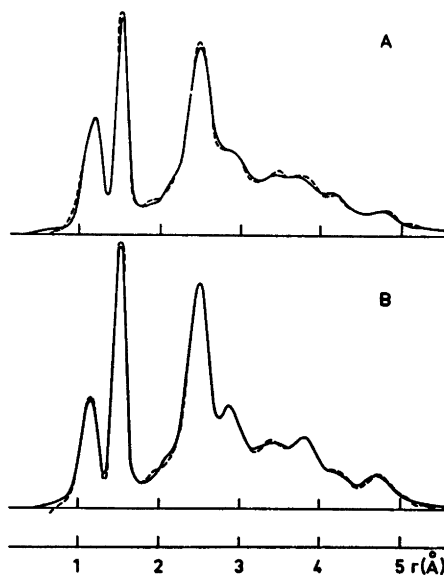


Fig. 3. Calculated (solid line) and experimental (dotted line) radial distribution curves. A: 2,2,6,6-tetramethyl-4-piperidone-1-oxyl (TAN), B: 2,2,6,6-tetramethyl-4-piperidone-1-hydroxyl (TAN-H). Artificial damping constant $k = 0.0009$ Å.

had to be modified somewhat. However, it could be shown that a chair conformation could be present according to the experimental RD curve.

Rozantsev³ has proposed that TAN should have a twisted boat conformation. His assumption is based on calculated values of the dipole moment. There are also other indications that TAN could have this form. A recent crystal structure investigation of TAN by Shibaeva and coworkers in the U.S.S.R.¹⁵ shows that

TAN has a twisted boat conformation with a 180° angle between the N—O and C=O directions. The structure is refined to an *R* value of 0.13 and the parameters should probably be further refined to obtain more accurate bond distances and angles. A crystal structure determination of cyclohexane-1,4-dione¹⁸ showed that this molecule had a twisted boat conformation. TAN is probably more analogous to cyclohexane-1,4-dione than TAN-H because of the sp^2 hybridized atoms in 1,4 positions. It was therefore also assumed that TAN should have this conformation, and the bond lengths mentioned above and the angles from cyclohexane-1,4-dione were used as starting parameters. There were indications that calculated RD curves of both conformations had a shape fairly similar to the experimental RD curve.

Least squares refinements on the chair form proceeded satisfactorily while the refinements on the twisted boat always resulted in unacceptable non-bonded interatomic distances. No matter what was tried, some interatomic distances would always come out too short.

Calculated and experimental molecular intensity curves are reproduced in Fig. 1. A comparison of the experimental and calculated RD curves is shown in Fig. 3.

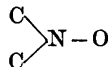
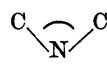
Table 1. Structure parameters for 2,2,6,6-tetramethyl-4-piperidone-1-oxyl (TAN) obtained by least squares refinement on the intensity data. Distances (r_a -values) and mean amplitudes of vibration (u -values) are given in Å, angles in degrees. The standard deviations given in parentheses have been corrected 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).

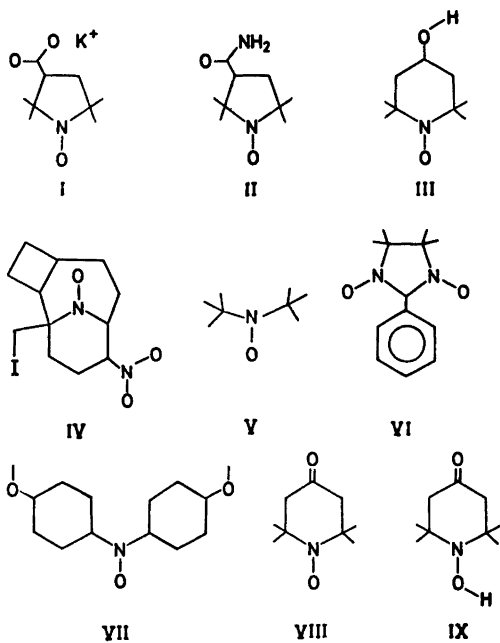
Distances	<i>r</i>	<i>u</i>
N(1)—O(7)	1.247 (11)	0.045
N(1)—C(2)	1.500 (20)	0.044
C(2)—C(3)	1.570 (36)	0.047
C(3)—C(4)	1.500 (38)	0.046
C(4)—O(8)	1.204 (11)	0.037
C(2)—C(9)	1.520 (20)	0.048
C(2)—C(11)	1.520 (20)	0.048
C—H	1.094 (9)	0.068
Angles	deg.	
C(2)—N(1)—C(6)	110 (5.3)	
C(2)—N(1)—O(7)	123 (3.5)	
N(1)—C(2)—C(3)	109 (5.4)	
C(3)—C(2)—C(9)	112 (3.6)	
C(3)—C(2)—C(11)	104 (3.6)	
C(2)—C(3)—C(4)	114 (3.6)	
C(3)—C(4)—C(5)	111 (7.0)	
C(3)—C(4)—O(8)	124 (3.5)	
N(1)—C(2)—C(9)	114 (3.6)	
N(1)—C(2)—C(11)	112 (3.6)	
C(2)—C(9)—H	112 (1.9)	

Table 2. Correlation matrix ($\times 100$) for the parameters. (The coefficients having absolute values less than 0.6 are not given.)

Parameters	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	R_{11}	R_{12}	R_{13}	R_{14}	R_{15}	R_{16}	R_{17}	R_{18}
R_1 N(1)—O(7)	100																	
R_2 N(1)—C(2)		100																
R_3 C(2)—C(3)			100															
R_4 C(3)—C(4)				100														
R_5 C(4)—O(8)					100													
R_6 C(2)—C(9)						100												
R_7 C(2)—N(1)—C(6)							100											
R_8 C(2)—N(1)—O(7)								100										
R_9 N(1)—C(2)—C(3)									100									
R_{10} C(3)—C(2)—C(9)										100								
R_{11} C(3)—C(2)—C(11)											100							
R_{12} C(2)—C(3)—C(4)												100						
R_{13} C(3)—C(4)—C(5)													100					
R_{14} C(3)—C(4)—O(8)														100				
R_{15} C(9)—C(2)—N(1)															100			
R_{16} C(11)—C(2)—N(1)																100		
R_{17} C(2)—C(9)—H																	100	
R_{18} C—H																		100

Table 3. Nitroxyl group parameters of free radicals whose structures have been determined. TAN-H is added for comparison.

Molecule	Ref.			N-O	N-C	Method
I	7	planar	114.5	1.26	1.50	X-ray
II	8	planar	114.8	1.267	1.48	X-ray
III	(9) 10	15.8	125.4	1.291	1.499	X-ray
IV	11	planar	121	1.308	1.496	X-ray
V	12	planar	136	1.28	1.512	el. diffr.
VI	15	planar	—	1.278	1.501	X-ray
VII	13	planar	124	1.23	1.44	X-ray
VIII	14	planar	121	1.30	1.46	X-ray
	this work	14	110	1.247	1.50	el. diffr.
IX	6	37	118	1.465	1.50	el. diffr.




RESULTS AND DISCUSSION

The bond lengths, angles, standard deviations, and u -values (vibrational amplitudes) are listed in Table 1. The correlation between parameters decides how well these parameters may be determined.¹⁹ In Table 2 the correlation coefficients $|r_{ij}| > 0.6$ are listed and the R_i , R_j refers to parameters shown in Table 1. The correlation between R_1 and the 17 other parameters is less than 0.6 and the same is the case for R_5 and R_{13} . These parameters are determined with greater accuracy than the other bond-distance parameters. R_2 and R_4 as well as R_3 and R_6 are greatly correlated and this explains why these are less accurately determined. The correlation between bond distances in TAN is greater than in TAN-H which makes the structure determination of the latter more accurate.

Except for the C(2)–C(3) the bond lengths are about what could be expected. The poor accuracy makes it doubtful to discuss the significance of this lengthening, but it may be pointed out that the 1.570 Å found here is close

to the 1.560 Å found in both TAN-H⁶ and cyclohexane-1,4-dione¹⁸ for the same distance. The C(2)–N–C(6) and C(3)–C(4)–C(5) angles are about equal in this structure while C(2)–N–C(6) was 118° in TAN-H. This change in angle contributes to the shortening of the distance between the axial methyl groups which in TAN is C(9)···C(10)=2.91 Å. The two distances from the nitroxyl oxygen to the methyl groups are O(7)···C(9)=3.35 Å and O(7)···C(11)=2.83 Å. The dihedral angles in the ring are C(5)–C(6)–N–C(2)=60°, N–C(2)–C(3)–C(4)=54°, and C(2)–C(3)–C(4)–C(5)=47°. The angle between the N–O bond and the plane defined by C(2)–N–C(6) is found to be

14°. A comparison of the  N–O parameters in the structures determined so far is shown in Table 3.

Pulse radiolysis studies have revealed that organic nitroxyl free radicals might have different steric hindrance in reactions with deoxy-nucleotide radicals.² Sterical hindrance seems also to be involved in the radiosensitizing effect of nitroxyls in bacteria.² Thus, in one bacterial species, where different nitroxyls had nearly equal sensitizing effects at high concentrations, the effect differed strongly at low concentrations of sensitizers.²

Evidence is accumulating that DNA is the vital target in irradiated bacteria and cells.²¹ It is therefore of interest that TAN may be bound covalently to irradiated native DNA, to the extent expected from the observed yield values in experiments where the four separate deoxy-nucleotides were irradiated in the presence of TAN.²⁰

Nearly all radiation induced binding of TAN to DNA seems to take place at the 5–6 position of the pyrimidine bases of DNA.²⁰ On a model of double stranded DNA it can be shown that TAN may interact with DNA radicals, located at such sites, without restrictive steric hindrance. It can also be shown that TAN, when bound to DNA with a conformation as in TAN-H (*i.e.* as a hydroxylamine derivative) may be located in the minor groove of the double helix of DNA without steric hindrance from neighbouring groups of DNA.

Acknowledgement. The authors are grateful to Dr. A. Almenningen for doing the electron diffraction experiments.

REFERENCES

- Emmerson, P. T. and Howard-Flanders, P. *Nature (London)* 204 (1964) 1005.
- Emmerson, P. T., Fielden, E. M. and Johansen, I. *Int. J. Radiat. Biol.* 19 (1971) 229.
- Rozantsev, E. G. *Free Nitroxyl Radicals*, Plenum, New York 1970.
- Nakken, K. F., Sikkeland, T. and Brustad, T. *FEBS Lett.* 8 (1970) 33.
- Nakken, K. F., Tomas, E. and Brustad, T. *Ninth Annual Meeting of the European Soc. for Radiation Biology; Book of Abstr.* (1972) 122.
- Andersen, P., Astrup, E. E., Frederichsen, P. S. and Nakken, K. F. *Acta Chem. Scand. A* 28 (1974) 671.
- Boeyens, J. C. A. and Kruger, G. J. *Acta Crystallogr. B* 26 (1970) 668.
- Turley, W. and Boer, F. P. *Acta Crystallogr. B* 28 (1972) 1641.
- Lajzerowicz-Bonneteau, P. J. *Acta Crystallogr. B* 24 (1968) 196.
- Berliner, L. J. *Acta Crystallogr. B* 26 (1970) 1198.
- Hawley, D. M., Ferguson, G. and Robertson, J. M. *J. Chem. Soc. B* (1968) 1255.
- Andersen, B. and Andersen, P. *Acta Chem. Scand.* 20 (1966) 2728.
- Hanson, A. W. *Acta Crystallogr.* 6 (1953) 32.
- Shibaeva, R. P., Atovmjan, L. O., Neigauz, M. G., Novakovskaya, L. A. and Ginzburg, S. L. *Zh. Strukt. Khim.* 13 (1972) 887.
- Hope, H. *Private communication.*
- Briere, R., Lemarie, H. and Rassat, A. *Bull. Soc. Chim. Fr.* (1965) 3273.
- Andersen, B., Seip, H. M., Strand, T. G. and Stølevik, R. *Acta Chem. Scand.* 23 (1969) 3224.
- Groth, P. and Hassel, O. *Acta Chem. Scand.* 18 (1964) 923.
- Seip, H. M., Strand, T. G. and Stølevik, R. *Chem. Phys. Lett.* 3 (1969) 617.
- Nakken, K. F., Tomas, E. and Brustad, T. *IAEA Panel on Modification of Radiosensitivity in Biological Systems*, Stockholm 1973. *To be published.*
- Ginoza, W. *Annu. Rev. Microbiol.* 21 (1967) 325.

Received October 17, 1973.