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Unwinding in an Undecamer Double-Helical DNA Fragment, after Binding of *cis*-$PtCl_2(NH_3)_2$ to a d(-GpTpG-) Sequence

Sir:

Binding of the antitumor drug *cis*-$PtCl_2(NH_3)_2$ (*cis*-DDP[15]) to DNA is supposed to be a main event in its mechanism of action.[1] Difunctional binding of *cis*-DDP to two adjacent guanine bases in the same strand of DNA appears to be the most frequently occurring lesion.[2] Other intrastrand binding modes of *cis*-DDP observed are to a d(-ApG-) fragment[3] and to two guanines, separated by a third base. The latter mode of binding was proposed— on the basis of the observation that base-pair mutations are caused by *cis*-DDP, in the *LacI* gene of *Escherichia coli*—to take place in d(-G-A-G-) and d(-G-C-G-) nucleotide sequences in particular.[4] Subsequently, Marcelis et al.[5] showed that, after reaction of the trinucleotide diphosphate d(GpCpG) with *cis*-DDP, *cis*-$Pt(NH_3)_2$(d(GpCpG)-*N7(1),N7(3)*)[6] was formed as the main (>90%) reaction product.

The distortion of the structure of DNA, after *cis*-DDP binding, is expected to be different for the various lesions. After investigating the distortion of a decamer double helix due to chelation at a d(-GpG-) fragment,[7] we now report an analogous study of a binding at a d(-GpTpG-) site in a undecanucleoside decaphosphate. It was decided not to use -GAG- because of the possibility of AG chelation,[3] whereas -GCG- was avoided because of the possible complications in case of self-association. It appeared that, in contrast to the d(-GpG-) case, two base pairs are dissociated, even at 0 °C. Moreover, the lowering of the melting temperature as a result of platination appears to be more severe in the d(-GpTpG-) case.

Experimental Section. The undecamers I, d(T-C-T-C-G-T-G-T-C-T-C), and II, d(G-A-G-A-C-A-C-G-A-G-A), were synthesized by using an improved phosphotriester method.[8] Strand I has the chelating d(-GpTpG-) sequence situated in the center,

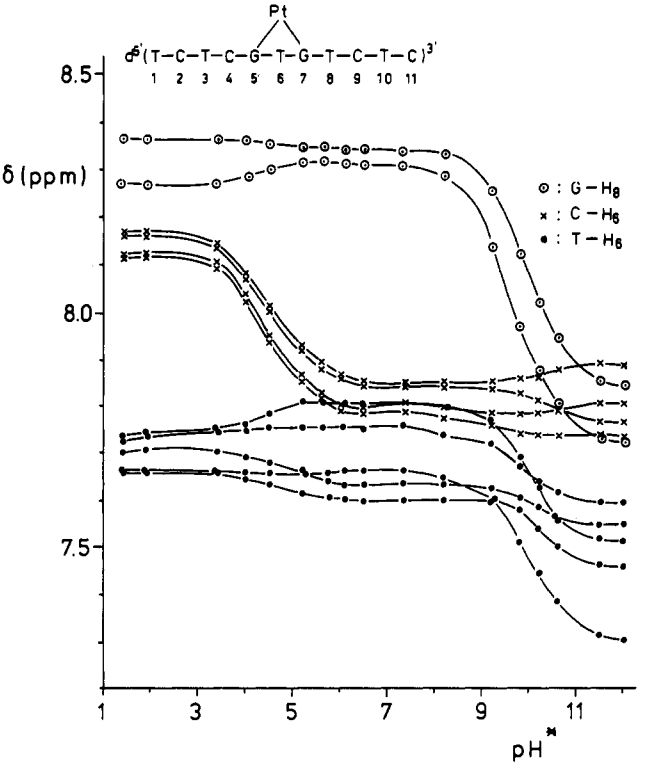

Figure 1. Chemical shifts (δ) vs. pH[15] (uncorrected meter readings) of nonexchangeable base protons of I–Pt: (○) guanine H8; (×) cytosine H6; (●) thymine H6. Chemical shifts are reported relative to DSS; T = 27 °C.

and no other rapidly reacting sites are present for platinum binding. A reaction mixture of equimolar amounts of I with *cis*-DDP was left for 7 days in the dark (0.05 mM aqueous solution, pH 6, 20 °C). The reaction products were separated with use of low-pressure anionic-exchange chromatography (DEAE A-25, eluent 0.0–0.8 M NaCl). A few small peaks (less than 10% each) appeared before the main reaction product, which represents more than 65% of the UV-absorbing material. Usually up to 20% unreacted undecamer (eluting after the main peak) was also present in the reaction mixture. Desalting of the compounds was performed by Sephadex G-25 gel filtration (eluent doubly distilled water). The resulting platinated single strand is abbreviated as I–Pt. In order to study the unplatinated and platinated double strands III and III–Pt, respectively, equimolar amounts of II were added to I and I–Pt, respectively.

For the study of the pH dependence of the chemical shifts (solvent 99.95% D_2O, T = 27 °C), proton NMR spectra of I–Pt were recorded at 300 MHz on a Bruker WM-300, selectively irradiating the HDO signal. All other spectra were recorded on

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(5) Marcelis, A. T. M.; den Hartog, J. H. J.; Reedijk, J. *J. Am. Chem. Soc.* **1982**, *104*, 2664.
(6) Following inorganic nomenclature, in formulas, the metal ion is listed first, followed by the ligands. The coordinating atoms of the oligonucleotide (numbering according to IUPAC-IUB recommendations: *Eur. J. Biochem.* **1983**, *131*, 9) can be indicated after the ligand, separated by a hyphen and italicized.
(7) den Hartog, J. H. J.; Altona, C.; van Boom, J. H.; van der Marel, G. A.; Haasnoot, C. A. G.; Reedijk, J. *J. Am. Chem. Soc.* **1984**, *106*, 1528.
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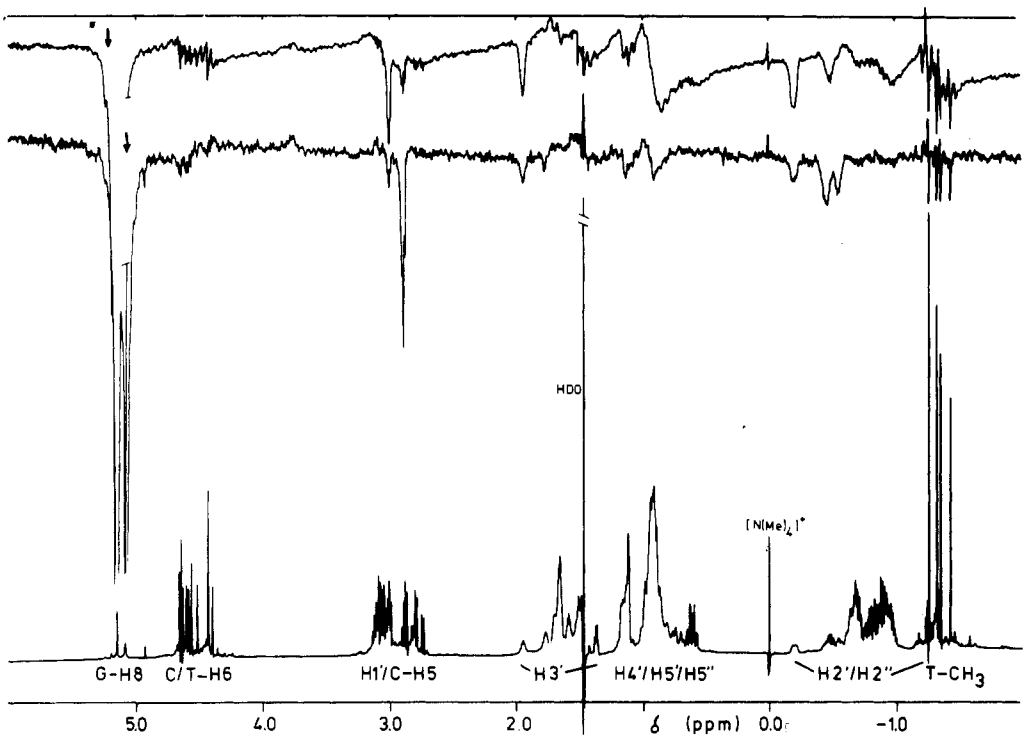

Figure 2. Proton spectrum of I–Pt, together with two NOE difference spectra. Arrows indicate the irradiated protons. (reference NMe_{4+}, 3.18 ppm downfield from DSS; T = 38 °C). The signal at 4.9 ppm is due to an impurity.

a Bruker WM-500, at 500 MHz. In case of the D_2O solutions a DASWEFT[9] pulse sequence was employed in order to reduce the residual HDO signal. For the observation of the imino proton signals in the double-stranded oligonucleotides samples were made up in H_2O/D_2O (93:7). A time-shared long pulse in combination with the data shift accumulation technique[10] was employed in order to reduce the H_2O signal. Chemical shifts are measured relative to NMe_4^+; $\Delta\delta$ is 3.18 ppm in relation to DSS. Circular dichroism (CD) spectra were recorded on a CNRS-Roussel-Jouan III dichrograph. For calculation of $\Delta\epsilon$, the estimated value of ϵ_{max} = 157 × 10^3 is used.

Results and Discussion. Undecamer Single Strands. In order to ascertain the platinum-binding sites in the single-stranded adduct I–Pt, the pH dependence of the base proton chemical shifts was studied.[5] The results (see Figure 1) clearly show that at low pH (pH <3) the N7 atoms of the two guanines are no longer accessible for protonation, proving platinum binding at these sites.[5] The deprotonation of the guanine N1 takes place above pH 8, which is slightly higher than in the GpG–platinum complexes.[7] This increase is not yet understood, although the deprotonation at thymine H3's might have an influence. Because of the apparent protonation of N3 of the four cytosine residues near pH 4.5, no platinum is coordinated here. The same holds for the thymine residues, because of deprotonation at pH >10.

The proton spectrum of I–Pt is redrawn in Figure 2, together with the results of two nuclear Overhauser effect (NOE) experiments. An observed NOE can be used as a measure of the relative distance to the irradiated proton. Hence, the relatively large NOE's of H2′ and H3′ apparent in the top spectrum, upon irradiation of H8 at 5.15 ppm (relative to NMe_4^+), is indicative for an anti conformation of the concomitant base. In contrast, irradiation of H8 at 5.08 ppm gives rise to a relatively large NOE at an H1′; therefore a syn conformation is most likely for this base with respect to its sugar ring. Unfortunately, no internucleotides NOE's are observed; therefore, it is impossible to assign the H8 protons to the specific nucleotide residues.

In a comparison of the d(-GpG-)-*cis*-Pt adduct[7] with the here described d(-GpTpG-)-*cis*-Pt one, two clear differences are apparent: (1) In the former, both bases adopt anti formations, whereas in the latter one base adopts the syn and one the anti geometry. (2) In the former, the H8 proton chemical shifts change more than 0.5 ppm (upfield and downfield shift changes) upon adding neighboring nucleotides to the d(GpG)-*cis*-Pt complex.[2,7] Comparison of I–Pt with d(GpCpG)-*cis*-Pt[11] shows that syn–anti equilibria change but that chemical shift changes are minor.

Undecamer Double Strands. The undecamer double strands III and III–Pt have been studied for their ability to form Watson–Crick hydrogen bonds and for information concerning the melting behavior of the duplexes.

In Figure 3a, the low-temperature imino proton spectrum of III is shown. Assignments are made by analogy with the results of our recently investigated decamer double strand[7] (that lacks the T-A base pair in the center) and were confirmed by 1-D NOE measurements (not shown). Interestingly, the imino proton of base pair T(1)-A(22) is observed and assigned (by NOE measurements), despite its tendency to exchange rapidly with water protons;[12] the resonance occurs at rather high field position for an A-T base pair (13.3 ppm) but is comparable to such a proton in analogous compounds.[13] When the temperature is increased, fraying at the ends of the duplex results in successive disappearance of several imino proton resonances and, between 53 and 58 °C, also the proton signals of the central part broaden and finally disappear.

In Figure 3b, spectra of III–Pt are shown. A comparison of the spectrum measured at pH 7 with the spectrum of III (Figure 3a) shows the following: first, three resonances in the A-T region are nearly isochronous, and second, around 11 ppm, a broad peak is observed. Because of the presence of the platinum bound to both G's in I–Pt, the central thymidine residue has to be "bulged out".[11] Therefore, one expects that the central T(6)-A(17) base

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(12) Measurements of the imino protons of d(A-T-C-C-T-A)-d(T-A-G-G-A-T) revealed only five protons at low temperature, a same T.A base-pair could not be detected (Haasnoot, C. A. G.; den Hartog, J. H. J.; Altona, C., unpublished results).
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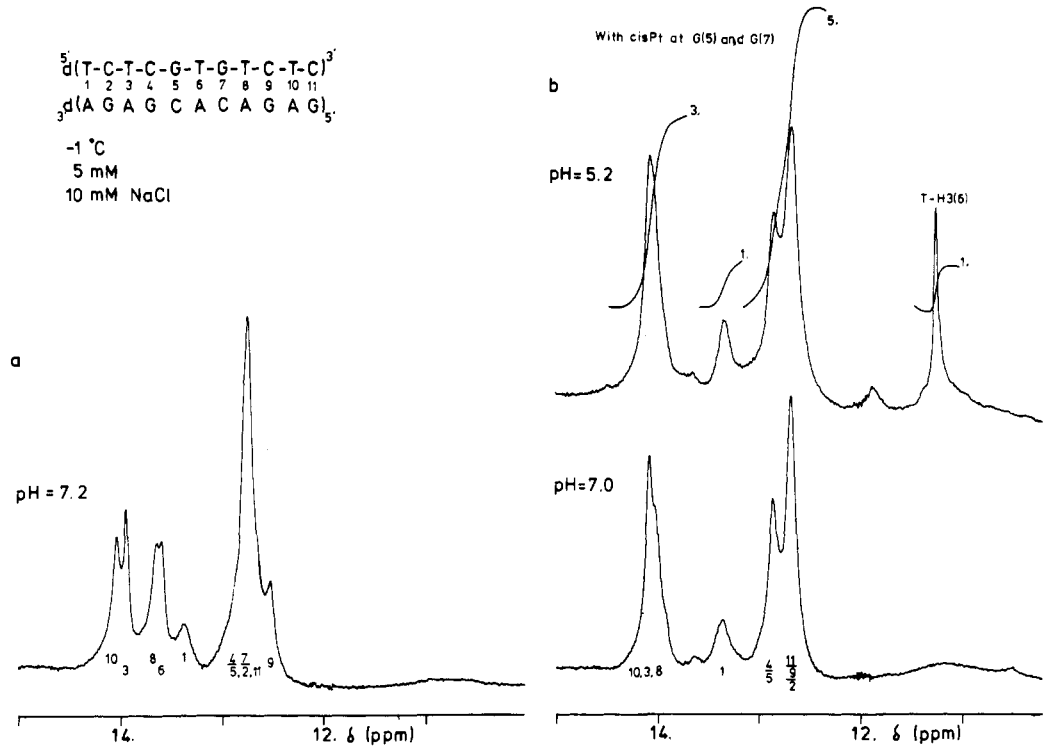

Figure 3. Low-field region of the 500-MHz proton spectra of III (a) and III–Pt (b). Chemical shifts (δ) are reported relative to DSS. A shorthand base-pair notation is used in the figure (as depicted in the upper left corner). The weak signal at ~12 ppm is not understood.

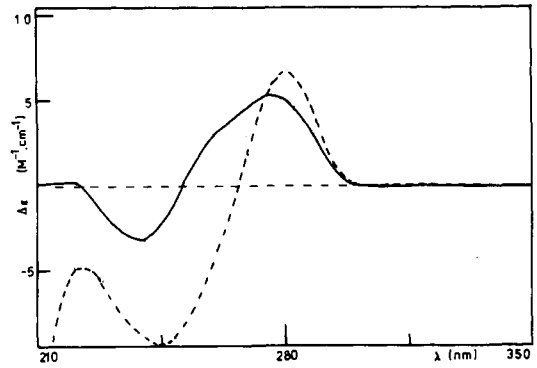

Figure 4. CD spectra of III (---) and III–Pt (—) at 2.5 °C.

pair is dissociated. In this case an H3 proton of a thymine base, not involved in hydrogen bonding, should be observable between 10 and 11 ppm at low temperature and slightly acidic pH.[13] Figure 3b (top spectrum) indeed shows a resonance at 11.23 ppm with an integrated intensity of one proton. The chemical shift of H3 in d(T-T-T-T) under these conditions is 11.2 ppm.[13] The apparent lack of shielding of H3(6) indicates that the thymine base indeed is "bulged out" from the stacked array of nucleobases. Further evidence for this comes from 1-D NOE experiments, which show that NOE effects are completely absent between H3(T6) and protons of neighboring bases.

The integration of the several peaks in Figure 3b (top spectrum) reveals three protons for the peak at 14.05 ppm. Furthermore, the resonance at 13.33 ppm (T(1)-A(22)) and the thymine proton, not involved in hydrogen bonding, both represent one proton, so all five T H3 imino protons are detected. Integration of the G H1 protons shows that the peaks at 12.83 and 12.66 ppm represent only five protons; hence, besides T(6)-A(17), also a G-C base pair is dissociated. The near-isochronicity of the A-T base pair resonances preclude unambiguous assignment of the remaining G-C base pairs. However, a tentative assignment can be based on the relatively large downfield shift of base pair T(8)-A(15) in III–Pt compared to the position in III. This appears to indicate that base pair G(7)-C(16) is dissociated. At increasing temperature, between 36 and 42 °C, remaining imino protons broaden and disappear. It should be realized that this is not an accurate measure of the melting temperature. The destabilization of the helix can be more accurately measured with CD spectroscopy. The melting curves of solutions of 6×10^{-6} M double strand reveal midpoint temperatures of 39 °C for III and 13 °C for III–Pt (0.1 M NaCl, trace Mg^{2+} added). Both melting temperatures are approximately 10 °C degrees higher for solutions prepared in 0.2 M NaCl. The CD spectra of III and III–Pt, measured at 2.5 °C, are shown in Figure 4. In contrast to the increase of $\Delta\epsilon$ of the band at 280 nm, due to platinum chelation by a d(-GpG-) sequence,[14] the platinum binding here (to d(-GpTpG-)) causes a decrease of 20% of this band. The decrease is accompanied by a very small hypsochromic shift (from 280 to 278 nm) and the appearance of a shoulder at 264 nm.

In conclusion, we have shown that the distortion of the undecamer double helix by platinum chelation by a d(-GpTpG-) sequence comparises the dissociation of two hydrogen bonds and a lowering of the duplex melting temperature by 26 °C. The distortion described here is much larger than that due to *cis*-DDP binding to two adjacent guanine bases,[7] which might be a reason that this lesion is relatively important, despite its less frequent occurrence in DNA.

Acknowledgment. This research was supported in part by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organisation for the Advancement of Pure Research (ZWO). Financial aid was also obtained through Grant MBL-83-1 of the Koningin Wilhelmina Fonds (Dutch Organisation for Fight Against Cancer), Amsterdam, The Netherlands. The authors are indebted to Dr. C. A. G. Haasnoot, P. A. W. van Dael, and W. Guyt for technical assistance with the recording of the 500-MHz spectra at the SON 500/200 HF-NMR facility at Nijmegen and to Johnson-Matthey

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(15) Abbreviations: *cis*-DDP, *cis*-diamminedichloroplatinum(II); undecamer I, d(T-C-T-C-G-T-G-T-C-T-C) numbering, T(1), C(2), ..., C(11); undecamer II, d(G-A-G-A-C-A-C-G-A-G-A) numbering, G(12), A(13), ..., A(22); I–Pt, *cis*-Pt$(NH_3)_2$(d(T-C-T-C-G-T-G-T-C-T-C)-*N7*(5),-*N7*(7)); III, I + II; II I–Pt, I–Pt + II; NOE, nuclear Overhauser enhancement; DSS, sodium 4,4-dimethyl-4-silapentanesulfonate.

Ltd. for their loan of K_2PtCl_4. C. Elie is thanked for recording CD spectra.

Registry No. d(T-C-T-C-G-T-G-T-C-T-C), 94891-00-6; *cis*-$PtCl_2(NH_3)_2$, 15663-27-1.

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Oxygen Donation to Manganese(III) Tetraphenylporphyrin Chloride. Low Reactivity of Hydroperoxides as Oxygen Donors to Manganese(III) Porphyrins

Sir:

Modeling of peroxidases and cytochromes P-450 have focused on the preparation of high-valent oxometalloporphyrins as the active intermediates in catalytic oxidations. For this purpose (tetraphenylporphinato)iron(III) chloride ($ClFe^{III}TPP$) has been used with the "oxene" donors PhIO,[1] percarboxylic acids,[2,3] hydroperoxides,[3] *N,N*-dimethylaniline *N*-oxides,[4] and oxaziridines.[5] These studies have been logically extended to $ClCr^{III}TPP$ and $ClMn^{III}TPP$ salts. A puzzling observation is that, although $ClFe^{III}TPP$, $ClCr^{III}TPP$, and $ClMn^{III}TPP$ appear to be roughly comparable catalysts for oxidations involving PhIO and *m*-$ClC_6H_4CO_3H$, $ClMn^{III}TPP$ is at best a very poor catalyst with alkyl hydroperoxide.[3,6] We show in this paper that the low reactivity of $ClMn^{III}TPP$, compared with $ClFe^{III}TPP$ and $ClCr^{III}TPP$, with alkyl hydroperoxides is due to its much greater sensitivity to the acidity of the leaving group (YOH) as evidenced by Brønsted β_{lg} values.

The kinetics of the reaction of percarboxylic acids and hydroperoxides with $ClMn^{III}TPP$ were studied in dried benzonitrile under anaerobic conditions at 30 °C. From past experience,[7] benzonitrile has proven to be a solvent of choice for studies of oxygen transfer to manganese(III) porphyrin salts. Reactions were carried out under the conditions of 50–400 turnovers of metalloporphyrin. The concentration range of $ClMn^{III}TPP$ and of oxygen donor was chosen on the basis of the reactivity of the latter. The higher valent oxomanganese porphyrin species was trapped with 2,4,6-tri-*tert*-butylphenol (TBPH),[8] and the reactions were followed by monitoring the increase in absorbance of 2,4,6-tri-*tert*-butylphenoxy radical (TBP·) at 630 nm (eq 1). Formation

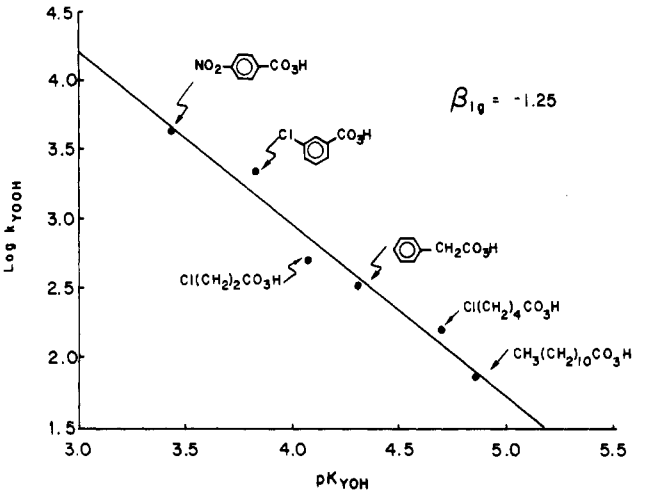


Figure 1. Plot of the log of the second-order rate constants (k_{YOOH}) for the reaction of $ClMn^{III}TPP$ with percarboxylic acids (YOOH) vs. pK_a of the carboxylic acid leaving groups (pK_{YOH}).

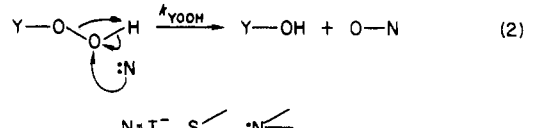
$$YOOH + ClMn^{III}TPP \xrightarrow[slow]{k_{YOOH}} YOH + O{=}Mn^{V}(Cl)TPP$$

$$2TBPH + O{=}Mn^{V}(Cl)TPP \xrightarrow{fast} 2TBP\cdot + H_2OMn^{III}(Cl)TPP \quad (1)$$

$$H_2OMn^{III}(Cl)TPP \xrightarrow{fast} H_2O + ClMn^{III}TPP$$

of TBP· was found to follow the first-order rate law at all [YOOH] employed. That oxygen transfer to $ClMn^{III}TPP$ is rate determining is shown by the linear dependence of the observed first-order rate constant (k_{obsd}) on [$ClMn^{III}TPP$] and its independence on [TBPH] and [YOOH] (when [TBPH] ≫ [YOOH] ≫ [$ClMn^{III}TPP$]). The species $H_2OMn^{III}(Cl)TPP$ has been shown, by a radiochemical technique,[7] to be very unstable in even wet benzonitrile so that its breakdown is expected to be quite rapid. Values of k_{YOOH} were determined as slopes of plots of k_{obsd} vs. [$ClMn^{III}TPP$]. The reactions with percarboxylic acids were found to be rapid and to involve heterolytic O–O bond cleavage.[9] Also, at completion of the turnover of all peracids, $ClMn^{III}TPP$ was found (spectrally) to be intact. Reactions of $ClMn^{III}TPP$ with alkyl hydroperoxides could not be detected. The hydroperoxides employed included those previously shown to react with $ClCr^{III}TPP$ and $ClFe^{III}TPP$, ranging from the previously found most reactive diphenylhydroperoxyacetonitrile to the least reactive *tert*-butyl and cumyl hydroperoxides.[5]

A dependence upon leaving group pK_a is an established characteristic for nucleophilic displacements upon the terminal oxygen of percarboxylic acids and hydroperoxides when accompanied by heterolytic O–O bond scission (eq 2).[10] For the reactions of eq



2, log k_{YOOH} is a linear function of the pK_a of YOH (eq 3; β_{lg}

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(9) $ClMn^{III}TPP$ (8.86×10^{-3} M) and $C_6H_5CH_2CO_3H$ (2.53×10^{-3} M) were allowed to react for 90 min in dry PhCN under N_2 when an excess of an etherial solution of CH_2N_2 was added. GC analysis showed that the peracid had been converted to the corresponding carboxylic acid, which was recoverable in 95% yield as the ester $PhCH_2CO_2CH_3$. Had the percarboxylic acid undergone homolysis, the resulting $PhCH_2CO_2\cdot$ would have undergone immediate decarboxylation: Barlett, P. D.; Ruchardt, C. *J. Am. Chem. Soc.* **1960**, *82*, 1756.
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