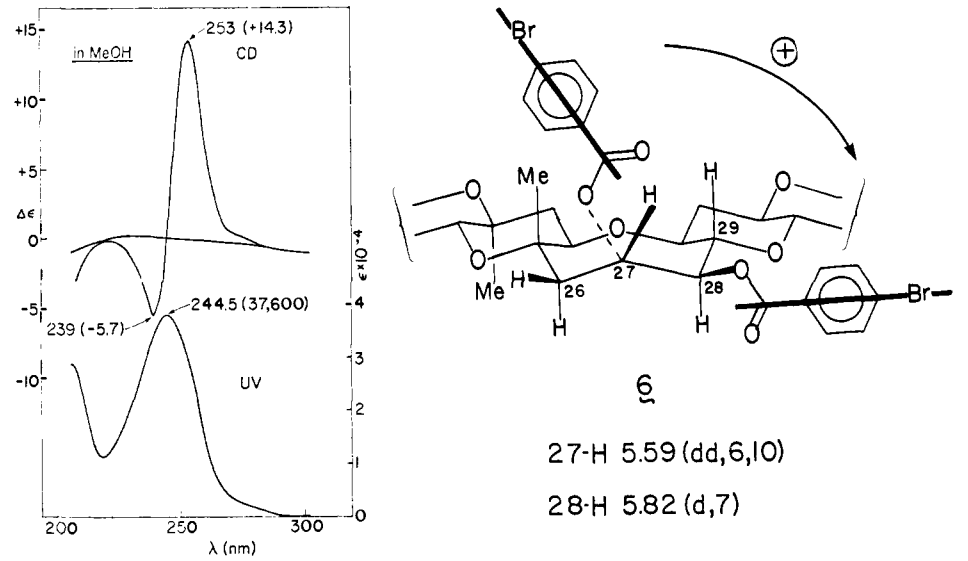


Figure 3. CD and UV spectra of **6**; positive exciton chirality of the 27,28-dibenzoate system in ring H.

CD[16] (MeOH) 225 nm ($\Delta\epsilon$ -3.93, ene-lactone $\pi\pi^*$), 257 (+6.77, ene-lactone $n\pi^*$), 329 (+0.16, enal $n\pi^*$); FTIR[17] (KBr pellet) 1737 cm^{-1} (lactone), 1691 (enal). NMR[18] data are as follows: 250-MHz ^1H NMR ($CDCl_3$) δ 1.04 (d, 3, J = 7 Hz, 13Me), 1.18 (s, 3, Me), 1.23 (s, 3, Me), 1.30 (s, 6, Me's), 1.31 (s, 3, Me), 1.97 (m, 3, 3-Me), 4.27 (ddq, 1, J = 10.6, 2.2 Hz, 4-H), 5.73 (dq. 1, J = 2.2, 1.0 Hz, 2-H), 5.78 (m, 2, 27-H and 28-H), 6.09 (d, 1, J = 1 Hz, 43-H_b), 6.32 (d, 1, J = 1 Hz, 43-H_a), and 9.53 (s, 1, 42-H); ^{13}C NMR ($CDCl_3$) δ 163.5 (C-1), 115.6 (C-2), 161.0 (C-3), 127.2 (C-27), 135.2 (C-28), 147.8 (C-41), 194.2 (C-42), and 135.7 (C-43); in addition 22 C-O signals were observed between δ 88.4 and 63.3, 12 CH_2's and 1 CH between δ 44.7 and 28.9, and 7 methyl signals appeared between δ 22.0 and 13.9.

It is to be noted that the UV and CD data are complementary, and hence both are necessary to reveal all of the transitions. The UV only shows a single short wavelength maximum corresponding to the enal, the other bands being too weak to be measured; in contrast, these weak UV transitions exhibit clear CD Cotton effects.

The absolute configuration of BTX-B (**1**) was determined by introducing a 1,2 dibenzoate system into the molecule via a 5-step sequence (Scheme I) and then applying the nonempirical dibenzoate chirality method:[19] (i) hydrogenation of 3.4 mg of BTX-B (**1**) with H_2/5% Pd-$BaSO_4$ in THF at 0 °C selectively reduced the 41-ene to give **2**; (ii) $NaBH_4$ reduction of the ene-lactone and aldehyde moieties gave the tetraol **3**; (iii) acetylation with Ac_2O/pyridine afforded the tetraacetate **4**; (iv) cis hydroxylation of the 27-ene located in the ring H was achieved with OsO_4 in THF.[20] The resultant diol **5** was derivatized with *p*-bromobenzyl chloride in pyridine to give 1.7 mg of the dibenzoate **6**. Each product was isolated and checked by ^1H NMR spectroscopy and D/CI-MS; the overall yield of the five-step sequence was 31%.

The absence of coupling between C-27 and C-28 protons in **6** suggests that their dihedral angle is close to 90°, thus indicating that the eight-membered ring adopts the crown conformation (Figure 3). Computer analysis using the Allinger MM2 force-field program also shows that the crown conformer is favored over the half-chair conformer by several kilocalories.[21]

As shown in Figure 3, the dibenzoate derivative **6** showed a typical split CD, and this established the chirality of the 27-OBz/28-OBz as being positive.[19] The absolute configuration of BTX-B is as shown in structure **1**.

Brevetoxin B is made up of a single carbon chain locked into a rigid ladderlike structure consisting of 11 contiguous trans-fused ether rings. There is no precedent for this extraordinary structure, and in addition, a plausible biogenetic scheme is not obvious. Structural studies of the minor toxins, BTX-A and BTX-C, are in progress.

Acknowledgment. The work at Cornell University was supported by NIH Grant CA 24487 and a training grant to Donna Van Engen. Studies at Columbia University were supported by NIH grant AI 10187.

Supplementary Material Available: Tables of fractional coordinates, thermal parameters, bond distances, bond angles, observed and calculated structure factors (25 pages). Ordering information is given on any current masthead page.

(16) Jasco J-40 CD.
(17) IBM FTIR/85.
(18) Bruker WM-250 NMR.
(19) (a) Harada, N.; Nakanishi, K. *J. Am. Chem. Soc.* **1969**, *91*, 3989-3991. (b) Harada, N.; Nakanishi, K. *Acc. Chem. Res.* **1972**, *5*, 257. (c) "Exiton Coupled Circular Dichroism-Application in Organic and Bioorganic Stereochemistry"; University Science Books: Mill Valley, Ca, in press.
(20) Molecular models show that OsO_4 can approach only from one side.
(21) Allinger's MM2 program was obtained from the Quantum Chemistry Exchange (QCPE Program No. 395); we are indebted to Professor W. C. Still for the computer calculations.

High-Energy Fragmentation of Chlorophyll *a* and Its Fully Deuterated Analogue by ^{252}Cf Plasma Desorption Mass Spectrometry

Jerry E. Hunt† and Ronald D. Macfarlane*

Chemistry Department, Texas A&M University College Station, Texas 77843

Joseph J. Katz*

Chemistry Division, Argonne National Laboratory Argonne, Illinois 60439

Ralph C. Dougherty*

Chemistry Department, Florida State University Tallahassee, Florida 32306

Received April 6, 1981

This report discusses results of the interaction of fast heavy ions (nuclear fission fragments from ^{252}Cf decay) with thin films of chlorophyll *a* (Chl *a*). Previous studies on Chl *a* by ^{252}Cf-plasma desorption mass spectrometry (^{252}Cf PDMS)[1] were directed to

†Chemistry Division, Argonne National Laboratory, Argonne, IL 60439.
(1) Hunt, J. E.; Macfarlane, R. D.; Katz, J.J.; Dougherty, R. C. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 1745-1748.

0002-7863/81/1503-6775$01.25/0

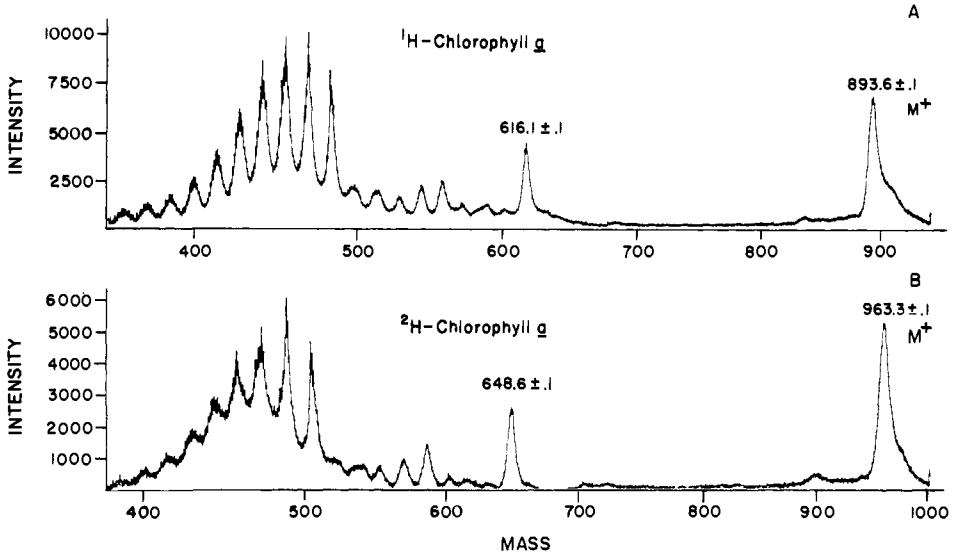


Figure 1. ^{252}Cf-PDMS spectra of (A), [^{1}H]chlorophyll *a* and (B) [^{2}H]chlorophyll *a*.[4] The mass scales are not identical in the two spectra.

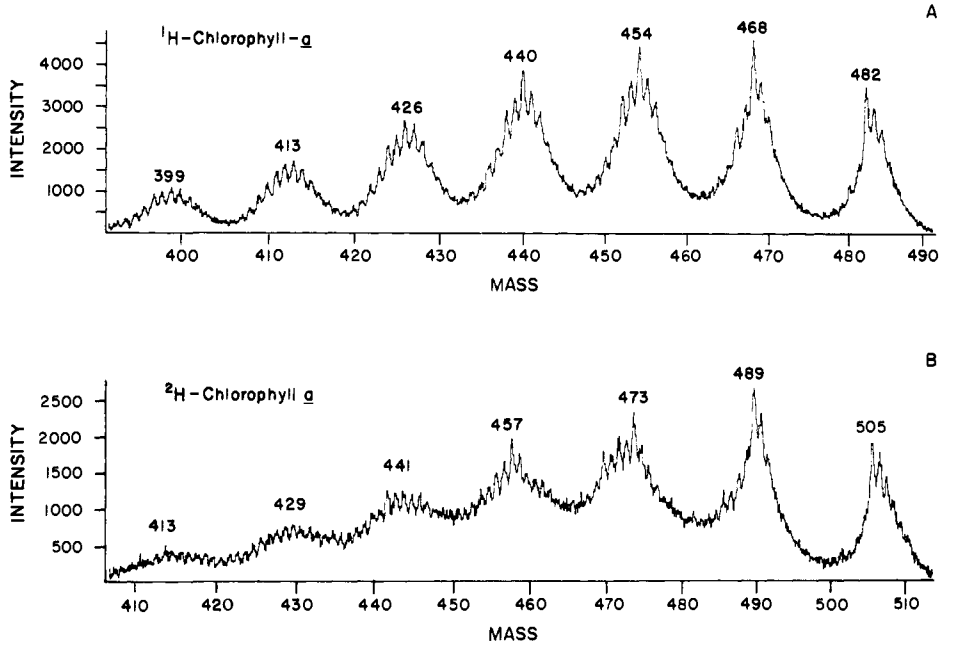


Figure 2. Detailed presentation of the mass fragmentation pattern in the mass range 400–500 u: (A) [^{1}H]Chl a; (B) [^{2}H]Chl *a*. The mass scales are not identical. The mass numbers correspond to the peak of maximum intensity.

the self-assembly of chlorophyll *a* and the formation of oligomers.[2] ^{252}Cf PDMS has been used as a "soft ionization" technique to produce intact molecular ions of other thermally labile nonvolatile molecules.[3] We show here that there is also an energetic part of the interaction, producing extremely high molecular excitations. A comparison of the ^{252}Cf-PDMS spectra of Chl *a* of ordinary isotopic composition ([^{1}H]Chl *a*) with those of essentially fully deuterated Chl *a*[4] ([^{2}H]Chl *a*) makes it possible to establish elemental compositions in the fragmentation pattern and to deduce plausible structures for the fragment ions produced. This is a new method for determining elemental compositions of ions that relies on hydrogen counting. The difference in mass between corresponding ions in the [^{1}H]Chl *a* and [^{2}H]Chl *a* spectra gives the number of hydrogens in the molecule. If we assume that all ions contain four nitrogens, the elemental composition for the ion in question can be determined.

Thin films of [^{1}H]Chl *a* and [^{2}H]Chl *a* (~25 μg/cm^2) were prepared by evaporation from CCl_4 solutions on 1.5 μm thick aluminized Mylar. The films were exposed in the PDMS spectrometer to a fission flux of 2000 s^{-1} cm^{-2} for a period of 1 h. The positive ion spectra in the mass region 350–1000 u (figure 1) are in sharp contrast to the fragmentation pattern of Chl *a* obtained by electron impact in a conventional mass spectrometer or by field desorption.[5] The latter show almost entirely small fragments formed by fragmentation of substituents on the Chl *a* macrocycle. In the ^{252}Cf PDMS experiments an intense molecular ion (M^+) is observed at 893.6 ± 0.1 u, which corresponds to the isotopically averaged molecular weight of [^{1}H]Chl *a*, and 963.3 ± 0.1 u, which corresponds to that of [^{2}H]Chl *a*. The mass difference for the molecular ions (69.7 ± 0.15) confirms as expected that 70 of the 72 hydrogen atoms in the [^{2}H]Chl *a* molecular ion are deuterium. From NMR studies it is known that the γ-methine and the C-10

(2) Katz, J. J.; Shipman, L. L.; Cotton, T. M.; Janson, T. R. "The Porphyrins"; Dolphin, D. Ed.; Academic Press: New York, 1978; Vol. V, pp 401–458.

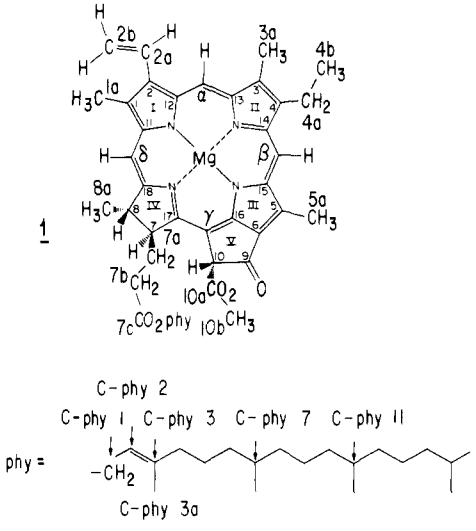
(3) Macfarlane, R. D.; Torgerson, D. F. *Science (Washington, D.C.)* **1976**, *191*, 920–925.

(4) Strain, H. H.; Thomas, M. R.; Katz, J. J. *Biochem. Biophys. Acta.* **1963**, *75*, 306–311.

(5) Dougherty, R. C.; Dreifuss, P. A.; Sphon, J.; Katz, J. J. *J. Am. Chem. Soc.* **1980**, *102*, 416–418.

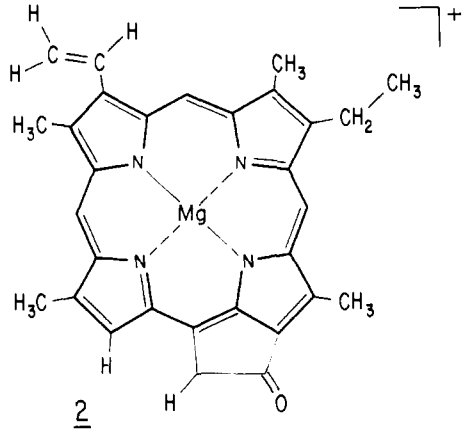
hydrogen atom on ring V of Chl *a* (**1**) are quickly exchanged with exchangeable hydrogen in the solvents during the chlorophyll extraction and purification process.[6] The shoulder to high mass of the molecule ion in Figure 1 has a visual aspect similar to isotope peaks; however, this shoulder is roughly 16 u higher in mass than Chl *a* and must be due to an ion–molecule reaction.

In addition to the molecular ions, an intense partially resolved fragmentation pattern was observed from 300–700 u, which was mass shifted in the deuterated analogue. The peak observed at 616 u corresponds to a molecular ion that has lost its aliphatic phytyl group and has added 2 hydrogen atoms to structure **1**. The



ions of lower mass are associated with the loss of additional side groups from the macrocycle and partial cleavage of rings IV and V. These ions form a periodic structure with a spacing of ~14 u for [^{1}H]Chl *a* and ~16 u for the deuterated analogue. Figure 2 shows the fragmentation pattern with an expanded mass scale, which demonstrates that each peak in the gross distribution is a composite of individual ions separated by 1 u. By comparing the spectra for [^{1}H]Chl *a* and [^{2}H]Chl *a* (d_{70}) in this mass region, the number of hydrogen atoms in selected fragment ions were determined from the corresponding mass differences. For example, the ion at 616 u in the [^{1}H]Chl *a* spectrum was at 649 u for the [^{2}H]Chl *a* (d_{70}) molecule, indicating that 33 deuterium atoms are present in the latter fragment. The chemical formula for the 616-u fragment ion then is $C_{35}H_{35}N_4O_5Mg$, the 2 labile hydrogen atoms making up the mass difference.

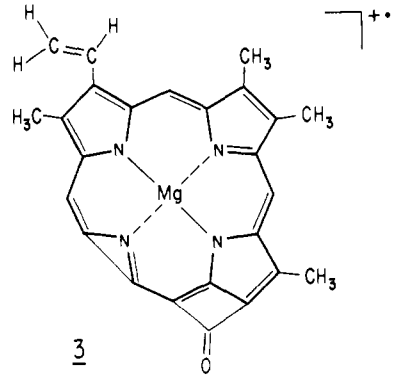
The ion at 481.7 u has been assigned structure **2** on the basis of its calculated elemental composition. It is clear from the



$C_{30}H_{25}N_4OMg$
obs. 481.7
calc. 481.872

spectrum in Figure 2 that a series of ions which differ in hydrogen content by up to 4 u must account for the 481.7-u peak. All of the polar side chains on the macrocycle in this fragment must have been lost leaving a stable macrocyclic structure to which only small aliphatic side groups were attached.

For the ions with mass 467 u and above, structures can be drawn that are consistent with electron-impact-induced fragmentation of the chlorin structure.[7] The fragment ions below mass 439 require cleavage of bonds to substituents bound directly to the macrocyclic ring. An example is the fragment ion at 414 u, which has an empirical formula $C_{25}H_{18}N_4OMg$ and an "atom bookkeeping structure" shown in **3**. The spectra in Figure 2 suggest



$C_{25}H_{18}N_4OMg$
obs. 414.5
calc. 414.76

that the series of ions that make up the 414-u peak differ in mass by up to 10 u. The "bookkeeping structure", **3**, serves only to indicate the extensive fragmentation that Chl *a* must have experienced to form this ion. Multiple cleavages of substituent side chains bound directly to aromatic rings required for the rationalization of the lighter fragments, e.g., the 414-u peak, are very unusual in other types of mass spectra. These reactions require the transfer of a minimum of 25 eV of excitation to the molecule. The time scale for these events must preclude complex rearrangements, and the fragmentation process should be restricted to simple bond cleavage.

The fragmentation reactions that were observed in the ^{252}Cf-PDMS spectrum of Chl *a* must have occurred on a short time scale (order 10^{-12}–10^{-14} s). The negative ^{252}Cf-PDMS spectrum of Chl *a* showed fragment ions with similar intensities and patterns to the positive ion spectrum. These ions must have been formed subsequent to the fragmentation reactions; otherwise a quasi-equilibrium-driven autoionization process would have occurred which would have neutralized part of the negative ion beam. Since all of the negative ion beam was deflected by an external magnetic field, this process evidently did not occur. The positive ion fragments were most likely formed in a similar way. Since the extensive fragmentation required a minimum of 25-eV exitation, the fragmentation must have been explosive (10^{-12}–10^{-14} s); otherwise redistribution of the energy would have formed doubly charged ions which would not have fragmented so extensively. Doubly charged ions were shown to be absent from the spectrum by the fact that all of the observed ions experienced the same deflection when an external magnetic field was applied perpendicular to the flight path. It is possible that doubly charged ions were formed and neutralized by charge exchange in the plasma; however, these ions could not have been responsible for the very high energy fragmentation reactions that were observed. Finally, the mass resolution for the fragment ions was comparable to that for molecule ions. If the fragmentation reactions occurred subsequent to ionization and at times greater than 10^{-14} s from the fission fragment pulse, the resulting metastable continuum would

(6) Dougherty, R. C.; Strain, H. H.; Katz, J. J. *J. Am. Chem. Soc.* **1965**, *87*, 104–109.

(7) Dougherty, R. C. "Biochemical Applications of Mass Spectroscopy"; Waller, G. W., Ed.; Wiley: New York, 1972, Vol. 1, pp 591–600; Vol. 2, pp 693–701.

have inevitably decreased the resolution for the fragment ions.

Chlorophyll *b*, pheophytin *a*, pyrochlorophyll *a*, and the pyrochlorophyll *a* synthetic linked pair[8] all exhibit this intense fragmentation structure, and there is evidence that cyanocobalamin[9,10] also shows the same remarkable fragmentation behavior.

Acknowledgment. This work was supported by the National Science Foundation and Robert A. Welch Foundation (J.E.H. and R.D.M.), the Division of Chemical Sciences, Office of Basic Energy Sciences of the U.S. Department of Energy (J.J.K.), and the National Institute of Health (R.D.M. and R.C.D.).

(8) Boxer, S. G.; Closs, G. L. *J. Am. Chem. Soc.* **1976**, *98*, 5406-5408.
(9) Macfarlane, R. D.; Vickrey, T. M; Hunt, J. E., to be published.
(10) Ens, W.; Standing, K. G.; Chait, B. T.; Field, F. H. *Anal. Chem.* **1981**, *53*, 1241-1244.

Infrared Spectroscopy of Oxidized Metalloporphyrins: Detection of a Band Diagnostic of Porphyrin-Centered Oxidation

Eric T. Shimomura, Martin A. Phillippi, and Harold M. Goff*

Department of Chemistry, University of Iowa Iowa City, Iowa 52242

William F. Scholz and Christopher A. Reed*

Department of Chemistry, University of Southern California Los Angeles, California 90007

Received September 2, 1980

A long-standing problem in the redox chemistry of metalloporphyrins is identifying whether the site of redox is metal centered or ligand centered. The most argued case is $[FeCl(TPP)]^+$ (TPP = tetraphenylporphyrinate), which for many years has been considered an iron(IV) complex.[1,2] More recently, a strong case has been made for its reformulation as an iron(III) radical cation.[3,4] Traditionally, choosing between metal or ligand oxidation has relied on solution-derived criteria, particularly UV-VIS spectroscopy, EPR spectroscopy, magnetic moment measurements, and electrochemical methods.[5-7] Such criteria are usually reliable for cases where metal oxidation is well separated in potential (> ~300 mV) from porphyrin ring oxidation but frequently lead to considerable ambiguity in the most interesting cases, namely, those of iron porphyrins. The importance of iron porphyrins which are oxidized above the ferric state lies in their appearance as intermediates in the hemoproteins peroxidase and catalase[8] and probably also in cytochrome P-450.[9] For example, it is generally accepted that the so-called compound I of horseradish peroxidase (HRP I), which is oxidized 2 equiv above iron(III), is an iron(IV) radical cation and not an iron(V) porphyrin.[10,11] UV-VIS spectroscopy,[10] Mössbauer spectroscopy,[12] and very recently ENDOR spectroscopy[13] have provided convincing support for this assignment. However, for many iron porphyrin complexes and other metalloporphyrins there remains considerable ambiguity over their formulation.

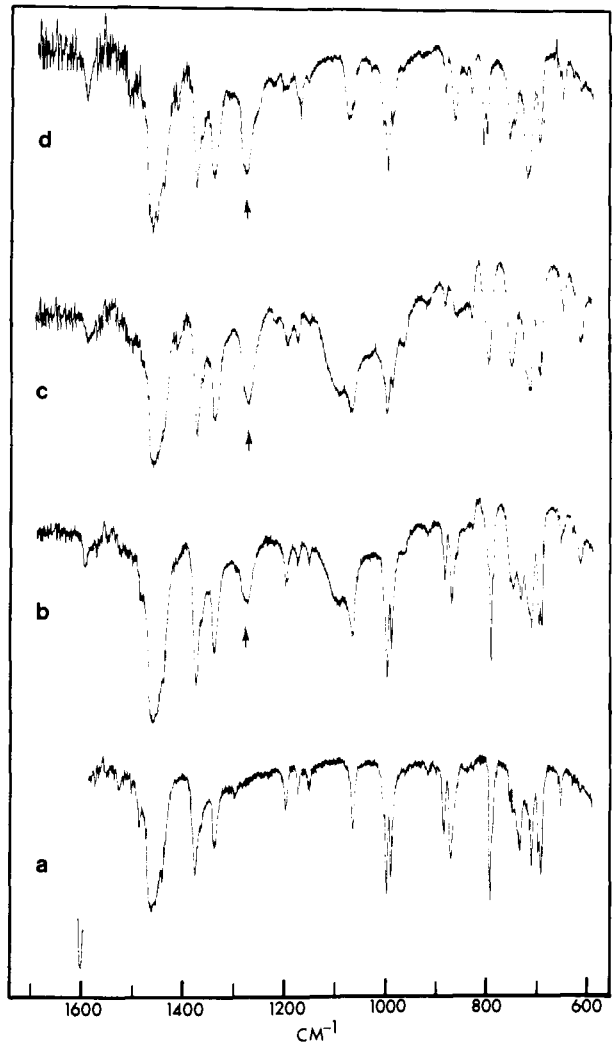


Figure 1. Infrared spectra of μ-oxo dimer iron tetraphenylporphyrin complexes. Compounds were examined as Nujol mulls and spectra were recorded at room temperature by using a single sodium chloride plate. (a) $Fe_2(TPP)_2O$; (b) $[Fe_2(TPP)_2O][ClO_4]$; (c) $[Fe_2(TPP)_2O][ClO_4]_2$; (d) $[Fe_2(TPP)_2O][SbCl_6]_2$.

In the course of our work in developing NMR techniques for characterizing oxidized metalloporphyrins[14,15] and developing preparative methods for their isolation,[3] we have discovered that IR spectroscopy provides a new criterion for identifying porphyrin radical cations. From the numerous cases we have studied, there is reason to believe that this criterion may have a unique generality. It also suggests that distinguishing between metal and ring oxidation in metalloporphyrins has significant chemical implications and is not a question of semantics.

In oxidized tetraphenylporphyrin complexes we find that a strong IR band in the region of 1270-1295 cm^{-1} seems to be diagnostic of the $TPP^{+\cdot}$ radical cation. In Figure 1 are displayed the Nujol mull IR spectra of the μ-oxo ferric dimer $Fe_2(TPP)_2O$ (a), its one-electron oxidation product $[Fe_2(TPP)_2O][ClO_4]$ (b), its two-electron oxidation product as the diperchlorate salt $[Fe_2(TPP)_2O][ClO_4]_2$ (c), and the bis(hexachloroantimonate) salt $[Fe_2(TPP)_2O][SbCl_6]_2$ (d). The perchlorate salts were prepared electrochemically as previously described[14,15] or by silver per-

(1) Felton, R. H.; Owen, G. S.; Dolphin, D.; Fajer, J. *J. Am. Chem. Soc.* **1971**, *93*, 6332-6334.
(2) Felton, R. H.; Owen, G. S.; Dolphin, D.; Forman, A.; Borg, D. C.; Fajer, J. *Ann. N.Y. Acad. Sci.* **1973**, *206*, 504-514.
(3) Gans, P.; Marchon, J.-C.; Reed, C. A.; Regnard, J.-R. *Nouv. J. Chim.* **1981**, *5*, 203-204.
(4) Phillippi, M. A.; Shimomura, E. T.; Goff, H. M. *Inorg. Chem.* **1981**, *20*, 1322-1325.
(5) Fuhrhop, J.-H. *Struct. Bonding (Berlin)* **1974**, *18*, 1-67.
(6) Felton, R. H. In "The Porphyrins"; Dolphin, D., Ed.; Academic Press: New York, 1978; Vol. V, pp 53-125.
(7) Fuhrhop, J.-H.; Kadish, K. M.; Davis, D. G. *J. Am. Chem. Soc.* **1973**, *95*, 5140-5147.
(8) Hewson, W. D.; Hager, L. P. In "The Porphyrins"; Dolphin, D., Ed.; Academic Press: New York, 1979; Vol. VII, pp 295-332.
(9) Groves, J. T.; Haushalter, R. C.; Nakamura, M.; Nemo, T. E.; Evans, B. J. *J. Am. Chem. Soc.* **1981**, *103*, 2884-2886.
(10) Dolphin, D.; Forman, A.; Borg, D. C.; Fajer, J.; Felton, R. H. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 614-618.
(11) Hanson, L. K.; Chang, C. K.; Davis, M. S.; Fajer, J. *J. Am. Chem. Soc.* **1981**, *103*, 663-670.
(12) Schulz, C. E.; Devaney, P. W.; Winkler, H.; Debrunner, P. G.; Doan, N.; Chiang, R.; Rutter, R.; Hager, L. P. *FEBS Lett.* **1979**, *103*, 102-105.
(13) Roberts, J. E.; Hoffman, B. M.; Rutter, R.; Hager, L. P. *J. Biol. Chem.* **1981**, *256*, 2118-2121.
(14) Phillippi, M. A.; Goff, H. M. *J. Am. Chem. Soc.* **1979**, *101*, 7641-7643.
(15) Phillippi, M. A.; Goff, H. M., submitted for publication.

0002-7863/81/1503-6778$01.25/0