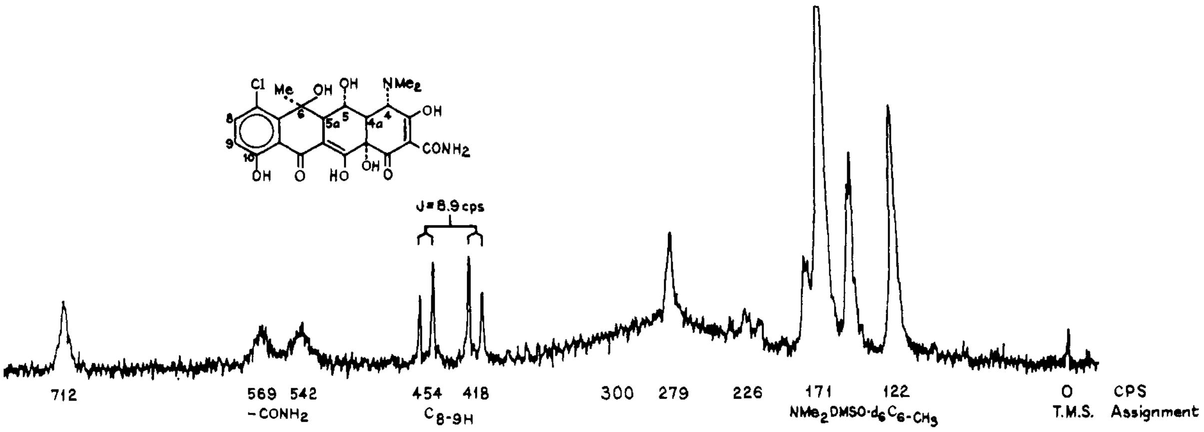

Figure 1. Nmr spectrum (60 Mc) of 5-hydroxy-7-chlortetracycline hydrochloride in dimethyl sulfoxide-d_6.

5-Hydroxy-7-chlortetracycline hydrochloride (IIc) crystallizes in small yellowish needles, $[\alpha]^{25}\mathrm{D}$ $-237°$ (c 0.880, 0.001 N MeOH·HCl). *Anal.* Found: C, 49.68; H, 5.12; N, 4.97; Cl, 13.05. The ultraviolet spectrum showed $\lambda_{\max}^{0.01N\text{HCl-MeOH}}$ 372, 266, and 229 mμ (ϵ 13,800 21,120, and 20,800, respectively); and the nmr spectrum in deuterated dimethyl sulfoxide (Figure 1) is fully consistent with formulation IIc, being identical with that of oxytetracycline below τ 5 and chlortetracycline above τ 5. Hydrogenolysis using 10% palladized charcoal in 50% aqueous dimethylformamide (containing a small amount of triethylamine) yielded 70% of oxytetracycline hydrochloride, which was identified by comparison (spectra and papergram mobility in three systems) with an authentic sample. Transformation of the antibiotic to oxytetracycline leaves only the position of the chlorine atom open to question. The aromatic region of the nmr spectrum (AB pattern at τ 2.43 and 3.04) clearly shows that, as expected, the chlorine atom remained at C_7 during the biological treatment. These findings are compelling evidence that the desired microbiological reactions have occurred in the anticipated fashion.

The antibiotic is less stable than oxytetracycline, chlortetracycline, and tetracycline in neutral solutions (Table I). This factor was a serious hindrance during the isolation studies. The addition of calcium ion satisfactorily overcomes this deficiency, and, when the antibiotic is dissolved in 80% propylene glycol solution at pH 7.5–7.6 and at least 2 molar equiv of calcium chloride is added,[3] the solution retains considerable bioactivity when stored at room temperature for weeks The antibiotic possesses one-half the bioactivity of chlortetracycline when measured against *Staphylococcus aureus* C209P by a turbidimetric procedure, and it is highly active orally and subcutaneously against experimental staphylococcal infections in mice.

Table I. Stability of Various Tetracyclines

pH	Bioactivity remaining after 2.5 hr.at 37°, % Tetracycline	Oxytetracycline	Chlortetracycline	5-Hydroxy-7-chlortetracycline
2.0	95	96	100	96
3.0	95	97	100	81
4.0	96	93	99	84
5.0	91	90	99	76
6.0	92	94	100	57
7.0	92	96	78	25

The preparation of 5-hydroxy-7-chlortetracycline provides a striking confirmation and extension of the tetracycline biosynthetic pathway presented in an earlier paper in this series.[1] Details of some of the biological studies briefly reported here will be given in a communication submitted elsewhere.

Acknowledgment. We wish to thank our colleagues in the Biochemical Research Section for performing numerous fermentations, bioassays, and large-scale isolations; the Organic Chemical Research Section for microanalyses; and J. T. Lancaster of our Central Research Division, Stamford, Conn., for nmr spectra.

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[3] We thank Dr. L. Leeson and co-workers of the Pharmaceutical Product Development Dept. of these laboratories for preparing these solutions.

Spectroscopic Evidence for Ionic Ground States in Molecular Donor–Acceptor Complexes[1]

Sir:

It has been suggested in both experimental and theoretical contexts that charge-transfer complexes between initially neutral organic molecules that are strong donors and strong acceptors may have essentially ionic, salt-like, ground states.[2] Polarized single-crystal spectro-

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[1] This work has been supported by grants from the National Science Foundation and the National Institutes of Health.

[2] (a) J. J. Weiss, *J. Chem. Soc.*, 245 (1942); *Phil. Mag.*, **8**, 1169 (1963); (b) H. Kainer, D. Bijl, and A. C. Rose-Innes, *Naturwissenschaften*, **41**, 303 (1954); D. Bijl, H. Kainer, and A. C. Rose-Innes, *J. Chem. Phys.*, **30**, 765 (1959); (c) H. Kainer and A. Uberle, *Chem. Ber.*, **88**, 1147 (1955); H. Kainer and W. Otting, *ibid.*, **88**, 1921 (1955); (d) R. Foster and T. J. Thomson, *Trans. Faraday Soc.*, **59**, 296 (1963); (e) R. Foster, *Photoelec. Spectrometry Group Bull.*, No. **15**, 413 (1963); (f) Y. Matsunaga, *J. Chem. Phys.*, **41**, 1609 (1964); (g) P. L. Kronick, H. Scott, and M. M. Labes, *ibid.*, **40**, 890 (1964) (further references are cited by these authors); (h) R. S. Mulliken, *J. Chim. Phys.*, **61**, 20 (1964); (i) H. M. McConnell, B. M. Hoffman, and R. M. Metzger, *Proc. Natl. Acad. Sci. U. S.*, **53**, 46 (1965); J. I. Krugler, C. G. Montgomery, and H. M. McConnell, *J. Chem. Phys.*, **41**, 2421 (1964).

scopic studies, which can allow one to examine simultaneously charge-transfer and "molecular" transitions and to resolve and distinguish between these transitions through their differing polarizations, hold the potential of providing important information in regard to this problem.[3] We are thus studying the specular reflection spectra[4] of single crystals of the N,N,N′,N′-tetramethyl-*p*-phenylenediamine (TMPD)–chloranil complex and report here our initial results, which give clear evidence for the existence of an ionic ground state.

Even visual inspection of polarized white light specularly reflected from well-formed TMPD–chloranil crystals, for which a preparative method has been developed as part of this work, indicates that the in-plane absorption is radically different from that expected for simple superposition of the TMPD and chloranil molecular absorptions. The spectra reported in Figure 1,[5] which were obtained as described elsewhere,[4c,d] confirm these qualitative conclusions. Thus the in-plane direction of face A[6] shows a band in the 500- to 600-mμ region, peaking at 560 mμ, and a second band with maximum reflectivity at 315 mμ. These peaks correspond closely to the parallel-polarized 565- and 325-mμ bands reported for the Wurster's blue cation, the oxidation product of TMPD.[9] Similarly, the band appearing on face B with peak reflectivity at 447 mμ may reasonably be associated with the chloranil anion absorption reported by Foster and Thomson[2d,e,10] to occur between 400 and 460 mμ. Although one must exercise care in inferring the relative magnitudes of the corresponding extinction coefficients from the heights of reflection bands, it will be observed that for the in-plane face A spectrum the 315-mμ band possesses a higher peak reflectivity than the 560-mμ band, which is the same order as the extinction coefficients of Wurster's blue in aqueous and rigid glass solutions.[9] Again, the in-plane face B reflectivity is reduced in comparison to face A, and the chloranil anion possesses lower extinction coefficients than Wurster's blue.[10]

Figure 1 also presents evidence for the existence, concurrently with the appearance of in-plane ionic absorptions, of an out-of-plane transition. Although the entire reflection band is not accessible to our present instrumentation, the long-wavelength dip and sharp rise in reflectivity noted for light polarized along the needle axis make clear that one here has in the near-infrared region at least a moderately intense absorption with the expected charge-transfer polarization.[11] There thus appears very little doubt that one is in fact dealing in this case with a solid charge-transfer complex possessing an ionic ground state.[12]

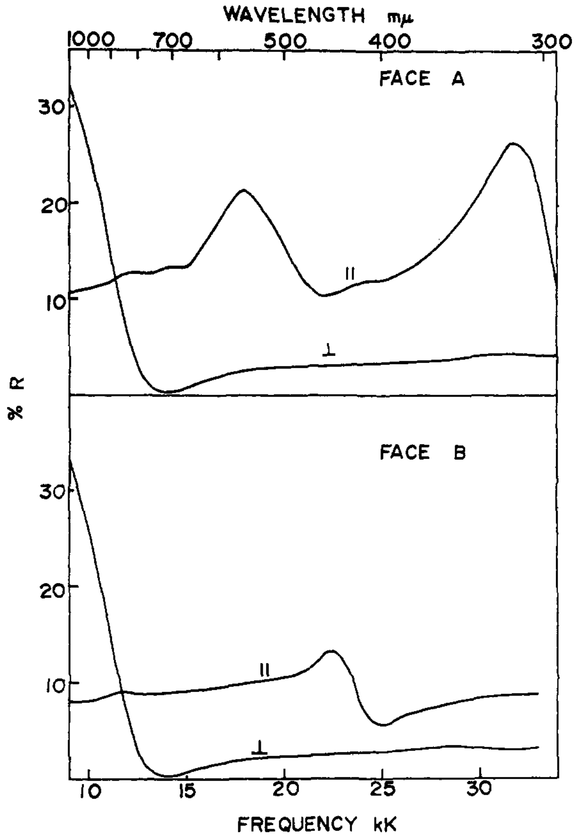

Figure 1. The reflection spectra obtained for two side faces of the single crystal of N,N,N′,N′-tetramethyl-*p*-phenylenediamine–chloranil.[6] The ∥ and ⊥ designations indicate that the spectra were obtained using polarized radiation whose electric vector vibrated parallel or perpendicular, respectively, to the molecular planes[7] (thus the ⊥ curve is that for light polarized parallel to the needle axis on each face).

That one also obtains an ionic ground state in solutions of this complex is not necessarily implied by the results obtained here, as the crystal environment could confer a stability which might be lacking in solution. A band assigned as the charge-transfer transition for this complex occurs at 870 mμ in cyclohexane solution and, although not always observed in more highly ionizing solvents, it is reported to occur at 843 mμ in acetonitrile.[10] The quinhydrone charge-transfer band has been found to undergo a change of energy of some 5 kK in the crystal in comparison to the solution ($\epsilon_{\max}$ occurs at 430 mμ in solution and at 550 mμ in the crystal).[13] If the TMPD–chloranil band occurring near 850 mμ in solution were to be perturbed similarly in the crystal, it would be found at about 1500 mμ. The appearance of the reflection band makes it reasonable to expect that the crystal absorption will peak at somewhat shorter wavelengths than this, but clearly considerably to the red of 850 mμ, and thus the crystal spectra are not

(3) L. J. Parkhurst, Thesis, Yale University, 1965.

(4) (a) B. G. Anex and W. T. Simpson, *Rev. Mod. Phys.*, **32**, 466 (1960); (b) B. G. Anex and A. V. Fratini, *J. Mol. Spectry.*, **14**, 1 (1964); (c) B. G. Anex, *Mol. Crystals*, **1**, 1 (1966); (d) L. J. Parkhurst and B. G. Anex, *J. Chem. Phys.*, in press.

(5) The spectra reported in Figure 1 are relative, and not absolute. On further refinement, some of these curves, especially the in-plane face A spectrum, may shift upward somewhat. The structural features reported have been checked on a number of crystals, however, and are quite reproducible.

(6) The present discussion utilizes Wallwork's preliminary crystal structure report[7] and makes the further assumption that the structure is, in common with other "charge-transfer" crystals,[8] such that molecules stack along the needle axis (*i.e.*, have their planes perpendicular to this axis in this instance). The spectral results of Figure 1 are consistent with this picture.

(7) S. C. Wallwork, *J. Chem. Soc.*, 494 (1961).

(8) See, for instance, H. Matsuda, K. Osaki, and I. Nitta, *Bull. Chem. Soc. Japan*, **31**, 611 (1958); D. S. Brown, S. C. Wallwork, and A. Wilson, *Acta Cryst.*, **17**, 168 (1964).

(9) A. C. Albrecht and W. T. Simpson, *J. Am. Chem. Soc.*, **77**, 4454 (1955).

(10) R. Foster and T. J. Thomson, *Trans. Faraday Soc.*, **58**, 860 (1962).

(11) B. G. Anex and L. J. Parkhurst, *J. Am. Chem. Soc.*, **85**, 3301 (1963).

(12) Although the present work supports the view that ionic states do exist, other studies in these laboratories (on, for instance, quinhydrone[13]) indicate that in cases involving less relative donor–acceptor power, one obtains un-ionized species in the ground state.

(13) Reference 11 and work yet to be published.

inconsistent with the complex also possessing an ionic ground state in solution.[14]

The measurements reported here are being extended and refined, and it is anticipated that they will be expanded to include other complexes having particular relevance to the question of ionic ground states.

[14] It should be noted in this regard, however, that ref 10 presents rather strong evidence for the complex being nonionic in solution.

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The Cage Recombination of Acetoxy Radicals to Yield Acetyl Peroxide

Sir:

The reactions within the solvent cage of radical pairs formed in the thermal decomposition of acetyl peroxide have been generally[1-3] credited with the formation of the observed methyl acetate and ethane. We wish to report evidence for an even more important cage reaction, the recombination of acetoxy radicals to yield acetyl peroxide.

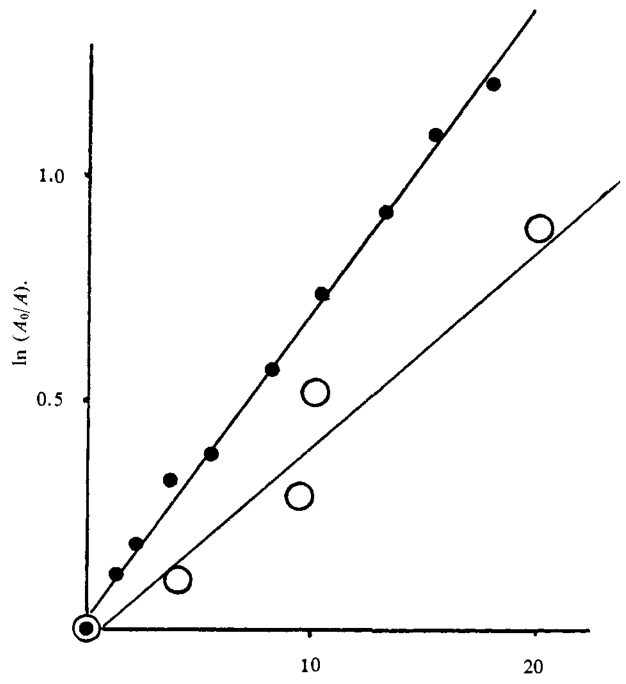

Figure 1. First-order rate plots for the disappearance of total acetyl peroxide (small circles, data obtained by infrared spectroscopy) and for scrambling of carbonyl label in recovered acetyl peroxide (large circles).

Acetyl peroxide initially labeled with oxygen-18 (0.6–1.5% excess), specifically in the carbonyl oxygen,[4] was recovered after partial decomposition at 80° in isooctane. Samples recovered after varying lengths of time were subjected to parallel treatment with samples of starting peroxide. A series of low-temperature recrystallizations from isooctane gave samples showing no infrared absorption in the 1700–1800 cm^{-1} region except that found in pure acetyl peroxide. These samples were identically treated with anhydrous methanolic sodium methoxide. The resulting methyl acetate was isolated by gas chromatography and assayed[5,6] for oxygen-18 by mass spectrometry. The starting peroxide gave methyl acetate from this treatment which invariably contained a level of label (calculated assuming one oxygen labeled per molecule) identical with that in the acetic anhydride from which it was made by treatment with unlabeled barium peroxide. The labeling of the methyl acetate therefore reflects the level of label in the carbonyl oxygen of the acetyl peroxide. Samples recovered after increasingly lengthy periods of heating gave methyl acetate containing steadily decreasing amounts of oxygen-18, without any decrease of total label in the peroxide. These data (Table I) show a kinetically first-order decrease in the fraction of recovered peroxide which is specifically labeled. The plot of Figure 1 (large circles) gives a rate constant for scrambling of label (k = 4.4 × 10^{-5} sec^{-1}, 80°) which can be compared with that for the over-all disappearance of acetyl peroxide, followed by infrared spectroscopy at 1800 cm^{-1}, in the same solvent (k = 7.2 × 10^{-5} sec^{-1}, 80°). The comparison suggests that *ca.* 38% of all radical pairs formed in the decomposition of acetyl peroxide recombined to give acetyl peroxide with scrambling of label.[7]

Table I. Specificity of Labeling in Acetyl Peroxide Recovered from Partial Decomposition at 80° in Isooctane

Time, sec	Carbonyl label, % excess	Fraction not scrambled
0	0.618	1.000
4080	0.574	0.896
8700	(0.541)[a]	0.749
9900	0.492	0.590
19800	0.439	0.410

[a] Normalized value calculated from a run showing 0.984% excess carbonyl-^{18}O from acetyl peroxide initially containing 1.124% excess.

The failure to observe exchange between labeled acetic acid and unlabeled acetyl peroxide combines with the observation of the first-order form of the rate

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[1] For a review see C. Walling, "Free Radicals in Solution," John Wiley and Sons, Inc., New York, N. Y., 1957, p 491 ff, or M. Szwarc in "Peroxide Reaction Mechanisms," J. O. Edwards, Ed., Interscience Publishers, Inc., New York, N. Y., 1962, p 153 ff.

[2] L. Herk, M. Feld, and M. Szwarc, *J. Am. Chem. Soc.*, **83**, 2998 (1961).

[3] W. Braun, L. Rajbenbach, and F. R. Eirich, *J. Phys. Chem.*, **66**, 1591 (1962).

[4] J. C. Martin and E. H. Drew, *J. Am. Chem. Soc.*, **83**, 1232 (1961).

[5] W. von E. Doering and E. Dorfman, *ibid.*, **75**, 5595 (1953); D. B. Denney and M. A. Greenbaum, *ibid.*, **79**, 979 (1957).

[6] We wish to thank Mr. J. Nemeth for carrying out the oxygen-18 analyses. These were run in parallel with quantitative direct oxygen analyses, using the same pyrolysis train. Failure to perform this control experiment led to an earlier report,[4] now recognized to be in error, of a specific attack by cyclohexene on acetyl peroxide. Spurious results in the isotopic assay of cyclohexanol (which will be discussed in a later publication) suggested incomplete scrambling of label in the formation of cyclohexyl acetate from acetyl peroxide and cyclohexene. In reality the scrambling of label in the ester is complete (J. C. Martin, E. H. Drew, and J. W. Taylor, to be published). For a discussion of this reaction see H. J. Shine, J. A. Waters, and D. M. Hoffman, *ibid.*, **85**, 3613 (1963).

[7] An earlier attempt[2] to use tracer techniques to detect this process used infrared spectroscopy to assay for carbonyl label in peroxide with *ca.* 0.6% enrichment and failed to detect scrambling in material recovered after 55% reaction. Presumably the technique of this paper provides a more sensitive assay method.