angles 115.0–115.9 (2)°), together with possibly greater F strain[12] in **3** than in **5**. The bridgehead carbon (C-5) of **3** is also flattened (CH_2–CH–CH_2 angles, 113.5–114.8 (3)°), leading to greater p character in the C(methine)–H bond.[13] This is corroborated by the coupling, $J_{^{13}C-H}$,[14] of the methine carbon and proton which was estimated as 121 ± 5 Hz for the hydrochloride **3**, compared with the $J \geqslant 140$ Hz observed for the bridgehead ^{13}C–H in quinuclidine hydrochloride. Both the ^{13}C nmr[15] and the ^1H nmr spectra indicated the unusual nature of the methine carbon and proton. Thus, the chemical shift for C-5 in the [3.3.3]amine hydrochloride (**3**) is at *lower field* (869 Hz upfield from dioxane) than the β_N carbons (915 Hz), whereas in quinuclidine hydrochloride (**5**) C-4 is at higher field (1083 Hz) than the β_N carbons (1000 Hz). The chemical shift for H-5 (δ 2.57 in $CDCl_3$–TMS) in the [3.3.3]-amine **2** is at lower field than the β_N CH_2's (δ 1.60), and this is also true for its hydrochloride **3** (δ 2.60 *vs.* 1.93), whereas H-4 in quinuclidine (**4**) (1.3–1.8) is in the same range as the β_N CH_2's (1.50) in that molecule.

The uv maximum for **2** is at an exceptionally long wavelength (240 nm, ϵ 2935 in ether!) for a saturated tertiary amine;[16] by contrast, quinuclidine (**4**) in diethyl ether solution shows only end absorption (above 200 nm). The spectrum of "manxine" (**2**) vapor exhibits extensive fine structure in the range 280–205 nm, with major absorption in the region 260–230 nm, at longer wavelength than that of quinuclidine in the vapor state.[17] The ultraviolet absorption of the amine **2** thus reflects the reduction in energy difference for the n → p transition between the ground state, where we observe a flattening of the nitrogen bridgehead in **3**, and the excited state, where, by analogy to quinuclidine (**4**), the nitrogen is expected to approach coplanar bonding.[17]

Acknowledgment. The authors are indebted to the National Science Foundation for financial support (Research Grant No. GP-8407X) and for assistance in the purchase of the 220-MHz spectrometer used in this work.[18]

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[12] H. C. Brown, D. H. McDaniel, and O. Häfliger in "Determination of Organic Structures by Physical Methods," Vol. 1, E. A. Braude and F. C. Nachod, Ed., Academic Press, New York, N. Y., 1955, Chapter 14. See in Figure 1 the interaction of H2A, H8A, and H9A with HN.

[13] See M. J. Sabacky, S. M. Johnson, J. C. Martin, and I. C. Paul, *J. Amer. Chem. Soc.*, **91**, 7542 (1969).

[14] For theoretical considerations, see N. Muller and D. E. Pritchard, *J. Chem. Phys.*, **31**, 768, 1471 (1959); K. Mislow, *Tetrahedron Lett.*, 1415 (1964); C. S. Foote, *ibid.*, 579 (1963).

[15] (a) ^{13}C spectra courtesy of Brucker Scientific, Inc., Elmsford, N. Y. (b) We also thank J. Jonas, R. L. Thrift, and J. R. Lombardi for very helpful discussions during this investigation.

[16] N. J. Leonard and D. M. Locke, *J. Amer. Chem. Soc.*, **77**, 437 (1955).

[17] A. M. Halpern, J. L. Roebber, and K. Weiss, *J. Chem. Phys.*, **49**, 1348 (1968).

[18] The X-ray structure analysis here reported was carried out as part of formal X-ray lecture and laboratory courses in crystal structure analysis given at the University of Illinois in the fall in 1970. We acknowledge the participation, interest, and enthusiasm of Carolyn I. Hejna, Alice C-Z. Shen, John Vandervoorn, Jr., and Stephen R. Byrn.

[19] Alfred P. Sloan Research Fellow, 1968–1970.

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Received May 26, 1971

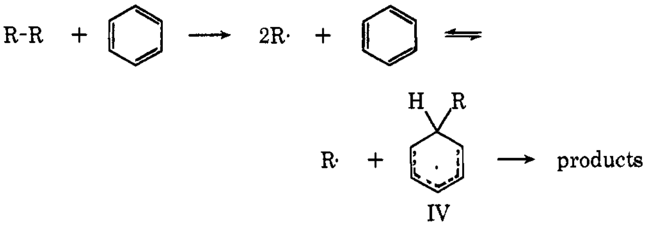
Chemically Induced Dynamic Nuclear Polarization and Substituent Effects in Some Homolytic Aromatic Substitution Reactions

Sir:

In homolytic aromatic substitution reactions any substituents in the substrate activate the ortho and para positions toward the attack of electrically neutral, free radicals.[1] However, upon investigating the corresponding reactions of the free radicals (R·) obtained by pyrolysis of bis(pentafluorobenzoyl) peroxide[2] (R–R) with the chemically induced dynamic nuclear polarization (CIDNP) method,[3] we found the substituent effects of *electrophilic* aromatic substitution.

In a typical experiment a 0.75 *M* solution of R–R in hexachlorobutadiene is decomposed in the presence of 1 *M* benzene at 160° in a 60-MHz nmr spectrometer. One observes the benzene resonance in emission, and other emission (E) and enhanced absorption (A) lines, attributed to the products pentafluorobenzoic acid (I), substituted 1,3- and 1,4-cyclohexadienes (II), and pentafluorobenzoates (III).

We assume that R· adds reversibly to benzene[4] to form 1-substituted cyclohexadienyl radicals (IV) still in the primary cage.[5] The resulting singlet radical pairs, R· + IV, can either react to afford the products I–III, or change their multiplicity to yield triplet pairs. The triplet pairs will separate and preferably cleave to the reactants, *i.e.*, benzene and R·.



Because the rates of multiplicity change are nuclear spin dependent,[6] CIDNP is expected for both the

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[1] G. H. Williams, "Homolytic Aromatic Substitution," Pergamon Press, London, 1960.

[2] M. W. Buxton, British Patent 977,963 (1964).

[3] J. Bargon, H. Fischer, and U. Johnsen, *Z. Naturforsch. A*, **22**, 1551 (1967); H. Fischer and J. Bargon, *Accounts Chem. Res.*, **2**, 110 (1969).

[4] From product studies, ^{19}F-CIDNP, and esr results we conclude tentatively that R· = pentafluorobenzoate. The role of pentafluorophenyl radicals is being further investigated. For other reversible additions to benzene see: T. Nakata, T. Tokumaru, and O. Simamura, *Tetrahedron Lett.*, 3303 (1967); O. Volkert and D. Schulte-Frohlinde, *ibid.*, 2151 (1968); M. L. Poutsma in "Methods of Free Radical Chemistry," E. S. Huyser, Ed., Vol. 1, Marcel Dekker, New York, N. Y., 1969, p 32, and references therein; J. Saltiel and H. C. Curtis, *J. Amer. Chem. Soc.*, **93**, 2056 (1971).

[5] The lifetime of the primary cage is considered to be 10^{-10}–10^{-9} sec [*cf.* L. Herk, M. Feld, and M. Szwarc, *ibid.*, **83**, 2998 (1961)]. Therefore, R· must add to benzenes with a rate constant on the order of 10^7–10^8 M^{-1} sec^{-1}. Consistently, addition rates of 10^9 M^{-1} sec^{-1} have been measured for hydroxyl and hydrogen radicals to benzenes and pyridines [*cf.* P. Neta and L. M. Dorfman, *J. Chem. Phys.*, **73**, 413 (1969); *Advan. Chem. Ser.*, No. 82, 222 (1968); B. Cercek and M. Ebert, *Trans. Faraday Soc.*, 1687 (1967)]. However, De Tar has estimated the unknown addition rate of benzoyloxy radicals to benzenes to be 35 M^{-1} sec^{-1} [D. F. De Tar, *J. Amer. Chem. Soc.*, **89**, 4058 (1967)] in which case spin correlation is lost. The fast reaction of pentafluorobenzoyloxy radicals with benzenes could be due to a π complex formation between the peroxide and the aromatic substrates which would provide a benzene molecule in the immediate vicinity when the radical pair is generated. Another case of spin-correlation conservation has been observed by R. Kaptein, *et al.*, during reactions of isobutyryl peroxide with $CBrCl_3$ (R. Kaptein, F. W. Verheus, and L. J. Oosterhoff in R. Kaptein, Thesis, University of Leiden, The Netherlands, 1971, p 41).

[6] (a) G. L. Closs, *J. Amer. Chem. Soc.*, **91**, 4552 (1969); G. L. Closs and A. D. Trifunac, *ibid.*, **91**, 4554 (1969); **92**, 2183, 2186 (1970);

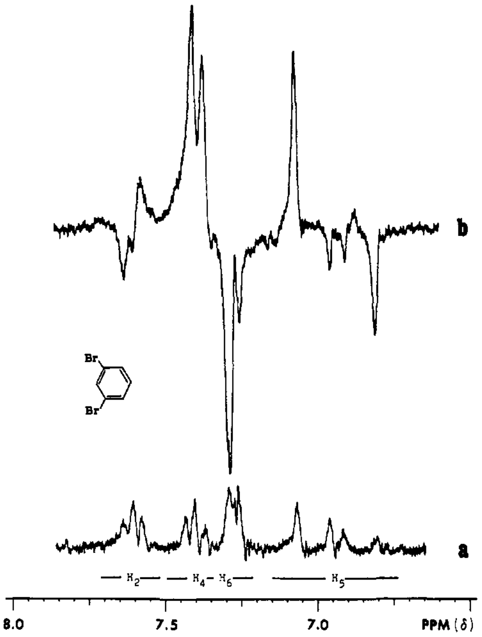

Figure 1. Nmr spectra (60 MHz) of *m*-dibromobenzene obtained before (a), during (b), and after (a), the decomposition of R–R. (Because *m*-dibromobenzene cannot be treated as a first-order nmr spectrum,[11] the analysis of its multiplet effect will be given elsewhere. For tetrasubstituted benzenes the rules of CIDNP[6] predict alternating emission and absorption lines for the four-line spectra, starting with absorption at low field if the two protons are ortho or para to one another, but with emission, if they are meta. Such spectra have been obtained for the corresponding compounds, even for those without heavy atom substituents if the reactions were carried out in weak magnetic fields.)

products and the reactants. The pentafluorobenzoate radical, R·, is likely to have a higher *g* value[7] than IV. Because of the *g*-value difference in the radical pair, one expects emission lines in the substrates and enhanced absorption lines in the products, respectively, for those protons which experienced positive hyperfine coupling constants a_i in IV, and the reverse for those with negative a_i.[6]

We have determined the absolute values of the a_i in IV by esr,[8] and because IV is an odd-alternant radical,[9] we attribute the following signs: $a_1 = +32.4 \pm 0.1$ G, $a_2 = a_6 = -8.8 \pm 0.1$ G, $a_3 = a_5 = +2.75 \pm 0.05$ G, and $a_4 = -12.95 \pm 0.1$ G. Since $|a_1 + a_3 + a_5| > |a_2 + a_4 + a_6|$, net emission results for benzene, whereas in the products both E and A are observed separately, depending on the signs of a_i.

With 1,3,5-trichlorobenzene the substrate resonance emits, and a lack of emission among the resonance lines of the products indicates radical attack at unsubstituted carbons, and thus only positive a_i.

With 1,3,5-tribromobenzene the substrate line shows enhanced absorption. Apparently here IV has a higher *g* value than R·, which is expected if bromines replace negatively coupled hydrogens.[10] The two bromines of *m*-dibromobenzene increase the *g* value of IV such that it matches that of R·, and the multiplet effect[2,6] (E and A for the same proton) is observed (Figure 1).

Figure 2. Nmr spectra of 2,4-dichlorobenzoyl chloride obtained before (a), during (b), and after (a), the decomposition of R–R.

However, if bromines replace positively coupled hydrogens, they do not increase the *g* value of IV.[10] Correspondingly, *o*- and *p*-dibromobenzene show energy polarization[6] (E), and 1,2,4-tribromobenzene shows the multiplet effect.

Because a_1 is the largest of all a_i, the strongest emission should result from the substrate protons attached to those carbons where the radical attack occurs. In para-disubstituted benzenes XC_6H_4Y, with X = NO_2, CN, $COCH_3$, COCl, COOH, and SO_2Cl and Y = OAlk, Alk, Br, Cl, F, $SSC_6H_4NO_2$, OC_6H_4-NO_2, the low-field protons[11] of the substrates always showed A and the high-field protons E. Therefore, these substrates are attacked ortho to the donor substituent. Similarly, from the CIDNP pattern we conclude that donor-substituted benzenes (anisole) are attacked in the ortho and para positions, but acceptor-substituted benzenes (nitrobenzene) are attacked in the meta positions. In multisubstituted benzenes the best donor substituents prevail in directing R· into their ortho and para positions, even overruling other nitro substituents and their contributions to resonance stabilization.[1]

In alkyl side chains, only the α protons show CIDNP (E). Donor substituents ortho or para to the alkyl groups favor side-chain attack. Fluorinated benzenes show ^{19}F-CIDNP, usually emission. Highly acceptor-deactivated molecules fail to show CIDNP in hexachlorobutadiene solution. Iodo, hydroxy, mercapto, vinyl, formyl, amino, and dimethylamino groups cause side reactions. Pyridines, naphthalenes, and fluorenes show similar CIDNP. The observed effects demonstrate the electrophilicity of R·.[12] CIDNP is built up

G. L. Closs, C. E. Doubleday, and D. R. Paulson, *ibid.*, **92**, 2185 (1970); (b) R. Kaptein and L. J. Oosterhoff, *Chem. Phys. Lett.*, **4**, 195, 214 (1969); (c) H. Fischer *ibid.*, **4**, 611 (1970).

(7) Usually, *g* values of oxy radicals [*g* = 2.0045, *cf.* H. C. Heller and T. Cole, *J. Amer. Chem. Soc.*, **84**, 4448 (1962)] are larger than those of IV [*g* = 2.002, *cf.* R. W. Fessenden and R. H. Schuler, *J. Chem. Phys.*, **39**, 2147 (1963)].

(8) J. Bargon and H. Hefter, submitted for publication.

(9) H. Fischer, *J. Chem. Phys.*, **37**, 1094 (1962); R. W. Fessenden and R. W. Schuler, *ibid.*, **38**, 773 (1963).

(10) J. Sinclair and D. Kivelson, *J. Amer. Chem. Soc.*, **90**, 5074 (1968).

(11) J. Martin and B. P. Dailey, *J. Chem. Phys.*, **37**, 2594 (1962).

(12) The benzoyloxy radical also has electrophilic properties [M. E. Kurz and M. Pellegrini, *J. Org. Chem.*, **35**, 990 (1970)].

in σ complexes, thus giving no evidence for π complexes.[13] In IV, the signs of the a_i are those expected for odd-alternant radicals, even in substituted IV.[10]

It follows from Figure 2 that CIDNP is useful in analyzing complex nmr spectra of aromatic molecules, because adjacent protons become oppositely polarized. Also, the absolute signs of the nmr coupling constants can be determined from the multiplet effect, which prevails at low magnetic fields.[6]

(13) Reference 1, p 50.

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Singlet Oxygen Analogs in Biological Systems. Peroxidase-Catalyzed Oxygenation of 1,3-Dienes

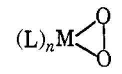
Sir:

The oxygenation of organic compounds by singlet molecular oxygen is a subject of considerable current interest. Exactly analogous processes of oxygenation take place in biological systems in reactions catalyzed by dioxygenases. Recently, the coupled oxygenation of 1,3-dienes by soybean lipoxidase, an enzyme whose mode of action is analogous to the "ene" reaction of singlet oxygen, has been reported.[1] Results on the direct oxygenation of 1,3-dienes by horseradish peroxidase are reported here.

A dilute suspension of 1,3-diphenylisobenzofuran (or other substrates discussed herein) was prepared by the addition of 0.1 *M* pH 4.0 acetate buffer (300 ml) to a solution of the compound (10 mg) in acetone (30 ml containing 5 μl of Tween 80). When the suspension was incubated with hydrogen peroxide (20 μl of 10 m*M* solution) and horseradish peroxidase (10 mg),[2] the yellow-green fluorescence of the isobenzofuran disappeared within minutes and *o*-dibenzoylbenzene was isolated in 65% yield.[3] Apart from 1,3-diphenylisobenzofuran, other 1,3-dienoid systems were also oxidized, although at a much reduced rate. After 4 hr of incubation under the above conditions, 2,3,4,5-tetraphenylfuran yielded 1,2-dibenzoylstilbene[4] (11%; 60% of starting material was recovered) and anthracene gave anthraquinone[4] (26%; 38% of starting material was recovered). Tetracyclone and 9,10-diphenylanthracene were not oxidized.[5] The oxygenation of the furans by peroxidase is directly analogous to the dye-sensitized photooxygenation reactions.[6,7] Further confirmation of the similarity between these modes of oxygenations is furnished by an analysis of the origins of the oxygen atoms in the products of these reactions.

When ^{18}O-enriched (7.9 ± 0.3%) 1,3-diphenylisobenzofuran was oxidized in dichloromethane solution to *o*-dibenzoylbenzene in methylene-blue-sensitized photooxygenation, the product had an ^{18}O enrichment of 7.9 ± 0.3%. Thus, photooxygenation of 1,3-diphenylisobenzofuran leads to the retention of the ethereal oxygen atom in the product. When the same sample of ^{18}O-enriched 1,3-diphenylisobenzofuran was oxidized by horseradish peroxidase and hydrogen peroxide, the ^{18}O enrichment in the product was 8.1 ± 0.3%, while incubation in buffer made up from $H_2{}^{18}O$ (5.0% enrichment) led to no incorporation (0.2 ± 0.3%) of ^{18}O into the product. These observations are consistent with the participation of the same mode of oxygenation in the enzyme-catalyzed and photooxygenation reactions. Although dye-sensitized photooxygenation of anthracene in aprotic solvents results in endoperoxide formation,[8] similarity between peroxidase-catalyzed and dye-sensitized oxygenations of anthracene can be demonstrated by methylene-blue-sensitized oxidation in methanol–benzene (1/9) which yields anthraquinone as the major product.

Although the catalysis of oxygenation by horseradish peroxidase can be attributed to the intermediacy of singlet molecular oxygen (failure of tetracyclone and 9,10-diphenylanthracene to react can be attributed to the lesser reactivity of these compounds, especially when in competition with quenching by groups on the enzyme), there are alternative explanations of which the participation of an enzyme-bound metal peroxy complex, discussed in detail below, offers an interesting as well as likely possibility. The ability of a transition metal peroxy complex (diperoxychromium(VI) oxide etherate) to oxygenate 1,3-dienes[9] in a reaction different from that of singlet oxygen[10] has been demonstrated. The existence of a peroxide–peroxidase complex containing the elements of an oxygen molecule bonded to a ferrous ion ("peroxidase compound III" which has also been considered to be identical with oxygenated ferroperoxidase) has been established.[12,13] The ease with which transition metal ions react with hydrogen peroxide to form peroxy complexes[14] suggests that peroxidase compound III and possibly other peroxide–peroxidase complexes have a peroxyheme structure of the general type **1**. The oxygenation reactions catalyzed by horseradish peroxidase may therefore be attributed to the formation of such a peroxy complex. The results discussed here do not, however, rule out other mechanisms of oxidation; in particular, the possibility that a dihydroxylation is involved cannot be excluded. In this context, the role of an oxoiron species



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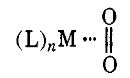

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[1] H. W.-S. Chan, *J. Amer. Chem. Soc.*, **93**, 2357 (1971).

[2] Type II, purchased from Sigma Chemical Co.; no reaction was observed when boiled enzyme was used.

[3] All products discussed were isolated by chromatography and shown to be identical with authentic material by melting point and ir and mass spectra.

[4] These incubations were performed on a scale five times that used for 1,3-diphenylisobenzofuran and with the addition of further aliquots of hydrogen peroxide after 1 and 2 hr.

[5] These compounds crystallized out of the emulsion in 0.5 hr, during which time no reaction was observed.

[6] C. Dufraisse and S. Ecary, *C. R. Acad. Sci.*, **223**, 735 (1946).

[7] J. Martel, *ibid.*, **244**, 626 (1957).

[8] C. Dufraisse and M. Gérard, *ibid.*, **201**, 428 (1935).

[9] H. W.-S. Chan, *Chem. Commun.*, 1550 (1970).

[10] 9,10-Diphenylanthracene is not oxidized by the chromium peroxy complex, but anthracene is oxygenated to anthraquinone; the oxygen atom of 1,3-diphenylisobenzofuran is also retained on conversion to *o*-dibenzoylbenzene.[11]

[11] H. W.-S. Chan, unpublished observations.

[12] I. Yamazaki and L. H. Piette, *Biochim. Biophys. Acta*, **77**, 47 (1963).

[13] H. S. Mason, *Proc. Int. Symp. Enzyme Chem., 1957*, 223 (1958).

[14] J. A. Connor and E. A. V. Ebsworth, *Advan. Inorg. Chem. Radiochem.*, 6, 279 (1964).