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]

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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 cata-

1 **2**

lyzed 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

(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).

as the active oxidant in mixed-function oxygenases has recently been suggested.[15]

By analogy with transition metal complexes of ethylene and acetylene,[16,17] where there is considerable decrease in C–C bond order with concomitant increase in metal–carbon bonding, structures **1** and **2** can be regarded as different (and extreme) forms representing a π complex between oxygen and a metal atom. The metal in structure **1** will have a formal oxidation state of two in excess of that in structure **2**. Thus, complexes of transition metals with molecular oxygen are often considered to have the former structure.[18,19] Catalysis by metalloenzymes that mimic singlet oxygen may therefore involve complexes of type **1** or **2** as the "enzyme-bound singlet-oxygen." The oxygenation reactions of diperoxychromium(VI) oxide etherate show that the oxygen moiety in peroxy complexes of metals in their higher oxidation states can have considerable "singlet" character. The chemical activity of metal–oxygen complexes containing oxygen bonded to metal atoms in their lower oxidation states is being examined.

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(15) K. B. Sharpless and T. C. Flood, *J. Amer. Chem. Soc.*, **93**, 2316 (1971).
(16) J. Chatt and L. A. Duncanson, *J. Chem. Soc.*, 2939 (1953).
(17) J. P. Collman and J. W. Kang, *J. Amer. Chem. Soc.*, **89**, 844 (1967).
(18) L. Vaska, *Science*, **140**, 809 (1963).
(19) J. Valentine, D. Valentine, Jr., and J. P. Collman, *Inorg. Chem.*, **10**, 219 (1971).

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Degradation of Saxitoxin to a Pyrimido [2,1-*b*]purine[1]

Sir:

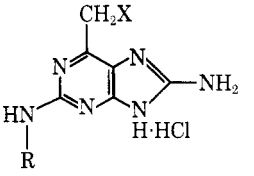
Paralytic shellfish poisoning is a severe form of food intoxication. The responsible toxic principle (LD_{50} 5–10 μg/kg (mouse), intraperitoneally) is saxitoxin, produced by the dinoflagellate *Gonyaulax catenella*[2] and accumulated in some otherwise edible species of shellfish. Previously,[3] we reported the isolation of 3-methyl-6,7-dihydro-5*H*-pyrrolo[1,2-*c*]pyrimidin-1-one from the reaction of saxitoxin with phosphorus and hydriodic acid. Saxitoxin has now been converted under a variety of mildly oxidative conditions to a crystalline product, $C_9H_{10}N_6O_2 \cdot HCl$ (**1**). This compound retains nine of the ten carbon atoms and six of the seven nitrogen atoms of saxitoxin.[3] We now wish to report the structure of this C_9 compound **1** and related degradation products.[4]

Saxitoxin, treated in dilute sodium hydroxide solution with 0.8% hydrogen peroxide at 25° followed by catalytic decomposition of excess peroxide and ion exchange chromatography, gave a 25% yield of crystalline salt **1**: $C_9H_{10}N_6O_2 \cdot HCl$; uv (pH 6) 324 ($\epsilon$ 21,000), 261 sh (4760), 236 (12,8000), 208 (22,650); nmr δ 5.23 (s, 2), 5.08 (t, 2, J = 7 Hz), 3.48 (t, 2, J = 7 Hz). These two triplets suggest the presence of a propionyl residue attached to an iminium nitrogen in **1**, *e.g.*, 1-(2-methoxycarbonylethyl)pyridinium bromide shows two triplets at δ 4.93 and 3.23 for its methylenes.

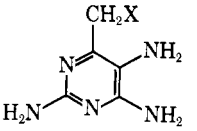
A 3-carbon residue was removed upon heating **1** in alkali, giving **2**, $C_6H_5N_6O \cdot HCl$: uv (pH 6) 308 (ϵ 10,720), 266 (7750), 228 (21,000), 206 (18,250); nmr δ 4.90 (s). The latter absorption is suggestive of the methylene singlet of benzyl alcohols. The C_6 product **2** was resistant to further acidic and alkaline hydrolytic conditions, indicating that the two guanidino groups[5] in saxitoxin probably are intact in the stable aromatic nucleus of **2**. Assuming a benzylic hydroxymethyl moiety in **2**, $C_5H_5N_6$ remains with which to construct a stable system containing two guanidines with no hydrogen attached to carbon. On this basis, the C_6 compound was assigned the probable structure 2,8-diamino-6-hydroxymethylpurine hydrochloride (**2**).

Reduction of **2** with phosphorus and hydriodic acid yielded the deoxy derivative, $C_6H_8N_6 \cdot HCl$, δ 2.51, formulated as 2,8-diamino-6-methylpurine hydrochloride (**3**). Synthesis of the deoxypurine **3** was accomplished in one step by condensing the known 6-methyl-2,4,5-triaminopyrimidine (**4a**) with cyanogen bromide.

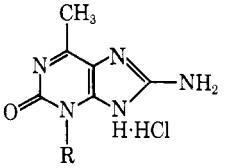
For the synthesis of **2**, the key intermediate was 6-hydroxymethyl-2,4,5-triaminopyrimidine (**4**) which was prepared *via* amination of 2,4-dichloro-6-methoxycarbonyl-5-nitropyrimidine,[6] followed by catalytic hydrogenation of the 5-nitro group and sodium borohydride reduction of the 6-ester function.[7] Condensation of **4** with cyanogen bromide gave 2,8-diamino-6-hydroxy-



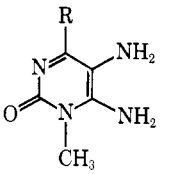
2, X = OH; R = H
3, X = R = H
a, X = H; R = CH_3



4, X = OH
a, X = H



5, R = CH_2CH_2COOH
a, R = CH_3



6, R = CH_3
a, R = H

(1) Supported in part by the U. S. Army Research Office, Durham, N. C.
(2) E. J. Schantz, J. M. Lynch, G. Vayvada, K. Matsumoto, and H. Rapoport, *Biochemistry*, **5**, 1191 (1966).
(3) W. Schuett and H. Rapoport, *J. Amer. Chem. Soc.*, **84**, 2266 (1962).
(4) Satisfactory elemental analyses and chromatographic and spectral characterization were obtained for all compounds mentioned in this communication; 60-MHz nmr spectra were taken in deuterium oxide with tetramethylsilane (δ = 0) as external standard; uv spectra [λ_{max} nm (ϵ)] were taken in water unless otherwise specified.
(5) A greater than 100 mol % yield of guanidine residues (as β-guanidinopropionic acid, guanidine, and **1**) has been obtained from the oxidation of saxitoxin (R. Oesterlin, Ph.D. Thesis, University of California, Berkeley). Details will be reported in a forthcoming publication.
(6) J. Clark and G. Ramage, *J. Chem. Soc.*, 2821 (1958).
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