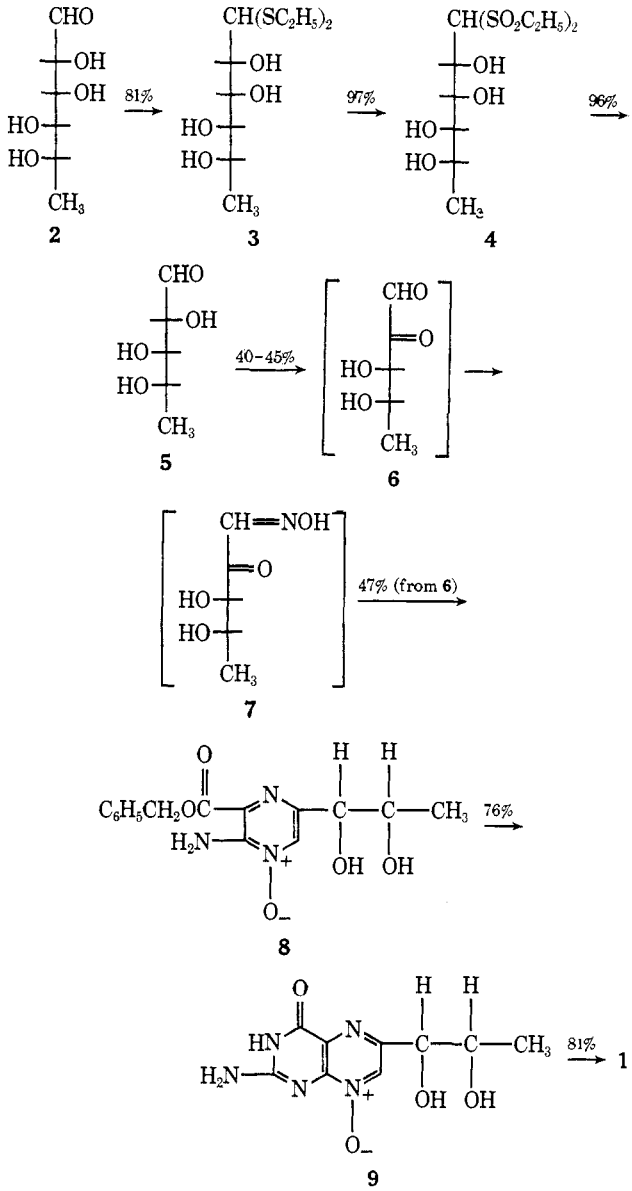
we have found that this oxidation to the bissulfone may be effected in 97% yield by *m*-chloroperbenzoic acid in anhydrous dioxane. Conversion of **4** to 5-deoxy-L-arabinose (**5**) can then be carried out in 96% yield with dilute aqueous ammonia at room temperature, followed by deionization with Amberlite-IR-120 and IR-4B resins (Scheme I).

Scheme I



The conversion of 5-deoxy-L-arabinose (**5**) to L-*erythro*-biopterin (**1**) was carried out as follows. Oxidation of 1.2 g of **5** in 6 ml of water and 75 ml of ethanol with 12.0 g of cupric acetate hydrate was carried out by boiling for 7 min, cooling immediately to 0°, filtering to remove excess reagent, and passing the filtrate through a 2.5 × 14 cm column of Dowex 50-WX4, using methanol as the eluting solvent. The crude osone, obtained by evaporation of the methanol,[23] was dissolved in 3 ml of water, the pH adjusted to 3.5, and 0.6 g of acetone oxime was added. After 6 hr at 50°, the reaction mixture was diluted with 15 ml of water, extracted with ether to remove excess acetone oxime, and evaporated. To the crude α-ketoaldoxime (**7**) was then added 1.1 g of benzyl α-aminocyanoacetate methanesulfonate,[25] and the mixture was stirred at room temperature for 36 hr. Dilution with ether and cooling and filtering then gave, as the final crystalline product of this series of *in situ* conversions, 0.46 g (47% yield from **6**) of 2-amino-3-benzyloxycarbonyl-5-(L-*erythro*-1′,2′-dihydroxypropyl)pyrazine 1-oxide (**8**), mp 165–166° (nmr (DMSO-d_6) δ 8.32(1)(s)(C_7–H), 7.53(2)(br s)(NH_2), 7.35(5)(s)(C_6H_5), 5.31(2)(s)(CH_2-C_6H_5), 4.53–3.22(4)(m)(CHOH–CHOH), 0.91(3)(d)(CH_3). *Anal.* Calcd for $C_{15}H_{17}N_3O_5$: C, 56.42; H, 5.37; N, 13.16. Found: C, 56.32; H, 5.28; N, 13.07). A suspension of 0.35 g of **8**, 0.22 g of guanidine hydrochloride, and 0.32 g of freshly prepared sodium methoxide in 4 ml of DMF was heated for 12 hr at 70–75° and diluted with 6 ml of water and the pH adjusted to 3–4 to give 0.21 g (76%) of biopterin 8-oxide (**9**), mp >300° (R_f 0.31 on Whatman No. 1, 30-cm path, butanol–acetic acid–water (50:15:35). *Anal.* Calcd for $C_9H_{11}N_5O_4$: C, 42.69; H, 4.38; N, 27.67. Found: C, 42.41; H, 4.41; N, 27.92). Heating a solution of 100 mg of **9** with a slight excess (70 mg) of sodium dithionite in a buffered aqueous (pH 7) solution for 30 min, followed by cooling and filtering, then gave 78 mg (81%) of pure L-*erythro*-biopterin; mass spectral, uv, nmr, and chromatographic behavior were identical with the authentic natural product. *Anal.* Calcd for $C_9H_{11}N_5O_3$: C, 45.56; H, 4.68; N, 29.53. Found: C, 45.49; H, 4.63; N, 29.65.

Acknowledgment. This work was supported by Grant No. CA12876 to Princeton University from the National Cancer Institute, National Institutes of Health.

(23) This reaction was monitored by conversion of the osone to iminodeoxyascorbic acid, followed by titration with iodine.[24] This is the poorest step in the overall conversion of 2 to 1; maximum yields achieved thus far in this oxidation have averaged 40–45%.

(24) (a) L. L. Salomon, J. J. Burns, and C. G. King, *J. Amer. Chem. Soc.*, **74**, 5161 (1952); (b) J. K. Hamilton and F. Smith, *ibid.*, 74, 5162 (1952).

(25) The condensation of an α-ketoaldoxime with esters of α-aminocyanoacetic acid has been described as the initial key step in a general and unequivocal synthesis of 6-substituted pterins: E. C. Taylor, K. L. Perlman, I. P. Sword, M. Sequin-Frey, and P. A. Jacobi, *J. Amer. Chem. Soc.*, **95**, 6407 (1973).

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Transition Metal Ion Inhibition of Enzyme-Catalyzed Phosphate Ester Displacement Reactions

Sir:

The action of oxovanadium(IV) and vanadium(V) ions and their uridine complexes led Lienhard and his colleagues[1] to suggest that they may act as possible transition state analogs. We have reached similar conclusions for the even more potent inhibition of acid phosphatases by transition metal oxyanions. Moreover, our results lead to significant conclusions about the potential utility of early transition metal oxyanions as structural and mechanistic probes of enzymatic reactions involving displacement reactions on phosphate esters.

(1) R. N. Lindquist, J. L. Lynn, Jr., and G. E. Lienhard, *J. Amer. Chem. Soc.*, **95**, 8762 (1973).

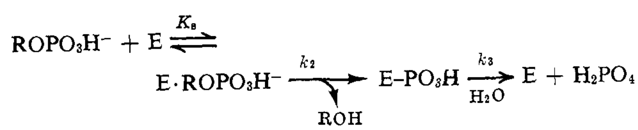
The acid phosphatases are relatively nonspecific enzymes which catalyze the hydrolysis of a variety of alkyl and aryl phosphate esters and have pH "optima" in the region of 4–6. While these phosphohydrolases have long been known to be subject to inhibition by ions such as molybdate,[2] extensive studies carried out under readily comparable conditions using purified acid phosphatases have not been reported nor has any convincing mechanistic explanation been presented. The results of such a study using both a plant and an animal acid phosphatase isoenzyme are presented in Table I. Of interest is the potent inhibition of the human liver acid phosphatase isoenzyme by L-(+)-tartarate, establishing this as a "tartarate-inhibitable acid phosphatase," a property which previously was regarded by many as a characteristic unique to the prostatic enzyme.[3] However, most noteworthy in Table I are the very small values, approaching 10^{-8} M, for competitive inhibition of the wheat germ and human liver acid phosphatases by the early transition metal oxoanions.

Table I. K_i Constants for Competitive Inhibition of Acid Phosphatase Isoenzymes[a,b]

Inhibitor	K_i (M), for acid phosphatase from: Human liver[c]	Wheat germ[d]
Phosphate	8.0×10^{-4}	1.3×10^{-3}
Arsenate	1.8×10^{-4}	6.1×10^{-4}
L-(+)-Tartarate	1.6×10^{-6}	$>10^{-2}$
Vanadate[e]	2.0×10^{-7}	6.7×10^{-6}
Molybdate[e]	3.6×10^{-8}	5.6×10^{-8}
Tungstate[e]	1.6×10^{-8}	6.1×10^{-8}

[a] All values were determined with p-nitrophenyl phosphate substrate at pH 5 in 0.05 M acetate buffer with added 0.1 M NaCl. The K_m (apparent) values were determined foı at least five different substrate concentrations and in the presence of at least four different inhibitor concentrations and were calculated using the Cleland HYPER program (W. W. Cleland, *Advan. Enzymol.*, **29**, 1 (1967)) which provides a statistical fit to the rectangular hyperbola; all data were also checked graphically using standard double-reciprocal plots. The substrate K_m values for the liver and wheat germ enzymes in the absence of inhibitor are 1.2×10^{-4} and 1.3×10^{-4} M, respectively. For the case of the wheat germ enzyme, calculations based on burst-titration data for the enzyme concentration established that the inhibitor concentration was always large (>50) relative to the enzyme concentration. [b] Each acid phosphatase was purified to the point where a single activity band was present on gel electrophoresis at several pH values; activity staining was done with methods using both α-naphthyl phosphate and p-nitrophenyl phosphate as substrates. [c] D. M. Rehkop and R. L. Van Etten, manuscript in preparation. [d] P. P. Waymack and R. L. VanEtten, manuscript in preparation. [e] Sources and formulas: Mallinckrodt sodium molybdate, $Na_2MoO_4 \cdot 2H_2O$; Fisher sodium tungstate, $Na_2WO_4 \cdot 2H_2O$; Research Organic/Inorganic sodium ortho vanadate, Na_3VO_4.

Recent evidence indicates that the mechanism of action of at least some acid phosphatases involves the formation of a covalent phosphoryl–enzyme intermediate and that in at least a portion of the pH range there is a rate-limiting dephosphorylation. Using a radioactively labeled substrate, p-nitrophenyl phosphate-^{32}P, Hickey and VanEtten succeeded in covalently labeling an isoenzyme of wheat germ acid phosphatase.[4] Following the complete alkaline hydrolysis of the protein, they obtained a near-stoichiometric yield of N'-phosphorylhistidine. This experiment with a substrate confirms the results of Igarashi, *et al.*, who found that purified rat liver acid phosphatase was inactivated by dye-sensitized photooxidation using Methylene Blue and who isolated radioactive phosphohistidine by a procedure involving incubation of the enzyme with [^{32}P] inorganic phosphate ion followed by alkaline hydrolysis of the protein.[5] Previous to this, N'-phosphohistidine was isolated *via* a similar sequence following incubation of glucose 6-phosphate with an insoluble microsomal acid phosphatase.[6] Other lines of kinetic evidence also bear importantly on the mechanism of action of acid phosphatases. Consistent with an earlier finding by Tsuboi and Hudson for human prostatic acid phosphatase,[7] Kilsheimer and Axelrod observed a common V_{max} value in the hydrolysis of substrates having widely varying alkyl, aryl, and even acyl leaving groups.[8] For the case of wheat germ acid phosphatase, the variation in rate with different leaving groups has been explored as a function of pH and supports the involvement of a covalent phosphoramidate intermediate.[9] These indications of a rate-limiting dephosphorylation step are consistent with the observation of a burst of p-nitrophenoxide ion when acid phosphatases are mixed with p-nitrophenyl phosphate at pH >7. This has been done with rat liver,[5] wheat germ,[4] and human prostatic[10,11] acid phosphatases. The available data best fit a mechanism of the following form for acid phosphatases. In this sequence the E·S complex, $E \cdot ROPO_3H^-$, breaks down with the expulsion of the alcoholic portion of the phosphate ester to form the covalent intermediate $E\text{–}PO_3H^-$. Depending on the specific acid phosphatase and the pH range under consideration, k_2 may be much faster than k_3 (particularly at more basic pH values). This is consistent with the detailed pH dependence of homogeneous prostatic acid phosphatase and the kinetic and practical limitations which must be placed upon burst-titration determinations of this enzyme.[12]



The apparent K_m values observed for substrates such as p-nitrophenyl phosphate probably are significantly smaller than the actual binding constants K_s. For acid phosphatases dephosphorylation may be rate limiting over much of the pH range around neutrality and therefore $K_m < K_s$.[13] This is also suggested by the fact that benzene phosphonic acid, $C_6H_5P(O)(OH)_2$, a close structural analog of the common aryl phosphate sub-

(2) *E.g.*, C. A. Bunton, B. L. Silver, and C. A. Vernon, *Proc. Chem. Soc. London*, 348 (1957).
(3) *Cf.* O. Bodansky, *Advan. Clin. Chem.*, **15**, 43 (1972).
(4) M. E. Hickey and R. L. VanEtten, *Arch. Biochem. Biophys.*, **152**, 423 (1972).
(5) M. Igarashi, H. Takahashi, and N. Tsuyama, *Biochim. Biophys. Acta*, **220**, 85 (1970).
(6) F. Feldman and L. G. Butler, *Biochim. Biophys. Res. Commun.*, **36**, 119 (1969).
(7) K. K. Tsuboi and P. B. Hudson, *Arch. Biochem. Biophys.*, **55**, 191 (1955).
(8) G. S. Kilsheimer and B. Axelrod, *J. Biol. Chem.*, **227**, 879 (1957).
(9) M. E. Hickey, P. P. Waymack, and R. L. VanEtten, manuscript in preparation.
(10) R. L. VanEtten and J. J. McTigue, Abstracts of the 164th National Meeting of the American Chemical Society, New York, N. Y., Aug–Sept 1972, Abstract BIOL 93.
(11) W. Ostrowski and E. A. Barnard, *Biochemistry*, **12**, 3893 (1973).
(12) J. J. McTigue and R. L. VanEtten, manuscript in preparation.
(13) For a discussion of a kinetically related case see B. Zerner and M. L. Bender, *J. Amer. Chem. Soc.*, **86**, 3669 (1964).

strates, is in fact a very poor inhibitor of the acid phosphatases, with K_i values >100 mM. Consequently the K_s/K_i ratios[14] for inhibition by transition metal ions may be expected to be even larger numbers than would be suggested by a comparison of K_m and K_i values.

There are several possible explanations for the potent inhibition of acid phosphatases by the transition metal oxides and these explanations differ significantly depending on the probable structural nature of the inhibitor ions present in dilute aqueous solution at these pH values. It has been stated that molybdate and tungstate are tetrahedral in aqueous solution.[15] If this is in fact the *exclusive* form of the ions in solution and at the enzyme active site then a rationale for the inhibition pattern would have to be made on the basis of the differing metal–oxygen bond lengths, which increase in the order P–O, As–O, V–O, Mo–O, and W–O (Table II). However, it seems very unlikely that the relatively small changes in ionic radii on going from phosphate or arsenate to the transition metal oxides can be used to satisfactorily explain the three or four orders-of-magnitude decrease in the enzyme–inhibitor dissociation constants. Rather, explanations based either on the resemblance of the hydrated transition metal oxides to the structure of the transition state or chelation and the resemblance of the chelated transition metal ion to the structure of the transition state must appear more likely.

Table II. Selected Bond Lengths and Interatomic Distances of Inhibitor Anions

Bond or molecule	Length (Å)	Bond or molecule	Length (Å)
P–O	1.52[a]	Mo–O	1.76[d,e]
As–O	1.65[a]	W–O	1.76[f]
V–O	1.70[b,c]	O(1) to O(6) of D-(−)-tartarate	4.57[g]

[a] W. H. Baur and A. A. Kahn, *Acta Crystallogr., Sect. B*, **26**, 1584 (1970). [b] P. Susse and M. J. Buerger, *Z. Kristallogr., Kristallgeometrie, Kristallphys. Kristallchem.*, **131**, 161 (1970). [c] The V–O bond lengths seem to be more subject to variation, and it has been stated in connection with a study on $FeVO_4$ that "the bond lengths [V^{5+}–O^{2-}] range from 1.66 to 1.81 Å depending on the environment:" B. Robertson and E. Kostiner, *J. Solid State Chem.*, **4**, 29 (1972). [d] L. O. Atovmyan and O. A. D'yachenko, *Zh. Strukt. Khim.*, **10**, 504 (1969); *J. Struct. Chem. (USSR)*, **10**, 416 (1969). [e] B. M. Gatehouse and P. Leverett, *J. Chem. Soc. A*, 849 (1969). [f] W. H. Zachariasen and H. A. Plettinger, *Acta Crystallogr.*, **14**, 229 (1961). [g] The longest O–O interatomic distance (corresponding to the "length" of the anion) was calculated from the data given by F. Stern and C. A. Beevers, *Acta Crystallogr.*, **3**, 341 (1950).

The isolation[4–6] of N'-phosphorylhistidine in experiments with acid phosphatases together with the known acid lability and alkaline stability of phosphoramidate derivatives such as the phosphohistidines[16] is consistent with the occurence of a phosphoramidate as a covalent intermediate. A good picture can be presented for the transition state in the reaction of water (for example) with a phosphoramidate intermediate.[17] This would be a trigonal byipyramidal species having an entering water and the leaving amine (such as imidazole) in axial orientations,[17] with somewhat extended P–O and P–N bonds, the relative lengths depending on the extent of bond making and breaking to the entering and leaving groups. Structurally such a transition state resembles (to a first approximation) trigonal bipyramidal cyclic phosphorus derivatives for which detailed crystallographic information is available.[18,19] A molecular model constructed to resemble a trigonal bipyramidal species and having slightly extended axial bonds bears a strong resemblance to the model of an octahedrally coordinated metal oxospecies. In this way, it may be possible to explain the apparent discontinuity between the K_i data (Table I) for the tetrahedral inhibitors such as phosphate and arsenate, and the early transition metal oxoanions.[20]

Vanadate, molybdate, and tungstate ions readily form chelates with a variety of donor groups including oxygen, nitrogen, and sulfur ligands.[22,23] The structures of many of these chelates are known in detail as the result of crystallographic and other studies. Numerous transition metal oxide hydrates[26] or chelates have octahedral or distorted octahedral coordination geometries with varying metal–oxygen bond distances,[24,25,27] while many other examples exist of tetrahedral, octahedral, distorted octahedral, trigonal bipyramidal, and even square pyramidal complexes and chelates involving vanadates, tungstates, and molybdates.[22,24–34] Taken as a whole these data indicate that the early transition metal oxoanions can form complexes and chelates with

(14) G. E. Lienhard, *Science*, **180**, 149 (1973). For earlier discussion of these concepts see R. Wolfenden, *Nature (London)*, **223**, 704 (1969); *Accounts Chem. Res.*, **5**, 10 (1972).

(15) (a) R. H. Busey and O. L. Keller, Jr., *J. Chem. Phys.*, **41**, 215 (1964); (b) V. Gonzalez-Vilchez and W. P. Griffith, *J. Chem. Soc. Dalton Trans.*, 1416 (1972).

(16) D. E. Hultquist, *Biochem. Biophys. Acta*, **153**, 329 (1968).

(17) N. K. Hamer, *J. Chem. Soc. C*, 404 (1966).

(18) W. C. Hamilton, S. J. LaPlaca, F. Ramirez, and C. P. Smith, *J. Amer. Chem. Soc.*, **89**, 2268 (1967).

(19) R. D. Spratley, W. C. Hamilton, and J. Ladell, *J. Amer. Chem. Soc.*, **89**, 2272 (1967).

(20) It is possible that the inhibition due to tartarate may be due to a resemblance to a transition state species, in that the length (Table II) is perhaps only ~ 1 Å greater than might be expected for the axial O–P–O bond length of a trigonal bipyramidal transition state. Interestingly, examination of molecular models of tartarate and octahedrally coordinated metal oxyanions reveal a surprisingly close resemblance to certain orientations of a trigonal bipyramid. However, the inhibition by tartarate is not a general feature of the acid phosphatases in that plant acid phosphatases (such as that from wheat germ) are not much inhibited by D- or L-tartarate. Indeed, Axelrod has suggested that this criterion can be used as a reliable way of differentiating between plant and animal acid phosphatases.[21]

(21) G. S. Kilsheimer and B. Axelrod, *Nature (London)*, **182**, 1733 (1958).

(22) *E.g.*, R. Puschel and E. Lassner in "Chelates in Analytical Chemistry," Vol. I, H. A. Flaschka and A. J. Barnard, Jr., Ed., Marcel Dekker, New York, N. Y., 1967, pp 265–326.

(23) These ions are also particularly noted for their tendency to form complex isopolyanions and heteropolyanions.[24] Fortunately, at the very dilute concentrations required for inhibition of these acid phosphatases, it is unlikely that polymerization of the molybdates, tungstates, or vanadates represents a significant problem; concentrations of $>10^{-4}$ M are required (at least in the pH range 5–7) before polymerization becomes significant.[25]

(24) D. L. Kepert in "Comprehensive Inorganic Chemistry," Vol. IV, J. C. Bailar, Jr., H. J. Emeleus, R. Nyholm, and A. F. Trotman-Dickenson, Ed., Pergamon Press, Oxford 1973, pp 607–672.

(25) D. L. Kepert, "The Early Transition Metals," Academic Press, New York, N. Y., 1972.

(26) W. P. Griffith, *Coord. Chem. Rev.*, **5**, 459 (1970).

(27) A. G. Swallow, F. R. Ahmed, and W. H. Barnes, *Acta Crystallogr.*, **21**, 397 (1966).

(28) B. Krebs, *Acta Crystallogr., Sect. B*, **28**, 2222 (1972).

(29) J. Fischer, L. Ricard, and P. Toledano, *J. Chem. Soc., Dalton Trans.*, 941 (1974).

(30) J. R. Knox and C. K. Prout, *Acta Crystallogr., Sect. B*, **25**, 1857 (1969).

(31 M. G. Drew and A. Kay, *J. Chem. Soc. A*, 1846 (1971).

(32) M. G. Drew and A. Kay, *J. Chem. Soc. A*, 1851 (1971).

(33) A. Kay and P. C. Mitchell, *J. Chem. Soc. A*, 2421 (1970).

(34) B. M. Gatehouse and P. Leverett, *J. Chem. Soc. A*, 1398 (1968); *cf.* M. Seleborg, *Acta Chem. Scand.*, **20**, 2195 (1966).

a variety of donors and involving a surprising range of geometries and bond lengths. It is proposed that the potent competitive inhibition of acid phosphatases by ions such as molybdate and tungstate is entirely consistent with the ability of these ions to rapidly and reversibly form chelates at the enzyme active site which resemble the trigonal bipyramidal transition state occurring in the hydrolysis of the phosphate ester or the phosphoryl enzyme intermediate. That is, inhibition occurs as the result of the ability of the ions to function as transition state analogs, consistent with one of the hypotheses advanced by Lienhard.[1] In this regard it is of considerable interest that molybdate ion has been found[35] to form a 1:1 complex with histidine in the pH range 5–7. Strong evidence exists implicating a critical histidyl residue at the active site of acid phosphatases.[4–6]

The ability to form complexes which resemble the trigonal bipyramidal transition states characteristic of many displacement reactions on phosphorus esters would mean that similar effects might be caused by these and related oxyanions in other biological reactions involving displacement reactions on phosphates. It is therefore satisfying to note a recent report of the inhibition of alkaline phosphatase by permanganate and periodate ions.[36] The periodate ion inhibition observed in the case of alkaline phosphatase cannot readily be explained as an oxidative process.[39] Two possible explanations can be advanced. Although not noted by the authors of that report[36] it is known that periodate forms very stable complexes and heteropolyanions with transition metals.[40,41] Thus, the periodatocobaltic ion was found to have a very high formation constant when studied in dilute alkaline solution,[42] even at 60°. While similar data are apparently not yet available for zinc, it seems very possible that the inhibition of alkaline phosphatase, a zinc metalloenzyme,[43] might be the result of the formation of a similar complex ion.

Alternatively, as we have noted for the case of the acid phosphatases, the strong inhibition may be due either to the reasonably close structural resemblance between octahedrally coordinated oxoanions and the transition state species, or to the presence of pentacoordinate oxoanions in aqueous solution which can then act as very close structural analogs of the trigonal bipyramidal transition state. In this regard it has been determined that the equilibrium[44] between IO_4^- and $H_4IO_6^-$ is reached very rapidly.[45] Kustin has advanced arguments for the intermediate occurence of pentacoordinate species in the aquation process.

A conclusion which should be apparent from the foregoing is that there may be a very broad range of uses of transition metal oxoanions as transition state analogs and mechanistic probes in reactions involving displacements on phosphate esters.

Acknowledgment. Supported in part by a research grant from the National Cancer Institute (CA 10585) and an institutional grant from the American Cancer Society. We thank Professors Richard Walton and Richard Wolfenden for helpful and stimulating discussions.

(35) J. T. Spence and J. Y. Lee, *Inorg. Chem.*, **4**, 385 (1965).

(36) J. T. Ohlsson and I. B. Wilson, *Biochim. Biophys. Acta*, **350**, 48 (1974). Not unexpectedly the inhibition by permanganate was complex; although the authors contend that oxidation "does not appear to be involved" they also noted that the "irreversibly inhibited" enzyme was completely restored by low concentrations of reducing agent. The MnO_4^- ion is tetrahedral in solution, with a Mn–O bond length[37] of 1.63 Å. This, together with the finding that 10^{-4} *M* ClO_4^- ion had only a slight inhibitory effect on alkaline phosphatase[36] is further evidence that a simple resemblance of the inhibitor anions to tetrahedral phosphate is not sufficient to cause the strong inhibition which seems to characterize transition metal ion inhibition. It may, however, as in the case of permanganate, facilitate an oxidation process in which tetrahedral permanganate resembles tetrahedral phosphate.[38]

(37) G. J. Palenik, *Inorg. Chem.*, **6**, 503 (1967).

(38) W. F. Benisek, *J. Biol. Chem.*, **246**, 3151 (1971).

(39) However, it must be kept in mind that the oxidizing potential of periodate is highly sensitive to changes in pH, with small decreases in pH greatly enhancing the oxidizing power. We find that periodate is not a simple competitive inhibitor of acid phosphatases and the results are probably due to simultaneous inhibition and oxidation of the enzyme.

(40) M. Dratovsky and L. Pacesova, *Russ. Chem. Rev.*, **37**, 243 (1968).

(41) H. Siebert, *Fortschr. Chem. Forsch.*, **8**, 479 (1967).

(42) M. W. Lister and Y. Yoshino, *Can. J. Chem.*, **38**, 45 (1960).

(43) D. Plocke and B. Vallee, *Biochemistry*, 1, 1039 (1962).

(44) C. Crouthamel, A. Hayes, and D. Martin, *J. Amer. Chem. Soc.*, **73**, 82 (1951).

(45) K. Kustin and E. Lieberman, *J. Phys. Chem.*, **68**, 3869 (1964).

(46) Recipient of a Research Career Development Award (GM 17,620) from the National Institute of General Medical Sciences, 1969–1973.

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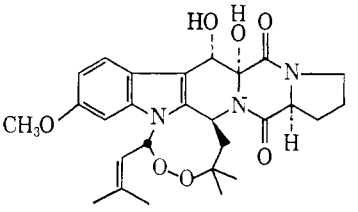
Structure of Verruculogen, a Tremor Producing Peroxide from *Penicillium verruculosum*

Sir:

A new mycotoxin, $C_{27}H_{33}N_3O_7$, that produced severe tremors when administered orally to mice or 1-day old cockerels was obtained from a strain of *Penicillium verruculosum* Peyronel isolated from peanuts.[1] Although several tremor producing mycotoxins have been reported none has been assigned complete stereostructures.[2–8] We wish to report the structure of verruculogen as the novel peroxide **1**.



1

P. verruculosum was cultured in Fernbach flasks with shredded wheat and Difco mycological broth supplemented with yeast extract. The toxin was extracted with chloroform and purified by chromatography and crystallization. The purified, crystalline material had mp 233–235 (dec) and *m/e* 511.236 (*m/e* calculated for $C_{27}H_{33}N_3O_7$, 511.232). The substance is neutral and has

(1) R. J. Cole, J. W. Kirksey, J. H. Moore, B. R. Blankenship, U. L. Diener, and N. B. Davis, *Appl. Microbiol.*, **24**, 248 (1972).

(2) A. Ciegler, *Appl. Microbiol.*, **18**, 128 (1969).

(3) C. T. Hou, A. Ciegler, and C. W. Hessetine, *Appl. Microbiol.*, **21**, 1101 (1971).

(4) B. J. Wilson and C. H. Wilson, *Science*, **144**, 177 (1964).

(5) B. J. Wilson, C. H. Wilson, and A. W. Hayes, *Nature (London)*, **220**, 77 (1968).

(6) M. Yamazaki, S. Suzuki, and K. Miyaki, *Chem. Pharm. Bull.*, **19**, 1739 (1971).

(7) M. Yamazaki, K. Susago, and K. Miyaki, *J. Chem. Soc., Chem. Commun.*, 408 (1974).

(8) D. T. Dix, J. Martin, and C. E. Moppett, *J. Chem. Soc., Chem. Commun.*, 1168 (1972).