

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.<sup>1</sup> In this regard it is of considerable interest that molybdate ion has been found<sup>35</sup> 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.<sup>4–6</sup>

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.<sup>36</sup> The periodate ion inhibition observed in the case of alkaline phosphatase cannot readily be explained as an oxidative process.<sup>39</sup> Two possible explanations can be advanced. Although not noted by the authors of that report<sup>36</sup> it is known that periodate forms very stable complexes and heteropolyanions with transition metals.<sup>40,41</sup> Thus, the periodatocobaltic ion was found to have a very high formation constant when studied in dilute alkaline solution,<sup>42</sup> 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,<sup>43</sup> 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 oxyanions and the transition state species, or to the presence of pentacoordinate oxyanions in aqueous solution which can then act as very close structural analogs of the trigonal bipyramidal transition state. In this regard it has been

(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  $\text{MnO}_4^-$  ion is tetrahedral in solution, with a Mn–O bond length<sup>37</sup> of 1.63 Å. This, together with the finding that  $10^{-4}$  M  $\text{ClO}_4^-$  ion had only a slight inhibitory effect on alkaline phosphatase<sup>36</sup> 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.<sup>38</sup>

(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).

determined that the equilibrium<sup>44</sup> between  $\text{IO}_4^-$  and  $\text{H}_4\text{IO}_6^-$  is reached very rapidly.<sup>45</sup> Kustin has advanced arguments for the intermediate occurrence of pentacoordinate species in the aequation process.

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

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(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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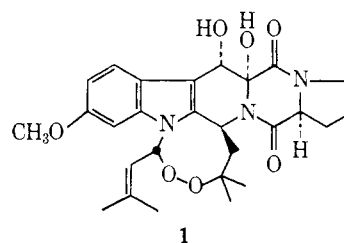
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### Structure of Verrucologen, a Tremor Producing Peroxide from *Penicillium verruculosum*

Sir:

A new mycotoxin,  $\text{C}_{27}\text{H}_{33}\text{N}_3\text{O}_7$ , 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.<sup>1</sup> Although several tremor producing mycotoxins have been reported none has been assigned complete stereostructures.<sup>2–8</sup> We wish to report the structure of verrucologen 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  $\text{C}_{27}\text{H}_{33}\text{N}_3\text{O}_7$ , 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. Hessestine, *Appl. Microbiol.*, **21**, 1101 (1971).

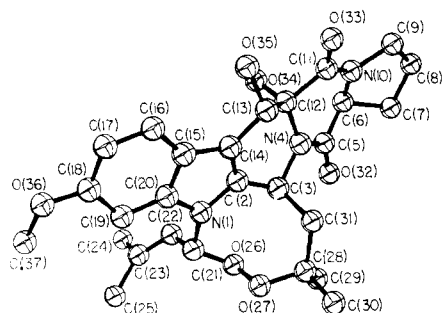
(4) B. J. Wilson and C. H. Wilson, *Science*, **144**, 177 (1964).

(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).



**Figure 1.** A computer generated perspective drawing of verruculogen (1). No hydrogen atoms are shown, and no absolute stereochemistry is implied.

a UV spectrum suggestive of a 6-*O*-methylindole with  $\lambda_{\text{max}}^{\text{EtOH}}$  226 (47,500), 277 (11,000), and 295 nm (9750).<sup>9</sup> The CD spectrum of verruculogen (1) in ethanol showed two Cotton effects corresponding to the first two uv bands; the third Cotton effect was not observed. The Cotton effect at 290 nm was positive ( $\Delta\epsilon = +0.16$ ). The Cotton effect at 265 nm was also positive ( $\Delta\epsilon = +0.56$ ).

The relative structure **1** was deduced from a single-crystal X-ray diffraction experiment. Large crystals of composition  $\text{C}_{27}\text{H}_{33}\text{O}_7\text{N}_3 \cdot \text{C}_6\text{H}_6$  could be grown by slow evaporation of a benzene-ethanol solution. Accurate, diffractometer determined cell constants were  $a = 9.88$  (1),  $b = 10.86$  (2), and  $c = 28.52$  (3) Å in the unambiguously determined space group  $P2_12_12_1$ . All unique reflections within a  $\theta$  sphere of  $63^\circ$  were measured using Ni-filtered Cu  $K\alpha$  X-rays (1.5418). The crystal darkened appreciably and periodically measured standard reflections decreased 20% during the course of data collection. Of the 2611 independent diffraction maxima investigated 2210 were judged observed after correction for background, Lorentz, and polarization effects. Phasing of the 336 largest normalized structure factors was accomplished with the tangent formula.<sup>10</sup> A plausible eight-atom fragment was recycled through the tangent formula and a 20-atom fragment was placed.<sup>10</sup> An electron density map with all the observed reflections finally showed all 43 non-hydrogen atoms of the verruculogen benzene complex. Full-matrix least-squares refinements with anisotropic temperature factors for all non-hydrogen atoms and 16 non-methyl hydrogens have converged to a standard crystallographic discrepancy index of 0.098.<sup>11</sup> Figure 1 is a computer generated drawing of the final X-ray model.<sup>11</sup> See paragraph at end of paper regarding supplementary material, including fractional coordinates, bond angles, and bond distances.

All bond distances and angles agree well with accepted values for given bond types. There are no inter-

(9) A. I. Scott, "Interpretation of the Ultraviolet Spectrum of Natural Products," Macmillan, New York, N. Y., 1964.

(10) G. Germain, P. Main, and M. M. Woolfson, *Acta Crystallogr., Sect. 8*, 24, 274 (1970).

(11) The following library of crystallographic programs was used: C. R. Hubbard, C. O. Quicksall, and R. A. Jacobson, "The Fast Fourier Algorithm and the Programs ALFF, ALFFDP, ALFFT and FRIEDEL," USAEC Report IS-2625, Iowa State University-Institute for Atomic Research, Ames, Iowa, 1971; W. R. Busing, K. O. Martin, and H. A. Levy, "A Fortran Crystallographic Least Squares Program," USAEC Report ORNL-TM-305, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1965; C. Johnson, "ORTEP, A Fortran Thermal-Ellipsoid Plot Program," U. S. Atomic Energy Commission Report ORNL-3794, Oak Ridge National Laboratory, Oak Ridge Tenn., 1965.

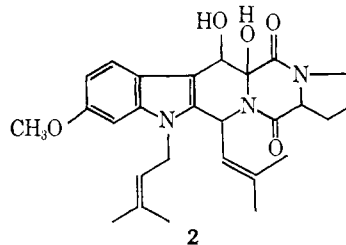
molecular contacts less than 3.40 Å save one —COH...O=C< distance of 2.66 Å. The indole portion of the molecule is planar and the diketopiperazine is folded into a boat conformation. The hydroxyls on carbons 12 and 13 are cis.

The C(21)–O(26) and C(28)–O(27) bond distances are 1.44 (1) and 1.45 (1) Å, respectively, while the O(26)–O(27) bond distance is 1.52 (1) Å. The C(21)–O(26)–O(27)–C(28) torsional angle is 155 (3)°.

The 100-MHz pmr spectrum of verruculogen (1) was obtained in chloroform- $d_1$  and dimethyl- $d_6$  sulfoxide, using TMS as internal standard at  $\delta$  0.00. Assignments of the absorptions listed below are made with reference to Figure 1. The spectrum taken in  $\text{CDCl}_3$  shows the following absorptions:  $\delta$  1.01 (s, 3 H,  $\text{CH}_3$  (29)), 1.72 (s, 6 H,  $\text{CH}_3$  (24, 25)), 1.99 (s, 3 H,  $\text{CH}_3$  (30)), 1.8–2.6 (bm, 6 H,  $\text{CH}_2$  (8, 7, 31)), 3.61 (t, 2 H,  $\text{CH}_2$  (9)), 3.82 (s, 3 H,  $\text{OCH}_3$  (37)), 4.13 (s, 1 H, OH (34)), 4.48 (m, 1 H, CH (6)), 4.79 (d,  $J = 3$  Hz, 1 H, OH (35)), 5.05 (d,  $J = 8$  Hz, 1 H, CH (22)), 5.64 (d,  $J = 3$  Hz, 1 H, CH (13)), 6.05 (d,  $J = 10$  Hz, 1 H, CH (3)), 6.58 (d,  $J = 2$  Hz, 1 H, CH (19)), 6.63 (d,  $J = 8$  Hz, CH (21)), 6.81 (d of d,  $J = 9$  Hz, 2 Hz, 1 H, CH (17)), 7.89 (d,  $J = 9$  Hz, 1 H, CH (16)). The spectrum taken in  $\text{DMSO}-d_6$  shows four sharp singlets in the methyl region at  $\delta$  0.95, 1.58, 1.70, and 1.99.

Double resonance experiments in both decoupling and INDOR modes show coupling between the protons on C(21) and C(22),  $J = 8$  Hz, and C(13) and O(35),  $J = 3$  Hz. The appearance of the C-3 methine proton as a doublet is consistent with the geometry of the molecule as shown in Figure 1.

Verruculogen (1) is clearly related to the family of compounds having a diketopiperazine ring formed from tryptophan and proline.<sup>12</sup> It is very closely related to the recently published gross structure of fumitremorgen B (2).<sup>7</sup> The close structural and spectral similarities



combined with the similar biological activity of verruculogen (1) and fumitremorgen B (2) strongly argue that the relative stereochemistry of 2 is the same as we have proposed for 1. We are unable to detect any of 2 in our *P. verruculosum* cultures although 1 most plausibly comes from 2 via a hydroperoxide.

We have recently isolated both verruculogen (1) and fumitremorgen B (2) from *Aspergillus caespitosus* cultures.

The LD<sub>50</sub> of verruculogen is 2.4 mg/kg (ip, Swiss mice) and the ED<sub>50</sub> for tremor response is 0.39 mg/kg (ip, Swiss mice). Oral doses were 40 times less effective in Swiss mice.

**Supplementary Material Available.** The fractional coordinates (Table I), bond distances (Table II), and important bond angles (Table III) will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary

(12) W. B. Turner, "Fungal Metabolites," Academic Press, New York, N. Y., 1971, p 320.

material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for \$3.00 for photocopy or \$2.00 for microfiche, referring to code number JACS-74-6785.

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### [CF<sub>2</sub>Cl<sup>-</sup>], Chlorodifluoromethide Ion. The Capture of an Elusive Species

Sir:

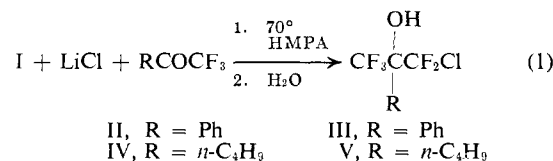
The concerted nature of difluorocarbene formation *via* the decomposition of chlorodifluoroacetic acid and its derivatives is presently accepted as there is no concrete evidence for the existence of the chlorodifluoromethide ion in the literature to date. Herein is reported evidence supporting the existence of the chlorodifluoromethide ion as a reactive intermediate in the decarboxylation of methyl chlorodifluoroacetate. Independent experiments show that the observed products do not result from the initial formation and subsequent reactions of difluorocarbene.

The usefulness of trichloroacetic acid and its derivatives as dichlorocarbene precursors has been known for years.<sup>1-4</sup> More recently, this interest has been extended to the potential use of chlorodifluoroacetic acid and its derivatives as synthetically useful sources of difluorocarbene. Alkali metal chlorodifluoroacetates have been used in the synthesis of steroid derivatives<sup>5</sup> and *gem*-difluorocyclopropanes,<sup>6</sup> in homologation reactions,<sup>7</sup> and in the preparation of 1,1-difluoroolefins.<sup>8</sup> Sodium ethoxide induced decomposition of ethyl chlorodifluoroacetate resulted in difluoromethylation of 2,3-dimethylindole.<sup>9</sup> Decomposition of lithium chlorodi-

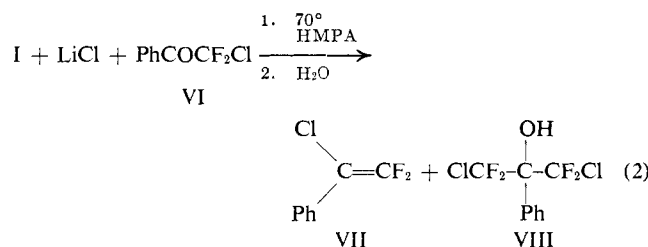
fluoroacetate in the presence of 9,10-phenanthrene quinone gave a hydroxy ketone which might possibly be a chlorodifluoromethide ion trapping product.<sup>10</sup> However, since initial studies by Hine and coworkers, it has been accepted that halodifluoromethide ions have no finite existence. In studies of both the hydrolysis of chlorodifluoromethane<sup>11</sup> and the decarboxylation of chlorodifluoroacetic acid,<sup>12</sup> it was concluded that difluorocarbene formation is a concerted process and that there is no intermediate formation of halodifluoromethide ion. Attempts to trap these carbanions even by protonation have met with little success.<sup>12</sup>

We have observed in this laboratory that lithium chloride initiates the facile decarboxylation of methyl chlorodifluoroacetate (I) in hexamethylphosphoramide (HMPA). A study of this decarboxylation in the presence of polyfluorinated ketones has been done, and the results are reported below.

Decarboxylation of I by lithium chloride in the presence of trifluoroacetophenone (II) in HMPA proceeded smoothly at 65 to 70°C.<sup>13</sup> After 20 hr, steam distillation resulted in isolation of 1-chloro-2-phenylpentafluoro-2-propanol (III) as the only product in 62% yield. Similarly, decarboxylation of I in the presence of 1,1,1-trifluoro-2-hexanone (IV) afforded 1-chloro-1,1-difluoro-2-trifluoromethyl-2-hexanol (V) as the only product in 39% yield. Decarboxylation of I in the presence of



chlorodifluoroacetophenone (VI), however, yielded an olefin, 1-chloro-1-phenyldifluoroethylene (VII), as the major product (50%) as well as 1,3-dichloro-2-phenyltetrafluoro-2-propanol (VIII) (18%) after steam distillation.



Two mechanistic interpretations have been considered to explain the observed results. The first interpretation is that the products in each case result from initial formation of the chlorodifluoromethide ion upon decarboxylation of I with subsequent attack of the carbanion at the carbonyl carbon atoms of ketones II, IV, and VI to form alcoholates IX, X, and XI, respectively (Scheme I). For reasons as yet unexplained, alcoholate XI can undergo an intramolecular S<sub>N</sub>2 displacement of chloride ion by oxygen to form oxirane

(10) M. Derenberg and P. Hodge, *J. Chem. Soc., Perkin Trans.*, 1056 (1972).

(11) J. Hine and P. B. Langford, *J. Amer. Chem. Soc.*, **79**, 5497 (1957).

(12) J. Hine and D. C. Duffey, *J. Amer. Chem. Soc.*, **81**, 1131 (1959).

(13) In one experiment methyl chloride was detected and identified by <sup>1</sup>H nmr and infrared spectroscopy. Quantitative analysis by <sup>1</sup>H nmr showed a 92% yield of methyl chloride. A yield of 88% CO<sub>2</sub> was determined by precipitation as barium carbonate.

(1) F. H. Verhoek, *J. Amer. Chem. Soc.*, **56**, 571 (1934).

(2) W. E. Parham and F. C. Loew, *J. Org. Chem.*, **23**, 1705 (1958).

(3) W. M. Wagner, *Proc. Chem. Soc., London*, 229 (1959).

(4) W. M. Wagner, *et al.*, *Recl. Trav. Chim. Pays-Bas*, **80**, 740 (1961).

(5) See, for example, L. H. Knox, E. V. Velarde, S. M. Berger, and D. H. Caudriello, *Chem. Ind. (London)*, 860 (1962); L. H. Knox, E. V. Velarde, S. M. Berger, D. H. Caudriello, P. W. Landis, and A. D. Cross, *J. Amer. Chem. Soc.*, **85**, 1851 (1963); C. Beard, N. H. Dyson, and J. H. Fried, *Tetrahedron*, **28**, 3281 (1966); C. Beard, I. T. Harrison, L. Kirkham, and J. H. Fried, *ibid.*, **28**, 3287 (1966); T. L. Popper, F. E. Carlon, H. M. Marigliano, and M. D. Yudis, *Chem. Commun.*, 277 (1968); P. Hodge, J. A. Edwards, and J. H. Fried, *Tetrahedron Lett.*, 5175 (1966); P. Crabbé, H. Carpio, E. V. Velarde, and J. H. Fried, *J. Org. Chem.*, **38**, 1478 (1973).

(6) J. M. Birchall, G. W. Cross, and R. N. Haszeldine, *Proc. Chem. Soc., London*, 81 (1960).

(7) P. Crabbé, H. Carpio, A. Cervantes, J. Iriarte, and L. Tökes, *Chem. Commun.*, 79 (1968); P. Crabbé, A. Cervantes, A. Cruz, E. Galeazzi, J. Iriarte, and E. V. Velarde, *J. Amer. Chem. Soc.*, **95**, 6655 (1973).

(8) See, for example, S. A. Fuqua, W. G. Duncan, and R. M. Silverstein, *J. Org. Chem.*, **30**, 2543 (1965); D. J. Burton and F. E. Herkes, *Tetrahedron Lett.*, 1883 (1965); F. E. Herkes, Ph.D. Thesis, University of Iowa, 1966; D. J. Burton and F. E. Herkes, *J. Org. Chem.*, **32**, 1311 (1967); **33**, 1854 (1968).

(9) C. W. Rees and C. E. Smithen, *J. Chem. Soc.*, 938 (1964).