The nucleus was then developed from **6** by acid-catalyzed regeneration[5] of the 3β-hydroxy-5-ene in **8a**[7] (mp 205–208 °C) (Scheme II) protected as the acetonide **8b**[7,8,13] (mp 130–131 °C) to allow tosylation at C-3 to form **8c**[7] (mp 69–70 °C). Oxygen was introduced at C-6 by hydroboration–oxidation (BH_3·THF, O °C, 1.5 h then 16 h at 22 °C) of **8c** to give **9**,[7] which underwent smooth elimination with Li_2CO_3 in dry dimethylacetamide (150 °C, 15 min) followed by Jones oxidation to give the 6-ketone **10**[7,8] (mp 228–229 °C) after silica gel chromatography.

Stereospecfic α-face hydroxylation (OsO_4, C_5H_5N; O °C, 3 h) of **10** gave the 2α,3α-diol **11**[7] (mp 216–218 °C) which was simultaneously deprotected and Bayer–Villiger oxidized in the final step. Thus, addition of **11** in CH_2Cl_2 to 3 equiv of ice-cold 0.6 M CF_3CO_3H[14] in moist CH_2Cl_2/CF_3CO_2H leads cleanly in 1 h at 22 °C to brassinolide **1**[7] in 74% yield[15,16] after recrystallization from aqueous methanol [mp 273–274 °C (lit.[1] mp 274–275 °C)]. The synthetic brassinolide in chemical ionization mass spectrometry (CH_4 reagent gas) showed ions at *m/e* 481 (100, M + 1), with four losses of H_2O at 463 (89), 445 (46), 427 (33), and 409 (21), and C-22–23 cleavage at 379 (36), 361 (45,379–H_2O).

The identity of synthetic with natural brassinolide was shown by ^{13}C NMR spectral coincidence (within 0.07 ppm) of all the lines observed in the CD_2Cl_2–CD_3OD (9:1) solution with those cited[1] for brassinolide. Biological activity of brassinolide is not diagnostic for side-chain stereochemistry since two synthetic stereoisomers (22*S*,23*S*,24*R* and 22*R*,23*R*,24*R*) were found[2] to be less potent but quite active at 10 ng/plant in pinto bean assays.[1] Extensive biological investigation of natural brassinolide was hindered by low availability, but we anticipate that more interesting studies may now be made possible by this work. Our beginning studies of its biological properties will be reported subsequently.

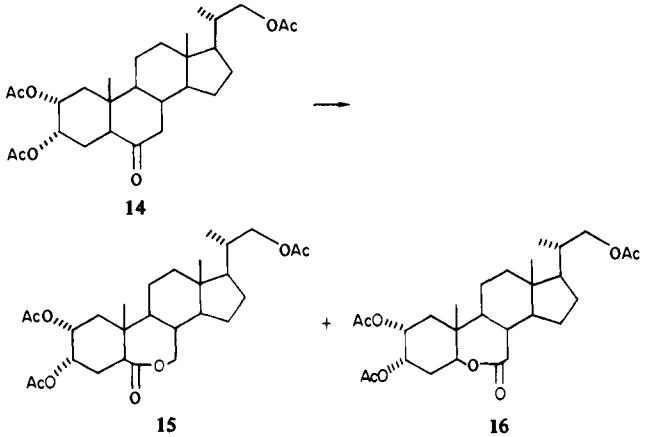
Acknowledgment. We thank Drs. M. Maddox (Syntex), J. Shoolery (Varian) and L. J. Durham (Stanford) for invaluable NMR spectroscopy data and G. Jamieson for mass spectroscopy data.

(13) Vicinal coupling of J = 8.5 Hz for H-22 to H-23 observed for **8b** is compatible with both threo and erythro relationships: Gregson, M.; Ollis, W. D.; Redman, B. T.; Sutherland, I. O.; Dietrichs, H. H. *Chem. Commun.* **1968**, 1394–1395.

(14) Prepared by adding $(CF_3CO)_2O$ (6.74 mL) to 30% aqueous H_2O_2 (1 g) in CH_2Cl_2 (7.4 mL) at 0 °C; dilution to 0.2 M in peracid results on addition of **11** in CH_2Cl_2. Oxidation[2] by *m*-$ClC_6H_4CO_3H$ is over 1000 times slower!

(15) Mother liquors contained some trifluoroacetates, recoverable as **1** by aqueous K_2CO_3 hydrolysis, with CH_3CO_2H for relactonization.

(16) Migration of C-5 leads to an isomeric 6-oxa-*B*-homo-7-one as the minor product in the "anomalous" Bayer–Villiger oxidation of 5α-6-keto steroids (ref 2 and 10 therein). We found by capillary GLC analysis that lactones **15** and **16** are produced in an 88:12 ratio when the CF_3CO_3H reagent[14] oxidizes **14**, in an alternate approach to the synthesis of brassinolide. The minor isomer from oxidation of **11** was not characterized.



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New Manganese(III)-Containing Acid Phosphatase. Evidence for an Intense Charge-Transfer Band and Tyrosine Phenolate Coordination

Sir:

Only a few enzymes, such as pyruvate carboxylase,[1] superoxide dismutase (SOD),[2] and diamine oxidase,[3] contain tightly bound manganese. Studies of the coordination chemistry of biological manganese have been limited and directed primarily to Mn(II) species.[4] However, recent magnetic susceptibility and electron spin resonance (ESR) experiments demonstrated that the metal bound to Mn–SOD of *E. coli* is trivalent.[5] We have isolated a Mn(III)-containing acid phosphatase and characterized the unique metal chromophore.

The purification and crystal preparation of the Mn–enzyme complex from the tuber of the sweet potato (Kintoki) and the enzymatic properties of the native enzyme will be fully described elsewhere. The manganese ion present at one atom per enzyme molecule (M_w 110 000) plays an essential role in the catalytic reaction of hydrolysis of phosphomonoesters and nucleotide phosphates.[6] This stable metalloenzyme is violet in color with an intense absorption band at 515 nm (ε 2460) attributed to the Mn ion directly coordinated with some amino acid residues. The extinction coefficient of the enzyme is significantly larger than that of the *E. coli* Mn–SOD complex [λ_{max} 473 nm (ε 400)].[2a] The ratio of Δε to ε is 2.1×10^{-4} for the characteristic visible band. The 550-nm extremum band (Δε −0.53) in the circular dichroism (CD) spectrum was used to determine Δε/ε. In a rough approximation, $\gamma = |\Delta\epsilon/\epsilon|$ can be utilized to estimate Kuhn's anisotropic factor, where Δε and ε are the CD($\epsilon_L - \epsilon_R$) and optical absorption in terms of extinction coefficients, respectively.[7] The ratio is typically $\geq 10^{-2}$ for magnetically allowed and electrically forbidden transitions of the d-d type. Therefore, the intense 515-nm band is assigned to an electrically allowed charge-transfer band from the ligand to the metal, which is expected for Mn(III) rather than Mn(II).[8,9]

Figure 1 shows the ESR spectra of the native (A) and denaturated (B) enzymes. The X-band ESR spectra were obtained at 293 K with a JES-FE-3X spectrometer. ESR signals were not obtained with the native violet enzyme. In contrast, the acid- and heat-treated colorless enzyme showed typical six-line ESR patterns due to the aquated Mn(II) ion (^{55}Mn, $I = {}^5/_2$) around $g = 2$. Similar ESR behavior has been observed in the Mn–SOD complex.[2a] Fee et al. reported that the absence of an observable ESR signal in the Mn–SOD complex is quite characteristic of a Mn(III) ($S = 2$) integral spin system with zero-field splitting of 1–2 cm^{-1}.[5] These visible and ESR results strongly indicate that the Mn valence state of the native acid phosphatase is trivalent, Mn(III).[10]

Figure 2 shows the resonance Raman spectrum of the native Mn-containing acid phosphatase. The present spectrum was

(1) Scrutton, M. C.; Utter, M. F.; Mildvan, A. S. *J. Biol. Chem.* **1966**, *241*, 3480–3487.

(2) (a) Keele, B. B.; McCord, J. M.; Fridovich, I. *J. Biol. Chem.* **1970**, *245*, 6176–6181. (b) Weissiger, R. A.; Fridovich, I. *Ibid.* **1973**, *248*, 3582–3592.

(3) Crabbe, M. J. C.; Waight, R. D.; Bardsley, W. G.; Barker, R. W.; Kelly, I. O.; Knowles, P. F. *Biochem. J.* **1976**, *155*, 679–687.

(4) Lawrence, G. D.; Sawyer, D. T. *Coord. Chem. Rev.* **1978**, *27*, 173–193.

(5) Fee, J. A.; Shapiro, E. R.; Moss, T. H. *J. Biol. Chem.* **1976**, *251*, 6157–6159.

(6) The Mn-removed apoenzyme was catalytically inactive. In addition, the enzyme activity was reduced in parallel with decrease in the 515-nm absorbance.

(7) Gillard, R. D. In "Physical Methods in Advanced Inorganic Chemistry"; Hill, H. A. O., Day, P., Ed.; Interscience: London, 1968; pp 167–213.

(8) (a) Dunn, T. M. In "Modern Coordination Chemistry"; Lewis, J.; Wilkins, R. G., Ed.; Interscience: New York, 1960; pp 229–300. (b) Dingle, R. *Acta Chem. Scand.* **1966**, *20*, 33–44.

(9) In the ferric enterobactin and tris(catecholate) complexes, it is known that a broad absorption band centered at ~500 nm is due to phenolate bound to Fe(III) and is assigned to a phenolate → Fe(III) charge-transfer transition: Gaber, B. P.; Miskowski, V.; Spiro, T. G. *J. Am. Chem. Soc.* **1974**, *96*, 6868–6873.

(10) An alternative interpretation for the loss of the ESR signal is that Mn(II) is tightly bound to protein and thereby immobilizes within its ligand field. In such a case, however, the intensely visible band would not appear.

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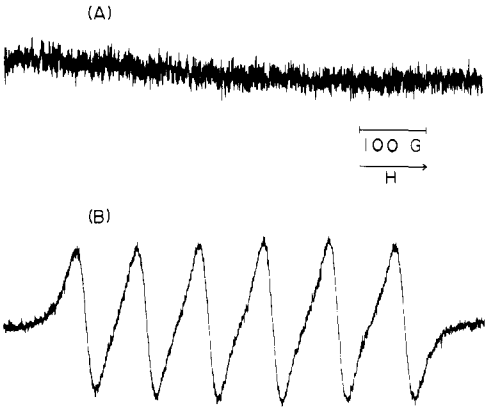

Figure 1. ESR spectra of the native enzyme (A) and the denaturated enzyme (B) at 20 °C. Sample B was obtained by mixing sample A (0.5 mM; 0.2 mL) in water with HCl (1.0 M; 0.05 mL) and then heating to 100 °C for 3 min.

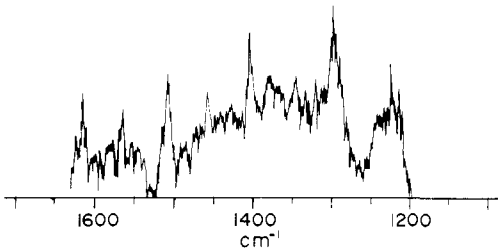

Figure 2. Resonance Raman spectrum of Mn-containing acid phosphatase. The sample concentration was 0.5 mM, and the spectrum was measured at pH 6.8 and 4 °C. Instrumental conditions were as follows: excitation, 514.5-nm line of Ar^+ laser; power, 20 mW at a sample point; time constant, 16 s; slit width, 200 μm; scan speed, 10 cm^{-1}/min.

excited by the 5145-Å line of an argon ion laser and was recorded on a JEOL-400 D Raman spectrometer equipped with a HTV-R 649 photomultiplier. The native enzyme exhibited prominent Raman lines at 1230, 1298, 1508, and 1620 cm^{-1}. These appear to be in resonance with the visible band and are probably due to internal vibrations of the coordinated amino acid residue. Repeated experiments established that these Raman bands are indeed reproducible. The four Raman lines of the Mn–enzyme complex resemble those of the Fe(III) complexes of transferrin (1174, 1288, 1508, and 1613 cm^{-1}),[9] protocatechuate 3,4-dioxygenase (1177, 1265, 1505, and 1605 cm^{-1}),[11] and *p*-cresol (1180, 1222, 1488, and 1618 cm^{-1}).[11] In these Fe(III) complexes, the four characteristic Raman lines have been assigned to vibrations of the coordinated phenolate anion. On the basis of the chemical similarity between Fe(III) and Mn(III) ions, the present Raman spectrum of the Mn–enzyme complex is interpretable in terms of the internal vibration of a coordinated tyrosine phenolate anion. Indeed, the amino acid composition of the violet enzyme revealed an abundance of tyrosine residues.

Sulfhydryl coordination was strongly suggested by the results of our *p*-chloromercuribenzoate binding study on the Mn-containing enzyme.[12] Mn(III) complexes of mercaptoamine and mercaptocarboxylate such as cysteamine, cysteine, and mercaptoacetic acid give intense absorption bands (ϵ 10^3–10^4) in the range of 480–670 nm. Both tyrosine(O) → Mn(III) and cysteine(S) → Mn(III) charge transfers may contribute to the intense 515-nm band of this Mn–enzyme complex. A Raman line due to Mn(III)–S(cysteine) stretching modes would be expected to appear near 350 cm^{-1} [13,14] but was not detected under our experimental condition because of the fluorescence of the sample.

In conclusion, the Mn-containing acid phosphatase is a classic example of tightly bound Mn(III). One of the characteristics of this Mn–enzyme complex is the intense charge-transfer band seen at 515 nm. The resonance Raman evidence indicates the coordination of a tyrosine phenolate anion to the Mn(III) active site. Investigations on the Mn chromophore of the acid phosphatase are under way.

Acknowledgment. Gratitude is due to Dr. S. Fujimoto for pertinent advice on the enzyme preparation, Dr. T. Kitagawa for resonance Raman measurements, and M. Ohara for comments on the manuscript. This study was supported in part by a grant from the Ministry of Education, Science, and Culture, Japan.

(11) Tatsuno, Y.; Saeki, Y.; Iwaki, M.; Yagi, T.; Nozaki, M.; Kitagawa, T.; Otsuka, S. *J. Am. Chem. Soc.* **1978**, *100*, 4614–4615.

(12) The binding of *p*-chloromercuribenzoate strongly inhibited the phosphatase activity and was concomitant with the loss of the violet color. The SH determination with the Ellman reagent showed that no free sulfhydryl groups were detected in the native enzyme, but 1 mol of SH/mol of enzyme was detected in the denaturated and Mn-removed enzyme.

(13) In iron–sulfur proteins, the Raman line near 350 cm^{-1} has been assigned to the Fe(III)–S stretching modes: (a) Long, T. V.; Loeher, T. M.; Alkins, J. R.; Lovenberg, W. J. *J. Am. Chem. Soc.* **1971**, *93*, 1809–1811. (b) Tang, S.-P. W.; Spiro, T. G.; Autanaitis, C.; Moss, T. H.; Holm, R. H.; Herskovitz, T.; Mortenson, L. E. *Biochem. Biophys. Res. Commun.* **1975**, *62*, 1–6.

(14) In the IR spectrum of the tris(*N*,*N*-diethylcarbamdithioato)manganese(III) complex, the Mn(III)–S band at ca. 370 cm^{-1} is extremely broad: Healy, P. C.; White, A. H. *J. Chem. Soc., Dalton Trans.* **1972**, 1883–1887.

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Stereochemistry of Chlorinolysis of the Phosphorus–Sulfur Bond in Thioloesters of Organic Phosphorus Thio Acids. Reaction of *S*-Methyl *tert*-(Butylphenylphosphino)thiolate with Halogens and Sulfuryl Chloride

Sir:

The reaction of organic phosphorus thioloesters with halogenating agents has been known for 20 years.[1] This reaction has been shown to proceed via different pathways, depending on the reaction medium. The reaction in nonaqueous solvents has been applied successfully in the synthesis of optically active 4-coordinated phosphorus compounds.[2] However, interpretation of the accumulated experimental facts on the basis of a frequently used scheme (Scheme I) leads to inconsistencies. Scheme I consists of electrophilic attack of the halogen on the sulfur atom with formation of the corresponding chlorosulfonium salt **2** (step a), which subsequently decomposes by nucleophilic attack of the halide anion on the phosphorus atom (step b).

The aim of this investigation was to examine the stereochemical course of the chlorinolysis reaction of organic phosphorus thioloesters by using a model which, due to the presence of a sterically crowded phosphorus atom, should reduce the rate of any intermediate step involving nucleophilic displacement at the reaction center, thus allowing the possibility to detect intermediates by spectroscopy.

It could be expected on the basis of Scheme I that halogenolysis of **1** (R = *t*-Bu; R′ = Ph; R″ = CH_3) should occur with inversion of configuration at the phosphorus atom, considering the preferences (apicophilicity vs. apicophobicity) of the groups bonded to phosphorus in an intermediate trigonal bipyramid. Contrary to this prediction, retention of configuration was observed[3] and

(1) (a) Saville, B. *Chem. Ind.* (*London*) **1956**, 660. (b) Stirling, C. J. M. *J. Chem. Soc.* **1957**, 3597.

(2) (a) Michalski, J.; Ratajczak, A. *Rocz. Chem.* **1963**, *37*, 1185. (b) Michalski, J.; Mikolajczyk, M.; Omelańczuk, J. *Tetrahedron Lett.* **1968**, 3565. (c) Krawiecka, B.; Skrzypczyński, Z.; Michalski, J. *Phosphorus Relat. Group V Elem.* **1973**, *3*, 177. (d) Hall, C. R.; Inch, T. D. *J. Chem. Soc., Perkin Trans. 1* **1979**, 1104.

(3) Retention of configuration is also observed on models not containing a sterically large *t*-Bu group at phosphorus.[2a,d]

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