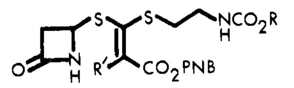
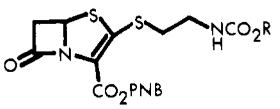


7a R′ = H, R = PNB
7b R′ = H, R = CH_2CCl_3
8a R′ = Br, R = PNB
8b R′ = Br, R = CH_2CCl_3



9a R = PNB
9b R = CH_2CCl_3

of **4** and *p*-nitrobenzyl-*N*-*p*-nitrobenzyloxycarbonylcysteaminyl-1,1-dithiomalonate[12] with sodium methoxide in methanol (25 °C, 24 h, 84%). Bromination of **7a** with NBS (1.1 equiv) (1 equiv of HMPA, THF, 25 °C, 0.3 h, 44%) afforded only the desired *Z* isomer **8a**.[13] Cyclization as before (1.1 equiv of LDA, 3.3 equiv. of $CuBr{\cdot}SMe_2$, THF, −78 to 0 °C, 3.0 h) gave penem **9a** in 55% yield: mp 151–153 °C; IR ($CHCl_3$) 1795, 1720 (br), 1690 (sh) cm^{-1}; NMR ($CDCl_3$) δ 3.1 (m, SCH_2), 3.27 (dd, J = 2, 16 Hz, H-6β), 3.56 (m, NCH_2), 3.84 (dd, J = 5, 16 Hz, H-6α), 5.19 (dd, J = 2, 5 Hz, H-5), 5.23 (s, CO_2CH_2Ar), 5.28 (br m, NH), 5.32 (s, CO_2CH_2Ar), 7.57 (m, ArH), 8.28 (m, ArH); λ_{max} (dioxane) 268 nm (ϵ 24 752), 318 (10 080).

The analogous sequence could also be conducted with the cysteamine nitrogen protected with the trichloroethyloxycarbonyl group. Thus **7b** was obtained analogously in 70% yield and brominated (1.1 equiv of NBS, 6 equiv of HMPA, THF, −78 to −20 °C, 1 h) to **8b**[13] in 70% yield (*Z*/*E* = 9). Cyclization of **8b** to **9b**[13] was best accomplished (52%) employing $CuI{\cdot}PBu_3$ (1 equiv) at −78 to −20 °C over a period of 5 h.

In conclusion, it has been demonstrated that a highly strained bicyclic β-lactam nucleus can be readily constructed in the presence of diverse functional groups utilizing a mild, facile, stereospecific oxidative addition reaction mediated by Cu(I) as the key transformation. The further application of this methodology for the synthesis of novel antibiotics will be reported in due course.

Acknowledgment. We are indebted to Professor Barry M. Trost for many fruitful discussions, to Mr. J. Smith and Mr. H. Flynn for mass spectral measurements, to Dr. B. Arison and Mr. H. Flynn for obtaining the 300-MHz NMR spectra, to Dr. J. A. McCauley and his associates for UV measurements, and to Mr. J. P. Gilbert and his staff for microanalytical determinations.

Supplementary Material Available: List of physical and spectroscopic properties of all new β-lactams (2 pages). Ordering information is given on any current masthead page.

References and Notes

(1) (a) R. Scartazzini, H. Peter, H. Bickel, K. Heusler, and R. B. Woodward, *Helv. Chim. Acta*, **55**, 408 (1972); (b) R. Scartazzini and H. Bickel, *Ibid.*, **55**, 423 (1972); (c) R. Scartazzini, J. Gostell, H. Bickel, and R. B. Woodward, *Ibid.*, **55**, 2567 (1972); (d) R. W. Ratcliffe and B. G. Christensen, *Tetrahedron Lett.*, 4649 (1973); (e) *ibid.*, 4653 (1973); (f) N. G. Steinberg, R. W. Ratcliffe, and B. G. Christensen, *ibid.*, 3567 (1974); (g) R. A. Firestone, N. S. Maciejewicz, and B. G. Christensen, *J. Org. Chem.*, **39**, 3384 (1974); (h) D. Borman, *Justus Liebigs Ann. Chem.*, 1391 (1974); (i) J. H. C. Nayler, N. F. Osborne, M. J. Pearson, and R. Southgate, *J. Chem. Soc., Perkin Trans. 1*, 1615 (1976); (j) R. B. Woodward, K. Heusler, I. Ernest, K. Burri, R. J. Friary, F. Haviv, W. Oppolzer, R. Paioni, K. Syhora, R. Wenger, and J. K. Whitesell, *Nouv. J. Chem.*, **1**, 85 (1977); (k) D. I. C. Scopes, A. F. Kluge, and J. A. Edwards, *J. Org. Chem.* **42**, 376 (1977); (l) S. Yamamoto, N. Haga, T. Aoki, S. Hayashi, H. Tanida, and W. Nagata, *Heterocycles*, **8**, 283 (1977); (m) R. Scartazzini and H. Bickel, *Ibid.*, **7**, 1165 (1977).

(2) (a) L. D. Cama and B. G. Christensen, *J. Am. Chem. Soc.*, **96**, 7582 (1974); (b) R. A. Firestone, J. L. Fahey, N. S. Maciejewicz, G. S. Patel, and B. G. Christensen, *J. Med. Chem.*, **20**, 551 (1977); (c) M. Narisada, H. Onoue, and W. Nagata, *Heterocycles*, **7**, 839 (1977); (d) Y. Hamashima, M. Narisada, M. Yoshioka, S. Uyeo, T. Tsuji, I. Kikkawa, and W. Nagata, Abstracts of the 176th National Meeting of the American Chemical Society, Miami Beach, Fla., Sept 1978, No. 14, Medicinal Chemistry.

(3) (a) R. N. Guthikonda, L. D. Cama, and B. G. Christensen, *J. Am. Chem. Soc.*, **96**, 7584 (1974); (b) J. Finkelstein, K. G. Holden, and C. D. Perchonock, *Tetrahedron Lett.*, 1629 (1978).

(4) I. Ernest, J. Gostell, C. W. Greengrass, W. Holick, D. E. Jackman, H. R. Pfaendler, and R. B. Woodward, *J. Am. Chem. Soc.*, **100**, 8214 (1978), and references therein.

(5) (a) L. D. Cama and B. G. Christensen, *J. Am. Chem. Soc.*, **100**, 8006 (1978); (b) M. Narisada, S. Uyeo, and W. Nagata, Abstracts of the 176th National Meeting of the American Chemical Society, Miami Beach, Fla., Sept 1978, No. 13, Medicinal Chemistry.

(6) For example, R. G. R. Bacon and A. Karim, *J. Chem. Soc., Perkin Trans. 1*, 272, 278 (1973), have reported the effective coupling of potassium phthalimide with both aryl and vinyl halides catalyzed by cuprous ions, and T. Kametani, K. Takahashi, M. Ihara, and K. Fukumoto, *Ibid.*, 389 (1976), have reported a novel, intramolecular cyclization of a copper(I) enamide and an aromatic bromide, and similarly with a pyrrole anion: T. Kametani, T. Ohsawa, M. Ihara, and K. Fukumoto, *ibid.*, 460 (1978).

(7) M. F. Semmelhack, P. M. Helquist, and J. D. Gorzynski, *J. Am. Chem. Soc.*, **94**, 9234 (1972).

(8) K. Clauss, D. Grimm, and G. Prossel, *Justus Liebigs Ann. Chem.*, 539 (1974).

(9) By analogy to the works of the Hoechst group[8,10] in which **4** was found to undergo facile displacements with sulfur nucleophiles and, more significantly, with a highly enolizable carbonyl compound, it was anticipated that thionomalonate **5** would also succeed, since compounds of this type readily form thiolate anions owing to the lower difference in dissociation energy between C=S and C—S (45 kcal/mol^{-1}) as compared with that between C=O and C—O (73 kcal/mol^{-1}).[11]

(10) H. W. Schnabel, D. Grimm, and H. Jensen, *Justus Liebigs Ann. Chem.*, 477 (1974).

(11) W. O. Foye, *J. Chem. Educ.*, **46**, 841 (1969).

(12) F. DiNinno and E. Linek, a manuscript describing the preparation of these and related compounds has been submitted for publication.

(13) All new compounds gave satisfactory spectroscopic analyses.

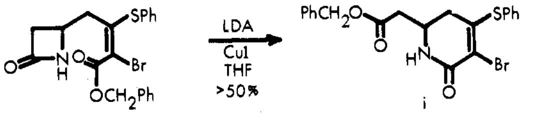
(14) It was found that in the absence of HMPA the undesired *E* bromide predominated (*Z*/*E* = 0.47).

(15) G. B. Kauffman and L. A. Teter, *Inorg. Synth.*, **7**, 9 (1963).

(16) Alternatively, penem **1** could be prepared in 52% yield when $CuBr{\cdot}SMe_2$[17] (2 equiv) was substituted for $CuI{\cdot}PBu_3$.

(17) H. O. House, C.-Y. Chu, M. M. Wilkins, and M. J. Umen, *J. Org. Chem.*, **40**, 1460 (1975).

(18) It was not possible to obtain a pure sample of the *E* bromide owing to its instability toward silica gel plate layer chromatography. The result of this decomposition was a deposition of *p*-nitrobenzyl alcohol, as the only identifiable substance, which presumably arises from an acid-catalyzed process similar to that indicated initially in Scheme I. Based on this finding, which was observed for all of the bromodithioketal azetidinone derivatives reported herein, the *E* and *Z* configurations were assigned. Diagnostically then was the uniformly downfield H-4 resonance of the *Z* bromide relative to the *E* bromide in the NMR spectrum, which allowed for the determination of the *E*/*Z* ratio prior to and after purification.



(19) T. N. Salzmann, unpublished results. In a related system depicted below, it was shown that compound i was the only product from the analogous reaction.

(20) (a) G. Albers-Schönberg, B. H. Arison, O. D. Hensens, J. Hirshfield, K. Hoogsteen, E. A. Kaczka, R. E. Rhodes, J. S. Kahan, F. M. Kahan, R. W. Ratcliffe, E. Walton, L. J. Ruswinkle, R. B. Morin, and B. G. Christensen, *J. Am. Chem. Soc.*, **100**, 6491 (1978), and references therein; (b) K. Okamura, S. Hirata, Y. Okamura, Y. Fukagawa, Y. Shimauchi, K. Kouno, T. Ishikura, and J. Lein, *J. Antibiot.*, **31**, 480 (1978).

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Derivatives of Heteropolyanions. 2. Metal–Metal-Bonded Derivatives

Sir:

We recently reported the preparation of several series of organic derivatives of the Keggin-structure (Figure 1) heteropolyanions $W_{12}SiO_{40}{}^{4-}$, $Mo_{12}SiO_{40}{}^{4-}$, and $W_{12}PO_{40}{}^{3-}$.[1] Extension of this work has led to the first heteropolyanions which contain metal-metal bonds, including $CpFe(CO)_2SnW_{11}PO_{39}{}^{4-}$ ($Cp = \pi\text{-}C_5H_5$), $(OC)_3Co(SnW_{11}SiO_{39})_2{}^{11-}$, $\pi\text{-}C_3H_5Pd(SnW_{11}SiO_{39})_2{}^{11-}$, and a high molecular weight completely inorganic polymer, $[(OC)_3CoGe_2W_{11}SiO_{40}{}^{5-}]_n$. Alternatively, these anions and the others reported here can be viewed as transition-metal complexes which contain heteropolyanion ligands.

These anions are prepared by reacting the "unsaturated" Keggin fragments $W_{11}SiO_{39}{}^{8-}$, $Mo_{11}SiO_{39}{}^{8-}$, or $W_{11}PO_{39}{}^{7-}$,

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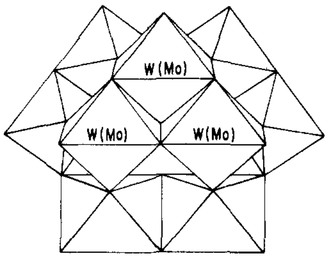

Figure 1. The Keggin structure, shared by $W_{12}SiO_{40}^{4-}$, $W_{12}PO_{40}^{3-}$, $Mo_{12}SiO_{40}^{4-}$, and many other heteropolyanions. It consists of four sets of three metal-centered octahedra symmetrically located around a central tetrahedrally coordinated heteroatom. This perspective does not show the heteroatom or all of the octahedra but was chosen to permit better correlation with Figures 2 and 3. For a better depiction and description of the Keggin structure as a whole, see ref 14.

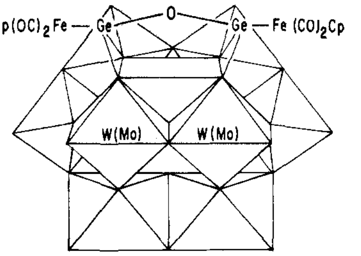

Figure 2. Proposed structure for $[CpFe(CO)_2Ge]_2W_{11}SiO_{40}^{4-}$, $[CpFe(CO)_2Ge]_2W_{11}PO_{40}^{3-}$, and $[CpFe(CO)_2Ge]_2Mo_{11}SiO_{40}^{4-}$.

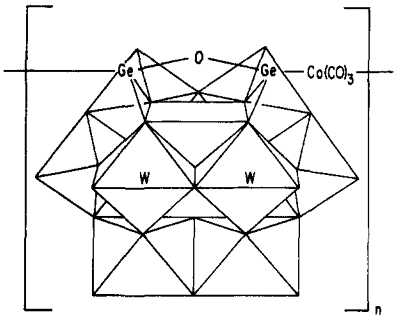

Figure 3. Proposed structure for $[(OC)_3CoGe_2W_{11}SiO_{40}^{5-}]_n$.

obtained by standard procedures,[2] with transition-metal complexes which contain trichlorostannane or trichlorogermane ligands. The trichlorostannanes react to add an L_nMSn^{3+} group to the W_{11} or Mo_{11} Keggin fragment; the resultant anion is a derivative of $W_{12}SiO_{40}^{4-}$, $Mo_{12}SiO_{40}^{4-}$, or $W_{12}PO_{40}^{3-}$ in which one tungsten- or molybdenum-centered octahedron has been replaced by a tin-centered octahedron which has the transition metal at the terminal octahedral vertex:

$$CpFe(CO)_2SnCl_3{}^{3} + Mo_{11}SiO_{39}^{8-} \rightarrow CpFe(CO)_2SnMo_{11}SiO_{39}^{5-}$$

$$(C_7H_8)_2RhSnCl_3{}^{4} + W_{11}PO_{39}^{7-} \rightarrow (C_7H_8)_2RhSnW_{11}PO_{39}^{4-}$$

Similar reactions have led to $CpFe(CO)_2SnW_{11}SiO_{39}^{5-}$, $CpFe(CO)_2SnW_{11}PO_{39}^{4-}$, $p\text{-}FC_6H_4Pt[P(C_2H_5)_3]_2SnW_{11}SiO_{39}^{5-}$, $CpW(CO)_3SnW_{11}SiO_{39}^{5-}$, and $[(C_6H_5)_3P]_2Ir(CO)(H)_2SnW_{11}SiO_{39}^{5-}$. In the last example, the phosphine ligands are cis (^{31}P and ^1H NMR analysis, τ 21.2 (H_1, t of d, $J_{H\text{-}P_{cis}} = 15$, $J_{H_1\text{-}H_2} = 4$ Hz), 22.2 (H_2, d of d of d, $J_{H\text{-}P_{trans}} = 108$, $J_{H\text{-}P_{cis}} = 15$, $J_{H_1\text{-}H_2} = 4$ Hz), whereas in the precursor, $[(C_6H_5)_3P]_2Ir(CO)(H)_2SnCl_3$[5], they are trans.

These anions and the others described here were isolated as trimethylammonium, tetramethylammonium, or trimethylsulfonium salts which were recrystallized from water and characterized by elemental analysis and by infrared and NMR spectroscopy. Where the anion contained organic substituents, integration of their ^1H NMR signal relative to that of the alkylammonium or alkylsulfonium cation served as a further check on composition.[6]

In several instances where the starting trichlorostannane transition-metal complex contained a readily dissociable ligand, the initially formed product underwent a spontaneous disproportionation reaction to give a complex containing two heteropolyanion ligands:

$$(OC)_4CoSnCl_3{}^{7} + W_{11}PO_{39}^{7-} \rightarrow [(OC)_4CoSnW_{11}PO_{39}^{4-}] \rightarrow (OC)_3Co(SnW_{11}PO_{39})_2^{9-}$$

The reaction byproducts include carbon monoxide and $CoW_{11}PO_{39}\cdot H_2O^{5-}$.[8] The reaction was run in air; precipitation of the initial crude product from aqueous solution as a trimethylammonium salt gave a mixture which exhibited infrared bands in the 2200-1900-cm^{-1} region that were typical of an $LCo(CO)_4$ species.[7] Several recrystallizations of this mixture from water converted it into golden yellow $[(CH_3)_3NH]_9(OC)_3Co(SnW_{11}PO_{39})_2$ which had only two infrared bands (2100 (w), 1950 cm^{-1} (s); dimethyl sulfoxide solution) in the metal carbonyl region, characteristic of a $t\text{-}L_2Co(CO)_3$ compound.[7] The remainder of the infrared spectrum was very similar to that of the parent $W_{12}PO_{40}^{3-}$ anion, except for splitting of the phosphate stretch into two bands, at 1050 and 1080 cm^{-1}. Similar spontaneous disproportionation reactions have given $(OC)_3(SnW_{11}SiO_{39})_2^{11-}$, $(OC)_2Fe(NO)(SnW_{11}SiO_{39})_2^{11-}$ (from $(OC)_3Fe(NO)SnCl_3$[9]; $(OC)_2Fe(NO)P(C_6H_5)_3SnCl_3$[10], however, leads to $(OC)_2Fe(NO)P(C_6H_5)_3SnW_{11}SiO_{39}^{5-}$ with no disproportionation), and $\pi\text{-}C_3H_5Pd(SnW_{11}SiO_{39})_2^{11-}$ (from $(C_6H_5)_3P(\pi\text{-}C_3H_5)PdSnCl_3$[11]).

Aqueous potassium cyanide degrades one heteropolyanion moiety in $(OC)_3Co(SnW_{11}PO_{39})_2^{9-}$ to give the potassium salt of an unusual stannoic acid, $K_5(OC)_3Co(SnW_{11}PO_{39})Sn(OK)_3$. Degradation of the remaining heteropolyanion ligand is prevented because $K_5(OC)_3Co(SnW_{11}PO_{39})Sn(OK)_3$ is relatively insoluble in water and rapidly precipitates. Reaction of this salt with $W_{11}SiO_{39}^{8-}$ at pH 5 gives $(OC)_3Co(SnW_{11}PO_{39})(SnW_{11}SiO_{39})^{10-}$

Reactions of the W_{11} or Mo_{11} Keggin fragments with transition-metal complexes containing trichlorogermane ligands under acidic conditions (pH ~4) follow a different stoichiometry than those involving trichlorostannane ligands:

$$CpFe(CO)_2SnCl_3 + W_{11}SiO_{39}^{8-} \rightarrow CpFe(CO)_2SnW_{11}SiO_{39}^{5-}$$

However

$$CpFe(CO)_2GeCl_3{}^{12} + W_{11}SiO_{39}^{8-} \rightarrow [CpFe(CO)_2Ge]_2W_{11}SiO_{40}^{4-}$$

The reactions of $Mo_{11}SiO_{39}^{8-}$ and $W_{11}PO_{39}^{7-}$ with $CpFe(CO)_2GeCl_3$ proceed analogously to give $[CpFe(CO)_2Ge]_2Mo_{11}SiO_{40}^{4-}$ and $[CpFe(CO)_2Ge]_2W_{11}PO_{40}^{3-}$, respectively. In these anions a $[CpFe(CO)_2Ge]_2O^{4+}$ unit rather than a $CpFe(CO)_2Ge^{3+}$ unit is replacing an MO^{4+} (M = tungsten or molybdenum) moiety in the parent heteroanion. This is the same stoichiometry that we previously observed for the reaction products of organotrichlorosilanes ($RSiCl_3$) with $W_{11}SiO_{39}^{8-}$, which have the general formula $(RSi)_2W_{11}SiO_{40}^{4-}$.[1] We are postulating a structure for the new anions (Figure 2) which is similar to that which we proposed in our discussion of the $(RSi)_2W_{11}SiO_{40}^{4-}$ series.[1] The structure in Figure 2 requires equivalence of the cyclopentadienyl groups, which is consistent with the observed NMR spectra.

If $(OC)_4CoGeCl_3$[13] is reacted with $W_{11}SiO_{39}^{8-}$, the anion $[(OC)_4CoGe]_2W_{11}SiO_{40}^{4-}$ is apparently formed initially. This, however, undergoes rapid disproportionation of the type discussed above for $(OC)_4CoSnW_{11}SiO_{39}^{5-}$. In the case of $[(OC)_4CoGe]_2W_{11}SiO_{40}^{4-}$, the presence of two $(OC)_4CoGe$ moieties gives rise to a polymer as the disproportionation product:

$$(OC)_4CoGeCl_3 + W_{11}SiO_{39}^{8-} \rightarrow [[(OC)_4CoGe]_2W_{11}SiO_{40}^{4-}] \rightarrow [(OC)_3CoGe_2W_{11}SiO_{40}^{5-}]_n$$

The presumed structure of the polymer is shown in Figure 3. Light-scattering molecular-weight determinations on the trimethylammonium salt in dimethyl sulfoxide gave an approximate molecular weight of one million. The infrared spectrum was consistent with a t-$L_2CO(CO)_3$ configuration as discussed above for $(OC)_3Co(SnW_{11}PO_{39})_2^{9-}$ and was strikingly similar to that of $W_{12}SiO_{40}^{4-}$ in the 1000-700-cm^{-1} region.

Further physical and chemical characterization of the species described above and extensions of this chemistry to new systems are in progress.

References and Notes

(1) W. H. Knoth, *J. Am. Chem. Soc.*, **101**, 759 (1979).
(2) C. M. Tourné, G. F. Tourné, S. A. Malik, and T. J. R. Weakley, *J. Inorg. Nucl. Chem.*, **32**, 3875 (1970).
(3) M. J. Mays and S. M. Pearson, *J. Chem. Soc. A*, 136 (1968).
(4) J. F. Young, R. O. Gilleard, and G. Wilkinson, *J. Chem. Soc.*, 5476 (1964).
(5) R. C. Taylor, J. F. Young, and G. Wilkinson, *Inorg. Chem.*, **5**, 20 (1966).
(6) Typical analyses follow. Calcd (found) for $[(CH_3)_3NH]_4C_5H_5Fe(CO)_2SnW_{11}PO_{39}\cdot 3H_2O$: C, 6.98 (7.05); H, 1.57 (1.51); N, 1.71 (1.74); Fe, 1.71 (1.76); Sn, 3.63 (3.66); W, 61.89 (61.95); H_2O, 1.65 (1.89); CH_3:C_5H_5 proton ratio, 7.2:1 (6.9:1). Calcd (found) for $[(CH_3)_3NH]_{11}(OC)_3Co(SnW_{11}SiO_{39})_2\cdot 4H_2O$: C, 6.69 (6.74); H, 1.86 (1.83); N, 2.38 (2.30); Co, 0.91 (0.86); Si, 0.87 (0.86); Sn, 3.67 (3.47); W, 62.57 (62.62); H_2O, 1.11 (1.24). Calcd (found) for $K_5(OC)_3Co(SnW_{11}PO_{39})Sn(OK)_3\cdot 12H_2O$: C, 0.99 (0.78); H, 0.67 (0.46); Co, 1.62 (1.57); K, 8.60 (8.49); Sn, 6.53 (6.24); W, 55.64 (55.72); H_2O, 5.94 (5.73). Calcd (found) for $[(CH_3)_3NH]_4[C_5H_5Fe(CO)_2Ge]_2W_{11}SiO_{40}$: C, 9.10 (9.06); H, 1.46 (1.53); N, 1.63 (1.61); O, 20.52 (20.51); Fe, 3.24 (3.30); Ge, 4.23 (3.84); Si, 0.82 (1.02); H_2O, 0.0 (0.16); CH_3:C_5H_5 proton ratio, 3.6:1 (3.5:1). Calcd (found) for $\{[(CH_3)_3NH]_5(OC)_3CoGe_2W_{11}SiO_{40}\}_x$: C, 6.59 (6.37); H, 1.53 (1.63); N, 2.13 (2.24); O, 20.98 (20.76); Co, 1.80 (1.92); Ge, 4.42 (4.03); Si, 0.86 (0.93).
(7) D. J. Patmore and W. A. Graham, *Inorg. Chem.*, **7**, 771 (1968).
(8) A. Komura, M. Hayashi, and H. Imanaga, *Bull Chem. Soc. Jpn.*, **49**, 87 (1976).
(9) A. R. Casey and A. R. Manning, *Chem. Commun.*, 674 (1970).
(10) A. R. Casey and A. R. Manning, *J. Chem. Soc. A*, 256 (1971).
(11) R. Mason, G. B. Robertson, P. O. Whimp, and D. A. White, *Chem. Commun.*, 1655 (1968).
(12) R. C. Edmondson, E. Eisner, M. I. Newlands, and L. K. Thompson, *J. Organomet. Chem.*, **35**, 119 (1972).
(13) D. J. Patmore and W. A. G. Graham, *Inorg. Chem.*, **6**, 981 (1967).
(14) T. J. R. Weakley, *Struct. Bonding (Berlin)*, **18**, 137–143 (1974).

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Spectrophotometric Determination of the Proton-Dependent Stability Constant of Ferric Enterobactin

Sir:

Although iron is an abundant element in the earth's crust, the insolubility of ferric ion at physiological pH ($K_{sp} \simeq 10^{-39}$) makes it very difficult for aerobic organisms to acquire the relatively large amounts of iron which they need for growth. In response to this environmental stress, microorganisms have evolved low-molecular-weight chelating agents called siderophores, which solubilize ferric ion and facilitate its transport into the cell.[1] Enteric bacteria such as *Escherichia coli* as well as *Salmonella typhimurium* produce the siderophore enterobactin (H_6ent), shown in Figure 1. It has been known for some time that this compound forms very stable complexes with iron(III). This is indicated by the low pH at which coordination of iron is completed,[2,3] the very low reduction potential of the ferric complex,[4] and the inability of EDTA to remove detectable amounts of iron from ferric enterobactin at pH 7.[5] Although enterobactin has been shown to form octahedral ferric complexes at neutral pH via coordination of the six catecholate oxygens,[6,7] the actual magnitude of the formation constant of ferric enterobactin has never been accurately determined. This datum is of particular importance in relation to current efforts to design specific iron chelators for the iron overload associated with the treatment of β-thalassemia and related human anemias.[8]

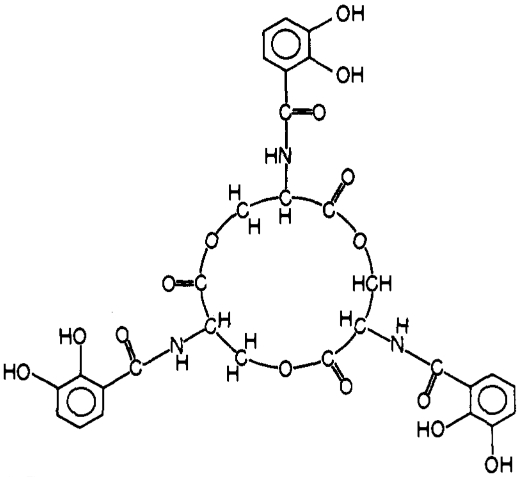

Figure 1. Structural formula of enterobactin.

We report here that the proton-dependent stability constant of ferric enterobactin has been determined spectrophotometrically by competition with EDTA. To do so, it was necessary to take advantage of the sixth-order hydrogen ion dependence of the reaction

$$Fe^{3+} + H_6ent \rightleftharpoons Fe(ent)^{3-} + 6H^+ \qquad (1)$$

Even though EDTA is totally incapable of competing with enterobactin for iron at pH 7, a measurable distribution of the metal between these two ligands can be obtained using less than a tenfold excess of EDTA at pH 5. Because the aqueous solubility of enterobactin itself is only approximately 0.1 mM,[9] very dilute solutions (0.08 mM) were used in the competition experiments; the enterobactin was added as 0.3 mL of a concentrated methanol solution, to give a final solution which was 3% methanol by volume.

The stability constant K^* has been determined, where K^* is defined as[10]

$$K^* = \frac{[Fe(ent)^{3-}][H^+]^6}{[Fe^{3+}][H_6ent]} = 10^{-9.7(3)} \qquad (2)$$

The ligand protonation constants of enterobactin are still unknown, but, if one assumes typical dihydroxybenzamide values of 8.4 and 12.1 for the two types of ligand -OH groups,[2] the proton-dependent constant, K^*, can be used to estimate the more standard formation constant:

$$K_f = \frac{[Fe(ent)^{3-}]}{[Fe^{3+}][ent^{6-}]} \simeq 10^{52} \qquad (3)$$

The visible spectrum of ferric enterobactin has also been recorded as a function of pH, as shown in Figure 2. These data have been used to determine the protonation constant, defined as