two equivalent rhodium atoms to be observed.[17] These data are consistent with a hexametal carbonyl cluster containing an interstitial boron for which two isomeric structures (Chart I) are possible. The spectroscopic observation of two hexametal products[13,14] demonstrates the production of both cis and trans isomers; however, the data does not definitively identify the most stable isomer.

To confirm the analysis and determine the structure of the most stable isomer,[14] a single-crystal X-ray diffraction study of the PPN (bistriphenylphosphineiminium) salt of the final product was carried out.[18] The structure is shown in Figure 2 and contains a distorted octahedral frame of two $Rh(CO)_2$ groups and four $Fe(CO)_3$ groups encapsulating a boron atom. The arrangement with Rh atoms in trans positions is the most stable structure. There are four short Rh–Fe bonds, av 2.730 (2) Å, and four longer Rh–Fe bonds, av 2.884 (2) Å. The longer bonds are bridged by semibridging CO groups: CO(13) and CO(15) to Rh(1) and CO(7) and CO(9) to Rh(2). The Fe–Fe distances, av 2.752 (2) Å, are normal and regular. The CO groups of each trans pair of iron atoms are eclipsed, while the rhodium pair is staggered. In the isoelectronic cluster, $[Fe_6(CO)_{16}C]^{2-}$, the Fe–Fe distances are in two groups, 2.66 (1)–2.74 (1) Å for unbridged bonds and 2.58 (1)–2.63 (1) Å for μ-CO bridged bonds;[15] this relationship is opposite to what we find for *trans*-$[Fe_4Rh_2(CO)_{16}B]^-$. Also in the all-iron analogue, the semibridging CO groups are much nearer to a symmetrical displacement. The metal–boron distances are av Rh–B, 2.03 (1) Å, and av Fe–B, 1.94 (1) Å.

The new boride, $[Fe_4Rh_2(CO)_{16}B]^-$, is directly related to the known closed carbide anion $[Fe_6(CO)_{16}C]^{2-}$ and thus constitutes another example of a metallaborane analogue of an organometallic species.[15,19] The boride is related in an isolobal sense to the carborane $C_2B_4H_6$ with *cis*-$[Fe_4Rh_2(CO)_{16}B]^-$ being analogous to 1,2-$C_2B_4H_6$ and *trans*-$[Fe_4Rh_2(CO)_{16}B]^-$ to 1,6-$C_2B_4H_6$. As the formation of *cis*-$[Fe_4Rh_2(CO)_{16}B]^-$ is rapid with respect to its isomerization to *trans*-$[Fe_4Rh_2(CO)_{16}B]^-$, we have been able to study the kinetics of the interconversion.[20] While a discussion of the results is not possible in this communication, a comparison of the isomerization rate with that of 1,2-$C_2B_4H_6$ is worth noting.[21,22] From the Arrhenius parameters for the isomerization of the boride and the stated conditions for the isomerization of the carborane,[21] we estimate that the transition-metal boride isomerizes 3×10^4 times faster than the carborane at 250 °C. The more facile rearrangement of the transition-metal system is expected. Note that if the formation of $[Fe_4Rh_2(CO)_{16}B]^-$ had been slow with respect to isomerization or if only crystalline products had been characterized, *trans*-$[Fe_4Rh_2(CO)_{16}B]^-$ would have been the sole product observed. Hence, in transition-metal cluster systems it is risky to base mechanistic conclusions on cluster product structure alone.[23]

(17) Assuming the relationship between J_{RhB} and J_{RhC} will be similar to that between J_{BH} and J_{CH} the observed J_{RhB} coupling constant is consistent with the range observed for J_{RhC}. Onak, T.; Leach, J. B.; Anderson, S.; Frisch, M. J. *J. Magn. Reson.* **1976**, *23*, 237. Brevard, C.; Granger, P. *Handbook of High Resolution Multinuclear NMR*; Wiley: New York, 1981; p 158.

(18) Crystal data: $C_{52}H_{30}BNO_{16}P_2Fe_4Rh_2$, monoclinic, $P2_1/c$, a = 11.554 (4) Å, b = 16.007 (6), c = 30.464 (13) Å, β = 97.11 (3)°, U = 5590 A^3, Z = 4, μ(Mo Kα) = 17.2 cm^{-1}, D(calcd) = 1.66 g cm^{-3}, T = 293 K, black specimen, 0.22 × 0.26 × 0.31 mm. Of 6442 data collected (Nicolet R3m, 4° ≤ 2θ ≤ 42°) and corrected for absorption, 6017 were independent, and 3846 were observed (5σF_o). Disorder in the Fe_4 plane of the anion exists as a low occupancy (~10%) alternative orientation for the four Fe atoms at a ~45° rotation about the Rh···Rh axis. Only the metal atom positions for the alternative position were incorporated in the disorder model. With all phenyl rings constrained to rigid, planar hexagons and all non-hydrogen atoms except for the minority Fe positions anisotropic: $R(F)$ = 4.28%, $R(wF)$ = 4.63%, GOF = 1.202, N_o/N_v = 6.2, Δ (ρ) = 0.39 e $Å^{-3}$.

(19) Grimes, R. N. *Acc. Chem. Res.* **1983**, *16*, 22. Kennedy, J. D. *Prog. Inorg. Chem.* **1984**, *32*, 519. Housecroft, C. E.; Fehlner, T. P. *Adv. Organomet. Chem.* **1982**, *21*, 57. Housecroft, C. E. *Polyhedron* **1987**, *6*, 1935. Fehlner, T. P. *N. J. Chem.* **1988**, *12*, 307.

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(21) Onak, T.; Drake, R. P.; Dunks, G. B. *Inorg. Chem.* **1964**, *3*, 1686.

(22) McKee, M. L. *J. Am. Chem. Soc.* **1988**, *110*, 5317.

(23) For a related example, see: Barreto, R. D.; Fehlner, T. P. *J. Am. Chem. Soc.* **1988**, *110*, 4471.

Acknowledgment. The support of the National Science Foundation is gratefully acknowledged.

Supplementary Material Available: Tables of atom coordinates, bond distances and angles, and anisotropic thermal parameters (12 pages); tables of observed and calculated structure factors (23 pages). Ordering information is given on any current masthead page.

Bio-Claisen Condensation Catalyzed by Thiolase from *Zoogloea ramigera*. Active Site Cysteine Residues

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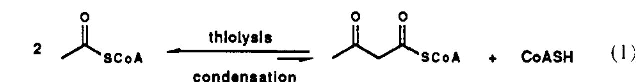
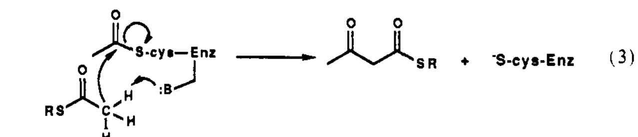
The enzyme acetoacetyl-CoA thiolase (acetyl-CoA: acetyl-CoA *C*-acetyl transferase EC 2.3.1.9) is a ubiquitous enzyme.[4] Our recent success in overproducing the biosynthetic thiolase from *Zoogloea ramigera* has facilitated the preliminary characterization and mechanistic investigation of this homotetramic enzyme, the first thiolase of established primary structure (subunit, 392 a.a.).[5] Thiolase catalyzes the condensation of two acetyl-CoA (AcSCoA) molecules to acetoacetyl-CoA (AcAcSCoA) (eq 1) via two steps. In the first half reaction (eq 2), the active site cysteine attacks AcSCoA to form an acetyl-S-enzyme intermediate and in the second half reaction (eq 3) this intermediate reacts with the anion





formed from the second acetyl-CoA molecule by enzymic deprotonation to complete the condensation. Herein we present evidence to demonstrate that the two cysteine residues, Cys-89 and -378, are essential for the first and second half reactions, respectively. The latter represents the catalytic base that has been sought after since Lynen's original proposal of the above mechanism in 1953.[6] A third cysteine residue (Cys-125) has also been found to be located close to Cys-89, and thus three of the total five cysteines (89, 125, 324, 378, 388) of the enzyme are in or near the active site.

Acetyl-S-enzyme Involving Cys-89. As described earlier, ^{14}C-iodoacetamide reacts with Cys-89 and inactivates the enzyme

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(4) Davis, J. T.; Moore, R. N.; Imperiali, B.; Pratt, A. J.; Kobayashi, K.; Masamune, S.; Sinskey, A. J.; Walsh, C. T.; Fukui, T.; Tomita, K. *J. Biol. Chem.* **1987**, *262*, 82 and references quoted therein.

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(6) (a) Lynen, F. *Federation Proc.* **1953**, *12*, 683. Also see: (b) Gehring, U.; Harris, J. I. *FEBS Lett.* **1968**, *1*, 150. (c) Gehring, U.; Riepertinger, C.; Lynen, F. *Eur. J. Biochem.* **1968**, *6*, 264. (d) Gilbert, H. F. *Biochemistry* **1981**, *20*, 5643. (e) Gilbert, H. F.; Lennox, B. J.; Mossman, C. D.; Carle, W. C. *J. Biol. Chem.* **1981**, *256*, 7371.

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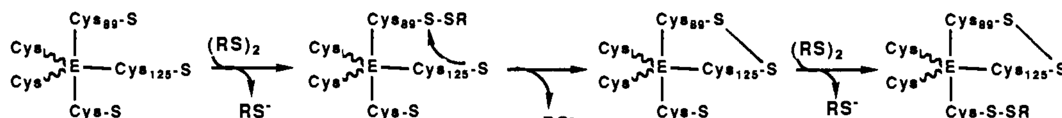
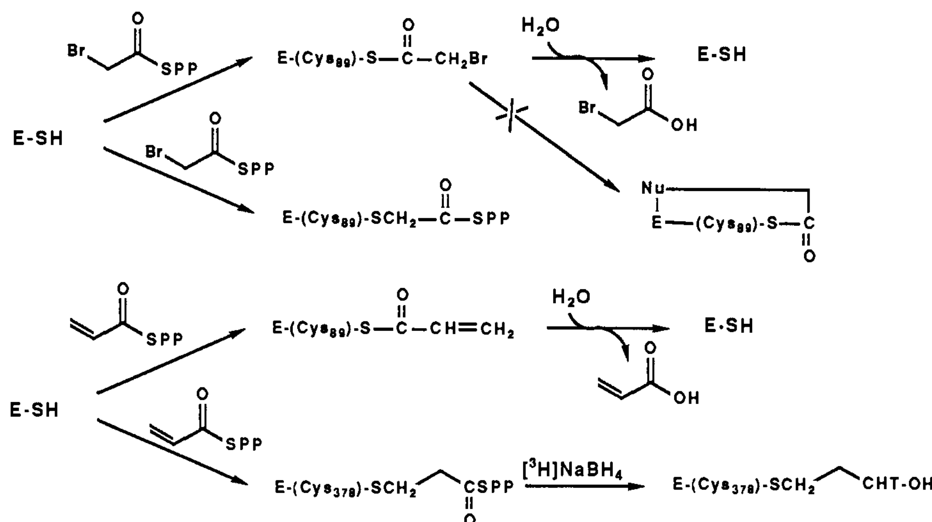
Scheme I



Scheme II



irreversibly, indicating that this residue is involved in the acetyl-S-enzyme formation.[4] This inference has now been verified. Incubation of thiolase with $^{14}CH_3COSCoA$, followed by acid precipitation, *denaturation*, tryptic digestion, radioactive peptide isolation, and sequencing, has identified the same residue as the active site nucleophile. Although the acetyl-S-enzyme is highly labile toward hydrolysis ($k \sim 5 \times 10^{-3}$ s^{-1}, at pH 7, 25 °C) compared with AcSCoA (2×10^{-7} s^{-1}), the C–C bond formation and thiol exchange reaction with Ac-S-enzyme proceed faster with rates equal to or greater than 71 and 810 s^{-1}, respectively.[7] The Cys-89 residue has been replaced with Ser, by site-directed mutagenesis following the standard procedure of Zoller and Smith.[8] The resulting Ser-89 mutant that has been overproduced and purified is shown by assay for enzyme activity to retain 1.5% of the k_{cat}/K_M of wildtype enzyme in the AcAcCoA thiolytic cleavage direction (reverse) and 0.045% of the k_{cat}/K_M in the forward Claisen condensation direction. This result is consistent with the view that a covalent Ac-O-enzyme is involved in the reactions of the mutant and, in fact, incubation with $^{14}CH_3COSCoA$ and tryptic digestion have provided a labeled Ser-89-containing peptide.

Intramolecular Disulfide Linkage between Cys-89 and Cys-125 (Scheme I). Native *Zoogloeal* thiolase reacts with excess 5,5′-dithiobis(2-nitrobenzoate) (DTNB) to liberate 3.08 equiv of thionitrobenzoate (TNB)/subunit, two cysteines reacting very rapidly ($k = 9.1$ min^{-1} M^{-1}, at pH 7.3, 25 °C) and the third 4–5 times more slowly. Isolation of the modified enzyme and addition of excess β-mercaptoethanol effects the release of 1 equiv of the TNB anion and restoration of enzyme activity. These results suggest that DTNB first reacts with the highly reactive Cys-89 (see above) to form the mixed disulfide which is converted to the intramolecular disulfide with a cysteine located proximate to Cys-89 and then another cysteine undergoes modification. The net result is the liberation of 3 equiv of TNB and formation of one intramolecular disulfide linkage.[9] The Ser-125 mutant enzyme prepared by site-directed mutagenesis liberates, upon incubation with excess DTNB, 2 equiv of TNB/subunit and incorporates 2 equiv of TNB/subunit into the mutant. This leads to the conclusion that the cysteine proximate to Cys-89 is Cys-125. The Ser-125 mutant is 47% as active toward thiolysis as the wildtype in terms of k_{cat}/K_M. Cys-125 is in or near the active site, but certainly not essential, and its exact function still remains unknown.

Active Site Catalytic Base Cys-378 (Scheme II). In our earlier search for the active site base in the second half reaction we used affinity labeled inactivators, such as bromoacetyl-S-pantetheine 11-pivalate (bromoacetyl-SPP) and its homologue.[5c] Each of these inactivators incorporates a SPP moiety, an effective CoA substitute, and also two electrophilic functionalities which should not only react with the highly reactive Cys-89 but may capture a "hidden" nucleophilic residue. As it turned out, *Zoogloeal* thiolase underwent two competitive reactions to provide acylated and alkylated enzyme. While the alkylation proceeded irreversibly, the acylated enzyme hydrolytically regenerated active enzyme for recycling and eventually all the thiolase was alkylated at *Cys-89*. The intramolecular alkylation which we had hoped for did not occur. Inactivation of enzyme with the new inactivator acryl-SPP[10] follows very similar pathways, again partitioning between acylation and Michael type alkylation. Unexpectedly, we discovered after NaB^3H_4 reduction of inactivated enzyme followed by tryptic digestion that the Michael reaction involved Cys-378 rather than Cys-89. The decreased energy requirement for the Michael reaction compared with the S_N2 type replacement (observed in earlier cases such as with bromoacetyl-SPP) and a favorable disposition of the acryl functionality for Cys-378 may account for this successful capture of a new residue. Interestingly, the classical suicide substrate 3-pentynoyl-SPP[11] has also been found to react with Cys-378 after its enzyme-catalyzed rearrangement to the 2,3-pentadienyl thiol ester. The region of primary sequence starting with Cys-378 is well conserved for all four thiolases whose structural genes have been sequenced.[12]

In order to assess if this nucleophile Cys-378 functions as a catalytic base we have replaced Cys with a Gly residue.[8] The resulting Gly-378 mutant enzyme exhibits virtually no catalytic activities, k_{cat}'s for both the forward and reverse directions being

(7) The rates given are the k_{cat} for the condensation reaction (71 s^{-1}) and for the thiolysis reaction (810 s^{-1}).

(8) Zoller, M.; Smith, M. *Methods Enzymol.* **1983**, *100*, 468.

(9) Cf. Izbicka-Dimitrijevic, E.; Gilbert, H. F. *Biochemistry* **1984**, *23*, 4218.

(10) For the synthesis of this inactivator, see supplementary material.

(11) Prepared by E. Differding, unpublished results.

(12) The three other thiolases are from *Alcaligenes eutrophus* [(a) Peoples, O. P.; Sinskey, A. J. *J. Biol. Chem.*, in press] and from rat mitochondria and peroxisome [(b) Arakawa, H.; Takiguchi, M.; Amaya, Y.; Nagata, S.; Hayashi, H.; Mori, M. *EMBO J.* **1987**, *6*, 1361. (c) Hijikata, M.; Ishii, N.; Kagamiyama, H.; Osumi, T.; Hashimoto, T. *J. Biol. Chem.* **1987**, *262*, 8151.]

less than 10^{-5} of those of the wildtype, respectively. Yet the mutant effects the first half reaction. Thus, the formation of the acetyl-S-enzyme mutant intermediate is evidenced from the incorporation of 0.82 equiv of ^{14}C from $^{14}CH_3COSCoA$ into the mutant. The acetyl incorporation, however, proceeds slowly, requiring 1 h incubation with $^{14}CH_3COSCoA$ for the mutant, as compared with less than 1 min for the wildtype. This is reflected in the rate of exchange of ^{32}P-CoASH with AcCoA by the acetyl-*S*-mutant enzyme. The observed *V*(exchange), 0.01 μM/(min·mg) (50 μM AcCoA, 50 μM CoASH), is compared with the *V*(exchange), 42 μM/(min·mg), under the same conditions for the exchange reaction with the wildtype.[13] These results are consistent with the view that the Cys-378 residue is involved in the proton abstraction and the reduced exchange rate observed is due at least in part to the decreased ionization of the Cys-89-SH to Cys-S$^-$ in the mutant. It was unexpected that the sulfuryl group might be responsible for the deprotonation.

Acknowledgment. We thank Dr. Edmond Differding for the synthesis of 3-pentynoyl-SPP and Dr. Friedrich Mayerl for the measurement of V_{max}(exchange) of the thiolase (wildtype) and preparation of [^{32}P]CoASH. This work was supported by a grant from the National Science Foundation (DMB-87-06273). S.F.W. is a SERC/NATO Postdoctoral Fellow.

Supplementary Material Available: Experimental procedures (including materials and methods) (5 pages). Ordering information is given on any current masthead page.

(13) Mayerl, F.; Walsh, C. T. unpublished results. V_{max}(exchange) with enzyme of 54 U/mg (forward reaction) is 86 μM/(min·mg).

Azophenolic Acerands: Amine-Selective Coloration and Crystal Structure of a Piperidinium Saltex†

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Received August 25, 1988

Studies on saltexation[1k] involving the Coulombic attractive force between oppositely charged hosts and guests will be expected to draw a new trend in molecular recognition, since this additional binding force will affect the stability and selectivity of the major complexes whose components, uncharged hosts and charged guests, are bound by ion-dipole interaction and/or hydrogen bonding. Indeed, such charge–charge interaction in saltexes[1] has been found to be favorable for lithium[2] and diamine[1k] selectivity of mono- and dibasic acerands, respectively. Azophenolic acerands **1**[2c,d] provide a good model to examine amine-selective saltexation because of their chromogenic property. Blue anionic ligands **2**$^-$ can be generated by dissociation (eq 1) or neutralization (eq 2) of yellow **1**. We report here the first systematic investigation of amine-selective coloration based on saltexation of **1**, a prototypical chromoacerand.[3]

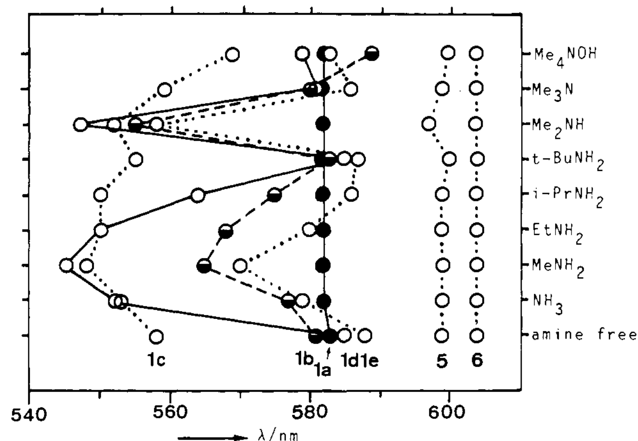
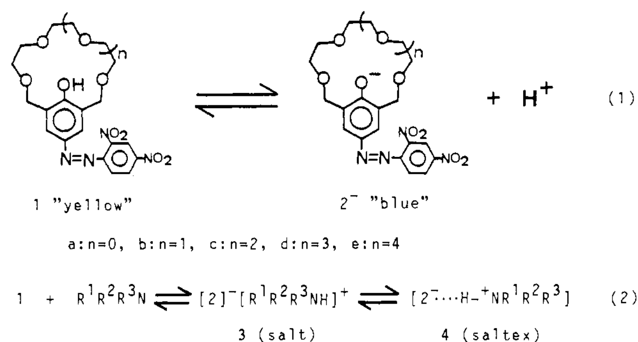


Figure 1. Absorption maxima of the colored salts of azophenols with amines.



For screening experiments, cycles **1** and open chain analogues **5** and **6**[4] were treated with ammonia and 11 simple alkylamines

† Dedicated to Professor Donald J. Cram, UCLA, on the occasion of his 70th birthday.

(1) We have suggested the term "saltex" in place of "salt complex" in ref 1k. For synthetic saltexes whose structures have been determined by X-ray crystallographic analysis: (a) Goldberg, I. *Acta Crystallogr., Sect. B* **1975**, *B31*, 2592–2600. (b) Newcomb, M.; Moore, S. S.; Cram, D. J. *J. Am. Chem. Soc.* **1977**, *99*, 6405–6410. (c) Behr, J. P.; Lehn, J. M.; Moras, D.; Thierry, J. C. *J. Am. Chem. Soc.* **1981**, *103*, 701–703. (d) Daly, J. J.; Schoenholzere, P.; Behr, J. P.; Lehn, J. M. *Helv. Chem. Acta* **1981**, *64*, 1444–1451. (e) Browne, C. M.; Ferguson, G.; McKervey, M. A.; Mulholland, D. L.; O'-Conner, T.; Parvez, M. *J. Am. Chem. Soc.* **1985**, *107*, 2703–2712. (f) Bradshaw, J. S.; Chamberlin, D. A.; Harrison, P. E.; Wilson, B. E.; Arena, G.; Dalley, N. K.; Lamb, J. D.; Izatt, R. M. *J. Org. Chem.* **1985**, *50*, 3065–3069. (g) Bradshaw, J. S.; Colter, M. L.; Nakatsuji, Y.; Spencer, N. O.; Brown, M. F.; Izatt, R. M.; Arena, G.; Tse, P.-K.; Wilson, B. E.; Lamb, J. D.; Dalley, N. K. *J. Org. Chem.* **1985**, *50*, 4865–4872. (h) Cheverier, B.; Moras, D.; Behr, J. P.; Lehn, J. M. *Acta Crystallogr.* **1987**, *C43*, 2134–2137. (i) Bradshaw, J. S.; McDaniel, C. W.; Skidmore, B. D.; Nielsen, R. B.; Wilson, B. E.; Dalley, N. K.; Izatt, R. M. *J. Heterocyclic Chem.* **1987**, *24*, 1085–1092. (j) McMurry, T. J.; Hosseini, M. W.; Garrett, T. M.; Hahn, F. E.; Reyes, Z. E.; Raymond, K. N. *J. Am. Chem. Soc.* **1987**, *109*, 7196–7198. (k) Kaneda, T.; Ishizaki, Y.; Misumi, S.; Kai, Y.; Hirao, G.; Kasai, N. *J. Am. Chem. Soc.* **1988**, *110*, 2970–2972.

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