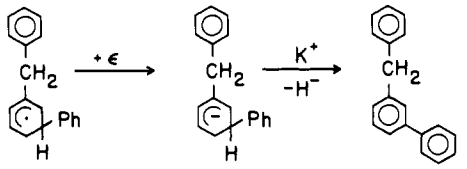
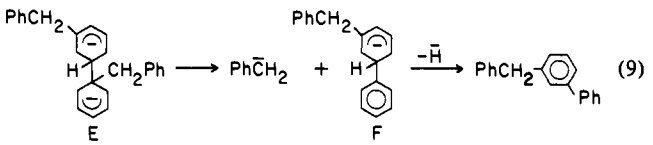
B and C, to yield the dianion E (eq 9), which then loses a benzyl



(8)

B + C ⇌

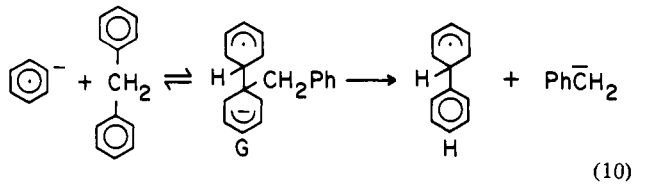


(9)

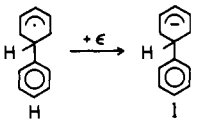
anion to produce the anion F; on loss of a hydride anion F yields 3-benzylbiphenyl. In both mechanisms the reaction is an ipso-aromatic substitution, and the leaving group is a benzyl anion.[9]

Gerson and Martin[10] showed that the diphenylmethane radical anion (−70 °C) possesses predominant and equal charges in the ortho and meta positions. If we assume a steric effect at the ortho position for attack by the radical anion, then the predominant meta attack, eq 1 and 6, appears reasonable.

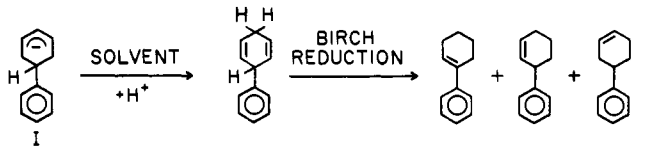
In order to determine whether we were dealing with an isolated case, we repeated the reaction of diphenylmethane with NaK in glyme–triglyme (0 °C, 3 h) in the presence of 1.1 molar equiv of benzene. We reasoned that the benzene radical anion should undergo the ipso reaction with diphenylmethane to yield toluene and biphenyl. After being quenched with water, the reaction mixture was subjected to GC (and GC/MS) analysis. The molar percentages of products for a typical run were toluene 56, biphenyl plus the hydrogenated biphenyls[11] 38, 3-benzylbiphenyl 2, and dihydro-3-benzylbiphenyl 6. Forty-eight percent of the diphenylmethane was recovered unchanged. The reaction between benzene and diphenylmethane can be formulated as an ipso nucleophilic substitution reaction which proceeds through the benzene radical anion as shown in eq 10–14. If the initial attack of the



(10)

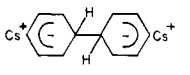


(11)



(12)

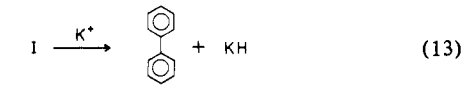
(9) Grovenstein and co-workers[2] formulate the dimerization of benzene in Cs K Na in THF at −70 °C as proceeding through the benzene radical anion to produce



which on quenching with water yields 1,1′,4,4′-tetrahydrobiphenyl.

(10) Gerson, F., Martin, W. B., Jr. *J. Am. Chem. Soc.* **1969**, *91*, 1883.

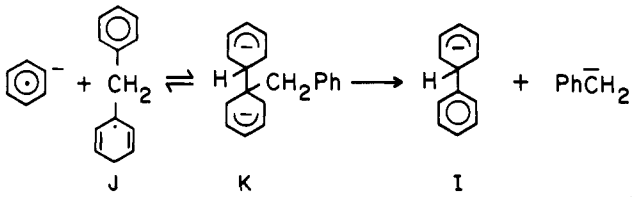
(11) A 168-mg sample of one peak (Dexsil 300 on 90/100 Anachrom Q) was isolated (preparative GC). ^1H and ^{13}C NMR spectra indicated the sample was a mixture of biphenyl and the phenylcyclohexenes in about equal amounts. Catalytic hydrogenation (Pd/C) afforded phenylcyclohexane (^{13}C NMR spectrum identical with authentic sample) and biphenyl. The GC/MS (OV101) column afforded better separation and indicated that a spectrum of hydrogenated biphenyls has been produced—in addition to biphenyl.



(13)

$$PhCH_2^- + H_2O \text{ (or solvent)} \rightarrow PhCH_3 + {}^-OS \quad (14)$$

benzene radical anion on diphenylmethane (eq 10) to form G is reversible,[12] then the driving force for the reaction could be the production of the stable benzyl anion and the phenylcyclohexadienyl radical[13] H by fragmentation of G. The Grovenstein mechanism[2,9] may also be written for the reaction of benzene with diphenylmethane and involves the formation of I through the intermediates J and K as shown in eq 15; I then follows the same



(15)

reaction scheme[14] (eq 12 and 13) previously postulated for the mechanism involving nucleophilic aromatic substitution.[15]

In summary, we have observed two cases of ipso-aromatic substitution. A choice between alternative mechanisms (e.g., eq 10 and 11 or eq 15) must await further evidence.

Acknowledgment. We thank Mr. L. L. Brown for determining the ^{13}C NMR spectra and Dr. E. W. Hagaman for their interpretation. C.J.C. acknowledges helpful discussions with Professor E. Grovenstein, Jr., Dr. M. Poutsma, and Professor F. Schell.

(12) The best models for the intermediates (or transition states) D and G are Meisenheimer complexes whose formation have been shown to be reversible under certain conditions. See: Servis, K. L. *J. Am. Chem. Soc.* **1965**, *87*, 5495; **1967**, *89*, 1508. Also, see a series of papers by Bernasconi and co-workers: Bernasconi, C. F.; Gandler, J. R. *Ibid.* **1978**, *100*, 8117.

(13) DeTar, D. F. *J. Am. Chem. Soc.* **1967**, *89*, 4058.

(14) For examples of further reductions of initial Birch–Hückel reduction products, see: Schlenk, W.; Bergman, E. *Liebigs Ann. Chem.* **1928**, *463*, 90. Hückel, W.; Breitschneider, H. *Ibid.* **1939**, *540*, 157. Birch, A. J. *J. Chem. Soc.* **1944**, 430. Krauch, H.; Kunz, W. "Reaktionen der Organische Chemie", 4th ed.; Huthig Verlag: Heidelberg, 1969. Birch, A. J.; Subba Rao, G. *Adv. Org. Chem.* **1972**, *8*, 1. Hückel, W.; Vevera, E. *Chem. Ber.* **1956**, *89*, 2105. Hückel, W.; Cramer, R.; Laufer, S. *Liebigs Ann. Chem.* **1960**, *630*, 89.

(15) A referee points out that Young and Bauld (Young, J. D.; Bauld, N. L. *Tetrahedron Lett.* **1971**, 2251) found that the diphenylmethane radical anion ($DPM^{-}\cdot$) is considerably more stable than the benzene radical anion ($B^{-}\cdot$) and that the proposed reaction 10 thus conflicts with the Young and Bauld results. If, however, $DPM^{-}\cdot$ is more stable, then $B^{-}\cdot$ should be more reactive. Young and Bauld do not specify the temperature employed, and they point out that radical anions "are not generated in high concentrations when the crown ether is omitted". We did not, in the experiments reported here, use crown ether as a solvent. In the one case in which glyme and 18-crown-6 were employed, the reaction did not proceed. We observed, in addition, a dependence of product upon temperature in several of the NaK reductions between −40 and 0 °C.

Interaction of Pyruvate–Thiamin Diphosphate Adducts with Pyruvate Decarboxylase. Catalysis through "Closed" Transition States[1]

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The enzymic decarboxylation of pyruvate has been proposed to proceed via the enzyme-bound adduct of the substrate with the coenzyme thiamin diphosphate (TDP, **1**).[2-4] This adduct, α-

(1) Supported by grants from the Atkinson Charitable Foundation and the Natural Sciences and Engineering Research Council of Canada.

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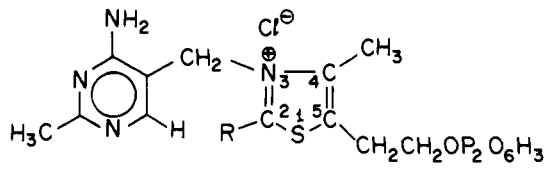

1, R = H

2, R = $-C(CO_2H)(OH)-CH_3$

3, R = $-C(CO_2C(CH_3)_3)(OH)-CH_3$

4, R = $-CH(OH)-CH_3$

5, R = $-C(CO_2C_2H_5)(OH)-CH_3$

lactylthiamin diphosphate (**2**), has eluded synthesis or isolation.[5] We have now developed syntheses of **2** and its esters. These compounds have been allowed to interact with wheat germ pyruvate decarboxylase apoenzyme. The results of these interactions provide kinetic information about the nature of the catalytic process.

Adducts of thiamin at C(2) have been prepared by ethoxide catalyzed condensation in ethanol with carbonyl group containing compounds.[9,10] However, the diphosphate moiety of TDP is cleaved to the monophosphate under those conditions. We have found that the use of sodium *tert*-butoxide as the catalyst in dimethylformamide solvent prevents cleavage of the diphosphate, with TDP solubilized as the bis(tetrabutylammonium) salt. We have thus prepared esters of **2**. Cleavage of an ester to give **2** must be accomplished under acidic conditions[11] that maintain the diphosphate. Concentrated aqueous acid (used for thiamin derivatives)[10,12] is therefore unsuitable. However the *tert*-butyl ester (**3**) can be cleaved in trifluoroacetic acid without perturbing the diphosphate.

The *tert*-butyl ester of pyruvate was prepared by reaction of pyruvic acid (in a pressure vessel) with excess isobutylene and a trace of H_2SO_4. Bis(tetrabutylammonium) thiamin diphosphate was prepared by titrating TDP with 10% aqueous tetrabutylammonium hydroxide (Eastman) and freeze-drying the resulting solution. To a solution of 10 mmol of this salt and 25 mmol of *tert*-butyl pyruvate in 40 mL of dry dimethylformamide at −5 °C was added 25 mmol of sodium *tert*-butoxide in 40 mL of dimethylformamide, over a 5-min period. Neutralization, after 30 min, with concentrated HCl (5.6 mL) and addition of ether (800 mL) gave complete precipitation of the product (**3**) (30% yield by ^{1}H NMR) and unreacted TDP. The precipitate was dissolved in methanol (700 mL) containing 10^{-3} M HCl. Addition of ether (1050 mL) gave a precipitate **3** and TDP (1:1) which was discarded. Addition of a further 2000 mL of ether gave a second precipitate, [**3**]:[TDP] = 3:1, which was collected by centrifugation and washed by resuspension in ether. One repetition of this extraction process yielded a sample (A) containing **3** and TDP in a 5:1 molar ratio and a small amount of NaCl. Traces of methanol and ether were removed by freeze-drying from water. No further purification (by chromatography)[13] was possible as

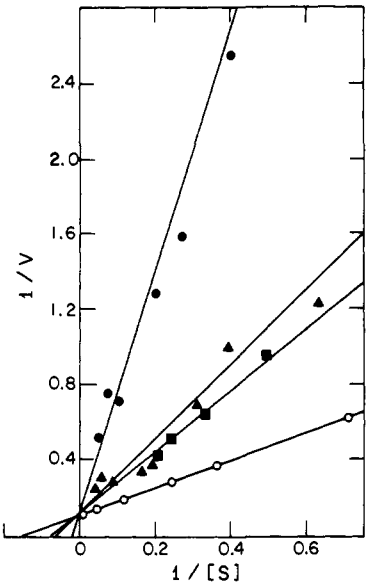

Figure 1. The activity of **1** (○), **3** (▲), **4** (■), and **5** (●) as cofactors with apopyruvate decarboxylase. The decrease in absorbance at 340 nm was measured for solutions (3 mL final volume) containing sodium phosphate buffer (20 mM, pH 6.0), sodium pyruvate (33 mM), NADH (3.3 μM), yeast alcohol dehydrogenase (0.55 mg), and the incubation mixture. The incubation mixtures (400 μL final volume) (maintained at 25 °C for 8 min) contained apopyruvate decarboxylase, $MgSO_4$ (3.3 mM), TDP or derivative, and sodium phosphate buffer (20 mM, pH 6.0). The K_m values (μM) are 6.4 (**1**), 12.9 (**4**), 15.9 (**3**), and 45 (**5**). Activity due to TDP present as an impurity in **3**, **4**, and **5** has been subtracted.

3 breaks down to TDP on the adsorbent. The presence of intact diphosphate (cleavage leads to monophosphate) is indicated by a broad resonance pattern at $\delta = -14.7$ in the proton-decoupled ^{31}P spectrum (relative to internal trimethyl phosphate in D_2O). The ^{1}H NMR spectrum of **3** includes a signal at δ 1.42 (s) due to the *tert*-butyl group. The resonance of the pyrimidine hydrogen of TDP occurs at δ 7.95 (s) which is well separated from that of **3** which occurs at δ 7.39 (s), allowing integration to be used as a measure of the relative amounts of these materials.

Product **2** was obtained when **3** (100 mg of A) was dissolved in trifluoroacetic acid (20 mL) at room temperature for 15 min. This solution was added dropwise to ether at −78 °C containing 8 mL of trifluoroacetic acid. After centrifugation of this solution, the solid pellet was dissolved in ice-cold water (30 mL) and freeze-dried at 0 °C to yield a white powder. The ^{31}P NMR revealed the presence of diphosphate only. ^{1}H NMR indicates complete absence of the *tert*-butyl group and that this sample (B) contains **2** and TDP (5:1). When allowed to decarboxylate, **2** gave a material whose ^{1}H NMR spectrum was identical with that of **4**. A sample of **4** produced in this manner gave the same enzymic activity as that observed with authentic **4** prepared unambiguously,[14] thus showing that **2** is indeed α-lactylthiamin diphosphate.

Enzyme studies were carried out on apopyruvate decarboxylase isolated from wheat germ.[16] If **2** binds to the apoenzyme, then, according to the Breslow mechanism,[3,4] it should be converted to TDP, acetaldehyde, carbon dioxide, and pyruvate. Since TDP dissociates slowly from the enzyme,[17] holoenzyme should be produced in a steady state with concentration proportional to

(2) Krampitz, L. O. "Thiamin Diphosphate and its Catalytic Functions"; Marcel Dekker: New York, 1970.
(3) Breslow, R. *Chem. Ind. (London)* **1957**, 893.
(4) Breslow, R.; McNelis, E. *J. Am. Chem. Soc.* **1959**, *81*, 3080.
(5) Reports of the isolation of **2** from the enzymic reaction[6-8] cannot be substantiated. Genuine samples of **2** (reported here) decompose to **4** under the reported conditions of isolation (hot methanol).
(6) Holzer, H.; Beaucamp, K. *Angew. Chem.* **1959**, *71*, 776.
(7) Holzer, H.; Beaucamp, K. *Biochim. Biophys. Acta* **1961**, *46*, 225.
(8) Holzer, H. *Ann. N.Y. Acad. Sci.* **1962**, *98*, 453.
(9) Risinger, G.; Gore, W. E.; Pulver, K. *Synthesis* **1974**, 659.
(10) Kluger, R.; Chin, J.; Smyth, T. *J. Am. Chem. Soc.* **1981**, *103*, 884.
(11) **2** is labile with respect to loss of CO_2 when the carbonyl group is ionized. (For this reason pyruvate itself cannot be used in the condensation step.)
(12) Crosby, J.; Stone, R.; Lienhard, G. E. *J. Am. Chem. Soc.* **1970**, *92*, 2891.
(13) Deus, B.; Ullrich, J.; Holzer, H. *Methods Enzymol.* **1970**, *18A*, 259. We have also found this breakdown occurs when using reverse-phase high-performance LC.
(14) We prepared **4** as a pure compound by condensing acetaldehyde with TDP followed by chromatographic isolation.[13] We found racemic **4** to give the same V_{max} as TDP but a 2-fold higher K_m (see Figure 1). Krampitz[15] has also shown that ^{14}C-labeled **4** binds to wheat germ apopyruvate decarboxylase. Since the enzyme is specific for coenzyme diphosphates (Morey, A. V.; Juni, E. *J. Biol. Chem.* **1967**, *243*, 3009), this confirms that the sample of **2** which served as the precursor of **4** was the diphosphate.
(15) Krampitz, L. O.; Suzuki, I.; Gruell, G. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1961**, *20*, 974.
(16) Singer, T. P.; Pensky, J. *J. Biol. Chem.* **1952**, *196*, 375.
(17) See ref 2, pp 18–25.

pyruvate decarboxylase activity (using the standard assay).[18] Using a range of concentrations of **2** (from sample B) from 1 to 53 μM, we find no activity due to its presence, although enzyme-bound **2** is an obligatory intermediate in the catalytic cycle.

Although the apoenzyme does not appear to bind **2**, it is activated by the *tert*-butyl ester of **2** (**3**) and the ethyl ester of **2** (**5**). These results are summarized in Figure 1. Presumably **3** and **5** are converted to the respective pyruvate esters and enzyme-bound TDP.[10] The different TDP derivatives give Lineweaver–Burk plots with different K_m values and V_{max} values that are identical within experimental error.

Scheme I shows a kinetic mechanism to account for the results in Figure 1. The species "X-TDP" are the active C(2) adducts **3**, **4**, and **5**. Assays of activity are done under conditions where a steady-state concentration of E·TDP has evolved.

Scheme I

$$\text{X—TDP} + \text{E} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{E·X—TDP}$$

$$\text{E·X—TDP} \xrightarrow{k_2} \text{E·TDP} + \text{X}$$

$$\text{E·TDP} \xrightarrow{k_3} \text{E} + \text{TDP}$$

Using steady-state equations for [E·TDP] and [E·X—TDP] and with $k_2 \gg k_3$ (since TDP dissociates very slowly from the holoenzyme), we obtain the Michaelis–Menten expression

$$1/v = (V_{max}/K_m)(1/s) + 1/V_{max}$$

where $K_m = k_1k_2/[k_3(k_{-1} + k_2)]$, v is a measure of [E·TDP], and V_{max} is a measure of $[E_{tot}]$. It is likely that $k_2 \gg k_{-1}$, making $K_m = k_1/k_3$. Since k_3 is independent of X, differences in K_m are due to differences in k_1.

Our observation of an apparent lack of affinity of the apoenzyme for **2** indicates either that the enzyme form which binds **2** during catalysis is not in equilibrium with the apoenzyme to a significant extent or there is a large kinetic barrier to its association with **2**. We find that the rate of association of **2** and apoenzyme must be slower than the rate of the nonenzymic decarboxylation of **2** ($t_{1/2}$ = 5 h,[10] yielding **4** which activates the enzyme) under our conditions.

Physical studies indicate that the TDP binding site of related enzymes is hydrophobic.[19-21] The order of K_m values for **3** and **5** suggests this also applies to wheat germ pyruvate decarboxylase apoenzyme. That is, large alkyl groups on the ester moiety promote association with the apoenzyme.

Lienhard has presented evidence, from model studies,[12] that suggests that the enzymic decarboxylation of **2** should occur most rapidly if its enzyme-binding site is hydrophobic. However, a polar site must be available initially to bind pyruvate and give **2**. This implies that the enzyme must assume at least two forms with different active-site polarities. We have suggested that the formation of **2** on an enzyme should be highly exergonic.[10] The energy that is thus made available could promote isomerization of the enzyme intermediate complex to the catalytically active form (for decarboxylation), placing **2** in a hydrophobic environment. Decarboxylation of enzyme-bound **2** would return the enzyme to another form which then catalyzes the release of acetaldehyde from **4**. This form is what binds **3**, **4**, and **5** and converts them to TDP. Thus, conclusions drawn from studies with **3** and **5** (and from other studies of apoenzymes that bind TDP)[19-21] should apply to the form of the enzyme that binds **4** (not **2**). Our results with **2**, however, suggest that in the normal catalytic cycle the pyruvate adduct is bound to a different, externally inaccessible, high-energy form of the enzyme which promotes decarboxylation of **2**.[22] It remains probable that hydrophobic catalysis is involved, but this has yet to be proven.

(18) Ullrich, J. *Methods Enzymol.* **1970**, *18A*, 109.
(19) Gutowski, J. A.; Lienhard, G. E. *J. Biol. Chem.* **1976**, *251*, 2863.
(20) Wittorf, J. H.; Gubler, C. J. *Eur. J. Biochem.* **1970**, *14*, 53.
(21) O'Brien, T. A.; Gennis, R. B. *J. Biol. Chem.* **1980**, *255*, 3302.
(22) The possibility of a situation in which a transition state would be bound to a "closed" form of an enzyme had been predicted by Wolfenden (Wolfenden, R. *Mol. Cell. Biochem.* **1974**, *3*, 207).

Isolation of a C,N-Bonded Formamido Complex and Its Isomerization to a C,O-Bonded Form on the Edge of a Triosmium Cluster. Crystal and Molecular Structure of a Urethane Derivative of the C,N-Bonded Formamido Complex

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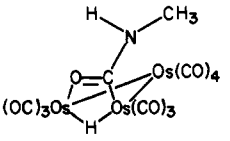
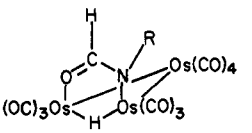
η^2-Bonded carbonyl groups either in mononuclear[1] or in polynuclear[2-4] systems are believed to be important in the homologation of carbon monoxide by metal catalysts. We have recently observed formation of a μ-η^2-carboxamido complex (**1**) in the treatment



1



2a, R = *p*-$CH_3C_6H_4$
b, R = Me

of $Os_3(CO)_{12}$ with primary aliphatic amines.[5,6] By contrast, an isomeric O,N-bonded formamido complex (**2a**) has been isolated as the principal product from the reaction of *p*-tolyl isocyanate with $H_2Os_3(CO)_{10}$.[7] We were interested in obtaining the methyl isocyanate complex (**2b**) to determine whether **1** and **2b** were thermally interconvertible and, if so, which would be the more stable.

The reaction of methyl isocyanate with $H_2Os_3(CO)_{10}$ at room temperature for 40 min in neat isocyanate was carried out under N_2 atmosphere by using Schlenk techniques. Solvent was removed from the mixture under vacuum. Hexane was then added to the dried powder to make a yellow solution which was subjected to column chromatography using a silica gel support. Starting with pure hexane and going in four stages to 80:20 hexane/dichloromethane, four bands were eluted.[8a] The first consists of a trace

(1) (a) Huffman, J. C.; Stone, J. G.; Krusell, W. C.; Caulton, K. G. *J. Am. Chem. Soc.* **1977**, *99*, 5829. (b) Manriquez, J. M.; McAlister, D. R.; Sanner, R. D.; Bercaw, J. E. *J. Am. Chem. Soc.* **1976**, *98*, 6733.
(2) (a) Parshall, G. W. "Homogeneous Catalysis"; J. Wiley: New York, 1980; p 77. (b) Masters, C.; Stone, F. G. A.; West, R. *Adv. Organomet. Chem.* **1979**, *17*, 61. (c) Cf. *Adv. Chem. Ser.* **1980**, *No. 178*, Kugler, E. L., Steffgen, F. W. Eds.
(3) (a) Wong, A.; Harris, M.; Atwood, J. D. *J. Am. Chem. Soc.* **1980**, *102*, 4530. (b) Demitras, G. C.; Muetterties, E. L. *Ibid.* **1977**, *99*, 2796. (c) Masters, C.; Van der Woude, C.; van Doorn, J. A. *Ibid.* **1979**, *101*, 1633. (d) Shore, L. I.; Schwartz, J. *J. Am. Chem. Soc.* **1977**, *99*, 5831.
(4) (a) Shriver, D. F.; Alich, A., Sr. *Coord. Chem. Rev.* **1972**, *8*, 15. (b) Kristoff, J. S.; Shriver, D. F. *Inorg. Chem.* **1974**, *13*, 499. (c) Butts, S. B.; Strauss, S. H.; Holt, E. M.; Stimson, R. E.; Alcock, N. W.; Shriver, D. F. *J. Am. Chem. Soc.* **1980**, *102*, 5093.
(5) (a) Kaesz, H. D.; Szostak, R.; Lin, Y. C.; Van Buskirk, G.; Humphries, A. P. "Abstracts of Papers", American Chemical Society/Chemical Society of Japan Chemical Congress, Honolulu, Hawaii, April 1979; American Chemical Society: Washington, DC; INOR 174. (b) A similar O,C-bonded complex was obtained from benzylamine and $Os_3(CO)_{12}$ in refluxing octane: Azam, K. A.; Yin, C. C.; Deeming, A. J. *J. Chem. Soc., Dalton Trans.* **1978**, 1201.
(6) The conventional vector between metal atoms of the double-bridged edge is omitted from our structure representations of trinuclear cluster complexes because we believe this better reflects the octahedral coordination around the metal atoms in question. Bonding interactions between metal atoms of a double-bridged edge are believed to take place through orbitals involving the bridging atoms. See: (a) Mason, R.; Mingos, D. W. P. *J. Organomet. Chem.* **1973**, *50*, 53. (b) Teo, B. K.; Hall, M. B.; Fenske, R. F.; Dahl, L. F. *Ibid.* **1974**, *70*, 413.
(7) Adams, R. D.; Golembeski, N. *J. Organomet. Chem.* **1979**, *171*, C21.
(8) (a) Since some of the products are related by proton shifts (see below), a reviewer asked whether the silica gel used in separation serves as a catalyst for interconversion. ^1H NMR spectrum of the crude reaction mixture demonstrates that the products and their distribution are unaffected by the chromatography. (b) For $t_{1/2}$ measurements the ratio of three different pairs of corresponding resonances in **3a** and **1**, namely, those of the methyl group, the bridging hydrogen atoms, and the OH and NH groups (see ref 15), were measured at the same time; all give consistent results.

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