Table II. Effect of Inhibitors on Firefly Luciferase Activity

Inhibitor	[Inhibitor]/[luciferase][a]	% activity of enzyme[b,f]
Control[c]	----	100
Ethyl 2-benzothiazolesulfonate (2)	30	48
	40	44
2 + luciferin (1)[d]	40	87
	200[e]	33
2-Benzothiazole sulfonic acid (3)	57	96
Methyl 4-nitrobenzenesulfonate	39	91
TPCK	40	14

[a] Recrystallized twice.[17] [b] From the initial flash of light emitted.[18] The light reaction was initiated by rapid injection of 0.2 ml of 0.02 *M* ATP into a solution of 2.0 ml of 0.05 *M* sodium phosphate buffer (pH 7.9), 0.1 ml of 0.1 *M* $MgSO_4$, 0.1 ml of ca. 1.2–5.8 × 10^{-3} *M* luciferin, and 0.015 ml of 1.6 × 10^{-5} *M* incubated luciferase solution (inhibitors added in THF, THF–*tert*-butyl alcohol (1 : 4), or methanol; concentration 0.5%). [c] Enzyme plus the appropriate organic solvent (0.5%). [d] Luciferin (1) was present in the luciferase–ester (2) solution ([1]/[2] = 2.9). [e] Not all initially soluble. [f] Percent activity of enzyme remaining after 1 hr incubation at 22°.

emission wavelength. Either this reagent attacks the first base or the enzyme becomes totally inactivated by attachment of a large group to the second base. The simple methylating agent methyl 4-nitrobenzenesulfonate[9] inhibits the enzyme, but slowly (Table II).

Our working hypothesis for these results is that the second basic center on the enzyme (Chart I, step e) displaces 2-benzothiazole sulfonate ion from the ethyl group of bound **2** faster than does the first basic center (Chart I, step b). Alkylation of the first center totally inhibits the enzyme, whereas alkylation of the second center modifies the enzyme so that red light is produced over the entire pH range from 6 to 8.6. A control experiment has shown that neither the observed shift of the emission wavelength to the red nor the irreversible inhibition of the enzyme are produced by 2-benzothiazole sulfonic acid (**3**) (Table II). Difference ultraviolet spectra of the inhibited enzyme and native luciferase do not show a benzothiazole chromophore indicating that compound **2** is not acting as a sulfonating agent. Work is in progress on the use of C-14 labeled **2** to identify the amino acid(s) being modified.

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References and Notes

(1) (a) T. A. Hopkins, H. H. Seliger, E. H. White, and M. W. Cass, *J. Am. Chem. Soc.*, **89**, 7148 (1967); (b) F. McCapra, Y. C. Chang, and V. P. Francois, *Chem. Comm.*, 22 (1968); (c) E. H. White, E. Rapaport, T. A. Hopkins, and H. H. Seliger, *J. Am. Chem. Soc.*, **91**, 2178 (1969); (d) E. H. White, E. Rapaport, H. H. Seliger, and T. A. Hopkins, *Bioorg. Chem.*, 1, 92 (1971); (e) N. Suzuki, M. Sato, K. Okada, and T. Goto, *Tetrahedron*, **28**, 4065 (1972); (f) N. Suzuki and T. Goto, *ibid.*, **28**, 4075 (1972); (g) E. H. White, J. D. Miano, and M. Umbreit, *J. Am. Chem. Soc.*, **97**, 198 (1975).

(2) The enzymic aspects of firefly bioluminescence were elucidated by W. D. McElroy et al. (M. DeLuca and W. D. McElroy, "Chemiluminescence and Bioluminescence," M. J. Cormier, D. M. Hercules, and J. Lee, Ed., Plenum Press, New York, N.Y., 1973, p 285).

(3) Alternatively, alteration of a distant site could cause a conformational change responsible for red light emission. The fact that inhibitor **2**, which leads to red light emission, is competitive with luciferin would appear to favor the two-base hypothesis, however,

(4) (a) H. H. Seliger and W. D. McElroy, *Radiat. Res., Suppl.*, **2**, 528 (1960); (b) H. H. Seliger and W. D. McElroyy, *Arch. Biochem. Biophys.*, **88**, 136 (1960); (c) H. H. Seliger and W. D. McElroy, *Proc. Nat. Acad. Sci. U.S.A.*, **52**, 75 (1964); (d) H. H. Seliger and R. A. Morton, "Photophysiology," Vol. IV, A. C. Giese, Ed., Academic Press, New York, N.Y., 1968, p 315; (e) other factors (e.g., temperature and substrate structure) can also lead to red light emission (E. H. White, H. Wörther, H. H. Seliger, and W. D. McElroy, *J. Am. Chem. Soc.*, **88**, 2015 (1966); footnote 2).

(5) (a) B. R. Baker, "Design of Active Site Directed Irreversible Enzyme Inhibitors," Wiley, New York, N.Y., 1967; (b) E. Shaw, "The Enzymes," 3rd ed, Vol. I, P. D. Boyer, Ed., Academic Press, New York, N.Y., 1970 pp 91–146; (c) L. A. Cohen, *ibid.*, pp 147–211; (d) E. Shaw, *Physiol. Rev.*, **50**, 244 (1970); (e) general chemical modification of enzymes is discussed in "Methods in Enzymology," Vol. XXV (Part B), C. H. W. Hirs and S. N. Timasheff, Ed., Academic Press, New York, N.Y., 1972, pp 387–651; (f) E. Shaw, *ibid.*, pp 655–660; (g) F. C. Hartman, *ibid.*, pp 661–671.

(6) (a) Reference 5b, p 142; (b) see also ref 5f.

(7) B. H. Havsteen, *Acta Chem. Scand.*, **27**, 3769 (1973).

(8) (a) In a few cases, the modified enzyme is active. B. H. Havsteen, ref 7, has reported that chymotrypsin modified by dimethyl malondilmidate retained its catalytic power. The substrate specificty of the enzyme shifted from amidase toward esterase activty after modification. (b) The exchange of metals in certain metalloenzymes leads to an active enzyme with altered specificity, B. L. Vallee and J. F. Riordan, *Annu. Rev. Biochem.*, **38**, 778 (1969); (c) J. E. Coleman and B. L. Vallee, *J. Biol. Chem.*, **235**, 390 (1960); (d) E. P. Kang, C. B. Storm, and F. W. Carson, *Biochem. Biophys. Res. Commun.*, **49**, 621 (1972).

(9) (a) Y. Nakagawa and M. L. Bender, *J. Am. Chem. Soc.*, **91**, 1566 (1969); (b) *Biochemistry*, **9**, 259 (1970).

(10) J. D. Rawn and G. E. Lienhard, *Biochem. Biophys. Res. Commun.*, **56**, 654 (1974).

(11) Examples of altered specificity are given in ref 5f and ref 8.

(12) Compound **2**, m.p. 71.5–72°, was synthesized from 2-benzothiazolesulfonyl chloride (R. O. Roblin and J. W. Clapp, *J. Am. Chem. Soc.*, **72**, 4890 (1950) and sodium ethoxide at 0°. Anal. Calcd for $C_9H_9NS_2O_3$: C, 44.43; H, 3.72. Found: C, 44.51; H, 3.64.

(13) (a) C. M. Suter, "The Organic Chemistry of Sulfur," Wiley, New York, N.Y., 1944; (b) J. Hine, "Physical Organic Chemistry," 2nd ed, McGraw-Hill, New York, N.Y., 1974.

(14) (a) R. T. Lee and W. D. McElroy, *Biochemistry*, **8**, 130 (1969); (b) J. L. Denburg, R. T. Lee, and W. D. McElroy, *Arch. Biochem. Biophys.*, **134**, 381 (1969); (c) R. T. Lee, J. L. Denburg, and W. D. McElroy, *ibid.*, **141**, 38 (1970).

(15) (a) G. Schoellmann and E. Shaw, *Biochem. Biophys. Res. Commun.*, **7**, 36 (1962); (b) G. Schoellmann and E. Shaw, *Biochemistry*, **2**, 252 (1963).

(16) Lee and McElroy, ref 14a, have shown that TPCK first binds reversibly with the luciferin binding sites of luciferase, and then reacts with sulfhydryl groups at or near these sites.

(17) A. A. Green and W. D. McElroy, *Biochim. Biophys. Acta*, **20**, 170 (1956).

(18) W. D. McElroy and H. H. Seliger, "Light and Life," Johns Hopkins University Press, Baltimore, Md., 1961, p 219 (for activity measurements, a phototube insensitive to red light was used).

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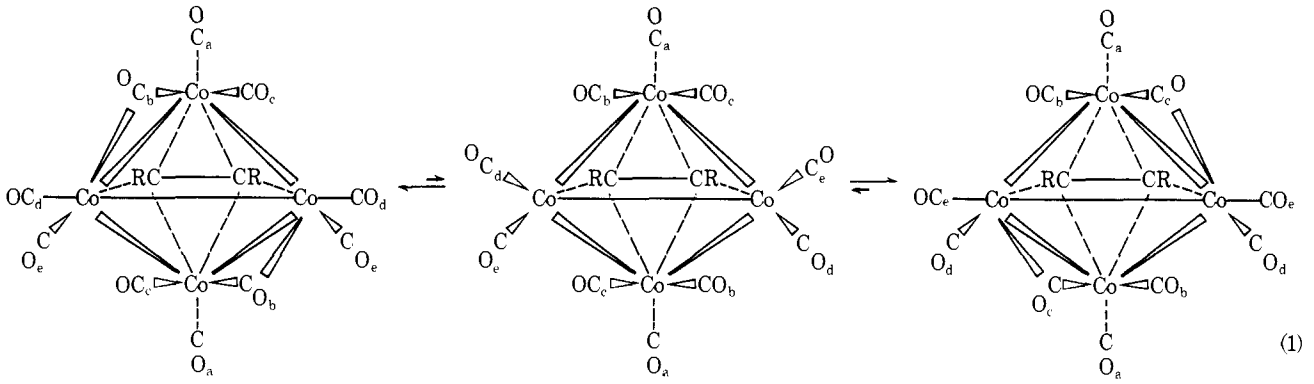
Tetracobalt Carbonyls in Solution

Sir:

We wish to report carbon-13 NMR studies of the two types of skeletal structures found for tetracobalt clusters. These observations demonstrate the dependence of carbonyl scrambling mechanisms upon the type of bridging carbonyl group.

The "butterfly" structures of the $RCCRCo_4(CO)_{10}$ series[1] presented the first skeletal type. These complexes have crystalline structures containing two asymmetrically bridging CO groups[2] (eq 1). The ^{13}C NMR[3] of (**1**), R = CO_2CH_3, and (**2**), R = Ph, were recorded (Table I) over a temperature range. At ca. −90° three resonances were observed. As the temperature is raised these signals broaden and eventually (−20°) coalesce to a broad resonance at ca. 203 ppm. Interaction with the cobalt nucleus (vide infra) maintains this broadening. It is apparent that at temperatures above ca. −70° CO scrambling over the entire Co_4 skeleton is occurring. In the region −104 to −70°, we believe the spectra are best interpreted in the following manner.

In the absence of tautomerism, five carbonyl resonances (one bridging and four terminal) are expected. Thus selective equilibration is occurring. The signal at lowest field is of correct intensity and shift to be due to the average of one bridging and one terminal environment, the remaining pair of signals resulting from averaged terminal carbonyl sites. Carbonyls CO_b (unsymmetric bridge) and CO_c equilibrate



1, R = CO_2CH_3
2, R = C_6H_5

(1)

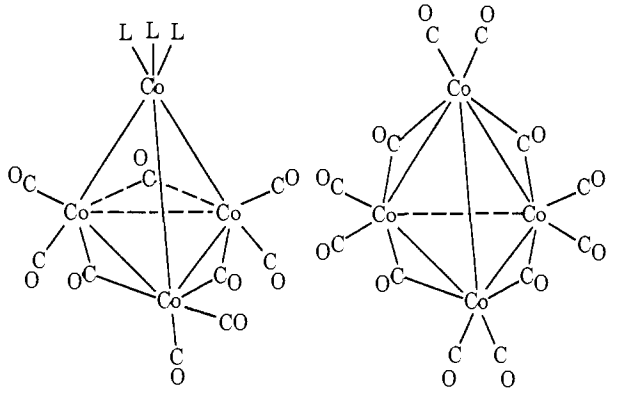
Table I. ^{13}C CO Chemical Shifts[a]

Complex	Carbonyl	Shift
1 −104°	Av	211.2 (4)
$CHFCl_2$	Av	198.2 (2)
	Av	191.3 (4)
2 −90°	Av	213.6 (4)
$CHFCl_2$	Av	198.7 (2)
	Av	193.5 (4)
3 −60°	B	243.1 (4)
CD_2Cl_2	T	195.9 (4)
	T	191.9 (4)
4 −70°	B	247.3 (3)
CD_2Cl_2	T	198.7 (3)
	T	180.9 (3)

[a] Shifts are measured downfield of TMS. B, T, and Av represent bridge, terminal, and averaged carbonyl environments, respectively. The relative signal intensities are displayed in parentheses.

by a rocking motion (eq 1). By the same motion environments CO_d and CO_e are also equivalenced. In this process the *bridging groups remain associated with the cobalt with which they have the shorter and probably stronger bond.* At higher temperatures this barrier is overcome and total equilibration is achieved.

A second skeletal type is exemplified by $Co_4(CO)_{12}$ (**3**) which possesses C_{3v} molecular symmetry in the crystal.[4]



C_{3v} **3**, L = CO
4, $L_3 = C_6H_5CH_3$

D_{2d} **3**

The infrared spectrum of (**3**) in solution has been variously reported to indicate structures of C_{3v}[5] and D_{2d}[6] symmetry. Subsequent ^{59}Co NMR measurements,[7,8] by indicating two cobalt environments populated in the ratio of 1:3, appear to support the C_{3v} structure. However, there appear to be significant discrepancies between the spectral data of the two reports.[9]

In the temperature range −100 to −60°, three ^{13}C resonances were observed for $Co_4(CO)_{12}$ corresponding to one bridging and two terminal environments (Table I). The three signals exhibited approximately equal intensities with or without the addition of the relaxing agent $Cr(acac)_3$. *If these intensities reflect the number of carbonyl groups in bridging and terminal environments*, then these results are only compatible with the D_{2d} structure; however, the factors which influence the relaxation times of ^{13}C nuclei in the presence of nuclei with high quadrupole moments are not well understood and, an intensity distortion is conceivable. It is relevant, however, that the spectra of two close relatives of **1** show no such intensity distortion. One of these cases, $Co_3Rh(CO)_{12}$, has already been reported.[10] $Co_4(CO)_9$(toluene) (**4**)[11] provides a second example. At −60° the ^{13}C NMR spectrum (Table I) of this cluster exhibits three signals of equal intensity (1 bridge and 2 terminal), fully consistent with its known structure. It must be concluded that the structure of $Co_4(CO)_{12}$ in solution remains uncertain.[12]

Spectra were also recorded over a range of temperatures. At −20° all signals were broadened, and at 10° total collapse was observed. Attempts to obtain a fast exchange spectrum were unsuccessful. Throughout the 40–70° region, no carbonyl resonance was observed and at higher temperatures decomposition occurred. Although the line widths of the resonances of carbon atoms coordinated to cobalt increase with temperature, this is insufficient to account for all the broadening observed.[14] The cluster therefore undergoes carbonyl scrambling, and our results are consistent with the type of process previously suggested for **3**[15] and observed for $Rh_4(CO)_{12}$.[13,16,17]

References and Notes

(1) U. Krüerke and W. Hübel, *Chem. Ber.*, **94**, 2829 (1961).
(2) L. F. Dahl and D. L. Smith, *J. Am. Chem. Soc.*, **84**, 2450 (1962).
(3) All samples were ca. 60–70% ^{13}C enriched and spectra were generally run in the presence of $Cr(acac)_3$.
(4) P. Corradini, *J. Chem. Phys.*, **31**, 1676 (1959); C. H. Wei and L. F. Dahl, *J. Am. Chem. Soc.*, **88**, 1821 (1966); F. A. Cotton, unpublished results.
(5) G. Bor, *Spectrochim. Acta*, **19**, 1209 (1963), and references therein.
(6) D. L. Smith, *J. Chem. Phys.*, **42**, 1460 (1965).
(7) E. A. C. Lucken, K. Noack, and D. F. Williams, *J. Chem. Soc. A*, 148 (1967).
(8) H. Haas and R. K. Sheline, *J. Inorg. Nucl. Chem.*, **29**, 693 (1967).
(9) The difference in the chemical shifts of the two signals is variously stated as 1147[7] and 133[8] ppm. Neither do the shifts against a standard agree.
(10) B. F. G. Johnson, J. Lewis, and T. W. Matheson, *J. Chem. Soc., Chem. Commun.*, 441 (1974).
(11) I. U. Khand, G. R. Knox, P. L. Pauson, and W. E. Watts, *J. Chem. Soc., Perkin Trans.*, 975 (1973).
(12) Both $Co_4(CO)_{12}$ and $Rh_4(CO)_{12}$, which has an established C_{3v} symmetry in solution,[13] exhibit similar ir spectra in solution, but since the former has three strong bands and the latter four, this similarity has probably been over-estimated. The infrared of (**3**) is unchanged between −80 and 25°.
(13) J. Evans, B. F. G. Johnson, J. Lewis, J. R. Norton, and F. A. Cotton, *J.*

Chem. Soc., Chem. Commun., 807 (1973).
(14) $Co_2(CO)_8$ exhibits one ^{13}C resonance between −100 and 20°. Linewidths of 15 Hz at −80° and 22 Hz at −30° were recorded. Only as room temperature was approached did broadening become marked (50 Hz at 20°).
(15) F. A. Cotton, *Inorg. Chem.*, **5**, 1083 (1966).
(16) F. A. Cotton, L. Kruczynski, B. L. Shapiro, and L. T. Johnson, *J. Am. Chem. Soc.*, **94**, 6191 (1972).
(17) The S.R.C. are acknowledged for financial support (to J.E. and T.W.M.).

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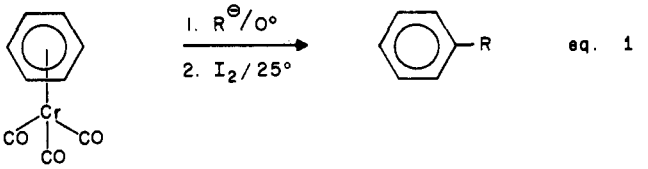
Reactions of Carbanions with π-Benzenechromium Tricarbonyl. Nucleophilic Aromatic Substitution for Hydride

Sir:

Coordination of a chromium tricarbonyl unit to an aromatic ring via π-bonding increases the reactivity of the ring toward attack by nucleophiles.[1-5] We have been interested in using the chromium tricarbonyl unit as an easily attached and removed activating group for nucleophilic aromatic substitution by carbanions.[6,7] Preliminary studies indicated that the substitution of tertiary carbanions for halide proceeded efficiently with π-halobenzenechromium tricarbonyl,[6] and minor products were observed in certain cases from formal substitution for hydride.[7,8] We now report conditions under which this side reaction becomes very efficient and promises a novel approach to coupling of carbon units with aromatic rings.

The method involves reaction of carbanions with π-benzenechromium tricarbonyl (**1**) to generate an intermediate



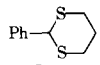
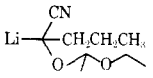
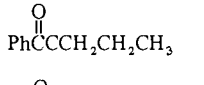
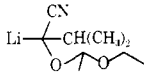
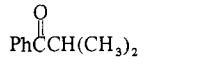
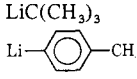
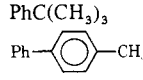
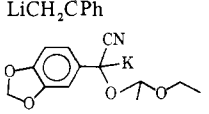
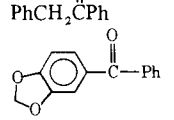
eq. 1

1

which can be converted to the free alkylbenzene by reaction with iodine (eq 1). Table I displays the results with a variety of carbanions. Certain of the carbanions were prepared by proton abstraction with lithium diisopropylamide (entries 1, 2, 4, 5, 8, 10-12, 16), potassium hexamethyldisilylazane (entry 9), potassium hydride (entries 15, 17), or *n*-butyllithium (entry 3), while others arose from reaction of the corresponding organic halide with the metal (Li or Mg; entries 7, 13, 14). *tert*-Butyllithium was obtained commercially. A solution of complex **1**[9] in tetrahydrofuran (THF) is added to the anion (1 mol equiv) in THF at −78°. The mixture is allowed to warm to higher temperature to allow interaction of the anion with the complex, and then excess iodine is added at −78°, as a solution in THF, followed by warming to 25°. The resulting chromium(III) salts are removed by aqueous washing, and the alkyl-arene is isolated from the organic solution. Most of the examples reported in Table I involve ca. 2 mmol scale; the following procedure at 25 mmol scale exemplifies potential preparative applications.

Lithium diisopropylamide[10] was generated from *n*-butyllithium (12.8 ml of a 1.95 *M* solution in hexane, 25.2 mmol) and diisopropylamine (3.84 ml, 27.5 mmol) in 50 ml of THF by mixing the reagents at −78° under argon and allowing the mixture to stir at 0° for 15 min. The pale yel-

Table I. Coupling of Carbanions with π-Benzenechromium Tricarbonyl

Entry	Carbanion	Product	Yield (%)[a]
1	$LiC(CH_3)_2CN$	$PhC(CH_3)_2CN$	94
2	$LiCH_2CN$	$PhCH_2CN$	68
3			93
4			90[b]
5			88[b]
6	$LiC(CH_3)_3$	$PhC(CH_3)_3$	97[c]
7			71
8	$LiC(CH_3)_2CO_2$-*t*-Bu	$PhC(CH_3)_2CO_2$-*t*-Bu	~10
9	$KC(CH_3)_2CO_2$-*t*-Bu	$PhC(CH_3)_2CO_2$-*t*-Bu	88
10	$LiC(CH_3)_2CO_2$-*t*-Bu	$PhC(CH_3)_2CO_2$-*t*-Bu	91[d]
11	$LiCH_2CO_2$-*t*-Bu	$PhCH_2CO_2$-*t*-Bu	87[d]
12	$LiCH(CH_3)CO_2$-*t*-Bu	$PhCH(CH_3)CO_2$-*t*-Bu	88[d]
13	$BrMgCH_2CH{=}CH_2$	$PhCH_2CH{=}CH_2$	<5
14	$ClMgC(CH_3)_3$	$PhC(CH_3)_3$	<5
15	$KCH_2CC(CH_3)_3$ (O)	$PhCH_2CC(CH_3)_3$ (O)	~20[e]
16	$LiCH_2CPh$ (O)	$PhCH_2CPh$ (O)	<5
17			<5[b,d]

[a] The yields are based on isolated material unless otherwise noted. The products were identified by comparison of spectral data and melting point (if solid) with published data or with data collected on a sample prepared by established procedures. [b] The final product was obtained by sequential acid and base hydrolysis according to the method of G. Stork and L. Maldonado, *J. Amer. Chem. Soc.*, 93, 5286 (1971). [c] This yield was determined by quantitative GLPC using an internal standard after careful concentration of the ether extract from the isolation procedure. [d] The medium is 1:1 THF:HMPA. [e] The medium is 1:5 THF:HMPA.