to occur intramolecularly. No incorporation of boron into II from the BCl_3 reagent occurs. This was shown from a study of the reaction of $^{10}BCl_3$ with I. A ^{11}B NMR signal of normal intensity was observed for II while no ^{11}B NMR signals were produced for either $Cl_3B_3O_3$ or its decomposition products. On a much slower time scale than the preparative time scale, $^{10}BCl_3$ reacts with II to enrich II with the ^{10}B isotope.

A possible pathway (Scheme I) for formation of II could occur through initial adduct formation between BCl_3 and the oxygen of the unique carbonyl of I,[6] resulting in reduction of the CO bond order thereby inducing the carbon to move to a μ_2 site[8] with the resulting exposed vertex boron accepting a chlorine atom. This halogen transfer would result in further reduction of CO bond order which could cause the carbon to move to a μ_3 position. With transfer of a second chlorine atom to boron, the BCl_2 group would move to a μ_2 site, and with the elimination of ClBO it could then move to a terminal site on the μ_3-carbon. The reaction of BH_3 with I is believed to give an initial complex, $(\mu\text{-H})_3(CO)_9Os_3(\mu_3\text{-BCOBH}_3)$. This reaction gives $(\mu\text{-H}_3)(CO)_9Os_3(\mu_3\text{-B-}\eta^2\text{-CH}_2)$ and $H_3B_3O_3$ with transfer of two H atoms of the coordinated BH_3 to the terminal carbon.[2]

For the synthesis of II, BCl_3 (0.132 mmol) was condensed into a CH_2Cl_2 solution (2 mL) of I (0.0605 g, 0.070 mmol). The solution was warmed to room temperature and stirred for 10 min. Volatiles were pumped from the reaction mixture, leaving behind a white residue consisting of a mixture of II and B_2O_3. Cluster II was extracted from this residue with CH_2Cl_2 which was then pumped away leaving a white solid, II, which was then washed with hexane. Yield of II: 95% (0.061 g, 0.067 mmol). Cluster II is stable under vacuum at room temperature but is very moisture-sensitive.

IR spectrum of II (ν_{CO}, CH_2Cl_2): 2020 (s (br)), 2089 (s) cm^{-1}. NMR spectra of II: ^1H NMR (CD_2Cl_2, 25 °C, $\delta(Si(CH_3)_4)$ 0.00) −19.43 (s) ppm; ^{11}B NMR (CD_2Cl_2, 25 °C, $\delta(BF_3\cdot OEt_2)$ 0.00) 57.4 (s, br) ppm; ^{13}C NMR (CD_2Cl_2, 25 °C, $\delta(Si(^{13}CH_3)_4)$ 0.00) 167.41 (3 C, s), 165.26 (6 C, d, J_{CH} = 11.3 Hz), 132.50 (1 C, s, br) ppm. Signals (3:6:1) of the carbon-13 NMR spectrum are consistent with the solid-state structure of II (Figure 1). Spin coupling (^{13}C–^1H) indicated in the signal of relative area 6 is consistent with hydrogen trans to carbon, implying the presence of Os–H–Os bridges.

The molecular structure of II (Figure 1)[10] has approximate C_s symmetry. The pseudo mirrow plane passes through B, C, Os(2), and the midpoint of the Os(1)–Os(3) bond. Positions of the hydrogen atoms, though not located, are implied as Os–H–Os bridges based on (1) Os–Os distances in the range observed for hydrogen-bridged osmium atoms[12,13] and (2) ^{13}C NMR spectrum discussed above. Structural parameters of the Os_3C core of the cluster (Figure 1) are consistent with those reported for other Os_3C units.[11,12] The B–C distance in II, 1.47 (2) Å, is comparable to the B–C distance in $(\mu\text{-H})_3(CO)_9Os_3(\mu_3\text{-BCO})$,[1] 1.469 (15) Å. These distances are significantly shorter than observed for a B—C single-bond distance, ca. 1.6 Å,[13,14] and can be attributed to some B=C character.

The μ_3-$CBCl_2$ unit of II is tilted only 15° from perpendicularity with respect to the Os_3 plane. The tilt angle is 60° for μ_3-BCH_2 in $(\mu\text{-H})_3(CO)_9Os_3(\mu_3\text{-B-}\eta^2\text{-CH}_2)$,[2,15] while tilt angles of 43° and 40° are observed for μ_3-CCH_2 in $(CO)_9Co_2Ru(\mu_3\text{-C-}\eta^2\text{-CRH})$ (R = *t*-Bu, Ph).[16]

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Registry No. I, 86727-98-2; II, 109801-61-8; BCl_3, 10294-34-5.

Supplementary Material Available: Listings of selected bond distances, selected bond angles, positional parameters, and anisotropic thermal parameters (5 pages); a listing of structure factor amplitudes (17 pages). Ordering information is given on any current masthead page.

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(16) Calculated from data in ref 4.

Transition-Metal Carbonyl Clusters as Novel Infrared Markers for Estradiol Receptor Site Detection

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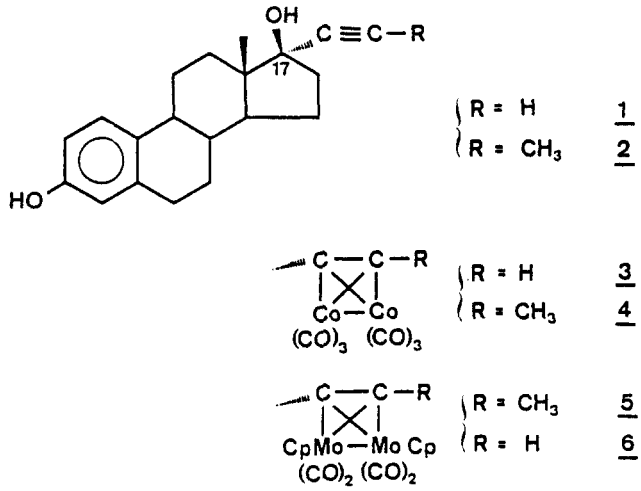
Summary: The first use of transition-metal carbonyl cluster fragments, such as $Co_2(CO)_6$ and $Cp_2Mo_2(CO)_4$ (Cp = η^5-C_5H_5), as infrared biological markers for the in vitro detection of femtomole concentrations of estradiol receptor sites in lamb uterine cytosol is described.

Despite the tremendous advances in the chemistry of transition-metal carbonyl clusters over the past 10 years and their considerable potential in fields such as hetero- and homogeneous catalysis,[1,2] and possibly even super-

conductivity,[3] there has been no report of their utility in a biochemical area. We present here the first use of this class of compounds as nonradiolabeled markers in estradiol receptor detection, a crucial step in early cancer diagnosis.[4] Problems with the use of radioisotopes in general, such as legal restrictions, health hazards, and radiolysis in solution, have prompted a worldwide search for new nonradiotracer procedures.[5] In an earlier paper,[6] we described some related work on estradiol derivatives labeled with mononuclear $Cr(CO)_3$ fragments. However, the photosensitivity of these organochromium steroids has made them difficult to use in routine clinical studies, and so we decided to see if the attachment of certain organometallic carbonyl clusters to the steroids might make them more convenient for biochemical purposes. Such polymetallic steroid complexes can indeed be synthesized[7] and are stable in air, light, and solution, even for the long periods of time necessary for biochemical incubations. These metal carbonyl derivatives exhibit intense ν(CO) bands in the IR at about 2000 cm^{-1}, a region where proteins do not absorb, and the basis of our new approach to hormone receptor assay is the detection of these IR bands at physiological levels (ca. 100 fmol/mg of protein) using modern FT-IR instrumentation.[8]

17β-Estradiol can be readily modified at several different positions, and we chose the 17α-position because of the known tolerance of the receptor sites to steroidal modification in this position.[9] 17α-Ethynylestradiol (**1**) and 17α-propynylestradiol (**2**)[10] were labeled with cobalt and molybdenum carbonyl clusters,[11] and then the products **3–6** were incubated in vitro with lamb uterine cytosol. The labeled complexes were produced in approximately 60–80% yields by reaction of the acetylenic steroids with either $Co_2(CO)_8$ or $Cp_2Mo_2(CO)_6$ (Cp = η^5-C_5H_5) in appropriate solvents.[12]



The IR-active ν(CO) bands of compound **6** in $CHCl_3$ are shown in Figure 1a; Figure 1b depicts the ν(CO) region of the difference spectrum obtained by subtraction of the spectrum of the precipitated protein from the in vitro incubation of compound **6** with lamb uterine cytosol at the same concentration level (1×10^{-8} M) as used in [6,7]^{3}H-17β-estradiol radiochemical assays[14] from that obtained under identical conditions except in the presence of excess diethylstilbestrol (DES) as well in order to eliminate the nonspecific binding.[15,16] The necessary subtraction scaling factor was determined from the subtraction of the ν(OH) harmonic at 6700 cm^{-1} which was used as the reference peak. Three of the four ν(CO) peaks expected for complex **6** are clearly discernible above the background at 1999 (m), 1920 (sh), and 1893 (s), cm^{-1} after the co-addition of 1000 scans while the band at 1825 cm^{-1} falls below the low-frequency cutoff of the detector used. These peaks provide for the first time a direct measure of the *specific binding* of an organometallic steroid (complex **6**) to the estradiol receptor sites, the concentration of which is estimated to be 300 fmol/mg of protein.[17] It should be mentioned that

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(7) Although different types of cobalt–steroid complexes were synthesized in the present work, the first ever report of such complexes appears to be by: Palyi, G.; Varadi, G.; Viziorosz, A.; Marko, L. *J. Organomet. Chem.* **1975**, *90*, 85.

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(10) More formal names for these acetylenic steroids are as follows: **1**, 17α-ethnynl-1,3,5(10)-estratriene-3,17β-diol; **2**, 17α-propynyl-1,3,5(10)-estratriene-3,17β-diol. Compound **1** is available commercially from Sigma, while **2** was prepared by the stereospecific reduction of estrone in THF solution with the Grignard reagent $CH_3C{\equiv}CMgBr$.

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(12) For example, a typical synthesis of **6** is as follows. Under an argon atmosphere, a solution of 0.5 g (1 mmol) of $Cp_2Mo_2(CO)_6$ in 20 mL of freshly distilled diglyme was heated at reflux for 3 h. The solvent was then evaporated off to afford $Cp_2Mo_2(CO)_4$. THF (30 mL) containing 0.34 g (1.15 mmol) of **1** was then added to this crude product. The mixture was heated at reflux for 4 h. After cooling and solvent removal, the crude product was chromatographed on silica gel by using ethyl acetate/hexane (1/3) as eluant. The dark red solid obtained (compound **6**) was recrystallized from ether/pentane (yield 60%; mp dec). Anal. Calcd for $C_{34}H_{34}O_6Mo_2$: C, 55.90; H, 4.69; Mo, 26.27. Found: C, 56.03; H, 4.89; Mo, 26.46. IR (in $CHCl_3$) ν(CO) 1985 (m), 1914 (sh), 1895 (s), 1825 (s) cm^{-1}. ^{1}H NMR (ppm, acetone-d_6): H-1, 7.10 d; H-2, 6.59 dd; H-4, 6.52 d; H-6α,β, 2.77 m; Me-13, 0.99 s; OH-3, 7.95 s; ≡CH, 7.03 s; Cp, 5.56 s. Mass spectroscopy: *m/e* 712 ($M^+ - H_2O$). $[\alpha]^{22}_D$: +202° ($CHCl_3$, *c* 4 g/L). The purity and authenticity of the order products were established by TLC, spectroscopic methods (^{13}C and ^{1}H NMR, FT-IR, mass), and elemental analysis. The relative binding affinity (RBA) values are as follows: **1**, 70; **2**, 44; **3**, 5; **4**, 12; **5**, 13; **6**, 16%. The procedure used to determine the binding affinities of such modified estrogens has been given previously,[6,13] except that the incubation time for compounds **2**, **4**, and **5** was 17 h rather than 3 h.

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(15) Hochberg, R. B. *Science (Washington, D.C.)* **1979**, *205*, 1138.

(16) The IR spectra were recorded on a Bomem Model DA3.02 spectrometer equipped with a liquid-nitrogen cooled In/Sb detector, a CaF_2 beamsplitter, and an evacuable sample chamber. The protein samples were pressed into 3-mm, self-supporting wafers weighing approximately 1–1.5 mg (which corresponds to ca. 0.5 mL of original cytosol). The protein wafers containing the organometallic moiety could be handled in the presence of air and light for up to 1 month without any apparent decomposition.

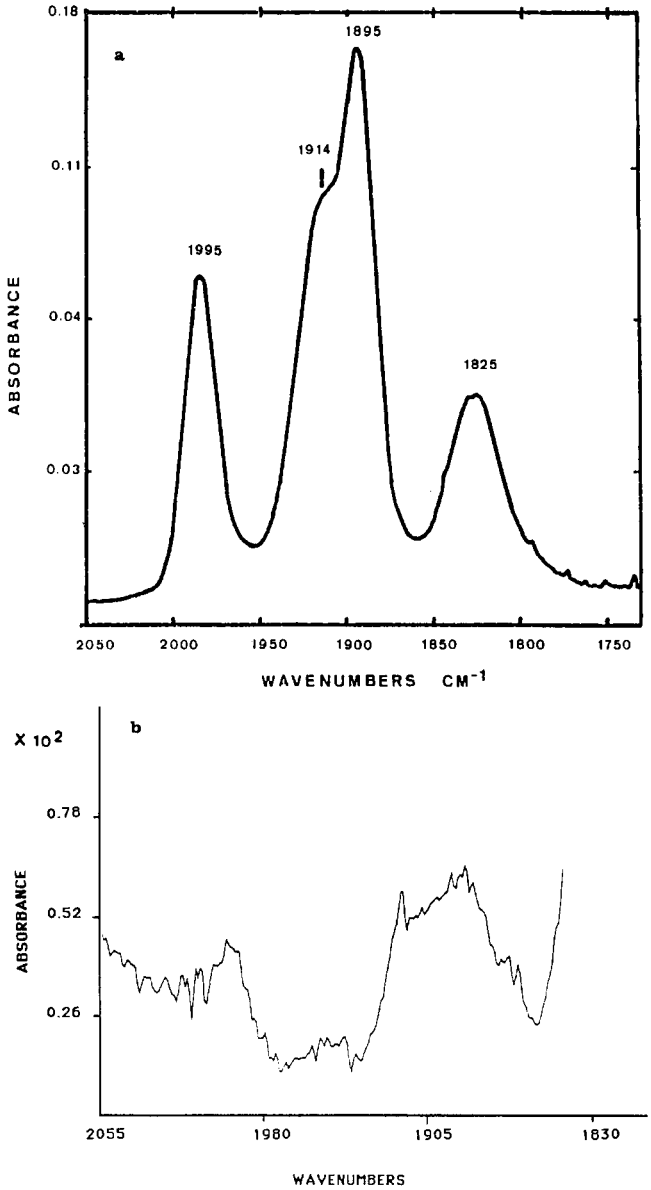

Figure 1. (a) FT-IR spectrum of compound **6** in $CHCl_3$ (30 scans at 2 cm^{-1} resolution). (b) FT-IR difference spectrum ν(CO) region obtained by subtracting the spectrum of precipitated protein, following incubation with compound **6** (1×10^{-8} M) in the presence of diethylstilbestrol (DES, 1×10^{-3} M), from that of precipitated protein incubated with compound **6** (1×10^{-8} M) in the absence of DES.

the choice of IR detector for these extremely low concentration measurements is a critical factor in detecting the ν(CO) peaks of the organometallic carbonyl cluster labels. We were unsuccessful by using the more conventional triglycine sulfate (TGS) and liquid-nitrogen-cooled mercury–cadmium–telluride (MCT) detectors and had to resort to the more sensitive liquid-nitrogen-cooled indium–antimonide (In/Sb) detector. Attempts to detect specific binding of a cobalt cluster labeled steroid through the agency of a doubly labeled species by exchange of some of the CO groups with ^{14}CO (specific activity = 50 mCi/mmol) failed essentially because the level of precision for ^{14}C detection proved to be insufficient.

The use of organometallic carbonyl cluster complexes as IR markers for biological systems augers well for the future since (a) the binding affinities when these types of molecules are attached to hormonal steroids are reasonably comparable to those of the free steroids themselves, (b) the labeled steroids are sufficiently soluble and stable in biological media for long periods of time, and (c) their ν(CO) peaks are strong enough to be detected by commercially available FT-IR spectrometers at about the femtomole concentration levels associated with hormone receptor sites by virtue of the fortuitous IR spectral window available in the case of proteins. Preliminary work on extending the applications of IR markers to other biological areas, e.g., other hormone receptors, mycotoxins, peptides and drugs, has already begun.

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Registry No. **1**, 57-63-6; **2**, 101915-79-1; **3**, 93122-00-0; **4**, 109282-00-0; **5**, 109282-01-1; **6**, 109306-28-7; e, 50-28-2.

(17) The specific-bound fractions were determined in parallel experiments by incubation of lamb uterine cytosol at 0 °C for 3 h with [6,7-^{3}H]-17β-estradiol (2×10^{-9} M; specific activity = 52 Ci/mmol).

Bimetallic μ-Malonyl Compounds. Synthesis, Characterization, and Reactivity of $(\eta^5\text{-}C_5Me_5)Re(NO)(PPh_3)(\mu\text{-}\eta^1,\eta^2\text{-}COCH_2CO)M(CO)_4$ (M = Re, Mn)

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Summary: Rhenaenolate, $(\eta^5\text{-}C_5Me_5)Re(NO)(PPh_3)(COCH_2Li)$, generated in situ from $(\eta^5\text{-}C_5Me_5)Re(NO)(PPh_3)(COCH_3)$ and *n*-BuLi, reacts with $M(CO)_5(OSO_2CF_3)$ (M = Re, Mn) to give the first μ-malonyl complexes $(\eta^5\text{-}C_5Me_5)Re(NO)(PPh_3)(\mu\text{-}\eta^1,\eta^2\text{-}COCH_2CO)M(CO)_4$ (**1-Re**, M = Re; **1-Mn**, M = Mn). The enolate anion of **1-Re** undergoes both diastereoselective alkylation and regioselective silylation chemistry to give **3** and **4**, respectively.

The role of acyl species in metal-catalyzed CO chemistry has for many years focused attention on the synthesis and properties of transition-metal acyl complexes. In view of the central place that carbonyl compounds claim in organic chemistry, a significant recent development is the increased utilization of acyl complexes for organic synthesis. A number of groups have now employed transition-metal enolate anions, derived by deprotonation of acyl complexes, as reagents for stereoselective carbon–carbon bond formation.[1] In contrast to simple metal acyl species, tran-

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