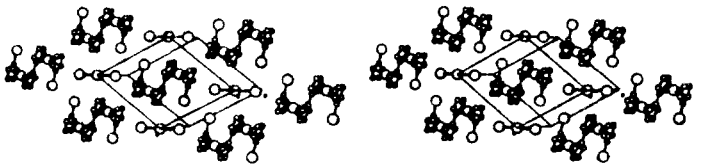

Figure 1. Stereoview showing the arrangement of 1',6'-diiodobiferrocenium cations and triiodide anions on the mirror plane of the unit cell. The separation between the iodocyclopentadiene iodine atom and the I_3^- ion is 3.98 Å, well within twice the van der Waals radius of iodine.

transfer. Within this series **1**[5,6] and **4**[7] are valence localized at 300 K on the Mössbauer time scale, **2**[6] and **3**[7] are valence delocalized from 300 to 4.2 K, and **5**,[8] **6**,[8] **7**,[9] and **8**[9] all show a temperature dependence such that they are localized below 200 K but become delocalized above 290 K. Structural characterization of **6** has shown that the cations and anions exist in segregated "slipped stacks" in the solid state.[10] It is likely that compounds **5** and **7** have similar structures. Compound **2** has been characterized structurally at 295 K and been found to have quite a different crystal structure.[11] The 1',6'-diiodobiferrocenium cation is centered about a site of crystallographically imposed $2/M$ symmetry in the unit cell and has the trans conformation found also for compound **6**. This symmetry requires that both iron atoms reside at environmentally equivalent positions in the unit cell. Figure 1 shows the alignment of the complex cations and I_3 anions on the crystallographic mirror plane. Close association between cations and anions was also found in the crystal structure of **6**. It is our conviction that rapid intramolecular electron transfer in **2** is supported by the symmetrical solid-state environment of both halves of the cation. Electron transfer in **2** occurs more rapidly than the Mössbauer and EPR time scales (rate $> 10^{10}$ s^{-1}) at 4.2 K, however, examination of the KBr-pellet IR spectrum of **2** and all the other salts listed above has shown that the cation is localized on the IR time scale (rate $<10^{13}$ s^{-1}).

All of the mixed-valence cations, 1-8, probably have the same trans conformation with a planar fulvalenide ligand. The magnitude of electronic coupling, i.e., the interaction of the d manifolds on the two iron ions as propagated by the fulvalenide ligand, is probably not very different from one cation to another. Furthermore, the vibronic coupling of the PKS model[12] is also probably not changing very much throughout the series. We suggest that it is the nature of the solid-state environment that determines the rate at which intramolecular electron transfer occurs. In the case of "valence localized" species the environment about the cation in the solid is not symmetric and the barrier for electron transfer is increased. If the I_3^- ion is fixed in position closer to one iron than the other, this environmental asymmetry reduces the rate of electron transfer. The Fe^{II} and Fe^{III} doublets in the Mössbauer spectra of **5**-**8** become a single average quadrupole-split doublet at temperatures of 275,[7] 245,[7] 275,[9] and 260 K,[9] respectively. At low temperatures the I_3^- ion (and substituents) are fixed in a position closer to one iron ion, but as temperature is increased the I_3^- ion becomes dynamically disordered such that on the Mössbauer and EPR time scales both metals experience equivalent environments. This motion of the I_3^- changes the potential energy curve for intramolecular electron transfer in the cation.

Two recent observations have been made which support this conclusion. We have found that the Mössbauer spectrum obtained upon heating a microcrystalline sample of **1** to 346 K consists of a single average quadrupole-split doublet. Recrystallization of microcrystalline samples of **7** and **8** by slow diffusion in CH_2Cl_2/hexane gives highly crystalline samples which exhibit one doublet at 300 K in their Mössbauer spectrum, whereas the microcrystalline samples each give a spectrum with two doublets. Two different polymorphs appear to exist in each case, one valence localized, the other delocalized on the Mossbauer time scale at 300 K. A crystallographic study[9] on delocalized crystals of **7** has shown considerable disorder of the I_3^- ion along the segregated stack of triiodide ions in the crystal structure.

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Registry No. 1, 39470-17-2; **2**, 56030-43-4; **3**, 88005-31-6; **4**, 88005-33-8; **5**, 78713-00-5; **6**, 78712-98-8; **7**, 96898-15-6; **8**, 96898-17-8.

Supplementary Material Available: Tables of atomic positional and thermal parameters and observed and calculated structure factors for 1',6'-diiodobiferrocenium triiodide (5 pages). Ordering information is given on any current masthead page.

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Metal Carbonyl Fragments as a New Class of Markers in Molecular Biology

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Although the utility of transition-metal carbonyl complexes in organic synthesis[1] and industrial catalysis[2] is now well established, their potential in biochemistry is only just being realized.[3] We report here an unprecendented use of these organometallic complexes in the important field of steroid hormone receptor assay.[4] Variations in the concentration of certain hormone receptors are clearly implicated in such cancers as breast carcinoma.[5] The

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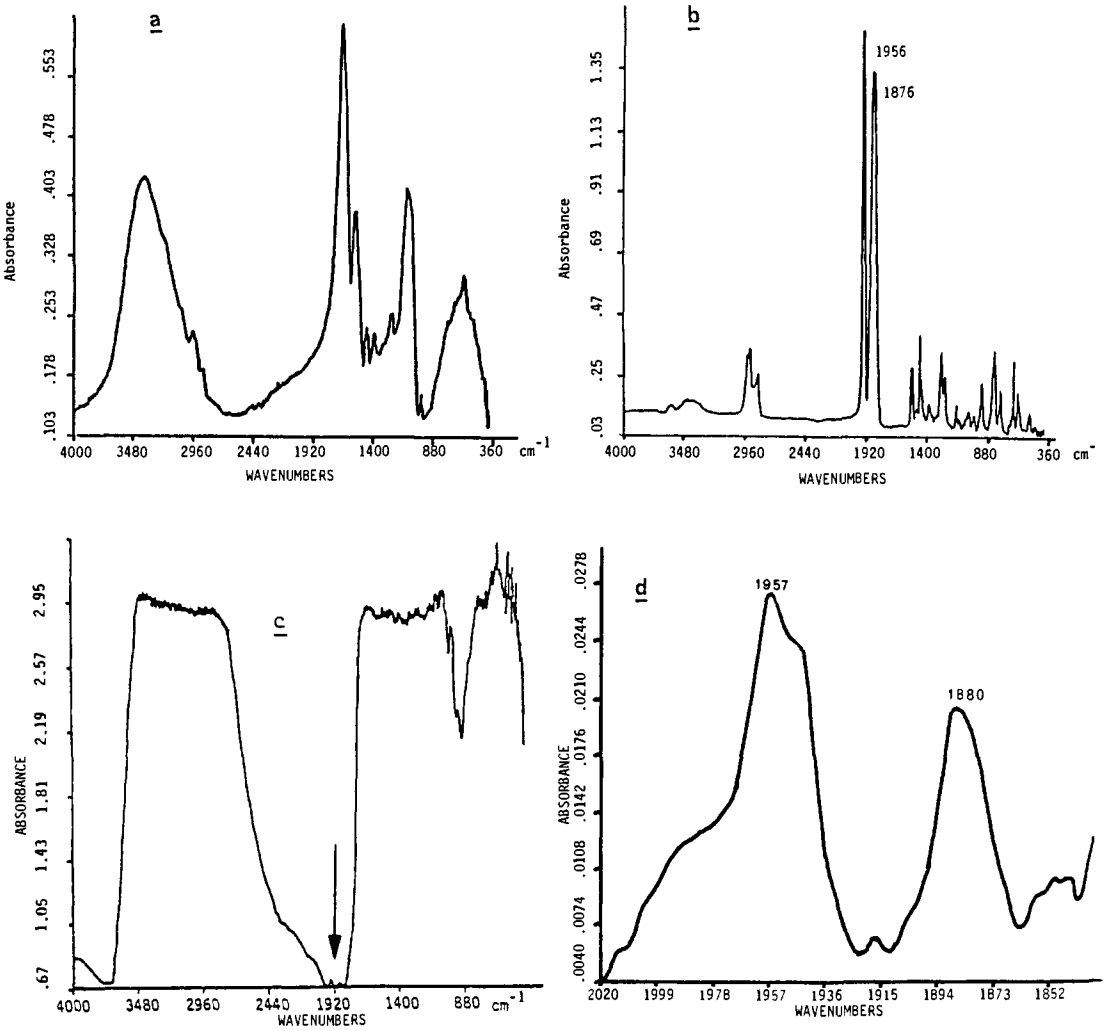

Figure 1. FT-IR spectra of (a) lamb uterine cytosol following incubation with 17β-estradiol and subsequent precipitation from solution with protamine sulfate (CsI minipellet), (b) compound **1** (CsI minipellet), (c) lamb uterine cytosol following incubation with [17α-^{3}H]-**1** and subsequent precipitation from solution with protamine sulfate (off-scale, pure minipellet), and (d) expansion of the ν(CO) region of the spectrum c and base-line correction.

Table I[a]

ligand	radioactivity, fmol/mL: bound without DES	bound with DES	specifically bound
[17α-^{3}H]-**1**	918	149	769
[6,7-^{3}H]-17β-estradiol	1134	126	1008

[a] Portions (400 μL) of lamb uterine cytosol were incubated at 0 °C for 3 h with either 6.3 10^{-9} M [6,7-^{3}H]-17β-estradiol (Amersham, England, S.A. 52 Ci/mmol) or 7.910^{-9} M [17α-^{3}H]-**1** (prepared as described[12] S.A. 4.58 Ci/mmol). Nonspecific binding was determined by using a 500-fold excess of unlabeled DES.[14] Bound fractions were determined by protamine sulfate precipitation.[11]

current methods of assaying utilize radiolabeled hormones.[6] However, despite the excellent detection properties of this technique, problems such as high costs, health hazards, limited variety of useable isotopes, and biochemical instability due to radiolysis have stimulated research into the feasibility of non-isotopic procedures for receptor assay.

In the infrared spectra of all proteins there is a spectral window at ~2000 cm^{-1} and it occurred to us that the introduction of metal carbonyls as probe molecules might be a convenient way of monitoring certain biochemical processes since these compounds have extremely intense ν(CO) peaks in the 2150–1800-cm^{-1} region.[7] To illustrate our organometallic labeling/infrared spectroscopy approach, we decided to apply it to estradiol receptors in lamb uterine cytosol. A series of modified estradiol–chromium tricarbonyl complexes were synthesized[8] and eventually compound **1** (mp 163 °C, $[\alpha]^{22}_D$ 45.8° in CH_2Cl_2 solution, concentration ~0.01 g mL^{-1}) proved to be the most suitable for our purpose because of its long-term stability in solution and its high receptor binding affinity (RBA = 28%).[10] The site of complexation (α or β) on the A ring of the steroid is also an important factor in receptor binding because for the β-Cr(CO)$_3$ analogue **2** the RBA values is reduced to 1.8, while for the free steroid **3** itself it is 35.

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(7) For example, see: Braterman, P. "Metal Carbonyl Spectra"; Academic Press: New York, 1975.

(8) 17β-Estradiol was heated with NaOH and Br(CH$_2$)$_3$OH in acetone.[9] After complexation by heating with Cr(CO)$_6$ 6 h at reflux in n-Bu$_2$O under argon and separation of the diastereomers (TLC using THF/petroleum ether, 2/3) **1** [[3-*O*-(3-hydroxypropyl)estradiol]tricarbonylchromium α] (mp 163 °C, 32%) and **2** [[3-*O*-(3-hydroxypropyl)estradiol]tricarbonylchromium β] (mp 157 °C, 25%) were obtained. The purity of the compounds was carefully checked by TLC, NMR, mass spectroscopy, and elemental analysis. The identification of the diastereomers **1** and **2** has been ascertained by chemical correlation with [3-*O*-(*tert*-butyldimethylsilyl)estradiol]dicarbonyl(thiocarbonyl) complex α for which a X-ray structural analysis has been carried out (Louer, M. manuscript in preparation).

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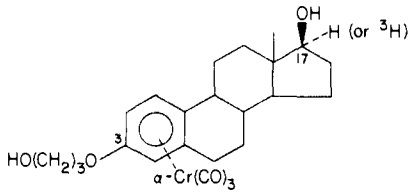

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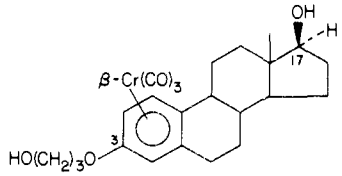

2

In order to prove the specific association of the chromium-labeled steroid for the estradiol receptor site, we prepared compound **1** tritium labeled at the 17α-position[12] and performed in vitro incubation experiments with lamb uterine cytosol (Table I). In this way, we were able to demonstrate that the radioactive hormone is bound specifically and reversibly to the uterine estrogen receptor. Furthermore, the amount of nonspecific binding is only slightly increased when compared to that of (^{3}H)estradiol itself. We also checked that compound **1**, as well as estradiol, binds poorly to, and is not displaced by DES from, high-capacity, low-affinity proteins found in nontarget tissues (e.g., rat lung). That the organometallic label does not decompose during the binding experiments is indicated by the following Fourier transform infrared (FT-IR) measurements.[13]

Figure 1a shows that FT-IR spectrum of lamb uterine cytosol used as a source of estradiol receptor following incubation with 17β-estradiol and precipitated from the cytosol by protamine sulfate technique.[11] The FT-IR spectrum of compound **1** is given in Figure 1b; the two ν(CO) peaks characteristic of the C_{3v} symmetry Cr(CO)$_3$ fragment are the strongest absorption present. Next, in Figure 1c, we show the FT-IR spectrum of the precipitated proteins following incubation with the organometallic tritiated compound **1** at approximately the same concentration ($\sim 10^{-8}$ M) as currently used in the radiochemical assays using estradiol itself.[6] The protein absorption are off-scale owing to the thickness of the minipellet but two very weak features can be discerned above the background at $\sim$1900 cm^{-1}. The computer expansion of this region shown in Figure 1d reveals the two ν(CO) peaks of the organometallic marker. Similar results were obtained with all the chromium tricarbonyl labeled estradiol molecules synthesized in this work. While the best signal-to-noise conditions for the spectra necessitated recording 10 000-30 000 scans at 4-cm^{-1} resolution (3-10 h), the two metal carbonyl peaks can just be detected above the background at 8-cm^{-1} resolution in $\sim$2 min. There is an excellent correlation (R = 0.98) between the area of the higher energy ν(CO) peak in the experiments with compound **1** and the weight of the minipellets, indicating that in principle it should be possible to extend this new method of protein receptor detection into the quantitative realm in the future.

Acknowledgment. This research was generously supported by operating grants from C.N.R.S. and P.I.R.M.E.D. (France), N.S.E.R.C. (Canada), and F.C.A.C. (Quebec) and travel grants under the auspices of a France-Quebec Exchange.

Registry No. 1, 93061-16-6; **2**, 93061-17-7; [17α-*H*]-**1**, 96648-81-6; **4**, 96648-82-7; **5**, 96687-90-0; 17β-estradiol, 50-28-2.

(12) As direct synthesis of ([6,7-^{3}H]-17β-estradiol)tricarbonylchromium complexes failed owing to radiolysis, [17α-^{3}H]-**1** was prepared by reduction with [^{3}H]NaBH$_4$ of the [3-*O*-(3-hydroxypropyl)estrone]tricarbonyl complex **4** obtained as follows: Estrone was heated with NaOH and Br(CH$_2$)$_3$OH in acetone.[9] After complexation by heating with Cr(CO)$_6$ 4$^1/_2$ h at reflux in *n*-Bu$_2$O under argon and separation of the diastereomers (TLC using ether/pentane, 10/1) compounds **4** (estrone analogue of **1**, mp 97 °C, 12%) and **5** (estrone analogue of **2**, mp 94 °C, 12%) were obtained. [17α-^{3}H]-**1** was obtained by heating 25 μM of **4** (20 h at 50 °C in 1.2 mL of 0.1 N NaOH/isopropyl alcohol, 1/5) in the presence of 6.25 μM of [^{3}H]NaBH$_4$ (Amersham, England, S.A. 16 Ci/mol). After hydrolysis, purification (TLC using ether), and crystallization (ether/pentane), the yellow solid obtained (0.64 mg) was identified as authentic compound **1** with a specific activity of 4.58 Ci/mol.

(13) FT-IR spectra were recorded on a Nicolet 6000 spectrometer equipped with a mercury-cadmium-telluride, liquid nitrogen cooled detector. Samples (1.5-2.0 mg) of the dried, white powders obtained from the protein precipitations of the cytosol were pressed into 3-mm minipellets.

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Bromate Oscillators: Elucidation of the Source of Bromide Ion and Modification of the Chemical Mechanism

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Although the FKN mechanism[1] is widely accepted, it is still debated which chemical reactions produce the bromide ion, the control intermediate of BZ oscillators. According to the FKN mechanism, bromide ion is generated in a reaction between the oxidized form of the catalyst, $M^{(n+1)+}$, and bromomalonic acid (BrMA) accumulated during the preoscillatory period. This multistep reaction appears in a concise form in step 5 of the Oregonator model:[2] $Z \rightarrow fY$ ($2Ce^{4+} \rightarrow f Br^-$).

This assumption has been supported by the observation that in a BZ system chemical oscillation starts only after the concentration of BrMA reaches a crucial value.[3] On the other hand, a number of experimental results cannot be reconciled with the above supposition. Some of these are the following: (a) in a BZ system with a high [MA]/[BrO_3^-] ratio (>30) oscillation starts without a preoscillatory period;[4] (b) there are many BZ systems where bromide ion cannot be formed by a reaction between $M^{(n+1)+}$ and an organic bromo compound because the $M^{(n+1)+}$ + >CHBr $\rightarrow M^{n+}$ + Br^- + CO_2 ... reaction is either slow or does not proceed at all, e.g., when the organic compound is malic acid or an aliphatic or cyclic diketone; (c) in the presence of bromo-complex-forming metal ions, e.g., Tl^{3+}, which complex the bromide ions generated in the BZ reaction, the rates of BrMA and bromide formation are practically equal;[5] (d) the measured rate of carbon dioxide evolution is 2 orders of magnitude higher than that calculated from the BrMA-Ce^{4+} reaction.[6]

Owing to problems concerned with the stoichiometric factor, f, of the Oregonator model, a few authors have looked for additional sources of bromide ion, i.e., for other reactions that may produce bromide ion. They assumed that bromide ion forms *also* in the reduction of oxybromine compounds.[7] A conclusion to this question, however, has not been given so far.

In order to understand this crucial point of the mechanism of BZ oscillators, we have performed reactions in BZ systems that contain both ^{82}Br-labeled BrMA and silver ions. In the starting reaction mixture the concentration of BrMA was above the crucial value[3] and equal with that of Ag^+. The initial composition of the system was the following: 0.08 M $KBrO_3$, 0.20 M malonic acid,

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