Modification of Estradiol at the 17-Position. Effect of Changing the OH Group for a Transition-Metal Carbonyl Cluster on the Estradiol Receptor Recognition

Domenico Osella,*,† Giorgia Dutto,† Gérard Jaouen,*,‡ Anne Vessiéres,‡ Paul R. Raithby,*,§ Laura De Benedetto,§ and Michael J. McGlinchey∥

Dipartimento di Chimica Inorganica, Chimica Fisica e Chimica dei Materiali, Università di Torino, Via P. Giuria 7, 10125 Torino, Italy, Ecole Nationale Supérieure de Chimie de Paris, URA CNRS 403, 11 rue Pierre et Marie Curie, 75231 Paris Cedex 05, France, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, U.K., and Department of Chemistry, McMaster University, Hamilton, Ontario L8S 4M1, Canada

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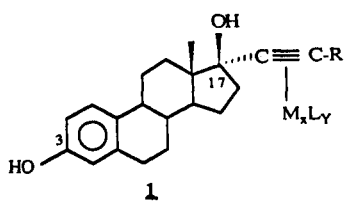
The reaction of $[Co_2(CO)_6(17\alpha\text{-ethynyl-}17\beta\text{-estradiol})]$ (**2a**) with $Fe(CO)_5$ in acetone produces $[FeCo(CO)_6(17\alpha\text{-ethynyl-}17\beta\text{-dehydroxyestradiol})]$ (**3a**) as the result of the dehydroxylation ability of the iron pentacarbonyl. The molecular structure of **3a** has been investigated not only by NMR spectroscopy but also by use of molecular modeling techniques. The structural data for the organometallic part of the molecule are derived from a single-crystal X-ray determination of the model complex $[FeCo(CO)_5(PPh_3)(H_3CC_2CH_2)]$, carried out at 123 K: triclinic, $P\bar{1}$ (No. 2), $a = 10.315(1)$ Å, $b = 10.579(1)$ Å, $c = 12.989(2)$ Å, $\alpha = 67.62(1)°$, $\beta = 89.44(2)°$, $\gamma = 79.30(2)°$, $V = 1287.1(7)$ Å^3, $Z = 2$, $R_F = 0.063$. The mixed-metal cluster $[FeCo(CO)_6(HC{=}C{=}CH_2)]$ was used as a model of the cation $[Co_2(CO)_6(HC{\equiv}CCH_2)]^+$, and its structure was rationalized by means of extended Hückel molecular orbital calculations. The biological results obtained from the incubation in vitro of the steroid–organometallic complex **3a** with lamb uterine cytosol (used as a source of estradiol receptor) indicate that the absence of the 17-OH group on estradiol prevents the formation of an irreversible (*covalent*) bond between the metal-labeled steroid and its specific receptor. The biochemical implications of such a change in reactivity are discussed.

Introduction

The mechanism of the mode of association of a hormone with its receptors is still largely unknown.[1] Currently, one of the most powerful tools for identifying the specific amino acids involved in the hormone–receptor recognition is the affinity labeling approach.[2] The anchoring of an organometallic fragment to a biomolecule can not only alter its reactivity but also provide valuable information on the nature of the recognition process. We have already shown that the coordination of selected organometallic fragments to 17α-alkynylestradiol (1) may induce an



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irreversible (covalent) binding of these organometallic hormones with the estrogen receptors.[3] To our knowledge, these complexes are the first affinity markers which incorporate the estradiol skeleton. The key step is the transformation of the 17β-OH functionality into the corresponding carbenium ion; this electron-deficient center is stabilized by the neighboring transition-metal cluster fragment, and it is this phenomenon which promotes cation formation. It was inferred that the nucleophilic sulfur residues of a coordination unit involving an acidic metal (presumably Zn^{2+}), cysteines 530 and 381, and histidines 524 and 516 (human estrogen receptor numbering)[4] in close vicinity to the estradiol binding site are good candidates for the establishment of such a covalent bond and the consequent receptor inactivation. For such a reaction to take place, it is necessary to have at the 17-position of the hormone both an OH group and an organometallic unit capable of dissipating the positive charge of the transient carbocation. Among the numerous 17α-alkynyl-17β-estradiol transition-metal complexes we have examined, the dicobalt clusters **2a** and **2b** have proven to be the most active affinity markers (*e.g.*, 80% of receptor inactivation is induced by incubation of lamb uterine cytosol in the presence of 10 nM of **2b** for 1 h).[5] In order to obtain further information on the mechanism of molecular recognition, we have synthesized the isoelectronic $[FeCo(CO)_6(17\alpha\text{-ethynyl-}17\beta\text{-dehydroxyestradiol})]$ complex **3a**, in which the steric bulk of the heterobimetallic fragment is very similar to that of the $Co_2(CO)_6$ homologue, but the 17β-OH functionality is no longer present. This obviously prevents the formation of a carbenium-ion-like species and, therefore, if the proposed mechanism is operative,

† Università di Torino.
‡ Ecole National Supérieure de Paris.
§ Cambridge University.
∥ McMaster University.
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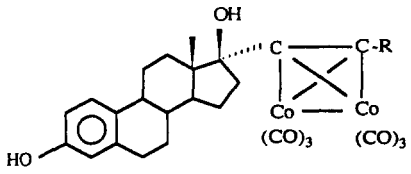
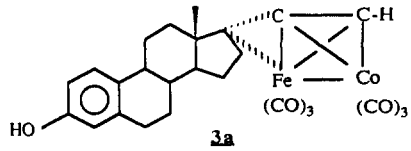


2a , R= H

2b , R= CH_3



3a

the relative binding affinity (RBA) and inactivation efficiency values of such a complex for the estradiol receptor should dramatically decrease.

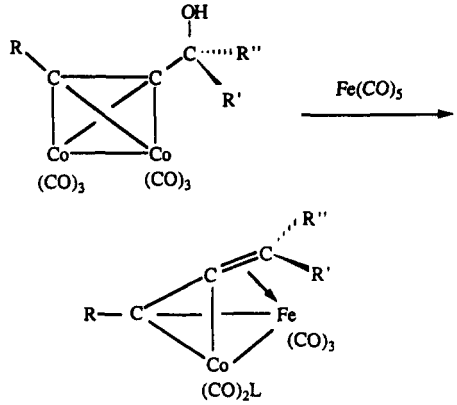
Results and Discussion

Synthesis and X-ray Structural Determination of a Model Complex. We have previously reported that hexacarbonyldicobalt–alkynol complexes $Co_2(CO)_6[\mu$-$RC{\equiv}CC(OH)R'R'']$ (**2**) react with $Fe(CO)_5$ to form heterometallic clusters of the type $FeCo(CO)_6[\mu$-$R{-}C{=}C{=}CR'R'']$ (**3**) as shown in Figure 1.[6] The driving force for the reaction is the well-known dehydroxylation ability of iron carbonyls toward α-unsaturated alcohols.[7]

In the absence of X-ray-quality crystals of the steroidal complex **3a**, we chose to model the structure by obtaining crystallographic data on the simpler molecule **3b** and subsequently using a molecular modeling program to graft the steroidal moiety onto the cluster fragment. The complex $[FeCo(CO)_6][CH_3C_2CH_2]$ (**3b**) is an oil, but its triphenylphosphine derivative $[FeCo(CO)_5(PPh_3)][CH_3C_2CH_2]$ (**4**) yielded crystals suitable for an X-ray crystallographic study. The spectroscopic data indicated that, as has been found in other closely related systems,[6] substitution of a CO by the phosphine occurred regiospecifically on the Co atom, and this result was confirmed by the X-ray diffraction study.

The crystal structure of $[FeCo(CO)_5(PPh_3)][CH_3C_2CH_2]$ (**4**) consists of discrete molecules separated by normal van der Waals distances. The molecular structure of **4** is illustrated in Figure 2, while selected bond parameters are listed in Table I. The overall molecular geometry of **4** closely resembles that of the isoelectronic complex $(OC)_3Fe(\mu$-p-$MeC_6H_4C{=}C{=}CH_2)W(CO)_3Cp$.[8] Both of these complexes represent rare examples of heteronuclear metal μ-allenyl compounds, and the structural parameters within the allenyl fragments in the two molecules are indeed very similar. In **4**, the C(1)–C(2) and C(2)–C(3) distances of 1.371(8) and 1.383(7) Å, respectively, show partial multiple-bond character with an angle of 137.0(4)° at the central carbon atom, C(1)–C(2)–C(3). The remainder of the bond parameters within the structure do not deviate significantly from the expected values.

The proton-coupled ^{13}C NMR spectrum of the parent compound **3b** shows for the CH_2 moiety two partially



3b: R = Me ; R'= R''= H ; L = CO

3c: R = H ; R'= Ph, R''= Me ; L= CO

3c': R = H ; R'= Me, R''= Ph ; L= CO

4 : R = Me ; R'= R''= H ; L= PPh_3

Figure 1. Synthesis of (μ-allenyl)–iron–cobalt clusters.

Table I. Selected Bond Distances (Å) and Bond Angles (deg) for 4

Fe(1)–Co(1)	2.565(1)	Fe(1)–C(21)	1.831(6)
Fe(1)–C(22)	1.811(7)	Fe(1)–C(23)	1.820(6)
Fe(1)–C(1)	2.041(5)	Fe(1)–C(2)	2.011(5)
Fe(1)–C(3)	2.195(6)	Co(1)–C(11)	1.822(6)
Co(1)–C(12)	1.809(6)	Co(1)–C(1)	2.035(6)
Co(1)–C(2)	1.955(6)	Co(1)–P(1)	2.237(1)
C(1)–C(2)	1.371(8)	C(1)–C(4)	1.519(8)
C(2)–C(3)	1.383(7)		
Co(1)–Fe(1)–C(1)	50.9(2)	Co(1)–Fe(1)–C(2)	48.8(2)
C(1)–Fe(1)–C(2)	39.5(2)	Co(1)–Fe(1)–C(3)	79.2(1)
C(1)–Fe(1)–C(3)	74.4(2)	C(2)–Fe(1)–C(3)	38.1(2)
Fe(1)–Co(1)–C(1)	51.1(1)	Fe(1)–Co(1)–C(2)	50.7(1)
C(1)–Co(1)–C(2)	40.1(2)	Fe(1)–Co(1)–P(1)	151.0(1)
C(1)–Co(1)–P(1)	102.5(1)	C(2)–Co(1)–P(1)	102.3(1)
Fe(1)–C(1)–Co(1)	78.0(2)	Fe(1)–C(1)–C(2)	69.1(3)
Co(1)–C(1)–C(2)	66.8(3)	Fe(1)–C(1)–C(4)	133.9(3)
Fe(1)–C(2)–Co(1)	80.6(2)	Fe(1)–C(2)–C(1)	71.4(3)
Co(1)–C(2)–C(1)	73.1(4)	Fe(1)–C(2)–C(3)	78.2(3)
Co(1)–C(2)–C(3)	131.0(4)	C(1)–C(2)–C(3)	137.0(4)
Fe(1)–C(3)–C(2)	63.8(3)		

overlapped doublets (the protons are not equivalent) having $^1J_{CH}$ values of 164.8 and 163.3 Hz, respectively, consistent with an sp^2 rehybridization of C(3). A similar $^1J_{CH}$ value (164 Hz) was reported for $(OC)_3Fe(\mu$-$CH_3{-}C{=}C{=}CH_2)W(CO)_2Cp$.[8] Unfortunately, attempts to obtain the more diagnostic J_{CC} couplings by means of the INADEQUATE sequence were frustrated by the broadening of the C_{ac} resonances induced by the quadrupolar Co nucleus.

Interestingly, the reaction of $Co_2(CO)_6[HC{\equiv}CC(OH)$-PhMe] with $Fe(CO)_5$ affords $FeCo(CO)_6[HCC_2CPhMe]$ as an mixture of stereoisomers, **3c** and **3c'**, in a 5:1 ratio (as evaluated by ^1H NMR integration of each group of resonances). Thus far, this mixture has resisted separation by fractional crystallization or by column or thin-layer chromatography. This stereoisomerism is easily rationalized in terms of the *syn* and *anti* orientations of the CR'R'' moiety adjacent to the intrinsically chiral FeCoCC' tetrahedral core[9] and gives rise to a diastereomeric pair. No interconversion could be observed on the NMR time scale up to 80 °C, at which temperature the complex starts to decompose.

$(HC{=}C{=}CH_2)FeCo(CO)_6$ vs $[(HC{\equiv}CCH_2)Co_2(CO)_6]^+$ Analogy. Aside from its intended use as a

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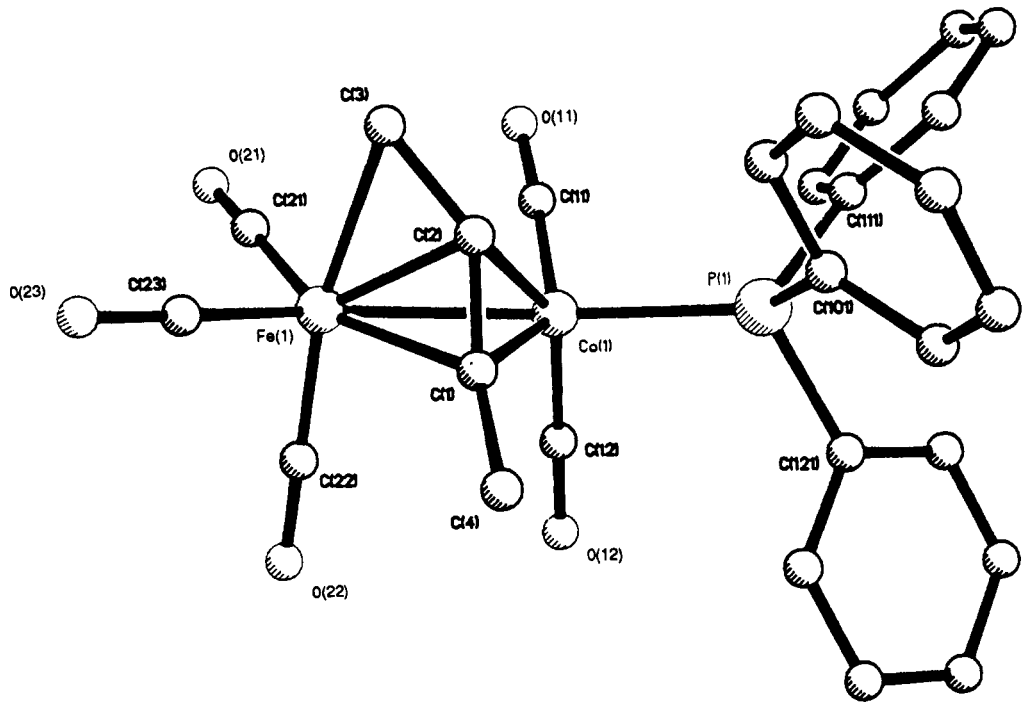
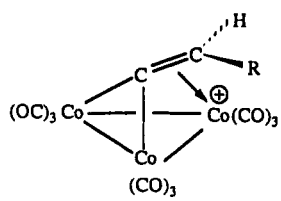


Figure 2. View of $[FeCo(CO)_5(PPh_3)(MeC{=}C{=}CH_2)]$ (**4**), showing the atom numbering.

template for the construction of a model of the steroid–$[FeCo(CO)_6]$ cluster **3a**, the molecular structure of **4** is significant in its own right. Making the not unreasonable assumption that the conformation of the allenyl moiety in $[FeCo(CO)_6][CH_3C_2CH_2]$ (**3b**) is essentially the same as that found crystallographically for the phosphine derivative **4**, one can relate this result to the longstanding problem of the stabilization of a carbocationic center α to a tetrahedral di- or tricobalt cluster.

More than 20 years ago, Seyferth suggested that, in the cation $[Co_3(CO)_9CCH_2]^+$ (**5**), the vinylidene capping fragment did not adopt an orientation perpendicular to the basal trimetallic plane but rather was tilted toward one of the cobalt vertices.[10] Subsequently, this proposal

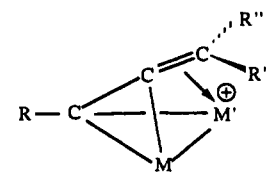


5 : R = H

6 : R = $CHMe_2$

received support not only from molecular orbital calculations at the extended Hückel level[11] but also from a series of elegant and ingenious NMR experiments.[12] In this latter study, it was shown that in the cationic cluster $[Co_3(CO)_9CCHCHMe_2]^+$ (**6**) the isopropyl methyls are rendered diastereotopic as a consequence of the chiral nature of the molecule; this can only arise if the capping C=CR′R″ fragment is bent away from the vertical alignment.

These concepts have also been applied to the analogous dicobalt–alkynyl systems **7**, and the variable-temperature NMR spectra of such cations have been explained in terms of the migration of the methylene terminus from one metal vertex to the other.[13] The direct interaction of an α-CH_2^+



7 : M = M′ = $Co(CO)_3$

8 : M = M′ = $Mo(CO)_2Cp$

substituent with a $CpM(CO)_2$ vertex, where M = Mo or W, in tetrahedral dimetallic clusters such as $[Cp_2Mo_2(CO)_4][\mu\text{-}HC{=}C{=}CH_2]^+$ (**8**) has been established crystallographically.[14] However, there are at present no X-ray structural data on cobalt cations such as **5** and **7**. There exist several mixed-metal systems containing an α-CR_2^+ cation together with such vertices as $(C_5H_5)Mo(CO)_2$ and $(C_5H_5)Fe(C_5H_4)$; however, in all these cases, the NMR, IR, and EHMO data favor the isomer in which the cationic center is *not* attached to the cobalt vertex. Indeed, the structures of two of these molecules have very recently been confirmed by X-ray crystallography.[15]

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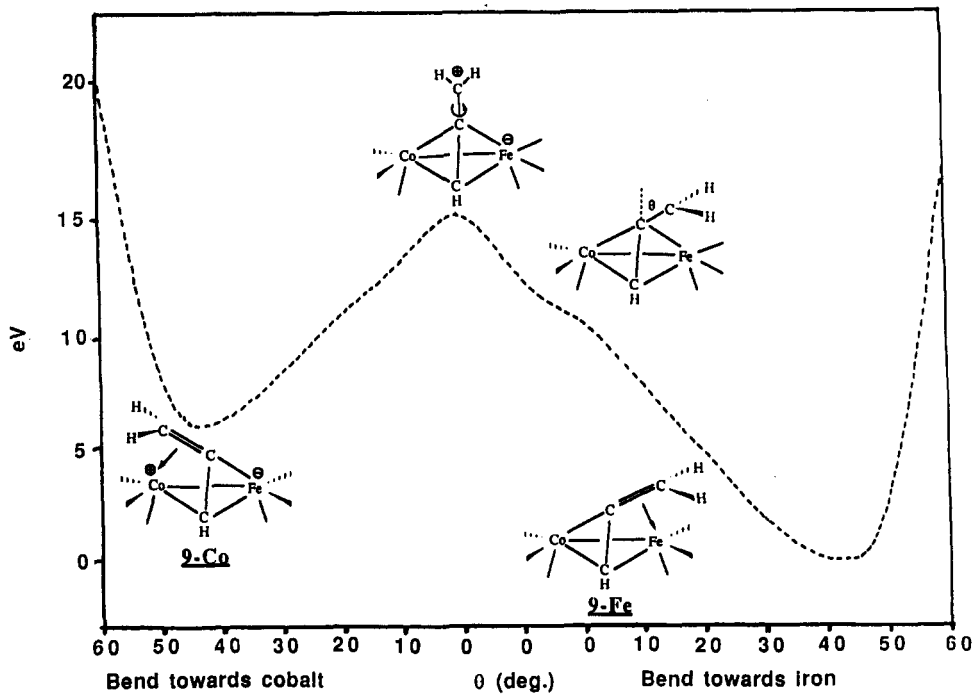

Figure 3. EHMO-calculated energy profile showing the effect of bending the capping vinylidene fragment in $[FeCo(CO)_6(HC{=}C{=}CH_2)]$ toward either the $Co(CO)_3$ or $Fe(CO)_3$ vertex.

The $FeCo(CO)_6$ complex **3b** may be regarded as the closest analogue of **7** to have been characterized in the solid state. It is precisely isoelectronic with $[Co_2(CO)_6(HC{\equiv}CCH_2)]^+$, and its structural parameters may with confidence be compared to the geometry calculated for **7**. Molecules such as **3b** and **7** have a high degree of electron delocalization, and any single valence-bond description is too oversimplified.[16] Instead, one may extend the EHMO picture already developed for $[Co_3(CO)_9CCH_2]^+$ (**5**)[10,17] to provide a more useful model of the bonding interactions in **3** and **7**.

Extended Hückel molecular calculations on $FeCo(CO)_6(HC{=}C{=}CH_2)$ were carried out on a model derived from the X-ray data for **4** in which the PPh_3 ligand was replaced by a CO and the methyl substituent by hydrogen. Figure 3 shows the energy profile of the system when the methylene originally leaning toward cobalt was allowed to undergo an antarafacial migration onto the iron vertex. It is readily apparent that there are two minima when the CH_2 moiety is tilted through approximately 45° from the vertical position toward either of the metal vertices. However, the interaction with the $Fe(CO)_3$ fragment is more stabilized to the tune of *ca.* 8 kcal mol^{-1}. Although continued bending toward the metal enhances the direct bonding interaction, ultimately it brings about steric interactions between the capping fragment and the carbonyls, resulting in a very steep increase in the energy. Since the hydrogens of the allenyl fragment in **4** were not located in the X-ray structural determination, these EHMO calculations assumed a planar CH_2 group but, if the methylene hydrogens are allowed to bend through 20° away from the iron vertex, the energy-minimized structure has an angle θ of 55°. In the X-ray crystal structure of **4**, the bend angle θ is 59°.

As shown in Figure 4, the crucial interaction involves overlap of the vacant p_z orbital on the methylene carbon with a filled metal orbital of d_{z^2} character. The in-phase combination is increasingly stabilized with greater bending of the capping moiety; in contrast, the out-of-phase combination of these two orbitals gives rise to the LUMO, which is destabilized by increased bending and so leads to a larger HOMO–LUMO gap.

Application of the 18-electron rule favors the attachment of the methylene to the $Fe(CO)_3$ vertex, since both metals satisfy the EAN count. In contrast, the cobalt-bonded isomer requires the zwitterionic structure **9–Co**. To the extent that we choose to believe the EHMO-derived charge distribution, this simple picture also comes through from the calculation, which places charges of +0.536 and −0.537 on the $Co(CO)_3$ and $Fe(CO)_3$ fragments, respectively, when the methylene leans toward the cobalt vertex. For comparison, in the thermodynamically favored iron-stabilized isomer **9–Fe**, the $Co(CO)_3$ and $Fe(CO)_3$ fragments bear charges of −0.037 and +0.035, respectively.

The strength of the localized interaction of the methylene moiety with the $Fe(CO)_3$ vertex in **3b** is also reflected in the low-temperature ^{13}C NMR spectra. At −80 °C, the $Co(CO)_3$ unit yields a sharp singlet, while the $Fe(CO)_3$ group exhibits three peaks in a 1:1:1 ratio. Cessation of the rotation of $M(CO)_3$ tripods, on the NMR time scale, as the result of localized electronic interactions has also been observed in the complexes **10**, **11**, and **12**.[18]

Synthesis and Characterization of the Steroid–$[FeCo(CO)_6]$ Complex 3a. Having established the basic molecular structure of $FeCo(CO)_6(RC{=}C{=}CR_2)$ derivatives, the next step was the synthesis of the steroidal

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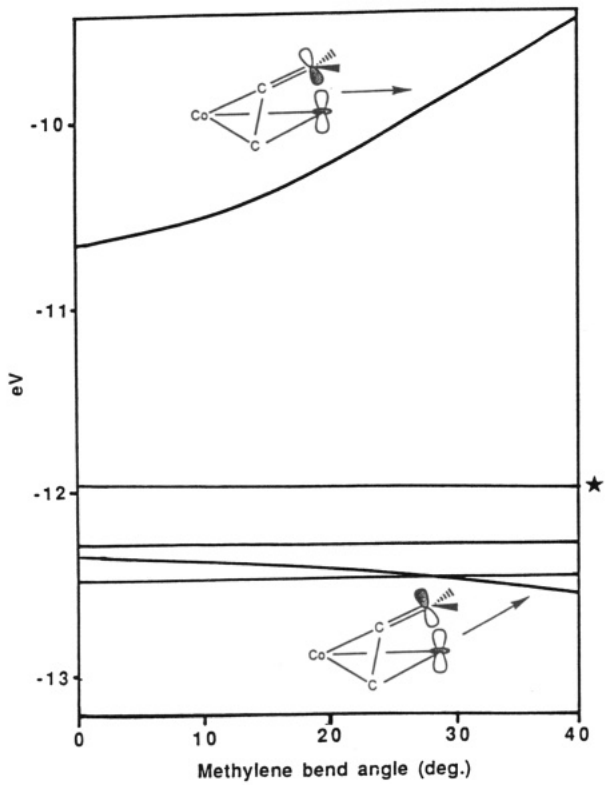
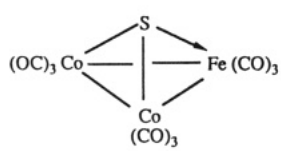


Figure 4. Orbital energy level diagram showing the effect of bending the vinylidene cap in $[FeCo(CO)_6(HC{=}C{=}CH_2)]$ toward the $Fe(CO)_3$ vertex. The HOMO is indicated by a star.



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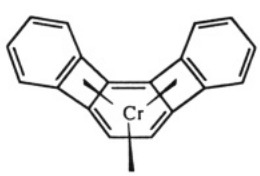

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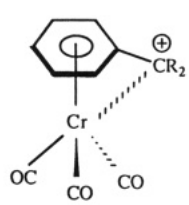

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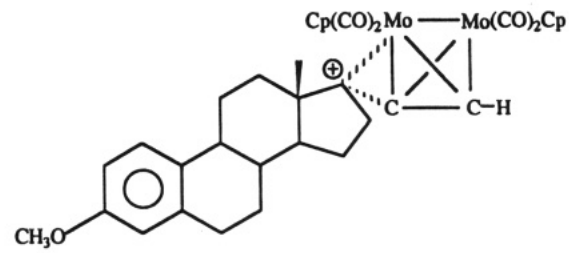
derivative $[FeCo(CO)_6(17\alpha\text{-ethynyl-}17\beta\text{-dehydroxyestradiol})]$ (**3a**) by treatment of $[Co_2(CO)_6(17\alpha\text{-ethynyl-}3,17\beta\text{-estradiol})]$ (**2a**) with $Fe(CO)_5$. Complex **3a** was readily identified by its ^1H and ^{13}C NMR spectra. In particular, comparison of the proton spectra of **3a** with those of the free ligand, *i.e.* 17α-ethynyl-$3,17\beta$-estradiol (**EE**), and of the $(17\alpha\text{-alkynylestradiol})Co_2(CO)_6$ complexes **2a** and **2b**[19] reveals not only the disappearance of the C(17)–O*H* peak at δ 4.3 but also the downfield shift of the C_{ac}–*H* absorption upon coordination to the heterobimetallic Fe–Co fragment (δ 6.24); in **2a** this acetylenic hydrogen is found at δ 6.75. Noteworthy is the marked deshielding of the C(17) nucleus in **3a** (135.9 ppm) attributable to the direct interaction with the Fe atom; this may be contrasted with the C(17) resonances in **EE** (87.6 ppm) and in the corresponding $Co_2(CO)_6$ complex **2a**, which is found at 85.2 ppm (see Table II).

In order to generate a viable molecular geometry for **3a**, one must obtain reasonable models for both the cluster moiety and for the hormonal steroid fragment. However, one must take into account not only the inherent chirality of the iron–cobalt cluster but also the fact that the iron vertex may bond to C(17) from either the α- or the β-face of the steroid; that is, the four stereoisomers **13a–d**, shown in Figure 5, must be considered. It is nevertheless interesting that the ^{13}C NMR spectrum of **3a** after chromatographic separation of the products indicates the presence of a single diastereomer.

Since there is considerable double-bond character in the linkage between C(20) and C(17), we cannot allow free rotation about this axis. To guide us here, we turn to the X-ray crystallographic data on the closest analogue of which we are aware. In the steroidal cation **14**, which is



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stabilized by interaction with a molybdenum atom, it is the β-face of the hormone which leans toward the metal. This places the C(18) methyl group *proximal* with respect to the $(C_5H_5)Mo(CO)_2$ vertex.[20] On the NMR time scale, this molecule is fluxional and the capping steroidal unit migrates between the two molybdenum sites.[21] In contrast, there is a considerable barrier to migration of the vinylidene capping group from iron to cobalt in **3b** or **3c**, and presumably in **3a** also.

A reasonable representation of the structure of **3a** can be obtained by use of the molecular modeling program PCMODEL,[22] which uses the MMX force field.[23] The organometallic fragment was derived from the X-ray structure of **4**, in which the phosphine was replaced by a carbonyl ligand with the Co–C length set at the average of the other cobalt–carbonyl bond distances, and the C_{ac}–H distance was taken from that found in $\{Co_2(CO)_4[P(OMe)_3]_2(HC_2H)\}$.[24] The steroidal moiety was taken from the X-ray crystallographic data on the $(C_5H_5)_2Mo_2(CO)_4$–mestranyl cation **14**,[20] and the two fragments were combined as in **14**. The initial structure was energy-

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(22) PC-MODEL, June 1990 version, available from Dr. K. Gilbert, Serena Software, Bloomington, IN.

(23) MMX is a version of MM2, modified by J. J. Gajewski and K. E. Gilbert, to accommodate transition-metal atoms. For more information, see program MMP2: Allinger, N. L.; Yuh, Y. H. *QCPE* **1980**, 395. Allinger, N. L.; Flanagan, H. L. *J. Comput. Chem.* **1983**, *4*, 399.

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Table II. Spectroscopic Data for Complexes 3a–c and 4

compd	IR,[a] ν(CO), cm^{-1}	^{1}H NMR,[b] δ, ppm	^{13}C NMR,[b] δ, ppm
3a	2083 m, 2037 vs, 2028 s, 2012 m, 1989 m, 1978 m	7.89 (s, 1), C_3–OH;[f] 6.97 (d, 1), C_1–H; 6.47 (dd, 1), C_2–H; 6.41 (d, 1), C_4–H; 6.24 (s, 1), C_{ac}–H[g]	210.3, $Fe(CO)_3$; 203.0, $Co(CO)_3$[h]
3b[c]	2084 m, 2041 vs, 2022 s, 2014 m, 1993 m, 1981 m	3.98–3.92 (each s, H, 2); 2.69 (s, Me, 3)	209.0, $Fe(CO)_3$; 202.2, $Co(CO)_3$; 121.0, 87.4, C_{ac}; 66.1, CH_2;[d] 18.5, Me[d]
3c, **3c'**	2089 m, 2041 vs, 2026 s, 2015 m, 1991 m, 1987 m	7.5–7.3 (each m, Ph 2);[e] 6.21, 5.95 (each s, H, 1);[e] 2.43, 2.23 (each s, Me, 3)[e]	209.0, $Fe(CO)_3$; 202.0, $Co(CO)_3$; 144.7, 113.8, C_{ac}; 128.5–125.0, Ph; 68.0, CH; 29.6–26.0, Me
4	2052 vs, 2003 s, 1989 vs, 1968 m	7.51 (m, Ph, 15); 3.96–3.94 (each s, H, 2); 2.07 (s, Me, 3)	211.2, $Fe(CO)_3$; 207.3, 205.1, $Co(CO)_2$; 122.3, 90.9, C_{ac}; 135.1–128.7, Ph; 67.6, CH_2; 19.6, Me

[a] In cyclohexane. [b] In $CDCl_3$ at 25 °C, except for ^{1}H NMR of **3a** recorded in acetone-d_6. [c] Previously reported in ref 6. [d] In proton-coupled spectrum: CH_2, $^1J_{CH}$ = 164.8 and 163.3 Hz; Me, $^1J_{CH}$ = 131.1 Hz. [e] Each pair of resonances gives an integrated intensity ratio of 5:1. [f] Temperature- and concentration-dependent resonance, which disappears when D_2O is added to acetone-d_6 solution. [g] The other resonances are almost identical with those of free EE. [h] For other resonances, see Table III.

Table III. $^{13}C\{^1H\}$ NMR Spectra of 17α-Ethynyl-3,17β-estradiol (EE), $Co_2(CO)_6$(17α-ethynyl-3,17β-estradiol) (2a), and $FeCo(CO)_6$(17α-ethynyl-17β-dehydroxyestradiol) (3a) Recorded in Acetone-d_6

compd	C_3	C_5; C_{10}	C_1; C_4; C_2	C_{17}; C_{13}; C_{18}	C_{20}	C_{21}
EE[a]	153.4	138.2; 132.7	126.5; 115.3; 112.8	87.6; 47.1; 12.7	80.0	74.0
2a[a]	153.4	138.2; 132.4	126.4; 115.3; 112.7	85.3; 48.8; 15.6	103.7	73.5
3a	153.4	138.0; 132.2	126.3; 115.2; 112.7	135.9; 48.9; 19.7	113.3	65.7

[a] Previously reported in ref 19.

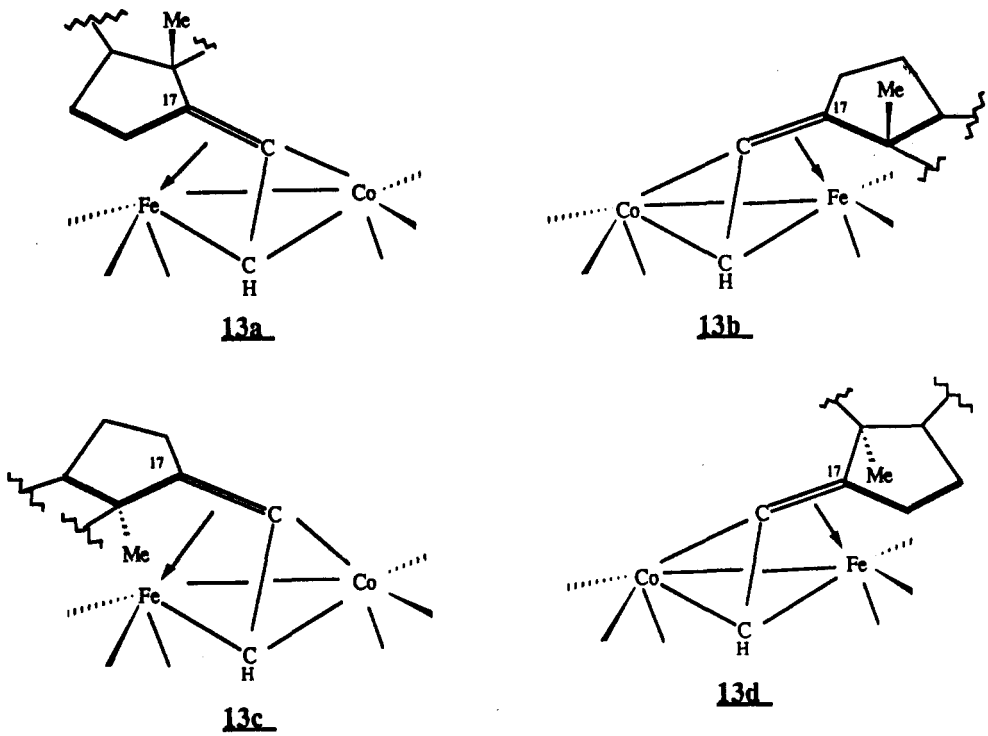

Figure 5. Diastereomers of [$FeCo(CO)_6$(17α-ethynyl-17β-dehydroxyestradiol)]. For clarity, only the D ring of the steroid is shown.

minimized (a) by allowing small oscillations about the C(20)–C(17) bond to find the most favorable conformation and (b) by adjusting the Fe–C(17) distance;[25] after multiple iterations, the global minimum was attained. Figure 6 shows the resulting structure. While recognizing the approximations inherent in such a procedure (*e.g.*, the fixed structure of the metal cluster fragment), we feel that this provides a reasonable model of a favorable diastereomer.

Biological Results and Conclusions. We have previously reported that for alkylnyl–dicobalt cluster derivatives, such as **2a** and **2b**, the relative binding affinity (RBA) value for the estradiol receptor can still be quite good. Compared to the natural hormone, *viz.* 3,17β-estradiol (RBA = 100%), [$Co_2(CO)_6$(17α-ethynyl-3,17β-estradiol)] (**2a**) exhibits an RBA value of 16%.[26] This can be contrasted with an RBA of only 3.4% when the 17-hydroxy functionality in estradiol itself is replaced by a hydrogen atom.[27] It thus seems essential to have OH

(25) In molybdenum-stabilized cations, the Mo–C_α bond length increases from 2.44 Å for a primary cation to 2.74 Å for a tertiary cation.

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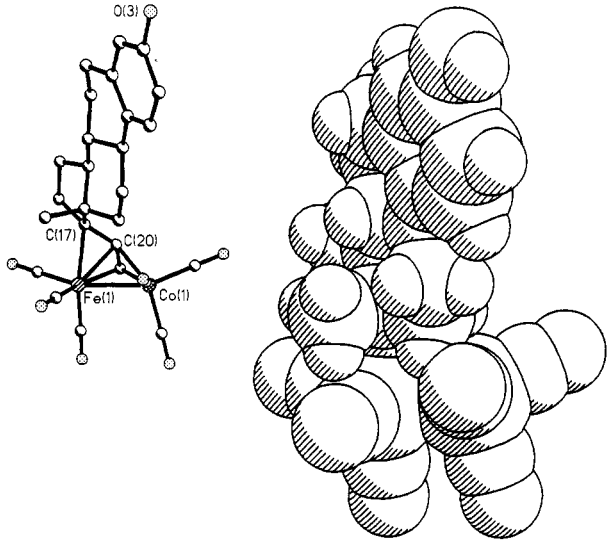

Figure 6. (a, left) View of one diastereomer of [$FeCo(CO)_6$-(17α-ethynyl-17β-dehydroxyestradiol)] (**3a**). Hydrogen atoms have been omitted for clarity. The structure was obtained by using a molecular modeling program to graft the steroidal fragment onto the organometallic cluster (see text). (b, right) Space-filling representation of the molecule depicted in Figure 6a.

groups at both the 3- and 17β-positions for good recognition of the receptor. Accordingly, the RBA for [$FeCo(CO)_6$-(17α-ethynyl-17β-dehydroxyestradiol)] (**3a**) was measured and found to be only 1.2%; this marked diminution relative to that observed for the dicobalt cluster **2a** is further evidence for the crucial role played by the 17β-OH substituent. Moreover, after incubation of the estradiol receptor in the presence of 1×10^{-8} M of **3a**, addition of tritiated estradiol brings about displacement of **3a**; this demonstrates that the FeCo complex **3a** binds to the estradiol receptor in a reversible manner.

To conclude, these data unequivocally demonstrate the need for both a 17β-OH group and an organometallic group to generate an alkylating functionality at the receptor binding site. When an organometallic unit is located in the α-position relative to the 17β-OH group, the generation of a carbenium ion in the presence of a preexisting acidic group (M^{2+}) is very easy, and the sulfur of a vicinal cysteine is readily and irreversibly linked.[3] The absence of a 17-OH group prevents this process of inactivation of the receptor. Moreover, if the organometallic fragment is directly bonded to the β-face of the steroid, and the molecule cannot rearrange so as to allow access to the 17β-position, then recognition by the specific receptor site is almost completely lost. These latest results provide further confirmation of our previous hypotheses relative to the affinity labeling of the estradiol receptor by 17α-alkynyl–organometallic complexes of 3,17β-estradiol.

Experimental Section

All reactions were carried out under an atmosphere of dry nitrogen. IR spectra were recorded on a Perkin-Elmer 580 B and NMR spectra on a JEOL EX 400 spectrometer. Elemental analyses were carried out at the University of Torino. The syntheses of the complexes **3a–c** were brought about by heating the appropriate alcohol with a 4-fold excess of $Fe(CO)_5$ in refluxing acetone according to the general procedure described previously.[6] [$FeCo(CO)_6$(17α-ethynyl-17β-dehydroxyestradiol)] (**3a**): Anal. Calcd for $C_{28}H_{23}O_7FeCo$: C, 55.54; H, 4.12; Fe, 9.93; Co, 10.48. Found: C, 55.76; H, 4.19; Fe, 9.97; Co, 10.51. It was not possible to crystallize the complex [$FeCo(CO)_6$][$CH_3C_2CH_2$] (**3b**) and obtain satisfactory consistent analyses. However, its formulation is substantiated by the EI mass spectrum recorded on an AEI MS 902 instrument: [M], m/z 336, followed by loss of six CO's. [$FeCo(CO)_6$][HC_2CPhMe] (**3c**, **3c′**): Anal. Calcd for $C_{16}H_9O_6FeCo$: C, 46.64; H, 2.20; Fe, 13.55; Co, 14.30. Found: C, 46.87; H, 2.26; Fe, 13.48; Co, 14.26. [$FeCo(CO)_5(PPh_3)$][CH_3C_2-CH_2] (**4**): Anal. Calcd for $C_{27}H_{20}O_5FeCoP$: C, 56.87; H, 3.54; Fe, 9.79; Co, 10.34. Found: C, 56.98; H, 3.63; Fe, 9.65; Co, 10.25.

Table IV. Crystal Data for 4

formula	$C_{27}H_{20}CoFeO_5P$
mol wt	570.2
cryst syst	triclinic
space group	$P\bar{1}$ (No. 2)
a, Å	10.315(1)[a]
b, Å	10.597(1)
c, Å	12.989(2)
α, deg	67.62(1)
β, deg	89.44(2)
γ, deg	79.30(2)
V, Å^3	1287.1(7)
Z	2
D(calc), g cm^{-3}	1.476
μ(Mo Kα), cm^{-1}	13.05
F(000)	584
T, K	123
radiation	Mo Kα
2θ limits, eg	$5.0 < 2\theta < 45.0$
data collection (hkl)	+10, ±11, ±13
no. of reflns	3458
no. of unique rflns	3240
no. of obsd rflns ($F_o \geq 4\sigma(K_o)$)	2906
R_F[b]	0.063
R_{wF}	0.071
w	$1/[\sigma^2(F_o) + 0.005F_o^2]$

[a] Unit cell dimensions obtained by a least-squares fit of the angular settings of 24 reflections ($20 \leq 2\theta \leq 25°$). [b] $R_F = \sum(|F_o| - |F_c|)/\sum(K_o)$. [c] $R_{wF} = [\sum w(|F_o| - |F_c|)^2/\sum w(F_o)^2]^{1/2}$.

Crystal Structure Determination of [$FeCo(CO)_5$-(PPh_3)($H_3CC_2CH_2$)] (4). Crystallographic data are collected in Table IV. Suitable red crystals were obtained by recrystallization from dichloromethane/n-hexane solution (1:5 v/v). With a crystal of dimensions 0.20 mm × 0.25 mm × 0.37 mm, accurate cell dimensions, at 123 K, were obtained by least-squares refinement of 24 accurately centered reflections ($20 < 2\theta < 25°$) on a Stoe-Siemens four-circle diffractometer equipped with graphite-monochromated Mo radiation. Data collection was performed by employing a 30-step ω/θ scan mode with a step width of 0.025° and step time in the range 1.0–4.0 s/step. No significant variations were observed in the three check reflections during data collection. An empirical absorption correction was based on 416 scan data (transmission factors 1.000 (maximum) and 0.775 (minimum)). The intensity data were converted to F_o after correction for Lorentz and polarization effects.

Structure Solution and Refinement. The space group was found to be $P\bar{1}$ (No. 2) from successful refinement. The positions of the Co and Fe atoms were determined by direct methods (TREF: SHELXTL PLUS).[28] The remaining non-hydrogen atoms were located from subsequent electron density difference syntheses. The structure was refined by full-matrix least squares with all non-hydrogen atoms, except the phenyl carbons, anisotropic. H atoms were placed in idealized positions (d(C–H) = 0.96 Å) and allowed to ride on the relevant carbons; the isotropic displacement parameters were fixed at 0.08 Å^2. The weighting scheme (Table IV) was introduced and gave reasonable agreement analyses. The maximum shift/error in the final cycle of refinement was 0.07, and a difference electron density synthesis showed features only in the range +0.85 to −0.88 Å^{-3}. The final R value is 0.063 from 2906 observed data ($F_o > 4\sigma(F_o)$) for 226 parameters. Atomic scattering factors for neutral atoms were obtained within

(28) SHELXTL PLUS, program version 4.0, Siemens Analytical Intruments, Madison, WI, 1990.

Table V. Atomic Coordinates (×10⁴) and Equivalent Isotropic Displacement Coefficients (Å × 10³)

	x	y	z	U(eq)[a]
Fe(1)	3172(1)	2537(1)	4882(1)	24(1)
Co(1)	1829(1)	4306(1)	3101(1)	21(1)
C(11)	298(6)	3676(5)	3497(4)	26(2)
O(11)	-640(4)	3241(4)	3795(3)	39(2)
C(12)	2484(5)	3653(5)	2063(4)	27(2)
o(12)	2952(4)	3220(4)	1416(3)	40(2)
C(21)	2170(5)	1197(5)	5433(4)	27(2)
O(21)	1554(4)	338(4)	5779(3)	40(2)
C(22)	4277(6)	1746(6)	4107(5)	34(2)
O(22)	5005(4)	1281(4)	3612(3)	43(2)
C(23)	4371(5)	1963(5)	6066(5)	30(2)
O(23)	5107(4)	1590(4)	6836(4)	44(2)
C(1)	3470(5)	4461(5)	3868(4)	25(2)
C(2)	2356(5)	4562(5)	4439(4)	25(2)
C(3)	2008(5)	3997(5)	5528(4)	23(2)
C(4)	4693(6)	5061(6)	3459(5)	37(2)
P(1)	1103(1)	6497(1)	1901(1)	20(1)
C(101)	1545(5)	7898(5)	2283(4)	21(1)
C(102)	1352(5)	7837(5)	3379(4)	24(1)
C(103)	1570(5)	8934(5)	3673(4)	28(1)
C(104)	1980(5)	10086(5)	2891(4)	29(1)
C(105)	2193(5)	10143(5)	1813(4)	28(1)
C(106)	1950(5)	9044(5)	1513(4)	26(1)
C(111)	-724(5)	7023(5)	1616(4)	23(1)
C(112)	-1408(4)	8305(5)	1590(4)	27(1)
C(113)	-2806(5)	8670(6)	1363(5)	34(1)
C(114)	-3506(6)	7767(6)	1139(4)	32(1)
C(115)	-2823(6)	6492(6)	1157(5)	34(1)
C(116)	-1445(5)	6138(6)	1373(4)	28(1)
C(121)	1736(5)	6893(5)	483(4)	22(1)
C(122)	875(5)	7533(5)	-495(4)	29(1)
C(123)	1403(6)	7828(6)	-1542(5)	34(1)
C(124)	2775(5)	7527(6)	-1622(5)	34(1)
C(125)	3629(6)	6898(6)	-644(5)	33(1)
C(126)	3102(5)	6578(5)	401(5)	29(1)

[a] Equivalent isotropic U, defined as one-third of the trace of the orthogoalized U_{ij} tensor.

the program SHELXTL PLUS,[28] and all calculations were performed using SHELXTL PLUS on a Micro-Vax II computer. Final atomic coordinates and equivalent isotropic displacement parameters are listed in Table V.

Biochemical Materials and Methods. Unlabeled steroids, charcoal (Norit A), and protamine sulfate (from salmon, grade X) were from Sigma. [6,7-^{3}H]Estradiol (specific activity 70 Ci/mmol) was purchased from CEA (Saclay, France).

Preparation of Lamb Uterine Cytosol and Binding Assays. Lamb uteri, used as a source of estrogen receptor, were obtained from the slaughterhouse of Mantes-la-Jolie, France, and kept frozen in liquid nitrogen until used. The lamb uteri were thawed and the minced tissues homogenized with an Ultra-Turrax in buffer A (0.05 M Tris-HCl, 0.25 M sucrose, 0.1% 3β-mercaptoethanol, pH 7.4). The homogenate was centrifuged at 40 000 rpm for 35 min in the TFT 65-13 rotor of a Kontron T-1160 ultracentrifuge. Aliquots of the supernatant (usually called cytosol) were incubated for 3 h at 0 °C with [^{3}H]estradiol (6×10^{-9} M) in the presence or absence of competing unlabeled steroids. Competitors were added at concentrations ranging from 7.5×10^{-10} to 8×10^{-8} M for the more efficient competitors and from 6×10^{-8} to 6×10^{-6} M for the less efficient ones. At the end of the incubation, separation of the free and bound fractions of the steroids was achieved by using protamine sulfate precipitation.[29] The bound fractions were collected on glass fiber paper (Whatman GF/C) under light vacuum and washed extensively. The filters were then transferred to a vial containing 10 mL of scintillation fluid (ACS, Amersham), and the radioactivity was measured in an LKB-1211 Rack-Beta scintillation counter. The percentage reduction in binding of [^{3}H]estradiol (Y) was calculated using the logit transformation of Y, where logit $Y = \ln(Y/1 - Y)$), vs the logarithm of the mass of competing steroid. The concentration of unlabeled steroid required to displace 50% of the bound [^{3}H]estradiol was calculated for the steroid tested, and the result is expressed as the relative binding affinity (RBA).

Receptor Inactivation Assays. Aliquots (3×200 μL) of lamb uterine cytosol were first incubated for 2 h at 0 °C in the presence of 1×10^{-8} M of **3a**; then [^{3}H]estradiol (final concentration 2.65×10^{-9} M) was added to each fraction, which was incubated again for 1 h at 25 °C. At the end of this second incubation, the fraction of [^{3}H]estradiol specifically bound to its receptor is measured by protamine sulfate precipitation (*vide supra*). This value corresponds to the fraction of the estradiol receptor reversibly bound to the organometallic hormone in the first incubation. This value is compared to that of a control, *i.e.* aliquots of uterine cytosol (3×200 μL) incubated under the same conditions of time and temperature, but in the presence of tritiated estradiol only.

EHMO Calculations. Molecular orbital calculations were perfomred via the extended Hückel method using weighted H_{ij}'s;[30] drawings were obtained by use of the program CACAO.[31] Orbital parameters were taken from ref 17.

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Supplementary Material Available: Anisotropic displacement parameters (Table S1), hydrogen atom coordinates and isotropic displacement parameters (Table S2), and bond distances and angles (Tables S3 and S4) (4 pages). Ordering information is given on any current masthead page.

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