Syntheses and Affinities of Novel Organometallic-Labeled Estradiol Derivatives: A Structure-Affinity Relationship

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Two series of novel estradiol derivatives, including cationic species, labeled with organometallic fragments $Cr(CO)_3$, Cp^*Ru^+, or Cp^*Rh^{2+} [$Cp^* = \eta^5$-$C_5(CH_3)_5$] either in the 17α-position or on the A-ring were synthesized, and their relative binding affinities (RBA) for the estradiol receptor were determined. The Ru(II) and the Rh(III) cationic derivatives were obtained as stable salts with the following counter anions (BF_4^-, PF_6^-, $CF_3SO_3^-$). The satisfactory RBA values obtained for most complexes belonging to the 17α series confirm that this position tolerates the presence of bulky neutral species. For instance, complex **4**, in which the organometallic fragment $Cr(CO)_3$ was attached to the phenyl ring of the 17α-phenylethynyl fragment, exhibited an RBA value of 24%, very similar to that of the uncomplexed estrogen derivative **3**. Surprisingly, the analogous cationic species **6** had no affinity for the estradiol receptor. This unprecedented result shows that the hormone binding site of the estrogen receptor does not tolerate the presence of a positive charge in the 17α-position of the steroid. On the other hand, the α-face of the A-ring of estradiol did tolerate positively charged organometallic fragments bearing bulky substituents althoug the RBA value tended to decrease with increasing charge. The counterion in these cationic derivatives also affected binding affinity. For instance, the Ru(II) species **7a** containing an $CF_3SO_3^-$ ion exhibited a reasonable RBA value (5.8%) compared to analogous species **13** with a PF_6^- ion (RBA of only 0.1%). Moreover, the triflate counteranion preserved the phenolic form of the A-ring of the estrogen derivative whereas the PF_6^- derivative was unstable and rapidly converted into the dienonylic form in buffer. The compared RBAs of the neutral and cationic species illustrate the preferences of the receptor hormone binding site in accepting or rejecting species of hydrophobic or hydrophilic character.

Introduction

The recognition process between a hormone and its receptor is an intriguing subject for both chemists and biochemists. Although several reports have described the amino acid sequences of various steroid hormone receptors (see refs 1-5 for example), the nature of the molecular interaction between the hormone and the active site at the molecular level is still unknown. However, it has been postulated that for steroid estrogens, the active site of the receptor is of hydrophobic nature.[6] In addition, it is known that the presence of OH groups at both C-3 and C-17 of the steroid molecule are essential for effective binding via hydrogen bonds to the hormone binding site.[7]

In our earlier studies we have shown that some organometallic hormones labeled with chromium carbonyl tripod, cobalt, or molybdenum clusters retain high receptor binding affinity and can be used as tracers for estrogen or progesterone receptor detection in conjunction with Fourier transform infrared spectroscopy[8,9] and as a means of obtaining suitable crystals for a X-ray studies.[10] Since selective complexation of either the α or β face of the estradiol A-ring is, to our knowledge, the only way of introducing a third dimension to the arene planar structure, these molecules could also be used to further our understanding of the hormone-receptor interaction with a view to performing a structure-activity study focussing on stereochemistry and charge. We have thus prepared several new neutral and cationic estradiol derivatives in which an organometallic moiety Cr, Ru, or Rh is bound either to the A-ring or at the 17α-position of the 17β-estradiol molecule and their affinity for the estradiol receptor studied, in vitro. In particular, organometallic fragments such as Cp^*Ru^+ and Cp^*Rh^{2+} ($Cp^* = \eta^5$-$C_5(CH_3)_5$) were used to introduce a positive charge either to the 17α-position or to the A-ring of the estradiol molecule, while preserving the phenolic character of the A-ring. Furthermore, the existence of radioisotopic ruthenium nuclei such as (^{97}Ru, ^{103}Ru), which are γ-ray emitters with half-life period of 2.9 d and 39.4 d, respectively, could extend the utility of these organometallic-labeled estrogens to the areas of nuclear medicine (imagery or therapy).[11]

Results and Discussion

A. Chemistry. (α) 17α-Position. Since previous studies had shown that substitution at the 17α-position of estradiol by bulky organometallic moieties does not

(1) Hollenberg, S. M.; Weinberger, C.; Ong, E. S.; Cerelli, G.; Oro, A.; Lebo, R.; Thompson, E. B.; Rosenfeld, M. G.; Evans, R. M. Primary structure and expression of a functional human glucocorticoid receptor cDNA. *Nature (London)* **1985**, *318*, 635-641.

(2) Green, S.; Walter, P.; Kumar, V.; Krust, A.; Bornert, J. M.; Argos, P.; Chambon P. Human oestrogen receptor cDNA: sequence expression and homology to v-erb A. *Nature (London)* **1986**, *320*, 134-139.

(3) Misahi, M.; Atger, M.; d'Auriol, L.; Loosfelt, H.; Meriel, C.; Fridlansky, F.; Guiochon-Mantel, A.; Galibert, F.; Milgrom, E. Complete amino acid sequence of the human progesterone receptor deduced from cloned cDNA. *Biochem. Biophys. Res. Commun.* **1987**, *143*, 740.

(4) Chang, C.; Kokontis, J.; Liao, S. Structural analysis of complementary DNA and amino acid sequences of human and rat androgen receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7211-7215.

(5) Arriza, J. L.; Weinberger, C.; Cerelli, G.; Glaser, T. M.; Handelin, B. L.; Housman, D. E.; Evans, R. M. Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor. *Science* **1987**, *237*, 268-275.

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(7) Raynaud, J. P.; Ojasoo, T.; Bouton, M. M.; Bignon, E.; Pons, M.; Crastes de Paulet, A. Structure-activity relationships of steroid estrogens. In *Estrogens in the Environment*; McLachlan, J. A., Ed.; Elsevier: Amsterdam, 1985; pp 24-42.

(8) Vessières, A.; Top, S.; Ismail, A. A.; Butler, I. S.; Louer, M.; Jaouen, G. Organometallic estrogens. Synthesis, interaction with uterine estrogen receptor and detection by infrared spectroscopy. *Biochemistry* **1988**, *27*, 6659-6666.

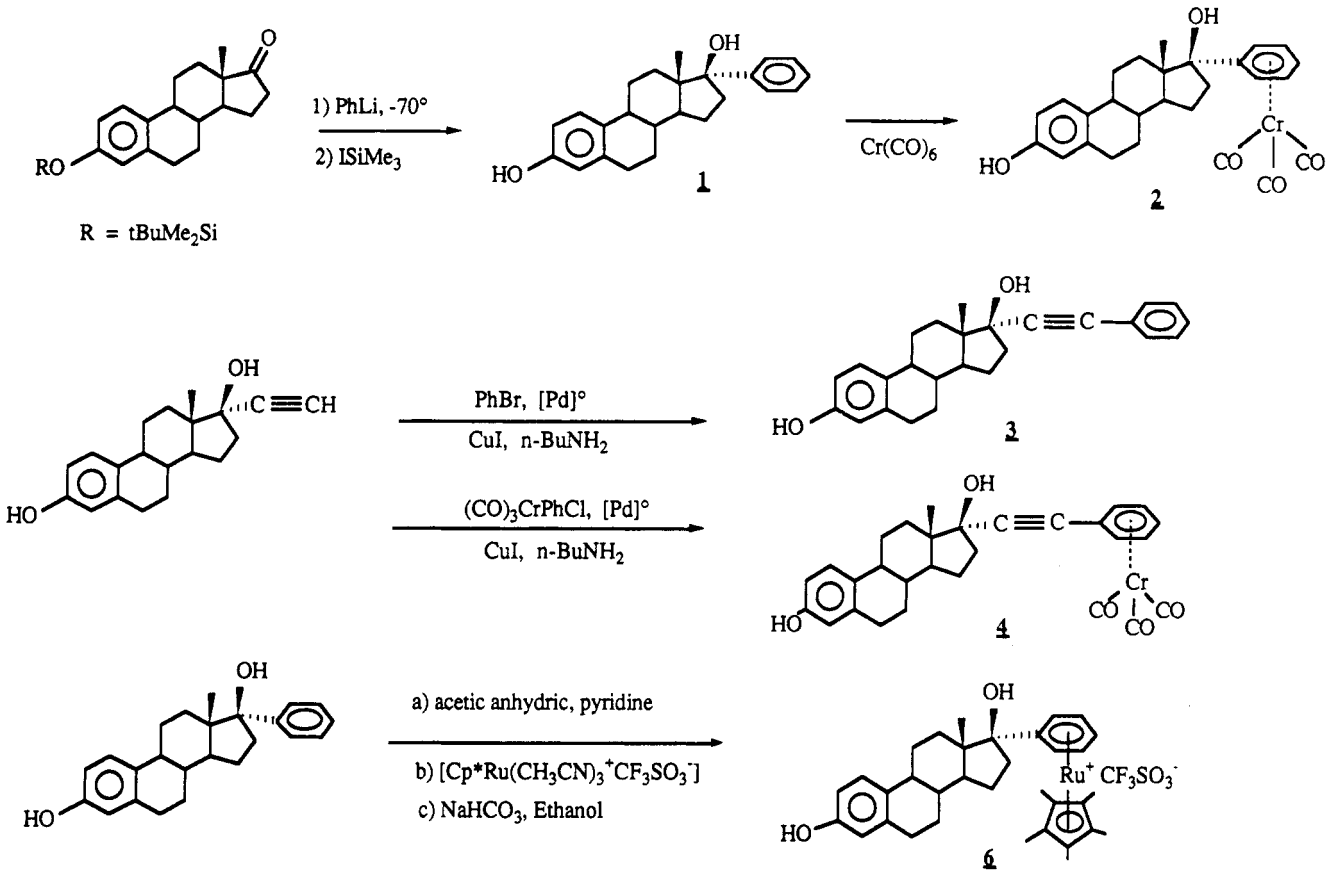
(9) Butler, I. S.; Vessières, A.; Jaouen, G. Application of organotransition metal carbonyl complexes as infrared markers. *Comments Inorg. Chem.* **1989**, *8*, 269-286.

(10) Jaouen, G. *Transition metal organometallics in organic synthesis*; Alper, H., Ed.; Academic Press: New York, 1978; Chapter 2, Vol. 2.

(11) Soine, W. H.; Guyer, C. E.; Knapp, F. F. Synthesis and biological evaluation of radiolabeled β-ruthenocenylalanine. *J. Med. Chem.* **1984**, *24*, 803-806.

0022-2623/92/1835-3130$03.00/0

Scheme I

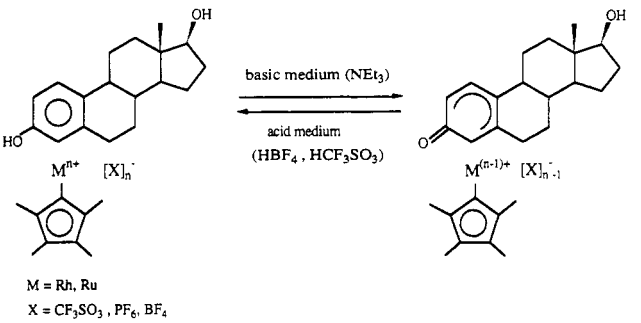


seriously alter binding to the estradiol receptor,[12] we investigated whether other factors such as electronic effects due to charged species might influence binding. Cp^*Ru^+ was introduced selectively into the phenyl ring of 17α-phenyl-17β-estradiol **1** to give [17α-[(Cp*Ru)phenyl]-17β-estradiol][CF_3SO_3] (**6**) in good yield.[13] For comparison purposes, the two neutral species (17α-phenyl-17β-estradiol)tricarbonylchromium (**2**) and [17α-(phenylethynyl)-17β-estradiol]tricarbonylchromium (**4**) were prepared and fully characterized by spectroscopic methods (see Scheme I).

(b) Complexation of the A-Ring by [Cp*M][X]*n*, M = Ru, Rh; X = CF_3SO_3, BF_4, PF_6, *n* = 1, 2. We have already shown that [3-*O*-(hydroxypropyl)-17β-estradiol]-α-tricarbonylchromium (**11a**) is able to recognize the estradiol receptor of lamb uterus cytosol with an RBA of 28% after 3-h incubation at 0 °C.[8] However, because the analog (17β-estradiol)-α-tricarbonylchromium is unstable in buffer, we prepared stable species by complexing the A-ring of the estradiol molecule by cationic fragments of the type $[Cp^*M][X]_n$. Such modification of the acidity of the hydroxyl group at C-3 which plays an essential role during the binding process[7] could affect binding affinity.

The introduction of $[Cp^*M][CF_3SO_3]_n$ on the A-ring of estradiol gives rise to two diastereoisomers (α,β)[Cp*M-(17β-estradiol)][CF_3SO_3]$_n$, M = Ru, *n* = 1: **7a**, **7b**; M = Rh, *n* = 2: **8a**, **8b**, depending on the side of complexation

Scheme II



with an (α,β) ratio (87:13) for the rhodium and (85:15) for the ruthenium derivative.[14,15] The formation of the α-isomer is favored due to the steric effects induced by "Cp*M" and the 13β-Me substituent of the estradiol molecule. [Cp*M(17β-estradiol)][X]$_n$ species were sensitive to the pH of the solution. In the presence of NEt_3 (basic medium) they lose the phenolic character of the A-ring in favor of the corresponding dienonylic form (α,β)[Cp*M(17β-estradienonyl)][X]$_{n-1}$, M = Ru, *n* = 1: **9a**; M = Rh, *n* = 2: **10a**, **10b**, while in the presence of HX, X = CF_3SO_3 (acidic medium), the initial species were recovered (Scheme II). In addition, in strongly coordinating

(12) Vessières, A.; Jaouen, G.; Gruselle, M.; Rossignol, J. L.; Savignac, M.; Top, S.; Greenfield, S. Synthesis and receptor binding of polynuclear organometallic estradiol derivatives. *J. Steroid Biochem.* **1988**, *30*, 301–305.

(13) Vichard, D.; Gruselle, M.; El Amouri, H.; Vaisserman, J.; Jaouen, G. Controlled and selective introduction of "Cp*Ru" to the 17α-position of the 17α-phenyl estradiol (Cp* = C_5Me_5). *Organometallics*, in press.

(14) El Amouri, H.; Gruselle, M.; Jackson, P. A.; Jaouen, G.; Vaisserman, J.: Stabilization of the dienonylic form of the A-ring of β-estradiol by the "Cp*Rh" fragment. X-ray structure of α-[(η^5-estradienonyl)RhCp*]BF_4 (Cp* = C_5Me_5). *Organometallics* **1990**, *9*, 2871–2873.

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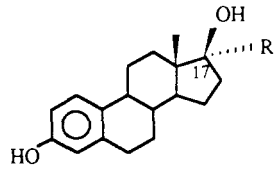
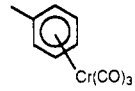
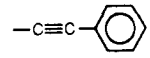
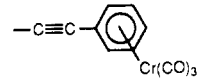
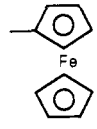
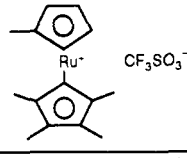
Table I. Structure and Estrogen Receptor Binding Affinity of 17α-Substituted Estradiol Derivatives



compound	R	RBA (%)[a]
1		25
2		11
3		27
4		24
5		15[b]
6		0

[a] Estrogen receptor binding affinity is determined in a competitive radioreceptor binding assay, using lamb uterine cytosol as a source of receptor and [^{3}H]estradiol as a tracer. Bound fractions were measured by protamine sulfate assay. Values are the average of two determinations. [b] Data from ref 19.

basic solvents such as DMSO α-[Cp*Rh(17β-estradiol)]-$[BF_4]_2$ was immediately transformed into the conjugated dienonylic form.[14]

The driving force for the above transformation is related to the high oxidation state of the metal center in the organometallic moiety, which pulls out electronic density from the A-ring and renders the phenolic group more acidic. The pK_a values for 17β-estradiol, **7a**, and **8a** were measured in MeOH/water (75/25) solution by alkalimetric titration using a pH meter and 0.1 N NaOH solution and were found to be 11.8, 7.3, and 2.4, respectively. As expected, the dicationic rhodium species **8a** as well as the monocationic ruthenium derivative **7a** were more acidic than estradiol itself.

B. Biochemistry: Receptor Binding Affinity of the Organometallic Hormones. The relative binding affinities of these species for the estradiol receptor are listed in Tables I and II. These values were determined by a competitive radiometric binding assay using lamb uterine cytosol as a source of receptor, [^{3}H]estradiol as a tracer and protamine sulfate precipitation for the separation of the free and bound fractions of the hormone.

(a) 17α-Position. Previous studies concerning the free ligands have shown that 17α-position fairly tolerates bulky substituents such as phenyl or arylalkynyl groups.[12,16] For example, derivatives **1** and **3** exhibit high relative binding affinity (RBA) values (25 and 27%, respectively) that agree well with the RBA reported by Salman et al.[16] For the organometallic hormones, the highest binding affinities were obtained for the neutral species (17α-phenyl-17β-estradiol)tricarbonylchromium (**2**) (RBA = 11%) and [17α-(phenylethynyl)-17β-estradiol]tricarbonylchromium (**4**) (RBA = 24%). Interestingly, compound **4** had an RBA value similar to that of the free bioligand **3** (as chromium carbonyl complexes are known to decomplex under certain conditions (UV light for example), we have checked by FT-IR spectroscopy that the $Cr(CO)_3$ tripod is still present on the organometallic hormone after protamine sulfate precipitation of the estrogen receptor as described in ref 8). It appears to us that the steric hindrance created by the complexation of the phenyl ring in **4** has no effect on the binding affinity. This is the first example in which an organometallic-labeled hormone displays an RBA value so close to that of the parent molecule. On the other hand, when $Cr(CO)_3$ was complexed directly to a 17α-phenyl group, the RBA value decreases significantly from 25% (RBA of **1**) to 11% (RBA of **2**). Similar results were obtained for 17α-ferrocenyl-17β-estradiol (**5**) (RBA = 15%)[19] but the analogous cationic species of ruthenium (**6**) had no affinity for the estradiol receptor. As the steric hindrance introduced by a Cp*Ru unit is comparable to that of the ferrocenyl group of complex **5**, we feel that the main reason for this loss in affinity is the positive charge of the Cp*Ru fragment. This result suggests that the active site that binds to the 17β-OH rejects cationic species as if it were itself positively charged, thereby implying that the amino acids in this region are protonated in a physiological environment. The presence of protonated amino acids, such as a lysine residue inside or at the vicinity of the active site of the estradiol receptor has already been hypothesized.[17]

(b) A-Ring Complexation. An important feature displayed by these organometallic labeled compounds is the possibility of complexation of either the α or β side of the steroid A-ring. In our mind this is the only way of discriminating between both faces of the molecules and of establishing an A ring stereochemistry–affinity relationship. The results obtained for the α and β ruthenium complexes **7a** and **7b** (Table II) confirm those previously found for the chromium complexes **11a** and **11b** where addition of a π-organometallic adduct on the α face in contrast to the β face of the A-ring does not prevent the binding of the complexes to the receptor. The neutral species **11a** displayed the highest binding affinity (RBA = 28%); the monocationic complex **7a** had an RBA of 5.7%, twice that of the analogous bicationic derivative **8a** with a 3-OH and three times that of the bicationic Rh complexes **12a** with a 3-*O*-hydroxypropyl. Thus, cationic species, in contrast to 17α-substituted derivatives, show reasonable to low affinity values for the estradiol receptor. It seems clear, however, that the increase in positive charge is detrimental to binding affinity. It is also possible that the increase in acidity of the phenolic A-ring of **8a** (pK_a = 2.4 compared to 11.8 for estradiol itself), which is in equilibrium with the corresponding dienonylic form **10a** (vide supra), could explain this low affinity. Indeed, we

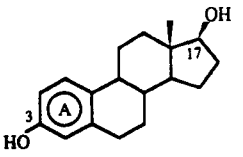
(16) Salman, M.; Reddy, B. R.; Delgado, P.; Stotter, P. L.; Fulcher, L. C.; Chamness, G. C. 17α-Substituted analogs of estradiol for the development of fluorescent estrogen receptor ligands. *Steroids* **1991**, *56*, 375–386.

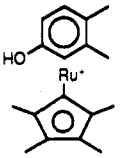
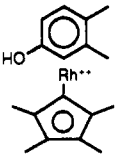
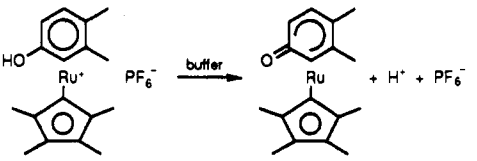
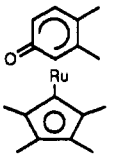
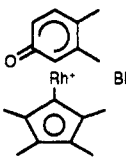
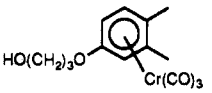
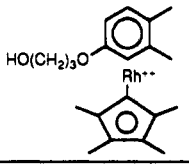
(17) Pakdel, F.; Katzenellenbogen, B. S. Human estrogen receptors mutants with altered estrogen and antiestrogen ligand discrimination. *J. Biol. Chem.* **1992**, *267*, 3429–3437.

(18) Busetta, B.; Hospital, M. Structure cristalline et moléculaire de l'oestradiol hemihydrate. *Acta Crystallogr. Sect. B: Struct. Crystallogr. Cryst. Chem.* **1972**, *B28*, 560–567.

(19) Vessières, A.; Vaillant, C.; Gruselle, M.; Vichard, D.; Jaouen, G. Affinity labelling of estradiol receptor by ferrocenyl tagging of estradiol 17α-position. *J. Chem. Soc. Chem. Commun.* **1990**, *11*, 837–839.

Table II. Structure and Estrogen Receptor Binding Affinity of Hormones with A-Ring Complexed by an Organometallic Moiety



compound	modification of A-ring of estradiol	side of complexation	RBA (%)[a]
7a, 7b		α β	5.8 0
8a		α	2.9
13a		α	0.1
9a		α	0.1
10a,b		α	0.44
11a,b		β α	0.46 28[b]
12a		β α	1.8[b] 1.8

[a] See footnote *a* in Table I. [b] Data from ref 8.

have undertaken an X-ray structural determination of α-[Cp*Rh(η^5-17β-estradienonyl][BF_4] (**10a**), which showed that the A-ring is of a dienonylic form and coordinated to the rhodium center via only five carbons with a typical C3–O3 double bond distance of 1.20 Å.[14] Furthermore, this species does not form any hydrogen bonds with its neighbors in contrast to the free estradiol hemihydrate.[18] Complex α-[Cp*Ru(17β-estradiol][PF_6] (**13a**) isolated in the phenolic form was found to transform easily in buffer solution into the corresponding dienonylic species [Cp*Ru(η^5-17β-estradienonyl)] (**9a**).[20] As expected for compounds having lost the 3-OH group essential for hydrogen bonding with the active site, **13a**, **9a**, and **10a** are poorly recognized by the estrogen receptor (RBA values less than 1%). It is noteworthy that the easy transformation of α-[Cp*Ru(17β-estradiol)][PF_6] (**13a**) is due to the counteranion PF_6^- which does not stabilize the phenolic OH at C-3 as observed for the analogous α-[Cp*Ru(17β-estradiol)][CF_3SO_3] (**7a**) where CF_3SO_3 coordinates to O17 to form infinite chains via intermolecular hydrogen bonding between O3 and O17 of two hormone subunits.[15]

Conclusion

As a general trend, the RBA values of the novel organometallic-labeled hormones described here decrease with presence and increase of positive charge. Furthermore, the introduction of the cationic species Cp^*Ru^+ to the A-ring of the estradiol molecule does not alter seriously the binding to the estradiol receptor as demonstrated by α-[Cp*Ru(17β-estradiol)][CF_3SO_3] (**7a**) (RBA = 5.7%). In contrast, when Cp^*Ru^+ is attached to the 17α-position, a complete loss in binding affinity is observed as illustrated by [17α-[(Cp*Ru)phenyl]-17β-estradiol][CF_3SO_3] (**6**). Interestingly the analogous neutral species (17α-phenyl-

(20) Vichard, D.; Gruselle, M.; El Amouri, H.; Jaouen, G. Synthesis and characterization of estradiol and estradienonyl derivatives of pentamethyl cyclopentadienylruthenium(II). *J. Chem. Soc. Chem. Commun.* **1991**, *1*, 46–48.

17β-estradiol)tricarbonylchromium (2) and [17α-(phenylethynyl)-17β-estradiol]tricarbonylchromium (4) showed good binding affinities. These results imply that the active site pocket responsible for binding to 17β-OH accepts species of hydrophobic character and rejects completely those of cationic nature and confirm the presence of positively charged amino acids, in particular a lysine residue, inside or at the vicinity of the active site pocket.

The complexation of the phenolic A-ring by $[Cp^*M][X]_n$ increases its acidity as indicated by the pK_a values. In a basic medium these species are transformed into the corresponding dienonylic compounds $[Cp^*M(\eta^5\text{-}17\beta\text{-estradienonyl}]^{n+}$, M = Ru ($n$ = 0), Rh (n = 1), **9a** and **10a**. The biochemical properties differed according to their stereochemistry. While the α-isomers exhibited satisfactory binding affinity values, the related β-species showed either low values or were inactive. Loss of the 3-OH function in the dienonylic species led to low RBA values.

Experimental Section

A. Chemistry. Chemicals, Materials, and Methods. Manipulations were carried out using a vacuum line under argon, and employing standard Schlenk techniques. Solvents were purified and dried prior to use by conventional distillation techniques under argon. IR spectra were recorded on a FT Bomem Michelson 100 spectrometer as KBr pellets. ^1H and ^{13}C NMR spectra were recorded on Brucker AM 250-MHz and 200-MHz instrument, and chemical shifts are relative to TMS (^1H, ^{13}C). ^{13}C data are proton-decoupled and reported downfield (positive) with respect to the reference standard. Elemental analyses were performed by the microanalysis department of the CNRS, ICSN, Gif-sur-Yvette, France and of CNRS, Vernaison, France. Syntheses of the following complexes have been described in previous papers: **5**,[19] **6**,[13] **9a**,[20] **10a,b**,[14] **11a,b**.[8]

17α-Phenyl-17β-estradiol (1). Compound **1** has already been prepared,[16] however we have followed a modified synthetic route, as described below. The 3-OH phenol group of estrone was first protected by the dimethyl-*tert*-butylsilyl group using the method previously described for the protection of estradiol.[21] A Schlenk tube containing 30 mL of THF was cooled to −78 °C. A volume of 4 mL (8 mmol) of a solution of phenyllithium in benzene/diethyl ether was added. A solution of 3-(dimethyl-*tert*-butylsiloxy)estrone (0.61 g, 1.6 mmol) in THF (30 mL) was slowly added, dropwise, to the cooled solution of phenyllithium (3 h). The stirring was maintained overnight while the temperature rose gradually to room temperature. Iodotrimethylsilane (5 drops) was then added to remove the protecting group. After 3 h, the mixture was poured into a NH_4Cl solution. After extraction by dichloromethane and solvent removal, the residue was chromatographed on silica gel plates using diethyl ether/pentane (1:1) as eluent. The colorless solid was collected and identified as 17α-phenyl-17β-estradiol (0.22 g, 39.5% yield): mp 225 °C; ^1H NMR (250 MHz, CD_3COCD_3, δ ppm) 7.90 (s, 1 H, 3-OH), 7.46, 7.30, and 7.21 (5 H, Ph), 6.98 (d, J = 8.3 Hz, 1 H, H-1), 6.53 (dd, J = 8.3, 2.6 Hz, 1 H, H-2), 6.49 (d, J = 2.6 Hz, 1 H, H-4), 2.75 (m, 2 H, H-6), 1.08 (s, 3 H, Me-13); mass spectrum (m/z) 348 $[M]^+$, 330 $[M - H_2O]^+$. Anal. ($C_{24}H_{28}O_2$) H, O; C: calcd, 82.80; found, 81.85.

(17α-Phenyl-17β-estradiol)tricarbonylchromium (2). 17α-Phenyl-17β-estradiol (0.17 g, 0.5 mmol) and $Cr(CO)_6$ (0.44 g, 2 mmol) were heated under reflux in dibutyl ether (50 mL) for 3.5 h. After filtration and solvent removal, the residue was chromatographed on silica gel plates using diethyl ether/pentane (3:2) as eluent. The yellow solid obtained was identified as 17α-(tricarbonylchromio)phenyl-17β-estradiol (**2**) (0.07 g, 29% yield): mp 204 °C dec; ^1H NMR (200 MHz, CD_3COCD_3, δ ppm) 8.02 (s, 1 H, 3-OH), 7.02 (d, J = 8.4 Hz, 1 H, H-1), 6.57 (dd, J = 8.4, 2.4 Hz, 1 H, H-2), 6.51 (d, J = 2.4 Hz, 1 H, H-4), 5.95 (d, J = 6.8 Hz, 1 H) and 5.62 (m, 4 H) (Ph), 4.02 (s, 1 H, 17-OH), 2.76 (m, 2 H, H-6), 1.08 (s, 3 H, Me-13); mass spectrum (m/z) 484 $[M]^+$, 428 $[M - 2CO]^+$, 400 $[M - 3CO]^+$, 348 $[M - Cr(CO)_3]^+$, 330 $[M - Cr(CO)_3 - H_2O]^+$.

17α-(Phenylethynyl)-17β-estradiol (3). Compound **3** has already been prepared,[16] however we have followed a modified synthetic route, as described below. This estrogen derivative **3** was prepared by the coupling method catalyzed by palladium(0), following the procedure described in ref 22. The reaction of 17α-ethynyl-17β-estradiol and bromobenzene gave 17α-(phenylethynyl)-17β-estradiol in a yield of 19%: mp 164 °C (lit.[16] 156–158 °C); ^1H NMR (200 MHz, CD_3COCD_3, δ ppm) 7.97 (s, 1 H, 3-OH), 7.42 and 7.33 (m and m, 2 H and 3 H, Ph), 7.11 (d, J = 8.3 Hz, 1 H, H-1), 6.61 (dd, J = 8.3, 2.5 Hz, 1 H, H-2), 6.54 (d, J = 2.5 Hz, 1 H, H-4), 4.49 (s, 1 H, 17-OH), 2.77 (m, 2 H, H-6), 0.94 (s, 3 H, Me-13); mass spectrum (m/z) 372 $[M]^+$, 253 $[M - H_2O - (C{\equiv}CPh)]^+$. Anal. ($C_{26}H_{28}O_2$) H; C: calcd, 83.37; found, 82.65.

[17α-(Phenylethynyl)-17β-estradiol]tricarbonylchromium (4). Owing to the low yield obtained for the free hormone **3**, direct complexation between **3** and $Cr(CO)_6$ was avoided and complex **4** was also prepared by the coupling method catalyzed by palladium(0) that was used to obtain **3**. The mixture of α-ethynylestradiol (0.296 g, 1 mmol), chlorobenzene tricarbonylchromium (0.747 g, 3 mmol),[23] tetrakis(triphenylphosphine)palladium (0.057 g, 0.05 mmol), CuI (0.010 g, 0.05 mmol), and *n*-butylamine (0.110 g, 1.5 mmol) in 30 mL of benzene was stirred at 70 °C for 20 h. The benzene was then evaporated under vacuum, and the products were extracted by diethyl ether. After filtration and evaporation of solvent, the residue was chromatographed on silica gel plates using diethyl ether/pentane (2:1) as eluent. 17α-[$(Cr(CO)_3$-phenylethynyl]-17β-estradiol was obtained as a yellow solid in a 24% yield (0.120 g): mp 196 °C; ^1H NMR (250 MHz, CD_3COCD_3, δ ppm), 7.94 (s, 1 H, 3-OH), 7.10 (d, J = 8.4 Hz, 1 H, H-1), 6.59 (dd, J = 8.4, 2.7 Hz, 1 H, H-2), 6.52 (d, J = 2.7 Hz, 1 H, H-4), 5.72 and 5.57 (m and s, 4 H and 1 H, Ph), 4.59 (s, 1 H, 17-OH), 2.75 (m, 2 H, H-6), 0.93 (s, 3 H, Me-13); mass spectrum (m/z) 508 $[M]^+$, 452 $[M - 2CO]^+$, 424 $[M - 3CO]^+$, 372 $[M - Cr(CO)_3]^+$. Anal. ($C_{29}H_{28}O_5Cr$) C, H.

(α,β)-[Cp*Ru(17β-estradiol)][CF_3SO_3] (7a, 7b). An amount of 0.22 g (0.72 mmol) of crystallized $[Cp^*RuCl]_4$ was maintained under reflux for 1 h in a mixture of CH_3CN/THF(10/10 mL) under argon. AgOTf (0.22 g, 0.864 mmol) was then added, and the yellow solution was filtered. To this solution was added 17β-estradiol (0.24 g, 0.936 mmol) in 10 mL of THF. After 12 h of reflux, the mixture was concentrated and complex **7a** (0.26 g, 0.38 mmol) was precipitated by addition of 40 mL of ether. **7b** (0.06 g, 0.091 mmol) was separated by fractional crystallization of the supernatent phase. **7a**: IR (KBr, cm^{-1}) 3415 (vs, ν OH associated), 1547-1466–1471 (vs, phenyl ring), 1285–1260 (vs), 1031 (vs), 638 (vs, CF_3SO_3); ^1H NMR (250 MHz, CD_3CN, δ ppm) 5.72 (d, J = 7.5 Hz, 1 H, H-1), 5.5 (dd, J = 7.5, 2.5 Hz, 1 H, H-2), 5.42 (d, J = 2.5 Hz, 1 H, H-4), 1.81 (s, 15 H, Cp*), 0.7 (s, 3 H, Me-18). **7b**: ^1H NMR (250 MHz, CD_3CN, δ ppm) 5.69 (d, J = 7.5 Hz, 1 H, H-1), 5.33 (dd, J = 7.5, 2.5 Hz, 1 H, H-2), 5.43 (d, J = 2.5 Hz, 1 H, H-4), 1.87 (s, 15 H, Cp*), 0.8 (s, 3 H, Me-18). Anal. ($C_{29}H_{39}O_5SF_3Ru$) C, H.

(α,β)-[Cp*Rh(17β-estradiol)][$CF_3SO_3]_2$ (8a, 8b). The preparation of **8a**, **8b** was carried out following the procedure described in ref 14. An amount of 0.257 g (1 mmol) of $AgCF_3SO_3$ in THF solution was added to an orange solution (0.154 g, 0.25 mmol) of $[Cp^*RhCl_2]_2$ in acetone. A white precipitate identified as AgCl suddenly formed, and the color of the mixture changed to yellow. The yellow solution of $[Cp^*Rh(solvent)_3][CF_3SO_3]_2$ was filtered off into a dry Schlenk tube. An amount of 0.136 g (0.5 mmol) of 17β-estradiol in 10 mL of THF was added dropwise via a canula to this solution, and the reaction mixture was left for 3 h. At this stage the reaction was stopped, and the reaction mixture was concentrated under vacuo. Addition of 20 mL of

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ether gave an oily precipitate which was separated, washed several times with ether, and dried under vacuum to give a light yellow powder identified as α-[Cp*Rh(17β-estradiol)][CF_3SO_3] (**8a**), while the supernatant phase gave the β-isomer **8b**. Overall yield (0.320 g, 79%) with α,β ratio 87:13. **8a**: (250 MHz, ^{1}H NMR, CD_3OD, δ ppm) 7.03 (d, J = 7.5 Hz, 1 H, H-1), 6.32 (dd, J = 7.5, 2.5 Hz, 1 H, H-2), 6.27 (d, 1 H, H-4), 0.8 (s, 3 H, Me-18), 2.09 (s, 15 H, Cp*). Anal. Calcd for $C_{30}H_{39}O_8F_6S_2Rh$: C 44.55, H 4.82; found: C 43.83; H 5.24. **8b**: (250 MHz, ^{1}H NMR, CD_3OD, δ ppm) 6.92 (d, J = 7.5 Hz, 1 H, H-1), 6.00 (dd, J = 7.5, 2.5 Hz, 1 H, H-2), 6.13 (d, J = 2.5 Hz, 1 H, H-4), 0.89 (s, 3 H, Me-18), 2.12 (s, 15 H, Cp*).

α-[Cp*Rh(3-*O*-(hydroxypropyl)-17β-estradiol)][BF_4]$_2$ (12a). As described for **8a**, [Cp*Rh(solvent)$_3$][BF_4]$_2$ was prepared in situ by mixing (0.16 g, 0.25 mmol) of [Cp*$RhCl_2$]$_2$ in acetone with $AgBF_4$ (0.200 g, 1 mmol) in THF solution. The resulting yellow solution was filtered into a new Schlenk tube. An amount of 0.165 g (0.5 mmol) of 3-*O*-(hydroxypropyl)estradiol in THF was added to this solution, and the reaction mixture was left for 2 h after which the light yellow solution was concentrated under vacuum. Addition of diethyl ether gave a creamy precipitate which was filtered, washed several times with diethyl ether, and then dried under vacuum. Identified as α-[Cp*Rh(3-*O*-(hydroxypropyl)-17β-estradiol)][BF_4]$_2$ (**12a**). Overall yield was 0.230 g, 62%. The supernatant phase gave the β-isomer **12b** (α,β ratio 87:13). **12a**: (250 MHz, ^{1}H NMR, CD_3COCD_3, δ ppm) 7.50 (d, J = 6.5 Hz, 1 H, H-1), 7.35 (dd, J = 6.5, 2.5 Hz, 1 H, H-2), 7.40 (d, J = 2.5 Hz, 1 H, H-4), 2.29 (s, 15 H, Cp*), 0.77 (s, 3 H, Me-18).

B. Receptor Binding Assays. Biochemicals, Materials, and Methods. Unlabeled steroids and protamine sulfate (from salmon grade X) were obtained from Sigma. [6,7-^{3}H]-17β-estradiol (52 Ci/mmol) was obtained from CEA Gif/Yvette, France.

Animal Tissues. Lamb uteri weighing approximately 7 g were obtained from the slaughterhouse at Mantes-la-Jolie, France. They were immediately frozen and kept frozen in liquid nitrogen prior to use.

Preparation of Lamb Uterine Cytosol. Lamb uteri were thawed and then minced. The resulting tissues were homogenized with an Ultra-Turrax in buffer A (0.05 M Tris-HCl 0.25 M sucrose, 0.1% β-mercaptoethanol, pH 7.4 at 25 °C). The homogenate was centrifuged at 105000g for 60 min in the 52 Ti rotor of a Beckman L5 ultracentrifuge. The protein concentration of the 105000g supernatent (cytosol) was determined by the method of Bradford.[24]

Protamine Sulfate Precipitation Assay. This technique was used for the separation of [^{3}H]-bound and free steroid.[8] Following incubation of aliquots of cytosol (200 μL containing 5 mg of protein/mL) with [^{3}H]estradiol, an equal volume of buffer containing 2.5 mg/mL protamine sulfate was added to each tube. The mixture was vortexed and allowed to stand at 0 °C for 10 min. The precipitate was filtered on glass fiber paper (Whatman GF/C) under light vacuum and washed with 40 mL of ice-cold buffer. The filter was then transferred to a scintillation vial and counted in 10 mL of ACS (Amersham). Radioactivity was measured in a LKB-1211 RackBeta with a counting efficiency of 30–40%.

Competitive Binding Assays. Aliquots of cytosol (200 μL) were incubated for 3 h at 0 °C with 2×10^{-9} M [^{3}H]estradiol in the presence or absence of competing unlabeled steroids (nine concentrations ranging from 1×10^{-10} to 1×10^{-6} M). Starting from a stock solution (10^{-3} M in absolute ethanol, serial dilutions of unlabeled steroids were performed in absolute ethanol just prior use. The percentage reduction in binding of [^{3}H]-labeled steroid (Y) was calculated by use of the logit transformation of Y.[8] The relative binding affinity (RBA) of the competitor is taken as the ratio of the concentrations of unlabeled estradiol/competitor required to inhibit half of the specific [^{3}H]-estradiol binding with the affinity of estradiol set at 100%.

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Synthetic Nonpsychotropic Cannabinoids with Potent Antiinflammatory, Analgesic, and Leukocyte Antiadhesion Activities

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Two strategies for the design of therapeutically useful cannabinoids have been combined to produce compounds with greatly increased antiinflammatory activity and with a low potential for adverse side effects. Enantiomeric cannabinoids with a carboxylic acid group at position 7 and with an elongated and branched alkyl sidechain at position 5′ have been synthesized and tested for antiinflammatory activity. They were effective when given orally at doses of 10 μg/kg in reducing paw edema in mice that had been induced by either arachidonic acid or platelet activating factor. Leukocyte adhesion to culture dishes was also reduced in peritoneal cells from mice in which the cannabinoids were orally administered in the same dose range as for the paw edema tests. Antinociception could be observed in the mouse hot plate assay; however, little stereochemical preference was seen in contrast to the above tests where the 3*R*,4*R* compounds are more active than the 3*S*,4*S* enantiomers. Finally, in agreement with earlier reports on the naturally occurring pentyl side chain acids, the synthetic acids showed little activity in producing catalepsy in the mouse, suggesting that they would be nonpsychtropic in humans.

Δ^1-Tetrahydrocannabinol (THC), the psychoactive component of marihuana, has been reported to exhibit activities in addition to its mood altering effects some of which may have therapeutic value.[1] Much effort has been expended over the years in seeking analogues that retain the properties of medicinal value and are devoid of the psychotropic effects. Novel approaches to this problem were recently reported by us. In one of them we showed

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