Synthesis of 17α-Ruthenocenyl-17β-oestradiol and its Potential as a Radiopharmaceutical Agent

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The synthesis of 17α-ruthenocenyl-17β-oestradiol and results of biochemical tests to determine its suitability as a radiopharmaceutical agent, are reported. 17α-Ruthenocyl-17β-oestradiol was obtained, in an overall yield of 29%, by addition of ruthenocenyl-lithium (prepared by treatment of ruthenocene with t-butyl-lithium) to the ketone function of protected oestrone, followed by the deprotection of the 3-OH function. It was characterized by X-ray crystallography: space group *P* 2_1 (monoclinic), *a* = 9.150(2) Å, *b* = 11.806(4) Å, *c* = 12.193(3) Å, β = 94.56(2)°, *V* = 1313(2) Å^3, *Z* = 2. The relative binding affinity (RBA) of this complex for the oestradiol-specific receptor was compared with that of oestradiol. 17α-Ruthenocyl-17β-oestradiol is still recognized by the oestradiol receptor with an RBA of 2%. Unlike its analogue, 17α-propynyl-$Co_2(CO)_6$-17β-oestradiol, it does not act as an affinity marker for the oestradiol receptor. This may be explained by the relative stability of the carbenium ion generated from it, which has a pK_{R^+} value of +0.73. 17α-Ruthenocyl-17β-oestradiol is however of potential interest as a radiopharmaceutical agent since ruthenium has radioactive isotopes emitting β- and γ-radiation useful in nuclear medicine. © 1997 John Wiley & Sons, Ltd.

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INTRODUCTION

Bio-organometallic synthesis (a term used first, to our knowledge, in Ref. 1) is proving to be one of the important future directions for the organometallic chemistry of transition metals.[2] Uses of bio-organometallics in the fields of immunology, receptorology and bioconversion, and even in the analysis of nucleotides, have been reported.[3, 4] In relation to the development of anti-oestrogens and aromatase inhibitors to treat oestrogen-dependent tumours, it has become clear following the pioneering work of Hochberg[5] that methods are needed to determine 'the receptor status of tumours and to localize oestrogen receptor-containing tumours'.[6] Derivatives of oestradiol, labelled either with gamma emitters such as iodine-131 and -123, or with the positron-emitting fluorine-18 isotope, have been prepared for this purpose.[7–11] These compounds bind reversibly to the receptor under study with equilibrium constants, K_d, on the order of 10^{-9}–10^{-10} M. The residence time of the imag-

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ing agent on the primary hormone-dependent tumour could be noticeably increased if the aforementioned equilibration could be eliminated by using radiopharmaceuticals capable of forming a covalent bond with the receptor.

It has been shown that the introduction of an organometallic complex at the 17α position of oestradiol leads, in specific cases, to complexes that act as affinity markers for the oestrogen receptor.[12–14] It would therefore seem to be an interesting prospect to study the hormone complex of ruthenium, which would possess the two characteristics mentioned above. In fact, the derivatives of ruthenocene containing [^{97}Ru] or [^{103}Ru] offer 'the desirable stable combination of a γ-emitter tightly bound to an aromatic organic moiety capable of considerable variation'.[15] Curiously, despite its potential and the pioneering work of Wenzel,[16–19] the radiochemistry of organometallic compounds[20] has undergone very little development in recent years,[21] owing to the difficulty in finding a stable combination of a γ-emitter and an organic vector with specific activity for a target organ.

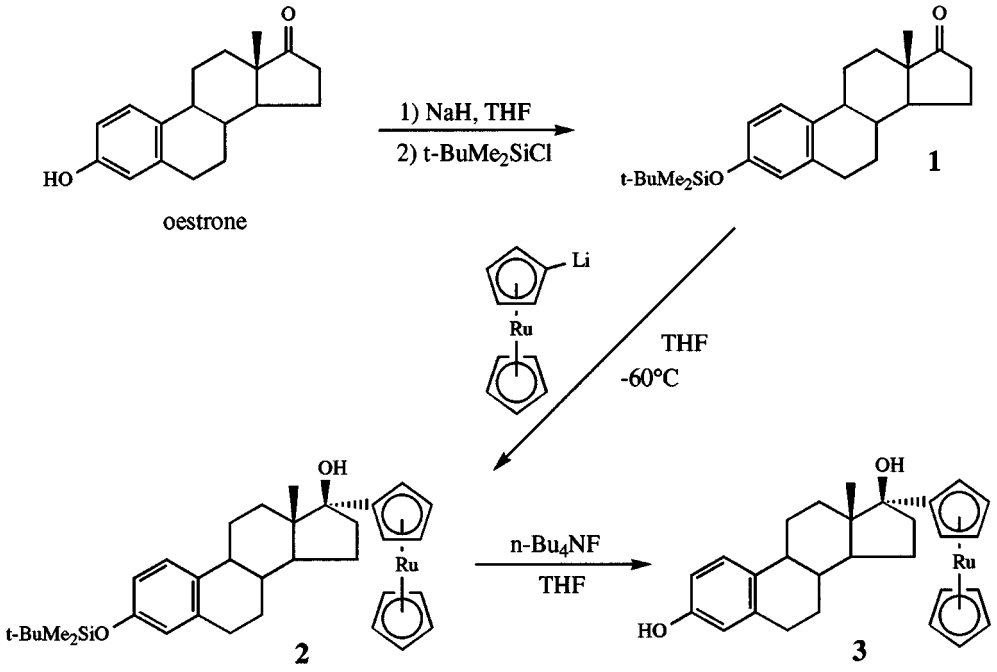
Here we describe the synthesis of 17α-ruthenocenyl-17β-oestradiol and report studies on its suitability as a radiopharmaceutical agent.

RESULTS AND DISCUSSION

Synthesis

In principle, 17α-ruthenocenyl-17β-oestradiol, **3**, can be prepared by the addition reaction of ruthenocenyl-lithium on the ketone function of an oestrone derivative (Scheme 1), a route necessitating a good method for generating the ruthenocenyl-lithium. The first lithiation of ruthenocene using n-butyl-lithium in hexane and in the presence of *N,N,N′,N′*-tetramethyl-ethylene-diamine (TMEDA) gave a mixture of mono- and di-ruthenocenyl-lithium.[22] This type of metallation has been extensively studied in the ferrocene series, and this latter study provided the information that the monolithiation of ferrocene by t-butyl-lithium in tetrahydrofuran (THF) at 0 °C, a method developed by Kagan and coworkers, is the most effective.[23] This method proved to be equally applicable for ruthenocene.[24, 25] We found that generation of ruthenocenyl-lithium by t-butyl-lithium is suitable for the reaction with the oestrone derivative. Scheme 1 shows the synthetic route to 17α-ruthenocenyl-17β-oestradiol, **3**.

The first step in the synthesis is to protect the



Scheme 1 Synthesis of 17α-ruthenocenyl-17β-oestradiol (**3**).

phenol function of the oestrone with the t-butyldimethylsilyl group. Ruthenocenyl-lithium, prepared by direct lithiation of the ruthenocene using t-butyl-lithium in THF at 0 °C, is then allowed to react with the protected oestrone at − 60 °C. The first product is the protected 17α-ruthenocenyloestradiol, **2**, which is then deprotected by reaction with n-Bu_4NF to give 17α-ruthenocenyloestradiol, **3**, with a overall yield of 29%. Despite efforts to refine and adjust the parameters of the reaction, it was not possible to increase the final yield. For addition reactions of organolithium compounds with oestrone, a low yield seems to be a general rule, as 17α-[η^6-C_6H_5)$Cr(CO)_3$]-17β-oestradiol **5**[26] and 17α-ferrocenyl-17β-oestradiol **4**[27] were also obtained in similar yields. It has been noted that the addition reaction on oestrone leads almost exclusively to the α isomer, because the methyl in the 13β position appears to prevent the reagent from reaching the β face of the steroid.[28–30] This selectivity has been confirmed by NMR spectroscopy, where the spectrum does indeed show the presence of a single isomer, identified by X-ray structural analysis as the α isomer.

Table 1 Summary of crystallographic data for **3**

Chemical formula	$C_{28}H_{32}O_2Ru,C_3H_6O$
Formula weight	559.7
Crystal system	Monoclinic
Space group	$p2_1$
Z	2
a (Å)	9·150(2)
b (Å)	11.806(4)
c (Å)	12.193(3)
β (deg)	94.56(2)
V (Å^3)	1313(2)
F(000)	584
ρ (calcd) (g cm^{-3})	1.42
μ (Mo Kα) (cm^{-1})	6.13
Diffractometer	CAD4
Monochromator	Graphite
Radiation	Mo Kα (0.71070)
Temperature (°C)	20
Scan type	$\omega/2\theta$
Scan range, θ (deg)	0.8 + 0.34 tan θ
2θ range (deg)	3–50
No. of reflections collected	2424
No. of reflections used (criteria)	1428(I>2σ(I))
R	0.045
R_w	0.052
Absorption correction[b]	Min, 0.82, max. 1.31
Weighting scheme	Unit weights
Rms (shift/ESD) (last ref.)	0.18
Least-squares parameters	318

[a] $R_w = [\sum_i W_i(F_0 - F_c)^2/\sum_i W_i F_0^2]^{1/2}$
[b] Difabs (Ref. 47).

Crystallographic structure of 17α-ruthenocenyl-17β-oestradiol, 3

It is well known that the 17β OH isomer of oestradiol has much higher recognition by the specific receptor than the substituted 17α isomer.[31] It is therefore important to confirm the structure of the complex formed. X-ray diffraction techniques were used to determine the structure of **3**. Slow evaporation of the solution of the compound in acetone gave monoclinic

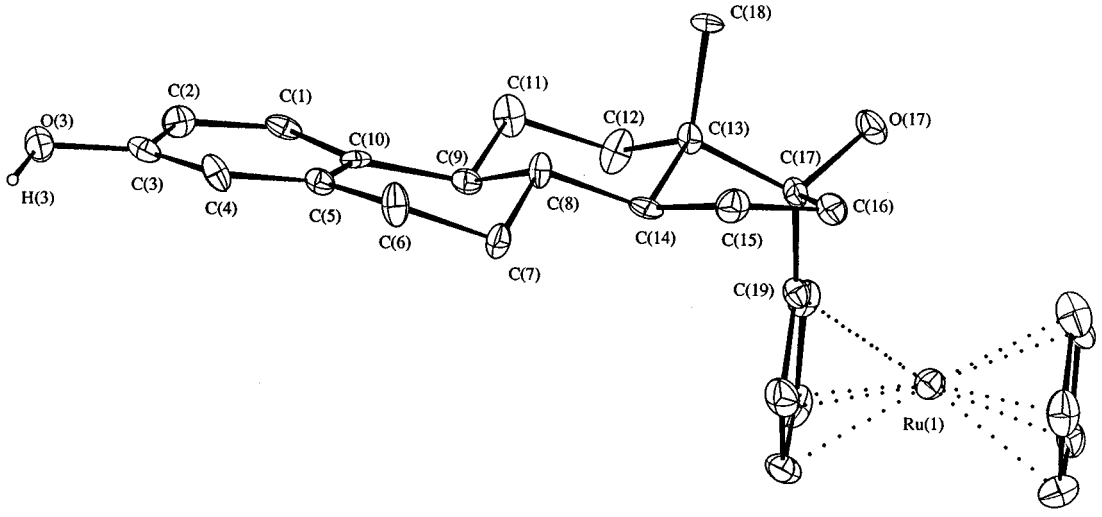


Figure 1 ORTEP drawing of 17α-ruthenocenyl-17β-oestradiol (**3**).

Table 2 Interatomic distances of **3**

Atoms	Distance (Å)	Atoms	Distance (Å)
C(1)–C(2)	1.38(2)	C(1)–C(10)	1.39(2)
C(2)–C(3)	1.38(2)	C(3)–O(3)	1.38(2)
C(3)–C(4)	1.37(2)	C(4)–C(5)	1.38(2)
C(5)–C(6)	1.52(2)	C(5)–C(10)	1.40(2)
C(6)–C(7)	1.50(2)	C(7)–C(8)	1.54(2)
C(8)–C(9)	1.55(2)	C(8)–C(14)	1.52(2)
C(9)–C(10)	1.51(2)	C(9)–C(11)	1.56(2)
C(11)–C(12)	1.51(2)	C(12)–C(13)	1.56(2)
C(13)–C(14)	1.58(2)	C(13)–C(17)	1.54(2)
C(13)–C(18)	1.56(2)	C(14)–C(15)	1.51(2)
C(15)–C(16)	1.54(2)	C(16)–C(17)	1.57(2)
C(17)–O(17)	1.47(1)	C(17)–C(19)	1.53(2)
Ru(1)–C(19)	2.16(1)		

crystals with a $P2_1$ space group. Crystallographic data are shown in Table 1, an ORTEP diagram in Fig. 1, and interatomic distances and bond angles are given in Tables 2 and 3.

Figure 1 shows clearly that the ruthenocenyl group is in position α as predicted. The skeleton of the steroid does not show any obvious deformation as compared with that of 17β-oestradiol.[32] The ruthenium atom lies centrally between two cyclopentadienyl rings, and the average C–Ru distance is 2.17 Å (2.15–2.20 Å). This distance is similar to that of ruthenocene (average 2.186 Å).[33] There is a close similarity between the structure of **3** and that of 17α-ferrocenyl-17β-oestradiol as obtained by Vichard *et al*.[27] As in 17α-ferrocenyl-17β-oestradiol, the ruthenocenyl group lies outside the hormone skeleton, no doubt to minimize interactions between the cyclopentadienyl and the D ring. It will be noted that the C(19)–C(17)–O(17) angle [104.1(10)°] is smaller than that for 17α-ferrocenyl-17β-oestradiol [107.3(8)°]. Moreover, the average C–Ru distance is 2.17 Å, whereas the average C–Fe distance in 17α-ferrocenyl-17β-oestradiol is 2.03 Å, reflecting the fact that the ruthenocenyl group is bulkier than the ferrocenyl group.

Figure 2 shows that 17α-ruthenocenyl-17β-oestradiol crystallizes with one molecule of solvent, here acetone. It is interesting to note that there is a hydrogen bond between the 3-hydroxyl and the 17-hydroxyl of another molecule. The O(17)–H(3)–O(3) angle is 159° and the O(17)–O(3) distance is 2.79 Å. The distance is similar to that found for oestradiol (2.77 Å)[28–30] and 17α-ferrocenyl-17β-oestradiol (2.80 Å).[27]

Affinity for the oestrogen receptor

The first question to be answered for a new modified hormone is its level of recognition by the specific receptor, in this case that of 17β-oestradiol. The recognition level is quantified as the relative binding affinity (RBA), a value that is measured competitively, comparing the extent to which the modified hormone and 17β-oestradiol bind to the receptor.[34] With the RBA value of 17β-oestradiol set by definition at 100%. 17α-ruthenocenyl-17β-oestradiol, **3**, has an RBA of

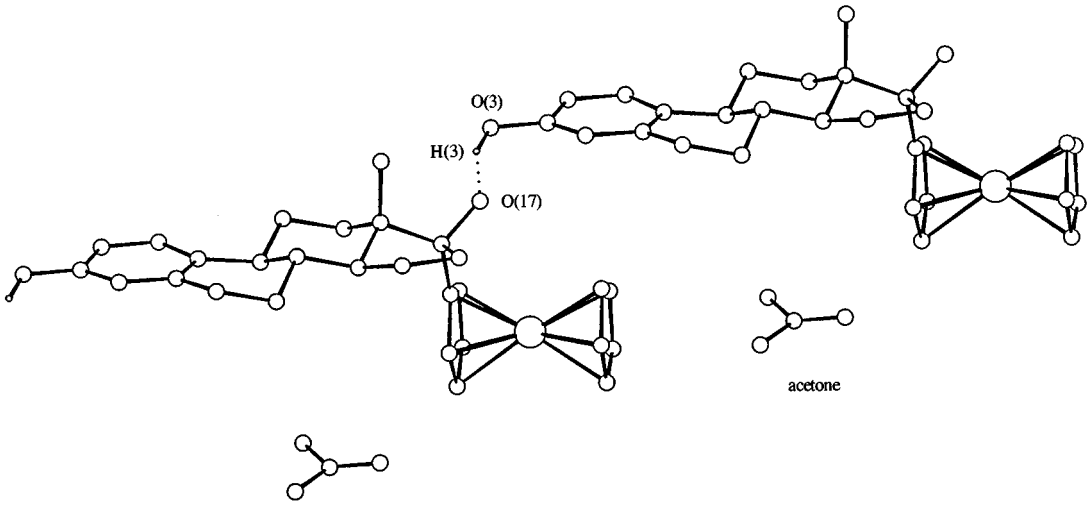


Figure 2 View of the packing of **3** in the solid state showing the intermolecular O–H···O hydrogen bonds which link the monomers.

2%. It is clear that the metal group lowers the recognition level markedly. This value can be compared with those of other hormones modified at position 17α. These data are collected in Table 4.

The RBA value of 17α-ruthenocenyl-17β-oestradiol, **3**, can be compared with those of 17α-ferrocenyl-17β-oestradiol, **4**, and in 17α-[η^6-C_6H_5)Cr(CO)$_3$]-17β-oestradiol, **5**, to the extent that the metal group is attached in the same position. It can be seen that **3** has a RBA value of 2%, and that **4** and **5** have values of 8% and 11%, respectively.[26, 37, 38] Of these three compounds, **3** has the lowest recognition level, the spread probably being due to the difference in the effect of steric crowding since the ruthenocenyl group is larger than the ferrocenyl group (average C–Ru and C–Fe distances are 2.17 Å and 2.03 Å, respectively). RBA values of 16% for 17α-[(–C≡C–η^5-C_5H_4)Re(CO)$_3$]-17β-oestradiol (**6**)[36] and 24% for 17α-[(–C≡C–η^6-C_6H_5)Cr(CO)$_3$]-17β-oestradiol (**7**)[26] show that the addition of a rigid spacer to position the metal group slightly further from the hormone skeleton, as well as the presence of an acetylene bond, improves the affinity of these compounds.

It has been suggested that 17α-ferrocenyl-17β-oestradiol, **4**, is an affinity marker, i.e. that it is capable of forming a covalent bond with the nuclear receptor of the hormone.[13] But this has never been proved by using the radioactive compound. However, the 17α-propynyl-17β-oestradiol complexed by the $Co_2(CO)_6$ unit **8** has been proved to act as an affinity marker.[14] The most likely hypothesis to account for this affinity marking is the presence of an acid site such as Zn^{2+} in the vicinity of the hormone–receptor association domain, giving rise to a carbenium ion in position α of the organometallic fragment and permitting the alkylation of a nucleophilic entity nearby.[12] If this is so, **3** could also act as an affinity marker, since its tendency to generate a carbenium ion is presumably comparable with or even superior than that of **4**. In this case the low RBA value of **3** would not be an impediment, to the extent that an irreversible covalent bond with the receptor is formed; moreover, the greater stability of **3** compared with the ferrocene derivative provides a further advantage, assuming of course that its stability is not so great as to be a detriment to its reactivity.

Another important point that must be considered is the specificity of the modified hormone in binding to the receptor at the sites specific for 17β-oestradiol. It has been shown that the specificity is inversely linked to the lipophilicity of the hormone.[37, 38] The lipophilicity of a compound can be assessed by its octanol/water partition coefficient (log $P_{o/w}$), measured by the

Table 3 Bond angles and fractional parameters of **3**
(a) Bond angles

Atoms	Angle (deg)	Atoms	Angle (deg)
		C(10)–C(1)–C(2)	121.4(12)
C(3)–C(2)–C(1)	120.9(13)	O(3)–C(3)–C(2)	118.7(14)
C(4)–C(3)–C(2)	117.7(12)	C(4)–C(3)–O(3)	123.5(13)
C(5)–C(4)–C(3)	122.3(12)	C(6)–C(5)–C(4)	118.9(11)
C(10)–C(5)–C(4)	120.0(11)	C(10)–C(5)–C(6)	120.9(10)
C(7)–C(6)–C(5)	113.6(12)	C(8)–C(7)–C(6)	111.2(11)
C(9)–C(8)–C(7)	107.3(10)	C(14)–C(8)–C(7)	109.3(11)
C(14)–C(8)–C(9)	107.9(11)	C(10)–C(9)–C(8)	111.8(10)
C(11)–C(9)–C(8)	109.2(10)	C(11)–C(9)–C(10)	115.6(10)
C(5)–C(10)–C(1)	117.2(11)	C(9)–C(10)–C(1)	121.8(11)
C(9)–C(10)–C(5)	120.7(10)	C(12)–C(11)–C(9)	111.9(12)
C(13)–C(12)–C(11)	109.3(13)	C(14)–C(13)–C(12)	107.4(12)
C(17)–C(13)–C(12)	115.7(12)	C(17)–C(13)–C(14)	98.0(10)
C(18)–C(13)–C(12)	113.1(13)	C(18)–C(13)–C(14)	113.1(12)
C(18)–C(13)–C(17)	108.7(11)	C(13)–C(14)–C(8)	110.9(11)
C(15)–C(14)–C(8)	118.7(12)	C(15)–C(14)–C(13)	102.5(10)
C(16)–C(15)–C(14)	105.0(12)	C(17)–C(16)–C(15)	104.8(11)
C(16)–C(17)–C(13)	104.2(11)	O(17)–C(17)–C(13)	112.2(10)
O(17)–C(17)–C(16)	108.4(11)	C(19)–C(17)–C(13)	115.4(11)
C(19)–C(17)–C(16)	112.6(12)	C(19)–C(17)–O(17)	104.1(10)

Table 3 Continued
(b) Fractional parameters

Atom	*x*/*a*	*y*/*b*	*z*/*c*	*U* (eq.)
Ru(1)	0.2170(1)	0.1984(3)	0.01918(9)	0.0391
C(1)	−0.291(1)	0.060(1)	0.618(1)	0.0290
C(2)	−0.323(2)	0.041(1)	0.725(1)	0.0397
C(3)	−0.286(2)	0.120(1)	0.806(1)	0.0380
O(3)	−0.316(1)	0.0955(9)	0.9126(7)	0.0480
C(4)	−0.229(1)	0.221(1)	0.775(1)	0.0304
C(5)	−0.195(1)	0.242(1)	0.669(1)	0.0300
C(6)	−0.137(2)	0.358(1)	0.640(1)	0.0430
C(7)	−0.064(2)	0.360(1)	0.533(1)	0.0406
C(8)	−0.159(2)	0.299(1)	0.441(1)	0.0320
C(9)	−0.167(1)	0.173(1)	0.4742(9)	0.0338
C(10)	−0.223(1)	0.159(1)	0.587(1)	0.0246
C(11)	−0.254(2)	0.106(1)	0.379(1)	0.0418
C(12)	−0.182(2)	0.115(1)	0.272(1)	0.0443
C(13)	−0.174(2)	0.242(1)	0.239(1)	0.0292
C(14)	−0.083(1)	0.306(1)	0.335(1)	0.0273
C(15)	−0.059(2)	0.421(1)	0.285(1)	0.0461
C(16)	−0.034(2)	0.397(1)	0.164(1)	0.0426
C(17)	−0.075(1)	0.269(1)	0.146(1)	0.0353
O(17)	−0.152(1)	0.2563(9)	0.0368(7)	0.0459
C(18)	−0.328(1)	0.296(2)	0.209(1)	0.0401
C(19)	0.061(1)	0.193(2)	0.1423(8)	0.0355
C(20)	0.205(1)	0.213(2)	0.195(1)	0.0490
C(21)	0.298(2)	0.118(2)	0.175(1)	0.0488
C(22)	0.209(2)	0.041(1)	0.111(1)	0.0466
C(23)	0.064(2)	0.088(1)	0.090(1)	0.0482
C(24)	0.176(1)	0.214(1)	−0.158(1)	0.0431
C(25)	0.324(2)	0.178(1)	−0.133(1)	0.0473
C(26)	0.398(2)	0.264(1)	−0.069(1)	0.0531
C(27)	0.297(2)	0.350(1)	−0.059(1)	0.0566
C(28)	0.159(2)	0.319(1)	−0.112(1)	0.0569
C(31)	0.392(2)	0.246(1)	0.519(1)	0.0504
C(32)	0.423(2)	0.306(2)	0.621(1)	0.0692
C(33)	0.319(2)	0.132(2)	0.516(2)	0.0809
O(34)	0.420(2)	0.293(1)	0.433(1)	0.0901

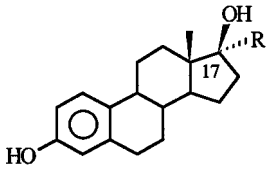
HPLC method developed by Minick *et al*.[39] The values measured for the complexes are listed in Table 4. It is clear that steroids modified by metallocene complexes are more lipophilic than 17β -oestradiol, and that the lipophilicity of **3** is slightly higher than that of **4**. A higher level of non-specificity for **3** than for 17β-oestradiol is thus to be expected. A precise measurement of the non-specific binding value can only be obtained by synthesizing **3** labeled with tritium.

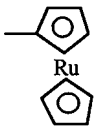
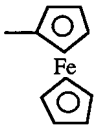
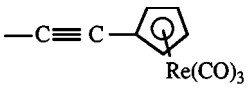
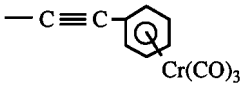
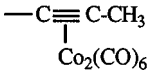
A test of **3** as an affinity marker was carried out by the method previously used for **8**.[13] Compound **3** was first incubated with lamb-uterus cytosol used as a source of oestrogen receptor. After elimination of non-bound compound **3**, tritiated oestradiol was added to the incubation medium to occupy the reversible binding sites of the receptor. The level of reversible binding sites not occupied by **3** was determined by radioactive counting. The results showed that the ruthenocenyl derivative **3** bound to the oestrogen receptor is completely displaced by tritiated oestradiol. This means that 17α-ruthenocenyl-17β-oestradiol, **3**, does not possess the property of an affinity marker.

Stability of the carbenium ion generated by compound 3

It has been suggested that the affinity marking property of organometallic hormones is due to the action of carbenium ions generated from

Table 4 Relative binding affinity (RBA) partition coefficients (log $P_{o/w}$) of some estradiol derivatives



Compound	R	RBA[a]	log$P_{o/w}$[b]
17β-Oestradiol	–H	100	3.30[c]
3		2	4.84
4		8[c]	4.71
5		11[d]	n.d.
6		16[d]	5.31[d]
7		24[4]	5.03[d]
8		12[c]	n.d.

[a] Oestrogen receptor binding affinity (RBA) is determined at 0 °C in a competitive radioreceptor binding assay, using lamb uterine cytosol as a source of receptor and [^{3}H]oestradiol as a tracer. Bound fractions were measured by protamine sulphate assay as described in Ref. 44.

[b] Octanol–water partition coefficients (log $P_{o/w}$) were determined by an HPLC method as described by Pomper *et al*. in Ref. 45.

[c] Value from Ref. 35. A value of 15% was reported for **4** in Ref. 13. A new measurement with a freshly purified sample gave an RBA value of 8%.

[d] Value from Ref. 36.

[e] Value from Ref. 14.

these complexes on a nucleophile at the association site. It is therefore of interest to measure the ability of organometallic hormones such as **3** to stabilize carbenium ions in position 17. Electron-impact mass spectrometry is the technique of choice to reveal the formation of such ions. Studies were carried out at 70 eV with a source temperature of 100 °C. 17β-Oestradiol, 17α-ethynyloestradiol, 17α-ruthenocenyl-17β-oestradiol (**3**) and 17α-ferrocenyl-17β-oestradiol (**4**) were chosen for this study. The sample was dissolved in acetone (1 μg μl^{-1}), then placed directly on the filament. Samples were run several times and averages calculated on the relative intensity of the molecular ion $M^{+\bullet}$ and the carbenium ion $[M-H_2O]^{+\bullet}$. Table 5 shows the results obtained.

The results show that the intensity of the carbenium ion $[M-H_2O]^{+\bullet}$ compared with the intensity of the molecular peak $[M]^{+\bullet}$ is 22% for the two compounds **3** and **4**. This value is only 1% and 2% for 17β-oestradiol and 17α-ethynyl-17β-oestradiol, respectively. It is clear therefore that the introduction for an organometallic fragment increases the ability to form a carbenium ion. The question of its real electrophilicity remains to be answered.

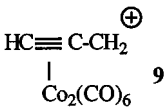
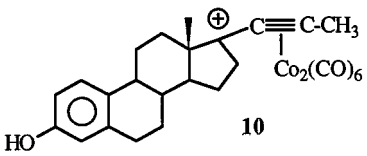
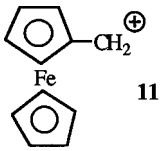
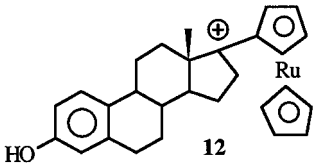
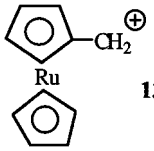
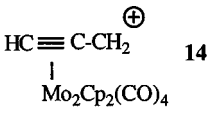
The stability of carbenium ions can also be quantified by measurement of pK_{R^+} values. Previous results have shown that hormonal compounds of the 17α-(organometallic moiety)-17β-oestradiol type often do not permit accurate measurement because of secondary reactions.[40] Owing to this difficulty, it was planned to measure the pK_{R^+} of the $[CpRu(\eta^5\text{-}C_5H_4CH_2)]^+$ ion **13** in addition to **12**, the carbenium ion generated by **3**. Table 6 lists the measured pK_{R^+} value along with the values found for other compounds.

A pK_{R^+} value of +1·52 was found for **13** the $[CpRu(\eta^5\text{-}C_5H_4CH_2]^+$ carbenium ion, and of +0.73 for **12**, the carbocation derivative from 17α-ruthenocenyl-17β-oestradiol, **3**. It is interesting to compare these values with those found for other carbenium ions: p$K_{R^+}=-5.5$ for **9**, $[Cp(\mu\text{–}HC{\equiv}CCH_2]^+$;[40] p$K_{R^+}=-6.0$ for **10**, the carbenium ion of Co generated from **8**; p$K_{R^+}=-0.68$ for **11**, $[CpFe(\eta^5\text{-}C_5H_4CH_2]^+$;[41] p$K_{R^+}=+3.0$ for **14** $[Mo_2Cp_2(CO)_4(\mu\text{–}HC{\equiv}CCH_2)]^+$.[40] These values indicate that the ruthenocenyl carbenium ions, **12** and **13**, are more stable, and therefore less reactive, than the cobaltocenyl and ferrocenyl carbenium ions **10** and **11**. Conversely, they are less stable than the

Table 5 Relative intensity of $M^{+\cdot}$ and $[M-H_2O]^{+\cdot}$ ions

Sample	$M^{+\cdot}$	Intensity (%)	$[M-H_2O]^{+\cdot}$	Intensity (%)
3	502	100	484	22
4	456	100	438	22
17β-Oestradiol	272	100	254	1
17α-Ethynyl-17β-oestradiol	296	100	278	2

Table 6 pK_{R^+} of some complexes

Compound	pK_{R^+}
 **9**	−5.5[a]
 **10**	−6.0
 **11**	−0.68[b]
 **12**	+0.73
 **13**	+1.52
 **14**	+3.0[a]

[a] Value from Ref. 40.

[b] A value of −0.17 has already been published for this compound.[41] In the latter case, the measurement was carried out in a 1:1 CH_3CN/water mixture. Our experiments were performed in a 1:9 CH_3CN/water mixture, and it is known that observed values are highly dependent on experimental conditons.

molybdenocenyl carbenium ion **14**. The relative stability of the ruthenocenyl ions could explain why **3**, like the molybdenum compound of oestradiol, does not act as an affinity marker, while the cobalt derivative **8** does possess this property.[42]

CONCLUSION

Our work has shown that the ruthenocenyl group can be attached to the 17β-oestradiol molecule in the 17α position. 17α-Ruthenocenyl-17β-oestradiol, **3**, is still recognized by the 17β-oestradiol receptor, with an RBA value of 2%. Compound **3** does not act as an affinity marker as its analogue 17α-propynyl-$Co_2(CO)_6$-17β-oestradiol (**8**) does. This property seems to be closely linked to the stability of the carbenium ion formed. An increase in stability as compared with the cobalt complex leads to a loss of reactivity. However, compound **3** is of potential interest as a radiopharmaceutical, since ruthenium has radioactive isotopes that emit β- and γ-radiation, and are therefore of value in nuclear medicine. Among these are ruthenium-97 ($t_{1/2}$=2.9 days, 216 keV, γ photon with 86% abundance), ruthenium-103 ($t_{1/2}$=39.4 days, γ-emitter) and ruthenium-105 ($t_{1/2}$=4.5 h, β-emitter). With this in mind, attempts can be made to synthesize new radioactive ruthenium compounds.[16–21]

EXPERIMENTAL

General procedures

All reactions were performed under a dry argon atmosphere using standard Schlenk techniques. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AM-250 and AM-200 spectrometers. Mass spectra were obtained on a Nermag R 10–10C mass spectrometer. CpRu(η^5-

$C_5H_4CH_2OH$) was prepared following the literature method.[43]

3-(t-Butyldimethylsiloxy)oestrone, 1

A solution of oestrone (2.70 g, 10 mmol) in THF (100 ml) was added dropwise to a suspension of sodium hydride (0.48 g, 12 mmol, 60% in mineral oil) in 40 ml of THF. The mixture was heated at reflux for 1 h and then 1.60 g (12 mmol) of t-butyldimethylsilyl chloride was added in one portion. Heating was maintained overnight. After cooling to room temperature, the mixture was hydrolysed with ice–water. After ether extraction and solvent removal, the residue was purified by crystallization in acetone: 2.62 g of 3-(*t*-butyldimethylsiloxy)oestrone was collected as white crystals (68% yield).

17α-Ruthenocenyl-17β-oestradiol, 3

t-BuLi (0.88 ml of a 1.7 M solution in hexane; 1.50 mmol) was added to a solution of ruthenocene (0.231 g, 1 mmol) in THF (10 ml) at 0 °C. After stirring for 10 min, the mixture was cooled to -60 °C. A solution of 3-(t-butyldimethylsiloxy)oestrone (**1**) (0.768 g, 2 mmol) in THF (50 ml) was slowly added. Stirring was continued overnight, during which time the temperature was allowed to rise slowly to room temperature. After hydrolysis with ice–water, ether extraction, and solvent removal, the residue was redissolved in THF (20 ml). Then 2.2 ml n-BuNF (1 M solution in THF) was added to deprotect the hormone complex. The mixture was hydrolysed with ice–water. After the work-up, the residue was chromatographed on silica-gel plates using ether/pentane (1:1) as eluent. 17α-Ruthenocenyl-17β-oestradiol (**3**; (0.146 g, 0.28 mmol; 29%) was obtained as a white solid. Crystallization in acetone gave white crystals, m.p. 238 °C, suitable for X-ray diffraction. Analysis: Calcd for $C_{28}H_{32}O_2Ru$, C_3H_6O: C, 66.52; H, 6.84. Found: C, 66.26; H, 6.70%. The mass spectrum (desorption chemical ionisation (DCI)/NH_3) showed peaks at m/z 502 $[M]^+$, 485 $[MH-H_2O]^+$, 232 $[Ru(C_5H_5)_2]^+$. ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.10 [d, 1H, J=8.3 Hz, H(1)], 6.60 [dd, 1H, J=8.3 and 2.7 Hz, H(2)], 6.55 (d, 1H, J=2.7 Hz, H(4)], 4.86 (s, 1H, OH), 4.63 [s, 5H, (η^5-C_5H_5)], 4.75–4.61–4.55–4.45 [m,m,m,m, 1H, 1H, 1H, 1H, (η^5-C_5H_4]), 2.80 [m, 2H, H(6)], 1.02 (s, 3H, Me-13). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 153.25 [C(3)], 138.26 [C(5)], 132.91 [C(10)], 126.47 [C(1)], 115.16 [C(4)], 112.59 [C(2)], 101.23 [C(17)], 81.64 [C(1) of (η^5-C_5H_4)], 72.95–70.61 [C(2) and C(5) of (η^5-C_5H_4)], 70.61 [(η^5-C_5H_5)], 70.24–70.12 [C(3) and C(4) of (η^5-C_5H_4)], 48.20 [C(14)], 44.97 [C(13)], 43.52 [C(9)], 39.16 [C(8)], 38.76 [C(12)], 33.71 [C(16)], 29.65 [C(6)], 27.29 [C(7)], 26.30 [C(11)], 23.42 [C(15)], 14.56 (Me-13).

X-ray crystallography

Suitable crystals of **3** were obtained from a slow evaporation of acetone solution. 2424 data points were collected at room temperature on a Nonius CAD4 diffractometer. Empirical absorption correction using DIFABS (min. 0.82, max 1.31) and anomalous dispersion terms were applied. The structures were solved by standard Patterson–Fourier techniques and refined by least-squares analysis using anisotropic thermal parameters for all non-hydrogen atoms. Except for the H(3) linked to O(3), located on a difference Fourier map, H atoms were calculated; all were introduced as fixed contributors. 1428 reflections with $1>2\sigma(I)$ were used to solve and refine the structure to R=0.045 and R_w=0.052, weighting scheme w=1, 318 least-squares parameters. The programs used were CRYSTALS and CAMERON.

Mass spectrometry

Generation of carbenium ions at position 17 of organometallic derivatives of oestradiol was studied by electron-impact mass spectrometry at 70 eV on a Nermag R 10–10C instrument attached to a Digital PDP11-23 Plus computer. Source temperature was 100 °C, filament intensity 200 μA. Samples were dissolved in acetone (1 μg μl^{-1}) and placed on the desorption filament according to the direct introduction method. The filament was then heated from 50 °C to 450 °C with the temperature gradient programmed at 10 mA s^{-1}. Samples were run several times and averages calculated of relative intensities of the molecular ion $M^{+\bullet}$ and the carbenium ion $[M-H_2O]^{+\bullet}$.

Determination of the pK_{R^+} values

The organometallic alcohol was dissolved in acetonitrile (1×10^{-3} M solutions except for the alcohols corresponding to **11** and **13**, where 1×10^{-2} M solutions were used); 50 μl of this organic solution were then added to 450 μl of

various concentrations of H_2SO_4 (due to solubility problems the measurement for **12** was carried out in a 1:1 (v/v) CH_3CN/water mixture). The UV measurements were performed at a selected wavelength after 15 min incubation at room temperature (30 min for **10**). Final calculations were done by using the Deno acidity function,[46] except for **12** and **13** where measurements performed in low H_2SO_4 dilutions require direct determination of the pH values and use of the formula $pH = pK_{R^+} - \log(C_{R^+}/C_{ROH})$.

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