Inorganica Chimica Acta, 56 (1981) 127–133

Metalloimmunoassay. V. Steroid–Platinum(II)–*o*-catecholato Complexes: A Novel Set of Metallohaptens

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Received March 17, 1981

The syntheses and characterization of steroid–Pt(II)–o-catecholato complexes from derivatives of estrone, estradiol and testosterone are reported. The new compounds possess high chemical stability and they are potentially suitable substrates for investigating Pt(II) effects in bioinorganic studies.

Introduction

Increasing attention has been devoted in the last decade to the preparation and/or isolation of metal complexes or metal ions bound to biological derivatives for medical, pharmacological and biological applications.

One particular use of metal atoms anchored to bioorganic structures has been recently reported for a non isotopic system, designated as metalloimmunoassay (MIA), in which a variety of metal atoms, in the form of their organometallic or coordination complexes replace radioisotopes as the labelling agents [1, 2]. The principle of MIA has been described in detail [3]. Briefly, the concept is based on competitive binding reactions in which metalloantigen and unlabelled antigen react with a fixed, limited quantity of specific antibodies. After separation of the free, unbound antigens from the antibody-antigen complexes the amount of metal present in the 'bound' and/or 'free' phase can be determined by suitable analytical methods such as emission, absorption and fluorescence spectrometry, electrochemical methods, neutron activation, *etc.* Preparation of a calibration curve plotted for standardised amounts of metalloantigen and unlabelled antigen provides the means of determining the quantity of analysed substances in unknown samples.

As described [3], one of the major requirements for MIA is the synthesis of stable metal derivatives covalently coupled to bioorganic molecules (metallohaptens or metalloantigens) for use in immunological reactions.

In earlier papers [1–4] we have already pointed out various approaches for anchoring transition metals to organic molecules of biological interest and we have indicated that the choice of a specific organometallic compound for use in immunological reactions is dependent upon the stability and solubility in biological fluids of the final product (*i.e.* metallohapten). A convenient approach for incorporating transition metals into organic structures has been achieved by the use of organometallic complexes having a free organic function [1–5]. We have recently reported a series of tetracoordinated Pd(II), Pt(II) [4, 6] and hexacoordinated Ir(III) [7]-functionalized-*o*-catecholato complexes, as a potential source for building tailor-made metallohaptens. In addition to the general stability encountered for the coordinatively saturated *o*-catecholato complexes [4, 7], the presence of a 4-substituted-*o*-catecholato ligand with a carboxylic acid or amine group confers to these complexes a very convenient handle for binding complementary functions by reaction on the coordinated ligand. We have already demonstrated the potential organic reactivity of the series of Pt(II) amine- and carboxyl-substituted-*o*-catecholato complexes, by coupling them with organic molecules used as models [4]. The successful results obtained so far with these complexes prompted us to extend their application to more elaborate organic structures.

In the present work we report the preparation and characterization of a number of steroid-Pt(II)-*o*-catecholato complexes from derivatives of estrone, estradiol and testosterone, conveniently functionalized in different positions of the steroid molecule. Reactions occurring on the organic function of the coordinated ligand or directly onto the coordination sphere of the metal by the reagents used during the coupling reaction preparation have been

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investigated. Some of the by-products have been isolated and characterized by IR, NMR, elemental analysis or mass spectra measurements. The results obtained so far have underlined the versatility of the Pt(II) functionalized-*o*-catecholato complexes for labelling complementary functionalized bioorganic molecules.

Immunological reactions with the novel platinum labelled estradiol complexes have been carried out and comparative studies in radioimmunoassays (to be described in a separate communication) have shown that the antigenicity of the metal-labelled antigens was dependent upon the position of the substitution in the estrogen molecule rather than upon the relative distance between the metal complex moiety and the hapten.

Experimental

Apparatus

All the reactions were performed in an atmosphere of nitrogen which had been purified by passing it through a column of R3-11 BASF deoxygenating catalyst and then drying it over molecular sieves. The subsequent work-up of the reaction mixture was carried out in air. Infrared spectra were recorded with a Perkin-Elmer 257 instrument or a Perkin Elmer 580 B, solid samples being run as KBr pellets or as otherwise stated. Proton NMR spectra were obtained by using a WP-60 Bruker spectrometer with $CDCl_3$ as solvent and tetramethylsilane as internal standard. Mass spectra measurements were carried out with MAT-711 (Varian) spectrometer. The elemental analyses were determined by Dr. F. B. Straus Microanalytical Laboratory, 10 Carlton Road, Oxford, OX2 7SA.

Solvents and Chemicals

All the solvents were deoxygenated prior to use, and the transfers were carried out with the flexible needle or syringe technique. Diethyl ether and tetrahydrofuran (THF) were purified as described in the literature [8].

N-Hydroxysuccinimide (NHS), N,N'-Dicyclohexylcarbodimide (DCC) (Fluka A.G. purum and puriss, grade products) were used throughout, triethylamine and iso-buthylchloroformate (Fluka A.G. puriss, and pract. respectively) were distilled prior to use. Estrone, estradiol and testosterone (Merck-Darmstadt) were used without further purification in the synthesis of carboxyl- and amine-functionalized steroid derivatives (details will be published elsewhere). Estradiol-17β-hemisuccinate (E_2-HS) was prepared) as described in the literature [9].

The complexes $Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}R)(P(C_6H_5)_3)_2$, where R = CO_2H, CH_2CO_2H, $CH_2CH_2CO_2H$, $CH_2\text{-}CH_2NH_2$, and the corresponding platinum carboxyl-substituted-*o*-catecholate active esters were prepared as previously reported [4, 6].

Preparation of $[Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}R\text{-}E)(PPh_3)_2$, E = $HNCH_2CH_2$-3-O-estra-1,3,5(10)-trien-17-one, R = CO (1), CH_2CO (2), CH_2CH_2CO (3); E = $HNCH_2\text{-}CH_2$-3-O-estra-1,3,5(10)-trien-17β-ol, R = CO (4), CH_2CO (5), CH_2CH_2CO (6); E = $HNCH_2$-17α-estra-1,3,5(10)-trien-3,17β-diol, R = CH_2CO (10), $CH_2\text{-}CH_2CO$ (11)

In a typical preparation, to a 3 ml THF solution containing 0.167 mmol of the appropriate amine-functionalized estrogen were added at 0 °C, 0.5 ml of triethylamine followed by a 5 ml THF solution containing 0.167 mmol of platinum carboxyl-substituted-*o*-catecholato active ester. The reaction mixture was stirred at 0 °C for 1 hr and overnight at 4 °C. After filtration, the solvent was removed at reduced pressure and the product kept *in vacuo* for 2 hr. The solid was washed with H_2O, dried *in vacuo*, dissolved in benzene/$CHCl_3$ (1:1) and filtered. The partially concentrated filtrate was chromatographed on a 20% deactivated neutral alumina eluting with benzene (compounds 1–3) or on a 15% deactivated alumina column eluting with ethyl acetate (compound **5**) or with benzene/$CHCl_3$ (1:1) compound **6, 7, 10, 11**). The eluted solution containing the product was concentrated at reduced pressure and rechromatographed through a small silica gel column with chloroform. The solution obtained was evaporated to dryness and the yellow (compound **1, 5**) or orange solid (compound **2, 3, 6, 7, 10, 11**) washed with ether. Complex **5** was also obtained through the formation of the Pt(II) mixed anhydride catecholate complex as intermediate (see discussion).

Preparation of $Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH_2CH_2NHCOCH_2\text{-}E)(PPh_3)_2$, E = 3-O-estra-1,3,5,(10)-trien-17-one (4), E = 3-O-estra-1,3,5(10)-trien-17β-hydroxy (8), E = CH_2CO-17β-O-estra-1,3,5(10)-trien-3-hydroxy (9)

(A) Estrogen Carboxyl-Substituted Active Ester Intermediates

In a typical preparation, a stoichiometric amount of DCC, dissolved in 1 ml of THF, was added to a 3 ml THF solution, containing 0.3 mmol of the appropriate carboxyl-substituted estrogen and a stoichiometric amount of NHS at 0 °C. The solution was stirred for 2 hr at 0 °C and overnight at 4 °C. The white precipitate formed in the reaction was filtered off and characterized as N,N'-dicyclohexylurea by comparison with an authentic sample. After removal of the solvent at reduced pressure, the product was dried *in vacuo*, dissolved in ethylacetate and filtered from a small amount of N,N'-dicyclohexylurea residue, the solvent evaporated and the

TABLE I. Physical and Analytical Data for Steroid-Platinum(II)–Catecholato Complexes and Isolated By-products. L = PPh_3, E_1 = 3-Hydroxyestra-1,3,5(10)-trien-17-one; E_2 = Estra-1,3,5(10)-triene-3,17β-diol; T = 17β-Hydroxy-4-androsten-3-one.

No.	Compound	Yield[a] %	M.P. °C	% Found (calcd)		
				C	H	N
1	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}COHNCH_2CH_2\text{-}3\text{-}O\text{-}E_1)L_2 \cdot H_2O$	12	148[b]	63.85(63.84)	5.31(5.20)	2.03(1.18)
2	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH_2COHNCH_2CH_2\text{-}3\text{-}O\text{-}E_1)L_2 \cdot 3H_2O$	32	152	62.12(62.22)	5.00(5.47)	1.14(1.13)
3	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH_2CH_2COHNCH_2CH_2\text{-}3\text{-}O\text{-}E_1)L_2 \cdot H_2O$	45	150	64.79(64.34)	5.64(5.41)	1.44(1.15)
4	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH_2CH_2NHCOCH_2\text{-}3\text{-}O\text{-}E_1)L_2$	46	146	65.24(65.18)	5.57(5.22)	1.35(1.19)
5	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}COHNCH_2CH_2\text{-}3\text{-}O\text{-}E_2\ L_2 \cdot H_2O$	12[c]	151[b]	63.42(63.72)	5.77(5.36)	1.59(1.18)
6	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH_2COHNCH_2CH_2\text{-}3\text{-}O\text{-}E_2)L_2 \cdot 4H_2O$	29	147	60.97(61.23)	5.33(5.71)	1.56(1.11)
7	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH_2CH_2COHNCH_2CH_2\text{-}3\text{-}O\text{-}E_2)L_2$	23	149	64.08(64.23)	5.58(5.57)	1.30(1.15)
8	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH_2CH_2NHCOCH_2\text{-}3\text{-}O\text{-}E_2)L_2$	53	128	66.44(65.07)	5.76(5.39)	1.33(1.19)
9	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH_2CH_2NHCOCH_2CH_2CO\text{-}17\beta\text{-}O\text{-}E_2)L_2 \cdot 2H_2O$	48	159	62.73(62.84)	5.27(5.52)	1.01(1.10)
10	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH_2COHNCH_2\text{-}17\alpha\text{-}E_2)L_2 \cdot H_2O$	14	188[b]	63.72(63.73)	5.22(5.36)	1.14(1.18)
11	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH_2CH_2COHNCH_2\text{-}17\alpha\text{-}E_2)L_2$	15	193[b]	64.84(64.96)	5.57(5.38)	1.29(1.18)
12	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH_2CH_2NHCOC{\equiv}C\text{-}17\alpha\text{-}E_2)L_2$	15	199	65.81(65.44)	5.40(5.16)	1.14(1.17)
13	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH_2CH_2NHCOC{\equiv}C\text{-}17\alpha\text{-}T)L_2$	28	193	66.05(65.55)	5.77(5.43)	1.13(1.16)
14	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CH_2CH_2NHCOOCH_2(CH(CH_3)_2L_2 \cdot 2H_2O$	10	119[b]	58.44(58.45)	5.00(5.12)	1.28(1.39)
15	$Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}COOCOOCH_2CH(CH_3)_2L_2$	38	205		[d]	
16	$[Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}CO)L_2]_2 0.4H_2O$	23	208[b]	60.13(60.61)	3.69(3.85)	
17	$(CH_3)_2CHCH_2OCOHNCH_2CH_2\text{-}3\text{-}O\text{-}E_2$	26[c]	[e]		[f]	

[a]Based on the metal functionalized catecholate. [b]Decomposition. [c]*5:* Based on *15; 17:* based on the aminofunctionalized estradiol-17β. [d]See text. [e]Isolated as colourless oil. [f]Molecular ion (high resolution mass spectrum) m/e 415.2589; $C_{25}H_{37}O_4N$ Calcd.: mol. wt. 415.62.

product filtered again with $CHCl_3$. After removing the solvent at reduced pressure, the estrogen carboxyl substituted active ester obtained was characterized by the IR absorptions at 1810 w, 1780 m and 1740 vs cm^{-1} and by the appearance of an NMR singlet at τ = 7.2($OCCH_2CH_2CO$) in addition to the typical estrogen proton resonances. Estradiol 17β-hemisuccinate active ester was additionally washed with ether and was characterized only by IR due to its low solubility in $CDCl_3$.

Reaction with the Pt(II)-amine-substituted-o-catecholato complex

In a typical preparation, a 2 ml THF solution, containing a stoichiometric amount of the appropriate active ester of the carboxyl-substituted estrogen, was added to a 4 ml THF solution, containing 0.215 mmol of the platinum(II)-amine substituted catecholato complex and 3.5 mmol of triethylamine at 0 °C. After being stirred at 0 °C for 2 hr and overnight at 4 °C, the reaction mixture was filtered and the solution evaporated to dryness. The solid was washed with H_2O, dried *in vacuo* and the orange product worked-up as for compounds *6, 7, 10, 11.*

Preparation of Pt(1,2-$O_2C_6H_3$-4-$CH_2NHCOC{\equiv}C$-17α-E)(PPh_3)$_2$, E = estra-1,3,5(10)-triene-3, 17β-diol (12), E = 17β-Hydroxy-4-androsten-3-one (13)

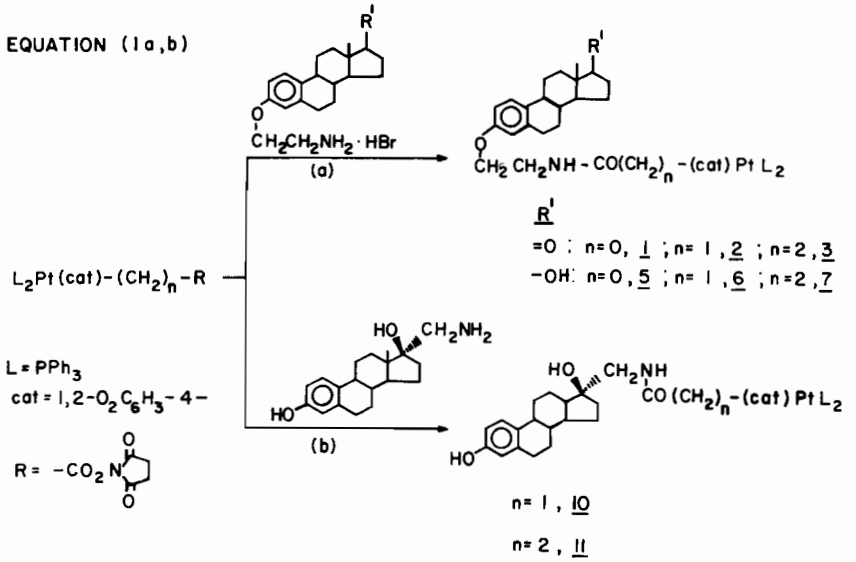
In a typical preparation, to a 2 ml THF solution containing 0.150 mmol of the appropriate carboxyl-functionalized steroid, were added at 0 °C 0.210 mmol of triethylamine immediately followed by 0.195 mmol of isobutylchloroformate. The reaction mixture was allowed to stir at 0 °C for 1.5 hr and then filtered under N_2. The filtrate was syringed at 0 °C to a 1.5 ml THF solution containing 0.131 mmol of the Pt(II) amine-substituted catecholato complex. Stirring was continued at 0 °C for 1 hr and overnight at room temperature. After filtration, the solvent was removed at reduced pressure and the product kept *in vacuo* for 2 hr. The solid was dissolved in ethyl acetate/$CHCl_3$ (1:1) and filtered. The partially concentrated filtrate was chromatographed on a 15% deactivated alumina column eluting with benzene/$CHCl_3$ (1:6) (compound **12**) or ethyl acetate (compound **13**). The eluted solution containing the product was concentrated at reduced pressure and finally chromatographed on a silica gel column eluting with $CHCl_3/CH_3OH$ (33:1).

TABLE II. Selected IR Bands[a] and NMR Data[b] for Steroid-Platinum(II)–Catecholato Complexes and Isolated Byproducts.

Compound	ν(amide-C=O) and ν(C–N–H) bending, cm^{-1}	ν(catecholato) cm^{-1}	Other frequencies ν, cm^{-1}	τ values						
				C_6H_5	estrogen[c] aromatic-H	$1,2\text{-}O_2C_6H_3$	$CH_2\text{-}3\text{-}O\text{-}E$[d]	$OCNCH_2$	$18\text{-}CH_3$	Other resonances
1	1640s, 1570w	1480vs, 1280s	1735s (ketone-C=O)	2.73(m)	3.32(m)	3.55(m)	6.0(m)	6.24(m)	9.09(s)	
2	1660s, 1570w	1485vs, 1270s	1740s (ketone-C=O)	2.74(m)	3.35(m)	3.76(m)	6.15(m)	6.5(m)	9.09(s)	6.56(s, $OCCH_2$)
3	1660s, 1565w	1485vs, 1270s	1740s (ketone-C=O)	2.75(m)	3.38(m)	3.75(m)	6.1(m)	6.5(m)	9.09(s)	
4	1680s, 1570w	1485vs, 1270s	1740s (ketone-C=O)	2.72(m)	3.33(m)	3.63(m)	5.55(s)	6.45(m)	9.08(s)	
5	1630s, 1575w	1480vs, 1280s		2.73(m)	3.39(m)	3.50(m)	6.14(m)	6.25(m)	9.22(s)	
6	1650s, 1570w	1485vs, 1270s		2.74(m)	3.34(m)	3.70(m)	6.03(t)	6.5(m)	9.22(s)	6.56(s, OCH_2)
7	1650s, 1570w	1485vs, 1270s		2.74(m)	3.35(m)	3.71(m)	6.06(t)	6.35(m)	9.22(s)	
8	1670s, 1570w	1485vs, 1270s		2.80(m)	3.35(m)	3.70(m)	5.60(s)	6.52(m)	9.19(s)	
9	1650s, 1565w	1485vs, 1265s	1730s(ester-C=O)	2.74(m)	3.41(m)	3.63(m)		6.55(m)	9.18(s)	
10	1635s, 1560w	1480vs, 1265s		2.73(m)	3.47(m)	3.70(m)			9.18(s)	
11	1640s, 1565w	1485vs, 1270s		2.73(m)	3.45(m)	3.69(m)			9.21(s)	
12	1630s, 1570w	1485vs, 1265s	2220m (C≡C)	2.73(m)	3.51(m)	3.75(m)		6.57(m)	9.17(s)	
13	1640s, 1570w	1485vs, 1270s	2220m (C≡C) 1660s (ketone-C=O)	2.85(m)		3.77(m)		6.56(m)	9.12(s)	4.27(s, OCCH=C) 8.81(s, 19-CH_3)
14		1485vs, 1270vs	1720s (uretane-C=O)	2.73(m)		3.75(m)				6.20(d, OCH_2) 9.10(d, CH_3)
15		1485vs, 1285vs	1782s, 1724s 1570m (O=C-O-C=O)	2.76(m)		3.68(m)				6.03(d, OCH_2) 9.05(d, CH_3)
16		1485vs, 1285	1745s, 1698s 1570s (O=C-O-C=O)	2.73(m)		3.4–3.9(m)				
17	1610m[e]		1720vs (uretane-C=O)[e]		2.78, 3.30(m)		5.96(t)	6.36(t)	9.15(s)	6.09(d, $COOCH_2$) 9.02(d, CH_3)

[a]As KBr pellets. [b]In $CDCl_3$ with Me_4Si as internal standard. [c]Part of the estrogen aromatic proton resonances occurring at τ = 2.65–2.9 are covered by the 30 H of the 2 PPh_3 ligands. [d]E = E_1 or E_2. [e]In $CHCl_3$.

EQUATION (1a,b)



The eluate was evaporated to dryness and the orange microcrystalline solid washed with ether.

Results and Discussion

Active esters of bis-phosphine-platinum(II)-4-carboxyl-substituted-*o*-catecholates, prepared by treatment of N-Hydroxysuccinimide (NHS), in the presence of N,N'-Dicyclohexylcarbodiimide (DCC), were reacted with estrone and estradiol-17β, transformed into their respective 3-O-aminoethyl- and 17α-amino-methyl-derivatives, according to eqn. 1a, b:

Physical and analytical data, selected infrared bands and NMR data for the estrogen-platinum complexes (compounds **1–3**, **5–7**, **10**, **11**) are reported in Table I and Table II. All the steroid-platinum complexes are very soluble in CH_2Cl_2, $CHCl_3$, THF and C_2H_5OH and only partially soluble in C_6H_6.

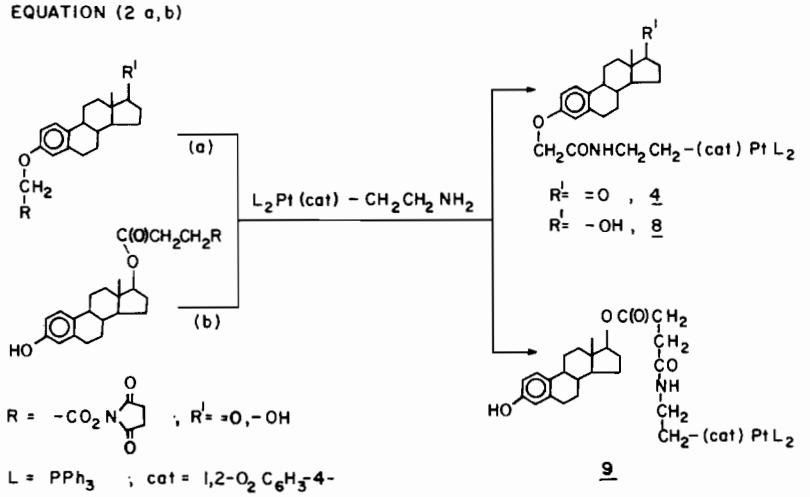
No variation of the strong infrared absorptions at *ca.* 1275 cm^{-1} and at 1480 cm^{-1}, due to the *o*-catecholato skeletal vibrations (the latter overlapping with the phosphine aromatic ring absorptions), were observed, therefore indicating the retention of the *o*-diolato ligand [4, 6, 7, 10]. The isolated, TLC pure products showed two new IR absorptions at approximately 1640 cm^{-1} and 1565 cm^{-1}, which are characteristic of an amide carbonyl group. NMR and analytical data are in agreement with the formation of 1:1 adducts. An additional strong absorption at 1740 cm^{-1} for compounds **1–3**, typical of the ν(C=O) of the estrone ketone group was observed.

In a similar fashion, active ester derivatives of 3-O-carboxymethyl ether-functionalized estrone and estradiol-17β-hemisuccinate have been prepared as intermediates and subsequently coupled with bis-phosphine Pt(II)-4-amine-substituted-*o*-catecholates, yielding the corresponding estrogen-platinum(II) complexes (**4**, **8**, **9**), according to eqn. 2a,b:

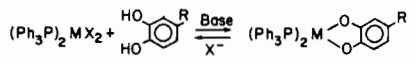
Analytical and spectroscopic data (Tables I and II) are consistent with the formation of estrogen-platinum(II)-*o*-catecholato-amide derivatives. The isolated products **4**, **8**, **9** show an IR and NMR pattern equivalent to the series **1–3**, **5–7**, **10**, **11**, with compound **9** presenting an additional strong IR absorption at 1730 cm^{-1}, characteristic of the ester carbonyl group of the estradiol-17β-hemisuccinate moiety.

It is noteworthy to point out that in the coupling reaction between the carboxyl substituted platinum-*o*-catecholates and the amine functionalized estrogens (eqn. (1a)), although a 1:9 excess of Et_3N was used, the presence of acid bromide in the reaction mixture lead to the formation of $[(Ph_3P)_2PtBr_2]$, in about 11% yield of pure isolated by-product: IR absorptions (CsJ pellets) at 221, 201 cm^{-1} for ν(Pt–Br), in addition to the overall IR pattern characteristic of the bis-phosphine-dihalogen platinum (II) square planar complexes, are also consistent with the bromide ligands in a *cis*-arrangement [11]. When the acid bromide, amine groups of the functionalized estrogens were previously unblocked with KOH in MeOH, or when a 1:20 excess of Et_3N was used in the coupling reaction, no formation of *cis*-$[(Ph_3P)_2PtBr_2]$ was observed. As we have previously reported [4, 6] the preparation of bis-(phosphine)Pd(II) and -Pt(II) functionalized-*o*-catecholato complexes is achieved by interaction between *cis*-$[(Ph_3P)_2MX_2]$, (M = Pd(II), Pt(II); X = halogen), and 4-substituted-*o*-catecholates in the presence of KOH, eqn. (3).

EQUATION (2 a,b)



EQUATION (3)



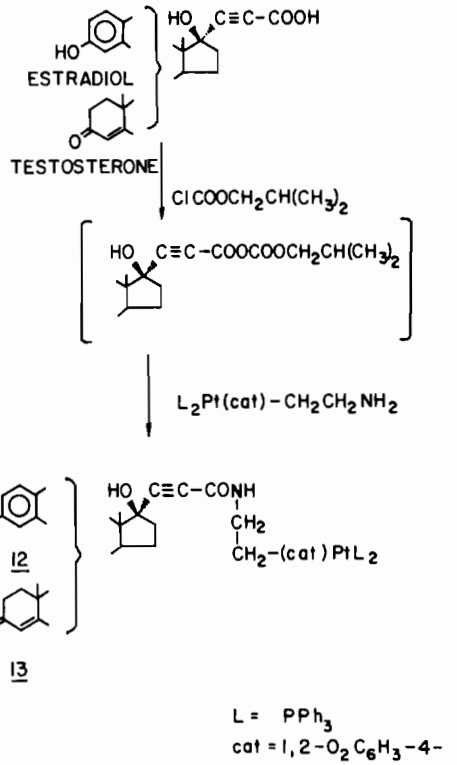
It appears also from previous observations [12] that an insufficient amount of base shifts the equilibrium reaction (3) to the starting dihalogen complex. In the present case, due to the use of Et_3N as a mild base, replacement of the *o*-catecholato ligand by the bromide ions, present in the reaction mixture, was likely to occur.

On the other hand the use of isobuthylchloroformate as an activating reagent for carboxyl groups appeared not to interact with the coordination sphere of the platinum(II) metal in our reaction conditions. Steroid-platinum derivatives (**12** and **13**) were synthesized by treatment of the 17α-carboxyl ethynyl-substituted estradiol and testosterone with isobutylchloroformate, which were subsequently coupled with the platinum(II) amine-substituted-*o*-catecholates, according to eqn. (4).

As already mentioned, although a slight excess of isobutylchloroformate was used, no formation of $[(Ph_3P)_2PtCl_2]$ was observed. Nevertheless, in addition to the expected steroid–platinum derivatives, reaction took place between the isobutylchloroformate reagent and the free amino function of the coordinated *o*-catecholato ligand (compound **14**). The formation of an uretane bond was easily detected by a strong IR absorption at 1720 cm^{-1}. NMR doublets at τ = 6.20 and τ = 9.10 are also consistent with the presence of the $COOCH_2CH(CH_3)_2$ moiety (Table II).

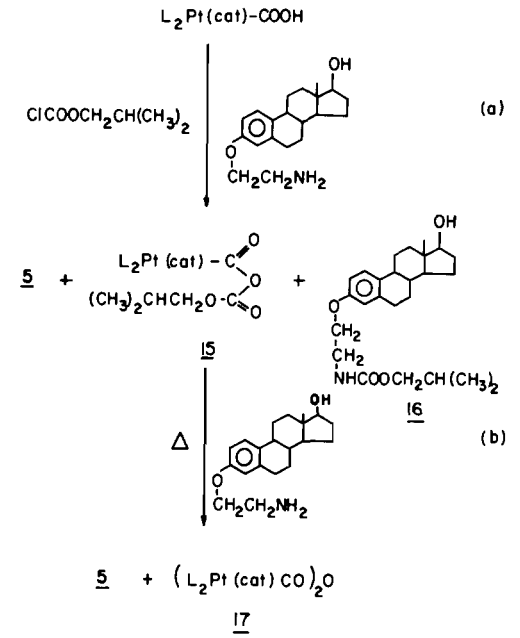
When $[(Ph_3P)_2Pt(1,2\text{-}O_2C_6H_3\text{-}4\text{-}COOH)]$ was used as the estrogen-labelling agent (eqn. 1a), very low yields of the estrogen–platinum derivatives were obtained. In addition to a number of side-products

EQUATION (4)



detected by TLC, a significant amount of the bis-(phosphine)Pt(II)-*o*-catecholato active ester derivative was recovered. This is in accordance with the known stability of α-aromatic or α-β unsaturated carboxyl 'active' esters (*i.e.* due to possible conjugation of the carbonyl group with an α-unsaturated carbon carbon bond). Activation of the carbonyl group with isobutylchloroformate and subsequently coupling it with amine ethyl-3-O-estradiol-17β did not improve the yield (eqn. 5a).

EQUATION (5 a, b)



L = PPh_3

cat = 1,2-O_2 C_6H_3-4-

Due to the excess of isobutylchloroformate used in the reaction, an uretane derivative of the estrogen (compound **16**) was isolated and the majority of the starting platinum(II)carboxylic acid-substituted-*o*-catecholato complex was transformed into the mixed anhydride intermediate **15** IR and NMR data (Table II) are in agreement with the presence of the $COOCH_2CH(CH_3)_2$ moiety for both **15** and **16** When the isolated compound **15** was again reacted with a 1 1 5 excess of the estradiol-3-O-amine derivative in refluxing THF, partial decomposition of the complex occurred, and, in addition to the formation of **5** in a 12% yield, the bis[platinum(II)-*o*-catecholato] symmetric anhydride **17** was isolated (eqn 5b) and characterized by analytical and spectroscopic measurements (Tables I and II)

Acknowledgement

We wish to thank the Italian CNR for research Fellowship to Dr O. Gandolfi (NATO G T 215.12/ 3-216 796)

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