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Electrochemical Differentiation of α- and β-Diastereoisomers of the Steroid Hormone Receptor Marker (3-(Benzyloxy)-17β-hydroxyestra-1,3,5(10)-triene)tricarbonylchromium

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Voltammetry at both conventional (linear diffusion) and microsized (radial diffusion) platinum electrodes has established that the α- and β-diastereoisomers of (3-(benzyloxy)-17β-hydroxyestra-1,3,5(10)-triene)tricarbonylchromium, $Cr(CO)_3$(steroid), exhibit oxidation processes in dichloromethane which differ in kinetic and thermodynamic aspects. The diastereoisomers therefore are distinguishable at an electrode surface as is also the case by hormone receptor sites. Electrochemical techniques may therefore provide a useful probe for ascertaining the bonding interactions in the hormone receptors known to be present in mammary tumors. An overall two-electron oxidation process, which depending on the electrochemical conditions may consist of two well-resolved one-electron steps or a single two-electron step, is observed for both diastereoisomers. At fast scan rates with conventional sized electrodes or with small radii disk microelectrodes, the first electron-transfer step for the 18-electron $Cr(CO)_3$(steroid) in the presence of Bu_4NClO_4 as the electrolyte is reversible and generates the 17-electron $[Cr(CO)_3(\text{steroid})]^+$ cation. Under these conditions a second irreversible one-electron transfer process producing a transient chromium(II) complex is observed at more positive potentials. With longer time domain experiments, an overall two-electron process is observed with 0.1 M Bu_4NClO_4 as the supporting electrolyte. The chemical step in this ECE reaction mechanism is the nucleophilic attack on the chromium(I) cation by the perchlorate anion. The second-order rate constants for this reaction were found to be 44 ± 9 and 85 ± 7 $M^{-1}\ s^{-1}$ for the α- and β-isomers, respectively, at 20 °C. In contrast, the first one-electron transfer step is chemically reversible under all conditions of scan rate and electrode size when Bu_4NPF_6 is substituted for Bu_4NClO_4 as the electrolyte. An E° (reversible half-wave potential) difference of 30 mV is observed between the diastereoisomers for the first one-electron charge-transfer process. Thus, the diastereoisomers are distinguished in the thermodynamic (redox) as well as kinetic sense with respect to reactivity of the $Cr(CO)_3$(steroid) cation. Controlled potential oxidative electrolysis of $Cr(CO)_3$(steroid) with either Bu_4NPF_6 or Bu_4NClO_4 as the electrolyte at all potentials results in a two-electron oxidation process with complete decomposition of the complex to form chromium(II), carbon monoxide, and a noncoordinated steroid for both α- and β-diastereoisomers.

Introduction

Coordination of a chromium tricarbonyl moiety to an organic aromatic ring via π-bonding increases the reactivity of the ring toward nucleophilic attack.[3-7] This can have important consequences in developing new methods in synthetic organic chemistry. The recognition of the role and use of such π-bonding in organometallic complexes containing biologically important ligands has attracted considerable academic and applied interest over the last 20 years.[8] In the steroid field, which is relevant to this paper, π-bonding to metal carbonyl moieties has provided access to both regio- and stereospecific pathways[9] for the synthesis of unsaturated steroids and D vitamins.[10] In the biological area of research, where a knowledge of estrogen action mechanisms and the elucidation of hormone-receptor sites are problems of vital importance,[11] chromium metal carbonyl π-complexes have been used as analytical markers for receptor and chemical immunological studies.[12] The ability of the metal carbonyl derivatives of the hormones to recognize their specific receptor site is significantly dependent on their stereochemistry. This and other details of the binding of organometallic hormone receptor markers have been monitored via their infrared absorption properties.[13-16]

While the X-ray crystal structure of the estradiol urea complex[17] has been established, the fine details of the structure of organometallic chromium tricarbonyl complexes are often unknown, particularly in solution. For example, attachment of the 12-electron chromium tri-

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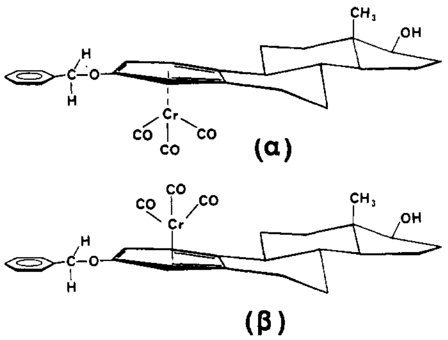

Figure 1. Diastereoisomers of α- and β-(3-(benzyloxy)-17β-hydroxyestra-1,3,5(10)-triene)tricarbonylchromium.

carbonyl moiety to a chiral hormone such as estra-1,3,5-(10)-triene-3,17β-diol or substituted forms of the estradiol hormone can occur on either the α- or β-faces of the steroid (Figure 1) so that diastereomeric mixtures may result. In a recent study, it was shown that with lamb uterine estrogen receptor sites the binding affinity to α- and β-diastereoisomers of tricarbonylchromium derivatives is significantly different.[15] Thus a detailed knowledge of structural features of the hormone markers is required. Elegant NMR configurational determinations of the structures in solution of a variety of steroid chromium tricarbonyl complexes have been published[14,18] utilizing multipulse COSY and SECSY two-dimensional experiments. However even these state of the art NMR techniques do not necessarily offer complete isomeric structural discernment, and application of other physical methods to studies of the structural variations of (steroid)chromium tricarbonyl complexes is therefore warranted. Recently, it has been shown that redox properties of tricarbonyl organometallic complexes[19-22] are often sensitive to structural variations. In this paper the oxidation at platinum electrodes of the α- and β-diastereoisomers of (3-(benzyloxy)-17β-hydroxyestra-1,3,5(10)-triene)tricarbonylchromium (Figure 1) is described. The electrochemical technique of cyclic voltammetry under conditions of linear diffusion at conventional sized platinum electrodes and steady-state voltammetry at microelectrodes illustrate that the complexes exhibit important thermodynamic and kinetic differences in their redox properties which can be related to their structural differences. We have thus established another possible physical technique of measurement with which to probe the influence of the structure of steroid complexes and their binding to steroidal hormone receptor sites which are known to be present in mammary tumors.[23-25]

Experimental Section

Reagents. Dichloromethane (Mallinckrodt Chrom AR, St. Louis, MO) was passed through a neutral alumina column of activity 1 prior to use in electrochemical experiments. The electrolytes tetrabutylammonium perchlorate (Bu_4NClO_4, G.F. Smith Chemical Co., Columbus, OH) and tetrabutylammonium hexafluorophosphate (Bu_4NPF_6, South Western Analytical Chemicals, Inc., Austin, TX) were dried over phosphorus pentoxide for at least 24 h before use. Oxidation of 5×10^{-4} M solution of ferrocene (Merck) at a platinum electrode was used to calibrate the Ag/AgCl reference electrode. The reversible half-wave potential of the ferrocene oxidation process was 0.520 ± 0.010 mV V vs Ag/AgCl at 20 °C in dichloromethane.

Electrodes, Instrumentation, and Procedures. Solutions were degassed with argon or nitrogen prior to use for at least 10 min to remove oxygen. All electrochemical experiments were done under a blanket of nitrogen or argon that was saturated with dichloromethane. Alumina was present in solution during the experiments to ensure that water was kept to a minimum level. In the presence of deliberately added water (mM level), electrochemical data are modified relative to that reported in this paper so it is important that solutions be as moisture free as possible. Glassware was cleaned and stored in drying ovens prior to use. Microelectrodes were constructed by sealing 25-, 10-, 5-, 2-, and 1-μm diameter platinum wires (Goodfellow, Cambridge, England) in evacuated Pyrex or soda glass tubes using a method related to that described previously in the literature.[26] The smaller 5-, 2-, and 1-μm wires initially contain a silver coating (Wollaston wire). Electrical contact was made to a copper wire via silver epoxy or soldering. The electrodes were ground with wet/dry paper and polished with alumina (5–0.05 μm) and water slurries on polishing cloth. Prior to use the electrodes were carefully dried to minimize contamination of solutions. For experiments at conventional size electrodes a Bioanalytical systems (BAS) platinum disk working electrode (1.6-mm diameter) was used. Conventional voltammetric experiments utilized a platinum wire counter electrode. The reference electrode was a Ag/AgCl electrode filled with CH_2Cl_2 (0.1 M Bu_4NClO_4) and saturated with LiCl. For voltammetric experiments, the reference electrode was separated from the test solution by a salt bridge containing CH_2Cl_2 and the electrolyte in use.

Experiments at conventional size electrodes were recorded by using either a BAS CV 27 voltammograph or an AMEL 551 potentiostat with a PAR 175 function generator. Controlled potential electrolysis experiments were done with a PAR 273 electrochemical system. The working electrode was a platinum basket, and the counter electrode was a platinum mesh separated from the solution by a salt bridge with a Vycor frit. Chromatographic and infrared monitoring was carried out during the course of the controlled potential electrolysis experiments as described previously.[27] Microelectrode experiments utilized a two-electrode configuration (platinum disk microelectrode and platinum wire reference electrode) and employed a PAR 175 function generator and a Keithley Model 480 picoammeter. A Houston Instruments Model 100 X-Y Recorder was used to record all experimental data.

Synthesis of α- and β-(3-(Benzyloxy)-17β-hydroxyestra-1,3,5(10)-triene)tricarbonylchromium. β-Estradiol (2.72 g, 10.0 mmol) and $Cr(CO)_6$ (4.4 g, 20.0 mmol) were heated under reflux in dibutyl ether (170 mL) for 8 h. After filtration and evaporation of solvent, the resulting yellow residue was dissolved in THF (20 mL). Following the method of Czernecki et al.,[28] this solution was carefully added to a suspension of 50% NaH (0.75 g, 15.5 mmol) and stirred for 5 h after which time benzyl bromide (8.5 g, 50 mmol) was introduced and the mixture heated under refluxing conditions for 5 h. The reaction mixture was left at ambient temperature overnight, then hydrolyzed with ice water, extracted with ether, and again washed with water and the ethereal solution dried over magnesium sulfate. The product was then chromatographed on a silica gel (Merck 7731) column and eluted with ether/petroleum ether (2:1). The first fraction was identified[14] as the α-isomer, mp 185 °C. Anal. Calcd for $C_{28}H_{30}O_5Cr$: C, 67.45; H, 6.06. Found: C, 66.10; H, 6.00. The second fraction was identified at the β-isomer,[12] mp 186 °C. Anal. Calcd for

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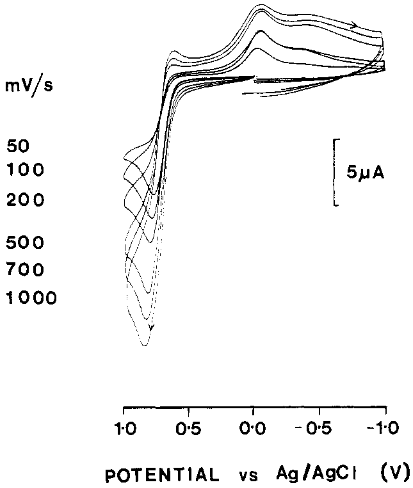

Figure 2. Cyclic voltammograms for 1×10^{-3} M β-(3-(benzyloxy)-17β-hydroxyestra-1,3,5(10)-triene)tricarbonylchromium, in CH_2Cl_2 (0.1 M Bu_4NClO_4) at 20 °C as a function of scan rate with the potential switched after the first oxidation process.

$C_{28}H_{30}O_5Cr$: C, 67.45; H, 6.06. Found: C, 67.51; H, 6.15. The crystalline material was stored under an argon atmosphere at 4 °C. The purity of the stored sample was confirmed by further microanalysis at the end of the 8-month period of experimental work and by periodic mass spectrometry (parent ion of correct mass (m/e = 499) is observed) and NMR measurements.[14]

Results and Discussion

(a) Voltammetry at Conventional Sized Disk Electrodes. In the presence of 0.1 M Bu_4NClO_4 as the supporting electrolyte, the first (least positive) oxidation process observed for both isomers of (3-(benzyloxy)-17β-hydroxyestra-1,3,5(10)-triene)tricarbonylchromium at conventional sized platinum electrodes at potentials around +0.7 to +0.8 V vs Ag/AgCl is chemically irreversible at slow scan rates (<100 mV s^{-1}) under conditions of cyclic voltammetry. The formulation $Cr(CO)_3$(steroid) is used in subsequent discussion to describe the steroid complex. As the concentration of perchlorate ion is reduced (while the total electrolyte concentration is maintained at 0.1 M with Bu_4NPF_6), the degree of chemical reversibility increases for both isomers of $Cr(CO)_3$(steroid). However, the degree of chemical reversibility is always greater for the α-isomer than for the β-isomer. In the absence of perchlorate ion, that is with 0.1 M Bu_4NPF_6 as the electrolyte, the first oxidation process corresponds to a completely chemical reversible one-electron oxidation process for both isomers even at a scan rate of 50 mV s^{-1}. With 0.1 M Bu_4NPF_6 as the electrolyte, a second (one) electron process is observed at more positive potentials. This second process observed in the potential region of +1.2 to +1.3 V vs Ag/AgCl is barely discernible at scan rates of 100 mV s^{-1} with 0.1 M Bu_4NClO_4 as the electrolyte. Clearly, the nature of the anion in the electrolyte is very important in determining the nature of the observed response. A third oxidation process of very positive potentials near the solvent limit corresponds to oxidation of the ligand[27] and is not discussed further.

Figure 2 shows cyclic voltammograms of the β-isomer in CH_2Cl_2 (0.1 M Bu_4NClO_4) as a function of scan rate. From this figure, it can be seen that, at slow scan rates, the process at around 0.8 V vs Ag/AgCl is chemically irreversible, and on the reverse (reductive) scan a complex reduction process(es) is observed at approximately 0 V vs Ag/AgCl. Clearly, a species can be formed after the first oxidation process, which is electroactive and which is a decomposition product of $[Cr(CO)_3(steroid)]^+$. As the scan rate is increased, process 1 becomes chemically reversible and concomitantly the reduction process observed around 0 V vs Ag/AgCl on the reverse scan decreases in magnitude. The cyclic voltammetry for the α-isomer is always qualitatively similar to that for the β-isomers but quantitatively different as will emerge after all relevant data are presented.

If the peak current of the first oxidation process is plotted as a function of the square root of the scan rate with 0.1 M Bu_4NClO_4 as the electrolyte, a nonlinear plot is observed at low scan rates, which is transformed to the linear relationship expected for a diffusion-controlled one-electron oxidation process at high scan rates. The scan rate at which the transition to a one-electron process is complete is dependent on whether the α- or β-isomer is being oxidized. As the scan rate is increased, the number of electrons involved (apparently) decreases from 2 to 1 in perchlorate media. In hexafluorophosphate media, data for the first oxidation process for both isomers are consistent with a diffusion-controlled one-electron process at all scan rates over the range 50–1000 mV s^{-1}.

The above observations are consistent with an ECE (electron transfer, chemical step, electron-transfer sequence) mechanism in the presence of perchlorate involving nucleophilic attack of the cation by the perchlorate ion followed by further oxidation of the product as in eq 1. The rate constant of the chemical step for perchlorate

$$\mathrm{Cr(CO)_3(steroid)} \underset{e^-}{\overset{-e^-}{\rightleftharpoons}} [\mathrm{Cr(CO)_3(steroid)}]^+ + \mathrm{ClO_4}^- \xrightarrow{k} \mathrm{product(s)} \xrightarrow{-e^-} \text{further oxidation} \qquad (1)$$

attack can be calculated at conventional size electrodes when linear diffusion conditions apply using a procedure developed by Nicholson and Shain[29,30] which involves comparing the measured peak current $(I_p)_K$ with the peak current $(I_p)_D$ expected for a reversible one-electron redox couple at the same scan rate. The peak current $(I_p)_D$ is calculated by using the equation[29,30]

$$(I_p)_D = 0.4463nFAC(Da)^{1/2} \qquad (2)$$

where $a = nFv/RT$, A = area of electrode, C = bulk concentration of species, D = diffusion coefficient, n = number of electrons (= 1), F = Faraday's constant, v = scan rate, R = universal gas constant, and T = temperature.

The following relationship applies where k' is the first-order (or pseudo-first-order in this case) rate constant of the chemical step

$$(I_p)_K/(I_p)_D = \frac{0.4 + k'/a}{0.396 + 0.469k'/a} \qquad (3)$$

and it is assumed that the diffusion coefficients of oxidized and reduced forms of the complex are equal. The difusiosn coefficient, D, was estimated from steady-state measurements at very small microelectrodes where it can be shown that the influence of the chemical step is insignificant (see later). The value of D obtained in this way was $(9.5 \pm 1.0) \times 10^{-6}$ cm^2 s^{-1}.

The analysis of data obtained from cyclic voltammetry at conventional sized electrodes requires careful correction of the peak currents, $(I_p)_K$, for ohmic iR drop which is not negligible in the highly resistive dichloromethane solvent. A convenient way of correcting for the influence of iR drop was to record voltammograms for the oxidation of fer-

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Table I. Pseudo-First-Order Rate Constant Calculated under Conditions of Linear Diffusion for a Conventional Sized Platinum Disk Electrode for Attack of the Perchlorate Anion on the α and β Chromium Tricarbonyl Steroid Cation Radicals in CH_2Cl_2 (0.1 M Bu_4NClO_4) at 20 °C

	α-isomer			β-isomer		
scan rate, V s^{-1}	$(I_p)_D$, μA	$(I_p)_K$, μA	(k_α),[b] s^{-1}	$(I_p)_D$, μA	$(I_p)_K$, μA	k_β,[c] s^{-1}
0.05	3.0	5.6	5.4	4.5	10.0	a
0.1	4.3	6.8	3.5	6.4	12.4	a
0.2	6.0	8.6	4.1	9.0	14.4	7.4
0.5	9.5	12.0	4.9	14.3	19.8	8.4
0.7	11.3	13.8	5.4	16.9	22.4	9.2
1.0	13.5	15.0	3.3	20.2	25.2	9.0

[a] Process is too close to being completely irreversible for accurate kinetic data to be obtained via the use of eq 3. [b] Average value = (4.4 ± 0.9) s^{-1}. [c] Average value = (8.5 ± 0.7) s^{-1}.

rocene under conditions identical with those used for oxidation of the chromium complex. In the case of ferrocene, any departure from a linear plot of peak current versus square root of scan rate is due to *iR* drop. The heterogeneous charge-transfer rate constant for oxidation of ferrocene is extremely fast,[31] and under the conditions of the present experiments this process would correspond to a reversible diffusion-controlled process. In the absence of *iR* drop a reversible process will exhibit a peak height which is directly proportional to the square root of scan rate.[29] The results of calculated value of the pseudo-first-order rate constants k_α' and k_β' using peak currents corrected for *iR* drop via reference to the ferrocene data are summarized in Table I. When 0.1 M Bu_4NClO_4 is used as the supporting electrolyte, k_α' and k_β' are 4.4 ± 0.9 and 8.5 ± 0.7 s^{-1}, respectively. Measured values of k_α' and k_β' are dependent on the concentration of perchlorate. When the medium is 0.05 M Bu_4NClO_4–0.05 M Bu_4NPF_6, the values are close to half those in 0.1 M Bu_4NClO_4 as required for a second-order process. Unfortunately, rate constants in dilute perchlorate concentrations are too slow to measure accurately by cyclic voltammetry. The calculated second-order rate constants k_α and k_β for the α- and β-isomers are 44 ± 9 and 85 ± 7 M^{-1} s^{-1}, respectively, for the reaction

$$[Cr(CO)_3(steroid)]^+ + ClO_4^- \xrightarrow{k} \text{products} \quad (4)$$

The reversible half-wave potentials, $(E^r_{1/2})$, of the two isomers for the reaction

$$Cr(CO)_3(steroid) \overset{E^r_{1/2}}{\rightleftharpoons} [Cr(CO)_3(steroid)]^+ + e^- \quad (5)$$

are also different. The $E^r_{1/2}$ values, which are closely related to the thermodynamically significant standard redox potentials ($E°$ values), were determined by taking the potential midway between the oxidation and reduction peak potentials when the voltammograms were chemically reversible (hexafluorophosphate data) or at fast scan rates (perchlorate data). For the α-isomer, $E^r_{1/2}$ was measured as (0.730 ± 0.005) V vs Ag/AgCl and for the β-isomer (0.760 ± 0.005) V vs Ag/AgCl in 0.1 M Bu_4NPF_6. The $E^r_{1/2}$ values for both isomers with 0.1 M Bu_4NClO_4 as the electrolyte obtained from fast scan rate data are in complete agreement with the values obtained with 0.1 M Bu_4NPF_6 as the electrolyte. So in the thermodynamic sense, the electrolyte is not important.

When the potential is scanned to values more positive than for the first oxidation step (Figure 3 shows the situation for the α-isomer), a well-defined second oxidation step is observed when 0.1 M Bu_4NClO_4 is the electrolyte at scan rates where the first process becomes reversible.

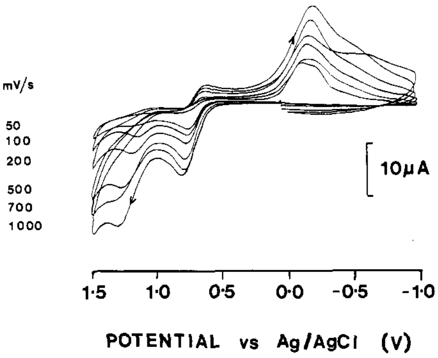

Figure 3. Cyclic voltammograms for 1 × 10^{-3} M α-(3-(benzyloxy)-17β-hydroxyestra-1,3,5(10)-triene)tricarbonylchromium in CH_2Cl_2 (0.1 M Bu_4NClO_4) at 20 °C as a function of scan rate with the potential switched at values beyond the first oxidation step.

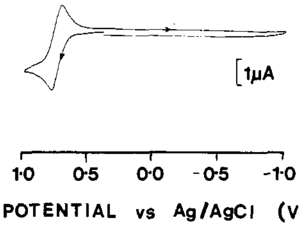

Figure 4. Cyclic voltammogram at a scan rate of 50 mV s^{-1} for the first oxidation process of 5 × 10^{-4} M β-(3-(benzyloxy)-17β-hydroxyestra-1,3,5(10)-triene)tricarbonylchromium in CH_2Cl_2 (0.1 M Bu_4NPF_6) at 17 °C.

This is consistent with the formation of a dication which rapidly decomposes to form a product(s) which can be reduced at a potential around 0 V vs Ag/AgCl. A similar response is also observed at 0 V vs Ag/AgCl when the first process is an overall irreversible two-electron step. This behavior is qualitatively similar to that observed for the oxidation of other chromium steroids[27] and $Cr(CO)_3$(benzene).[32] The second process can be written as

$$[Cr(CO)_3(steroid)]^+ \rightarrow [Cr(CO)_3(steroid)]^{2+} \rightarrow \text{products} \quad (6)$$

The postulated dication is a 16-electron species and probably more readily attacked by perchlorate (or hexafluorophosphate) than the 17-electron $[Cr(CO)_3(steroid)]^+$ complex to form an unstable 18-electron, 7-coordinate species such as $Cr(CO)_3(steroid)(ClO_4)$.

Figure 4 shows a cyclic voltammogram of the β-isomer in 0.1 M Bu_4NPF_6 electrolyte. Under conditions where the scan direction is reversed prior to reaching the second step and the first oxidation step is reversible, the (stripping) reduction process(es) observed on the reverse scan

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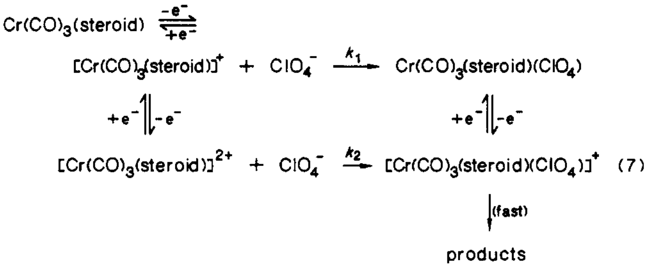
Table II. Apparent Number of Electrons Transferred in the Limiting Current Region and Other Data at Platinum Microdisk Electrodes of Different Radii for the First Oxidation Process for the α- and β-Isomers of $Cr(CO)_3$(steroid) in CH_2Cl_2 (0.1 M Bu_4NClO_4) at 20 °C

electrode, radius, μm	α-isomer				β-isomer			
	$E_{1/2}$,[b] V	$E_{1/4} - E_{3/4}$, mV	$i_{(l)}$, pA	n_{app}[a]	$E_{1/2}$,[b] V	$E_{1/4} - E_{3/4}$, mV	$i_{(l)}$, pA	n_{app}[a]
12.5	0.740	53	3370	1.5	0.755	50	3600	1.9
5.0	0.730	58	1260	1.4	0.770	50	1000	1.3
2.5	0.738	58	500	1.1	0.770	56	500	1.0
1.0	0.747	56	180	1.0	0.775	55	155	1.0
0.5	0.748	56	90	1.0	0.768	56	77	1.0

[a] n_{app} is assumed to be one for the 0.5-μm radius platinum disk electrode. The scan rate used for all experiments was 50 mV s^{-1}. [b] V vs Ag/AgCl.

at around 0 V vs Ag/AgCl is absent. This supports the idea that the species reduced at 0 V vs Ag/AgCl arises from a product formed by attack of electrolyte anion on the dication $[Cr(CO)_3(steroid)]^{2+}$. The process(es) around 0 V vs Ag/AgCl are always observed if the scan direction is reversed at potentials more positive than the second process. Results are consistent with the simplified reaction scheme given in eq 7. If Bu_4NPF_6 electrolyte is used, then



nucleophilic attack occurs with PF_6^-. Under these conditions, the analogous k_1 and presumably k_2 rate constants presumably are slower than when ClO_4^- is used as an electrolyte.

(b) Voltammetry at Microdisk Electrodes. It has been shown by Fleischmann et al.[33] that microelectrodes used in the steady-state mode (sigmoidal rather than peak shaped curves are obtained) offer a convenient way of measuring chemical rate constants of ECE mechanisms. Under steady-state conditions, *iR* drop and charging current problems are minimal and the apparent number of electrons observed, n_{app}, for an ECE process as determined from the magnitude of the limiting current will decrease from 2 at sufficiently large electrodes to 1 at sufficiently small electrodes. That is, the influence of the chemical step following the first charge-transfer process will diminish as the electrode radius is decreased. The equivalent situation is achieved at fast scan rates with voltammetry at conventional sized electrodes. The relationship for a spherical microelectrode of radius r_s is given in where $\Delta n = n_{app} - n_1$, D = the diffusion coefficient which

$$\frac{1}{\Delta n} = \left[\left(\frac{D}{n_2^{\,2}k'}\right)^{1/2}\frac{1}{r_s} + \frac{1}{n_2}\right] \qquad (8)$$

is assumed to be the same for all species, n_2 = number of electrons involved in the second transfer step (1 in this case), and k' is the first-order (or pseudo-first-order) rate constant of the chemical step.

In this work, disk rather than spherical microelectrodes were used. However, it can be assumed that the limiting currents at a disk electrode is equivalent to that at a sphere of radius $(2/\pi)r_d$, where r_d is the radius of the disk.[34,35]

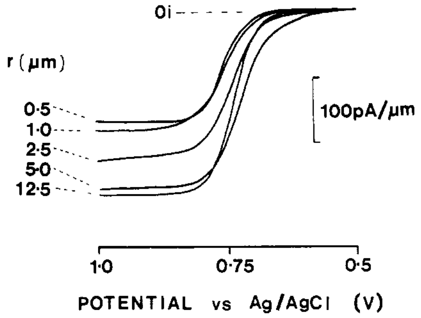


Figure 5. Voltammograms at platinum microdisk electrodes at various radii for the first oxidation process of 5 × 10^{-4} M α-(3-(benzyloxy)-17β-hydroxyestra-1,3,5(10)-triene)tricarbonylchromium in CH_2Cl_2 (0.1 M Bu_4NClO_4) at 20 °C. The current scale is normalized to the radius of the electrode so that the wave height is proportional to the apparent number of electrons transferred, at 20 °C. The scan rate is 50 mV s^{-1}.

Steady-state voltammograms were recorded on both isomers on platinum microdisk electrodes, ranging in radius from 0.5 to 12.5 μm. A typical series of steady-state voltammograms is shown for oxidation process 1 for the α-isomer in Figure 5. The limiting current is normalized to the electrode radius to allow for the change in the electrode size. After normalization, the increase in the apparent number of electrons is obvious as the electrode size increases. Results for both isomers are summarized in Table II.

Although qualitative confirmation of the ECE-type mechanism was possible from the microelectrode work, quantitatively useful data could not be obtained at microelectrodes. Adsorption of the product(s) after the first or second step(s) on the electrode surface (which causes stripping peaks around 0 V vs Ag/AgCl on the reverse scans of cyclic voltammograms) leads to a lack of reproducibility at microelectrodes which is not evident at conventional sized electrodes. Electrochemical cleaning of the electrodes by pulsing the potential between plus and minus 3 V vs Ag/AgCl prior to the experiment gave the results presented in Table II. However, even under these conditions, the reproducibility is not high enough for quantitative calculations of the rate constant. Data in Table II confirm that the $E^{r}_{1/2}$ value calculated for the reaction in eq 6 from data at microelectrodes for the α- and β-isomers differ by about 30 mV under conditions of radial diffusion and that the first charge-transfer process is a reversible one-electron step.

The enhanced problem of electrode fouling under steady-state experiments at microelectrodes relative to conventional size electrodes is intriguing. Presumably the large current densities obtained at microelectrodes compared to larger electrodes leads to more severe fouling

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[33] Fleischmann, M.; Lasserre, F.; Robinson, J. *J. Electronanal. Chem.* **1984**, *177*, 115.

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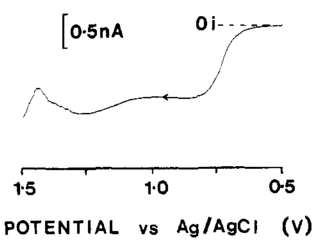

Figure 6. Voltammogram at a 5-μm radius platinum microdisk electrode for the oxidation of 5 × 10^{-4} M α-(3-benzyloxy)-17β-hydroxyestra-1,3,5(10)-triene)tricarbonylchromium in CH_2Cl_2 (0.1 M Bu_4NClO_4) over an extended potential range at 20 °C. The scan rate is 50 mV s^{-1}.

problems (electrode blockage). Figure 6 shows a voltammogram of the α-isomer obtained at a 5-μm radius platinum disk electrode when the potential is scanned to more positive potentials than was the case in Figure 5. When the potential is scanned into the region of the second oxidation peak, the electrode becomes totally fouled and useful data on this second step could not be obtained at microelectrodes. Indeed, holding the potential at a constant value between processes one and two always leads to the current decaying to zero. This implies that a film is formed which completely covers the microelectrodes and blocks the electrode response. It is this compond(s) which is stripped from the electrode at around 0 V vs Ag/AgCl on the reverse (reductive) scan of cyclic voltammograms at conventional sized electrodes.

(c) Controlled Potential Electrolysis. Controlled potential electrolysis of both diastereoisomers at a platinum gauze basket electrode with the potential held between values observed for oxidation processes one and two occurs with the transfer of two electrons per molecule. Electrode fouling makes bulk electrolysis experiments very slow. Infrared monitoring during the course of electrolysis (carbonyl bands) demonstrates that all carbonyl groups are lost. Chromatographic analysis confirms that free steroid is recovered quantitatively and that no organometallic complex remains. Thus the overall process occurs as in eq 9.

$$Cr(CO)_3(\text{steroid}) \rightarrow Cr(II) + 3CO\uparrow + \text{free steroid} + 2e^- \qquad (9)$$

Conclusions

Both voltammetric studies at conventional and microsized platinum electrodes lead to the conclusion that the one-electron oxidation of the 18-electron α-isomer of $Cr(CO)_3$(steroid) is thermodynamically easier than the β-complex. The $E°$ difference of the two diastereoisomers is (30 ± 10) mV, and the 17-electron cation generated from the β-diastereoisomer is more prone to attack by the perchlorate ligand than the α-isomer. On examining spatial alternative conformational structures (e.g. Figure 7) there are a number of possible conformational arrangements of α- and β-diastereoisomers which may or may not be altered on oxidation to the formally chromium(I) 17 electron state. Presumably, steric factors provide greater accessibility of the perchlorate ion to a 17-electron transition-state complex. However, in view of the fact that the exact conformation of the oxidized or reduced chromium complexes are unknown, further speculation on the origin of the thermodynamic and kinetic differences is not warranted.

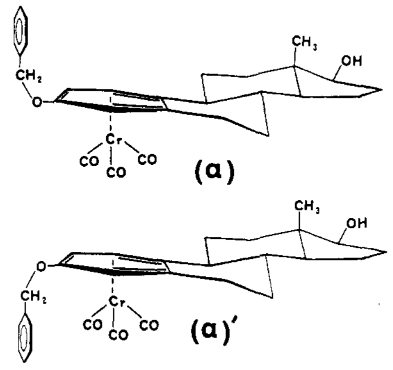

Figure 7. Exo (α) and endo (α′) spatial arrangements of α conformational isomers.

While in the past, Fourier transform infrared studies of the carbonyl moiety have been used to probe hormone receptor binding,[14] this work leads to the observation that electrochemical studies might prove useful in adding to existing knowledge concerning the binding action of steroids on receptor sites[12,14] in cells by utilizing the $Cr(CO)_3$(steroid) complexes as electrochemical markers.

The electrochemical oxidation of estradiol and other hormone steroids and their organometallic carbonyl steroid complexes (chromium, molybdenum, and tungsten) has been previously reported.[27] In this work, it was also established that either a single irreversible two-electron oxidation process or two one-electron oxidation steps could be observed depending on the conditions and the complex. However, no consideration of the influence of the diastereoisomers was considered. In the present work the diastereoisomers α- and β-(3-(benzyloxy)-17β-hydroxyestra-1,3,5(10)-triene)tricarbonylchromium are shown to be distinguishable in both the oxidation potential and chemical reversibility of the first oxidation process. Consequently, previous studies, which were undertaken on mixtures of the diastereoisomers, may need careful reevaluation with respect to isomeric details.

Acknowledgment. We thank Chemical and Micro-Analytical Services Pty. Ltd. of North Essendon, Victoria Australia for provision of their microanalysis service free of charge.

Registry No. α-[$Cr(CO)_3$(steroid)], 98688-30-3; β-[$Cr(CO)_3$(steroid)], 98757-32-5; α-[$Cr(CO)_3$(steroid)]$^+$, 122189-74-6; β-[$Cr(CO)_3$(steroid)]$^+$, 122291-03-6; steroid, 14982-15-1; CH_2Cl_2, 75-09-2; Pt, 7440-06-4; Bu_4NClO_4, 1923-70-2; Bu_4NPF_6, 3109-63-5; Cr(II), 22541-79-3; CO, 630-08-0.