25, 113599-35-2; **26**, 113599-36-3; **27**, 113599-37-4; **28**, 113599-38-5; **29**, 113599-39-6; **30**, 113599-40-9; **31**, 113599-41-0; **32**, 113599-42-1; **33**, 113599-43-2; **34**, 77027-48-6; **35**, 113599-44-3; **36**, 113599-45-4; **37**, 113599-46-5; **38**, 113599-47-6; **39**, 113599-48-7; **40**, 113599-49-8; **41**, 113599-50-1; **42**, 113599-51-2; **43**, 113599-52-3; **44**, 113599-53-4; **45**, 113599-54-5; **46**, 113666-81-2; **47**, 113666-82-3; 3-isopropyltetronic acid, 113599-55-6; (23*R*,22*R*)-6-(ethylenedioxy)-20,22,23-trihydroxy-2α,3α-(isopropylidenedioxy)-5α-23-ergosten-28-oic acid γ-lactone, 113599-56-7; (22*R*,23*R*,24*R*)-22,28-diacetoxy-2α,3α-23-trihydroxy-5α-ergostan-6-one, 113599-57-8; (22*R*,23*R*,24*S*)-22-acetoxy-2α,3α,23-trihydroxy-5α-ergostan-6-one, 113599-58-9.

Polarographic and Spectroscopic Examination of the Reaction of the Anabolic Steroid Oxymetholone with Methanol and Ethanol

Alan M. Bond,* Dainis Dakternieks, Patrick P. Deprez,† and Petr Zuman‡

Division of Chemical and Physical Sciences, Deakin University, Waurn Ponds 3217, Victoria, Australia

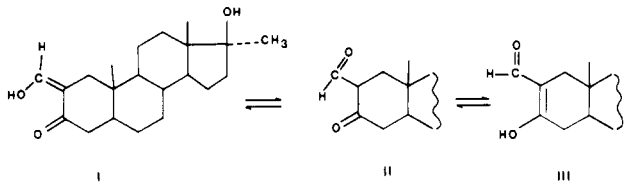
Received May 1, 1987

Studies employing electrochemical reduction at mercury electrodes, carbon-13 nuclear magnetic resonance spectroscopy, mass spectrometry, and other techniques have contributed to an understanding of the solution chemistry of the anabolic steroid oxymetholone (17-hydroxy-2-(hydroxymethylene)-17-methyl-5α,17β-androstan-3-one). The compound can exist in three tautomeric forms, I–III. In mixtures of aqueous buffer and acetonitrile, the geminal diol of form III is electrochemically reducible. In mixtures containing methanol or ethanol, a hemiacetal VI is formed, which is in slowly established equilibrium with the hydrate. Two separate peaks in differential pulse polarography show establishment of the equilibria between the hydrate and the hemiacetal, whereas UV spectra show overlapping peaks. An approximate value $K = 0.29$ has been found for the equilibrium constant K = [hemiacetal][H_2O]/[hydrate][CH_3OH]. Interaction of oxymetholone with alcohols was confirmed by mass spectra of reaction products. ^{13}C NMR spectra indicate that addition of methanol also occurs in chloroform solutions. Electrochemical studies enhance the observation of an unexpected chemical reaction.

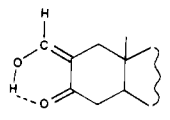
Introduction

Oxymetholone (17-hydroxy-2-(hydroxymethylene)-17-methyl-5α,17β-androstan-3-one) is a commonly prescribed anabolic steroid used in treating anemias. Clinical assessments of oxymetholone have been made to establish the degree of anabolism and androgenicity of this steroid,[1-6] and information on side effects has been documented. Despite the wide use of this steroid in pharmaceutical preparations and significant studies on side effects,[4-6] relatively little is known about the solution chemistry of this compound.

Oxymetholone[7] is a 1,3-dicarbonyl compound, which can exist in three tautomeric forms I–III. There is no quantitative information available regarding the positions of these equilibria and on solvent effects on them. Nevertheless, the structure of oxymetholone is usually presented as I, which in nonhydroxylic media is stabilized by formation of an intramolecular hydrogen bond (IV).



I II III



IV

The very broad relatively low intensity band in the infrared spectrum at 2500–3000 cm^{-1} has been attributed[8] to intramolecularly bonded enolic OH, a peak at 3350 cm^{-1} to the hydroxy group, and the strong peak at 1615 cm^{-1} to the C=O stretching in CO—C=C—OH. On the other hand, the proton NMR spectra have been interpreted[9] to favor, in some solvents, the formyl enol form III.

In hydroxylic solvents able to form intermolecular hydrogen bonds, the role of intramolecular hydrogen bonds is usually negligible[10] whereas covalent solvation, resulting in formation of hydrates and hemiacetals, may occur in nonhydroxylic solvents. As aldehydes form hydrates and hemiacetals more readily than ketones,[11] nucleophilic addition of the solvent will favor the formyl groups in structures II and III. As conjugation deactivates the carbonyl function toward hydrate and hemiacetal formation,[11] stronger hydration and reaction with alcohols is predicted to involve structure II rather than III. In this structure, the presence of the electron-withdrawing carbonyl groups in the β-position can be expected to enhance the reactivity of the formyl group toward nucleophilic addition. The only other reported[8] nucleophilic attack on oxymetholone, that of hydroxide ions, is also assumed to involve structure II. Resulting deformylation resembles that of other 3-formyl ketones.[12,13] On the other hand,

† Present address: CSIRO Division of Oceanography, G.P.O. Box 1538, Hobart, Tasmania 7001.

‡ On leave from Department of Chemistry, Clarkson University, Potsdam, NY 13676.

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α,β-unsaturated carbonyl compounds resembling structures I and III are not measurably hydrated on the carbonyl groups and do not add neutral alcohol molecules. Addition of a hydroxide[14,15] or alkoxide[16,17] ion occurs to the ethylene bond rather than to the carbonyl group. The electronic spectrum in 95% ethanol shows[8] a maximum at 285 nm with log ϵ 4.05, which indicates that in such solutions a species with a conjugated system CO—C=C predominates (i.e., structures I or III) in the equilibrium.

In the electrochemical investigation of oxymetholone, tautomeric form II can be expected to be reducible at rather negative potentials, comparable, e.g., with reduction of phenylacetaldehydes.[18] Both forms I and III involve an α,β-unsaturated carbonyl group which should be accessible to a relatively easy reduction. The ketone system in tautomer I differs from other anabolic steroids reducible at mercury electrodes[19-21] in the exocyclic position of the double bond. To the best of our knowledge, no known precedent exists for reduction of the unsaturated aldehyde–enol form III. All aldehydic steroids studied so far, such as cymarine,[22] hollarhimine and related compounds,[23] or aldosterone,[24] bear the formyl group of an sp^3 carbon. In this study an examination of the electrochemical reduction of oxymetholone at mercury electrodes in aqueous–organic solvent mixtures and organic solvents has been carried out in combination with spectroscopic investigations in order to contribute to characterization of the solution chemistry of the compound. Greatest attention has been paid to water–alcohol mixtures, as such mixtures are commonly used for the polarographic[25] and spectrophotometric[8] studies on anabolic steroids. The results suggest that an unexpected reaction occurs relative to that predicted from considerations presented above.

Experimental Section

(a) Chemicals and Reagents. All chemicals used were of reagent grade purity unless otherwise stated. Oxymetholone was obtained from Parke-Davis and Syntex and checked for purity by thin-layer chromatography and GC–MS. Acidic solutions up to pH 2 were dilute solutions of hydrochloric acid. For medium pH range, 0.1 M acetate buffers were used, and for higher pH values, 0.1 M phosphate buffers were prepared. These electrolytes were mixed in equal volume (50% v/v) with the organic solvent prior to final "pH" measurement. Such mixtures containing 50% of the organic solvent used for electrochemical experiments are referred to as the "blank".

(b) Instrumentation and Procedures. (i) Electrochemical. Polarograms were obtained with a PAR Model 174A (Princeton Applied Research Corporation, Princeton, NJ) polarographic analyzer and recorded on a Houston Omnigraphic Model 2000 X-Y recorder.

The polarographic cell was of 10–20-mL volume. The working electrode was either a conventional controlled drop time dropping mercury electrode or a PAR static mercury drop electrode (SMDE) Model 303. Aqueous Ag/AgCl (saturated NaCl or 3 M KCl) or methanolic Ag/AgCl (saturated LiCl) reference electrodes were employed along with a platinum auxiliary electrode in a three-electrode system. In differential pulse polarography, the modulation amplitude used was -50 mV. Peak heights in differential pulse polarography (DPP) were measured as a difference from a tangent fit of the base line. The scan rate used for the polarographic measurements was 2 mV/s, and data were recorded in compliance with the criteria set out in the literature.[26] All polarographic measurements were made at 25 ± 1 °C.

In all electrochemical studies, a freshly prepared stock solution of oxymetholone was made up in the given solvent at a concentration of 10^{-2} or 10^{-3} M. At known times, appropriate volumes of stock solution of the steroid were transferred to the polarographic cell, which contained 10 mL of the blank. Oxygen was removed by a vigorous stream of high-purity nitrogen, which was also introduced above the surface of the solution during the recording of polarograms.

Controlled potential electrolysis and coulometric measurements were performed at 25 ± 1 °C by using a PAR Model 173 potentiostat/galvanostat and PAR Model 179 digital coulometer. The working electrode used as a Hg pool (≈3.5-cm diameter). The reference electrode was Ag/AgCl (saturated NaCl), and the auxiliary electrode consisted of platinum mesh which was immersed in a glass jacket containing the blank and separated from the test solution by a salt bridge. After addition of 1×10^{-5} mol of the steroid into a 20-mL volume of the blank, the resultant total coulomb count corrected for background was obtained. Extractions of the electrolysis products were made with 2 × 10 mL aliquots of chloroform, and the volume was reduced to approximately 0.5 mL for examination by gas chromatographic–mass spectrometric methods.

A Metrohm pH meter E 520 (Herisau, Switzerland), used for measuring pH to ±0.05 pH units, was calibrated against an aqueous solution of 0.05 M potassium hydrogen phthalate (pH 4.00). The measured pH values for solutions containing 50% of organic solvent are denoted as "pH".

(ii) Spectroscopic. Carbon-13 spectra (proton decoupled) were recorded in $CDCl_3$ solution and referenced against internal TMS by using a JEOL GX-270 spectrometer. The number of protons attached to each carbon was determined from 45°, 90°, and 135° DEPT experiments. A COSY experiment was used to check assignments and to determine that the proton with δ_H 8.68 is attached to the carbon with δ_C 188.01.

Spectrophotometric data were obtained with a Pye-Unicam S.P. 800 spectrophotometer. The KBr disk technique was used throughout for infrared measurements on solid samples with a Unicam SP1000 infrared spectrophotometer.

Gas chromatographic–electron-impact mass spectrometric (GC–MS) experiments were performed with a Finnigan 3200 Series gas chromatograph–mass spectrometer by using the following conditions and procedures. An OV 101 capillary column was used. The carrier gas was helium at a flow rate of 2–4 mL/min. Quadrupole measurements were carried out at low resolution (0–800 amu). The ion source was maintained at 35 °C, the electron energy was 70 eV, and the scan time was 1 ms/amu.

Results and Discussion

The reaction of oxymetholone with alcohols was studied partly in water–alcohol mixtures by using electrochemical and spectrophotometric methods and partly in chloroform solutions by ^{13}C NMR spectroscopy. The structure of the product isolated from methanolic solutions was investigated by IR and mass spectral methods.

(A) Studies of Water–Alcohol Mixtures. (a) Polarographic Investigation of the Reaction of Oxy-

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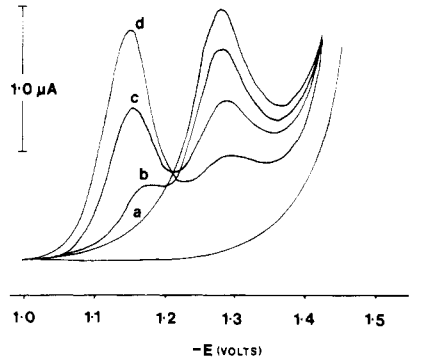


Figure 1. Differential pulse polarograms of 1.39×10^{-4} M oxymetholone obtained in 50% v/v methanol–aqueous acetate buffer (0.1 M) at "pH" 5.1. Prior to a 100-fold dilution and addition to the aqueous buffers, the oxymetholone had been stored in methanol for (a) 10 min; (b) 2 h 20 min; (c) 9 h 51 min; (d) 26 h. T = 25 °C. Pulse amplitude = −50 mV. Drop time = 0.5 s (dropping mercury electrode).

metholone with Methanol. A freshly prepared stock solution of oxymetholone added to a "pH" 5.1 buffer gave a single wave at −1.3 V vs Ag/AgCl in a differential pulse polarogram. Methanolic stock solutions more than 0.5 h old gave two waves at −1.15 and −1.3 V vs Ag/AgCl when added to the blank. With increasing aging of the stock solution, the height of the peak at −1.15 V vs Ag/AgCl increased while that at −1.3 V vs Ag/AgCl decreased (Figure 1) until after 5 days' standing the stock solution in 100% methanol when added to the aqueous buffer containing 50% methanol yielded only the peak at −1.15 V vs Ag/AgCl. During the change in peak heights with time, the reduction potentials for the two peaks remain virtually the same. Existence of an "isosbestic point" (Figure 1) indicates absence of accumulation of an intermediate or side product in the course of the reaction.

The peak at −1.3 V vs Ag/AgCl in fresh solutions and the peak at −1.15 V vs Ag/AgCl in aged solution are linear functions of concentration in the range between 5×10^{-6} M and 1×10^{-3} M. Figure 2 shows a polarogram of a stock oxymetholone solution 5 days after the steroid was dissolved in methanol. When the aged stock solution is added to a buffer containing 50% methanol and polarograms are recorded as a function of time, the height of the peak at −1.15 V vs Ag/AgCl decreases and this peak is gradually replaced by the peak at −1.3 V vs Ag/AgCl. The latter peak predominates when equilibrium is reached in buffered solutions containing 50% methanol. This behavior indicates the presence of an equilibrium between a form A of oxymetholone, which is reduced at −1.3 V vs Ag/AgCl and which is predominant in both methanolic stock solutions prepared from solid oxymetholone and equilibrated solutions buffered at "pH" 5.1 containing 50% methanol, and a form B of oxymetholone, which is reduced at −1.15 V vs Ag/AgCl and which predominates in the equilibrium position present in 100% methanolic solutions. As the equilibrium between A and B is established slowly over a period of hours and days, the changes in polarographic waves reflect changes of concentration of species A and B in the bulk of the solution rather than in the vicinity of the electrode. It seems probable that species A results from interaction of oxymetholone with water, species B from interaction with methanol.

Form II can react with both water and methanol and form a hydrate V (R = H) or hemiacetal (R = CH_3). Whereas it is known[27] that electrochemical reduction of α-hydroxy ketones results in a cleavage of the C–O bond,

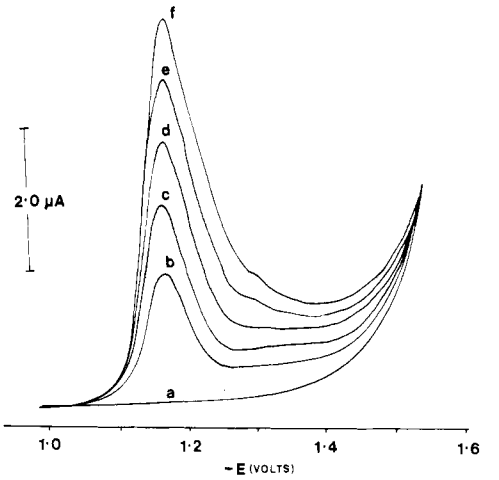
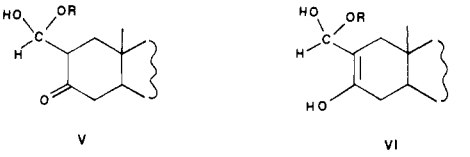


Figure 2. Differential pulse polarograms recorded in a solution of 50% v/v methanol–aqueous acetate buffer (0.1 M) at "pH" 5.3 showing the reduction peak of oxymetholone after reaction with methanol for 5 days. Concentration after 100-fold dilution: (a) blank; (b) 1×10^{-4} M; (c) 2×10^{-4} M; (d) 3×10^{-4} M; (e) 4×10^{-4} M; (f) 5×10^{-4} M. T = 25 °C. Pulse amplitude = −50 mV. Drop time = 0.5 s (dropping mercury electrode).

no system has been reported in which a β-hydroxy ketone is reduced.



V VI

Form III can also add both water and methanol and form the hydrate VI (R = H) or hemiacetal (R = CH_3). For these compounds the C–OR band is adjacent to an ethylenic group (RO—C—C=C) where electronic interaction similar to that in α-hydroxy ketones (RO—C—C=O) is possible. To our knowledge, reduction of γ-hydroxy α,β-unsaturated compounds has not been reported, but the reductive cleavage of the C–OR bond seems plausible on the basis of analogy with reduction of α-hydroxy ketones.[27] Facilitation of the reduction of the C–OCH_3 (at −1.15 V) when compared with C–OH (at −1.3 V vs Ag/AgCl) also resembles the behavior of α-hydroxy and α-alkoxy ketones.

Form I is an α,β-unsaturated ketone, which are unreactive to addition of water and neutral alcohol molecules. Even if the reactivity of the ethylenic bond toward the addition of ROH might be increased by the presence of the hydroxy group on the methylenic bond, addition would occur in the β-position yielding the hydrate or hemiacetal, the electroreducibility of which was questioned above.

Thus the equilibrium between A and B can be ascribed to transacetalization of the type recently described by Guthrie.[28] Form III seems to be the most probable participant in this reaction.

Participation of methanol in the reaction of form III is further supported by the polarographic behavior of oxymetholone in a "pH" 5.0 acetate buffer containing 50% acetonitrile. In such a solution a single wave at −1.3 V vs Ag/AgCl was observed. This wave is time independent, indicating the absence of a chemical reaction with solvent.

(b) Spectrophotometric Examination of the Reaction of Oxymetholone with Methanol. A freshly prepared 1×10^{-4} M solution of oxymethlone in methanol shows a broad absorption band in the UV region with a maximum of 282 nm (Figure 3). This band is within 24

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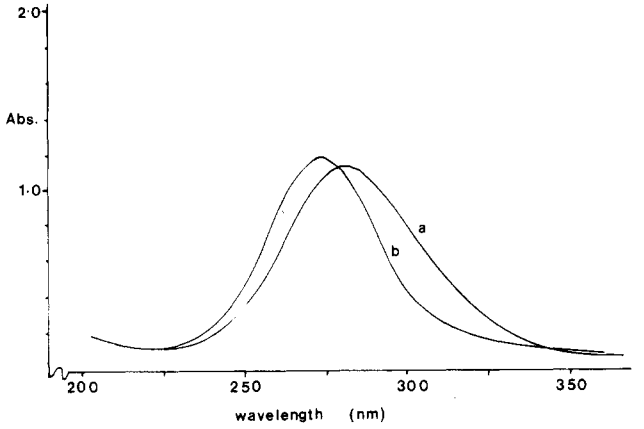

Figure 3. Spectrophotometric data for oxymetholone (10^{-4} M) in 100% methanol. Curve a is the response for a freshly prepared solution and curve b the response after being left for 24 h. T = 25 °C.

h gradually replaced by a band at 275 nm. An isosbestic point observed at 280 nm indicates (similarly as polarographic studies) that reaction does not involve accumulation of an absorbing intermediate or of a side product. Application of the Woodward–Hoffmann rules[29] does not allow distinction of the difference of 7 nm between A_{max} of the structure VI for R = H and R = CH_3. Molar absorptivity is of the order of 1.1×10^4 mol^{-1} cm^{-1}, which is in general agreement with the literature.[8]

(c) Rates and Equilibrium Constants for the Reactions of Oxymetholone with Methanol. Because of superior resolution, the rate and equilibria of the reaction of oxymetholone with methanol are more easily monitored by differential pulse polarography than by UV spectrophotometry.

From reaction mixtures containing 1×10^{-3} M and 9.4×10^{-3} M oxymetholone in pure methanol, samples were taken at chosen time intervals and examined immediately in a "pH" 5.0 acetate buffer containing 50% methanol. A plot of the logarithm of the peak current against time indicates that reaction 1 follows first-order or pseudo-

$$A + CH_3OH \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B + H_2O \qquad (1)$$

first-order kinetics. Under the conditions used, the conversion of A into B is pratically quantitative and the reverse reaction with rate constant k_{-1} can be neglected, due to the low concentration of water. From the slope of the logarithmic plot it follows that the pseudo-first-order rate constant k_f' is given by $k_f' = k_1[CH_3OH] = (3.3 \pm 0.17) \times 10^{-5}$ s^{-1}.

To follow the reverse reaction, we first aged a 1.0×10^{-1} M solution of oxymetholone until only the form B was present, as confirmed by the presence of a single peak at −1.15 V on the polarographic curve. Aliquots of this aged stock solution were then introduced into an acetate buffer at "pH" values between 4.0 and 5.5 and containing 50% methanol. From the linear logarithmic plot of the peak current at −1.15 V as a function of time, the pseudo-first-order rate constant, k_r', of the reverse reaction $k_r' = k_{-1}[H_2O] = (11 \pm 0.17) \times 10^{-5}$ s^{-1} has been found. This value was practically pH-independent over the range studied. It is understood that the forward reaction was followed in 100% methanol whereas the reverse reaction

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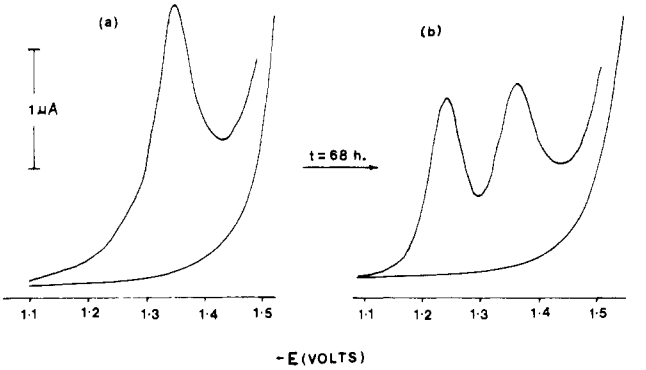

Figure 4. Differential pulse polarographic examination of solutions of oxymetholone dissolved in 8.9 mL of methanol and 1.1 mL of water. Curve a shows the polarogram for a 100-μL aliquot of the 10^{-2} M stock solution obtained immediately after being dissolved and examined in 10 mL of 50% v/v methanol–acetate buffer at "pH" 4.7. Curve b shows the polarogram under the same conditions for a 100-μL aliquot of a 10^{-2} M stock solution after being left to react for 68 h. Drop time = 0.5 s (static mercury drop electrode). T = 25 °C. Pulse amplitude = −50 mV.

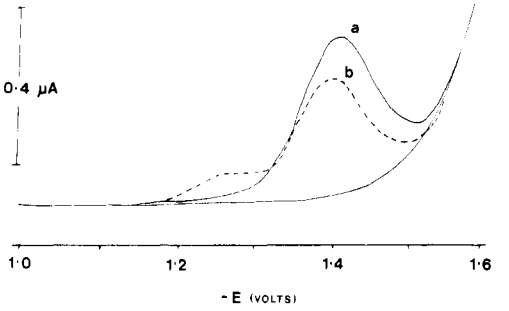

Figure 5. Differential pulse polarographic examination of solutions of oxymetholone dissolved in ethanol. Curve a is a 100-μL aliquot of a 3×10^{-2} M solution of a freshly dissolved sample examined in 10 mL of 50% v/v ethanol–aqueous acetate buffer, "pH" 5.75. Curve b is a 100-μL aliquot taken from the stock solution and examined after 20 h. Polarographic parameters are as in Figure 4.

was followed in 50% methanol–50% water (v/v). Nevertheless, as a first approximation the equilibrium constant K for reaction 1 can be calculated as

$$K = k_f'/k_r' = [B][H_2O]/[A][CH_3OH] = 0.29 \qquad (2)$$

This equation indicates that equilibrium concentrations of forms A and B will be equal when $[CH_3OH]:[H_2O]$ is approximately 3.6:1. To prove the validity of the above assumptions, we prepared a reaction mixture by mixing 8.9 mL of methanol with 1.1 mL of water and dissolving oxymetholone in this reaction mixture to a concentration of 1×10^{-2} M. The polarographic curve obtained when 0.1 mL of the freshly prepared reaction mixture was added to 10 mL of "pH" 4.9 acetate buffer containing 50% methanol indicates (Figure 4a) the presence of only form A, which is reducible at −1.3 V vs Ag/AgCl. When the reaction mixture was kept for 68 h to achieve establishment of the equilibrium and the polarographic curve recorded in the buffer as above, the peak at −1.3 V vs Ag/AgCl decreased by about 34% and the heights of peaks at −1.3 V vs Ag/AgCl and −1.15 V vs Ag/AgCl were in the ratio of 1.05:1.00, close to 1.00:1.00 predicted by equation (Figure 4b).

(d) Reaction of Oxymetholone with Ethanol. A reaction of the type in eq 1 attributed to the formation of a hemiacetal from a hydrate also occurs in ethanol. Aliquots from freshly prepared stock solutions in 100% ethanol added to a buffer, pH 5.0, containing 50% (v/v)

ethanol show a peak of −1.4 V vs Ag/AgCl (Figure 5a). After the stock solution was aged for 20 h, a decrease of a peak at −1.4 V vs Ag/AgCl was accompanied by an increase of a new peak at −1.25 V vs Ag/AgCl (Figure 5b). A smaller decrease in concentration of form A indicates that, in the presence of ethanol, equilibrium 1 is either more slowly established or less shifted in favor of B than in methanolic solutions.

(e) Course of Electroreduction of Oxymetholone. Because of the stability of oxymetholone in acetonitrile, initial electroreduction experiments were carried out in buffered acetate solutions containing 50% acetonitrile using stock solutions obtained by dissolving oxymetholone in acetonitrile. For these solutions, the dc polarographic limiting current remained practically constant between pH 3.5 and 5.3 and corresponds to a one-electron reduction ($n = 1$) on the basis of comparison of the limiting current per unit concentration with standard reference materials of known n value. At pH values greater than about 6, the limiting current decreased with increasing pH with the shape of a dissociation curve having an inflection point at a pH value of about 6.5. At pH 7.1, a small wave was observed of −1.65 V vs Ag/AgCl (which might correspond to the reduction of the aldehydic group in form II). Cyclic voltammetry indicated the complete irreversibility of the electrode process at all pH values. The peak potentials of differential pulse polarograms, E_p, are shifted to more negative potentials with increasing pH. The plot of E_p against "pH" was linear over the range pH 2.0 to 6.5 with a slope of 98 mV/"pH", with E_p at pH 5 being −1.3 V vs Ag/AgCl. The interpretation of the pH dependence is made difficult by the uncertainty related to the definition of the "pH" scale and the irreversibility of the process.

A stock solution of oxymetholone was freshly prepared in methanol and added to buffers containing 50% methanol, and differential pulse polarographic curves were recorded after approximately the same time intervals. At "pH" 5.0, a single peak was observed at −1.3 V vs Ag/AgCl. With decreasing pH, a peak at potentials about 0.15 V more positive increased with increasing pH, until at pH 1.1 it predominated. This indicates acid catalysis of the transacetalization, resulting in conversion of form A into B. At pH greater than 6, the height of the peak at −1.3 V decreased and was gradually replaced by a peak at −1.5 V vs Ag/AgCl. At pH greater than 8, the height of this peak gradually decreased and the current–voltage curves showed only a small peak at about −1.65 V vs Ag/AgCl (probably due to reduction of the saturated aldehydic group; see above). The plot of E_p vs pH was linear with a slope of 91 mV/"pH". This slope may not reflect solely the effect of activity of hydrogen ions, since pK_a values of acetatic and phosphoric acids are differently affected by alcohols.

The electrochemical behavior as a function of pH indicates that reduction of oxymetholone can occur in two forms of species A. The more easily reduced conjugate acid is reduced at pH values smaller than about 6, its conjugate base at higher pH values.

Controlled potential reductive electrolysis of a freshly prepared solution of oxymetholone was carried out in 50% methanol–"pH" 5.0 acetate buffer by using a mercury pool electrode, and an n value of 1.0 ± 0.1 was obtained by coulometry. Measurement of the limiting current by using a dropping mercury electrode also enabled the determination of $n = 1.0 \pm 0.2$ to be obtained via reference to standard materials of known n value. The reduction product, extracted with chloroform, was examined by gas chromatography–mass spectrometry (GC–MS) and found to have the parent ion at m/e 632, which is strongly indicative of formation of a dimer accompanying electron transfer.

Controlled potential electrolysis of oxymetholone freshly dissolved in an aqueous "pH" 4.5, 0.1 M acetate buffer containing 50% v/v acetonitrile, followed by isolation of the product and mass spectral analysis of the product of reduction, showed the parent ion at m/e 646. The breakdown pattern is identical with that for the reduction product obtained from electrolysis of oxymetholone in aqueous methanol, but the difference in m/e value indicates the formation of a different but closely related dimeric product. The chemical nature of the dimeric species formed after reductive electrolysis is unknown, and of course the products may be different at a mercury pool electrode from those at the dropping mercury electrode.[30] The reduction process for oxymetholone in a buffered acetonitrile–water mixture at a mercury pool electrode also involves one electron per steroid molecule as experimentally determined by coulometry. This result is the same as obtained at the dropping mercury electrode.

(f) Characterization of Isolated Product from Reaction with Alcohol. The product of reaction of oxymetholone with pure methanol was isolated by evaporation of methanol (with nitrogen) from a solution left to react over a period of 7 days. The dried product was stored in a desiccator prior to examination by infrared mass spectroscopic methods, and results were compared with a sample of oxymetholone.

The infrared spectra of oxymetholone and its product of reaction with methanol show major differences. The infrared spectrum of oxymetholone is similar to that reported in the literature[8] except for minor detail. The characteristic stretch observed at 1620 cm^{-1} in this work was previously reported at 1615 cm^{-1}, with a shoulder at 1630 cm^{-1} in ref 29. The infrared spectrum of the product of reaction with methanol shows a very sharp and intense peak at 1580 cm^{-1} attributable to the ring stretch,[29,31] a very sharp peak of medium intensity at 1675 cm^{-1}, and a sharp peak of medium intensity at 1725 cm^{-1}, which may be indicative of the presence of an α,β-unsaturated aldehyde group. The appearance of a medium sharp peak at 1260 cm^{-1} is assigned to asymmetric C–O–C stretching, with the symmetric C–O–C stretch being at 1085 cm^{-1}. The intense and sharp peak at 1205 cm^{-1} in the parent steroid spectrum due to the ketone bending and stretching is not present in the product spectrum. Characteristic to both oxymetholone and product are the asymmetric and symmetric methyl stretches in the region between 2800 and 3000 cm^{-1} respectively. Also common to both compounds are the free hydroxyl absorption bands between 3400 and 3500 cm^{-1}. From the above findings it can be concluded that there is a small but by no means conclusive degree of evidence in the solid-state mixture of the unsaturated aldehyde form, and a hemiketal form is present.

Mass spectra were also obtained from the parent steroid and product of reaction with methanol. The breakdown pattern for oxymetholone was identical with that reported in the literature,[8,32] with the parent ion at m/e 332 (MW 332). The mass spectrum of the product gave a parent molecular ion at m/e 346, thereby confirming that the reaction with methanol occurs with a net addition of one carbon atom and two hydrogen atoms as proposed.

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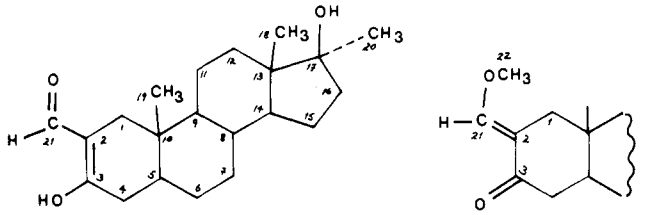
Table I. ^{13}C NMR Data for (a) Oxymetholone in $CDCl_3$ and (b) Changes Observed 5 Days after Addition of Methanol



(a) (b)

δ (^{13}C)	carbon type	assignment
	(a) Oxymetholone	
11.27	CH_3	19
13.83	CH_3	18
20.80	CH_2	11
23.17	CH_2	15
25.73	CH_3	20
27.99	CH_2	6
31.10	CH_2	7
31.47	CH_2	12
35.22	CH_2	16
35.39	quat	10
36.24	CH	8
37.33	CH_2	1
38.83	CH_2	4
40.41	CH	5
45.32	quat	13
50.45	CH	14
53.32	CH	9
81.48	quat	17
107.76	quat	2
183.55	quat	3
188.01	CH	21
	(b) Changes after 5 Days	
61.30	CH_3	22
159.21	CH	21
201.40	quat	3
114.04	quat	2

Analogously, mass spectra of the product isolated from oxymetholone reacting with ethanol gave the parent peak with overall addition of two carbon atoms and four hydrogen atoms, again confirming the stoichiometry of the postulated process.

B. Carbon-13 NMR Studies of Chloroform Solutions. ^{13}C NMR data in aqueous or methanolic media cannot be obtained because of the relative insolubility of oxymetholone in these solvents. However, oxymetholone is very soluble in chloroform, to which solution methanol can be added, and ^{13}C NMR spectra can be used for monitoring of reactions occurring. The proton-decoupled $^{13}C\{H\}$ spectrum of oxymetholone in $CDCl_3$ shows 21 carbon atoms. Assignments shown in Table I were assisted by DEPT experiments (which confirmed the number of protons attached to each of the carbon atoms) and by tables compiled by Blunt and Stothers[33] on ^{13}C NMR spectra of steroids. The formyl enol structure is used in this assignment as being the most probable form. Methanol was added to the above solution, and $^{13}C\{H\}$ spectra were recorded at various time intervals. The spectrum recorded 1 h after methanol addition shows that the resonances attributed to carbon atoms 21 and 3 are broadened. This broadening is consistent with methanol inducing exchange consistent with tautomerism.

The $^{13}C\{H\}$ spectrum recorded 5 days after methanol addition is significantly altered (Table I). The most apparent changes are that the resonances originally assigned to carbon atoms 21, 3, and 2 are now barely detectable whereas three new resonances appear at 159.2, 201.4, and 114.0 ppm. Furthermore, a new resonance at 61.3 ppm is observed, and this is assigned to the methyl group (carbon 22) of the reaction product of oxymetholone with methanol. Assignment of the peaks in the latter reaction product was assisted by further DEPT experiments. Addition of water changes the equilibrium position with the result that there is an increase in intensity of resonances due to carbon atoms 21, 3, and 2. This result is consistent with electrochemical data where the methanol and water reactions were found to be reversible.

Conclusions

Interaction of alcohols with keto steroids has been reported for 18-hydroxycorticosterone,[34] but little is known about hydration and hemiacetal and hemiketal formation for other steroids. On the basis of electrochemical investigations, the presence of two forms, A and B, of oxymetholone which are in equilibrium in water–alcohol mixtures has been demonstrated. Electrochemical behavior is consistent with form A being a hydrate of the enol aldehyde III, form B a hemiacetal of the same species. In water–methanol, water–ethanol, and water–acetonitrile solutions, the tautomeric form II is electroinactive in the potential range studied. Absence of waves characteristic for reduction of α,β-unsaturated ketones indicates that in such solutions form I is not predominant and hence probably not physiologically active.

Polarographic measurements enable following of the establishment of the equilibrium between forms A and B, which is not feasible by spectrophotometry, as the two characteristic absorption bands overlap. Limited solubility of oxymetholone in water–alcohol mixtures prevents the study of such equilibria by NMR. However, in chloroform solutions, ^{13}C NMR studies also indicate addition of methanol.

In the isolated product of the reaction with methanol and ethanol, infrared and mass spectra indicated formation of the hemiketal VI. Formation of a hydrate has been considered in the interpretation of the alkaline hydrolysis of some β-formyl ketones,[12,13] but in these cases a geminal diol anion was formed by addition of hydride ion rather than water or alcohol. Hence, on the basis of electrochemical data, an unexpected chemical reaction has been observed, which may play a role in the physiological activity of oxymetholone.

Acknowledgment. Assistance from S. Carr with some preliminary NMR experiments is gratefully acknowledged, as is the generous donation of oxymetholone by Parke-Davis and Syntex. We thank E. Mocellin for recording the dc polarograms for verification of the number of electrons involved in the charge-transfer step at the dropping mercury electrode. P.Z. expresses thanks to Deakin University for support during his stay.

Registry No. I, 434-07-1; II, 105767-05-3; III, 113274-93-4; V (R = H), 113274-94-5; V (R = CH_3), 113274-95-6; V (R = Et), 113274-96-7; B, 23775-55-5; MeOH, 67-56-1; EtOH, 64-17-5; 2-(ethoxymethylene)-17β-hydroxy-17-methyl-5α-androstan-3-one, 113301-81-8.

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