Analytica Chimica Acta, 120 (1980) 31—39
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POLAROGRAPHIC ANALYSIS FOR CORTICOSTEROIDS
Part 5. Reduction Mechanism of Halogen-containing Corticosteroids and Analysis of some Preparations

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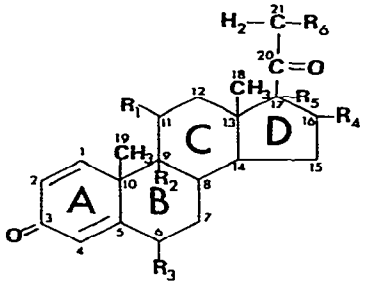
(Received 9th June 1980)

SUMMARY

In fluprednisolone and chloroprednisone acetate, the polarographic reduction of the carbon—halogen bond in position 6 occurs first. The carbanion—enolate formed is reduced at the dropping mercury electrode at more negative potentials than the conjugate acid. Controlled potential electrolysis at a mercury pool electrode where the carbanion—enolate can be protonated, yields the unsaturated ketone. Polarographic reduction of clobetasol-17-propionate and of clobetasone-17-butyrate results in cleavage of the C—Cl bond in the side-chain. This process is followed by reduction of the α,β-unsaturated ketone in the A-ring. Analytical methods for the determination of these compounds in ointments, creams and eye/ear drops gave results with standard deviations of 1—2%.

Investigations [1] of polarographic reduction of corticosteroids bearing a fluorine atom in position 9 of the steroid ring indicated that the carbon—fluorine bond in such molecules does not undergo protolytic cleavage. Consequently, current—voltage curves of dexamethasone, betamethasone and triamcinolone resemble closely those of prednisolone in all media studied. In contrast, corticosteroids bearing a halogen atom in position 6 or 21 show waves corresponding to reductive hydrolysis of the carbon—halogen bond.

Polarographic reduction of the C—F bond in fluprednisolone (I) and of the C—Cl bond in chlorprednisone acetate (II) in position 6 as well as the cleavage of the C—Cl bond in clobetasol propionate (III) and clobetasone butyrate (IV) is discussed in this paper. In position 6, the halogen atom is in a position adjacent to a —CH=CH—CO—CH=CH— conjugated system; in position 21, it is in the α-position to a carbonyl group.



	R_1	R_2	R_3	R_4	R_5	R_6
I Fluprednisolone	HOH	H	F	H	OH	OH
II Chlorprednisone acetate	O	H	Cl	H	OH	OCOCH
III Clobetasol propionate	HOH	F	H	CH_3	$OCOC_3H_7$	Cl
IV Clobetasone butyrate	O	F	H	CH_3	$OCOC_4H_9$	Cl
V Prednisolone	HOH	H	H	H	OH	OH
VI Prednisone	O	H	H	H	OH	OH

In organic compounds bearing a halogen atom on an sp^3 carbon, aliphatic or alicyclic, in the majority of cases a 2-e process takes place; this has been likened to an S_N2 displacement reaction from the rear of the carbon—halogen bond by the electron-rich mercury surface [2—6]. In α-haloketones the reduction of the C—X bond is facilitated when compared with that of the corresponding α-halohydrocarbons and occurs at potentials more positive than that of the carbonyl group [7—14]. It had been realized rather early [15, 16] that whereas a vinylic C—X bond is more difficult to reduce than an aliphatic bond, the reduction of an allylic C—X bond is facilitated. In compounds bearing both vinylic (Br^1) and allylic (Br^2) halogen atoms, such as $Br^1CH{=}CH{-}CH_2Br^2$, the allylic is reduced first, at more positive potentials [17]. No systematic study on the role of the extension of the conjugated allylic system on the reducibility of the C—X bond in straight-chain compounds has been reported.

In the steroid series, most of the reductions of α-haloketones studied were those of α-bromoketones [18—21]. Whereas several compounds bearing chlorine or fluorine and the carbonyl group in the steroid skeleton have been studied [19, 22], only a single reduction of a 21-chloro-20-ketosteroid [19] has been reported.

The reduction of a carbon—halogen bond of the allylic type has been reported for some 6-fluoro-, 6-chloro- and 6-bromo-Δ^4-3-ketosteroids [19—24]. In some instances [22, 24], the possibility of the reduction of the carbon—halogen bonds (including those involving fluorine) occurring prior to the reduction of the —CO—CH=CH— group has been proposed.

In none of these cases has the proposed mechanism actually been proved experimentally. It is the aim of this paper to offer such an approach. Moreover, none of the individual compounds I—IV has been investigated before by means of polarography.

EXPERIMENTAL

Apparatus

The polarographic curves were recorded on a Bruker E 310 modular electrochemical system and a PAR 174 polarograph, both of which were equipped with a drop timer and a Houston Model 2000 X—Y recorder.

For voltammetric curves at the HMDE, the PAR polarograph was equipped with a model 303 SMDE (static mercury drop electrode). A water-jacketed 10-ml polarographic cell (Metrohm EA 880-T-5) was employed with a dropping mercury electrode (t = 6.7 s, m = 0.92 mg s^{-1}), a Metrohm EA 436 Ag/AgCl/saturated KCl reference electrode and a platinum wire auxiliary electrode. The potential of the reference electrode was —52 mV vs. SCE when the bridge was filled with 0.03 M $(CH_3)_4$ NOH in methanol. The cell was kept at a temperature of 20 ± 0.2°C. Controlled potential electrolysis was carried out with a Metrohm E524 coulostat and a Metrohm E525 integrator. Oxford P7000 micropipets (50 and 100 μl) were used for additions of small volumes of sample or standard to the supporting electrolyte. An Orion fluoride-selective electrode (model 94-09A) and an Orion chloride-selective electrode (model 94—17A) were used for fluoride and chloride determinations. An $Hg/HgSO_4$/saturated K_2SO_4 reference electrode (Metrohm EA 441/2) was used for chloride measurements to prevent diffusion of chloride from the reference electrode compartment. The potential of this reference electrode was —470 mV vs. SCE when the bridge was filled with 0.03 M $(CH_3)_4NOH$ in methanol. The mercury pool electrode used had an area of 7.1 cm^2.

Chemicals

The steroids were used as supplied by the manufacturers: prednisolone and prednisone (Nogepha); fluprednisolone (Upjohn); chloroprednisone acetate (Organon); clobetasol-17-propionate and clobetasone-17-butyrate (Glaxo). The solvents and materials for the supporting electrolytes were: methanol (Nanograde, Mallinckrodt); dimethylformamide (DMF-Uvasol); tetramethylammonium hydroxide ($(CH_3)_4NOH$; 10% zur Polarographie; Merck); toluene (Baker analyzed reagent). The toluene was distilled twice before use; all other reagents were used without further purification.

Procedures

Polarographic curves. The methanolic solution of 0.03 M $(CH_3)_4NOH$ (10 ml) was deaerated by a stream of nitrogen for about 10 min and the curve of the supporting electrode was recorded in the differential pulse polarographic mode with a scan rate of 2 mV s^{-1}, pulse amplitude of 100 mV and a controlled drop-time of 2 s. Then 100 μl of the freshly-prepared 0.02 M solution of the corticosteroid in methanol was added, resulting in a 2×10^{-4} M solution. After an additional deaeration for 1 min, the differential pulse polarographic curve was recorded.

Controlled potential electrolysis. A portion (20 ml) of a 10^{-3} M solution of corticosteroid in a methanolic solution of 0.1 M $(CH_3)_4NOH$ was deaerated for 15 min. Then a potential was applied which was about 50 mV more negative than the peak potential of the first peak in the differential pulse polarogram until complete electrolysis was achieved; this took about 6 h. Deaeration by a stream of nitrogen was continued during the electrolysis for stirring and to prevent invasion of oxygen.

After complete electrolysis the fluoride or chloride concentration was measured with the appropriate selective electrode and the content was computed with the aid of a calibration curve, which was obtained under similar conditions. Then the solution was diluted 5 times and the differential pulse polarographic curve was recorded. These curves were compared with the curves of 2×10^{-4} M solutions of the original steroids and with those of 2×10^{-4} M prednisolone (V) and prednisone (VI).

Analysis of ointments. A sample (1 g) of the ointment was dissolved in 5.0 ml of toluene. Then 10 ml of 0.02 M $(CH_3)_4NOH$ in a mixture of DMF and water (8:1, v/v) was deaerated by purging by nitrogen for 10 min, and a differential pulse polarographic curve of the supporting electrolyte was recorded. An aliquot (1 ml) of the toluene solution of the ointment was then added, so that the final concentration of the steroid was between 1×10^{-5} and 1×10^{-3} M, the solution was deaerated for 1 min, and the differential pulse polarogram was recorded. A small volume of a 0.02 M solution of the corticosteroid in DMF was added, the solution was deaerated again for 1 min and the polarographic curve was again recorded. The volume was chosen so that the concentration of the steroid after the standard addition would approximately double. If this resulted in a change in the total volume of more than 1%, a correction for the volume change was introduced into the calculation of the sample concentration using the standard addition method. The height of the most positive peak was measured.

Analysis of creams. Treatment of 1 g of the creams with 5.0 ml of methanol resulted in disintegration of the creams and complete dissolution of the active constituents. The supporting electrolyte consisted of 0.03 M $(CH_3)_4NOH$ in methanol. The curve of 10 ml of the supporting electrolyte was recorded as above and 1 ml of the methanolic solution of the cream was added. The differential pulse polarogram was recorded and evaluated by standard addition as above.

RESULTS AND DISCUSSION

Fluprednisolone (I)

The differential pulse polarographic curve of a methanolic 2×10^{-4} M solution of fluprednisolone containing 0.03 M tetramethylammonium hydroxide, shows a reduction peak at $E_p = -1.34$ V, a second reduction peak at $E_p = -1.67$ V and a third peak at -2.00 V (Fig. 1a). Of these three peaks, only the third peak corresponding to the reduction of the side-chain in position 17 [1] was identical to the one observed for prednisolone. Peaks at -1.55 and -1.75 V corresponding to two 1-e reduction waves of prednisolone (V) were absent from the curves obtained for fluprednisolone.

Under the assumption that the carbon—halogen bond is reduced first [22, 24], waves of prednisolone would be expected to follow the first reduction step. To interpret the absence of such waves, it was first necessary

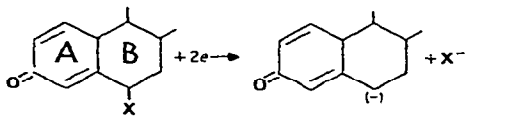
to determine if the C—F bond in 6-position is actually cleaved in the first reduction step and then to find an explanation for the absence of peaks at −1.55 and −1.75 V.

Controlled-potential electrolysis was used to prove the nature of the process occurring in the first wave. Because the first reduction peak on the voltammogram of fluprednisolone recorded for a hanging mercury drop electrode occurred in the same potential range as the first peak on the differential pulse polarographic curves, it was assumed that the product formed in controlled-potential electrolysis at a mercury pool electrode will be identical [25] to that formed at the dropping mercury electrode.

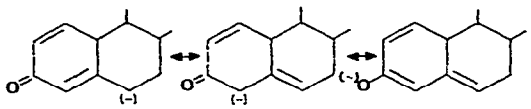
Electrolysis performed at the potential corresponding to the limiting current of the first reduction step at a mercury pool electrode gave a linear plot of log i vs. time. From the slope of this dependence, a value n = 1.96 was found. In the solution after completed electrolysis, fluoride ion concentration was determined by a fluoride-selective electrode. The concentration of fluoride produced was equal to the initial molar concentration of fluprednisolone. Furthermore, comparison of wave-heights of fluprednisolone with benzophenone and naphthoquinone obtained by d.c. polarography indicated a 2-e process. Thus the reduction peak of fluprednisolone (I) at −1.34 V corresponds to a carbon-fluorine bond fission involving the transfer of two electrons.

When the differential pulse polarographic curve of the solution was recorded after complete electrolysis of the fluprednisolone (I) at the pool electrode (Fig. 1a, broken line), the resulting curve showed peaks at −1.55, −1.75 and −2.00 V. These peak potentials and the relative heights of these three peaks were identical to those obtained in the same supporting electrolyte for prednisolone (V). Thus, prednisolone is the final electrolysis product of fluprednisolone, but is not formed as the primary product, and so is not shown on the polarographic curve of fluprednisolone, but in a relatively slow reaction which can take place during the hours needed for exhaustive electrolysis.

Study of the simple α-substituted ketones and related compounds by Lund [26] has indicated that reductive cleavage of the carbon—halogen bond results in the formation of a carbanion—enolate. Such a process can be assumed to occur in the reduction of fluprednisolone (I) (X = F):



(1)



(2)

The rate of protonation of the carbanion—enolate, stabilized by conjugation (2), as indicated, is slow when compared with the "time window" of

about 60 ms in differential pulse polarography. Hence, the carbanion—enolate remains unprotonated in the course of polarographic measurement and is reduced at −1.67 V. The more negative potential of this reduction when compared with the first reduction step of prednisolone (at −1.55 V) is in agreement with the generally observed more negative reduction of the conjugate base than that of the corresponding acid.

A possibility of the formation of a carbanion—enolate has been mentioned in the case of reduction of α-haloketones [14, 18, 20, 23], but here (e.g. for Δ^4-2-bromo-3-ketosteroids [13]) no reduction wave was observed for the carbanion formed in the available potential range.

At the mercury pool electrode the carbanion—enolate formed has sufficient time to be protonated to form prednisolone (V), the presence of which was confirmed. The system represents another interesting example of differences between reduction processes at electrodes with renewable surface and at a pool electrode [25].

Reduction of the side-chain in position 17 is practically unaffected by changes occurring in rings A and B. Thus, the third reduction peak of fluprednisolone at −2.0 V (Fig. 1a, full line) occurs at the same potential and has the same shape as the corresponding peak of prednisolone, either added to the same supporting electrolyte or formed by exhaustive electrolysis (Fig. 1a, broken line).

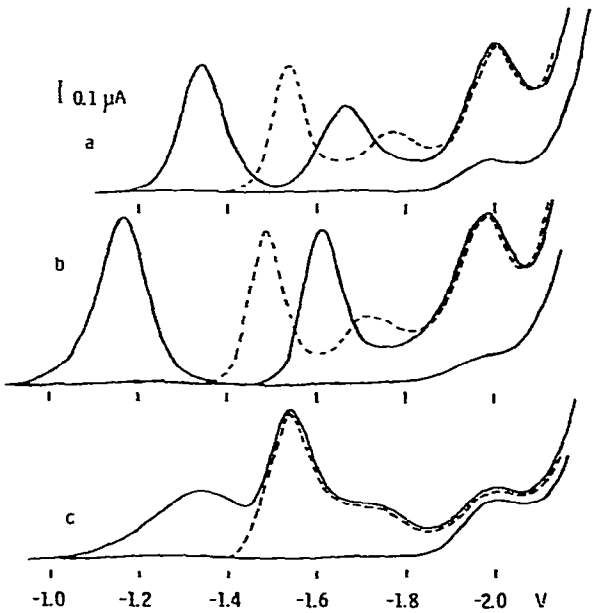


Fig. 1. Differential pulse polarographic curves of 2×10^{-4} M fluprednisolone (a), chlorprednisone acetate (b) and clobetasol propionate (c) in 0.03 M tetramethylammonium hydroxide in methanol before and after (broken line) complete electrolysis performed at 50 mV more negative than the peak potential of the most positive peak.

Chlorprednisone acetate (II)

Differential pulse polarographic curves, and the behaviour of the product of an exhaustive electrolysis at the mercury pool at the potential corresponding to the limiting current of the first reduction peak of solutions of clorprednisone acetate (II), resembled closely the patterns observed for fluprednisolone (I).

The reduction with $E_p = -1.17$ V (Fig. 1b, full line) thus corresponds to fission of the carbon—chlorine bond, resulting in formation of the corresponding carbanion—enolate according to reaction (I) (X = Cl). Reduction of the carbon—chlorine bond in position 6 occurs at more positive potentials than that of the carbon—fluorine bond in fluprednisolone. This follows the generally observed [20] sequence in which the reduction potential of the carbon—halogen bond becomes gradually more negative C—I > C—Br > C—Cl > C—F. Reduction of the carbanion—enolate derived from chlorprednisone acetate at −1.61 V occurs at more positive potentials than that of the carbanion—enolate of fluprednisolone, similarly as for the observed difference in peak potentials for prednisone/prenisolone and cortisone/hydrocortisone [1].

Values of n determined coulometrically at the mercury pool ($n = 1.98$) and by comparison of wave-heights of the first reduction step of chlorprednisone acetate with wave-heights of benzophenone and naphthoquinone obtained by d.c. polarography indicated a 2-e reduction step for the carbon—chlorine bond fission. Furthermore, electrolysis at potentials corresponding to the limiting current of the first reduction peak with the mercury pool electrode, yielded a concentration of chloride ions equimolar with the initial chlorprednisone acetate concentration, as was determined with the chloride-selective electrode.

The current—voltage curve after exhaustive electrolysis (Fig. 1b, broken line) is identical with that of prednisone (VI). The peak at −2.00 V, corresponding to the reduction of the side-chain in position 17, remains again practically unaffected by the presence and reduction of groups in rings A and B.

Clobetasol-17-propionate (III) and clobetasone-17-butyrate (IV)

In both these compounds the initial reduction involves the carbon—chlorine bond fission in position 21, *vicinal* to the electron-withdrawing keto group in position 20 (Fig. 1c, full line). This reduction occurs in a broad peak at −1.3 V, preceding the reduction peaks of the carbonyl group in position 3 at −1.55 V and −1.75 V for clobetasol (III) and −1.52 V and −1.72 V for clobetasone (IV) similarly to the first two reduction peaks of prednisolone (V) and prednisone (VI), respectively. Reduction in the side-chain does not affect reduction in ring A and thus both peaks of the reduction of the carbonyl group in position 3 are observed also in the solution, resulting in exhaustive electrolysis at a mercury pool (Fig. 1c, broken line).

Further evidence for the nature of the process at −1.3 V was obtained from coulometric data and comparison of d.c. polarographic wave-heights,

indicating $n = 2$. Hence, the smaller height of this peak in differential pulse polarography is due to a slow electrode process rather than to a lower number of electrons transferred. Moreover, determination of concentration of chloride ions in the solution after electrolysis indicated formation of one equivalent of chloride per clobetasol (III) or clobetasone (IV) molecule.

The adjacent conjugated system in ring A facilitates reduction of the carbon—chlorine bond in position 6 ($E_p = -1.17$ V) evidently more than the presence of a single carbonyl group in position 20 facilitates reduction of such a bond in position 21 (−1.3 V).

Reduction of the carbonyl group in the side-chain in position 17 does not occur, even after prolonged electrolysis. This can be attributed to the fact that the fission of the carbon—chlorine bond in position 21 yields a compound with a single —OR group adjacent to the carbonyl group. Such compounds, if reduced at all, undergo reduction at such negative potentials that their waves cannot be observed in methanolic media.

Applications

Some pharmaceutical preparations containing one of the examined corticosteroids were analyzed by the methods described in Experimental. Results are summarized in Table 1. For calculations, the most positive peak in the differential pulse polarographic curves was used. The contents were determined by the standard addition method. In solutions which were 10^{-5} M or less in steroid, a slight deviation from linearity of peak height with concentration occurred. At such low concentrations it was necessary to use a calibration curve. In the analysis of ointments, the electro-inactive constituents did not affect the polarographic behaviour of the corticosteroids to be determined.

Adremycine eye/ear drops were analyzed in the same way as ointments, using DMF—water mixture. Instead of dissolving in toluene, nevertheless, 0.5 ml of the liquid was directly used for analysis. This sample contains

TABLE 1

Assays of the dosage forms and standard deviations based on 5 determinations with separate sampling

Constituents	Content (mg g^{-1})	Name	Source	Found (mg g^{-1})	R.s.d. (%)
Clobetasol propionate	0.5	Dermovate ointment	Glaxo	0.50	1.2
Clobetasone butyrate	0.5	Eumovate ointment	Glaxo	0.50	1.3
Chlorprednisone acetate	1[a]	Adremycine eye/ear drops	Organon	1.03[a]	1.8
Neomycin sulphate	5[a]				
Paraffin					
Clobetasol propionate	0.5	Dermovate cream	Glaxo	0.50	1.2
Clobetasone butyrate	0.5	Eumovate cream	Glaxo	0.50	1.1

[a]Content in mg ml^{-1}.

liquid paraffin, which has been shown [27] to complicate the determination of $\Delta^{1,4}$-3-ketosteroids by an increase of the base line. Because, however, the reduction peak of the chlorine in position 6 occurs at potentials about 350 mV more positive than that of the reduction peak of the $\Delta^{1,4}$-3-keto group, the presence of paraffin does not affect the reduction peak of the C—Cl bond in chlorprednisone.

Inspection of Table 1 indicates that the content found follows closely the declared values. Standard deviations are in the range 1—2%; analysis takes about 15 min to carry out.

The authors thank Dr. C. Burgess (Glaxo Operations U.K.) for kindly supplying some of the examined preparations and pure compounds.

REFERENCES

1 H. S. de Boer, J. den Hartigh, H. H. J. L. Ploegmakers and W. J. van Oort, Anal. Chim. Acta, 102 (1978) 141.
2 P. J. Elving and B. Pullman, Adv. Chem. Phys., 3 (1961) 1.
3 F. L. Lambert and K. Kobayashi, J. Am. Chem. Soc., 82 (1960) 5324.
4 P. Zuman, Substituent Effects in Organic Polarography, Plenum Press, New York, 1967, p. 184.
5 P. J. Elving, Record Chem. Progr. (Kresge—Hooker Sci. Lib.), 14 (1953) 99.
6 C. L. Perrin, Progr. Phys. Org. Chem., 3 (1965) 165 (256).
7 P. J. Elving and C. E. Bennett, J. Electrochem. Soc., 101 (1954) 520.
8 P. J. Elving and R. Vanatta, Anal. Chem., 27 (1955) 1908.
9 P. J. Elving and J. T. Leone, J. Am. Chem. Soc., 79 (1957) 1546.
10 M. Hali and E. Harris, Anal. Chem., 41 (1969) 1130.
11 M. M. Romer and P. Zuman, J. Electroanal. Chem., 64 (1975) 258.
12 W. A. Szafranski and P. Zuman, J. Electroanal. Chem., 64 (1975) 255.
13 P. Zuman and S. Wawzonek, Prog. Polarogr., 1 (1962) 303.
14 A. M. Wilson and N. L. Allinger, J. Am. Chem. Soc., 83 (1961) 1999.
15 M. v. Stackelberg and W. Stracke, Z. Elektrochem., 53 (1949) 118.
16 P. Zuman, Chem. List., 48 (1954) 94.
17 P. Zuman, Substituent Effects in Organic Polarography, Plenum Press, New York, 1967, p. 185.
18 V. Delaroff, M. Bolla and M. Legrand, Bull. Soc. Chim. Fr., (1961) 1912.
19 P. Kabasakalian and J. McGlotten, Anal. Chem., 34 (1962) 1440.
20 P. Zuman, Talanta, 12 (1965) 1337.
21 P. Zuman, Substituent Effects in Organic Polarography, Plenum Press, New York, 1967, p. 332.
22 A. J. Cohen, Anal. Chem., 35 (1963) 128.
23 O. Hrdý, Collect. Czech. Chem. Commun., 27 (1962) 2447.
24 M. J. Heasman and A. J. Wood, J. Pharm. Pharmacol., 23 (1971) 176.
25 P. Zuman, in M. M. Baizer (Ed.), Organic Electrochemistry, M. Dekker, New York, 1973, p. 153.
26 H. Lund, Acta Chem. Scand., 13 (1959) 192.
27 H. S. de Boer, P. H. Lansaat and W. J. van Oort, Anal. Chim. Acta, 116 (1980) 69.