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Determination of estradiol and estriol by single-sweep polarography

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Abstract

A sensitive and rapid single-sweep polarographic assay was developed for the determination of estradiol or estriol The assay involves a controlled nitration using 0 4 M sodium nitrite solution at 100°C in a water-bath for 30 min The nitrated estradiol or estriol is determined directly in the reaction mixture by polarography Because of the strong adsorption ability of nitro derivatives, the sensitivity in single-sweep polarography is extremely high The detection limits are as low as 6×10^{-8} and 4×10^{-8} M, respectively The procedure was applied to the determination of estriol in the urine of pregnant women

Keywords Polarography, Estradiol, Estriol, Steroids, Urine

Estradiol and estriol (Fig 1) are important steroid hormones, the concentrations of which and their changes in women are closely related to fertility, infertility and many diseases The determination of steroid hormones has mainly been accomplished by spectrophotometric and more recently by chromatographic techniques [1–4] Because these two compounds generally show no electrochemical reduction behaviour in a 0 01 M borate electrolyte, it is difficult to determine them directly at a dropping mercury electrode by polarography

Recent work [5–7] has demonstrated that nitrated phenyl and phenolic compounds give well defined waves for the reduction of the nitro group in the potential range −300 to −800 mV (vs SCE) Therefore, the principle of controlled nitration can be applied to analyses for a wide variety of phenyl-ring-containing drugs which may lack functional groups amenable to electroanalytical methods This work involves the controlled nitration of estradiol and estriol under optimum conditions followed by polarography of the nitro derivatives Because nitro derivatives are strongly adsorbed on the surface of mercury electrode, the polarographic determination is very sensitive The detection limits are as low as 6×10^{-8} and 4×10^{-8} M for estradiol and estriol, respectively The two nitro derivatives have almost the same peak potential ($E = -600$ mV), and therefore they cannot be determined separately without prior separation

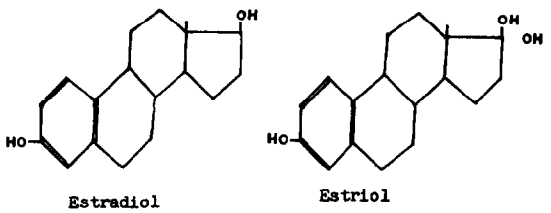

Fig 1 Structural formulae of estradiol and estriol

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EXPERIMENTAL

Apparatus

An EG & G Princeton Applied Research (PAR) Model 174A polarographic analyser was used, together with an RE 0089 *X–Y* recorder (PAR) The working electrode was a Model 303 static mercury drop electrode used in the HMDE mode A Model JP-2 oscillopolarograph (Chengdu Instrumental Factory) was used to record normal and derivative single-sweep polarograms with a mercury flow-rate of 2 0 mg s^{-1}, a drop time of 7 s and a scan rate of 250 mV s^{-1} All polarograms were obtained using a conventional three-electrode configuration, a dropping mercury electrode (DME), a platium wire counter electrode and a saturated calomel reference electrode (SCE) All potentials were measured versus SCE

Reagents

All reagents were of analytical-reagent grade All solutions were prepared with distilled, deionized water

Estradiol and estriol standard chemicals were purchased from Sigma Stock solutions of estradiol or estriol were prepared by dissolving the reagents in 80 ml of absolute ethanol and diluting to 100 ml with distilled water A 0 05 M borate buffer solution (pH 10 5) was prepared by adjusting the pH of a sodium tetraborate solution with dilute sodium hydroxide Sodium nitrite solution (2 M) was prepared by dissolving sodium nitrite in 1×10^{-4} M sulphuric acid Urine samples were collected from Hubei Medical College and were stored in a refrigerator

Nitration of estradiol or estriol

A 1 0-ml volume of estriol or estradiol was pipetted into a 10-ml volumetric flask and 2 0 ml of 2 M sodium nitrite solution were added The stoppered flask was heated in a water-bath at 100°C for 30 min and then allowed to cool to room temperature in a cold water-bath

Polarographic procedure

To a 10-ml volumetric flask 1 ml of nitration sample was added together with 2 ml of 0 05 M borate buffer and the pH was adjusted to 10 5 by addition of sodium hydroxide solution or hydrochloric acid, followed by dilution to volume with distilled water The resulting solution was transferred into the polarographic cell Single-sweep polarography was applied with a scan rate of 250 mV s^{-1} The polarograms were recorded from −350 to −850 mV All experiments were performed at ambient temperature and it was not necessary to remove dissolved oxygen from the solutions

RESULTS AND DISCUSSION

Nitration conditions

To investigate the transformation of the aromatic ring of steroids, a series of nitrated derivatives of estradiol have been synthesized, including 2- and 4-nitro-17β-estradiol by the direct nitration of 17β-estradiol [8,9] There are many factors that may affect the reaction rate of estradiol or estriol with sodium nitrite, such as the reaction temperature, the amount of sodium nitrite and the concentration of the two compounds in the samples

The reaction rate of estradiol or estriol with sodium nitrite was examined to investigate the effect of the amount of sodium nitrite on the

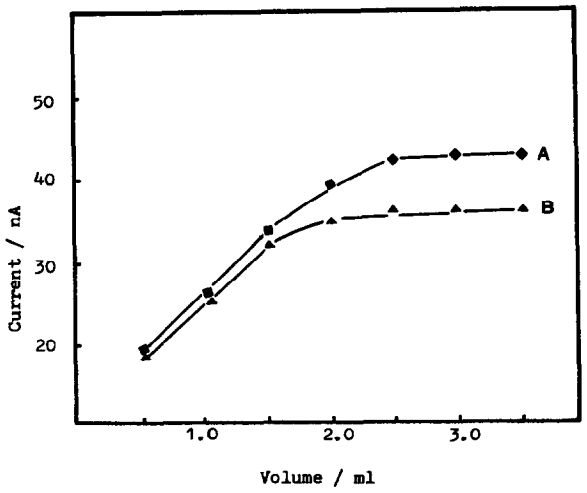

Fig 2 Effect of volume of sodium nitrite on peak current (A) 0 1 μM estradiol, (B) 0 1 μM estriol Conditions 2 M sodium nitrite, 30 min, 100°C

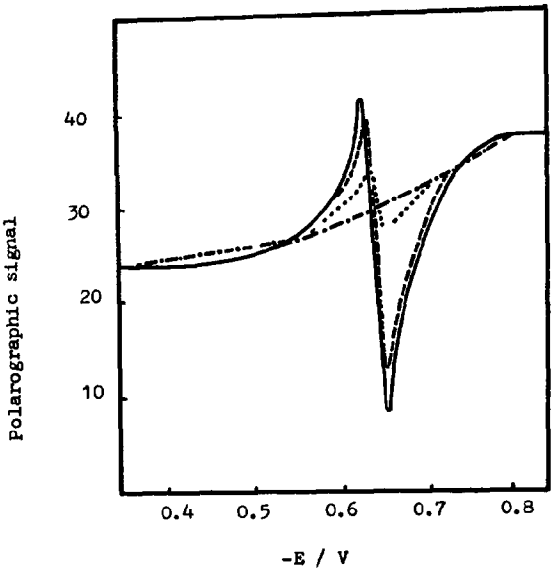

Fig 3 Derivative linear-sweep polarograms of estriol 0 01 M borate, 0 05 M sodium nitrite and 1 μM estriol – – , 25°C, , 40°C, - - - - - -, 85°C, ——, 100°C

peak current Polarographic analysis (Fig 2) shows that the peak current increases with an increase in the amount of sodium nitrite when the reaction time is fixed at 30 min at 100°C The peak current exhibits a maximum with 2 ml of 2 M sodium nitrite

The optimum reaction time for nitration was determined by reacting 5×10^{-7} M estradiol or estriol at 100°C for 10, 20, 30, 40 and 60 min, nitration for 30 min was found to be sufficient

Figure 3 shows the dependence of the reaction temperature on the peak current The peak current increases as the reaction temperature increases, nitration at 100°C yielded essentially one nitro derivative with high sensitivity for both compounds The results indicated that nitration for 30 min at 100°C gave the optimum yield of the nitro derivatives The polarograms show a distinct peak for the derivative at −0 6 V

Choice of supporting electrolyte

The choice of the electrolyte and its concentration are critical with regard to the peak current in single-sweep polarography The response was examined in the presence of various supporting electrolytes, such as acetic acid–sodium acetate, disodium hydrogenphosphate–potassium dihydrogenphosphate, borate buffer and sodium hydroxide The best results, with respect to peak enhancement and peak shape, were obtained using borate solution, and interferences from other compounds were minimized by the use of 0 01 M borate buffer at pH 10 5

For each compound, 5×10^{-6} M samples were analysed by polarography in 0 01 M borate buffer in the pH range 5 5–13 The dependence of the peak potential on pH showed that both derivatives were reduced at more negative potentials with increase in pH At pH 13, it was possible to distinguish a mixture of the nitro derivative of estradiol and estriol, but there was only a 60 mV separation of the peaks and the peak current for both compounds is low at this pH Table 1 indicates that the use of pH 10 5 buffer is the best for the polarographic determination of both compounds

Voltammetric behaviour

Cyclic voltammograms obtained for a solution 1×10^{-6} M estriol in borate electrolyte at pH 10 5 are shown in Fig 4 The cathodic peak due to the reduction of nitrated estriol can be clearly seen On the reverse scan, there is no corresponding anodic peak in the polarogram On

TABLE 1

Effect of pH on peak current

Current [a]	pH						
	8 50	9 08	9 55	10 50	11 05	12 12	13 08
I_p^1 (μA)	0 60	1 03	1 24	1 47	1 38	1 19	1 10
I_p^2 (μA)	0 68	0 72	0 84	1 09	1 02	0 98	0 95

[a] I_p^1, 5×10^{-6} M estriol, I_p^2, 5×10^{-6} M estradiol

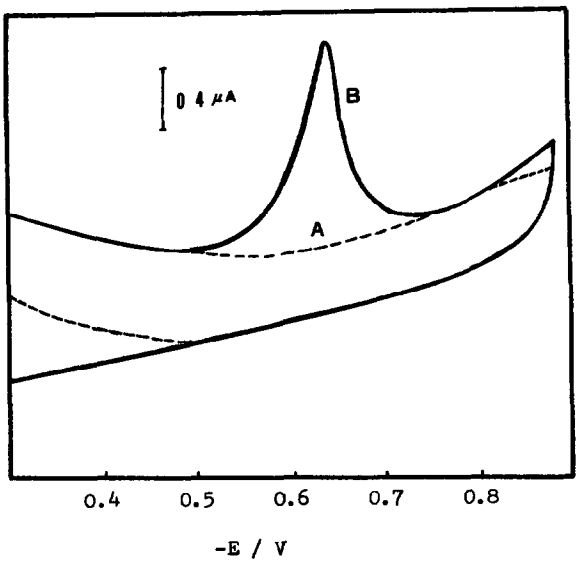

Fig 4 Cyclic voltammograms (A) 0 01 M borate, (B) 0 01 M borate plus 1 μM estriol

varying the potential scan rate, the cathodic peak current increased rectilinearly with scan rate All these effects are typical of an irreversible reduction process of an adsorbed species

Calibration

Typical calibration graphs are shown in Fig 5, the peak current of nitrated estriol or estradiol

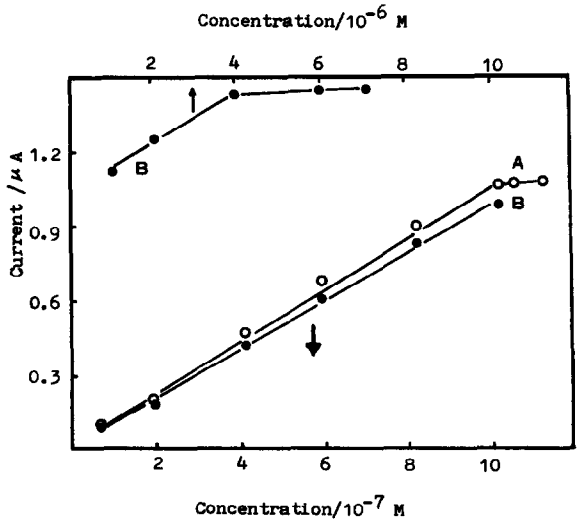

Fig 5 Calibration graphs in 0 01 M borate (A) estriol, (B) estradiol

increases linearly with concentration in the ranges 4×10^{-6}–1×10^{-7} M and 1×10^{-6}–1×10^{-7} M, respectively At concentrations higher than 4×10^{-6} and 1×10^{-6} M, respectively, curvature of the calibration graphs is observed This curvature presumably indicates that a limiting value of the amount of the nitro-estrogen on the electrode surface has been attained under the prescribed conditions Further increases in concentration were not accompanied with an increase in the amount of the nitro-estrogen at the electrode owing to surface saturation and the peak current remained constant The detection limits were 6×10^{-8} and 4×10^{-8} M for estradiol and estriol, respectively

Determination of estriol in urine

On the basis of the above study, a sensitive method for the determination of estriol in urine was developed Considerable problems were encountered in the nitration of estriol in urine extracts because of co-extracted impurities Optimum extractability was established by extraction into diethyl ether, benzene, ethanol and light petroleum from digested urine, and the highest recoveries for estriol were obtained according to the separation procedure reported by Brown et al [10]

Urine samples (2–3 l per 24 h) were obtained from a pregnant woman A 10 ml volume of urine sample was added to a 150 × 15 mm i d glass test-tube and heated to 100°C on a boiling water-bath Then 1 5 ml of concentrated hydrochloric acid were added and the mixture was heated on a water-bath at 100°C for 60 min The test-tube was allowed to stand at room temperature for 30 min to complete the digestion of the urine sample

Extraction and nitration of estriol were performed by the recommended method and the above-mentioned procedure, respectively The resulting sample solution was transferred to the polarographic cell, 0 05 M borate buffer was added and single-sweep polarography was applied Quantification was based on a calibration graph The limit of detection for estriol in urine under these conditions is ca 0 5 μg ml^{-1} Three individual assays of one sample yielded an aver-

age estriol concentration of 4 2 μg ml^{-1}, which is in agreement with reported results [11]

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