

## THE CONVERSION OF LYNESTRENOL TO POLAROGRAPHICALLY ACTIVE SUBSTANCES BY MIXTURES OF SULFURIC ACID AND METHANOL

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Lynestrenol (19-nor-17 $\alpha$ -pregn-4-en-20-yn-17-ol) is a progestational agent<sup>1</sup> and is used alone (Orgametril and Exluton\*) and in combination with mestranol (Lyndiol, Ovanon and Orgaluton) or ethynylestradiol (Pregnon 28) in oral contraceptives and other preparations. Lynestrenol in formulations can be determined by t.l.c.<sup>2-11</sup>, followed by comparison of spot areas<sup>10</sup> or a spectrophotometric finish<sup>2-9,11</sup>, by column chromatographic methods<sup>12,13</sup>, by g.l.c.<sup>14</sup>, by a radiochemical method<sup>15</sup>, by i.r. spectrometry<sup>16</sup>, and by titration<sup>17</sup>.

In the work described here, a polarographic determination for lynestrenol was sought. Since the compound itself is not reducible at a dropping mercury electrode<sup>18-20</sup>, as can be deduced from its structure, lynestrenol must be converted to a polarographically active substance by some chemical method. A variety of methods has been proposed for the polarographic determination of steroids by chemical derivatization<sup>19,21-23</sup>, including nitrosation, oxidation, and formation of addition compounds. None of these methods seems to be adequate for lynestrenol. A new method of chemical modification was found for lynestrenol: the compound gives by treatment with concentrated sulfuric acid or mixtures of this acid with methanol, a number of reaction products, of which some are polarographically reducible. To establish if this conversion could form the basis of a quantitative method for lynestrenol, the influence of sulfuric acid concentration and time of treatment has been studied.

### EXPERIMENTAL

#### *Apparatus*

The polarographic curves were run with a Princeton Applied Research Corporation Model 174 Polarographic Analyzer, with a drop timer and a Houston Omnigraphic Model 2200-3-3 X-Y Recorder. A thermostat (Tamson TX 9-100) was employed to keep the polarographic cell (Metrohm EA 876, volume 20 ml) at a temperature of 25°C.

\* Orgametril, Exluton, Lyndiol and Orgaluton are registered trade names of Organon Nederland B.V., Oss (The Netherlands) and Pregnon 28 and Ovanon of Nourypharma, Oss (The Netherlands).

A three-electrode system was used, consisting of a dropping mercury working electrode, a platinum wire auxiliary electrode (Metrohm EA 202) and a saturated calomel reference electrode (SCE, Metrohm EA 404).

### Reagents

The reagents were of analytical quality, unless stated otherwise and were used without further purification. Lynestrenol was of commercial quality\*. The mercury used was of polarographic quality (Merck, Darmstadt). The nitrogen employed was deoxygenated previously by an alkaline solution of pyrogallol<sup>24</sup>.

A 0.005% (w/v) Triton X-100 (Rohm & Haas, Philadelphia) solution in distilled water was used in order to suppress polarographic maxima.

Reaction solutions I to IV were prepared, consisting respectively of mixtures of 20, 40, 60 and 80% (v/v) concentrated sulfuric acid (Baker, Deventer) and methanol (Merck, Darmstadt). Reaction solution V consisted of concentrated sulfuric acid without any addition (95.7% (w/w) H<sub>2</sub>SO<sub>4</sub>, 4.3% (w/w) H<sub>2</sub>O). The mixtures were prepared by dilution of the necessary volume of concentrated sulfuric acid with methanol to 250 ml. The volume of the acid was determined from the weight and the density.

### Procedure

Approximately 10 mg of lynestrenol was weighed accurately, dissolved in one of the reaction solutions I–V and made up to 100 ml in a volumetric flask. Then the lynestrenol–sulfuric acid solution was transferred to a 50-ml buret.

The Triton X-100 solution (10 ml), 25 ml of methanol and a quantity (0–4 ml) of concentrated sulfuric acid depending on the reaction solution used, were pipetted into a 50-ml volumetric flask. At predetermined intervals, 5 ml of the lynestrenol–sulfuric acid solution were added from the buret into this mixture in order to stop the chemical reaction. After cooling to room temperature, the solution was made up to 50 ml with methanol. All polarographic solutions finally consisted of 10% (v/v) concentrated sulfuric acid and 20% (v/v) of the Triton X-100 solution in methanol and contained approximately 0.5 mg of modified lynestrenol.

Oxygen dissolved in the polarographic solution was removed by bubbling for 10 min with a nitrogen flow, which had been presaturated with vapors of a solution of the polarographic composition in a gas bubbler. Then the lynestrenol solution was polarographed. The polarographic curves were recorded from –350 to –1100 mV vs. SCE in the differential pulse mode at a sensitivity of 1, 2 or 5  $\mu$ A full scale. The modulation amplitude was 100 mV, the scan-rate 2 mV s<sup>-1</sup> and the drop time 1 s throughout the experiments. The height of the mercury column was kept constant at 70 cm.

## RESULTS AND DISCUSSION

The experiments showed that at least three polarographically reducible derivatives can be formed from lynestrenol in the sulfuric acid–methanol mixtures, depending on acid concentration and time of reaction. The compounds A, B and

\* Courteously supplied by Organon Nederland B.V., Oss (The Netherlands).

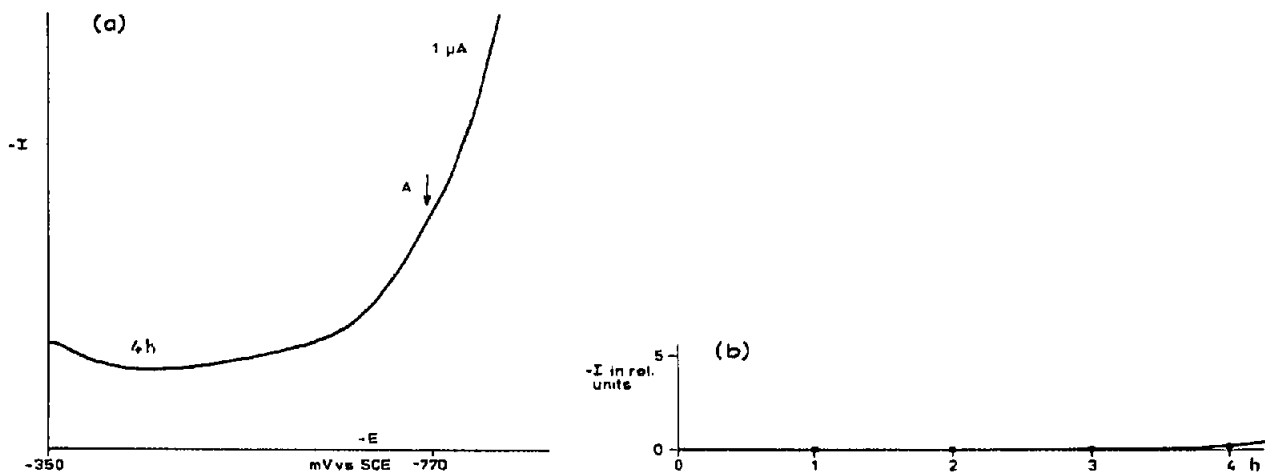


Fig. 1. (a) Polarographic curve of solution containing 20% sulfuric acid. (b) Peak current vs. time in 20% sulfuric acid.  $E_p = -770$  mV vs. SCE.

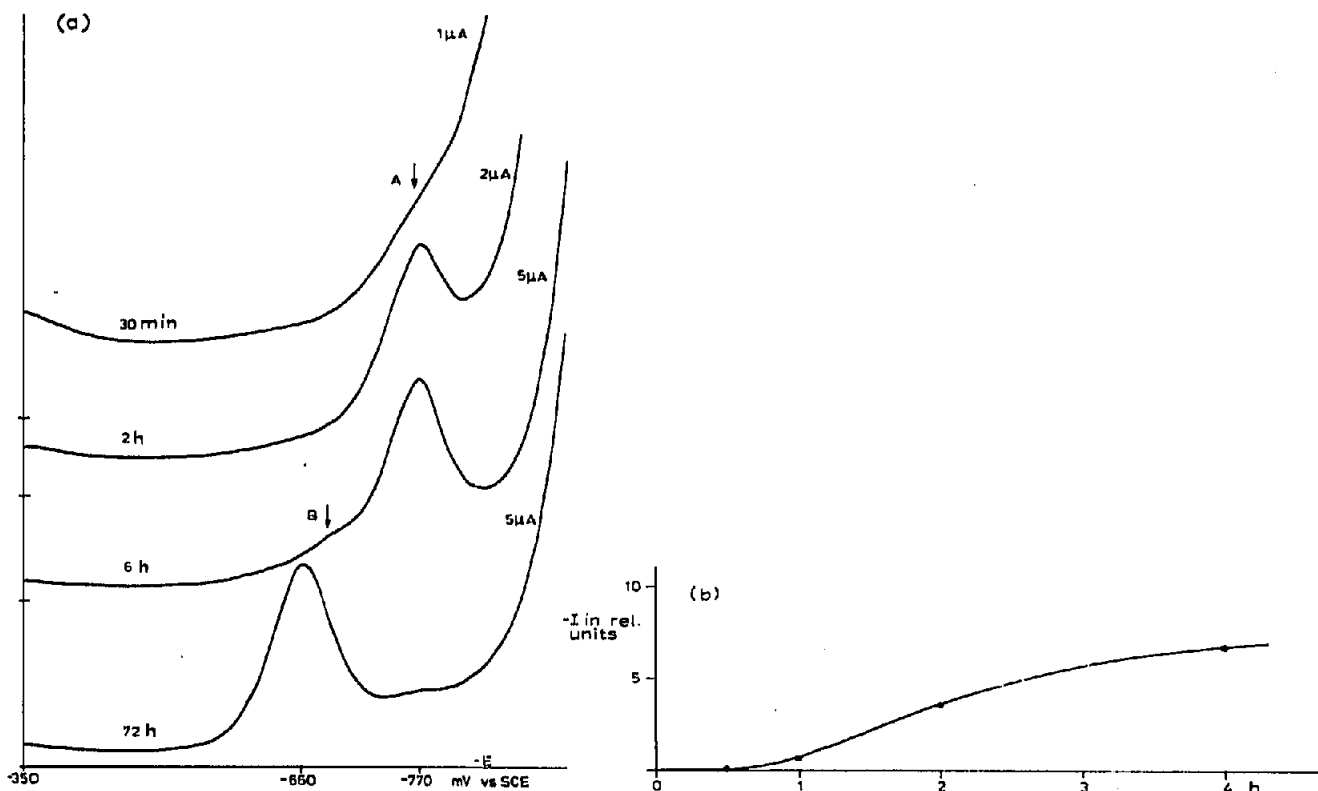


Fig. 2. (a) Conversion of compound A to compound B with time in 40% sulfuric acid. (b) Peak current vs. time in 40% sulfuric acid.  $E_p = -770$  mV vs. SCE.

C have peak potentials ( $E_p$ ) respectively at  $-770$ ,  $-660$  and  $-550$  mV vs. SCE in the polarographic solutions used. After 4 h, the polarographic curve of solution I ( $20\%$   $\text{H}_2\text{SO}_4$ ) started to show a minor wave, owing to the formation of compound A (Fig. 1a). The peak current ( $I_p$ ) is plotted vs. time in Fig. 1b.

With increased acid concentration (solution II:  $40\%$   $\text{H}_2\text{SO}_4$ ), compound A is formed much sooner. After about 6 h, the polarographic curve shows a new compound B, which was not detectable before. After a reaction time of 72 h, compound A is almost completely converted into B (Fig. 2a). Figure 2b shows the peak current of compound A as a function of time for the first 4 h.

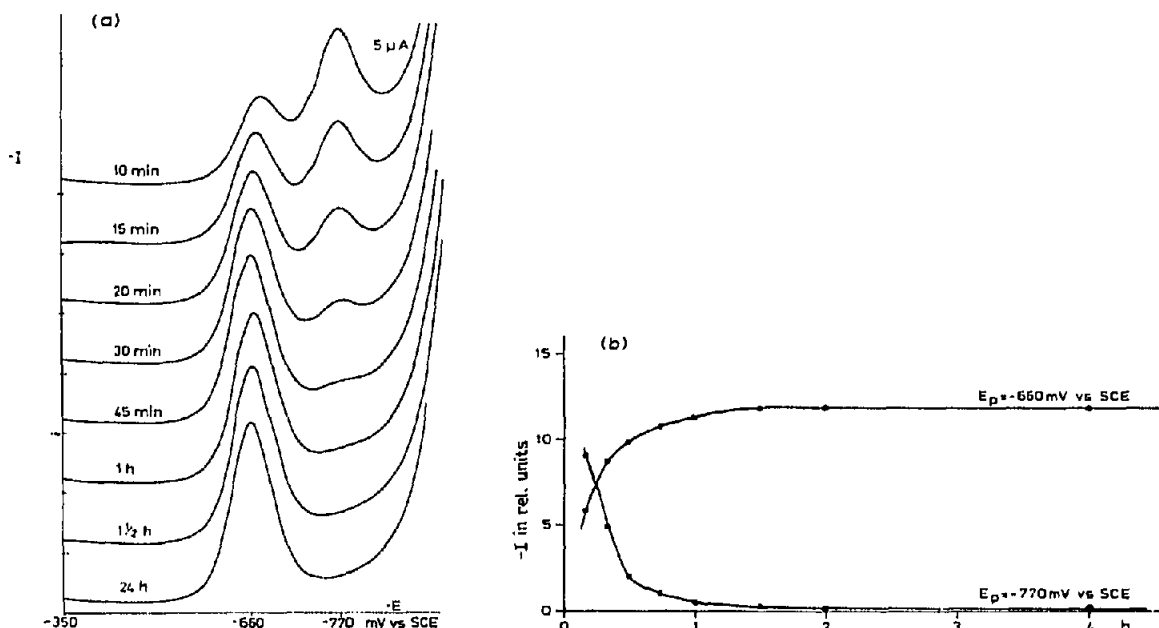


Fig. 3. (a) Development of compound B with time in  $60\%$  sulfuric acid. (b) Peak currents vs. time in  $60\%$  sulfuric acid.

In the first stage of the reaction in solution III ( $60\%$   $\text{H}_2\text{SO}_4$ ), both compounds A and B are present. In this medium, A is converted into B much faster than in solution II. After about 1.5 h, only compound B is detectable (Fig. 3a). The peak currents of compounds A and B are plotted as a function of time in Fig. 3b.

In the polarographic curves of solution IV ( $80\%$   $\text{H}_2\text{SO}_4$ ) only compound B appears (Fig. 4a). Compound A may be formed, but this compound is completely converted to B within 10 min. The peak current for compound B was found to be independent of time (Fig. 4b).

In solution V (undiluted concentrated sulfuric acid), compound B is initially the only detectable compound. However, after 4 h, a compound C appears in the polarographic curve (Fig. 5a). The peak current for compound B was plotted vs. time for the first 4 h, and was found to be independent of time (Fig. 5b).

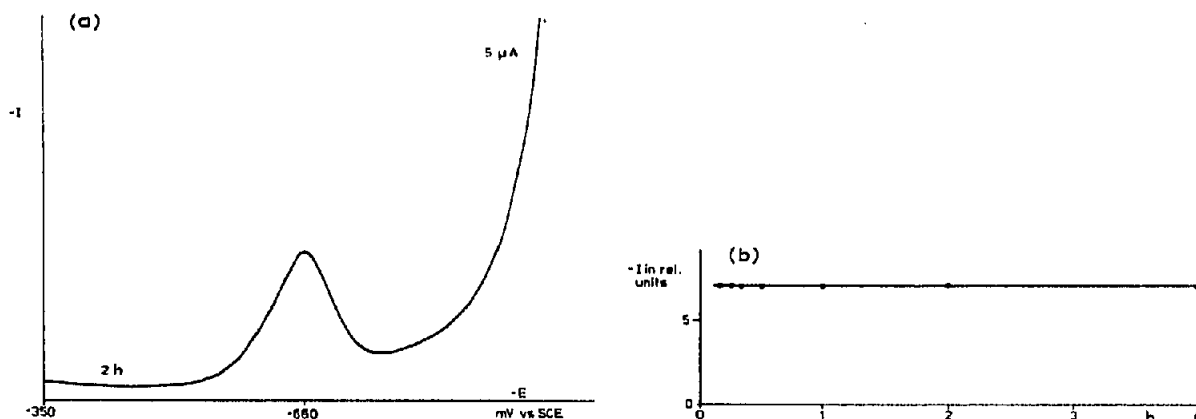


Fig. 4. (a) Development of compound B in 80% sulfuric acid. (b) Peak current *vs.* time in 80% sulfuric acid.  $E_p = -660$  mV *vs.* SCE.

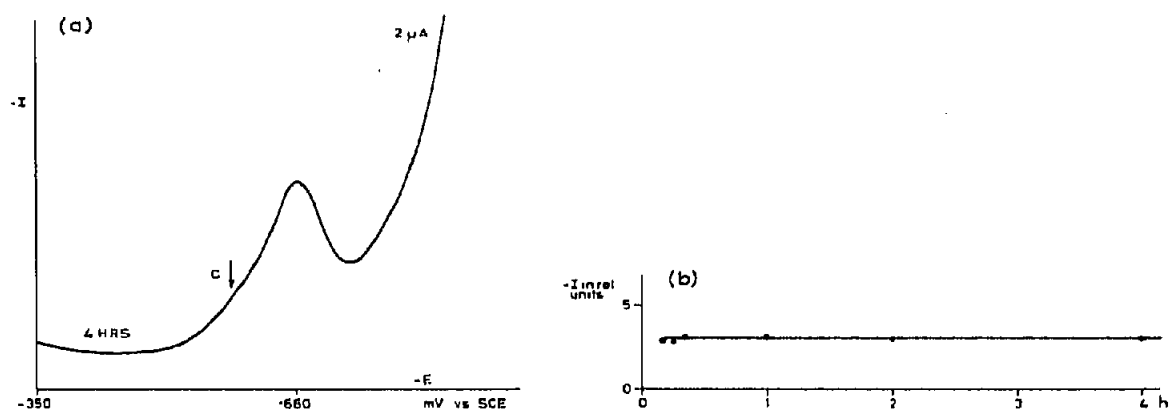


Fig. 5. (a) Development of compound B in 100% sulfuric acid. (b) Peak current *vs.* time in 100% sulfuric acid.  $E_p = -660$  mV *vs.* SCE.

The results obtained with the five reaction solutions are presented in Fig. 6 as plots of peak current *vs.* time. For purposes of comparison, the peak currents were corrected for a polarographic blank, and calculated for an analytical concentration of 10 mg of lynestrenol in 100 ml of reaction solution and a current sensitivity of 5  $\mu$ A full scale. Fig. 6 shows that the polarographic peak-current of compound B can serve for the determination of lynestrenol if solutions III–V are used. The  $I_p$  values for compound B are constant after 1.5 h or sooner. The sensitivity of the determination depends on the acid concentration of the reaction solution and decreases in the order III to V. When solution I or II is used, the  $I_p$ –time curves of compound A ( $E_p = -770$  mV *vs.* SCE) rise slowly at first. Among other things, this is caused by the slow rate of dissolution of the steroid in these media.

Work is now in progress to establish the chemical constitution of the polarographically reducible compounds and the reaction mechanism.

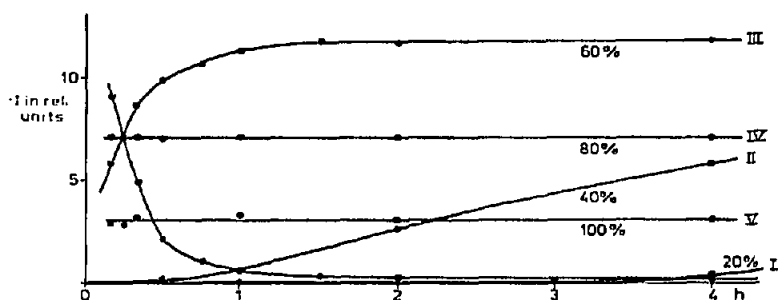


Fig. 6. Peak current vs. time for samples of lynestrenol in solutions I-V.  $E_p = -770$  mV vs. SCE for curves I and II.  $E_p = -660$  mV vs. SCE for curves III-V. Sensitivity  $5 \mu\text{A}$  f.s.d.

Spectroscopic data (to be published) suggests that the structure of compound B is 19-nor-pregna-14,16-dien-20-one. The  $17\alpha$ -ethynylcarbinol group of the steroid is rearranged<sup>25</sup> to a  $\Delta^{16}$ -20-one group and the double bond at  $C_4$  is moved through the steroid backbone to  $C_{14}$ . The application of the method for the determination of lynestrenol in formulations is also being examined.

The influence of acids on other steroids containing a  $17\alpha$ -ethynyl-carbinol-group will also be investigated.

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#### SUMMARY

Lynestrenol (19-nor- $17\alpha$ -preg-4-en-20-yn-17-ol) can be converted to polarographically active substances by treatment with sulfuric acid-methanol solutions. Maximal sensitivity is obtained in 60% sulfuric acid solutions but development of the active compound requires 1.5 h; slightly poorer sensitivity is found in 80% sulfuric acid solutions but the reaction requires only 10 min.

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