POLAROGRAPHIC ESTIMATION OF AMPHENONE B AND SU 4885

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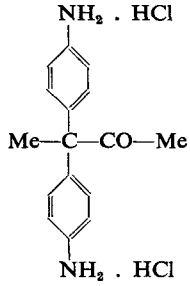
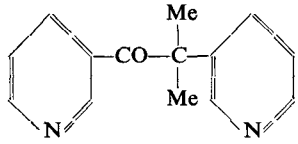
The polarographic behaviour of the compounds Amphenone B (3,3-bis(*p*-aminophenyl)-butan-2-one) and Su 4885 (1,1-dimethyl-2-oxo-ethylenebis-3-pyridine) in aqueous medium is described. Both substances are reduced at the dropping mercury electrode by two electrons and produce well-developed diffusion cathodic waves of a height proportional to the concentration of the depolariser. Su 4885 can be estimated polarographically also in adrenal homogenates in Ringer-Krebs phosphate buffer.

THE insecticide DDD, the active component of which is 1,1-dichloro-2, 2-bis(*p*,-chlorophenyl)ethane, causes a decrease of the corticosteroid excretion and extensive atrophy of adrenals, accompanied by destruction of the adrenal cortical tissue[1,2].

Among other substances capable of inhibiting the production of some physiologically active corticosteroids by direct action in the adrenal glands are the compounds Amphenone B (3,3-bis(*p*-aminophenyl)-butan-2-one dihydrochloride)[3] (I) and Su 4885 (1,1-dimethyl-2-oxo-ethylenebis-3-pyridine)[4] (II). These compounds have been utilised in the study of the biochemistry of the corticosteroid metabolism and physiology of the adrenal cortex, as well as for the diagnosis and experimental therapy of some endocrine disorders. Amphenone B inhibits the enzymatic oxygenation of the steroid molecule at the 11-, 17- and 21-positions[4–6] and Su4885 acts as a specific inhibitor of 11-hydroxylation[6–8]. In contrast to the effect of DDD, the adrenals do not atrophy but hyperplasia develops.



I



II

Hitherto no attention had been paid to the analytical estimation of Amphenone B or Su 4885 nor to their polarographic behaviour.

EXPERIMENTAL

The polarographic curves were recorded on a Heyrovský polarograph, Type V 301 (ČZ Brno). The measurements were made in a Kalousek vessel with a separate saturated calomel reference electrode. The rate of

mercury flow through the capillary used at the reservoir height $h = 60$ cm. was $m = 3{\cdot}05$ mg.sec.$^{-1}$ and the drop time was $t = 2{\cdot}6$ sec. (in 0·1 N LiCl). The galvanometer sensitivity was $2{\cdot}2 \cdot 10^{-9}$A/mm. The half-wave potentials were measured with an accuracy of $\pm$ 10 mV.

As the supporting electrolyte 0·1 N lithium chloride, McIlvain's buffer or 0·1 N lithium hydroxide were used. The oxygen was removed from the solution by bubbling a stream of nitrogen through it for 3 minutes before the curve was recorded. The standard aqueous stock-solution of Amphenone B and Su 4885 was $5 \cdot 10^{-3}$M.

RESULTS

The compounds are polarographically reduced at a dropping mercury electrode in a solution of 0·1 N lithium chloride. One very well-developed cathodic wave was recorded. The polarographic waves are limited by diffusion; this was confirmed by studying the dependence of the limiting

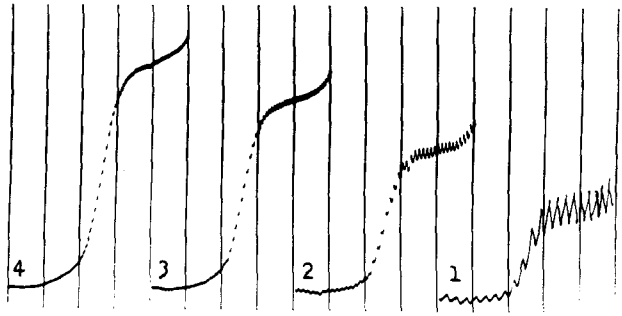


FIG. 1. Dependance of the height of the amphenone wave on the height of the mercury reservoir.

$3{\cdot}5 \times 10^{-4}$ M Amphenone B in 0·1 N LiCl; from about—1·0 V against standard calomel electrode, 200 mV/absc., height of the reservoir h = 1. 15 cm. 2. 30 cm. 3. 55 cm. 4. 80 cm., sensitivity 1 : 15.

current on the square root of the height of the mercury reservoir (Fig. 1). The dependence is linear from the origin. The temperature coefficients $1/i \,.\, \triangle i/\triangle T$ of the waves of both substances also provide evidence of diffusion character of limiting current (see Table I).

The two-electron reduction, common also in other ketones, was confirmed by comparing the height of the waves with the height of a thalium (I) wave under analogous conditions. Amphenone B is reduced at a more

TABLE I

THE VALUES OF SOME POLAROGRAPHIC CONSTANTS

	Half-wave potential $E_{\frac{1}{2}}$ V(SCE) in 0·1 N LiCl	Diffusion current constant $d = id/m^{2/3}t^{1/6}c$	No. of electrons n	Diffusion coefficient D . 10^6 (cm.^{2}sec.$^{-1}$)	Temperature coefficient $1/i \,.\, \Delta i/\Delta T$ grad^{-1} per cent
Amphenone B	−1·40	1·8	2	2·2	1·59
Su 4885	−1·22	2·3	2	3·7	1·28

negative potential than Su 4885 which would be expected as the carbonyl group of the latter compound conjugates with the aromatic pyridine nucleus while in Amphenone B the carbonyl is isolated from the aromatic system. The difference of 180 mV between the half-wave potentials of the two substances in 0·1 N LiCl makes it possible to estimate them simultaneously.

When investigating the influence of pH of the solution over the range of pH 3 to 8 (McIlvain buffer) and pH 12 respectively (LiOH) the half-wave potential of Su 4885 is shifted by 83·6 mV per pH unit to the more negative potentials (Fig. 2). The effect of pH on Amphenone B could not be

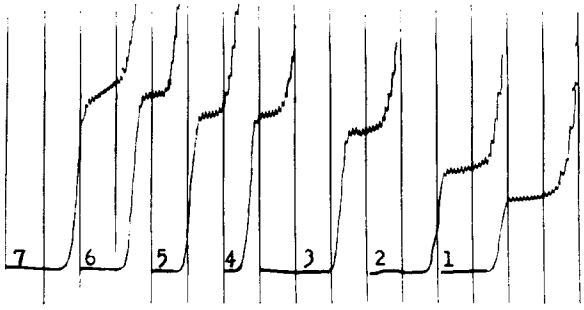


FIG. 2. Effect of pH on the Su 4885 wave.
$2{\cdot}5 \times 10^{-4}$ M Su4885 in McIlvaine buffer; pH = 1. 2·2. 2. 3·0. 3. 4·0. 4. 5·0. 5. 6·0. 6. 7·0. 7. 8·0, from about 1 to 3. — 0·4 V, 4 to 8. — 0·8 V, h = 60 cm. 200 mV/absc, sensitivity 1:15.

ascertained because the dihydrochloride of this substance in the buffered aqueous solution did not produce measurable polarographic waves. Neither the shape nor the half-wave potential of the Su 4885 wave in 0·1 N LiCl were markedly influenced by the presence of deformable ions Ca^{++} and Cs^{+}.

The height of the polarographic wave of Amphenone B and Su 4885 in 0·1 N lithium chloride and of the latter compound also in other media studied is a linear function of the depolariser concentration (Fig. 3).

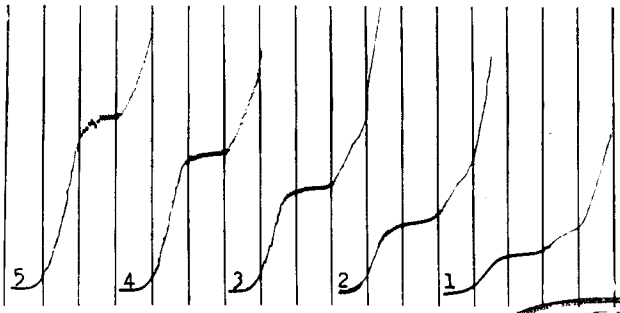


FIG. 3. Dependance of the height of the wave on the concentration of Su4885 in 0·1 N LiCl.
1. $0{\cdot}6 \times 10^{-4}$M. 2. $1{\cdot}2 \times 10^{-4}$M. 3. $1{\cdot}8 \times 10^{-4}$M. 4. $2{\cdot}4 \times 10^{-4}$M. 5. $3{\cdot}0 \times 10^{-4}$M of Su4885. 200 mV/absc., h = 60 cm., sensitivity 1:20, from about — 1·0 V against standard calomel electrode.

This fact can be utilised for the analytical estimation of these compounds. That the height of the wave does not change with time is also of advantage analytically. Polarographic curves rarely show maxima which can be suppressed by a gelatine solution but not by acid fuchsin, and then at higher concentrations only.

The effect of the hydrogen ion concentration in McIlvain's buffer on the height of the wave was studied in Su 4885. On the acid side the height of the wave rises sharply with increasing pH, but over the range of pH 4 to 8 changes in the hydrogen ion concentration have less influence. (Fig. 4).

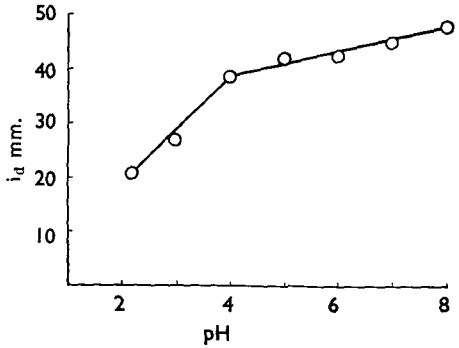


FIG. 4. Effect of pH on the height of polarographic wave of Su4885 in McIlvaine buffer.

These findings are of importance in the polarographic estimation of Su 4885 in aqueous solutions and of Amphenone B in neutral aqueous non-buffered solutions with the usual accuracy of polarographic estimation, of 2 per cent. In addition to the possibility of estimating these compounds in the pure crystalline state and in pharmaceutical preparations, the polarographic method of estimating Su 4885 can be used when investigating its fate during its action as the biochemical inhibitor of the oxygenation of the steroid molecule in the adrenal cortical tissue. For this purpose Ringer-Krebs phosphate buffer either alone or with homogenised adrenals proved very satisfactory.

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REFERENCES

1. Brown, Griffin and Smith, *Metabolism*, 1955, **4**, 542.
2. Brown, Griffin and Smith, *Endocrinology*, 1953, **53**, 116.
3. Allen and Corwin, *J. Amer. chem. Soc.*, 1950, **72**, 114.
4. Allen, *J. org. Chem.*, 1950, **15**, 435.
5. Rosenfeld and Bascom, *J. biol. Chem.*, 1956, **222**, 565.
6. Jenkins, Meakin and Nelson, *Endocrinology*, 1959, **64**, 572.
7. Chart, Sheppard, Allen, Bencze and Gaunt, *Experientia*, 1958, **14**, 151.
8. Liddle, Island, Lance and Harris, *J. clin. Endocrin. Metab.*, 1958, **18**, 906.