885. *Polarography of 1-Amino-4-methylthioxanthone and of Miracil D.*

By D. C. Munro.

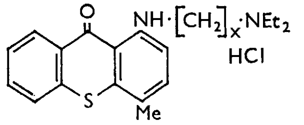
At the dropping-mercury cathode, 1-amino-4-methylthioxanthone and its *N*-substituted derivatives give a single reduction wave in acid solution; in alkaline solutions containing ethanol a distorted second wave is observed. Values of half-wave potential show that reducibility is insensitive to alkylation of the amino-group.

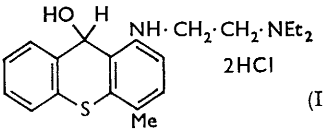
In acid solutions at concentrations of 10^{-4}M and below, the shape of the wave is consistent with a two-electron reduction to the corresponding thioxanthenol; the failure of this correlation at higher concentrations is discussed.

In 0·2N-hydrochloric acid containing 50% of ethanol, Miracil D gives a linear relation between diffusion current and concentration from 8 to 200 mg./l. This provides a method for determination of Miracil D within ±5%.

For many organic compounds, reducibility can be studied by polarographic techniques which can also be used for quantitative determination. The importance of oxidation–reduction processes to biological systems is well known, and Mason [1] showed that, for two homologous series of acridine antimalarials, the variation in ability to inhibit the enzyme diamine-oxidase *in vitro* ran parallel to the variation in polarographic half-wave potential. A study has therefore been made of the polarographic reduction of Miracil D and some related thioxanthones. Miracil D (1-2′-diethylaminoethylamino-4-methylthioxanthone monohydrochloride; lucanthone hydrochloride, B.P.) (I; $x = 2$) has been found useful in the treatment of some schistosome infections; its precursor, 1-amino-4-methylthioxanthone, and three of its homologues (I; $x = 3$, 4, and 5), have also been studied.

(I)





(II)

These thioxanthones have very small solubilities in water, and extensive use has been made of media containing 50% of ethanol. However, there is some uncertainty in glass-electrode readings of acidity in ethanol–water mixtures, so the variation of half-wave potential with pH for Miracil D has been examined in a series of aqueous buffers. The four homologous compounds have been compared in aqueous solution at pH 7·4.

Experimental

The instrument used was a Cambridge Instrument Co. polarograph with photographic recording of current–potential variation. Potential was increased mechanically at 0·3 v/min. A 2 ml. cell was used; a bridge of 2½% agar jelly saturated with potassium chloride served to connect this with the saturated calomel anode. All potentials given are those measured at 18° relative to this anode. Oxygen was removed from solutions by cylinder nitrogen previously saturated with the appropriate solvent mixture.

In our study of Miracil D two supporting electrolyte solutions were used, each containing 50% v/v of ethanol. They were (*a*) 0·2N-hydrochloric acid, and (*b*) sodium acetate–acetic acid buffer.

Quantitative extraction and determination of Miracil D in measured portions of solution was carried out as follows. The solution (20 ml.) in a 250 ml. tap-funnel was treated with N-sodium hydroxide (6 ml.) and extracted successively with 10, 10, 5, 5, 5, and 5 ml. portions of chloroform; the combined extracts were evaporated on a steam-bath. The residue was dried

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[1] Mason, *J.*, 1950, 351.

(NaOH) for 12 hr., then treated with a measured volume (usually 5 ml.) of the ethanolic hydrochloric acid described above and set aside in a stoppered flask for 2 days to achieve complete dissolution. The concentration was determined on the polarograph.

Diffusion-current values reported are mean values of two or three polarograms from each solution. For Miracil D the current–concentration proportionality was found to be $1{\cdot}28 \times 10^{-2}$ μA l./mg. or 4·83 μA l./millimole: the capillary, with 50 cm. height of mercury, delivered 1·447 mg./sec. at 20° with drop times of 3·85 sec. in water on open circuit, and in the ethanolic hydrochloric acid of 3·32 sec. on open circuit and 3·59 sec. at −0·8 v.

Solutions of Miracil D in less acid solutions were prepared by adding measured volumes of an aqueous solution (2012 mg./l.) to the appropriate buffer solution.

The half-wave potential of the reduction of Miracil D was studied in buffer solutions based on acetate (pH 2·0, 4·6), phosphate (pH 5·5, 7·4), and borate (pH 8·5, 9·5), without addition of ethanol. Buffers containing acetate (pH 4·6) and borate (pH 8·5) with addition of ethanol to 50% v/v were used in comparing the half-wave potentials given by 1-amino-4-methylthioxanthone and Miracil D.

Solutions of 1-amino-4-methylthioxanthone in the ethanolic acetate and borate media were prepared by stirring small amounts of the solid with 5 ml. of the solvent and, after 24 hr., decanting the saturated solution.

The three compounds homologous with Miracil D were prepared from 1-amino-4-methylthioxanthone [2] by alkylation by standard methods. They were dissolved in water to give solutions in the range $1{\cdot}6$—$2{\cdot}0 \times 10^{-3}$M, and appropriate volumes of these solutions were diluted with the phosphate buffer to give solutions at pH 7·4 of $5{\cdot}0 \times 10^{-5}$M.

The thioxanthenol (II) from Miracil D (1-2′-diethylaminoethylamino-4-methylthioxanthenol dihydrochloride) was prepared as follows. Miracil D (0·2 g.) in water (30 ml.) with concentrated hydrochloric acid (10 ml.) was heated to boiling; small amounts of zinc dust were added during 10 min., and the mixture was filtered and cooled; the white *thioxanthenol dihydrochloride* (II) separated. The mother-liquors acquired a deep blue colour by aerial re-oxidation of the product. After two recrystallisations from 2N-hydrochloric acid the solid appeared as a white powder, insoluble in benzene or ligroin, very soluble in methanol or ethanol, and having m. p. 196—206° (Found: C, 58·0; H, 6·8; N, 6·5; S, 7·4; Cl, 17·00. $C_{20}H_{26}ON_2S,2HCl$ requires C, 57·8; H, 6·8; N, 6·75; S, 7·7; Cl, 17·1%). Solutions for polarographic examination were made as described above for 1-amino-4-methylthioxanthone.

RESULTS AND DISCUSSION

Determination of Miracil D.—In 0·2N-ethanolic hydrochloric acid, Miracil D gives a reduction wave ($E_{\frac{1}{2}} = -0{\cdot}86$ v) which, although close to the hydrogen wave, gives consistent values for the diffusion current over the concentration range investigated (1000—1 mg./l.). The reduction wave ($E_{\frac{1}{2}} = -1{\cdot}17$ v) is better separated from the hydrogen wave in the ethanolic acetate medium where a similar range of concentration has been examined, but a graph showing the variation of log current with log concentration gives a line having a slope less than unity, so that in the ethanolic acetate medium there is no range of direct proportionality between diffusion current and concentration.

For solutions in ethanolic hydrochloric acid, the logarithmic plot is a straight line of unit slope (see Figure). Direct proportionality between diffusion current and concentration is observed over the concentration range 8—203 mg./l. within ±3%, and determination of Miracil D may be carried out in this medium.

TABLE 1. *Determination of Miracil D in ethanolic hydrochloric acid.*

Concn. (mg./l.)	8·3	14·6	20·4	24·8	29·1	43·7	58·2	132	203
Concn. found (mg./l.)	8·4	14·2	20·8	24·2	29·6	43·6	57·6	137	208
Error (%)	+1	−3	+2	−2	+2	0	−1	+4	+2

In order to find the accuracy of this procedure, a series of standard solutions were made up containing Miracil D in the ethanolic hydrochloric acid. The concentrations are compared in Table 1 with those determined polarographically.

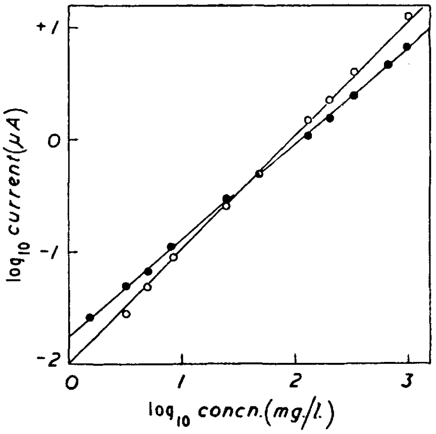
[2] Hammick and Munro, *J.*, 1952, 1077.

In ethanolic hydrochloric acid, therefore, it is possible to estimate Miracil D polarographically within the above limits of concentration to a fair degree of accuracy. However, for biological systems it may be desirable to separate the thioxanthone from other reducible materials. To investigate the possibility of this estimation when the thioxanthone is extracted from preferentially water-soluble materials, solutions were made up to give a series of known concentrations in aqueous solution. Each solution was made alkaline and extracted with chloroform. After evaporation and dissolution of the residue in a measured amount of ethanolic hydrochloric acid, diffusion currents were measured and the concentration was calculated. In Table 2 are compared the concentrations set up with determinations made by the above technique.

TABLE 2. *Results of extraction and determination of Miracil D.*

Initial concn. (mg./l.)	8·05	13·2	18·1	24·3	31·1	41·6	72·8	104·0	145·6
Concn. found (mg./l.)	7·88	12·2	17·2	22·3	29·3	40·3	71·8	96·0	142·1
Error (%)	−2	−8	−5	−8	−6	−3	−1	−8	−2

The results show a mean loss of 5% of the original concentration due to the extraction and evaporation procedure; a superimposed scatter of ±3% is attributed to the errors in the measurement of polarographic diffusion current.



Plot of log *current against* log *concentration for Miracil D in acid solutions containing* 50% *of ethanol.*

○ *Ethanolic hydrochloric acid.*

● *Ethanolic acetate.*

Variation of Half-wave Potentials.—The half-wave potentials of Miracil D have been measured in various aqueous buffer solutions; the measurements are for concentrations 1—50 × 10^{-5}M for pH 4·6 and below, and for the range 1—3 × 10^{-5}M for pH 5·5 and above. In an aqueous buffer of pH 9·5 no waves were obtained owing to the insolubility of the free base of Miracil D at this alkalinity. These half-wave potentials are independent of concentration, and the variation with pH is close to 0·058 v/pH unit (see Table 3); at 18°, this is the theoretical value for reduction with uptake of one hydrogen ion per electron.

TABLE 3. *Half-wave potentials of Miracil D in aqueous buffer solutions.*

pH	0·80	2·33	4·64	5·53	7·44	8·45	9·45
$E_{\frac{1}{2}}$ (v)	−0·780	−0·885	−1·030	−1·095	−1·195	−1·230	—

The polarographic reductions of Miracil D and of 1-amino-4-methylthioxanthone have been compared in solutions containing 50% of ethanol, owing to the insolubility of the latter substance in all aqueous solutions except the most acid. In acetate (pH 4·6) with ethanol, both give a single reduction wave. In borate (pH 8·5) with added ethanol, both gave a second wave of distorted shape for which consistent measurements of half-wave potential cannot be made. It appears from Table 4 that the reducibility of the thioxanthone system is scarcely affected by substitution of a basic alkyl side-chain for hydrogen

TABLE 4. *Effect of* N-*alkylation on half-wave potential in ethanolic media.*

1-Amino-4-methylthioxanthone	$E_{\frac{1}{2}}$ (v)	−1·19 (acetate)	−1·36 (borate)
Miracil D	$E_{\frac{1}{2}}$ (v)	−1·17 (acetate)	−1·33 (borate)

on the 1-amino-group. Even less effect is produced by variation of side-chain length, as shown in Table 5 by the half-wave potentials of Miracil D and three homologues, measured in M/15-phosphate buffer (pH 7·4) without ethanol.

TABLE 5. *Effect of side-chain length on half-wave potential at* pH 7·4.

Compound	(I; $x = 2$) (Miracil D)	(I; $x = 3$)	(I; $x = 4$)	(I; $x = 5$)
$E_{\frac{1}{2}}$ (v)	−1·23	−1·23	−1·22	−1·22

The Reduction Process.—From the shape of a polarographic wave it is sometimes possible to estimate the number of electrons involved in the reduction. From the beginning to the end of the wave, the tangent at the half-wave point describes a potential increment (ΔE) which is related[3] to the electron number by the equation $\Delta E = -4\boldsymbol{RT}/n\boldsymbol{F}$ volts. The potential increment of the reduction wave given by Miracil D in the ethanolic hydrochloric acid has been measured for the concentration range 0·1—27·0 × 10^{-4}M (5—1000 mg./l.) and is seen to vary (Table 6). At lower concentrations, the value corresponds with a two-electron process, but at about 5 × 10^{-4}M and above (200 mg./l.) the potential increment tends towards that of a one-electron process.

TABLE 6. *Variation of electron number with concentration of Miracil D.*

10^4c (M)	0·13	0·22	0·66	1·28	3·50	5·4	9·0	27
ΔE (v)	0·05	0·055	0·055	0·05	0·075	0·08	0·09	0·09
n (electrons)	2·0	1·8	1·8	2·0	1·3	1·25	1·1	1·1

That the reduction in acid solution is fundamentally a 2-electron reduction of the thioxanthone grouping is supported by the reduction of Miracil D in hydrochloric acid by zinc dust to give the colourless thioxanthenol (II): this change involves two electrons. The standard electrode potential of zinc relative to the saturated calomel electrode is −1·01 v at 25°, and this is a little greater than the half-wave potential of Miracil D under similar conditions of acidity.

The thioxanthenol gives no waves at the acidity of the ethanolic acetate medium, although in the ethanolic borate solution a distorted reduction wave may be present at high negative potentials, and merged with the wave from the base electrolyte. In the absence of an anodic wave from the reduced species, in this case the thioxanthenol, the values for electron number obtained from the wave shape are not precise; however, their trend shows that the characteristics of the reduction process change with increasing concentration. The decline in apparent electron number may be accounted for in the following way.

The reduction of an unsaturated grouping may take place in two stages, with the intermediate formation of a free radical:

at E_1 $$\mathrm{X} + \mathrm{H}^+ + e \longrightarrow \mathrm{XH}\cdot \quad (R_1)$$

and at E_2 $$\mathrm{XH}\cdot + \mathrm{H}^+ + e \longrightarrow \mathrm{XH}_2 \quad (R_2)$$

Under conditions of polarographic reduction, when E_2 is larger than E_1, the formation of two waves is observed, each representing a one-electron reduction. But when E_1 is larger than E_2 the two reduction stages take place together, to give a single two-electron wave.

The results suggest that, for Miracil D, E_1 and E_2 have similar values whose precise differences depends upon pH, so that in acid solutions, low concentrations give a two-electron wave, but in alkaline media such as the ethanolic borate solution a separation into two one-electron waves may take place.

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[3] Munro, *Nature*, 1957, **180**, 540.

With high concentrations of intermediate free radical at the surface of the mercury drop, the radicals may disproportionate or dimerise:

$$2XH\cdot \longrightarrow X + XH_2 \qquad (R_3)$$

$$2XH\cdot \longrightarrow (XH)_2 \qquad (R_4)$$

Such reactions serve to diminish the importance of the second reduction stage (R_2), leaving the first one-electron reduction (R_1) as the predominant electrode reaction.

The polarographic reduction of the structurally similar xanthone system has been studied by Whitman and Wiles,[4] who report that the reduction characteristics, measured at $9{\cdot}5 \times 10^{-4}$M, are those of a one-electron process; no results are reported for lower concentrations. These authors account for the one-electron character of xanthone waves in terms of radical dimerisation (R_4).

For Miracil D, the transition from a two-electron wave to a one-electron wave is marked at concentrations above 5×10^{-4}M (188 mg./l.); also above this point the failure of current–concentration proportionality becomes apparent. The evidence available is insufficient to choose between radical disproportionation (R_3) or dimerisation (R_4) as the more important process for thioxanthones.

The use of ethanolic hydrochloric acid mixtures was based on preliminary experiments by Dr. G. W. Meacock whose help is gratefully acknowledged. The author is indebted to Dr. D.Ll. Hammick, F.R.S., for valuable discussions throughout the preparation of this paper.

Department of Inorganic and Structural Chemistry,
The University, Leeds, 2.
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[4] Whitman and Wiles, *J.*, 1956, 3016.