1060. *Physicochemical Properties of Some Chemotherapeutic Thioxanthones.*

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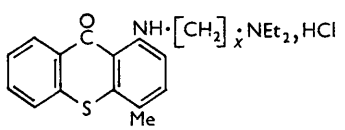
For Miracil D and three homologous compounds, the basicites have been measured and at pH 7·4 lipoid partition coefficients, surface activities, and protein affinities, which may be relevant to biological activity.

Many of the compounds used to combat the parasitic infections of malaria and schistosomiasis have structural features in common. The acridine and quinoline antimalarials mepacrine (Atebrin) and pamaquin (Plasmoquine), also the xanthone and thioxanthone drugs Miracil B and Miracil D (lucanthone), have each a reducible heterocyclic nucleus with an aliphatic diamine side-chain. It was the observation of a relation between acridines and xanthones which led Mauss [1] to the preparation of xanthones and thioxanthones, and hence to the discovery among the latter of activity against *Schistosoma haematobium* infections.

Properties such as basicity and reducibility, associated with the structural groupings common to all these compounds, may contribute to the activity against the different parasitic organisms. In fact some acridines are effective against *S. haematobium* infections. A series of thioxanthone compounds have been studied in a manner earlier applied to antimalarials, to see whether similar relations are to be found

In a series of investigations on acridine antimalarials, Hammick and Mason [2] measured several physicochemical parameters for correlation with antimalarial activity. The values of these parameters have now been found for the thioxanthone series which includes Miracil D (lucanthone; 1-2′-diethylaminoethylamino-4-methylthioxanthone hydrochloride) (I; $x = 2$).

Compounds homologous with Miracil D, and having three and four methylene groups in the side-chain (I; $x = 3$, 4), have been described by other workers,[1,3] who prepared them from the chloromethylthioxanthone by treatment with the diamine appropriate to the side-chain. The present work has made use of 1-amino-4-methylthioxanthone which was readily available in pure form from an earlier study; [4] and these compounds, together with the pentyl homologue (I; $x = 5$) have been prepared, but in small yield, by interaction of 1-amino-4-methylthioxanthone with the appropriate ω-bromoalkyldiethylamine hydrobromide.

(I) 

Miracil D and its homologues may be characterised as picrates. The hydrochlorides proved difficult to obtain with definite proportions of hydrogen chloride, the monohydrochlorides containing more than, and the dihydrochlorides less than, the stoicheiometric amount of acid. The monohydrochlorides were used for physical measurements, the uncertainty in concentration of the base, due to variable hydrogen chloride content in the salt, is less than 3%.

The importance of basicities in relation to various types of biological activity, exemplified by acridines, has been surveyed by Albert [5] for results obtained in water and in 50% aqueous ethanol.

Measurements have been made for Miracil D and its homologues, but the basicities of these thioxanthones cannot be measured in aqueous solution owing to the insolubility of the free bases. In 50% v/v ethanol, however, $\sim 10^{-3}$M-solutions of Miracil D base can be

[1] Mauss, *Chem. Ber.*, 1948, **81**, 19.
[2] Hammick and Mason, *J.*, 1950, 345, 348.
[3] Archer and Suter, *J. Amer. Chem. Soc.*, 1952, **74**, 4303.
[4] Hammick and Munro, *J.*, 1952, 1077.
[5] Albert, "The Acridines," Edward Arnold and Co., London, 1951, pp. 113—119, 251, 287, 298.

prepared; and the changes in apparent pH on addition of sodium hydroxide give a measure of the basicities. These are listed in the Table.

The basicities of the basic aromatic amino-group vary little, and the dissociation constants are little different from that of 1-amino-4-methylthioxanthone (apparent pK_a 3·0). For the aliphatic basic centre, the values obtained show a progressive increase in basicity as the number of methylene groups separating the two basic centres is increased.

The values of pK_a determined in 50% ethanol are generally about 0·8 unit (individually 0·43—1·46 unit) lower than those in water.[6] An acidity of pH 7·4, which is the mean acidity of the human bloodstream, appears therefore to correspond with an apparent pH of 8·2—8·3 in 50% ethanol.

Miracil D itself has apparent $pK_{2a} = 8·25$, while the homologous compounds have decidedly higher values. This suggests that substantial amounts of Miracil D are present as the free base in solution at pH 7·4, while the homologues are present as first conjugate acids.

This conclusion is supported by their behaviour in aqueous buffers without ethanol. At pH 7·4, 5×10^{-5}M-solutions of Miracil D slowly yielded crystals of the free base. Comparable solutions of the homologues did not do this, although from more alkaline solutions the free bases were precipitated.

Physicochemical properties of Miracil D series.

Compound	Basicities in 50% EtOH pK_{a1}	Basicities in 50% EtOH pK_{a2}	Surface activities at 5×10^{-5}M γ (dynes/cm.)	Surface activities at 5×10^{-5}M $\Delta\gamma$	Protein affinity	Lipoid affinity
I; $x = 2$ (Miracil D)	3·4	8·25	71·12	2·46	1·0	9×10^3
I; $x = 3$	3·7	8·87	70·89	2·69	2·0	4×10^3
I; $x = 4$	3·5	9·22	68·40	5·18	1·9	4×10^3
I; $x = 5$	3·5	9·41	66·95	6·63	1·7	5×10^3
1-Amino-4-methylthioxanthone ...	3·0	—	—	—	—	—

Surface activities and protein and lipoid affinities have been measured by procedures similar to those used by Hammick and Mason. The results do not relate to any single molecular species, but rather in each case to an equilibrium mixture of free base with its first conjugate acid; however, material in the bloodstream is present as a similar equilibrium mixture, and it is the properties of this mixture that are of significance in relation to therapeutic effect.

Surface activities have been assessed by the extent to which the compounds lower the surface tension of an aqueous buffer of pH 7·4. Values have been obtained by the drop-weight method. An increase in activity with chain length is observed, but owing to the presence in differing proportions of the first conjugate acid and the free base, the results do not agree well with Traube's rule, which suggests an approximately three-fold increase in surface tension lowering for each successive methylene group. The small difference between the surface activity of Miracil D and that of the first succeeding homologue may be accounted for by the relatively greater proportion of free base in the case of the former.

In the animal body, extraction of drugs from their centre of action by complex formation with proteins may be important in determining relative potencies. Wormall and Dewey [7] have indicated that complex formation with proteins may be assessed by precipitation methods. This, with the use of albumin as a representative protein, since it is quantitatively the major protein in blood plasma, provides the basis for a convenient laboratory method for assessment of protein affinities. Hammick and Mason [2] found that the precipitation method, applied to acridine antimalarials, gave results comparable with those from more elaborate dialysis procedures.

Solutions of Miracil D and homologues in phosphate buffer of blood pH were treated

[6] Albert and Goldacre, *J.*, 1946, 706.
[7] Wormall and Dewey, *Biochem. J.*, 1946, **40**, 119.

with a solution of blood albumin. After precipitation of albumin by addition of ethanol, approximately half the original amount of thioxanthone remained in solution. For each compound, the proportion of concentration remaining in solution related to the corresponding proportion measured for Miracil D gives the protein affinity in a form independent of the uncertain concentration of the albumin solution.

The ratios of extracted to residual concentration were of the same order as those observed by Hammick and Mason for acridine antimalarials. A minimum extraction of the compound with two methylene groups in the side-chain is observed, but there is little variation with side-chain length.

The partition coefficients between phosphate buffer of blood pH and acid-free oil have been determined in order to find the relative extents to which Miracil D and homologues may be extracted by fatty tissues in the body. Hammick and Mason used castor oil, which has a constitution sufficiently similar to that of animal fats and is liquid at room temperature; their procedure has been followed in studying the thioxanthone series.

Volumes of buffer containing known amounts of the thioxanthones were shaken with oil; after separation of the liquid layers the concentrations remaining in the aqueous layer were determined, and the partition coefficients calculated (Table 1). For the four compounds examined, the partition coefficients are high. A minimum extraction by oil is observed with the compounds having three and four methylene groups.

Experimental

Preparation of Solutions.—Miracil D and its homologues, as monohydrochlorides, were dried for several days under a vacuum over sulphuric acid. Weighed quantities were dissolved in water to give 1·6—2·0 $\times$ 10^{-3}M-solutions. These were used in the titrations to find the basicities. Solutions in M/15-phosphate buffer of pH 7·4 (obtained by mixing M/15-KH_2PO_4, 1 vol. with M/15-Na_2HPO_4, 4 vol.) were prepared by diluting the aqueous solutions (5—10 ml.) with the volume of phosphate buffer (200—300 ml.) calculated to give 5 $\times$ 10^{-5}M-solutions. 1-Amino-4-methylthioxanthone hydrochoride was dissolved in ethanol to give a 2·05 $\times$ 10^{-3}M-solution.

Basicities.—The solution (10 ml.) of the compound to be examined was treated with appropriate amounts of ethanol and water to give 50 ml. of solution containing 50% v/v of ethanol, and cooled to room temperature (18°). In the case of Miracil D and its homologues as monohydrochlorides, one equiv. of acid was added to give a solution equivalent to the dihydrochloride. A glass electrode was used to observe changes in pH after addition of successive small amounts of 0·02N-sodium hydroxide; stirring and protection from atmospheric carbon dioxide were provided by nitrogen bubbled through the liquid.

Surface-tension Measurements.—A stalagmometer of Pyrex glass, with a capillary air leak, had a drop time of 20 sec. The weights of 5 drops were found, for water, for M/15-phosphate buffer of pH 7·38, and for 5 $\times$ 10^{-5}M-solutions of Miracil D and its homologues in the phosphate buffer. Results were calculated on the basis of direct proportionality between surface tension and drop weight, with the value 72·75 dynes/cm. at 20° for the surface tension of water. The surface tension of the M/15-phosphate buffer solution was found to be 73·58 dynes/cm. at 20°.

Protein Affinities.—Blood albumin was obtained from B.D.H. Albumin (20 g.) with water (400 ml.) was stored for 3 days at 0—5°. Undissolved solid was removed by filtration and at the centrifuge. 5 $\times$ 10^{-5}M-Solutions of the thioxanthones in the M/15-phosphate buffer were employed; for Miracil D, a saturated solution (*ca.* 2·2 $\times$ 10^{-5}M) was used. The thioxanthone in M/15-phosphate buffer (20 ml.) was mixed with the albumin solution (20 ml.); after 30 min. at room temperature, ethanol (40 ml.) was added and the precipitated material was removed by centrifugation (3500 r.p.m.), by boiling the solution, and by extraction of the thioxanthone by chloroform. The extract was evaporated to dryness, and the residue redissolved in 0·1N-hydrochloric acid (10 ml.). 2M-Sodium acetate (1·0 ml.) was added and the concentration of the thioxanthone estimated colorimetrically. Reference solutions were obtained from each initial solution of thioxanthone in the phosphate buffer by a parallel extraction procedure.

For each of the four homologues, the ratio (loss in concentration)/(residual concentration)

was calculated. The protein affinities were obtained by dividing this ratio by that obtained for Miracil D.

Lipoid Affinities.—Castor oil (B.P.) was made acid-free by shaking a weighed quantity of oil with water and sodium carbonate (1 mol. per equiv. of acidity). The oil was recovered, after filtration, by extraction with ether; from the dried extract ether was removed by distillation and by heating the residue at 100°/15 mm.

Preliminary experiments were conducted in order to determine the initial concentration and the quantity of castor oil required to give a residual concentration in the aqueous layer of about 2×10^{-5}M.

A weighed quantity of acid-free oil (15—40 mg.; *d* 0·963) and the solution (50 ml.) of the thioxanthone in M/15-phosphate buffer were shaken together for 2½ hr. The resulting emulsion was broken on the centrifuge at 3500 r.p.m. and the aqueous layer removed and filtered. The residual concentration in the aqueous layer was determined colorimetrically.

Preparation of Thioxanthones.—The homologues of Miracil D were prepared by heating 1-amino-4-methylthioxanthone [4] (1 mol.) with the ω-bromoalkyl-diethylamine hydrobromide (2 mol.) in ethanol for 96—168 hr. The product was separated from unchanged 1-amino-4-methylthioxanthone by 2N-hydrochloric acid, which precipitated the latter while retaining the diethylaminoalkylaminothioxanthone in solution: these were characterised as picrates.

The bromopropyldiethylamine hydrobromide was obtained from trimethylene dibromide.[8] For the two higher homologues tetrahydrofuran and tetrahydropyran, respectively, yielded the corresponding chloroalkyl acetates by treatment with acetyl chloride in the presence of zinc chloride; [9] on treatment with diethylamine and subsequent hydrolysis these gave the hydroxyalkyldiethylamines; distillation with hydrobromic acid yielded the bromoalkyldiethylamine hydrobromides.

1-2′-*Diethylaminoethylamino*-4-*methylthioxanthone picrate*, crystallised from ethanol, had m. p. 142° (Found: C, 55·1; H, 4·1; N, 12·6. $C_{20}H_{24}N_2OS,C_6H_3N_3O_7$ requires C, 54·8; H, 4·8; N, 12·3%). 1-3′-*Diethylaminopropylamino*-4-*methylthioxanthone picrate* (from ethanol) had m. p. 173·5° (Found: C, 55·9; H, 5·1; N, 12·0. $C_{21}H_{26}N_2OS,C_6H_3N_3O_7$ requires C, 55·6; H, 5·0; N, 12·0%). The monohydrochloride (from ethanol) had m. p. 173° (Mauss [1] gives m. p. 173°; Archer and Suter [3] give m. p. 164—165°) (Found: N, 6·9; Cl, 11·5. Calc. for $C_{21}H_{26}N_2OS,HCl$: N, 7·2; Cl, 9·1%). The hygroscopic dihydrochloride (from methanol and ether) had m. p. 148° (Found: Cl, 14·3. Calc. for $C_{21}H_{26}N_2OS,2HCl$: Cl, 16·6%). 1-4′-*Diethylaminobutylamino*-4-*methylthioxanthone picrate* (from ethanol) had m. p. 128·5° (Found: C, 56·4; H, 5·0; N, 11·8. $C_{22}H_{28}N_2OS,C_6H_3N_3O_7$ requires C, 56·3; H, 5·2; N, 11·7%). The monohydrochloride (from methanol–ether) had m. p. 201° (Found: N, 7·2; Cl, 10·1. Calc. for $C_{22}H_{28}N_2OS,HCl$: N, 6·9; Cl, 8·75%). The hygroscopic dihydrochloride (from methanol–ether) had m. p. 210° (Archer and Suter [3] give m. p. 153—155°) (Found: Cl, 13·4. Calc. for $C_{22}H_{28}N_2OS,2HCl$: Cl, 16·25%). 1-5′-*Diethylaminopentylamino*-4-*methylthioxanthone picrate* (from ethanol) had m. p. 134° (Found: C, 57·0; H, 5·1; N, 11·8. $C_{23}H_{30}N_2OS,C_6H_3N_3O_7$ requires C, 56·9; H, 5·4; N, 11·45%). The *monohydrochloride* (from ethanol) had m. p. 190° (Found: N, 6·35; Cl, 8·1. $C_{23}H_{30}N_2OS,HCl$ requires N, 6·7; Cl, 8·5%). The hygroscopic dihydrochloride (from methanol–ether) had m. p. 156° (Found: Cl, 13·8. Calc. for $C_{23}H_{30}N_2OS,2HCl$: Cl, 15·6%).

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The University, Leeds, 2. [*Received, May* 31*st*, 1961.]

[8] Marvell, Zartman, and Bluthardt, *J. Amer. Chem. Soc.*, 1927, **49**, 2301.
[9] Cloake and Pilgrim, *J. Amer. Chem. Soc.*, 1939, **61**, 2667.