THE ABSORPTION SPECTRA AND IONIC DISSOCIATION OF THIOURACIL DERIVATIVES WITH REFERENCE TO THEIR ANTI-THYROID ACTIVITY

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A number of derivatives of thiourea and especially the substituted thiouracils inhibit thyroid activity in animals[1,2]; in particular thiouracil and methylthiouracil have been used in the treatment of hyperthyroidism in man. Miller, Roblin and Astwood[3] studied the reaction between iodine and 2-thiouracil and found that at *p*H 7·4 in the presence of sodium bicarbonate a disulphide from two molecules of thiouracil was formed. The absorption of iodine was such that tyrosine and casein were protected from iodination under these conditions in the presence of 2-thiouracil. This was held to support the hypothesis that thio-derivatives may prevent hormone synthesis in the thyroid gland by blocking the iodination of hormone precursors. Doubts, however, have been expressed by Rimington and Lawson[4] concerning the validity of this reaction ($2\ RSH + I_2 \rightleftharpoons RS.SR + 2\ HI$) as a mechanism owing to the activity of sulphonamide derivatives which have no free – SH group.

Williams and Kay[5] found that the activity of thiouracil was distinctly decreased or in some instances lost by the addition of methyl or ethyl substituents on the nitrogen atoms or by the addition of substitutents on the sulphur atom. Since both of these alterations to the molecule are connected directly or indirectly with the ionisable hydrogen atom it seems probable that ionisation is an important factor in thiouracil activity. It was therefore decided to investigate the dissociation of 2-thiouracil and 2-thio-4-methyluracil and to try and determine the probable structure of the resulting ion. With the latter object in view the methylated thiol structures corresponding to 2-thio-4-methyluracil, namely, 2-methylmercapto-4-methyl-6-oxypyrimidine and 1 : 4-dimethyl-2-methylmercapto-6-oxypyrimidine, were prepared and their ultra-violet absorption spectra determined under varying *p*H conditions. At the same time the dissociation of the compounds prepared was investigated by means of an electrometric titration.

Experimental

Absorption Spectra. The absorption curves were determined at varying *p*H values using a Beckman photoelectric spectrophotometer. Samples of 2-thiouracil and 2-thio-4-methyluracil of appropriate strengths were dissolved in potassium dihydrogen phosphate-sodium hydroxide buffer solutions and examined in a 1 cm. cell. Solutions of high and low *p*H were obtained by dissolving in carbon dioxide-free water and adding sodium hydroxide or hydrochloric acid until the required *p*H was obtained as indicated by *p*H meter; solutions obtained in this manner were used immediately.

Electrometric Titrations. These were carried out in general by running N/10 aqueous sodium hydroxide into solutions of thiouracil

derivatives in 50 per cent. aqueous ethyl alcohol, the *p*H of the solution being determined at intervals by a standard Cambridge *p*H meter. A typical result is shown for 2-thio-4-methyluracil:

Per cent. neutralised	10	20	30	40	50	60	70	80
pK_a	8·6	8·5	8·5	8·6	8·5	8·5	8·4	8·4

The temperature throughout the *p*H measurements was 18°±2°C. The results are not corrected for the effects of alcohol.

Preparation of the methylated derivatives. 2-Methylmercapto-4-methyl-6-oxypyrimidine was prepared by methylation of 2-thio-4-methyluracil in sodium ethylate using methyl iodide according to List[6]. After crystallisation from alcohol it had m.pt. 220°C. Di-methylation of 2-thio-4-methyluracil, by the method of Wheeler and MacFarland[7] produced 1:4-dimethyl-2-methylmercapto-6-oxypyrimidine which after crystallisation from alcohol had m.pt. 94°C. The constitution of the methylated compounds was proved by Wheeler and MacFarland (*loc. cit.*) since on heating with hydrochloric acid 1:4-dimethyl-2-methylmercapto-6-oxypyrimidine gave 1:4-dimethyluracil.

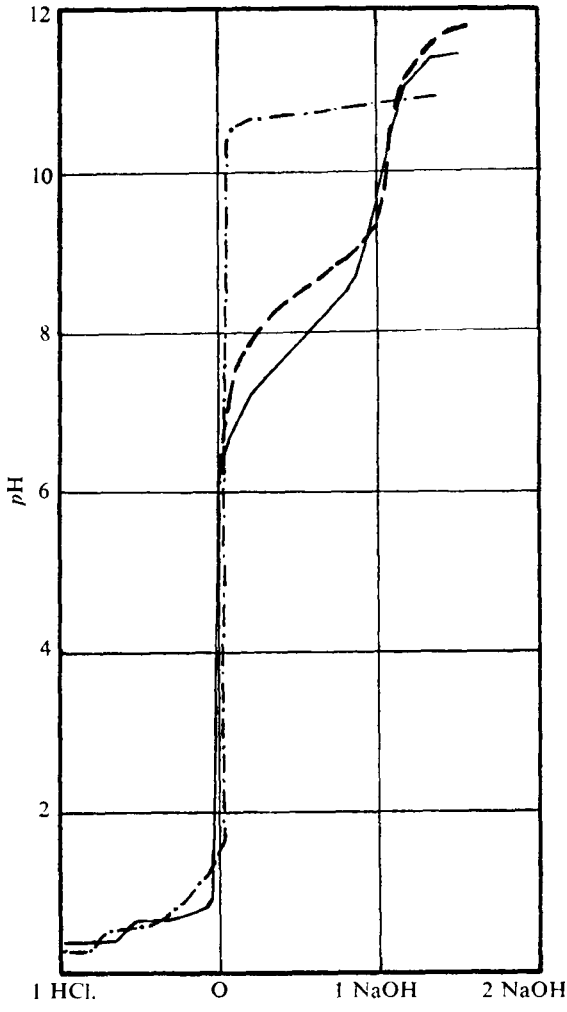


FIG. 1. Electrometric titration curves:
– – – – 2-thio-4-methyluracil;
——— 2-methylmercapto-4-methyl-6-oxypyrimidine;
–·–·– 1:4-dimethyl-2-methylmercapto-6-oxypyrimidine.

RESULTS

The results in general are of interest in showing the close correlation between dissociation as shown by the *p*H curves and changes in absorption

spectrum, i.e. structural changes are due primarily to ionisation. Thus 2-thio-4-methyluracil possesses a potentiometric titration curve (Fig. 1) in aqueous ethyl alcohol with a break corresponding to an acidic dissociation

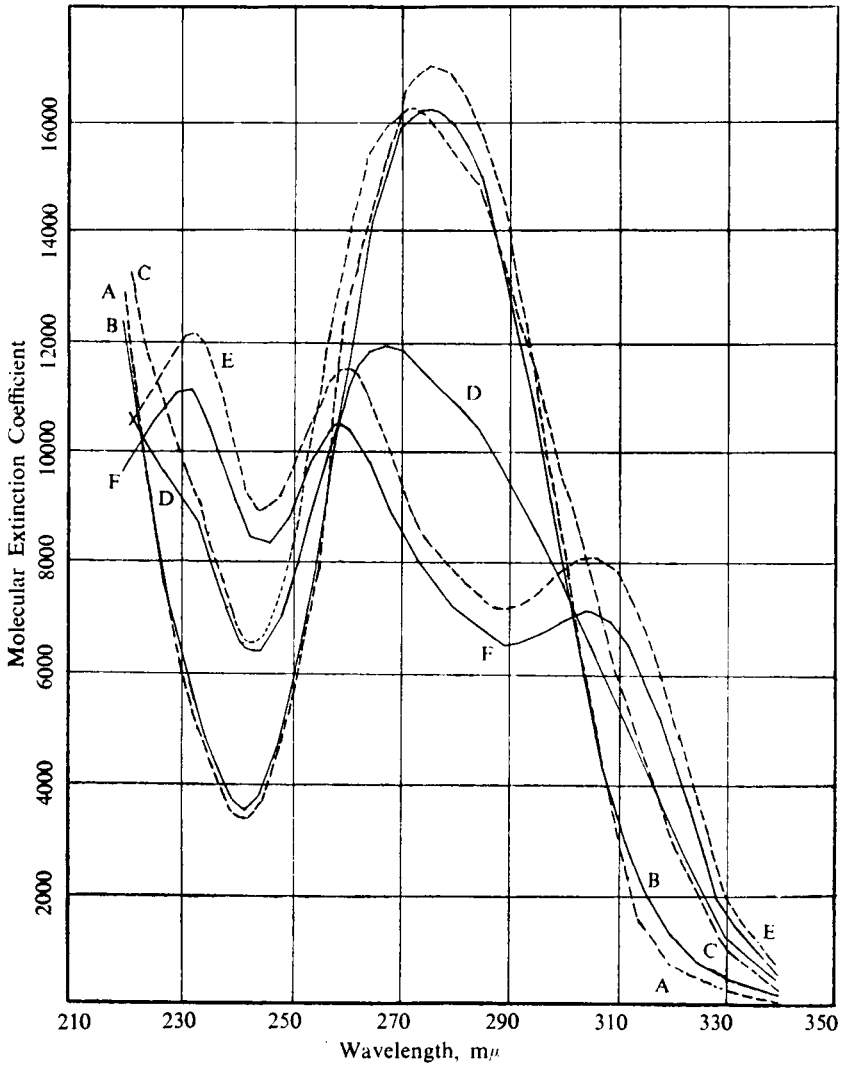


FIG. 2. Absorption spectra of 2-thio-4-methyluracil. A, at *p*H 2; B, at *p*H 7; C, at *p*H 8; D, at *p*H 9; E, at *p*H 10; F, at *p*H 12.

constant of *p*H 8·5. The spectra of this compound (Fig. 2) show minor differences only at *p*H values up to 7, and at *p*H 8 the main peak λ_{max} *ca.* 270 mμ ε_{max} *ca.* 16,000 is still not appreciably changed. At higher *p*H values, corresponding to increasing ionisation three peaks become evident, a transition curve occurring at *p*H 9.

2-Methylmercapto-4-methyl-6-oxypyrimidine does not show any main peak ε_{max} *ca.* 16,000 as for 2 : thio-4-methyluracil, and the changes are not so fundamental (Fig. 3). A break in the *p*H curve (Fig. 1) between *p*H 0 and *p*H 2 corresponds to a change in absorption spectra at these

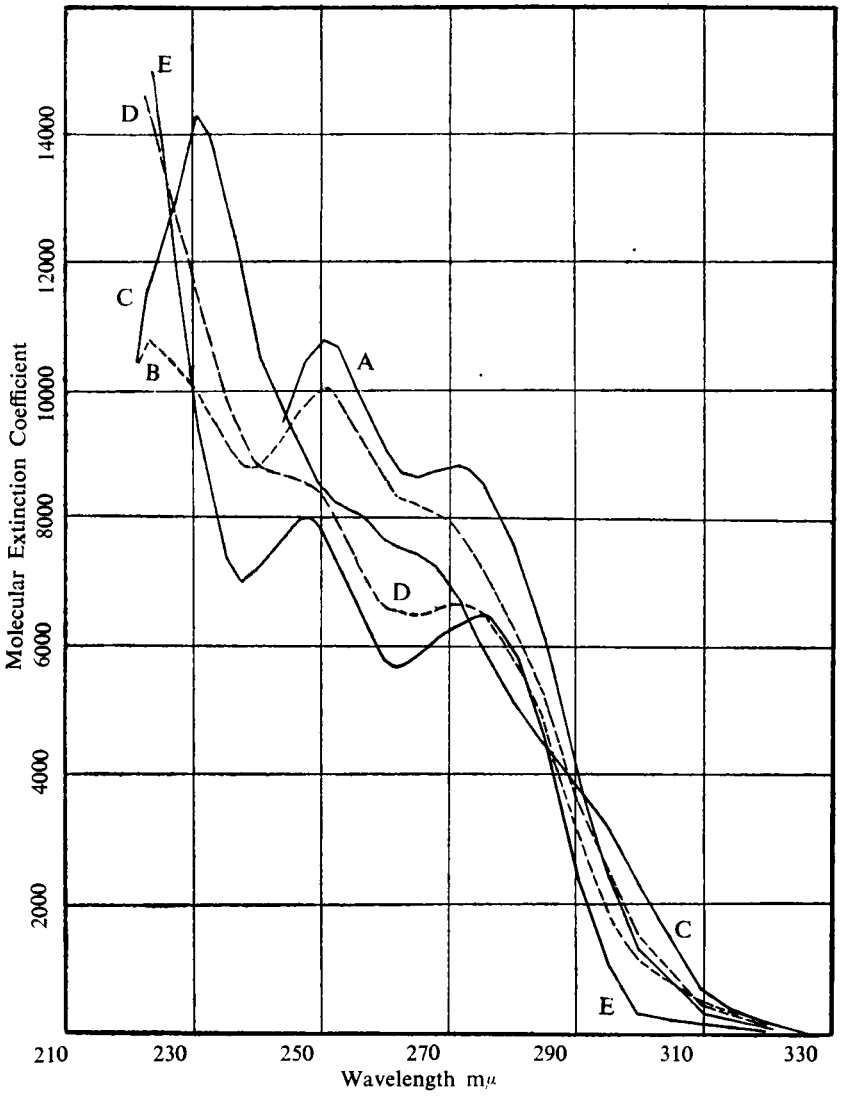


FIG. 3. Absorption spectra of 2-methylmercapto-4-methyl-6-oxypyrimidine. A, at *p*H 0; B, at *p*H 1; C, at *p*H 2; D, at *p*H 8; E, at *p*H 9.

values. The electrometric titration results for this compound show it to be a weak acid, having a pK_a value of 7·9.

1 : 4-Dimethyl-2-methylmercapto-6-oxypyrimidine shows absorption changes only at *p*H values less than 2 (Fig. 4), again corresponding to a

break in the electrometric titration curve (Fig. 1). The spectrum of 2-thiouracil (Fig. 4) is in general agreement with the graphical results of Elion, Ide and Hitchings[8] and of Miller, Roblin and Astwood[3].

It was not possible directly to calculate the dissociation constants from absorption measurements (see e.g. Morton and Tipping[9] for violuric acid, Stuckey[10] for barbituric acid), as the relationship between absorption maxima and ionisation was not linear. It is obvious from this, and from

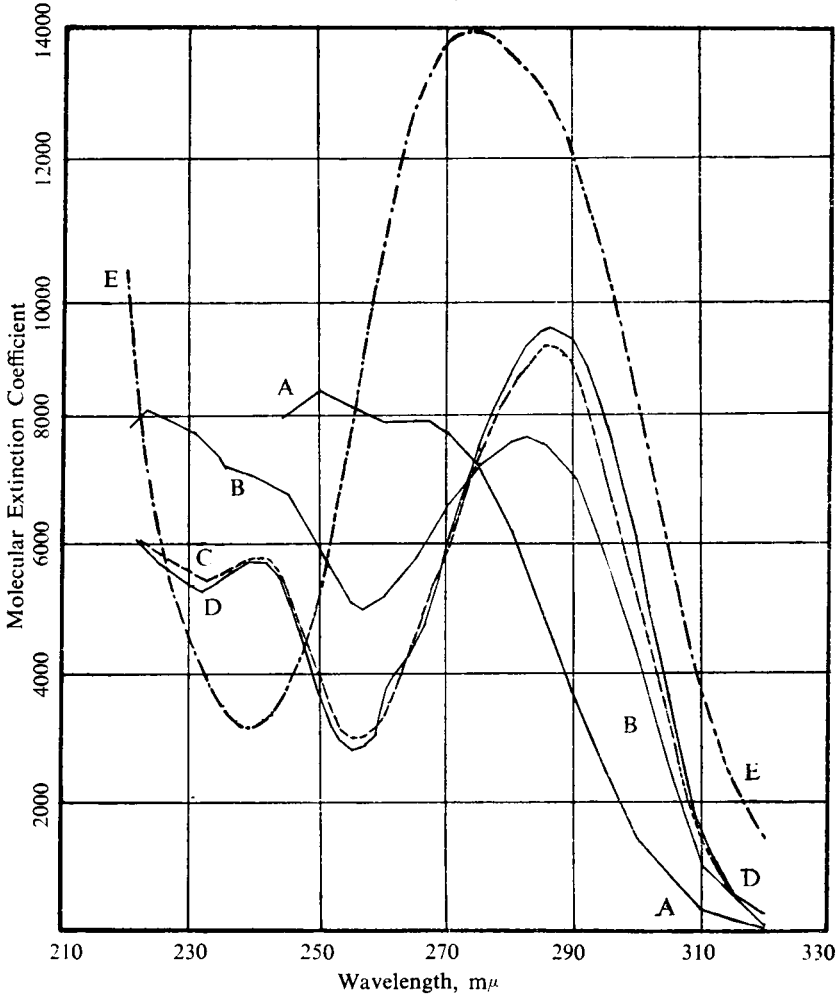


FIG. 4. Absorption spectra of 1 : 4-dimethyl-2-methylmercapto-6-oxypyrimidine. A, at *p*H 0; B, at *p*H 1; C, at *p*H 2; D, at *p*H 12. E, 2-Thiouracil, at *p*H 7.

the fundamental spectrum changes, that variations in *p*H must be causing spectrum changes associated with resonance effects or with more than one group in the molecule.

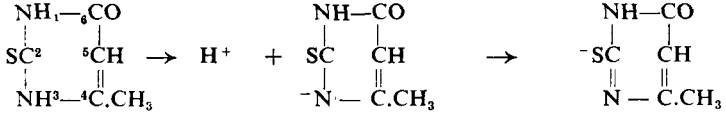
Discussion

Tautomerism in 2-thio-4-methyluracil (I) can theoretically occur in more than one part of the molecule, notably involving the hydrogen attached to the nitrogen atom in the 3-position producing a $\Delta^{2,3}$ structure, or involving the hydrogen attached to the nitrogen atom in the 1-position producing either a $\Delta^{1,2}$ or a $\Delta^{1,6}$ structure. The potentiometric titration curve for this compound (Fig. 1) shows a single dissociating group with a pK_a value in aqueous ethyl alcohol of 8·5, but the fact that the absorption

TABLE I

	*p*H	λ_{max} mμ	ϵ_{max}
2-Thiouracil	7	274	13,940
2-Thio-4-methyluracil	2	275	16,980
	7	275	16,160
	8	272	16,100
	9	267	11,800
	10	232·5	12,180
		260	11,500
		305	8,050
	12	232·5	11,070
		260	10,430
		305	7,080
2-Methylmercapto-4-methyl-6-oxypyrimidine ...	0	250	10,800
		270	8,810
	1	222·5	10,730
		250	10,000
	2	230	14,260
	8	272·5	6,600
	9	247·5	7,980
		275	5,960
1 : 4-Dimethyl-2-methyl-mercapto-6-oxypyrimidine	0	250	8,320
	1	222·5	8,040
		282·5	7,620
	2	241	5,750
		285	9,050
	12	241	5,740
		286	9,420

changes do not show a linear relationship between peak values and ionisation indicates that spectrum changes are not directly associated with a single ionic structure.

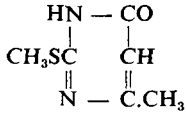


I II III

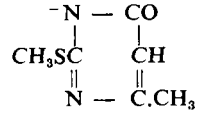
The absorption spectra of 2-thio-4-methyluracil show a main broad peak *ca.* 270 mμ with relatively little change at *p*H values up to 8. This peak corresponds to the undissociated form I, by analogy with other pseudo-acids, e.g., barbituric acid derivatives, the thio-ketone form being present in aqueous acid solution. It is noteworthy that Schneider and Halverstadt[11] found from a study of dipole moments in dioxan that 2-thiouracil possessed the structure corresponding to I. The change in

spectrum accompanying dissociation (from *p*H 7 to *p*H 10) is fundamental, with the splitting of the main band into three subsidiary bands; at *p*H 10 the change is virtually complete, the absorption maxima at higher *p*H values showing a slight decrease only, common to the spectra of many pseudo-acids in alkaline solution (cf. Stuckey[10]), the main characteristics being retained. The reason for the main spectrum change is not easily interpreted, but it is probable that this follows ionic rearrangement, predominantly with the production of structures II and III with associated resonance effects. This is supported by the fact that *p*H measurements show only one dissociating group. Any second dissociation with the addition of a further double link (from subsequent tautomerism) would produce the basic pyrimidine structure with three double links; this would have characteristically lower absorption values, which are not actually found. Methylation, with the production in the first instance of 2-methylmercapto-4-methyl-6-oxypyrimidine (IV), gives support to the supposition that the nitrogen in the 3-position is the dissociating group. The spectra of IV in alkaline solution show, broadly, a similar pattern to 2-thio-4-methyluracil in alkaline solution.

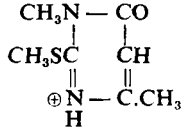
The spectrum of 2-thiouracil (Fig. 4) agreed with the graphical results of Elion, Ide and Hitchings[8] and of Miller, Roblin and Astwood[3]. The peak values in acid solution were considerably lower than for the 4-methyl compound and lower than for 2-thiothymine. Although the introduction of a methyl group in the 4-position has no direct effect on the tautomerism it must obviously have some effect other than the normal weighting although exactly what is causing the increase in ε_{max} is rather obscure. The parent uracil has ε_{max} 11,000 at 258 mμ. (Loofbourow, Stimson and Hart[12]) so that the replacement of oxygen by sulphur has caused only a slight increase in ε_{max}.



IV



V



VI

2-Methylmercapto-4-methyl-6-oxypyrimidine (IV, Fig. 3) shows an absorption curve at *p*H 2 with a maximum at 230 mμ, but lacking any peak values at longer wavelengths; at this *p*H, ionisation accompanied by the splitting off of a hydrogen atom does not take place to any extent and the spectrum will be that of the undissociated structure IV. There is a change in spectrum with the development of two subsidiary peaks in alkaline solution (*p*H 9) corresponding to dissociation as shown by the electrometric titration curve. The spectrum at *p*H 9 is due to the ion (V) with the possibility of resonance with a fully unsaturated pyrimidine ion having a 1:6 double link. The *p*H curve for 2-methylmercapto-4-methyl-6-oxypyrimidine shows a slight break with an inflexion corresponding to a radical change in the spectra *ca.* *p*H 1 and

*p*H 0, undoubtedly due to the partial conversion of the tertiary nitrogen to a $=\overset{\oplus}{N}H-$ structure.

1:4-Dimethyl-2-methylmercapto-6-oxypyrimidine shows no change from *p*H 2 to *p*H 12, which is to be expected in the absence of an ionisable hydrogen atom. The break in the *p*H curve between *p*H 0 to *p*H 1 corresponds, as with the monomethylated compound IV, to the addition of a proton to the tertiary nitrogen in the 3-position (VI).

The iodine-absorption theory of anti-thyroid activity is supported by the work of Albert, Rawson, Merrill, Lennon and Riddell[13], who found that the loss of thyrotropic activity occurring during exposure of a pituitary extract to iodine, was restored by treatment of the iodinated hormonal material with 2-thiouracil. The production of a disulphide compound incorporating the thiol form of the thiouracil molecule (III) in Astwood's iodination experiments, together with the findings of Williams and Kay[5], suggest that any *mechanism* of iodine absorption depends on ionisation and subsequent ion tautomerism. In view of this it appears that ionisation is necessary for antithyroid activity in thiouracil derivatives. The present work has shown that 2-thio-4-methyluracil has a pK_a value in 50 per cent. aqueous alcohol of 8·5; in order to make this figure applicable to an aqueous solution it is necessary to apply a correction. Mizutani[14] found values for ΔpK_a between water and 50 per cent. aqueous ethyl alcohol to be of the order of 0·9 to 1·2, with an approximate average of 1·1, for the numerous acids studied. It is unfortunate that a more precise correction cannot be applied since a small difference in pK_a in the region pK_a 6·6—8·0 corresponds to a big difference in percentage dissociation at *p*H 7·3; the very limited solubility of 2-thio-4-methyluracil, however, makes an accurate determination in aqueous solution a matter of difficulty. Assuming ΔpK_a to be 1·1 units, the corrected figure for 2-thio-4-methyluracil is, therefore, 7·4. This corresponds to a dissociation of 44 per cent. at a blood *p*H of 7·3. On the limited evidence available an appreciable dissociation of thiouracil derivatives at *p*H 7·3 would seem to be necessary for iodine absorption in anti-thyroid activity.

The peak absorption values in acid solution shown by 2-thio- and 2-thio-4-methyluracil provide a useful property for the analytical estimation of these compounds. This, together with the dissociation properties of thiouracil derivatives, is the subject of further investigation.

SUMMARY

1. The ultra-violet absorption spectra of 2-thiouracil, 2-thio-4-methyluracil, 1:4-dimethyl-2-methylmercapto-6-oxypyrimidine and 2-methylmercapto-4-methyl-6-oxypyrimidine have been determined at varying *p*H values.

2. Electrometric titration curves have been plotted for 2-thio-4-methyluracil, 2-methylmercapto-4-methyl-6-oxypyrimidine and 1:4-dimethyl-2-methylmercapto-6-oxypyrimidine, and pK_a values for these compounds in aqueous ethyl alcohol have been recorded.

3. The close connection between change in *p*H and change in ultraviolet light absorption for the compounds studied shows that absorption changes are due primarily to ionisation and subsequent ionic rearrangement.

4. On the basis of the iodine oxidation theory, the probable ionic nature of the iodine absorption mechanism suggests that thiouracil derivatives which are appreciably ionised at *p*H 7·3 are most likely to exhibit anti-thyroid activity.

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