THE COLORIMETRIC DETERMINATION OF METHYLTHIOURACIL AND PROPYLTHIOURACIL IN TABLETS USING 2 : 6-DICHLOROQUINONE-CHLOROIMIDE

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THE recent paper by Bergren and Kirsten[1] on the errors involved in the titrimetric determination of propylthiouracil in tablet form, due to the presence of magnesium stearate, has prompted us to report on the colorimetric determination of methylthiouracil and propylthiouracil using the chloroimide reaction. One of us (McAllister[2,3,4,5]) has already described the chloroimide reaction for antithyroid compounds of both the thiouracil and mercaptoimidazole series, and methods have been elaborated for the quantitative determination of these. During the course of this work much information has been accumulated on the reaction involved, and we have been able to isolate the coloured product of the reaction with propylthiouracil in crystalline form. The present paper describes methods for the colorimetric determination of methylthiouracil and propylthiouracil in tablet form together with other work not previously reported.

PROCEDURES

Reagents. 1. 0·4 *per cent. solution of* 2:6-*dichloroquinone chloroimide in aldehyde-free absolute ethanol.* The colour reagent will keep for about 4 weeks if stored in a brown bottle.

2. *Buffer-chloride solution, p*H 8·0. To 50 ml. of 0·2 M boric acid in 0·2 M potassium chloride add 4 ml. of 0·2 N sodium hydroxide. Then add 100 ml. of a 20 per cent. solution of sodium chloride in water. Make the volume of the solution to 200 ml. with distilled water. Check the *p*H of the solution and adjust it to 8·0 by the addition of 0·1 N sodium hydroxide.

3. *Aldehyde-free absolute ethanol.* Prepared according to the method of Callow, Callow and Emmens.[6] To 500 ml. of absolute ethanol add 2 g. of *m*-phenylenediamine and allow the mixture to stand for 1 week in a dark cupboard. The prolonged digestion time may be replaced by boiling for 1 hour under a reflux condenser.[7] Distil the mixture in an all-glass still. If stored in a dark bottle, the solution will keep indefinitely.

4. *Chloroform B.P.*

5. *Standard solutions.* (*a*) *Propylthiouracil.* Dissolve 25 mg. of the compound in 5 ml. of aldehyde-free absolute ethanol. It is advisable to use a small beaker for the purpose to minimise deposition of the substance on the sides of the beaker due to evaporation. Add 100 ml. of distilled water, and allow to stand for 10 minutes. Make the volume to 250 ml. with distilled water. 1 ml. of this solution contains 100 μg. of propylthiouracil. The solution will keep for about 2 days. (*b*) *Methylthiouracil.* Weigh out 100 mg. of 4-methyl-2-thiouracil. Add 80 ml. of water and 0·8 ml. of concentrated ammonia solution and mix until

dissolved. Make the final volume to 1 l. with distilled water. 1 ml. of this is equivalent to 100 μg. of methylthiouracil. This solution will not keep.

Standard graphs. Dilute both standards suitably and take amounts up to 100 μg. of each. Adjust the volume of each to 5 ml. with water. Apply the same procedure to these as is given in the section dealing with the analysis of tablets.

Methods of Assay

(*a*) *Methylthiouracil.* One tablet of methylthiouracil containing 100 mg. of the active compound is crushed in 80 ml. of water and 0·8 ml. of concentrated ammonia solution (sp.gr. 0·880) added. The solution is stirred for 20 minutes, and the volume then made up to 1 l. with distilled water. The solution is then filtered to remove undissolved tablet base. For the assay this solution is diluted 1 in 4 with distilled water and 1, 2, 3, and 4 ml. taken. The volume of each of these containing 25, 50, 75 and 100 μg. of methylthiouracil respectively, is adjusted to 5 ml. with water. 5 ml. of the buffer-chloride solution, *p*H 8·0, and 0·1 ml. of the 0·4 per cent. chloroimide reagent are added to each. The solutions are well mixed and the colour reaction in each allowed to proceed for 45 minutes at room temperature. Then 10 ml. of chloroform is added to each and the tubes well shaken. Once all of the yellow colour in each has been extracted the chloroform extracts are allowed to settle, and the aqueous supernatant liquid in each removed by suction. The chloroform extracts are then filtered through small Whatman No. 42 filter-papers. The optical density in each is read in a Spekker absorptiometer, using a Spekker filter (violet, O.B.1). The concentration of methylthiouracil in each is determined by reference to a standard graph.

(*b*) *Propylthiouracil.* A tablet containing 25 mg. of the active compound is crushed in a beaker with a glass rod and 5 ml. of aldehyde-free absolute ethanol added. The solution is stirred well and allowed to stand for 20 minutes. Stirring is maintained during this period. The volume of the solution is then made up to 250 ml. with distilled water and filtered. Dilute this solution 1 in 4 with water and take 1, 2, 3 and 4 ml. of it. This gives a range of 25, 50, 75 and 100 μg. of propylthiouracil. Adjust the volume of each of these to 5 ml. with water and proceed as described under methylthiouracil. The colour is the same therefore the same absorptiometer filter is used as for methylthiouracil.

Results. The recovery values obtained in the analysis of tablets are as follows:—methylthiouracil, 100 mg.; recovered 86, 98, 96·2, 94·3 per cent.; propylthiouracil, 25 mg.; recovered 96, 100, 97·2, 96 per cent.

The colour reaction. In a suitably buffered solution, 2 : 6-dichloroquinone-chloroimide condenses with methylthiouracil[2] and propylthiouracil[3] with the formation of a yellow-coloured complex. The latter is removable from the reaction mixture by means of chloroform. The solvent extraction renders the colour reaction specific for thiouracils and mercaptoimidazoles.[5]

Preparation of the Thiouracil Complexes

(*a*) *Propylthiouracil.* 50 mg. of 6-*n*-propylthiouracil was dissolved in 10 ml. of aldehyde-free absolute ethanol and 100 ml. of the buffer-chloride mixture, *p*H 8·0, added. The mixture was cooled in ice and 20 ml. of a 0·4 per cent. solution of 2 : 6-dichloroquinone chloroimide in aldehyde-free absolute ethanol added slowly, and with constant stirring. The orange-red solution was then extracted with chloroform. The latter was then taken down to near dryness *in vacuo*. From this mixture the propylthiouracil–chloroimide complex was isolated in orange-red needles; m.pt. 172° C., with decomposition. These dissolved readily in chloroform to give intensely coloured yellow solutions.

(*b*) *Methylthiouracil.* A similar procedure applied to 4-methyl-2-thiouracil gave very concentrated solutions of the pigment, but in our hands, the pigment could not be crystallised from the reaction mixture.

As far as we are aware the products of the reactions above have not been previously isolated. Fearon[8] working on the 2 : 6-dichloroquinone chloroimide reaction for uric acid was unsuccessful in an attempt to isolate the coloured product, but his work was hindered by the insolubility of both the reactants.

Specificity of the colour reaction. This has already been reported in some detail by one of us (McAllister[2,3,5]). Here we have investigated the effect of magnesium stearate and lactose on the colour development with methylthiouracil and propylthiouracil and have found no interference. As regards the presence of magnesium stearate in tablets, it may be noted that in the initial dissolving of the thiouracils any stearate present is removed during the removal of insoluble tablet base by filtration.

Summary

1. A colorimetric method for the determination of methylthiouracil and propylthiouracil in tablet form is presented.

2. Recovery values obtained with the method are given.

3. The isolation of the coloured complex formed in the reaction between propylthiouracil and 2 : 6-dichloroquinone chloroimide is described.

References

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