A COLORIMETRIC METHOD FOR THE DETERMINATION OF 1-METHYL-2-MERCAPTOIMIDAZOLE

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Previous colorimetric methods for the determination of antithyroid compounds of the thiouracil type have been based upon the bluish-green colour which is given when compounds containing the C=S group react with Grote's reagent[1]. This colour reaction has been criticised by a number of workers (Williams *et al.*[2], Chesley[3], Christensen[4], and Olson *et al.*[5]) all of whom have stressed the instability of the colour reagent, which necessitates not only the frequent preparation of fresh reagent, but also the inclusion of standards with each set of unknowns. In view of this we have been interested in the development of a suitable colour reaction for compounds of the thiouracil type, which would be capable of adaptation on a quantitative basis.

The writer[6] has shown that in buffered solution at *p*H 8·0, thiouracil and its 4-methyl derivative condense with 2:6-dichloroquinone-chloroimide with the formation of yellow coloured complexes. These latter are readily removed from the aqueous phase at this *p*H by means of chloroform, a process which distinguishes them from the colourations given by a number of other compounds in the test, mainly some amino-acids, purines and the ureides of certain dicarboxylic acids. This colour reaction has been made the basis of a method for the determination of methylthiouracil in urine (McAllister[7]) and has also been applied, with certain modifications, to the determination of propylthiouracil in urine (McAllister[8]).

The recent interest in antithyroid compounds of the mercaptoimidazole type has prompted us to examine the behaviour of a number of these in the test. It has been found that the mercaptoimidazoles also give coloured products which can be readily removed from the reaction mixture by means of chloroform. The reaction of certain substituted mercaptoimidazoles in the colour reaction has recently been reported by the writer[9]. The present paper deals with this in more detail, and describes a method for the determination of 1-methyl-2-mercaptoimidazole.

The Colour Reaction

When small amounts of certain mercaptoimidazoles in a borate buffer, *p*H 8·0, are treated with an 0·4 per cent. solution of 2:6-dichloroquinone-chloroimide in aldehyde-free absolute ethanol, intense colourations are produced. These are readily removed from the reaction mixture by shaking with chloroform.

Test. An amount of the compound up to 1 mg. is taken, in 5 ml. of water, and mixed with 5 ml. of buffer-chloride solution, *p*H 8·0 (described

in the text). Then 0·1 ml. of an 0·4 per cent. solution of 2:6-dichloroquinone-chloroimide in aldehyde-free absolute ethanol is added. The colour reaction is allowed to develop for 20 minutes at room temperature. 5 ml. of chloroform is then added, and the mixture well shaken. The chloroform extracts are then allowed to settle, and examined. The colours given by various mercaptoimidazoles are shown in Table I.

TABLE I

CHLOROIMIDE REACTION FOR MERCAPTOIMIDAZOLES
*p*H 8·0

Compound (1 mg. for each test)	Colour Reaction
2-Mercaptoimidazole	Rapid ; deep red
4-Methyl-2-mercaptoimidazole	Rapid ; deep red
4-Amino-methyl-2-mercaptoimidazole	Rapid ; orange
1-Methyl-2-mercaptoimidazole	Rapid ; yellow.

Results. By the above technique, the various mercaptoimidazoles tested gave chloroform-soluble coloured complexes. Under similar conditions, 2-thiouracil, methylthiouracil and propylthiouracil, give yellow colours similar to that given by 1-methyl-2-mercaptoimidazole, but the reaction products of the former group are not so soluble in chloroform. Of a very large number of compounds tested, only thiourea reacted in the test to give an orange-violet colour, the violet component of which was removable from the reaction mixture by chloroform.

Specificity. Chloroform has been shown to be a selective solvent for the products of the reaction at *p*H 8·0, with 2-thiouracil, methylthiouracil and propylthiouracil, and certain mercaptoimidazoles (McAllister[6,7,8,9] and this paper).

Other compounds which react at this *p*H with the chloroimide reagent are some free amino acids, some water-soluble vitamins, some purines, some thio-compounds, and certain ureides of dicarboxylic acids. At *p*H 8·0, however, the reaction with other compounds is considerably slowed down, and with the exception of thiourea, none of the coloured products is removable from the reaction mixture by the solvent. The results obtained by applying the colour reaction to various compounds are given in Table II.

Mechanism. Fearon was unsuccessful in his attempt to isolate the yellow complex formed in the chloroimide reaction with uric acid, due to the low solubility of both the reactants. In this work we have been aided by the fact that the coloured products can be taken up in chloroform, and in work aimed at elucidating the mechanism of the colour reaction for the antithyroid compounds, we have been able to isolate the coloured product of the reaction with propylthiouracil in crystalline form. The necessary analysis and structural characteristics of this compound are being determined at present and will be reported on elsewhere. Later we hope to be able to isolate the product of the reaction with the 1-methyl-

2-mercaptoimidazole. Fearon[10] has shown that the colour reaction for thiourea is catalysed by copper, and that the yellow product formed in the chloroimide reaction for uric acid is turned red by the addition of

TABLE II

BEHAVIOUR OF VARIOUS COMPOUNDS IN THE TEST
*p*H 8·0

Compound (1 mg. of each)	Colour Reaction	Solubility of Coloured Product in Chloroform
Glucose	No reaction	—
Lactose	No reaction	—
Fructose	No reaction	—
Barbituric acid	Rapid violet	Insoluble
Barbitones	No reaction	—
Sulphonamides	No reaction	—
Ascorbic acid	No reaction	—
Pantothenic acid	No reaction	—
Pyridoxin	Blue, decomposes	Insoluble
Riboflavine	No reaction	—
Aneurine	Yellow, turns brown	Insoluble
Thiourea	Orange violet	Violet component soluble
Acacia	No reaction	—

silver salts. In this work I have not observed any catalysis of the reaction by metals, nor any silver-binding NH group effect as noted by Fearon. *p*H is a critical factor. At *p*H 10, the reagent tends to undergo a spontaneous decomposition with the formation of a reddish mixture of pigments. Gibbs[11,12], in work on the reaction of phenols with the reagent, noted that this decomposition was accelerated by exposure to light, and Fearon noted that the inclusion of 10 per cent. of sodium chloride in the buffer mixture repressed this. In this work, due to the lower *p*H value used, I have not encountered any reagent decomposition during the test, but have also included chloride in the buffer.

QUANTITATIVE ASPECTS

These have been examined with the 1-methyl-2-mercaptoimidazole. When the colour reaction is applied to 10 to 100μg. the colour system obeys Beer's law both in the aqueous and chloroform phases. In actual practice, the colour is extracted from the reaction mixture with chloroform in order to eliminate possible interference from other compounds. In such instances, the colour is measured in a Spekker absorptiometer, readings being taken against pure chloroform, and using a Spekker violet filter. Reagent blanks give negligible readings and the graphs obtained by plotting concentrations against absorptiometer readings are reproducible. By absorptiometer measurements, the yellow colour has been

found to be stable for at least 40 minutes in the aqueous phase and for the same time in chloroform. For 1-methyl-2-mercaptoimidazole, the sensitivity of the colour reaction is about 10 μg.

DETERMINATION OF 1-METHYL-2-MERCAPTOIMIDAZOLE

Reagents. 1. 0·4 *per cent. 2 : 6-Dichloroquinone-chloroimide.*—A 0.4 per cent. solution in aldehyde-free absolute ethanol. Stored in a brown bottle, the reagent will keep for about 4 weeks.

2. *Buffer-Chloride Solution, pH 8·0.*—To 50 ml. of a 0·2 M solution of boric acid in 0·2 M potassium chloride add 4 ml. of 0·2 N sodium hydroxide, followed by 100 ml. of a 20 per cent. aqueous solution of sodium chloride. Since the latter depresses the *p*H, the *p*H of the solution should then be adjusted to 8·0 by the addition of 0·1 N sodium hydroxide.

3. *Standard Solution of 1-Methyl-2-Mercaptoimidazole.* 20 mg. of the pure compound is dissolved in distilled water and the volume of the solution made to 200 ml. This solution slowly deteriorates on standing and should be prepared fresh. For the preparation of standard reference graphs, the above standard is suitably diluted, and amounts equivalent to 10, 20, 40, 50, 80, and 100 μg. taken. The volume of each of these is then adjusted to 5 ml. with distilled water, and treated in exactly the same manner as the unknowns; described below.

4. *Chloroform B.P.*

All chemicals used should be of A.R. standard and all-glass distilled water used throughout.

Procedure. The material to be analysed is dissolved in water, and if acid in reaction should be neutralised before analysis. The solution is then made up to a suitable volume and an amount containing approximately 50 μg. in 5 ml. taken. 5 ml. of the buffer-chloride solution, *p*H 8·0, is then added followed by 0·1 ml. of the 0·4 per cent. chloroimide reagent. After mixing, the solution is allowed to stand at room temperature for 20 minutes. 10 ml. of chloroform is then added, and the mixture shaken until all the yellow colour passes into the chloroform. The latter is allowed to settle and the aqueous supernatant liquid removed by suction. The chloroform extract is filtered through a small No. 42 Whatman filter paper, and read in a Spekker absorptiometer against chloroform, and using a Spekker violet filter. The amount of the compound present is then determined by reference to a standard graph.

Results. The results shown in Table III are typical of those obtained using the method. The material was tablets containing 2 mg. of 1-methyl-2-mercaptoimidazole in each. For each analysis one of the tablets was dissolved in water and the solution made to 100 ml. After filtering, 2·5 and 1 ml. of the filtrate were taken and analysed. These amounts corresponded to 50 and 20 μg. of the compound respectively.

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TABLE III

ANALYSIS OF PROPRIETARY TABLETS

Amount Present mg.	Amount Found mg.	Recovery per cent.
2·0	1·986	99·3
	1·984	99·2
2·0	2·000	100·0
	1·986	99·3
2·0	1·974	98·7
	1·985	99·25

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