A METHOD FOR THE DETERMINATION OF 2-CARBETHOXYTHIO-1-METHYLGLYOXALINE

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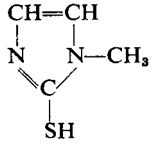
PREVIOUS papers[1,2] described the estimation of the antithyroid substance 2-mercapto-1-methylglyoxaline (I) by means of the chloroimide reaction, as well as the detection of various 2-mercaptoglyoxalines with the iodobismuthous acid test. In the former procedure, small amounts of the compound in a buffer solution (*p*H 8·0) are treated with 2:6-dichloroquinone-chloroimide, which results in the formation of yellow condensation products. At this *p*H the reaction product is extractable with chloroform, a property which makes the reaction specific for 2-mercaptoglyoxalines as well as thiouracils[3].

We have been examining recently the possibility of applying these methods to the estimation of 2-carbethoxythio-1-methylglyoxaline (II) in tissues and body fluids, and the present communication is part of this work. The 2-carbethoxythio-derivative is an antithyroid substance with a prolonged action which was introduced by Lawson, Rimington and Searle[4].



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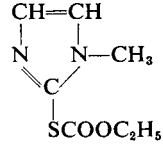


(II)

PROCEDURE

Reagents: (1) Solution of 2:6-dichloroquinone-chloroimide 0·4 per cent. in aldehyde-free absolute ethanol. The solution will keep for about 4 weeks if stored in a dark bottle.

(2) N Hydrochloric acid.

(3) N Sodium hydroxide.

(4) Standard solution of 2-carbethoxythio-l-methylglyoxaline. Dissolve 100 mg. of the pure compound in about 190 ml. of distilled water and make to 200 ml. The solution keeps indefinitely.

(5) Standard solution of 2-mercapto-l-methylglyoxaline. Dissolve 100 mg. of the pure compound in 100 ml. of water. The solution keeps indefinitely.

(6) 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, and 100 ml. of a 20 per cent. aqueous solution of sodium chloride. Make to 200 ml. with distilled water. Adjust *p*H to 8·0 with 0·1 N sodium hydroxide.

The analytical procedure involves the alkaline hydrolysis of 2-carbethoxythio-1-methylglyoxaline to the 2-mercapto-1-methylglyoxaline. For photometric measurements, the calibration curve is prepared using the 2-mercapto-1-methylglyoxaline as follows.

Preparation of Calibration Curve: The standard solution of 2-mercapto-1-methylglyoxaline is suitably diluted and amounts equivalent to 20, 40, 60, 80 and 100 μg. taken. The volume in each is adjusted to 5 ml. with distilled water, and 5 ml. of the buffer added to each. A blank consisting of 5 ml. of water is treated likewise. To the contents of each tube is then added 0·1 ml. of the chloroimide reagent from a dry pipette, and the tubes mixed by inversion. Colours are allowed to develop at room temperature for 20 minutes. 10 ml. of chloroform is then added to each, and the tubes shaken until all of the yellow colour passes into the chloroform phase. The contents are then allowed to settle, and the aqueous supernatant liquid in each removed by suction. The chloroformic solutions are then filtered through small, Whatman No. 42 filter papers to remove traces of water. The density of each is then determined in a Spekker absorptiometer, readings being taken against the blank using Chance glass filters (OBI). The relationship between optical density and concentration is linear.

Results Obtained with 2-Carbethoxythio-1-Methylglyoxaline

When 100 μg. amounts of the 2-carbethoxythio-derivative were subjected to the above procedure, the colours obtained were equivalent to about 10 μg. of 2-mercapto-1-methylglyoxaline. This was probably due to mild alkaline hydrolysis taking place during the development of the colour. Acid hydrolysis of the 2-carbethoxythio- derivative was then examined as follows.

TABLE I

Alkaline hydrolysis of 2-carbethoxythio-1-methylglyoxaline (100 μg.) Temperature 37° C. Strength of alkali 0·25 N sodium hydroxide

Time of incubation at 37° C. minutes	2-mercapto-1-methylglyoxaline found μg
Control (not heated)	35·6
10	58·0
15	61·0
20	62·3
30	60·0
45	60·0
60	61·5

To a series of tubes containing 100 μg. of the compound in a volume of 3 ml. of water was added 1 ml. of N hydrochloric acid. The tubes were then stoppered and placed in an incubator at 37° C. for various periods. After incubation, the tubes were cooled, and 1 ml. of N sodium hydroxide added to neutralise the acid. 5 ml. of buffer was then added and the colours developed as described previously. Acid hydrolysis in this manner was found to proceed irregularly as determined by the amounts of the 2-mercapto-1-methylglyoxaline obtained. Approximately 20 minutes incubation in 0·25 N acid was needed to give the theoretical amount (61·8 μg.) of 2-mercapto-1-methylglyoxaline from 100 μg. of 2-carbethoxythio-1-methylglyoxaline, but the process was not reproducible, and even gave different values in duplicate series. We have been unable to find a reason for this.

Alkaline hydrolysis was then carried out in exactly the same manner, 1 ml. of N sodium hydroxide being of course used in place of 1 ml. of N acid. After incubation the mixtures were neutralised and colour development carried out as previously. A control was carried through with each set of tubes. This consisted of 100 μg. of the 2-carbethoxythio-derivative in a volume of 4 ml. containing 1 ml. of N sodium hydroxide. Immediately after the addition of alkali 1 ml. of N hydrochloric acid was added and the colour developed as before. Results are shown in Table I.

As will be seen from the above results, alkaline hydrolysis with 0·25 N sodium hydroxide at 37° C. proceeds smoothly. Since 100 μg. of 2-carbethoxythio-1-methylglyoxaline are equivalent to 61·8 μg. of 2-mercapto-1-methylglyoxaline, the values obtained are well within the experimental error of the method. A series of 2-mercapto-1-methylglyoxaline standards were then prepared each in a volume of 3 ml. water. 1 ml. of N sodium hydroxide was added to each, and the tubes incubated at 37° C. for 25 minutes. After cooling, the alkali was neutralised with 1 ml. of N hydrochloric acid, and the colours developed as described previously. The optical densities of these were then compared against an untreated series with the results shown in Table II. As will be seen the compound was stable to incubation at 37° C. in 0·25 N sodium hydroxide. These results were found to be reproducible, and agreed well in duplicate series.

TABLE II

STABILITY OF 2-MERCAPTO-1-METHYLGLYOXALINE TO 0·25 N ALKALI AT 37° C.

Amount μg.	Optical density in chloroform	
	Untreated	Incubated in 0·25 N sodium hydroxide for 25 minutes at 37° C.
20	0·110	0·105
40	0·240	0·240
60	0·320	0·330
80	0·450	0·430

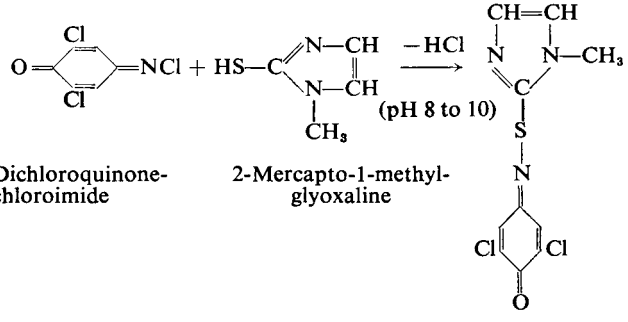
COLORIMETRIC DETERMINATION OF 2-CARBETHOXYTHIO-1-METHYLGLYOXALINE

The material should be dissolved in distilled water. This is diluted further to give a concentration of about 50 μg. per ml. 2 ml. of this solution is placed in a glass-stoppered test tube, and 1 ml. of water and 1 ml. of N sodium hydroxide added. The tube is stoppered, shaken gently, and placed in a beaker of water maintained at 37° C. in an incubator, for 15 or 20 minutes. The tube is cooled, and 1 ml. of N hydrochloric acid added followed by 5 ml. of the buffer (*p*H 8·0). 0·1 ml. of the chloroimide reagent is added and the contents of the tube mixed by inversion, and allowed to stand at room temperature for 20 minutes. After this time, 10 ml. of chloroform is added, and the tube shaken until the yellow colour is extracted by the chloroform. After settling, the aqueous layer is removed, and the chloroform extract filtered through a small No. 42 Whatman filter paper. The optical density is determined in a Spekker absorptiometer, readings being taken against a similarly treated blank solution and using Chance glass filters (OBI). Results in terms of 2-mercapto-1-methylglyoxaline are interpolated from a standard curve, which is prepared as described previously. Amount of

the 2-carbethoxythio-1-methyglyoxaline present initially is obtained by multiplying μg. of 2-mercapto-1-methylglyoxaline found by 1·61.

NOTE ON THE MECHANISM OF THE CHLOROIMIDE REACTION

In a previous paper[1] it was indicated that the thiol group is the main essential for colour formation in the chloroimide reaction for the 2-mercaptoglyoxalines, and this is confirmed by the relatively negative finding with 2-carbethoxythio-1-methylglyoxaline reported here. We have tentatively ascribed the reaction to be due to a condensation between the chloroimide grouping in the quinone and the thiol hydrogen in position 2 in the glyoxaline ring, with the elimination of hydrogen chloride. This condensation is in accordance with that formulated for the phenols by Gibbs[5].



2:6-Dichloroquinone-chloroimide

2-Mercapto-1-methyl-glyoxaline

(yellow)

THE IODOBISMUTHOUS ACID REACTION

The iodobismuthous acid test for certain 2-mercaptoglyoxalines described in a previous paper[2] gives a negative reaction with 2-carbethoxy-thio-1-methylglyoxaline, presumably due to the absence of the thiol grouping. If however a few mg. of the 2-carbethoxythio-derivative is heated in 1 ml. of N hydrochloric acid for a few mintues over a small flame, then cooled, the characteristic deep red precipitate is formed when the solution is treated with the iodobismuthous acid reagent.

This work is part of a study being carried out at the request of the British Schering Ltd., to whom thanks are due for a gift of pure 2-carbethoxythio-1-methylglyoxaline.

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