

A COLOUR REACTION FOR CERTAIN MERCAPTOIMIDAZOLES USING IODOBISMUTHOUS ACID

By RONALD A. MCALLISTER

From the Biochemical Laboratory, Royal Samaritan Hospital, Glasgow

Received November 26, 1951

THE present writer^{1,2} has already described the colorimetric determination of 1-methyl-2-mercaptoimidazole using 2 : 6-dichloroquinone chloroimide. The reaction has also been applied to the determination of propylthiouracil in urine (McAllister³), and to the determination of these compounds in tablet form (McAllister and Howells⁴). Further work aimed at investigating the binding of mercaptoimidazoles on plasma proteins prompted the need for a confirmatory colour reaction for 1-methyl-2-mercaptoimidazole, other than the chloroimide reaction mentioned above. For this purpose the reactions of this compound with metals were examined. As would be expected, mercuric salts precipitated the mercaptoimidazole from aqueous solution. In common with certain other organic thio-compounds, e.g., thiourea and dimercaptothiodiazole, the 1-methyl-2-mercaptoimidazole was found to react with bismuth salts with the formation of yellow complexes of the metal. Of the bismuth salts examined, bismuth sulphate (acid) was the only one found to give a stable colour, but this colour reaction, as well as being non-specific, was also found to be insufficiently sensitive to be used on an analytical basis. It was found, however, that when an excess of iodide ions was present in the reaction mixture, an intense red colour was produced and red needles crystallised out. This colour reaction would appear to be due to the formation of a complex iodide. Since slightly acid solutions of bismuth salts react with iodides, with the formation of yellow iodo-bismuthous acid, the latter would appear to be the active colour reagent. The reaction forms the basis of the colour test for certain mercaptoimidazoles reported here.

One interesting outcome of this work has been that no other metallic or acid radical in place of iodide has been found to simulate the colour reaction. This has resulted in the use of a bismuth mercaptoimidazole compound as a specific colour reagent for iodides, and this has been reported in detail elsewhere (McAllister⁵).

Reagents. (1) Freshly prepared 10 per cent. solution of potassium iodide, Analar. (2) Bismuth sulphate (acid).

To prepare the reagent, which must be made up fresh, add 50 mg. of bismuth sulphate to 5 ml. of the 10 per cent. aqueous solution of potassium iodide. Mix well, then add 1 ml. of N sulphuric acid. Set aside for a few minutes to allow the undissolved bismuth salt to settle. Use the clear supernatant liquid for the test.

Test. Place a small crystal of the drug, or if in tablet form use one tablet, in the depression of a spot test plate. Add 1 drop of the reagent; the crystal immediately turns a scarlet colour, which upon standing or

RONALD A. McALLISTER

on agitation, forms a red microcrystalline precipitate, if the 1-methyl-2-mercaptoimidazole or other derivative shown in Table I is present. If the test is applied to the compound in tablet form, the addition of a further drop of the reagent results in the coloured complex diffusing out to form a red ring round the tablet.

The addition of an excess of iodide ions to an acid solution of a bismuth salt results in the formation of yellow iodobismuthous acid. When this solution is added to a mercaptoimidazole of the type shown, an insoluble red complex iodide is produced.

The coloured complex is practically insoluble in water, and dilute acids. Reducing agents, e.g., hydrazine sulphate, have no effect, but oxidising agents rapidly destroy it with the liberation of iodine. As would be expected, alkalis destroy it giving a colourless solution, probably due to the labile nature of the sulphhydryl group under such conditions. When in suspension in aqueous solutions, the red complex exhibits a slight red fluorescence, which is quenched when the mixture is extracted with an organic solvent such as ethyl acetate, the resulting solution having an orange-yellow colour. The data presented in Table I show the results

TABLE I
THE IODOBISMUTHOUS ACID REACTION FOR MERCAPTOIMIDAZOLES

Compound	Colour reaction
1-Methyl-2-mercaptoimidazole ..	Scarlet: forms red needles
4-Methyl-2-mercaptoimidazole ..	Orange: not very sensitive
2-Mercaptoimidazole	Red: sensitivity about the same as for the 4-methyl derivative
4-Amino-methyl-2-mercaptoimidazole	No reaction

Sensitivity of the test for the 1-methyl derivative is in the region of 100 μ g.

obtained when the reaction was applied to various synthetic mercaptoimidazoles. The following compounds gave no colour reactions:— 2-thiouracil, 4-methyl-2-thiouracil, propylthiouracil, thiourea, 1-cystine, *p*-ethylsulphonylbenzaldehyde thiosemicarbazone, *p*-acetylaminobenzaldehyde thiosemicarbazone, lactose, lactates, and sulphonamides. It will be seen that the colour test shows a high degree of specificity. In connection with the reaction with various mercaptoimidazoles, it is of interest to note that the colour response was best with the 1-methyl-2-mercaptoimidazole showing a varying response to the others, and none with the 4-amino-methyl derivative. Although insufficient data are presented, further work in this connection might give sufficient information for the test to be used for orientation purposes.

SUMMARY

A colour reaction for certain mercaptoimidazoles is described. This is based upon the red coloured complex iodide which is produced, when a slightly acid solution of iodobismuthous acid is added to their solutions.

A COLOUR REACTION FOR MERCAPTOIMIDAZOLES

REFERENCES

1. McAllister, *Nature, Lond.*, 1951, **167**, 863.
2. McAllister, *J. Pharm. Pharmacol.*, 1951, **3**, 506.
3. McAllister, *J. Clin. Path.*, 1951, **4**, 432.
4. McAllister and Howells, *J. Pharm. Pharmacol.*, 1952, **4**, 259.
5. McAllister, *Nature, Lond.*, in the press.