

### Colour Reaction for Certain Mercaptoimidazoles

IN a recent communication<sup>1</sup>, I described a new colour reaction for the antithyroid drugs, 2-thiouracil and 4-methyl-2-thiouracil. This is based upon the yellow colour which is given when small concentrations of the drugs are treated with 2:6-dichloroquinone-chloroimide at pH 8.0. Although some free amino-acids, purines and other urinary solutes give coloured products in the reaction, these latter are insoluble in chloroform, whereas within certain concentration limits the coloured products of the reaction with the drugs are readily extractable from the aqueous phase by this solvent. By this means the colour reaction has been made specific, and has been applied to the colorimetric determination of methylthiouracil in urine.

Recently, Stanley and Astwood<sup>2</sup> and others have described the clinical use of antithyroid compounds of the mercaptoimidazole type, which have been shown to have a much higher *in vivo* activity than the thiouracils. In view of this we have been interested in the behaviour of certain mercaptoimidazoles in the above colour reaction, as a potential method for their determination.

Compounds of this type examined were 2-mercaptoimidazole, 4-methyl-2-mercaptoimidazole, and 4-amino-methyl-2-mercaptoimidazole (hydrochloride). These were dissolved in distilled water, and the pH of their solutions brought to 8.0 by the cautious addition of decinormal sodium hydroxide. After suitable dilution, aliquots of the solutions were taken and mixed with 2 ml. of a borate buffer, pH 8.0, and 0.1 ml. of a 0.4 per cent solution of 2:6-dichloroquinone-chloroimide in aldehyde-free absolute ethanol. Colour formation in each case was very rapid. Once the solutions had stood for 10 min. at room temperature, the solubility of the coloured complexes in chloroform was determined by shaking the solutions with 5 ml. of this solvent (see table). The amount of compound tested in each case was 0.5 mgm.

Compound	Colour reaction	Solubility in chloroform
2-Mercaptoimidazole	Rapid; deep red	Soluble
4-Methyl-2-mercaptoimidazole	Rapid; deep red	Soluble
4-Amino-methyl-2-mercaptoimidazole	Rapid; orange	Soluble

The sensitivity of the colour reaction for each of the compounds tested was in the region of 10 µgm. Of the three compounds tested, the 2-mercaptoimidazole and the 4-methyl-2-mercaptoimidazole gave the best results as based upon the stability of their coloured products. The 4-amino-methyl-2-mercaptoimidazole gave an orange-coloured product which tended to decompose. Imidazole-4,5-dicarboxylic acid gave no colour in the reaction.

In spite of the preliminary nature of this work, it would appear that other compounds of the mercaptoimidazole type would behave analogously in the reaction, since the introduction of a methyl group at position 4 in the 2-mercaptoimidazole made no appreciable difference in the colour reaction. Since chloroform exhibits a selective solvent action in this instance, as it does in the case of the thiouracil reaction, it is believed that the colour reaction for the mercaptoimidazoles could be made the basis of a useful method for their determination. Whether

this would be applicable to the determination of the compounds in urine is, of course, based upon whether these are excreted as such. A more detailed description of this work will be published elsewhere.

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<sup>1</sup> McAllister, R. A., *Nature*, **166**, 789 (1950).

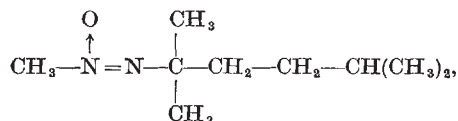
<sup>2</sup> Stanley, M. M., and Astwood, E. B., *Endocrin.*, **44**, 588 (1949).

### Purely Aliphatic Azoxy Compounds with a Methyl Group attached to the Nitrogen Atom

ALIPHATIC azoxy compounds were first synthesized by one of us in 1931<sup>1,2</sup>, but all contained only tertiary alkyl groups attached to nitrogen.

In view of the work being carried out at Cambridge by Lythgoe and collaborators<sup>3</sup> on the natural product, macrozamin, which apparently contains an azoxy group with a methyl group directly attached, it seems worth while to make available our results on the synthesis of an azoxy compound containing such a group.

We have synthesized methyl-2-azoxy-2,5-dimethylhexane,



by the condensation of N-methylhydroxylamine with 2-nitroso-2,5-dimethylhexane by a method similar to that reported by one of us<sup>4</sup>.

A suspension was made of 8.0 gm. (0.145 mole) of powdered potassium hydroxide in 100 ml. of dry ether, and to this was added with stirring over a 20-min. period 9.3 gm. (0.112 mole) of N-methylhydroxylamine hydrochloride. The ether was removed with an aspirator at 60 mm. pressure to leave a slightly yellow, curdy solid. To this was added at once 16.0 gm. (0.112 mole) of *bis*-2-nitroso-2,5-dimethylhexane. The mixture was heated to the melting point of the nitroso compound, whereupon a vigorous reaction ensued which required rapid cooling. When the reaction had subsided, the mixture was heated to 85° for 1 hr., and then to 110° for an additional 2 hr. After cooling, 40 ml. of water and 50 ml. of ether were added and the layers separated. The aqueous layer was extracted again with 25 ml. of ether. The combined ether extract was dried over sodium sulphate. After distilling the ether, the residue was distilled at 35 mm. pressure and gave the fractions shown in the accompanying table.

Fraction	Vapour temp. (°C.)	Weight of fraction	Colour of distillate
1	32°-91°	0.3 gm.	Light blue
2	91°-103°	2.3 gm.	Very light blue
3	103°-105°	8.7 gm.	Light blue tint