# LETTERS TO THE EDITORS

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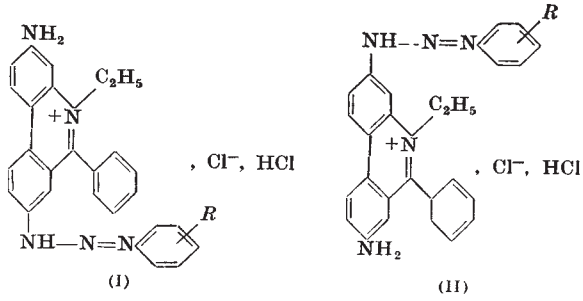
## Metamidium: a New Trypanocidal Drug

THIS communication describes a new trypanocide, which has been found to possess considerable potentiality as a curative and as a prophylactic drug, both in laboratory experiments and in field trials carried out in Africa during the past year.

In following up the long-standing interest in these laboratories in the trypanocidal activity of both phenanthridinium salts[1] and the aromatic diamidines[2], we decided to prepare the *p*-amidinophenyldiazoamino-derivative[3] of 2 : 7-diamino-10-ethyl-9-phenylphenanthridinium chloride (homidium chloride). The diazoamino linking group was selected because of the structural relationship our product would then bear to the recently introduced curative trypanocidal drug 'Berenil', *p*-amidinophenyldiazoamino-*p*-benzamidine[4].

The coupling reaction between diazotized *p*-aminobenzamidine (1 mol.) and homidium chloride in the presence of sodium acetate (7 mol.) at a temperature of 0–5° C. yields a mixture of two isomeric *p*-amidinophenyldiazoamino-derivatives, which can be separated by precipitation from water with sodium chloride, followed by fractional crystallizations from water and from methanol. The relatively water-insoluble isomer, m.p. 287–289° (dec.), was purple and the other, m.p. 236–240° (dec.), red. Both isomers were self-indicating on paper electrophoresis in 3 *N* acetic acid, the purple isomer being distinctly more mobile than the red; this provided an invaluable means of following the progress of separation procedures.

Since in 2 : 7-diamino-10-methyl-9-phenylphenanthridinium chloride (dimidium chloride) it is the




7-amino-group which reacts preferentially on acetylation[5], we provisionally assigned the structure (I; $R$ = *p*-amidino) to the predominating purple isomer and (II; $R$ = *p*-amidino) to the red isomer. Work to provide unambiguous proof of structure continues. A diazoamino structure was expected from the method of preparation and the expected microanalytical figures were obtained for both isomers. Infra-red spectra ruled out the remote possibility that the isomers were due to the existence of two stable tautomeric forms of the diazoamino-group in one or other of the structures (I) or (II). The presence of a diazoamino-group in each isomer was confirmed by the rapid evolution of nitrogen on boiling in 6 *N* sulphuric acid—the red isomer yielded nearly the theoretical volume of nitrogen, while the purple regularly yielded only about 65 per cent (the reason for this deficiency is at present unexplained).

The very promising results obtained on testing the isomers (I and II; $R$ = *p*-amidino) as trypanocides led us to study extensively structure/activity relationships among similar pairs of isomers, particularly those in which the substituent $R$ and the group in the phenanthridine-10 position were varied. As a result, we found that the most promising product was a mixture of the two isomers (I and II; $R$ = *m*-amidino)[6], first designated M & B 4404, and later

Table 1. TOXICITY AND THERAPEUTIC ACITIVITY AGAINST *T. congolense* IN MICE

| Compound having structure I or II | Substituent $R$ in structures I or II | *LD*50 (mgm./gm.) subcutaneous | *CD*50 (mgm./gm.) subcutaneous | | |
|---|---|---|---|---|---|
| | | | Strain of *T. congolense* | | |
| | | | Laboratory (*a*) | Kenya (*b*) | Sakwa XXIV (*c*) |
| Purple isomer | *p*-amidino | 1·0 | 0·002 | | |
| Red isomer | ,, | 0·30 | 0·00075 | | |
| Purple isomer | *m*-amidino | 0·43 | 0·00003 | | |
| Red isomer | ,, | 0·26 | 0·000002 | | |
| Compound | | | | | |
| Metamidium chloride hydrochloride | | 0·23 | 0·000008 | 0·003 | 0·00004 |
| Metamidium suramin salt | | >5·0 | 0·000027 | | |
| Homidium chloride | | 0·07 | 0·00003 | Not curative at 0·01 | 0·0015 |

(*a*) Isolated in Tanganyika in 1933. (*b*) Isolated in Kenya in June 1956. (*c*) Isolated at Sakwa (Kenya) in December 1956.

Table 2. PROPHYLACTIC ACTIVITY AGAINST THE LABORATORY STRAIN OF *T. congolense*

| Compound having structure I or II ($R$ = *m*-amidino) | Dose (mgm./gm.) subcutaneous | Number of mice protected out of number challenged | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2 | 3 | 4 | 8 | 9 | 12 | 16 | 20 | 26 |
| | | (weeks after dosing) | | | | | | | | |
| Purple isomer | 0·025 | | 5/17 | 1/8 | 0/11 | | 8/8 | 5/7 | | |
| Red isomer | 0·025 | | 11/11 | | 6/6 | | | | | |
| Compound | | | | | | | | | | |
| Metamidium chloride hydrochloride | 0·025 | | | 12/12 | 12/12 | | 6/6 | 10/11 | 6/10 | 0/5 |
| | 0·005 | | | 4/4 | 6/6 | 5/6 | | | | |
| | 0·001 | 6/6 | | 5/6 | | 0/3 | | | | |
| Homidium chloride | 0·025 | 1/10 | | | | | | | | |
| Berenil | 0·06 | 0/10 | | | | | | | | |

assigned the common name metamidium chloride hydrochloride.

In metamidium the two isomers are present in the same relative proportions (approximately 55 per cent purple isomer and 45 per cent red isomer; Campbell, H., and Muggleton, D. F., private communication) as are formed in the coupling reaction between diazotized *m*-amino-benzamidine and homidium chloride. Both isomers have been separated by methods similar to those described above for the *p*-amidino series, to give a purple isomer, m.p. 258–260° (dec.), and a red isomer, m.p. 244–245° (dec.), both in the form of their chloride hydrochloride salts. Metamidium chloride hydrochloride is 12·5 per cent w/v soluble in water at 25°, the *p*H of a 1 per cent solution being 6·65.

The suramin salt[7] of metamidium, designated M & B 4427, was precipitated and dried *in vacuo* at room temperature to give a dark purple powder, which was less than 20 μgm./ml. soluble in water at 20° C. For biological evaluation this solid was presented as a 10 per cent w/v ball-milled aqueous suspension.

The foregoing compounds were examined for activity against a laboratory strain of *Trypanosoma congolense* which gave an acute infection in mice. A single subcutaneous injection was administered to mice with an infection in the peripheral bloodstream of from 1–10 trypanosomes per high-power field. Ten mice were used for each dose, and five untreated controls which died within 5–7 days were included in each experiment. Homidium chloride was used as a standard.

Wet blood smears were examined three times a week for four weeks, and the number of animals clear of trypanosomes for that period (that is, cured) was noted for each dose. The *CD*50 (the dose which cured 50 per cent of the mice) was then obtained graphically for each compound using de Beer's method[8]. The subcutaneous *LD*50 for each compound was also obtained in mice.

The results of these therapeutic and toxicity experiments are given in Table 1, together with the results obtained with metamidium and homidium against two more recently isolated strains of *T. congolense*, which we obtained through the courtesy of the East Africa Trypanosomiasis Research Organization, Tororo, and the Veterinary Research Laboratory, Kabete. These experiments were carried out less than twelve months after the strains were isolated.

These results show that metamidium chloride hydrochloride was more active than homidium chloride against all three strains of *T. congolense*.

Prophylactic experiments in mice were also carried out with some of the compounds. In these experiments a single subcutaneous injection of the substance was given to a large batch of mice and groups of them were challenged intraperitoneally with approximately 65,000 *T. congolense* (laboratory strain) per mouse at various times after dosing. No mouse was challenged more than once.

The mice were examined for trypanosomes for four weeks after challenge, as in the therapeutic experiments, and those which remained clear of trypanosomes for this period were considered to have been protected. Untreated controls were infected at each challenge and these became positive for trypanosomes within five days.

The results of these experiments are given in Table 2.

Metamidium chloride hydrochloride completely protected mice for up to sixteen weeks at about one-ninth of the *LD*50. This was an interesting result because neither the parent compound, homidium chloride, nor the drug 'Berenil', with which metamidium shares some structural similarities, has any appreciable prophylactic activity at one-third of the *LD*50.

Of the two isomers of metamidium, the red was more active than the purple both therapeutically and prophylactically, but there was little difference in their toxicities.

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W. R. Wragg
K. Washbourn
K. N. Brown
J. Hill

Research Laboratories,
May and Baker, Ltd.,
Dagenham, Essex.
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[4] British Patent 728,457/1955; Milne, A. H., Robson, J., and Lwebandiza, T., *Vet. Rec.*, **62**, 280 (1955). Jensch, H., *Arzneimitt l Forsch.*, **5**, 634 (1955).
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[6] British Provisional Patent Application No. 21814/56.
[7] cf. Williamson, J., and Desowitz, R. S., *Nature*, **177**, 1074 (1956).
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## A Rate-Governing Reaction of Protein Synthesis

Rabbit reticulocytes synthesize hæmoglobin *in vitro*. In order to maintain a high rate for at least four hours there needs to be added to the saline solution in which the cells are suspended a mixture of amino-acids, glucose, iron and an iron-chelating agent. The rate then (in four hours) is about five times that in saline alone. Glucose, iron and chelating agent have little effect on the rate unless the amino-acid mixture is added with them[1].

When in a reaction mixture complete except for one amino-acid, and that amino-acid is added in the carbon-14-labelled form and in varying concentration, the rate of its incorporation was found to vary as a first approximation with the concentration according to the equation $\frac{v}{V} = \frac{[A]}{[A] + K}$, where $v$ is the observed rate at any given concentration, $V$ is the maximum rate, $[A]$ is the concentration of the labelled amino-acid added to the reaction mixture and $K$ is constant, which is formally identical with $K_m$ in the Michaelis–Menten equation, that is to say, it is the equilibrium constant for the combination of substrate with enzyme. In the present instance $K = \frac{[B]\,[A]}{[BA]}$, where $B$ is a substance in the cells with which $[A]$ combines, and the rate of incorporation (fraction of the maximum) is proportional to $\frac{[BA]}{[B][BA]}$. The term $[BA]$ governs the rate of protein synthesis.