

ethylene-diimine bis-salicylaldehyde, *o*-phenylene bis-dimethylarsine have been found to have room temperature magnetic moments 2.10–2.72 B.M.¹¹, it is known that the Co(II) planar complexes have not been well characterised. A few papers dealing with spectral studies of planar Co(II) complexes have been published¹². As the spectra at $\sim 8300\text{ cm}^{-1}$ was not taken, no definite conclusion could be drawn about Co(II) complex of *m*-TAS.

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STUDIES ON THE COPPER COMPLEX OF ISONICOTINIC ACID HYDRAZIDE— A NEW ANTIVIRAL DRUG

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ABSTRACT

Detailed studies have been carried out to elucidate the precise nature of the complex formed between copper and isoniazid as this complex is now shown to be active against animal viruses. IR and ESR data provide definite proof for the existence of both cupric and cuprous complexes with isoniazid. A possible structure of cuprous-isoniazid complex is proposed.

1. INTRODUCTION

ISONICOTINIC acid hydrazide (isoniazid) is one of the potent drugs used in the treatment of tuberculosis. The bactericidal action of isoniazid is well understood¹. Cupric ions enhance the activity of isoniazid *in vitro* against *Mycobacterium tuberculosis*²⁻⁴. Cupric ions form 1:1 (monodentate) and 1:2 (bidentate) complexes (chelates) with isoniazid⁵⁻⁷. Rieber and Bemski⁷ reported that under aerobic conditions, not more than 20% of Cu²⁺ is reduced during the complex formation with isoniazid, whereas the report of Krivis and Rabb⁸ showed that approximately 92% of the Cu²⁺, which is precipitated as copper-isoniazid complex, exists in cuprous form.

In the light of the above conflicting reports, the present study was undertaken to determine the precise nature of the copper-isoniazid complex, as

this drug is a strong inhibitor of the *in vitro* reverse transcriptase activity of Rous sarcoma virus and is now shown to be active against both DNA and RNA animal viruses⁹⁻¹⁰. In this report, we present data to show the formation of two different complexes between copper and isoniazid, depending upon the oxidation state of the metal ion.

2. MATERIALS AND METHODS

Isoniazid (Sigma Chemical Co., USA), vanillin (B.D.H.) and other reagents used were of analytical grade.

2.1. Preparation of the Copper-Isoniazid Complexes

Cupric and cuprous complexes were prepared separately by mixing equal volumes of the equimolar solutions^{7,8}. For cuprous-isoniazid complex, freshly prepared cuprous chloride was used. The precipitate obtained in each case was

washed extensively with distilled water, dried and stored at 4° C.

2.2. Paper Chromatography

Ascending paper chromatography was done using Whatman No. 1 paper with isopropanol : glacial acetic acid : water (12 : 3 : 5; v/v/v) as the solvent system.

2.3. Estimation of Isoniazid

Isoniazid was estimated by the method of Kelly and Poet¹¹. The yellow colour, developed by the addition of 0.3 ml of 2% vanillin solution in 1 N NaOH to 3 ml of 1 N H₂SO₄ containing either isoniazid or copper-isoniazid complexes was measured at 380 nm.

2.4. Reverse Transcriptase Assay

15 µl of purified SR strain of Rous sarcoma virus (suspended in 100 µl of a solution containing 8 mM Tris buffer of pH 8.1, 8 mM MgCl₂, 200 µM each of dATP, dCTP and dGTP), was exposed to 40 µM isoniazid, cuprous-isoniazid or cupric-isoniazid complex separately and incubated at 37° C for 15 min. Five µl of 1% NP-40 and 10 µl of ³H-TTP (10 µCi/ml, Sp. Act. 23.9 Ci/mmol) were then added. The reaction mixture was incubated at 37° C for 60 min. and after the addition of 0.5 ml of 2% sodium pyrophosphate and 2 ml of 10% TCA, the precipitate was filtered, washed, dried and the radioactivity was determined in a Beckman LS-100 liquid scintillation spectrometer.

2.5. IR Spectrophotometry and ESR Spectrometry

IR spectra of isoniazid, cuprous-isoniazid and cupric-isoniazid complexes were recorded in UR 10 Carl Zeiss Jena double beam IR spectrophotometer at 25° C. The KBr pellet technique was followed as described by Tu and Reinos¹².

ESR spectra of both the complexes were recorded in an ESR spectrometer at about 9,300 MHz with 100 KHz modulations. All recordings were carried out at 25° C. A marker of $g = 2.0036$ was used to calibrate the magnetic field.

3. RESULTS AND DISCUSSION

A few characteristic properties of the two complexes are given in Table I. The precipitation of the cupric-isoniazid complex takes a little more time than that of the cuprous-isoniazid complex, indicating that initially, 1 : 1 (monodentate) complex is formed between Cu²⁺ ions and isoniazid which later form 1 : 2 (bidentate) complex⁷. On the other hand, Cu⁺ ions and isoniazid form only 1 : 1 (monodentate) complex. This was proved by estimating the amount of bound isoniazid present in both the complexes. The amount of isoniazid

in the cupric-isoniazid complex was twice that present in the same concentration of the cuprous-isoniazid complex (Table I). Microanalysis of both the complexes further proved this point. (Data not shown).

TABLE I
Characteristic properties of the two complexes formed between copper and isoniazid

	Cuprous-isoniazid	Cupric-isoniazid
1. Precipitation time	5-7 min	10-12 min.
2. Colour and nature	Yellow, thin and powdery	Green, clumped and flocculent
3. R _f value	0.73	0.64
4. Stoichiometry	1 : 1 (Monodentate)	1 : 2 (Bidentate)
5. Melting point	~250° C d.	~280° C d.
6. Reverse transcriptase inhibition (40 µM)	45%	95%

The chemical structures of the complexes and the involvement of the groups of isoniazid side chain in the complex formation were ascertained by IR spectrophotometry. The IR spectra of isoniazid, cuprous-isoniazid and cupric-isoniazid complexes are presented in Fig. 1. The absorption between

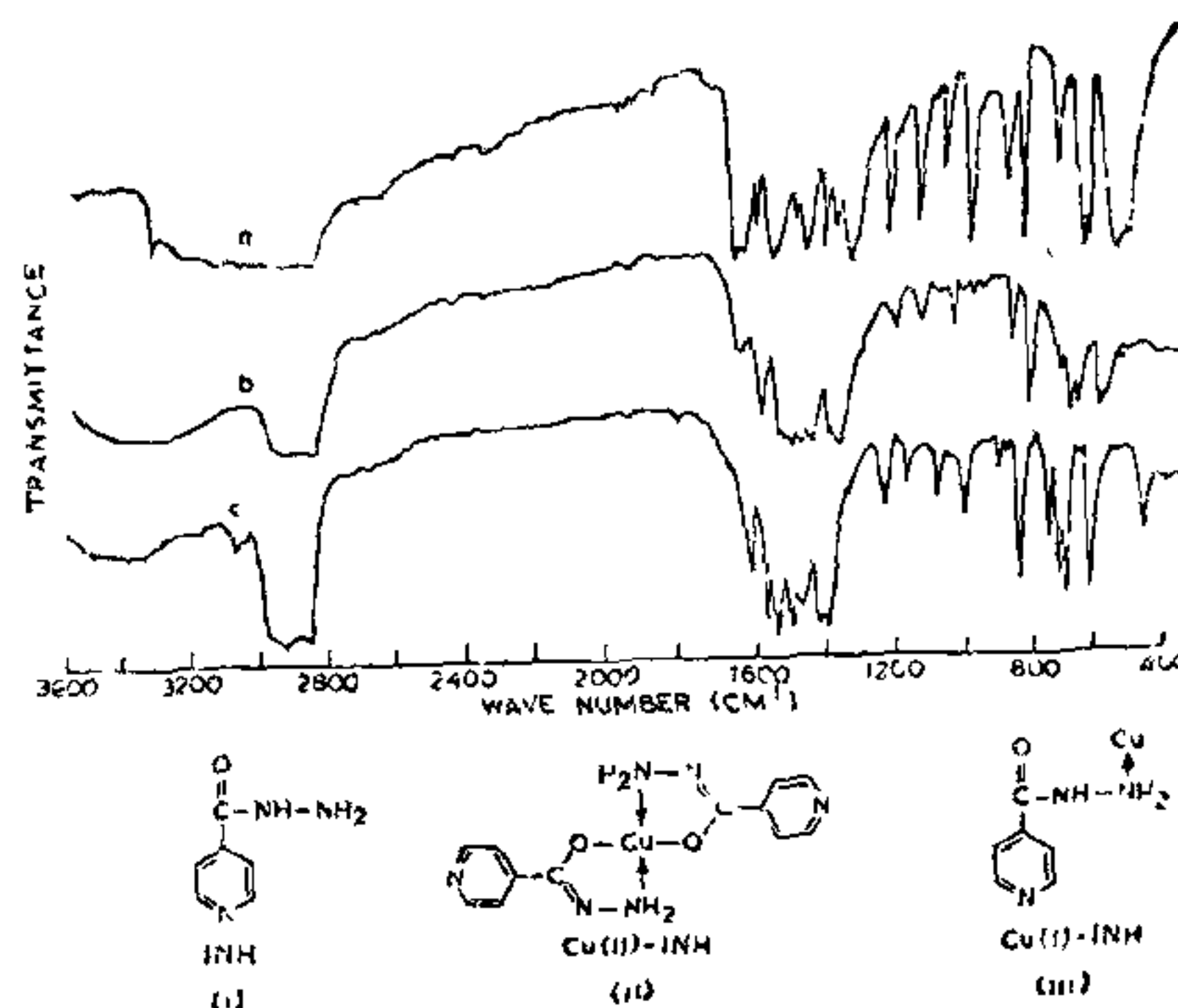


FIG. 1. IR spectra of isoniazid (a), cuprous-isoniazid complex (b), and cupric-isoniazid complex (c). A possible structure of cuprous-isoniazid complex is shown in (iii). Structure (ii) is redrawn from Fallab and Erlenmayer¹³. (i) INH-isonicotinic acid hydrazide.

1650-1680 cm⁻¹ in the IR spectrum of cuprous-isoniazid complex indicates the presence of -C=O group of -CO.NH.NH₂, and the hydrogen bonded -NH group of isoniazid is indicated by the absorption in the region 2950-3200 cm⁻¹. The absence of the sharp absorption peak at 3300 cm⁻¹ by -NH₂ group indicates the involvement of -NH₂ group in the

complex formation. The IR spectrum of cupric-isoniazid complex indicates the presence of only -NH group. Both -NH_2 and -C=O groups of isoniazid side chain are involved in the complex formation, since the corresponding peaks disappear from the cupric-isoniazid complex spectrum^{12,13}. The presence of intact pyridine nucleus of isoniazid by the aromatic C=C stretching vibrations at 1580 cm^{-1} in both the cases indicates the non-involvement of the pyridine nucleus in the complex formation.

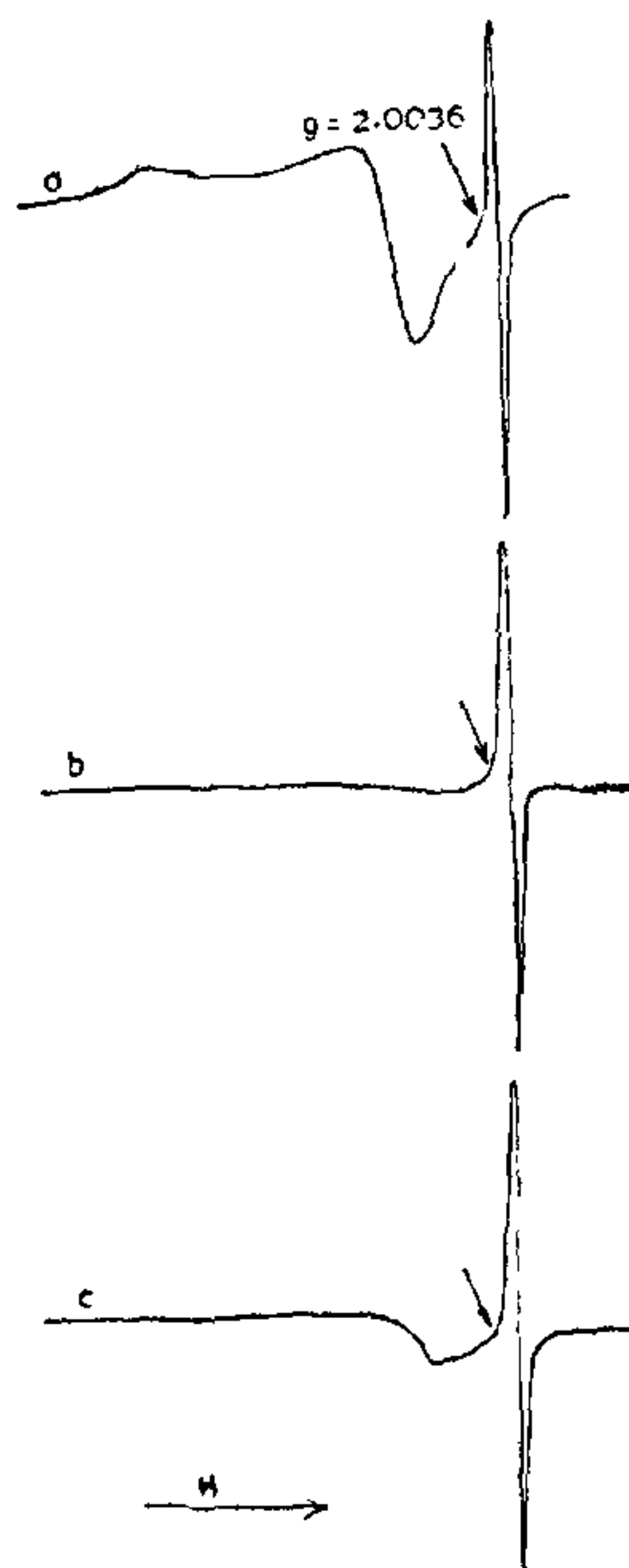


FIG. 2. ESR spectra of cupric-isoniazid complex (a), cuprous-isoniazid complex (b), and oxidized cuprous-isoniazid complex (c).

The ESR spectra of the complexes (Fig. 2) rules out the possibility of any significant reduction of Cu^{2+} to Cu^+ during the complex formation as proposed by Krivis and Rabb⁸. The cupric-isoniazid complex gave an absorption around 3200 G with a g value of 2.30 (as was obtained by Rieber and Bemski⁷), the complex showing less detail and a slight shift towards the lower magnetic field as compared with the best resolved hyperfine structure, with four lines superposed on the large absorption by cupric ions alone. On the other hand, under similar conditions, cuprous-isoniazid complex did not show any absorption. However, a very weak signal was obtained when cuprous-isoniazid complex was

subjected to oxidizing conditions. These results show that there is no significant reduction of Cu^{2+} to Cu^+ during the complex formation, and Cu^+ ions themselves are capable of forming another complex with isoniazid. Oxidation of Cu^+ to Cu^{2+} may occur under aerobic conditions to an extent of not more than 5%, since isoniazid serves as an antioxidant.

The present results, therefore, support the finding of Rieber and Bemski⁷, that the complex formation between cupric ions and isoniazid does not involve reduction of cupric ions and a 1 : 2 (bidentate) complex is formed.

The inhibition of the *in vitro* reverse transcriptase activity of Rous sarcoma virus and also the transforming ability of the virus in chick embryo fibroblast cell culture by the cupric-isoniazid complex, is significantly more than either isoniazid or cuprous-isoniazid complex^{9,10}. Furthermore, cupric-isoniazid complex is least toxic to the animal cells (primary chick embryo fibroblast and vero cells) grown in stationary cultures, as compared with either isoniazid or cuprous-isoniazid complex (Unpublished results). A detailed study on the nature of the physiologically active molecular complex is now under investigation.

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