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Keyphrases

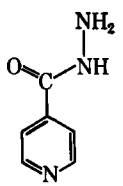
Tolbutamide tablets—disintegration
Corn starch grains—channel formation
Disintegration time—starch concentration

Influence of Metallic Ions on the Antituberculous Activity of Isonicotinoyl Hydrazones

By V. A. E. VOYATZAKIS*, G. S. VASILIKIOTIS†, G. KARAGEORGIOU‡, and IR. KASSAPOGLOU‡

Eleven isonicotinoyl hydrazones were prepared and they were tested *in vitro* as antitubercular agents. The effects of cupric and cobalt ions on their activity were investigated.

THE SPECIFIC activity of isonicotinic acid hydrazide (isoniazid I) against tubercle bacilli suggests interference with an essential metabolite. It has been shown (1, 2) that isoniazid combines with cupric ions to give 1:1 Cu-isoniazid complex (II) and 1:2, respectively. It also has been claimed that the action of isoniazid against *M. tuberculosis in vitro* is increased when copper is supplied in excess of that normally present in the medium (2).



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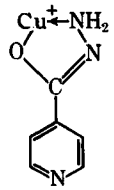


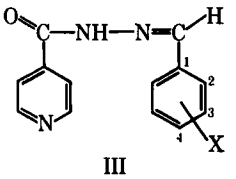
II

Moreover it has been shown (3) that 8-hydroxyquinoline (oxine) and related substances are toxic to bacteria only when traces of iron or copper ions are present in the medium. In this case the 1:1 metal complex has been shown to be the true toxic agent (4). The hypothesis was put forward that oxine is active due to the combination with these metal ions in the medium and that the complexes thus formed catalyze the oxidation of essential cell constituents. Cobalt can prevent injury to the cells, and it was suggested that this ion may be an essential cell constituent whose function is to protect, from oxidative destruction a vitally important chemical group. However, Albert (5) pointed out that while chelation might play some part in the activity of isoniazid, there must be some more important factor since the closely related picolinic acid hydrazide was less effective against tubercle bacilli but showed a metal affinity 10^3 to 10^6 times that of isoniazid.

Youatt (6) who investigated the effect of cupric ions on the uptake of hydrazides, found that copper prevented the development of strains resistant to isoniazid and increased the sensitivity to hydrazides of a strain which was already resistant to isoniazid. Stimulation of isoniazid uptake was still observed if the cells were exposed to copper ions and then washed before the addition of the isoniazid. This suggests that copper may first be bound to the cell and that chelation may occur on or in the cell.

Among the most active derivatives of isoniazid are its hydrazones. Fox and Gibas (7) have shown that 1-isonicotinoyl-2-isopropylidene hydrazone was very active against tubercle bacilli. Shchukina *et al.* (8) synthesized a number of isonicotinoyl hydrazones from aldehydes and ketones. Their biological tests, as antitubercular agents indicated that some of these are more active *in vivo* and far less toxic in mice. Sah and Peoples (9) reported also that a large number of isonicotinoyl hydrazones possess very high *in vivo* activity against *M. tuberculosis*, H 37 Rv, higher than that of streptomycin, and at least of the same order of magnitude as isoniazid. Recently Chakravarty, Bose, and Bose (10) synthesized some isonicotinoyl hydrazones which showed antituberculous activity comparable to that of isoniazid (Table I).

In this paper there is reported: (*a*) the synthesis of 11 isonicotinoyl hydrazones derived from substituted aromatic aldehydes and ketones having the general formula (III); (*b*) their microbiological examination *in vitro* as antitubercular agents, and (*c*) the influence of cupric and cobalt ions on their activity.



III

EXPERIMENTAL

Preparation of Isonicotinoyl Hydrazones—The hydrazones used in this study were synthesized according to the method of Sah and Peoples (9). All of them were recrystallized twice from methanol

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TABLE I—ISONICOTINOYL HYDRAZONE DERIVATIVES

Compd.	R	X	Compd.	R	X
A-1	H	H	A-7	H	4—OH
A-2	H	2—OH	A-8	H	4—NO_2
A-3	H	2—Cl	A-9	CH_3	4—OH
A-4	H	2—NO_2	A-10	CH_3	4—CH_3O
A-5	H	3—OH	A-11	CH_3	4—CH_3
A-6	H	4—$N(CH_3)_2$			

before use and their melting points were in agreement with those given in the literature (9); their infrared absorption spectra have already been reported (11).

Microbiological Examination—Test organism. Stock strains: (*a*) A strain (M K1) of *M. tuberculosis* isolated from a patient before any treatment. This strain was sensitive to isoniazid in concentration of 0.1 γ/ml. and was catalase and peroxidase positive; (*b*) A strain (M K2) of *M. tuberculosis* isolated from patient treated with isoniazid. It was resistant to isoniazid (10 γ/ml.) and was catalase and peroxidase negative. *In vitro* test: Compounds were incorporated with Lowenstein-Jensen medium before its coagulation at 80°. Their concentration is expressed in γ/ml. of medium. Thereafter, they were inoculated with a culture of *M. tuberculosis* grown in the same medium and incubated at 38° for 28 days. Control tubes containing only strain culture were maintained for comparison. All these specimens were incubated at 38° for 28 days again. In each case cultures of both strains were tested with 0.1 γ, 1 γ, and 10 γ/ml. of compound and then again with the same concentration but with the addition, separately of 1 γ, 10 γ, 20 γ, 40 γ, and 80 γ of cupric or cobalt ions, derived respectively from $CuSO_4$ or $CoSO_4$ solutions. Stability tests were performed to find out the rate at which compounds and isoniazid became inactivated by incubation at 38°. The results suggest that the proportionate loss of activity was of about the same order for all compounds and isoniazid.

RESULTS AND DISCUSSION

Strain M K1 was sensitive to all of these compounds in concentration of 0.1 γ/ml., while strain M K2 was resistant to them even in 10 γ/ml. So the results mainly concern the resistant strain M K2 and the activity of these compounds is comparable to isoniazid. Cupric ion alone, in concentration above 60 γ/ml., inhibited completely the development of cultures in both cases while cobalt ion was ineffective up to 80 γ/ml. In mixtures with isonicotinoyl hydrazones, the first notable influence of Cu^{2+} appeared in the range of 20 γ/ml. added to 0.1 γ/ml. of these compounds. Results are summarized in Table II. There was a complete inhibition, in all cases, by the addition of 40 γ/ml. of cupric ions. About the same results were obtained with 1 γ/ml. and 10 γ/ml. of these compounds.

TABLE II—EFFECT *In Vitro* OF Cu^{2+} (20γ/ML.) ON THE BACTERIOSTATIC ACTIVITY OF ISONICOTINOYL HYDRAZONES (0.1 γ/ML.) BY USING ISONIAZID RESISTANT STRAIN M K2

Compd.	Inhibition
A-1	++[a]
A-2	+++
A-3	+++
A-4	++++
A-5	++
A-6	+++
A-7	++
A-8	++
A-9	—[b]
A-10	+++
A-11	—
Cu^{++} (60 γ/ml.) alone	+++++

[a] Each + represents 20% of inhibition. [b] No inhibition at all.

TABLE III—EFFECTS OF COBALT IONS ADDED TO HYDRAZONES

Compd.	Inhibition	Compd.	Inhibition
A-1	++[a]	A-7	—[b]
A-2	+++	A-8	++
A-3	+++	A-9	++
A-4	++	A-10	+
A-5	++	A-11	++
A-6	+++	Co^{2+} (80 γ/ml.) alone	[c]

[a] Each + represents 20% of inhibition. [b] No inhibition at all. [c] 10–20 colonies observed against ~500 of the control tube.

Effects of cobalt ions (40 γ/ml.) added to a concentration of 0.1 γ/ml. of hydrazones are summarized in Table III.

By adding 80 γ/ml. of Co^{2+}, to the tested compounds, still 10–20 colonies were observed against to 400–500 of the control tube.

In the mixtures used in this study, the concentration of free cupric ions was well below that which would cause inhibition by them alone. There is also a possibility of formation of cupric chelates with hydrazones and then the concentration of free Cu^{2+} will be even lower and will depend of course on its affinity to the ligand, on pH, ratio of ligand to metal, stability constants, and acid dissociation constants of the ligand.

Since the results of the inhibition due to added cupric ions to the strains studied were independent of the concentration of the compound used (between 0.1 γ/ml. and 10 γ/ml.) there is some evidence that free cupric ion may be responsible for the stimulation of isonicotinoyl hydrazone uptake as well as in the case of isoniazid (6).

Thus, development of the resistant strain M K2 was supressed and the critical quantity of cupric ions, under the experimental conditions was 20 γ/ml. even in the presence of 0.1 γ/ml. of the drug. Of course there are variations of the activity of these drugs and research on their Cu complex stability constants is continuing and the results shall be published in due time.

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Keyphrases

Isonicotinoyl hydrazones—synthesis
Metallic ions–isonicotinoyl hydrazones—complexes
Antitubercular activity—cupric, cobalt ions effect

Potential Diazo Reagents for Colorimetric Determination

By TIBOR URBÁNYI and JOSEPH A. MOLLICA

The diazonium derivatives of 27 substituted aromatic amines were investigated as reagents for colorimetric determination. The phenolic moiety used in the study was the estrogen, estradiol. Their applicability was determined on the basis of speed, sensitivity, color stability, and reproducibility of the coupling reaction. Using these criteria, the diazotization product of 4-amino-6-chloro-*m*-benzenedisulfonamide appeared to be the most suitable colorimetric reagent for the compounds containing a phenolic hydroxyl group.

A RAPID AND SENSITIVE method for the determination of estrogens is based on their ability to couple with diazotized amines. This communication considers the results of investigations regarding the suitability of various substituted aromatic amines as analytical reagents for compounds having coupling capability. Under the experimental condition employed, 4-amino-6-chloro-*m*-benzenedisulfonamide appeared to be the most promising reagent. The mechanism for the formation of its diazonium compound and its application to the analysis of several estrogens was reported previously (1, 2).

The following criteria for suitability were established: the coupling reaction should be rapid at room temperature, the color produced should be relatively stable, and the method should be sensitive. Since speed, sensitivity, and reproducibility were the objectives, the same reaction conditions were employed for all amines. Although these conditions were not optimum for all amines tested, this procedure was selected since the goal was to find suitable analytical reagents and not to investigate the reaction for each amine.

EXPERIMENTAL

A Beckman DU spectrophotometer was used to determine the absorbance values, and a Cary model 11 spectrophotometer was used to record the absorption spectra.

All compounds were of reagent grade quality and were used without further purification.

Reagents and Solutions—Aromatic amines: a 7×10^{-3} *M* solution of the amine was prepared in either methanol or 1 *N* hydrochloric acid. Sodium nitrite, 1% aqueous solution; hydrochloric acid, 1 *N*; sodium acetate 2 *N*; sodium hydroxide 0.1 *N* and 1 *N*. The estradiol solution was prepared as follows: about 50 mg. of estradiol, accurately weighed, was dissolved in 50 ml. of methanol and then this solution was diluted 10-fold with 0.1 *N* sodium hydroxide; concentration approximately 0.1 mg./ml.

Procedure—Into a 10-ml. volumetric flask were pipeted 1 ml. of the aromatic amine solution, 1 ml. of sodium nitrite solution, and 1 ml. of 1 *N* hydrochloric acid. The solution was mixed well and allowed to stand for 1 to 2 min. Then, 1 ml. of estradiol solution and 2 ml. of sodium acetate solution were added, mixed, and the resulting solution allowed to stand for exactly 6 min. The contents of the flask were diluted to volume with 1 *N* sodium hydroxide solution. A blank solution was prepared in the same manner except that 1 ml. of 0.1 *N* sodium hydroxide was used in place of the estradiol solution. The absorbance of the sample solution was measured against the blank solution at the wavelength of the absorption maximum in 1-cm. cells.

DISCUSSION

The coupled products yielded absorption spectra with maxima in the range 450–550 mμ. The spectra were all similar to the spectrum of 4-amino-6-chloro-*m*-benzenedisulfonamide and estradiol which was published previously (2). The apparent molar absorptivity values in Table I were obtained by dividing the absorbance of the coupled solution, measured at the wavelength of absorption maximum, by the concentration of the amine employed. The molar absorptivity values reported are only apparent values since for many of the compounds investigated, the absorbance continued to increase after the 6-min. reaction time. This is due to either incomplete coupling and/or side reactions of the diazotized amine (3, 4). As stated in the introduction, the purpose of this study was not to investigate the mechanism of reaction, but to find a suitable analytical reagent. However, some conclusions regarding the effect of substituent groups on color intensity can be drawn. Comparisons of the molar absorptivity values indicate that substitution strongly influences the intensity of the coupled product, *e.g.*, 3 and 4, 13 and 15, 23 and 24; not

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