METABOLISM OF ETHIONAMIDE, A SECOND-LINE ANTITUBERCULAR DRUG

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ABSTRACT

Several derivatives and possible metabolites of ethionamide (2-ethyl thioisonicotinamide), an active tuberculostatic second-line drug have been chemically synthesized. Of all the compounds prepared, ethionamide sulphoxide showed more antitubercular activity than ethionamide when tested against M. tuberculosis $H_{37}R_V$ *in vitro. The N-methyl and N-ethyl derivatives of ethionamide were fairly active but all the other derivatives were much less active than the parent compound. Ethionamide is converted to various metabolites on administration to experimental animals; the urinary metabolites of the drug in guinea pigs have been identified as ethionamide sulphoxide, the N-methyl pyridones of ethionamide and ethionamide sulphoxide, and the N-methyl pyridone derivatives of the desulphurated compounds, viz., 2-ethyl isonicotinamide and 2-ethyl isonicotinic acid, in addition to the parental compound. The distribution of ethionamide and its major metabolite (ethionamide sulphoxide) in various tissues of the guinea pig as well as their excretion pattern in urine after the oral administration of the drug have also been studied.*

Key words: Ethionamide; Antitubercular drug.

1. INTRODUCTION

Ethionamide (2-ethylthioisonicotinamide) is an active, tuberculostatic second-line drug, which plays an important role in the retreatment of pulmonary tuberculosis, resistant to one or more of the classical drugs [1]. The drug possesses very high activity and relatively low toxicity, thus offering good scope for the treatment of tuberculosis, especially in cases of infection caused by organisms resistant to the primary drugs, *viz.*, isoniazid or streptomycin. The structure of ethionamide shows similarity to other antitubercular compounds, notably the thiosemicarbazones and isoniazid. Unlike these drugs, the metabolism of thioamides has not been investigated, although the

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behaviour of the $-CSNH_2$ group in thioureas and thiosemicarbazones is to some extent understood [2, 3].

This paper deals with the isolation and characterization of the metabolites after the administration of ethionamide to guinea pigs. These metabolites and a few other derivatives of the drug have been synthesized chemically and their antitubercular activities *in vitro* have been tested.

2. Experimental

(*a*) *Chemicals*

Ethionamide was purchased from May and Baker Ltd., Dagenham, U.K. and was recrystallized from alcohol before use. Silica gel for thin layer chromatography was purchased from E. Merck, Darmstadt, Germany. Other chemicals used were all of analytical grade.

(*b*) *Synthesis of the metabolites and derivatives of ethionamide*

(1) *Ethionamide sulphoxide.*—Ethionamide sulphoxide was prepared according to the method of Libermann *et al.* [4]. 2·2 ml of hydrogen peroxide was added to 3·32 g of ethionamide in 7 ml of pyridine. The temperature was maintained below 40° for 3–4 hr and the pyridine was removed by evaporation under vacuum. Fine crystals of ethionamide sulphoxide were formed. These were recrystallised twice from methyl alcohol (Melting point-144-145° C; yield 75–80%).

(2) *N-methyl ethionamide sulphoxide.*—This and the following few metabolites were prepared according to the method of Yamamoto and Yamaguchi [5]. 500 mg of ethionamide sulphoxide was dissolved in a minimal amount of acetone followed by the addition of methyl iodide. The reaction was allowed to take place at room temperature in the dark. After 30–40 min, the solution was concentrated by evaporation in the desicator. The residue was dissolved in a minimal amount of acetone and left for crystallization. Only a pasty mass was obtained and the melting point could not be checked (yield 60%).

(3) *N-methyl ethionamide sulphoxide pyridone.*—The N-methyl ethionamide sulphoxide (220 mg) thus obtained was dissolved in water and oxidzed by potassium ferricyanide in alkaline conditions. The pyridone formed was extracted several times with ether after adjusting the pH to 4–5, with dilute hydrochloric acid. The ether extracts were pooled, evaporated to dryness and recrystallized from acetone-alchol. (Melting point

122° C; yield 65%). The product was found to be highly fluorescent (greenish yellow in colour) under ultraviolet light.

(4) *N-methyl ethionamide.*—1 g of ethionamide was dissolved in the minimal amount of acetone and 0·75 ml of methyl iodide was added in regulated amounts. The flask containing the reactants was kept at 37° C for 2–3 hr with frequent agitation. After cooling, fine crystals of N-methyl ethionamide separated out. The crystals were collected by filtration and recrystallized twice from acetone (Melting point 203° C; yield 85%).

(5) *N-methyl ethionamide 6-pyridone.*—N-methyl ethionamide 6-pyridone was prepared by dissolving N-methyl ethionamide (300 mgs) in water and to that was added 1·24 g of potassium ferricyanide in water followed by 30% sodium hydroxide. The temperature of the mixture was maintained below 20° C and the solution was allowed to stand for 80 m'n without stirring. After adjusting the pH to 4–5 with dilute hydrochloric acid, the resulting solution was extracted with ether for 8–10 hrs continuously and the pyridone was obtained after removing the ether by distillation. This product was recrystallized from alcohol-acetone. (Melting point 141° C; yield 80–85%) The product is highly fluorescent giving blusih purple fluorescence under ultraviolet light.

(6) *2-Ethyl isonicotinamide.*—1 g of ethionamide was dissolved in 10% aqueous solution of sodium hydroxide and allowed for the desulphuration reaction to take place (30 min). After adjusting the pH to 4–5 with dilute hydrochloric acid, the solution was evaporated under reduced pressure. The crude residue thus obtained was extracted with alcohol. The alcoholic solution was evaporated to dryness under vacuum and the residue was recrystallized from methanol (Melting point 130° C; yield 90%).

(7) *N-methyl 2-ethyl isonicotinamide.*—500 mg of 2-ethyl isonicotinamide was dissolved in acetone and methyl iodide was added. The methylation reaction was allowed to take place for 1 hr at 37° C with frequent agitation. Few crystals of N-methyl 2-ethyl isonicotinamide (white crystals) separated out. The crystals were separated by filtration, and recrystallized before use (Melting point 192° C; yield 75%).

(8) *N-methyl 2-ethyl isonicotinamide 6-pyridone.*—100 mg of N-methyl-2-ethyl isonicotinamide was dissolved in water and an aquous solution of potassium ferricyanide (1·24 g/5 ml) and 30% sodium hydroxide were added. The reaction was allowed to take place; care was taken to see that the temperature was maintained below 20° C. The pyridone thus formed was

extracted with ether many times. The ether extracts were pooled and the ether was removed by distillation. The product remained pasty and hence the melting point could not be checked. (Yield 55%). The product showed yellow fluorescence under ultraviolet light.

(9) *2-ethyl isonicotinic acid.*—1 g of ethionamide was dissolved in 20 ml of 10% aqueous solution of sodium hydroxide and boiled for 3 hr under reflux condenser. After cooling, the solution was brought to pH 4–5 with dilute hydrochloric acid and evaporated to dryness. The crude residue was extracted with ethanol. The alcoholic solution was evaporated to dryness and the residue was recrystallized from methanol (Melting point 233° C; yield 70–75%).

(10) *N-methyl-2-ethyl isonicotinic acid.*—400 mg of 2-ethyl isonicotinic acid was dissolved in acetone followed by regulated addition of methyl iodide. The contents in a flask were incubated at 37° for some time with frequent agitation. Methyl iodide and acetone were removed by distillation and the crude sample was recrystallized from acetone (Melting point 130° C; yield 60%).

(11) *N-methyl 2-ethyl isonicotinic acid 6-pyridone.*—100 mg of N-methyl 2-ethyl isonicotinic acid (prepared as described above), was dissolved in water. To this solution an aqueous solution of potassium ferricyanide (1·24 g/5 ml) and 30% sodium hydroxide were added and the temperature was maintained below 20°. The solution was allowed to stand for 70 min without stirring. After adjusting the pH to 4–5 with dilute hydrochloric acid, the resulting solution was extracted with ether for 8–10 hr continuously and the pyridone was obtained after removing the ether by distillation. The product remained pasty; hence the melting point could not be checked (yield 55%). The product showed yellow fluorescence under ultra-violet light.

(12) *N-ethyl ethionamide.*—1 g of ethionamide was dissolved in acetone and ethyl iodide was added in regulated amounts. The reaction was allowed to take place at 37° for 2–3 hr with frequent agitation. After cooling, fine crystals of N-ethyl ethionamide separated out. The crystals were separated and recrystallized from acetone (Melting point 152° C; yield 60%).

(*c*) *Treatment of animals*

Guinea pigs (700–800 g), random bred were divided into several groups (each group containing a minimum of 5 animals were used in all the experiments). The animals were fed *ad libitum* on stock diet from Hindustan Lever Ltd., Bombay, and were housed in metabolic cages for the collection of urine

samples at different time intervals. Ethionamide dissolved in 10% citric acid containing sucrose was given orally at a dose of 140 mg/kg body weight of animals.

(*d*) *Treatment of tissues*

The animals were bled at various time intervals (by cardiac puncture) and the blood samples were saved for the separation of serum. The liver, kidney, spleen, lungs and pancreas were removed immediately and the tissue homogenates were prepared in 1·15% potassium chloride (2 volumes) using MSE homogeniser (Nelco). The concentrations of ethionamide and ethionamide sulphoxide in the tissue homogenates as well as in the serum were estimated.

(*e*) *Estimations of ethionamide and ethionamide sulphoxide*

Estimations of ethionamide and ethionamide sulphoxide were carried out with a slight modification of the method of Bieder *et al.* [5, 6.] The estimations in tissue extracts, serum and urine samples were carried out by the same procedure.

(*f*) *Treatment of the urine samples*

The volume of the urine samples collected at different time intervals was noted. The samples were processed for the extraction of ethionamide and ethionamide sulphoxide as described above. After estimating the amount of ethionamide and ethionamide sulphoxide, the aqueous (containing methanol), chloroform -and acid layers were concentrated and the concentrates were separately chromatographed on thin layers of silica gel, using the solvent system butanol: acetic acid: water (4: 1: 5). The fluorescent spots in all the different plates were located by ultraviolet light and the R_F values were calculated. The spots from each plate were eluted with methanol, chloroform and dilute hydrochloric acid respectively and the fluorescence spectra were recorded on a Perkin-Elmer fluorescence spectrophotometer.

(*g*) *Organism for testing antituberculous activity*

Mycobacterium tuberculosis var. *hominis* $H_{37}R_V$ (Strain No. 7416) was obtained from the National collection of Type cultures (England). For growth inhibition studies the bacilli were grown on the synthetic liquid medium [7].

Stock solutions of 5–10 mgs/ml of all the metabolites synthesized were prepared by dissolving a known quantity in ethanol and serial dilutions were made such that the final concentrations would be 0·1 to 50 μg/ml of the medium.

Suitable controls were included for the alcohol concentration. The tubes were inoculated with the strain $H_{37}R_V$ taking care to see that the size of the inoculum was the same in all the tubes. The experiment was done in triplicates. The tubes were incubated at 37° C for three weeks, the culture reading being noted at weekly intervals. At the end of three weeks, the dry weight of the bacilli was recorded.

3. Results

(i) *Antituberculous activity of the derivatives and possible metabolites of ethionamide.*—The growth inhibiting effects of ethionamide and some of the probable metabolites synthesized, on *M. tuberculosis* $H_{37}R_V$ *in vitro* are shown in Table I.

TABLE I

In vitro *growth inhibitory effect of the metabolites (synthetic) of ethionamide on* M. tuberculosis $H_{37}R_V$

Metabolites	M.I.C.* (μg/ml)
Ethionamide (parent drug)	0·5
Ethionamide sulphoxide	0·1
N-methyl ethionamide	1·0
N-ethyl ethionamide	1·0
2-ethyl isonicotinamide	25·0
2-ethyl isonicotinic acid	50·0
N-methyl 2-ethyl isonicotinamide	—
N-methyl 2-ethyl isonicotinic acid	—
N-methyl ethionamide sulphoxide	50·0
Pyridone of N-methyl ethionamide	50·0
Pyridone of N-methyl ethionamide sulphoxide	10·0
Pyridone of N-methyl 2-ethyl isonicotinamide	25·0
Pyridone of N-methyl 2-ethyl isonicotinic acid	50·0

* M.I.C. = Minimal inhibitory concentration, to bring about complete inhibition of growth. These compounds were synthesized as described under "Experimental". The *in vitro* antituberculous activity was determined using *M. tuberculosis* $H_{37}R_V$, in Youman's medium The percentage inhibition was calculated by the determination of dry weights of cells after 21 days growth at 37° C. Each value represents an average of at least 3–4 independent determinations. — represents no inhibition upto 100 μg/ml.

The results indicate that ethionamide sulphoxide is at least 5 times more effective than the parent drug in inbiting the growth of *M. tuberculosis* $H_{37}R_V$. On the other hand, the other probable metabolities and derivatives of ethionamide required concentrations higher than the parental compound for growth inhibition. The desulphurated derivatives of ethionamide (N-methyl 2-ethyl isonicotinamide and N-methyl 2-ethyl isonicotinic acid) had no growth inhibiting effects upto 100 μg/ml.

(ii) *Excretion of ethionamide and its metabolites.*—After oral administration of ethionamide to guinea pigs, the excretion of the free drug as well as the metabolites was studied. After a single dose of oral administration, excretion of ethionamide was observed upto 72 hr in the urine. Although several metabolites derived from ethionamide were found in the urine at the different time intervals, the major metabolitic was identified (by comparing R_F value in different solvent systems and ultraviolet and visible absorption spectral characteristics) as ethionamide sulphoxide.

Among the other metabolites, at least 4 of them showed fluorescence and they were identified as N-methyl ethionamide pyridone, N-methyl ethionamide sulphoxide pyridone, N-methyl 2-ethylisonicotinamide pyridone and N-methyl 2-ethyl isonicotinic acid pyridone, with the help of the authentic samples synthesized (Table 2). Some of the non-fluorescent metabolites could not be identified. However, from the formation and excretion of some of the pyridones, it could be concluded that sulphoxidation, desulphuration and deamination followed by methylation are evident in the metabloism of ethionamide in guinea pigs.

Since ethionamide sulphoxide appeared to be the major metabolite and its *in vitro* antituberculous activity was more than that of the parent compound itself, the distribution of ethionamide and its sulphoxide in various tissues as well as their excretion in urine after the oral administration of the drug was studied. The results are presented in Tables III and IV. As far as the distribution of the drugs in various tissues is concerned, it can be seen from Table III that the level of ethionamide was higher in liver than in other tissues and the sulphoxide could not be detected in tissues other than liver.

The level of ethionamide in all the tissues reached the maximum within an hour of administering it and then strated declining. Ethionamide sulphoxide concentrations reached the maximum at 6 hr after the administration of the drug and after that the level strated declining. At 24 hr, the presence of sulphoxide could not be detected even in liver. Thus after oral

TABLE II

Identification of the fluorescent metabolites of ethionamide

Hours after administration	Colour of fluorescence	R_F	Excitation maxima	Emission maxima	Identification
24 ..	Bluish purple	0·64	340	410	N-methyl ethio-
48 ..	Bluish purple	0·61	340	405	namide pyridone
60 ..	Bluish purple	0·66	340	405	
Authentic (N-methyl ethionamide pyridone) ..	Bluish purple	0·63	340	405	
24 ..	Yellowish green	0·16	330	460	Ethionamide
48 ..	Yellowish green	0·15	335	460	sulphoxide
Authentic (Ethionamide sulphoxide N-methylated pyridone) ..	Yellowish green	0·15	335	460	N-methylated pyridone
24 ..	Yellow	0·52	320	370	N-methyl 2-ethyl
48 ..	Yellow	0·58	320	370	isonicotinamide
60 ..	Yellow	0·56	315	365	pyridone
Authentic (N-methyl 2-ethyl isonicotinamide pyridone)	Yellow	0·55	320	370	
24 ..	Yellow	0·35	330	430	N-methyl 2-ethyl
48 ..	Yellow	0·32	330	430	isonicotinic acid
60 ..	Yellow	0·35	330	425	pyridone
Authentic (N-methyl 2-ethyl isonicotinic acid pyridone)	Yellow	0·35	330	430	

The urine samples were extracted with chloroform and re-extracted with hydrochloric acid. The acidic, aqueous and chloroformic layers were concentrated and the concentrates were applied on thin layer of silica gels, on different plates and the chromatograms were developed using Butanol : Acetic Acid : Water : : 4 : 1 : 5. The air dried chromatograms were observed under ultraviolet light and the fluorescent spots were marked. After calculations of the R_F values, the fluorescent spots were eluted with 0.1 N hydrochloric acid, methanol and chloroform from the respective plates and fluorescence spectra were recorded. From the comparison of spectra to those of the authentic samples, the metabolites excreted were identified.

TABLE III

Distribution of ethionamide and ethionamide sulphoxide in various tissues and blood (serum) of guinea pigs after oral administration of ethionamide

Tissue	Concentration of ethionamide and ethionamide sulphoxide	Time after administration of ethionamide (in hrs)									
		1		3		6		12		24	
		Ethionamide	Ethionamide sulphoxide	Ethionamide	Ethionamide sulphoxide	Ethionamide	Ethionamide sulphoxide	Ethionamide	Ethionamide sulphoxide	Ethionamide	Ethionamide sulphoxide
Liver ..	μg gm of tissue	7·27	1·96	4·27	2·42	2·89	3·20	0·98	0·61	0·17	0
Kidney ..	do.	1·95	0	1·46	0	0·76	0	0·1	0	0	0
Spleen ..	do.	0·88	0	0·40	0	0·20	0	0·43	0	0	0
Lungs ..	do.	5·21	0	3·20	0	1·0	0	0·32	0	0	0
Pancreas	do.	0·25	0	0·55	0	0·47	0	0	0	0	0
Serum ..	μg ml	58·73	9·74	13·00	2·6	5·89	2·9	1·1	1·5	0·71	0·45

Guinea pigs were administered 140 mgs kg of ethionamide orally and the distribution of ethionamide and ethionamide sulphoxide in various tissues at different time intervals was followed. The tissues were homogenised in 1·15% KCl and the estimation of ethionamide and its sulphoxide were followed as described under "Experimental".

administration of ethionamide, only very low levels of both the unmetabolised drug and the sulphoxide were distributed.

TABLE IV

Excretion of ethionamide and ethionamide sulphoxide in urine after an oral administration of ethionamide

Time intervals (hrs)	Total excretion of ethionamide* (μg)	Total excretion of ethionamide sulphoxide (μg)
12	1016	860
24	750	202
36	698	178
48	464	132
60	286	77
72	165	54

Ethionamide was administered orally to guinea pigs weighing 700–800 g at a dose of 140 mgs/kg. The urine samples were collected at different time intervals. The details for the estimation of ethionamide and ethionamide sulphoxide is described in the text under "Experimental".

* Total excretion per animal.

It can be seen from Table IV that there was a total urinary excretion of 1016 μg of the unchanged drug and 860 μg of ethionamide sulphoxide within 12 hours and this corresponds to less than 2% of the drug administreed. The level comes down considerably after 12 hours and very little excretion of the unchanged drug and the sulphoxide was observed after 72 hr. The bulk of the drug might have been eliminated (unadsorbed) through the faeces, since the drug was administered orally; the faecal contents were, however, not estimated.

4. DISCUSSION

Ethionamide, a second line antitubercular drug is effective against isoniazid resistant and streptomycin resistant strains of *M. tuberculosis* and therefore occupies an important place in the chemotherapy of tuberculosis.

The *in vitro* activity of the drug in experimentally infected animals is greater than its *in vitro* activity which indicates that the drug exercises its antituberculous effect through the metabolites [8]. From the results presented here the major metabolite of ethionamide in guinea pigs appear to be ethionamide sulphoxide; the sulphoxide has also been identified as a metabolite in man [9, 10] and rabbit [11]. This is very significant because ethionamide sulphoxide showed more antibacterial activity *in vitro* against *M. tuberculosis* $H_{37}R_V$ than ethionamide itself. The ready formation of the sulphoxide in animals and man may thus be important in exerting the antituberculous activity of the drug.

In addition to the sulphoxide, several other metabolites like the pyridone of ethionamide sulphoxide and the pyridone of the desulphurated derivatives are excreted in the urine of guinea pigs, administered with a single dose of ethionamide. Most of these metabolites had antituberculous activity *in vitro*, equal to or less than ethionamide itself. Earlier studies [10, 12, 13] have demonstreated the formation of ethionamide sulphoxide 2-ethyl isonicotnamide, 2-ethylisonicotinic acid, N-methyl ethionamide and its pyridone in human. The present studies reported here show for the first time the formation of metabolites like the pyridones of N-methyl-2-ethyl isonicotinc acid, N-methyl-2-ethyl isonicotinamide and N-methyl-ethionamide sulphoxide.

The distribution of ethionamide and its sulphoxide in the animal was also studied. The maximum levels of ethionamide sulphoxide were found in the liver, 6 hours after the administration of the drug; on the other hand, the highest levels in the serum were reached within 1 hour. Our recent studies [14] have shown the presence of an enzyme, a mono-oxygenase, located in the microsomal fraction of the liver, which is capable of converting ethionamide to its sulphoxide. Although the enzyme was present in various tissues and the serum of guines pigs, the highest activities were always found in liver (15). Thus the formation of ethionamide sulphoxide may form an important step in the antituberculous activity of the drug.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

[1] Goodman, S. and Gillman, A. *The Pharmacological Basis of Therapeutics*, 4th edn., Macmillan, London, 1970, pp. 1331–1333.

[2] Arita, T. .. *J. Pharm. Soc. (Japan)*, 1956, **76**, 987–990.

[3] Shibata, I. .. *Jap. J. Med. Prog.*, 1958, **45**, 455.

[4] Libermann, D., Rist, N. and Grunbach, M. F. *C.R. Acad. Sci.*, Ser. D 1963, **257**, 307–308.

[5] Yamamoto, M. and Yamaguchi, Y. *Jap. J. Chest Dis.*, 1964, **8**, 1415–1418.

[6] Bieder, A., Burnel, P. and Mazeau, L. *Ann. Pharm. Fr.*, 1963, **21**, 375–387.

[7] Bieder, A., Burnel, P. and Mazeau, L. *Ann. Pharm. Fr.*, 1966, **24**, 493–500.

[8] Youmans, G. P. and Karlson, A. G. *Amer. Rev. Tuberc.*, 1947, **55**, 529–534.

[9] Rist, N., Grumbach, F. and Libermann, D. *Amer. Rev. Tuberc.*, 1959, **79**, 1–5.

[10] Bieder, A. and Mazeau, L. *Ann. Pharm. Fr.*, 1962, **20**, 211–216.

[11] Bieder, A. and Mazeau, L. *Therapie*, 1964, **19**, 897–907.

[12] Kane, P. O. .. *Nature (London)*, 1962, **195**, 495–496.

[13] Yamamoto, M., Yamaguchi, W., Kumazawa, Y. and Takahashi, Y. *Jap. J. Chest. Dis.*, 1962, **6**, 1036–1041.

[14] Johnston, J. P., Kane, P. O. and Kirby, M. R. *J. Pharm. Pharmacol.*, 1967, **19**, 1–9.

[15] Prema, K. and Gopinathan, K. P. *Biochem. J.*, 1974, **143**, 613–624.

[16] Prema, K. .. Studies on the metabolism of ethionamide, a second-line antitubercular drug, 1974, ch. 2, pp. 38–62, *Thesis* (unpublished), Indian Institute of Science.