IN VITRO STUDIES ON THE TUBERCULOSTATIC ACTIVITY OF CYANO-ALIPHATIC ACID HYDRAZIDES AND RELATED COMPOUNDS. PART I

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Following the announcement of high antituberculosis action of *iso*-nicotinyl hydrazide, various aliphatic, aromatic and heterocyclic carboxylic acid hydrazides, their derivatives and related compounds have been reported and examined for their action on *Mycobacterium tuberculosis*[1,2,3,4,5]. Amongst the aliphatic acid series, acetic acid hydrazide has been reported to possess no appreciable antituberculosis activity. Elsewhere it is mentioned that cyanacetic acid hydrazide has been found to possess a high activity against human tuberculosis, although no experimental data as to its order of activity *in vitro* has been presented[6]. The above findings suggest that the introduction of a CN group in the acetic acid hydrazide molecule has produced a distinct and specific effect against the mycobacterium. In view of this, work was undertaken in these laboratories to study the influence of the introduction of the CN group in various aliphatic acid hydrazides and related compounds for evaluation as antituberculosis agents. It was also considered to be of interest to study the effect of the modifications of CN group in cyanacetic acid hydrazide into easily derivable groups. The present communication describes the results of our preliminary investigation in this series.

Compounds tested for inhibition of growth of *Myco. tuberculosis* H37Rv in modified Dubos medium containing Tween 80 are listed in the following tables. Compounds which failed to inhibit the growth at a concentration of 1 mg. per cent. are listed as inactive.

Experimental

Materials and Methods

Medium. The culture medium was Medical Research Council—modified Dubos at *p*H range 7·4 to 7·6, containing potassium dihydrogen phosphate, 0·1 per cent.; disodium hydrogen phosphate, 0·625 per cent.; sodium citrate, 0·15 per cent.; magnesium sulphate, 0·06 per cent.; Tween 80, 0·05 per cent.; casein hydrolysate (enzymatic), 0·2 per cent., in glass-distilled water.

Each 5 ml. of the medium was distributed in 25-ml. screw-cap bottles and autoclaved at 10 lb. for 10 minutes. The *p*H was maintained at 7·4 to 7·6 and the media was incubated, to prove sterility, for 72 hours before use.

Strain. A virulent strain of *Mycobacterium tuberculosis*—H37Rv (Human) was employed.

TABLE I

ALIPHATIC ACID HYDRAZIDES AND RELATED COMPOUNDS

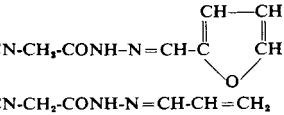
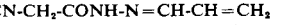
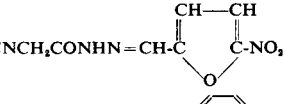
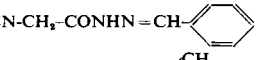
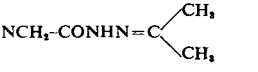
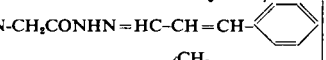
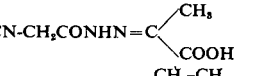
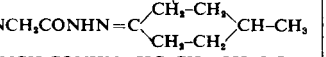
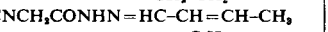
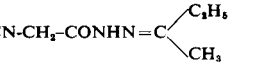
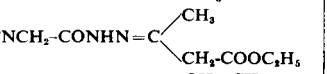
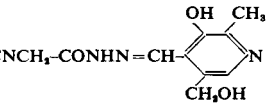
Structure	Minimum inhibitory concentration mg. per cent.
$CNCH_2CONHNH_2$	0·125–0·0625
$CNCH_2CH_2CONHNH_2$	0·25 –0·125
$CNCH_2CONHNHCOCH_2CN$	1·0
$CNCONHNHCOCN$	inactive
$CNCH_2CONHNHCOCH_3$	0·25
$CNCH_2CONHNNHCHO$	inactive
$CN-CH(NO)-CONHNH_2$	inactive
$CN-CH(NHCOCH_3)-CONHNH_2$	inactive
$CN-CH(CH_2C_6H_4Cl)-CONHNH_2$	0·5
$CNCH_2COOH$	inactive
$CNCH_2CONH_2$	inactive
$HOOCCH_2CONHNH_2$, HCl	0·5
$CH_2(CONHNH_2)_2$	inactive
$NH{=}C(NH_2)-CH_2CONH_2$, HCl	inactive
$NH{=}C(NH_2)-CH_2-CONHNH_2$	inactive
$NH{=}C(NH_2)-CH_2CONHOH$	inactive
$NH_2CH_2CONHNH_2$, 2HCl	1·0
$NO_2CH_2CONHNH_2$	inactive
$CH_3-CH_2CONHNH_2$	inactive
$(CH_3)_2(Cl)-N-CH_2CONHNH_2$	1·0–0·5
$CH_3-C(=N-NH-COCH_2CN)-CH_2CONHNH_2$	inactive
$C_6H_5-CH(OH)-CONHNH_2$	inactive
$C_6H_5-CH(OH)-CONHNH-CO-CH(OH)-C_6H_5$ ..	inactive
$C_6H_5OCH_2CH_2CONHNH_2$ ..	inactive
p-$Cl-C_6H_4-CH_2CONHNH_2$	inactive
$C_6H_5-CH(CONHNH_2)_2$	inactive
p-$NH_2-C_6H_4-CH{=}CH-CONHNH_2$ ..	0·5
o-$CH_3O-C_6H_4-OCH_2CONHNH_2$ ..	inactive
o-$C_6H_4(CONHNH_2)(OCH_2CONHNH_2)$	inactive
$Cl-C_6H_3(CONHNH_2)(OCH_2CONHNH_2)$	0·5

Inoculum. 0·05 ml. of a 14 day-old culture of H37Rv in modified Dubos medium was used for 5 ml. medium containing the drug.

Procedure. Solutions of the compounds in distilled water or in a minimum quantity of propylene glycol and water were filtered through a Seitz filter and tested in the final concentration of 1, 0·5, 0·25, 0·125,

TABLE II

HYDRAZONES OF CYANACETIC ACID HYDRAZIDES

Compound No.	Structure	Minimum inhibitory concentration mg. per cent.
1		inactive
		inactive
3		inactive
4		0·0625
5		1·0
6		0·0312–0·0156
7		0·5
8		inactive
9		1·0
10		inactive
11		inactive
12		1·0

0·0625 etc., mg. per cent. in 5-ml. of Dubos medium, distributed in 25 ml. screw-cap bottles.

Each test series with serial drug dilution had two controls without any drug and was inoculated with 0·05 ml. of 14 days' culture of H37Rv and were incubated at 37° C. for 14 days.

Results. The bottles were examined visually for growth after 7 and 14 days of incubation at 37° C. and readings were recorded when complete inhibition was shown on the 14th day of incubation, while the controls showed good growth. As an inhibitory control of known efficacy,

sodium *p*-aminosalicylic acid was tested in each set of experiments, in which inhibitory concentration was uniformly found to be at 0·03 mg. per cent.

Summary

1. Certain cyano-aliphatic acid hydrazides, their hydrazones and related compounds have been examined for their *in vitro* activity against tubercle bacilli.

2. Extension of the CN group by a methylene group retains appreciably the antibacterial activity of cyanoacetic acid hydrazide.

3. Modification of the CN group into easily derivable groups, viz., carboxy, amidino, aminomethyl, etc. reduced considerably the antibacterial activity of cyanacetic acid hydrazide.

4. Certain hydrazones of cyanacetic acid hydrazide retain or increase the original activity.

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