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STUDIES IN THE CHEMOTHERAPY OF TUBERCULOSIS: PART VII. THE OXIDATION AND REDUCTION PRODUCTS OF THIOSEMICARBAZONES

BY

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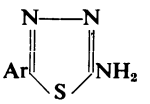
A degree of antituberculous "activity" in experimental animals has been detected with certain benzaldehyde thiosemicarbazones (I) by a number of workers (Domagk, Behnisch, Mietzsch, and Schmidt, 1946; Hoggarth, Martin, Storey, and Young, 1949). One compound of this type (*p*-acetylaminobenzaldehyde thiosemicarbazone) has had extensive clinical trials in Germany, and these trials have been critically reviewed by Hinshaw and McDermott (1950). In man, therapeutic effect is accompanied by a considerable risk of toxic side-effects, and we have made attempts to improve upon the activity and reduce the toxicity of these drugs by chemical modification of them.

TABLE I

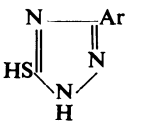
ANTITUBERCULOUS TESTS ON SOME 2-AMINO-5-PHENYL-1:3:4-THIADIAZOLES (A) AND 3-PHENYL-1:2:4-TRIAZOLE-5-THIOLS (B)

Doses given twice daily by syringe and catheter

Compounds of the forms:



(A)



(B)

No.	Form	Ar	Dose (mg. per 20 g. mouse)	Increased mean survival time (days)	Increase required for significance (days)
6252	A	phenyl	0.25	−0.4	1.1
			0.5	−1.1	
6289	A	*p*-anisyl	2.0	−0.8	1.1
6560	A	*p*-nitrophenyl	0.25	−0.6	1.0
			0.5	−0.2	
6794	B	phenyl	10.0	−0.9	1.6
6719	B	*p*-anisyl	2.0	−0.6	1.4
			4.0	−0.1	
7262	B	*p*-chlorophenyl	8.0	0.1	1.6

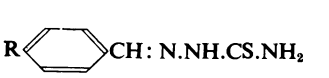
Experimental Methods

The test method consisted of the infection of mice by the intravenous route, and their treatment with drugs administered twice daily by syringe and catheter or by mixing with the food, at doses ranging downwards from the maximum tolerated ; the method has already been described in detail (Martin, 1946 ; Hoggarth and Martin, 1948). Drugs were given for a period of about two weeks.

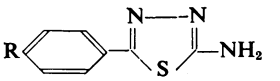
Results

It is characteristic of aldehyde thiosemicarbazones as a class that, under mild oxidizing conditions, cyclization takes place, with formation either of 2-amino-5-phenyl-1 : 3 : 4-thiadiazoles (II), or of 3-phenyl-1 : 2 : 4-triazole-5-thiols (III) (Young and Eyre, 1901 ; De and Roy-Choudhury, 1928). We have developed improved methods for the preparation of these cyclized compounds (Hoggarth, 1949) and have examined in tuberculous mice the examples listed in Table I. During the course of the chemical investigations, a number of related cyclic structures were synthesized, and some twenty compounds belonging to the following closely related classes were also examined. No compound showed any activity.

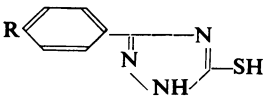
2-alkylamino-5-phenyl-1 : 3 : 4-thiadiazoles
2-amino-5-phenyl-1 : 3 : 4-oxadiazoles
2-alkylamino-5-phenyl-1 : 3 : 4-oxadiazoles
4-alkyl-3-phenyl-4 : 1 : 2-triazole-5-thiols
5-hydroxy-3-phenyl-1 : 2 : 4-triazoles
5-amino-3-phenyl-1 : 2 : 4-triazoles
4 : 5-diamino-3-phenyl-4 : 1 : 2-triazoles



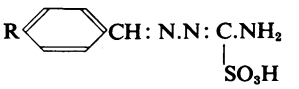
(I)



(II)



(III)



(IV)

(V)

(VI)

Whilst the oxidation of thiosemicarbazones with cold " perhydrol " in acetic acid was being studied, a group of novel sulphonic acids (IV) was isolated (Hoggarth, 1951, patent protection pending). Examination of the sulphonic acids listed in Table II showed that certain of these compounds had " activity " about equal to that of the corresponding thiosemicarbazones. We have also shown (Hoggarth and Young, 1950) that mild reduction of thiosemicarbazones gives 1-benzylthiosemicarbazides (V). These compounds were also examined and found to show

TABLE II

ANTITUBERCULOUS TESTS ON SOME 1-AMINO-4-PHENYL-2 : 3-DIAZABUTA-1 : 3-DIENE-1-SULPHONIC ACIDS

Doses given mixed with powdered food; the dose quoted is the estimated daily intake per 20 g. mouse per day

Compounds of the form: $ArCH{:}\ N.N{:}\ C(SO_3H).NH_2$

No.	Ar	Dose (mg.)	Increased mean survival time (days)	Increase required for significance (days)
8243	phenyl	20.0	3.4	1.2
8244	*p*-anisyl	20.0	−0.2	1.2
9111	*p*-ethylsulphonylphenyl	16.0	10.4	1.7
9669	*p*-acetylaminophenyl	16.0	4.4	1.3
10487	*p*-tolyl	20.0	−0.2	
		10.0	−0.1	2.2
		4.0	−0.4	
10488	*p*-chlorophenyl	10.0	1.8	1.8
		4.0	−1.7	

"activity" in general about equal to that of the parent thiosemicarbazones (Table III). No activity was found among a group of closely related compounds which included the types listed below. The first two classes are positional isomers of the reduction products of thiosemicarbazones, and the second two classes are derived by reduction of substituted benzaldehyde semicarbazones and acetophenone thiosemicarbazones respectively.

2-benzylthiosemicarbazides

4-benzylthiosemicarbazides

1-benzylsemicarbazides

1 : 1′-phenylethylthiosemicarbazides

The previously mentioned sulphonic acids (IV) are readily hydrolysed and yield, according to the conditions, either benzaldehyde semicarbazones, which have been found to be inactive (Hoggarth, Martin, Storey, and Young, 1949), or benzaldehyde azines (VI). The results of the examination of some fifteen azines and related derivatives of hydrazine were also negative except for slight, though reproducible, activity with a dibenzoylhydrazine (1-*p*-aminobenzoyl-2-benzoyl-hydrazine, No. 8137). The hydrazine derivatives examined in this connexion were of the classes listed below. The last group of compounds arose from the first group by a facile cyclization.

1 : 2-dibenzoylhydrazines

benzhydrazides

2 : 5-diphenyl-1 : 3 : 4-oxadiazoles

TABLE III

ANTITUBERCULOUS TESTS ON SOME 1-BENZYLTHIOSEMICARBAZIDES

Doses given twice daily by syringe and catheter or mixed with powdered food. In the latter case the dose quoted is the estimated daily intake per 20 g. mouse

Compounds of the form: $ArCH_2NH.NH.CS.NH_2$

No.	Ar	Dose (mg. per 20 g. mouse)	Increased mean survival time (days)	Increase required for significance (days)
7314	phenyl	0.5	0.9	1.6
		1.0	−0.1	1.6
		0.4 mg. per day in food	1.3	1.3
7316	*p*-dimethylaminophenyl	5.0	4.3	1.6
		5.0	6.4	1.3
		5.0 mg. per day in food	7.0	1.3
7315	*p*-anisyl	2.0	2.9	2.5
		4.0	3.9	2.5
		5.0	6.1	1.5
		6.0	4.5	2.5
		5.0 mg. per day in food	8.7	1.7
7317	*p*-hydroxyphenyl	5.0	−1.1	1.8
		5.0	−2.0	2.5
		10.0	0.6	1.5
		10.0	1.0	2.5
		10.0 per day in food	1.7	1.3
9112	*p*-ethylsulphonylphenyl	10.0	9.8	1.7

DISCUSSION

The narrowness of the limits within which " activity " is found with compounds related to the thiosemicarbazones (which was noted in Part V of this series, Hoggarth, Martin, Storey, and Young, 1949) is again emphasized. The positive effects observed in tuberculous mice with 1-benzylthiosemicarbazides (Table III) were very similar to those previously reported for the parent thiosemicarbazones, and in view of the fact that these reduction products are very readily reoxidized to thiosemicarbazones (Hoggarth and Young, 1950) this was not surprising. It was also to be expected that the corresponding reduction products of benzaldehyde semicarbazones and acetophenone thiosemicarbazones would show no activity. The very low " activity " of 1-*p*-hydroxybenzylthiosemicarbazide (No. 7317) was, however, unexpected, since the corresponding thiosemicarbazone (No. 6082), previously examined, had shown high " activity." Behnisch, Mietzsch, and Schmidt (1950) have reported in general terms that 1-benzylthiosemicarbazides show antituberculous activity.

The results with the 1-amino-4-phenyl-2 : 3-diazabuta-1 : 3-diene sulphonic acids (Table II) do not parallel those with the parent compounds. Thus No. 8243, derived from an " inactive " thiosemicarbazone, produced a positive effect, whilst

No. 8244, which is similarly derived from an " active " parent, did not. It is possible that *in vivo* the 1-benzylthiosemicarbazides undergo oxidation to thiosemicarbazones, but it is much less likely that the sulphonic acids are reduced to the parent compounds. The sulphonic acids may therefore produce their effects by a mechanism distinct from that of the thiosemicarbazones, and the lack of parallelism in the tests with the sulphonic acids as compared with the corresponding parent compounds would not then be surprising. The oxidative cyclizations so characteristic of thiosemicarbazones do not appear to take place *in vivo*. Extracts from the blood of animals dosed with thiosemicarbazones do not show absorption bands corresponding to either of the known types of cyclization product (Spinks, 1949).

SUMMARY

Some sixty compounds derived from thiosemicarbazones by a variety of oxidative and reductive processes, or closely related to such compounds, were examined in tuberculous mice. Two groups of " active " compounds were found—viz., 1-benzylthiosemicarbazides and 1-amino-4-phenyl-2 : 3-diazabuta-1 : 3-diene sulphonic acids ; the activity was of the same order as that found in the parent series of thiosemicarbazones.

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