were refluxed in dry ethanol (100 ml.) until a clear solution was obtained. Excess ethanol and halide were distilled under reduced pressure, and the residue was extracted with water and filtered. Treatment of the filtrate with saturated $NaHCO_3$ afforded the product which was filtered off and crystallized from ethanol or aqueous ethanol. In two cases (**44** and **46**), the hydrochlorides of the product separated from the reaction mixture on cooling.

Acknowledgment.—The authors wish to thank Mrs. B. Richardson, Mr. D. Gell, and Mr. D. C. Mills for technical assistance.

Antiviral Chemotherapy. II. Structure–Activity Relationships in a Series of Isothiazolealdehyde and Ketone Thiosemicarbazones[1a]

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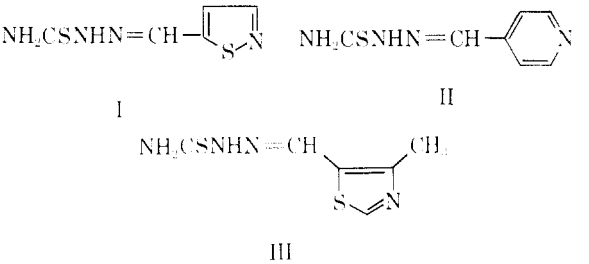
A series of isothiazolealdehyde and ketone thiosemicarbazones has been synthesized and tested for antiviral activity. Several of these compounds, in particular 4-bromo-5-formyl-3-methylisothiazole thiosemicarbazone,[1b] gave high protection to mice infected intracerebrally with the I.H.D. strain of neurovaccinia. Structure–activity relationships in the series are discussed.

We have reported previously[1a] that oral administration of 4-formylpyridine thiosemicarbazone affords marked protection to mice infected intracerebrally with neurovaccinia. It was further demonstrated that nuclear substitution abolished or reduced antiviral activity and that, of several possible metabolites, only 3-mercapto-5-(4-pyridyl)-1,2,4(H)-triazole had comparable activity. The present communication is concerned with another line of development, *viz.*, the synthesis and evaluation of thiosemicarbazones of heterocyclic carbonyl compounds chemically analogous to 4-formylpyridine thiosemicarbazone.

Our preliminary investigations showed that thiosemicarbazones derived from simple thiophene, furan, imidazole, and pyrazole carbonyl compounds were of little interest. Furthermore, 5-formyl-4-methylthiazole thiosemicarbazone, which was reported to be highly active against an intranasal infection of vaccinia in mice,[2] was only marginally active in our tests using the intracerebral route for infection. However, isothiazolecarbonyl thiosemicarbazones showed promising activity and accordingly a series was prepared and tested (Table I).

It is clear that antiviral activity is markedly dependent on the position of the thiosemicarbazone moiety in the isothiazole ring (compare **1**, **2**, and **3**). If isothiazole is regarded as electronically and geometrically related to pyridine, *e.g.*, the –S– of the former replacing –CH=CH– of the latter, activity in the two series follows a similar pattern Thus, 5-formylisothiazole thiosemicarbazone (I) and the analogous 4-formylpyridine thiosemicarbazone (II) are both active, whereas thiosemicarbazones of 4-formylisothiazole and 3-formylpyridine are only weakly active, and 3-formylisothiazole and 2-formylpyridine thiosemicarbazones are totally inactive.[1a] The weak activity of the formylthiazole thiosemicarbazone (III) is explainable by its formal analogy to the weakly active 3-formylpyridine thiosemicarbazone.



I II

III

In the 5-formylisothiazole series, substitution by halogen in the 4-position increases activity (compare **2**, **11**, **12**, and **13**) with a decrease in toxicity, particularly the bromo derivative **12**. A 3-methyl substituent decreases toxicity slightly without decreasing activity (**2** and **4**), and a combination of a 3-methyl and a 4-chloro or -bromo substituent leads to highly active compounds with low toxicity (**15** and **16**). 4-Nitro and 4-methyl substituents have little effect on activity (compare **4** and **19**, and **2** and **5**) but a carboxy substituent (**18**) completely abolishes activity. Compounds substituted in the side chain are, in general, inactive (**7**, **8**, **21**, and **27–39**) with the exception of the highly active 5-acetyl-3-methyl derivative (**9**) and the marginally active 4-bromo-5-formyl-3-methylisothiazole 2-methylthiosemicarbazone (**26**). Cyclization of the side chain of an active thiosemicarbazone to a mercaptotriazole (compare **4** and **41**, and **16** and **42**) or an aminothiadiazole (compare **4** and **43**) considerably reduced or eliminated activity.

Six compounds (**9**, **11–13**, **15**, and **16**) satisfied our criterion for significant activity, namely, they more than doubled the mean survival time. Of these, three were of relatively low toxicity (**12**, **15**, and **16**) and one (**16**, M&B 7714) was eventually selected for more extended biological evaluation[3] and clinical trials against smallpox.[4]

No activity was shown by any of the thiosemicarbazones against murine infections of influenza virus,

(1) (a) Part I: D. H. Jones, R. Slack, S. Squires, and K. R. H. Wooldridge, *J. Med. Chem.*, **8**, 676 (1965). (b) M&B 7714.

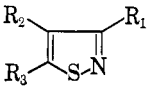
(2) E. Campaigne, R. L. Thompson, and J. E. Van Werth, *J. Med. Pharm. Chem.*, **1**, 577 (1959).

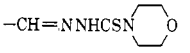
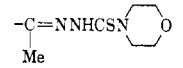
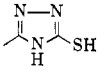
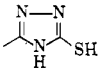
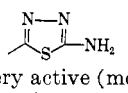
(3) R. Slack, K. R. H. Wooldridge, J. A. McFadzean, and S. Squires, *Nature*, **204**, 587 (1964).

(4) J. A. McFadzean, A. R. Rao, and S. Squires, *Proc. N. Y. Acad. Sci.*, in press.

TABLE I

THE ANTIVIRAL ACTIVITY OF ISOTHIAZOLEALDEHYDE AND KETONE THIOSEMICARBAZONES AND RELATED COMPOUNDS AGAINST NEUROVACCINIA IN MICE



No.	R_1	R_2	R_3	Oral LD_{50}, mg./g.	Daily dose, (mg./g.) × 4	Mean survival time, days: Treated	Mean survival time, days: Controls	Activity[a]
1[b]	$-CH=NNHCSNH_2$	H	H	0.2	0.05	6.6	6.0	0
2[c]	H	H	$-CH=NNHCSNH_2$	0.5	0.12	9.3	5.3	++
3[d]	Me	$-CH=NNHCSNH_2$	H	0.15	0.04	8.8	6.0	+
4[d]	Me	H	$-CH=NNHCSNH_2$	0.7	0.15	9.0	5.4	++
5[c]	H	Me	$-CH=NNHCSNH_2$	0.27	0.07	10.0	5.3	++
6[b]	$-(CMe)=NNHCSNH_2$	H	H	0.5	0.125	4.4	5.0	0
7[e]	H	H	$-C(Me)=NNHCSNH_2$	0.5	0.125	4.7	5.0	0
8[d]	Me	$-C(Me)=NNHCSNH_2$	H	1.33	0.33	4.7	5.0	0
9[d]	Me	H	$-C(Me)=NNHCSNH_2$	0.11	0.03	10.2	4.9	+++
10[b]	$-CH=NNHCSNH_2$	Br	H	>4.0	1.0	5.5	5.0	0
11[c]	H	Cl	$-CH=NNHCSNH_2$	0.5	0.12	10.8	5.0	+++
12[c]	H	Br	$-CH=NNHCSNH_2$	>4.0	1.0	11.5	5.0	+++
13[c]	H	I	$-CH=NNHCSNH_2$	2.0	0.5	10.0	4.9	+++
14[b]	$-C(Me)=NNHCSNH_2$	Br	H	1.47	0.37	4.0	6.0	0
15[c]	Me	Cl	$-CH=NNHCSNH_2$	>4.0	1.0	12.2	6.0	+++
16[d]	Me	Br	$-CH=NNHCSNH_2$	>4.0	1.0	12.7	4.8	+++
17[c]	Me	I	$-CH=NNHCSNH_2$	>4.0	1.0	10.3	6.0	++
18[c]	Me	CO_2H	$-CH=NNHCSNH_2$	>4.0	1.0	4.9	5.0	0
19[e]	Me	NO_2	$-CH=NNHCSNH_2$	>4.0	1.0	9.4	5.0	++
20[e]	Me	H	$-C(CH_2CO_2Et)=NNHCSNH_2$	>4.0	0.5	5.4	5.3	0
21[d]	Me	Br	$-C(Me)=NNHCSNH_2$	0.07	0.02	4.7	4.6	0
22[e]	Me	H	$-CH=NNHCSNHCH_2CH_2OH$	>4.0	1.0	5.5	5.3	0
23[e]	Me	H	$-CH=NNHCSNHCH_2CH_2OEt$	>4.0	1.0	5.0	5.1	0
24[e]	Me	H	$-CH=NNHCSNH(CH_2)_3OMe$	1.26	0.31	6.1	5.3	0
25[e]	Me	$-C(Me)=NNHCSNH(CH_2)_2OH$	H	>4.0	1.0	5.9	5.0	0
26[e]	Me	Br	$-CH=NN(Me)CSNH_2$	>4.0	1.0	9.1	6.4	++
27[e]	Me	H	$-CH=NNHCSNMe_2$	3.16	0.79	5.6	8.0	0
28[e]	Me	Br	$-CH=NNHC(SMe)=NH\cdot HI$	0.93	0.23	7.6	6.0	+
29[e]	Me	Br	$-CH=NNHCSNHMe$	>4.0	1.0	5.4	5.3	0
30[e]	Me	Br	$-C(Me)=NNHCSNHMe$	>4.0	1.0	4.6	4.6	0
31[e]	Me	Br	$-CH=NNHCSNMe_2$	>4.0	1.0	5.7	6.0	0
32[e]	Me	Br	$-CH=NNHCSNHCH_2CH_2OH$	>4.0	1.0	5.5	5.3	0
33[e]	Me	Br	$-C(Me)=NNHCSNH(CH_2)_2OH$	>4.0	1.0	6.0	5.3	0
34[e]	Me	Br	$-CH=NNHCSNHCH_2CH_2OEt$	>4.0	1.0	5.0	5.0	0
35[e]	Me	Br	$-C(Me)=NNHCSNH(CH_2)_3OMe$	2.71	0.68	4.7	5.3	0
36[e]	Me	Br		>4.0	1.0	5.0	5.0	0
37[e]	Me	Br		0.91	0.23	4.5	4.6	0
38[e]	Me	Br	$-CH=NNHCSNHPh$	>4.0	1.0	5.9	6.0	0
39[e]	Me	Br	$-CH=NNHCSNHCH_2Ph$	>4.0	1.0	5.3	5.0	0
40[e]	Me	Br	$-CH=NNHCONH_2$	>4.0	1.0	6.0	5.1	0
41[f]	Me	H		>4.0	1.0	5.9	5.1	0
42[f]	Me	Br		2.71	0.68	7.4	5.1	+
43[f]	Me	H		>4.0	1.0	6.1	5.1	0

[a] 0 = inactive, + = marginal activity, ++ = active, +++ = very active (more than doubles the mean survival time). [b] Ref. 8. [c] Ref. 7. [d] Ref. 11. [e] See Table II. [f] Described in Experimental section.

Rift Valley fever virus, or encephalomyocarditis (Columbia SK) virus.

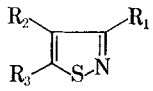
Experimental

Biological Methods.—The same technique was employed as described previously.[1a] The mice were infected intracerebrally with a suitable dilution of the virus (*Neurovaccinia* I.H.D. strain), and the test compound was administered orally once daily for 4 days. Mortalities were recorded daily, and the mean survival time was determined.

Syntheses.[5]—All melting points were determined on an Electrothermal instrument and are corrected.

(5) Microanalyses were performed by Mr. S. Bance and his staff.

TABLE II



No.	R_1	R	R_3	M.p., °C.	Formula	Calcd., % C	H	N	S	Found, % C	H	N	S
7	H	H	$-C(Me)=NNHCSNH_2$	204–205[a]	$C_6H_8N_4S_2$	36.0	4.0		32.0	35.9	3.6		31.9
19	Me	NO_2	$-CH=NNHCSNH_2$	191–192[a]	$C_6H_7N_5O_2S_2$				26.1				25.8
20	Me	H	$-C(CH_2CO_2Et)=NNHCSNH_2$	152–154	$C_{10}H_{14}N_4O_2S_2$	42.0	4.9			42.4	5.3		
22	Me	H	$-CH=NNHCSNHCH_2CH_2OH$	188–190	$C_8H_{12}N_4OS_2$	39.4	4.9		26.2	39.0	5.1		26.3
23	Me	H	$-CH=NNHCSNHCH_2CH_2OEt$	145–147	$C_{10}H_{16}N_4OS_2$	43.8	5.9	20.2		44.2	5.9	20.6	
24	Me	H	$-CH=NNHCSNH(CH_2)_3OMe$	134–139	$C_{10}H_{16}N_4OS_2$			20.7				20.7	
25	Me	$-C(CH_3)=NNHCSNHCH_2CH_2OH$	H	182–185	$C_9H_{14}N_4OS_2$	41.9	5.4		24.8	42.1	5.5		24.4
26	Me	Br	$-CH=NNCSNH_2$ (Me)	223–224[a]	$C_7H_9BrN_4S_2$[b]				21.8				22.1
27	Me	H	$-CH=NNHCSNMe_2$	168–170[a]	$C_8H_{12}N_4S_2$	42.0	5.3		28.2	42.0	5.1		28.0
28	Me	Br	$-CH=NNHC(SMe)=NH\cdot HI$	197–201[a]	$C_7H_{10}BrIN_4S_2$				15.1				14.7
29	Me	Br	$-CH=NNHCSNHMe$	234–236[a]	$C_7H_9BrN_4S_2$[c]	28.7	3.1			28.6	3.3		
30	Me	Br	$-C(CH_3)=NNHCSNHMe$	181–184	$C_8H_{11}BrN_4S_2$[d]			18.2	20.9			18.4	20.7
31	Me	Br	$-CH=NNHCSNMe_2$	180–183[a]	$C_8H_{11}BrN_4S_2$			18.2	20.9			18.2	20.8
32	Me	Br	$-CH=NNHCSNHCH_2CH_2OH$	208–211[a]	$C_8H_{11}BrN_4OS_2$				19.8				19.7
33	Me	Br	$-C(Me)=NNHCSNHCH_2CH_2OH$	176–179	$C_9H_{13}BrN_4OS_2$	32.1	3.8		19.0	32.5	4.3		19.1
34	Me	Br	$-CH=NNHCSNHCH_2CH_2OEt$	171–175	$C_{10}H_{15}BrN_4OS_2$[e]	34.2	4.3			34.5	5.2		
35	Me	Br	$-C(Me)=NNHCSNH(CH_2)_3OMe$]	81–85	$C_{11}H_{17}BrN_4OS_2$			15.4	18.1			15.9	17.8
36	Me	Br		193–195	$C_{10}H_{13}BrN_4OS_2$			16.1				16.4	
37	Me	Br		163–165	$C_{11}H_{15}BrN_4OS_2$			15.4	17.6			15.5	17.9
38	Me	Br	$-CH=NNHCSNHPh$	189–193[a]	$C_{12}H_{11}BrN_4S_2$	40.6	3.1		18.0	41.1	3.1		18.0
39	Me	Br	$-CH=NNHCSNHCH_2Ph$	191–193	$C_{13}H_{13}BrN_4S_2$			15.2	17.3			15.7	17.4
40	Me	Br	$-CH=NHCONH_2$	239–241[a]	$C_6H_7BrN_4OS$	27.9	2.7		12.2	27.7	2.7		12.4

[a] Decomposed. [b] *Anal.* Calcd.: Br, 27.4. Found: Br, 27.0. [c] *Anal.* Calcd.: Br, 27.4. Found: Br, 27.3. [d] *Anal.* Calcd.: Br, 26.0. Found: Br, 25.8. [e] *Anal.* Calcd.: Br, 22.5. Found: Br, 22.5.

General Method for the Preparation of Thiosemicarbazones (See Table II).—The carbonyl compound (0.1 mole) was heated with a solution of the thiosemicarbazide (0.1 mole) in ethanol or methanol (200–400 ml.) containing a few drops of glacial acetic acid. The mixture was refluxed for 2–3 hr. and then concentrated to *ca.* 100 ml. The thiosemicarbazone obtained on cooling was recrystallized from ethanol, aqueous ethanol, or ethanol and dimethylformamide. In a few cases, the carbonyl compound in methanol was added to a solution of the thiosemicarbazide in concentrated HCl and methanol. The thiosemicarbazone separated on standing and was recrystallized from a suitable solvent.

The thiosemicarbazides[6] and some of the carbonyl compounds[7,8] were prepared by published methods; the remainder are described below.

5-Acetylisothiazole.—Isothiazole-5-carboxylic acid[7] and thionyl chloride gave isothiazole-5-carbonyl chloride (91%), b.p. 82° (26 mm.).

Anal. Calcd. for C_4H_2ClNOS: C, 32.5; H, 1.4; S, 21.7. Found: C, 32.7; H, 1.5; S, 21.7.

The acid chloride and NH_3 gave isothiazole-5-carboxamide (64%), needles from water, m.p. 172–174°.

Anal. Calcd. for $C_4H_4N_2OS$: C, 37.5; H, 3.2; S, 25.0. Found: C, 37.5; H, 3.2; S, 24.8.

Isothiazole-5-carboxamide and $POCl_3$ at 100° for 1.5 hr. afforded 5-cyanoisothiazole (75%), needles from petroleum ether (b.p. 40–60°), m.p. 47–48°.

Anal. Calcd. for $C_4H_2N_2S$: N, 25.4; S, 29.1. Found: N, 25.0; S, 28.8.

5-Cyanoisothiazole and methylmagnesium iodide in ether were refluxed for 4 hr. to give 5-acetylisothiazole (69%), needles from petroleum ether (b.p. 60–80), m.p. 27°.

Anal. Calcd. for C_5H_5NOS: C, 47.2; H, 4.0; S, 25.2. Found: C, 47.3; H, 3.7; S, 25.0.

5-Formyl-3-methyl-4-nitroisothiazole Thiosemicarbazone (19). 3-Methyl-4-nitroisothiazole-5-carboxylic acid[9] and thionyl chloride gave the acid chloride (52%), b.p. 117–118° (9 mm.).

Anal. Calcd. for $C_5H_3ClN_2O_3S$: C, 29.1; H, 1.5; S, 15.5. Found: C, 29.5; H, 1.6; S, 15.6.

Lithium tri-*t*-butoxyaluminum hydride[10] (27.0 g., 0.105 mole) in ether (200 ml.) and diethylene glycol dimethyl ether (150 ml.) was added dropwise during 90 min. to a solution of 3-methyl-4-nitroisothiazole-5-carbonyl chloride (14.5 g., 0.07 mole) in the glycol ether (30 ml.) at −70° under nitrogen. After a further 5 min. the mixture was poured on to ice (*ca.* 600 g.) and extracted with three 150-ml. portions of ether. The extract was washed with 2 *N* Na_2CO_3 solution and water and dried ($MgSO_4$). Evaporation of the ether afforded the crude aldehyde; 2,4-dinitrophenylhydrazone, yellow prisms from benzene, m.p. 236–238°.

Anal. Calcd. for $C_{11}H_8N_6O_6S$: C, 37.5; H, 2.3; S, 9.1. Found: C, 37.8; H, 2.4; S, 9.1.

Treatment of the crude aldehyde with thiosemicarbazide afforded the thiosemicarbazone, yellow prisms from ethanol, m.p. 191–192° dec.

5-Hydroxymethyl-3-methyl-4-nitroisothiazole.—From the mother liquors of the preparation of the above thiosemicarbazone the hydroxymethyl compound crystallized as pale yellow plates from benzene–petroleum ether (b.p. 60–80°), m.p. 89–91°.

Anal. Calcd. for $C_5H_6N_2O_3S$: C, 34.5; H, 3.5; S, 18.4. Found: C, 34.8; H, 3.6; S, 18.2.

3-Mercapto-5-(4-bromo-3-methylisothiazol-5-yl)-1,2,4(H)-triazole (42).—Potassium thiocyanate (7.2 g., 0.074 mole) was added to a solution of 4-bromo-3-methylisothiazole-5-carboxyhydrazide[11] (17.5 g., 0.074 mole) in warm 1 *N* HCl (85 ml.). The reaction mixture was heated on the steam bath for 30 min., cooled, treated with 2 *N* NaOH solution (200 ml.), and kept at room temperature for 7 days. Acidification afforded the mercaptotriazole (9.2 g., 45%), prisms from ethanol, m.p. 263° dec.

Anal. Calcd. for $C_6H_5BrN_4S_2$: Br, 28.9; S, 23.1. Found: Br, 28.7; S, 22.9.

3-Mercapto-5-(3-methylisothiazol-5-yl)-1,2,4(H)-triazole (41), m.p. 294–296° dec., was prepared similarly.

Anal. Calcd. for $C_6H_6N_4S_2$: C, 36.4; H, 3.0; N, 28.3. Found: C, 36.4; H, 3.3; N, 28.4.

2-Amino-5-(3-methylisothiazol-5-yl)-1,3,4-thiadiazole (43).—5-Formyl-3-methylisothiazole thiosemicarbazone[11] (17.0 g., 0.085 mole) and acetic anhydride (80 ml.) were refluxed for 45 min., cooled, and poured onto ice to give 5-formyl-3-methylisothiazole N^4,S-diacetylthiosemicarbazone (23 g., 95%), prisms from dilute acetic acid, m.p. 185–186°.

Anal. Calcd. for $C_{10}H_{12}N_4O_2S_2$: C, 42.2; H, 4.2; S, 22.5. Found: C, 42.6; H, 4.2; S, 22.8.

The diacetyl compound was added during 30 min. to a solution at 0° of peracetic acid, prepared by adding 30% H_2O_2 (44 g.) dropwise to glacial acetic acid (220 g.) at 0°. The mixture was stirred for a further 30 min. at 0° and then the filtrate was cooled to give 2-acetamido-5-(3-methylisothiazol-5-yl)-1,3,4-thiadiazole (8.0 g., 41%), needles from dilute acetic acid, m.p. >320°.

Anal. Calcd. for $C_8H_8N_4OS_2$: C, 40.0; H, 3.3; N, 23.3. Found: C, 39.9; H, 3.5; N, 23.4.

The acetamido compound was refluxed with 4 *N* HCl (75 ml.) until a clear solution was obtained (1 hr.). The solution was cooled and neutralized with 2 *N* NH_4OH to give the aminothiadiazole (3.0 g., 45%), needles from dilute ethanol, m.p. 250–252°.

Anal. Calcd. for $C_6H_6N_4S_2$: C, 36.4; H, 3.0; S, 32.2. Found: C, 36.6; H, 3.0; S, 32.1.

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