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Some Heterocyclic Aldehyde Thiosemicarbazones Possessing Anti-viral Activity*

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Introduction

Although no chemotherapeutic agent has yet been shown to inhibit virus infections in man, the most active compounds in tests with laboratory animals appear to be certain thiosemicarbazones.[1] With the exception of benzaldehyde thiosemicarbazone and some of its derivatives, most of the thiosemicarbazones possessing the highest activity are those in which the thiosemicarbazone group, $=NNHCSNH_2$, is separated by two carbon atoms from a nitrogen or sulphur atom,[2] such as thiosemicarbazones of certain α-oximinoketones and 2-thiophenecarboxaldehydes. In view of these results it was of interest to prepare for anti-viral screening thiosemicarbazones of thiazole and imidazole aldehydes, in which any position of the carbonyl-thiosemicarbazone group on the heterocyclic ring was such that the desired structure was obtained.

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Discussion

Synthesis

Thiazole aldehydes were prepared by the Newman-Caflisch[3] modification of the McFadyen-Stevens[4] synthesis. The monocyclic thiazole esters needed as intermediates were prepared by the reaction of appropriate α-halocarbonyl compounds with thioamides. Ethyl 2-benzothiazolecarboxylate was most conveniently prepared directly from ethyl oxalate and 2-aminobenzenethiol,[5] but was also prepared by esterification of the acid obtained by permanganate oxidation of 2-hydroxymethylbenzothiazole. The required hydrazides were obtained by reaction of the ethyl esters with hydrazine hydrate in the presence of a little alcohol to ensure a homogeneous solution. In the conventional reflux procedure,[6] the 5-carbethoxythiazoles gave poor yields of hydrazides, often with the formation of red colours and accompanied by high-melting compounds, presumably diacylhydrazines. Similar observations were reported by Erlenmeyer, Mengesen, and Prijs,[7] who obtained improved results by using methyl 5-thiazolecarboxylate in place of the ethyl ester. Indeed, we found that the methyl ester of 4-methyl-5-thiazolecarboxylic acid too, gave better yields than its ethyl ester under reflux conditions, but that smooth reactions and much improved yields could be obtained from the ethyl esters of 5-thiazolecarboxylic acids if the reaction mixtures were allowed to stand at room temperature for one to five days instead of being heated. No difficulty was experienced in the reactions of the 2- and 4-carbethoxythiazoles with hydrazines at elevated temperatures. The data are given in Table I.

Yields of the aldehydes in the McFadyen-Stevens reaction varied according to the position of the formyl group on the thiazole ring, decreasing in the order 5- > 4- > 2-. Because of the low yields of 2-thiazolecarboxaldehydes, 2-benzothiazolecarboxaldehyde was more conveniently prepared by selenium dioxide oxidation[8] of 2-hydroxymethylbenzothiazole. Some discussion of this carbinol is in order. Zubarovskii reported a m.p. of 90–100° for the crude product[8] from the reaction of glycolic acid and 2-aminobenzenethiol, and a m.p. of 101° after recrystallization from toluene.[9] On repeating this work, we could not obtain a sharply-melting product by repeated recrystallizations from

Table I. Preparation of thiazolecarboxyhydrazides

$$ThCOOR + N_2H_4.H_2O \xrightarrow{C_2H_5OH} ThCONHNH_2$$

Ester used[a]	Ester, moles	$N_2H_4.H_2O$, moles	Abs. EtOH, ml	Temp.	Reaction time	Hydrazide yield, %	Hydrazide m.p., °C
5-ThCOOC$_2$H$_5$	0·063	0·12	4·5	Room	1 day	61	161–162[b]
	0·032	0·040	6	Reflux	2·5 h	<4	—
4-CH$_3$-5-ThCOOC$_2$H$_5$	0·018	0·036[c]	15	Room	2 days	42	167·0–167·8[d]
	0·380	0·758	31[e]	Reflux	24 h[f]	21[g]	167·2–167·7
4-CH$_3$-5-ThCOOCH$_3$	0·088	0·20	0[h]	Reflux	2 h	51[g]	166·5–167·1
	0·688	0·743	200	Reflux	3 h	30[i]	—
4-CH$_3$-2-CH$_3$S-5-ThCOOC$_2$H$_5$	0·092	0·204	30	Room	5 days	84	160·5–161·3[j]
	0·009	0·020	3	Reflux	2 h	48	148–152
4-ThCOOC$_2$H$_5$	0·136	0·275	30	Reflux	1 h	90[k]	143–144[l]
4-CH$_3$-2-ThCOOC$_2$H$_5$	0·082	0·098	20	Reflux	0·5 h	79[k]	147·4–148·8[m]
2-BenzoThCOOC$_2$H$_5$	0·100	0·120	30[n]	Reflux	0·25 h	95	175–175·6[o]

[a] Th = thiazole. [b] After recrystallization from ethanol, m.p. 163–164° (reference 10: 164°). [c] An additional 1·5 ml of $N_2H_4.H_2O$ was added after one day. [d] Reference 11: 166°. [e] Methanol was used as solvent in this experiment. [f] Other attempts, using shorter heating times, also gave low yields. [g] Only high melting solids were obtained on concentration of the mother liquor. [h] Twenty ml of abs. ethanol added at end of heating period. [i] Unchanged ester obtained on concentration of the mother liquor. [j] After recrystallization from abs. ethanol, m.p. 162·2–162·5°; *Anal.* Calcd. for $C_6H_9ONS_2$: N, 20·67. Found: N, 20·69. [k] A small portion of the product was obtained from the mother liquor. [l] Reference 6: 143–144°. [m] Reference 10: 149°. [n] 95% ethanol. [o] Reference 5.

benzene, toluene, or xylene. Mixtures melting over a range of 2–4° in the region from 95°–102° were obtained. Recrystallization from carbon tetrachloride, on the other hand, yielded well-defined crystals melting at 93–94°. The identification of this material with 2-hydroxymethylbenzothiazole was confirmed by elemental and infrared analysis, oxidation both to the corresponding aldehyde and carboxylic acid and conversion of the latter to its ethyl ester. Recrystallization of the pure carbinol from toluene produced the higher melting mixture, from which the carbinol could again be isolated by recrystallization from dilute ethanol, but not from carbon tetrachloride. The identity of the contaminant was not investigated.

Although Seyhan and Avan[12] reported no reaction when 2-methylbenzimidazole was heated with selenium dioxide in moist dioxan for 1 h, we obtained approximately a 10 per cent yield of 2-benzimidazolecarboxaldehyde by heating for several hours, and were able to recover most of the unreacted starting material. When the corresponding alcohol, 2-hydroxymethylbenzimidazole, was used in place of the methyl compound, the yield of crude aldehyde was increased to 77 per cent. The aldehyde was most conveniently isolated and purified as its hydrochloride. No aldehyde could be obtained by oxidation of 2-methylbenzimidazole with chromic acid in acetic anhydride, nor by reaction of 2-chloromethylbenzimidazole with hexamethylenetetramine.

Thiosemicarbazones of several other aromatic and aliphatic aldehydes were prepared for evaluation in addition to the thiazole and imidazole derivatives. These are described in the experimental section.

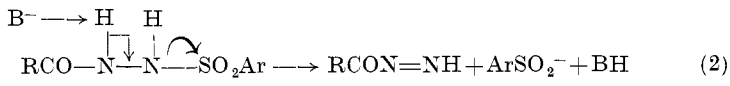
Mechanism of the McFadyen-Stevens Reaction

The general equation for this reaction is:

$$RCONHNHSO_2Ar + OH^- \longrightarrow RCHO + ArSO_2^- + N_2 + H_2O \qquad (1)$$

Low yields of aldehyde have commonly been encountered when R is electron-deficient, as in the *o*- and *p*-nitrobenzoyl derivatives[13] and heteroyl derivatives.[11,14,15] It has been suggested[13,15] that electron-withdrawing effects in R lead to increased formation of the diacylhydrazide, thus lowering the yield of aldehyde.

McFadyen and Stevens[4] suggested that the first step in the reaction leading to the aldehyde was the formation of an acyldiimide, which Dhar, *et al.*[16] proposed could occur by a bimolecular elimination (equation 2). The decomposition of the acyldiimide



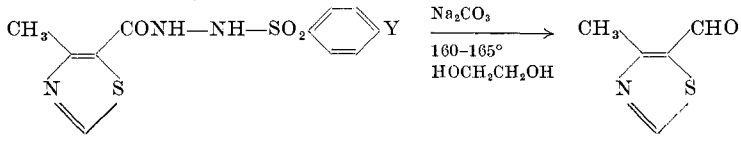
$$B^- \longrightarrow H \quad H$$
$$RCO{-}N{-}N{-}SO_2Ar \longrightarrow RCON{=}NH + ArSO_2^- + BH \qquad (2)$$

$$RCON{=}NH \xrightarrow{\Delta} RCHO + N_2 \qquad (3)$$

(equation 3) relates the whole process to the synthesis of aldehydes by the oxidative decomposition of acylhydrazides.[17,18]

The above mechanism suggests that electron-withdrawing substituents on the benzenesulphonyl group might increase the yield of aldehyde by facilitating the elimination step. Therefore the effects of *para* substituents on the benzenesulphonyl group on the yield of aldehyde in the conversion of 1-arylsulphonyl-2-(4-methyl-5-thiazolecarbonyl)hydrazine were investigated. The results are shown in Table II. The yield of aldehyde decreased

Table II. Conversion of *p*-substituted benzenesulphonylhydrazides to aldehyde

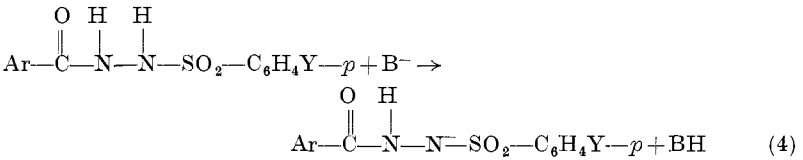


$$CH_3\text{-}C(CONH{-}NH{-}SO_2{-}C_6H_4{-}Y){=}N\text{-}CH{=}C(S\text{-}) \xrightarrow[160\text{–}165^\circ\ HOCH_2CH_2OH]{Na_2CO_3} CH_3\text{-}C(CHO){=}N\text{-}CH{=}C(S\text{-})$$

Y	Sulphonyl hydrazide, moles	Reaction time, min	Yield, %	m.p., °C[a]
H	0·0337	10	37	73·7–75·0
H	0·0337	5	42	71·5–73·7
H	0·0337	2	47	69 – 73·5
CH_3O	0·0337	2	51	68 – 74
Br	0·0237	2	37	65 – 73
NO_2	0·0281	2	25	59 – 70
NO_2	0·0339	2	23	64 – 72

[a] Crude. After recrystallization from 63–99° petroleum ether, m.p. 74·5–75·3° (Reference 11: 75°).

with the variation of the *para* substituent in the order CH_3O > H > Br > NO_2. These results are contrary to what was expected on the basis of the previously proposed mechanism. They may be



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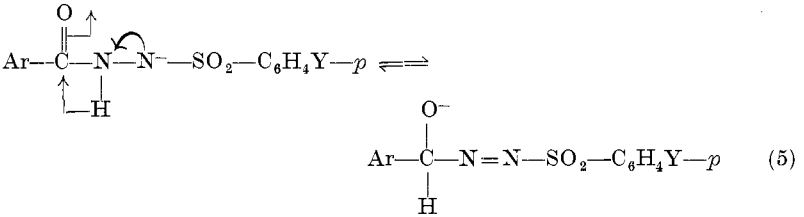

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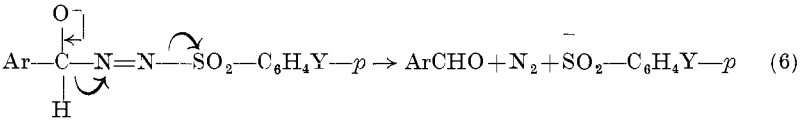
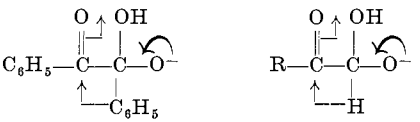


(6)

due to increased conversion to diacylhydrazide in the negatively-substituted benzenesulphonyl derivatives, as was suggested for the negatively-substituted aroylhydrazides.[13] On the other hand, these results are in line with a second possible mechanism for the McFadyen-Stevens reaction.



(I) (II)

Sulphonamides are acidic substances, and the expected initial reaction of a base with a sulphonylhydrazide would be rapid extraction of a proton from the sulphonamide nitrogen (equation 4).* The resulting anion may participate in a 1,2-hydride shift, as

* Newman and Caflisch[3] recently reported that the sodium salt of 1-benzene sulphonyl-2-benzoylhydrazine decomposed in ethylene glycol at 160° in the presence of a surface-active catalyst to give benzaldehyde in high yield.

shown in equation 5.* Such shifts should be inhibited when Y is electron-withdrawing and promoted when Y is electron-donating. The resulting sulphonylazoalkoxide ion could finally decompose to the aldehyde, nitrogen and the arylsulphinate ion (equation 6). Further experimental work to test this mechanism is under way.

Anti-viral evaluation

The International Health Division (IHD) strain of vaccinia virus was used in tests to determine the anti-viral activity of the thiosemicarbazones. In some experiments the virus was inoculated intracerebrally while in others the nasal route was employed. The inoculum for intracerebral injection consisted of an appropriate dilution of infected mouse brain tissue. Intranasal inoculations were made with diluted ascitic fluid from infected, tumour-bearing mice. A suspension of the Lettré strain of Ehrlich ascites tumour cells was injected intraperitoneally into 20 to 25 g Taconic Swiss mice. Vaccinia virus was inoculated by the same route 6 days later and ascitic fluid removed after an incubation period of 3 days. After storage overnight at 4°C, the fluid was shell-frozen, thawed at room temperature, and centrifuged at 3,000 rev/min for 15 min. The supernate was removed and stored at 4°C; when diluted, this constituted the inoculum. The quantities of virus administered in the different experiments were sufficient to produce fatal infections in from 75 to 100 per cent of untreated mice.

Taconic Swiss white female mice, weighing from 12 to 14 g, were distributed in groups of 10 in solid-bottom, stainless steel cages. Test compounds were mixed into ground Purina dog checkers and fed *ad lib.* for a period of one week, commencing one day before the inoculation of virus. All inoculations were made while the animals were under ether anaesthesia. The inoculum was 0·03 ml. Infected mice were observed for a period of 13 days.

The results obtained from the experiments are expressed in terms of the increased survival rate, in per cent of treated as compared to untreated mice. Thus, if 5 of 10 treated and 5 of 20 untreated animals survived, the survival rates would be 50 and

* The mechanism proposed here is similar to mechanisms involving 1,2-shifts proposed for other base-catalyzed reactions, such as the benzilic acid rearrangement of intermediate (I)[19] and the internal Cannizzaro reaction,[20] (II).

25 per cent, respectively, or an increased survival rate of 25 per cent.

The results of anti-viral tests made in mice inoculated by the cerebral route are presented in Table III and those obtained from tests with intranasally inoculated animals are presented in Table IV.

Table III. Anti-viral activity of thiosemicarbazones in mice inoculated intracerebrally with vaccinia virus

Thiosemicarbazone	Concn. in diet, %	Excess survivors, %[a]
3,4-Diethoxybenzaldehyde	0·1	10
2,4-Dihydroxybenzaldehyde	0·1	5
2-Hydroxy-3-methoxybenzaldehyde	0·04	0
	0·1	−10
o-Nitrobenzaldehyde	0·04	10
2,3-Dimethoxybenzaldehyde	0·04	10
2,4-Dichlorobenzaldehyde	0·1	−5
	0·04	0
o-Methoxybenzaldehyde	0·1	10
2,6-Dichlorobenzaldehyde	0·04	30
Propionaldehyde	0·1	12
*Iso*butyraldehyde	0·1	10
Heptaldehyde	0·1	−10
Isatin	0·1	67
	0·03	80
	0·02	29

[a] Per cent survivors in treated group minus per cent survivors in control group.

It has been observed that vaccinia infection induced in mice by the intranasal inoculation of virus may respond more readily to thiosemicarbazones than infections produced by intracerebral inoculation of the virus. This is illustrated by the results obtained in tests with 2,6-dichlorobenzaldehyde thiosemicarbazone. When fed in the diet in a concentration of 0·04 per cent, the survival rate of intracerebrally inoculated mice was increased by 30 per cent as compared to increases of 45 and 65 per cent in intranasally

Table IV. Anti-viral activity of thiosemicarbazones in mice inoculated intranasally with vaccinia virus

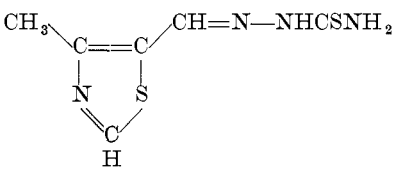
Thiosemicarbazone	Concn. in diet, %	Excess survivors, %[a]
2,4-Dichlorobenzaldehyde	0·025	24
2,6-Dichlorobenzaldehyde	0·1	55
	0·05	65
	0·025	45
	0·01	44
Propionaldehyde	0·01	34
*Iso*butyraldehyde	0·01	34
Hexaldehyde	0·01	14
Heptaldehyde	0·01	−14
4-Nitro-2-thenaldehyde	0·1	57
	0·01	34
	0·0025	10
2-Benzimidazolecarboxaldehyde	0·1	−10
	0·05	0
	0·01	0
Isatin 2-Benzothiazolylhydrazone	0·1	0
	0·025	0
Glyoxal (Dithiosemicarbazone)	0·05	0
	0·01	0
4-Methyl-5-thiazolecarboxaldyhyde	0·1	60
	0·05	90
	0·025	70
	0·01	80
4-Thiazolecarboxaldehyde	0·1	10
	0·025	−10
	0·01	10
4-Methyl·2-methylthio-5-thiazolecarboxaldehyde	0·1	50
	0·025	10
2-Benzothiazolecarboxaldehyde	0·1	60
	0·05	40
	0·025	40
	0·01	30
2,3·Butanedione oxime	0·1	76
	0·05	66
	0·01	56
	0·025	0
Isatin	0·2	75
Benzaldehyde	0·05	50
	0·01	40

[a] Per cent survivors in treated group minus per cent survivors in control group.

infected animals fed 0·025 and 0·05 per cent, respectively, of the compound.

4-Methyl-5-thiazolecarboxaldehyde thiosemicarbazone demonstrated greater anti-vaccinal activity than any other compound and appears to be as effective against intranasally induced infection as 2,3-butanedione oxime thiosemicarbazone. Neither isatin nor benzaldehyde thiosemicarbazone appears to be as effective as the latter compound against this type of infection. 2-Benzothiazolecarboxaldehyde thiosemicarbazone exhibited a moderate degree of activity but the 2-benzimidazole analogue was inactive. None of the aliphatic derivatives produced significant protection in either type of test. It may be noted that glyoxal thiosemicarbazone has been reported to be carcinostatic for certain tumours in mice.[21]

The results reported here indicate that thiosemicarbazones may exert a greater protective effect against intranasally-induced than against intracerebrally-induced vaccinia infection in the mouse. Small quantities of virus are lethal when administered by the cerebral route while massive doses are required to produce fatal disease when inoculated by the nasal route. In either case, the protection afforded animals by thiosemicarbazones can be reduced or eliminated by increasing the dose of virus administered to the mice.



(III)

With few exceptions, unsubstituted heterocyclic and benzaldehyde thiosemicarbazones have been found to exhibit greater anti-vaccinal activity in mice than substituted derivatives.[22, 23] In most thiosemicarbazones with anti-vaccinal activity, the thiosemicarbazone group, $=NNHCSNH_2$, is separated by two carbon atoms from nitrogen or sulphur atoms.[2] Compounds with this configuration, however, may be inactive and benzaldehyde thiosemicarbazones form an exception to this rule.

4-Methyl-5-thiazolecarboxaldehyde thiosemicarbazone (III) was found to be an outstanding chemoprophylactic agent against vaccinia infection in mice.

Experimental

α-Chloroacetoacetic esters. Ethyl α-chloroacetoacetate was prepared by the method of Allihn.[24] Since no yield and few procedural details were reported, they are given here for convenience. A flask containing 261 g (2·02 moles) of freshly distilled ethyl acetoacetate was cooled in ice, and 271 g (2·03 moles) of freshly distilled sulphuryl chloride was added dropwise with agitation over a period of about 75 min, keeping the temperature below 15°. Stirring and cooling were continued for about one hour, and the residual hydrogen chloride and other volatile materials were removed by warming under aspirator vacuum. The product was fractionated *in vacuo*, using a well shielded pump, to yield 294 g (88·5 per cent) of colourless oil boiling at 79–82°/10 mm.

The corresponding methyl ester[11] was obtained similarly in 78 per cent yield, b.p. 62–69°/10 mm, from commercial samples of methyl acetoacetate and sulphuryl chloride.

4-Methyl-5-thiazolecarboxylic esters. These esters were prepared from the corresponding α-chloroacetoacetic esters and thioformamide by the excellent thiazole synthesis procedure described by Buchman and Richardson.[25] Considerably better yields were obtained than have been reported previously for these compounds. Ethyl 4-methyl-5-thiazolecarboxylate,[26] b.p. 90–90·5°/3 mm, was obtained in 76 per cent yield. The following modification in the procedure was made in the preparation of methyl 4-methyl-5-thiazolecarboxylate.[11] In the precipitation of the product with alkali carbonate, the pH was raised no higher than 5·0 to avoid contamination of the product with a red oil. The solid ester was then obtained by filtration, rather than by extraction, in a yield of 70 per cent, m.p. 73·8–74·8°. After recrystallization from 63–99° petroleum ether, the melting point was 74·5–75·5° (Reference 26: 75°; reference 11: 76°).

Ethyl 5-thiazolecarboxylate. Thirty-four g (0·41 mole) of 95 per cent thioformamide hydrate* was added, with cooling, to 49·7 g (0·330 mole) of ethyl chloromalonaldehydate[27] in 10 ml of

* Kindly supplied by Merck, Inc.

ethanol. The mixture was heated on the steam bath in an open flask for about 30 min (hood), then placed in the refrigerator for about 1·5 h. The ester[10] was isolated by the Buchman-Richardson procedure;[25] 11·0 g (21 per cent) of colourless oil boiling at 107–108°/10 mm was obtained. No ester could be obtained from the ether washings of the acidified reaction mixture. When the reaction mixture was not heated, but kept below 20° during the initial reaction, then stored in the refrigerator for six days before isolation, only a 12·5 per cent yield was obtained.

Ethyl 4-methyl-2-thiazolecarboxylate. To 19·1 g (0·139 mole) of bromoacetone[28] in 10 ml of ethanol was added 18·2 g (0·137 mole) of ethyl 2-thiooxamate[29], in portions, with cooling. On warming to room temperature a vigorous exothermic reaction set in, and when it had abated somewhat, the spongy mixture was heated on a steam bath for 5–10 min, then allowed to stand at room temperature until the next day. After treatment with 80 ml of 10 per cent sodium carbonate solution, the mixture was extracted with three 25-ml portions of chloroform. The extracts were dried over sodium sulphate and distilled at reduced pressure to yield 14·4 g (61·5 per cent) of the ester, b.p. 122·/5 mm.

Anal. Calcd. for $C_7H_9NO_2S$: C, 49·11; H, 5·30; N, 8·18. Found: C, 48·96; H, 5·59; N, 8·06.

On treatment of the distillation forerun and a small amount of oil coming over after collection of the main cut with hydrazine hydrate in absolute ethanol, 1·6 g of the hydrazide, m.p. 148·5–149·3°, was obtained, bringing the total yield of ester to 69 per cent. This ester had been prepared previously,[10,30] using chloroacetone, but no yields were reported.

Ethyl 4-methyl-2-methylthio-5-thiazolecarboxylate. A mixture of 93·9 g (0·462 mole) of crude ethyl 2-mercapto-4-methyl-5-thiazolecarboxylate[31]* and 600 ml of acetone was warmed to dissolve the ester. After cooling nearly to room temperature, a solution of 36·0 g (0·260 mole) of potassium carbonate in 60 ml of water was stirred in, followed by 33 ml (75 g; 0·53 mole) of methyl iodide. The mixture began to reflux; stirring was continued for a 2·5-h period, during the last 1·5 h of which the mixture was refluxed on a steam bath. When cool, the mixture

* A sample was kindly supplied by Dr. J. J. D'Amico of the Monsanto Chemical Company.

was diluted with 1 l. of water and extracted with four 200-ml portions of ether; the extracts were dried over sodium sulphate and distilled at reduced pressure. After a red forerun, 74·4 g (74 per cent) of ethyl 4-methyl-2-methylthio-5-thiazolecarboxylate, boiling at about 117°/0·15 mm, m.p. 31–32·5°, was obtained. A portion dissolved in 30–60° petroleum ether and cooled in the refrigerator produced slender prisms melting at 32·8–33·0°.

Anal. Calcd. for $C_8H_{11}NO_2S_2$: N, 6·45. Found: N, 6·31.

2-Hydroxymethylbenzothiazole. A mixture of 47·5 g (0·625 mole) of glycolic acid (purified, Fisher) and 62·7 g (0·501 mole) of 2-aminobenzenethiol (American Cyanamid) was heated to gentle boiling so that the water formed distilled slowly into a Dean-Stark moisture trap. After 1·5 h of heating at 120–170°, the mixture was allowed to cool to below 100° and treated with a mixture of 150 ml of concentrated hydrochloric acid and 450 nil of water. The mixture was warmed on the steam bath to effect solution, then filtered to remove a small amount of yellow solid. With ice-cooling, the addition of 300 ml of 20 per cent sodium hydroxide solution caused the precipitation of 67·3 g of the carbinol, m.p. 91–94°. The addition of 15 ml more base precipitated an additional 6·2 g, m.p. 86–89°, bringing the total yield to 89 per cent. Recrystallization of the carbinol from carbon tetrachloride yielded colourless needless melting at 93–94°.

Anal. Calcd. for C_8H_7NOS: N, 8·48. Found: N, 8·59. The infrared spectrum showed no band in the carbonyl region, and contained hydroxyl bands at 3145 and 1075 cm^{-1}, not present in the spectrum of 2-methylbenzothiazole.

2-Benzothiazolecarboxylic acid. A suspension of 5·0 g (0·030 mole) of 2-hydroxymethylbenzothiazole in 100 ml of water containing 2 g of sodium carbonate was heated to 50°, and a solution of 6·3 g (0·040 mole) of potassium permanganate in 200 ml of water, also previously heated to 50°, was added in small portions, with agitation, over a period of 15 min. The mixture was kept at $50 \pm 2°$ for an additional 20 min by occasional application of steam, allowed to cool, and filtered to remove manganese dioxide. The yellow solution was brought to pH 3 (Hydrion paper) by the addition of 10 per cent hydrochloric acid, and the white solid which precipitated was removed, washed with water, and dried in a

vacuum desiccator. The crude acid weighed 4·5 g and melted at 92–92·5° (dec.).

Ethyl 2-benzothiazolecarboxylate. The acid was converted directly, without purification, to the acid chloride,[32] m.p. 114–116° after recrystallization from dry benzene. The acid chloride (1·5 g) was refluxed in 3 ml of absolute ethanol for ½ h, 2·5 ml of water was added, and the solution was allowed to cool to precipitate 1·3 g (82 per cent) of the ethyl ester, m.p. 69·8–70·5°, as previously reported.[5] The melting point was not changed by mixing with an authentic sample of the ester.

Hydrazides. These were obtained by treatment of the esters, usually dissolved in a small amount of absolute ethanol, with an excess of hydrazine hydrate. In the room temperature reactions, the hydrazides slowly crystallized from solution. In the reflux reactions, the solutions were cooled to room temperature at the end of the heating period to cause crystallization. The crystals were then removed and washed with absolute ethanol. Commercial absolute ethanol was preferred both as solvent and for washing purposes, since the hydrazides, particularly those with the carboxyhydrazide group in the 4- or 5-position, were usually much more soluble in water and dilute ethanol (even 95 per cent ethanol) than in the absolute ethanol. The preparatory data are summarized in Table I.

Sulphonylhydrazides. The hydrazide was suspended or dissolved in pyridine (1 ml/0·001 mole). About 10 per cent excess of the sulphonyl chloride was added dropwise or, in the case of the *p*-bromo and *p*-nitro derivatives, in small portions to the stirred, ice-cooled mixture, which was usually stirred for 10–15 min longer, then poured, with stirring, into five volumes of ice water, usually containing an amount of hydrochloric acid equivalent to the pyridine used (or a slight excess), to prevent the formation of gums. The solid obtained was washed with water or ethanol and recrystallized. The compounds, being difficultly soluble in most common solvents, were generally dissolved in excess hot *iso*propyl alcohol and the solutions were concentrated before cooling. Preparative data are given in Table V.

4-Methyl-5-thiazolecarboxaldehyde.[11] A stirred mixture of 0·034 moles of the sulphonylhydrazide in 50 ml of redistilled ethylene glycol was immersed in an oil bath heated to 175°. When the

Table V. Sulphonylhydrazides

R	Y	Yield, % (recryst.)	m.p., °C	Formula	Nitrogen, % Calcd.	Nitrogen, % Found
		R-CONHNH-SO_2-C_6H_4Y-*p*				
4-CH_3-5-Th-[a]	H	75	170·5–171[b]	$C_{11}H_{11}N_3O_3S_2$	—	—
	CH_3O[c]	68	164·3–164·9	$C_{12}H_{13}N_3O_4S_2$	12·83	12·63
	Br	44	194–194·3 (dec)	$C_{11}H_{10}BrN_3O_3S_2$	11·17	11·31
	NO_2	51	213–214 (dec)[d]	$C_{11}H_{10}N_4O_5S_2$	16·36	16·62
4-CH_3-2-CH_3S-5-Th-	H	81	184·5–185·1[e]	$C_{12}H_{13}N_3O_3S_3$	12·23	12·32
4-Th-	H	87	213–215[f]	$C_{10}H_9N_3O_3S_2$	14·84	14·96
4-CH_3-2-Th-	H	83	214·5–215·6 (dec)	$C_{11}H_{11}N_3O_3S_2$	14·13	13·98
2-BenzoTh-	H	82	207·8–209·0 (dec)	$C_{14}H_{11}N_3O_3S_2$	12·60	12·80[g]

[a] Th = thiazole. [b] Reference 11: 170°. [c] *p*-Methoxybenzenesulphonyl chloride was prepared by the method of Morgan and Cretcher.[33] [d] m.p. of analytical sample, 214·5–215·5° (dec). [e] m.p. of analytical sample, 185·3–186·3°. [f] Reference 6: 213–214°. [g] Calcd: C, 50·45; H, 3·33. Found: C, 50·37; H, 3·37.

temperature of the bath had equilibrated to 160–165°, 3 g of ground soft glass and 1·96 g (0·0185 mole) of anhydrous reagent sodium carbonate were added to the solution. After stirring at a bath temperature of 159–166° for the desired time, the oil bath was quickly replaced by an ice bath; the cooled mixture was treated with 250 ml of saturated sodium chloride solution and filtered. The residue on the filter was washed with 30 ml of chloroform in four portions, and the filtrate and washings were shaken together and separated. The aqueous phase was extracted four more times with 30-ml portions of chloroform. The combined chloroform solutions were dried over sodium sulphate, concentrated on a steam bath, and distilled *in vacuo* (10 mm).

In the case of the *p*-bromo derivative, only 0·024 mole was used, and proportionally smaller amounts of all the other materials were employed. Similarly, with the *p*-nitro compound, in addition to a regular-sized run, a proportionally smaller run was made. The results are summarized in Table II.

4-Methyl-2-methylthio-5-thiazolecarboxaldehyde. A solution of 27·5 g (0·080 mole) of 1-benzenesulphonyl-2-(4-methyl-2-methylthio-5-thiazolecarbonyl)-hydrazine in 120 ml of ethylene glycol was treated at 160–165° with 7 g of ground soft glass and 4·67 g (0·044 mole) of anhydrous sodium carbonate, stirred for 2–3 min at that temperature, then cooled in ice at 8°. The mixture was filtered to remove a mixture of glass and organic solid, which was washed with 10 per cent sodium carbonate solution and water, dried, and extracted at room temperature with 150 ml of chloroform in three portions. Evaporation of the extracts yielded 5·9 g of aldehyde. A second crop of 0·3 g was precipitated by adding 500 ml of sodium carbonate solution to the glycol solution and cooling in the refrigerator overnight; an additional 0·4 g was obtained by extractions of the mother liquor with chloroform, evaporation of the extracts, and washing the oily crystals with a 2 : 3 acetone–water mixture. The combined product (6·6 g; 48 per cent) melted at 99–101°; after recrystallization from dilute ethanol yellow needles melting at 101·6–102·1° were obtained.

Anal. Calcd. for $C_6H_7NOS_2$: N, 7·99. Found: N, 8·29.

The basic aqueous solution, after the chloroform extraction, was acidified with acetic acid to precipitate 1·4 g of 1,2-bis(4-methyl-2-methylthio-5-thiazolecarbonyl)hydrazine, m.p. about

209–213°, which after recrystallization from 95 per cent ethanol (Darco) melted at 217–217·6°.

Anal. Calcd. for $C_{12}H_{14}N_2O_2S_4$: N, 14·96; mol. wt., 375. Found: N, 15·20; mol. wt., 351 (Rast).

4-Thiazolecarboxaldehyde. As in the preparation of 4-methyl-5-thiazole-carboxaldehyde, 30·6 g (0·108 mole) of 1-benzenesulphonyl-2-(4-thiazolecarbonyl) hydrazine in 150 ml of ethylene glycol was decomposed with 10 g of ground glass and 6·30 g (0·0594 mole) of sodium carbonate, using a 7-min reaction time. The dried chloroform extract was concentrated to a volume of about 50 ml and cooled to deposit 0·9 g of colourless needles, m.p. 186–187°. After recrystallization from ethanol, then from water, colourless needles of unchanged starting material, m.p. 214–215°, were obtained.

The chloroform solution was further concentrated, and after removing a second crop of crystals, was distilled at reduced pressure, affording 2·51 g (21 per cent) of 4-thiazolecarboxaldehyde, b.p. 99–104°/14 mm, m.p. 63–65° (Reference 6: 65–66°).

*4-Methyl-2-thiazolecarboxaldehyde.** Using the procedure outlined for 4-methyl-5-thiazolecarboxaldehyde, 10·0 g (0·337 mole) of 1-benzenesulphonyl-2-(4-methyl-2-thiazolecarbonyl)hydrazine was decomposed, using a 3-min reaction time. On distillation, only 0·1 g of the aldehyde was collected as a yellow oil while the bath temperature was raised to 280°/10 mm. The aldehyde was converted to the thiosemicarbazone for analysis (see Table VI).

2-Benzothiazolecarboxaldehyde. (a) *By the McFadyen-Stevens Reaction.* A mixture of 24·0 g of 1-benzenesulphonyl-2-(2-benzothiazolecarbonyl)hydrazine and 110 ml of ethylene glycol was heated to 160–163° and treated with 6·5 g of ground soft glass and 4·20 g (0·0396 mole) of anhydrous sodium carbonate, stirred at that temperature for 3 min, then cooled in ice. The filtered glycol solution (the filter residue yielded no aldehyde on extraction with hot petroleum ether) was treated with 500 ml of 10 per cent sodium carbonate solution, filtered to remove a small amount of solid, and extracted with three portions of chloroform. The residue obtained by evaporating the chloroform was extracted with

* Since this work was completed, the preparation of this aldehyde by a different route, in about 10 per cent yield, b.p. 49–50°/3 mm, was reported by Beyer, Hess, and Liebenow.[34]

Table VI. Thiosemicarbazones

Aldehyde	Yield %	m.p., °C	Recrystn. solvent	Formula	Nitrogen, % Calcd.	Found
4-Methyl-5-thiazolecarboxaldehyde	94	229·3–229·7 (dec.)[a]	Acetic acid	$C_6H_8N_4S_2$	27·97	27·69
4-Methyl-2-methylthio-5-thiazolecarboxaldehyde	89	213–214 (dec.)	*n*-Propyl alcohol	$C_7H_{10}N_4S_3$	22·74	23·05
4-Thiazolecarboxaldehyde	90[b]	185–185·5 (dec.)[c]	Water	$C_5H_6N_4S_2$[d]	30·08	29·96
4-Methyl-2-thiazole-carboxaldehyde	—[e]	224 dec.[f]	Ethanol	$C_6H_8N_4S_2$	27·97	28·19
2-Benzothiazolecarboxaldehyde	97	243–244 (dec.)[g]	Acetic acid	$C_9H_8N_4S_2$	23·71	23·27
2-Benzimidazolecarboxaldehyde	90[b]	258·5–259 (dec.)	Ethanol	$C_9H_9N_5S.H_2O$[h]	27·43	27·58[i]
4-Nitro-2-thenaldehyde[j]	91	247–248 (dec.)	DMF[k]-water	$C_6H_6N_4O_2S_2$	24·33	24·25
o-Methoxybenzaldehyde	95	210·5–211 (dec.)[l]	Acetic acid	$C_9H_{11}N_5OS$	20·08	19·81
o-Nitrobenzaldehyde	95	247–247·5 (dec.)[m]	Pyridine-water	$C_8H_8N_4O_2S$	24·99	25·22
2,4-Dihydroxybenzaldehyde	91	238 (dec.)[n]	Water-methanol	$C_8H_9N_3O_2S$	19·90	20·05
2-Hydroxy-3-methoxybenzaldehyde	95	231·8–232·4 (dec.)[p]	Acetic acid	$C_9H_{11}N_3O_2S$	18·65	19·07
2,3-Dimethoxybenzaldehyde	93	222·5–223 (dec.)[q]	*Iso*amyl alcohol	$C_{10}H_{13}N_3O_2S$	17·56	17·40
3,4-Diethoxybenzaldehyde	91	172·8–173·7	Ethanol	$C_{12}H_{17}N_3O_2S$	15·72	15·87
2,4-Dichlorobenzaldehyde	95	235·5–236·5 (dec.)[r]	Acetic acid	$C_8H_7N_3Cl_2S$	16·94	17·14
2,6-Dichlorobenzaldehyde[s]	81	235·7–236 (dec.)[t]	Acetic acid	$C_8H_7N_3Cl_2S$	16·94	17·40
Propionaldehyde	85	158–158·5[u]	Water	$C_4H_9N_3S$	32·03	32·18
*Iso*butyraldehyde	84	87·5–88·3	Petr. ether-chloroform	$C_5H_{11}N_3S$	28·84	28·76
Hexaldehyde	89	89·5–90	Petr. ether-chloroform	$C_7H_{15}N_3S$	24·25	24·42
Heptaldehyde	93	66–67[v]	Petr. ether-chloroform	$C_8H_{17}N_3S$	22·43	22·45
Glyoxal[w]	92	> 330 (dec.)[x]	Moist DMF[k]	$C_4H_8N_6S_2$	41·14	40·72

[a] Reference 44: 233° dec. [b] Approximate value; forms a hydrate. [c] Reference 45: 189–190° dec. [d] Dried for analysis at 140° *in vacuo* with P_2O_5. [e] Small scale preparation; yield not determined. [f] Reference 34: 207°. [g] Reference 46: 242–243° dec. [h] Dried at 60° *in vacuo* with P_2O_5. [i] Calcd.: S, 12·56. Found: S, 12·63. [j] Aldehyde prepared by the method of Gever[47] except that the 4-isomer was crystallized from an ether solution of the mixture of isomers by chilling, rather than from *iso*propyl alcohol. [k] Dimethylformamide. [l] Reference 48: 216°. [m] Reference 46: 247–248° dec. [n] Reference 46: 235–237° dec. [p] Reference 46: 226–227° dec. [q] Reference 46: 225–226° dec. [r] Reference 46: 242–244° dec. [s] Aldehyde prepared by Dr. Burton Appleton. [t] Reference 46: 235–236·5° dec. [u] Reference 49: 154–156°. [v] Reported by Neuberg and Neimann,[50] but m.p. not given. [w] Dithiosemicarbazone. [x] Reference 50: dec. > 300°.

about 50 ml of boiling hexane in three portions; the combined hexane solutions were boiled with Darco, filtered hot, and evaporated to dryness, yielding 0·7 g (6 per cent) of the crude aldehyde, m.p. 67–70°. After several recrystallizations from petroleum ether (63–99°), the melting point was raised to 72–73°, unchanged on admixture with the product obtained by the method below.

(b) *By oxidation of the carbinol.* To a hot solution of 3·4 g (0·031 mole) of freshly prepared selenium dioxide[35] in a mixture of 25 ml of dioxan and 1 ml of water was added 5·0 g (0·030 mole) of 2-hydroxymethylbenzothiazole, and the mixture was refluxed for 30 min; 1·2 g (0·015 mole) of selenium was recovered by filtration after cooling. The solution was poured into ice water. Part of the aldehyde was obtained by filtration, and the remainder was obtained by extraction with chloroform. The total yield was 4·1 g (83 per cent),† m.p. 65–72°. Recrystallization from petroleum ether yielded yellow opaque crystals melting at 72–73°.

2-Methylbenzimidazole. Several procedures[36–38] for the preparation of this compound by condensation of acetic acid or anhydride with *o*-phenylenediamine were examined. The following, a modification of the method of Rupe and Porai-Koschitz,[39] was the simplest and gave by far the best yield. Equimolar amounts of *o*-phenylenediamine and acetic anhydride were cautiously mixed (acetic acid could be used as a solvent to modify the initial reaction). The mixture was then distilled through a short (4 cm) Vigreux column. The last amounts of liquid materials were removed by distilling at aspirator vacuum until solid began to form in the column. The residue was then distilled, or recrystallized from water containing a little ammonia to neutralize residual acid. Yields were 86–89 per cent of material melting above 175° (References 37, 38; 176°).

Oxidation of 2-methylbenzimidazole with selenium dioxide. Equimolar amounts of 2-methylbenzimidazole and selenium dioxide were refluxed in moist dioxan for 2 h, then warmed on a steam bath overnight. After treatment with hydrochloric acid, two crops of solid were obtained on neutralization. At pH 5 (Hydrion paper), about a 10 per cent yield of crude aldehyde was

† Zubarovskii[8] previously obtained a 32·5 per cent yield by this method, using a crude preparation of the carbinol melting at 90–100°.

obtained, which after recrystallization from ethanol, melted at about 230° (dec.). Further neutralization of the mother liquor yielded 80 per cent of the original 2-methylbenzimidazole.

No aldehyde could be obtained on oxidation of 2-methylbenzimidazole with chromic acid in acetic anhydride; only a small amount of material, which was apparently starting material, was obtained on neutralization.

2-Benzimidazolecarboxaldehyde. A mixture of 10·0 g (0·068 mole) of 2-hydroxymethylbenzimidazole,[40] 7·5 g (0·068 mole) of selenium dioxide,* 150 ml of dioxan and 5 ml of water was heated at reflux for 4 h. The hot solution was decanted from the solid, and, on cooling, 2·4 g of crude aldehyde separated as an ivory-coloured powder. An additional 5·2 g of aldehyde was obtained by extracting the solid remaining in the reaction flask with 4N hydrochloric acid and precipitating with ammonia. The total yield was 77 per cent; 4·0 g (0·051 g-atom) of selenium was recovered. After recrystallizing several times from pyridine and pyridine-water mixtures (Darco), the aldehyde, a white solid, melted at 235° (dec.), as previously reported.[42]

Anal. Calcd. for $C_8H_6N_2O$: C, 65·73; H, 4·14; N, 19·17. Found: C, 66·25; H, 4·39; N, 19·20.

The aldehyde was most conveniently isolated as the hydrochloride. The reaction mixture, prepared as above, was allowed to cool and was filtered. The residue was treated with 50 ml of concentrated hydrochloric acid, and the mixture was heated to boiling. The hot solution was filtered through a sintered-glass funnel and then cooled in ice. The hydrochloride separated as the dihydrate, in colourless needles (9·3 g; 63 per cent). When heated from 119°, the product melted at 120·7–121·8° with decomposition. When heated from room temperature, it darkened, but did not melt below 200°. A second recrystallization from concentrated hydrochloric acid raised the melting point to 121·2–122·5° (dec., heated from 119°).

Anal. Calcd. for $C_8H_7ClN_2O.2H_2O$: C, 43·96; H, 5·07; N, 12·82; Cl, 16·22. Found: C, 44·03; H, 5·22; N, 13·00; Cl, 16·38.

* Selenium dioxide was prepared and sublimed immediately by the convenient procedure of Hill, Soth, and Ricci.[35] The sublimed material was used as much as two months after preparation without apparent ill effect (cf. Kaplan[41] on reaction of methylquinolines with aged selenium dioxide).

Thiosemicarbazones. The aldehyde (if a solid, in ethanol solution) was added dropwise to a boiling solution of an equivalent amount of thiosemicarbazide in aqueous alcohol or water, which, for the heterocyclic derivatives, contained a small amount of acetic acid as a catalyst;[43] the mixture was then heated for 0·5 h at reflux. A quite pure product was usually obtained, in high yield, by this procedure. A summary of the preparative data on thiosemicarbazones in given in Table VI.

In the preparation of 2-benzimidazolecarboxaldehyde thiosemicarbazone, the hydrochloride of the aldehyde was dissolved in water and added dropwise to a hot aqueous solution of thiosemicarbazide and sodium acetate.

Isatin 2-benzothiazolylhydrazone. A solution of 6·70 g (0·0406 mole) of 2-hydrazinobenzothiazole (Eastman) and 5·78 g (0·0393 mole) of isatin in 35 ml of 95 per cent enthanol was heated for 2 h. Water (35 ml) was added to the hot solution to produce 5·4 g (47 per cent) of the hydrazone, which on recrystallization from moist pyridine yielded golden-yellow needles melting at 315–315·5° (dec.).

Anal. Calcd. for $C_{15}H_{19}N_4OS$: N, 19·04. Found: N, 19·11.

Summary. In a study of thiosemicarbazones as anti-viral agents. thiosemicarbazones of thiazole and imidazole aldehydes were synthesized, 2-Benzimidazolecarboxaldehyde was prepared by selenium dioxide oxidation of 2-hydroxymethylbenzimidazole. Thiazole aldehydes were prepared by the Newman-Caflisch modification of the McFadyen-Stevens synthesis. Yields of 4-methyl-5-thiazolecarboxaldehyde from 1-benzenesulphonyl-2-(4-methyl-5-thiazolecarbonyl)hydrazines decreased with increasing electron-withdrawing capacity of a substituent in the *para* position of the benzenesulphonyl group, a result which was unexpected in view of the previously proposed mechanism for the McFadyen-Stevens reaction, and a possible alternate mechanism is suggested. Monocyclic thiazole esters needed as starting materials for the McFadyen-Stevens synthesis were prepared by reactions of α-chlorocarbonyl compounds with thioamides. 2-Benzothiazolecarboxylic acid was conveniently prepared by oxidation of 2-hydroxymethylbenzothiazole, and was converted via the acid chloride to the ethyl ester.

As vaccinia virus antagonists in mice, the thiosemicarbazones of 2- and 5-thiazolecarboxaldehydes were among the most active compounds tested, but 4-thiazolecarboxaldehyde thiosemicarbazone was much less active. 2-Benzimidazolecarboxaldehyde thiosemicarbazone was inactive. These and previous data with thiophene and pyridine derivatives indicate that

compounds in which the carbonylthiosemicarbazone group is adjacent to a ring sulphur atom are likely to be active, while an adjacent pyridine-type ring nitrogen inhibits activity.

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