90. *Antituberculous Compounds. Part V. 2-Sulphanilamido-5-alkyl-1 : 3 : 4-oxadiazoles and -thiadiazoles and Related* iso*Thiosemicarbazones and* iso*Thioureas.*

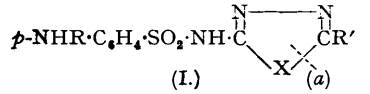
By J. D. Brooks, P. T. Charlton, P. E. Macey, D. A. Peak, and W. F. Short.

Following the observation of the specific *in vitro* activity, against *Mycobacterium tuberculosis*, of 2-sulphanilamido-5-methyl-1 : 3 : 4-oxadiazole (I; X = O, R = H, R′ = Me), a number of derivatives and analogues of this compound have been prepared. These include N^4-acyl derivatives and higher alkyl homologues (R′ = amyl, undecyl, and heptadecyl). Similar activity is shown by corresponding thiadiazoles (I; X = S).

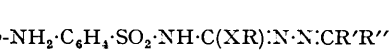
Structurally analogous *iso*thiosemicarbazones have also been prepared. Although these were generally active *in vitro*, a selected compound showed no activity *in vivo* in contrast to the closely related, therapeutically active benzaldehyde thiosemicarbazones (Domagk, *Nordisk Medicin*, 1948, **39**, 1322).

A series of *iso*thioureas has also been prepared. *N*-Aryl*iso*thioureas showed high *in vitro* activity only in the absence of serum. Cyclic *iso*thioureas, such as 2-aminobenzthiazoles and 2-alkylthiopyrimidines, were without interest.

The work to be described in the present paper arose from the observation made in 1944 that 2-sulphanilamido-5-methyl-1 : 3 : 4-oxadiazole (I; X = O, R = H, R′ = Me), while virtually inactive against organisms normally sensitive to sulphonamides, exhibited a highly specific activity *in vitro* against *Mycobacterium tuberculosis*. The compound, first prepared in 1943, has since been described (Tappi and Migliardi, *Arch. Sci. Biol.*, 1941, **27**, 170; Tappi, *Rec. Trav. chim.*, 1943, **62**, 207; not available in abstract form in this country until 1944), but without



(I.) (*a*)



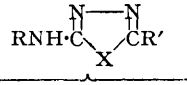
(II.)

reference to its antituberculous activity. Complete inhibition of the growth of *M. tuberculosis* occurs at dilutions of 1 in 10,000—50,000, 4-aminosalicylic acid showing the same activity under the same conditions of test. Although this activity is of a comparatively low order, the low toxicity of the compound and its apparent persistence in high concentrations in the blood-stream gave promise of chemotherapeutic activity. An extensive series of *in vivo* tests failed to indicate any such activity, and this inactivity was later accounted for by the demonstration that the Bratton–Marshall estimation of blood-levels is misleading in this case, the compound being in fact rapidly metabolised to an inactive substance. Details of this work will be published elsewhere.

Before the explanation of this negative result was obtained, derivatives and homologues had been prepared in order to elucidate the structural features of activity and in the hope of increasing the activity. The compounds prepared are summarised in Table I, together with their *in vitro* activities. In the subsequent discussion figures in parentheses following the name of a compound refer to the number of the compound in this or other tables.

TABLE I.

5-Amino-2-alkyl-1 : 3 : 4-oxadiazoles and -thiadiazoles.



Compound. -1 : 3 : 4-oxadiazole or -thiadiazole.	X.	R′.	R″.	M. p.	Formula.	Found N, %.	Req. N, %.	Activity * in absence of serum.
(1) 2-Sulphanilamido-5-methyl-	O	Me	$NH_2 \cdot C_6H_4 \cdot SO_2 \cdot$	175°	$C_9H_{10}O_3N_4S$	21·5	22·0	10—50
(2) 2-(3 : 5-*Di-iodosulphanilamido*)-5-*methyl-*	O	Me	$NH_2 \cdot C_6H_2I_2 \cdot SO_2 \cdot$	228—229	$C_9H_8O_3N_4I_2S$	11·3	11·1	1
(3) 2-*N*⁴-Acetylsulphanilamido-5-methyl-	O	Me	$AcNH \cdot C_6H_4 \cdot SO_2 \cdot$	212	$C_{11}H_{12}O_4N_4S$	—	—	<1
(4) 2-N⁴-*Hexoylsulphanilamido-5-methyl-*	O	Me	$C_5H_{11} \cdot CO \cdot NH \cdot C_6H_4 \cdot SO_2 \cdot$	203	$C_{15}H_{20}O_4N_4S$	15·9	15·9	5
(5) 2-N⁴-*Dodecoylsulphanilamido-5-methyl-*	O	Me	$C_{11}H_{23} \cdot CO \cdot NH \cdot C_6H_4 \cdot SO_2 \cdot$	124	$C_{21}H_{32}O_4N_4S$	12·7	12·8	5
(6) 2-N⁴-*Stearoylsulphanilamido-5-methyl-*	O	Me	$C_{17}H_{35} \cdot CO \cdot NH \cdot C_6H_4 \cdot SO_2 \cdot$	128—129	$C_{27}H_{44}O_4N_4S$	10·7	10·8	1
(7) 2-*Toluene*-p-*sulphonamido-5-methyl-*	O	Me	$CH_3 \cdot C_6H_4 \cdot SO_2 \cdot$	152	$C_{10}H_{11}O_3N_3S$	16·7	16·6	1
(8) 2-*Amino-5-amyl-*	O	C_5H_{11}	H	151	$C_7H_{13}ON_3$	27·2	27·1	1
(9) 2-*Amino-5-undecyl-*	O	$C_{11}H_{23}$	H	150—151	$C_{13}H_{25}ON_3$	17·3	17·6	1
(10) 2-*Amino-5-heptadecyl-*	O	$C_{17}H_{35}$	H	143	$C_{19}H_{37}ON_3$	13·5, 13·3	13·0	<1
(11) 2-N⁴-*Acetylsulphanilamido-5-amyl-*	O	C_5H_{11}	$AcNH \cdot C_6H_4 \cdot SO_2 \cdot$	186	$C_{15}H_{20}O_4N_4S$	15·9	15·9	1
(12) 2-N⁴-*Acetylsulphanilamido-5-amyl-*	S	C_5H_{11}	$AcNH \cdot C_6H_4 \cdot SO_2 \cdot$	201—202	$C_{15}H_{20}O_3N_4S_2$	15·3	15·2	1
(13) 2-*Sulphanilamido-5-amyl-*	O	C_5H_{11}	$NH_2 \cdot C_6H_4 \cdot SO_2 \cdot$	148—149	$C_{13}H_{18}O_3N_4S$	18·3	18·1	50
(14) 2-*Sulphanilamido-5-amyl-*	S	C_5H_{11}	$NH_2 \cdot C_6H_4 \cdot SO_2 \cdot$	182	$C_{13}H_{18}O_2N_4S_2$	17·4	17·2	50
(15) 2-*Sulphanilamido-5-undecyl-*	O	$C_{11}H_{23}$	$NH_2 \cdot C_6H_4 \cdot SO_2 \cdot$	105—107	$C_{19}H_{30}O_3N_4S$	14·1	14·2	100
(16) 2-*Sulphanilamido-5-heptadecyl-*	O	$C_{17}H_{35}$	$NH_2 \cdot C_6H_4 \cdot SO_2 \cdot$	91—93	$C_{25}H_{42}O_3N_4S$	11·4	11·7	10

* Dilution (in thousands) at which complete inhibition of the growth of *M. tuberculosis* (human virulent strain) was maintained for 4 weeks in modified Long's medium (by the floating-pellicle method). Under the same conditions of test 4-aminosalicylic acid gave a value of 10 in the absence of serum.

(1) Preparation by Dr. H. Kitchen. Found : C, 42·55; H, 4·4. Calc. for $C_9H_{10}O_3N_4S$: C, 42·5; H, 4·0%. Tappi and Migliardi (*loc. cit.*) and Tappi (*loc. cit.*) record m. p. 172°. (3) Found : S, 10·6. Calc. for $C_{11}H_{12}O_4N_4S$: S, 10·8%. Tappi (*loc. cit.*) records m. p. 213°. (4), (5), (6), and (7) Preparations by Dr. R. P. Hullin. (8) Colourless needles by precipitation of its solution in hydrochloric acid with dilute ammonia solution.

2-N^4-*Hexoyl-*, *-dodecoyl-*, and *-stearoyl-sulphanilamido-5-methyl-*1 : 3 : 4-*oxadiazoles* (4, 5, 6) were prepared by conventional methods from 2-sulphanilamido-5-methyl-1 : 3 : 4-oxadiazole. The activity was in all cases lower than that of the parent compound and the N^4-acetyl derivative (3) was completely inactive, indicating the necessity for a free N^4-amino-group. This was further shown by the low activity of 2-*toluene*-p-*sulphonamido-5-methyl*-1 : 3 : 4-*oxadiazole* (7), prepared from 2-amino-5-methyl-1 : 3 : 4-oxadiazole (Stollé and Fehrenbach, *J. pr. Chem.*, 1929, **122**, 289; De, *J. Indian Chem. Soc.*, 1930, **7**, 651) and toluene-*p*-sulphonyl chloride. Iodination of the 2-sulphanilamido-compound with iodine monochloride afforded a *di-iodo*-derivative (2) of low activity, again possibly due to the weakened basicity of the N^4-amino-group.

The homologous series, 2-*amino-5-amyl-*, *-undecyl-*, and *-heptadecyl-*1 : 3 : 4-*oxadiazoles* (8, 9, 10), was prepared from the corresponding 1-acylthiosemicarbazides by the action of lead monoxide in boiling ethanol (cf. Stollé and Fehrenbach, *loc. cit.*). The yields were progressively poorer in the case of the higher homologues, particularly the heptadecyl compound for which cyclisation was retarded by the formation of an insoluble lead salt of the 1-stearoylthiosemicarbazide. All three compounds were of low activity. Their *sulphanilyl* derivatives (13, 15, 16), prepared by conventional methods, showed activity which was of a high order in the case of the undecyl compound. No *in vivo* tests were carried out in view of the realisation at this time of the rapid inactivation of the methyl homologue in the blood-stream. One thiadiazole was prepared. 1-Hexoylthiosemicarbazide was cyclised with benzenesulphonic acid to 2-*amino-5-amyl*-1 : 3 : 4-*thiadiazole* and thence converted into the N^4-*acetylsulphanilyl* and *sulphanilyl* derivatives (12, 14). The last-named showed the same activity as its oxygen analogue; its acetyl derivative exhibited the same weak activity as 2-N^4-*acetylsulphanilamido-5-amyl*-1 : 3 : 4-*oxadiazole* (11).

In view of the unfavourable *in vivo* properties of this series, modifications of the structure were sought. A possible line of approach appeared to be the preparation of uncyclised forms of the molecule in which fission may be regarded to have taken place at the bond (*a*) in (I). This would lead to 4-sulphanilyl-3-alkyl*iso*semicarbazones (II; X = O, R = alkyl, R′ and R″ = H or alkyl) from the oxadiazoles and to 4-sulphanilyl-3-alkyl*iso*thiosemicarbazones (II; X = S, R = alkyl, R′ and R″ = H or alkyl) from the thiadiazoles. *In vitro* tests with 2-sulphanilamido-5-methyl-1 : 3 : 4-thiadiazole (I; X = S, R = H, R′ = Me), a supply of which was kindly made available to us by the Therapeutic Research Corporation of Great Britain Ltd., had shown that it possessed activity similar to that of the oxadiazole and that it was subject to the same *in vivo* limitations. Results with the amyl homologues (12, 14) confirmed that oxygen and sulphur were interchangeable in the molecule. Since 3-alkyl*iso*thiosemicarbazones were more readily accessible than 3-alkyl*iso*semicarbazones, it was proposed to prepare a series of sulphanilyl derivatives of the former. The first compound prepared, acetone 3-ethyl*iso*-thiosemicarbazone (17; Table II) had, itself, fair activity but its 4-*sulphanilyl* derivative (18) was almost completely devoid of activity. Further work was therefore directed towards examination of the 3-alkyl*iso*thiosemicarbazones themselves. These were prepared smoothly in all cases by the method of Baird, Burns, and Wilson (*J.*, 1927, 2527) and their properties and activities are summarised in Table II. Benzaldehyde 3-ethyl*iso*thiosemicarbazone (19) was as active as the acetone analogue and, in a series of 3-alkyl homologues, high activity was reached with the butyl derivative (20), falling to zero with the hexadecyl derivative (22). The butyl derivative was not seriously affected by serum but no *in vivo* activity could be demonstrated in guinea-pigs. No favourable effect was produced by the introduction of a 3-(2-diethylaminoethyl) group (23) or of a 4-phenyl or 4-*p*-butoxyphenyl group (24, 25, 26).

Late in 1946, the first information regarding parallel work which had been proceeding in Germany became available in this country. Behnisch (*Elberfeld Ann. Res. Reports*, No. 17, p. 2; FD 20/46) reported the activity of various substituted benzaldehyde thiosemicarbazones. In particular, *p*-pyrrolidino- and *o*-nitro-benzaldehyde thiosemicarbazones were mentioned as active, the latter having been selected for clinical test. The 3-ethyl*iso*thiosemicarbazones (27, 29) corresponding to these two compounds were therefore prepared together with *p*-dimethyl-aminobenzaldehyde thiosemicarbazone (28). None of these compounds showed promising activity, nor did *o*-nitrobenzaldehyde thiosemicarbazone itself. This is in agreement with the recent findings of Hoggarth, Martin, Storey, and Young (*Brit. J. Pharmacol.*, 1949, **4**, 248) who found no *in vivo* activity with *o*-nitrobenzaldehyde thiosemicarbazone or with 3-alkyl*iso*thio-semicarbazones in general. Subsequent publications (Domagk, Behnisch, Mietzsch, and Schmidt, *Naturwiss.*, 1946, **33**, 15; Domagk, *Zentr. Gynäk.*, 1947, **69**, 833) show that their approach to the thiosemicarbazones was almost identical with our own, starting in their case from the observation of the activity of 2-sulphanilamido-5-ethyl-1 : 3 : 4-thiadiazole (I; X = S,

TABLE II.

3-Alkylisothiosemicarbazones and 2-alkylisothioureas.

Compound. -iso*thiosemicarbazone.*	RN:C(SR')·NHR''. R.	R'.	R''.	Hydrohalide. M. p.	Formula.	Found N, %.	Req. N, %.	Activity.‡ In absence of serum.	In presence of serum.
(17) Acetone 3-ethyl-	Me_2C:N–	Et	H	153—154°	$C_6H_{13}N_3S$,HCl *	21·35	21·5	10	10
(18) *Acetone 4-sulphanilyl-3-ethyl-*	Me_2C:N–	Et	·SO_2·C_6H_4·NH_2-*p*	—	—	—	—	1	—
(19) Benzaldehyde 3-ethyl-	PhCH:N–	Et	H	193—194	$C_{10}H_{13}N_3S$,HCl	17·3	17·2	10	10—50
(20) Benzaldehyde 3-butyl-	PhCH:N–	C_4H_9	H	185—186	$C_{12}H_{17}N_3S$,HCl *	15·7	15·5	100 (500)	50
(21) Benzaldehyde 3-octyl-	PhCH:N	C_8H_{17}	H	176	$C_{16}H_{25}N_3S$,HCl *	12·8	12·8	10	10
(22) Benzaldehyde 3-hexadecyl-	PhCH:N–	$C_{16}H_{33}$	H	162—163	$C_{24}H_{41}N_3S$,HCl *	9·8	9·6	<1	—
(23) Benzaldehyde 3-(2-diethylaminoethyl)-	PhCH:N–	NEt_2·CH_2·CH_2·	H	192	$C_{14}H_{22}N_4S$,2HCl *	15·5	15·95	10	10
(24) *Benzaldehyde 4-phenyl-3-ethyl-*	PhCH:N–	Et	Ph	—	—	—	—	50 (100)	10
(25) Benzaldehyde 4-phenyl-3-(2-diethylaminoethyl)-	PhCH:N–	NEt_2·CH_2·CH_2·	Ph	—	—	—	—	10	5—10
(26) *Benzaldehyde* 4-p-*butoxyphenyl-3-ethyl-*	PhCH:N–	Et	*p*-C_4H_9O·C_6H_4·	—	—	—	—	10	—
(27) *p*-Pyrrolidinobenzaldehyde 3-ethyl-	*p*-$[CH_2]_4$>N·C_6H_4·CH:N–	Et	H	245 †	$C_{14}H_{20}N_4S$,HCl *	17·7	17·9	(50)	—
(28) *p*-Dimethylaminobenzaldehyde 3-ethyl-	*p*-Me_2N·C_6H_4·CH:N–	Et	H	230—232	$C_{12}H_{18}N_4S$,HCl *	19·8	19·5	1	—
(29) *o*-Nitrobenzaldehyde 3-ethyl-	*o*-NO_2·C_6H_4·CH:N–	Et	H	169·5—170·5	$C_{10}H_{12}O_2N_4S$,HCl *	19·2	19·4	(10)	—
-iso*thiourea.*									
(30) *N*-Phenyl-*S*-ethyl-	Ph	Et	H	—	—	—	—	>500	1—5
(31) *N*-Phenyl-*S*-butyl-	Ph	C_4H_9	H	—	—	—	—	100	10 (50)
(32) *N*-Phenyl-*S*-octyl-	Ph	C_8H_{17}	H	—	—	—	—	50	5
(33) *N*-*p*-Butoxyphenyl-*S*-ethyl-	*p*-C_4H_9O·C_6H_4·	Et	H	—	—	—	—	1—5	1—5
(34) *NN'*-Diphenyl-*S*-ethyl	Ph	Et	Ph	—	—	—	—	100 (500)	1—5
(35) *S*-Methyl-	H	Et	H	115—117	$C_2H_6N_2S$,HI	—	—	—	<1
(36) *S*-Butyl-	H	C_4H_9	H	109—110	$C_5H_{12}N_2S$,HI *	11·0	10·8	—	5
(37) *S*-Octyl	H	C_8H_{17}	H	96—96·5	$C_9H_{20}N_2S$,HBr *	10·85, 10·7	10·4	—	1

* New compound. † With decomposition.

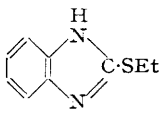
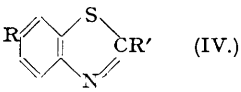
‡ See Notes to Table I. Figures in parentheses represent dilutions at which partial inhibition occurred.

(17) Needles from acetone. (18) See Experimental. (19) Baird *et al.* (*loc. cit.*) record m. p. 195°. (20) Colourless rods from ethanol–acetone. (21) Shining plates from acetone. (22) Needles from acetone–methanol. (23) Hygroscopic plates from acetone–ethanol. (24) The free base crystallised from ethanol in large pale yellow plates, m. p. 78° (Found: N, 14·85. $C_{16}H_{17}N_3S$ requires N, 14·85%). (25) The *Reineckate*, crystallised from 40% acetone, had m. p. 166—167° (decomp.) (Found: N, 20·7. $C_{24}H_{33}N_{10}S_5Cr$ requires N, 20·8%). For test this was converted into an aqueous solution of the hydrochloride. (26) The free *base* crystallised from ethanol in plates, m. p. 90° (Found: N, 12·2. $C_{20}H_{25}ON_3S$ requires N, 11·8%). It was feebly basic. (27) Reddish-brown needles from ethanol. (28) The *dihydrochloride* crystallised from ethanol containing a little hydrogen chloride in yellow needles, m. p. 219—220° (decomp.) (Found: N, 17·85. $C_{12}H_{20}N_4Cl_2S$ requires N, 17·3%), converted into red needles of the monohydrochloride by boiling with ethanol. (29) Irregular crystals from ethanol containing a little hydrogen chloride. (30) The picrate, yellow needles from 70% ethanol, had m. p. 199·5° (Found: N, 17·4. Calc. for $C_{15}H_{15}O_7N_5S$: N, 17·1%). Bertram (*Ber.*, 1892, **25**, 55) records m. p. 196°. (31) The *picrate*, yellow plates from ethanol, had m. p. 144° (Found: N, 16·2. $C_{17}H_{19}O_7N_5S$ requires N, 16·0%). (32) The *picrate*, minute yellow needles from ethanol, had m. p. 130·5° (Found: N, 14·5. $C_{21}H_{27}O_7N_5S$ requires N, 14·2%). (33) The *picrate*, large yellow prisms from ethanol, had m. p. 162—163° (Found: N, 14·9. $C_{19}H_{23}O_8N_5S$ requires N, 14·55%). (34) The free base, crystallised from aqueous ethanol, had m. p. 73° (Found: N, 11·1. Calc. for $C_{15}H_{16}N_2S$: N, 10·95%. Will (*Ber.*, 1881, **14**, 1490) records m. p. 79° and Rathke (*ibid.*, p. 1777) records m. p. 73°. (35) Bernthsen and Klinger (*Ber.*, 1878, **11**, 492) record m. p. 117°. (36) Plates from chloroform. (37) Plates from chloroform–ether.

R = H, R′ = Et). It is now clear that, notwithstanding the apparent logic of the approach, the activities of the thiadiazoles and thiosemicarbazones are in fact entirely unrelated. Thus, while the oxa- and thia-diazoles are equally active, semicarbazones, in contrast with the corresponding thiosemicarbazones, are entirely inactive (Behnisch, Mietzsch, and Schmidt, *Angew. Chem.*, 1948, **60**, 113; Hoggarth *et al.*, *loc. cit.*). Further, while the sulphanilamido-group is essential to the activity of the oxa- and thia-diazoles, it greatly reduces the activity in the case of *iso*thiosemicarbazones as noted above. The fact that the *iso*thiosemicarbazones are more closely related structurally to the thiadiazoles than are the thiosemicarbazones is therefore without significance. The latter structure is apparently more favourable in this series to the development of *in vivo* activity, in spite of the approximately equivalent *in vitro* activity of the two types of compound. Our own subsequent trials have confirmed the high *in vivo* activity of selected members of the thiosemicarbazone group (Domagk, *Nordisk Medicin*, 1948, **39**, 1322; Hoggarth *et al.*, *loc. cit.*).

As a further simplification of the basic structure, *S*-alkyl*iso*thioureas, prepared by standard methods, were next examined. Very high *in vitro* activity was shown by *N*-phenyl- and *NN′*-diphenyl-*S*-ethyl*iso*thioureas (30, 34). This was reduced in the former case by substitution of *S*-butyl and *S*-octyl groups (31, 32) for ethyl or by introduction of a *p*-butoxy-group into the *N*-phenyl substituent (33). This series was not extended, since poor activity in the presence of serum precluded *in vivo* activity.

2-Ethylthiobenziminazole (III), which may be regarded as a cyclic *iso*thiourea, was prepared from 2-mercaptobenziminazole (Billeter and Steiner, *Ber.*, 1887, **20**, 231) by interaction with ethyl iodide. It was only weakly active. 2-Aminobenzthiazoles may also be regarded as cyclic *iso*thioureas, and the claims of Freedlander and French (*Proc. Soc. Exp. Biol. Med.*, 1947, **66**, 362) that *in vivo* activity had been demonstrated with such compounds and with related 2-alkoxybenzthiazoles prompted us to examine a selection of these compounds.

(III.)   (IV.)

2 : 6-Diaminobenzthiazole (IV; R = R′ = NH_2), stated to be one of the most active compounds, was prepared by the method of Kaufmann and Schultz (*Arch. Pharm.*, 1935, **273**, 31). It had relatively low *in vitro* activity and no *in vivo* activity. 2-Amino-6-butoxybenzthiazole (IV;

TABLE III.

2-Alkylthiopyrimidines.

Pyrimidine.	B. p.	M. p.	Formula.	Found N, %.	Req. N, %.
(38) 2-Mercapto-4 : 6-dimethyl-	—	209—212°	—	—	—
(39) 2-Methylthio-4 : 6-dimethyl-	113—114°/15 mm.	27—28	$C_7H_{10}N_2S$	18·2	18·2
(40) 2-*Methylthio*-4-*phenyl*-6-*methyl*-	154—160°/1 mm.	—	$C_{12}H_{12}N_2S$	13·0	13·0
(41) 4-Hydroxy-2-methylthio-6-methyl-	—	224—225	—	—	—
(42) 4-Hydroxy-2-methylthio-5-methyl-	—	234·5—236	—	—	—
(43) 4-*Hydroxy*-2-*butylthio*-5-*methyl*-	—	105—106	$C_9H_{14}ON_2S$	14·5, 14·3	14·1
(44) 4-*Hydroxy*-2-*octylthio*-5-*methyl*-	—	88—89	$C_{13}H_{22}ON_2S$	11·1	11·0
(45) 4-Chloro-2-methylthio-6-methyl-	118—120°/12 mm.	34—34·5	—	—	—
(46) 4-*Chloro*-2-*methylthio*-6-*methyl*-	132°/15 mm.	20—23	$C_6H_7N_2ClS$	15·9	16·0
(47) 4-*Chloro*-2-*butylthio*-5-*methyl*-	124—126°/1 mm.	—	$C_9H_{12}N_2ClS$	12·6	12·9
(48) 4-*Chloro*-2-*octylthio*-5-*methyl*-	144—148°/2 mm.	—	$C_{13}H_{21}N_2ClS$	10·5	10·2
(49) 4-Amino-2-methylthio-6-methyl-	—	135—136	—	—	—
(50) 4-*Amino*-2-*methylthio*-5-*methyl*-	—	130—131	$C_6H_9N_3S$	27·4	27·1
(51) 4-*Amino*-2-*butylthio*-5-*methyl*-	—	85—86	$C_9H_{15}N_3S$	21·5	21·3
(52) 4-*Amino*-2-*octylthio*-5-*methyl*-	—	85—86	$C_{13}H_{23}N_3S$	16·7	16·6

(38) Evans (*J. pr. Chem.*, 1893, **48**, 489) records m. p. 210°. (39) Hale and Williams (*J. Amer. Chem. Soc.*, 1915, **37**, 594) record m. p. 24°. Wheeler and Merriam (*Amer. Chem. J.*, 1904, **32**, 356) record m. p. 23—24° and b. p. 123—125°/14 mm. (41) Wheeler and Merriam (*ibid.*, 1903, **29**, 478) record m. p. 219°. (42) *Idem, ibid.*, record m. p. 233°. (43) Rods from ethanol. (44) Rhombic plates from methanol. (45) Wheeler and McFarland (*loc. cit.*) record b. p. 147°/32—35 mm., needles (from light petroleum), m. p. 39—40°. (49) Backer and Grevenstuk (*Rec. Trav. chim.*, 1945, **64**, 115) and Hull, Lovell, Openshaw, and Todd (*J.*, 1947, 41) record 136—137° and 133·5—135° respectively. (50) Clusters of rods from water. (51) Thin plates from benzene. (52) Irregular plates from light petroleum.

R = OBu, R′ = NH_2), probably identical with the "*p*-butoxyaminobenzthiazole" described in F.P. 742,398, was prepared by the same procedure and was also devoid of activity. Attempted preparation of 6-amino-2-butoxybenzthiazole by the reduction of 6-*nitro*-2-*butoxybenzthiazole* with stannous chloride led to simultaneous chlorination with the formation of x-*chloro*-6-*amino*-2-*butoxybenzthiazole dihydrochloride*, also quite inactive.

Finally, as a further variant of alkyl*iso*thioureas, a number of substituted 2-alkylthiopyrimidines were prepared. These are summarised in Table III. In the preparation of 2-mercapto-4-phenyl-6-methylpyrimidine (Merkatz, *Ber.*, 1919, **52**, 869) as an intermediate for the 2-*methylthio*-derivative (40), an alkali-insoluble by-product was obtained and identified as *di*-(4-*phenyl*-6-*methyl*-2-*pyrimidyl*) *disulphide*, the same product being produced by oxidation of the mercaptopyrimidine with iodine. Condensation of the appropriate 2-alkyl*iso*thiourea with ethyl α-formylpropionate according to the method of Wheeler and Merriam (*Amer. Chem. J.*, 1903, **29**, 478) afforded 4-hydroxy-2-methylthio-, -2-*butylthio*-, and -2-*octylthio*-5-*methylpyrimidines* (42, 43, 44). These were converted smoothly into the 4-*chloro*-derivatives (46, 47, 48) by phosphorus pentachloride and phosphorus oxychloride (Wheeler and McFarland, *Amer. Chem. J.*, 1909, **42**, 431) and thence by ethanolic ammonia into the 4-*amino*-derivatives (50, 51, 52). The corresponding 2-methylthio-6-methylpyrimidines (41, 45, 49) were also prepared. All compounds except the intermediate chloro-derivatives were tested *in vitro*. Activities are not recorded since in no case were the compounds active at a dilution greater than 1 in 1000 in the presence of serum.

Experimental.

N^4-*Acyl Derivatives of* 2-*Sulphanilamido*-5-*methyl*-1 : 3 : 4-*oxadiazole*.—These were prepared by the condensation of 2-sulphanilamido-5-methyl-1 : 3 : 4-oxadiazole with the corresponding acid anhydride in benzene solution in the case of the *hexoyl* derivative, and with the corresponding acid chloride in pyridine solution in the case of the *dodecoyl* and *stearoyl* derivatives. The *toluene*-p-*sulphonyl* derivative was similarly prepared in pyridine. The products were crystallised from ethanol.

2-*Amino*-5-*alkyl*-1 : 3 : 4-*oxadiazoles*.—1-Acylthiosemicarbazides were prepared by heating a mixture of powdered thiosemicarbazide (1 mol.) with the appropriate acid anhydride (1 mol.) at 80° and finally at 110—120°. The product was triturated with ether or ethanol–ether and crystallised from ethanol. This afforded an impure product which was, however, satisfactory for the next stage. The crude acylthiosemicarbazide (10·0 g.) was stirred and heated under reflux with absolute ethanol (150—200 c.c.) and lead monoxide (3 mols.) for 15—30 hours. The lead sulphide and unchanged lead oxide were filtered off, the ethanol was removed *in vacuo*, and the solid residue triturated with aqueous sodium hydroxide to remove unchanged acylthiosemicarbazide. The residue was crystallised from ethanol–ether. In the case of 1-stearoylthiosemicarbazide the yield was poor owing to the formation of an insoluble lead salt of the thiosemicarbazide. Some improvement was effected by conducting the reaction in propanol instead of ethanol.

2-*Sulphanilamido*-5-*alkyl*-1 : 3 : 4-*oxadiazoles*.—The 2-amino-5-alkyloxadiazole was heated with acetylsulphanilyl chloride (1 mol.) in dry pyridine at 100° for 20—45 minutes, and the mixture poured into excess of dilute hydrochloric acid. The crude N^4-acetyl derivative was filtered off and hydrolysed in the usual manner with boiling 2N-sodium hydroxide. The crude product, obtained by acidification with hydrochloric acid, was purified by crystallisation from ethanol or aqueous ethanol.

2-*Sulphanilamido*-5-*amyl*-1 : 3 : 4-*thiadiazole*.—1-Hexoylthiosemicarbazide (22 g.) and benzenesulphonic acid (30 g., 1·25 mols.) were heated on the steam-bath for 15 minutes. The melt was cooled and dissolved in water, and the clear solution basified with ammonia. The precipitated solid was crystallised from ethanol, affording 2-*amino*-5-*amyl*-1 : 3 : 4-*thiadiazole* (17 g.) as needles, m. p. 195° (Found : N, 24·8. $C_7H_{13}N_3S$ requires N, 24·6%). The N^4-*acetylsulphanilyl* and *sulphanilyl* derivatives (see Table I) were prepared by the same methods as for the oxadiazoles and were both crystallised from ethanol.

2-(3 : 5-*Di-iodosulphanilamido*)-5-*methyl*-1 : 3 : 4-*oxadiazole*.—2-Sulphanilamido-5-methyloxadiazole (2·5 g.) was dissolved in water (15 c.c.) and concentrated hydrochloric acid (4 c.c.), and a solution of iodine monochloride (3·3 g.) in concentrated hydrochloric acid (3 c.c.) added. The solution soon became turbid and deposited a dark oil which solidified when warmed and scratched. The solid was filtered off, dried, and crystallised from ethanol. Yield, 3·0 g. (see Table I).

Benzaldehyde 4-p-*Butoxyphenylthiosemicarbazone*.—Following the general method for the preparation of aryl*iso*thiocyanates (*Org. Synth.*, Coll. Vol. I, 1941, p. 448), *p*-butoxyaniline (33 g.) was mixed with carbon disulphide (24 g.) with ice-cooling. Considerable heat was evolved, and the mixture solidified. Ethanol (36 c.c.) was added and after 2 hours the solid was broken up and cooled to 10°. Concentrated aqueous ammonia (33 g.) was then added at 10—15° in small portions with vigorous shaking. The solid dissolved and the ammonium salt of the dithiocarbamate crystallised. After being kept overnight, the crystals were filtered off, washed with ether (2 × 20 c.c.), and dissolved in water (400 c.c.). Addition of a solution of lead nitrate (70 g.) in water (200 c.c.) caused rapid precipitation of lead sulphide. The reaction was completed by boiling and the *p*-butoxyphenyl *iso*thiocyanate (11·25 g., 27%) isolated by extraction with ether as a mobile, odourless oil. The crude product (4·25 g.) in absolute ethanol (6 c.c.) was treated with hydrazine hydrate (1·2 c.c. of 90%) in ethanol (1 c.c.) with cooling. 4-*p*-Butoxyphenylthiosemicarbazide separated immediately in plates (4·2 g.), m. p. 175°. The crude product was heated under reflux for 3 hours with ethanol (60 c.c.) and benzaldehyde (2·2 g.). After filtration from a little undissolved solid, the solution on concentration afforded *benzaldehyde* 4-p-*butoxyphenylthiosemi-*

carbazone (4·5 g.) as pale yellow needles, m. p. 164—165° after recrystallisation from ethanol (Found : N, 12·9. $C_{18}H_{21}ON_3S$ requires N, 12·85%).

3-*Alkyl*iso*thiosemicarbazones*.—These were prepared from the corresponding thiosemicarbazones by alkylation with sodium ethoxide and the appropriate alkyl halide in ethanol (Baird, Burns, and Wilson, *loc. cit.*). The hydrochlorides were formed by precipitation of the crude bases in ether with hydrogen chloride and by crystallisation of the product from a suitable solvent (see Table II).

Acetone 4-N^4-*Acetylsulphanilyl*-3-*ethyl*iso*thiosemicarbazone*.—Acetone 3-ethyl*iso*thiosemicarbazone (3·2 g.) was stirred with 50% ethanol (25 c.c.) and sodium hydrogen carbonate (2·1 g.), and acetylsulphanilyl chloride (5·13 g., 1·1 mols.) was added at 20°. The viscous oil, which separated on stirring of the mixture overnight, afforded a granular solid on trituration with ether containing a little ethanol. Crystallisation from ethanol yielded crude *acetone* 4-N^4-*acetylsulphanilyl*-3-*ethyl*iso*thiosemicarbazone* (0·67 g.) as prisms, m. p. 167—168° raised by repeated recrystallisation from ethanol and from acetic acid to 183—184° (Found : N, 15·8. $C_{14}H_{20}O_3N_4S_2$ requires N, 15·7%).

When sodium hydroxide was used instead of sodium hydrogen carbonate the sole product obtained was ethyl acetylsulphanilate, plates (from aqueous ethanol), m. p. 121—122° (Found : N, 5·9. Calc. for $C_{10}H_{11}O_4NS$: N, 5·8%). Crossen, Jenkins, and Rogers (*Pharm. Arch.*, 1941, **12**, 26) quote m. p. 121°. Hydrolysis afforded sulphanilic acid, identified by colour reactions and analysis (Found : N, 8·0. Calc. for $C_6H_7O_3NS$: N, 8·1%).

Acetone 4-*Sulphanilyl*-3-*ethyl*iso*thiosemicarbazone*.—The foregoing compound (0·17 g.) was heated with 2·5N-sodium hydroxide (1 c.c.) at 100° for 1 hour. Acidification to pH 4 afforded a precipitate of *acetone* 4-*sulphanilyl*-3-*ethyl*iso*thiosemicarbazone* (0·14 g.), prisms (from ethanol), m. p. 186—187° (Found : N, 17·9. $C_{12}H_{16}O_2N_4S$ requires N, 17·8%).

N-p-*Butoxyphenylthiourea*.—*p*-Butoxyphenyl *iso*thiocyanate (7·0 g.) in ethanol (20 c.c.) was treated with gaseous ammonia with cooling until no further crystalline material separated. The product (7·5 g.) was crystallised from ethanol, giving N-p-*butoxyphenylthiourea* as rods, m. p. 163° (Found : N, 12·4. $C_{11}H_{16}ON_2S$ requires N, 12·5%).

S-*Alkyl*iso*thioureas*.—These were prepared from the appropriate thiourea and alkyl halide in refluxing ethanol. The resulting alkyl*iso*thiourea hydrohalides were tested as such or converted into picrates and thence into aqueous solutions of the hydrochlorides for test.

2-*Ethylthiobenziminazole*.—2-Mercaptobenziminazole (Billeter and Steiner, *loc. cit.*) (1·5 g.), suspended in ethanol (5 c.c.), was heated under reflux with ethyl iodide (1·6 g.) for 2 hours, complete dissolution occurring after 15 minutes. The ethanol was evaporated *in vacuo* and the crystalline residue (2·88 g.) dissolved in water, the solution filtered, and the free base precipitated with sodium carbonate solution. Recrystallisation of the resulting white solid from aqueous ethanol afforded 2-*ethylthiobenziminazole* as needles, m. p. 173·5—174·5° (Found : N, 15·9. $C_9H_{10}N_2S$ requires N, 15·7%).

2-*Amino*-6-*butoxybenzthiazole*.*—A solution of bromine (10 g.) in glacial acetic acid (13 c.c.) was added slowly with stirring to a solution of *p*-butoxyaniline (8·25 g.) and ammonium thiocyanate (9·5 g.) in 95% acetic acid (50 c.c.). The yellow solid which separated overnight was filtered off and the filtrate diluted with water. Addition of aqueous sodium hydroxide caused first the separation of a dark oil, which was discarded, and then a solid (2·2 g.), m. p. 101°. Repeated crystallisation from carbon tetrachloride afforded 2-amino-6-butoxybenzthiazole as prisms, m. p. 119° (Found : N, 12·7. Calc. for $C_{11}H_{14}ON_2S$: N, 12·6%). F.P. 742,398 cites m. p. 121° for "*p*-butoxyaminobenzthiazole".

x-*Chloro*-6-*amino*-2-*butoxybenzthiazole Dihydrochloride*.*—2-Chloro-6-nitrobenzthiazole was prepared by the method of Hofmann (*Ber.*, 1880, **13**, 10) from 2-chlorobenzthiazole (Scott and Watt, *J. Org. Chem.*, 1937, **2**, 148). This (10 g.) was dissolved in warm butanol (250 c.c.), a solution of sodium (1·07 g.) in butanol (50 c.c.) added, and the mixture boiled under reflux for 20 hours. After filtration, the solution was evaporated to a small volume, affording 6-*nitro*-2-*butoxybenzthiazole* (4·15 g.), needles (from chloroform), m. p. 60° (Found : N, 11·2. $C_{11}H_{12}O_3N_2S$ requires N, 11·1%). The foregoing (10 g.) was added to a preheated solution of crystalline stannous chloride (32 g.) in concentrated hydrochloric acid (40 c.c.) at such a rate as to maintain the temperature at 70—80°. The reaction was completed by boiling under reflux for 30 minutes. The solution was then poured into 6N-sodium hydroxide (200 c.c.), and the solution extracted with butanol (4 × 50 c.c.). The combined extracts were dried (K_2CO_3) and hydrogen chloride passed in. The precipitated solid (2·5 g.) was recrystallised from aqueous ethanol, giving x-*chloro*-6-*amino*-2-*butoxybenzthiazole dihydrochloride* as needles, m. p. 268° (decomp.) (Found : N, 8·5, 8·7; Cl, 33·4. $C_{11}H_{15}ON_2Cl_3S$ requires N, 8·5; Cl, 32·3%). It dissolved in water to give a solution of low pH which could be adjusted to pH 6 without precipitation.

Di-(4-*phenyl*-6-*methyl*-2-*pyrimidyl*) *Disulphide*.—In the preparation of 2-mercapto-4-phenyl-6-methylpyrimidine (Merkatz, *loc. cit.*) a fraction was obtained which was insoluble in ethanol and in dilute hydrochloric acid or sodium hydroxide. It was purified by exhaustive extraction with N-hydrochloric acid. Crystallisation from benzene afforded *di*-(4-*phenyl*-6-*methyl*-2-*pyrimidyl*) *disulphide* as yellow rods, m. p. 185·5—186° (Found : N, 13·9; *M*, 397. $C_{22}H_{18}N_4S_2$ requires N, 13·9; *M*, 402). The same compound, m. p. 184—184·5° not depressed by the above, was formed when a solution of the mercaptopyrimidine in dilute sodium hydroxide was oxidised with a slight excess of aqueous iodine solution.

4-*Hydroxy*-2-*alkylthio*-5-*methylpyrimidines*.—The appropriate *S*-alkyl*iso*thiourea hydrohalide (1 mol.) was dissolved in one equivalent of 2·5N-sodium hydroxide, and, if necessary, ethanol was added to dissolve precipitated oil or solid. Crude ethyl sodioformylpropionate (1 mol.) was then added with sufficient water to give a clear solution. After 24 hours the solution, filtered if necessary, was acidified with acetic acid, and the product dried and crystallised (see Table III).

4-*Chloro*-2-*alkylthio*-5-*methylpyrimidines*.—The 4-hydroxypyrimidine was boiled under reflux with a mixture of phosphorus pentachloride (1 mol.) and an equal weight of phosphorus oxychloride for 45 minutes. Phosphorus oxychloride was distilled off *in vacuo* and the residue poured on crushed ice. The crude solid or oil was isolated with ether and distilled (see Table III).

* Experiments by Mr. J. L. Lowe.

4-*Amino*-2-*alkylthio*-5-*methylpyrimidines*.—The chloropyrimidine was heated under pressure at 135—150° with 10% ethanolic ammonia (*ca.* 8—10 parts by weight) for 6—8 hours. The ammonium chloride which separated on cooling was filtered off and the filtrate evaporated. The residue was dissolved in dilute acetic acid, and the product precipitated as a solid by the addition of aqueous sodium hydroxide and crystallised from a suitable solvent (see Table III).

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