Chemical Constitution and Fungistatic Action of Organic Sulphur Compounds

By W. H. DAVIES AND W. A. SEXTON, *Imperial Chemical Industries Limited,
Dyestuffs Division, Research Laboratories, Blackley, Manchester*

(Received 4 February 1946)

Elementary sulphur and some of its inorganic compounds have long been of practical importance for the control of parasitic and saprophytic fungi, but the use of organic compounds of sulphur is of more recent origin. Compounds such as tetramethylthiuram disulphide and certain metallic salts of dithiocarbamic acids are now of commercial importance in this connexion (Tisdale & Flenner, 1942; Roberts, 1942). This paper records observations made over some years on the relation of chemical constitution to fungistatic action in this and closely related classes of organic sulphur compounds.

The fungicidal properties of the xanthic acid derivatives were first mentioned in the patent literature (Böhringer, 1929). The only recorded systematic investigation of homologous xanthates is that of Goldsworthy, Carter & Green (1942) where a series of cuprous salts was examined. The fungicidal properties of salts of dithiocarbamic acids and of their oxidation products, the thiuram sulphides, have been mentioned in the patent literature since 1931. Goldsworthy, Green & Smith (1942) examined metallic salts of three dialkyldithiocarbamic acids, but apart from this paper the few references in the scientific literature are to individual compounds, such as tetramethylthiuram disulphide, which have found commercial applications as fungicides. Limited investigations of the fungicidal activity of thiocyanates and isothiocyanates are recorded by Walker (1937), Wilcoxon & McCallan (1935) and Walker, Morell & Foster (1937). The first mention of the fungicidal properties of benzthiazole derivatives was in a patent (Grasselli, 1932) which disclosed that the 1-hydroxy, 1-amino and 1-thiol derivatives were fungicides. The fungicidal activity of the 1-thiol and 1-phenyl derivatives was investigated by Everitt & Sullivan (1940).

METHODS

Preparation of compounds. The compounds examined were prepared by conventional methods. Those which are new are listed, together with criteria of purity, in Table 1.

Fungistatic tests. The testing was carried out partly in the laboratories of Prof. H. Raistrick at the London School of Hygiene and Tropical Medicine and partly in the Hawthorndale Laboratories, Jealott's Hill Research Station, Imperial Chemical Industries Limited.

During the period covered by the investigation there were certain minor variations in technique, but a standard fungistatic substance, sodium salicylanilide, was included in all tests, and this showed no variation in activity, thus checking the reliability of the method for comparing the activities of the various compounds under investigation.

The method consisted, briefly, of inoculating an agar nutrient medium containing known amounts of the substance under test with spores of the fungi and observing the growth visually. A method was thereby provided of comparing fungistatic but not necessarily fungicidal activity. In earlier tests the following four organisms were used: *Cladosporium herbarum* Link., *Botrytis cinerea* Pers., *Fusarium caeruleum* (Lib.) Sacc., *Penicillium digitatum* Sacc.; but in later tests only the following two were employed: *Fusarium graminearum* Schwabe, *Penicillium digitatum*.

The concentrations of test substance in the media formed a fivefold dilution series and are referred to by their serial dilution numbers as shown below:

Concentration in medium	Dilution no.
1:500	1
1:2,500	2
1:12,500	3
1:62,500	4
1:312,500	5
1:1,561,500	6
1:7,807,500	7

In recording the results the figures given in brackets after a compound are the appropriate dilution numbers related to concentration as shown above, and indicating the highest concentration which permitted growth, usually only restricted growth.

Table 1. *Characteristics of new compounds tested*

Compound	Physical characteristics	Elementary analysis (%)	
		Found	Required
$\text{Me}_2\text{N} \cdot \text{CS} \cdot \text{SC}_4\text{H}_9(n)$	b.p. 157–159°/20 mm.	S, 36.9	36.2
$\text{Me}_2\text{N} \cdot \text{CS} \cdot \text{SCH}_2\text{Ph}$	b.p. 220°/20 mm., m.p. 40–41°	S, 30.5	30.2
$\text{Me}_2\text{N} \cdot \text{CS} \cdot \text{SC}_4\text{H}_9\text{OH}$	Undistillable liquid	S, 38.1	38.7
$\text{Me}_2\text{N} \cdot \text{CS} \cdot \text{SCH}_2 \cdot \text{CH} : \text{CH}_2$	b.p. 140°/20 mm.	S, 40.6	39.8
$\text{CH}_2 \begin{array}{c} \text{CH}_2 - \text{CH}_2 \\ \diagup \quad \diagdown \\ \text{CH}_2 - \text{CH}_2 \end{array} \text{N} \cdot \text{CS} \cdot \text{SMe}$	b.p. 164–166°/16 mm., m.p. 31–32°	S, 36.6	36.6
$(\text{Et}_2\text{NCS})_2\text{S}$	m.p. 33–34°	C, 45.7 H, 7.6 N, 10.5 S, 36.1	45.6 7.6 10.6 36.4
$\text{Me} \begin{array}{c} \diagup \\ \text{HO} - \text{C}_6\text{H}_4 - \text{NCS} \end{array}$	b.p. 146–149°/3 mm., m.p. 52–54°	N, 8.45	8.5
$\text{Cl} \begin{array}{c} \diagup \\ \text{HO} - \text{C}_6\text{H}_4 - \text{NCS} \end{array}$	m.p. 58–59°	N, 8.0	7.7
$\text{HOOC} \begin{array}{c} \diagup \\ \text{C}_6\text{H}_4 - \text{SCN} \end{array}$	m.p. 177–179° (dec.)	C, 53.4 H, 2.8 N, 7.8	53.6 2.8 7.8
$\text{HOOC} \begin{array}{c} \diagup \\ \text{C}_6\text{H}_4 - \text{SCN} \end{array}$	m.p. 220–221° (dec.)	C, 54.1 H, 2.95 N, 7.75	53.6 2.8 7.8

RESULTS

An examination of the effects of all the compounds on the different organisms revealed no marked specificity of action except that *Penicillium digitatum* was frequently more resistant than the others. Results, except where otherwise stated, relate to this organism, which was common to all the tests. As standards of comparison, several well-known fungicides were included, with the following results:

	Dilution no.
Salicyl anilide	4
8-Hydroxyquinoline	6
Phenylmercuriacetate	6
Ethylmercurichloride	8

Xanthates and xanthyl sulphides:

The most active compound tested was potassium methylxanthate (5) and against *Botrytis cinerea* and *Fusarium caeruleum* it was fungistatic up to (7). As the homologous series of xanthates was ascended ($R = \text{Et}$, C_4H_9 (*n*- and *iso*-) $\text{C}_6\text{H}_{11}(n)$, $\text{C}_{12}\text{H}_{25}(n)$) the activity decreased, the highest member giving full growth of *Penicillium digitatum* at (1), though at this concentration it afforded complete control of *Botrytis cinerea*. This is in contrast to the findings of Goldsworthy, Carter & Green (1942) on the

cuprous salts, who found maximum activity with the ethyl and butyl derivatives. In our tests the nature of the metal ($M = \text{Na}$, K , Ni , Zn) caused little change in the activity. The xanthyl mono- and disulphides ($R = \text{Me}$, Et and $\text{C}_4\text{H}_9(n)$) had activities approximately equal to the corresponding xanthate salts.

Thiuram sulphides and related types:

Thiuram disulphide ($R' = R'' = \text{H}$) had very low activity (< 2), whereas the dimethyl ($R' = \text{Me}$, $R'' = \text{H}$) and tetramethyl ($R' = R'' = \text{Me}$) derivatives were active at (3–4). Tetraethylthiuram disulphide ($R' = R'' = \text{Et}$) had about the same activity as the tetramethyl analogue, but further increase in molecular weight ($R' = \text{Ph}$, $R'' = \text{Me}$; $R'R'' = -(\text{CH}_2)_5-$) gave compounds less fungistatic than thiuram sulphide itself. The monosulphides ($R' = R'' = \text{Me}$; $R' = R'' = \text{Et}$) had about the same activity as the corresponding disulphides.

Dithiocarbamic esters: $\text{R}'\text{R}''\text{N} \cdot \text{CS} \cdot \text{SR}'''$.

Esters of dithiocarbamic acid were much less active than the corresponding thiuram sulphides. In the dimethyldithiocarbamates ($R' = R'' = \text{Me}$) the methyl ester was assessed at (2). Of the other esters tested ($R''' = \text{C}_4\text{H}_9$, CH_2Ph , $\text{C}_2\text{H}_4\text{OH}$, $\text{CH}_2 : \text{CH} \cdot \text{CH}_2$)

the allyl derivative was the most active (4). Of the methyl esters of other dithiocarbamates

($R''' = \text{Me}$, $R' = R'' = \text{Et}$, $R' = R'' = \text{cyclo-C}_6\text{H}_{11}$,
 $R' \cdot R'' = -(\text{CH}_2)_6$, $R' = \text{Ph}$, $R'' = \text{H}$),

the phenyl derivative $\text{PhNH} \cdot \text{CSSMe}$ was the most active (2), and two other esters ($R''' = \text{Et}$, $R''' = \text{CH}_2\text{Ph}$) of the same acid had slightly increased activity (3).

Thiocarbamic and carbamic esters:

($R'\text{NHCSOR}''$, $R'\text{NHCO} \cdot \text{SR}''$ and $R'\text{NHCOOR}''$)

Methyl phenylthioncarbamate showed a low activity (about 2) equal to that of the dithio ester. Increasing the size of the ester grouping

($R'' = \text{Et}$, C_3H_7 (*n*- and *iso*-),
 C_4H_9 (*n*- and *iso*-), $\text{C}_{12}\text{H}_{25}$ (*n*))

slowly decreased the fungistatic powers. Substitution of the methyl ester in the aromatic nucleus ($R' = p\text{-MeC}_6\text{H}_4$, $p\text{-Cl} \cdot \text{C}_6\text{H}_4$) caused no change in activity.

Comparison of sulphur derivatives with oxygen analogues

In some of the series examined it was observed that replacement of sulphur by oxygen did not markedly affect the fungicidal activity. These results are summarized in Table 2.

Table 2. Effect of replacing sulphur by oxygen

Compound	Highest dilution permitting growth	Compound	Highest dilution permitting growth
$\text{PhNHCS} \cdot \text{SMe}$	2	$(\text{EtO} \cdot \text{CS})_2\text{S}$	4-5
$\text{PhNHCS} \cdot \text{OMe}$	2	$\text{EtO} \cdot \text{CS}$	4
$\text{PhNHCO} \cdot \text{SMe}$	2	EtOCO	
$\text{PhNHCO} \cdot \text{OMe}$	3	$(\text{EtO} \cdot \text{CO})_2\text{S}$	4
$\text{PhNHCS} \cdot \text{SEt}$	3	$\text{Me}_2\text{N} \cdot \text{CS}$	5-6
$\text{PhNHCS} \cdot \text{OEt}$	2	$\text{MeO} \cdot \text{CO}$	
$\text{PhNHCO} \cdot \text{OEt}$	3	$\text{MeO} \cdot \text{CS}$	4
		$\text{MeO} \cdot \text{CO}$	

It is of interest that $\text{PhNHCO} \cdot \text{OEt}$, which has been found to be of high potency in preventing germination of seeds of certain green plants (Templeman & Sexton, 1945) by interference with cell division, showed no abnormal effects on fungi.

Thiocyanates and isothiocyanates (RSCN and RNCS)

The lower aliphatic thiocyanates ($R = \text{Et}$, C_4H_9 , (*n*)) and isothiocyanates ($R = \text{Et}$, C_4H_9 , C_6H_5) had very low activity. Phenyl thiocyanate, on the other hand, was active to 4; substitution in the phenyl group

($R = o$ -, *m*- and *p*- $\text{HOOC} \cdot \text{C}_6\text{H}_4$, $p\text{-HO} \cdot \text{C}_6\text{H}_4$,
 $p\text{-AcO} \cdot \text{C}_6\text{H}_4$, $p\text{-NH}_2 \cdot \text{C}_6\text{H}_4$, $p\text{-AcNH} \cdot \text{C}_6\text{H}_4$)

did not affect the activity greatly, but the *p*-dimethylamino derivative was less active (<3) and the *p*-chlorophenyl more active (5).

Phenyl isothiocyanate was less active (3) than the thiocyanate, but substitution in the nucleus enhanced the activity in the following order:

$p\text{-Me} < p\text{-Cl} = p\text{-MeO} < m\text{-COOH} < 4\text{-HO} \cdot 2 \cdot 6\text{-Me}_2$
 $< p\text{-COOH} < p\text{-HO} = p\text{-AcO} = m\text{-AcO}$
 $= 3\text{-Cl} \cdot 4\text{-HO} = 4\text{-HO} \cdot 3\text{-Me}.$

These last compounds gave only a trace of growth at (6), thus being at least equal to 8-hydroxyquinoline and phenylmercuriacetate.

Benzthiazole derivatives:

The compounds tested were all derivatives of 1-hydroxy (I), 1-thiol (II) or 1-aminobenzthiazole (III). The thiol derivative was assessed at (3) and the hydroxy and amino compounds at (2). Methylation of the thiol group in II did not reduce the activity, but other alkyl groups (Et , C_3H_7 (*n*), $\text{C}_4\text{H}_9\text{OH}$) caused slight reduction. 4-Chloro-1-thiolbenzthiazole was less active than (II), but 4-chloro-1-methyl-thiolbenzthiazole was, surprisingly, more active (4). The 5-ethoxy and 5-nitro derivatives of (II) showed low activity (2) and the disulphide from II was practically inactive. Substitution in the amino group of III (Me , Ph) decreased the activity, but the 5-ethoxy derivative permitted growth only at (2). The most active compound in the series was 1-thiocyanobenzthiazole (5), a fact which is not surprising in view of its high chemical reactivity (Davies & Sexton, 1944).

DISCUSSION

No generalizations can be made from the results obtained, but two points are noted by way of comment:

(1) The lower xanthate derivatives and the hydroxyarylisothiocyanates must be regarded as amongst the most highly fungistatic synthetic organic substances known, the activity of the latter being at least equal to that of the best arylmercuri-salts, e.g. phenylmercuriacetate, though falling short of the more powerful ethylmercuri-salts.

(2) It appears from the results with quite a number of compounds, notably thiocarbamic acid derivatives, that sulphur *per se* makes no contribution to the fungistatic activity. Comparisons between sulphur and oxygen analogues in some cases are not possible because of the non-existence or extreme instability of the oxygen compounds. In these

cases the sulphur atoms permit the synthesis of unique molecular types, but it is probably the molecule as a whole and not some special attribute of the sulphur atoms which is responsible for the biological effect.

SUMMARY

A series of xanthic, dithiocarbamic, thiocyanic and isothiocyanic acid derivatives, together with some oxygen analogues and benzthiazole derivatives,

have been prepared and their relative fungistatic activities recorded. In many of the compounds the sulphur atoms can be replaced by oxygen without marked loss of fungistatic power. The lower xanthates and certain substituted arylisothiocyanates have very high fungistatic activities.

The authors are indebted to several colleagues for the preparation of certain compounds, for helpful discussions and also for the carrying out of the biological testing.

REFERENCES

- Böhringer (1929). G.P. 485059.
 Davies, W. H. & Sexton, W. A. (1944). *J. chem. Soc.* p. 11.
 Everitt, E. L. & Sullivan, M. X. (1940). *J. Wash. Acad. Sci.* **30**, 125.
 Goldsworthy, M. C., Carter, R. H. & Green, E. L. (1942). *Phytopathology*, **32**, 497.
 Goldsworthy, M. C., Green, E. L. & Smith, M. A. (1942). *Phytopathology*, **32**, 6.
 Grasselli (1932). B.P. 407691.
 Roberts, J. W. (1942). *J. industr. engng Chem.* **34**, 497.
 Templeman, W. G. & Sexton, W. A. (1945). *Nature, Lond.*, **156**, 630.
 Tisdale, W. H. & Flenner, A. L. (1942). *J. industr. engng Chem.* **34**, 501.
 Walker, J. C. (1937). *Phytopathology*, **27**, 142.
 Walker, J. C., Morell, S. & Foster, H. H. (1937). *Amer. J. Bot.* **24**, 536.
 Wilcoxon, F. & McCallan, S. E. A. (1935). *Contr. Boyce Thompson Inst.* **7**, 333.

The Composition of Bovine Cervical Mucins and their Reaction with Oxidizing Agents

By E. BOYLAND, *Veterinary Laboratory, Ministry of Agriculture and Fisheries, New Haw, Weybridge*

(Received 17 December 1945)

Woodman & Hammond (1925) found that the appearance of bovine cervical mucin differs according to the physiological condition of the cow, and suggested that the nature of the mucin could be used as a guide to the reproductive state of the animal. The physical properties of the three types of vaginal mucin have been investigated by Scott Blair and his colleagues (Scott Blair, Folley, Malpress & Coppen, 1941; Scott Blair, Cowie & Folley, 1942), who have shown that the mucin of pregnant animals is stiff and plastic rather than elastic; that of oestrus shows a particular type of elasticity, while the dioestrous mucin is viscous but not elastic. The degree of flow elasticity can be measured with the 'oestroscope' and used for diagnosis of oestrus in cows. The mucin present in oestrus appears to contain very little protein and is readily differentiated from the mucins of dioestrus and pregnancy. No easy chemical test differentiating the two types of mucin, which might serve as a pregnancy diagnosis reaction, has been found.

Pathogenic organisms infecting the uterus and vagina of animals may be protected by the mucin

present which, being viscous, slows down the diffusion of drugs. Drugs applied to the genital tract would penetrate more rapidly to infecting organisms if the viscosity of the mucin were decreased. The mucins can be dissolved by oxidizing agents particularly hypochlorite. In the present work a study has been made of some chemical properties of these mucins.

EXPERIMENTAL

Each sample of mucin was obtained from the vicinity of the cervix of a normal cow. Oestroscope determination and microscopical examinations were carried out on all samples, which were discarded if they contained an excessive number of cells. Mucin was stored in the refrigerator without added preservative. The dioestrous and pregnancy samples were pooled from samples taken on different days, but mucin from different cows was not mixed. The amount of mucin obtained from cows in oestrus (20–50 ml.) was usually sufficient for many determinations to be made on a single sample.