BIOLOGICAL ACTIVITY OF ARYL THIOL DERIVATIVES:

PART 1. Substituent Effects on the Herbicidal Activity of Aryl Thiol Derivatives.

PART 2. A Synthetic Route to Polyhydroxylated Aryl Thiols: Ring Substitution of Phenols by Iodine-Thiourea Complexes.

A Thesis

Presented to

the Department of Chemistry,

School of General Studies,

Australian National University

In Partial Fulfilment

of the Requirements for the Degree

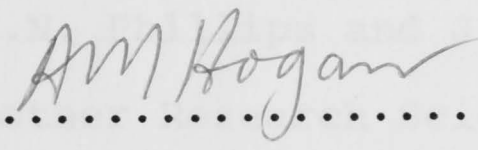
Master of Science

by

Allan Michael Hogan, B.Sc.

February, 1974.

Except where it is acknowledged to have been done by others, all work described in this thesis was performed by me



A.M. Hogan

ACKNOWLEDGEMENTS

I wish to extend my sincerest appreciation to my supervisor at CSIRO, Dr. R.L.N. Harris, for his advice and encouragement. I would also like to thank Drs. J.N. Phillips and J.L. Huppatz for helpful discussions, and the other Research Scientists for their interest and advice.

My thanks are due also to Mr. A.E. Geissler of the Plant Chemotherapy Section for his generous technical support in the herbicidal assays, the members of the Biological Group in the Section for conducting the fungal assays, and Mr. C. Edwards of the Division of Mathematical Statistics, CSIRO, for the regression analyses.

I would also like to thank Dr. J.A. Elix of the Chemistry Department at the A.N.U. for his help and advice during my visits there.

Last, but in no way least, I wish to thank my dear wife for her patience and unfailing good humour while this thesis was being written...

S U M M A R Y

PART 1.

The post-emergent herbicidal activity of series of p-substituted aryl thiolacetates and thiocyanates was measured on five plant species. It was found that both electron-donating and electron-withdrawing substituents enhanced the activity, the p-dimethylamino compound being up to 50 times more active than the unsubstituted compound in the thiolacetate series. A relationship between the order of activity and electronic and lipophilic effects of the substituents has been established and the results are interpreted in terms of the possible mode of action of the compounds.

PART 2.

The reaction of polyhydroxylated phenols with iodine-thiourea complexes in aqueous solution has been examined. Resorcinol treated in this way yielded S-(2,4-dihydroxyphenyl)isothiouronium iodide. Phloroglucinol and pyrogallol treated in this way, yielded the corresponding isothiouronium derivatives. Results for a number of substituted phenols as well as 1-naphthol and 2-naphthol are reported.

Spectrophotometric studies on the mechanism suggest that it proceeds via the formation of a cationic sulphenyl iodide intermediate which, through a process of electrophilic attack, substitutes into the aryl ring.

CONTENTS

PART 1. SUBSTITUENT EFFECTS ON THE HERBICIDAL ACTIVITY OF ARYL THIOL DERIVATIVES.

INTRODUCTION

Sulphur is essential for the growth and metabolism of all living organisms and appears in cells in a number of forms, the most active of which is the sulphydryl group (SH).

The most abundant source of active SH groups is cysteine residues (1) which are natural components of protein and therefore of enzymes and certain hormones. Other common sources are the pantothenic acid derivative coenzyme A (2) (an important precursor in fatty acid synthesis) and the peptide glutathione (GSH) (3).

A number of important biological processes in plants have been shown to be thiol dependent. Sulphydryl groups are required in photosynthetic phosphorylation[1] as well as the reverse reaction, the hydrolysis of adenosine-5'-triphosphate in chloroplasts by light (photosynthesis).[2] The reduction of carbon dioxide to carbohydrates has also been shown to be thiol dependent.[3] The enzyme responsible, ribulose-1,5-diphosphate carboxylase is a SH requiring enzyme having ninety-six sulphydryl groups of which only a few need to be blocked to produce full inhibition. Electron transport[4], transpiration[5], protoplasmic streaming[6] and resistance to frost[7] are among other processes for which evidence has been tendered implicating sulphydryl groups.

Obviously then, by exploiting the reactivity of the SH group, a number of avenues are open for direct

chemical control of cellular mechanisms. The SH group may be blocked by alkylation using active halogen compounds such as iodoacetamide[8], or acylation, by introducing carbamates and organophosphates etc. The commercial use of dithiocarbamates as acaricides, fungicides and insecticides is but one example of interference with SH groups by exploitation of their reactivity towards acylating agents.

Arylation[9] of SH groups, particularly with quinones and quinonamines is also very effective since thiols undergo addition reactions. Suitably substituted quinone compounds have found use as antibiotics e.g. Phoenicin and flaviolin.[10] Apart from these methods, oxidation to disulphides using iodine will also inactivate thiols. Most of these approaches however have been fairly well explored as seen by abundant references to them in the literature.

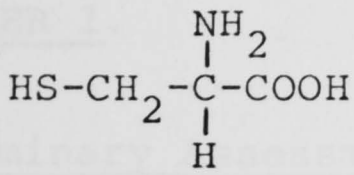
Another approach which has received relatively little attention is the design of specific thiol competitors. Such compounds could be highly nucleophilic thiols protected by acyl or alkyl groups enabling them to be transported intact through the cell membrane. Once inside the cell, the protecting group could be displaced and the free sulphydryl group thus unmasked could compete with natural thiols already in the cell and so disrupt the cellular chemistry.

For some years now, certain arylthioesters with the general structure (4) have been reported as exhibiting biological activity, and indeed the more active arylthiolacetates such as (5) are patented for their biotoxic properties.[11] The use of arylthiocyanates such as

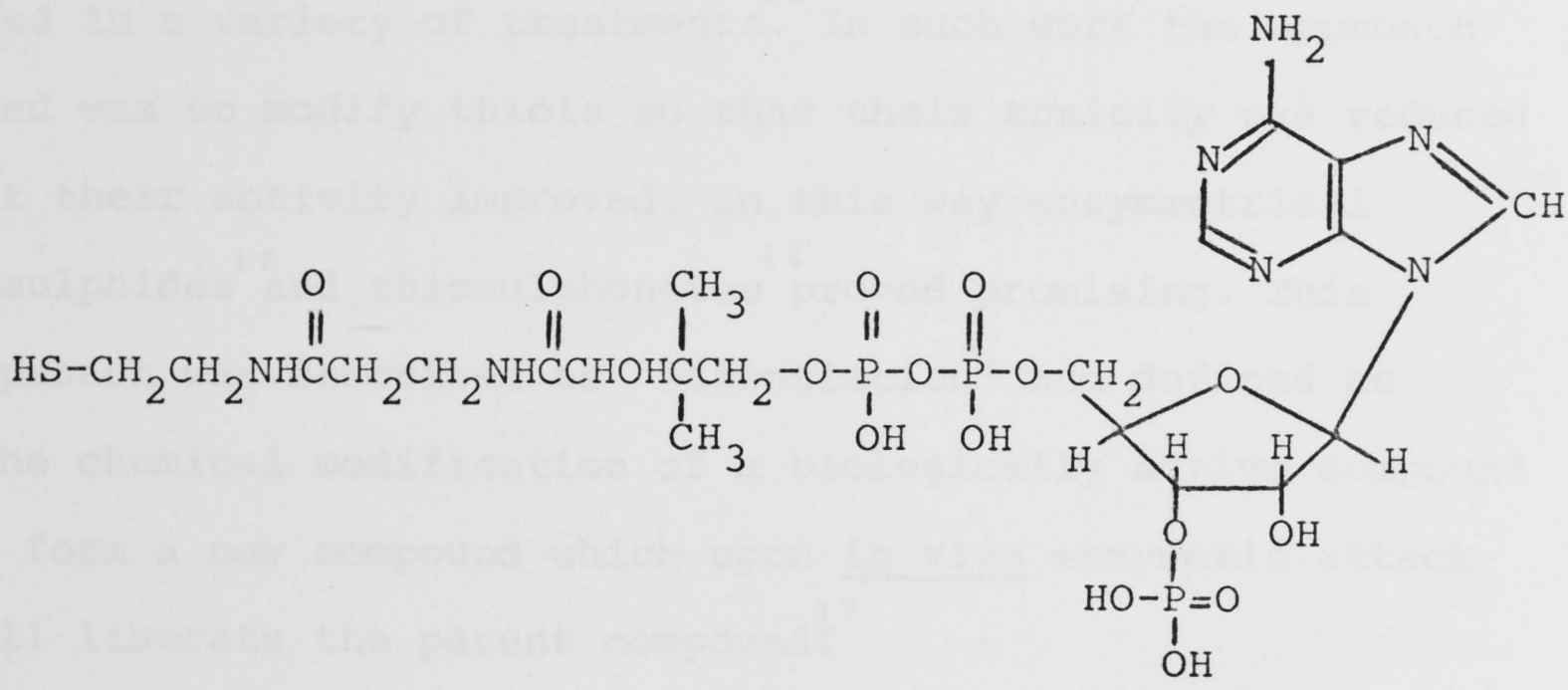
(6) as fungicides is likewise well documented.[12]

It is conceivable that such compounds might conform with the "protected thiol" concept since a similar mode of activity has been proposed to explain the fungicidal properties of the structurally analogous dithiocarbamate group, for example in tetramethylthiuram disulphide (7).[13]

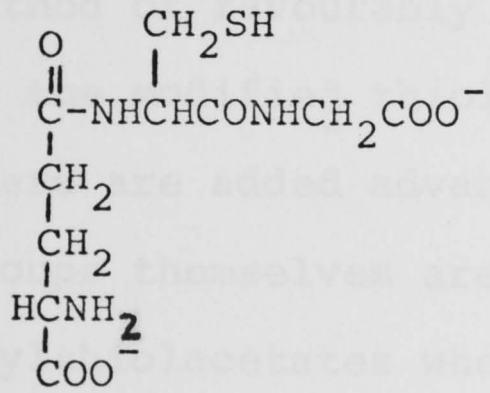
To gain a further insight into the mode of action of arylthioesters, a study was required to determine possible structure-reactivity relationships. Accordingly, a number of arylthioesters were prepared and screened for biological activity. The results obtained were assessed quantitatively and interpreted in terms of possible modes of action.



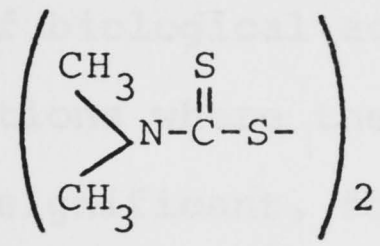
(1)



(2)



(3)



(7)

CHAPTER 1.

Preliminary Assessment of Protecting Groups and Aromatic Substituents.

Earlier references to the concept of protected thiols have occured in medical work where thiols have been used in a variety of treatments.[14] In such work the approach used was to modify thiols so that their toxicity was reduced but their activity improved. In this way unsymmetrical disulphides[15] and thiosulphonates[16] proved promising. This approach was described as "latentiation" and defined as "the chemical modification of a biologically active compound to form a new compound which upon *in vivo* enzymatic attack will liberate the parent compound."[17]

This idea is very attractive when applied to protected aryl thiols, i.e. arylthioesters. It provides a method of favourably influencing the absorption and transport of the modified thiol to the site of biological activity. There are added advantages in situations where the protecting groups themselves are biologically significant, for example, arylthiolacetates where the acyl group as well as the parent thiol may contribute to the chemical disruption of cellular metabolism.

An important factor in the biological activity of these compounds is therefore the reactivity of the thiolester bond (S-X). Despite its relative ease of hydrolysis it is quite stable in aqueous solution at neutral

pH and physiological temperatures, a property which permits thioester containing compounds to function in cellular metabolism.

Compared to oxygen analogues, the bonding of a carbonyl group to sulphur compounds results in longer and weaker bonds than with corresponding oxygen compounds. This results in less interelectronic repulsion and more facile bond cleavage in nucleophilic displacement reactions at the carbonyl carbon atom. The smaller tendency of sulphur compared to oxygen to utilise p-electrons to form double bonds causes a greater localisation of charge in the carbonyl group and an electron deficiency around the carbonyl carbon. Thus the contribution of the aromatic substituent R, and the protective group X, to the biological activity of compounds with the structure (4) are inter-dependent since the inductive and mesomeric effects of R and X influence the nucleophilicity of the sulphur atom and the strength of the S-X bond.

Since structure-reactivity relationships may be more easily correlated in a series of related compounds, it was necessary to define the limits of R and X consistent with biological activity. Accordingly, a number of compounds were prepared which had a common substituent R but varying protective groups X. In this way, the more reactive toxophoric groups (SX) were identified and used in a series of compounds in which the the protective group was constant but the aromatic substituent R was varied. Compounds obtained in this manner were then screened for biological

activity. The results obtained formed the basis of a structure-reactivity study.

The Protective Group X.

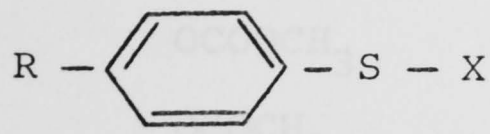
The biological activity of arylthiolacetates and thiocyanates has been referred to earlier. Certain substituted phenyl-O-methylthiocarbonates also have been found to possess biological activity and are patented as plant-growth regulators[18]. A common feature of all these compounds is that they contain an electrophilic carbon atom adjacent to a sulphur atom and could therefore be expected to be cleaved in a hydrolytic reaction to yield the parent thiol. As an initial guideline therefore, this feature was used as a selection basis for other groups in the series. These groups are listed in column "X" in Table 1.

The different series were each characterised by an R substituent the first of which was the p-dimethylamino group, selected because of the activity of (5)[11]. The lipophilic character (i.e. ease of cell penetration) conferred by chloro substituents and the ready availability of p-chlorothiophenol dictated the choice of the p-chloro substituent for R in the second series. The third series was characterised by the hydroxy group, selected because of the known herbicidal activity of (6)[12].

Initially compounds were screened for both pre- and post-emergent herbicidal activity on five plant species. However the pre-emergent tests were not significant

TABLE 1

STRUCTURES

4. 

5.	R = $N(CH_3)_2$	X = $COCH_3$
6.	OH	CN
8.	$N(CH_3)_2$	CN
9.	$N(CH_3)_2$	COC_6H_5
10.	$N(CH_3)_2$	$CONHCH_3$
11.	$N(CH_3)_2$	$CON(CH_3)_2$
12.	$N(CH_3)_2$	$COOCH_3$
13.	$N(CH_3)_2$	$CSNHC_6H_5$
14.	Cl	$COCH_3$
15.	Cl	CN
16.	Cl	$COCH_2Cl$
17.	Cl	$CO(CH_2)_4CH_3$
18.	Cl	COC_6H_5
19.	Cl	$CONHCH_3$
20.	Cl	$CONHCH_2CH_3$
21.	Cl	$CON(CH_3)_2$
22.	Cl	$COOCH_3$
23.	Cl	$CSNHCH_3$
24.	Cl	CSNHcyclohexane
25.	Cl	CONHcyclohexane
26.	OH	$COCH_3$
27.	OH	COC_6H_5
28.	OH	$CONHCH_3$

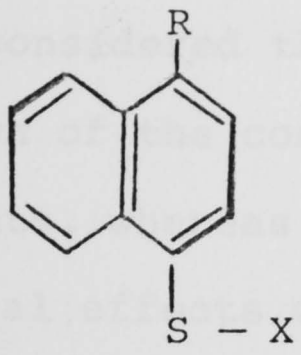
TABLE 1

(continued)

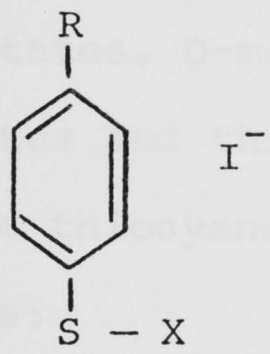
29.	R = OH	X = $CON(CH_3)_2$
30.	$OCOOCH_3$	$COOCH_3$
31.	$OCOCH_3$	$COCH_3$
32.	OC_6H_5	COC_6H_5
33.	$OCOOCH_3$	$COCH_3$
34.	OCH_3	$COCH_3$
35.	OCH_3	CN
36.	OCH_3	$COOCH_3$
37.	CH_3	$COCH_3$
38.	CH_3	CN
39.	CH_3	$COOCH_3$
40.	H	$COCH_3$
41.	H	CN
42.	H	$COOCH_3$
43.	F	$COCH_3$
44.	Br	$COCH_3$
45.	Br	$COOCH_3$
46.	$COCH_3$	$COCH_3$
47.	$COCH_3$	CN
48.	$COCH_3$	$COOCH_3$
49.	CN	$COCH_3$
50.	CN	CN
51.	CN	$COOCH_3$
52.	NO_2	$COCH_3$
53.	NO_2	CN
54.	NO_2	$COOCH_3$
55.	$COCH_3$	$CON(CH_3)_2$

TABLE 1

(continued)



56.	R = OH	X = $CON(CH_3)_2$
57.	OH	$CSNHC_6H_5$



58.	R = $\overset{+}{N}(CH_3)_3$	X = $COCH_3$
59.	"	$CONHCH_3$
60.	"	$CON(CH_3)_2$
61.	"	COC_6H_5

since no compounds were active in preventing seed germination. It was considered that contact with the soil caused rapid breakdown of the compounds before they were incorporated into the plants, whereas in the post-emergent tests the contact herbicidal effects were quite significant and reproducible results were obtained since the compounds were absorbed into the plants intact.

Synthesis of Compounds

The thiolacetates, O-methylthiocarbonates, thio- and dithiocarbamates and thiobenzoates were prepared by standard methods. The thiocyanates were prepared by one of three general methods:

(a) Thiocyanation of an aromatic ring using potassium thiocyanate and bromine in methanol[19]

(b) the Sandmeyer reaction

(c) thiocyanation of an aromatic ring with chlorosulphurylthiocyanate.

This latter method involves the generation of chlorosulphurylthiocyanate[21] from the action of sulphuryl chloride on lead thiocyanate in acetic acid. It is closely related to the procedure using chlorothiocyanogen developed by Bacon and Guy[20] which requires the use of chlorine gas. The use of sulphuryl chloride is undoubtedly more convenient and appears an attractive general alternative to known procedures.

Biological Activity

Table 2 contains a summary of the herbicidal activities of the compounds tested. The actual procedures used for the determination of activity are described in the Experimental Section (page 41).

The thiolacetates (5,26,) and the thiocyanates (6,8,15) exhibited satisfactory activity as did the O-methyl thiocarbonates (12,22). N-methylthiocarbamates (10,19,28), N,N-dimethylthiocarbamates (11,21,29) and S-(p-chlorophenyl)-N-ethylthiocarbamate (20), were inactive despite showing some antifungal activity (see Appendix A). This last observation parallels that of indolyl thiocarbamates which have exhibited similar trends in their biological activity.[22] The dithiocarbamates (13,23,24) showed similar properties to the thiocarbamates. p-Chlorothiobenzoate (18) was slightly herbicidal but p-dimethylaminophenylthiobenzoate(9) was completely inactive. The activity of S-(p-chlorophenyl)-thiohexanoate (17) was only marginally better than the thiolacetate (14) despite the enhanced lipophilicity conferred by the hydrocarbon chain attached to the carbonyl carbon. A similar improvement was obtained by (16) which contained a halogen on the α-carbon. S-(4-hydroxynaphthyl)-N,N-dimethyl-thiocarbamate (56) which is the analogue of the corresponding 4-hydroxyphenyl compound(29), showed no increase in herbicidal activity over (29) despite the increased lipophilicity due to the presence of an extra aromatic ring.[23] S-(4-hydroxy-naphthyl)-N-phenyldithiocarbamate (57) while inactive as a herbicide was slightly antifungal (see Appendix A).

A number of other compounds were obtained by disubstitution of 4-hydroxythiophenol. Apart from the dibenzoate derivative (32) which was inactive, the di-O-methylcarbonate (30), the diacetate (31) and 4-methoxy-carbonyloxyphenyl-O-methylthiocarbonate (33) all exhibited significant herbicidal activity(see Table 2).

The Aromatic Substituent R

The results obtained from the series shown in Table 2, gave some guidelines as to the type of protecting groups required for high herbicidal activity. To determine quantitatively the effects of different aromatic substituents on the herbicidal activity, three series of arylthioesters were prepared in which the aromatic substituent R varied throughout the series but the protective group X remained constant. The three protective groups selected were the acetyl , nitrile and O-methylcarbonyl groups. The same R substituents were generally used in each of the series which were then screened as before. The results aresummarised in Table 3, (page 16).

It is apparent that electron-donating substituents ($N(CH_3)_2$, OCH_3, CH_3 etc.) generally increase the activity of the otherwise unsubstituted phenylthiolacetate. Such substituents would certainly increase the nucleophilicity of the sulphur atom. The behaviour exhibited by the p-bromo and p-chloro substituted compounds may well be the result of increased lipophilicity conferred on the compounds by the halogen atom. Significantly,the p-fluoro compound is totally inactive under the conditions of this test. The

TABLE 2

POST-EMERGENT HERBICIDAL ACTIVITY

FOR SERIES WHERE X VARIES,

R IS CONSTANT

Structure No.	PLANT SPECIES L	S	B	M	P	No.	L	S	B	M	P
5.	4	6	3	4	3	23.	0	0	0	0	0
6.	1	2	1	0	0	24.	0	1	0	0	1
8.	4	4	2	4	1	25.	0	0	0	0	0
9.	0	0	0	0	0	26.	2	3	2	3	2
10.	0	0	0	0	0	27.	0	0	0	0	0
11.	0	0	0	0	0	28.	0	0	0	0	0
12.	4	5	3	4	2	29.	0	0	0	0	0
13.	0	0	0	0	0	30.	2	2	1	2	0
14.	1	2	1	0	0	31.	2	2	2	2	2
15.	2	3	1	1	1	32.	0	0	1	1	0
16.	1	1	1	1	1	33.	3	3	1	2	2
17.	2	2	1	1	2	56.	0	0	0	0	0
18.	1	1	1	0	1	57.	0	0	0	0	0
19.	0	0	0	0	0	58.	0	0	0	0	0
20.	0	0	0	0	0	59.	0	0	0	0	0
21.	0	0	0	0	0	60.	0	0	0	0	0
22.	2	0	1	0	1	61.	0	0	0	0	0

(Key to ratings is on page 15)

KEY TO TABLES 2 AND 3

Plant Species:

L = linseed

S = sugarbeet

B = buckwheat

M = mustard

P = peas

Herbicidal Rating:

0 = < 70% kill at 8Kg/hectare

1 = > 70% " " 8Kg/ha

2 = > " " " 4Kg/ha

3 = > " " " 2Kg/ha

4 = > " " " 1Kg/ha

5 = > " " " 0.5Kg/ha

TABLE 3

POST-EMERGENT HERBICIDAL ACTIVITY

FOR SERIES WHERE R VARIES

X IS CONSTANT

	X = $COCH_3$						X = CN						X = $COOCH_3$					
R	No.	L	S	B	M	P	No.	L	S	B	M	P	No.	L	S	B	M	P
$N(CH_3)_2$	5.	4	6	3	4	3	8.	4	4	2	4	1	12.	4	5	3	4	2
OH	26.	2	3	2	3	2	6.	1	2	1	0	0	----------					
OCH_3	34.	2	3	2	3	1	35.	2	1	3	2	1	36.	2	4	2	2	0
CH_3	37.	0	1	1	1	0	38.	0	0	0	0	0	39.	1	1	1	1	1
H	40.	0	0	0	0	0	41.	0	0	0	0	0	42.	0	0	0	0	0
F	43.	0	0	0	0	0	----------						----------					
Cl	14.	1	2	1	0	0	15.	2	3	1	1	1	22.	2	1	0	1	0
Br	44.	1	3	1	2	1	----------						45.	2	0	1	0	1
$COCH_3$	46.	2	2	1	2	1	47.	1	1	3	2	2	48.	2	2	2	2	0
CN	49.	3	4	0	3	1	50.	0	0	0	0	0	51.	0	0	0	0	0
NO_2	52.	2	4	2	3	1	53.	1	2	1	3	1	54.	0	0	0	0	0

(Key to ratings is on page 15)

negative inductive effect of the fluorine in deactivating the sulphur is not compensated by any lipophilic character. A more surprising result is the considerable activity of the p-nitro compound (52). The p-nitro substituent with its high negative inductive and mesomeric effects would certainly deactivate the sulphur, implying that the mode of action for this compound differs from those with electron-donating groups. Similar comments apply to the thiocyanate and O-methylthiocarbonate series. In view of their overall superior herbicidal activity, the thiol-acetate and thiocyanate series were singled out for further study.

CHAPTER 11. PART A

Derivation and Application of a Biological Hammett Equation.

The chemical modification of growth obviously requires the chemical substance to arrive at the physiological site through which its actions are manifested. Externally applied herbicides especially may be structurally altered, destroyed or removed by a variety of environmental factors such as leaching, volatilisation, photodecomposition etc. and are less available to the plant. The actual entry of a herbicide into a plant is further controlled by other biochemical, morphological and physiological factors, while internally, the absorption at inactive sites, complex formation and metabolic alteration are further hindrances. The successful arrival of a herbicide at its site of action is dependent in part on its structural configuration, chemical composition and physical properties. Thus, the quantity of herbicide applied to the soil or above-ground parts of the plant, which becomes available internally for reaction with growth controlling processes is conceivably a very small percentage of the amount originally applied.

In view of this, several factors must be assessed if a true estimation of structure-reactivity relationships in a series of compounds is to be achieved. For a given class of related compounds such as the arylthiolacetates and thiocyanates, the biological activity is a function of the spatial requirements, lipophilic, electronic and other properties which vary progressively through the series.[24,28]

Thus:

$$\text{Activity} = f(\text{lipophilic}) + f(\text{electronic}) + f(\text{steric}) + f(\text{degradation}) + \ldots \quad (1)$$

The electronic effects of the substituents may be expressed in terms of the Hammett substituent constant σ.[31] This constant represents the transmitted inductive and mesomeric effects of the substituent and depends solely on its nature and position.

The original Hammett equation, $\log ({}^{k}/k_{o}) = \sigma\rho$, (where k and k_o are equilibrium constants or rate constants for the reaction of substituted and unsubstituted compounds respectively; and ρ is a reaction constant) is a purely empirical relationship which was evolved to explain the substituent effects on the ionization of the appropriate benzoic acids. The extension of the Hammett equation to other benzenoid reactions has been successful in explaining structure-reactivity relationships.[31]

Whilst the measured toxic concentration gives some idea of the intrinsic toxicity of the compound, the true toxicity is dependent on the phase distribution of the compound as well.[25] Collander[26] has shown that the partition of a given series of compounds in a water/immiscible solvent system may be calculated from another system, and that the relative order of partition is generally constant. Further, from work on Nitella cells the rate of movement of a great variety of organic compounds through cellular material is approximately proportional to the logarithm of their partition coefficients between an organic solvent and water.[27] From these findings, Hansch and Fujita[28,29] have derived a constant π ,

which is a free-energy term relating the partition of a substituted member of a series to that of the unsubstituted parent member in a reference two-phase system, usually n-octanol/water,(eq: 2)

$$\pi = \log P_x - \log P_H \qquad (2)$$

Although values for various substituents in different series of compounds have been published for these parameters[29,30], to avoid variation occuring between series due to changes in the protective group, experimental determination of these parameters for the substituted arylthiolacetates and arylthiocyanates was carried out, (see Table 4, page 21).

In this work, the steric factors and the overall relationship between the protective group ($-COCH_3$ or $-CN$) and the substituent are generally constant. In view of this, it may be assumed that the main factors influencing herbicidal activity are variations in electronic and lipophilic effects, and equation (1) can be modified to:-

$$\text{Activity} = f(\text{electronic}) + f(\text{lipophilic}) \qquad (1a)$$

A quantitative form of equation (1a) has been derived by Hansch and Fujita[28] wherein the activity is expressed as a function of σ coupled with a quadratic function of π:

$$pI_{50} = \log 1/C = -k\pi^2 + k'\pi + \rho\sigma + k'' \qquad (3)$$

where $C = LD_{50}$
k, k' and k'' are constants
π = lipophilic constant of substituent
σ = Hammett substituent constant
ρ = Hammett reaction constant

TABLE 4

PARTITION COEFFICIENTS

for

p-SUBSTITUTED PHENYL THIOLACETATES AND THIOCYANATES

Substituent	$P_{SCOCH_3} \times 10^2$	$^*P_{SCN} \times 10^2$
$N(CH_3)_2$	3.46	8.34
OH	0.74	1.58
OCH_3	1.91	2.52
CH_3	3.70	2.44
H	1.98	1.74
F	1.96	1.53
Cl	8.65	7.34
Br	9.00	----
$COCH_3$	0.93	0.82
CN	0.73	0.46
NO_2	0.95	0.82

* The Author wishes to thank Dr.L.T. Oswald for supplying values of P_{SCN}

This equation has been used successfully to explain substituent effects on the biological activity of carcinogenic compounds in mice[28], auxin activity of phenoxyacetic acids[32] and the hypnotic effect of barbiturates.[33]

A linear relationship between biological activity and σ is obtained from equation (3) when lipophilic effects are negligible. Such a relationship is expressed in the form of a biological Hammett equation:

$$pI_{50} = \log 1/C = \rho\sigma + k \qquad (4)$$

Hansch parameters (π), Hammett constants (σ) and herbicidal activity (LD_{50} in moles/hectare) for the p-substituted phenylthiolacetates and phenylthiocyanates are shown in Table 5, page 23.

A plot of pI_{50} v σ (Fig.1, page 24) shows that activity is enhanced by both electron donating and electron withdrawing substituents and that apart from the chloro and bromo derivatives, the activity of the compounds approximates a V-shaped curve with a minimum near =0. As previously noted however, consideration of electronic effects alone is a broad approximation since lipophilic properties of the compounds are also important factors and must be considered in the final toxicity ratings. The relative contributions of electronic and lipophilic factors to the total activity may be determined by regression analysis of the Hansch equation. However, corrections for lipophilicity while standardising the high activity of the chloro and bromo derivatives would not be expected to significantly effect the overall shape of the curve since the variations in lipophilicity between other

TABLE 5

HANSCH PARAMETERS (π), HAMMETT CONSTANTS (σ) AND POST_EMERGENT HERBICIDAL ACTIVITY (LD_{50} IN MOLES PER HECTARE) FOR A SERIES OF p-SUBSTITUTED PHENYLTHIOLACETATES

Substituent	π	σ	LD_{50}				
			Linseed	Sugarbeet	Buckwheat	Mustard	Peas
$N(CH_3)_2$	0.24	-0.60	1.55	2.07	4.10	2.63	5.85
OH	-0.43	-0.36	7.02	8.90	15.8	10.1	17.5
OCH_3	-0.015	-0.27	6.46	3.84	9.0	10.5	21.8
CH_3*	0.27	-0.17	32.2	29.0	38.1	28.2	25.0
H *	0.00	0.00	88.9	91.6	110	120	109
F *	-0.005	0.06	46.4	41.1	78.3	62.7	51.3
Cl	0.64	0.23	17.2	11.0	24.7	24.7	15.3
Br	0.66	0.23	14.0	9.9	17.2	12.6	17.8
$COCH_3$	-0.33	0.52	14.4	16.0	19.6	15.0	30.1
CN	-0.43	0.63	9.10	2.54	26.5	9.6	21.0
NO_2	-0.32	0.78	6.65	4.47	13.1	6.1	26.1

* necessary to apply > 8Kg/ha to obtain LD_{50} values

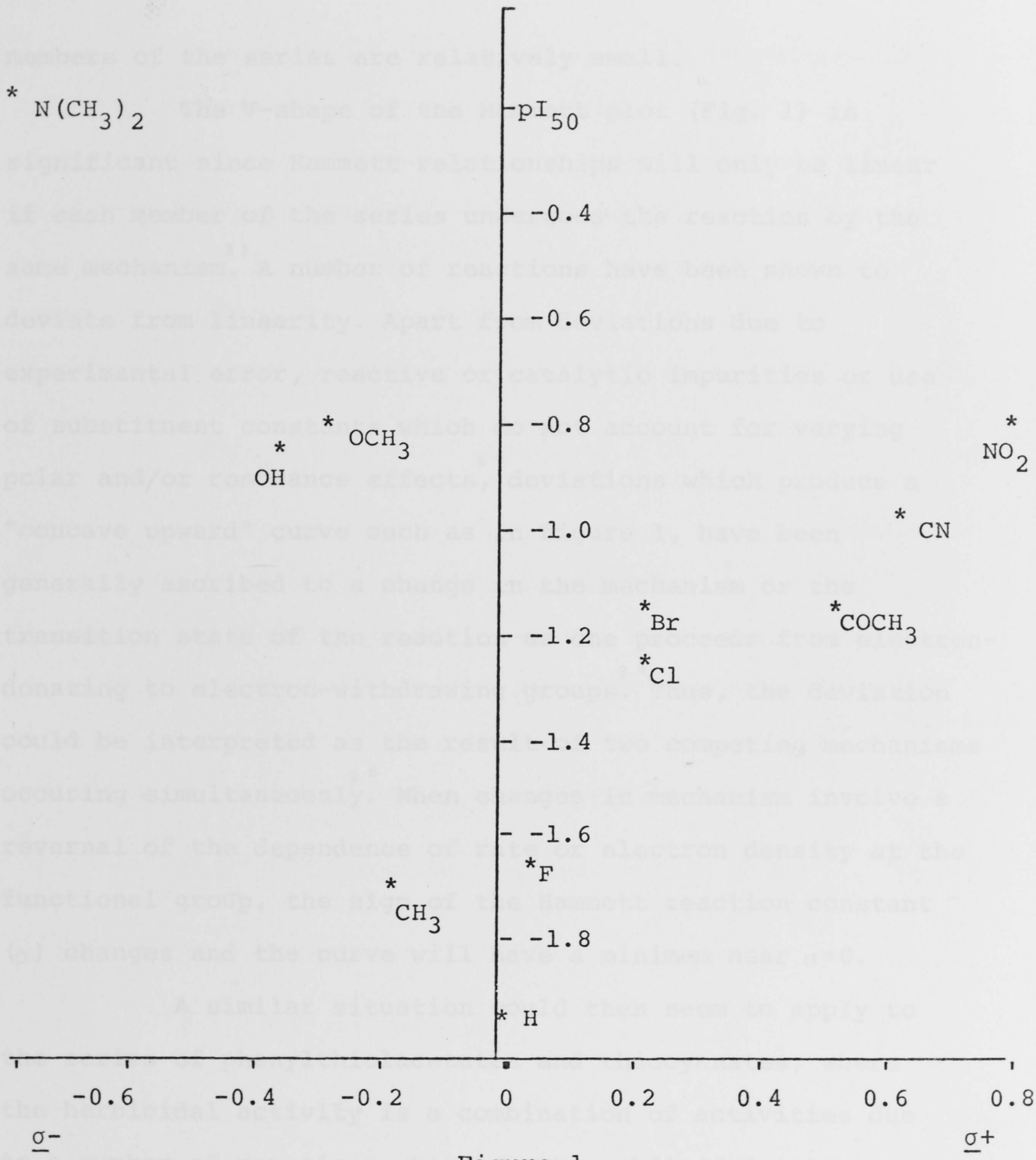


Figure 1.

Post-emergent herbicidal activity of p-substituted phenyl thiolacetates on linseed

pI_{50} = -log LD_{50} in kg. per hectare

σ = Hammett substituent constant

members of the series are relatively small.

The V-shape of the Hammett plot (Fig. 1) is significant since Hammett relationships will only be linear if each member of the series undergoes the reaction by the same mechanism.[31] A number of reactions have been shown to deviate from linearity. Apart from deviations due to experimental error, reactive or catalytic impurities or use of substituent constants which do not account for varying polar and/or resonance effects,[34] deviations which produce a "concave upward" curve such as in Figure 1, have been generally ascribed to a change in the mechanism or the transition state of the reaction as one proceeds from electron-donating to electron-withdrawing groups.[34] Thus, the deviation could be interpreted as the result of two competing mechanisms occuring simultaneously.[35] When changes in mechanism involve a reversal of the dependence of rate or electron density at the functional group, the sign of the Hammett reaction constant (ρ) changes and the curve will have a minimum near $\sigma=0$.

A similar situation would then seem to apply to the series of phenylthiolacetates and thiocyanates, where the herbicidal activity is a combination of activities due to a number of reactions which may be subdivided into two groups (a) and (b), each group having an opposite dependence on the electron density at the thiolacetate and thiocyanate groups. A linear Hammett relationship can no longer be expected between activity and σ and so the Hansch equation must be modified accordingly.

The basis of the Hansch approach to structure-activity correlations is the assumption that most biologically active chemical agents exert their activity through a key rate-controlling reaction (a) occuring at an "active site". In the ideal situation where the amount of compound reaching the active site in a given time is directly proportional to the extra cellular concentration (C)

$$\text{rate of biological response} \propto Ck_a \qquad (5)$$

where k_a is the rate constant for reaction (a). The present study suggests that the herbicidal activity of phenyl-thiolacetates could arise from two key reactions (or groups of reactions). If it is assumed that the observed biological activity is the sum of activities due to the two key reactions (a) and (b) acting independently, then

$$\text{rate of biological response} \propto C(k_a + k_b) \qquad (6)$$

At conditions of equivalent response

$$C(k_a + k_b) = \text{constant}$$

$$\frac{1}{C} = K(k_a + k_b) \qquad (7)$$

where K is constant

k_a and k_b are rate constants for reactions (a) and (b) and for a given series of related compounds the Hammett equation may be applied for each reaction

i.e. $$\log \frac{k_a(X)}{k_a(H)} = \rho_a\sigma$$

$$k_a(X) = k_a(H)\, e^{\rho_a\sigma} \qquad (8)$$

Similarly $$k_b(X) = k_b(H)\, e^{\rho_b\sigma} \qquad (9)$$

Substituting equations (8) and (9) in equation (7)

$$\frac{1}{C} = K\left[k_a(H)\, e^{\rho_a\sigma} + k_b(H)\, e^{\rho_b\sigma} \right] \qquad (10)$$

and if the observed dependence of the rate of reaction (b) on σ is the reverse of that for reaction (a)

$$\frac{1}{C} = K\left[k_a(H)\, e^{\rho_a\sigma} + k_b(H)\, e^{-\rho_b\sigma} \right] \qquad (11)$$

$$= Kk_a(H)\, e^{\rho_a\sigma}\left[1 + \frac{k_b(H)}{k_a(H)}\, e^{-(\rho_a + \rho_b)\sigma}\right] \qquad (12)$$

In equation (12), K, $k_a(H)$, $k_b(H)$, ρ_a and ρ_b are all constants for a given series of compounds in a given biological system. The equation can therefore be simplified to

$$\frac{1}{C} = Kk_a(H)\, e^{\rho_a\sigma}\left[1 + \alpha e^{-\beta\sigma}\right] \qquad (13)$$

$$\text{where } \alpha = \frac{k_b(H)}{k_{a(H)}}$$

$$\beta = \rho_a + \rho_b$$

Expressing the equations in logarithmic form and expanding $\ln(1 + \alpha e^{-\beta\sigma})$ as a power series in $\alpha e^{-\beta\sigma}$

$$pI_{50} = \log \frac{1}{C} = K' + \rho_a\sigma + \alpha e^{-\beta\sigma} - \frac{\alpha^2 e^{-2\beta\sigma}}{2} + \frac{\alpha^3 e^{-3\beta\sigma}}{3} \qquad (14)$$

or alternatively, from equation (11)

$$\frac{1}{C} = Kk_b(H)\ e^{-\rho_b\sigma}\left[\frac{k_a(H)}{k_b(H)}\ e^{(\rho_a + \rho_b)\sigma} + 1\right] \qquad (15)$$

i.e. using the notation in equation (13) and taking logarithms

$$pI_{50} = K'' - \rho_b\sigma + \frac{e^{\beta\sigma}}{\alpha} - \frac{e^{2\beta\sigma}}{2\alpha} + \frac{e^{3\beta\sigma}}{3\alpha} \qquad (16)$$

The penetration of chemical to the active sites in a biological system in practice does not reflect the ideal situation referred to above.[36] In the given series of related compounds the penetration process can be related to the lipophilic effects of the substituents expressed in terms of the appropriate Hansch π parameters.[28]

Substituting Hansch's π terms in equation (14)

$$\log \frac{1}{C} = K' - k'\pi^2 + k''\pi + \rho_a\sigma + \alpha e^{-\beta\sigma} - \frac{\alpha^2 e^{-2\beta\sigma}}{2} + \frac{\alpha^3 e^{-3\beta\sigma}}{3} \qquad (17)$$

A similar equation can be derived in which activity is described in terms of reaction (b)

$$\log \frac{1}{C} = K'' - k'\pi^2 + k''\pi - \rho_b\sigma + \frac{e^{\beta\sigma}}{\alpha} - \frac{e^{2\beta\sigma}}{2\alpha^2} + \frac{e^{3\beta\sigma}}{3\alpha^3} \qquad (18)$$

Equations (17) and (18) represent the general situation where two significant reactions (a) and (b) are independently contributing to the observed biological activity. The π terms are the same as those in the Hansch equation and predict a quadratic dependence of activity on π. There are now

a series of σ terms, the significance of which will depend primarily on the value of α. When one reaction is more significant than the other, $\alpha = \frac{k_a(H)}{k_b(H)} \rightarrow 0$ or ∞. That is, the the polynomial terms in equations (17) and (18) reduce to zero, leading to the normal Hansch equation for the predominant reaction. When both reactions are of comparable significance, the polynomial terms become important and observed activity receives a contribution from both reactions. The activity - σ plot (ignoring lipophilic contributions) will then appear as a hyperbola (Fig. 2) which can be approximated to two straight lines of opposite slope (ρ_a,$-\rho_b$) intersecting at the point where both reactions are contributing equally (Fig. 2). When lipophilic effects are considered, the equations for these two lines are

$$\log \frac{1}{C} = K' - k'\pi^2 + k''\pi + \rho_a\sigma \qquad \text{when } \sigma \text{ is +ve} \qquad (19)$$

$$\log \frac{1}{C} = K'' - k''\pi^2 + k''\pi - \rho_b\sigma \qquad \text{when } \sigma \text{ is -ve} \qquad (20)$$

Extrapolation of the two lines in Figure 2 would be unrealistic in a complex biological system. Further increase in σ +ve values or decrease in σ -ve values would increase the rate of reactions (a) and (b) eventually to a point where they no longer represented the rate-limiting step in a long chain of events between penetration of the chemical and the ultimate biological response. Under these conditions a gradual increase in the dependence of activity on σ might be expected and an activity - σ curve would

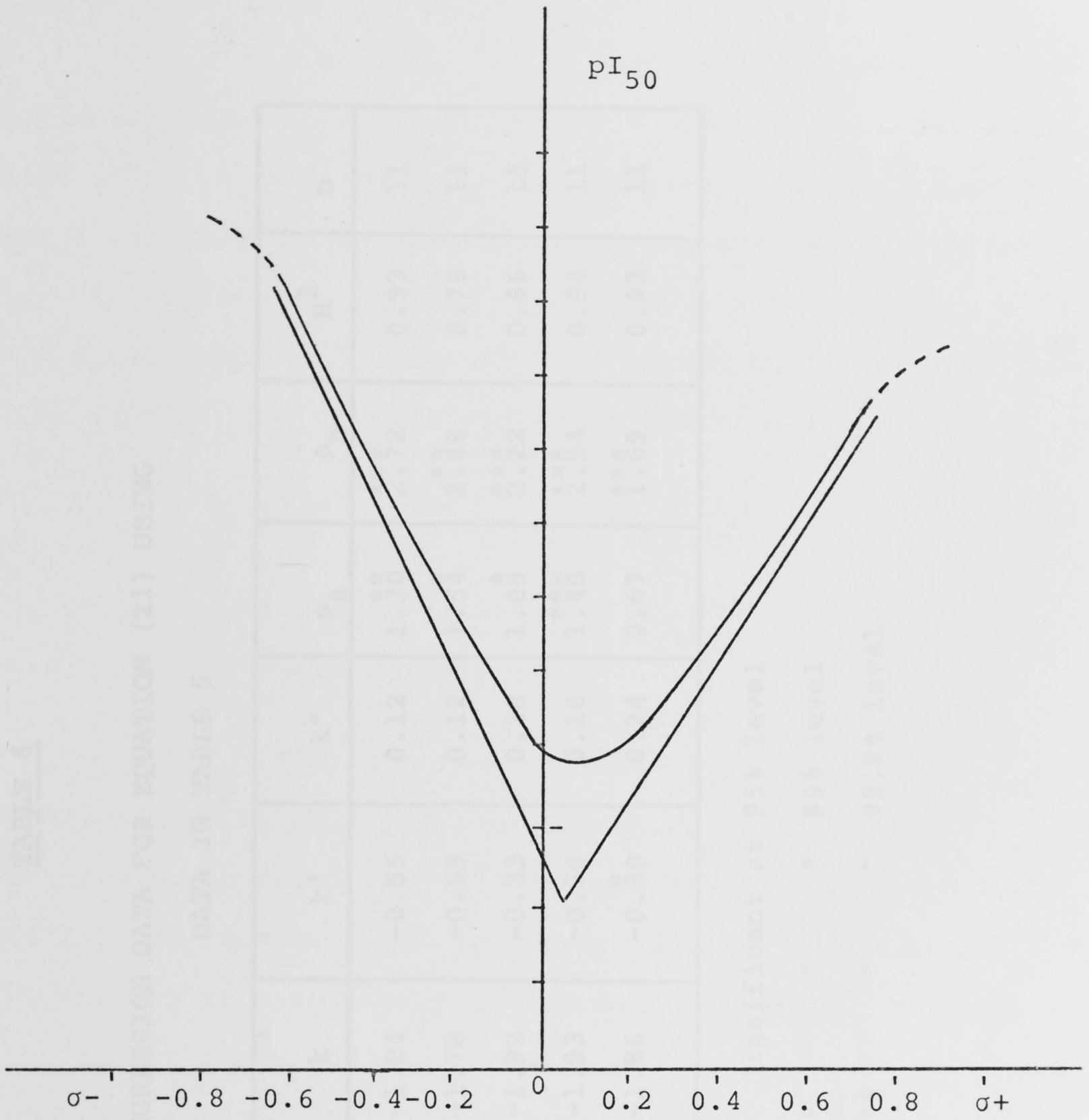


Figure 2.

TABLE 6

REGRESSION DATA FOR EQUATION (21) USING

DATA IN TABLE 5

Plant Species	k	k'	k"	ρ_a	ρ_b	R^2	n
linseed	-1.84	-0.55	0.12	1.30**	2.72***	0.93	11
sugarbeet	-1.78	-0.69	0.12	1.54*	2.48**	0.78	11
buckwheat	-1.92	-0.33	0.28	1.09*	2.22***	0.86	11
mustard	-1.93	-0.56	0.16	1.46***	2.54***	0.94	11
peas	-1.86	-0.80*	0.24	0.67	1.69***	0.93	11

* Significant at 95% level

** " " 99% level

*** " " 99.9% level

flatten out (broken lines in Fig. 2).

Returning to the herbicidal activity of the arylthiolacetates, a comparison of Figures 1 and 2 suggests that over the range of compounds studied, a special case of the two-straight-line approximation (equations 19 and 20) may apply where the lines intersect at $\sigma = 0$. Then $K' = K''$ and $k_a(H) = k_b(H)$. To simplify regression analysis, equations (19) and (20) can be combined as in equation (21)

$$\log \frac{1}{C} = k - k'\pi^2 + k''\pi + \rho_a\sigma - \rho_b\sigma \qquad (21)$$

where $\rho_a = 0$ when σ is -ve

$\rho_b = 0$ when σ is +ve

When the data in Table 5 is fitted to equation (21) by multiple regression analysis, good correlation is found for all five species of plants studied. The derived constants and regression data is given in Table 6. The results show that with the exception of peas, the most significant factor affecting herbicidal activity in the compounds studied is the electronic effect of the substituent. The increased significance of lipophilic factors in peas is accountable by the exceptionally hard, waxy leaf surfaces in this plant which hinder the penetration of herbicides of low lipophilicity. Furthermore, again with the exception of peas, the contribution to activity by +ve and -ve σ terms is comparable for each plant species. In peas those reactions of the herbicide facilitated by electron-withdrawing substituents in the benzene ring no longer contribute

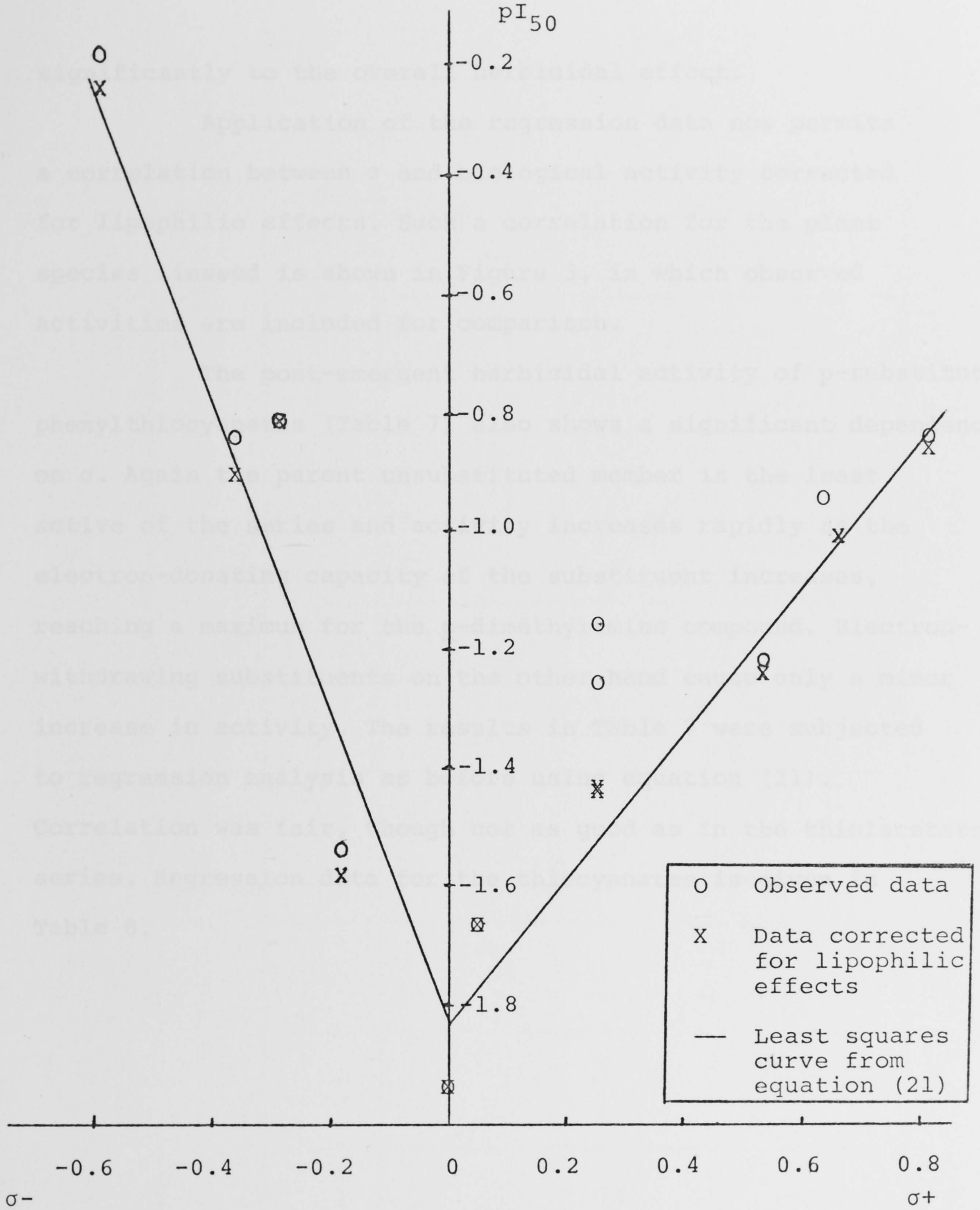


Figure 3

Post-emergent herbicidal activity of p-substituted phenyl-thiolacetates on linseed

significantly to the overall herbicidal effect.

Application of the regression data now permits a correlation between σ and biological activity corrected for lipophilic effects. Such a correlation for the plant species linseed is shown in Figure 3, in which observed activities are included for comparison.

The post-emergent herbicidal activity of p-substituted phenylthiocyanates (Table 7) also shows a significant dependence on σ. Again the parent unsubstituted member is the least active of the series and activity increases rapidly as the electron-donating capacity of the substituent increases, reaching a maximun for the p-dimethylamino compound. Electron-withdrawing substituents on the other hand cause only a minor increase in activity. The results in Table 7 were subjected to regression analysis as before using equation (21). Correlation was fair, though not as good as in the thiolacetate series. Regression data for the thiocyanates is given in Table 8.

TABLE 7

HANSCH PARAMETERS (π), HAMMETT CONSTANTS (σ) AND POST-EMERGENT HERBICIDAL ACTIVITY (LD_{50} IN MOLES PER HECTARE) FOR A SERIES OF p-SUBSTITUTED PHENYLTHIOCYANATES

Substituent	π	σ	LD_{50}				
			Linseed	Sugarbeet	Buckwheat	Mustard	Peas
$N(CH_3)_2$	0.68	-0.60	3.66	3.59	5.20	2.46	3.31
OH	-0.04	-0.36	10.5	12.6	11.4	10.8	16.9
OCH_3	0.16	-0.27	11.0	4.00	9.64	3.84	40.1
CH_3*	0.15	-0.17	226	95.3	190	122	118
H *	0.00	0.00	217	233	263	250	268
F *	-0.06	0.06	66.2	73.7	214	73.7	101
Cl	0.62	0.23	9.69	14.4	27.9	16.4	20.3
$COCH_3$	-0.33	0.52	23.1	18.6	33.8	15.1	23.8
CN	-0.58	0.63	45.8	10.2	300	36.7	277
NO_2	-0.33	0.78	19.3	20.4	48.3	18.2	34

* necessary to apply > 8Kg/ha to obtain LD_{50} values.

TABLE 8

REGRESSION DATA FOR EQUATION (21) USING

DATA IN TABLE 7

Plant Species	k	k'	k"	ρ_a	ρ_b	R^2	n
linseed	-2.05	-0.81	0.30	0.96	1.86	0.62	10
sugarbeet	-1.97	-0.08	0.08	1.03	2.08	0.65	10
buckwheat	-2.23	-1.37	-0.22	0.62	2.11	0.74	10
mustard	-2.03	-0.08	0.39	0.39	2.74	0.67	10
peas	-2.10	+0.17	0.66	0.66	2.33	0.69	10

PART B

Interpretation of Results

The increase in herbicidal activity in p-substituted phenylthiolacetates and thiocyanates by both electron-donating and electron-withdrawing substituents has been explained in terms of two mechanisms of action for the compounds operating simultaneously and having an opposite dependence on electron density at the thiolacetate and thiocyanate group.

An activity which is enhanced by electron-withdrawing substituents is not hard to visualise. In studies on the antifungal and antibacterial activity of arylthiolacetates, Yamagishi[37] concluded that their mode of action involves acyl transfer to amino and/or thiol functions in enzymes. This conclusion was supported by qualitative comparisons of the trans-acylating ability of several substituted phenylthiolacetates in chemical systems.

These acyl transfer reactions, involving nucleophilic attack at the carbonyl group in the thioester and expulsion of the phenylthio- anion would be greatly facilitated by the presence of electron-attracting groups in the benzene ring (Fig. 4). Similarly thework of Patchornik et al.[38] on the selective cyanylation of sulphydryl groups in proteins and peptides has shown that a process of nucleophilic attack at the SCN group is responsible; again facilitated by electron-withdrawing groups.

While the arylthiolacetates and thiocyanates may be considered to be acylating and cyanylating agents, this does not explain the full herbicidal activity since the most active members of the series are those which contain electron-donating substituents. This suggests that reactions involving the participation of the arylthiol moiety as a nucleophile are important. The more electron-donating the substituent, the more nucleophilic the thiol and ultimately the higher the activity. This supports the earlier contention of the protected thiol concept for compounds of the general type (4), where the unmasking of the nucleophilic thiol *in vivo* allows it to compete with biologically important nucleophilic sites and thus disrupt the cellular chemistry of the plants.

Reactions which might be expected in this context are outlined in Figure 5, although verification of these modes of action would require experiments with isolated enzyme systems in which these particular reactions play an activity-determining role.

An increase in $\sigma+$ for substituents could be expected to bring increased post-emergent herbicidal activity. To achieve this, the *p*-dimethylamino substituted compounds (5,9, 10,11) were quaternized with methyl iodide to yield the corresponding trimethyl ammonium derivatives (58,59,60,61 respectively). The trimethyl ammonium substituent has a $\sigma+$ value of +0.85 (c.f. *p*-nitro $\sigma+ = 0.78$). The post-emergent herbicidal activity of these latter compounds was negligible, even the thiolacetate derivative was inactive. These observations suggest that the intrinsic activity of the

charged species (58-61) is not realised because of problems associated with their transport across lipid membranes.

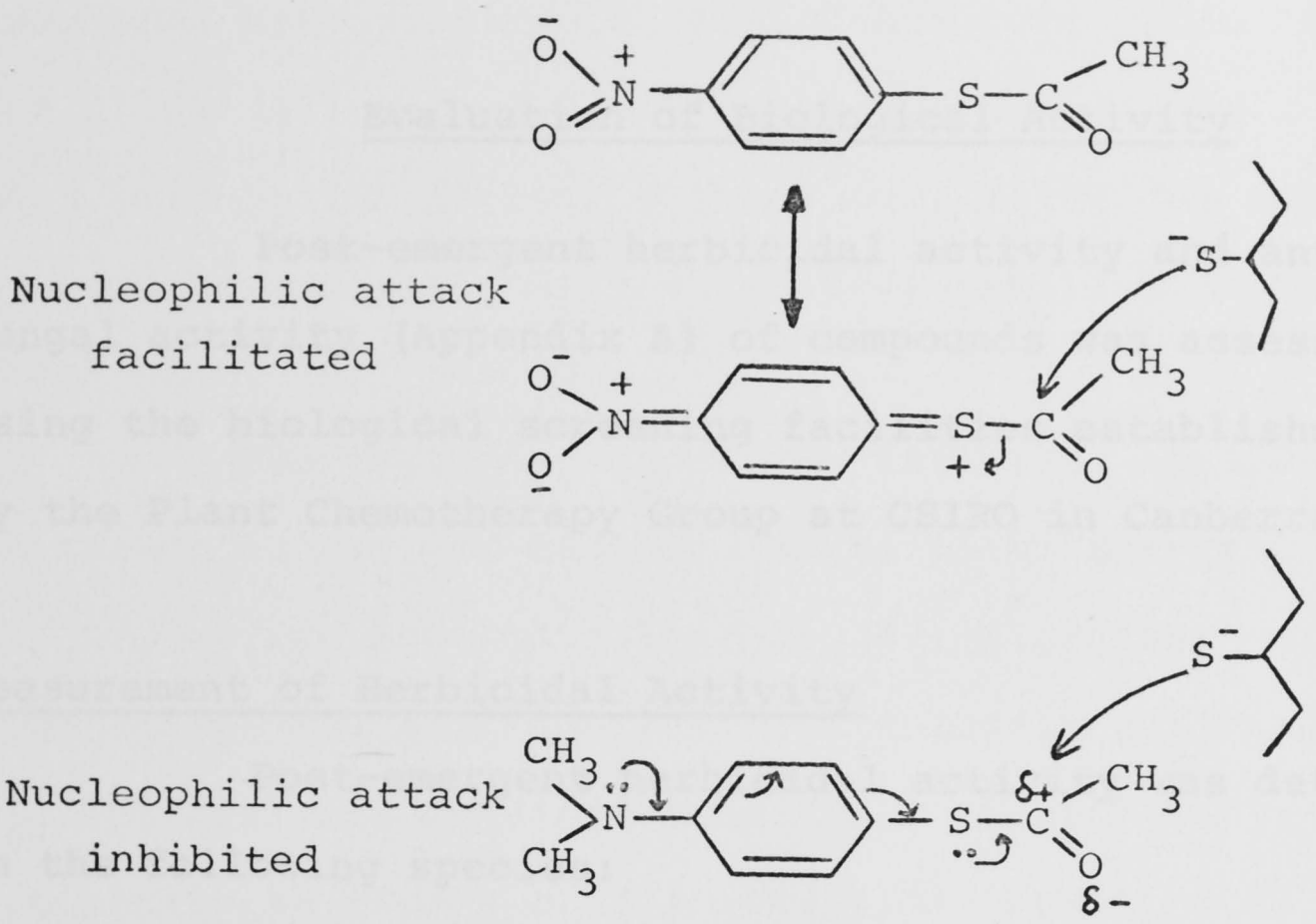


Figure 4.

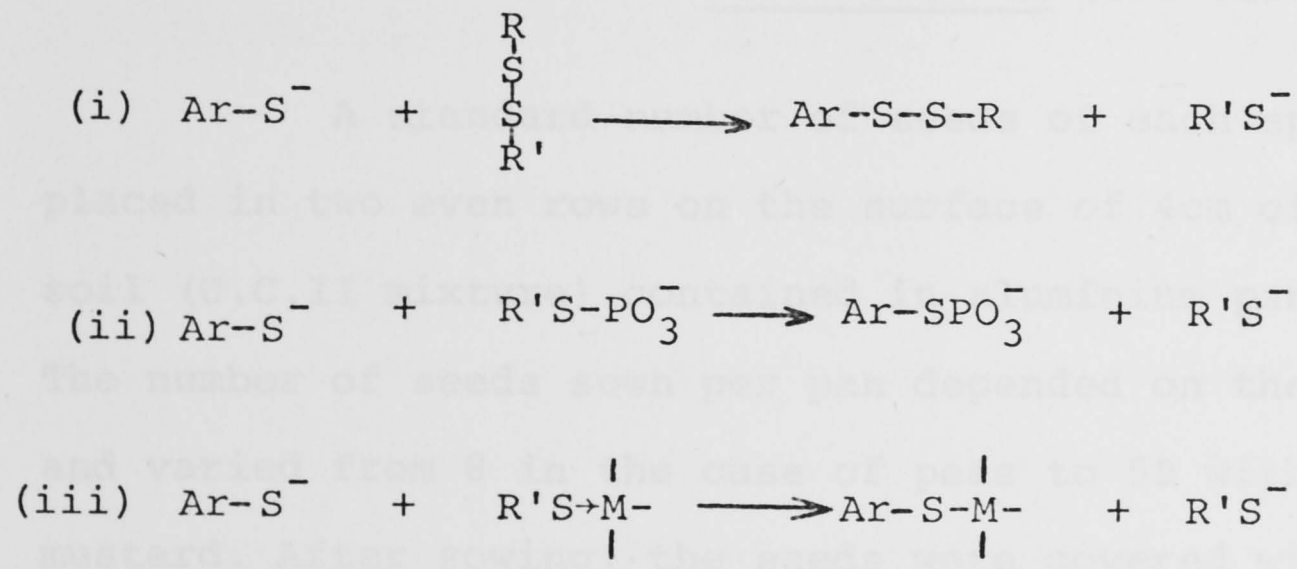


Figure 5

EXPERIMENTAL

Evaluation of Biological Activity

Post-emergent herbicidal activity and anti-fungal activity (Appendix A) of compounds was assessed using the biological screening facilities established by the Plant Chemotherapy Group at CSIRO in Canberra.

Measurement of Herbicidal Activity

Post-emergent herbicidal activity was determined on the following species:

Linseed	Linum usitatissimum
Sugarbeet	Beta Vulgare (cv. Hilleshog polyploide)
Buckwheat	Fagopyrum esculentum (cv. Tokyo)
Mustard	Sinopis altoa
Peas	Pisum sativum (cv. Victory Freezer)

A standard number of seeds of each species was placed in two even rows on the surface of 4cm of moist uniform soil (U.C.II mixture) contained in aluminium pans (20cm x 10cm). The number of seeds sown per pan depended on their size and varied from 8 in the case of peas to 50 with white mustard. After sowing, the seeds were covered with 1cm of soil. The test species were treated two weeks after planting. Sufficient of the test compound to give the required application rate was dissolved in acetone and diluted 50% with water and the solution carefully sprayed evenly over the leaves of the plants with a hand spray. Analysis of the

phytotoxicity was carried out 7 days after spraying. Throughout the experiments, the plants were grown at 24±1 ^{o}C with illumination from fluorescent tubes (1500 ft.c. at plant height) for 14-hour day periods. Water was applied as required directly to the soil surface taking care not to splash plant leaf surfaces.

The test compounds were initially applied at rates equivalent to 8Kg per hectare. Where plant survival exceeded 80%, higher application rates (16, 32, 64 Kg/ha) were applied Phytoxicity (as % of control) was visually estimated and LD_{50} ratings were obtained from the data by Probit analysis. Phytoxicity in excess of 80% invariably resulted in plant death; when less than 20%,plants usually recovered over periods in excess of the 7 day test period. Ratings measured in this manner were found to be reproducible.

Pre-Emergent Herbicidal Testing

The testing procedure is similar to that for post-emergent testing except that the test compound is applied in solution to the soil as soon as the seeds have been planted. If less than 80% germination occurs, the concentration of the test solution is decreased and the test repeated. In this way, scoring is similar to that for the post-emergent testing.

CHEMICAL SYNTHESIS

The NMR spectra were measured on a Varian A60 instrument using tetramethyl silane as internal reference. Mass spectra* were recorded on an AEI MS9 instrument using a direct insertion probe. Melting points were measured on a Büchi melting point apparatus and are uncorrected.

(i) p-Substituted Thiophenols

Thiophenol, p-chloro-, p-bromo-, p-fluoro- thiophenols and ortho- and para- thiocresols were obtained commercially. p-Nitrothiophenol was prepared by a literature method.[39,40]

p-Dimethylamino-, p-hydroxy-, p-methoxy and p-cyano- thiophenols were obtained by reduction of the correspondingly substituted phenylthiocyanate by sodium in liquid ammonia at -30°C as described by Laufer.[41]

p-Acetylthiophenol

S-(p-acetylphenyl)-N,N-dimethylthiocarbamate (55) (20mmole) was refluxed for 2 hrs. under nitrogen in 10% aqueous sodium hydroxide solution. The solution was then filtered quickly under nitrogen and the filtrate cooled on ice for 30 min. after which time the thiophenate solution was used *in situ*, or neutralised with 10% HCl and the free thiol extracted in solvent ether, the organic phase separated, dried ($MgSO_4$), the solvent removed and the thiol used in non aqueous solution.

* The Author wishes to thank the members of the Mass Spectrometry Unit in the Division of Entomology, CSIRO Canberra, for recording the mass spectra.

(ii) p-Substituted Phenylthiocyanates

These compounds were prepared by one of three general methods:

(a) thiocyanation of an aromatic ring using potassium thiocyanate and bromine in methanol[19],

(b) diazotization of an appropriately substituted amino benzene, followed by thiocyanation using the Sandmeyer reaction[43],

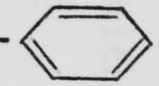
(c) thiocyanation with chlorosulphurylthiocyanate (see p. 11). The following is typical:

4-Methoxyphenylthiocyanate (35)

Sulphuryl chloride (20 m.mole) was added dropwise to lead thiocyanate (10 m.mole) suspended in acetic acud (20 ml). The mixture was stirred at room temperature for 1hr., after which time, anisole (20 m.mole) was added and the reaction stirred for a further 48hrs. Evolution of sulphur dioxide was observed during the reaction. The mixture was filtered to remove the lead chloride and the filtrate poured into water. The aqueous mixture was extracted three times with solvent ether, and the organic phase neutralised with sodium bicarbonate and dried ($MgSO_4$). The solvent was then removed leaving crude 4-methoxyphenylthiocyanate. The pure product was obtained by distillation.

Structures of products were confirmed by I.R. and mass spectrometric data. A summary of experimental details appears in Table 9.

TABLE 9

EXPERIMENTAL DATA FOR p-SUBSTITUTED PHENYLTHIOCYANATES, (R—  —SCN)

R	Method	Yield	M.P/B.P ^{o}C mm.Hg	M.P./B.P. ^{o}C (lit.) mm Hg.	Reference
$N(CH_3)_2$	a.	65%	72-73	73-74	44.
OH	a.	90%	51-52	52-53	45.
OCH_3	c.	70%	109/0.5	85/0.07	45.
CH_3	b.	75%	73/1.5	73/1.5	46.
H*	-	-	-	-	-
F*	-	-	-	-	-
Cl*	-	-	-	-	-
$COCH_3$	b.	77%	78	80	43.
CN	b.	75%	124-126	127-128	47.
NO_2	b.	72%	125-126	128-129	47.

* compounds prepared by Dr. R.L.N. Harris.

(iii) p-Substituted Phenylthiolacetates

Two methods were used to prepare these compounds:

(a) Acetylation of the appropriate thiophenol with acetyl chloride in the presence of a base,

(b) Reductive acetylation of the corresponding thiocyanate using zinc dust in 50% acetic acid-acetic anhydride at room temperature for 24hr.

p-Fluorophenylthiolacetate (43) and p-cyanophenyl-thiolacetate (49) had not been reported previously; the former was obtained as a colourless oil, while the latter was obtained as colourless needles, (Table 10).

The purity of the products was established by glc and the structures confirmed by NMR and mass spectrometry.

4-Acetoxyphenylthiolacetate was obtained by treating 4-mercapto-phenol with acetic anhydride in 2% NaOH solution.[44] (Table 10)

4-chlorophenylchlorothiolacetate (16) was obtained as in (a) above except that chloroacetyl chloride was used.[50]

4-chlorophenylthiohexanoate (17) was prepared as in (a) except that n-hexoyl chloride was used.

(iv) p-Substituted Phenyl-O-methylthiocarbonates

Compounds in this series were prepared by acylation of the corresponding thiophenol using chloromethyl-formate as described in a patent awarded to Farbenfabriken Bayer A.-G.[48] Several compounds in the series had not been reported previously. They are presented with confirmatory mass spectrometric data in Table 11.

TABLE 10

EXPERIMENTAL DATA FOR p-SUBSTITUTED PHENYLTHIOLACETATES

Structure	Method	M.P./B.P.$_{mmHg}$ °C	M.P./B.P.$_{mmHg}$ °C (lit.)	High Resolution Mass Spectra	Reference
5.	b.	81	80		51
14.	a.	32	32		53
16.	a.	76	82		50
17.	a.	220/720	-	$C_{12}H_{15}ClOS$ requires 242.05322 found 242.05296	-
26.	a.	82	86		52
31.	see text	57	66		58
34.	a.	136/9	137/9		51
37.	a.	127/11	125/11		51
40.	a.	-	-		-
43.	a.	-	-	C_8H_7FOS requires 170.02017 found 170.025025	-
44.	a.	52	52		54
46.	a.	60	62		55
49.	b.	67	-	C_9H_7NOS requires 177.02480 found 177.02392	-
52.	a.	78	78		56

TABLE 11

EXPERIMENTAL DATA FOR p-SUBSTITUTED PHENYL-O-METHYLTHIOCARBONATES

Structure	M.P./B.P.$_{mmHg}$ ^{o}C	M.P./B.P.$_{mmHg}$ ^{o}C (lit. value)	High Resolution Mass Spectra	References
12.	55-57	50-52		18
22.	104/0.2	119-121/1.5		18
30.	64-65	-	$C_{10}H_{10}O_5S$ requires 242.02490 found 242.02450	
36.	88-90/0.02	119-121/1.5		18
39.	72/0.05	134/10		18
42.	64-65/0.05	101-102/5		18
45.	90/0.05	-	$C_8H_7BrO_2S$ requires 245.935009 found 254.93487	-
48.	59	-	$C_{10}H_{10}O_3S$ requires 210.035071 found 210.03563	-
51.	106-108	-	$C_9H_7NO_2S$ requires 193.019755 found 193.01964	-
54.	139-140	-	C H NO S requires 213.009585 found 213.00903	-

(iv) S-(p-substituted phenyl)thiobenzoates (9,18,27)

An appropriately substituted aryl thiol was treated with 10% excess of benzoyl chloride in aqueous sodium hydroxide solution.[49] Products were purified by recrystallisation fron aqueous ethanol and structures confirmed by I.R. and NMR. Other physical data is presented in Table 12. The dibenzoate product (32) was obtained similarly except that two equivalents of benzoyl chloride were used in the reaction.

(v) S-(p-substituted phenyl)thiocarbamates (10,19,28)

The appropriate aryl thiol was treated with methylisocyanate in benzene for 4hrs, at room temperature. Compound (20) was obtained using ethylisocyanate.

N,N-dimethylthiocarbamates were obtained by treating the appropriate aryl thiol with dimethylcarbamoyl chloride in benzene in the presence of triethylamine (20% excess), at room temperature for 2hrs. Products were isolated by standard procedures. Compounds in this group are (11,21,29,55,56)

(vi) Dithiocarbamates (13,23,24,57)

These compounds were obtained by treating the appropriate aryl thiol with the desired isothiocyanate in benzene (containing a catalytic amount of triethylamine) for 24hr. at room temperature. Products were obtained by standard procedures.

(vii) Quaternary Ammonium Compounds (58,59,60,61)

The corresponding p-dimethylamino compound was

refluxed with an excess of methyl iodide for 24 hrs. The remaining methyl iodide was removed in vacuo to yield the trimethylammonium salt.

Measurement of Partition Coefficients

Partition coefficients (P) for the series of p-substituted phenylthiolacetates and thiocyanates were measured in the reference system n-octanol-water using the equation

$$P = \frac{C_{octanol}}{C_{water}}$$

Concentrations were measured in the aqueous phase using a Bausch and Lomb 505 spectrophotometer by comparison with a standard concentration-absorbance curve and concentrations in the octanol phase obtained by difference. Measurements were made at 20-25^{o}C and were determined for octanol-water ratios of 1:5 and 1:10. Partition was effected by mechanical agitation of the mixture for 24hrs. and the phases were separated by centrifugation for 15min at ∿1000G. Partition coefficients thus determined are listed in Table 4, page 21.

TABLE 12

EXPERIMENTAL DATA FOR THIOBENZOATES, THIO- and DITHIOCARBAMATES and QUATERNARY AMMONIUM COMPOUNDS

Structure	M.P./B.P.$_{mmHg}$	M.P./B.P.$_{mmHg}$	High Resolution Mass Spectra / Micro Analysis	References
9.	139	136		59.
10.	176	-	$C_{10}H_{14}N_2OS$ requires 210.08269 found 210.08284	
11.	123	123		42.
13.	140	-	$C_{15}H_{16}N_2S_2$ requires 288.075499 found 288.07564	
18.	72	73		60.
19.	125	-	C_8H_8ClNOS requires 210.001516 found 210.00129	
21.	109	110		61.
23.	104	104		57.
24.	66	-	$C_{13}H_{16}ClNS_2$ requires 285.041276 found 285.04139	
27.	128	-	$C_{13}H_{10}O_2S$ requires 230.040156 found 230.04028	
28.	177	-	$C_8H_9NO_2S$ requires 183.035405 found 183.03553	
29.	210	194		48.
32.	161	161		62.

TABLE 12

(continued)

Structure	M.P./B.P.$_{mmHg}$ °C	M.P./B.P.$_{mmHg}$ °C (lit.)	High Resolution Mass Spectra / Micro Analysis	Reference
55.	107	107		42.
56.	77	-	$C_{13}H_{13}NO_2S$ requires 247.066706 found 247.06695	-
57	132	-	$C_{17}H_{13}NOS_2$ requires 311.04386 found 311.04409	-
58.	164	-	$C_{11}H_{13}INOS$ requires / found C 39.53 / C 39.6 H 3.92 / H 4.06 N 4.19 / N 4.26	-
59.	159	-	$C_{11}H_{17}IN_2OS$ requires / found C 37.51 / C 37.6 H 4.86 / H 4.92 N 7.95 / N 7.85	-
60.	152	-	$C_{12}H_{19}IN_2OS$ requires / found C 39.35 / C 39.4 H 5.23 / H 5.30 N 7.65 / N 7.71	-
61.	171	-	$C_{16}H_{18}INOS$ requires / found C 48.13 / C 48.2 H 4.54 / H 4.61 N 3.51 / N 3.45	-

REFERENCES

1. Haugaard, N., Lee, N.H., Kostrzewa, R. and Haugaard, E.S., Biochim. biophys. Acta., 1969, 172, 198.

2. Gibbs, M., Ann. Rev. Pl. Physiol., 1967, 20, 341.

3. Sugiyama, T., Akasawa, T., Nakayamo, N., Tanaka, Y., Archs. Biochem. Biophys., 1968, 125, 107

4. Asahi, T., and Masaki, S., J. Biochem. Tokyo, 1966, 60, 90.

5. Zelich, I., Ann. Rev. Pl. Physiol., 1969, 20, 341.

6. Kamiya, N., Ann. Rev. Pl. Physiol., 1960, 11, 329.

7. Mazura, P., Ann. Rev. Pl. Physiol., 1969, 20, 437.

8. Smythe, C.V., J. Biol. Chem., 1936, 114, 601.

9. Albert, A.,"Selective Toxicity" Methuen, 3rd.Edition, 1965, p.274.

10 Rebstock, "Antibiotics" Burger, Ed. In "Medicinal Chemistry" 2nd,Edition, Interscience, 1960.

11. Farbenfabriken Bayer A.-G. Netherlands Patent, 6601856, (1966) (Chem. Abst., 66 : 28547 (1967))

12. Noguchi, T, Muratama, T., Kosaka, S., Kaji,A. and Kano, S., Japan Patent 5695(1963) (Chem. Abst., 60:P7387e)

13. Summers, L.A., Rev. Pure and Appl. Chem, 1968, 18, 1.

14. Goodman, L.S. and Gibman, A., Ed. "The Pharmacological Basis of Theraputics" 3rd. Ed. The Macmillan Co. New York, N.Y., 1965, p. 1312.

15. Field,L. and Barbee, R.B., J. Org. Chem., 1969, 34, 1792.
Field, L. and Buckman, J.D. ibid, 1967, 32, 3467.
Field, L. and Kim, H., J. Med. Chem., 1967, 10, 1166.

16.Field, L., Ferretti, A., Grenshaw, R. and Owen, T.C., J. Med. Chem., 1964, 7, 39.

17. Harper, N.J., J. Med. Chem., 1959, 1, 467.

18. Farbenfabriken Bayer A.-G. Netherlands Patent 6,408,287. (Chem Abst. 63:11439 (1965))

19. Kazuo Konishi, Takeda Kenkyusho Nempo, 1965, 24, 233.

20. Bacon, R. and Guy, R., J. Chem. Soc. (London), 1960, 318.

21. Harris, R.L.N., Unpublished Results.

22. Beveridge, Susan, Thesis, Australian National University, Canberra, 1969.

23. Rich, S. and Horsfall, J.G., "Fungitoxicity of Carbamic and Thiocarbamic Acid Esters" Bulletin 639, The Connecticut Agricultural Experiment Research Station, New Haven, March 1961, p. 16.

24. Gabbot, P.A., In "Progress in Photosynthesis Research" (Metzner, H., Ed.) 1969, Vol. 13, p. 1777. Tübingen.

25. Ferguson, J., Proc. Royal Soc.(London), 1939, 127(B), 387.

26. Collander, R., Acta Chemica Scandinavica, 1951, 5, 774.

27. Collander, R., Physiol. Planatorum, 1954, 7, 420.

28 Hansch, C. and Fujita, T., J. Amer. Chem Soc., 1964, 86, 1616.

29. Hansch, C. and Fujita, T., J. Amer. Chem. Soc., 1964, 86, 5175.

30. Iwasa, J., Fujita, T., Hansch, C., J. Med. Chem., 1965, 8, 150.

31. Jaffe, H.H., Chem. Rev., 1953, 53, 191.

32. Hansch, C., Maloney, P.P, Fujita, T. and Muir, R., Nature, 1962, 194, 178.

33. Hansch, C. and Anderson, S.M., J. Med. Chem., 1967, 10, 745.

34. Schreck, J., J. Chem. Education, 1971, 48, 103.

35. Harris, R.L.N., Hogan, A.M., Geissler, A.E., (in the press)

36. Hansch, C. and Clayton, J., J. Pharm. Sci. 1973, 62, 1.

37. Yamagishi, S., Yakugaku Zasshi, 1958, 78, 1138.

38. Degani, Y., Neumann, H., Patchornik, A., J. Amer. Chem. Soc., 1970, 92(23), 6969.

39. Waldron, W.R., Reid, E.E., J. Amer. Chem. Soc., 1923, 2401.

40. Brogart, M. and Stull, A.,"Organic Synthesis" Coll. Vol.1, p. 220

41. Laufer, R., (Consolidation Coal Co.) U.S. Patent 3,129,262. (Chem. Abst. 61:1799, (1964))

42. Newman, M.S. and Karnes, H.A., French Patent 1,521,896 (Chem. Abst. 71:12814 (1969))

43. Pohloudek-Fabini, R. and Luess, K.D., Arch. Pharm., 1966, 229(10), 866.

44. Brewster, R.Q. and Schroeder, W., "Organic Synthesis" Coll. Vol. II, p.574.

45. Aritsune, K., Nippon Kagaku Zasshi, 1961, 82, 382.

46. Farbenfabriken Bayer A.-G. German Patent 1,195,743. (Chem. Abst. 64:15795 (1966))

47. Riemeschneider, R., Wojahn, F., Orlick, G., J. Amer. Chem. Soc. 1951, 73, 5905.

48. Newman, M. and Karnes, H., J. Org. Chem. 1966, 31(12), 3980.

49. Vogel, A.I., "Elementary Practical Organic Chemistry" Longmans, London, 1962, p. 453.

50. Sumrell, G., Zief, M., Huber, E., Ham, G. and Schramm, G., J. Amer. Chem. Soc. 1959, 81, 4313.

51. Baliah, V. and Ganapathy, K., Trans. Far. Soc. 1963, 59, 1784.

52. Bordwell, F., Boutan, P., J. Amer. Chem. Soc., 1956, 78, 854.

53. Morgenstern, J., Kunz, D., Mayer, R., Pharmazie, 1969, 24(8), 450.

54. Herz, H., Tarbel, D., J. Amer. Chem. Soc. 1953, 75, 4657.

55. Overberger, C.G., Lebouits, A., J. Amer. Chem. Soc., 1956, 78, 4792.

56. Newman, Joyce K., Spectrochim Acta (A), 1969, 25(4), 897.

57. Haynes, G., Legator, M., (Shell International Research) Belgian Patent 633,277 (Chem. Abst. 61:P3022c)

58. Zincke and Ebel, Berichte, 47, 1105.

59. Wuyts, Y., Vangendertaelen, A., Bull. Soc. Chim. Belg., 1921, 30, 323.

60. Berezin, G.H., Harris, G., U.S. Patent 3,219,679 (1965) (Chem, Abst. 64:8097).

61. Tilles, H., Williamson, T.B., (Stauffer Chemical Co.) U.S. Patent 3,265,563. (Chem. Abst. 66:Pc2346k)

62. Magnusson, S., Christian, J.E., Jenkins, G., J. Am. Pharm. Assoc. (Sci. Ed.) 1947, 36, 257.

APPENDIX A

Antifungal Activity of Some Aryl Thiol Derivatives

Antifungal Testing.

The in vitro testing which is reported here, was carried out on Monolinia fructicola (brown rot). The germination ratings are determined by the lowest concentration of the applied compounds which score a 50% germination inhibition in comparison with control cultures i.e. LD_{50}.

Thus:

LD_{50}	>	150 ppm	rates	0
from		50-150 ppm	"	1
"		15-50 ppm	"	2
"		5-15 ppm	"	3
"		1.5-5 ppm	"	4 etc.

In vivo Fungal Assays:

These assays involve testing against six species of fungi (species underlined). The infected medium (in brackets) is dipped into a solution of the compound being tested and the extent of fungal growth inhibition is assessed against controlled medium, i.e. medium which is infected but not dipped. In the case of E. cichoracearum and Fus. oxysporum, the infected medium was grown in soil treated with the test compound.

APPENDIX A (continued)

P. cinnamomi	(lupins)	no control of infection at 8ppm	-
		partial control " " "	+
		full control " " "	++
E. cichoracearum	(cucumbers)	phytotoxic at 8Kg/ha	P
		no control " "	-
		partial control " "	+
		full control " "	++
Fus. oxysporum	(tomatoes)	as for E. cichoracearum	
Penicilli	(oranges)	phytotoxic in 2000ppm dip	P
		no control	-
		partial control	+
		full control	++

M. fructicola (peaches) as for Penicilli

Rhiz. solani (cotton) as for Fus. oxysporum except 16Kg/ha

Compound	Germination	In Vivo					
Structure No.)	M. fruct.	Penicilli	P.cin.	E.cich.	M.fruct.	Fus. oxy.	R.sol.
10.	3	-	-	-	-	-	++
11.	1	-	-	-	-	-	++
13.	1	-	-	-	-	-	-
18.	4	-	++	-	-	-	-
19.	3	+	++	-	+	-	-
20.	2	-	-	+	-	-	-
21.	1	+	-	-	-	-	-
23.	3	-	++	-	-	-	-
24.	2	-	++	-	-	-	++
28.	2	-	-	-	-	+	+
56.	0	-	-	-	+	-	-
57.	2	-	-	-	-	-	+

Antifungal Activity of Some Aryl Thiol Derivatives

PART 2. A SYNTHETIC ROUTE TO POLYHYDROXYLATED ARYL THIOLS: RING SUBSTITUTION OF PHENOLS BY IODINE-THIOUREA COMPLEXES

INTRODUCTION

In view of the general conclusions reached in Part 1 relating to aromatic substituent effects on biologocal activity, it is reasonable that activity associated with hydroxyarylthiol derivatives (such as 4-thiocyanatophenol and 4-hydroxyphenylthiolacetate) might be due to the enhanced nucleophilic character bestowed by the hydroxy groups. Thus, compounds containing two or even three such groups in the aromatic ring might be expected to exhibit even higher biological activity under similar conditions.

The paucity of information in the literature relating to polyhydroxylated thiophenols stimulated a study of the synthesis and properties of these compounds and their derivatives. The S-acyl and related compounds were considered especially to be of potential biological interest.[1] The high reactivity and hydrophilic properties of these thiols could be anticipated to pose problems of isolation, and a method for their synthesis <u>in situ</u> under basic conditions (suitable for subsequent reactions) was desirable.

Results and Discussion

Reactions of Phenols with Iodine-Thiourea Complexes

A novel reaction for the generation of highly reactive pyrrolyl thiols has been described[2]. In this reaction, the pyrrole was treated with iodine and thiourea to yield the S-(pyrrolyl)isothiouronium salt (1) which in turn was hydrolysed to the thiol.

The frequently quoted comparison of the reactivity of polyhydroxylic phenols with that of pyrroles suggested that an extension of the iodine-thiourea reaction to phenols might produce the analogous isothiouronium derivatives (2). The stability of these compounds together with their ease of hydrolysis under basic conditions seemed an attractive route for the synthesis of polyhydroxylated thiophenols.

Some S-(hydroxyaryl)isothiouronium salts have been reported previously. Compounds such as S-(2,5-dihydroxyphenyl)isothiouronium chloride (3) have been prepared by the reaction of quinones with thiourea[3]. Others, for example S-(3-methyl, 4-hydroxyphenyl)isothiouronium bromide (4) have been obtained by reacting diformamidinium disulphide dihydrobromide with simple phenolic esters such as acetates[4]. An alternative method of producing these compounds involving the reaction of diazonium salts with thiourea is unsuitable because of anomalous reactions of aminophenols with nitrous acid[5].

It was decided in the present work to attempt the extension of the iodine-thiourea substitution reaction to

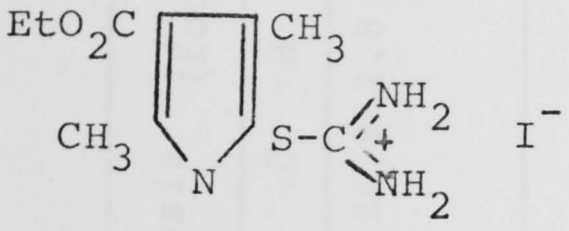
mono and polyhydroxylated phenolic compounds. Accordingly, a series of phenols was reacted with iodine and thiourea in aqueous solution under varying conditions in an attempt to introduce the isothiouronium function into the aromatic ring.

It is apparent from the summary of results in Table 1, that simple phenols do not readily undergo ring substitution in this reaction and that best results are obtained with polyhydroxylic phenols such as resorcinol, (5) which yielded S-(2,4-dihydroxyphenyl)isothiouronium iodide (6).

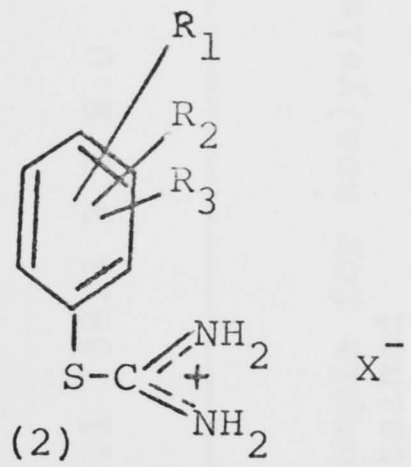
An interesting sidelight to the application of this reaction to resorcinol, was that by increasing the reaction time to 3-4 hours, the isothiouronium function reacted with the adjacent hydroxy group to form (via the imino intermediate (7)) 6-hydroxy-1,3-benzoxathiol-2-one (8). The good yield for the oxathiolone makes this reaction a very convenient route to this important class of biologically active compound.[6]

Pyrogallol (9) and phloroglucinol (11) when treated under similar conditions to resorcinol yielded S-(2,3,4-trihydroxyphenyl)isothiouronium iodide (10) and S-(2,4,6-trihydroxyphenyl)isothiouronium iodide (12) respectively.

Unlike 1-naphthol (13) which formed the expected S-(4-hydroxynaphth)isothiouronium iodide (14), 2-naphthol (15) gave a mixture of products, namely the naphtho-(1,2-d)-1,3-oxathiol-2-one (16), bis(2-hydroxynaphthyl)monosulphide (17), and bis(2-hydroxynaphthyl)disulphide (18). The formation of these products indicates that although 2-naphthol undergoes substitution in the 1- position, the isothiouronium derivative is not stable under acidic conditions.

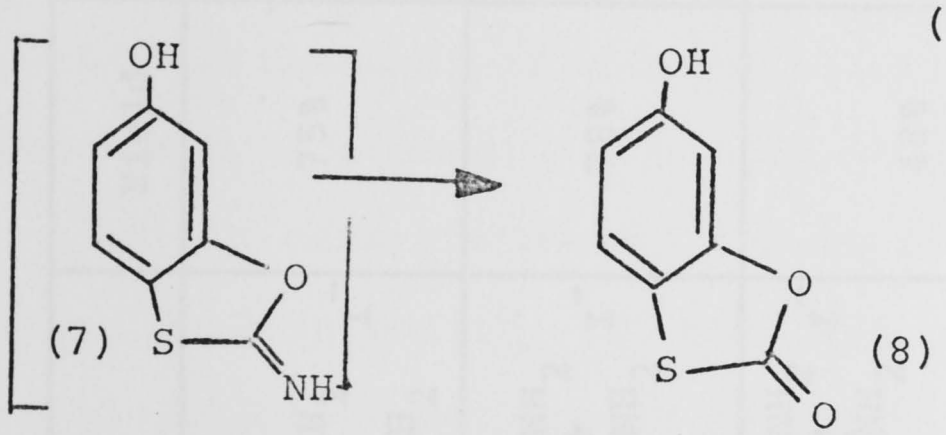


(1)

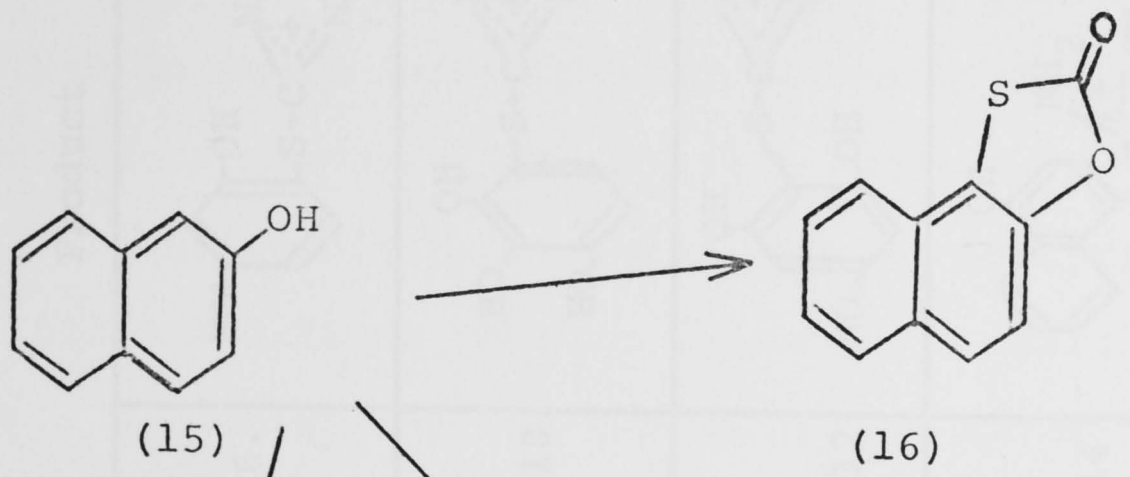


(2)

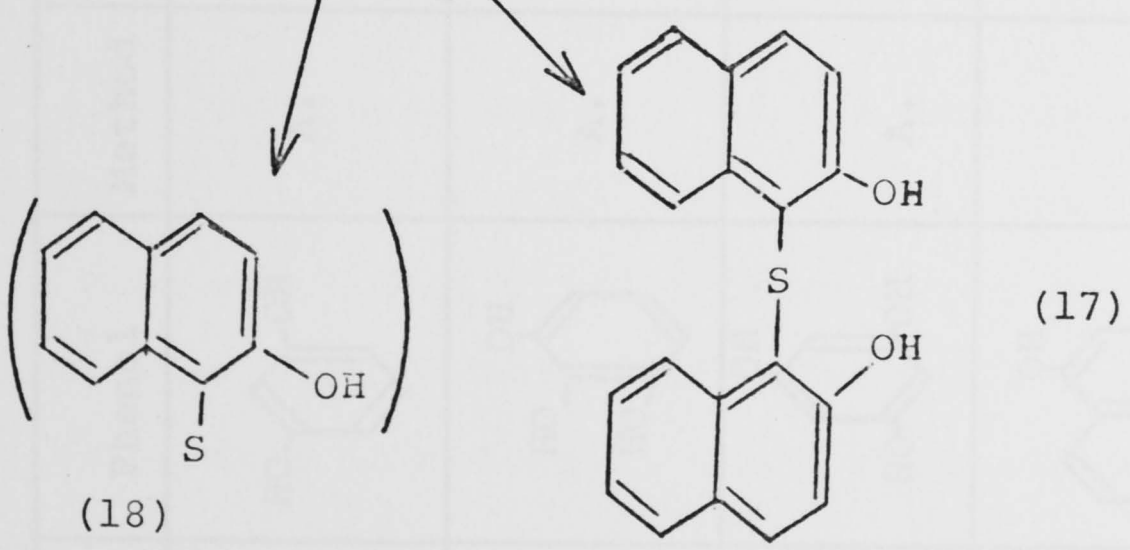
	R_1	R_2	R_3	X
(3)	2-OH	5-OH	H	Cl
(4)	3-CH_3	4-OH	H	Br



(7) (8)

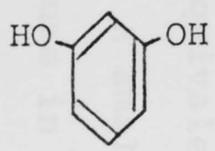
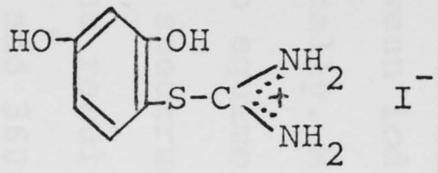
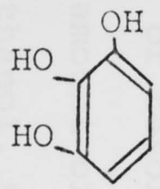
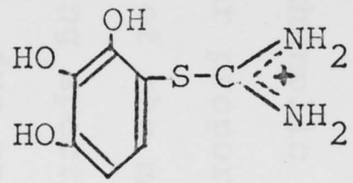
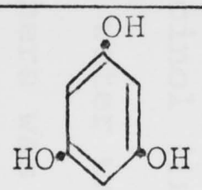
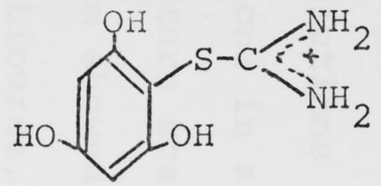
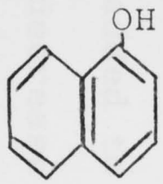
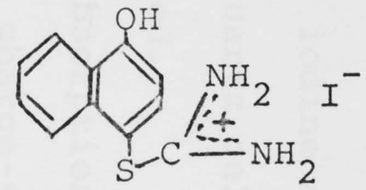
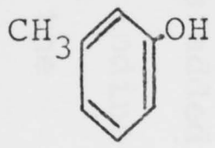
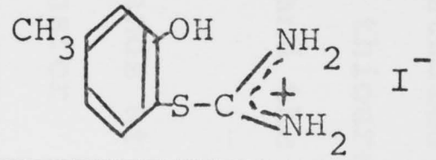


(15) (16)



(18) (17)

Table 1

Comp. No.	Phenol	Method	Product	Yield	Analysis (required) C	H	N	(found) C	H	N
5.		A.	6. 	75%	26.9	2.9	8.4	26.9	2.8	8.2
9.		A.	10. 	78%	25.6	2.8	8.5	25.8	2.8	9.3
11.		A.	12 	48%	25.6	2.8	8.5	25.8	2.7	8.5
13.		C.	14 	62%	38.2	3.2	8.1	38.6	3.1	8.0
20.		B.	19. 	30% + 60% NH_4I	pure sample for analysis not obtained					

The reaction failed with the various nitro- and aminophenols as well as with o- and p-cresol. Ammonium iodide was the only product isolated by the usual work-up technique (isolation of salts). An exception was the reaction with m-cresol, the product of which was found to contain approximately 30% of S-(2-methyl,4-hydroxyphenyl)-isothiouronium iodide (19) (NMR) and 60% ammonium iodide.

All attempts to repeat the reaction in anhydrous solvents such as methanol, ethanol, dimethylformamide, benzene and ethyl acetate etc. were unsuccessful. However, a 20% water in methanol or ethanol solution was sufficient to produce some product, albeit in lower yields.

In order to assess the importance of water, the reaction between iodine, thiourea and resorcinol was studied spectroscopically. Methanolic solutions of iodine and thiourea were mixed in equimolar proportions in a quartz cell and the ultra-violet spectrum of the mixture scanned.

The resulting spectrum showed absorption bands at 242nm, 298nm and 360nm, due to thiourea, a charge-transfer band of a 1:1 molecular complex of iodine and thiourea and the triiodide ion respectively.[7]

Continuous scanning of the mixture for three hours showed no decrease in intensity of the bands, even when a mole equivalent of resorcinol (in methanol) was added. However, when a small amount of water was added to an iodine-thiourea mixture in methanol, there was a decrease in the intensity of the bands as time progressed. The 360nm band of I_3^- was monitored for this purpose.

The reaction was repeated several times using methanol-water solvents of varying water content. The reaction rate increased with increasing aqueous content. For a given proportion of methanol and water in the solvent, the inclusion of resorcinol in the initial reaction mixture produced an increase in reaction rate above that normally obtained for that particular solvent. As the reactions progressed it was noticeable that the $\lambda_{max.}$ underwent a hypsochromic shift of 2-4nm probably as a result of the increasing ionic strength of the solvent. A plot of $\log\frac{I_o}{I_t}$ v. time (where I_o is the initial absorption and I_t is the absorption at time t sec.) produced a series of straight lines (Fig. 1) whose gradients depended on the water content of the reaction solvent.

Mechanism of the Reaction

It is proposed that iodine and thiourea react initially to form a charge-transfer complex[7] (a) (λ_{max} 298nm), which is in redox equilibrium with the diformamidinium species (b) (λ_{max} 360nm for I_3^-), and a sulphenyl iodide species (c)[8]. In (b) the iodine is partially reduced (to I^-, I_3^-) and in the latter all the iodine is present as I^{-1}, (Scheme 1).

Reaction with resorcinol may occur either by electrophilic substitution with the sulphenyl iodide species (c) (path A), or by cleavage of the disulphide bond in (b) through nucleophilic attack by the C-4 centre in resorcinol, (path B). This latter pathway is similar to that proposed by Bhattacharya for the reaction between diformamidinium disulphide dihydrobromide and resorcinol.[4]

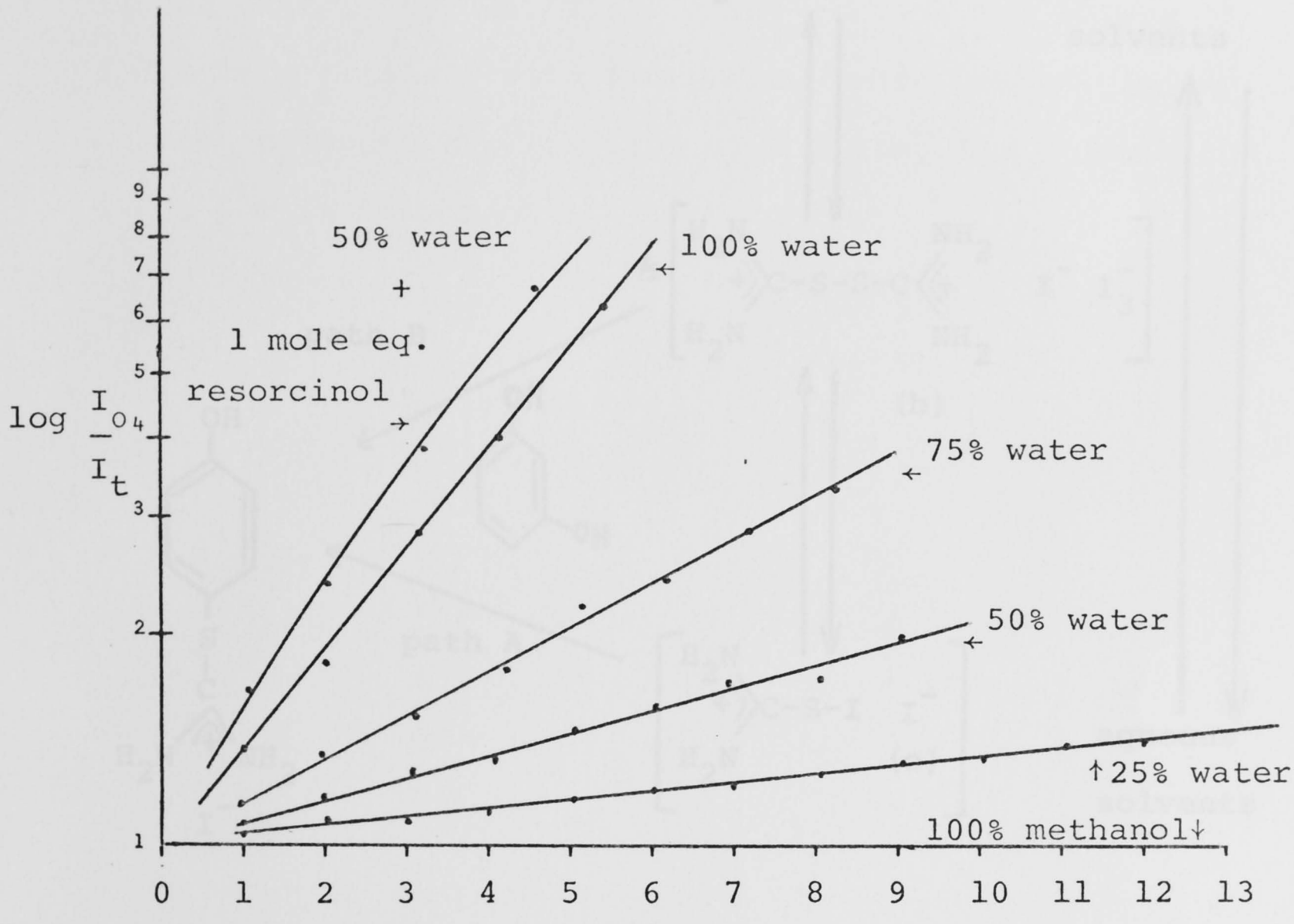
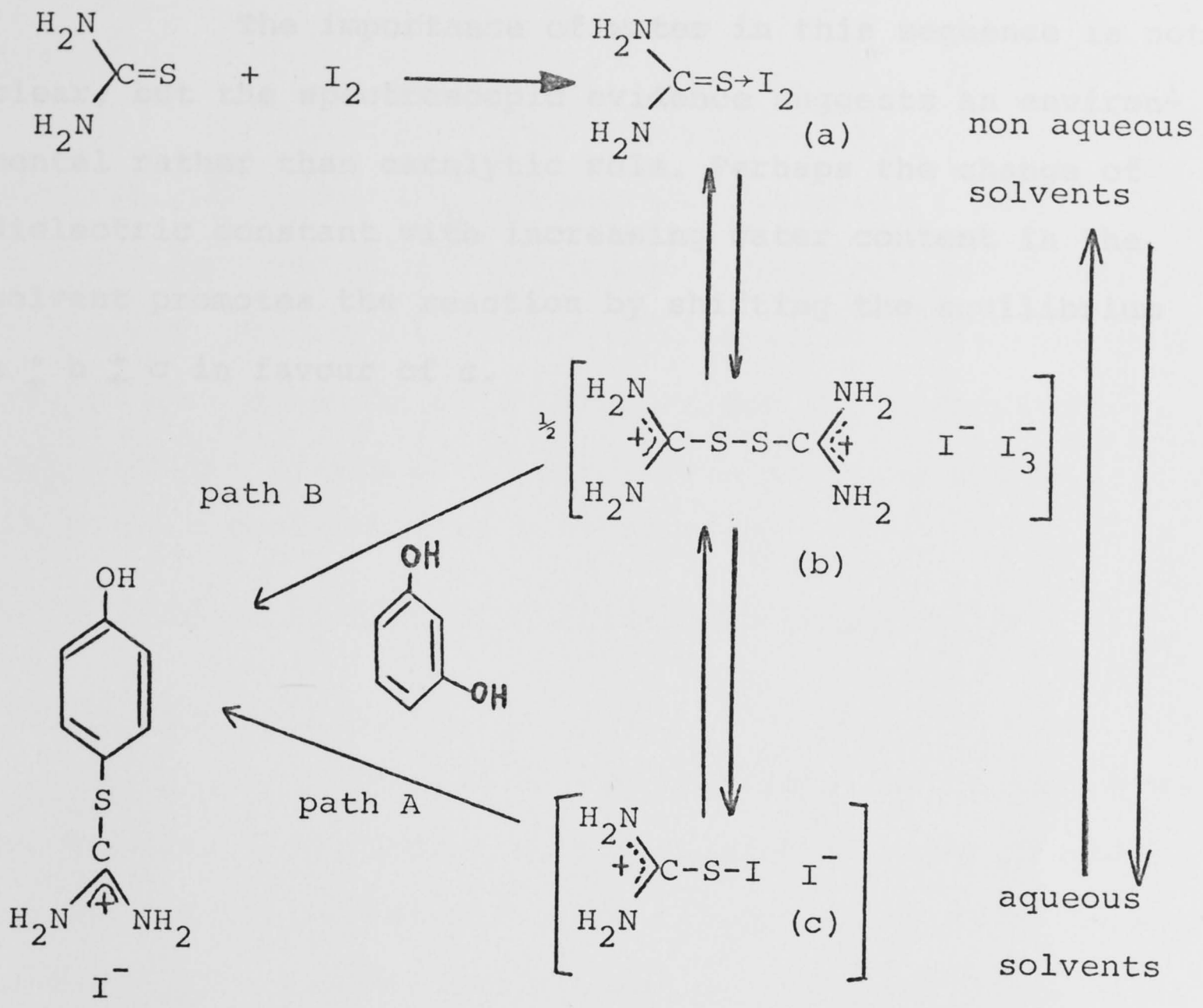

50% water
+
1 mole eq.
resorcinol
100% water
75% water
50% water
25% water
100% methanol
log I0/It
1
2
3
5
6
7
8
9
0 1 2 3 4 5 6 7 8 9 10 11 12 13
time 1 unit = 48 sec.

Figure 1.



Scheme 1

The importance of water in this sequence is not clear, but the spectroscopic evidence suggests an environmental rather than catalytic role. Perhaps the change of dielectric constant with increasing water content in the solvent promotes the reaction by shifting the equilibrium $a \rightleftarrows b \rightleftarrows c$ in favour of c.

EXPERIMENTAL

General

Microanalyses were determined by the Australian Microanalytical Service, Melbourne. NMR spectra were measured on a Varian A60 instrument using tetramethylsilane as internal reference. Ultra-violet spectra for mechanistic studies were measured on a Perkin-Elmer 402 instrument equipped with an automatic repetitive scanning option. All phenols were obtained commercially.

Preparation of S-(hydroxyaryl)isothiouronium Salts.

General Procedure

(A) The phenol (100 m.mole), iodine (100 m.mole) and thiourea (100 m.mole) were suspended in water (10 ml) and the mixture warmed for a few minutes until a red solution developed. The mixture was allowed to stand for 24hrs after which time the supernatant liquid was decanted (hydriodic acid) and the residual solid slurried with ethyl acetate and filtered. The product was washed with ether until colourless and air dried. Isothiouronium salts obtained by this method were essentially pure (NMR) and were recrystallised for analysis from methanol-ethylacetate-ether.

(B) This procedure was essentially as described in A above except that the reaction mixture was heated on an oil bath at 120^{0}C for two hrs. The products were isolated as in A above.

(C) This procedure was as for A except that the reaction mixture was heated for 5 hrs. on a steam bath. Products were isolated as above.

6-Hydroxy-1,3-benzoxathiol-2-one (8)

Resorcinol (50 m.mole), iodine (50 m.mole) and thiourea (50 m.mole) were suspended in 100 ml of water and heated on a steam bath for 4hrs. The crude product began to crystallise from solution after about 3½ hrs. The pure product was obtained by recrystallisation from boiling water, m.p. 158-160°C, (lit. value 160°C)[6] yield: 5.58g, 70%; molecular wt. M^+ at m/e 168.

Reactions of 2-Naphthol

2-Naphthol was treated as in method C. When the reaction was cooled, a solid product deposited and was collected by filtration. The solid was then dissolved in chloroform and was found to be a mixture (3 spots tlc). The phenolic material was extracted by washing the chloroform layer with 10% aqueous sodium hydroxide . Evaporation of the remaining chloroform solution yielded naphtho-(1,2-d)-1,3-oxathiol-2-one (16).The alkaline washings were neutralised with 10% HCl and the solid product collected and oven dried. The dry solid was found to be a mixture of two compounds (tlc). These were separated by fractional crystallisation from chloroform-petroleum ether to yield:

bis(2-hydroxy-1-naphthyl) sulphide m.p. 166°C (lit. 166°C)[9]
Mol. Wt. (mass spec) M^+ at m/e 318.

bis(2-hydroxy-1-naphthyl) disulphide m.p. 215°C, (lit. 211°C)[9]
Mol. Wt. (mass spec) M^+ at m/e 350

Naphtho-(1,2-d)-1,3-oxathiol-2-one (5.8g, 29%) m.p. 121-123°C
Mol. Wt. (mass spec) M^+ at m/e 202.

Spectroscopic Studies

Solutions of iodine, thiourea and resorcinol were made up in methanol at concentrations of 10^{-3}M so that final concentrations of approximately 10^{-4}M were achieved in the quartz cells. This latter concentration was found to be satisfactory for the limits of the instrument. A repetitive scan attachment was used in conjunction with the instrument and this enabled regular auromatic scanning over fixed ranges of wavelength and at fixed intervals of time. For the purposes of the experiment, a time interval of 48 sec. was used, while the wavelength range was 280nm-380nm. The band at λ_{360} due to I_3^- was monitored to obtain values of I_o and I_t which in turn were used to compile Figure 1.

REFERENCES

1. Fiedler and Kaben, Pharmazie, 1966, 21(3), 178.

2. Harris, R.L.N., Tet. Letters, 1968, 37, 4045.

3. Burton and David, J. Chem. Soc., 1952, 2193.

4. Bhattacharya, A., J. Ind. Chem. Soc. 1966, 43(12) 742.

5. Busch, M. and Schulz, K., J, Prakt. Chem.,1938, 150, 173.

6. Raab, W., Klin. Exp. Dermatol., 1968, 231(3), 250.

7. Lang, R.P., J. Amer. Chem. Soc. 1962, 84, 1185.

8. Kharasch, N., "Organic Sulphur Compounds", Vol. 1., N. Kharasch, Ed., Pergamon Press, Oxford, 1961, p.387.

9. Tsukamoto, H., et al., J. Pharm. Soc. Japan., 1953, 73, 1083.