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Theoretical studies on the biocidal activity of phenylthiocyanates

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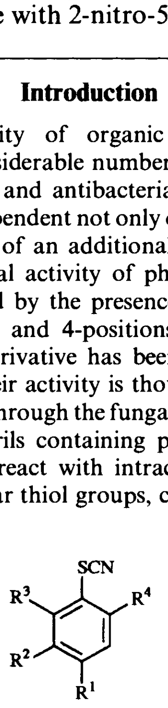
Molecular orbital calculations are reported on the structure and electronic properties of phenylthiocyanates using both the semi-empirical AM1 and MNDO methods and the *ab initio* 3-21G and 6-31G** methods. The fungicidal activity of these molecules which is thought to arise from their reaction with intracellular thiols does not appear to correlate with simple molecular properties such as the calculated atomic charges at the sulfur and cyano-carbon atoms. Reaction coordinate calculations at the AM1-SM1 level in water, using methanethiolate as the attacking nucleophile, strongly suggest that nucleophilic attack will occur at the cyano-carbon in preference to the sulfur atom, in line with experimental data obtained from the reactions of glutathione with 2-nitro-5-thiocyanatobenzoic acid in aqueous solution.

Introduction

The biological activity of organic thiocyanates is well established and a considerable number have been patented as antivirals, antifungals and antibacterials.[1] In many cases the degree of activity is dependent not only on the presence of sulfur but also the presence of an additional activating group. For example, the fungicidal activity of phenylthiocyanate (**1a**) is substantially enhanced by the presence of electron attracting substituents at the 2- and 4-positions of the aromatic ring and the 2,4-dinitro-derivative has been patented as a potent antifungal agent.[2] Their activity is thought to arise from their ability to readily pass through the fungal cell walls which consist of loosely knitted fibrils containing protein, polysaccharides and lipids, and then react with intracellular species such as glutathione and cellular thiol groups, causing the cell function to be impaired.[3]



1a R^1–R^4 = H
b $R^1 = NO_2$; $R^2 = CO_2H$; $R^3 = R^4 = H$
c $R^1 = NH_2$; R^2–R^4 = H

The mechanism of the intracellular reaction has not been fully elucidated but there are two main possibilities which involve nucleophilic attack by glutathione or a cellular thiol group (Cell-SH) either (1) at the sulfur atom of the phenylthiocyanate

$$\text{RSCN} + \text{Cell-SH} \longrightarrow \text{Cell-SSR} + \text{HCN} \qquad (1)$$

resulting in displacement of cyanide or (2) at the cyano-carbon

$$\text{RSCN} + \text{Cell-SH} \longrightarrow \text{Cell-SCN} + \text{RHS} \qquad (2)$$

resulting in the displacement of a thiol. Experimentally, alkyl thiocyanates containing the ethyl, octyl or benzyl group readily liberate cyanide in the presence of enzyme bound glutathione[4,5] supporting eqn. (1). However, other studies on the reactions of the water soluble 2-nitro-5-thiocyanatobenzoic acid (ArSCN, **1b**) with glutathione (G-SH), have shown that the liberation of cyanide occurs by a more complex mechanism involving the initial displacement of 2-nitro-5-sulfanylbenzoic acid or its anion [eqn. (3)] and its subsequent reaction with the starting

$$\text{ArSCN} + \text{G-SH} \longrightarrow \text{G-SCN} + \text{ArSH} \qquad (3)$$

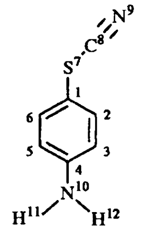
thiocyanate to give a symmetrical disulfide [eqn. (4)].[6]

$$\text{ArSCN} + \text{ArSH} \longrightarrow \text{ArSSAr} + \text{HCN} \qquad (4)$$

The present studies were initiated to explore theoretically the two possible reaction pathways using molecular orbital methods. Two approaches, involving the following approximations, were investigated where: (*i*) the transition state is assumed to occur early in the passage along the reaction coordinate and is essentially reactant-like, and can be assessed simply from the electronic properties of the phenylthiocyanate itself calculated at the semi-empirical or *ab initio* levels; (*ii*) the transition state occurs later along the reaction coordinate and can be identified by calculating the change in reaction enthalpy as the thiol approaches and the respective nucleophile departs (in this approach the entropy has been assumed to be constant because an identical number of reactants and product molecules are involved in each case).

Method of calculation

Calculations were carried out with full geometry optimisation on the free phenylthiocyanates using both the semi-empirical MNDO[7] and AM1[8] methods of the MOPAC program[9] and the *ab initio* 3-21G method[10] and 6-31G** methods[10] of the GAMESS program.[11] The numbering convention adopted for the calculations is shown in Scheme 1.



Scheme 1 Numbering convention adopted for phenylthiocyanate (**1a**) and the 4-amino-derivative (**1c**)

Reaction coordinate calculations were carried out both in the gas phase at the AM1 level[7] and in aqueous solution using the AM1-SM1 method[12] of the AMSOL program[13] with full optimisation of all geometric variables (keyword 'derinu').[13] Methanethiolate was adopted as the incoming nucleophile with attack centred at the sulfur atom of the phenylthiocyanate on the one hand and at the cyano-carbon of the same molecule on the other. The initial distance between the attacking sulfur and the atom under attack was set at 10 Å, with the geometry and energy of the reaction assessed as a function of distance between the approach of the nucleophile and the departure of the leaving group.

Results and discussion

Early transition state model

A search of the Cambridge Structural Database[14] shows few experimental structures available on arylthiocyanates for comparative purposes with the exception of the 4-amino-derivative (**1c**).[15] Initial calculations were carried out to assess the potential of both the semi-empirical MNDO[7] and AM1[8] methods to reproduce the experimental geometry of this structure (**1c**) before analysing the electronic properties such as the atomic charges at the key sulfur and cyano-carbon atoms of phenylthiocyanate itself (**1a**). The results of both calculations show similar trends in terms of the calculated bond lengths and angles but both the Ph–S and S–CN bond lengths are substantially underestimated relative to the crystal structure (Table 1). A similar overestimation of the bond lengths at sulfur has been reported also for methylthiocyanate at the MNDO level.[16] The calculated angles show a better correlation with experiment with the key Ph–S–CN only slightly overestimated by around 5° and a predicted tetrahedral sp^3 amino-group with C^3–C^4–N^{10}–H^{11} torsion angles close to the experimental data in both cases (Table 1). However, the torsion angles at the SCN substituent are significantly different with the MNDO method predicting an almost orthogonal conformation for the CN group relative to the plane formed between the sulfur atom and the aromatic ring in line with the crystal structure, whereas the AM1 method appears to underestimate this value (Table 1).

In an alternative approach, the *ab initio* 3-21G method[10] was adopted, again with full geometry optimisation, to assess the accuracy of a representative *ab initio* treatment. In this case, the calculated bond lengths and angles do not appear to hold any advantage over the semi-empirical methods with the key Ph–S and S–CN bond lengths substantially overestimated relative to the crystal structure (Table 1). The predicted torsion angle of the CN group relative to the plane formed between the sulfur atom and the aromatic ring is very similar to that predicted by the AM1 method and dissimilar to that found in the crystal structure (Table 1). However, the large observed torsion angle for this group may arise from the effect of the local electrostatic field found in the crystal. A trigonal sp^2 conformation is predicted for the amino-group in contradiction to the experimental data. However, although the dipole moment at the *ab initio* level at 6.0 D is overestimated, it is nonetheless closer to the experimental value of 5.2 D[17,18] than the values of 3.54 and 4.16 D obtained with either the MNDO or AM1 methods respectively (Table 1).

None of the methods explored here gave particularly good results in terms of the key Ph–S and S–CN bond lengths and accordingly all three methods were adopted to probe the structure and electronic properties of the parent phenylthiocyanate (**1a**). The calculated structures show similar features to those predicted for the 4-amino-derivative (**1c**) with the semi-empirical methods showing much shorter Ph–S and S–CN bond lengths than the 3-21G method (Table 2). The torsion angles of the CN group relative to the plane formed between the sulfur atom and the aromatic ring are very similar to those calculated for (**1c**) by all three methods. The dipole moments of the semi-empirical methods, however, are closer to the reported experimental values of 3.0[19] and 3.6[20] than the *ab initio* method (Table 2).

An analysis of the charge distributions at the key sulfur and cyano-carbon atoms by all three methods suggests that nucleophilic attack would predominately occur at the sulfur atom since this is strongly positive, whereas the carbon atom carries a negative charge (Table 3). Because the calculated charge distributions could be an artifact of the geometry produced by the three methods, a final more thorough calculation was carried out at the 6-31G** level, again with full geometry optimisation. The results in this case show a much better correlation for the expected Ph–S and S–CN bond lengths, but the overall charge distribution still shows the same pattern with the sulfur atom strongly positive (Tables 2 and 3). All these results suggest that nucleophilic attack is likely to occur at sulfur in apparent contradiction with the experimental results obtained for glutathione in solution.

Table 1 Calculated *versus* experimental geometry of 4-aminophenylthiocyanate (**1c**)

Geometric parameter[a]	Method of calculation AM1	MNDO	3-21G	Experiment[b]
C^1–C^2	1.396	1.412	1.378	1.383
C^2–C^3	1.388	1.402	1.379	1.369
C^3–C^4	1.416	1.421	1.395	1.391
C^4–C^5	1.418	1.420	1.396	1.395
C^5–C^6	1.386	1.402	1.379	1.374
C^6–C^1	1.402	1.412	1.379	1.386
C^1–S^7	1.700	1.699	1.852	1.786
S^7–C^8	1.613	1.628	1.741	1.698
C^8–N^9	1.170	1.164	1.141	1.137
C^4–N^{10}	1.392	1.415	1.370	1.377
C^1–C^2–C^3	121.0	120.8	120.0	120.4
C^2–C^3–C^4	120.5	120.5	121.2	121.0
C^3–C^4–C^5	118.2	118.7	117.8	118.1
C^4–C^5–C^6	120.4	120.3	120.8	121.1
C^5–C^6–C^1	121.0	121.0	120.3	119.8
C^6–C^1–C^2	118.9	118.8	119.9	119.6
C^2–C^1–S^7	125.8	120.5	123.1	120.2
C^1–S^7–C^8	104.6	104.9	100.7	99.9
S^7–C^8–N^9	180.0	180.0	178.4	178.6
C^2–C^1–S^7–C^8	−24.9	−91.9	−25.9	−75.4
C^3–C^4–N^{10}–H^{11}	−23.1	−31.1	−0.1	−26.1
Dipole moment[c]	4.16	3.54	6.00	5.16,[d] 5.22[e]

[a] Bond lengths in angstroms, angles in degrees. [b] Ref. 15. [c] In Debyes. [d] Ref. 17. [e] Ref. 18.

Table 2 Calculated geometry of phenylthiocyanate (**1a**)

Geometric variable[a]	Method of calculation AM1	MNDO	3-21G	6-31G**
C^1–C^2	1.400	1.413	1.377	1.385
C^2–C^3	1.393	1.405	1.384	1.385
C^3–C^4	1.394	1.407	1.383	1.385
C^4–C^5	1.397	1.406	1.384	1.385
C^5–C^6	1.395	1.406	1.383	1.385
C^6–C^1	1.402	1.411	1.380	1.387
C^1–S^7	1.701	1.701	1.850	1.796
S^7–C^8	1.614	1.627	1.740	1.703
C^8–N^9	1.170	1.164	1.141	1.137
C^1–C^2–C^3	119.8	120.9	119.2	119.4
C^2–C^3–C^4	121.3	119.6	120.4	120.6
C^3–C^4–C^5	118.9	120.1	119.8	119.7
C^4–C^5–C^6	120.3	120.2	120.1	120.3
C^5–C^6–C^1	121.5	120.3	119.4	119.6
C^6–C^1–C^2	119.1	118.9	111.1	120.5
C^2–C^1–S^7	123.8	120.5	122.6	123.0
C^1–S^7–C^8	104.7	105.3	101.0	101.6
S^7–C^8–N^9	178.0	177.1	178.5	177.4
C^2–C^1–S^7–C^8	25.7	82.9	25.5	26.3
Dipole moment[b]	3.01	3.42	4.27	4.40

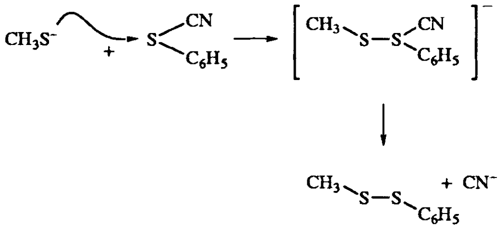
[a] Bond lengths in angstroms, angles in degrees. [b] In Debyes, experimental value 3.01 (ref. 19); 3.62 (ref. 20).

Table 3 Calculated atomic charges in phenylthiocyanate

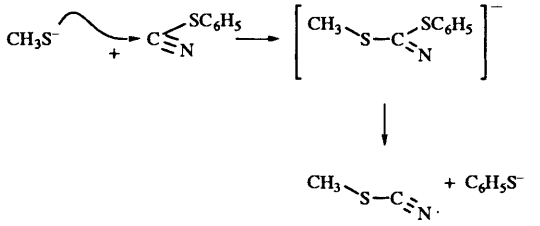
Atom label	AM1	MNDO	3-21G	6-31G**
C^1	−0.288	−0.258	−0.424	−0.225
C^2	−0.109	0.004	−0.203	−0.120
C^3	−0.122	−0.074	−0.226	−0.140
C^4	−0.116	−0.018	−0.238	−0.147
C^5	−0.122	−0.076	−0.229	−0.138
C^6	−0.101	0.009	−0.204	−0.136
S^7	0.530	0.366	0.755	0.347
C^8	−0.378	−0.247	−0.090	0.097
N^9	0.005	−0.052	−0.457	−0.389
H^2	0.145	0.069	0.281	0.188
H^3	0.137	0.070	0.259	0.167
H^4	0.139	0.066	0.255	0.163
H^5	0.138	0.069	0.256	0.164
H^6	0.142	0.071	0.266	0.171

Reaction coordinate calculations

The energetics of the nucleophilic displacement reaction were next explored on both possible reaction pathways using the AM1 method in preference to the MNDO method because it appeared to give a better structural correlation with the more accurate 6-31G** method for phenylthiocyanate. The reactions were simplified, by using methanethiolate as attacking nucleophile in place of glutathione or other intracellular thiols, as shown (Schemes 2 and 3).



Scheme 2 Attack of methanethiolate at the sulfur atom of phenylthiocyanate with displacement of cyanide



Scheme 3 Attack of methanethiolate at the cyano-carbon of phenylthiocyanate with displacement of phenylthiolate

In terms of the thermodynamics of the reactions, full structure optimisation of all the molecular species involved in the gas phase strongly suggest that reaction (2) is favoured by a substantial margin (Table 4). However, because the activation energies of the processes are likely to be significantly different, both reactions were explored in detail. A preliminary analysis for the gas phase reactions shows that as methanethiolate approaches the sulfur atom of phenylthiocyanate a reaction cluster or intermediate is predicted which is substantially lower in energy than the reactants themselves. As the CN group is displaced from the intermediate, the energy rises sharply as the S–CN bond is stretched beyond the bonding limit, and then it begins to level off at around 5 Å. There does not appear to be a definite transition state for this gas phase nucleophilic process and the reaction is strongly endothermic. A similar result has been reported for the reaction of pyridine with methyl chloride where the lack of a transition state has been attributed to the absence of solvent in the calculation.[21] A reaction cluster is also predicted for the alternative nucleophilic attack of methanethiolate at the cyano-carbon, though a clearly defined transition state is found in this case.

Table 4 Calculated heats of formation of the reactants and products formed from the attack of methanethiolate on phenylthiocyanate

		Heat of formation/kcal mol^{-1}	
		AM1 (gas phase)	AM1-SM1 (water)
Reactants	$CH_3S^- + C_6H_5SCN$	36.59	−47.00
Products	$CH_3SSC_6H_5 + CN^-$	75.00	−13.41
	$CH_3SCN + C_6H_5S^-$	27.38	−55.04

The calculated enthalpies of the nucleophilic displacement reaction in the gas phase clearly show that reaction (2) is favoured by a substantial margin over reaction (1). However, because the presence of water is likely to have a substantial effect on the course of the nucleophilic reaction, especially on the energies of the two transition states, the reactions were repeated using the AM1-SM1 method.[12] Here, free energy of solvation terms are added to the AM1 Fock operator to account for aqueous solvation effects. There are two terms considered, one accounts for the electric polarisation of the solvent using a distributed monopole representation of the solute charges, while the other is proportional to the solvent accessible surface area.[12]

In the first reaction explored (Scheme 2) with the AM1-SM1 method,[12] as methanethiolate approaches the sulfur atom of phenylthiocyanate the energy of the reactants rises, from a starting point of −47.0 kcal mol^{-1},† as charge is transferred from the anion to the substrate. This energy is considerably lower than that calculated for the starting point of the gas phase reaction at 36.6 kcal mol^{-1} (Table 4). At a distance of 3.0 Å, an early transition state is predicted which is 7.52 kcal mol^{-1} higher in energy than the reactants with a residual charge of −0.941 at the nucleophile; this transition state is clearly reactant-like in nature. As the distance narrows, the energy falls and at 2.6 Å the charge at the nucleophile falls to −0.812. With a further decrease in distance, a reactant cluster or intermediate is again predicted which is substantially lower in energy that the reactants themselves (as in the gas phase run) with predicted S–S and C–CN bond lengths of 2.20 and 1.64 Å, respectively, and with a total atomic charge of −0.666 now distributed over the atoms of the methanthiol group.

However, as the CN group is displaced from this intermediate, the energy rises sharply as the S–CN bond is gradually stretched so that at a distance of 2.0 Å, a charge of −0.618 has been transferred to the cyano-group. With further stretching, a second much higher energy transition state is formed which is 36.9 kcal mol^{-1} higher in energy than the reactants with a charge of −0.996 now at the cyano-group; this transition state is clearly product-like in nature. With further increases in distance, the energy slowly falls and more charge is transferred to the departing cyano-group so that at 5.0 Å the group is an anion with a total charge of −1.00. The illustration (Fig. 1) shows the heat of formation as a function of the change in the S–S distance (left hand side) and the S–CN distance (right hand side) from those found in the low energy cluster at 2.20 and 1.64 Å, respectively. The products are some 33.6 kcal mol^{-1} higher in energy than the reactants (Table 4).

In the alternative reaction at the cyano-carbon (Scheme 3), the energy of the reactants rises smoothly as methanethiolate approaches. In this case, at a S–CN distance of 4.0 Å, the

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† 1 cal = 4.184 J.

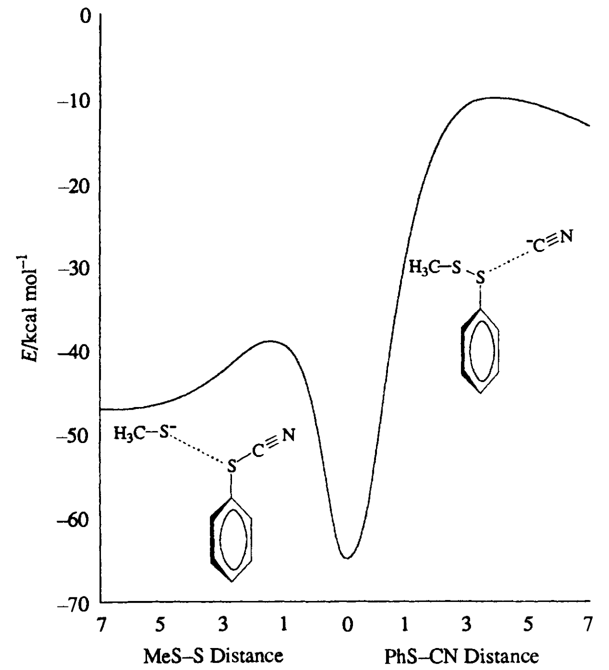

Fig. 1 Calculated energy pathway for the reaction of methanethiolate with phenylthiocyanate at the sulfur atom (see text for distance definition)

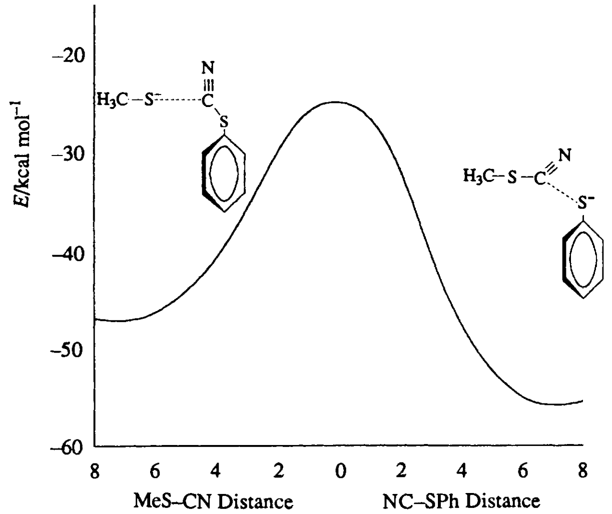

Fig. 2 Calculated energy pathway for the reaction of methanethiolate with phenylthiocyanate at the cyano-carbon (see text for distance definition)

nucleophile is anionic with a charge of -0.999, but at 3.0 Å, significant charge is transferred from the thiolate to the phenylthiocyanate so that the net charge on the nucleophile falls to -0.775. The energy continues to rise as the distance between the thiolate and the cyano-carbon narrows further, and at 2.20 Å, a transition state results which is some 21.1 kcal mol^{-1} higher in energy than the reactants, with a NC–SC_6H_5 bond length of 1.63 Å. The energy gradually falls as phenylthiolate is displaced, and at 2.05 Å the charge on the leaving group is -0.670, while at 4.00 Å the group is strongly anionic with a total charge of -0.999. The illustration in this case (Fig. 2) shows the heat of formation as a function of the change in the S–CN distances (left hand side) and the S–SC_6H_5 distance (right hand side) from those found in the transition state. The products here are some -8.04 kcal mol^{-1} lower in energy than the reactants (Table 4).

The calculated enthalpies of the nucleophilic displacement reaction in water therefore show that reaction (2) is favoured by a substantial margin over reaction (1), in line with the results found for glutathione with the related 2-nitro-5-thiocyanatobenzoic acid, suggesting that entropic factors may be reasonably constant. The AM1-SM1 method appears to give a reasonable account, therefore, of the expected nucleophilic reactions of methanethiolate with phenylthiocyanate, though there is no experimental evidence to support the formation of a reaction cluster in the attack at sulfur.

Conclusions

The calculated results obtained at the semi-empirical or *ab initio* levels clearly show that the transition state of the nucleophilic reactions of intracellular thiols with phenylthiocyanate cannot be adequately represented by a simple picture of the electronic properties of the substrate alone. However, reaction coordinate calculations at AM1-SM1 level in water, using methanethiolate as the attacking nucleophile, strongly suggest that nucleophilic attack will occur at the cyano-carbon in preference to the sulfur atom, in line with experimental data obtained from the reactions of glutathione with 2-nitro-5-thiocyanatobenzoic acid in aqueous solution.

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