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Evidence for the formation of isothiocyanate during sulfurisation of phosphines and phosphites using xanthane hydride

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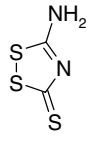
Abstract—Contrary to a previous report, the sulfurisation of triphenylphosphines and trialkyl phosphites by 3-amino-1,2,4-dithiazole-5-thione (xanthane hydride) does not yield carbon disulfide and cyanamide as the additional reaction products but unstable thiocarbamoyl isothiocyanate which has been trapped with nucleophiles.

There is an increasing use of phosphorothioate analogues of oligonucleotides in nucleic acid research.[1] The stability of the phosphorothioate linkage, its resistance to hydrolysis by nucleases and the improved pharmacokinetic profiles of these analogues have led to their incorporation into therapeutic oligonucleotides.[2] Therefore, the synthesis of oligonucleotide phosphorothioate analogues is of considerable interest. Their synthesis is normally achieved by the sulfurisation of the corresponding nucleotide–phosphite through the reaction of the P(III) analogue, supported on a solid support, with an organic sulfurising agent which is present in an organic solvent. For sulfurisation of phosphorus(III) compounds a number of reagents have been designed and tested in recent years. One of them is 3-amino-1,2,4-dithiazole-5-thione (**1**) (xanthane hydride), which appears to have an optimal combination of properties that suggest it will be an advantageous alternative to existing sulfurising reagents.[3]



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In reporting the use of 3-amino-1,2,4-dithiazole-5-thione (**1**) as a sulfurisation agent Tang et al. proposed[3] that carbon disulfide and cyanamide were additional reaction products and that the reaction likely proceeded via nucleophilic attack at the sulfur adjacent to the amino group, to generate an intermediate phosphonium ion. Although this suggestion has been widely accepted, no evidence was provided for the proposed reaction products or the mechanism. Both the proposed site of nucleophilic attack and the presumed products appeared to us to be questionable and so we thought warranted further investigation.

As models for nucleotide phosphites we have used both phosphines and simple phosphites. A reaction mixture of triphenylphosphine and, separately, trimethyl phosphite with **1** in acetonitrile were analysed by ^{1}H, ^{13}C and ^{31}P and by GC–MS. All attempts to detect carbon disulfide and cyanamide or carbodiimide failed. The ^{1}H and ^{13}C NMR spectra of the crude by-products in deuterium oxide showed major signals that were more typical of a H_2N–(C=S)–N grouping, that is, two broad singlets at 7.85 and 8.43 ppm in the ^{1}H NMR and 186.7 ppm in the ^{13}C NMR. This was not consistent with the proposed products and mechanism[3] and an alternative reaction pathway must occur.

A possible reaction pathway involves nucleophilic attack of the phosphorus at sulfur next to the thiocarbonyl group of **1** and subsequent decomposition of the

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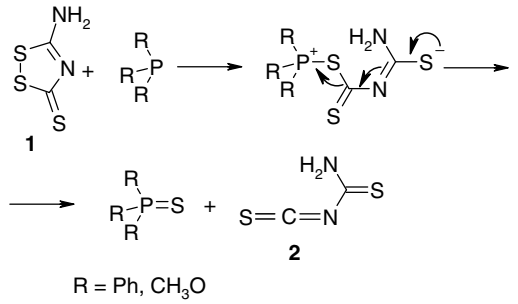


Scheme 1.

phosphonium intermediate formed into triphenylphosphine sulfide or trimethyl thiophosphate and thiocarbamoyl isothiocyanate (**2**) (Scheme 1).

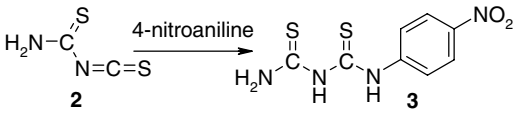
Thiocarbamoyl isothiocyanate (**2**) itself has not been described in the literature, presumably because of its high reactivity and instability. *N*,*N*-Dimethylthiocarbamoyl isothiocyanate has been characterised and is stable for a couple of days[4] at −15 °C; at an ambient temperature *N*,*N*-dimethylthiocarbamoyl isothiocyanate readily undergoes[4,5] dimerisation ([4+2] cycloaddition) to give 2-dimethylamino-5-dimethylthiocarbamoyl-1,3,5-thiadiazin-4,6-dithione.

To demonstrate[6] the presence of the reactive thiocarbamoyl isothiocyanate during the course of the sulfurisation reaction we added an external nucleophile, 4-nitroaniline, to the reaction mixture to act as a trap. After reaction work-up, 1-(4-nitrophenyl)dithiobiuret (**3**) was obtained in almost quantitative yield, which is the expected product of nucleophilic addition of 4-nitroaniline to thiocarbamoyl isothiocyanate (Scheme 2).

The kinetics of the sulfurisation reaction were also studied spectrophotometrically. The absorbance due to **1** decreased exponentially with time from which was obtained a pseudo first-order rate constant which varied linearly with the concentration of the substituted triphenylphosphines to give the corresponding second-order rate constant. These second-order rate constants were high. For example, for triphenylphosphine $k = 1.32 \times 10^4\ \mathrm{l\,mol^{-1}\,s^{-1}}$ and trimethyl phosphite $k = 3.03 \times 10^3\ \mathrm{l\,mol^{-1}\,s^{-1}}$.

A Hammett plot for the data, σ_p^- against the second-order rate constants for the seven substituted triphenylphosphines is linear, and the slope generates a reaction constant $\rho = -0.86$.

The interpretation of the Hammett ρ-value in terms of charge distribution in the transition state requires a reference reaction, ideally a corresponding ρ-value for an equilibrium reaction. There are a limited number of Hammett correlations in the literature which relate to



Scheme 2.

reactions of phosphorus(III) species, but they include kinetic values measured for the reaction of: aryldiethyl phosphines with ethyl iodide[7] $\rho = -1.0$; for the reaction of triarylphosphines with elemental sulfur[8] $\rho = -2.5$ and for the reaction of triarylphosphines and triarylphosphites with[9] diphenyl trisulfide $\rho = -1.1$. In determining possible transition state structures these values can be compared with equilibrium data measured for the proton transfer to phosphines, measured in nitromethane using Taft substituent constants[10] $\rho^* = -2.6$ which is very similar to the reaction constant determined for the protonation of amines[11] $\rho = -2.77$.

Given the product analysis, it is likely that the sulfurisation reaction proceeds by initial nucleophilic attack of phosphorus(III) on the disulfide linkage to generate a phosphonium ion intermediate as shown in Scheme 1. Either formation or breakdown of the intermediate could be the rate limiting step and both steps would have transition states with positively charged phosphorus compared with the reactant state. The relatively small reaction constant measured here of about −1.0 indicates the development of a partial positive charge on phosphorus in the transition state. If the first step is rate limiting, then the observed ρ-value suggests an early transition state with a little build up of charge on phosphorus. If the second step is rate limiting, then the reaction constant is the sum of two values for the two steps (Scheme 1), probably with opposite signs. The reaction constant for the formation of intermediate ρ_I is expected to be about −2.8. It is therefore unlikely that the second step is rate limiting and the observed ρ-value is indicative of rate limiting formation of the phosphonium ion with less than half of the unit positive charge developed on phosphorus in the transition state as a result of bond formation.

This conclusion is supported by the activation parameters for the sulfurisation reaction which were determined from kinetic data measured in acetonitrile at four temperatures: $\Delta H^{\neq} = 13.3\ \mathrm{kJ\,mol^{-1}}$ and $\Delta S^{\neq} = -121\ \mathrm{J\,mol^{-1}\,K^{-1}}$. The entropy of activation is very negative and is consistent with a bimolecular association step leading to the transition state. This is consistent with the first step being rate limiting but may also contain a contribution from the entropy change associated with increased solvation of charge in the transition state.

Acknowledgements

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6. 3-Amino-1,2,4-dithiazole-5-thione (**1**) (1 g, 6.7 mmol) was dissolved in 400 ml of acetonitrile under a nitrogen atmosphere at 30 °C and a solution containing 1.75 g (6.7 mmol) of triphenylphosphine in 100 ml of acetonitrile was added in one portion. The reaction mixture was stirred for 5 min and then 0.92 g (6.7 mmol) of 4-nitroaniline in 20 ml acetonitrile was added. After 15 h, the acetonitrile was removed and the solid residue was quickly extracted with 3×40 ml of 3% aqueous potassium hydroxide solution. After filtration, the insoluble material which was practically pure triphenylphosphine sulfide (1.85 g, 94%) was recovered. The filtrate was immediately neutralised by concentrated HCl (pH = 3). Precipitated 1-(4-nitrophenyl)dithiobiuret was filtered off and dried. Yield 1.65 g (97%). Mp 168–170 °C, ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.97 (AA′XX′, J 9.05, 2H, Ar–2H), 8.25 (AA′XX′, J 9.1, 2H, Ar–2H), 9.1 and 9.42 ($2 \times$ br s, 2H, NH_2), 11.01 (br s, 1H, NH), 13.22 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 123.3, 124.6, 143.7, 144.3, 177.3, 179.2. m/z (ESI) 255.0016 (M^-, $C_8H_7N_4O_2S_2$ requires 255.0005).
   The same procedure was carried with trimethyl phosphite instead of triphenylphosphine. Yield of 1-(4-nitrophenyl)-dithiobiuret was 1.35 g (80%).
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