Synthesis and kinetics of decomposition of some novel *S*-nitrosothiols

Andrew P. Munro and D. Lyn H. Williams

Abstract: The *S*-nitrosothiols 2-acetamido-2-deoxy-*S*-nitroso-1-thio-β-D-glucopyranose 3,4,6-triacetate (GPSNO) and *S*-nitroso-*N*-carbamyl-D,L-penicillamine (SNCP) were synthesized by *S*-nitrosation of the corresponding thiols, isolated, and fully characterized. The nitrosothiol (TGSNO) from 1-thioglycerol was obtained as a red gelatinous liquid, which decomposed rapidly at room temperature and so was not characterized. The kinetics of decomposition of GPSNO showed that there is a surprisingly large thermal pathway overlaid with a Cu^{2+}/RS^{-} catalyzed reaction. The results strongly suggest that the product disulfide complexes Cu^{2+} (for which there is some spectral evidence), leading to incomplete conversion by that route. Ascorbate also acts as a Cu^{2+} reductant. Another *S*-nitroso sugar, *S*-nitroso-1-thio-β-D-glucose (SNTG), behaved very similarly from solutions generated and used in situ. The decomposition of TGSNO shows induction periods suggesting that slow initial generation of Cu^{+} (the true catalyst) is taking place. There appears to be also a significant alternative pathway (analogous to that found for GPSNO), where the rate appears to be independent of $[Cu^{2+}]$, but very unusually this pathway is effectively halted by addition of EDTA either at the start of the reaction or at a later time. Reaction schemes are put forward to account for these unusual reaction characteristics.

Key words: *S*-nitrosothiols, nitric oxide, ascorbate, copper catalysis.

Résumé : On a synthétisé les *S*-nitrosothiols, 3,4,6-triacétane de 2-acétamido-2-désoxy-*S*-nitroso-1-thio-β-D-glucopyranose (GPSNO) et *S*-nitroso-*N*-carbamyl-D,L-pénicillamine (SNCP), en procédant à la *S*-nitrosation des thiols correspondants, on les a isolés et caractérisés complètement. Le nitrosothiol (TGSNO) obtenu à partir du 1-thioglycérol est un liquide gélatineux rouge qui se décompose rapidement à la température ambiante et il n'a pas été caractérisé. La cinétique de décomposition du GPSNO montre qu'il existe une voie réactionnelle thermique importante qui est recouverte par une réaction catalysée par Cu^{2+}/RS^{-}. Les résultats suggèrent fortement que le disulfure formé se complexe avec le Cu^{2+} (quelques données spectrales supportent ces hypothèses); cette interaction provoque une conversion incomplète par cette voie. L'ion ascorbate agit aussi comme réducteur du Cu^{2+}. Un autre *S*-nitrososucre généré en solution et utilisé in situ, le *S*-nitroso-1-thio-β-D-glucose (SNTG), se comporte d'une façon très semblable. La décomposition du TGSNO présente des périodes d'induction qui suggèrent une génération initiale lente du Cu^{+} (le vrai catalyseur). Il semble exister une voie de réaction alternative importante (analogue à celle observée pour le GPSNO) dans laquelle la vitesse semble indépendante de $[Cu^{2+}]$; il est toutefois surprenant de réaliser que cette voie est effectivement interrompue par l'addition d'EDTA soit au début de la réaction soit plus tard. Des schémas réactionnels sont proposés pour expliquer ces caractéristiques réactionnelles inusitées.

Mots clés : *S*-nitrosothiols, oxyde nitrique, ascorbate, catalyse par le cuivre.

[Traduit par la Rédaction]

Introduction

One aspect of the continuing intense and widespread interest in the "nitric oxide story" concerns the chemistry of *S*-nitrosothiols, RSNO. Not only are these compounds under active consideration as possible nitric oxide donors for medical therapeutic use, but also currently there is much interest in establishing the part played by them in vivo, possibly in the storage and transport of NO in the body. *S*-Nitrosoglutathione GSNO (1), *S*-nitrosoproteins (2), and the *S*-nitroso derivative of hemoglobin (3) have been identified as naturally occurring compounds, and there are literature hypotheses regarding their role in the storage and transport of NO around the body (2, 4). A recent suggestion that the interconversion between the Fe-NO and side chain S-NO forms in nitrosated hemoglobin, in some way controls blood pressure (3), has attracted much interest. The biological (5) and chemical (6) properties of *S*-nitrosothiols have recently been reviewed.

Formerly, it was believed that most RSNO compounds (with a few exceptions, notably GSNO and *S*-nitroso-*N*-acetyl penicillamine, SNAP) were too unstable in the pure state to allow their isolation and characterization, but in recent years an increasing number have been shown to be stable at room temperatures, although others decompose during extraction. In general, the corresponding *O*-nitroso compounds, the alkyl nitrites, are much more widely stable and of course

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This paper is dedicated to Jerry Kresge in recognition of his many achievements in chemistry.

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well known. Fairly dilute (1×10^{-3} to 1×10^{-4} M) solutions of all RSNO are easily generated from thiols and nitrous acid in an equilibrium process, which lies very much on the side of RSNO, and are usually sufficiently stable (even for those examples which cannot be obtained in the pure state) to allow experiments to be successfully carried out in situ.

For most RSNO compounds that have been studied, the thermal decomposition reaction is rather slow at room temperature, with half lives of many tens of hours. However, we have shown (7, 8) that in solution, in phosphate buffer at pH 7.4, RSNO species decompose giving NO and RSSR, in a copper-catalyzed process. The effective reagent is Cu^+, generated in situ by thiolate reduction of Cu^{2+} (eqs. [1]–[3]), which in many cases occurs when both RS^- and Cu^{2+} are present only at the trace impurity level. In contrast to the corresponding photochemical reaction, there is no EPR evidence for intermediate thiyl radical formation. There is a large reactivity range that results from the presence or otherwise of suitable substituents (e.g., $-NH_2$) which will allow bidentate binding by Cu^+ to occur. If the reactions are carried out aerobically then the ultimate fate of NO is NO_2^- after initial oxidation of NO, although at very low NO concentrations, such as those encountered in vivo, oxidation of NO by oxygen is very slow and probably will not compete with other NO reactions.

[1] $2Cu^{2+} + 2RS^- = 2Cu^+ + RSSR$

[2] $RSNO + Cu^+ = RS^- + Cu^{2+} + NO$

[3] $4NO + 4OH^- + O_2 = 4NO_2^- + 2H_2O$

[4] $RSNO + R'SH = R'SNO + RSH$

Added thiols can produce a variety of effects. If the thiol is structurally different from that from which RSNO is derived, then transnitrosation (eq. [4]) can occur, leading to the formation of a new R′SNO (9). If the added thiol is the same as that from which RSNO is derived, then at low concentration of added thiol, catalysis can occur (increasing the rate of Cu^+ formation), whereas at higher concentrations, thiol complexation of Cu^{2+} can occur, often resulting in the appearance of substantial induction periods, during which Cu^+ is being generated from very low concentrations of free Cu^{2+} (8, 10). The relative importance of each reaction (reduction and complexation) is governed by the thiol concentration and structure. At very much higher added thiol concentrations, a quite different reaction or series of reactions occurs, leading principally to ammonia formation, together with some nitrous oxide (11, 12).

Recently, we have shown that ascorbate ion can bring about two reactions with *S*-nitrosothiols (13): (*i*) at low ascorbate concentration, the copper catalyzed reaction is dominant in which ascorbate takes over the role of the thiolate and acts as a reducing agent for Cu^{2+} (this reaction can be completely halted by EDTA addition), and (*ii*) at much higher ascorbate concentration, a different reaction occurs, unaffected by the presence of Cu^{2+} or EDTA, and which leads to NO and *thiol* (and not diulfide) formation; under these conditions, all the evidence suggests that ascorbate acts as a nucleophile and undergoes electrophilic nitrosation in the same way as do nitrous acid and alkyl nitrites — the former being a very well known reaction.

In this paper we describe the synthesis and characterization of a *S*-nitrosothiol derived from a thio sugar and examine its decomposition together with that of the *S*-nitroso derivative of 1-thioglycerol (prepared in solution) to nitric oxide, both of which turn out to show some unusual features.

Experimental section

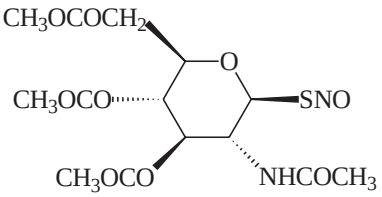
Materials

An aqueous solution of sodium nitrite (0.11 g in 1.65 cm^3) was added dropwise over 2 min with vigorous stirring to a solution of a commercial sample of 2-acetamido-2-deoxy-1-thio-β-D-glucopyranose 3,4,6-triacetate (0.30 g in 1:1 methanol: 1 M HCl, 3.3 cm^3, and containing concentrated sulfuric acid 0.17 cm^3). The mixture became pale red almost immediately, was stirred for 1 h, and the precipitate collected by filtration. The pink solid was washed with ice-cold water and air-dried for 24 h. (65% yield). The solid was stable at room temperature but decomposed on heating, so no melting point could be recorded. Elemental analysis calcd. for $C_{14}H_{20}N_2O_9S$: C 42.9, H 5.15, N 7.1%; found: C 43.0, H 5.03, N, 6.6%. The UV-visible spectrum showed λ_{max} (H_2O) 343 and 557 nm, ε 450 and 17 $dm^3\ mol^{-1}\ cm^{-1}$ respectively. The infrared spectrum included bands at 1572 cm^{-1} (NO stretch) and 654 cm^{-1} (CS stretch). The complex ^1H NMR spectrum was consistent with the expected structure **1**.

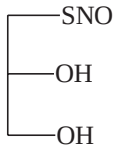
Attempts to prepare the *S*-nitroso derivative of 1-thioglycerol (3-mercapto-1,2-propanediol) TGSNO (structure **2**) resulted in the formation of a red gelatinous liquid, which decomposed rapidly at room temperature, but which could be kept for a short while below 4° in the dark. It was, however, too unstable to purify and characterize. However, a solution prepared from equimolar quantities of 1-thioglycerol and sodium nitrite gave a UV-visible spectrum with λ_{max} 333 and 544 nm, ε 881 and 21 $dm^3\ mol^{-1}\ cm^{-1}$, respectively. Similarly, the *S*-nitroso derivative of 1-thio-β-D-glucose SNTG (structure **4**) was too unstable to isolate, but we obtained the characteristic UV-visible spectrum with λ_{max} 342 and 557 nm with ε 436 and 14 $dm^3\ mol^{-1}\ cm^{-1}$, respectively. These spectral characteristics are very similar to those found for GPSNO.

The same synthetic procedure gave a 54% yield of the *S*-nitroso derivative SNCP (structure **3**) of *N*-carbamyl penicillamine (provided as a gift by the former Wellcome company). This was a stable green solid with red reflections, very similar in appearence to the closely related and very well known *S*-nitroso-*N*-acetyl penicillamine (SNAP). Elemental analysis calcd. for $C_6H_{11}N_3O_4S$: C 32.6, H 4.98, N 19.0%; found: C 32.3, H 4.86, N 18.4%. The UV-visible spectrum showed λ_{max} (H_2O) 340 and 590 nm, ε 853 and 20 $dm^3\ mol^{-1}\ cm^{-1}$. The infrared spectrum included bands at 1494 cm^{-1} (NO stretch) and 661 cm^{-1} (CS stretch). The ^1H NMR spectrum was in accord with the expected structure and included the characteristic (14) downfield shift of the proton signals in the vicinity of the sulfur atom upon nitrosation.

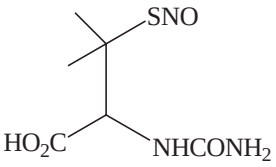
All other materials were commercial samples of the highest available purity grade.



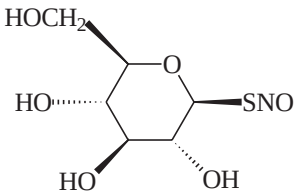
1 (GPSNO)



2 (TGSNO)

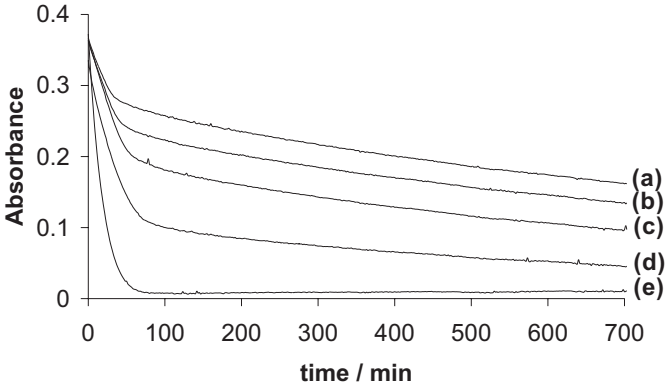


3 (SNCP)



4 (SNTG)

Fig. 1. Absorbance–time plots for the decomposition of GPSNO (1×10^{-3} M) in the presence of Cu^{2+}: (*a*) no added Cu^{2+}; (*b*) [Cu^{2+}] 0.1×10^{-5} M; (*c*) [Cu^{2+}] 0.5×10^{-5} M; (*d*) [Cu^{2+}] 1.0×10^{-5} M; (*e*) 5.0×10^{-5} M.



Spectral measurements

These were all carried out in aqueous phosphate buffer, pH 7.4, at 25° in a conventional spectrophotometer operating in the time-drive mode at around 340 nm or in the spectral scan mode. Typical concentrations of GPSNO were ~1×10^{-3} mol dm^{-3}. Solutions of TGSNO and SNTG were prepared at the same concentration by reaction of equimolar amounts of 1-thioglycerol or 1-thio-β-D-glucose and sodium nitrite in mildly acidic solution, immediately prior to investigations of the decomposition characteristics at pH 7.4.

Nitric oxide and nitrite ion analysis

Nitric oxide was determined in the reaction solutions, under nitrogen, using a commercial World-Precision ISO-NO specific electrode, calibrated with standard ascorbic acid-sodium nitrite solutions. Nitrite ion was determined by a modification of the Griess method (8).

Results

2-Acetamido-2-deoxy-*S*-nitroso-1-thio-β-D-glucopyranose 3,4,6-triacetate (GPSNO), 1

We have prepared GPSNO by direct *S*-nitrosation of the corresponding thiol, using a modification of the method used by Field et al. (15) for the first synthesis of SNAP. It was characterized by satisfactory elemental analyses and by the observation of the NO and CS stretching frequencies in the infrared spectrum and also by observation of the characteristic UV-visible absorbances at 343 and 557 nm. The pink solid is quite stable at room temperature. Analysis of an aqueous solution of GPSNO by Ellman's method (16) showed that the thiol level resulting from the equilibration of GPSNO in solution was 1.9% when the total GPSNO concentration was ~5×10^{-3} mol dm^{-3}.

In solution at pH 7.4, the decomposition of GPSNO showed the rather unusual absorbance–time plots shown in Fig. 1 for a series of solutions containing different concentrations of added Cu^{2+}. To separate any metal ion catalyzed process from any other reaction, we carried out two experiments with and without the addition of the general metal ion chelator EDTA, and these traces are given in Fig. 2. The faster reacting component of the reaction (which is Cu^{2+} catalyzed) is completely attenuated, leaving the other component unchanged. Addition of the specific Cu^{+} chelator neocuproine generated the same pattern of behaviour (not shown).

The effect of ascorbate in low concentration was also investigated, and the results are presented graphically in Fig. 3. Clearly ascorbate catalyzes the reaction, and also results in the quantitative decomposition of GPSNO observed now as a single process.

Repeat scans of the decomposition (shown in Fig. 4) at high concentration of added Cu^{2+} (5×10^{-5} mol dm^{-3}) showed the clear development of a small absorbance at around 280 nm, which can be interpreted as the Cu^{2+} complex of the

Fig. 2. Absorbance–time plots for the decomposition of GPSNO (1×10^{-3} M) in the presence of Cu^{2+} (1×10^{-5} M) (*a*) without EDTA, (*b*) with EDTA (1×10^{-3} M).

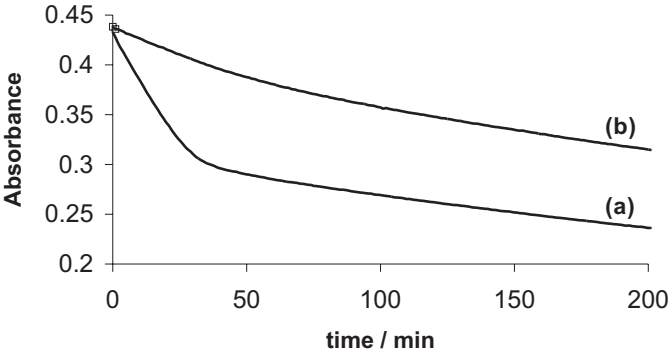
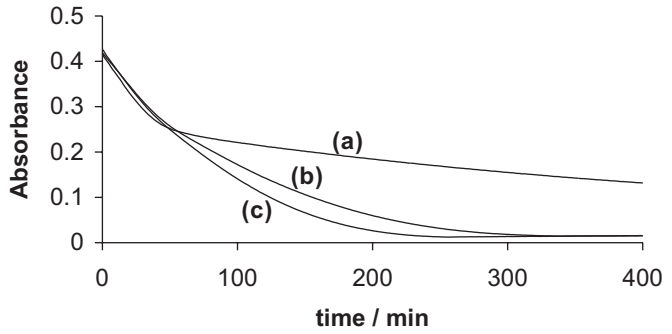


Fig. 3. Absorbance–time plots for the decomposition of GPSNO (1×10^{-3} M) in the presence of Cu^{2+} (1×10^{-5} M) with (*a*) no ascorbate, (*b*) ascorbate (5.0×10^{-5} M), (*c*) ascorbate (1.0×10^{-4} M).



disulfide derived from GPSNO. When the reaction was carried out anaerobically, NO was detected using the NO–electrode system in the solution at ~30%. The same experiment carried out aerobically gave a value of 51% of the theoretical maximum of nitrite anion.

There was a very rapid reaction between GPSNO and sulfite ion at pH 7.4 in the presence of added EDTA, yielding a second-order rate constant of 8630 M^{-1} s^{-1}. No nitric oxide or nitrite ion were detected in these reaction products.

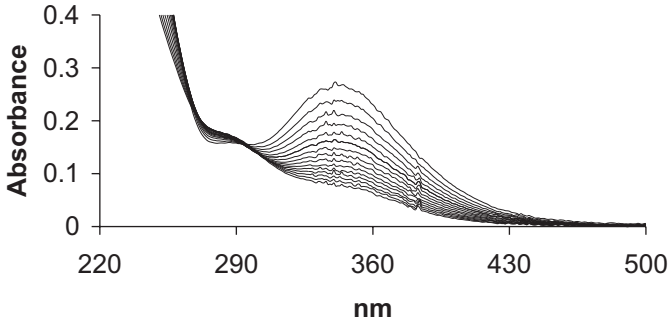
Attempts to isolate the *S*-nitroso derivative of 1-thio-β-D-glucose were unsuccessful, even though it was clear from the development of a red colour that *S*-nitrosation had occurred. The decomposition of solutions generated from the thiol and nitrous acid were examined spectrophotometrically at pH 7.4 and showed very similar behaviour to that shown in Fig. 1 for GPSNO. In the same way, the addition of EDTA completely suppressed the Cu^{2+}-catalyzed reaction leaving a significant thermal reaction.

S-Nitroso 1-thioglycerol (TGSNO), 2

All attempts to effect the nitrosation of 1-thioglycerol (3-mercapto-1,2-propanediol) using the known procedures generated an unstable red gelatinous liquid. It is almost certain that this is the *S*-nitroso derivative of 1-thioglycerol and solutions prepared from equimolar amounts of 1-thioglycerol and nitrous acid gave the expected UV-visible spectrum so characteristic of *S*-nitrosothiols.

The decomposition behaviour is shown in Fig. 5 and reveals a similar pattern of behaviour to that found earlier for

Fig. 4. Repeat scans (4 min intervals) for the decomposition of GPSNO (1×10^{-3} M) with added Cu^{2+} (5×10^{-5} M). Absorbance increases at 280 nm and decreases at 350 nm.



GPSNO in that it appears that there are two reactions: one catalyzed by added [Cu^{2+}] and the other not. In contrast, however, to the results for GPSNO, the spectral traces of the Cu^{2+}-dependent reaction show evidence of induction periods which are shorter as the [Cu^{2+}] and the concentration of the added 1-thioglycerol (not shown) is increased. In marked contrast to the behaviour of GPSNO, however, the effect of added metal ion chelator EDTA to solutions of TGSNO (Fig. 6) progressively halted *both* components of the reaction (the Cu^{2+}-dependent and Cu^{2+}-independent pathways), leaving a virtually stable solution of TGSNO over at least 12 h. Addition of neocuproine produced the same effect. When EDTA was added at various time points after the start of the reaction, decomposition was immediately halted at those time points. The NO-probe gave a value of ~45% for NO production and the nitrite analysis, and when the reaction was carried out aerobically, gave a figure of 97% of the theoretical maximum.

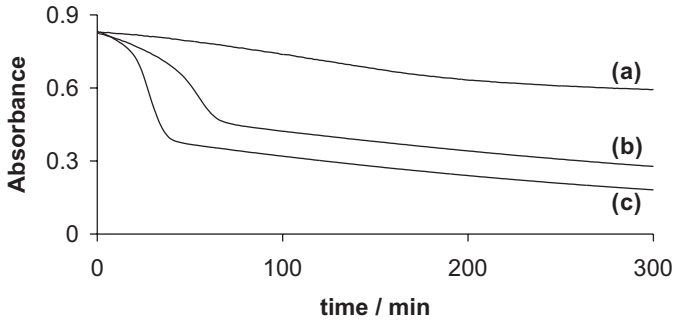
Ascorbic acid, added in low concentration (1×10^{-5} to 1×10^{-4} M) also catalyzed the decomposition of TGSNO and resulted in quantitative reaction, just as in the case of GPSNO. At much higher ascorbate concentration (0.04–0.24 M) and in the presence of EDTA, the reaction was fully first order in TGSNO concentration, and there was an excellent linear relationship between the observed first-order rate constant and ascorbate concentration, yielding a value of 1.59×10^{-3} M^{-1} s^{-1} for the second-order rate constant. Under these conditions NO (or NO_2^- if aerobic) is formed, together with the thiol product.

Repeat scans (not shown) of the reaction carried out in the presence of added Cu^{2+} (5×10^{-5} M) clearly showed the disappearence of the absorbance at around 340 nm, and there was some evidence of the development of a small absorbance in the 260 nm region, which disappeared during the course of the reaction.

S-Nitroso-*N*-carbamyl penicillamine (SNCP), 3

The *S*-nitroso derivative of *N*-carbamyl penicillamine (SNCP) was synthesized, fully characterized, and shown to be a stable solid at room temperature. In aqueous solution, 1.5% free thiol was determined by Ellman's method, reflecting again the equilibration in solution. The thiol was a gift from the former Wellcome company, and the quantity of SNCP synthesized did not allow enough material for the study of its decomposition characteristics. However, some rate measurements were carried out on the decomposition of

Fig. 5. Absorbance–time plots for the decomposition of TGSNO (1×10^{-3} M) with (*a*) no added Cu^{2+}, (*b*) Cu^{2+} (0.5×10^{-5} M), (*c*) Cu^2 (1×10^{-5} M).



SNCP generated and used in situ from equimolar quantities of the thiol and nitrous acid. Good first-order plots were obtained for experiments with added Cu^{2+} in the range 0.3–1.0 $\times 10^{-5}$ M, and the measured first-order rate constants were linearly dependent on [Cu^{2+}].

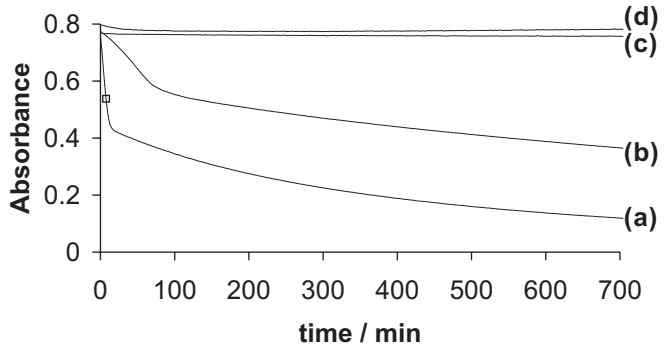
Discussion

GPSNO (1)

The successful synthesis of GPSNO and and of SNCP together with the demonstration of their apparent indefinite stability in the solid state has added further examples to the small list of stable *S*-nitrosothiols. It is likely that it, along with all RSNO species tested, will show the general characteristics of vasodilation and inhibition of platelet aggregation in vivo. Testing is underway. It is possible that this sugar derivative may have significant advantages (e.g., of solubility in water) over SNAP and GSNO for in vivo administration in order to effect these properties. A related sugar derivative, which does not have the stability of GPSNO in the pure state, has been shown (17) to have vasodilatory properties, including when the application is made transdermally to human cutaneous vascular smooth muscle.

The characteristics of in vitro decomposition and NO release of GPSNO are somewhat unusual compared with those of other *S*-nitrosothiols, particularly those derived from cysteine and related compunds. Figure 1 shows the pattern. It is clear that there are two reactions here, an initial faster reaction which is Cu^{2+}-catalyzed (and which does not go to completion unless the [Cu^{2+}] is relatively high –5×10^{-5} M in this case), and a slower reaction which is unaffected by the [Cu^{2+}], shows no signs of stopping at incomplete conversion, and which appears to be a thermal reaction. This interpretation is supported by the evidence in Fig. 2 which shows the disappearance of the copper-promoted reaction when EDTA is present (7), leaving the thermal reaction unaffected. The same result was obtained (not shown) when the specific Cu^{+} chelator neocuproine was added, at the same concentration, instead of EDTA, confirming our earlier suggestions that in these copper ion catalyzed reactions, the true catalyst is Cu^{+} (8), generated by thiolate ion reduction of Cu^{2+}. Thiolate ion is likely to be present in all RSNO solutions because of the reversibility of the *S*-nitrosation process (18). In this case we have measured a thiol level of ~2% in a solution of GPSNO (~5×10^{-3} M), which is a fairly typical figure, and which is high enough to effect Cu^{2+} reduction.

Fig. 6. Absorbance–time plots for the decomposition of TGSNO (1×10^{-3} M) with added Cu^{2+} (1×10^{-5} M) (*a*) without EDTA, (*b*) with EDTA (1×10^{-5} M), (*c*) with EDTA (2×10^{-5} M), (*d*) with EDTA (5×10^{-5} M).



We estimate the first-order rate constant (assuming the decomposition to be a first-order process) for the decomposition of GPSNO by the thermal route to be ~3×10^{-5} s^{-1}, i.e., with a half life of about 6–7 h. This compares with a value for the half life for the thermal decomposition of *S*-nitroso cysteine (one of the more unstable nitrosothiols in the pure state) of ~55 h at the same temperature (8). Other related structures e.g., SNAP, behave similarly.

The copper-catalyzed part of the reaction shows an interesting feature in that reaction without added Cu^{2+} appears to cease after only less than 30% conversion. As the added Cu^{2+} increased so does the % conversion, until at 5×10^{-5} M added Cu^{2+} reaction is essentially quantitative and is so rapid as to effectively swamp the thermal reaction. We have not observed this pattern of behaviour with other *S*-nitrosothiols. Incomplete conversion, however, has been noted earlier in the glutathione (GSH)/Cu^{2+} promoted decomposition of GSNO, in the presence of added oxidized glutathione, GSSG (19), which was interpreted in terms of complexation of Cu^{2+} by the added GSSG. For the GSNO reaction, there was spectroscopic evidence of such a complex with a shoulder at 250 nm and at higher concentration, a peak of low extinction at 620 nm, characteristic of a Cu^{2+} complex, for which a 1:1 structure has been proposed (20). Here with GPSNO decomposition we find (Fig. 4) the development of a small absorbance at around 280 nm which could reasonably be ascribed to the formation of the Cu^{2+} complex of the disulfide.

The derivative from 1-thio-β-D-glucose (generated in situ) behaved in a very similar manner, suggesting that the basic sugar unit provides sites which are available in the product disulfides for coordination of Cu^{2+}, and which allows a significantly larger rate of thermal decomposition of *S*-nitroso sugars generally when compared with simpler cysteine derivatives.

We detected nitric oxide in the decomposition reaction when it was carried out anaerobically. With relatively long reaction times it is extremely difficult to determine NO quantitatively, because of its reactivity, particularly towards traces of oxygen. Under these circumstances a measured yield of ~30% NO is not unusual. A little more difficult to explain is the relatively low yield of nitrite anion (51%)

determined when the reaction was carried out aerobically. We have no obvious explanation at this stage.

When ascorbic acid was added at 5×10^{-5} and 1×10^{-4} M, reaction proceeds to completion, implying that Cu^{2+} bound to the disulfide product is accessible for reduction by ascorbate but not by thiolate.

As part of another study which is looking at reactions involving nucleophilic attack at the nitroso nitrogen atom,[2] we discovered that sulfite ion is generally very reactive towards *S*-nitrosothiols, bringing about complete decomposition in seconds or fractions of seconds in most cases. The reaction forms the thiol and not the disulfide product, and does not generate nitric oxide (or nitrite ion in the presence of air), and by analogy with the corresponding reaction of nitrous acid (21), probably forms hydroxylamine disulfonate.

GPSNO shows the same characteristics in reaction with sulfite ion with a second-order rate consant of 8630 M^{-1} s^{-1}, which means that for reaction of GPSNO (1×10^{-3} M) and sulfite (2×10^{-2} M), the half life of reaction is only 4×10^{-3} s. Since reaction is so rapid and thiol products are readily analysed by the Ellman procedure, this reaction could be the basis for a quantitative analytical procedure for the determination of *S*-nitrosothiols.

TGSNO (2)

Unfortunately, it appears that TGSNO is not sufficiently stable at room temperature to enable a complete characterization and to allow standard solutions to be prepared from it. The red liquid, which could be kept at 0° for some weeks, is almost certainly the *S*-nitroso compound, and the UV-visible spectrum supports this. TGSNO was chosen for this study because preliminary in vivo testing experiments with solutions generated and used in situ had shown some unusual results which could result in some beneficial therapeutic properties.[3]

The decomposition features shown in Fig. 5 are in some ways similar to those found for GPSNO, in that there appear to be two reactions, one copper ion catalyzed and the other not. The copper-catalyzed reaction, however, does show induction periods, not evident in the reactions of GPSNO. Induction periods of this kind have been observed on a number of previous occasions (8, 10), and have been interpreted as the relatively slow initial formation of Cu^{+} (from Cu^{2+} and RS^{-}) with the regeneration of both reactants (eq. [2]). As expected, the induction period is reduced as the $[Cu^{2+}]$ is increased. It is also effectively removed by addition of thioglycerol (RS^{-}), not shown. Nitrite ion was formed quantitatively when reactions were carried out aerobically, and a substantial amount of NO (45%) was measured when oxygen was excluded.

One major difference between the reactions of TGSNO and GPSNO is shown in Fig. 6, where it can be seen that the addition of EDTA effectively stops *both* reactions. Reaction can also be halted when the EDTA is added during the course of the reaction, rather than at the beginning. The non-Cu^{2+}-catalyzed reaction cannot therefore, for TGSNO, be a thermal reaction, if it is halted by the addition of EDTA. It is not immediately obvious what is going on here. There are clearly two reactions taking place, one catalyzed by "free" Cu^{2+} and the other not, yet both are stopped by EDTA addition. A possible explanation is that the slower reaction *is* a copper-promoted reaction, but the effective catalyst, Cu^{+}, is obtained by thiolate reduction of the RSSR–Cu^{2+} complex, which would be expected to be a slower process than the thiolate reduction of free Cu^{2+}, and which would lead to complete, rather than partial decomposition of TGSNO. Further work is needed to clarify this point.

Some evidence of RSSR–Cu^{2+} complex formation is evident from the repeat scan experiments (not shown), but the complex does not appear to be as stable as that generated from GPSNO. Ascorbate also acts as a reducing agent at low concentration, resulting in complete decomposition of TGSNO, implying that, as for GPSNO, Cu^{2+} bound to the disulfide product is accessible for reduction by ascorbate.

At higher concentrations of addded ascorbate, the role of ascorbate changes from that of a reducing agent to that of a nucleophile, which undergoes electrophilic nitrosation by TGSNO, resulting in the formation of NO (becoming NO_2^{-} if aerobic) and the thiol 1-thioglycerol (cf. the reaction of nitrous acid with ascorbic acid (22)). Its reactivity is comparable to that of SNAP and *S*-nitroso-*N*-acetylcysteine.[4] A similar reaction has been reported (13) for the reaction of TGSNO with 1-thioglycerol at high [1-thioglycerol], where RS^{-} now acts as a nucleophile, leading to ammonia formation.

SNCP (3)

Because of a shortage of material, we were unable to carry out a full kinetic analysis on the decomposition of SNCP. However, we found good first-order behaviour over a range of $[Cu^{2+}]$ and obtained a value of 780 dm^3 mol^{-1} s^{-1} for the second-order rate constant k_2 (defined by rate = $k_2[RSNO][Cu^{2+}]$), which compares with a value of 20 dm^3 mol^{-1} s^{-1} for the closely structurally related SNAP. It is tempting to account for the reactivity difference in terms of the relative abilities of the *N*-$COCH_3$ and *N*-$CONH_2$ groups to bind Cu^{+} in an intermediate leading to NO formation, but this does not take account of the relative thiolate concentrations in both cases, which can dramatically affect reactivity in the ability to generate Cu^{+} from Cu^{2+} (10).

Conclusions

We have prepared and characterized two new stable *S*-nitrosothiols, GPSNO and SNCP, and have examined the decomposition in aqueous buffer (pH 7.4) of GPSNO, TGSNO, and SNTG (the latter two generated and used in situ). All reactants decomposed by two pathways, (*i*) GPSNO by a significant thermal pathway in parallel with a Cu^{2+}-catalyzed reaction, which tends to stop at incomplete conversion, as the Cu^{2+} catalyst is tied up by complexation with the disulfide product, and (*ii*) TGSNO by a Cu^{2+} pathway, which also stops at incomplete conversion probably for the same reason, together with a component which may arise by copper

[2] A.P. Munro and D.L.H. Williams. To be published.
[3] A.R. Butler. Personnal communication.
[4] A.J. Holmes and D.L.H. Williams. To be published.

catalysis where the true catalyst Cu^{+} is generated from the product disulfide–Cu^{2+} complex. Both compounds showed the "normal" reactivity with relatively high concentrations of reactive nucleophiles such as ascorbate and sulfite.

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References

1. B. Gaston, J. Reilly, J.M. Darzen, J. Fackler, P. Ramdev, D. Arnelle, M. Mullins, D.J. Sugarbaker, C. Chee, D.J. Singel, J. Loscalzo, and J.S. Stamler. Proc. Natl. Acad. Sci. U.S.A. **90**, 10 957 (1993).
2. J.S. Stamler, O. Jaraki, J. Osborne, D.I. Simon, J. Keaney, J. Vita, D. Singel, C.R. Valeri, and J. Loscalzo. Proc. Natl. Acad. Sci. U.S.A. **89**, 7674 (1992).
3. L. Jia, C. Bonaventura, J. Bonaventura, and J.S. Stamler. Nature (London), **380**, 221 (1996).
4. R.M. Clancy, D. Levartovsky, J. Leszczynska-Piziak, J. Yegudin, and S.B. Abramson. Proc. Natl. Acad. Sci. U.S.A. **91**, 3680 (1994).
5. A.R. Butler and P. Rhodes. Anal. Biochem. **249**, 1 (1997).
6. S. Oae, and K. Shinhama. Org. Prep. Proc. Int. **15**, 165 (1983).
7. J. McAninly, D.L.H. Williams, S.C. Askew, A.R. Butler, and C. Russell. J. Chem. Soc. Chem. Commun. 1758 (1993).
8. S.C. Askew, D.J. Barnett, J. McAninly, and D.L.H. Williams. J. Chem. Soc. Perkin Trans. 2, 741 (1995).
9. D.J. Barnett, A. Rios, and D.L.H. Williams. J. Chem. Soc. Perkin Trans. 2, 1279 (1995).
10. A.P. Dicks, P.H. Beloso, and D.L.H. Williams. J. Chem. Soc. Perkin Trans. 2, 1429 (1997).
11. S.P. Singh, J.S. Wishnock, M. Keshive, W.M. Deen, and S.R. Tannenbaum. Proc. Natl. Acad. Sci. U.S.A. **93**, 14 428 (1996).
12. A.P. Dicks, E. Li, A.P. Munro, H.R. Swift, and D.L.H. Williams. Can. J. Chem. **76**, 789 (1998).
13. A.J. Holmes and D.L.H. Williams. Chem. Commun. 1711 (1998).
14. B. Roy, A. du Moulinet d'Hardemare, and M. Fontcave. J. Org. Chem. **59**, 7019 (1994).
15. L. Field, R. Dilts, R. Ravichandran, P.G. Lenhert, and G.E. Carnahan. J. Chem. Soc. Chem. Commun. 249 (1978).
16. P.W. Riddles, R.L. Blakeley, and B. Zerner. Anal. Biochem. **94**, 75 (1979).
17. A.R. Butler, R.A. Field, I.R. Greig, F.W. Flitney, S.K. Bisland, F. Khan, and J.J.F. Belch. Nitric Oxide: Biol. Chem. **1**, 211 (1997).
18. P.H. Beloso and D.L.H. Williams. J. Chem. Soc. Chem. Commun. 89 (1997).
19. H.R. Swift. Ph.D. thesis. University of Durham, U.K. 1996. pp. 59–61.
20. K. Varnagy, I. Sovago, and H. Kozlowski. Inorg. Chim. Acta, **151**, 117 (1988).
21. S.B. Oblath, S.S. Markowitz, T. Novakov, and S.G. Chang. J. Phys. Chem. **86**, 4853 (1982).
22. C.A. Bunton, H. Dahn, and L. Loewe. Helv. Chim. Acta, **43**, 303 (1960); **43**, 317 (1960); **43**, 320 (1960); and earlier papers.