Organosulfur oxoacids. Part 2. A novel dimethylthiourea metabolite — Synthesis and characterization of the surprisingly stable and inert dimethylaminoiminomethane sulfonic acid

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Abstract: A new metabolite of the biologically active thiocarbamide dimethylthiourea (DMTU) has been synthesized and characterized. DMTU's metabolic activation in the physiological environment is expected to be dominated by S-oxygenation, which produces, successively, the sulfenic, sulfinic, and sulfonic acids before forming sulfate and dimethylurea. Only the sulfinic and sulfonic acids are stable enough to be isolated. This manuscript reports on the first synthesis, isolation, and characterization of the sulfonic acid: dimethylaminoiminomethanesulfonic acid (DMAIMSOA). It crystallizes in the orthorhombic *Pbca* space group and exists as a zwitterion in its solid crystal form. The negative charge is delocalized over the sulfonic acid oxygens and the positive charge is concentrated over the planar N–C–N framework rather than strictly on the sp^2-hybridized cationic carbon center. As opposed to its sulfinic acid analogue, DMAIMSOA is extremely inert in acidic environments and can maintain its titer for weeks at pH 6 and below. It is, however, reasonably reactive at physiological pH conditions and can be oxidized to dimethylurea and sulfate by mild oxidants such as aqueous iodine.

Key words: thiourea, metabolites, bioactivation.

Résumé : On a effectué la synthèse d'un nouveau métabolite du thiocarbamide de la diméthylthiourée (DMTU), un produit biologiquement actif. On s'attend à ce que l'activation métabolique du DMTU dans un environnement physiologique devrait être dominée par une S-oxygénation qui conduit à la formation successive d'acides sulfénique, sulfinique et sulfonique avant de donner un sulfate et de la diméthylurée. Seuls les acides sulfinique et sulfonique sont suffisamment stables pour être isolés. Dans ce travail, on rapporte la première synthèse, l'isolation et la caractérisation de l'acide sulfonique, l'acide diméthylaminoiminométhanesulfonique (ADMAIMSO). Il cristallise dans le système orthorhombique, groupe d'espace *Pbca* et dans sa forme cristalline solide il existe sous la forme de zwitterion. La charge négative est délocalisée sur les oxygènes de l'acide sulfonique et la charge positive est concentrée sur le squelette planaire N–C–N plutôt que strictement sur le centre carboné cationique d'hybridation sp^2. Par opposition à son analogue l'acide sulfinique, le ADMAIMSO est extrêmement inerte dans des environnements acides et il peut maintenir son titre pour plusieurs semaines à des pH de 6 ou moins. Il est toutefois assez réactif dans des conditions de pH physiologique et il peut être oxydé en diméthylurée et en sulfate par des oxydants doux, tel une solution aqueuse d'iode.

Mots-clés : thiourée, métabolites, bioactivation.

[Traduit par la Rédaction]

Introduction

N,*N*′-Dimethylthiourea (DMTU) is a well-known sulfur-based radical scavenger.[1–4] It is a small, highly diffusible molecule that efficiently scavenges toxic oxygen metabolites and reduces oxidative injury in many biologic systems. There have been very few studies undertaken on the fate and nature of the metabolites formed after such antioxidants have mediated oxidative injury.

Our bodies have evolved several very efficient methods of handling a large numbers of xenobiotics.[5] Our bodies, however, do not have a consistent answer to sulfur-based xenobiotics. Nearly all relevant sulfur chemistry in the human body is of organic origin. The range of physiological effects associated with organic sulfur chemistry spans from therapeutic to toxic.[6] There is no other group of compounds that displays such a wide range of biological activity. While the action and effects of organosulfur compounds on human health is well-documented, the mechanisms by which these effects are expressed are not known. Drug design and the

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ability to predict physiological effects is dependent upon our ability to understand the mechanism of metabolic activation of the relevant drug.

Sulfur compounds, such as DMTU, undergo a variety of metabolic reactions in the physiological environment such as oxidations, reductions, hydrolysis, and conjugations.[7] Sulfur, in most organic configurations, however, is nucleophilic, and nucleophilic atoms are usually susceptible to metabolic oxidations.[8] Thus, the oxidation of sulfur-containing compounds represents a very important aspect of sulfur metabolism. Oxidations of sulfur compounds appear to be involved in many cellular functions, including the reductive degradation of polypeptide hormones and proteins, regulation of protein synthesis, maintenance of intracellular redox potential, and protection of the cell from oxidative damage.[9] The aerobic physiological environment encourages S-oxygenation as the major metabolic activation pathway for most organosulfur compounds where the sulfur center is successively oxygenated, sometimes all the way to the sulfate, culminating in the cleavage of the attendant C–S bond.[10,11]

The molecular basis for S-oxygenation is not well-understood, although S-oxygenation of xenobiotics by microsomes supplemented with NADPH and oxygen has been known for years. In general, metabolism of chemically stable compounds depends on enzyme catalysis, and both the microsomal cytochrome P-450 system and the flavin-containing monooxygenases have been implicated in S-oxygenations catalyzed by microsomes.[8,12,13]

DMTU, although in most situations is effective in decreasing oxidant-mediated injury, has, however, inexplicably failed, on occasions, to reduce injury in some biological systems where oxygen metabolites were ostensibly causing damage.[14,15] It has also been experimentally shown that increasing the DMTU dose in rats did not increase protection and may, in fact, be associated with more injury. DMTU should exercise its physiological effects after metabolic activation and it is generally accepted that these physiological effects are derived from its metabolites.

In a previous publication from this laboratory,[16] we synthesized the first stable metabolite of DMTU, dimethylaminoiminomethanesulfinic acid (DMAIMSA). This metabolite was surprisingly very reactive and its aerobic decomposition in slightly basic to basic environments produced dithionite and a cascade of reactive oxygen species that were genotoxic, and could explain some of the inadvertent toxicity associated with DMTU. The general progress of the oxidation of a sulfur center initially proceeds through the sulfenic acid (unstable except in sterically hindered sulfenic acids),[17–19] then to the sulfinic acid (e.g., DMAIMSA), and then the sulfonic acid before cleavage of the C–S bond to form sulfate and an organic residue.[20] The existence of DMAIMSA had always been conjectured, but no one had managed to prepare and characterize this metabolite until the work of Otoikhian and co-workers.[16,21,22]

Since the sulfonic acid (dimethylaminoiminomethanesulfonic acid, DMAIMSOA) is expected to be the next metabolite after formation of DMAIMSA, we undertook to synthesize and characterize this metabolite, and hopefully, from its reactivity, be able to rationalize some of the observed conflicting physiological effects of DMTU. We report in this manuscript on this oxidation metabolite of DMTU. Despite its similarity to DMAIMSA, DMAIMSOA proved to be very different from DMAIMSA in terms of structure and reactivity.

Table 1. Crystal data and structure refinement for DMAIMSOA.

Empirical formula	$C_3H_8N_2O_3S{\cdot}H_2O$
Formula weight	170.19
Temperature (K)	295(2)
Wavelength (Å)	0.71073
Crystal system	Orthorhombic
Space group	*Pbca*
Unit cell dimensions	
a (Å)	12.533(1)
c (Å)	9.624(1)
c (Å)	12.918(1)
α (°)	90
β (°)	90
γ (°)	90
Volume (Å^3)	1558.1(2)
Z	8
Density (calculated; g/cm^3)	1.451
Absorption coefficient (cm^{-1})	3.81
F(000)	720
Crystal size (mm^3)	0.20 × 0.40 × 0.44
θ range for data collection (°)	3.10–25.00
Limiting indices	$0 \le h \le 14$
	$0 \le k \le 11$
	$-15 \le l \le 0$
Reflections collected	1356
Independent reflections	1356
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	1356/0/102
Goodness-of-fit on F^2	1.022
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0462$, $wR_2 = 0.1032$
R indices (all data)	$R_1 = 0.0838$, $wR_2 = 0.1205$
Largest diff. peak and hole (e Å^{-3})	0.241 and –0.307

Table 2. Atomic coordinates (×10^4) and equivalent isotropic displacement parameters (Å^2 × 10^3) for $SO_3C(NHMe)_2{\cdot}H_2O$. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(eq)
S	3569(1)	278(1)	6551(1)	37(1)
O(1)	3597(2)	–1207(3)	6619(2)	60(1)
O(2)	3329(2)	811(3)	5538(2)	50(1)
O(3)	4444(2)	985(3)	7046(2)	58(1)
O(4)	4487(3)	2529(4)	4164(3)	66(1)
N(1)	2636(2)	1527(3)	8142(2)	39(1)
N(2)	1469(2)	372(3)	7079(2)	41(1)
C(1)	2427(3)	783(3)	7335(3)	32(1)
C(2)	1825(3)	2042(4)	8854(3)	49(1)
C(3)	1158(3)	–468(4)	6185(3)	57(1)

Experimental

Materials

DMTU, hydrogen peroxide, and acetonitrile (Sigma-Aldrich) were purchased and used without any further purification.

Some batches of DMTU were damp and oily and could not form DMAIMSOA upon applying the synthetic procedure outlined below. Instead, the product formed slowly turned yellow upon exposure to the atmosphere and at ambient temperatures. The consistency of the DMTU batches was extremely important for the generation of high and consistent yields of DMAIMSOA. Aqueous iodine solutions were prepared by dissolving iodine crystals (Sigma-Aldrich) in distilled water followed by filtration and storage in the dark. Standard solutions of sodium hydroxide were purchased from Fisher Scientific.

Synthesis of DMAIMSOA

N,*N*-Dimethylaminoiminomethanesulfonic acid (DMAIMSOA) was prepared according to a modified form of a synthetic procedure given in the literature for the synthesis of dimethylaminoiminomethanesulfinic acid (DMAIMSA).[16] *N*,*N*′-Dimethylthiourea (DMTU; 10.42 g, 0.10 mol) were dissolved in 80 mL of a 50% acetonitrile–water solution, which was chilled in an acetone–CO_2 ice bath at –50 °C. To this ice-cold solution, 3 equiv (0.30 mol) of hydrogen peroxide, which was measured as a 30.61 mL aliquot of a 30% concentrated solution, were added dropwise with the rate of addition maintained such that the temperature of the reaction mixture did not exceed –30 °C. The 3:1 ratio of hydrogen peroxide to DMTU had to be strictly maintained to avoid the production of mixtures of oxoacids. The frozen mixture was allowed to sit until it melted and it was then stirred at room temperature for 2 h. The resulting long, colorless, needle-like crystals were filtered and washed twice with a 50% acetonitrile–water solution and deionized water and then dried in a desiccator. Typical yields before recrystallization were approximately 90%. Further recrystallization and purification was performed using the same strength of acetonitrile solution. Most of the product was lost during the washing and recrystallization of DMAIMSOA, thus giving a lower overall yield since it is highly soluble in water.

Methods

UV–vis spectra were taken on a PerkinElmer Lambda 2S spectrophotometer equipped with a thermostatable compartment. Most experiments were performed at 25 ± 0.5 °C. Distilled and deionized water were utilized to prepare all standard solutions used in these experiments. The reaction between DMAIMSOA and HOCl was monitored by following the absorbance of DMAIMSOA at 215 nm where an absorptivity coefficient of 9025 $(mol/L)^{-1}$ cm^{-1} had been deduced. The reaction between DMAIMSOA and iodine (as well as iodate) was followed by the use of the iodine–triiodide isosbestic point, which had been experimentally determined as 465 nm in this work and other previous studies from our laboratory using the same spectrophotometer.[21] Only freshly prepared iodine solutions were utilized. A Hi-Tech Scientific SF-DX2 stopped-flow spectrophotometer was used to follow the kinetics of DMAIMSOA oxidations and hydrolysis. KinetAsyst 2.1 software (High-Tech Scientific) was used for data acquisition and analysis. Temperature control was maintained with a NesLab RTE-101 thermostat bath. Some reaction solutions were degassed with argon and tightly capped to reduce the effects of dissolved oxygen.

Description of the X-ray structural analysis of $C_3H_8N_2O_3S{\cdot}H_2O$

A colorless crystal of $C_3H_8N_2O_3S{\cdot}H_2O$ was wedged in a glass capillary and then optically aligned on the four-circle of a Siemens P4 automated X-ray diffractometer. The reflections that were used for the unit cell determination were located and indexed by the automatic peak search routine provided by XSCANS.[23] $C_3H_8N_2O_3S{\cdot}H_2O$ crystallizes in the centrosymmetric space group *Pbca* (D_{2h}^{15}, No. 61). The final lattice parameters and orientation matrix were calculated from a nonlinear least-squares fit of the orientation angles of at least 35 reflections at 22 °C. The lattice parameters and other pertinent crystallographic information are summarized in Table 1 for $C_3H_8N_2O_3S{\cdot}H_2O$.

Intensity data were measured with graphite-monochromated Mo Kα radiation (λ = 0.710 73 Å) and variable ω scans. Background counts were measured at the beginning and at the end of each scan with the crystal and counter kept stationary. The intensities of three standard reflections were measured after every 100 reflections and did not show any evidence of crystal decay. The raw data were corrected for Lorentz-polarization effects.

Initial coordinates for the nonhydrogen atoms were determined by a combination of direct methods and difference Fourier calculations with the use of SHELXTL 6.1.[24] The crystallographic asymmetric unit contains a molecule of water that is located in a general position and is hydrogen bonded to the O(2) atom. The hydrogen atoms of the water molecule were located and their positions and isotropic temperature parameters were refined. Idealized positions for the remaining hydrogen atoms were included as fixed contributions using a riding model with isotropic temperature factors set at 1.2 times that of the adjacent nonhydrogen atom. The positions of the methyl hydrogen atoms were optimized by a rigid rotating group refinement with idealized angles. Full-matrix least-squares refinement, based upon the minimization of $\Sigma w_i\ |F_o^2 - F_c^2|^2$ with weighting $w_i^{-1} = [\sigma^2(F_o^2) + (0.0528P)^2 + 0.880P]$ where $P = (\max(F_o^2, 0) + 2F_c^2)/3$, converged to give the final discrepancy indices[25] provided in Table 1. A correction for secondary extinction was applied. The maximum and minimum residual electron density peaks in the final difference Fourier map were 0.241 and –0.307 e $Å^{-3}$, respectively (Table 2). The linear absorption coefficient, atomic scattering factors, and anomalous dispersion corrections were calculated from values found in the *International Tables of X-ray Crystallography*.[26]

Results and discussion

Description of the molecular structure of DMAIMSOA ($SO_3C(NHMe)_2$)

The molecular structure of DMAIMSOA was determined by X-ray crystallography. The perspective view is shown in Fig. 1. Like its sulfinic acid analogue,[16] it exists in its zwitterionic form in solid crystal form. The positive charge is delocalized over the N(2)–C(1)–N(1) framework with the sp^2 hybridization at the central carbon atom. Bond angles around this carbon atom add up to 360°. The C(1)–N bond distances of 1.291(4) and 1.306(4) Å are significantly shorter than a C–N bond distance of 1.47 Å, indicating considerable double bond character in both of them. The whole

Fig. 1. Perspective view of the molecular structure of DMAIMSOA with the atom labeling scheme. Thermal ellipsoids are scaled to enclose 30% probability.

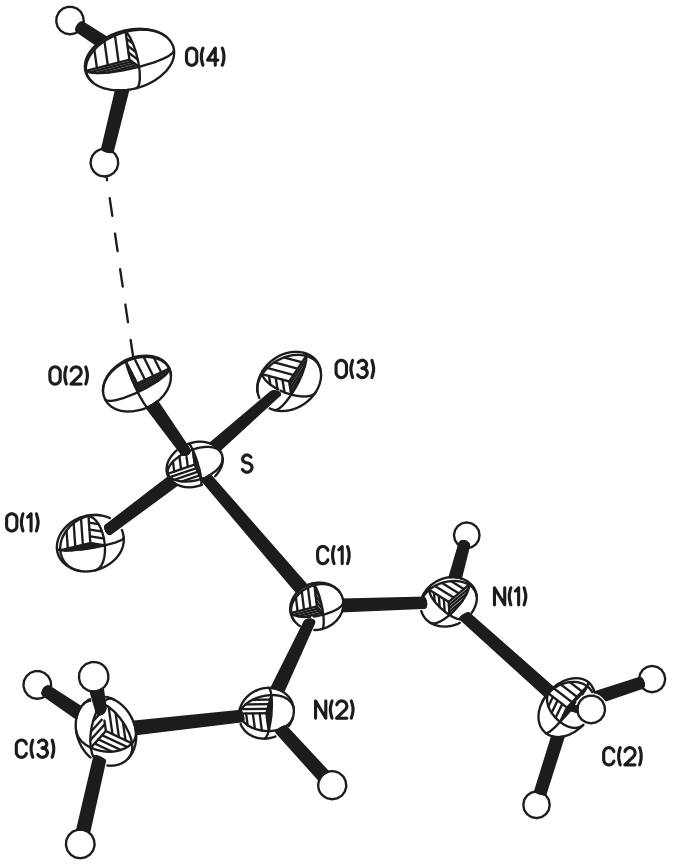
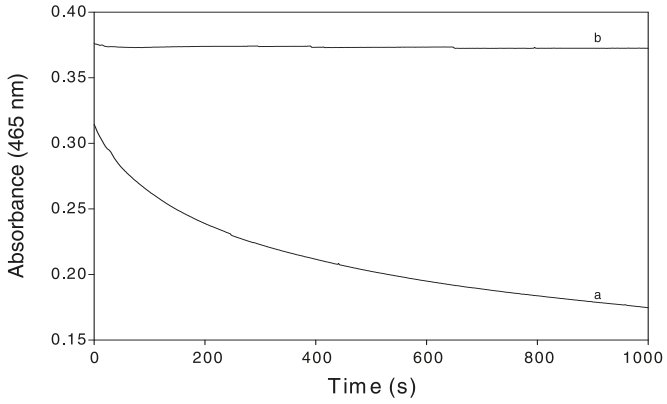


Table 3. Interatomic distances (Å) and bond angles (°) for DMAIMSOA.

Interatomic distances (Å)	
S—O(1)	1.433(3)
S—O(2)	1.437(3)
S—O(3)	1.440(3)
S—C(1)	1.820(3)
N(1)—C(1)	1.291(4)
N(1)—C(2)	1.458(4)
N(2)—C(1)	1.306(4)
N(2)—C(3)	1.463(5)
Bond angles (°)	
O(1)–S–O(2)	114.7(2)
O(1)–S–O(3)	115.2(2)
O(2)–S–O(3)	113.3(2)
O(1)–S–C(1)	104.6(2)
O(2)–S–C(1)	104.2(1)
O(3)–S–C(1)	103.1(2)
N(1)–C(1)–N(2)	123.9(3)
N(1)–C(1)–S	115.9(2)
C(1)–N(2)–C(3)	127.7(3)
C(1)–N(1)–C(2)	123.8(3)
N(2)–C(1)–S	120.1(3)

three-bond network, after the s-bond framework, then comprises four electrons, both derived from the nitrogen p-orbitals, which interact with the vacant p_π-orbitals of the formally cationic carbon center, imparting partial double bond character to both bonds, and hence their deviation from the expected value for a single bond. The negative charge is delocalized on the three oxygen atoms on the sulfonic acid group. The S–O bonds are also nearly equivalent, ranging between 1.433(3) and 1.440(3) Å. These are shorter than a normal S–O bond owing to the delocalization of the negative charge over the three oxygen atoms. These three S–O bonds are approximately 0.04 Å shorter than the two S–O bonds in DMAIMSA, which are at approximately 1.48 Å. The three oxygens form a nearly symmetric triangular base around the sulfur center except for the oxygen atom in close proximity to the methyl group, which is slightly displaced from this pyramidal shape by about 2° (Table 3).

Fig. 2. Comparison traces of iodine oxidation of DMAIMSA and DMAIMSOA showing the relative inertness of DMAIMSOA to oxidation. Iodine solutions with DMAIMSOA held their titer for hours with no measurable consumption of iodine observed. $[I_2]_o$ = 5.24×10^{-4} mol/L, (a) $[DMAIMSA]_o = 2.0 \times 10^{-4}$ mol/L, and (b) $[DMAIMSOA]_o = 2.0 \times 10^{-4}$ mol/L.

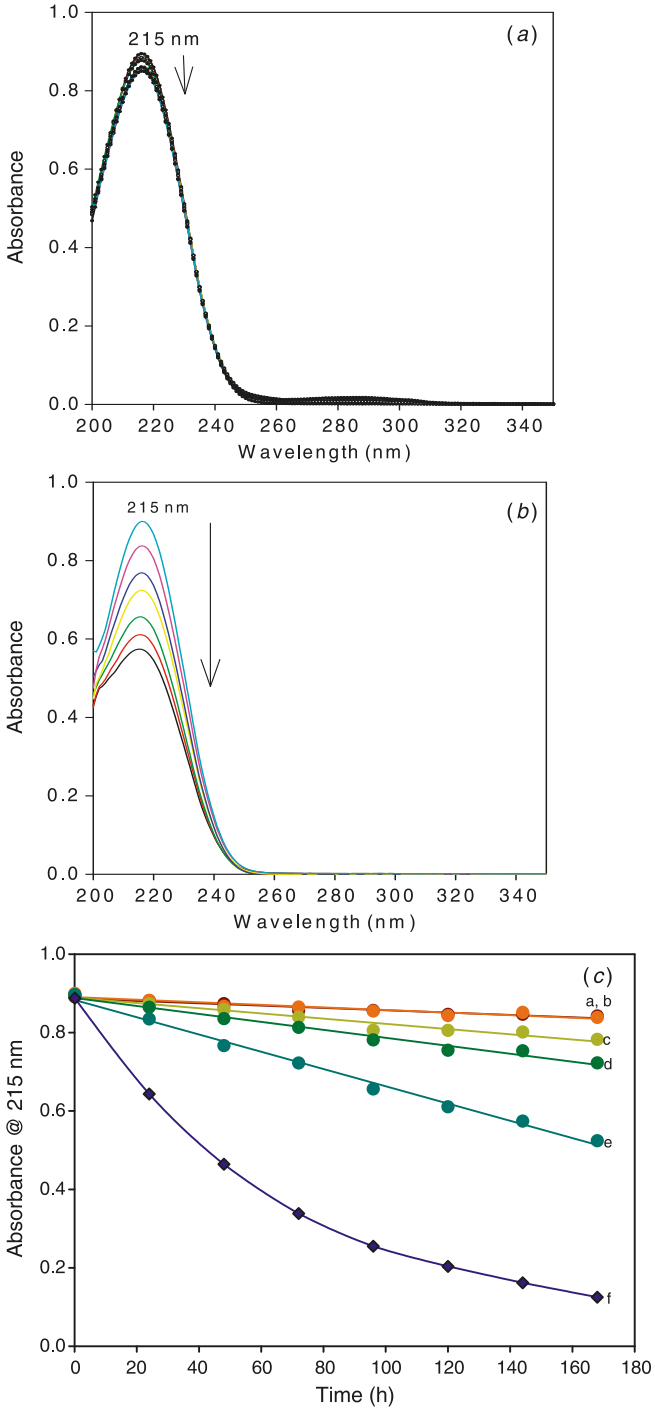


The C–S bond, at 1.820(3) Å, is 0.03 Å longer than a typical C–S bond of 1.79 Å, but is noticeably shorter than the C–S bonds of 1.880 Å in DMAIMSA and 1.867 Å in unsubstituted aminoiminomethanesulfinic acid (AIMSA, thiourea dioxide). Both sulfinic acids are highly reactive with easy cleavage of the C–S bond to release highly reducing sulfur-based leaving groups.[27] DMAIMSOA is not densely packed, and has a density of 1.451 g cm^{-3}, which is comparable to that exhibited by DMAIMSA of 1.496 g cm^{-3}. Although DMAIMSOA shows hydrogen bonding through O(2) to the single water of crystallization in the unit cell, it is incapable of supporting strong and extensive hydrogen bonding, which would have been expected to increase its density as was observed with aminoiminomethanesulfonic acid (AIMSOA, thiourea trioxide) with a density of 1.948 g cm^{-3}.[28]

Reactivity of DMAIMSOA

DMAIMSA is known to be highly reactive, and its aerobic decomposition in mildly acidic and basic environments quickly and rapidly produces dithionite.[16] Its oxidation to dimethylurea and sulfate is facile. Figure 2 shows a simple experiment carried out to evaluate the rates of oxidation of DMAIMSA and DMAIMSOA in slightly acidic media (pH 5.0) by the weak biological oxidant iodine. While DMAIMSA is oxidized at a reasonable rate with bimolecular kinetics with a rate constant of 20.5 $(mol/L)^{-1}$ s^{-1},

Fig. 3. (*a*) UV spectral of incubated DMAIMSOA in water at a slightly acidic pH of 6 showing its inertness. The spectra scan was taken at 24 h intervals for 7 days. There is very little change in the absorbance of DMAIMSOA. [DMAIMSOA] = 1.0×10^{-4} mol/L. (*b*) UV spectra scan of the same DMAIMSOA solution as in Fig. 3*a* at a physiological pH of 7.4 phosphate buffer. At this slightly basic pH there is a noticeable decomposition of DMAIMSOA. (*c*) The effect of pH on the rate of decomposition of DMAIMSOA in 0.05 mol/L phosphate buffer. Substantial decomposition starts at pH conditions greater than 7. [DMAIMSOA] = 1.0×10^{-4} mol/L; pH (a) 6.2 (unbuffered), (b) 6.2, (c) 6.6, (d) 7.0, (e) 7.4, and (f) 8.2.



DMAIMSOA remains inert to oxidation at this pH. This was a noticeable difference in the reactivities of these aminosulfur oxoacids. This discrepancy in their reactivities has brought about a new school of thought that suggests, strongly, that in acidic media, the sulfonic oxoacid is unlikely to be an intermediate in the oxidation of the thiouredo group to sulfate and an organic residue.[29]

Figure 3 also displays the inertness of DMAIMSOA. In Fig. 3*a*, DMAIMSOA solutions are dissolved in water at a slightly acidic pH of 6 and allowed to sit, capped, and in the dark for a week. Other aminosulfur oxoacids show extensive decomposition in aqueous solutions.[16] Figure 3*a* shows that, even after 7 days, there is very little change in the absorbance of DMAIMSOA monitored at 215 nm. Figure 3*b*, on the other hand, shows the same DMAIMSOA solution, this time in a pH 7.4 buffer, which is slightly basic and is the physiological pH. At these conditions, there is noticeable decomposition of DMAIMSOA. The slight change in pH from Figs. 3*a* to 3*b* can impart a remarkable change in reactivity. Figure 3*c* shows, much more vividly, the rates of decomposition of DMAIMSOA at various pH conditions. In all traces, the pH was maintained by phosphate-type buffer solutions. The phosphate anions in this buffer mixture are not innocent, and are known to increase the rate of decomposition of aminosulfur oxoacids owing to their nucleophilic nature. Figure 3*c* shows that substantial decomposition commences at pH conditions greater than 7. While there is no decomposition observed around pH 6 (Fig. 3*c*, trace a), at pH 8.2 (Fig. 3*c*, trace f), however, complete decomposition of DMAIMSOA is attained within 3 min.

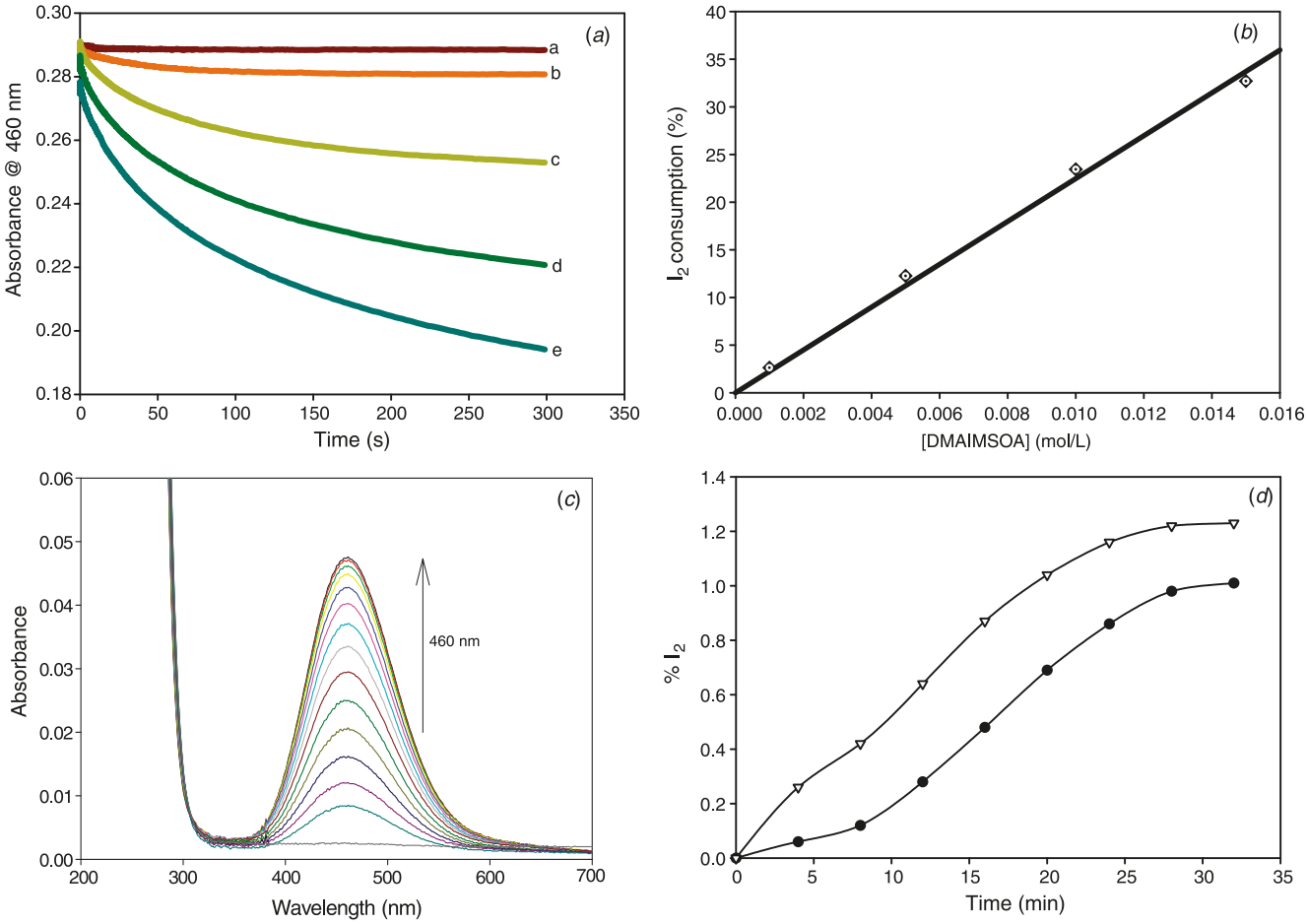
Reactions with iodine and acidic iodate

It is not possible to spectrophotometrically observe I_2–DMAIMSOA reactions at high pH conditions because most of the aqueous iodine is converted into hypoiodous acid, which has no absorption in the visible range in neutral and acidic environments. Iodine, however, exists mostly in the aqueous molecular form and its consumption can be followed at 460 nm (see Fig. 4*a*). Figure 4*a* shows an initially reasonably fast rate of iodine consumption, which quickly slows down and shuts itself down about 300 s into the reaction. Figure 4*a* has an expanded scale, and shows that very little aqueous iodine is actually consumed. These I_2–DMAIMSOA solutions can be left overnight and the absorbance values reread after 24 h. After 24 h, the ratio of iodine consumed is shown in Fig. 4*b*. The linearity shows that the only reaction occurring is the consumption of iodine by DMAIMSOA. The expected stoichiometry for the oxidation of DMAIMSOA by iodine is 1:1

$$[1] \quad (\mathrm{MeHN})(\mathrm{MeN}{=})\mathrm{CSO_3H} + \mathrm{I_2(aq)} + 2\mathrm{H_2O} \rightarrow (\mathrm{MeHN})_2\mathrm{C}{=}\mathrm{O} + \mathrm{SO_4^{2-}} + 2\mathrm{I^-} + 4\mathrm{H^+}$$

The percent consumption of iodine should allow for the calculation of the fraction of DMAIMSOA that is "oxidizable" because these fractions in Fig. 4*b* remain basically invariant on further incubation of the reaction solutions. This percentage iodine consumption is calculated on the basis of the stoichiometry in eq. [1] in which a 1:1 mol ratio of DMAIMSOA–I_2 is considered to be 100%. Using the plot in Fig. 4*b* and reaction stoichiometry in eq. [1], a simple calcu-

Fig. 4. (*a*) Absorbance traces showing the consumption of aqueous iodine by DMAIMSOA in unbuffered solutions. Despite the initial reasonably fast rate of consumption, very little aqueous iodine is consumed. [I_2(aq)] = 3.82 × 10^{-4} mol/L, [DMAIMSOA] = (a) 0.0, (b) 0.001, (c) 0.005, (d) 0.010, and (e) 0.015 mol/L. (*b*) A linear plot showing the percent consumption of iodine in Fig. 4*a* after 24 h of I_2–DMAIMSOA incubation. (*c*) Multiple spectral scan measurements of the reaction of DMAIMSOA and acidic iodate taken every 2 min showing the formation of iodine at 460 nm. A very small amount of iodine is formed indicating that at these conditions only a negligible fraction of DMAIMSOA is oxidizable. [DMAIMSOA] = 0.025 mol/L, [IO_3^-] = 0.025 mol/L, and [H^+] = 0.05 mol/L. (*d*) Comparison traces of the percent formation of iodine in the reactions of freshly prepared (closed circles) and 3-day-old (open triangles) DMAIMSOA solutions with acidic iodate. [DMAIMSOA] = 0.025 mol/L, [IO_3^-] = 0.025 mol/L, and [H^+] = 0.05 mol/L.



lation shows that 0.77% of DMAIMSOA is oxidized after 24 h of reaction time. This shows that, at these conditions, only a very small fraction of DMAIMSOA is oxidized or oxidizable. In the acidic environment, iodate can also be used to evaluate the response of DMAIMSOA to oxidation. By using excess iodate concentrations, the amount of iodine produced can be utilized to calculate the amount of DMAIMSOA oxidized (see Fig. 4*c*). Initially, iodate oxidizes DMAIMSOA to produce iodide (reaction stoichiometry in eq. [2]), which is subsequently oxidized by the excess iodate to give iodine (reaction in eq. [3]) as the final reduction product of iodate.

$$[2]\quad IO_3^- + 3(MeHN)(MeN{=})CSO_3H + 3H_2O \rightarrow I^- + 3(MeHN)_2C{=}O + 3SO_4^{2-} + 6H^+$$

Followed by the Dushman reaction:[30]

$$[3]\quad IO_3^- + 5I^- + 6H^+ \rightarrow 3I_2 + 3H_2O$$

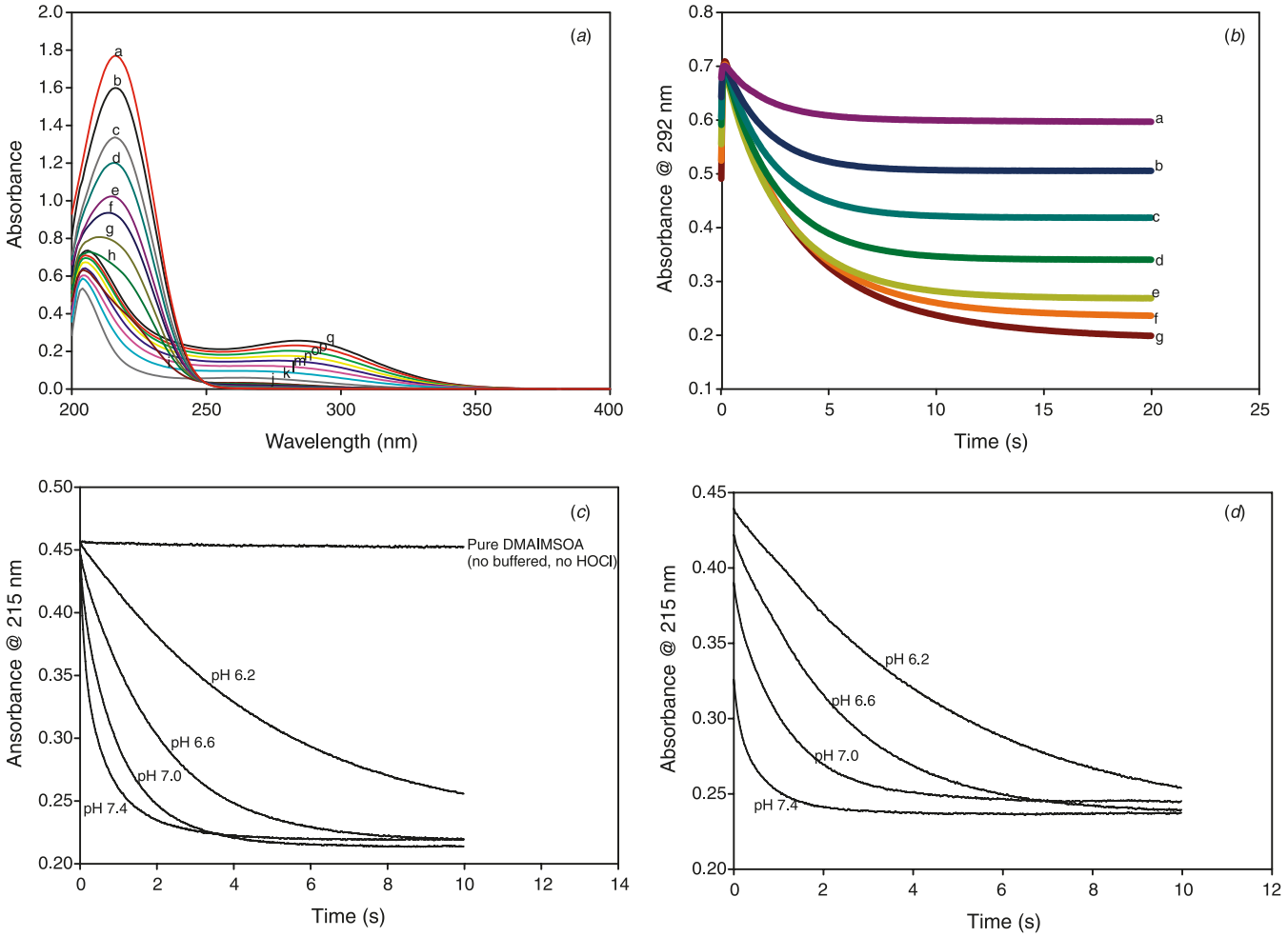
The addition of 5(eq. [2]) and eq. [3] eliminates iodide to give the overall stoichiometry that gives iodine as the final product:

$$[4]\quad 2IO_3^- + 5(MeHN)(MeN{=})CSO_3H + 4H_2O \rightarrow I_2 + 5(MeHN)_2C{=}O + 5SO_4^{2-} + 8H^+$$

One can thus correlate the amount of iodine formed to the amount of DMAIMSOA consumed through the 5:1 ratio. Figure 4*c* shows that very little iodine is formed, also confirming that very little DMAIMSOA is oxidized under these conditions. IO_3^- is inert in basic environments, and thus this type of correlation can only be effected in this acidic environment. Previous work in our laboratory[27,31] and by others[32] had always conjectured that such aminosulfur oxoacids initially undergo an irreversible, entropy-driven hydrolysis to give a reducing sulfur species, which in DMAIMSOA's case is bisulfite:

$$[5]\quad (MeHN)(MeN{=})CSO_3H + H_2O \rightarrow (MeHN)_2C{=}O + HSO_3^- + H^+$$

Fig. 5. (*a*) UV spectral scans of the HOCl–DMAIMSOA reaction after 48 h of reaction time at pH 7.4 in 0.05 mol/L phosphate buffer. [DMAIMSOA] = 2.0×10^{-4} mol/L, [HOCl] = (a) 0 (not buffered), (b) 0 (buffered), (c) 5.00×10^{-5}, (d) 7.50×10^{-5}, (e) 1.00×10^{-4}, (f) 1.25×10^{-4}, (g) 1.50×10^{-4}, (h) 1.75×10^{-4}, (i) 2.00×10^{-4}, (j) 3.00×10^{-4}, (k) 4.00×10^{-4}, (l) 5.00×10^{-4}, (m) 6.00×10^{-4}, (n) 7.00×10^{-4}, (o) 8.00×10^{-4}, (p) 9.00×10^{-4}, and (q) 1.00×10^{-3} mol/L. (*b*) Absorbance traces at 292 nm showing variation of DMAIMSOA with HOCl at a pH of 7.4 in 0.05 mol/L phosphate buffer. [HOCl] = 2.0×10^{-3} mol/L, [DMAIMSOA] = (a) 2.0×10^{-4}, (b) 4.0×10^{-4}, (c) 6.0×10^{-4}, (d) 8.0×10^{-4}, (e) 1.0×10^{-3}, (f) 1.2×10^{-3}, and (g) 1.4×10^{-3} mol/L. (*c*) Absorbance traces at 215 nm showing the effect of pH on the HOCl–DMAIMSOA reaction in 0.05 mol/L phosphate buffer for a freshly prepared DMAIMSOA. [HOCl] = 3.0×10^{-3} mol/L and [DMAMSOA] = 5.0×10^{-5} mol/L. (*d*) Absorbace traces of the same reaction in Fig. 5*c* but with a 7-day-old incubated DMAIMSOA.



The oxidations observed in Figs. 4*a*–4*c* are effectively the oxidation of bisulfite released in the reaction in eq. [5]. The extent of the reaction in eq. [5] (a sort of pseudoequilibrium constant since eq. [5] is assumed to be irreversible) is strongly dependent on pH, and any value derived for this reaction has to be associated with a specific pH value. Further studies are needed to evaluate the general base catalysis that can be evoked by the anions that make up the various buffers that might be used to maintain the pH. It is anticipated that the type and concentrations of the buffers themselves would influence the observed extent of the reaction in eq. [5]. The reaction in eq. [5] is an extremely slow process, which proceeds at a faster rate in basic conditions. Figure 4*d* shows that the reaction in eq. [5] is a prerequisite for the observed oxidations in Figs. 4*a* and 4*c*. Some DMAIMSOA reaction solutions were used immediately after preparation (Fig. 4*d*, dark circles), while other solutions were incubated for 3 days before being used in the DMAIMSOA–IO_3^- reactions (Fig. 4*d*, open triangles). It is evident that after 30 min more of the DMAIMSOA is observed to have been oxidized with the incubated solutions (1.23%) over those that were not incubated (1.03%).

Reactions in basic environments

For these experiments, hypochlorous acid was used as the oxidant. $^-$OCl has an absorption peak at 292 nm, and does not interfere with the DMAIMSOA peak at 215 nm as HOI does. Thus, the reaction between HOCl and DMAIMSOA can be followed at both 215 and 292 nm. Figure 5*a* shows a series of spectral scans of the HOCl–DMAIMSOA reaction after 48 h of reaction time at pH 7.4 in a phosphate buffer. Activity is observed at both wavelengths, showing a very viable and facile reaction. The stoichiometry of this reaction was derived to be 1:1 using barium sulfate precipitation.

[6] $(MeHN)(MeN{=})CSO_3H + HOCl + H_2O \rightarrow (MeHN)_2C{=}O + SO_4^{2-} + Cl^- + 3H^+$

Absorbance traces in Fig. 5*b* were monitored at 292 nm, and show that the reaction essentially goes to completion and is over within 20 s. Any rate constant derived from these data is only relevant for this pH condition and for this buffer strength and concentration. The final observed rate of reaction is derived from a complex interaction of the buffer anions with DMAIMSOA and their effect in facilitating the reaction in eq. [5]. The positively charged carbon center is susceptible to nucleophilic attack with the result that the C–S bond is weakened, facilitating its cleavage. Figures 5*c* and 5*d* show that the incubation of solutions is not an important factor for reactions run in basic environments. Freshly prepared solutions gave nearly the same reaction times as those incubated for a week. Examination of the initial absorbance readings for the incubated solutions, however, show that the initial DMAIMSOA absorbances are depressed from the values recorded for unincubated solutions. The data, taken at 215 nm in Figs. 5*c* and 5*d*, are more difficult to explain if indeed the reaction in eq. [5] is irreversible. Thus, in basic environments, some equilibrium is attained, although some irreversible entropically favored decomposition also still proceeds. The reaction of HOCl with HSO_3^- is essentially diffusion controlled, and would not show the type of kinetics observed in Figs. 5*b*–5*d*.

Conclusions

Owing to the inertness of aminoiminomethanesulfonic acid (AIMSOA) and DMAIMSOA, previous assertions from our research work had suggested that in these types of compounds that contain the thiouredo group, oxidation can proceed directly from the sulfinic acid to sulfate, bypassing the sulfonic acid.[10,31,33] Evidence for this involves the direct observation of an untrapped sulfoxyl anion radical in aerobic decompositions of aminosulfinic acids.[34] The inordinately long C–S bond in aminoiminomethanesulfonic acid (AIMSA) and DMAIMSA is easily cleaved by thermal activation or by the presence of nucleophiles. In fact, high yield guanidine formation is effected by the reaction of aminosulfinic acids with amines.[35–37] The nucleophilic amine center attacks the positive carbon center, culminating in the cleavage of the C–S bond and the formation of a C=N double bond.

The present synthesis and characterization, however, does not preclude the formation of the sulfonic acid as one of the stable intermediates in the oxidation of a thiouredo group in the basic environment and in the presence of basic anions or nucleophiles. Thus, in the physiological environment, metabolic activation of thiocarbamides, where S-oxygenation occurs at pH 7.4, the sulfonic acid is a viable intermediate, which can later be oxidized further to give sulfate and urea-type organic residue.

Supplementary data

Supplementary data for this article are available on the journal Web site (canjchem.nrc.ca). CCDC 778932 contains the X-ray data in CIF format for this manuscript. These data can be obtained, free of charge, via http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax +44 1223 336033; or deposit@ccdc.cam.ac.uk).

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