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The Oxidation of Trialkyl Trithioarsenites, $(RS)_3As$, by Octasulfur/Triethylamine and Dioxygen

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Trialkyl trithioarsenites, $(RS)_3$ As, bearing terminal carboxy (—COOH) or carboxylate ($—COO^-Na^+$) or zwitterionic ($—CH(NH_3{}^+)COO^-$) groups were studied for their ability to be oxidized by octasulfur, S_8, and by dioxygen. The carboxy-bearing trithioarsenites are not oxidized by S_8 to the corresponding As(V) esters, $(RS)_3As{=}S$, probably because they cannot open the octasulfur ring, but they react with S_8 in the presence of triethylamine, which acts as an activator of S_8. The carboxylate-bearing trithioarsenites, however, do react under drastic conditions with S_8. In both cases colored insoluble solids were obtained which could not be characterized. The carboxy-bearing trithioarsenites are oxidized by air in a hydrocarbon chain-length depended manner, while the carboxylate-bearing trithioarsenites are not, if at all, oxidized by air to arsenic(III) oxide, As_2O_3, and disulfide, RSSR. The zwitterion-bearing tris(penicillamine), tris(cysteine), and tris(glutathione) trithioarsenites are air oxidized slower than their corresponding parent thiols and this may be of biochemical significance.

Keywords Activation; binding; dioxygen; octasulfur; trialkyl trithioarsenites

INTRODUCTION

Inorganic arsenic can enter into a cell either as arsenate or as arsenite. Whichever the mechanism of the entry, once inside arsenate can mimic the reactions of phosphate[1] or it can be reduced to arsenite by arsenate reductase (E.C.1.20.4.1)[2] or biological thiols, e.g., cysteine or glutathione[3–5] according to Eq. (1):

$$H_3AsO_4 + 5RSH \rightarrow (RS)_3As + RSSR + 4H_2O\ . \qquad (1)$$

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Similarly, arsenite inside a cell can react with these thiols according to Eq. (2):

$$H_3AsO_3 + 3RSH \rightarrow (RS)_3As + 3H_2O. \tag{2}$$

The fate of these trialkyl trithioarsenites, when formed inside a cell, has not been studied, except that the eukaryote *S. cerevisiae* pumps As(III) probably as As(glutathione)$_3$ into a vacuole thus detoxifying the arsenite.[6] The same ester was found to be transported out of hepatocytes by MRP2/cMOAT and into bile in rats.[7] As(glutathione)$_3$ has been found to be an inhibitor of yeast[8,9] and *S. cerevisiae*[10] glutathione reductase (E.C. 1.8.1.7), yeast and bovine glutathione peroxidase (E.C. 1.11.1.9),[10] and equine glutathione S-transferase (E.C. 2.5.1.18),[10] while As(cysteine)$_3$ inhibits thioredoxine reductase (E.C. 1.8.1.9).[11] The inhibition may or may not involve transthiolation (Eq.(3)), e.g. the transfer of As(III) to a dithiol[12] or to closely spaced-SH (cysteine) groups on an enzyme which may form such a dithiol.

$$(RS)_3As + 3R'SH \rightleftharpoons (R'S)_3As + 3RSH. \tag{3}$$

Conceivably, trialkyl trithioarsenites can be oxidized by dioxygen. It is known that certain liquid aliphatic trithioarsenites, e.g., (EtS)$_3$As,[13] (n-PrS)$_3$As,[13,14] and (n-BuS)$_3$As,[14] are very sensitive towards atmospheric oxygen (65% As_2O_3 is produced[14] when air is bubbled through (n-BuS)$_3$As for 2 h). However, the oily ester As(SCH$_2$CH$_2$OH)$_3$ is "fairly stable" (p. 827) towards air oxidation.[15] The stability of the biologically relevant As(cysteine)$_3$ and As(glutathione)$_3$ towards dioxygen has not been studied but their close analogue, Me-As(cysteine)$_2$, is unstable under aerobic conditions.[16]

It was found that *aromatic* trithioarsenites, (ArS)$_3$As, are oxidized by dioxygen to As_2O_3 and disulfides according to Eq. (4):

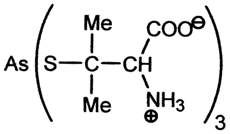
$$2(ArS)_3As + 3/2O_2 \rightarrow As_2O_3 + 3ArSSAr, \tag{4}$$

and the extent of the oxidation depends on the solvent, the nature of Ar (substituted phenyl, 2-pyridyl or 2-pyridyl N-oxide), the nature of the *para* substituent,[17] and the nature of E in (PhE)$_3$As (Se$\gg$S).[13]

The action of other oxidizing elements on trithioarsenites has not been studied much. Thus, alcoholic iodine decomposed (PhS)$_3$As to its disulfide[18] and octasulfur in boiling carbon disulfide was said[19,20] to oxidize (p-MePhS)$_3$As to (p-MePhS)$_3$As=S. However, we showed[21] that the As(III) in this and other aromatic trithioarsenites is not nucleophilic towards octasulfur and such As(V) esters cannot be produced by reacting trithioarsenites with octasulfur. Moreover, it was found[21] that As_2O_3 and ArSSAr were produced during the attempted oxidations by octasulfur, obviously according to Eq. (4). When the octasulfur ring was opened in the presence of 10–20 mol% triethylamine, the esters $(ArS)_3As$ gave colored, insoluble solids always accompanied by As_2O_3.

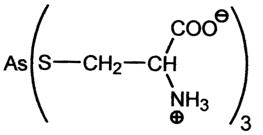
In this article we describe our results on the reaction of the aliphatic trithioarsenites **1–3**, **5**, and **6** with octasulfur in the absence and in the presence of triethylamine, as an activator of octasulfur, and on the air oxidation of aliphatic trithioarsenites bearing carboxy, **1–4**, carboxylate, **5–8**, and zwitterionic, **9–11**, groups.

$As(S[CH_2]_xCOOH)_3$		$As(S[CH_2]_xCOONa)_3$	
1	$x = 1$	**5**	$x = 1$
2	$x = 2$	**6**	$x = 2$
3	$x = 10$	**7**	$x = 10$
4	$x = 15$	**8**	$x = 15$



$As(SC(Me)_2CH(COO^{\ominus})NH_3^{\oplus})_3$

9



$As(SCH_2CH(COO^{\ominus})NH_3^{\oplus})_3$

10

$As(glutathione)_3$

11

RESULTS AND DISCUSSION

Preparation and Properties of the Trithioarsenites 1–11

The preparation of the esters $(RS)_3As$ is usually done by reacting the liquid and water-sensitive $AsCl_3$ with the sodium salt of a thiol, the latter being prepared from the thiol and sodium metal.[13,14] The use of the solid and easily handled As_2O_3 for the preparation of $As(SCH_2COOH)_3$ in water was reported in 1904[22] and we developed the method in order to prepare a wide variety of aromatic and aliphatic trithioarsenites in water, methanol, or ethanol solvent with very satisfactory yields.[5] Previously,[5] **5** and **6** have been prepared by reacting As_2O_3 with $HSCH_2COONa$ in water or the "Na_3AsO_3" with the $HSCH_2CH_2COOH$ in water. Now, the compounds **5–8** have been prepared by neutralizing **1–4** with an equivalent amount of NaOH. An excess of NaOH should be avoided because the trithioarsenites are not stable in alkaline solutions.[5,23]

The trithioarsenites **4**, **7**, and **8** could not be studied because of their low solubilities.

Reaction of the Trithioarsenites 1–3, 5, and 6 with Octasulfur

As in the case of aromatic trithioarsenites,[21] the acidic esters **1–3** did not react with octasulfur in chloroform/methanol solutions, indicating that the As(III) is not nucleophilic enough to open the octasulfur ring. The reaction can, however, be effected by a catalytic amount (~20 mol%) of triethylamine, which reacts with an acidic sulfur-containing impurity (e.g., H_2S, SO_2), found even in recrystallized octasulfur,[24a] giving an anion which, in turn, opens the octasulfur ring giving Impurity-S-S-S_6-S^-. The basic environment of the carboxylate-bearing **5** and **6** in water is sufficient to open the octasulfur ring presumably by activating the previously mentioned impurity, or by direct reaction of HO^- on S_8, which is known to give a variety of products, e.g., S^{2-} and SO_3^{2-}.[24b]

The penultimate sulfur shown in bold, Impurity-S-**S**-S_6-S^-, can now be attacked by the As(III) of the esters **1–3**, **5**, and **6** in a manner analogous to that postulated for the dithioarsonites, Ar-As$(SPh)_2$[25] and the aromatic trithioarsenites, $(ArS)_3As$[21] to give colored precipitates. The precipitates were insoluble in all common organic solvents and aqueous hydrochloric acid and were decomposed by aqueous sodium hydroxide. Those obtained from **1–3** contained less triethylamine compared to those obtained from the aromatic trithioarsenites.[21] From the color and the solubilities, the orange solids may be mixtures of As_4S_6 (orange) and oligomeric (?) As_4S_4 rings having RS-groups.

While the solids obtained from the aromatic trithioarsenites in chloroform always contained As_2O_3 produced at the beginning of the reaction,[21] the aliphatic trithioarsenites **1–3**, **5**, and **6** dissolved in nondeaerated solvents reacted with octasulfur producing traces only of As_2O_3 (Table I). Control experiments (dissolution of **5** or **6** and 10% As_2O_3 in water, evaporation and drying) showed that As_2O_3 was clearly detectable by IR, implying that it was not converted to, e.g., NaH_2AsO_3/$NaAsO_2$, by the alkaline environment. Thus, the aromatic trithioarsenites studied[21] seems to be more vulnerable to As_2O_3 production than the aliphatic ones during the S_8/Et_3N treatment. The mechanism of the As_2O_3 production has not been elucidated.

Air Oxidation of the Trithioarsenites 1–3, 5–7, and 9–11

The polar esters **1–11** are grouped in three classes: acidic **1–4**, basic **5–8**, and zwitterionic **9–11**. From Table II it is clear that the acidic **1–3** are oxidized by air much faster than the other two classes and moreover the oxidative decomposition to As_2O_3 and, presumably, disulfide is diminished with the chain length of the carboxy thiol.

TABLE I Conditions for the Reaction of Some Aliphatic Thioesters, $(RS)_3As$, (0.2 mmol) With Octasulfur (0.6 mmol Sulfur Atoms) and Some Characteristics of the Obtained Insoluble, Orange Solids. N.D. = Not Detected by IR

Ester	Solvent temperature, °C	Et_3N, mol%	Time, h	Weight, mg	As_2O_3	RS/Et_3N, molar ratio	RS content, % by weight
1	$CHCl_3$/MeOH 1:1 25	20	24	26	N.D.[a]	1:0.5	11
2	$CHCl_3$/MeOH 1:1 25	20	24	25	N.D.[b]	1:0.7	8
3	$CHCl_3$/MeOH 1:1 25	20	24	22	N.D.[a]	1:0.6	13
5	H_2O 100	0	9	21	N.D.[c]	—	1
6	H_2O 100	0	9	10	N.D.[c]	—	2

[a] Traces of As_2O_3 were detected in the supernatant.
[b] <10% As_2O_3 was detected in the supernatant.
[c] As_2O_3 was not detected in the supernatant.

TABLE II Percent of Oxidative Decomposition of Some Trialkyl Trithioarsenites, $(RS)_3As$, When Stirred in Air at R.T., and Air Oxidation of Some Parent Thiols Under the Same Conditions. N.D. = Not Detected by IR

				% oxidative decomposition from		
Ester	Solvent	Molarity M	Time days	precipitated As_2O_3	As_2O_3 in supernatant (by IR)	RSSR from RSH, %
1	$CHCl_3$/MeOH 1:1	0.17	4	40	N.D.	–
2	$CHCl_3$/MeOH 1:1	0.17	4	32	N.D.	–
3	$CHCl_3$/MeOH 1:1	0.17	4	22	~ 7	–
5	H_2O	0.06	3	N.D.	~ 10	–
6	H_2O	0.06	3	N.D.	N.D.[b]	–
7	H_2O	0.06[a]	3	N.D.	N.D.[b]	–
9	H_2O	0.01	7	0	<5	41[c]
10	H_2O	0.004[a]	7	0	0	24[d]
11	H_2O	0.025	7	0	<2	13[e]

[a] Light suspension.
[b] Very weak peak at ~840 cm^{-1} ($NaAsO_2$?).
[c] 0.35 M.
[d] 0.40 M.
[e] 0.08 M.

The basic **5–7** in water are autoxidized very slowly, if at all (Table II). Only in the case of **5** did we detected As_2O_3, while an extremely weak peak at ~840 cm^{-1}, which can be attributed to $NaAsO_2$ (which, when pure, exhibits[26] strong, sharp peaks at 831 and 848 cm^{-1}), was seen in the IR spectra of the air oxidized **6** and **7**. Control experiments (e.g, an aqueous solution of **7** containing As_2O_3 was freeze dried) showed that a 10% As_2O_3 can be seen in the solid state IR spectrum.

The rates of air oxidation of D-penicillamine,[27] L-cysteine,[28] and reduced glutathione[28] have a maximum at pH ~ 7.5 and are slow at acidic and not so slow at alkaline pH. We found that unbuffered aqueous solutions of DL-penicillamine (pH ~ 5), cysteine (pH ~ 6), and reduced glutathione (pH ~ 4) after 7 days stirring in air were oxidized to their disulfides to an extent of 41, 24, and 13%, respectively (Table II).

A dilute solution of **9** in water was oxidized by air after 7 days stirring (Table II), giving apart from As_2O_3 (detected by IR), disulfide, and free DL-penicillamine, detected by ^1H NMR. However, no reliable percentages of oxidative decomposition could be obtained from the ^1H NMR spectrum because **9** is sparingly soluble whereas penicillamine and its disulfide are soluble in water. From the IR spectrum a <5% oxidative decomposition was estimated (Table II).

An unbuffered suspension of **10** in water did not produce (IR analysis) any As_2O_3 after stirring in air for 7 days, while an unbuffered aqueous solution of **11**, stirred in air, produced <2% As_2O_3 (by IR) and 14% oxidized glutathione by (^1H NMR). The latter value is similar to that obtained from the air oxidation of free reduced glutathione.

Recently, we have studied the air oxidation of aromatic trithioarsenites, $(ArS)_3As$, to As_2O_3 and ArSSAr, and we found that with electron-donating *para* substituted phenyl groups the autoxidation in nonpolar solvents was slow while with electron-withdrawing groups, e.g., chloro and nitro, the autoxidation was much faster.[17] These results were explained by assuming binding of triplet dioxygen to As(III), the binding being more favorable with contracted[29,30] 4d orbitals of As(III), to give a diradical, $(ArS)_3\dot{A}s{-}O{-}\dot{O}$, or a cyclic arsenadioxirane, $(ArS)_3As(O_2)$, intermediate.[17] Any of these intermediates can give the As(V) esters $(ArS)_3As{=}O$ which by expelling a disulfide, ArSSAr, are reduced to [ArS—As=O]. The latter, then, oxidatively gives As_2O_3 and more ArSSAr. Compounds of the type [RS—As=O], e.g., [(arsenate reductase)-cys-S—As=O], was postulated to hydrolytically give (arsenate reductase)-cys—SH and H_3AsO_3.[31] The enzyme prevents the oxidation of cystein to cysteine, while in our case such an inhibition is not possible and a disulfide, ArSSAr, is produced. In solvents containing methanol, the rate of the air oxidation generally increased probably because a zwitterion $(ArS)_3As^{+}\text{-}O{-}O^{-}$ was formed,[17] which, by hydrogen bonding with methanol, was more easily attacked[32] by another $(ArS)_3As$ to produce two molecules of $(ArS)_3As{=}O$. In moist solvents the water accelerates the air oxidation of some aromatic trithioarsenites probably because a pentacoordinated arsenic(V) intermediate, $(ArS)_3(HO)As{-}OOH$, is formed.

The other route of air oxidation of the trithioarsenites is the "hydrolysis-oxidation" one, in which stepwise hydrolysis (Eq. (5)) leads to $As(OH)_3$ and thiol:

$$\mathrm{(RS)_3As} \underset{\mathrm{RSH}}{\overset{\mathrm{H_2O}}{\rightleftharpoons}} \mathrm{(RS)_2As{-}OH} \underset{\mathrm{RSH}}{\overset{\mathrm{H_2O}}{\rightleftharpoons}} \mathrm{SR{-}As(OH)_2} \underset{\mathrm{RSH}}{\overset{\mathrm{H_2O}}{\rightleftharpoons}} \mathrm{As(OH)_3}. \quad (5)$$

The arsenous acid dehydrates to As_4O_6 while the thiol is oxidized by dioxygen to its disulfide:

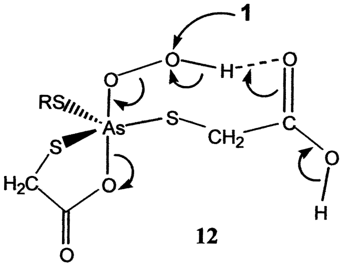
$$\mathrm{2RSH + 1/2O_2 \rightarrow RSSR + H_2O}. \quad (6)$$

This route, if followed at all, should be a minor one for aromatic trithioarsenites because thiols in a neutral environment are not autoxidized to their disulfides in the absence of radical initiators.[33] The autoxidation of thiols takes place in alkaline media because it is the anion RS^- which reacts with O_2 in the rate determining step, and it is faster in nonprotic than in protic solvents.[34]

The nonpolar liquid aliphatic trithioarsenites, mentioned in the introduction, e.g., $(RS)_3As$, $R = Et$, n-Pr, n-Bu,[13,14] are sensitive to air oxidation giving As_2O_3 and presumably RSSR.[14] Their autoxidation cannot be explained by the "hydrolysis-oxidation" route and, therefore, can go only by a diradical, $(RS)_3As^{\cdot}{-}O{-}O^{\cdot}$ or a arsenadioxirane, $(RS)_3As(O_2)$, intermediate. The hydroxyl-bearing liquid ester, $As(SCH_2CH_2OH)_3$, is "fairly stable" to air oxidation[15] and its autoxidation can proceed via an intermediate analogous to **12**.

A likely explanation of the oxidative decomposition of **1** is based on the formation of the hydroperoxy intermediate **12** having a 5-membered and an 8-membered structure. Nucleophilic attack by As(III) of another **1** on the hydroperoxy oxygen (see also ref. 32) will afford two $(RS)_3As{=}O$ molecules, which will decompose to As_2O_3 and RSSR as described above. The size of the ring system for **2** and **3** can explain the diminished rates of their air oxidation (Table II). The alternative "hydrolysis-oxidation" route cannot a priori be excluded, but the protic solvent and the absence of base to give the mercaptide ion[34] point towards a very slow autoxidation of the carboxy-bearing free thiols generated by Eq. (5).

The very small decomposition of the water-soluble **5** and **6** can be explained by the prevention of attack by As(III) of another **5** or **6** at the



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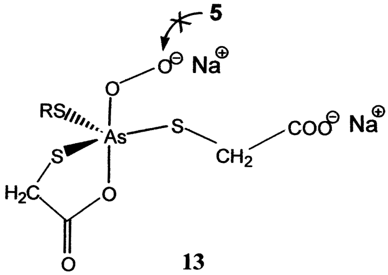

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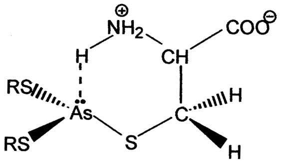

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negatively charged hydroperoxy oxygen as shown in **13**. The probable micellar structure of **7** in water makes the detailed understanding of its unreactivity more difficult.

The detection of free DL-penicillamine during the air oxidation of **9** implies that detachment of the penicillamine molecule had taken place according to Eq. (5). Then, the facile air oxidation of the penicillamine will give its disulfide. Overall, therefore, the data indicate that the binding of penicillamine to As(III) protects it from oxidation, and the air oxidation of **9** most likely proceeds via the "hydrolysis-oxidation" route. A very dilute suspension of **10** in water is not autoxidized, probably meaning that hydrolysis according to Eq. (5) does not take place and that the O_2 cannot coordinate to As(III) in **10**. This maybe due to hydrogen bonding of the $-NH_3^+$ group to As(III) as shown in **14**. Then, O_2 cannot displace the $-NH_3^+$ group, and therefore oxidative decomposition can not take place. The finding that aqueous solutions of **11** in air produce As_2O_3 and GSSG imply that **11** in water is most likely autoxidized by the "hydrolysis-oxidation" route and the intermediates, e.g., $(GS)_2As{-}OH$, are not hydrolysed to $As(OH)_3$.

Summarizing, the rate of oxidation of the biochemically interesting thiols in unbuffered aqueous solutions is penicillamine > cysteine > glutathione, and their trithioarsenites are air oxidized at a slower rate probably by the "hydrolysis-oxidation" route. Therefore, **9–11** themselves do not produce reactive oxygen species probably because the dioxygen binding is not favored.

EXPERIMENTAL

11-mercaptoundecanoic acid and 16-mercaptohexadecanoic acid were from Aldrich and were used without further purification. L-glutathione (reduced form) was from Serva. The trialkyl trithioarsenites **1**, **2**, **5**, **6**, and **9–11** were prepared according to Ioannou and colleaques.[5] Octasulfur was recrystallized from carbon disulfide.[35] AR grade solvents and solutions were deaerated by boiling, flushing with nitrogen, stoppering, and cooling to r.t.

Arsenic(III) oxide was detected by IR (sharp peak at 802–804 cm^{-1}).[26] IR spectra were obtained on a Perkin-Elmer model 16PC FT-IR spectrometer. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were run on a Bruker DPX Advance spectrometer. Elemental analyses were obtained through the Center of Instrumental Analyses, University of Patras, Patras, Greece.

Preparation of the Trithioarsenites 3, 4, 7, and 8

Tris(10-carboxydecyl) trithioarsenite, 3

A suspension of 11-mercaptoundecanoic acid (1.308 g, 6 mmol) and arsenic(III) oxide (198 mg, 1 mmol As_2O_3) in deaerated methanol (10 mL) was stirred at r.t. for 2 days. Centrifugation and washing with methanol (1 × 1.5 mL) gave the product, **3**, (1.334 g, 92%) as a white solid, insoluble in MeOH, Me_2CO, Et_2O, and petroleum ether (peth) and sparingly soluble in $CHCl_3$ and CS_2. M.p. 85–87°C. Calculated for $C_{33}H_{63}O_6S_3As$ (M_r726.94): C, 54.52; H, 8.74; S,13.23%; found; C, 54.10; H, 8.72; S, 13.53% IR (KBr): 3434 w, broad, 2916 vs, 2850 s, 1700 s, 1470 mw, 1432 mw, 1288 mw, 1256 w, 1230 w, 1106 w, 938 w, 720 w, 682 w.

Tris(15-carboxypentadecyl) trithioarsenite, 4

A suspension of 16-mercaptohexadecanoic acid (433 mg, 1.5 mmol) and arsenic (III) oxide (50 mg, 0.25 mmol As_2O_3) in deaerated methanol (10 mL) was stirred at r.t. for 2 days. Centrifugation and washing with methanol (1 × 1.5 mL) gave the product, **4**, (428 mg, 91%) as a white solid, insolube in MeOH, Me_2CO, $CHCl_3$ peth, and p and sparingly soluble in CS_2. M.p. 96–98°C. Calculated for $C_{48}H_{93}O_6S_3As$ (M_r 937.33): C, 61.50; H, 10.00; S, 10.26%; found: C, 61.84; H, 10.20; S, 10.36% IR (KBr): 3432 w, broad, 2918 vs, 2850 s, 1700 m, 1470 mw, 1436 w, 1292 w, 1254 w, 1216 w, 948 w, 718 w, 670 w.

Trisodium Salt of tris(carboxymethyl) trithioarsenite, 5

To a solution of the acid **1**.1/2H_2O (209 mg, 0.6 mmol) in methanol (5 mL) a solution of sodium hydroxide (72 mg, 1.8 mmol) in methanol

(1 mL) was added and stirred at r.t. for 4 h. Centrifugation and washing with methanol (1 × 1.5 mL) gave the salt **5** (210 mg, 85%), m.p. 240–241°C dec. (lit.[5] 238–240°C dec). IR (KBr): same as in ref. 5.

Trisodium Salt of tris(α-carboxyethyl) trithioarsenite, 6

To a solution of the acid **2** (195 mg, 0.5 mmol) in methanol (5 mL), a solution of sodium hydroxide (60 mg, 1.5 mmol) in methanol (1 mL) was added and stirred at r.t. for 1 h. The solution was concentrated until opalescence, warmed, transferred to a centrifuge tube and the product precipitated by adding acetone (6 mL). Washing with acetone (1 × 2 mL) and drying gave the salt **6** (234 mg, 92%), m. p. 212°C dec. (lit.[5] 210°C dec.) IR (KBr): same as in ref. 5.

Trisodium Salt of tris(10-carboxydecyl) trithioarsenite, 7

To a suspension of **3** (145 mg, 0.2 mmol) in methanol (5 mL) a solution of sodium hydroxide (24 mg, 0.6 mmol) in methanol (5 mL) was added and stirred at r.t. for 2 days. Concentration to a small volume, centrifugation of the fluffy solid, and washing with methanol (1 × 1.5 mL) gave the product **7** as a white solid, (137 mg, 86%). M.p. 254–259°C dec. It is insoluble in peth, CS_2, $CHCl_3$ Et_2O, Me_2CO, and slightly soluble in MeOH and H_2O. Calculated for $C_{33}H_{60}O_6S_3AsNa_3$ (M_r 792.89): C, 49.98; H, 7.63%; found; C, 49.70; H 7.60%. IR (KBr): 3422 mw, broad, 2918 s, 2848 vs, 1560 vs, 1470 m, 1442 m, 1422 m, 1300 w, 924 w, 718 w, 698 w.

Trisodium Salt of tris(15-carboxypentadecyl) trithioarsenite, 8

To a suspension of **4** (188 mg, 0.2 mmol) in methanol (5 mL) a solution of sodium hydroxide (24 mg, 0.6 mmol) in methanol (5 mL) was added and stirred at r.t. for 2 days. Centrifugation of the fluffy solid gave the product **8** as a white powder (194 mg, 96%), insoluble in MeOH, Me_2CO, $CHCl_3$, and H_2O. M.p. 257–264°C. dec. Calculated for $C_{48}H_{90}O_6S_3AsNa_3$ (M_r 1003.28): C, 57.50; H, 9.04%; found: C 57.27; H, 9.19%. IR (KBr): 3422 mw, broad, 2918 vs, 2850 vs, 1560 vs, 1468 m, 1440 ms, 1420 ms, 1300 w, 1260 w, 924 w, 718 mw.

Reaction of the Trithioarsenites 1–3 with S_8/Et_3N

A solution of **2** (98 mg, 0.25 mmol) and octasulfur (8 mg, 0.25 mmol sulfur atoms) in chloroform/methanol 1:1 (6 mL) was stirred in air at r.t. Octasulfur was always detected by TLC (silica gel, petroleum ether, R_f 0.80) and the solution gradually deposited a solid. After 24 h, centrifugation gave 9 mg of a yellowish solid, which, by IR, was octasulfur contaminated by traces of **2** and As_2O_3.

To a solution of **1**, **2**, or **3** (0.2 mmol) in chloroform/methanol 1:1 (5 mL), octasulfur (19 mg, 0.6 mmol sulfur atoms) was suspended. Triethylamine (6 μl, 0.04 mmol, 20 mol%) was added and the solution was stirred at r.t. for 24 h. A white suspension was formed which in ~1.5 h became yellow and in ~2 h an orange solid formed. Centrifugation gave the orange solid, which was insoluble in all solvents and decomposed by aqueous sodium hydroxide. To weighed samples of the solids containing known amounts of benzylarsonic acid (as internal standard), D_2O and solid NaOH were added, and the decolorized solutions were analyzed by ^{1}H NMR. The results are shown in Table I. Evaporation of the chloroform/methanol supernatant gave a solid, which was examined for As_2O_3 by IR. Data are also shown in Table I.

Reaction of the Trithioarsenites 5, 6 with S_8

To **5** or **6** (0.2 mmol) dissolved in water (1.5 mL) in a centrifuge tube, octasulfur (0.6 mmol sulfur atoms) was added and stirred in an oil bath at 110°C for 9 h. After cooling at r.t., centrifugation, washing with water (1.5 mL), acetone (1.5 mL), and carbon disulfide (1.5 mL) gave an orange solid insoluble in all solvents and decomposing in aqueous sodium hydroxide. The percentage of the RS groups in the solid was determined by ^{1}H NMR, as previously mentioned. The supernatants were combined, evaporated (rotary 50°C), and dried to a solid which was examined, by IR, for As_2O_3. Data are shown in Table I.

Air Oxidation of the Trithioarsenites 1–3

A solution of **1**, **2**, or **3** (0.5 mmol) in chloroform/methanol 1:1 (3 mL), was stirred in air for 4 days at r.t., and the solvent was replenished from time to time. The solution was evaporated and dried. An addition of acetone (3 mL) and centrifugation left pure As_2O_3 (by IR) from which the oxidative decomposition of the esters was calculated (Table II).

Air Oxidation of the Trithioarsenites 5–7

The ester **5** or **6** (0.25 mmol) dissolved (while **7** gave a light suspension) in water (4 mL) and was stirred at r.t. for 3 days. Then, acetone (40 mL) was added and centrifuged. The solid and the supernatant, after evaporation and drying, were analyzed by IR for As_2O_3. The results are shown in Table II.

When a 0.01 M aqueous solution of **7** was stirred at r.t. for 24 h an opalescence developed in 10 min and gradually a white solid formed. Freeze drying and IR (KBr) examination revealed that about half the —COONa groups have been protonated by ($CO_2 + H_2O$) giving the water insoluble **3**. As_2O_3 was not present in the solid.

Relative Rates of Air Oxidation of the Trithioarsenites 9–11 and Their Parent Thiols

A 0.01 M solution of **9** in water was stirred in air for 7 days. Evaporation (rotary, 50°C) and drying gave a solid which, by IR, had $<5\%$ As_2O_3. ^1H NMR of a saturated solution of the solid in D_2O showed the presence of disulfide and peaks due to free DL-penicillamine.[27,36] A 0.35 M solution of DL-penicillamine in water was air oxidized to its disulfide to an extent of 25% in 3 days and 41% in 7 days (^1H NMR analysis).

The ester **10** (10 mg) was suspended in water (6 mL) and stirred in air for 7 days. Evaporation and drying gave a white solid which, by IR, did not contain As_2O_3. A 0.4 M solution of cysteine in water stirred in air precipitated cystine corresponding to a 10% oxidation in 3 days and a 24% oxidation in 7 days.

A solution of **11** (25 mg) in water (1 mL) was stirred in air for 7 days. Evaporation and drying gave a solid which, by IR, contained $<2\%$ As_2O_3. The ^1H and ^{13}C NMR spectra of the solid in D_2O showed the presence of **11**[3,4,7] and 14% of glutathione disulfide. A 0.08 M solution of reduced glutathione in water that was stirred in air for 7 days was oxidized to glutathione disulfide to an extent of 13% (^1H NMR analysis).

Control experiments (solutions or emulsions of **9–11** and 10% by weight As_2O_3 were freezed-dried) showed that As_2O_3 was very clearly seen in their IR spectra.

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