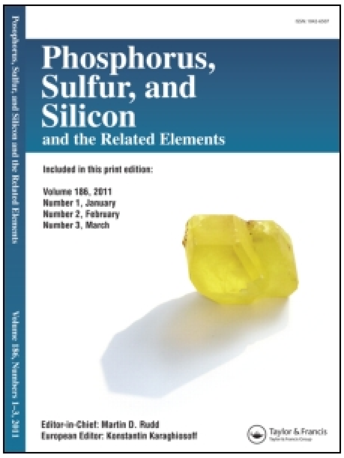

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REACTION OF ARSENIC(III) OXIDE, ARSENOUS AND ARSENIC ACIDS WITH THIOLS†

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Arsenic(III) oxide and arsenous acid in water or aqueous ethanolic solutions react, at room temperature, with a variety of lipophilic and hydrophilic thiols giving quantitatively triaryl and trialkyl trithioarsenites, $(ArS)_3As$ and $(RS)_3As$. Aqueous solutions of arsenic acid react with certain thiols giving quantitatively mixtures of trithioarsenites and disulfides, RSSR. These reactions may help towards the elucidation of the biochemistry of arsenous and arsenic acids.

Key words: Arsenic(III) oxide, arsenous acid, arsenic acid, triaryl trithioarsenites, trialkyl trithioarsenites.

INTRODUCTION

The reaction of arsonic and arsinic acids, $RAsO(OH)_2$, $R_2AsO(OH)$, with thiols, R'SH, to give $RAs(SR')_2$ and R_2AsSR', respectively[1] attracted wide interest because it was implicated in the mode of action of organoarsenic chemotherapeutic agents[2,3] and in the detoxification of arsenic via methylation-reduction steps.[4]

There are only a few isolated reports on the reaction of As_2O_3 and H_3AsO_3 with thiols. Thus, As_2O_3 reacted with thioglycolic acid[5] and thiophenol[6] and H_3AsO_3 reacted with glutathione,[7] cysteine and substituted thiophenols[8] to give trialkyl and triaryl trithioarsenites, $(RS)_3As$ and $(ArS)_3As$. These reactions did not attract[9] the preparative significance they deserved and the chemistry of trithioarsenites remains largely unexplored.[8-11] Finally, the literature[2,12,13] leaves one with the impression that arsenic acid is unreactive towards thiols, although its reduction by methyl thioglycolate[14] and glutathione[7] has recently been reported.

Herein we report on the reactions of As_2O_3, H_3AsO_3 and H_3AsO_4 with lipophilic and hydrophilic monothiols which have preparative significance and, probably, biochemical implications.

RESULTS AND DISCUSSION

Arsenic(III) oxide reacted in ethanolic or in aqueous solutions with lipophilic and hydrophilic thiols giving quantitatively thioesters of arsenous acid:

$$As_2O_3 + 6RSH \rightarrow 2As(SR)_3 + 3H_2O \quad (1)$$

When the product was insoluble, e.g. **1** and **9**, the supernatant gave negative

†Dedicated to the memory of Professor A. Galinos.

nitroprusside test[15] for free thiol but when the product was soluble, e.g. **4**, **5** and **10**, sampling gave positive nitroprusside test indicative of an equilibrium:

$$As(SR)_3 + H_2O \rightleftharpoons HOAs(SR)_2 + RSH \qquad (2)$$

The water soluble products (except **4**) were precipitated by adding acetone. The esters **1** and **4** · $1/2H_2O$ were previously obtained[5,6] by reacting As_2O_3 with the thiols without solvent.

The reaction of H_3AsO_3 with lipophilic thiols, e.g. thiophenol, 2-naphthalenethiol and ethyl thioglycolate was very fast and usually complete in 30 min. The reaction was pH dependent: with thiophenol as substrate an optimum pH $\sim$ 7 was found based on the yield of **1** (90–95%) while at pH 14 and 10 the yields were 8 and 61%, respectively. Since the pK_1 of H_3AsO_3 is 9.29[16] and that of aromatic thiols is 6–8[17] it seems that the reaction is favoured by the unionized forms of the reagents, Equation (3).

$$As(OH)_3 + 3RSH \rightarrow As(SR)_3 + 3H_2O \qquad (3)$$

This observation corroborates the mechanism proposed by Chadaeva *et al.*[11]

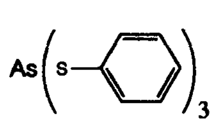
The stoichiometry of the reaction was found to be as in Equation (3) using excess PhSH. When excess As(III) was used the reaction did not stop at any intermediate compound, $(HO)_2AsSPh$ or $HOAs(SPh)_2$, but $As(SPh)_3$ was quantitatively (based on the PhSH used) obtained. The driving force for such a behaviour probably is the insolubility of the final ester, $As(SPh)_3$.

Arsenous acid did not react with methanolic solutions of 2-mercaptothiazoline, 2-mercapto-1-methylimidazole, and 2-mercaptobenzimidazole probably because the compounds are in their keto form.

The reaction of alkaline H_3AsO_3 with hydrophilic thiols, e.g. thioglycolic acid, 3-mercaptopropionic acid, L-cysteine, and L-glutathione, was analogous to that of As_2O_3 except that the sodium salts **5**, **7** and **11** (but see Reference 7), and the zwitterion, **8**, were obtained based on the pK values of the —COOH group(s)[18] for thioglycolic acid, cysteine and glutathione. The product with L-cysteine, **8**, precipitated during the reaction while the other products **5**, **7** and **11** were precipitated by acetone. The produce with DL-penicillamine, if formed after 1 h reaction, could not be precipitated.

With L-cysteine as substrate the yield of precipitated **8** was found to be pH dependent: optimum pH range was 8–4 (yields 88–77%) while at pH 12 and 10 the yields were 40 and 76%, respectively. At pH 7 the stoichiometry was found to be 1:3 and with excess As(III) the reaction did not stop at any intermediate compound. With excess L-cysteine 2 mM H_3AsO_3 gave visible needles after 1 h at RT.

All the prepared water soluble trithioarsenites, when dissolved in water, gave positive test for free thiol probably because of equilibrium (2). The trithioarsenites were not hygroscopic, or only slightly so, and (except **3**) were apparently stable towards oxidation by air. Compound **3** was very unstable in air giving As_2O_3 and the disulfide $(SCH_2COOEt)_2$. Such an instability was previously noted[9,19] for tripropyl and tributyl trithioarsenites while triaryl trithioarsenites are very stable.[19] The UV and IR spectra of **1** and **8**, respectively have been published[8] and the stretching frequencies in the IR of $(RS)_3As$ and $(ArS)_3As$ have been assigned.[19]



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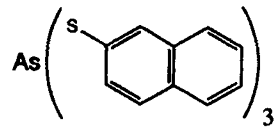


2

$As(SCH_2COOEt)_3$

3

$As(SCH_2COOH)_3$

4

$As(SCH_2COONa)_3$

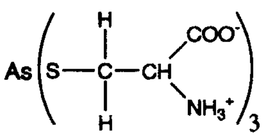
5

$As(SCH_2CH_2COOH)_3$

6

$As(SCH_2CH_2COONa)_3$

7



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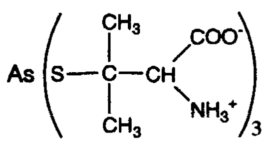

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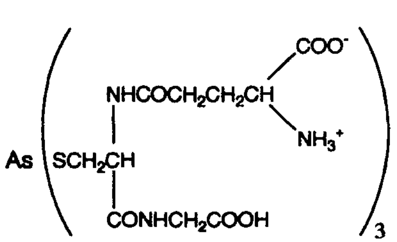

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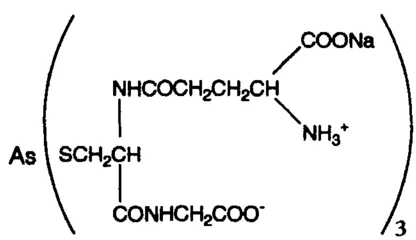

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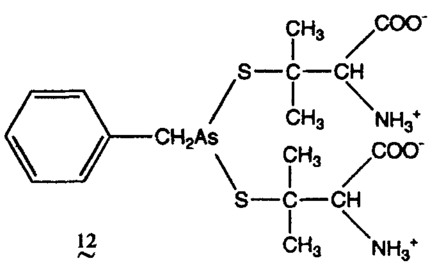

12

The AsS—C stretching in **1** and **2** was very strong, for **4** was medium strong, for **5**, **8** and **9** was very weak, while for **3**, **6**, **7**, **10** and **11** was too weak for positive assignment. The ^{1}H-NMR spectrum of **4** gave a sharp singlet at δ 3.75 clearly separated from the singlet of thioglycolic acid at δ 3.70. The ^{1}H-NMR spectrum of **10** has been published.[7]

The usual methods for the preparation of trithioarsenites is the reaction between $AsCl_3$ and RSNa or RSH + base (see Reference 9 for a summary of the methods used). The reaction of As_2O_3 or H_3AsO_3 with aliphatic and aromatic thiols in aqueous or alcoholic solutions is the simplest way to prepare trithioarsenites, and except for some isolated cases,[5-8] it has not been used. The yields are quantitative and the purity of the products excellent.

The reaction can be used for the separation of cysteine from other amino acids and derivatives, e.g. penicillamine or glutathione. Arsenous acid can be used as a blocking group for thiols. Deblocking can be achieved by pH adjustment (alkaline[8,15] or acidic[8]) while oxidative deblocking by O_2 at pH < 7,[8] or H_2O_2/H_2O or I_2/H_2O/organic solvent leads to disulfide and arsenic acid. The latter can be removed from aqueous solutions using magnesia mixture.

Kamai's group[9,10] has studied some reactions of trithioarsenites, and we observed that "transthiolation" of the water soluble trithioarsenites, e.g. **4** or **11**, takes place with a thiol which gives an insoluble product.

$$As(SR)_3 + 3PhSH \xrightarrow{H_2O} As(SPh)_3 \downarrow + 3RSH \tag{4}$$

Although thiophenol reacted "instantaneously" with H_3AsO_3, it reacted slower with AsO_4^{3-} according to:

$$H_3AsO_4 + 5RSH \rightarrow As(SR)_3 + RSSR + 4H_2O \tag{5}$$

The rate was pH dependent being slow at pH $= pK_3 = 11.53$, fast at pH $= pK_2 = 6.98$ and fastest at pH $= pK_1 = 2.22$.[20] This behaviour is in agreement with the statement[21] that arsenic acid is a weak oxidizing agent, but more strongly oxidizing in acid than in alkaline solution. Ethyl thioglycolate slowly reduced Na_2HAsO_4 (magnesia mixture test) in H_2O/EtOH 1:1. The reaction was slow because the NaOH which was produced at the early stages converted the Na_2HAsO_4 to the less reactive Na_3AsO_4. The slowness of the reductions of alkaline arsenic acid and the instability of $As(SCH_2COOMe)_3$ have previously been noted.[14]

Hydrophilic thiols, e.g. thioglycolic acid, 3-mercaptopropionic acid, L-cysteine and L-glutathione, quantitatively reduced arsenic acid (magnesia mixture test). The reduction at various initial pHs is best followed by ^{1}H-NMR because the signals due to trithioarsenite, e.g. **5** and **10**,[7] are clearly separated from those due to their corresponding disulfides. From the thiols examined penicillamine did not reduce Na_2HAsO_4 at 2:1 molar ratio after 2 h, while at 5:1 molar ratio 62% reduction occurred after 9 days. Penicillamine quantitatively reduced benzylarsonic acid to **12** after 7 days.

Mechanism for the reduction of arsonic acids to $RAs(SR')_2$ by thiols have been proposed by Barber,[22] Cohen *et al.*,[15] and Cullen *et al.*[23,24] They differ in the structure of the intermediate, being $RAs(OH)_2$, $RAs(SR')_4$ and $RAs(OH)_2$ via $RAs(SR')_2(OH)_2$, respectively.

For the reaction of arsenic acid with thiophenol and L-cysteine the reduction step, As(V) $\rightarrow$ As(III), was slower compared to the reaction of H_3AsO_3 with the same thiol as judged from the time required for the appearance of the insoluble products, **1** and **8**. The reduction step was also pH dependent, as discussed above, being faster in acidic solutions. The reaction does not stop at the H_3AsO_3 stage as judged from the reaction of Na_2HAsO_4 with PhSH at 1:2 molar ratio where an equimolar mixture of **1** and PhSSPh was produced (TLC analysis). This result differs from that obtained with glutathione[7] where the products were H_3AsO_3 and oxidized glutathione.

Arsenic acid or suitable arsenoxides can be used for site directed disulfide (intra-chain or inter-chain) formation in peptide synthesis.[25]

Our results may help towards the elucidation of some aspects of the poorly understood[4,7,26,27] biochemistry of arsenous acid (e.g. entrance into a cell may occur via transthiolation) and arsenic acid (e.g. unreactivity[3,13] towards enzymes may be due to isolated crucial —SH group(s)).

EXPERIMENTAL

2-Naphthalenethiol (Merck), thioglycolic acid, reduced L-glutathione free acid (Serva) and the other thiols (Aldrich) were used without further purification. Ethyl thioglycolate (b.p. 65°C/15 mm Hg) and sodium thioglycolate monohydrate[5] (m.p. 220°C dec.) were prepared from thioglycolic acid. Benzylarsonic acid was prepared from sodium arsenite and benzyl chloride.[28] Nitroprusside tests in buffered with saturated $NaHCO_3$ solutions gave cherry-red colour for all water soluble thiols except penicillamine which developed a blue-green colour in 30 s. Test for AsO_4^{3-} was done with magnesia mixture.[29] The % As was determined after wet digestion with conc. H_2SO_4 and H_2O_2.[30] TLC, using silica gel H (Merck), were run on microslides. Visualization was effected by I_2 vapors or by spraying with 35% H_2SO_4 and charring. IR and ^{1}H-NMR spectra were obtained on a Perkin Elmer model 16PC FT-IR and a Varian model T-60A spectrometers. Optical rotations were measured on a Schmidt and Haensch Polatronic Universal polarimeter using a 5 cm cell.

All reactions were done at room temperature. The products were dried in vacuo over P_2O_5.

Reactions of As_2O_3 with thiols

a) With thiophenol

As_2O_3 (0.5 mmol) reacted for 20 h with thiophenol (3 mmol) in 5 ml of 95% EtOH to give, **1**, (100%) as a white solid pure by TLC (petroleum ether, R_f 0.13). **1** is soluble in CH_2Cl_2, $CHCl_3$, CCl_4, AcOEt and insoluble in H_2O, EtOH, Et_2O and petroleum ether. M.p. 93–5°C [lit.[8,9] 95°C]. IR (KBr): 748 vs and 690 s. ^{1}H-NMR ($CDCl_3$) δ: 7.4 (broad s, C_6H_5).

b) With ethyl thioglycolate

As_2O_3 (0.5 mmol) dissolved completely after 20 h in a solution of 3 mmol of ethyl thioglycolate in 4 ml of absolute EtOH. Removal of the solvent gave an oil and traces of a white solid (As_2O_3 by IR) which were separated by dissolving the oil in Et_2O. The product **3** (91%) was a colourless oil, pure by TLC (Et_2O/hexane 1:1, R_f 0.60), soluble in Et_2O and $CHCl_3$. Nitroprusside test: weakly positive. IR: 2982 m, 1732 s, 1290 m, 1124 m, 1026 m. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (t, J = 7 Hz, 9H, CH_3), 3.60 (s, 6H, SCH_2), 4.25 (q, J = 7 Hz, 6H, CH_2CH_3). The ester **3** after 12 h at RT gave a solid (As_2O_3 by IR) and an oil which by TLC (Et_2O/hexane 1:1) was a mixture of **3** and $(SCH_2COOEt)_2$, R_f 0.75.

c) With thioglycolic acid

1.45 mmol of As_2O_3 and 8.70 mmol of thioglycolic acid in 5 ml of degassed H_2O were stirred for 48 h. The product did not precipitate by adding Me_2CO, MeCN or dioxane. Evaporation and drying gave **4** (98%) as a white amorphous hard solid which was recrystallized from boiling Et_2O by adding petroleum ether (98% recovery). **4** is soluble in Me_2CO, MeOH, H_2O, slightly soluble in boiling Et_2O and insoluble in $CHCl_3$, MeCN. Nitroprusside test: positive. M.p. 111–4°C. % As: found 21.28, calcd for $C_6H_9AsO_6S_3$ 21.51% and for $C_6H_9AsO_6S_3 \cdot 1/2H_2O$[5] 20.97%. IR(KBr): 3013 broad s, 1694 s, 1418 s, 1274 s, 1196 s, 898 m, 876 m, 776 m, 662 m. ^{1}H-NMR (D_2O) δ: 3.75 (s, 6H, CH_2).

d) With sodium thioglycolate

As_2O_3 (0.3 mmol) dissolved after 2 days stirring in a solution of 3 mmol of $HSCH_2COONa \cdot H_2O$ in 1 ml of degassed H_2O. The product **5** was precipitated (100%) by adding Me_2CO. **5** is soluble in H_2O and insoluble in Me_2CO, Et_2O, $CHCl_3$, MeOH, DMF and DMSO. Nitroprusside test: positive. M.p. 238–240°C dec. % As: found 18.08, calcd for $C_6H_6AsO_6S_3Na_3$ 18.10%. IR (KBr): 1584 s, 1400 s, 1224 m, 774 w. ^{1}H-NMR (D_2O) δ: 3.60 (s, 6H, CH_2).

e) With 3-mercaptopropionic acid

To 4.85 mmol of As_2O_3 a solution of 29.10 mmol of 3-mercaptopropionic acid in 30 ml of degassed H_2O was added and stirred for 2 days. The insoluble white product, **6**, was centrifuged and dried. Yield 91%. **6** is soluble in Me_2CO, Et_2O, MeOH and DMSO, slighty soluble in H_2O and insoluble in $CHCl_3$.

It dissolves in aqueous $NaHCO_3$ giving positive nitroprusside test. M.p. 102–3°C. %As: found 19.00, calcd for $C_9H_{15}AsO_6S_3$ 19.23%. IR (KBr): 3024 broad m, 1696 s, 1426 m, 1400 m, 1256 s, 1202 m, 920 m. ^{1}H-NMR (DMSO-d_6): 2.50(t, J = 6 Hz, 6H, CH_2COOH), 2.95, (t, J = 6 Hz, 6H, SCH_2).

f) With DL-penicillamine

To a solution of 3 mmol of DL-penicillamine in 10 ml of degassed H_2O 0.5 mmol of As_2O_3 was added, purged with N_2 and stirred for 7 days. Centrifugation, washing with acetone (1 × 4 ml) and drying gave the product **9** (80%) as a white solid. **9** is sparingly soluble in H_2O, MeOH, DMF and DMSO and soluble in warm DMSO (from which does not precipitate on cooling) and in aqueous $NaHCO_3$ giving positive nitroprusside test. M.p. 140–2°C dec. %As: found 14.18, calcd for $C_{15}H_{30}AsN_3O_6S_3$ 14.43%. IR (KBr): 2972 broad s, 1620 s, 1576 s, 1494 s, 1388 s, 1122 m, 778 w.

g) With L-glutathione

0.2 mmol of As_2O_3 dissolved in a solution of 1.2 mmol of reduced L-glutathione free acid in 4 ml of degassed H_2O after 4 days stirring under N_2. Addition of 20 ml of Me_2CO and leaving at +4°C for 48 h gave a sticky white precipitate. This was triturated with 20 ml of MeOH to give a crystalline solid (100%). The product, **10**, is soluble in H_2O and in warm DMSO, sparingly soluble in warm DMF and insoluble in MeCN, MeOH, Et_2O, $CHCl_3$ and Me_2CO. Nitroprusside test: positive. M.p. 147–150°C dec. %As: found 6.78, calcd for $C_{30}H_{48}AsN_9O_{18}S_3$ 7.54%. $[\alpha]_D^{20}$ − 10° (c 0.4, H_2O). IR(KBr): 3280 broad s, 1652 vs, 1532 vs, 1408 s, 1228 s. ^{1}H-NMR (D_2O) δ: 2.3 (m, 6H, glu β-CH_2), 2.6 (m, 6H, glu γ-CH_2), 3.4 (m, 6H, $AsSCH_2$), 4.1 (m, 12H, gly CH_2, glu CH, cys CH).

Reactions of H_3AsO_3 with thiols

a) With thiophenol

The pH of a solution of Na_3AsO_3 (0.25 mmol) in 1 ml of H_2O was adjusted to 8 (phenolophthalein) with HCl and a solution of thiophenol (0.75 mmol) in 1 ml of 95% EtOH was added. After 2 h, centrifugation, washing with H_2O (2 × 1 ml) and absolute ethanol (1 × 1 ml) gave the product, **1**, (93%), pure by TLC.

b) With 2-naphthalenethiol

Prepared as in **1** above in quantitative yield. It can be recrystallized from petroleum ether (100 ml/g): recovery 70%. The product, **2**, is soluble in CH_2Cl_2, $CHCl_3$, CCl_4, Et_2O, Me_2CO, sparingy soluble in petroleum ether and insoluble in EtOH and H_2O. TLC (petroleum ether, R_f 0.15). M.p. 89–91°C. % As: found 13.45, calcd for $C_{30}H_{21}AsS_3$ 13.57%. IR (KBr): 820 vs, 800 vs, 738 vs. ^{1}H-NMR ($CDCl_3$) δ: 7.8 (broad m, $C_{10}H_7$).

c) With ethyl thioglycolate

3 was obtained in 84% yield by the same procedure used to prepare **1**.

d) With thioglycolic acid

0.453 mmol of As_2O_3 and 2.718 mmol of NaOH were dissolved in 0.5 ml of H_2O and 2.718 mmol of $HSCH_2COOH$ in 0.3 ml of H_2O was added. Heat was evolved from the neutralization of the thioglycolic acid from the Na_3AsO_3. After 1 h Me_2CO (22 ml) was added to precipitate the product ($HSCH_2COONa$ is, however, precipitated under the same conditions), which after centrifugation gave the product as the sodium salt, **5** (97%).

e) With 3-mercaptopropionic acid

Prepared in 90% yield by the same procedure used to prepare **5**. The product **7**·H_2O is soluble in H_2O, MeOH and insoluble in Et_2O, Me_2CO. Nitroprusside test: positive. M.p. 210°C dec. %As: found 16.10, calcd for $C_9H_{12}AsO_6S_3Na_3\cdot H_2O$ 15.82%. IR (KBr): 3406 broad m, 1564 s, 1432 m, 1400 m, 1312 w, 1276 w, 668 w. ^{1}H-NMR (D_2O): 2.60 (t, J = 6 Hz, 6H, CH_2COONa), 3.05 (t, J = 6 Hz, 6H, SCH_2).

f) With L-cysteine

To an aqueous solution of Na_3AsO_3 (0.25 mmol/ml), adjusted to pH 8, an aqueous solution of L-cysteine (0.75 mmol/ml) was added. After 1 h, centrifugation and washing with H_2O (1 × 2 ml) and EtOH (1 × 2 ml), gave **8** (94%). The zwitterion, **8**, is soluble in 1 M NaOH and 0.5 M HCl, and insoluble in H_2O, EtOH, DMF, DMSO, Me_2CO, $CHCl_3$. Nitroprusside test: weakly positive. M.p. 238–240°C dec. [lit.[8] 235 and 245°C]. $[\alpha]_D^{20}$ +48° (c 0.5, 0.5 M HCl) [lit.[8] +38° (c 0.5, 0.5 M HCl)]. IR (KBr): similar to that published.[8]

g) With DL-penicillamine

Under the same conditions, as with cysteine, after 1 h reaction no precipitate was obtained by adding Me_2CO, MeOH or EtOH. When to this solution was added an equivalent amount of PhSH in EtOH 82% $(PhS)_3As$ was obtained.

h) With L-glutathione

To a solution of As_2O_3 (1 mmol) and NaOH (6 mmol) dissolved in 1 ml of H_2O a solution of 6 mmol of L-glutathione in 15 ml of H_2O was added and stirred for 2 h. Acetone (70 ml) was added and the system was left at +4°C for 2 days. The precipitated gum was triturated with 50 ml of MeOH and the white, crystalline solid weighed 2.239 g (100.5% as **11**$\cdot 3H_2O$). The product **11**$\cdot 3H_2O$ is soluble in H_2O and insoluble in MeCN, CH_2Cl_2, AcOEt, MeOH, DMSO and DMF. It does not precipitate from H_2O with MeCN, DMSO or DMF. Nitroprusside test: positive. M.p. 183–5°C dec. $[\alpha]_D^{20}$ +10° (c 0.4, H_2O). %As: found 6.59, calcd for $C_{30}H_{42}AsN_9O_{18}S_3Na_3 \cdot 3H_2O$ 6.73%. IR (KBr): 3444 broad s, 1646 s, 1595 s, 1538 s, 1402 m. Its ^{1}H-NMR spectrum resembled that published.[7]

Transthiolations

When an aqueous solution of **5** or **11** was treated with 3 mol of thiophenol per mole of ester in EtOH, **1** was obtained in 85–90% yields. When 0.5 ml of a 0.2 M aqueous solution of **10** was treated with 0.5 ml of a 0.6 M aqueous solution of cysteine no precipitation of **8** was observed after 15 min at RT. Addition of 0.3 mmol thiophenol in EtOH gave after 15 min at RT 71% of **1**.

Reactions of H_3AsO_4 with thiols

a) With thiophenol

Using 5 mol of PhSH in EtOH per mole of H_3AsO_4 at pH equal to pK_1, pK_2 and pK_3 of H_3AsO_4 we obtained, after 24 h, a mixture of the products [**1** + PhSSPh] in yields of 76, 64 and 61%, respectively. TLC (petroleum ether: $(PhS)_3As$ R_f 0.20, PhSSPh R_f 0.59). With 2 mol of PhSH per mole Na_2HAsO_4 after 4 h at RT a mixture of **1** plus PhSSPh was obtained.

b) With ethyl thioglycolate

Na_2HAsO_4 (0.25 mmol) in 1 ml of H_2O and 1.25 mmol of ethyl thioglycolate in 1 ml of absolute EtOH were stirred for 2 days. Centrifugation gave a white solid (40 mg) which was a hydrated salt of arsenic acid (0.17 mmol AsO_4^{3-} by magnesia mixture precipitation) and a supernatant from which 52 mg of **3** plus $(SCH_2COOEt)_2$ were isolated as a colorless oil.

c) With thioglycolic acid

Na_2HAsO_4 was quickly (1–2 h) and quantitatively reduced by thioglycolic acid in D_2O (1:5 molar ratio) giving **5** (singlet at δ 3.70) and disulfide (singlet at δ 3.60) while $HSCH_2COONa$ gave a singlet at δ 3.35. No precipitate with magnesia mixture was obtained when the reduction was done at initial pH 2.2, 7.0 and 11.5 for 24 h.

d) With 3-mercaptopropionic acid

3-Mercaptopropionic acid quantitatively reduced AsO_4^{3-} (magnesia mixture test) at initial pH 2.2, 7.0 and 11.5 in 24 h. At pH 2.2 the products, **6** and $(SCH_2CH_2COOH)_2$, were quantitatively precipitated while at pH 7.0 and 11.5 we obtained less than the expected weight because of the solubility of salts of **6** and $(SCH_2CH_2COOH)_2$.

e) With L-cysteine

L-Cysteine and arsenic acid reacted as in the case of 3-mercaptopropionic acid.

f) With DL-penicillamine

DL-Penicillamine slowly reduced Na_2HAsO_4 in D_2O (5:1 molar ratio). The singlet (δ 3.70) diminished and a new singlet appeared at δ 3.68. The singlet at δ 1.55 moved to 1.65, the singlet at δ 1.45 diminished and a new singlet appeared at δ 1.40. After 9 days magnesia mixture test showed 62% reduction had taken place.

g) With L-glutathione

Using 1.25 mmol of reduced glutathione monosodium salt per 0.25 mmol of H_3AsO_4, after 3 h reaction addition of acetone precipitated 429 mg (95%) of a mixture of products (**11**$\cdot 3H_2O$ and hydrated oxidized glutathione sodium salt), $[\alpha]_D^{20}$ −35° (c 0.4, H_2O).

Reaction of benzylarsonic acid with DL-penicillamine

To a solution of 2 mmol of DL-penicillamine in 7 ml of degassed H_2O 0.5 mmol of benzylarsonic acid was added, sealed under N_2 and stirred for 7 days. The solid arsonic acid slowly dissolved giving the product **12** as a white solid. Centrifugation and washing with acetone gave a solvate which after drying gave the product **12**$\cdot 2H_2O$ as a white powder (232 mg, 94%). The product is insoluble in H_2O and MeOH, sparingly soluble in DMF and moderately soluble in DMSO. M.p. 175–8°C dec. %As: found 14.79, calcd for $C_{17}H_{27}AsN_2O_4S_2 \cdot 2H_2O$ 15.06%. IR (KBr): 3416 broad s, 3062 s, 2964 s, 1634 vs, 1508 vs, 1384 vs, 1334 s. The supernatant after evaporation and drying gave 170 mg of a white solid [mp 143–5°C dec., IR (KBr): qualitatively similar to **12**$\cdot 2H_2O$] which is hydrated disulfide of penicillamine.

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