^{13}C NMR Spectral Analysis of Mono and Diphenylarsine Adducts of Glutathione in DMSO

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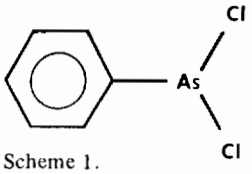
Phenyldichloroarsine (Scheme 1; ϕ–As(Cl)$_2$) is a vesicant analog of Lewisite [1]. Organic arsenicals are known to deactivated sulfhydryl containing enzymes because of their ability to react with the essential thiol groups of those enzymes [4–7].

We have recently shown that ϕ–As(Cl)$_2$ can be absorbed by erythrocytes and that it probably reacts with internal glutathione (GSH) [2, 3]; the structure of the adduct is depicted in Fig. 1. Related compounds such as diphenylchloroarsine (Scheme 2; ϕ_2–As(Cl)) may be of interest to determine whether



Scheme 1.

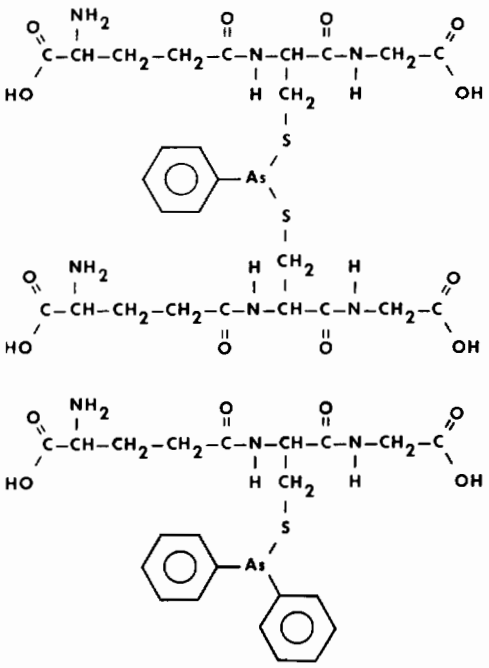


Fig. 1. Primary structures of ϕ–As(GS)$_2$ and ϕ_2–As(GS).

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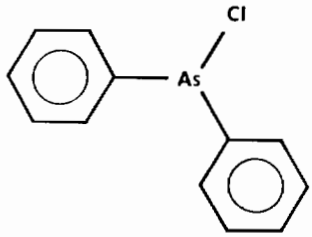

Scheme 2.

the addition of another bulky group on the arsenic atom has any effect on the reactivity and stability of the organic arsenical or a given adduct of that arsenical.

In this article we present ^{13}C chemical shift data for ϕ–As(Cl), ϕ–As(Cl)$_2$, and for their adducts with glutathione. These data were determined for the samples in DMSO because in the course of the study we found that ϕ_2–As(GS) had limited solubility in H_2O and that it was unstable under these conditions. This article also represents the first reported synthesis of ϕ_2–As(GS).

Experimental

Materials

GSH was purchased from Sigma Chemical, Co., St. Louis, Mo. ϕ–As(Cl)$_2$ was purchased from Research Organic/Inorganic Chemical Corp., Sun Valley, Calif., and purified as described earlier [2]. ϕ_2–As(Cl) was obtained from Pfaltz and Bauer, Inc., Waterbury, Conn. and used without further purification. All solvents used were reagent grade or better.

Methods

ϕ_2–As(GS) and ϕ–As(GS)$_2$ were prepared using identical methods [2, 8]. The respective arsenical was dissolved in absolute ethanol and to the solution a quantitative amount of GSH was added. The reaction was further stirred for 1 h and the ethanol was removed under a stream of nitrogen. The residue was used for NMR studies. NMR samples were prepared by either dissolving ϕ_2–As(Cl) or ϕ–As(Cl)$_2$ in DMSO or by taking up the respective organic arsenical–glutathione adduct in DMSO.

Proton-decoupled, natural abundance ^{13}C NMR spectra of the various compounds were recorded on either a JEOL FX-90Q or an IBM NR 200. Chemical shifts are given downfield relative to Me_4Si.

Results and Discussion

Table I gives the ^{13}C chemical shift data for ϕ_2–As(Cl), ϕ–As(Cl)$_2$, GSH, ϕ_2–As(GS), and ϕ–As(GS)$_2$ in DMSO. These samples were run in DMSO

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TABLE I. ^{13}C Chemical Shift Data for Mono and Diphenylarsine Adducts of Glutathione[a]

Carbon atoms	Compounds				
	ϕ_2–As(Cl)	ϕ–As(Cl)$_2$	GSH	ϕ_2–As(GS)	ϕ–As(GS)$_2$
Aromatic	141.9	147.8		139.1	138.1
	131.2	129.1		131.6	131.8
	129.0	128.5		128.2	129.8
	127.9	127.0			129.0
Cys C^{α}			54.7	54.6	53.9
Glu C^{α}			52.7	52.9	51.8
Gly C^{α} [b]			–	–	–
Glu C^{β}			26.2	26.2	26.0
Cys C^{β}			25.7	32.6	33.3
Glu C^{γ}			30.9	31.3	31.0

[a]All samples were recorded in DMSO. Sample concentrations were ~50 mg/ml and the spectra were recorded with about ~1500 scans using block averaging. [b]Overlapped with the DMSO peak.

because of the limited solubility of the organic arsenicals and ϕ_2–As(GS) in H_2O and because we found that in time ϕ_2–As(GS) decomposed in H_2O (NMR spectral changes were noticed overnight) but appeared to be stable in DMSO. We have previously synthesized ϕ–As(GS)$_2$ and reported its chemical shift data in H_2O (pH ~7.0) [2, 3]. In that case the C^{α} and C^{β} resonances of the Cys residue were split into doublets indicating a chemical shift nonequivalence for the two attached glutathione residues. This phenomenon was not observed for the sample in DMSO and this could be partially attributed to the lower field strength of the instruments used. There was no attempt to adjust the pH of the samples although when samples were either dissolved or suspended in H_2O the pH was found to be less than 2, indicating that peptide samples were probably fully protonated.

Compound ϕ_2–As(GS) has never been synthesized before; we believe that we do indeed have this compound on the basis of the carbon chemical shift data and on the basis of the integration data. Formation of ϕ_2–As(GS) adduct appears to produce about a 2 ppm upfield shift for the nonprotonated aromatic carbon atom attached to As and a 6.9 ppm downfield shift for C^{β} of Cys (Table II). Integration of the aliphatic carbon atoms of the attached glutathione molecule (excluding Gly C^{α}) relative to carbon atoms of the phenyl rings of ϕ_2–As(GS) indicate a ratio of 2.3/1 for ϕ and GS, respectively.

The ^{13}C chemical shift data in Table I and the ^{13}C chemical shift difference data in Table II indicate that there appears to be little difference in the ^{13}C chemical shifts for glutathione attached to the mono and diphenylarsine derivative. On the other hand, the aromatic regions for the mono and diphenyl derivatives do exhibit some unusual chemical shift differences. We are unable to explain why a large stability difference exists between these two glutathione adducts, aside from the obvious steric factors.

TABLE II. ^{13}C Chemical Shift Differences for the Carbon Atoms of ϕ_2–As(GS) and ϕ–As(GS)$_2$ and Glutathione

Carbon atom	ϕ_2–As(GS) – GSH	ϕ–As(GS)$_2$ – GSH
Cys C^{α}	−0.1	−0.2
Glu C^{α}	+0.2	−0.1
Glu C^{β}	0.0	−0.2
Cys C^{β}	+6.9	+7.6
Glu C^{γ}	+0.4	+0.1

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