Carbohydrate Research, 196 (1990) 141–146
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

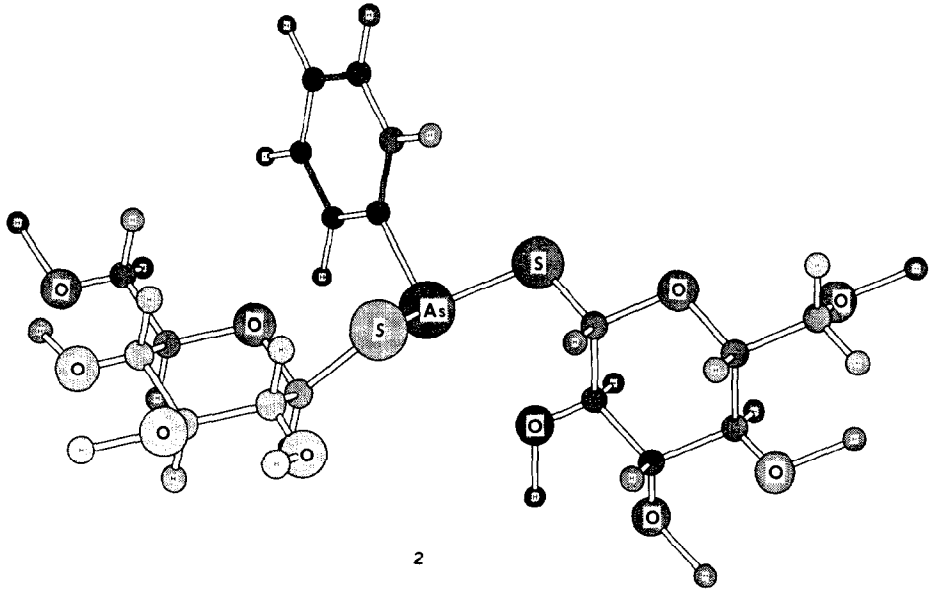
Preparation, structure, and solution dynamics of phenyldichloroarsine–thio sugar adducts

KILIAN DILL, SUNGHO HU,
Department of Chemistry, Clemson University, Clemson, SC 29634 (U.S.A.)

RICHARD J. O'CONNOR, AND EVELYN L. MCGOWN
Chemistry Branch, Biophysical Research Division, Letterman Army Institute of Research, Presidio of San Francisco, CA 94129 (U.S.A.)

(Received April 12th, 1989; accepted for publication, July 15th, 1989)

Metal-ion derivatives of 1-thio-β-D-galactose and 1-thio-β-D-glucose have been used or tested for medicinal purposes[1–4]. The most common derivative, auranofin, the gold derivative of 1-thio-β-D-glucose, has undergone worldwide testing and has been used therapeutically against rheumatoid arthritis[1–3,5]. The drug unfortunately has some serious clinical drawbacks[1–3,5]. More recently, copper derivatives of 1-thioglucose were synthesized and tested for anti-inflammatory activity[4]. Also, dimethylarsinous acid ester derivatives of 1- and 6-thiogalactose,



2

0008-6215/90/$ 03.50

and 1- and 6-selenogalactose have been synthesized and tested as potential carcinostatic agents[6,7].

Trivalent arsenic reacts preferentially with sulfhydryl groups and the most-stable adducts formed are those with molecules which contain vicinal sulfhydryl groups. These 1:1 adducts are the basis for several recent antidote structural studies[8–10]. In order to study further the interactions between trivalent organic arsenicals and biologically relevant molecules, we investigated the structure and solution dynamics of the 1:2 adducts formed between phenyldichloroarsine (PDA) and the two thio sugars, 1-thio-β-D-glucopyranose and 1-thio-β-D-galactopyranose (**1** and **2**).

EXPERIMENTAL

Materials and methods. — 1-Thio-β-D-glucose (Na^+ form) and 1-thio-β-D-galactose (Na^+ form) were purchased from Sigma. Methanol-d_4 and deuterium oxide were obtained from Merck Sharpe & Dohme. Phenyldichloroarsine was purchased from Research Organic/Inorganic Chemical Co., Sun Valley, CA, and purified by vacuum distillation.

The thio sugar–PDA adducts were prepared by reacting each sugar with PDA in a 2:1 ratio according to methods developed in our laboratories for other arsenical adducts[11–13]. The thio sugars (214 mg) were added to 4 mL of methanol-d_4 in a 10-mm n.m.r. tube to form a suspension. To this, 65 μL of PDA (d = 1.65 g/mL) was added and the mixture thoroughly mixed until all of the components had reacted, resulting in the dissolution of the solid. The sample was degassed with gaseous N_2 and a ^{13}C-n.m.r. spectrum was recorded in order to determine the extent of the reaction. The methanol-d_4 was then removed under vacuum and replaced with D_2O. The pH* (uncorrected meter reading) of each sample was adjusted to neutrality with NaOD or DCl and the solvent removed under vacuum and then replaced with D_2O to ensure the near-total removal of all exchangeable protons from the sample. These methods were necessary because of the very limited solubility of phenyldichloroarsine in aqueous media; we have found that the maximum solubility is ~20 mM, and in order to achieve saturation, the sample must be heated and stirred over a long period of time. Pertinent analytical information was also obtained for these compounds from the aromatic and anomeric regions of the ^1H-n.m.r. spectra taken in methanol-d_4. **1**: 7.85 (2 H), 7.44 (1 H), and 7.42 (2 H); 4.75 (1 H; $^3J_{H\text{-}H}$ = 9.15) and 4.68 (1 H; $^3J_{H\text{-}H}$ = 8.15). **2**: 7.72 (2 H), 7.37 (1 H), and 7.28 (2 H); 4.81 (1 H; $^3J_{H\text{-}H}$ = 7.91) and 4.70 (1 H; $^3J_{H\text{-}H}$ = 9.22).

The proton-decoupled, natural abundance ^{13}C-n.m.r. spectra of the thio sugar–PDA adducts were recorded with a Varian XL-300 spectrometer operated at 75.4 MHz, using 10-mm sample-tubes at 30°. The data were collected in 4992 addresses (using a window of 16 502 Hz) with a recycle time of 5 s. Spin–lattice relaxation times (T_1 values) were determined using the partially relaxed Fourier-transform method with 11 τ values. An exponential fit of the data was performed using

the Varian programs. All spectra are referenced to Me_4Si using the reported chemical shifts of C-6 of the sugar moieties as an internal standard[14].

RESULTS AND DISCUSSION

Figure 1 shows the proton-decoupled, natural-abundance ^{13}C spectra of the thio sugar–PDA adducts. Table I contains the ^{13}C-chemical shift data for these adducts, as well as the respective normalized spin–lattice relaxation time data (NT_1). Assignments in the spectra were based on comparison of our data with data

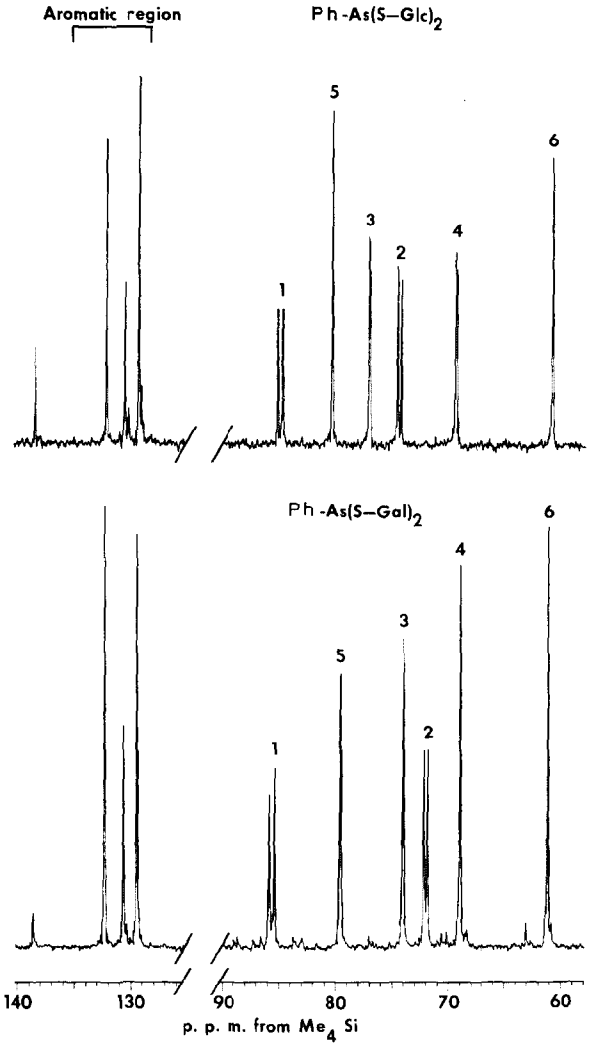


Fig. 1. Proton-decoupled, natural-abundance ^{13}C-n.m.r. spectra of **1** and **2**. Concentrations of both samples were 273 mM in D_2O at neutral pH*. [A] Spectrum required 500 transients. [B] Spectrum required 512 transients.

published by Berman *et al.*[14] for various thio sugars. Upon scrutinizing the intensities of the resonances in Fig. 1, it becomes obvious that the adducts contain 2 sugars/PDA. Additional spectral evidence also confirms the molar ratio of the adduct: (*a*) The extreme differences in the linewidths[15] and small, but substantial, differences in the chemical shifts of "free PDA" and PDA involved in the adduct, (*b*) the chemical shift differences for C-1 and C-2 of the thio sugars involved in the adducts when compared to the solution chemical shifts of the nonreacted species[14], and (*c*) the fact that we observe predominantly the ^{13}C resonances of a single species in our spectra.

There are several spectral features in Fig. 1 which merit discussion. One is that the chemical shift of C-1 of the sugar molecules is about 20 p.p.m. upfield, compared to what is known for the oxy counterparts[14,16]. The second is that, for the two bound thio sugars, the carbon atoms closest to the arsenic atom (C-1 and C-2) exhibit a chemical-shift nonequivalence. This can only result because of the development of a chiral center at the arsenic atom (it also contains a lone pair of electrons) through hindered rotation of the sugars about the As–S–anomeric bond.

The NT_1 data obtained can provide qualitative and quantitative information about the dynamics of the molecule in solution. This assumes that a model for the molecular motion may be determined and an equation used to evaluate the data. Table I clearly shows that the NT_1 values of the various carbon atoms are, in some cases, vastly different. For instance, compare the NT_1 values of the *p*-carbon atoms with the *m*- and *o*-carbon atoms of the phenyl ring. Furthermore, in the case of compound **2**, the NT_1 value of C-6 is substantially different from the NT_1 values of the other pyranose carbon atoms of **2**.

TABLE I

CARBON-13-N.M.R. CHEMICAL SHIFT DATA[a] AND SPIN–LATTICE RELAXATION TIME DATA[b] FOR COMPOUNDS **1** AND **2**

Carbon atom	*Compound*	
	1	**2**
1	86.6 (0.55)	87.1 (0.58)
	86.2 (0.56)	86.6 (0.57)
2	76.1 (0.56)	73.4 (0.59)
	75.8 (0.55)	73.3 (0.58)
3	78.6 (0.57)	75.3 (0.58)
4	71.0 (0.57)	70.3 (0.49)
5	81.8 (0.58)	80.8 (0.57)
6	62.3 (0.64)	62.4 (0.96)
Phenyl[c] carbons	139.9	139.9
	133.7 (1.14)	133.6 (1.19)
	132.1 (0.40)	131.9 (0.44)
	130.8 (1.08)	130.7 (1.16)

[a]Sample concentrations were 273 mM in D_2O near neutral pH*. [b]NT_1 values given in seconds within parentheses. [c]NT_1 values are given only for the protonated carbon atoms.

In order to evaluate our data quantitatively, the following model for the motion of our molecules is proposed. The overall motion of the molecule is considered to be isotropic. There is substantial internal motion about As–phenyl bond, but there is only one degree of freedom and this can be simply modeled. Thus, the *p*-carbon atom of the phenyl group truly reflects the overall correlation-time of the molecule. There is limited motion about As–S–sugar bond. This is based on the NT_1 values of the pyranose carbon atoms (very similar to the NT_1 value of the phenyl ring) and on the chemical-shift differences observed for carbohydrate carbon atoms of these adducts (see later).

For protonated carbon atoms at this field strength (7.0 T), the dipolar relaxation-mechanism should dominate the spin–lattice relaxation time[17]. Furthermore, such a small molecule should readily fall into the extreme-narrowing range-condition of equations depicting T_1-relaxation. Under these two conditions, the following equation is applicable for the analysis of our results:

$$(T_1^{DD})^{-1} = \hbar^2 \gamma_C^2 \gamma_H^2 N r_{CH}^{-6} (\tau_R)$$

In this equation, T_1^{DD} is the spin–lattice relaxation time, which is dominated by a dipolar mechanism. It is a function of nuclear constants, carbon–hydrogen distances (dipole–dipole interactions), the number of directly bonded hydrogen atoms (N), and the overall molecular reorientation time of the molecule (τ_R). The use of this equation indicates that the overall molecular correlation time (τ_R) of this complex is 1.11×10^{-10} s at 30°. Using the relationship $\tau_R = (6D_{iso})^{-1}$, the isotropic diffusion constant (D_{iso}) was determined to be 1.50×10^9 s^{-1}.

The T_1 data for the phenyl group indicates that there is substantial movement of the phenyl ring about the phenyl–arsenic bond. The movement would not affect the relaxation time of the *p*-carbon atom of the phenyl ring, but would affect the *o*- and *m*-carbon atoms because of their movement about the rotational axis. Such motions have been previously correlated, using equations that relate the overall molecular motions, the observed T_1 values, and the geometry of the molecule. The following equation may be used to assess the motion of the phenyl ring[18–20]:

$$(T_1^{DD})^{-1} = \hbar^2 \gamma_C^2 \gamma_H^2 N r_{CH}^{-6} (\tau_R)[A + 6B/(6 + \rho) + 6C/(6 + 4\rho)]$$

For this equation, $\rho = D_{int}/D_{iso}$, $A = 0.25\,(3\cos^2\theta - 1)^2$, $B = 0.75\,(\sin^2 2\theta)$, and $C = 0.75\,(\sin^4\theta)$. D_{int} represents the internal diffusion constant and θ is the angle between the C-H bond in question and the axis of internal rotation. A solution of this equation using the average parameters given in Table I and the determined τ_R and D_{iso} values yield a D_{int} value of 9.63×10^9 s^{-1}. This value then indicates that the phenyl ring is rotating about the As–phenyl axis ~6.4 times faster than the overall tumbling rate of the molecule. The value determined in this study for the thio sugar–arsenical adducts is slightly larger than those determined for the motions of phenyl rings in other phenyl-containing dithiarsolanes[18] and diarsolanes[19] and

also for diphenyl dichalcogen[20]. Our results indicate that the overall tumbling of this molecule is retarded by the presence of the carbohydrate residues, possibly because of the hydrogen bonding with water molecules, which could provide additional frictional drag.

As mentioned earlier, the NT_1 value of C-6 of compound **2** is substantially larger than the NT_1 values of the other pyranose carbon atoms of **2**. Such a larger NT_1 value could easily be explained on the basis of fast internal motion about the C-5–C-6 bond. This does not appear to be the case for compound **1**. The only rationale for such a result must be an interaction between O-6 and another oxygen atom on the pyranose ring either through an internal hydrogen-bond or mediated through a water molecule.

ACKNOWLEDGMENT

K. D. thanks the National Research Council for their financial support [Senior Research Award 1988–89].

REFERENCES

1 J. C. DELAFUENTE AND T. G. OSBORN, *Clin. Pharm.*, 3 (1984) 121–127.
2 P. DAVIS, *Clin. Rheum. Dis.*, 10 (1984) 369–383.
3 K. L. BLOCKA, H. E. PAULUS, AND D. E. FURST, *Clin. Pharmacokinet.*, 11 (1986) 133–143.
4 L. GARUTI, M. ROBERTI, G. GIOVANNINETTI, R. GAGG, J. DEFAYE, AND H. DRIGUEZ, *Pharm. Acta Helv.*, 63 (1988) 202–205.
5 M.-L. HU, C. J. DILLARD, AND A. L. TAPPEL, *Agent Actions*, 25 (1988) 132–138.
6 J. R. DANIEL AND R. A. ZINGARO, *Phosphorus Sulfur*, 4 (1978) 179–185.
7 J. R. DANIEL AND R. A. ZINGARO, *Carbohydr. Res.*, 64 (1978) 69–79.
8 K. DILL, E. R. ADAMS, R. J. O'CONNOR, AND E. L. MCGOWN, *Magn. Reson. Chem.*, 25 (1987) 1074–1077.
9 R. J. O'CONNOR, E. L. MCGOWN, K. DILL, AND S. F. HALLOWELL, *Magn. Reson. Chem.*, 27 (1989) 669–675.
10 V. L. BOYD, J. W. HARBELL, R. J. O'CONNOR, AND E. L. MCGOWN, *Chem. Res. Toxicol.*, 2 (1989) 301–306.
11 K. DILL, E. R. ADAMS, R. J. O'CONNOR, S. CHONG, AND E. L. MCGOWN, *Arch. Biochem. Biophys.*, 257 (1987) 293–301.
12 K. DILL, R. J. O'CONNOR, AND E. L. MCGOWN, *Inorg. Chim. Acta*, 138 (1987) 95–97.
13 K. DILL, E. R. ADAMS, R. J. O'CONNOR, AND E. L. MCGOWN, *Chem. Res. Toxicol.*, 2 (1989) 181–185.
14 E. BERMAN, M. E. DAMAN, AND K. DILL, *Carbohydr. Res.*, 116 (1983) 144–149.
15 R. J. O'CONNOR, E. L. MCGOWN, E. R. ADAMS, AND K. DILL, *USAMRDC Letterman Army Inst. Rep.*, no. 343 (1988).
16 K. DILL, E. BERMAN, AND A. A. PAVIA, *Adv. Carbohydr. Chem. Biochem.*, 43 (1985) 1–49.
17 R. S. NORTON, A. O. CLOUSE, R. ADDLEMAN, AND A. ALLERHAND, *J. Am. Chem. Soc.*, 99 (1977) 79–83.
18 D. W. AKSNES AND T. A. HOLAK, *Org. Magn. Reson.*, 17 (1981) 285–289.
19 D. W. AKSNES AND K. RAMSTAD, *Org. Magn. Reson.*, 23 (1985) 253–258.
20 M. BALDO, A. FORCHIONI, K. J. IRGOLIC, AND G. C. PAPPALARADO, *J. Am. Chem. Soc.*, 100 (1978) 97–100.