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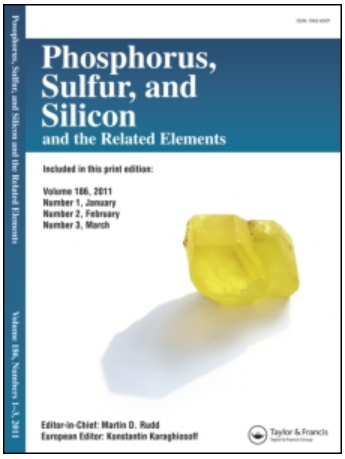

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DIMETHYLARSINOUS ACID ESTERS OF 1-THIO- AND -SELENOGALACTOSE. A NEW CLASS OF POTENTIAL CARCINOSTATIC AGENTS

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DIMETHYLARSINOUS ACID ESTERS OF 1-THIO- AND -SELENOGALACTOSE. A NEW CLASS OF POTENTIAL CARCINOSTATIC AGENTS

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The syntheses of 2,3,4,6-tetra-*O*-acetyl-1-*S*-dimethylarsino-1-thio-β-D-galactopyranose (**4**), 1-*S*-dimethylarsino-1-thio-β-D-galactopyranose (**5**), and 2,3,4,6-tetra-*O*-acetyl-1-*Se*-dimethylarsino-1-seleno-β-D-galactopyranose (**8**) are reported. An attempted preparation of 1-*Se*-dimethylarsino-1-seleno-β-D-galactopyranose (**10**) is also described. The compounds were characterized by nmr, uv, and mass spectroscopy, as well as elemental analysis. The ^{1}H nmr spectra of these compounds were not exceptional except for the slight down-field shift of the $-AsMe_2$ resonance noted in the selenium derivative as compared to the sulfur compound. Ultraviolet spectra of these compounds showed a relatively intense absorption which can be attributed to an $n \rightarrow \sigma^*$ transition associated with the As–X (X = S, Se) bond. The λ_{max} for the Se compounds show a slight red shift relative to analogous S compounds. The mass spectra of these compounds showed immediate loss of the aglycon, $-XAsMe_2$, in all cases. Other fragments can be ascribed to subsequent fragmentation of the sugar portion of the molecule. Biochemical testing was carried out on compounds **4** and **5** and both displayed carcinostatic activity against mouse lymphocytic leukemia. A hypothesis is advanced to account for the observed carcinostatic action of dimethylarsino compounds as a group.

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INTRODUCTION

In recent years much interest has been focused on the synthesis and characterization of chalcogen-containing biomolecules, i.e., sulfur- and selenium-containing amino acids,[1] proteins,[2] purines,[3] and pyrimidines.[4] These compounds are interesting not only from a synthetic point of view, but also because of their novel biological properties. The importance of sulfur, present as a sulfhydryl or disulfide moiety in proteins or enzymes cannot be overstated. The thiol group occurs at the active site of a number of enzymes[5] and the disulfide group is important in maintaining tertiary protein conformation.[6] Selenium has also attracted much attention since the discovery of the Se-containing enzyme, glutathione peroxidase.[2] Additionally, the chemotherapeutic activity of mercaptopurines and selenopurines is well known.[3,7]

In view of the extensive work which has been reported on chalcogen-containing compounds, it is noteworthy that the synthesis and chemical and biological characterization of sulfur and selenium containing carbohydrates have been explored to such a limited extent. The lack of activity in this field is, however, understandable since only a few naturally occurring sulfur sugars are known,[8] and the occurrence of naturally occurring seleno sugars has yet to be firmly established.

The pioneering work of Adley and Owen[9] and Whistler[10–12] in the field of sulfur-containing sugars has stimulated interest in the field. Observations of the biological activity of gold salts of 1-thio-glucose[13] and Whistler's study of 5-thioglucose[14] have spurred further investigation of the biological activity of sulfur-containing sugars.

Recent work by Zingaro *et al.*,[15–19] on thio and seleno sugars where the chalcogen is bonded to a Group V element (phosphorus, arsenic, or antimony) has revealed not only much useful synthetic chemistry, but also that these compounds, especially the sulfur-arsenic bonded species display carcinostatic activity. Interest in this class of compounds, which had been limited to glucose derivatives, led to the preparation of 1-galactose derivatives containing the $-SAs(CH_3)_2$ and $-SeAs(CH_3)_2$ groups. The preparation, purification, and characterization of these compounds is reported herein. The results of biological testing of some of these compounds is also reported.

DISCUSSION

The synthesis of the 1-thiogalactose derivatives is similar to the synthesis of the epimeric 1-thioglucose

† Abstracted in part from the Ph.D. dissertation of J. R. Daniel, Texas A&M University, 1977.

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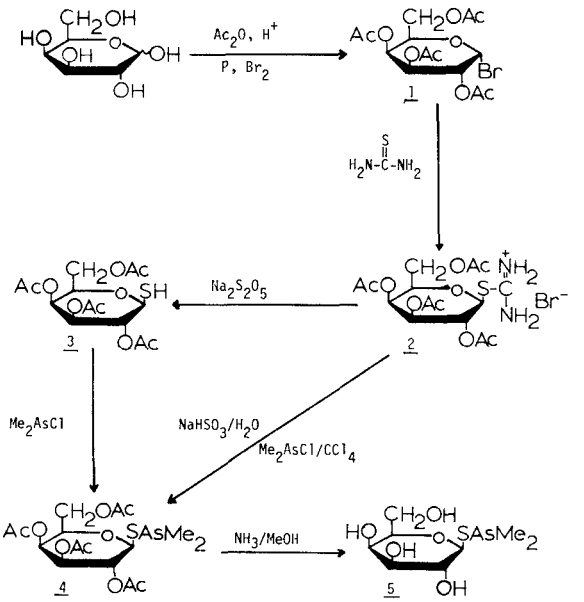
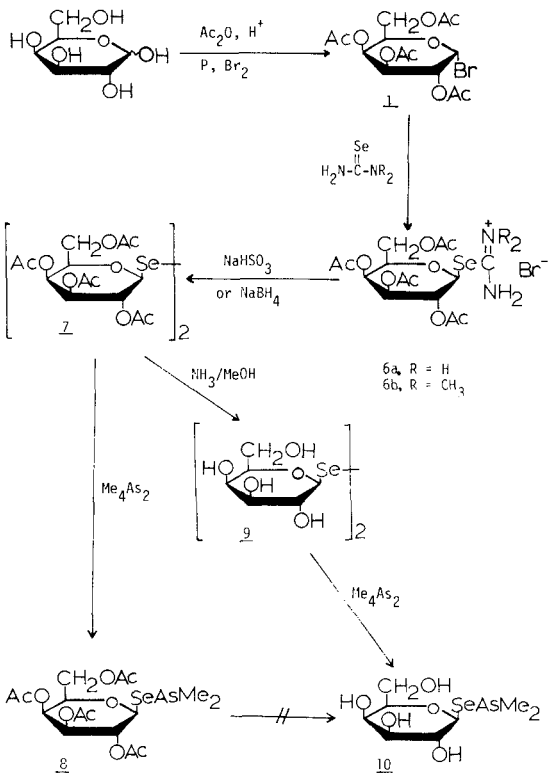
derivatives.[15] Acetobromogalactose (**1**)[20] is prepared by the reaction of galactose pentaacetate, generated *in situ*, with red phosphorus and bromine. The bromosugar then undergoes reaction with thiourea in acetone to produce the crystalline thioureide (**2**)[21] which may be reduced to the acetylated 1-thiogalactose (**3**)[21] which then undergoes reaction with dimethylchloroarsine[22] to produce the acetylated S–As bonded sugar (**4**). Alternatively, the thioureide (**2**) may be reduced *in situ* and extracted into chloroform where it reacts with dimethylchloroarsine to produce (**4**). The deacetylation of **4** gives the free hydroxy S–As bonded derivative (**5**), again attesting to the stability of the sulfur-arsenic linkage toward basic hydrolysis, compared with the selenium-arsenic linkage. These reactions are summarized in Scheme 1.



SCHEME 1 Reactions leading to 1-*S*-dimethylarsino-1-thio-β-D-galactopyranose derivatives.

The selenium derivatives were prepared by reactions analogous to those reported for the 1-selenoglucose compounds.[15] The reaction of **1** in acetone with selenourea[23] produces the selenoureide (**6a**) which exists as an unstable hygroscopic solid. Attempts to isolate and purify the 1-selenoureide always resulted in its decomposition. Consequently, the crude material was used for further preparations. The selenoureide is reduced by $NaHSO_3$ and then oxidized in air to produce the acetylated galactosyl-1,1′-diselenide (**7**). This slightly hygroscopic yellow solid could also be prepared by the reaction of **1** with *N,N*-dimethylselenourea[24] in DMF to produce the dimethylselenoureide (**6b**) which may be reduced with sodium borohydride to yield, after air oxidation, impure **7**. Compound **7** can be purified by chromatography on silica gel. Under nitrogen, **7** is cleaved by tetramethyldiarsine[25] to produce crude **8** which is purified by column chromatography. The reaction of **7** to produce **8** is much less efficient than in the glucose series. The deacetylation of **7** gives **9** which reacts with tetramethyldiarsine to yield the free hydroxy Se–As bonded compound (**10**). Due to the tendency of **10** to decompose, a satisfactory elemental analysis was not obtained, but all spectroscopic and chromatographic evidence supports the formulation of **10** as shown in Scheme 2. It is not possible to convert **8** directly to **10** due to the instability of the Se–As bond toward basic hydrolysis, as previously noted. The Se–As bonded compounds are generally less stable to both oxidation and hydrolysis than the S–As bonded species. These reactions are shown in Scheme 2.

The *X*-dimethylarsino (X = S, Se) galactose derivatives reported in this work were characterized by



SCHEME 2 Reactions leading to 1-*Se*-dimethylarsino-1-seleno-β-D-galactopyranose derivatives.

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nuclear magnetic resonance (nmr) spectroscopy, ultraviolet (uv) spectroscopy, mass spectroscopy, and elemental analysis. The nmr spectra of the sugar acetates were unexceptional except for the slight downfield shift of the arsenic methyl resonances noted in the selenium isologue (1.5 δ) relative to the sulfur derivative (1.35 δ). This deshielding effect of selenium in similar compounds has been previously noted.[26] No really satisfactory explanation is presently at hand as both electro-negativity arguments and $p\pi$-$d\pi$ back-bonding schemes appear unattractive. Further work on this problem is needed. In the deacetylated sulfur and selenium derivatives the $-As(CH_3)_2$ resonances appear at 1.4 δ and 1.8 δ, respectively. The nmr data for all compounds is consistent with a $^4C_1$ chair conformation for the pyranose ring.

The ultraviolet spectra of these compounds were investigated because of the dearth of information on organic compounds containing a Group VA to Group VIA element single bond. In particular, no literature reference was found which treats the spectra of organic thio- or selenoarsenites. The S–As derivatives were found to exhibit an absorption maxima at 222-223 nm ($\log \epsilon \approx 3.70$) and the Se–As chromophore absorbed at λ_{max} 237 nm ($\log \epsilon \approx 3.70$). The red shift in the λ_{max} of the Se compound relative to the S compound may be explained by the use of a simple molecular orbital theory, similar to that used for disulfides and diselenides.[27,28] The transition which is being observed is an $n \rightarrow \sigma^*$.

The mass spectra of the acetylated sugar derivatives do not show a molecular ion. Immediately upon electron impact the aglycon, $-XAs(CH_3)_2$ (X = S, Se), is lost and the highest *m/e* ion noted in the mass spectra is the 2,3,4,6-tetra-*O*-acetylpyranonium ion (*m/e* 331). This ion is among the most abundant in the mass spectra and is common to the spectra of all hexopyranose pentaacetates.[29] The free hydroxy derivatives can be interpreted on the basis of a common, but branched fragmentation pathway. A molecular ion is observed for the sulfur derivative, but not for the selenium derivative. A major fragmentation pathway for both of these derivatives appears to involve a ring fragmentation reaction with initial loss of a molecule of glyoxal.

The elemental analyses of all compounds were satisfactory except for 1-*Se*-dimethylarsino-1-seleno-β-D-galactopyranose. Due to the extremely hygroscopic nature of this compound coupled with its tendency to decompose with deposition of elemental selenium, a satisfactory analysis was never obtained. However, nmr and mass spectroscopic data support the formulation of this compound as indicated.

Biochemical Testing

The synthesis of the types of compounds which are the subject of this paper is being carried out in large degree because of the activity they have demonstrated against tumor cells in both *in vivo* and *in vitro* test systems. A number of these derivatives have been tested including two which are the subject of this paper, *viz.*, 2,3,4,6-tetra-*O*-acetyl-1-*S*-dimethylarsino-1-thio-β-D-galactopyranose and 1-*S*-dimethylarsino-1-thio-β-D-galactopyranose. The results of these tests, as reported by the National Cancer Institute are summarized in Table I.

The test animals appear to tolerate fairly high doses of the compounds (50 mg/kg body weight to 200 mg/kg body weight) very well. Toxic effects are noted only at very high dosages. A variety of compounds containing the $X–AsMe_2$ moiety (X = S, Se) have been found

TABLE I
Tumor screening data for some dimethylarsinous acid esters of 1-thiogalactose[a,b,c]

Compound	Dose[d]	Toxicity day survivors	Control body weight change	Animal weight difference	T/C (%)
4	50	6/6	1.2	−0.2	111
4	100	6/6	1.2	−0.8	129
4	200	6/6	1.2	−1.7	124
5	50	6/6	1.4	−2.2	125
5	100	6/6	1.4	−5.1	–
5	200	2/6	1.4	−7.2	–

[a]Route:intraperitoneal; [b]Interval:1 injection/day; [c]No. of injections:9; [d]mg/kg body weight/injection.

to exhibit carcinostatic activity and these are discussed elsewhere.[30]

A Possible Interpretation of Carcinostatic Action of Arsenicals

Three apparently unrelated pieces of information will be used in the formulation of this hypothesis: (1) some arsenicals (both three-coordinate and four-coordinate) are effective mitotic (cell division) poisons, (2) the importance of the sulfhydryl-disulfide cycle in cell division, and (3) the high affinity of arsenic for sulfur.

That arsenicals are effective mitotic poisons is a well established fact.[31-36] This body of work has shown that various arsenic compounds, primarily cacodylic acid, are effective in inhibiting cell division both *in vivo* and *in vitro*. An amino acid derivative, *S*-dimethylarsinocysteine, has also been shown to be a mitotic poison in mouse fibroblasts.[36]

A number of studies have shown that the intracellular sulfhydryl-disulfide cycle is critical in the regulation of cell division. Swann[37,38] reports that in its resting state the level of free SH in the cell is low and the level of free disulfide is high. The converse appears to be true for bound (protein) sulfur moieties. In its active, dividing state the level of intracellular free SH (presumably glutathione) is high and the level of free disulfide is low. Swann has postulated that these elevated levels of free SH act as the mitotic initiator and that these sulfhydryl compounds coordinate the mechanism of division. This concept is also supported by the studies of Együd and Szent-Györgi.[39]

It was further suggested by Dustin[40] that the spindle protein which is formed during cell division may contain a high concentration of SH groups which become exposed during the transformation of the nuclear globular protein to the fibrous spindle protein. This view is also held by Jellum and Eldjarn.[41]

The high levels of free SH discussed so far are characteristic of actively dividing normal cells. This phenomenon has also been observed in tumorous cells. Two studies by Dijkstra[42,43] have shown that an increase in acid soluble SH accompanies the development and appearance of tumors in animals which were fed carcinogenic dyes, but these increases were not seen in animals which were fed non-carcinogenic dyes. In addition, glutathione appears to be the main sulfur-containing constituent present in the blood of patients with untreated chronic granulocytic leukemia.[44]

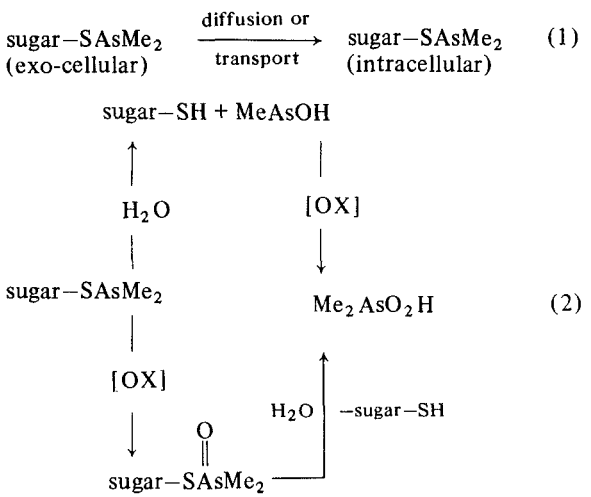
Furthermore, the enzyme which controls the level of glutathione, glutathione reductase, is present at elevated levels in the plasma, liver, and serum of tumor-bearing rats, compared with normal animals.[45,46] The clear inference in the foregoing discussion is that when cancer occurs, there appears to develop a defect in the SH/SS cycle which causes the cell to maintain constantly elevated levels of free SH and this becomes a contributing factor in uncontrolled cell division.

The chemical affinity of arsenic for sulfur has long been recognized and is a reasonable chemical expectation since both are "soft" atoms. Possibly, the best known organic arsenic-sulfur compounds are the heterocycles formed in the reaction of various arsenicals with 2,3-dimercaptopropanol (BAL). BAL is an effective antidote for heavy metal poisoning by many metals, e.g., As, Hg, and Pb. These compounds are extremely stable and are excreted from the organisms, thus bringing about detoxification. The reactions of arsenicals with sulfhydryl compounds have been reviewed by Johnstone[47] and by Peters.[48]

In light of the preceding discussion the following hypothesis is made: Various arsenicals can enter the cell and react with free SH groups, thus lowering their intracellular concentration. This allows the cells to stop their rapid, continuous division. Alternatively, rather than acting on the specific free SH compound (possibly glutathione) the arsenicals may react with SH groups of the enzyme responsible for maintaining the high free SH level. This dimethylarsenylated enzyme is inactive and this leads to a lowering of free intracellular SH levels.

In the specific case of the *S*-dimethylarsinothio sugars discussed in the present work, the sugar derivative may enter the cell and then be hydrolyzed and/or oxidized to cacodylic acid. The cacodylic acid may then react directly with the free SH component or may react with and deactivate the enzyme responsible for maintaining the high free SH concentration. These reactions are summarized in a general way below.

$$\underset{\text{(exo-cellular)}}{\text{sugar}-\text{SAsMe}_2} \xrightarrow[\text{transport}]{\text{diffusion or}} \underset{\text{(intracellular)}}{\text{sugar}-\text{SAsMe}_2} \qquad (1)$$



(2)

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$$Me_2AsO_2H + 3ESH \longrightarrow Me_2AsSE + (ES)_2 + 2H_2O \quad (3)$$

or

$$Me_2AsO_2H + E(SH)_3 \longrightarrow \begin{matrix} S \\ | \\ S \end{matrix}\!\!>\!E\text{–}SAsR_2 + 2H_2O \quad (4)$$

E = protein or enzyme.

This scheme could explain the carcinostatic action of arsenicals. Clearly more work is needed to establish this point and isotopic labelling studies would prove very valuable in this regard. Separate and double-labelling studies involving ^{35}S and ^{74}As could help clarify this matter.

EXPERIMENTAL

General Methods

Evaporations were performed under reduced pressure on a Büchi Rotavapor-R rotary evaporator. Melting points were determined using a Büchi SMP-20 melting point apparatus and are uncorrected. Nmr spectra were measured at 60 MHz with a Varian T-60 nmr spectrometer. Chemical shifts refer to an internal standard of tetramethylsilane (δ = 0.00) for organic solutions and an internal standard of *t*-butyl alcohol (δ = 1.2) for aqueous solutions.

Mass spectra were recorded by Dr. Ronald Grigsby, Department of Biochemistry, Texas A&M University with a Dupont CEC21-110 high resolution spectrometer operating at an ionizing potential of 70 eV and an ion current of 200 μA. The accelerating potential was 6 kV and the source temperature ranged from 210° to 220°. Thin layer chromatography was performed on Bakerflex silica gel 1B TLC plates which were visualized in an iodine chamber. Column chromatography was done on a 1.5 cm x 41 cm column of Merck silica gel 60 (70–230 mesh).

*2,3,4,6-Tetra-*O*-acetyl-1-*S*-dimethylarsino-1-thio-β-*D*-galactopyranose* (**4**). 2,3,4,6-Tetra-*O*-acetyl-1-*S*-dimethylarsino-1-thio-β-D-galactopyranose was prepared by a modification of the method described by Zingaro and Thomson[15] in the synthesis of the *gluco* epimer.

Sodium bisulfite (5.6 g, 0.05 moles) was dissolved in water (90 ml) and the solution was heated to 40°. 2-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-thiopseudourea hydrobromide (**2**)[21] (20 g, 0.04 moles) was added to the warm solution with stirring. Immediately, dimethylchloroarsine (6 ml, 0.06 moles) dissolved in dichloromethane (75 ml) was added. Diethylamine (10–12 ml) was then added dropwise until the mixture was basic to litmus and stirring was continued for 15 minutes. The organic phase was then separated, dried over anhydrous magnesium sulfate, and the solvent was evaporated under reduced pressure. The resulting syrup was taken up in absolute methanol, treated with activated carbon, and filtered through Celite. The solvent was removed under reduced pressure. The syrup was taken up in dry diethyl ether and chromatographed on silica gel (diethyl ether eluent). Product-containing fractions were identified by TLC (R_f = 0.40, silica gel: $HCCl_3$). These fractions were combined and the solvent removed under reduced pressure to give pure **4** as a colorless syrup in 81% yield.

Nmr (60 MHz, chloroform-*d*): 1.35 δ (6-proton singlet, $As(CH_3)_2$), 1.95, 2.1, 2.2 δ (3-, 6-, 3-proton singlets, OAc), 3.6–4.3 δ (3-proton multiplet, H-5, H-6, H-6′), 4.3–5.6 δ (4-proton multiplet, H-1, H-2, H-3, H-4). uv (CH_3OH): λ_{max} 223 nm (log ϵ 3.61).

Anal. $C_{16}H_{25}O_9SAs$, Calcd: C, 41.03; H, 5.38. Obs: C, 41.07; H, 5.32.

*1-*S*-Dimethylarsino-1-thio-β-*D*-galactopyranose* (**5**). 2,3,4,6-Tetra-*O*-acetyl-1-*S*-dimethylarsino-1-thio-β-D-galactopyranose (**4**) (11.0 g, 0.025 moles) was dissolved in absolute methanol (100 ml) and the solution was cooled in an ice-salt bath. A rapid stream of ammonia gas was passed into the stirred solution for 1 h. and it was then stored at 5–10° for 20 h. The solvent was removed under reduced pressure and the syrup obtained was recrystallized three times by dissolving in chloroform, adding diethyl ether to permanent turbidity, and cooling in a freezer. This gave the product, melting at 92–4°, in 68% yield. Nmr (60 MHz, deuterium oxide): 1.4 δ (6-proton singlet, $As(CH_3)_2$), 3.3–4.1 δ (6-proton multiplet, H-2, H-3, H-4, H-5, H-6, H-6′), H-1 is obscured by the solvent HOD peak at 4.6 δ. uv (CH_3OH): λ_{max} 222 nm (log ϵ 3.78).

Anal. $C_8H_{17}O_5SAs$, Calcd: C, 32.00; H, 6.00. Obs: C, 31.67; H, 5.71.

*Bis(2,3,4,6 tetra-*O*-acetyl-β-*D*-galactopyranosyl) 1,1′-Diselenide* (**7**). 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl bromide (**1**) (20.5 g, 0.05 moles) and *N,N*-dimethylselenourea[24] (7 g, 0.05 moles) were dissolved in *N,N*-dimethylformamide (100 ml). The reaction mixture was heated to 50°, with stirring, for 3 h. The formation of the selenopseudoureide was observed by TLC (R_f = 0.81, silica gel: MeOH). The reaction flask was then flushed with nitrogen and cooled in an ice bath. A solution of sodium borohydride (2.0 g, 0.05 moles) in DMF (30 ml) was added dropwise and the reaction was stirred under nitrogen at 0° for 1.5 h. The reaction was then warmed slowly to room temperature and stirred under nitrogen overnight. The nitrogen flow was discontinued and air was bubbled through the solution for 2 h. The solvent was removed under reduced pressure and the dark residue was taken up in chloroform. The organic layer was washed with several volumes of water, dried over magnesium sulfate, treated with activated carbon, filtered and the solvent was removed under reduced pressure. The yellow syrup obtained was placed in a vacuum desiccator where it crystallized; mp = 46–8°. Yield 15.4 g (77%). Nmr (60 MHz, chloroform-*d*): 1.95, 2.1, 2.2 δ (3-, 6-, 3-proton singlets, OAc), 3.7–4.4 δ (3-proton multiplet, H-5, H-6, H-6′), 4.6–5.6 δ (4-proton multiplet, H-1, H-2, H-3, H-4).

Anal. $C_{28}H_{38}O_{18}Se_2$, Calcd: C, 40.98; H, 4.63. Obs: C, 41.17; H, 4.86.

*Bis(β-*D*-galactopyranosyl)-1,1′-Diselenide* (**9**). *Bis*(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1,1′-diselenide (**7**) (4.0 g, 0.005 moles) was dissolved in methanol (100 ml). This solution was cooled in an ice salt bath and ammonia gas was bubbled through the stirred solution for 45 minutes. This solution was stored overnight at 0°. The solvent was removed under reduced pressure and the syrup so obtained was triturated several times with hot chloroform to remove acetamide (a deacetylation product). The syrup was then dissolved in methanol, and diethyl ether was added to the solution to induce crystallization. This suspension was cooled and the product was collected by filtration at reduced pressure in a glove box under nitrogen.

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The solid was stored in a vacuum desiccator with phosphorus pentoxide. This compound is extremely hygroscopic. Yield: 1.3 g (54%). The compound was homogeneous as indicated by TLC (R_f = 0.78, silica gel:MeOH). The melting point was poorly defined, but liquefaction occurred in the region 82–7°. Poorly defined melting points are characteristic of free hydroxy sugars. The nmr data and elemental analysis indicate that 0.5 molecules of methanol of solvation are present in the solid. Nmr (60 MHz, D_2O): 3.3 δ (1.5 proton singlet, methanol of solvation) 3.6–4.2 δ (6-proton multiplet, H-2, H-3, H-4, H-5, H-6, H-6′), H-1 is obscured by the solvent HOD peak at 4.6 δ.

Anal. $C_{12}H_{22}O_{10}Se_2 \cdot 0.5CH_3OH$, Calcd: C, 30.35; H, 4.67. Obs: C, 30.25; H, 4.98.

2,3,4,6-Tetra-O-acetyl-1-Se-dimethylarsino-1-seleno-β-D-galactopyranose (8). The acetylated galactosyl diselenide (7) (2.0 g, 0.0025 moles) was dissolved in chloroform (30 ml) and this solution was de-gassed by bubbling nitrogen through it for 15–20 minutes. The solution was placed in a glove box under nitrogen and tetramethyldiarsine[25] (1.5 ml, 0.011 moles) was added *via* a syringe. The reaction mixture was allowed to stand under nitrogen at room temperature for 1 h. The solvent was removed under reduced pressure and the syrupy residue was dissolved in benzene:acetone (99:1) and chromatographed on silica gel using the same solvent mixture (R_f = 0.58, silica gel:diethyl ether). Fractions were monitored by thin-layer chromatography and those containing the desired compound (R_f = 0.27, silica gel; benzene:acetone; 99:1) were combined. If required, these combined fractions were re-chromatographed to obtain pure 8 as a light yellow syrup. Yield = 1.5 g (76%). Nmr (60 MHz, chloroform-*d*): 1.48 δ (6-proton singlet, $As(CH_3)_2$), 1.9, 2.0, 2.1 δ (3-, 6-, 3-proton singlets, OAc), 3.6–4.3 δ (3-proton multiplet, H-5, H-6, H-6′), 4.6–5.6 δ (4-proton multiplet, H-1, H-2, H-3, H-4). uv (CH_3OH): λ_{max} 237 nm (log ε 3.69).

Anal. $C_{16}H_{25}O_9SeAs$, Calcd: C, 37.30; H, 4.89. Obs: C, 36.92; H, 4.98.

Deacetylation of 8. (i) 2,3,4,6-Tetra-*O*-acetyl-1-*Se*-dimethylarsino-1-seleno-β-D-galactopyranose (8) (6.2 g, 0.012 moles) was dissolved in 60 ml of absolute methanol. This solution was cooled in an ice-salt bath and a rapid stream of anhydrous ammonia was passed through the solution for 1 h. The flask was stoppered and stored at 5° for 20 h. The solvent was removed under reduced pressure and the yellow syrup obtained was treated with 40 ml of hot chloroform. This suspension was cooled to 0° and the chloroform was removed by decantation. The syrup was again treated with hot chloroform, cooled and again decanted. The yellow syrup was then subjected to reduced pressure to remove the last traces of moisture. The yellow hygroscopic solid so obtained was recrystallized from methanol. Nmr spectroscopy in D_2O showed no $-As(CH_3)_2$ resonances. Thus, treatment of the tetra-acetate with ammonia in methanol does remove the acetate esters, but at the same time this treatment ruptures the Se–As bond, leading to the formation of *bis*(β-D-galactopyranosyl)diselenide (9).

(ii) *Bis*(β-D-galactopyranosyl)-1,1′-diselenide (9) (0.7 g. 0.001 moles) was suspended in 75 ml of dichloromethane. The flask was flushed for 20 minutes with dry nitrogen, and tetramethyldiarsine[25] (1.0 ml, 0.01 moles) was delivered, *via* syringe, into the flask. The reaction mixture was stirred at room temperature under nitrogen for 2 days. A light yellow solid formed and was collected by filtration. The yield of 0.6 g represented a 100% conversion. The melting point of this solid is 88–90°. On further heating to 95–100° the melt deposits red selenium. Nmr (60 MHz, D_2O): 1.8 δ, (6-proton singlet, $As(CH_3)_2$), 3.3–4.0 δ (6-proton multiplet, H-2, H-3, H-4, H-5, H-6, H-6′). H-1 is obscured by the solvent HOD peak. Due to the extremely hygroscopic nature of this compound along with its tendency to decompose with the deposition of elemental selenium a satisfactory analysis was never obtained. The best analysis corresponds roughly to the sugar monohydrate.

Anal. $C_8H_9O_6SeAs$, Calcd: C, 24.46; H, 4.90. Obs: C, 27.67; H, 5.16.

(iii) A reaction similar to (ii) was carried out in DMF to insure homogeneity of the reaction mixture. Similar results were obtained.

(iv) *Bis*(β-D-galactopyranosyl)diselenide (9) (0.9 g, 0.002 moles) was suspended in 10 ml of absolute ethanol and the flask was flushed with dry nitrogen for 20 minutes. Hypophosphorous acid (50%, 0.5 ml, 2.5-fold excess) was added *via* syringe and the reaction mixture was stirred at room temperature for 1 h. Dimethylchloroarsine[22] (0.4 ml, 0.004 moles) was added *via* syringe and the reaction mixture was stirred for 18 h. under a nitrogen atmosphere. Dry diethyl ether was added to the reaction mixture and a light yellow to white solid precipitated. The very hygroscopic solid was filtered and subjected to analysis by nmr spectroscopy. The nmr indicated that the desired product had been formed, but was impure. All attempts at purification by recrystallization or chromatography failed and resulted in the formation of a less pure product.

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