

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

## Ethyl Glycosides of 2-Acetamido-2-deoxy-1-thio-D-galactose

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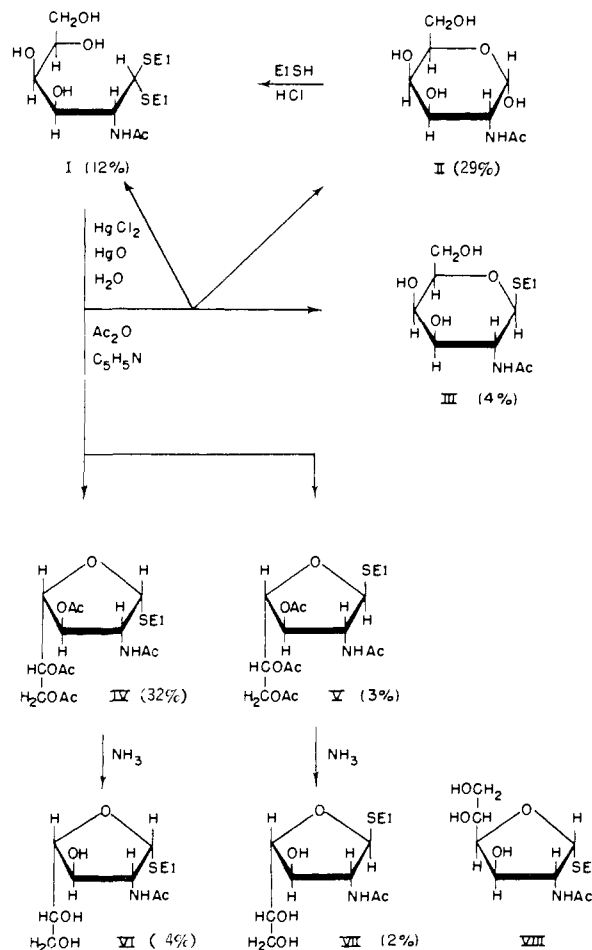
The products obtained from the action of an aqueous mercuric chloride solution, in the presence of mercuric oxide, on 2-acetamido-2-deoxy-D-galactose diethyl dithioacetal (I) were isolated by successive carbon, cellulose and silicate column chromatography, and characterized as ethyl 2-acetamido-2-deoxy-1-thio- $\alpha$ -D-galactofuranoside (ethyl 2-acetamido-2-deoxy-1-thio-D-glycero- $\beta$ -L-arabino-hexofuranoside) (VI), ethyl 2-acetamido-2-deoxy-1-thio- $\beta$ -D-galactofuranoside (ethyl 2-acetamido-2-deoxy-1-thio-D-glycero- $\alpha$ -L-arabino-hexofuranoside) (VII) and ethyl 2-acetamido-2-deoxy-1-thio- $\beta$ -D-galactopyranoside (III). The tri-O-acetyl derivatives (IV and V) of VI and VII are also described.

The first synthetic thioglycosides, phenyl 1-thio- $\beta$ -D-glucopyranoside and phenyl 1-thio- $\beta$ -lactopyranoside were prepared by Fischer and Delbrück,<sup>2</sup> by treating the corresponding acetylated  $\alpha$ -D-glycopyranosyl bromides with sodium thiophenoxide. Schneider and Sepp<sup>3</sup> obtained an ethyl 1-thio-D-glucoside by treating an aqueous solution of D-glucose diethyl dithioacetal, at room temperature, with one mole of mercuric chloride and neutralizing the acid formed, from time to time, with alkali. Green and Pacsu<sup>4</sup> modified the method and added mercuric oxide to neutralize the hydrochloric acid as it was formed. They favored the furanose structure on the basis of rotatory value and ease of acid hydrolysis. Periodate oxidation<sup>5</sup> gave definitive evidence for the furanose structure of this glycoside. Pacsu and Wilson<sup>4</sup> demonstrated that under mild acidity D-glucose diethyl dithioacetal could undergo the following sequence of reactions: D-glucose diethyl dithioacetal  $\rightarrow$  ethyl 1-thio- $\alpha$ -D-glucofuranoside  $\rightarrow$  ethyl 1-thio- $\beta$ -D-glucopyranoside  $\rightarrow$  ethyl 1-thio- $\alpha$  (and  $\beta$ )-D-glucopyranoside.

This method of partial demercaptalation was applied by Wolfrom, Olin and Polglase<sup>6</sup> to the synthesis of ethyl 2-acetamido-2-deoxy-1-thio- $\alpha$ -D-glucopyranoside. Ethyl 2-acetamido-2-deoxy-1-thio- $\alpha$  (and  $\beta$ )-D-glucopyranosides were prepared by Hough and Taha<sup>7</sup> by mercaptalation of 2-acetamido-2-deoxy-D-glucose at room temperature.

We wish to report herein the synthesis of ethyl 2-acetamido-2-deoxy-1-thio- $\alpha$ -D-galactofuranoside (ethyl 2-acetamido-2-deoxy-1-thio-D-glycero- $\beta$ -L-arabino-hexofuranoside) (VI), ethyl 2-acetamido-2-deoxy-1-thio- $\beta$ -D-galactofuranoside (ethyl 2-acetamido-2-deoxy-1-thio-D-glycero- $\alpha$ -L-arabino-hexofuranoside) (VII), ethyl 2-acetamido-2-deoxy-1-thio- $\beta$ -D-galactopyranoside (III), and the fully acetylated derivatives of the anomeric ethyl 2-acetamido-2-deoxy-1-thio-D-galactofuranosides (IV and V), all isolated in crystalline form. The starting material, 2-amino-2-deoxy- $\alpha$ -D-galactose hydrochloride, was prepared from chondroitin sulfate essentially by the procedure of Wolfrom and

Onodera,<sup>8</sup> converted to the N-acetyl derivative<sup>9</sup> and thence to 2-acetamido-2-deoxy-D-galactose diethyl dithioacetal (I)<sup>8</sup> by the method of Wolfrom and Anno,<sup>10</sup> for the analogous D-glucose derivative. Treatment of substance I with aqueous mercuric chloride and mercuric oxide<sup>4,6</sup> produced a reaction



mixture from which the components were isolated by successive carbon and cellulose column chromatography to yield 2-acetamido-2-deoxy-D-galactose (II) and ethyl 2-acetamido-2-deoxy-1-thio- $\beta$ -D-galactopyranoside (III), m.p. 231–231.5°,  $[\alpha]_{\text{D}} -20^\circ$  (methanol). The residual sirupy material was acetylated and subjected to silicate column

(8) M. L. Wolfrom and K. Onodera, *THIS JOURNAL*, **79**, 4737 (1957).

(9) S. Roseman and J. Ludowieg, *ibid.*, **76**, 301 (1954).

(10) M. L. Wolfrom and Kimiko Anno, *ibid.*, **74**, 6150 (1952).

(1) Rockefeller Foundation Fellow, 1956–1957; National Science Foundation Research Associate, 1957–1958, under Grant NSF-C4494 to The Ohio State University.

(2) F. Fischer and K. Delbrück, *Ber.*, **42**, 1476 (1909).

(3) W. Schneider and Johanna Sepp, *ibid.*, **49**, 2054 (1916).

(4) J. W. Green and E. Pacsu, *THIS JOURNAL*, **59**, 115 (1937); E. Pacsu and R. J. Wilson, Jr., *ibid.*, **61**, 1450 (1939).

(5) M. L. Wolfrom, S. W. Waisbrot, D. I. Weisblat and A. Thompson, *ibid.*, **66**, 2063 (1944).

(6) M. L. Wolfrom, S. M. Olin and W. J. Polglase, *ibid.*, **72**, 1724 (1950).

(7) L. Hough and M. I. Taha, *J. Chem. Soc.*, 2042 (1956).

extrusion chromatography to produce ethyl 2-acetamido-tri-*O*-acetyl-2-deoxy-1-thio- $\alpha$ -D-galactofuranoside (IV), m.p. 87.5–88.5°,  $[\alpha]_D +99^\circ$  (chloroform), and its  $\beta$ -anomer V, m.p. 114–115°,  $[\alpha]_D -107^\circ$  (chloroform). Ethyl 2-acetamido-2-deoxy-1-thio- $\alpha$ -D-galactofuranoside (VI), m.p. 81–83°,  $[\alpha]_D +150^\circ$  (water),  $+129.5^\circ$  (methanol), and its  $\beta$ -anomer VII, m.p. 108–110°,  $[\alpha]_D -135^\circ$  (methanol), were obtained upon treatment of their acetates with methanolic ammonia.

The ring structures of these thioglycosides were assigned on the basis of sodium metaperiodate oxidation data (Table I). The oxidation conditions used were those recommended by Jeanloz and Forchielli<sup>11</sup> and by Masamune, Yosizawa and Haga<sup>12</sup> for glycosides of 2-acetamido-2-deoxyhexoses. The original data of Wolfrom, Waisbrot, Weisblat and Thompson,<sup>5</sup> for the periodate oxidation of ethyl 1-thio- $\alpha$ -D-glucopyranoside, are reproduced in their entirety in Fig. 1. In the original publication the last three points were omitted. These data show that the terminal glycol unit in this substance is the first point of attack and this serves to establish the furanoside structure in it. The data of Fig. 1, Table I and those of Hough and Taha, allow the following conclusions to be made. 1-Thiohexofuranosides rapidly liberate 1 mole of formaldehyde while the 1-thiohexopyranosides do not; the glycol splitting is the most rapid reaction; a subsequent reaction is caused by the presence in the molecule of the thioethoxyl group, resulting in the uptake of about one further mole of oxidant in a reaction the nature of which is obscure<sup>13,14</sup>; long periods of oxidation may result in a further slow "overoxidation" of unknown nature.

TABLE I

OXIDATION OF ETHYL 2-ACETAMIDO-2-DEOXY-1-THIO-D-GLYCOSIDES (0.1 M)					
By sodium metaperiodate at 0–3°, pH 4.5, in the dark					
Compound	Time, min.	Oxidant <sup>a</sup>		Formaldehyde <sup>a</sup> Found <sup>c</sup>	Formic acid <sup>a</sup> Found <sup>d</sup>
		Added	Used <sup>b</sup>		
VIII <sup>e</sup>	30	3	1.80	0.99	0.00
	60	3	1.92	..	
	120	3	2.01	0.98	0.02
	480	3	2.05	..	
	30	1	1.00	0.99	
VI	30	0.5	0.50	.48	
	30	3	1.63	.98	
	120	3	1.89	..	0.00
	480	3	1.98	..	
	30	1	1.00	0.98	
VII	30	0.5	0.50	.49	
	30	3	1.80	.98	
III <sup>f</sup>	30	1	1.00	.97	
	30	2	2.00	.00	

<sup>a</sup> Moles per mole of sample. <sup>b</sup> Determined iodometrically. <sup>c</sup> By dimedon assay. <sup>d</sup> Titrable acidity, phenolphthalein end-point. <sup>e</sup> 2-Acetamido-2-deoxy-1-thio- $\alpha$ -D-glucopyranoside. <sup>f</sup> 0.05 M.

(11) R. W. Jeanloz and E. Forchielli, *J. Biol. Chem.*, **188**, 361 (1951).

(12) H. Masamune, Z. Yosizawa and M. Haga, *Tohoku J. Exptl. Med.*, **65**, 79 (1956); *C. A.*, **51**, 13006a (1957).

(13) L. Hough and M. I. Taha, *J. Chem. Soc.*, 3994 (1957).

(14) W. A. Bonner and R. W. Drisko, *THIS JOURNAL*, **73**, 3699 (1951).

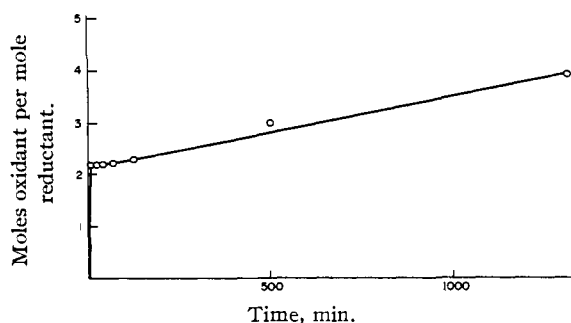


Fig. 1.—Oxidation of ethyl 1-thio- $\alpha$ -D-glucopyranoside with sodium metaperiodate; maximum formaldehyde formed, 0.9 mole, at 1 hr.

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### Experimental

**Preparation of 2-Acetamido-2-deoxy-D-galactose Diethyl Dithioacetal (I).**—This substance, reported by Wolfrom and Onodera,<sup>8</sup> was obtained in improved yield by utilizing the procedure employed by Wolfrom and Anno<sup>10</sup> for the synthesis of 2-acetamido-2-deoxy-D-glucose diethyl dithioacetal. 2-Acetamido-2-deoxy- $\alpha$ -D-galactose<sup>8,9</sup> (10 g.) was dissolved in 40 ml. of hydrochloric acid (sp. gr. 1.18) and stirred with 40 ml. of ethanethiol for 24 hr. at 0°. The solution was then neutralized with an excess of basic lead carbonate, and after the addition of 500 ml. of water, it was filtered and the precipitate was washed with 1000 ml. of water. The combined filtrate and washings were concentrated under reduced pressure to a sirup and further dried by repeated evaporation with methanol. The residue was extracted with methanol at room temperature. The solvent was removed and the residue was crystallized from methanol-ether; yield 11 g. (74%), m.p. 160–162°. The substance was recrystallized from ethanol-chloroform-ether; m.p. 164–165°,  $[\alpha]_D^{20} -39^\circ$  (c 0.5, ethanol),  $-52^\circ$  (c 0.5, methanol). Wolfrom and Onodera<sup>8</sup> reported m.p. 163–165°,  $[\alpha]_D^{20} -32^\circ$  (ethanol).

**Ethyl 2-Acetamido-2-deoxy-1-thio- $\beta$ -D-galactopyranoside (III).**—Solution of 9.3 g. of 2-acetamido-2-deoxy-D-galactose diethyl dithioacetal in 400 ml. of water was effected at 70°. After cooling to 50°, washed yellow mercuric oxide,<sup>4</sup> prepared from 10 g. of mercuric chloride, was suspended in the solution, to which 4.02 g. (0.5 equiv.) of mercuric chloride dissolved in 300 ml. of water was added dropwise, with stirring, over a period of 40 min. The stirring was continued for an additional 40 min., 6 ml. of pyridine was added and the mixture was filtered through an asbestos pad. The filtrate was evaporated under reduced pressure and the resultant sirup was dried by repeated evaporation from alcohol solution. The residual sirup was dissolved in 60 ml. of ethanol and filtered from a small amount of insoluble material which separated upon standing overnight. Ethyl ether was then added to incipient turbidity and the solution was made basic with two drops of pyridine. After standing for a week, a small amount (12%) of starting material (I) separated and was removed by filtration. The filtrate was concentrated to a sirup which was dissolved in 50 ml. of water and chromatographed on a carbon<sup>15</sup> (Nu-char C unground)<sup>16</sup> column (100 × 15 mm., diam.) and developed successively with water and 400 ml. each of 5, 10, 30 and 60% ethanol solutions. The solvent in the effluent from each concentration of developer was removed under reduced pressure. The distribution of material was determined by paper chromatography using Whatman No. 1 filter paper, with 1-butanol, ethanol and water (40:11:19 by vol.) as developer and periodate-permanganate-benzidine indicator.<sup>17</sup> The sirups from the water and 5% effluents, comprising most of the material, contained an un-

(15) R. Whistler and D. F. Durso, *ibid.*, **72**, 677 (1950).

(16) A product of the West Virginia Pulp and Paper Co., New York, N. Y.

(17) M. L. Wolfrom and J. B. Miller, *Anal. Chem.*, **28**, 1037 (1956).

known substance,  $R_f$  0.68, and 2-acetamido-2-deoxy- $\alpha$ -D-galactose,  $R_f$  0.17. It was dissolved in 50 ml. of a mixture of 1-butanol-ethanol-water (40:11:19 by vol.) and placed on a cellulose powder<sup>18</sup> column (500  $\times$  50 mm., diam.) which had been prewashed with the developing solvent, and developed with the same solvent mixture. The effluent was collected in thirty 60-ml. portions and each were examined by paper chromatography to indicate the presence of an unknown substance,  $R_f$  0.68, in fractions 9-13 and of 2-acetamido-2-deoxy- $\alpha$ -D-galactose,  $R_f$  0.17, in fractions 14-30. After concentration to a low volume, no crystalline material was obtained from combined fractions 9-11. 2-Acetamido-2-deoxy- $\alpha$ -D-galactose (II) crystallized from combined fractions 14-30; yield 1.8 g. (29%). The sirupy residue from combined fractions 12-13 was diluted with 5 ml. of absolute ethanol and 10 ml. of anhydrous ether and allowed to crystallize in the refrigerator; yield 260 mg. (4%) of substance III, m.p. 208-210°. Pure material was obtained after several recrystallizations from methanol-ethanol-ether, m.p. 231-231.5°,  $[\alpha]_D^{20} -20^\circ$  (*c* 1, methanol); X-ray diffraction data<sup>19</sup>: 12.4m, 11.1m, 8.71s(2), 7.88m, 6.90w, 4.66w, 4.44s(1), 4.13m, 3.96w, 3.71s(3), 3.57m, 3.45m, 3.28vw, 3.09w, 2.89m, 2.65m, 2.47w, 2.39vw, 2.33vw, 2.24m, 2.05w, 1.81vw.

*Anal.* Calcd. for  $C_{10}H_{19}NO_5S$ : C, 45.27; H, 7.22; N, 5.28; S, 12.07. Found: C, 45.20; H, 7.06; N, 5.46; S, 11.88.

Periodate oxidation (Table I) together with the above data, indicate that substance III is ethyl 2-acetamido-2-deoxy-2-amino-1-thio- $\beta$ -D-galactopyranoside.

**Ethyl 2-Acetamido-tri-*O*-acetyl-2-deoxy-1-thio- $\alpha$  (IV)- and  $\beta$  (V)-D-galactofuranoside.**—The mother liquor from the preparation of substance III and the residue from fractions 9-11 from the above cellulose column chromatography were combined and evaporated under reduced pressure to a sirup, dissolved in 30 ml. of water, and this was passed through a column of Amberlite MB-3<sup>20</sup> (150  $\times$  60 mm., diam.) and washed with 200 ml. of water. The combined effluents and washings were concentrated to a sirup and dried, by repeated evaporation from ethanol under reduced pressure, followed by storage over phosphorus pentoxide in a desiccator; yield 3.5 g. This sirup was acetylated with a mixture of 30 ml. of acetic anhydride and 30 ml. of pyridine at room temperature for 24 hr. The acetylation mixture was then poured into 300 ml. of ice and water and extracted with chloroform. The solution was washed successively with water, sodium bicarbonate solution, and water and evaporated under reduced pressure to a sirup which was dried by repeated evaporation from ethanol; yield 5.1 g. An amount of 3.6 g. of this material was dissolved in benzene and divided into two portions which were placed on columns (200  $\times$  55 mm., diam.) of Magnesol-Celite<sup>21</sup> (5:1 by wt.) and developed with 2000 ml. of benzene-ethanol (100:1 by vol.). Upon extrusion and streaking with permanganate indicator (1% potassium permanganate in 10% sodium hydroxide), two zones appeared. These zones were sectioned and eluted with acetone and the resulting acetone solutions were evaporated to sirups and further dried by repeated evaporation with ethanol. Evaporation of the benzene effluent produced a third fraction, which was combined with the material from the lower column zone. These fractions were each dissolved in ether and separated from a small amount of insoluble material by centrifugation. Crystalline material from the upper zone (100-140 mm. from the column top), compound V, was obtained upon concentration of the ether solution; yield 220 mg. (3%), m.p. 112-113°. The pure substance

(18) Whatman cellulose powder.

(19) Interplanar spacing, Å.,  $CuK\alpha$  radiation. Relative intensity, estimated visually: s, strong; m, medium; w, weak; v, very. First three strongest lines are numbered; (1) strongest, duplicate numbers approximately equal.

(20) A monobed ion-exchange resin produced by Rohm and Haas Co., Resinous Products Division, Philadelphia, Pa.

(21) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, THIS JOURNAL, 67, 527 (1945).

was obtained on recrystallization from ethanol-ether-petroleum ether; m.p. 114-115°,  $[\alpha]_D^{20} -107^\circ$  (*c* 1, chloroform); X-ray powder diffraction data<sup>19</sup>: 7.95s(1), 6.61m, 6.24s(3), 5.83m, 4.82w, 4.56s(2), 4.31w, 4.12m, 3.88s(2), 3.68m, 3.41vw, 3.13w, 3.02vw, 2.76vw, 2.65w, 2.40vw, 2.02vw.

*Anal.* Calcd. for  $C_{16}H_{25}NO_8S$ : C, 49.09; H, 6.43; N, 3.58; S, 8.17. Found: C, 48.81; H, 6.57; N, 3.68; S, 8.09.

The above data, together with the periodate oxidation data (Table I) on the *O*-deacetylated substance VII, indicate that this compound (V) is ethyl 2-acetamido-tri-*O*-acetyl-2-deoxy-1-thio- $\beta$ -D-galactofuranoside.

The material from the bottom zone (140-200 mm. from the column top), combined with the benzene effluent material, was crystallized from ether-petroleum ether to yield the crystalline compound IV; yield 2.5 g. (32%), m.p. 86-88° with preliminary softening. Pure material was obtained by recrystallization from ether-petroleum ether; m.p. 87.5-88.5° with preliminary softening,  $[\alpha]_D^{20} +99^\circ$  (*c* 1, chloroform); X-ray powder diffraction data<sup>19</sup>: 12.2vw, 9.64s(1), 7.42w, 6.52vw, 5.97vw, 4.85s(2), 4.68m, 4.51s(3), 4.30w, 4.16w, 3.98m, 3.73m, 3.54m, 3.27w, 3.16w, 3.08vw, 2.91vw, 2.51w.

*Anal.* Calcd. for  $C_{16}H_{25}NO_8S$ : C, 49.09; H, 6.43; N, 3.58; S, 8.17. Found: C, 49.25; H, 6.57; N, 3.53; S, 8.05.

These data, together with the periodate oxidation data on the deacetylated derivative VI (Table I), identify compound IV as ethyl 2-acetamido-tri-*O*-acetyl-2-deoxy-1-thio- $\alpha$ -D-galactofuranoside.

**Ethyl 2-Acetamido-2-deoxy-1-thio- $\alpha$ -D-galactofuranoside (Ethyl 2-Acetamido-2-deoxy-1-thio-D-glycero- $\beta$ -1-arabino-hexofuranoside) (VI).**—Ethyl 2-acetamido-tri-*O*-acetyl-2-deoxy-1-thio- $\alpha$ -D-galactofuranoside (IV, 0.62 g.) was dissolved in 15 ml. of methanol through which a rapid stream of anhydrous ammonia was passed for 30 min. at 0°. After standing for 2 hr. at room temperature, the solution was concentrated under reduced pressure to a sirup and further dried by repeated evaporation of its methanol solution. The sirup was dissolved in 10 ml. of water, and passed through a column of Amberlite MB-3<sup>20</sup> (80  $\times$  8 mm., diam.) and the column was washed with 100 ml. of water. The effluent and washings were evaporated to a sirup and this was dried by repeated distillation with ethanol under reduced pressure. The sirup was crystallized from ethanol-ether-petroleum ether; yield 320 mg. (76%), m.p. 79-81°. Pure material was obtained upon further recrystallization from ethanol-ether-petroleum ether; m.p. 81-83°,  $[\alpha]_D^{20} +150^\circ$  (*c* 1, water),  $+129.5^\circ$  (*c* 1, methanol); X-ray powder diffraction data<sup>19</sup>: 15.5m, 10.2vw, 7.78s(2), 6.51s(3), 4.98m, 4.75m, 4.53m, 4.29m, 4.13s(1), 3.99m, 3.75m, 3.42w, 3.28w, 2.93w, 2.84w, 2.75vw, 2.67vw, 2.57w, 2.50vw, 2.36vw, 2.28w, 2.07w, 2.00vw, 1.86vw.

*Anal.* Calcd. for  $C_{10}H_{19}NO_5S$ : C, 45.27; H, 7.22; N, 5.28; S, 12.07. Found: C, 45.31; H, 7.41; N, 5.47; S, 12.35; periodate oxidation data in Table I.

**Ethyl 2-Acetamido-2-deoxy-1-thio- $\beta$ -D-galactofuranoside (Ethyl 2-Acetamido-2-deoxy-1-thio-D-glycero- $\alpha$ -1-arabino-hexofuranoside) (VII).**—Ethyl 2-acetamido-tri-*O*-acetyl-2-deoxy-1-thio- $\beta$ -D-galactofuranoside (V, 170 mg.) was deacetylated by the procedure described above for the  $\alpha$ -anomer. The material was crystallized from ethanol-ether-petroleum ether; yield 87 mg. (76%), m.p. 106-108°. Pure material was obtained on further recrystallization from ethanol-ether-petroleum ether; m.p. 108-110°,  $[\alpha]_D^{20} -135^\circ$  (*c* 1, methanol); X-ray powder diffraction data<sup>19</sup>: 11.2w, 9.66s(1), 7.94vw, 6.73w, 6.33w, 5.85vw, 4.75w, 4.58s(2), 4.44m, 4.25w, 4.10m, 3.95m, 3.34s(3), 3.18vw, 3.11vw, 3.01vw, 2.79m, 2.59vw, 2.15w, 1.98vw.

*Anal.* Calcd. for  $C_{10}H_{19}NO_5S$ : C, 45.27; H, 7.22; N, 5.28; S, 12.07. Found: C, 45.39; H, 7.28; N, 5.49; S, 12.04; periodate oxidation data in Table I.

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