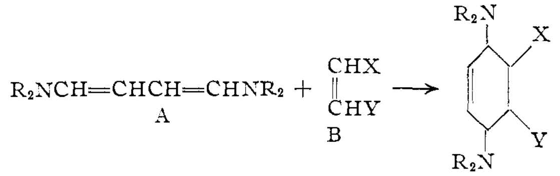
DIELS–ALDER ADDUCTS DERIVED FROM 1,4-BIS-(DIALKYLAMINO)-1,3-BUTADIENES



A		B			Nitrogen, %		
R_2N–	Structure[a]	X	Y	M.p. or b.p. (mm.), °C.	Theory	Found	Yield of adduct, %
$(CH_3)_2N$–	*t–t*	CN	CN[b]	136–137.5	25.7	25.5	46
	c–t			145.5–147.5	25.7	25.4	55
C_4H_8N–[f]	*t–t*	CN	CN[b]	157.5–159	20.7	20.6	..
	c–t			195–196.5	20.7	20.5	..
C_4H_8ON–[g]	*t–t*	CN	CN[b]	197–197.5	18.5	18.4	50
$(CH_3)_2N$–	*c–t*	H	CN	110–112 (0.5)[c]	21.7	21.2	30[d]
$(CH_3)_2N$–	*c–t*	H	$COOC_2H_5$	103–107 (1.0)[e]	11.7	11.8	21

[a] *c–t* = *cis,trans*; *t–t* = *trans,trans*. [b] Fumaronitrile. [c] $n^{20}D$ 1.4986. [d] Foreruns contained $(CH_3)_2NCH_2CH_2CN$ (picrate m.p. 154–155°; mixed m.p. with authentic sample which had m.p. 156.5–157.5°, 155–156.5°). [e] $n^{20}D$ 1.4848. [f] N-Pyrrolidyl. [g] N-Morpholyl.

pressure and the solid residue was washed twice with ethanol and pressed dry on porous plate (12 g., m.p. 139–142°). Recrystallization from ethanol gave the pure product, m.p. 145.5–147.5°, neut. equiv. 110 (calcd. for $C_{12}H_{18}N_4$, 109).

cis,cis-**3,6-Bis-(dimethylamino)-4-cyclohexene-*cis,trans*-1,2-dicarbonitrile.**—A mixture of *trans,trans*-1,4-bis-(dimethylamino)-1,3-butadiene (14 g., 0.10 mole), fumaronitrile (7.8 g., 0.10 mole) and benzene (30 g.) was warmed to 50° and stirred for 30 minutes. A mildly exothermic reaction which ensued within 5 minutes maintained the temperature at 51–52° during the next 15 minutes. The mixture was allowed to stand overnight at room temperature. Benzene was evaporated under reduced pressure, the solid was filtered, washed twice with ethanol and pressed dry on porous plate (10.2 g., m.p. 135–137°). Two recrystallizations from ethanol gave the pure product, m.p. 136–137.5°, neut. equiv. 110 (calcd. for $C_{12}H_{18}N_4$, 109).

The fumaronitrile adducts were less stable when dissolved in ethanol than when dissolved in benzene. Recrystallizations were carried out quickly to avoid large losses.

cis,trans- (or *trans,cis*)-**3,6-Di-(1-pyrrolidino)-4-cyclohexene-*cis,trans*-1,2-dicarbonitrile.**—A mixture of *cis,trans*-1,4-dipyrrolidino-1,3-butadiene[2] (3.3 g., 0.0172 mole), fumaronitrile (1.4 g., 0.018 mole) and benzene (15 ml.) was heated to boiling, then allowed to cool. The precipitate was recrystallized from ethanol, m.p. 195–196.5°, neut. equiv. 134 (calcd. for $C_{16}H_{22}N_4$, 135). Phthalonitrile (m.p. and mixed m.p. 139–141°) was isolated upon distillation of a reaction mixture prepared as above.

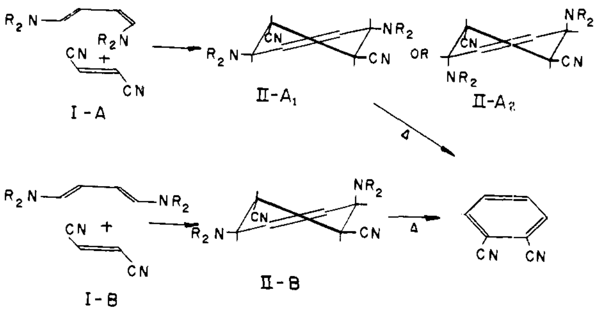


Fig. 1.

cis,cis-**3,6-Di-(1-pyrrolidino)-4-cyclohexene-*cis,trans*-1,2-dicarbonitrile.**—A mixture of *trans,trans*-1,4-dipyrrolidino-1,3-butadiene[4] (4.0 g., 0.0208 ml.), fumaronitrile (1.6 g., 0.205 mole) and benzene (20 ml.) was warmed to 80° then allowed to cool. The mixture was reheated to 80° and again allowed to cool. The mixture was allowed to stand at room temperature for 1 week. The solid which separated was recrystallized from ethanol, m.p. 157.5–159°, neut. equiv., 133 (calcd. for $C_{16}H_{22}N_4$, 135).

Phthalonitrile (m.p. and mixed m.p. 139–141°) was isolated upon distillation of a reaction mixture prepared as above.

PHILADELPHIA, PENNA.

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

Dithioacetals of D-Glucuronic Acid and 2-Amino-2-deoxy-D-galactose

BY M. L. WOLFROM AND K. ONODERA[1]

RECEIVED APRIL 11, 1957

Thioacetals and acetylated thioacetals of D-glucuronic acid and of 2-amino-2-deoxy-D-galactose have been prepared in crystalline form. D-Glucurono-3,6-lactone diethyl dithioacetal reacts with methanolic hydrogen chloride to yield a crystalline dehydration product.

Dithioacetals of reducing sugars have been utilized for sugar identification since their discovery by Fischer.[2] Aldoses may be identified conveniently as their acetylated dithioacetals.[3] Simultaneous hydrolysis and thioacetal formation ("mercaptolysis") has been useful in the elucidation of oligosaccharide and polysaccharide structures[4–6] especially when followed by reductive desulfurization.[7,8] Thioacetals of D-galacturonic acid have

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[1] National Science Foundation Research Associate under Grant NSF-G584 to The Ohio State University; recipient of a travel grant from The Rockefeller Foundation.

[2] E. Fischer, *Ber.*, **27**, 673 (1894).

[3] M. L. Wolfrom and J. V. Karabinos, THIS JOURNAL, **67**, 500 (1945).

[4] M. L. Wolfrom, J. C. Sowden and E. N. Lassettre, *ibid.*, **61**, 1072 (1939).

[5] C. Araki and S. Hirase, *Bull. Chem. Soc. Japan*, **26**, 463 (1953).

[6] A. N. O'Neill, THIS JOURNAL, **77**, 6324 (1955).

[7] M. L. Wolfrom and J. V. Karabinos, *ibid.*, **66**, 909 (1944).

[8] R. U. Lemieux and M. L. Wolfrom, *Advances in Carbohydrate Chem.*, **3**, 355 (1948).

been reported by Campbell and Link[9] and those of 2-amino-2-deoxy-D-glucose (D-glucosamine, chitosamine) have been reported by Wolfrom and co-workers.[10] We report herein the extension of these techniques to D-glucuronic acid and to 2-amino-2-deoxy-D-galactose (D-galactosamine, chondrosamine). The dithioacetals obtained constitute reference substances for possible "mercaptolysis" products of polysaccharides containing these units.

D-Glucurono-3,6-lactone (I)[11] yielded the diethyl dithioacetal, isolated as the sodium salt (IV, anhydrous and monohydrated), on treatment with hydrochloric acid and ethanethiol. This substance was further characterized as its lactone triacetate II and amide III.

2-Amino-2-deoxy-D-galactose hydrochloride (VII) is difficult to prepare[12] in crystalline form from its most available source, chondroitinsulfate C from cartilage. We describe herein our procedure for accomplishing this end. This hydrochloride was allowed to react with ethanethiol in hydrochloric acid solution and, after chloride ion removal[13] with ion exchange resin, 2-amino-2-deoxy-D-galactose diethyl dithioacetal (VIII) was obtained in crystalline form as the free base. Acetylation yielded *N*-acetamido-tetra-*O*-acetyl-2-deoxy-D-galactose diethyl dithioacetal (IX) of melting point 137–139° and $[\alpha]_{\mathrm{D}}$ −17° in chloroform. These constants differ grossly from those (184° and −22°) recorded by Kent and co-workers[14] for what was purported to be the same substance. *N*-Acetamido-2-deoxy-D-galactose (V), previously reported by Stacey,[15] was prepared by an adaptation of the procedure employed for the corresponding D-glucose derivative by Inouye and co-workers.[16] Reaction with ethanethiol yielded the crystalline *N*-acetamido-2-deoxy-D-galactose dithioacetal (VI). Hough and Taylor[17] have obtained 2-acetamido-tetra-*O*-acetyl-D-galactose 1,1-bis-(diethyl sulfoxide) without preparing the acetylated dithioacetal.

Dimler and Link[18] subjected D-galacturonic acid to simultaneous thioacetalation ("mercaptalation") and esterification. Application of their procedure to D-glucurono-3,6-lactone (I) led to the formation, in low yield, of a crystalline product (m.p. 141–143°, $[\alpha]_{\mathrm{D}}$ +169° in ethanol) which was likewise obtained on treating the diethyl dithioacetal (sodium salt, IV) with methanolic hydrogen chloride. This substance exhibited the analysis required for an anhydro-D-glucuronolactone diethyl dithioacetal. It absorbed bromine, but this is not diagnostic for unsaturation since the presence of the dithioacetal function imparts this ability. It decolorized permanganate but did not reduce Fehling solution, which it turned from blue to green. The blue color of 2,6-dichlorophenolindophenol (Tillman reagent) was turned to green by the substance. The product exhibited strong infrared absorption at 1755 (potassium bromide) and 1760 cm.$^{-1}$ (Nujol mull), whereas D-glucurono-3,6-lactone absorbed at 1735 cm.$^{-1}$ (Nujol) and sodium D-glucuronate diethyl dithioacetal absorbed at 1595 cm.$^{-1}$ (Nujol). In the ultraviolet spectral region the neutral aqueous solution exhibited no selective absorption, but on being made alkaline a strong band appeared at 377 mμ which shifted to a much lower band at 332 mμ on acidification. These wave lengths would appear to be too long to indicate a reductone[19] and the structure of this crystalline substance remains unknown.

Experimental

Sodium D-Glucuronate Diethyl Dithioacetal (IV) and Its Monohydrate.—D-Glucurono-3,6-lactone (I, 3 g.) was suspended in concd. hydrochloric acid (37.5%, 3 ml.) and was shaken with ethanethiol (3 ml.) for 30 min. at 0°. The reaction mixture was poured into 6 ml. of cold water and was extracted with two 30-ml. portions of ethyl acetate. The extract was concentrated under reduced pressure to a thick sirup which was dissolved in methanol. The methanolic solution was concentrated under reduced pressure to a sirup which was further dried in a vacuum desiccator. The product was again dissolved in methanol (20 ml.) and the solution was neutralized with a concd. solution of sodium hydroxide in methanol. The neutralization was completed gradually and the crystallizing dish was scratched to initiate crystallization. The crystals (thin plates) were collected and washed with a small amount of cold methanol; yield 1.58 g. Recrystallization was effected from methanol; m.p. 115–118° (preliminary softening at about 70°),[20] $[\alpha]^{26}_{\mathrm{D}}$ −37° (*c* 4.1, water). This substance was found to be a monohydrate; X-ray powder diffraction data: 9.33[21] vw,[22] 7.41w, 6.33vw, 5.28s, 4.73w, 4.56vw, 4.23w, 3.78m, 3.60vw, 3.44w, 3.36w, 2.76vw, 2.66w. Sodium D-glucuronate diethyl dithioacetal monohydrate was soluble in hot ethanol and methanol and was insoluble in acetone and ethyl acetate.

Anal. Calcd. for $C_{10}H_{19}NaO_6S_2 \cdot H_2O$: C, 35.25; H, 6.22; S, 18.84; Na, 6.76. Found: C, 35.46; H, 6.26; S, 18.75; Na, 6.78.

On drying the above monohydrate at 80° and 1–2 mm., the crystalline residue was the anhydrous form, m.p. 115–118°; X-ray powder diffraction data: 9.48vw,[21,22] 7.64vw, 6.76w, 5.63vw, 5.31s, 4.85w, 4.52vw, 4.31vw, 3.93vw, 3.77w, 3.59vw, 3.06w, 2.96vw, 2.85vw, 2.69w, 2.58vw, 2.49vw, 2.43vw, 2.29w.

Anal. Calcd. for $C_{10}H_{19}NaO_6S_2$: C, 37.25; H, 5.94; S, 19.90; Na, 7.13. Found: C, 37.00; H, 5.97; S, 20.04; Na, 7.19.

Reaction Product of D-Glucurono-3,6-lactone Diethyl Dithioacetal with Methanolic Hydrogen Chloride.—(a) D-Glucurono-3,6-lactone (10 g.) was suspended in a mixture of dry *N* hydrogen chloride in abs. methanol (60 ml.) and ethanethiol (15 ml.) and was shaken at room temperature for about 20 min., after which a clear solution resulted. The reaction mixture was allowed to stand for 1.5 hr. and then was refluxed for 30 min. This solution was concen-

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[9] H. A. Campbell and K. P. Link, *J. Biol. Chem.*, **120**, 471 (1937).

[10] M. L. Wolfrom, R. U. Lemieux and S. M. Olin, THIS JOURNAL, **71**, 2870 (1949); M. L. Wolfrom and Kimiko Anno, *ibid.*, **74**, 6150 (1952).

[11] Currently commercially available in the United States and produced by the Corn Products Refining Co., 17 Battery Place, New York 4, N. Y.

[12] A. B. Foster and M. Stacey, *Advances in Carbohydrate Chem.*, **7**, 256 (1952).

[13] H. Zinner, *Chem. Ber.*, **86**, 495 (1953).

[14] M. W. Whitehouse, P. W. Kent and C. A. Pasternak, *J. Chem. Soc.*, 2315 (1954).

[15] M. Stacey, *ibid.*, 272 (1944).

[16] Y. Inouye, K. Onodera, S. Kitaoka and S. Hirano, THIS JOURNAL, **78**, 4722 (1956).

[17] L. Hough and T. J. Taylor, *J. Chem. Soc.*, 978 (1956).

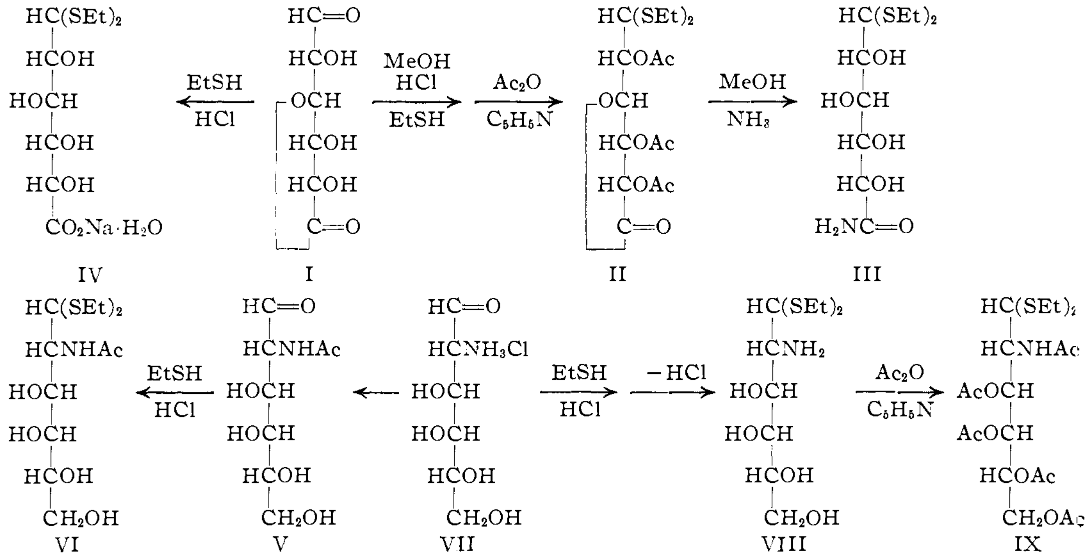
[18] R. J. Dimler and K. P. Link, THIS JOURNAL, **62**, 1216 (1940).

[19] F. Smith, *Advances in Carbohydrate Chem.*, **2**, 101 (1946).

[20] All melting points were observed on a Köfler melting point stage.

[21] Interplanar spacing, Å., CuKα radiation.

[22] Relative intensity, estimated visually; s, strong; m, medium; w, weak; vw, very weak.



trated under reduced pressure to a thick sirup which was dissolved in ethyl acetate. The ethyl acetate extract was washed with a saturated aqueous sodium bicarbonate solution, with water, and dried. The sirup obtained upon solvent removal was dissolved in a small amount of abs. ethanol. This solution yielded crystals on standing at room temperature for a few days; yield 0.53 g., m.p. 125–128°. Recrystallization was effected from abs. ethanol or abs. methanol–petroleum ether; m.p. 141–143°, $[\alpha]^{25}\text{D}$ +169° (*c* 1.1, 95% ethanol); X-ray powder diffraction data: 11.22m[21,22] 7.95m, 7.06w, 4.87s, 4.65w, 4.38vw, 4.13vw, 3.79vw, 3.75w, 3.58m, 3.13vw, 3.08w, 2.68vw, 2.63w, 2.47vw, 2.38vw, 2.32vw, 2.22w. This substance was easily soluble in ethanol, methanol, acetone and ethyl acetate; it was insoluble in ether.

Anal. Calcd. for $C_{10}H_{16}O_4S_2$: C, 45.45; H, 6.10; S, 24.27. Found: C, 45.48; H, 6.04; S, 24.20.

The product absorbed bromine water (as do also dithioacetals) and decolorized potassium permanganate solution in a few minutes at room temperature. The blue color of 2,6-dichlorophenolindophenol (slightly alkaline) was rapidly turned to green but was not decolorized. The blue color of Fehling solution also was turned to green but no reduction occurred on warming. Its neutral, aqueous solution (*c* 0.003) showed a very weak, broad absorption in the ultraviolet spectral region. When this solution was made just alkaline it became faintly yellow in color and showed selective absorption at 377 mμ (absorbance 0.562) which shifted on acidification to 332 mμ (absorbance 0.167). In Nujol mull, the substance showed strong absorption at 1760 cm.$^{-1}$ (1755 cm.$^{-1}$ in potassium bromide). D-Glucurono-3,6-lactone absorbed at 1735 cm.$^{-1}$ (Nujol) and sodium D-glucuronate diethyl dithioacetal absorbed at 1595 cm.$^{-1}$ (Nujol).

(b) Sodium D-glucuronate diethyl dithioacetal (1 g.) was refluxed with *N* hydrogen chloride in abs. methanol (15 ml.) for 30 min. After cooling, the sodium chloride precipitate was removed by filtration and the filtrate was concentrated under reduced pressure with three additions of abs. methanol. The resulting sirup was dissolved in abs. methanol and the insoluble material (sodium chloride) was removed. The filtrate was concentrated under reduced pressure to a sirup which was further dried in a vacuum desiccator. The residue was recrystallized from ethanol; yield 50 mg., m.p. 140–142°. No depression in melting point on admixture with the specimen described in (a) was observed. The crystals also showed identical X-ray powder diffraction data as those described in (a).

Tri-*O*-acetyl-D-glucurono-3,6-lactone Diethyl Dithioacetal (II).—D-Glucurono-3,6-lactone (5 g.) was added to a mixture of dry *N* hydrogen chloride in abs. methanol (30 ml.) and ethanethiol (8 ml.). The reaction mixture was kept at room temperature, with occasional shaking, for 1.5 hr. (the lactone dissolved in about 20 min.). After refluxing for 5 min., the reaction mixture was concentrated under reduced pressure to a thick sirup which was dissolved in ethyl acetate. The extract was washed with a saturated aqueous sodium bicarbonate solution and then with water. The dried solution was concentrated under reduced pressure to a sirup which was dried in a vacuum desiccator over phosphorus pentoxide.

The above dried residue was treated with a mixture of acetic anhydride (25 ml.) and pyridine (12.5 ml.) for 16 hr. at 10 to 15°. The mixture was poured into an ice and water mixture (500 ml.) to give rise to a precipitated resinous substance which was separated and crystallized from 95% ethanol; yield 2.32 g., m.p. 108–110°. A chloroform extract of the water phase gave no further crystalline material. One recrystallization from the above solvent gave a pure substance, m.p. 113–114°, $[\alpha]^{25}\text{D}$ +58° (*c* 4.1, chloroform); X-ray powder diffraction data: 9.03m,[21,22] 7.14m, 6.78w, 5.60m, 5.26vw, 5.06vw, 4.74vw, 4.50w, 4.14m, 4.00w, 3.80w, 3,54m. Tri-*O*-acetyl-D-glucurono-3,6-lactone diethyl dithioacetal was soluble in chloroform, acetone, ethyl acetate, ether, ethanol and methanol; it was insoluble in petroleum ether.

Anal. Calcd. for $C_{16}H_{24}O_8S_2$: C, 47.04; H, 5.92; S, 15.70. Found: C, 46.97; H, 6.30; S, 15.73.

D-Glucuronamide Diethyl Dithioacetal (III).—Tri-*O*-acetyl-D-glucurono-3,6-lactone diethyl dithioacetal (II, 0.5 g.) was dissolved in 30 ml. of abs. methanol. Dry ammonia was then passed into the solution at 0° for 20 min. and this was followed by maintaining the solution at room temperature for 2 hr. The reaction mixture was concentrated under reduced pressure to a sirup which was dissolved in acetone, and petroleum ether was added to incipient opalescence. The mixture was maintained at 10 to 15° for approximately a month during which time crystals slowly formed; yield 60 mg., m.p. 128–130°. Two recrystallizations from acetone gave the pure substance, m.p. 131–132°, $[\alpha]^{23}\text{D}$ −33° (*c* 0.4, water); X-ray powder diffraction data: 13.43vw,[21,22] 10.65s, 8.59w, 5.81s, 5.30w, 4.71s, 4.54vw, 4.30s, 3.79s, 3.52w, 3.38w, 3.23w, 2.89w, 2.81w, 2.45w, 2.20w. D-Glucuronamide diethyl dithioacetal was soluble in ethanol, methanol and acetone; it was insoluble in ether.

Anal. Calcd. for $C_{10}H_{21}NO_5S_2$: C, 40.11; H, 7.07; N, 4.68; S, 21.42. Found: C, 40.30; N, 7.11; N, 4.50; S, 21.04.

Preparation of 2-Amino-2-deoxy-D-galactose Hydrochloride (VII).—The crude sodium chondroitinsulfate[23] (50 g.) was purified according to an adaptation of the method of Summers,[24] by shaking its solution in 50 ml. of water for 30 min. with 30 g. of Magnesol[25]–Celite[26] (5:1 by wt., pre-

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[23] A product of the Wilson Laboratories, Chicago, Ill.; essentially sodium chondroitinsulfate C from cartilage, compare K. Meyer and M. M. Rapport, *Science*, **113**, 596 (1951).

[24] C. G. Summers, Ph.D. dissertation (M. L. Wolfrom, preceptor), The Ohio State University, 1955.

[25] A magnesium acid silicate produced by the Westvaco Division of Food Machinery and Chemical Corp., South Charleston, W. Va.

[26] A siliceous filter-aid produced by Johns-Manville Co., New York, N. Y.

viously washed with acetone and dried at 40°). This treatment was repeated. The filtered solution was passed twice through a column containing 60 g. of Magnesol–Celite (5:1 by wt.). To the clear light yellow filtrate (525 ml.) obtained on filtering the effluent through asbestos, 250 ml. of concd. hydrochloric acid was added and this was refluxed for 12 hr. Water (500 ml.) was added to the hydrolyzate and the cooled solution was filtered through sintered glass. The filtrate was concentrated under reduced pressure to a sirup which was redissolved in water and was again concentrated. This procedure was thrice repeated and in the final process the solution was treated with active carbon. The resulting sirup was dissolved in 300 ml. of methanol and some inorganic material was removed by filtration. The filtrate was concentrated under reduced pressure to a thick sirup which was dissolved in 100 ml. of hot abs. methanol. The solution deposited crystals on standing at 10 to 15° and a second crop was obtained on concentrating the mother liquor to 30 ml.; yield 7.7 g. (5.5 g. + 2.2 g.) of a product assaying 70 ± 10% 2-amino-2-deoxy-D-galactose.[27]

The above crude crystals were dissolved in 5 parts of water, and 3 volumes of abs. ethanol were added. Upon maintaining the mixture at 10 to 15°, the separation of two different types of crystals could be noted, the one being larger in shape as well as in amount; yield 3 g. A portion of the larger crystals was separated mechanically; $[\alpha]^{30}\mathrm{D}$ +92° (*c* 1, water, final value, upward mutarotation), X-ray powder diffraction data in agreement with those reported[28] for 2-amino-2-deoxy-β-D-galactose hydrochloride.

Acetone was added to the above filtrate to incipient opalescence and the mixture was maintained at 10 to 15° for several days to complete crystallization; yield 3.5 g., $[\alpha]^{26}\mathrm{D}$ +92° (*c* 0.9, water, final value). The crystals were of a smaller shape than the β-D-form. X-Ray powder diffraction data were in agreement with those reported[28] for 2-amino-2-deoxy-α-D-galactose hydrochloride.

2-Amino-2-deoxy-D-galactose Diethyl Dithioacetal (VIII).—2-Amino-2-deoxy-D-galactose hydrochloride (VII, 500 mg.) was shaken with concd. hydrochloric acid (3 ml.) and ethanethiol (2 ml.) in a sealed tube for 10 min. at 0° and was then maintained at 10 to 15° for 2 days. The reaction mixture was shaken further for 1 hr. at 0° and then was poured into 30 ml. of cold water. The solution was passed through a 1.8 (diam.) × 20 cm. column of Amberlite IR4B[29] (carbonate form) and 300 ml. of the effluent was collected. This was concentrated to dryness under reduced pressure and the residue was dissolved in hot abs. ethanol. The residue obtained on solvent removal from the filtered solution was crystallized from ethanol; yield 120 mg., m.p. 133–139°. Recrystallization was effected from the same solvent; m.p. 155–157°, $[\alpha]^{30}\mathrm{D}$ +24° (*c* 0.44, water); X-ray powder diffraction data: 14.03s,[21,22] 9.94vw, 8.82s, 7.78w, 5.41vw, 4.91s, 4.59s, 4.19s, 3.99vw, 3.59m, 3.34m, 3.08w, 2.91vw, 2.72vw, 2.51vw. 2-Amino-2-deoxy-D-galactose diethyl dithioacetal exhibited a negative test for chloride ion and a positive ninhydrin test. It was soluble in ethanol and methnol and was insoluble in ethyl acetate and acetone.

Anal. Calcd. for $C_{10}H_{23}NO_4S_2$: C, 42.08; H, 8.12; N, 4.91; S, 22.47. Found: C, 42.16; H, 8.21; N, 4.99; S, 22.43.

2-Acetamido-2-deoxy-D-galactose Diethyl Dithioacetal (VI).—2-Amino-2-deoxy-α-D-galactose hydrochloride (1 g.) was added to 5 ml. of abs. methanol in which 0.11 g. of sodium had been dissolved. The mixture was shaken and the precipitate that formed was removed by filtration. Acetic anhydride (1 ml.) was added to the filtrate and the solution was maintained at 10 to 15° for 1 day. It was then concentrated under reduced pressure to a sirup. Several portions of abs. methanol were added to the sirup and removed by distillation under reduced pressure. The residue was again treated with acetic anhydride in abs. methanol as described above and the resultant sirup, which gave a slightly positive ninhydrin reaction, was dissolved in 30 ml. of water and passed through a 2 (diam.) × 20 cm. column of Dowex 50[30] (H^+ form). The combined effluent (200 ml.) was concentrated under reduced pressure to dryness; yield 0.68 g., m.p. 100–115°. Stacey[15] reports the m.p. 120–122° for 2-acetamido-2-deoxy-α-D-galactose.

The above-described preparation of 2-acetamido-2-deoxy-D-galactose hydrochloride (0.65 g.) was dissolved in concd. hydrochloric acid (4 ml.) and was shaken with ethanethiol in a sealed tube at 0° for 30 min. The reaction mixture was maintained for 24 hr. at 10 to 15°, with occasional shaking, and then was shaken at 0° for 30 min. The mixture was poured into 50 ml. of cold water and the resulting solution was passed through a 1.8 (diam.) × 20 cm. column of Amberlite IR4B[29] (carbonate form). The combined effluent (300 ml.) was concentrated under reduced pressure to dryness and the residue was crystallized from ethyl acetate; yield 220 mg., m.p. 160–163°. Recrystallization was effected from the same solvent; m.p. 163–165°, $[\alpha]^{30}\mathrm{D}$ −32° (*c* 0.53, ethanol); X-ray powder diffraction data: 10.65m,[21,22] 8.32s, 5.36s, 4.95w, 4.28m, 4.06m, 3.68vw, 3.61s, 3.44vw, 3.08w, 2.68vw. This substance was easily soluble in ethanol and methanol and was soluble in hot acetone and ethyl acetate.

Anal. Calcd. for $C_{12}H_{25}NO_5S_2$: C, 43.98; H, 7.70; N, 4.28; S, 19.58. Found: C, 44.27; H, 7.81; N, 4.33; S, 19.46.

2-Acetamido-tetra-*O*-acetyl-2-deoxy-D-galactose Diethyl Dithioacetal (IX).—2-Amino-2-deoxy-D-galactose hydrochloride (VII, 1.0 g.) was allowed to react with ethanethiol (4 ml.) in concd. hydrochloric acid (3 ml.), and the reaction mixture was treated as described above for the preparation of the dithioacetal. The resultant crude product was treated with a mixture of acetic anhydride (3 ml.) and pyridine (6 ml.) at 10 to 15° for 24 hr. and then at room temperature for another 24 hr. The mixture was poured into 80 ml. of ice and water and was extracted with chloroform. A crystalline product was obtained upon evaporating the dried chloroform extract after washing with a saturated aqueous sodium bicarbonate solution and with water. The residue was extracted with hot ligroin (b.p. 65–110°) from which crystals were deposited on cooling; m.p. 127–132°. Recrystallization was effected from ethyl acetate–petroleum ether; yield 110 mg., m.p. 137–139°, $[\alpha]^{30}\mathrm{D}$ −17° (*c* 0.71, chloroform); X-ray powder diffraction data: 9.61m.[21,22] 7.94s, 6.42w, 6.07vw, 5.80w, 5.54s, 4.30m, 4.06m, 3.91vw, 3.79vw, 3.63m, 3.40vw, 3.29w, 2.95vw, 2.88vw, 2.75w. Kent and co-workers[14] report m.p. 184° (from ethyl acetate) and $[\alpha]^{20}\mathrm{D}$ −22° (*c* 0.36, chloroform).

2-Acetamido-tetra-*O*-acetyl-2-deoxy-D-galactose diethyl dithioacetal was easily soluble in chloroform, acetone, ether, ethyl acetate, ethanol and methanol; it was soluble in hot ligroin (b.p. 65–110°) and was insoluble in hot petroleum ether (b.p. 30–65°).

Anal. Calcd. for $C_{20}H_{33}NO_9S_2$: C, 48.47; H, 6.71; N, 2.83; S, 12.94. Found: C, 48.58; H, 6.55; N, 2.73; S, 12.97.

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[27] C. J. M. Rondle and W. T. J. Morgan, *Biochem. J.* (*London*), **61**, 586 (1955); analysis by Dr. R. A. Gibbons of this Laboratory.

[28] I. Werner, *Mikrochemie*, **39**, 133 (1952).

[29] A cation exchange resin produced by the Resinous Products and Chemical Co., Philadelphia, Pa.

[30] An ion exchange resin produced by The Dow Chemical Co., Midland, Mich.