2-Carboxy-4-hydroxy-*cis*-3,6-endomethylenehexahydrobenzoyl-DL-valine γ-Lactone.—Two and eight-tenths grams (0.0095 mole) of the above methyl ester was added to 9.8 ml. (0.020 mole) of 1.95 *N* sodium hydroxide, and the mixture was warmed on the steam-bath for a few minutes until complete solution. The liquid was cooled and then acidified with concentrated hydrochloric acid. The mixture was then filtered to give 2.4 g. (89%) of material with m.p. of 187–189°. The m.p. rose to 191.5–192.5° after three recrystallizations from water.

Anal. Calcd. for $C_{14}H_{19}O_5N$: C, 59.8; H, 6.81; N, 4.98; neut. equiv., 281. Found: C, 59.7; H, 6.49; N, 4.94; neut. equiv., 280.

Methyl Ester of 2-Carboxy-4-hydroxy-*cis*-3,6-endomethylenehexahydrobenzoic Acid γ-Lactone (III).—Alder and Stein[23] isolated this ester in a 15% yield following esterification with sulfuric acid as a catalyst. The use of hydrogen chloride proved to be more satisfactory.

A mixture of 20 g. (0.11 mole) of the previously described *cis*-lactonic acid and 200 ml. of methanol was saturated with dry hydrogen chloride and refluxed for one hour. It was then resaturated with hydrogen chloride and refluxed for a total of three hours. The residue from evaporation of the methanol was dissolved in chloroform and extracted once with water and once with potassium carbonate solution. The chloroform solution of the ester was dried over sodium sulfate and evaporated to give a residue of 18.5 g. (86%) of the methyl ester with a m.p. of 80–82°. The m.p. was raised to 82–83° after several recrystallizations from ethyl acetate–hexane. The previously reported value[17] was 85°.

2-Carboxy-4-hydroxy-*trans*-3,6-endomethylenehexahydrobenzoyl-DL-valine γ-Lactone.—The acid chloride of the lactonic acid[21] was prepared in benzene from 5.0 g. (0.027 mole) of the acid prepared in turn by saponification of the above methyl ester and 6.0 g. (0.029 mole) of phosphorus pentachloride. The benzene and the phosphorus oxychloride were removed from the solution under reduced pressure in a water-bath kept at 60°. Small amounts of benzene were then added and evaporated to ensure removal of all phosphorus oxychloride. To the residue there was added, with vigorous shaking, a cold solution of 6.5 g. (0.055 mole) of DL-valine in 50 ml. (0.10 mole) of 2 *N* potassium carbonate. The resulting solution was acidified in the cold with concentrated hydrochloric acid. Upon the addition of about 20 ml. of ethyl acetate to the oily mixture, there appeared a precipitate which was filtered off and dried; m.p. 210–218°. The material was purified at room temperature by solution in absolute ethanol, addition of ethyl acetate to prevent layering, and then precipitation with hexane. Repetition of this procedure finally gave crystals with a m.p. 230–231°.

Anal. Calcd. for $C_{14}H_{19}O_5N$: C, 59.8; H, 6.81; N, 4.98; neut. equiv., 281. Found: C, 59.9; H, 6.42; N, 4.88; neut. equiv., 281.

Antibacterial Tests.—The compounds were tested against *Escherichia coli* in the medium of Gray and Tatum[24] and against *Lactobacillus arabinosus* in a "synthetic" medium[7] at concentrations of substance of 1.00, 0.100, and 0.010 mg./ml., by Daniel Atkinson. All tubes showed at 20 hr. virtually the same growth as in controls.

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[23] K. Alder and G. Stein, *Ann.*, **514**, 24 (1934).

[24] C. H. Gray and E. L. Tatum, *Proc. Natl. Acad. Sci.*, **30**, 404 (1944).

AMES, IOWA RECEIVED JULY 23, 1951

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA]

The Degradation of Sugars by Means of their Disulfones[1]

BY D. L. MACDONALD AND HERMANN O. L. FISCHER

When D-glucose diethyl mercaptal pentaacetate was oxidized with monoperphthalic acid in ether, D-*arabo*-3,4,5,6-tetraacetoxy-1,1-bis-(ethanesulfonyl)-hexene-1 (I) was the main product of the reaction. When this was treated wtih hydrazine in methanol, a splitting of the molecule took place, and D-arabinose and bis-(ethanesulfonyl)-methane could be isolated in good yield. In an analogous manner, D-lyxose could be prepared from D-galactose. The double bond in the *arabo*-stereoisomer readily adds ammonia, to give predominantly a disulfone derivative of the glucosamine series.

In his first paper on sugar mercaptals, Emil Fischer[2] described briefly the attempted oxidation of these compounds to the disulfones using potassium permanganate. Since that time nothing appears to have been published on this type of sugar derivative, although the thioglycosides have been oxidized to the corresponding sulfoxides[3] and some monosulfones of sugars have been prepared and their properties studied.[4]

If the acetylated mercaptals of glucose, mannose or galactose are oxidized with monoperphthalic acid in ether, readily crystallizing 1,1-disulfones can be obtained in yields varying with the particular sugar employed. The present work deals with the disulfones derived from the above mentioned hexoses, and at the time that the results of this work were first announced,[1] it was learned that Seidman and Link[5] had oxidized sugar mercaptals with peracetic acid because of their desire to investigate the pharmacological properties of sulfone-containing sugar derivatives.

When D-glucose diethyl mercaptal pentaacetate was oxidized in our laboratory with monoperphthalic acid in ether, the main product was a compound, isolated in a yield of 60–70%, whose analyses corresponded with the formula of an unsaturated disulfone, D-*arabo*-3,4,5,6-tetraacetoxy-1,1-bis-(ethanesulfonyl)-hexene-1 (I). Tests for the presence of a double bond proved negative with tetranitromethane and, as expected, with bromine in acetic acid; however, the compound slowly gave a positive test with osmium tetroxide. The presence of a double bond was further substantiated by the isolation of the same compound in a yield of 55–60% by oxidation of D-mannose diethyl mercaptal pentaacetate. The analogous oxidation of D-galactose diethyl mercaptal pentaacetate likewise gave in good yield (85%) an unsaturated disulfone,

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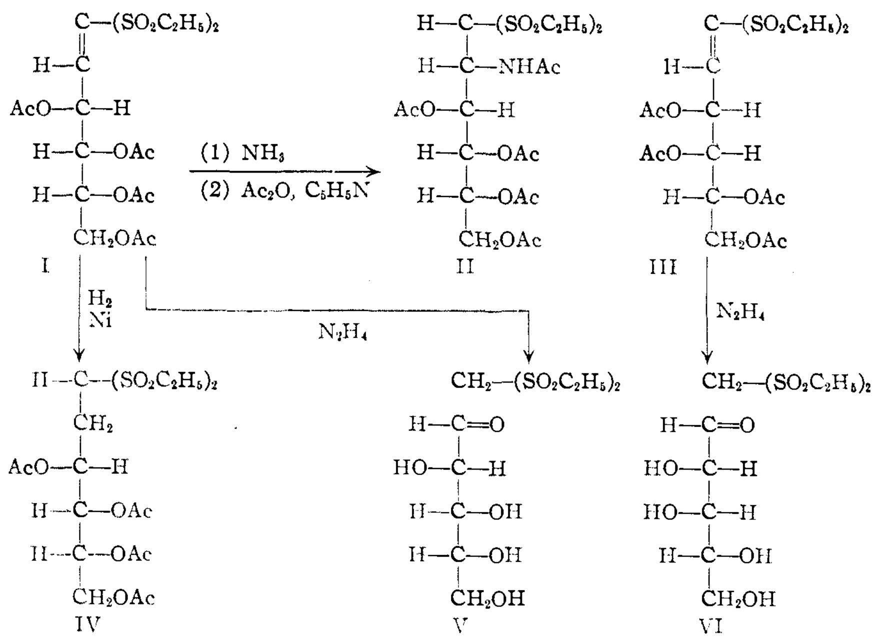
[1] Presented in part before the Division of Sugar Chemistry at the 118th National Meeting of the American Chemical Society, Chicago, Illinois, September, 1950.

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

[3] F. Micheel and H. Schmitz, *ibid.*, **72**, 992 (1939).

[4] F. Wrede and W. Zimmerman, *Z. physiol. Chem.*, **148**, 65 (1925); W. A. Bonner and R. W. Drisko, THIS JOURNAL, **70**, 2435 (1948); **73**, 3699, 3701 (1951).

[5] M. Seidman, Ph.D. Thesis, Summaries of Doctoral Dissertations, University of Wisconsin Press, Madison, Wisconsin, Vol. 12, 1950–1951.



D - *lyxo* - 3,4,5,6 - tetraacetoxy - 1,1 - bis - (ethanesulfonyl)-hexene-1 (III); it too gave the same reactions with the above reagents for double bonds. Another qualitative test, which appears to be dependent upon the presence of a double bond in these two compounds, is their behavior in dry pyridine solution: such a solution turns to a cherry red in color after a short period of standing.

These unsaturated disulfones appeared to be quite unstable to alkali. When treated with sodium hydroxide in ethanol, a small amount of bis-(ethanesulfonyl)-methane was obtained, but no other products of the reaction could be isolated. However, by treatment of these unsaturated disulfones obtained from hexoses with hydrazine hydrate in methanol, followed by splitting the anticipated hydrazone with benzaldehyde in the usual way, and subsequently deionizing the solution, the corresponding pentoses were isolated in crystalline condition in excellent yield. Thus, D-arabinose, having $[\alpha]^{24}\text{D}$ -102 to $-103°$ (equilibrium in water) was obtained in yields of 72% from the disulfone from glucose, and D-lyxose, showing $[\alpha]^{24}\text{D}$ -13.5 to $-14.0°$, was obtained in yields of 54–58% from the disulfone from galactose. Oxidation of D-glucose diethyl mercaptal pentaacetate, followed by degradation using hydrazine, without purification of the intermediate disulfone, gave D-arabinose in a yield of 57–60%, exhibiting $[\alpha]^{25}\text{D}$ $-103.3°$; the corresponding galactose derivative gave D-lyxose in yields of 55–60% with $[\alpha]^{25}\text{D}$ $-13.4°$. The accepted specific rotation of D-arabinose at equilibrium in water is $-104.5°$ while that of D-lyxose is $-13.8°$.

This degradation of hexoses to pentoses compares favorably with the well known methods of Ruff and Wohl; using modern techniques, the Ruff degradation gives arabinose and lyxose in yields of about 45%[6] while the Wohl degradation gives arabinose in yields of about 65%.[7]

The bis-(ethanesulfonyl)-methane which would result from a scission of the sugar molecule between carbon atoms one and two was isolated in yields of 86–90% as described in the experimental section. Thus it is apparent that the action of hydrazine results in a clean-cut splitting of the molecule. It is of interest to note that bis-(ethanesulfonyl)-methane has been obtained previously from a sugar mercaptal: it results from the oxidation of ethyl thiostreptobiosaminide diethyl mercaptal hydrochloride with hydrogen peroxide in acetic acid.[8]

Some sulfone groups have been found to be readily removable by Raney nickel,[9] but the sulfone groups in D-*arabo*-3,4,5,6-tetraacetoxy-1,1-bis-(ethanesulfonyl)-hexene-1 are quite resistant to this reagent, the only product isolable being the saturated disulfone (IV). By carrying out the hydrogenation as described in the experimental section, the pure saturated disulfone could be obtained in a yield of 73%. When the hydrogenation was carried out on D-*lyxo*-3,4,5,6-tetraacetoxy-1,1-bis-(ethanesulfonyl)-hexene-1, no crystalline material could be obtained.

In contrast to the clean-cut splitting of the molecule which hydrazine brings about, ammonia was found to react readily with the double bond, similar to its addition to α,β-unsaturated monosulfones.[10] When D-*arabo*-3,4,5,6-tetraacetoxy-1,1-bis-(ethanesulfonyl)-hexene-1 was dissolved in aqueous ammonia or in methanol saturated with ammonia, the addition of ammonia was, of course, accompanied by deacetylation. Reacetylation gave only one product, in good yield, the condition of the experiment apparently favoring the formation of one isomer, namely, D-*gluco*-2-acetamino-3,4,5,6-tetraacetoxy-1,1-bis-(ethanesulfonyl)-hexane (II). That this isomer possessed the glucosamine and not the mannosamine configuration was conclusively shown by the synthesis of the same compound by oxidation of D-glucosamine diethyl mercaptal pentaacetate[11] with monoperphthalic acid in ether.

As shown in Fig. 1, the ultraviolet absorption spectra of the two unsaturated disulfones exhibit broad maxima in the 270 mμ region. In the case of the disulfone derived from glucose (curve I), this maximum disappears on reduction (curve IV) and on conversion to the glucosamine derivative

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[6] H. G. Fletcher, Jr., H. W. Diehl and C. S. Hudson, This Journal, **72**, 4546 (1950).

[7] O. Braun, *Org. Syntheses*, **20**, 14 (1940).

[8] F. A. Kuehl, Jr., E. H. Flynn, N. G. Brink and K. Folkers, This Journal, **68**, 2096 (1946).

[9] R. Mozingo, D. E. Wolf, S. A. Harris and K. Folkers, *ibid.*, **65**, 1013 (1943).

[10] See for instance: F. G. Bordwell and W. H. McKellin, *ibid.*, **72**, 1985 (1950); C. M. Suter, "Organic Chemistry of Sulfur," John Wiley and Sons, Inc., New York, N. Y., pp. 725–728.

[11] M. L. Wolfrom, R. U. Lemieux and S. M. Olin, This Journal, **71**, 2870 (1949).

(curve II). Similar compounds such as vinyl ethyl sulfone and bis-(ethanesulfonyl)-methane are transparent in this region.[12]

Up to the present, the isolation of D-*gluco*-2,3,4,-5,6 - pentaacetoxy - 1,1 - bis - (ethanesulfonyl)-hexane by oxidation of D-glucose diethyl mercaptal pentaacetate has not been successful, although we believe it to be among the products of oxidation. Work on the preparation of this compound as well as the preparation of the corresponding *manno*- and *galacto*-stereoisomers is in progress.

Experimental[13]

D-*arabo*-3,4,5,6-Tetraacetoxy-1,1-bis-(ethanesulfonyl)-hexene-1 (I) (a) From Glucose.—To an ice-cold solution of 20.0 g. of D-glucose diethyl mercaptal pentaacetate[14] in 200 ml. of dry ether, was added monoperphthalic acid in ether[15] (20% excess over the required four moles) and the solution cooled in ice for 1 hour and then allowed to stand at room temperature for an additional 16 hours. The ether was removed under reduced pressure and the residue extracted with four 75-ml. portions of chloroform. The chloroform was washed with saturated aqueous sodium bicarbonate, then with water, dried over sodium sulfate and then removed under reduced pressure to give 19.9 g. of crystalline material, m.p. 134–146°. Two recrystallizations from diisobutyl ketone, followed by one from isopropyl alcohol and one from methyl ethyl ketone by addition of petroleum ether (b.p. 35–60°), gave a constant m.p. of 160–162° and $[\alpha]^{23}$D +3.8° (*c* 5.0, U.S.P. chloroform); yield 12.9 g. (64%);

Anal. Calcd. for $C_{10}H_{16}O_8S_2(CH_3CO)_4$ (500.5): C, 43.19. H, 5.64; S, 12.81; CH_3CO, 34.40. Found: C, 43.13; H, 5.60; S, 12.70; CH_3CO, 34.8.

The material gives only slowly a reaction with osmium tetroxide in water, the black precipitate reaching its maximum intensity in about one week. When the material is dissolved in dry pyridine, the solution becomes dark reddish-brown after a few hours. No reaction was observable with tetranitromethane nor, as anticipated, could the addition of bromine be effected.

(b) **From Mannose.**—Five grams of D-mannose diethyl mercaptal pentaacetate[16] in 50 ml. of dry ether was oxidized exactly as in the case of the glucose derivative. The product was recrystallized four times from diisobutyl ketone and then from isopropyl alcohol, yielding 2.83 g. (56%), $[\alpha]^{24}$D +3.7° (*c* 1.5, U.S.P. chloroform), m.p. 159–161°, undepressed on admixture with the above described disulfone.

D-*lyxo*-3,4,5,6-Tetraacetoxy-1,1-bis-(ethanesulfonyl)-hexene-1 (III).—Fifteen grams of D-galactose diethyl mercaptal pentaacetate[17] was oxidized with monoperphthalic acid exactly as described for the glucose derivative. The material obtained by concentration of the chloroform extract was recrystallized from acetone by addition of petroleum ether (b.p. 35–60°) and then twice recrystallized from isopropyl alcohol giving 12.94 g. (85%), m.p. 119–120°, $[\alpha]^{25}$D +20.3° (*c* 1.1, U.S.P. chloroform).

Anal. Calcd. for $C_{10}H_{16}O_8S_2(CH_3CO)_4$ (500.5): C, 43.19; H, 5.64; S, 12.81; CH_3CO, 34.40. Found: C, 42.84; H, 5.75; S, 13.01; CH_3CO, 33.8.

Like the glucose derivative, this compound gives reactions with osmium tetroxide and with pyridine, and no reaction with the tetranitromethane.

D-*arabo*-3,4,5,6-Tetraacetoxy-1,1-bis-(ethanesulfonyl)-hexane (IV).—One gram of the D-*arabo*-3,4,5,6-tetraacetoxy-1,1-bis-(ethanesulfonyl)-hexene-1 (I) was shaken with 10 g. of Raney nickel[18] in 50 ml. of dioxane for 90 minutes, at 70° and a hydrogen pressure of 45 p.s.i. The catalyst was removed by centrifugation and washed with dioxane, and the combined solution and washings were concentrated under reduced pressure. Addition of ethanol followed by concentration *in vacuo* gave a crystalline residue which, on recrystallization from isopropyl ether, gave 0.82 g. of material, m.p. 102–103°. Pure material was obtained by recrystallizing again from isopropyl ether; yield 0.73 g. (73%), m.p. 103–104°, $[\alpha]^{23}$D +22.1° (*c* 3.0, U.S.P. chloroform).

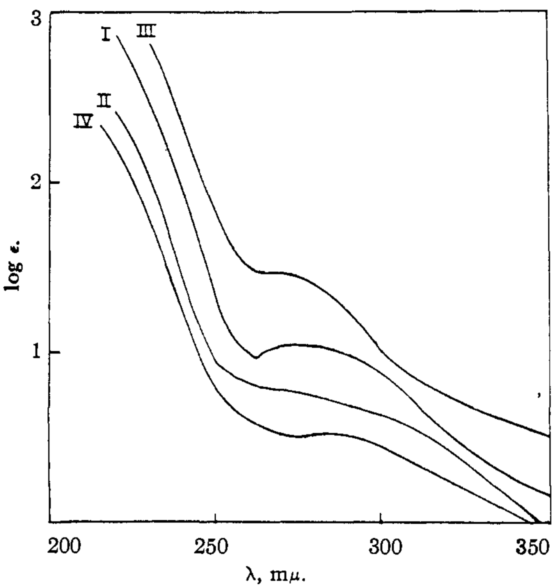

Fig. 1.—Absorption spectra in 95% EtOH, 1-cm. cell, concentration 1 mg./ml. Curve numbers refer to the formulas.

Anal. Calcd. for $C_{10}H_{18}O_8S_2(CH_3CO)_4$ (502.5): C, 43.02; H, 6.02; S, 12.76; CH_3CO, 34.26. Found: C, 43.00; H, 5.94; S, 12.62; CH_3CO, 34.02.

This compound gave no reaction with either osmium tetroxide or pyridine. On deacetylation with ammonia in methanol, followed by reacetylation, the pure starting material was recovered in 84% yield after recrystallization from isopropyl ether. When the reduction was carried out for 4.5 hours using 5% palladium-on-charcoal, in 95% ethanol, at 70° and 45 p.s.i., the same compound was obtained but in a yield of only 35%.

D-*gluco*-2-Acetamino-3,4,5,6-tetraacetoxy-1,1-bis-(ethanesulfonyl)-hexane (II) (a) From D-Glucosamine.[19]—Because of the difficulties experienced in the crystallization of D-glucosamine diethyl mercaptal pentaacetate,[12] the sulfone was prepared in the following manner. To an ice-cold solution of 0.75 g. of N-acetyl D-glucosamine diethyl mercaptal[12] in 3.5 ml. of dry pyridine was added 3.5 ml. of acetic anhydride, and after standing in an ice-bath for one hour, the solution was allowed to stand at room temperature for 20 hours. The product was poured into 40 ml. of ice-water, allowed to stand one hour with frequent shaking, extracted five times with 5-ml. portions of chloroform, and the chloroform extract washed twice with saturated aqueous sodium bicarbonate, then with water and dried (sodium sulfate). The chloroform was removed at reduced pressure, and the resulting sirup dissolved in 15 ml. of dry ether, and oxidized with monoperphthalic acid in ether (20% excess, based on the N-acetylglucosamine mercaptal used). After 60 hours, the product was worked up as in the case of the other oxidations and recrystallized three times from ethanol, giving 1.04 g. (81%), m.p. 176.5–178°, $[\alpha]^{25}$D + 13.6° (*c* 4.1, U.S.P. chloroform).

Anal. Calcd. for $C_{10}H_{16}O_8S_2N(CH_3CO)_5$ (559.6): C, 42.92; H, 5.94; N, 2.50; S, 11.46. Found: C, 43.05; H, 6.13; N, 2.25; S, 11.31.

(b) **From D-Glucose.**—One gram of D-*arabo*-3,4,5,6-tetraacetoxy-1,1-bis-(ethanesulfonyl)-hexene-1 (I) was dissolved with swirling in 20 ml. of concentrated aqueous ammonia and allowed to stand at room temperature for 20 hours. The solution was concentrated at reduced pressure, absolute alcohol was added and removed at reduced pressure and the process repeated several times. The product was acetylated with pyridine (3 ml.) and acetic anhydride (3 ml.) for

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[12] E. A. Fehnel and M. Carmack, *ibid.*, **71**, 231 (1949).

[13] All melting points taken in capillaries with Anschütz thermometers. Microanalyses by Microchemical Laboratory, University of California, and by Dr. A. Elek, Los Angeles, California.

[14] M. L. Wolfrom, THIS JOURNAL, **51**, 2188 (1929).

[15] H. Böhme in *Org. Syntheses*, **20**, 70 (1940).

[16] N. W. Pirie, *Biochem. J.*, **30**, 374 (1936).

[17] M. L. Wolfrom, THIS JOURNAL, **52**, 2464 (1930).

[18] A. A. Pavlic and H. Adkins, *ibid.*, **68**, 1471 (1946).

[19] Experimental work by Mr. R. Dorner.

18 hours at room temperature, and then poured into ice, giving 0.99 g. of crystalline material. Recrystallization from ethanol gave 0.95 g. (84%) of pure material, m.p. 177–178.5°, undepressed on admixture with material prepared from D-glucosamine, $[\alpha]^{25}$ +13.6° (*c* 4, U.S.P. chloroform).

Anal. Calcd. for $C_{10}H_{18}O_8S_2N(CH_3CO)_5$ (559.6): C, 42.92; H, 5.94; N, 2.50; S, 11.46; CH_3CO, 38.46. Found: C, 42.88; H, 5.94; N, 2.71; S, 11.44; CH_3CO, 38.91.

The same material was also obtained by substituting methanolic ammonia for aqueous ammonia in the above procedure.

D-Arabinose.—Five grams of the D-*arabo*-3,4,5,6-tetraacetoxy-1,1-bis-(ethanesulfonyl)-hexene-1 (I) was added to 100 ml. of methanol and to this there was added 4.5 ml. of 85% hydrazine hydrate in water. The material dissolved rapidly and, after four hours at room temperature, the solvent was removed at reduced pressure, water (50 ml.) was added, and the mixture extracted four times with 20-ml. portions of chloroform. To the aqueous layer was added 35 ml. of benzaldehyde, 0.5 g. of benzoic acid and 40 ml. of ethanol and the mixture was refluxed for 3 hours. After cooling, the mixture was extracted with three 20-ml. portions of chloroform and then with 20 ml. of ether, and then passed consecutively through columns of Duolite C-3H and Duolite A-2[20] and washed with about 400 ml. of water, after which the effluent gave a negative Fehling test. The percolate was concentrated at reduced pressure (bath temperature below 60°) to a volume of about 10 ml., charcoal was added and the water removed from the filtered solution in a vacuum oven at 40°.[21] To complete the crystallization of the D-arabinose 5 ml. of hot methanol was added, and after 24 hours at +4° the crystalline residue was washed twice with cold methanol by decantation and dried *in vacuo* over calcium chloride. In a typical experiment, there was obtained 1.10 g. (73%) of D-arabinose $[\alpha]^{23}D$ −103.0° (equilibrium, *c* 2, water).

The first chloroform extract was dried with Na_2SO_4 and, after removal of the solvent at reduced pressure, the crystalline residue was recrystallized from water giving 1.73 g. (87%) of bis-(ethanesulfonyl)-methane m.p. 102–103°, undepressed on admixture with an authentic sample.

The degradation could also be carried out on the crude disulfone; 5.00 g. of D-glucose diethyl mercaptal pentaacetate was oxidized with monoperphthalic acid as described, and the crude disulfone obtained after concentration of the chloroform extract was degraded exactly as in the case of the pure material. In a typical experiment there was obtained 0.88 g. of D-arabinose (58%) having $[\alpha]^{25}D$ −103.3°.

D-Lyxose.—Five grams of D-*lyxo*-3,4,5,6-tetraacetoxy-1,1-bis-(ethanesulfonyl)-hexene-1 (III) was degraded exactly as in the case of the *arabo*-isomer. To effect crystallization of the D-lyxose, the sirup obtained after concentration in the vacuum oven was dissolved in 3 ml. of hot methanol, seeded, and left at +4°. After crystallization had progressed considerably (24 hours), 3 ml. of isopropyl alcohol was added and after a further 24 hours at +4° the crystals were washed twice by decantation with cold isopropyl alcohol. In a typical experiment there was obtained 0.86 g. (57%) of D-lyxose, $[\alpha]^{24}D$ −14.0° (equilibrium, *c* 3.8, water).

Working up the chloroform extract containing the bis-(ethanesulfonyl)-methane gave, after recrystallization from water, 1.80 g. of material (90%), m.p. 102–103°.

When the degradation was carried out on the crude disulfone obtained by oxidation of 5.00 g. of D-galactose diethyl mercaptal pentaacetate, there was obtained 0.93 g. (60%) of D-lyxose showing $[\alpha]^{25}D$ −13.4° (equilibrium, *c* 4.0, water).

Acknowledgments.—The authors are particularly grateful to Miss B. Rolander for carrying out many of the combustion analyses, and to Mr. R. Dorner for the preparation of D-*gluco*-2-acetamino-3,4,5,6 - tetraacetoxy - 1,1 - bis - (ethanesulfonyl)-hexane from D-glucosamine.

BERKELEY 4, CALIFORNIA RECEIVED NOVEMBER 12, 1951

[20] Manufactured by the Chemical Process Company, Redwood City, California; column dimensions 1.4 × 21 cm.

[21] *Cf.* W. Z. Hassid, M. Doudoroff and H. A. Barker, THIS JOURNAL, **66**, 1416 (1944).

[CONTRIBUTION FROM THE BIOLOGY DIVISION, OAK RIDGE NATIONAL LABORATORY]

The Separation of Sugars by Ion Exchange[1]

BY JOSEPH X. KHYM AND LEONARD P. ZILL

The separation of sugars has been achieved by the elution of their borate complexes from strong-base anion exchangers with boric–borate buffers. Disaccharides are readily separated from the monosaccharides and the components of hexose or pentose mixtures are easily separated. Hexose–pentose mixtures can be analyzed by the techniques presented. The results are consistent with current concepts of the structures of sugar–borate complexes and the reactions of free sugars in aqueous solutions.

Introduction

The lack of simple quantitative methods for the analysis of sugar mixtures has long been recognized. Until recently,[2] the assay of one or more components of a sugar mixture has been somewhat involved and, in some cases, impossible. Although newer methods have overcome to a large extent the fundamental empiricism of reporting sugars as total and reducing sugars, they have certain difficulties of methodology.

The experiments to be described had as their objective the development of a procedure for the quantitative analysis of mixtures of sugars using the technique of ion exchange in a fashion similar to that used in separating nucleotides and related compounds.[3]

Sugars, being very weak electrolytes, have little tendency to react with ion exchangers. However, it has long been known that certain polyhydroxy compounds react with borate ion (from boric acid or its salts) with great ease and rapidity to form borate complexes which are negatively charged ions.[4] Although within a given class of sugars

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[1] Biology Division, Oak Ridge National Laboratory, operated under Contract Number W-7405-Eng-26 for the Atomic Energy Commission, Oak Ridge, Tennessee.

[2] S. Udenfriend and M. Gibbs, *Science*, **110**, 708 (1949); W. W. Binkley and M. L. Wolfrom, Chromatography of Sugars and Related Substances, Sugar Research Foundation, Inc., *Scientific Report Series* #10 (1948); B. W. Lew, M. L. Wolfrom and R. M. Goepp, Jr., THIS JOURNAL, **68**, 1449 (1946); M. Ikawa and C. Niemann, *J. Biol. Chem.*, **180**, 923 (1949); M. Ikawa and C. Nieman, *Arch. Biochem. Biophys.*, **31**, 62 (1951); Z. Dische, L. B. Shettles and M. Osnos, *Arch. Biochem.*, **22**, 169 (1949); S. Hestrin and J. Mager, *Nature*, **158**, 95 (1946); L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 2511 (1949); S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).

[3] W. E. Cohn, THIS JOURNAL, **72**, 1471 (1950); E. Volkin, J. X. Khym and W. E. Cohn, *ibid.*, **73**, 1533 (1951).

[4] J. Böeseken in "Advances in Carbohydrate Chemistry," edited by W. W. Pigman and M. L. Wolfrom, Academic Press, Inc., New York, N. Y., 1949, Vol. 4, p. 189.