Periodate Oxidations.—All oxidations were carried out in unbuffered 0.08 *M* sodium metaperiodate in the dark. Samples of 200–300 mg. (accurately weighed) were used and in all cases approximately 8 moles of oxidant per mole of compound was used in a total volume of 100 ml. Consumption of oxidant was followed[15] by adding 1-ml. samples withdrawn at intervals to 5 ml. of 0.066 *M* phosphate buffer *p*H 7.4–7.5 containing 1 ml. of 20% aqueous potassium iodide. Iodine liberated was titrated after 10 minutes with 0.01 *N* sodium arsenite. At intervals the consumption of oxidant was also checked by adding a 1-ml. aliquot of the reaction to 10 ml. of 2 *N* sulfuric acid containing 1 ml. of 20% aqueous potassium iodide and titrating the iodine liberated with 0.1 *N* thiosulfate. In all cases the possibility of iodine consumption by the products of the reaction was checked by adding 5 ml. of 0.1 *N* iodine to an aliquot of the reaction mixture and titrating with 0.1 *N* thiosulfate after 10 minutes.

Volatile acid was determined on 5-ml. samples of the reaction mixture. Two ml. of ethylene glycol and 2 ml. of concentrated sulfuric acid were added and the sample steam distilled until 50-ml. aliquots of the distillate required only one drop of 0.005 *N* sodium hydroxide to give a pink color with phenolphthalein.

Formaldehyde was determined by adding 50 ml. of the reaction mixture to 100 ml. of a 0.4% aqueous solution of dimedon (5,5-dimethyl-1,3-cyclohexanedione) and adjusting the *p*H to 6.5 with 3 *N* sodium hydroxide. The precipitate was removed by filtration, washed with water and air-dried and then recrystallized from methanol. Melting points were compared with a sample prepared from formaldehyde. The results obtained with the various materials expressed as moles per mole of compound were

Compound	IO_4^- consumed 1 hr.	IO_4^- consumed 24 hr.	IO_4^- consumed 96 hr.	HCOOH produced 1 hr.	HCOOH produced 96 hr.	HCHO produced 1 hr.	HCHO produced 96 hr.
III	3.97	4.06	4.10	3.03	2.94	0.87	0.94
IV	3.84	4.14	4.08	2.76	2.87	0.96	.89
XI	4.09	4.16	4.80	3.10	3.19	1.04	.98
V	2.01	3.92	4.15	0.96	3.03	0.0	.86
X	1.95	4.01	4.06	0.92	2.98	0.1	.78

Paper chromatography was carried out by the descending method at room temperature using Whatman #1 paper with butanol–acetic acid–water (4:1:5 v./v.) as the solvent. Ammoniacal silver nitrate or periodate–benzidine[16] were used to detect polyhydroxy compounds. The R_f's of the various materials were: III, 0.41; IV, 0.42; XI, 0.42; V, 0.63; X, 0.67. The 1-deoxy-1,1-bis-(ethylsulfonyl)-hexitols III and IV invariably showed two components on chromatography, but it is probable that the materials are actually homogeneous and that the second component was formed during the preparation of the solution from which samples were withdrawn for chromatography. For instance, a sample of III was dissolved in 5% aqueous acetic acid by cautious warming and this solution was spotted on the paper. The developed chromatogram showed two spots, R_f 0.41 and 0.63; no streaking was observed, indicating that little or no interconversion had occurred during chromatography. If a sample of III is prepared for chromatography by heating at 100° for one minute in a dilute acetic acid solution, only the faster moving component is found. Chromatography of a second sample, prepared by first heating in dilute acetic acid and then making the solution slightly basic with ammonia shows that the component with R_f 0.63 is relatively stable to basic conditions; a reaction time of 4 to 5 days is required before this component is completely degraded to D-arabinose. Chromatography of a sample prepared by suspending III in dilute aqueous ammonia and acidifying after 5 minutes shows that in this time, the crystalline material is completely degraded to D-arabinose.

1-Deoxy-1,1-bis-(ethylsulfonyl)-D-galactitol could be dissolved in dilute acetic acid in the cold, and showed but one component on chromatography. However, it showed a behavior similar to that of the other hexitol derivatives when treated as above.

(15) G. D. Greville and D. H. Northcote, *J. Chem. Soc.*, 1945 (1952).

(16) M. Viscontini, D. Hoch and P. Karrer, *Helv. Chim. Acta*, **38**, 642 (1955).

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Some Oxidation and Reduction Products of 2,4-*O*-Ethylidene-D-erythrose[1]

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Certain improvements in the synthesis of 4,6-*O*-ethylidene-D-glucose are described, and the compound has been used as a convenient starting material for the preparation of D-erythronolactone and some erythritol derivatives.

Recently, there has been considerable interest in D-erythrose and its oxidation and reduction products, particularly with reference to their use as intermediates in the synthesis of other sugars and their conversion to various phosphate esters. For instance 2,4-*O*-ethylidene-D-erythrose has been used as an intermediate in the preparation of D-erythrose 4-phosphate[3] and D-erythritol 4-phosphate.[4] It has also been used as a starting material for the preparation of certain glycosides of D-erythrose,[5] a diethylidene octose,[6] and certain labeled pentoses.[7] D-Erythronolactone has recently been used as an intermediate in the preparation of D-ribulose derivatives,[8] and for the preparation of certain phosphates of D-erythronic acid.[9]

A suitable starting material for all of the above syntheses is 4,6-*O*-ethylidene-D-glucose, the preparation of which has been described.[10,11] However, the procedure of Hockett, Collins and Scattergood gives a product which is difficult to purify to constant melting point. This difficulty, which results from the presence of ammonium sulfate in the product, has been overcome and the improved procedure is described below. The periodate oxidation of 4,6-*O*-ethylidene-D-glucose to form 2,4-*O*-ethylidene-D-erythrose using sodium bicarbonate to

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(2) National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.

(3) C. E. Ballou, H. O. L. Fischer and D. L. MacDonald, THIS JOURNAL, **77**, 5967 (1955).

(4) D. L. MacDonald, H. O. L. Fischer and C. E. Ballou, *ibid.*, **78**, 3720 (1956).

(5) C. E. Ballou, Abstr. Am. Chem. Soc., 134th Meeting, Chicago, Ill., 1958, p. 9-D.

(6) R. Schaffer, THIS JOURNAL, **81**, 2838 (1959).

(7) H. L. Frush and H. S. Isbell, *J. Research Natl. Bur. Standards*, **51**, 307 (1953).

(8) D. L. MacDonald, J. D. Crum and R. Barker, THIS JOURNAL, **80**, 3379 (1958).

(9) R. Barker and F. Wold, manuscript in preparation.

(10) B. Helferich and H. Appel, *Ber.*, **64**, 1841 (1931).

(11) R. C. Hockett, D. V. Collins and A. Scattergood, THIS JOURNAL, **73**, 599 (1951).

neutralize the formic acid produced has been described previously.[3,7,12] The product so obtained is often heterogeneous, as evidenced by paper chromatography, and the contaminants may interfere with the subsequent steps, in particular, in the crystallization of D-erythronolactone. In the present report, we have carried out the periodate oxidation at *p*H 4, in a manner similar to that recently described by Schaffer,[6] with consistently good results. The 2,4-*O*-ethylidene-D-erythrose so obtained was oxidized using peroxypropionic acid, and, after hydrolysis, D-erythronolactone was obtained in yields of 60–65%, based on 4,6-*O*-ethylidene-D-glucose. Alternatively, the 2,4-*O*-ethylidene-D-erythrose could be reduced without isolation to give 2,4-*O*-ethylidene-D-erythritol (1,3-*O*-ethylidene-L-erythritol) in yields of 80%. Hydrolysis gives erythritol in yields of 75%. Also described is 2-*O*-acetyl-1,3-*O*-ethylidene-4-*O*-trityl-L-erythritol which was prepared during the work on L-erythritol 4-phosphate.

Experimental[13]

4,6-*O*-Ethylidene-D-glucose was prepared by the method of Hockett, *et al.*[11] modified in the following fashion. D-Glucose (180 g.) was treated with 135 ml. of paraldehyde containing 1 ml. of concentrated sulfuric acid in a 2-liter reagent bottle. The reaction was shaken until it became semi-solid (approximately 30 minutes) and then set at room temperature for 3 days. The mixture was then slurried in 600 ml. of absolute ethanol and the *p*H adjusted to 6.5–7.0 (indicator paper) with 1 *N* ethanolic potassium hydroxide. The solids were brought into solution by heating, and the *p*H kept at 6.5 by the addition of more alkali. Ten grams of charcoal was added and the solution filtered with suction through a layer of Celite which was then washed with hot ethanol. The filtrate on standing overnight deposited 100–120 g. of crystalline material, m.p. 179–181°, identical with that reported.[11] The filtrate was concentrated to dryness to remove the excess paraldehyde and from the crystalline residue more material of the same m.p. was obtained by recrystallization from ethanol; total yield 140–160 g. (70–80%). The compound showed $[\alpha]^{20}D$ −2.37° at equilibrium (*c* 19.7, water).

2,4-*O*-Ethylidene-D-erythrose.—4,6-*O*-Ethylidene-D-glucose (103 g., 0.5 mole) was dissolved in 250 ml. of water and added dropwise over a period of 30 minutes to a well-stirred solution of 216 g. (1.01 moles) of sodium metaperiodate in 2 liters of water. The temperature of the reaction was kept below 10° with an ice-bath and the *p*H maintained at approximately 4 (indicator paper) by the dropwise addition of 60 ml. of 8 *N* sodium hydroxide (0.5 mole). At this time the reaction mixture gave a faint test for periodate.[14] The *p*H was then adjusted to 6.5 by the addition of more 8 *N* sodium hydroxide (approx. 60 ml.), causing rapid hydrolysis of the formyl group. The reaction mixture was concentrated at 40–45° and the resulting solids were dried overnight at room temperature and 0.1 mm. pressure, and then thoroughly extracted with three 200-ml. portions of hot ethyl acetate. The combined filtered extracts were dried over sodium sulfate and concentrated to give 65–72 g. (89–99%) of a colorless or slightly yellow amorphous glass. This glass was chromatographically homogeneous and was shown to be 95–100% pure by hypoiodite titration.[15] The rotation was $[\alpha]^{20}D$ −36.8° at equilibrium (*c* 8.25, water).

Anal. Calcd. for $C_6H_{10}O_4$ (146.1): C, 49.31; H, 6.90. Found: C, 49.56; H, 7.02.

D-Erythronolactone.—2,4-*O*-Ethylidene-D-erythrose (50 g.) was dissolved in 200 ml. of dry ethyl acetate, and a 200% excess of peroxypropionic acid[16] added (250 ml. of 4 *M* peracid). The solution was refluxed for 6 hours and then concentrated to dryness. Water was added and the solution concentrated, and this process was repeated to remove propionic acid. The sirup so obtained was dissolved in 700 ml. of water and the resulting solution concentrated to 300 ml. at atmospheric pressure, at approximately 100°, to effect hydrolysis of the ethylidene group, and removal of the acetaldehyde. The solution was then concentrated to dryness and the resulting sirup heated *in vacuo* at 70–90° for 1 hour. On cooling, D-erthronolactone crystallized spontaneously. Recrystallization from three parts of isopropyl alcohol yielded approximately 25 g. of material, m.p. 100–102°. Further crops were obtained by concentrating the mother liquors to dryness, heating for a short time at 70° *in vacuo*, and crystallizing from isopropyl alcohol. The total yield of material, m.p. 100–102°, was 28–34 g. Recrystallization from isopropyl alcohol (3 parts) or ethyl acetate (20 parts) gave 22–26 g., m.p. 103–104, $[\alpha]^{20}D$ −72.6° (*c* 4.5, water), in agreement with the values (103° and −73°) reported.[17] Further pure material (4–8 g.) was obtained from the mother liquors, total yield 65–75%.

Anal. Calcd. for $C_4H_6O_4$ (118.1): C, 40.68; H, 5.12. Found: C, 40.85; H, 5.27.

2,4-*O*-Ethylidene-D-erythritol.—4,6-*O*-Ethylidene-D-glucose (103 g., 0.5 mole) was oxidized to 2,4-*O*-ethylidene-D-erythrose as described above. After the hydrolysis of the formate group had been completed, the *p*H of the solution was adjusted to 10, whereupon 40 g. of sodium borohydride was added portionwise over a period of one hour. The temperature of the well-stirred reaction mixture was maintained below 15°, and the stirring was continued for a half-hour after all the reducing agent had been added. The reaction mixture was neutralized with dilute sulfuric acid, concentrated, and dried at room temperature and 0.1 mm. pressure overnight. The solid residue was extracted with four 200-ml. portions of boiling chloroform, and the hot chloroform extracts were filtered, then combined and reduced in volume to 200 ml. On standing overnight, 53 g. (73%) of 2,4-*O*-ethylidene-D-erythritol crystallized in large prisms, m.p. 99–100°. Concentration of the mother liquors yielded a further 4.5 g. of material of the same m.p. A sample sublimed for analysis had m.p. 99.5–100.5°, and showed $[\alpha]^{24}D$ −54.7 (*c* 2, water).

Anal. Calcd. for $C_6H_{12}O_4$ (148.2): C, 48.63; H, 8.17. Found: C, 48.58; H, 8.08.

2-*O*-Acetyl-1,3-*O*-ethylidene-4-*O*-trityl-L-erythritol.—Trityl chloride (9.7 g.) was added to a solution of 2,4-*O*-ethylidene-D-erythritol (5.00 g.) in dry pyridine (50 ml.). After 24 hours at room temperature, the solution was cooled to 0°, 8 ml. of acetic anhydride was added, and the mixture left at room temperature overnight. The excess of acetic anhydride was decomposed by the addition of chipped ice, and the solution concentrated to a crystalline residue. This was dissolved in methylene chloride (150 ml.) and washed with 1 *N* aqueous sulfuric acid, 1 *M* aqueous potassium carbonate and water, and then dried (sodium sulfate). Concentration of the solution, followed by crystallization from absolute ethanol (4 parts) gave 11.53 g. (79%) of material, m.p. 102–103°, $[\alpha]^{20}D$ −39.1° (*c* 2.1, chloroform).

Anal. Calcd. for $C_{27}H_{28}O_5$(432.5): C, 74.98; H, 6.53. Found: C, 74.82; H, 6.48.

Erythritol.—2,4-*O*-Ethylidene-D-erythrose (14.6 g., 0.1 mole) was dissolved in 50 ml. of water and treated with a solution of 1.1 g. of sodium borohydride in 15 ml. of 0.1 *N* potassium hydroxide. The solution was left at room temperature for 1 hour, then acidified with 1 *N* hydrochloric acid and passed through a column of Dowex 50 (H^+). The eluate and wash water were combined and boiled for half an hour to hydrolyze the ethylidene group, and then taken to dryness. Boric acid was removed by six concentrations from 50-ml. portions of methanol.[18] Crystallization oc-

(12) D. A. Rappoport and W. Z. Hassid, THIS JOURNAL **73**, 5524 (1951), carried out the same oxidation on 4,6-*O*-ethylidene-L-glucose.

(13) Microanalyses were carried out by the Microchemical Laboratory, University of California. Unless otherwise stated the solvents were reagent grade, and all evaporations were carried out at water-pump pressure.

(14) Filter paper sprayed with a 0.5% solution of benzidine in acetic acid–ethanol (1:4) makes a satisfactory test paper.

(15) G. M. Kline and S. F. Acree, *Ind. Eng. Chem., Anal. Ed.*, **2**, 413 (1930).

(16) J. d'Ans and W. Frey, *Ber.*, **45**, 1845 (1912); R. Barker and D. L. MacDonald, THIS JOURNAL, **82**, 2297 (1960).

(17) O. Ruff, *Ber.*, **32**, 3672 (1899).

(18) L. P. Zill, J. X. Khym and G. M. Cheniae, THIS JOURNAL, **75**, 1339 (1953).

curred spontaneously during this process. Recrystallization from 35 ml. of absolute ethanol yielded 10.1 g. (83%) of material, m.p. 119–120°.

Anal. Calcd. for $C_4H_{10}O_4$ (122.1): C, 39.34; H, 8.25. Found: C, 39.57; H, 8.08.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY, ST. LOUIS, MO.]

Concerning the Synthesis of D-Mannosamine and D-Glucosamine from D-*arabo*-3,4,5,6-Tetraacetoxy-1-nitro-1-hexene

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The action of methanolic ammonia on D-*arabo*-3,4,5,6-tetraacetoxy-1-nitro-1-hexene yields both 2-acetamido-1,2-dideoxy-1-nitro-D-mannitol and 2-acetamido-1,2-dideoxy-1-nitro-D-glucitol, with the former predominating in a ratio of about 6:1. The action of hydrochloric acid on the sodium salts of these two compounds (Nef reaction and *N*-acetyl hydrolysis) yields, respectively, D-mannosamine hydrochloride and D-glucosamine hydrochloride in high yield.

Considerable biochemical interest in D-mannosamine (2-amino-2-deoxy-D-mannose) has been aroused by the recent observation that it occurs naturally as a structural component of *N*-acetylneuraminic acid.[1] Consequently, the development of reasonable methods for the laboratory synthesis of the aminosugar assumes new importance. Of the methods developed to date[2–5] the preparation based on D-arabinose reported by O'Neill[5] appears to be the best route to D-mannosamine. This method involves the conversion of D-arabinose to D-*arabo*-3,4,5,6-tetraacetoxy-1-nitro-1-hexene,[6] reaction of the latter with alcoholic ammonia to give 2-acetamido-1,2-dideoxy-1-nitro-D-mannitol and, finally, application of the Nef reaction followed by acetylation to give D-mannosamine pentaacetate. The latter was converted to D-mannosamine hydrochloride by hydrolysis with hydrochloric acid.

We had independently studied the synthetic route reported by O'Neill but had reached somewhat different conclusions regarding the course of the reaction of D-*arabo*-3,4,5,6-tetraacetoxy-1-nitro-1-hexene with ammonia. In view of this, and since we had developed a somewhat more direct route from the acetylated sugar nitroölefin to D-mannosamine hydrochloride, it seems appropriate to record briefly our experiences with the synthesis.

Contrary to the findings of O'Neill, who concluded that the addition of ammonia to D-*arabo*-3,4,5,6-tetraacetoxy-1-nitro-1-hexene is stereospecific and gives only the D-*manno* isomer, we found rather that the addition is simply stereoselective and that the yield of D-*manno*:D-*gluco* isomer is approximately 6:1. We observed further that the 2-acetamido-1,2-dideoxy-1-nitro-D-mannitol (m.p. 172–173°, $[\alpha]^{25}D$ −16.8°) does not depress the melting point of 2-acetamido-1,2-dideoxy-1-nitro-D-glucitol (m.p. 155–156°, $[\alpha]^{25}D$ −12.7°), so that slight contamination of the former by the latter is difficult to detect. The epimeric substances are, however, readily separated by fractional crystallization from absolute ethanol, in which the D-*gluco* isomer is considerably more soluble than is its epimer.

Application of the Nef reaction, using hydrochloric acid rather than sulfuric acid, to the epimeric acetamidonitroalcohols, followed by heating to hydrolyze the amide linkage yields, respectively, D-mannosamine hydrochloride and D-glucosamine hydrochloride in high yield. The acidic hydrolysis of the *N*-acetyl group in *N*-acetyl-D-glucosamine was observed to be much slower than in *N*-acetyl-D-mannosamine.

The addition of ammonia to the acetylated sugar nitroölefins is reminiscent of its similar addition to the acetylated sugar disulfone olefins.[7] It is noteworthy, however, that whereas the addition to D-*arabo*-3,4,5,6-tetraacetoxy-1,1-bis-(ethanesulfonyl)-1-hexene apparently gives exclusively a product with the D-*gluco* configuration, the addition to the double bond of D-*arabo*-3,4,5,6-tetraacetoxy-1-nitro-1-hexene gives preponderantly a product with the D-*manno* configuration. The stereospecificity in the former case has been explained by Hough and Taylor[8] on the basis of neighboring group participation, whereas the latter case has been rationalized by O'Neill on the basis of rotational conformation. It is apparent that no single explanation is appropriate for both cases.

We are at present examining the addition of ammonia and various amines to other sugar nitroolefins and plan to report on these studies at a later date.

Experimental

2-Acetamido-1,2-dideoxy-1-nitro-D-mannitol and 2-Acetamido-1,2-dideoxy-1-nitro-D-glucitol.—Fifteen grams of D-*arabo*-3,4,5,6-tetraacetoxy-1-nitro-1-hexene,[6] m.p. 114–115°, was covered with 150 ml. of absolute methanol and the mixture was cooled to 0°. Anhydrous ammonia was bubbled into the mixture to approximate saturation, during which operation the acetylated nitroölefin dissolved. The resulting solution was protected from moisture with a drying-tube and allowed to warm to room temperature. After standing overnight, the solution was concentrated in a stream of dry air to a semi-crystalline mass. Filtration, washing with absolute ethanol and recrystallization from absolute ethanol (about 300 ml.) yielded 5.3 g. of pure 2-

(1) D. G. Comb and S. Roseman, THIS JOURNAL, **80**, 497, 3166 (1958).

(2) P. A. Levene, *J. Biol. Chem.*, **36**, 73 (1918); **39**, 69 (1919).

(3) R. Kuhn and W. Kirschenlohr, *Ann.*, **600**, 115 (1956); R. Kuhn and W. Bister, *ibid.*, **602**, 217 (1957).

(4) C. T. Spivak and S. Roseman, THIS JOURNAL, **81**, 2403 (1959).

(5) A. N. O'Neill, *Can. J. Chem.*, **37**, 1747 (1959).

(6) J. C. Sowden and H. O. L. Fischer, THIS JOURNAL, **69**, 1048 (1947); J. C. Sowden, U. S. Patent 2,530,342 (Aug. 29, 1947).

(7) D. L. MacDonald and H. O. L. Fischer, THIS JOURNAL, **74**, 2087 (1952).

(8) L. Hough and T. J. Taylor, *J. Chem. Soc.*, 970 (1956).