4B. Chromatographic examination in solvent E showed two sugars corresponding to 2,3,4-tri-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-xylose. The sirupy mixture of methylated sugars obtained in this way was separated on large sheets of filter paper (Whatman No. 1) using solvent E to give 3,4-di-*O*-methyl-D-xylose (0.143 g.) and 2,3,4-tri-*O*-methyl-D-glucose (0.237 g.).

Identification of 3,4-Di-*O*-methyl-D-xylose.—The R_g values (movement relative to that of 2,3,4,6-tetra-*O*-methyl-D-glucose) for this sirupy product using solvents B and D corresponded to those of a known sample of 3,4-di-*O*-methyl-D-xylose. *Anal.* Calcd. for $C_7H_{14}O_5$: OCH_3, 34.8. Found: OCH_3, 34.4. The 3,4-di-*O*-methyl-D-xylose (0.111 g.) in water (2 ml.) was oxidized with excess bromine in the dark for 50 hr. The bromine was removed by aeration, the solution neutralized with silver carbonate and the residual silver removed with hydrogen sulfide. The final sirup was heated at 15 mm. pressure at 90° for 3 hours to lactonize the acid. On seeding with 3,4-di-*O*-methyl-δ-D-xylonolactone, the sirup crystallized. Recrystallization from ether yielded 3,4-di-*O*-methyl-δ-D-xylonolactone,[26] m.p. 67° and $[\alpha]^{25}\mathrm{D}$ −22.3° (*c* 1.5 in water). The X-ray diffraction pattern was identical with that of an authentic sample.

Identification of 2,3,4-Tri-*O*-methyl-D-glucose.—The sirupy material (0.237 g.) obtained above had the same R_g values (0.79, 0.53, 0.87) in solvent B, D and E, respectively, as a known sample of 2,3,4-tri-*O*-methyl-D-glucose and showed $[\alpha]^{25}\mathrm{D}$ +69° (*c* 1.3 in water); reported value +66.8°.[27] *Anal.* Calcd. for $C_9H_{18}O_6$: OCH_3, 41.9. Found: OCH_3, 41.5. The trimethylglucose (0.139 g.) was oxidized with nitric acid (15 ml.) (d. 1.2) for 3 hr. at 90°. The acid was removed by successive distillation with water, ethanol and methanol. The sirup was heated for 3 hr. at 90° *in vacuo* and converted to the corresponding ester lactone by refluxing for 6 hr. with 2% methanolic hydrogen chloride. The product was recovered in the usual way and distilled. The 2,3,4-tri-*O*-methyl-D-glucosaccharo-1,5-lactone-6-methyl ester[28] (0.081 g.), b.p. (bath temp.) 140–150° (0.01 mm.), crystallized upon nucleation, m.p. 105–106°, $[\alpha]^{22}\mathrm{D}$ +108° in methanol (*c* 0.75) changing in 20 hr. to +58°. *Anal.* Calcd. for $C_{10}H_{16}O_7$: OCH_3, 50.0. Found: OCH_3, 49.6.

(b) **From 2-*O*-(4-*O*-Methyl-D-glucopyranosyluronic Acid)-D-xylose (Fraction III).**—The fully methylated acid (0.593 g.) obtained above gave upon reduction with lithium aluminum hydride as already described, methyl 2-*O*-(2,3,4-tri-*O*-methyl-D-glucopyranosyl)-3,4-di-*O*-methyl-D-xylopyranoside (0.523 g.). *Anal.* Calcd. for $C_{17}H_{32}O_{10}$: OCH_3, 47.0. Found: OCH_3, 46.4.

Hydrolysis of this methylated disaccharide and separation of the cleavage products as described above gave (a) 3,4-di-*O*-methyl-D-xylose (76 mg.), identified as 3,4-di-*O*-methyl-δ-D-xylonolactone, m.p. 67°, $[\alpha]^{25}\mathrm{D}$ −22.3° in water, and (b) 2,3,4-tri-*O*-methyl-D-glucose (132 mg.) identified chromatographically as 2,3,4-tri-*O*-methyl-D-glucosaccharo-1,5-lactone-6-methyl ester, m.p. 105–106°.

Acknowledgment.—The authors wish to thank Dr. J. K. N. Jones, Queen's University, Kingston, for a sample of 3,4-di-*O*-methyl-δ-D-xylonolactone and Dr. Fred Smith, University of Minnesota, for a sample of 2,3,4-tri-*O*-methyl-D-glucosaccharo-1,5-lactone-6-methyl ester.

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[26] S. P. James and F. Smith, *J. Chem. Soc.*, 739 (1945).

[27] S. Peat, E. Schlüchterer and M. Stacey, *ibid.*, 581 (1939).

[28] F. Smith, *ibid.*, 1724 (1939).

[CONTRIBUTION FROM THE ORGANIC CHEMISTRY SECTION, DIVISION OF CHEMISTRY, NATIONAL BUREAU OF STANDARDS]

Sodium Borohydride Reduction of Aldonic Lactones to Glycitols[1]

BY HARRIET L. FRUSH AND HORACE S. ISBELL

RECEIVED DECEMBER 19, 1955

Two procedures are presented for the sodium borohydride reduction of aldonic lactones to glycitols in high yield. In the first procedure, the lactone is reduced at low temperature in aqueous solution, in the presence of sufficient buffer to maintain an acid medium during the first part of the reduction. When all of the lactone has been reduced to the sugar stage or beyond, the *p*H is adjusted to approximately 9, and the reaction mixture is allowed to stand overnight. Cation exchange resin, boric acid, carbon dioxide and sodium acid oxalate were found to be satisfactory buffers. The method has been applied to the preparation of C^{14}-labeled glycitols in yields of 90 to 98%. Directions are given for the preparation of D-mannitol-2-C^{14}. In the second procedure, the lactone is treated with borohydride in anhydrous alcohol. Since the ester that may be formed by alcoholysis, like the original lactone, is reducible, the yield of the glycitol is high.

Labeled glycitols are required as intermediates for the synthesis of D-fructose-1,6-C^{14}, D-glyceraldehyde-3-C^{14}, L-sorbose-6-C^{14}, sedoheptulose-1-C^{14} and other substances needed for biological research. Prior to this investigation, labeled aldonic lactones had been converted to glycitols in yields up to 80%[2] by sodium amalgam reduction to the sugar, followed by high-pressure catalytic hydrogenation. Sodium borohydride[3,4] had also been employed to reduce the lactones in one step to either the aldoses or the glycitols in yields of 25 to 67%.[5,6] Because of the greater convenience of reduction by sodium borohydride, efforts have been made to improve the yields in the reduction of lactones to glycitols by this reagent.

In the previously reported reductions, the aldose was obtained by adding the sodium borohydride to the lactone in a solution maintained at *p*H 3 to 4 by the addition of acid; the glycitol by adding the lactone to an excess of the borohydride. In the latter case, the hydride solution, which was strongly alkaline at the beginning of the reaction, had a *p*H of 8 after complete addition of the lactone. Since, in alkaline solution, sodium borohydride is reported to reduce aldoses nearly quantitatively to glycitols,[7] it seemed probable that the lower yields in the reduction of lactones to glycitols might be due to con-

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[1] Part of a project on the development of methods for the synthesis of radioactive carbohydrates, sponsored by the Atomic Energy Commission.

[2] H. S. Isbell and J. V. Karabinos, *J. Research Natl. Bur. Standards*, **48**, 438 (1952).

[3] H. I. Schlesinger and H. C. Brown, THIS JOURNAL, **62**, 3429 (1940).

[4] R. F. Nystrom and W. G. Brown, *ibid.*, **69**, 1197, 2548 (1947); S. W. Chaikin and W. G. Brown, *ibid.*, **71**, 122 (1949).

[5] M. L. Wolfrom and H. B. Wood, *ibid.*, **73**, 2933 (1951).

[6] M. L. Wolfrom and K. Anno, *ibid.*, **74**, 5583 (1952).

[7] (a) M. Abdel-Akher, J. K. Hamilton and F. Smith, *ibid.*, **73**, 4691 (1951); (b) B. Lindberg and A. Misiorny, *Svensk Papperstidn*, **55**, 13 (1952); *C. A.*, **46**, 7942 (1952); (c) P. S. Skell and J. G. Crist, *Nature*, **173**, 401 (1954). Reference a reports a negative Fehling test after reduction, and isolation of the glycitol acetates in 70–92% yield; references b and c report a nearly quantitative gasometric technique.

version of part of the lactone in alkaline solution to the non-reducible sodium aldonate. A procedure has now been found that restricts this side reaction and gives glycitols in yields of 90 to 98%. The products can be crystallized directly without the intermediate separation of the more readily crystallized acetates.[7a] A second procedure gives somewhat lower yields, but may prove useful for reduction of water-insoluble materials.

In the first method, the reaction is conducted at a low temperature in aqueous solution in the presence of an amount of buffer sufficient to maintain an acid medium during the reduction of the lactone to the sugar, but insufficient to maintain acidity toward the end of the reaction. The solution is finally adjusted with alkali to *p*H 9, approximately, and allowed to stand overnight. Of numerous buffers employed, cation exchange resin, boric acid and carbon dioxide have been found to be the most convenient.[8] By a slight modification, the method can be applied in the reduction of numerous alkali-sensitive materials such as the dialdehydes obtained by periodate oxidation of polysaccharides.

The second method of reduction employs anhydrous alcohol as solvent. Water must be excluded in order to avoid hydrolysis of the lactone and formation of the non-reducible alkali salt. Since the ester formed by alcoholysis, like the original lactone, is reducible, the yield of the glycitol is high. The reduction method appears promising for water-insoluble substances, but it has not been so thoroughly investigated as the first method described.

Experimental

Reduction of Lactones in the Presence of Cation Exchange Resin.—One millimole of lactone, 2 ml. of Amberlite IR-120[9] and 10 ml. of 0.05 *M* aqueous boric acid were placed in a small flask and cooled in an ice-bath. With efficient stirring of the lactone solution, 10 ml. of freshly prepared 0.3 *M* sodium borohydride[10] was added dropwise in 2 or 3 minutes. Stirring was continued for 30 minutes, and a second 10-ml. portion of the borohydride solution then added as before. After an additional 30 minutes the *p*H was adjusted to approximately 9 by means of a solution of sodium hydroxide, and the reduction mixture was stored overnight at a low temperature (about 5°). It was then passed through a column containing 10 ml. of Amberlite IR-120, and the effluent and washings were freeze-dried (preferably) or concentrated under reduced pressure to a thin sirup[11] In order to remove boric acid as volatile methyl borate, the residue was repeatedly dissolved in methanol, and the solvent distilled under reduced pressure.[12] When a test for boric acid with acidified turmeric paper was negative, one or two more evaporations were carried out. The residual acid or lactone was estimated by adding an excess of 0.01 *N* sodium hydroxide and titrating the solution, after ten minutes, with 0.01 *N* hydrochloric acid in the presence of phenolphthalein indicator.[13]

In order to isolate the glycitol, the neutralized solution was passed through a column containing 5 ml. of mixed cation and anion exchange resin (Amberlite IR-120 and Duolite A-4).[14] The effluent and washings were carefully concentrated at low temperature under reduced pressure, and the residual sirup was dissolved in a small amount of methanol or ethanol, filtered, seeded with the glycitol and allowed to crystallize in slow motion. With highly soluble glycitols the addition of 2-propanol or diisopropyl ether is advantageous as crystallization proceeds. In a radioactive preparation, the product was recrystallized, and the residual glycitol in the combined mother liquors was separated by use of the non-radioactive carrier. The products, as reported, were chromatographically pure.

If determination or recovery of unreduced acid is considered unnecessary, the above procedure can be considerably shortened; it then consists of reduction, passage through mixed cation and anion exchange resins, freeze-drying or evaporation, removal of boric acid as methyl borate, filtration and crystallization from a suitable solvent.

The following substances were reduced by the method described, and the indicated yields of the corresponding glycitol were determined by titration of the residual acid or lactone: D-xylono-γ-lactone, 97.2%; D-arabono-γ-lactone, 95.8%; D-glucono-γ-lactone, 98.8%; D-glucono-δ-lactone, 93.8%; D-gulono-γ-lactone, 95.2%; D-galactono-γ-lactone, 97.2%; D-*glycero*-D-*gulo*-heptono-γ-lactone, 98.6%; and lactobiono-δ-lactone, 97.0%.

Reduction of Lactones in the Presence of Boric Acid.—One millimole of lactone and 5 ml. of 0.4 *M* boric acid were cooled in an ice-bath, and 10 ml. of 0.3 *M* sodium borohydride was added dropwise with stirring during a period of 30 minutes. After standing an additional 30 minutes at 0°, the solution was made alkaline (approximately *p*H 9) by the addition of sodium hydroxide and stored at about 5° overnight. It was then treated by the method previously described.

When 1 millimole of D-mannono-γ-lactone-1-C^{14}, having an activity of 46.3 microcuries (μc.), was reduced in the presence of boric acid, the residual acid corresponded to 0.76% of the lactone used. After treatment of the solution as outlined before, 44.7 μc. of D-mannitol-1-C^{14} was obtained without carrier, and an additional 1.1 μc. of D-mannitol-1-C^{14} with carrier, to give a radiochemical yield of 98.9%.

The following substances were reduced according to the boric acid procedure, and the indicated yields of the glycitol were determined by titrating the residual acid or lactone: D-xylono-γ-lactone, 94.9%; L-rhamnono-γ-lactone, 95.8%; D-gulono-γ-lactone, 96.3%; and D-galactono-γ-lactone, 96.5%. Copper reduction tests showed the absence of sugar in all cases.

Reduction of Lactones in the Presence of Carbon Dioxide.—One millimole of lactone and 5 ml. of ice-water were cooled in an ice-bath. A stream of carbon dioxide was bubbled through the solution, and one millimole of sodium borohydride dissolved in 10 ml. of water added dropwise during 30 minutes. The flow of carbon dioxide was then discontinued, and a solution of 3 millimoles of sodium borohydride in 10 ml. of water added dropwise during the next 30 minutes. The reaction mixture was then adjusted to *p*H 8.5 to 9 by means of sodium hydroxide and stored overnight at a low temperature. The unreduced acid was determined, and the glycitol isolated by the procedure described previously.[15]

D-Mannono-γ-lactone-1-C^{14}, when reduced by this method, gave a radiochemical yield of D-mannitol-1-C^{14} of 98.3%. By titration of the residual acid, it was found that D-glucono-γ-lactone, D-galactono-γ-lactone and lactobiono-δ-lactone were reduced to the extent of 90.4, 90.7 and 94.1%, respectively.

Reduction of Lactones in Anhydrous Alcohol.—One millimole of finely powdered lactone was added to an ice-cold solution of 2-millimoles of sodium borohydride and 0.1 millimole of sodium methylate in 10 ml. of methanol, contained in a flask equipped with a magnetic stirrer and a drying tube. The mixture was stirred until the lactone dis-

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[8] Sodium acid oxalate was used in our early work, and one reduction in the presence of this buffer is reported here. However, removal of the oxalate makes this method more laborious than later procedures.

[9] Rohm and Haas Co., Resinous Products Division, Philadelphia 5, Pa.

[10] Metal Hydrides, Inc., Beverly, Mass.

[11] Because of the danger of anhydride formation from sugar alcohols in the presence of acids, care must be taken to avoid overheating.

[12] L. P. Zill, J. X. Khym and G. M. Cheniae, This Journal, **75**, 1339 (1953).

[13] Since a copper-reduction test has invariably shown the absence of reducing sugar at the end of the reaction, the titration is used as a measure of the completeness of the reduction of the lactone to the glycitol.

[14] Chemical Process Co., Redwood City, Calif. With partially lactonized acids, the free acid, which is not reduced, can be reclaimed from the mixed resins by elution with 10% aqueous acetic acid.

[15] The sodium borohydride reduction of carbon dioxide to formic acid has been reported by T. Wartik and R. K. Pearson, This Journal, **77**, 1075 (1955). Any formic acid produced in this process would be volatilized along with the methyl borate.

solved, and then allowed to come gradually to room temperature. After 18 hours, the alcohol was removed in a current of air. The residue was dissolved in water and the solution passed through a column containing 5 ml. of cation exchange resin. The column was washed with water, and the combined effluent was evaporated under reduced pressure to a sirup. The boric acid was removed by the method previously described, and the glycitol crystallized from a suitable solvent.

In a preparation of D-mannitol-1-C^{14} from 238 μc. of D-mannono-γ-lactone-1-C^{14}, 192 μc. of D-mannitol-1-C^{14} was obtained without carrier, and an additional 10 μc. by use of 200 mg. of non-radioactive D-mannitol as carrier. The radiochemical yield was thus 85%.

In a similar reduction of one millimole of methyl D-galactonate, the unreduced ester was determined by saponifying with dilute alkali and titrating the excess. The result showed a reduction of 87.6%.

Reduction of D-glucono-γ-lactone in methanol was carried out similarly, except that granulated aluminum was added to the reaction mixture. The reduction, as determined by titration of residual acid or lactone, was 92.6%.

Two reductions in absolute ethanol were performed by adding one millimole of lactone to 10 ml. of absolute ethanol containing 3 millimoles of sodium borohydride and a small quantity of granulated aluminum. The mixture was stirred overnight at room temperature, and treated as previously described. By titrating the residual acid it was found that D-glucono-γ-lactone, D-arabono-γ-lactone and D-*glycero*-D-*gluco*-heptono-γ-lactone were reduced to the extent of 87.8, 89.2 and 95.4%, respectively.

Preparation of D-Mannitol-2-C^{14} by Reduction of D-Mannono-γ-lactone-2-C^{14} in the Presence of Sodium Acid Oxalate.—To two millimoles (356 mg.) of D-mannono-γ-lactone-2-C^{14} having an activity of 892 μc., and 4 millimoles of sodium acid oxalate (520 mg.), in an ice-bath, was added 10 ml. of ice-water. With continuous stirring of the lactone solution, 8 millimoles (320 mg.) of sodium borohydride in 20 ml. of water was added dropwise during a period of 1 hr. The ice-bath was removed, and the flask was allowed to stand at room temperature for 3 hr. The solution was then passed through a column containing 20 ml. of cation exchange resin, the effluent was mixed with 0.5 g. of calcium carbonate, and the precipitated calcium oxalate was removed by filtration. The filtrate was passed through a column containing 10 ml. of cation exchange resin, and the effluent and washings were concentrated under reduced pressure to a thick sirup. Boric acid was removed, and the mannitol-2-C^{14} brought to crystallization by the methods previously described. After recrystallization, 348 mg. (851 μc.) of D-mannitol-2-C^{14} was obtained, and an additional 26 μc. by use of 200 mg. of non-radioactive carrier. The total radiochemical yield, 877 μc., corresponds to 98.3% of the activity of the lactone originally used.

Radioactivity Measurements.—Known concentrations of the materials in formamide solution were counted with a windowless proportional counter.[16] The count was converted to microcuries by means of a factor based on the NBS carbon-14 standard. Samples were counted to a probable error of about 1% (10,000 counts).

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[16] A. Schwebel, H. S. Isbell and J. D. Moyer, *J. Research Natl. Bur. Standards*, **53**, 221 (1954).

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[Contribution No. 1348 from the Sterling Chemistry Laboratories, Yale University]

The Reaction of Some 5,6-Anhydro Derivatives of Glucose and Idose with Organo-lithium Compounds[1]

By James English, Jr., and M. Frank Levy[2]

Received January 3, 1956

The reaction of 1,2-*O*-isopropylidene-3-*O*-benzyl-5,6-anhydro-D-glucose and 1,2-*O*-isopropylidene-3-*O*-benzyl-5,6-anhydro-L-idose with methyllithium is described.

As a part of a program directed toward the synthesis of compounds containing carbon-to-carbon bonds between sugars and other molecules,[3] 1,2-*O*-isopropylidene-3-*O*-benzyl-5,6-anhydro-D-glucose[4] (I) was treated with excess methyllithium in ether solution. An exothermic reaction occurred and there was obtained by high vacuum distillation a colorless levorotatory sirup II having the analysis corresponding to the formula $C_{16}H_{20}O_4$. This was treated with hydrogen and Raney nickel (and palladium-on-charcoal) under conditions known to cleave benzyl ethers of this type.[5,6] A mixture was obtained containing crystalline material III that could be purified by crystallization and sublimation *in vacuo*. On the basis of the following evidence the structure 1,2-*O*-isopropylidene-5,6-dideoxy-D-glucose has been assigned to III. Fehling solution is not reduced by III until after hydrolysis in acid; a steam distillate from such an hydrolysate yields iodoform, indicating the presence of the 1,2-*O*-isopropylidene group. Although the free sugar has not yet been obtained in crystalline form, a periodate titration of a solution obtained from hydrolysis of a weighed pure sample of III showed an uptake of 3 moles of periodate per mole of III. This, together with the isolation of propionaldehyde (as its dimedon derivative) from the periodate reaction, establishes the presence of a 6-carbon chain with four adjacent hydroxyl groups and a terminal ethyl group. Carbon–hydrogen analysis, active hydrogen and molecular weight determinations are in good agreement with the proposed structure.

McSweeny and Wiggins[7] have reported the preparation of 1,2-*O*-isopropylidene-5,6-dideoxy-D-glucose by reaction of the corresponding 5,6-anhydro sugar with carbon disulfide followed by treatment with Raney nickel. Their product melted at 78°, $[\alpha]^{21}\text{D}$ $-31.4°$; III melts at 79.0–79.6°, $[\alpha]^{21}\text{D}$ $-21.9°$ and shows a depression in melting point on mixing with McSweeny and Wiggins com-

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[1] We are indebted to the Research Corporation for a grant in support of this work.

[2] Taken from the thesis submitted by M. Frank Levy to Yale University in partial fulfillment of the requirements for the Ph.D. degree.

[3] J. English, Jr., and P. Griswold, This Journal, **67**, 2039 (1945); **70**, 1390 (1949).

[4] M. H. Adams, R. E. Reeves and W. F. Goebel, *J. Biol. Chem.*, **140**, 653 (1941); A. S. Meyer and T. Reichstein, *Helv. Chim. Acta*, **29**, 152 (1946).

[5] C. E. Ballou and H. O. L. Fischer, This Journal, **76**, 3191 (1954).

[6] A. S. Meyer and T. Reichstein, *Helv. Chim. Acta*, **29**, 192 (1946).

[7] G. P. McSweeny and L. F. Wiggins, *Nature*, **168**, 874 (1951).