[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

Preparation of D-Arabinose from D-Glucose with Hypochlorite[1,2]

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D-Arabinose can be prepared from D-glucose by a two-stage, but single batch, hypochlorite oxidation. D-Glucose is first converted to D-gluconate by oxidation at *p*H 11 with 3 moles of hypochlorite per mole of sugar. In about 20 hr. the *p*H is adjusted to 4.5–5.0 and about 1.4 moles of hypochlorite per mole of original D-glucose is added. The D-gluconate is further oxidized to D-arabinose, which can be isolated in 35% crystalline yield from the reaction products after their treatment with anion and cation exchange resins and concentration to small volume.

Useful methods for shortening the carbon chain in sugars are described in the carbohydrate literature. The classical methods are those of Weerman, Ruff and Wohl. In the method of Weerman[3] an aldonamide is degraded by sodium hypochlorite, but under conditions in which the newly formed sugar of one less carbon is also attacked and yields are consequently low. In an example of the Ruff[4] degradation, calcium D-gluconate is oxidized with hydrogen peroxide in the presence of ferric acetate to produce a yield of about 36–40% D-arabinose. The yield can be raised to 44% by addition of ion-exchange resins, as shown by Fletcher, Diehl and Hudson.[5] The Wohl[6] degradation normally starts with the sugar oxime and involves removal of the cyanide group from the acetylated nitrile, which with D-gluconic acid nitrile produces D-arabinose in 84% yield. More recently other degradation methods have been introduced. One of these[7] also starts with the sugar aldoxime which is treated with 2,4-dinitrofluorobenzene to give the next lower aldose, 2,4-dinitrophenol and hydrogen cyanide. Another[8] uses an aldose diethyl mercaptal which on treatment with perpropanoic acid followed by ammonia is degraded to the aldose of one less carbon and bis-(ethylsulfonyl)-methane. By this method D-arabinose is obtained in 80–83% yield from D-mannose diethyl mercaptal.

Experience in this Laboratory has suggested that variation of oxidation potential and alteration in composition of hypochlorite solution with *p*H might be used with advantage to produce D-arabinose from D-glucose in a single batch reaction if proper control were exercised over both *p*H and oxidant concentration. It is well established that D-glucose is readily converted to D-gluconate ion by hypohalite oxidation in alkaline solution. Reexamination of the hypochlorite oxidation shows that in 1–4% D-glucose solutions and with 3 moles of oxidant per mole of sugar, highest yields of D-gluconate are obtained at *p*H 11. Since there is no reason to assume that this oxidation proceeds differently from those with other alkaline hypohalites, the reaction probably proceeds primarily through oxidation of β-D-glucose and by way of the glucono-δ-lactone. Further hypochlorite oxidation of the lactone or D-gluconate ion has not been thoroughly examined. Since D-arabinose is present in comparatively large amounts in hypochlorite oxidations of D-glucose at near neutrality, a further investigation of the possible origin of D-arabinose from D-gluconolactone or D-gluconate is made. It is observed that hypochlorite oxidation of either D-glucono-γ-lactone or D-gluconate solutions leads to D-arabinose and that maximal amounts of the pentose appear in oxidations at *p*H 4.5 to 5.0 and at hypochlorite levels of 2 moles of oxidant per mole of carbohydrate.

Consequently the two oxidations of D-glucose to D-gluconate and D-gluconate to D-arabinose can be joined together sequentially into a single batch, two-stage reaction to convert D-glucose to D-arabinose. The first stage yield is about 80%, while the yield from the second stage is 65–70%. The over-all yield as obtained in practice is 35%. Recommended conditions are a 20-hr. treatment of D-glucose at *p*H 11 with 3 moles of hypochlorite per mole of sugar, adjustment of the *p*H to 4.5–5.0 and further oxidation with an additional 1.4 moles of hypochlorite. At the end of 10 hr. the reaction mixture is freed of salts through crystallization and treatment with anion and cation exchange resins and the residue concentrated to produce crystalline D-arabinose.

Experimental

Oxidation of D-Glucose.—One per cent. solutions of D-glucose were oxidized in the dark at 25° with 2 moles of hypochlorite per mole of D-glucose. Oxidations were at *p*H levels of 3, 5, 7, 9, 11 and 13. The *p*H levels were checked frequently and corrected, when necessary, by addition of hydrochloric acid or sodium hydroxide. The oxidant was consumed most rapidly at *p*H 7. Conversion of hypochlorite to chlorate was 19% at this *p*H and from less than 1 to 6% at other *p*H values.

The number and amount of reaction products varied with the conditions of oxidation. Paper chromatography with irrigant A, ethyl acetate–pyridine–water (8:2:1 v./v.), or irrigant B, ethyl acetate–acetic acid–formic acid–water (18:3:1:4 v./v.), using aniline hydrogen phthalate as the spray reagent,[9] showed a decrease of D-glucose from *p*H 7 to 11, at which point it reached a minimum and was apparently higher again at *p*H 13. At *p*H values below 7 large amounts of D-glucose remained unoxidized. Some small amount of D-arabinose was formed at these low *p*H levels. D-Arabinose yields appeared highest at *p*H 3, and decreased as the *p*H of oxidation was increased. At *p*H 11, no D-arabinose was detected. Other components were evident in small amounts, being in greatest abundance at *p*H 7. With permanganate-

(1) This is paper number 6 in a series concerning "Action of Oxidants on Carbohydrates." The previous paper is R. L. Whistler and R. Schweiger, THIS JOURNAL, **81**, 3136 (1959).

(2) Journal Paper No. 1412 of the Purdue University Agricultural Experiment Station.

(3) R. A. Weerman, *Rec. trav. chim.*, **37**, 16 (1917).

(4) O. Ruff, *Ber.*, **31**, 1573 (1898); **32**, 550, 3677 (1899); **34**, 1362 (1901); O. Ruff and G. Ollendorf, *ibid.*, **33**, 1798 (1900).

(5) H. G. Fletcher, Jr., H. W. Diehl and C. S. Hudson, THIS JOURNAL, **72**, 4546 (1950).

(6) A. Wohl, *Ber.*, **26**, 730 (1893); **32**, 3666 (1899); A. Wohl and E. List, *ibid.*, **30**, 3101 (1897).

(7) F. Weygand and R. Löwenfeld, *ibid.*, **83**, 559 (1950).

(8) D. L. MacDonald and H. O. L. Fischer, *Biochim. et Biophys. Acta*, **12**, 203 (1953).

(9) S. M. Partridge, *Nature*, **164**, 443 (1949).

periodate spray reagent,[10] an acid and a lactone appeared as the main oxidation products at *p*H 9 and 11. These had chromatographic flow rates identical to authentic D-gluconic acid and its lactone. Acidic material was separated from non-acidic material by passage through an anion exchange column of Amberlite[11] IR-45(OH) followed by elution with dilute ammonium hydroxide. Ammonium ions were then removed by passing the solution through Amberlite IR-120(H). The solution was treated with charcoal, filtered and concentrated under reduced pressure to a colorless sirup. This sirup was methylated with diazomethane in ether–methanol solution. The methyl ester was mixed with phenylhydrazine in ethanol and heated on a steam-bath for 1 hr. and then allowed to stand overnight at 0°. Crystals that formed were filtered, washed and dried, m.p. 196–197°; after recrystallization from ethanol–water, m.p. 198–199°, undepressed when mixed with authentic D-gluconophenylhydrazide.

To determine the optimum *p*H for the formation of D-gluconic acid, oxidations were conducted at *p*H levels of 7, 9, 11 and 13. In each, when all oxidant was consumed, the small amount of chlorate formed was reduced with sulfur dioxide, the mixture was passed through a column of Amberlite IR-120(H), concentrated to a small volume and sulfate ions removed by precipitation as barium sulfate. The solution was again passed through a column of Amberlite IR-120 (H) and concentrated to a sirup, which was diluted with water and stirred with Amberlite IR-45(OH) for about 20 hr. The resin was filtered, washed and extracted with dilute ammonium hydroxide. Ammonium ions were removed from the extract by Amberlite IR-120(H). Traces of hydrochloric acid which might still be present were removed by stirring the extract with silver carbonate. After filtration, the excess silver ions were removed by precipitation with hydrogen sulfide. The solution was concentrated to a sirup and diluted to 25 ml. and analyzed. The amount of free acid present was determined by direct titration of a portion with 0.1 *N* sodium hydroxide. Determination of both free acid and lactone was made on a second aliquot which was heated with excess sodium hydroxide and the excess alkali titrated with 0.1 *N* oxalic acid. A third portion was methylated with diazomethane in ether–methanol and then heated with phenylhydrazine in ethanol. The phenylhydrazide was filtered and weighed. After recrystallization, the melting point, m.p. 199°, was undepressed when mixed with authentic D-gluconophenylhydrazide. The yields obtained by these three determinations in per cent. of theory are:

*p*H	7	9	11	13
Free acids	21.4	51.7	58.8	72.5
Lactone and acid	34.6	72.0	81.0	95.5
Phenylhydrazide	..	23.4	28.8	22.4

Oxidation of D-Glucono-γ-lactone.—One gram of D-gluconolactone was dissolved in 50 ml. of water, the *p*H adjusted to 3 and the solution mixed with 50 ml. of 0.45 *N* sodium hypochlorite solution adjusted to *p*H 3. Similar reaction mixtures were prepared at *p*H 5, 7, 9 and 11, and each was kept at 25° in the dark. Oxidant was consumed in about 2 hr. at *p*H 7, but consumption required 20 to 30 hr. at *p*H 3, 5 and 9, and several days at *p*H 11. Only a very small percentage of hypochlorite was converted to chlorate at both high and low *p*H values, but conversion at *p*H 5 was 6%, and at *p*H 7 about 11%.

Paper chromatographic analysis with irrigant A or B, using aniline hydrogen phthalate as the spray reagent showed D-arabinose as the principal reducing substance, but small amounts of other reducing substances were also present. From visual examinations of the paper chromatograms, the largest yield of D-arabinose seemed to be obtained near *p*H 5. At higher *p*H values the yield decreased rapidly.

To isolate D-arabinose, the oxidation mixture at *p*H 5 was passed through columns of Amberlite IR-120(H) and Amberlite IR-45(OH) to remove both cations and anions. The effluent was treated with charcoal and concentrated under reduced pressure to a sirup which crystallized. For recrystallization the product was dissolved in a very little water, ethanol was added and the solution was placed in a desiccator. After 2 days, the crystals were filtered on a fritted glass disk, washed with ethanol and dried; $[\alpha]^{25}D$ −106.3 ± 1.3° (*c* 1.2 in water), m.p. 156–157°, undepressed when mixed with authentic D-arabinose.

To ascertain more exactly the optimum *p*H value for D-arabinose production, additional oxidations were made at intervals over a narrow *p*H range. Six portions of D-gluconolactone were oxidized as described above, but at *p*H 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0, respectively. After the oxidant had been consumed a portion of each mixture was chromatographed on paper both with irrigants A and B and sprayed with aniline hydrogen phthalate. D-Arabinose seemed in highest yields in oxidations conducted at *p*H levels of 3.5, 4.0, 4.5 and 5.0. At higher *p*H values yields were conspicuously less. In addition to D-arabinose, other reducing compounds were present in trace amounts in oxidations conducted at all *p*H levels. A substance with an $R_{glucose}$, (R_g), value of 5.65 in irrigant A and 4.48 in B gave a brownish-gray spot and was present in oxidations conducted at *p*H 5.0, 5.5 and 6.0 in increasing amount. Another substance present in these oxidation mixtures had an R_g value of 3.82 in irrigant A and 3.11 in irrigant B and gave a yellow spot. The substance was present in least amount in the oxidation conducted at *p*H 5.0. A third trace component with an R_g value of 3.13 in irrigant A and 2.32 in irrigant B gave an olive-brown spot, the intensity of which decreased slightly as the *p*H of the oxidation decreased.

Each oxidation mixture was passed through columns of both Amberlite IR-120(H) and Amberlite IR-45(OH). Effluents were treated with charcoal, filtered and concentrated under reduced pressure. Paper chromatographic separation of the concentrates from oxidations conducted at *p*H 3.5, 4.0 and 4.5 showed only D-arabinose, while concentrates from other *p*H levels gave evidence of a trace of a second component with aniline hydrogen phthalate spray.

For estimation of the amount of D-arabinose present in each concentrate an aliquot was analyzed by the method of Willstätter and Schudel[12] with the results as shown.

*p*H	3.5	4.0	4.5	5.0	5.5	6.0
D-Arabinose, %	37.9	38.6	38.8	26.5	23.1	17.3

To determine the optimum molar amount of oxidant the following determinations were made: 1% solutions of D-gluconolactone maintained between *p*H 4.5 and 5.0 were oxidized with 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 moles of oxidant per mole of lactone. Chlorate formation was 2% when 2 moles of oxidant were used per mole of lactone.

After the reaction mixtures were passed through columns of both cation and anion exchange resins and were concentrated to small volumes, they were chromatographed with irrigant A. In addition to D-arabinose, trace amounts of other reducing substances were evident when aniline hydrogen phthalate was used as spray. One of these had an R_g value of 2.2 (red) and another an R_g value of 2.55 (yellowish-brown). The amount of each decreased as the concentration of oxidant increased. Two other trace components with R_g values of 3.74 (yellowish-brown) and 4.58 (yellow) appeared in the sample oxidized with 3 moles of oxidant per mole of lactone and increased as the oxidant concentration was raised. D-Arabinose seemed to be most free of trace components when prepared with 2.5 moles of oxidant per mole of lactone. Permanganate–periodate spray showed no further components.

Determination of D-arabinose by the Willstätter-Schudel method gave the results shown.

Moles oxidant per mole D-gluconolactone	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
D-Arabinose, %	23.6	39.2	42.5	41.5	42.9	32.4	29.9	41.9

Correction of the results for the trace reducing component will be slight except possibly for the two highest oxidant levels.

From the sirup derived by oxidation with 2 moles of oxidant per mole of lactone there could be crystallized D-arabinose in 68% of the yield expected from the above table.

(10) R. U. Lemieux and H. F. Bauer, *Anal. Chem.*, **26**, 920 (1954).

(11) Product of Rohm and Haas Co., Philadelphia, Penna.

(12) R. Willstätter and G. Schudel, *Ber.*, **51**, 780 (1918).

Paper chromatograms were made of oxidation products from oxidations at various *p*H levels and were irrigated with irrigants A, B and C, butanol–pyridine–water (3:1:1.4 v./v.), and sprayed with permanganate–periodate, with hydroxylamine–ferric chloride,[13] and with ammoniacal silver nitrate.[14] Evidence was obtained for several lactones and acids, and components were observed which moved with rates identical to those for D-gluconolactone, D-arabinolactone, D-erythronolactone and glycolic acid.

Oxidation of D-Gluconate.—One gram of D-gluconolactone was dissolved in 50 ml. of water and sufficient sodium carbonate was added to bring the *p*H to 10. The solution was then held at 60° for 30 minutes and the *p*H maintained at 10 by addition of sodium carbonate. At the end of this saponification, hypochlorite oxidation was performed in the same manner as that described for D-gluconolactone. D-Arabinose produced at different *p*H levels of oxidation was determined by the method of Willstätter and Schudel with the results shown.

*p*H	3.5	4.0	4.5	5.0	5.5
D-Arabinose, %	55.4	61.3	61.4	62.3	59.5

Conditions for Oxidation of D-Glucose to D-Arabinose.—One gram of D-glucose was dissolved in 20 ml. of water and adjusted to *p*H 9. To this was added 50 ml. of 0.45 *N* sodium hypochlorite solution (2 moles of oxidant per mole D-glucose unit) at *p*H 9. Similar oxidations were conducted at *p*H 10 and 11. The *p*H was checked frequently and corrected, when necessary, by addition of sodium carbonate or sodium hydroxide. After about 30 hr. at *p*H 9 all oxidant was consumed, but after 2 days small amounts of hypochlorite still remained in reaction mixtures at *p*H 10 and 11. At the end of the above time periods the solutions were adjusted to *p*H 4.5–5.0 by addition of concentrated hydrochloric acid. Then 20 ml. of 1.12 *N* sodium hypochlorite at *p*H 4.5–5.0 was added to each mixture and each was maintained in the dark at 25°. When all of the oxidant was consumed each mixture was passed through columns of cation and anion exchange resins, treated with charcoal, filtered, concentrated to a colorless sirup and diluted to 50 ml. with water.

Chromatographic analysis showed D-arabinose as the main oxidation product and also gave evidence of some unoxidized D-glucose, which appeared in greater amount in oxidations initiated at *p*H 9 than in those initiated either at *p*H 10 or 11.

In an attempt to lower the amount of D-glucose in the reaction product another series of three oxidations was performed with the initial *p*H at 11 but with 3 moles of hypochlorite per mole of D-glucose. After consumption of 2.18 moles of hypochlorite in the first, 2.28 moles in the second and 2.41 moles in the third, the oxidation mixtures were adjusted to *p*H 4.5–5.0 and sufficient hypochlorite in 20 ml. was added to raise the level of each to 2 moles of hypochlorite per mole of original D-glucose. The total volume of each was 100 ml. After the oxidant was consumed, D-arabinose, together with any unoxidized D-glucose, was determined as previously described by passage of the solution through cation and anion exchange columns followed by Willstätter–Schudel titration. Calculations for D-arabinose in per cent. of theory were:

Initial moles hypochlorite	2.2	2.3	2.4
D-Arabinose, %	50.4	48.6	46.0

Chromatographic analysis showed that all three samples contained small amounts of D-glucose, although at the higher oxidant levels only a trace was evident. Hence, the amounts of D-arabinose present probably did not differ essentially from the values calculated.

To determine the possible catalytic effect of potassium bromide, a series of oxidations was conducted in the presence of 0.1 g. of potassium bromide. Initial oxidations were at *p*H 11 as described, but with hypochlorite levels of 1.7, 2.0 and 2.5 moles per mole of D-glucose, respectively. Second oxidations at *p*H 4.5–5.0 were with 2 moles of hypochlorite present per mole of original D-glucose in a total volume of 100 ml.

Moles hypochlorite init. oxidn.	1.7	2.0	2.5
D-Arabinose, %	13.9	12.5	11.8

In one further experiment at 2.5 moles of hypochlorite per mole of D-glucose, but in the presence of 0.1 g. of potassium iodide at *p*H 11, the calculated yield of D-arabinose was also in the range of 12% of theory. On chromatography of the oxidized solution no D-glucose was detected.

Preparation of D-Arabinose from D-Glucose.—Eight grams of D-glucose was dissolved in 300 ml. of water. To this was added 3 moles of sodium hypochlorite per mole of D-glucose unit in 200 ml. of water which had been adjusted to *p*H 11 by the addition of sodium bicarbonate. The mixture was kept at 25° in the dark and the *p*H frequently checked and corrected, when necessary, by the addition of sodium hydroxide. In about 20 hr., when about 2.4 moles of sodium hypochlorite per mole of D-glucose was consumed, the solution was brought to a *p*H of 4.5–5.0 by the addition of hydrochloric acid. Then 1.4 moles of sodium hypochlorite per mole of original D-glucose in 100 ml. of solution at *p*H 4.5–5.0 was added to the carbohydrate. The mixture was kept at 25° in the dark and the *p*H maintained between 4.5 and 5.0 by the addition of sodium bicarbonate. After 10 hr. when the oxidant was consumed, the solution was concentrated under reduced pressure until salt crystallized in large amounts. Then 3-4 volumes of methanol and 1 volume of ethyl ether were added. After 10 min. the salts were filtered and the filtrate was treated with charcoal and with Amberlite IR-120(H) and refiltered. The clear filtrate was stirred with Amberlite IR-45(OH) for 24 hr., treated with charcoal, again filtered and concentrated under reduced pressure to an almost colorless sirup which crystallized immediately. The crystals were stirred in 10 ml. of hot methanol to dissolve any sirup which might be present and the mixture was refrigerated for a day. A small volume of ethanol was added and the mixture was again refrigerated for a day. The resulting crystals were filtered and washed with a mixture of methanol and ethanol, then with acetone and finally with ether; m.p. 140–144°, $[\alpha]^{25}D$ −103.7 ± 1.5° (*c* 2.2 in water), yield 2.3 g. or 35%. For recrystallization the crude crystals were dissolved in a very small amount of water, the mixture treated with charcoal, filtered and put into a desiccator to concentrate to a thin sirup of 4-ml. volume. Then 10–15 ml. of hot methanol was added and the mixture kept several days at 0° for crystal formation; finally ethanol was added and the solution again refrigerated for several days. Crystalline D-arabinose, m.p. 153.5–156°, was isolated in a yield of 2.02 g. or 30.3%.

Another recrystallization yielded 1.74 g. or 26.1%, m.p. 158.5–160°, $[\alpha]^{25}D$ −104.5 ± 3° (*c* 1.9 in water).

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(13) M. Abdel-Akher and F. Smith, THIS JOURNAL, **73**, 5859 (1951).
(14) S. M. Partridge, *Nature*, **158**, 270 (1946).