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# REMOVAL OF BORIC ACID AND RELATED COMPOUNDS FROM SOLUTIONS OF CARBOHYDRATES WITH A BORON-SELECTIVE RESIN (IRA-743)

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## ABSTRACT

A boron-selective resin (IRA-743) was used to remove boric acid from synthetic solutions of carbohydrates. A commercially available polystyrene–divinylbenzene resin, which contains (covalently attached) 1-deoxy-1-methylamino-D-glucitol functional groups, absorbed >11 mg of boric acid per mL of resin. Optimum conditions are described for use of this resin in a model system and in two practical applications: the quantitative removal of boric acid from a preparative-scale (>100 g) ketose-synthesis mixture and in the removal of boric acid from a solution of borohydride-reduced sugars (alditols) prior to acetylation and g.l.c. analysis.

## INTRODUCTION

Boric acid and other boron-containing reagents, such as sodium borohydride and sodium tetraborate, are valuable reagents in carbohydrate chemistry and are frequently used in the preparation of synthetic sugars and their derivatives. Sugar alcohols, for example, may be produced by the borohydride reduction[1] of reducing sugars in aqueous solution. Unavailable or valuable ketoses are often synthesized by the high-yielding isomerization of readily available aldoses in alkaline solutions containing boric acid[2,3] or sodium tetraborate[4]. In all of these cases, purification of the synthetic product requires complete removal of the boron-containing compounds from the final mixture. In most instances, this is accomplished by the conversion of all boron species into boric acid, which is then removed by evaporation as the volatile methyl ester. This process requires the repeated dissolution of the sample in methanol, followed by the evaporation of those solutions at elevated temperatures and diminished pressures. In addition to being a time-consuming and technically difficult process, this repeated methanol treatment may lead to the for-

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mation of side-products[3,5] and a difficult-to-remove methanol residue in the final product. Because of these problems, we have investigated a boron-selective resin for the removal of boric acid and other borates from carbohydrate solutions.

## RESULTS AND DISCUSSION

Amberlite IRA-743 is a synthetic resin that is highly selective for borate anions and boric acid. Although the resin was originally developed for the removal of phytotoxic borates from irrigation waters, we have recognized that such a resin may be used to solve difficult purification problems in sugar chemistry. The structure of the resin (Fig. 1) consists of spherical beads of polystyrene–divinylbenzene copolymer, with 1-deoxy-1-methylamino-D-glucitol residues attached[6]. It has long been known[7–9] that boron compounds react with polyols to form relatively stable products. These reactions, however, are not well understood because in most cases a wide variety of structurally diverse complexes may result from the interaction of the starting reactants. One possible complex of this type (Fig. 1) is the result of the reaction of boric acid with the 1-deoxy-1-methylamino-D-glucitol residue of the IRA-743 resin, to yield an anionic, tetrahedral borate-complex. By the formation of such complexes, resins of this type are able to remove boric acid efficiently from dilute aqueous solutions[6].

*Model system.* — In order to determine whether IRA-743 would also efficiently remove boric acid from solutions containing reducing sugars (which also form strong complexes with boric acid[7]), the following model system was developed (Table I). We compared the relative abilities of IRA-743, a strongly basic anion-exchange resin (IRA-400), and the traditional methanol procedure for the removal of boric acid from solutions with or without added carbohydrates. This model was developed to simulate our reaction conditions[2,3] for isomerizing aldoses into ketoses. In the model system, boric acid was first removed by repeated addition and evaporation of methanol after treatment of the solution with cation-exchange resin (Experiment 1), resulting in the removal of practically all boric acid. In the

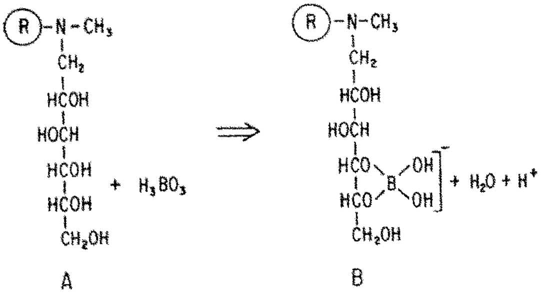

Fig. 1. Structure and reactivity of Amberlite IRA-743 resin. (A) 1-deoxy-1-methylamino-D-glucitol group reacts with free boric acid to form hypothetical tetrahedral borate complex, shown in B. R = polystyrene–divinylbenzene resin

remaining Experiments 2–8, test solutions were treated with cation exchanger, followed by an additional treatment with either IRA-743 or IRA-400 to remove boric acid. The strongly basic anion exchanger (IRA-400) was examined first (Experiment 2). Because it is well documented[10,11] that the hydroxide form of these resins irreversibly absorbs and degrades reducing sugars, we used the less-reactive[10,11] carbonate form. (Weakly basic anion-exchange resins have negligible affinity[12] for boric acid and hence were not used here). This resin absorbed a substantial amount of boric acid, but was subsequently shown (Experiment 3) to absorb fructose irreversibly from solution as well. The boron-selective resin (Experiment 4) removed boric acid as efficiently as methanol treatment. When fructose was present (Experiment 5), the capacity of the resin was slightly diminished, but none of the fructose was absorbed. The remaining Experiments 6–8 clearly demonstrated that IRA-743 is capable of completely removing boric acid from solutions of glucose and/or fructose, without any detectable sugar absorption. The removal of boric acid from the solution in Experiment 8 is clearly demonstrated by high-performance liquid-chromatographic (l.c.) analysis (Fig. 2).

*Large-scale purification of ketoses.* — The following study was performed to determine this resin's ability to remove boric acid quantitatively from large (preparative) quantities of a synthetic ketose, maltulose (4-*O*-α-D-glucopyranosyl-D-fructose). Because a preliminary study[13] indicated that the resin-regeneration method could alter its absorptive properties, we investigated that effect here also. The maltulose sample prepared by the isomerization[3] of maltose contained boric acid and enough sodium hydroxide to titrate the solution to pH 11. The approximate sugar composition before resin treatment was maltulose, 90%; maltose, 7%;

TABLE I

REMOVAL OF BORIC ACID FROM TEST SOLUTIONS BY RESIN OR METHANOL TREATMENT

| *Experiment* | *Initial composition*[a] *(mg)* | | | *Treatment used*[b] | *% Removal of components*[c] | | |
|---|---|---|---|---|---|---|---|
| | *Boric acid* | *Fru* | *Glc* | | *Boric acid* | *Fru* | *Glc* |
| 1 | 17 | 0 | 0 | $H^+$ resin[d] + MeOH[e] | >98 | | |
| 2 | 17 | 0 | 0 | $H^+$ resin + 1 mL of IRA-400 ($CO_3^{2-}$) | 75 | | |
| 3 | 17 | 50 | 0 | $H^+$ resin + 1 mL of IRA-400 ($CO_3^{2-}$) | 65 | 45 | |
| 4 | 17 | 0 | 0 | $H^+$ resin + 2 mL of IRA-743 | >98 | | |
| 5 | 17 | 50 | 0 | $H^+$ resin + 2 mL of IRA-743 | 85 | 0 | |
| 6 | 17 | 50 | 0 | $H^+$ resin + 4 mL of IRA-743 | >98 | 0 | |
| 7 | 17 | 0 | 50 | $H^+$ resin + 4 mL of IRA-743 | >98 | | <2 |
| 8 | 17 | 25 | 25 | $H^+$ resin + 4 mL of IRA-743 | >98 | 0 | 0 |

[a] All test solutions contained the specified number of mg of boric acid, fructose (Fru), and glucose (Glc) and were titrated to pH 11 with sodium hydroxide (M). Final volume was 1 mL. [b] See Experimental section. [c] Determined by l.c. [d] 1 mL, IR-120($H^+$) resin. [e] Five successive treatments with methanol (see Experimental section).

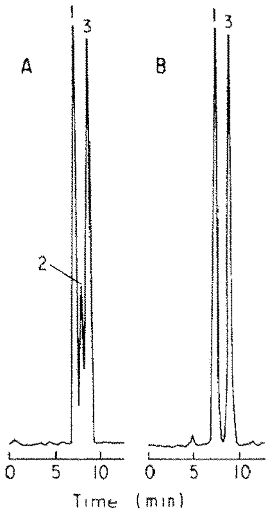

Fig. 2 L.c. analysis of experimental solutions containing boric acid and reducing sugars (A) Separation of glucose (1), boric acid (2), and fructose (3) in standard solution before treatment with IRA-743 resin. (B) Same sample, after treatment with IRA-743 (see Experimental 8, Table I) HPX-87C column at 85° Mobile phase, $H_2O$ at 0.8 mL/min Injection size 20 $\mu$L. Refractive index detection at 4X

and monosaccharides, 2–3%. Three equal portions of the crude synthetic maltulose mixture, each containing 120 g of total carbohydrate and ~21 g of boric acid, were passed through columns of IR-120 ($H^+$) resin, to remove sodium ions, and then through a column of IRA-743, as shown in Table II. In Trial 1, the boron-selective resin was used directly as received from the manufacturer. The effluent from this column showed a normal sugar-distribution, >99% of the sugar loaded onto the column (no absorption), and only 216 p.p.m. of boron (a decrease of >99% of the original boron level). In a second experiment (Trial 2), the IRA-743 resin had been pretreated by the manufacturer's suggested regeneration procedure: treatment with aqueous sulfuric acid and then aqueous sodium hydroxide, followed by washing with distilled water. Use of this resin resulted in the irreversible absorption of ~8% of the carbohydrate and the degradation of another similar quantity (8.2%) to non-sugar, unidentified products. Analysis of the sugar distribution from this second trial indicated that a substantial amount of maltulose had been lost, probably via alkaline "peeling" reactions, as these would explain the presence of larger quantities of glucose, and the large amount of unidentified products (possibly saccharinic acids).

The preceding degradation-reactions were prevented when the IRA-743 resin was regenerated by a slightly modified procedure. In addition to the manufacturer's suggested regeneration scheme, the resin was subjected to treatment with carbon dioxide-saturated water. When this resin was used (Trial 3, Table II), the resulting sugar syrup was recovered in excellent yield, and it contained no measurable levels of boric acid.

TABLE II

EFFECT OF PRETREATMENT OF IRA-743 RESIN ON ITS AFFINITY FOR BORIC ACID AND CARBOHYDRATES

| *Trial* | *IRA-743 resin pretreatment* | *mg $H_3BO_3$/mL resin*[a] | *Composition of purified syrup (%)*[b] | | | | | *Carbohydrate lost during purification (% of total)* | *Boron, p.p.m.*[c] |
|---|---|---|---|---|---|---|---|---|---|
| | | | *Fru* | *Glc* | *Maltose* | *Maltulose* | *U*[d] | | |
| 1 | None | 9.0 | <0.1 | 1.8 | 7.8 | 89.6 | <1 | <1 | 216 |
| 2 | Regeneration Method A | 9.0 | 0.2 | 12.9 | 9.7 | 69.0 | 8.2 | 7.6 | |
| 3 | Regeneration Method B | 11.0 | 0.5 | 1.9 | 6.8 | 89.4 | 1.4 | 1.5 | <5 |

[a] Ratio of mg of boric acid in sample to mL of IRA-743 resin used in column. [b] Determined by l.c. [c] Determined by ICP analysis (commercial laboratory) [d] Unidentified compounds in final syrup (total sample dry wt. minus wt. of known sugars, determined by l.c.

The use of IRA-743 resin, as described in Trial 3 of Table II, is therefore recommended for the efficient removal of boric acid from analytical and preparative-scale sugar solutions. Under these conditions, we found that the resin could remove at least 11 mg of boric acid per mL of resin before regeneration was required. Use of IRA-743 resins in these applications also had additional benefits. They functioned as weakly basic anion-exchangers (removing strong acids from solution) and were also especially effective in decolorizing the dark solutions used in this study. Moreover, they prevented the formation of byproducts[3,5] associated with the use of methanol to remove boric acid from ketose solutions.

*Use of IRA-743 resin in the preparation of sugar derivatives for g.l.c. analysis.* — Another application for the boron-selective resin was found in the alditol acetate procedure for g.l.c. analysis of reducing sugars. In this method, sugars are first reduced by sodium borohydride, the excess of borohydride is converted into boric acid, which is removed, and the alditols are then acetylated. In that process, it has been shown[14,15] that failure to remove all boric acid by five separate additions and evaporations of methanol results in incomplete acetylation of the alditols. As it would be an advantage in these analyses to avoid the tedious methanol treatments, we have now shown that a single, convenient treatment with IRA-743 resin accomplishes the same goal. Thus, a standard solution that contained six common sugars and erythritol was first treated with sodium borohydride (to reduce aldoses to alditols) and then with cation-exchange resin ($H^+$ form) to convert all boron compounds into boric acid. An aliquot of this solution was treated by the traditional methanol procedure to remove boric acid, and an internal standard, methyl 2,3,4,6-tetra-*O*-acetyl-$\alpha$-galactopyranoside, was added. Following acetylation, all components of the mixture were readily separated and detected by g.l.c. (Fig. 3A). A second aliquot was treated similarly, except that boric acid was not removed prior to acetylation. As expected, the boric acid prevented the acetylation of the alditols and when this mixture was chromatographed (Fig. 3B), only the (pre-acetylated) internal standard gave an acceptable g.l.c. peak. Treatment of identical sample aliquots with IRA-743 resin (in lieu of methanol treatment), for appropriate intervals prior to acetylation, produced samples that gave excellent g.l.c. peak-area sensitivities, as demonstrated in Fig. 3C. All alditols gave maximum chromatographic peak-areas (relative to the internal standard) after 20–30 min of resin treatment. Increasing the weight ratio of resin to original borohydride from 200:1 (as in Fig. 3) to 500:1 did not affect relative peak-area sensitivities, but allowed acetylation to be complete after only 10 min of incubation.

A sample of corn-bran hemicellulose was hydrolyzed and analyzed by the alditol acetate procedure, using the new IRA-743 resin treatment. The calculated carbohydrate content: arabinose, 26.9%; xylose, 46.3%; mannose, 1.5%; galactose, 7.2%; and glucose, 3.3%, agreed well (within experimental error) with a duplicate sample analyzed by the alditol acetate procedure in which methanol had been used to remove boric acid (arabinose, 28.5%; xylose, 47.9%; mannose, 1.5%; galactose, 7.8%; and glucose, 3.1%).

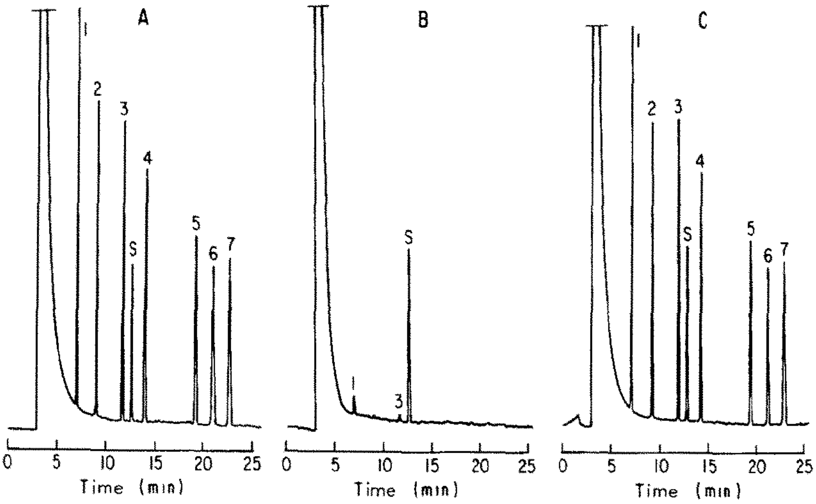

Fig. 3. Capillary g.l.c analysis of reducing sugars as alditol acetates. (A) Standard mixture of monosaccharides treated by the conventional[14] procedure. Boric acid removed prior to acetylation of alditols by repeated methanol treatment. (B) Same procedure except boric acid was not removed before acetylation of alditols. (C) Same procedure as A, except that prior to acetylation of alditols, boric acid was removed by treatment of alditol solution with IRA-743 resin for 30 min, rather than by methanol treatment. Numerals refer to the peracetates of the following alditols: 1, erythritol; 2, rhamnitol; 3, arabinitol; 4, xylitol; 5, mannitol, 6, galactitol; and 7, glucitol. S = methyl 2,3,4,6-tetra-*O*-acetyl-$\alpha$-D-galactopyranoside.

In conclusion, the boron-selective resin IRA-743 may be used to remove boric acid (and any boron compound that can be converted to boric acid) quantitatively from both analytical and preparative-scale carbohydrate solutions, and may allow the use of boron reagents in the synthesis of edible ketose and sugar alcohol-type syrups.

## EXPERIMENTAL

*General.* — All chemicals were of reagent-grade quality. All carbohydrates, and Amberlite IR-120($H^+$) and Amberlite IRA-400 ($Cl^-$) resins, were purchased through Sigma Chemical Company*. Amberlite IRA-743 (free-base form) was either kindly provided by the Rohm and Haas Company, Philadelphia, PA, or was purchased through Sigma Chemical Company. IRA-400($Cl^-$) resin was converted into the carbonate form by treating the resin in a glass column with 2M sodium carbonate until the effluent was chloride-free. Boron-selective resin (IRA-743) was regenerated by two separate methods. *Method A*: A sulfuric acid solution (200 g sulfuric acid + 1,800 g distilled water) was slowly percolated through a glass

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*Reference to brand or firm name does not constitute endorsement by the U.S Department of Agriculture over others of a similar nature not mentioned.

column containing 1 L of IRA-743. The resin was then washed with 2 L of $H_2O$, followed by a solution of sodium hydroxide (256 g, 50% sodium hydroxide + 2,944 g $H_2O$). The resin was then washed with deionized water until no further decrease in pH of the effluent was observed. *Method B*: The preceding method (*A*) was followed, and then the resulting resin was suspended in deionized water (2 L) in a large Erlenmeyer flask. Carbon dioxide gas (generated from Dry Ice) was bubbled into the flask for 30 min, the solution was decanted, 2 L of fresh, deionized water was added, and the process was repeated two more times. At this point, an aqueous slurry of the resin should have a pH value of 4.5–5.0. The resin (in an aqueous slurry) may be vacuum degassed for 5 min prior to packing in columns, to prevent the formation of $CO_2$ gas bubbles.

*Model system.* — For each experiment, a solution (20 mL) was prepared that contained the amount of boric acid, fructose, and glucose as specified in Table I. Before final dilution, each solution was titrated to pH 11.0 with M sodium hydroxide solution. A 1-mL aliquot of this solution was treated by first removing sodium ions with IR-120($H^+$) resin and then with five successive additions and evaporations of methanol to remove boric acid[14] (Experiment 1). In the remaining Experiments 2–8, a 1-mL aliquot of each test solution was eluted through a glass column that contained 1 mL of IR-120($H^+$) layered on top of a second resin, as described in Table I. Each sample was carefully washed through the column with deionized water and collected in a final volume of 10 mL. These solutions, and the sample from Experiment 1 (dissolved in 10 mL of $H_2O$) were clarified by filtration (0.2 $\mu$m, Nylon 66) and analyzed by l.c. with a chromatographic system that consisted of a Dupont 870 pump module with a heated column-compartment (85°), a Rheodyne fixed-loop (20-$\mu$L) injector, a Waters R-401 Differential Refractometer, a Hewlett-Packard 3390A recording integrator, and a Biorad HPX-87C column, eluted with filtered (0.2 $\mu$m, Nylon 66) distilled wter at 0.6 mL/min.

*Large-scale purifications of sugar solutions.* — Three samples of isomerized maltose syrups, prepared as previously described[3], each containing ~120 g of carbohydrate, 22 g of boric acid, and 640 meq of sodium hydroxide in 2 L of dark-brown solution, were each slowly (5 mL/min) passed through a column of IR-120($H^+$) resin (7 L) and then eluted with an additional 2 L of $H_2O$. The resulting, dark solutions (pH 2.8) were each applied to a column of IRA-743 resin (2 L) that had been previously treated as described in Table II and earlier. Flow rates were adjusted to 2 mL/min for optimal boron-removing efficiency. For each trial (Table II), the sample was eluted from the column with an additional 3.5 L of deionized $H_2O$. Effluents were colorless, except for Trial 2, which was light brown. The final pH values for the eluates from Trials 1, 2, and 3 were 4.8, 7.8, and 5.0, respectively. Each solution was then evaporated to dryness under diminished pressure and analyzed by l.c. (IBM Amino-column) as previously described[3,13]. Boron levels were determined by inductively-coupled plasma spectroscopy at a commercial laboratory (Galbraith Laboratories, Knoxville, TN).

*Modified g.l.c. procedure for alditol acetates using IRA-743 resin.* — A standard solution of erythritol, L-rhamnose, D-xylose, L-arabinose, D-mannose, D-glucose, and D-galactose (1 mg/mL of each) was prepared. A 5-mL aliquot of this solution was treated with sodium borohydride (25 mg; 60 min at room temperature) and then with Dowex 50($H^+$) resin (2.5 g) to remove sodium ions from the solution, which was then filtered and diluted to 50 mL with deionized water. A 2.5-mL aliquot was evaporated and treated with five separate additions and evaporations of methanol to remove boric acid[14]. The final residue was dissolved in methanol–water (1:1; 0.1 mL), transferred to a reaction vial, and evaporated in a stream of dry air. A 1-mL aliquot of methyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside solution (0.5 mg/mL in acetone) was added, the solution was again evaporated to dryness, and then the sample was acetylated (1:1 pyridine–acetic anhydride; 0.4 mL, for 10 min at 100°). The sample was then chromatographed on a Perkin–Elmer 900 Series GC Chromatograph using an SP-2330-coated, fused-silica capillary column [30 m × 0.32 mm (i.d.)] supplied by Perkin–Elmer. An injected sample-size of 1 μL with a sample splitter (150:1) was used, and the oven temperature was kept for 4 min at 225° and then raised at 16°/min to 245° where it was kept for 20 min. Helium was the carrier gas. An additional 2.5-mL aliquot of the borohydride-reduced, standard solution was also acetylated and chromatographed, but without prior removal of boric acid. Another 25-mL aliquot was stirred with 2.5 g of IRA-743 resin (regenerated by method *B*) and 2.5-mL aliquots of the solution were removed after 10, 30, and 60 min. The residues were acetylated, treated with internal standard, and chromatographed.

Corn-bran hemicellulose (10 mg) was hydrolyzed in 2M trifluoroacetic acid (containing 2 mg of erythritol) for 1.5 h at 120°. The solution was evaporated under diminished pressure to remove the acid and the dry sample was redissolved in water (2 mL). The sample was treated with sodium borohydride (10 mg) for 1 h at room temperature and then acidified with Dowex 50($H^+$) resin (1.0 g). The resin was removed by filtration and the solution was divided into two equal parts. One half was treated with 2.0 g of IRA-743 resin, which was removed (by filtration) after 15 min of incubation. The solution was then evaporated. The other half was subjected to additions and evaporations of methanol as described before. Both samples were then acetylated and chromatographed.

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