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Note

Synthesis of D-[5-^{3}H]mannose*

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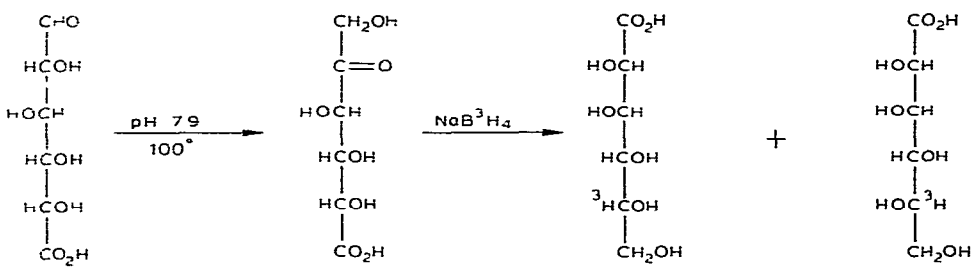
During studies on the mechanism of biosynthesis of alginic acid in bacteria, guanosine 5′-(α-D-[5-^{3}H]mannopyranosyl diphosphate) (GDP-[5-^{3}H]Man) was required. Although an enzymic synthesis of D-[5-^{3}H]mannose, which is the starting material for the synthesis of the sugar nucleotide, is available[1], a more convenient, chemical synthesis was sought.

Barium D-*lyxo*-5-hexulosonate (**2**) was isolated after heating an aqueous solution of D-glucuronic acid (**1**), pH 7.0, for 90 min at 100°, followed by fractionation of the reaction products by anion-exchange chromatography[2]. The barium salt of **2** was converted to the sodium salt and reduced with sodium borotritide. The resulting mixture of D-[5-^{3}H]mannonic acid (**3**) and L-[5-^{3}H]gulonic acid (**4**) was converted to the corresponding lactones. Reduction of the lactones with diborane[3] yielded a mixture containing ^{3}H-labeled D-mannose, L-gulose, and hexitols. D-[5-^{3}H]Mannose



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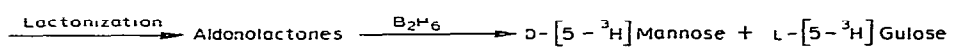

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was isolated from the mixture by paper electrophoresis in borate buffer, followed by paper chromatography.

EXPERIMENTAL

General. — Sodium borotritide (sp. activity > 100 Ci/mol) was purchased from New England Nuclear, Boston, MA 02118. Paper chromatography was performed on Whatman No. 1 paper in 3:3:1 (v/v) ethyl acetate–acetic acid–water (*A*) or 5:2 (v/v) ethanol–M ammonium acetate, pH 3.6 (*B*); electrophoresis was carried out on Whatman No. 3 paper in 50mM borate buffer, pH 9.2 (*C*). Radioactive compounds were located with a 4 π strip-counter and eluted with water. Radioactivity was measured with a Searle Analytic, Mark II, liquid-scintillation system Model 6880 (Searle Radiographics Inc., Des Plaines, IL 60018) in Aquasol, a 1,4-dioxane-based scintillation mixture from New England Nuclear. Analytical gas-liquid chromatography (g.l.c.) was performed with a Perkin–Elmer 900 gas chromatograph in a column of OV-17; compounds were examined as butaneboronic acid derivatives[+]. All evaporations were conducted under reduced pressure at 40°. Carbohydrates were detected with alkaline silver nitrate[5]. M Diborane in tetrahydrofuran was purchased from Alfa Div., Ventron Corp., Danvers, MA 01923. Tetrahydrofuran was purified by distillation from lithium aluminum hydride, and stored over molecular sieves.

Barium D-lyxo-*5-hexulosonate.* — An aqueous solution of D-glucuronic acid (**1**) (pH 7.0) was isomerized by heating for 90 min at 100°, and the reaction products were separated by ion-exchange chromatography on Dowex 1 with M acetic acid[2]. The major product, D-*lyxo*-5-hexulosonic acid was precipitated as the barium salt, converted into the sodium salt by treatment with Dowex 50 (H^+), followed by neutralization with M sodium hydroxide (**2**). The sodium salt of **2** was reduced with sodium borohydride; the two products obtained were characterized by g.l.c. as D-mannonic acid (**3**) and L-gulonic acid (**4**).

D-[*5-*^{3}*H*]*Mannose.* — Barium D-*lyxo*-5-hexulosonate (20 mg) was suspended in water (1 mL) and stirred with Dowex 50 (H^+, 50–100 mesh) until the pH reached 2.0. The resin was removed by decantation, and the solution was neutralized with M sodium hydroxide. Sodium borotritide (4 mg, 0.10 mmol, 12.5 mCi) in water (0.2 mL) was added at 25° over a period of 10 min, and the mixture was stirred for a further 10 min. Dowex 50 (H^+, 50–100 mesh) was added to decompose the excess of sodium borotritide and remove sodium ions. The resin was filtered off, and the filtrate was evaporated to dryness. Methanol was repeatedly added and evaporated from the residue to remove boric acid, and the resulting borate-free aldonolactones were dried *in vacuo* in the presence of potassium hydroxide pellets overnight. The dry residue was suspended in dry tetrahydrofuran (2 mL), and a solution of M diborane in tetrahydrofuran (1 mL) was added dropwise with stirring under anhydrous conditions. Stirring was continued for a total of 1 h, water was then slowly introduced until the evolution of hydrogen ceased, and the solution was evaporated to dryness. After removal of boric acid as described above, the mixture was subjected to paper electro-

phoresis in solvent *C*. The most slowly migrating component, comprising 30% of the total radioactivity, had the mobility of D-mannose. A more rapidly migrating, heterogeneous component accounted for 60% of the total radioactivity. The most rapidly migrating component represented the remaining 10% of the incorporated tritium. The component that corresponded to D-mannose was eluted, desalted as described above, and purified by chromatography in solvent *A*. Its identity was confirmed as D-mannose by paper chromatography in solvents *A* and *B*, and by oxidation with hypobromite to a compound that corresponded to D-mannonic acid upon g.l.c. analysis. The specific activity, determined by scintillation spectroscopy and quantitative analysis[6], was 1.33×10^8 d.p.m./μmol.

The [^{3}H]mannose was shown to belong to the D series by its ability to be converted into GDP-[^{3}H]Man in a series of reactions catalyzed by hexokinase (EC 2.7.1.1) and enzymes present in an extract of *Arthrobacter viscosus*[1]. There was no loss of label upon conversion of the GDP-[^{3}H]Man into guanosine 5'-(α-D-[^{3}H]mannopyranosyluronic acid pyrophosphate) with an extract of *Pseudomonas aeruginosa*[1], thus demonstrating the absence of label at C-6 of the D-mannosyl residue. To establish the position of the tritium atom, the D-[^{3}H]mannose was converted into the methyl glycoside, mixed with methyl α-D-[U-^{14}C]mannopyranoside, and the mixture was degraded by successive periodate oxidation, hypobromite oxidation, and acid hydrolysis[7]. The only ^{3}H-labeled fragment was glyceric acid; the ^{3}H to ^{14}C ratio of this compound was twice that of the undegraded mixture of methyl glycosides. These results established the location of the label at C-5 of the D-[^{3}H]mannose, since a lower ^{3}H to ^{14}C ratio would otherwise have been found[7].

The heterogeneous, electrophoretically separated component was eluted, desalted, and shown by chromatography in solvent *A* to separate into three ^{3}H-labeled substances having the chromatographic mobilities of L-gulose, D-glucitol, and D-mannitol. The compound that corresponded to L-gulose (presumably L-[5-^{3}H]-gulose) contained 30% of the total radioactivity.

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