

224. *The Synthesis of Laminaribiose (3- β -D-Glucosyl D-Glucose) and Proof of its Identity with Laminaribiose isolated from Laminarin.*

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Laminaribiose has been synthesised by the interaction of 1 : 2-5 : 6-diisopropylidene glucose and tetra-acetyl glucosyl bromide, proof of its formulation as 3- β -D-glucosyl D-glucose being thus obtained. The synthetic material is identical with laminaribiose prepared from laminarin. Various derivatives of the sugar are described including the α - and the β -octa-acetate, hepta-acetyl laminaribiosyl bromide, hepta-acetyl methyl- β -laminaribioside, and methyl- β -laminaribioside.

LAMINARIBIOSE was first studied by Barry (*Sci. Proc. Roy. Dublin Soc.*, 1941, **22**, 423), who isolated it as the osazone from the mixture of sugars produced by the action of the juice of *Helix Pomatia* on the polysaccharide laminarin. The free sugar, m. p. 161—162°, was obtained by the partial hydrolysis of laminarin with mineral acid. After removal of the glucose by fermentation and of the oligosaccharides by precipitation with alcohol, the disaccharide was obtained as an amorphous powder which crystallised when its aqueous solution was slowly evaporated. Earlier work by the same author (*ibid.*, 1939, **22**, 59) had shown that laminarin was composed of glucose residues mutually linked through the

1 : 3-positions by β -linkages. Since the disaccharide was hydrolysed by emulsin giving rise only to glucose, Barry formulated it as 3- β -D-glucosyl D-glucose. Laminaribiose was further studied by Connell, Hirst, and Percival (*J.*, 1950, 3494), and its identity has now been confirmed by synthesis as the result of the condensation of 2 : 3 : 4 : 6-tetra-acetyl glucosyl bromide with 1 : 2-5 : 6-diisopropylidene glucose.

Gakhokidze (*J. Gen. Chem. U.S.S.R.*, 1946, 16, 1923) had previously claimed the synthesis of the α -linked analogue of laminaribiose by the condensation of 2 : 3 : 4 : 6-tetra-acetyl glucose with 4 : 6-benzylidene 1 : 2-isopropylidene glucose followed by alkaline and acid hydrolysis. The formulation of this as 3- α -D-glucosyl D-glucose was established by the same author in a later paper (*ibid.*, 1949, 19, 2100). Preliminary experiments revealed that reactions of this type are by no means easy to carry out and the presence of an effective internal desiccant has been found to be essential. Helferich, Bohn, and Winkler (*Ber.*, 1930, 63, 989) used calcium chloride in their synthesis of gentiobiose by the interaction of tetra-acetyl glucosyl bromide and 1 : 2 : 3 : 4-tetra-acetyl glucose, and Reynolds and Evans (*J. Amer. Chem. Soc.*, 1938, 60, 2559) found the proprietary material "Drierite" enabled gentiobiose octa-acetate to be obtained in 75–80% yield. Haskin, Hann, and Hudson (*ibid.*, 1941, 63, 1724) have also used "Drierite" in similar reactions.

In the present work 3- β -D-glucosyl D-glucose was synthesised by the condensation of tetra-acetyl glucosyl bromide with 1 : 2-5 : 6-diisopropylidene glucose in benzene solution in the presence of silver carbonate, iodine, and "Drierite." Deacetylation, followed by removal of the isopropylidene group, and separation of the products on a cellulose column gave in 9.5% yield the crystalline α -form, laminaribiose, m. p. 198–201°, $[\alpha]_D^{25}$ \rightarrow +18.6° in water (compare Connell, Hirst, and Percival, *loc. cit.*).

For comparison with the synthetic material, laminaribiose was prepared from laminarin. After partial hydrolysis of the polysaccharide, laminaribiose was separated from the other products on a charcoal-"Filter Cel" column of the type described by Whistler and Durso (*J. Amer. Chem. Soc.*, 1950, 72, 677). The crystalline product had m. p. 199–202°, and gave no depression of m. p. on admixture with the synthetic material. The two samples gave identical osazones and octa-acetyl derivatives, and their X-ray powder photographs were indistinguishable. The formulation of natural laminaribiose as 3- β -D-glucosyl D-glucose is, therefore, definitely established. In the course of these experiments crystalline hepta-acetyl methyl- β -laminaribioside and methyl- β -laminaribioside were prepared.

After this work had been completed a synthesis of 3- β -glucosyl glucose was reported by Freudenberg and Oertzen (*Annalen*, 1951, 574, 37), who used the same starting materials for the synthesis and obtained the crystalline β -form, m. p. 188–192°, $[\alpha]_D^{+7}$ \rightarrow 20.8° in water, of laminaribiose, but were not in a position to compare directly the natural and the synthetic disaccharide. There is, moreover, little overlap between their work and ours since their experimental methods and the derivatives they describe are not the same as those recorded in the present paper.

EXPERIMENTAL

1 : 2-5 : 6-Diisopropylidene 3-(β -Tetra-acetyl D-Glucosyl) D-Glucose.—Pure 1 : 2-5 : 6-diisopropylidene glucose (10 g.), silver carbonate (15 g.), and "Drierite" (30 g.; dehydrated at 240° during 2 hours) were shaken in dry benzene (80 c.c.) for 12 hours. Iodine (3 g.) was added followed by an equimolecular proportion of 2 : 3 : 4 : 6-tetra-acetyl glucosyl bromide (15.7 g.) in dry benzene (80 c.c.), added slowly during 1 hour with constant shaking in the dark. The shaking (with occasional release of carbon dioxide from the flask) was continued until a test sample gave no precipitate with alcoholic silver nitrate (70 hours). After filtration through "Filter Cel" the unchanged diisopropylidene glucose (2.8 g.) was removed by repeated extraction with water (4 \times 100 c.c.). The aqueous extracts showed only diisopropylidene glucose when examined on a paper chromatogram. The solution was dried (Na_2SO_4), treated with a little charcoal, and filtered. Removal of the solvent gave a colourless glass (A) (16.8 g.).

3- β -D-Glucosyl D-Glucose.—The acetyl groups were removed by shaking a solution of (A) in methanol (80 c.c.) for 6 hours with 0.1N-sodium methoxide (10 c.c.) at room temperature, followed by neutralisation with aqueous oxalic acid. Removal of most of the methanol at 40°/15 mm. was followed by the removal of the isopropylidene groups by treatment of the

residue with oxalic acid (0.01N; 150 c.c.) at 100°. After neutralisation with barium carbonate and filtration, the remaining ions were removed by Amberlite 1R-100H and 1R-4B ion-exchange resins. Evaporation of the water left a colourless syrup (B) (10.3 g.) (Found: Ac, nil). Examination on the paper chromatogram with pyridine showed the presence of much glucose (R_G 1.0) and of substances with R_G values 3.1, 2.1, 0.75 (laminaribiose), 0.4, and 0.25.

A column of powdered cellulose (50 \times 2.8 cm.) was prepared, washed, and tested as described by Hough, Jones, and Wadman (*J.*, 1949, 2511). The syrup (B) (10.2 g.) was dissolved in butanol-pyridine-water (2 : 1 : 1). The elution was started with butanol (300 c.c.) followed by butanol-water-pyridine (6 : 1 : 1). The disaccharide appeared after elution of the column by butanol (300 c.c.) and butanol-water-pyridine (6 : 1 : 1) (2130 c.c.), and was present in the next 600 c.c. Removal of the solvent gave a white glass (1.242 g.; equivalent to a yield of 9.5% of theory); this crystallised slowly when a concentrated solution in ethanol was kept at 0°, and formed needles (1.17 g.), m. p. 196—199°. Further recrystallisation gave material, m. p. 204—206°, $[\alpha]_D^{16} + 24.9^\circ$ (20 minutes), $+18.6^\circ$ (9 hours, constant) in water (*c.* 2.5). Estimation by hypiodite oxidation according to the method described by Hirst, Hough, and Jones (*J.*, 1949, 928) indicated that the material was 99.5—100% pure (Found: C, 42.0; H, 6.6. Calc. for $C_{12}H_{22}O_{11}$: C, 42.1; H, 6.5%).

3- β -Glucosyl Glucosazone.—The disaccharide (0.075 g.) on suitable treatment gave laminaribiosazone, which recrystallised from hot water containing some pyridine as long needles, m. p. 199—201°, $[\alpha]_D^{15} - 76.0^\circ$ in ethanol (*c.* 0.5) {Connell, Hirst, and Percival (*loc. cit.*) recorded $[\alpha]_D - 71.5^\circ$ for natural laminaribiosazone; Barry and Dillon (*Proc. Roy. Irish Acad.*, 1941, 22, 423) gave -79.6° , and Freudenberg and Oertzen, -71° } (Found: C, 54.2; H, 6.4; N, 10.4. Calc. for $C_{24}H_{32}O_6N_4$: C, 55.4; H, 6.2; N, 10.7%). The osazone acetate was prepared by Muir and Percival's method (*J.*, 1940, 1480); it formed a yellow amorphous solid.

Laminarin (20 g.) was hydrolysed by heating it with aqueous oxalic acid (750 c.c.; 0.1N) at 100° for 7 hours. After neutralisation with calcium carbonate and filtration, the solution was evaporated at 40°/15 mm. to 100 c.c. Examination of the product on a paper chromatogram [solvent: benzene-*n*-butanol-pyridine-water (1 : 5 : 3 : 3)] indicated the presence of glucose (R_G 1.0), laminaribiose (R_G 0.75), and four unknown substances having R_G 0.45 and less. The mixture was separated on a column (25 \times 4 cm.) of equal parts of charcoal and "Filter Cel" (Whistler and Durso, *loc. cit.*). The charcoal and "Filter Cel" had been thoroughly mixed, and a suspension in water was poured in small quantities into the column, the lower end of which was closed with a tightly pressed layer of cotton wool. The water was allowed to drain through between the additions. The column was washed with water (1500 c.c.) before use. Water was used as the eluant and after the removal of glucose and a portion of the unknown substance having R_G 0.45 (750 c.c.), a mixture of laminaribiose (1.03 g.) and the rest of the unknown of R_G 4.5 (200 c.c.) was obtained. Laminaribiose alone was present in the next 800 c.c. and its complete removal (2.62 g.) was effected by changing the eluant to water-ethanol (10 : 0.7) (1200 c.c.). The impure fraction of laminaribiose was passed through a smaller column (12 \times 4 cm.). The first fraction contained the unknown sugar, R_G 0.45, and the laminaribiose (0.37 g.) was obtained after addition of 7% of alcohol to the eluant. The total yield of laminaribiose was 2.99 g. After crystallisation it had m. p. 204—206° not depressed on admixture with synthetic laminaribiose; $[\alpha]_D^{18} + 26.5^\circ$ (15 minutes), $+19.5^\circ$ (40 hours, constant) in water (*c.* 2.8). Through the kindness of Dr. C. A. Beevers, X-ray powder photographs of the natural and the synthetic product were obtained, a copper target and 1 hour's exposure (25 mA; 50 kv) being used; the two substances gave identical photographs (Found: C, 42.1; H, 6.8. Calc. for $C_{12}H_{22}O_{11}$: C, 42.1; H, 6.5%).

Octa-acetyl β -Laminaribiose.—Laminaribiose (0.53 g.) (from laminarin) and anhydrous powdered sodium acetate (0.26 g.) were heated with acetic anhydride (3 c.c.) at 100°. After 20 minutes the powder had dissolved, and heating was continued for a further 20 minutes. The addition of water (15 c.c.) caused the precipitation of an oil which was separated from the supernatant liquor (C). This oil solidified very slowly when kept under cold water. The solid (0.31 g.; m. p. 135°) was crystallised by the addition of methanol to its solution in chloroform, followed by concentration. Crystallisation was initiated by the addition of ether, followed later by light petroleum (b. p. 40—60°). After five recrystallisations octa-acetyl β -laminaribiose was obtained as prismatic needles, m. p. 160—161°, $[\alpha]_D^{18} - 28.8^\circ$ in chloroform (*c.* 2.5) (Found: C, 49.7; H, 5.7; Ac, 50.7. $C_{28}H_{38}O_{19}$ requires C, 49.6; H, 5.6; Ac, 50.7%).

Synthetic disaccharide (0.22 g.) was treated as above and after three recrystallisations from methanol-ether gave octa-acetyl β -laminaribiose (0.08 g.), m. p. 156—158° not depressed on admixture with the material just described; $[\alpha]_D^{16} - 25.3^\circ$ in chloroform (*c.* 2.2).

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Octa-acetyl α-Laminaribiose.—(a) *Laminaribiose from laminarin.* The liquor (C) (see previous paragraph) was neutralised (sodium hydrogen carbonate) and extracted with chloroform. On evaporation of the chloroform, crude octa-acetyl laminaribiose was obtained as a yellow oil (0.6 g.). This material was isomerised by heating it with acetic anhydride (3 c.c.) and fused zinc chloride (0.04 g.) (cf. Hudson and Johnson, *J. Amer. Chem. Soc.*, 1915, **37**, 1270). After 45 minutes the mixture was cooled and water (20 c.c.) added. On the repeated addition of water and decantation, the precipitated oil solidified. After four recrystallisations of the brown solid (0.38 g.) from chloroform–ethanol *octa-acetyl α-laminaribiose* was obtained as prismatic needles containing one molecule of ethanol of crystallisation; it had m. p. 77–78°; $[\alpha]_D^{17} + 20^\circ$ in chloroform (c, 3.6), calculated for the ethanol-free compound (Found: Ac, 47.5, 47.8. $C_{28}H_{38}O_{19} \cdot C_2H_6O$ requires Ac, 47.5. $C_{28}H_{38}O_{19}$ requires Ac, 50.7%). The rotation figures for the α- and the β-octa-acetate indicate that the separation of the two isomers is not quite complete (compare Hudson and Johnson, *ibid.*, 1917, **39**, 1272).

(b) *Synthetic laminaribiose.* The sugar (0.075 g.) was acetylated by the procedure described by Kruger and Roman (*Ber.*, 1936, **69**, 1832) and Nicolas and Smith (*Nature*, 1948, **161**, 349) (yield of crude octa-acetate, 51%). Recrystallisation from ethanol gave a product having m. p. 77–79°, not depressed on admixture with the octa-acetate from natural laminaribiose described above, $[\alpha]_D^{18} + 21^\circ$ in chloroform (c, 1.8) (Found: C, 50.0; H, 6.3; Ac, 47.9. Calc. for $C_{28}H_{38}O_{19} \cdot C_2H_6O$: C, 49.7; H, 6.1; Ac, 47.5%). Attempts to remove the ethanol from the crystals only led to its replacement by other solvents; for instance recrystallisation from methanol gave a *product* having $[\alpha]_D^{18} + 21.5^\circ$ in chloroform (c, 2.56), m. p. indefinite, containing one molecular proportion of methanol of crystallisation (Found: C 49.0; H, 6.2; Ac, 48.9. $C_{28}H_{38}O_{19} \cdot CH_4O$ requires C, 49.0; H, 6.0; Ac, 48.5%). Similar results were obtained with benzene.

Hepta-acetyl Laminaribiosyl Bromide.—Laminaribiose octa-acetate (0.6 g.; a mixture of the α- and the β-form) was added with shaking to glacial acetic acid (2 c.c.) saturated with hydrogen bromide at 0° (Latham, May, and Mosettig, *J. Org. Chem.*, 1950, **15**, 884). After 5 minutes, cooling was discontinued but shaking was continued until all the octa-acetate had dissolved (10 minutes). The viscous oil which separated was kept at 15° for 3 hours. Addition of dry toluene (20 c.c.) followed by its removal at 40–50°/12 mm. gave after two such operations a pale yellow solid (0.48 g., 83%). Crystallisation of this from chloroform–ethanol gave white needles (0.44 g.), m. p. 165–167°. After four recrystallisations from chloroform–ether–light petroleum *hepta-acetyl laminaribiosyl bromide* was obtained having m. p. 180.5–181.5° $[\alpha]_D^{18} + 85.0^\circ$ in chloroform (c, 3.0) (Found: C, 45.15; H, 4.9; Br, 10.8. $C_{26}H_{35}O_{17}Br$ requires, C, 44.6; H, 5.0; Br, 11.4%).

Hepta-acetyl Methyl-β-laminaribioside.—Hepta-acetyl laminaribiosyl bromide (0.32 g.), dry methanol (10 c.c.), and dry benzene (10 c.c.) [dried by shaking it with “Drierite” (3 g.) for 2 hours] were shaken in the dark with dry silver carbonate (0.5 g.) and iodine (0.1 g.) until a test sample gave no precipitate with silver nitrate (20 hours) (compare Reynolds and Evans, *J. Amer. Chem. Soc.*, 1940, **62**, 66). Chloroform (50 c.c.) was added, and after filtration aided by “Filter Cel,” followed by evaporation of the solvent, a red syrup was obtained which crystallised on the addition of ethanol (yield 0.255 g., 86%). Hepta-acetyl methyl-β-laminaribioside after three recrystallisations from ethanol had m. p. 164–165°, but on further heating it resolidified and melted again at 179–180°. In another experiment the substance was obtained in the first instance as needles, m. p. 183°; nevertheless on recrystallisation it showed the double melting point, 164–165° and 179–180°, and had $[\alpha]_D^{18} - 45^\circ$ in chloroform (c, 1.7) (Found: C, 50.0; H, 6.0; OMe, 4.3; Ac, 48.0. $C_{27}H_{35}O_{18}$ requires C, 49.8; H, 5.9; OMe, 4.8; Ac, 47.7%).

Methyl-β-laminaribioside.—Hepta-acetyl methyl-β-laminaribioside (0.408 g.) in dry methanol (10 c.c.) was boiled with sodium methoxide (1 c.c.; 0.1N) for 1 hour (Zemplén, *Ber.*, 1936, **69**, 1827). Filtration through charcoal and removal of the solvent gave a colourless syrup, which crystallised after 4 days under alcohol. Three recrystallisations from ethanol–ether gave *methyl-β-laminaribioside* as fine needles, m. p. 165–166°, $[\alpha]_D^{19} - 28^\circ$ in water (c, 2.5) (Found: C, 41.9; H, 7.0; OMe, 7.8. $C_{13}H_{24}O_{11} \cdot H_2O$ requires C, 41.7; H, 6.95; OMe, 8.3%).

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