34. *The Structure of Laminarin. Part III.*[1] *Synthesis of Structural Oligosaccharides.*

By Stanley Peat, W. J. Whelan, and J. M. Evans.

The chemical syntheses of eight oligosaccharides are described. Three are reducing trisaccharides based on β-D-glucose and contain 1,3- and 1,6-glycosidic bonds. The others consist of D-mannitol substituted at $C_{(1)}$ by glucose or laminaridextrins. Some of these sugars have previously been found in partial acid hydrolysates of laminarin and baker's yeast glucan.

The picture of the structure of laminarin which emerges from studies [1,2] of the oligosaccharides formed by its partial acid hydrolysis is that of chains of β-D-glucose members linked mainly by 1,3-bonds, but to a lesser degree by 1,6-links also. Furthermore, they showed that a unit of D-mannitol was an integral component of about half of the laminarin chains (see also ref. 3) and that this mannitol unit was most probably joined glucosidically to the chain through the primary alcohol group of the mannitol (*i.e.*, through $C_{(1)}$ or $C_{(6)}$, these positions being equivalent because of the centre-point symmetry of the hexitol). Later studies by Smith and his co-workers [4] suggest that the mannitol is also substituted at $C_{(2)}$ and that mannose is present.

The identification of the oligosaccharide fragments was based in part on comparison with authentic specimens which were prepared by chemical synthesis. The present paper records the details of these definitive syntheses.

The " trisaccharide fraction " of the partial acid hydrolysate of laminarin contained three reducing sugars identified [1,2] as laminaritriose (Ia), 3-*O*-β-gentiobiosylglucose (Ib)

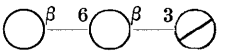
[1] Part II, *J.*, 1958, 729.
[2] Part I, *J.*, 1958, 724.
[3] Peat, Whelan, and Lawley, *Chem. and Ind.*, 1955, 35; Unrau and Smith, *ibid.*, 1957, 330; Anderson, Hirst, Manners, and Ross, *J.*, 1958, 3233.
[4] Goldstein, Smith, and Unrau, *Chem. and Ind.*, 1959, 124; Smith and Unrau, *ibid.*, 1959, 636.

and 6-*O*-β-laminaribiosylglucose (Ic) as well as two non-reducing " trisaccharides " containing mannitol, namely, 1-*O*-β-laminaribiosylmannitol (If) and 1,6-di-*O*-β-glucosylmannitol (Id). The last-named was obtained from the laminarin hydrolysate in such relatively small yield that no structural significance could be attached to its presence.[1]

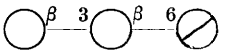
The methods employed in the synthesis of the " trisaccharides " (Ia), (Ib), (Ic), (Id),

Oligosaccharides related to laminarin.

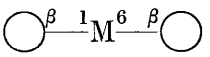
(Ia) Laminaritriose



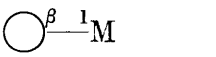
(Ib) 3-*O*-β-Gentiobiosylglucose



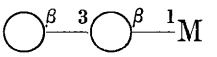
(Ic) 6-*O*-β-Laminaribiosylglucose



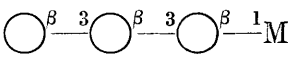
(Id) 1,6-Di-*O*-β-glucosylmannitol



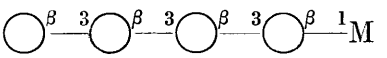
(Ie) 1-*O*-β-Glucosylmannitol



(If) 1-*O*-β-Laminaribiosylmannitol



(Ig) 1-*O*-β-Laminaritriosylmannitol



(Ih) 1-*O*-β-Laminaritetraosylmannitol

Key. ○ = glucose. ⊘ = reducing glucose unit. M = mannitol.

and (If), as well as of 1-*O*-β-glucosylmannitol (Ie) and the higher oligosaccharides (Ig) and (Ih) are described below. The glucosylmannitol (Ie) is found in the laminarin hydrolysate and also occurs in the free state, together with (Id), in *Fucus vesiculosus*.[5] The latter (Id) has been previously synthesised by Lindberg.[6] The work now described also assisted in the elucidation [7] of the structure of yeast glucan, since partial acid hydrolysis of the latter yielded the reducing trisaccharides (Ia), (Ib), and (Ic).

The syntheses were all based on the Koenigs–Knorr reaction, whereby an α-1-bromosugar acetate (*A*) is condensed, in the presence of silver oxide, with a " monosaccharide " (*B*), partially acylated in such a way that it carries a free hydroxyl group in the appropriate position.[8] A β-glycosidic linkage is thus established between *A* and *B*, the condensation being accompanied by optical inversion. The specific components used in each condensation are shown in Table 1.

TABLE 1. *Koenigs–Knorr condensations.*

Component *A*	Component *B*	Deacylated product
Hepta-*O*-acetyl-α-laminaribiosyl bromide	1,2,4,6-Tetra-*O*-acetyl-β-glucose	Laminaritriose (Ia)
Hepta-*O*-acetyl-α-laminaribiosyl bromide	1,2,3,4-Tetra-*O*-acetyl-β-glucose	6-*O*-β-Laminaribiosylglucose (Ic)
Hepta-*O*-acetyl-α-gentiobiosyl bromide	1,2,4,6-Tetra-*O*-acetyl-β-glucose	3-*O*-β-Gentiobiosylglucose (Ib)
2,3,4,6-Tetra-*O*-acetyl-α-glucosyl bromide	2,3,4,5-Tetra-*O*-benzoylmannitol	1-*O*-β-Glucosylmannitol (Ie) and 1,6-di-*O*-β-glucosylmannitol (Id)
Hepta-*O*-acetyl-α-laminaribiosyl bromide	1,2,3,4-Tetra-*O*-acetyl-β-mannose	6-*O*-β-Laminaribiosylmannose *
Deca-*O*-acetyl-α-laminaritriosyl bromide	1,2,3,4-Tetra-*O*-acetyl-β-mannose	6-*O*-β-Laminaritriosylmannose *
Trideca-*O*-acetyl-α-laminaritetraosyl bromide	1,2,3,4-Tetra-*O*-acetyl-β-mannose	6-*O*-β-Laminaritetraosylmannose *

* Converted into the corresponding mannitol derivative by reduction with sodium borohydride.

The properties of the synthetic oligosaccharides are reported in Table 2. Four of the five trisaccharides gave crystalline acetyl derivatives, one trisaccharide was obtained in insufficient amount for acetylation, and the other sugars gave amorphous esters. Four methods were used to confirm that the sugars had the structures expected from their

[5] Lindberg, *Acta Chem. Scand.*, 1953, **7**, 1119.
[6] Lindberg, *Acta Chem. Scand.*, 1953, **7**, 1218.
[7] Peat, Whelan, and Edwards, *J.*, 1958, 3862.
[8] Evans, Reynolds, and Talley, *Adv. Carbohydrate Chem.*, 1951, **6**, 27.

method of synthesis. These were (i) paper-chromatographic examination of the disaccharide products of partial hydrolysis with acid and with emulsin (almond β-glucosidase), (ii) comparison of the specific optical rotations of the sugars and their acetates with calculated values, and, for the series of laminaridextrins containing mannitol, the demonstration that the plots of (iii) R_M value of the sugars and (iv) $[M]_D$ of the sugars and their acetates against degree of polymerisation were linear and ran parallel to the corresponding relation for the laminaridextrins proper. These methods have been explained in Parts I [2] and II.[1] All the synthetic oligosaccharides behaved in the expected manner. The disaccharide products of partial acidic and enzymic hydrolysis are listed in Table 2.

TABLE 2. *Properties of the chemically synthesized oligosaccharides.*

	$[\alpha]_D$ in H_2O			Acetate $[\alpha]_D$ in $CHCl_3$		Partial hydrolysis products *	
	Found	Calc.	M. p.	Found	Calc.	Acid	Enzyme
Laminaritriose (Ia)	2·5°	—	121°	−40·8°	—	L	L
3-*O*-β-Gentiobiosylglucose (Ib)	−4·2	−3·2°	—	—	—	L, G	L
6-*O*-β-Laminaribiosylglucose (Ic)	−6·0	−3·5	216—217	−27·4	−24·2°	L, G	G
1-*O*-β-Glucosylmannitol (Ie)	−19·8	—	syrup	—	—	—	—
1,6-Di-*O*-β-glucosylmannitol (Id)	−23·5 †	—	136—138	−7·7	—	—	—
1-*O*-β-Laminaribiosylmannitol (If)	−24·3	−24·3	145	−17·9	—	—	—
1-*O*-β-Laminaritriosylmannitol (Ig)	−26·0	−26·3	amorph.	−24	−28·3	—	—
1-*O*-β-Laminaritetraosylmannitol (Ih)	−28·1	−27·1	amorph.	−33·1	−35·6	—	—

* L = laminaribiose G = gentiobiose

† Previously reported values of $[\alpha]_D$ are −14·0° [5] and −16·5°.[1]

EXPERIMENTAL

Methods.—The analytical and chromatographic methods have already been described.[1,2,7,9] The Koenigs–Knorr condensations were carried out as follows, the method being based on that of Reynolds and Evans.[10] The hydroxy-compound, *B*, was dissolved in dry alcohol-free chloroform. Anhydrous calcium sulphate (preheated at 240° for 2 hr.), silver oxide (except where stated), and iodine were then added. The mixture was placed in a cold room at 0—2° and the bromo-sugar, *A*, in chloroform, was added in portions during 1—2 hr. with stirring, the flask being protected against the atmosphere by a calcium chloride tube. The flask was shaken for 72 hr., except where stated, the contents then filtered through a pad of Celite no. 535, and the filtrate and chloroform washings of the pad evaporated to a dry syrup under diminished pressure. Except in two instances (see below) the syrup (2% in dry methanol or methanol–acetone) was deacetylated with barium methoxide at a final concentration of 15mN, and then fractionated chromatographically after a preliminary examination on a paper chromatogram. The first condensation experiment described below gives details of the quantities of reagents used and of the charcoal–chromatographic procedure and is typical of the other experiments.

Synthesis of Laminaritriose (Ia).—1,2,4,6-Tetra-*O*-acetyl-β-D-glucose (m. p. 126—127°, lit.,[11] m. p. 126—127°; 2·2 g.) was dissolved in chloroform (20 ml.), and calcium sulphate (10 g.), silver oxide (1 g.), and iodine (0·5 g.) were added, followed by hepta-*O*-acetyl-α-laminaribiosyl bromide (m. p. 176—177°, lit.,[12] m. p. 180—181°; 2·2 g.) in chloroform (20 ml.). The deacetylated mixture was fractionated on charcoal–Celite (100 × 5 cm.) which was eluted with water (500 ml.), 7·5% ethanol (1 l.), and 15% ethanol, fractions of 50 ml. being collected. Glucose was in fractions Nos. 5—9, laminaribiose in fractions Nos. 24—29, and laminaritriose (100 mg., 6·3%) in fractions Nos. 38—42. The properties of laminaritriose and of its acetate are given in Table 2.

Synthesis of 3-O-β-*Gentiobiosylglucose* (Ib).—β-Gentiobiose octa-*O*-acetate was synthesized from glucose [10] and converted into the hepta-*O*-acetyl bromide (m. p. 143—144°, lit.,[13] m. p. 144°). The bromide (7·3 g.) was condensed with 1,2,4,6-tetra-*O*-acetyl-β-D-glucose (8 g.). The subsequent operations were (i) deacetylation, (ii) fractionation on charcoal–Celite (112 × 5 cm.) the trisaccharide being desorbed with 25% ethanol but not by 15% ethanol, and (iii) two

[9] Peat, Whelan, and Roberts, *J.*, 1957, 3916.

[10] Reynolds and Evans, *J. Amer. Chem. Soc.*, 1938, **60**, 2559.

[11] Freudenberg, von Hochstetter, and Engels, *Ber.*, 1923, **58**, 666; Freudenberg and Plankenhorn, *Annalen*, 1938, **536**, 257; Adams, Reeves, and Goebel, *J. Biol. Chem.*, 1941, **140**, 653.

[12] Bächli and Percival, *J.*, 1952, 1243.

[13] Brauns, *J. Amer. Chem. Soc.*, 1927, **49**, 3170.

fractionations on thick filter paper in butanol–acetic acid–water (4 : 1 : 5, by vol.) to yield the sugar (117 mg., 2·2%), whose properties are reported in Table 2.

Synthesis of 6-O-β-*Laminaribiosylglucose* (Ic).—1,2,3,4-Tetra-*O*-acetyl-β-D-glucose (m. p., 128—129°, lit.,[14] m. p. 128—129°; 3·5 g.) was condensed with hepta-*O*-acetyl-α-laminaribiosyl bromide (3·5 g.) for 55 hr. The resulting acetate crystallized in needles from ethanol and was unchanged in m. p. when recrystallized, the final yield being 3·7 g. (81%). The properties of the *acetate* and the free sugar are in Table 2 (Found: C, 49·8; H, 5·7. $C_{40}H_{54}O_{27}$ requires C, 49·7; H, 5·6%).

Synthesis of 1-O-β-*Glucosylmannitol* (Ie) *and* 1,6-*Di*-O-β-*glucosylmannitol* (Id).—2,3,4,5-Tetra-*O*-benzoyl-D-mannitol[15] (syrup, 8·4 g.) was condensed with 2,3,4,6-tetra-*O*-acetyl-α-D-glucosyl bromide (10 g.) for 72 hr. The syrup was deacetylated with sodium ethoxide and fractionated on charcoal–Celite (150 × 5 cm.). 1-*O*-β-Glucosylmannitol was desorbed with 7·5% ethanol and from some of the early fractions a component migrating on paper faster than glucose was removed by thick-paper chromatography. The total yield was 1·02 g. As found previously,[1,5] a crystalline acetate could not be prepared. 1,6-Di-*O*-β-glucosylmannitol was desorbed with 20% ethanol. It formed a *dodeca-acetate* (Found: OAc, 51·6. $C_{42}H_{58}O_{28}$ requires OAc, 51·2%). The properties of these sugars are recorded in Table 2.

Synthesis of 1-O-β-*Laminaribiosylmannitol* (If).—1,2,3,4-Tetra-*O*-acetyl-β-D-mannose (m. p. 135—136°, lit.,[16] m. p. 135—136·5°; 5·4 g.) was condensed with hepta-*O*-acetyl-α-laminaribiosyl bromide (6·6 g.) for 72 hr. The syrup was deacetylated and fractionated on charcoal–Celite (150 × 5 cm.), removing glucose and laminaribiose as usual and a trisaccharide (2·743 g., 57%), presumably 6-*O*-β-laminaribiosylmannose, with 15% ethanol. This was dissolved in water (50 ml.) and mixed with 10% sodium borohydride (50 ml.). After 5 hr. at room temperature the sugar had lost its copper-reducing power and the residual borohydride was destroyed with dilute acetic acid. Sodium borate was removed by adsorption of the sugar on charcoal–Celite,[9] and the product (1·97 g.) was examined as in Table 2. The *dodeca-acetate* softened at 125—126° before melting at 145° (Found: C, 49·9; H, 5·7; OAc, 51·3. $C_{42}H_{58}O_{28}$ required C, 49·9; H, 5·7; OAc, 51·1%).

Synthesis of 1-O-β-*Laminaritriosylmannitol* (Ig).—Laminaritriose (15 g.) was converted into deca-*O*-acetyl-α-laminaritriosyl bromide (5·58 g.) by the general method.[17] This compound, not previously described, was an amorphous white powder having $[\alpha]_D$ 44·8° (*c* 0·36 in chloroform). The bromide (5·4 g.) was condensed with 1,2,3,4-tetra-*O*-acetyl-β-D-mannose (3 g.) in presence of silver carbonate for 72 hr. The syrup was deacetylated and fractionated on charcoal–Celite (150 × 5 cm.), mannose being removed with water, laminaritriose with 15% ethanol, and the synthetic tetrasaccharide (1·03 g., 29%) with 25% ethanol. This was reduced with sodium borohydride, and the product (0·65 g.) recovered as before. It formed an amorphous acetate (see Table 2).

Synthesis of 1-O-β-*Laminaritetraosylmannitol* (Ih).—Laminaritetraose (5 g.) was converted into trideca-*O*-acetyl-α-laminaritetraosyl bromide (4·1 g.). This substance, not previously reported, was a white amorphous powder having $[\alpha]_D$ 20·0° (*c* 0·23 in chloroform). The bromide (3·99 g.) was condensed with 1,2,3,4-tetra-*O*-acetyl-β-D-mannose (1·75 g.) in presence of silver carbonate for 72 hr. The syrup was deacetylated and the sugar mixture (2·83 g., 100 ml.) reduced with sodium borohydride (1 g., 30 ml.). After destruction of the residual borohydride the mixture was fractionated on charcoal–Celite (100 × 5 cm.). The unchanged reactants were removed and the synthetic pentasaccharide (1·23 g.) was desorbed with 50% ethanol. It was contaminated with laminaritetraitol and was purified by thick filter paper chromatography in butanol–pyridine–water (6 : 4 : 3, by vol.). The pure sugar (0·19 g., 7·3%) formed an amorphous acetate (see Table 2). A partial acid hydrolysate contained non-reducing substances having R_F values corresponding to mannitol, 1-*O*-β-glucosyl-, 1-*O*-β-laminaribiosyl-, and 1-*O*-β-laminaritriosyl-mannitol.

We thank Dr. J. R. Turvey for help in this work and the Department of Scientific and Industrial Research for a maintenance grant (to J. M. E.).

UNIVERSITY COLLEGE OF NORTH WALES,
BANGOR, NORTH WALES.

[*Received, June 3rd,* 1959.]

[14] Helferich and Klein, *Annalen*, 1926, **450**, 219.
[15] Müller, *Ber.*, 1932, **65**, 1051.
[16] Reynolds and Evans, *J. Amer. Chem. Soc.*, 1940, **62**, 66.
[17] Fischer and Fischer, *Ber.*, 1910, **43**, 2534.