Fig. 1 shows the result when birch extracts were sprayed with diazotized sulphanilic acid. With this spray, vanillic acid gives an orange spot and syringic acid a red spot. Both extracts showed strong spots of each of these acids, whereas the control (which had not been inoculated) gave only very faint spots. Vanillic acid alone was released from spruce sawdust by both fungi. Using 2 : 4-dinitrophenylhydrazine, both fungi gave faint spots of vanillin and syringaldehyde from birch sawdust and of vanillin from spruce sawdust; but similar spots were given by the controls.

These results are of interest as they conform with chemical analyses of lignin[4], which show the occurrence of guaiacyl and syringyl groups in hardwood lignin and of guaiacyl groups alone in softwood lignin.

These compounds are released probably by the action of extracellular enzymes produced by the wood-rotting fungi. It is possible that such molecules can thus be rendered available to micro-fungi under natural conditions, as it has been shown that such fungi do not themselves release these substances from sawdust.

M. E. K. Henderson

Macaulay Institute for Soil Research,
Craigiebuckler, Aberdeen.
Dec. 7.

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Synthesis of Oligosaccharides by Growing Cultures of *Betacoccus arabinosaceous*

Koepsell *et al.*[1] have shown recently that oligosaccharides are formed when the dextransucrase of *Leuconostoc mesenteroides* (*NRRL B*-512) acts on sucrose in the presence of certain simple sugars, which serve as chain-initiators. We have observed a similar phenomenon with the dextransucrase and with growing cultures of *Betacoccus arabinosaceous* (Birmingham strain). When grown on a medium containing sucrose (10 per cent), yeast extract and inorganic salts, this organism produces a dextran in which the principal glucosidic linkages are α-1 : 6 and the branch points involve positions 1 and 3 [2]. Fructose, traces of glucose and a ketose-containing disaccharide can be detected in the medium. The degree of branching in the polysaccharide is dependent, *inter alia*, on the magnesium content of the medium; dextran samples produced from magnesium-rich and magnesium-deficient media have been proved to have average chain-lengths of 6–7 and 40–50 anhydroglucose units, respectively[3].

It has now been shown that very profound reductions in the molecular size of the dextran produced by the growing organism can be effected by the addition of suitable simple sugars to the culture medium; in extreme cases, di-, tri- and tetrasaccharides are the principal products. This work was a logical development of studies in which glucose, maltose, *iso*maltose and other sugars were found to serve as chain-initiators in transglucosylations by the enzyme *Betacoccus arabinosaceous* dextransucrase (cf. Koepsell *et al.*[1]).

The addition of glucose (40 per cent w/v) to the standard culture medium almost completely inhibited growth of the organism, whereas with 10 per cent glucose there was good growth, accompanied by the formation of polymeric material. However, with 20 per cent glucose dextran production was noticeably depressed and *iso*maltose, *iso*maltotriose and their higher homologues were prominent on paper chromatograms of the culture solution.

The addition of 10–50 per cent maltose (w/v) to the standard medium (40 c.c., to which another 4 gm. of sucrose was afterwards added) had a similar effect; at the latter concentration the amount of product which was insoluble in 66 per cent ethanol was negligible. Paper chromatographic analysis of the solution revealed the presence of a large amount of panose, together with unchanged maltose, higher aldosaccharides, fructose, a ketose-containing disaccharide and a ketose-containing trisaccharide. Fractionation of the mixture on a charcoal column[4] gave a trisaccharide fraction (3·5 gm.) and a mixed oligosaccharide fraction (4·5 gm., eluted by 15–20 per cent ethanol); crystallization of the former from aqueous methanol afforded panose, melting point and mixed melting point 222–224° (decomp.) $[\alpha]_D^{21}$ + 153·9° (equil.; in water).

Chromatographic evidence was also obtained of: (*a*) non-reducing products (presumably methyl glycosides) in the oligosaccharide range when methyl α-D-glucopyranoside (25 per cent) was added to the standard medium; (*b*) an unidentified trisaccharide on the addition of cellobiose (30 per cent); (*c*) a ketose-containing disaccharide on the addition of fructose (25 per cent); and (*d*) probable tri- and tetra-saccharides when lactose (10 per cent) was incorporated in a modified medium containing only 2 per cent sucrose.

These results with growing cultures, like those obtained with dextransucrase itself, point to a multi-chain mechanism of synthesis and throw light on the structural requirements for chain-initiation. In addition, they provide a convenient route for the preparation of appreciable quantities of higher saccharides (and their glycosides) with a predetermined range of molecular weight, which might well prove to be useful commercially for the production of gums, anti-staling agents for bread, plasticizers, etc. We have shown also that they simplify the difficult preparation of dextran-free dextransucrase by providing a source in which the enzyme is accompanied by only traces of the polysaccharide.

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R. W. Bailey
S. A. Barker
E. J. Bourne
M. Stacey

Chemistry Department,
University,
Birmingham 15.
Feb. 8.

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