ing of the experimental points, particularly when the slope increases greatly would make it hazardous to utilize an apparent decrease in molecular weight for calculation of a solvation layer.

Flow birefringence studies of Edsall and Foster [7] indicate that γ-globulin and serum albumin, too, are little affected by high glycerol concentrations. It has also been shown by optical rotation and sedimentation studies that γ-globulin retains its native conformation in ethylene glycol-water mixtures up to a glycol concentration of at least 80 % by volume except that some aggregation occurs. Ethylene glycol is similarly inert toward β-lactoglobulin [8]. From the data presented as Fig. 3, however, it appears that there is an increase in molecular weight because aggregation occurs upon addition of ethylene glycol (even in small amounts) to aqueous solutions of creatine-phosphotransferase. The tolerance exhibited by this enzyme towards glycerol is higher: Changes begin only after approximately 50 volume percent organic solvent have been attained. In the case of bovine serum albumin, dissolved in glycerol – water mixtures, the results are similar (Fig. 4). Again, these solvents cannot be employed to gain information on the degree of hydration of the protein molecules.

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# Studies on Glucomannans from Norwegian Spruce *

## 4. Enzymic Hydrolysis

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The glucomannans from coniferous woods have been extensively studied during the last decade. The results, reviewed by Aspinall [1] show an essentially linear structure with (1→4)-linked β-D-glucose and β-D-mannose residues, usually in the proportion 1:3-4. The native polysaccharides also contain *O*-acetyl groups [2-4]. In some preparations glucose and mannose residues are to some extent substituted in the sixth position by α-D-galactopyranose residues [1].

There are indications that besides the galactosidic side-chains additional branching occurs in some coniferous wood glucomannans but conclusive evidence for this is lacking. It is further not known whether or not the glucose and mannose residues are arranged according to a regular pattern. The isolation and characterisation of the oligosaccharides formed upon enzymic hydrolysis of a glucomannan using a specific enzyme would give information of this. A crude hemicellulase has previously been used by Perila and Bishop [5] for enzymic hydrolysis of Jack pine (*Pinus banksiana* Lamb.) glucomannan.

A glucomannan from Norwegian spruce (*Picea abies* Karst.), containing 27 % glucose, 72 % mannose, and 1 % galactose, was accordingly hydrolysed with a commercial cellulase preparation from *Penicillium chrysogenum notatum* Astra 200 and the products were fractionated by paper and carbon column chromatography. The results are summarised in Table 1. Inspection of the table shows that the enzymic preparation was not specific for (1→4)-β-D-glucosidic linkages and the purpose of the investigation was therefore not attained. The glucosidic linkages were, however, more readily hydrolysed than the mannosidic, as indicated by the relatively high

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*Table 1.* (1→4)-β-linked glucose (G) and mannose (M) containing oligosaccharides from enzymic hydrolysis of Norwegian spruce glucomannan.

| Saccharide | Yield mg | % | $R_G$ | $M_G$ | m.p. | $[\alpha]_D^{20}$ | G:M |
|---|---|---|---|---|---|---|---|
| Monomers | 26 | 1.6 | — | — | — | — | 3:1 |
| M→M | 279 | 17.2 | 0.64 | 0.64 | 203–205° | −8.3° | |
| G→M | 71 | 4.1 | 0.90 | 0.56 | — | + 6.0° | 1:1 |
| M→G | 229 | 14.1 | 0.50 | 0.41 | 201–204° | + 18.6° | 1:1 |
| G→G | 86 | 5.3 | 0.65 | 0.26 | 235–238° | + 24.7° | — |
| M→M→M | 479 | 29.5 | 0.33 | 0.53 | 220–221° | −21.8 | — |
| M→M→G | 99 | 6.1 | 0.26 | 0.38 | — | — | 1:2 |
| G→M→M | 21 | 1.3 | 0.42 | 0.60 | — | — | 1:2 |
| M→G→M | 97 | 6.0 | 0.36 | 0.49 | — | — | 1:2 |
| M→M→M→M | 202 | 12.4 | 0.15 | 0.54 | — | −29.5° | — |
| M→M→M→M→M | 40 | 2.5 | — | 0.45 | — | −37.6° | |

yields of oligosaccharides, terminated by a reducing glucose residue and of (1→4)-β-mannooligosaccharides. Mannopentaose has previously been isolated from a partial hydrolysate of ivory nut mannan [6] but not from wood glucomannans. The lability of mannosidic linkages towards the enzyme was confirmed by digesting the isolated mannooligosaccharides with the enzyme. Mannopentaose and -tetraose were readily hydrolysed while mannotriose and mannobiose were fairly stable showing central mannosidic linkages to be less stable than terminal.

*Experimental.* The glucomannan was isolated from preextracted (10 % potassium hydroxide) Norwegian spruce wood meal by extraction with 17 % sodium hydroxide containing 3 % boric acid [7]. It was purified by removal of residual lignin by the chlorine-ethanolamine method [8] and by precipitation with barium hydroxide [9].

A suspension of the glucomannan (18.5 g) and *Penicillium chrysogenum notatum* Astra 200 cellulase (1.0 g) in citrate buffer (1000 ml) of pH 5.1 (9.7 parts of 0.1 M citric acid and 10.3 parts of 0.2 M disodium hydrogen phosphate) was kept at 40° for 3 days. Insoluble material was removed by centrifuging and the centrifugate deionised (Dowex 50W and Dowex 3 resins), concentrated and poured into ethanol. The precipitate (1.54 g) was discarded while unprecipitated material was concentrated to a syrup (3.61 g) of mainly lower oligosaccharides.

The oligosaccharide mixture was fractionated and the components characterised as described in Part 3 [10]. The proportion and sequence of glucose and mannose in the heterooligosaccharides was established by paper chromatography following partial and total hydrolysis before and after reduction of samples with sodium borohydride. The results are given in Table 1. For paper chromatography the solvent system ethyl acetate, pyridine, water (2:1:2) was used and for paper electrophoresis 0.1 M borate buffer of pH 10. In addition to the oligosaccharides given in the table there was obtained 1.18 g of higher oligosaccharides which were not examined further.

Enzymic digestion of isolated oligosaccharides was performed under the same conditions used in the hydrolysis of the polysaccharide.

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