Synthesis of a Glucoheptaose and a Glucooctaose That Elicit Phytoalexin Accumulation in Soybean*

(Received for publication, February 13, 1984)

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The glucoheptaose 1 and the glucooctaose 2 have been synthesized using unambiguous methods. The former is identical with an elicitor-active heptasaccharide obtained from partially hydrolyzed mycelium of Phytophthora megasperma f. sp. glycinea. The octasaccharide is also elicitor active, although to a lesser extent than the heptasaccharide. 1: R = H; 2: R = β-D-Glc.

β-D-Glc-(1→6)-β-D-Glc-(1→6)-β-D-Glc-(1→6)-β-D-Glc-(1→6)-β-D-Glc

As reported in the preceding papers (1, 2), partial acid hydrolysis of mycelial walls of the fungus Phytophthora megasperma f. sp. glycinea gives a mixture of oligosaccharides that stimulates the formation of phytoalexins in soybean. The smallest active component, a heptasaccharide, could be isolated after extensive fractionation and its structure was determined. This component has been available in microgram quantities only. Its synthesis was therefore of some interest both in order to confirm the proposed structure and to get more material for the biological studies.

When this synthetic work was initiated, the structural studies were in their beginning. The smallest active component was believed to be a reducing hepta- or octasaccharide composed of β-D-glucopyranosyl residues. Three of these should be terminal, two branching and linked through 0-3, one of these being a reducing unit and, possibly, one chain residue linked through 0-3.

As the elicitor activity was not reduced on treatment of the active oligosaccharide with exo-1, 3-β-D-glucosidase or endo-1,3-β-D-glucanase, it was further assumed that the oligosaccharide contained a main chain of (1→6)-linked β-D-glucopyranosyl residues, two of which were further linked through O-3. A polysaccharide that would give this type of oligosaccharide on acid hydrolysis had been isolated from yeast, which lent further support to this assumption (3).

Of the various alternative structures, we chose to prepare heptasaccharide 1 and octasaccharide 2. As these might have proved to be inactive, the syntheses were so designed that it should be possible to use the various intermediates in the synthesis of other, isomeric oligosaccharides. A preliminary report on the synthesis of 1 and 2 has been published (4).

EXPERIMENTAL

The syntheses are summarized in the scheme. For the following discussion the glycosyl residues in 1 and 2 are numbered from the reducing end as 1–5 (linear part), 3′ and 4′ (first branch), and 5′ (second branch).

Treatment of gentiobiose with benzaldehyde and zinc chloride yielded 1,2,3,5-di-O-benzylidene-6-O-(4,6-O-benzylidene-β-D-glucopyranosyl)-α-D-glucofuranose (4), a building block for glucosyl residues 1,2, and 3,4 in the target molecules. Substance 4 was obtained as a mixture of phenyl exo- and endo-isomers in the 1,2-O-benzylidene ring as evident from the signals in the 1H NMR spectrum of the benzylidene proton at δ 5.88 and 6.09, respectively (5). The configuration in the six-membered benzylidene rings was equatorial phenyl. Substance 4 was purified via its crystalline acetate and obtained as the endo-isomer. As reported under "Experimental," pure exo- and endo-isomers of 4 and of substances prepared from it could be obtained by crystallization or chromatography and were often used in the subsequent syntheses. Isomerization, however, occurred during hydrolysis under mild conditions and glycosidation reactions and all syrupy reaction products containing the 1,2-O-benzylidene ring were consequently exo-endo mixtures.

* This work was supported in part by the National Swedish Board for Technical Development and the Swedish Natural Science Research Council. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 Portions of this paper (including "Experimental," a scheme, and "Appendix") are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 84M-0443, cite the authors, and include a check or money order for $4.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
Partial acetylation of 4 yielded a mixture of 2- and 3-O-acetyl derivatives, which were separated by chromatography on silica gel. On treatment of 4 with 2,3,4,6-tetra-O-benzoyl-a-D-glucopyranosyl bromide (6), silver trifluoromethane sulfonate (silver triflate), and powdered 4 Å molecular sieves in dichloromethane, however, glucosidation of the 3-position was regiospecific, and protection of the 2-position was therefore not needed. The remaining free hydroxyl group was acetylated to yield the trisaccharide (7) which contains the glycosyl residues 1,2 and 3′ of heptasaccharide 1. Analogously, regiospecific glycosylation of 4 using hepta-O-benzoyl-a-laminarabiosyl bromide (3) followed by acetylation yielded the tetrascarharide derivative 5 containing glycosyl residues 1,2,3′ and 4′ of octasaccharide 2.

The trisaccharide derivative 7 was used for the synthesis of a tetrasaccharide derivative 9, containing glycosyl residues 3, 4, 5 and 5′ of the two target molecules. After acetylation, the 4,6-O-benzylidene group of 7 was selectively removed by treatment with dichloroacetic acid/aqueous acetic acid, giving 8. Glycosylation of 8 with 2,3,4,6-tetra-O-benzoyl-a-D-glucopyranosyl bromide, under the same conditions as those described above, was regioselective and, after acetylation, yielded essentially the 6-O-glycosyl derivative 9. The 1,2-O-benzylidene groups in 9 were hydrolysed off by treatment with 90% aqueous trifluoroacetic acid; the product was then benzoylated and reacted with hydrogen bromide in acetic acid/methylene chloride. The yields in previous and subsequent steps were good or moderate, but in this step the yield of the glycosyl derivative 10 was only 24% and it had to be purified by preparative TLC. The reason for the low yield is most probably that 1→4 linkages were cleaved during the reaction conditions. Other conditions for synthesis of 10 or the corresponding chloride, including hydrogen bromide in acetic acid or dichloromethane, trimethylsilyl bromide, neat, or in toluene and titanium tetrachloride or titanium tetra-bromide were tested with unsatisfactory results.

Selective hydrolysis of the 4,6-O-benzylidene group in 5 was performed as above. Monoglycosylation of the product 6, and also of the above trisaccharide 8 using glycosyl bromide 10, was regiospecific, giving reaction in the 6-position, exclusively. The protected hepta- (11) and octasaccharide (12) were deblocked by treatments first with 90% aqueous trifluoroacetic acid then with sodium methoxide in methanol, yielding heptasaccharide 1 and octasaccharide 2, respectively.

When 2,4,6-trimethylpyridine was used as an acid scavenger in the glycosylation step aiming at producing 5, an orthoester but no glycoside was formed. The orthoester could, however, be transformed into the tetrascarharide derivative 5 by treatment with trifluoromethylsulfonic acid in dry toluene.

All products gave 1H NMR (270 or 400 MHz) spectra in agreement with the postulated structures. For those with 4 or less sugar residues, there were only a few ambiguities in the assignments of signals to individual hydrogen atoms. For the hepta- and octasaccharide derivatives, most signals could also be assigned but there were several ambiguities. The free hepta- and octasaccharides were further reduced to the alditols and subjected to methylation analyses (7), which also were in agreement with the postulated structures.

As will be reported in the following publication (8), the synthetic heptasaccharide 1 and the elicitor-active heptasaccharide from the mycelium hydrolysate were chemically indistinguishable and had the same biological activity. The octasaccharide had about one-third of this activity.

Acknowledgment—We are indebted to the Department of Organic Chemistry at the University of Hamburg for the use of a 270-MHz NMR spectrometer.

REFERENCES
Synthesis of Phytoalexin Accumulation Elicitors

EXPERIMENTAL

General methods - All reactions were carried out under anhydrous conditions. Hydrobromic acid, triethylamine, and toluene were distilled from metallic sodium, benzyl alcohol from calcium hydride, and dry tetrahydrofuran from calcium hydride. The solvents were distilled from sodium in a nitrogen atmosphere and used without further purification. Acetonitrile was dried over calcium hydride and stored over 4Å molecular sieves. Dry tetrahydrofuran was used without further purification.

1. Synthesis of Phytalexin Accumulation Elicitors

2. Preparation of Phytalexin Accumulation Elicitors

3. Characterization of Phytalexin Accumulation Elicitors

4. Conclusion

5. References

SUPPORTING MATERIAL TO SYNTHESIS OF A GLYCEROLIPID AND A GLYCOSIDE THAT ELICIT PHOTOCLEAVABLE ACCUMULATION IN DIATOMS


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