Synthetic Studies on Rhynchosporoside and Related Substances†

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The stereoselective synthesis of the 1-O-α-D-glucopyranosides and 1-O-α-D-cellobiosides of 3-deoxy-2(R)- and 2(S)-glycerols to determine the complete stereochemistry of rhynchosporoside, which is a host selective phytotoxic from Rhynchosporium secalis, is described in detail.

Rhynchosporium secalis is the causal microorganism for scald disease in barley and other grasses.2) A phytotoxic compound, named rhynchosporoside, was isolated by Auriol et al.3) in 1978, and it was proposed that its structure was 1-O-α-D-glucopyranosyl-, 1-O-α-D-cellobiosyl- or 1-O-α-D-cellotriosyl-3-deoxy-glycerol (1) in 1980.4) Sugawara et al.5) reported the stereoselective synthesis of di- and triglycoside derivatives related to rhynchosporoside. Biological tests on barley (Hordeum vulgare) using the synthetic products revealed that the cellobiosyl and cellotriosyl-3-deoxy-2(R)-glycerols possessed stronger phytotoxicity than their 2(S) isomers.6) Recently, Nicolaou et al.7) also reported the synthesis of the tri-, tetra- and pentaglycosides of propanediol, of which the 2(R) glycoside retained biological activity against barley. We describe here the stereoselective synthesis of 1-O-α-D-glucopyranosyl- and 1-O-α-D-cellobiosyl-3-deoxy-2(R)- and 2(S)-glycerols (2, 3, 6, 7).

RESULTS AND DISCUSSION

For the unambiguous synthesis of 2, 3, 6,
and 7, chiral epoxides 4, 5, 8, and 9, shown in Scheme 1, were designed as versatile key intermediates. The ring opening reaction with nucleophiles (Nu~) should lead to the formation of rhynchosporoside and related substances. To obtain glucopyranosides (4, 5), methyl 2,3,4,6-tetra-O-benzyl-1-deoxy-1-thio-\beta\text{-}D-glucopyranoside (12) was prepared from pentaacetyl glucose in a 81.2% overall yield. The methythio group was easily converted into chloride via a hydroxyl group (13) with thionyl chloride and dimethylformamide8) in a quantitative yield. The glycosylation of 2,3-O-isopropylidene-sn-glycerol (15) with glucopyranosyl chloride (14) was performed in the presence of tetrabutylammonium chloride and diisopropylammonium9) to give the a-anomer (16), selectively. The signal for the anomic proton of the major product (48.8%, from 13) in the \textsuperscript{1}H-NMR spectrum was observed at δ 4.13 ppm (J = 4.8 Hz), supporting the \alpha-D-configuration at C-1 of 6. After deprotection of the isopropylidene group, the diol (17) was converted into 3-\textit{p}-toluenesulfonate (18) to be led
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to the 2-(R)-epoxide (4). The ring-opening reaction with lithium aluminum hydride followed by deprotection of the benzyl ether gave 2 in a 50.0% yield from 16. The diol (17) was also tosylated on C-2 to give the 2(S)-epoxide (5). The ring-opening reaction and debenzylation gave 3 in a 32.3% yield from 16. The structures of the α,β-configurations in 2 and 3 were well supported by both 1H- and 13C-NMR data such as δ 4.93 ppm (H-1') and 98.8 (C-1') for 2 and 4.94 (H-1') and 98.3 (C-1') for 3. These synthetic sequences of cellobiosides were also applied for the synthesis of cellobiosides 6 and 7. The protection of allyl β-D-glucopyranoside (23) with benzaldehyde dimethyl acetal and then benzyl bromide gave allyl 2,3-di-O-benzyl-4,6-benzylidene-β-D-glucopyranoside (25), which was further converted into allyl 2,3-di-O-benzyl-β-D-glucopyranoside (26) by partial deprotection in a 63.1% yield from 23. The selective alkylation10) of 26 with bis(tri-^-butyltin) oxide and benzyl bromide gave a single product in a high yield (97.6%). The 13C-NMR spectrum showed a deshielded signal at δ 10.3 ppm that was assigned to C-6', which supported the expected structure of allyl 2,3,6-tri-O-benzyl-β-D-glucoside (27). After acetylation on C-4' (28) and deallylation on C-1' (29), the hydroxyl group was converted to chloride (30) in a 79.1% yield from 27. The glycosylation of 15 with 30 was performed as described in the case of 14 to give the α-anomer (31) in a 68.1% yield. After deacetylation on C-4', the isopropylidene group of 31 was exchanged with a cyclohexylidene group (34) in a 63.3% yield from 31, because the isopropylidene group was not stable enough during the next acidic glycosylation. The glycosylation of 1,2-O-cyclohexylidene-3-O-(2,3,6-tri-O-benzyl-α-D-glucopyranosyl)-sn-glycerol (35) with tetraacetyl glucopyranosyl chloride was performed with silver triflate in the presence of activated molecular sieves to give the β-anomer (36) in a high yield (92.0%). The 13C-NMR spectrum showed the presence of two anomic carbons at δ 100.1 ppm and 97.4 that were assigned to βC-1'b and αC-1'a, respectively. After re-placement of the acetyl group by benzyl ether (38) and then deprotection of glycerol to give 3-O-{2,3,6-tri-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-α-D-glucopyranosyl}-sn-glycerol (39) in a 52.9% yield from 36, the diol (39) was subjected to selective tosylation on C-3 (40), which was, in turn, converted into the 2(R)-epoxide (8). The ring-opening reaction with lithium aluminum hydride and then deprotection of the benzyl ether gave 6 in a 60.8% yield from 39. The diol (39) was also converted into 2-O-tosylate (42) in order to provide the 2(S)-epoxide (9). Ring-opening, and then deprotection gave 7 in a 35.1% yield from 39. These structures were supported by the 13C-NMR spectra which showed signals at δ 102.8 ppm (βC-1'b) and 98.6 (αC-1'a) for 6, and 102.8 (βC-1'b) and 98.1 (αC-1'a) for 7, respectively.

Biological testing by means of the cut leaf assay2) showed the phytotoxicity of 6 at the concentration of 0.3 mM/liter against barley, however, the other compounds (2, 3, 7) did not show any activity at 3.0 mM/liter. This unambiguously showed that the configuration at C-2 of the 3-deoxyglycerol part of rhynchosporoside should be R rather than S. The biological significance of both the configuration at anomeric carbons and the number of glycosyl moieties will be discussed in a following paper.

EXPERIMENTAL

General. Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were determined with a Perkin-Elmer Model 241MC polarimeter, in chloroform, unless noted otherwise. Column chromatography was performed on columns of Silica Gel Merck (70 ~ 230 mesh; E. Merck, Darmstadt, Germany). Thin-layer chromatography (TLC) was performed on plates (layer thickness, 0.25 mm) precoated with Silica Gel 60 F254 (E. Merck). IR spectra were recorded with an EPI-G2 Hitachi spectrophotometer, using KBr pellets for crystalline samples and neat films for liquid samples. 1H-NMR spectra were recorded with a Varian EM-360, JEOL JNM-FX90Q or FX400 NMR spectrometer, using either tetramethylsilane in chloroform-d or sodium 3-(trimethylsilyl)propionate-d4 in deuterated water as an internal standard. 13C-NMR spectra were recorded with a JNM-FX100 FT NMR
2,3,4,6-Tetra-O-benzyl-D-glucopyranosyl chloride (14). To a solution of 13 (2.4 g) in dichloroethane (20 ml) and dimethylformamide (0.1 ml) was added dropwise thionyl chloride (0.66 ml) at 0°C. The mixture was stirred for 16 hr at 20°C and then filtered through silica gel (5 g). The filtrate was evaporated in vacuo to give a quantitative yield of 14, Rf 0.44 in 19:1 toluene-ethyl acetate.

1,2-O-Isopropylidene-3-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-sn-glycerol (16). A mixture of 14 (4.6 g), 15 (1.1 g), tetrabutylammonium chloride (2.3 g) and N,N-diisopropylethylamine (1.2 g) in dichloroethane (50 ml) was stirred for 16 hr at 85~95°C under argon. The reaction mixture was cooled to 20°C and then diluted with dichloromethane (30 ml). The organic layer was washed with water, dried over anhydrous magnesium sulfate and then evaporated in vacuo. The residue was crystallized from diisopropyl ether to give 16 (2.7 g, 48.8%), Rf 0.24 in 9:1 toluene-ethyl acetate, [α]D +37.8° (c 0.19). NMR data (CDCl3): 1H, δ 1.44 (3H, s, CCH3), 1.38 (3H, s, CCH3), 1.01 (3H, s, CCH3).

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NMR spectrometer operated at 25.0 MHz. The δ values are expressed in ppm as to the standard.

Methyl 2,3,4,6-tetra-O-acetyl-1-deoxy-1-thio-β-D-glucopyranoside (10). To a solution of 2,3,4,6-tetra-O-acetyl glucopyranosyl acetate (160 g) and tri-n-butylmethylsulfide (145 g) in dichloromethane (11) was added dropwise tin(IV) chloride (27.4 g) at 0~5°C with stirring, followed by stirring for 16 hr at 20°C. The reaction mixture was poured into ice-cold aqueous sodium bicarbonate and then filtered through celite. The filtrate was washed with saturated saline, dried over anhydrous magnesium sulfate and then evaporated in vacuo. The residual oil was crystallized from diisopropyl ether to give 10 (140 g, 90%), mp 95.0~95.5°C, Rf 0.54 in 3:1 toluene-ethyl acetate, [α]D -13.6° (c 0.77). NMR data (CDCl3): 1H, δ 2.16 (3H, s, SCH3), 2.09 (3H, s, COCH3), 2.08 (3H, s, COCH3), 2.03 (3H, s, CCH3), 2.01 (3H, s, CCH3).

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2,3,4,6-Tetra-O-benzyl-α-D-glucopyranosyl chloride (14). A mixture of 13 (2.4 g) in dichloroethane (20 ml) and dimethylformamide (0.1 ml) was added dropwise thionyl chloride (0.66 ml) at 0°C. The mixture was stirred for 16 hr at 20°C and then filtered through silica gel (5 g). The filtrate was evaporated in vacuo to give a quantitative yield of 14, Rf 0.44 in 19:1 toluene-ethyl acetate.
pyranosyl)-sn-glycerol (5). To a solution of 20 (1.34 g) in 2,3-Anhydro-1-O-(2,3,4,6-tetra-O-benzyl-a-D-glucopyranosyl)-sn-glycerol (19). To a solution of 17 (2.0 g) in 45.37; H, 7.62.

Anal. Found: C, 74.22; H, 7.07.

1-O-(a-D-Glucopyranosyl)-3-deoxy-2(R)-deoxyglycerol (2). A mixture of 21 (600 mg) and 10% palladium on active carbon (300 mg) in ethanol (10 ml) was stirred for 2 days at 20°C under hydrogen. A quantitative yield of 2 was obtained after chromatography on Sephadex LH-20 (50 g) in methanol, \( R_f \) 0.16 in 4:1 chloroform-methanol, \([\delta]_D +91.9^\circ \) (c 0.19, methanol). NMR data (DCl_3): \( \delta \) 55.50 (1H, s, CHPh).

Anal. Found: C, 62.32; H, 6.54.

1-O-Benzoyl-3-O-(2,3,4,6-tetra-O-benzyl-a-D-glucopyranosyl)-sn-glycerol (19). To a solution of 17 (2.0 g) in pyridine (10 ml) was stirred for 12 hr at 20°C under reflux. Processing and chromatography on silica gel (30 g) in 2:1 toluene-ethyl acetate afforded 21 (560 mg, 97.0%), \( R_f \) 0.46 in 2:1 toluene-ethyl acetate, \([\delta]_D +27.9^\circ \) (c 0.27).

NMR data (CDCl_3): \( \delta \) 5.51 (1H, m, H-1), 1.18 (3H, d, \( J=6.8^\circ \), CHCH_3).

13C, \( \delta \) 59.82 (C-1', \( 1J_{CH}=169.7Hz \)), 73.39 (C-3'), 73.22 (C-1), 72.11 (C-5'), 71.81 (C-2'), 69.94 (C-4'), 66.90 (C-2), 60.87 (C-6'), 18.40 (C-3).


1-O-Benzoyl-3-O-(2,3,4,6-tetra-O-benzyl-a-D-glucopyranosyl)-sn-glycerol (20). A solution of 19 (2.2 g, 95.4%), \( R_f \) 0.38 in 5:1 toluene-ethyl acetate, \([\delta]_D +26.7^\circ \) (c 0.23). NMR data (CDCl_3): \( \delta \) 5.82 (1H, d, \( J=8.0^\circ \), CHCH_3).

Anal. Found: C, 74.87; H, 6.76. Calcd. for C_37H_40O_7: C, 74.05; H, 7.06.

1-O-(2,3,4,6-Tetra-O-benzyl-a-D-glucopyranosyl)-3-deoxy-2(R)-deoxyglycerol (3). A mixture of 22 (632 mg) and 10% palladium on active carbon (300 mg) in ethanol (10 ml) was stirred for 2 days under hydrogen and then processed as described for 21 to give 3 (152 mg, 60%) after chromatography on Sephadex LH-20 (50 g) in methanol, \( R_f \) 0.16 in 4:1 chloroform-methanol, \([\delta]_D +84.2^\circ \) (c 0.19, methanol). NMR data (DCl_3): \( \delta \) 5.94 (1H, d, \( J=3.9^\circ \), CH-1), 1.21 (3H, d, \( J=6.3^\circ \), \( CH_3 \)).

13C, \( \delta \) 59.82 (C-1', \( 1J_{CH}=169.7Hz \)), 73.38 (C-3'), 72.65 (C-1'), 72.07 (C-2'), 71.73 (C-5'), 69.89 (C-4'), 66.51 (C-2), 60.86 (C-6'), 18.41 (C-3).


Ally 1,4,6-0-benzylidene-β-D-glucoanoside (24). A mixture of 23 (144.6 g), benzaldehyde dimethyl acetal (107.0 g) and p-toluenesulfonic acid (1.0 g) in dimethylformamide (500 ml) was stirred for 2 hr at 60°C under reduced pressure (20 mmHg) in order to remove methanol. The reaction mixture was neutralized with triethylamine and then evaporated in vacuo. The residue was poured into saturated sodium bicarbonate and then extracted with chloroform. The organic layer was washed with water, dried over magnesium sulfate and then evaporated to dryness. A saturated solution of sodium methoxide in methanol (20 ml) was added to the mixture under nitrogen followed by stirring for 2 hr. Processing and chromatography on silica gel (60 g) in 9:1 toluene-ethyl acetate afforded 20 (0.89 g, 97.4%), \( R_f \) 0.38 in 5:1 toluene-ethyl acetate, \([\delta]_D +27.9^\circ \) (c 0.22).

NMR data (CDCl_3): \( \delta \) 5.60 (1H, m, H-1), 1.20 (3H, d, \( J=6.7^\circ \), \( CH_3 \)).

NMR data (CDCl_3): \( \delta \) 5.51 (1H, m, H-1), 1.18 (3H, d, \( J=6.8^\circ \), CHCH_3).

13C, \( \delta \) 59.82 (C-1', \( 1J_{CH}=169.7Hz \)), 73.38 (C-3'), 72.65 (C-1'), 72.07 (C-2'), 71.73 (C-5'), 69.89 (C-4'), 66.51 (C-2), 60.86 (C-6'), 18.41 (C-3).

Anal. Found: C, 74.52; H, 6.76. Calcd. for C_37H_40O_7: C, 74.52; H, 6.76.
isopropyl ether, Rf 0.56 in chloroform, $[\alpha]_D^0 = -34.6^\circ$ (c 0.28). NMR data (CDCl$_3$): $^1$H, $\delta$ 5.56 (1H, s, CH$_2$Ph).

Anal. Found: C, 73.97; H, 6.60. Calcd. for C$_{30}$H$_{32}$O$_6$: C, 73.75; H, 6.60.

 Allyl 2,3-di-O-benzyl-\beta-D-glucopyranoside (26). A solution of 25 (100 g) in acetic acid (500 ml) was stirred for 6 hr at 100°C and then evaporated in vacuo to give a syrup which was diluted with chloroform. The organic layer was washed with aqueous sodium bicarbonate, dried over magnesium sulfate and then evaporated in vacuo. The residue was crystallized from ethyl acetate–petroleum ether to give 26 (80 g, 97.6%), m.p 63–64°C, Rf 0.22 in 1:1 toluene–ethyl acetate, $[\alpha]_D$ +10.8° (c 0.19). NMR data (CDCl$_3$): $^1$H, $\delta$ 5.56 (1H, s, CH$_3$CO).

Anal. Found: C, 73.19; H, 6.94. Calcd. for C$_{30}$H$_{34}$O$_6$: C, 74.08 (C-5), 73.59 (CH$_2$Ph), 71.44 (C-4), 70.28 (C-6 and C-3), 84.02 (C-2), 83.9 (C-3), 81.68 (C-2), 75.20 (CH$_2$Ph), 74.76 (CH$_2$Ph), 74.58 (CH$_2$Ph, 74.08 (C-5), 73.59 (CH$_2$Ph), 71.44 (C-4), 70.28 (C-6 and OCH$_2$CH$_2$).

Anal. Found: C, 68.83; H, 6.88. Calcd. for C$_{25}$H$_{26}$O$_6$: C, 68.98; H, 7.05.

 Allyl 2,3,6-tri-O-benzyl-\beta-D-glucopyranoside (27). A mixture of 26 (16.0 g) and bis(tri-n-butyl)amine (18.0 g) in toluene (300 ml) was stirred for 16 hr under reflux with continuous azotropic removal of water and then concentrated to a half volume. After cooling to 20°C, tetra-n-butylammonium bromide (2.0 g) and benzyl bromide (7.2 ml) were added. The mixture was stirred for 16 hr at 100°C and then evaporated in vacuo. The residue diluted with water, dried on anhydrous magnesium sulfate and then evaporated in vacuo. The solid was chromatographed on silica gel (800 g) in 10:1 toluene–ethyl acetate to give 27 (19.0 g, 97.0%), crystals from petroleum ether, mp 36–37°C, Rf 0.33 in 10:1 toluene–ethyl acetate, $[\alpha]_D$ -24.2° (c 0.26). NMR data (CDCl$_3$): 2H, $\delta$ 1.86 (3H, s, CH$_3$CO).

Anal. Found: C, 69.29; H, 6.98. Calcd. for C$_{35}$H$_{42}$O$_9$: C, 69.10; H, 6.94. Calcd. for C$_{33}$H$_{40}$O$_{8}$: C, 70.19; H, 6.56. Calcd. for C$_{35}$H$_{42}$O$_{9}$: C, 70.19; H, 6.56.

4-O-Acetyl-2,3,6-tri-O-benzyl-D-glucopyranose (28). A solution of 27 (7.4 g), palladium(II) chloride (1.7 g) and sodium acetate (1.8 g) in acetic acid (57 ml) with water (3 ml) was stirred for 14 hr at 60°C and then filtered. The filtrate was diluted with chloroform, washed with aqueous sodium bicarbonate and water and then dried over anhydrous magnesium sulfate. The organic layer was evaporated in vacuo to give crystalline 29 (4.2 g, 84.2%) from diisopropyl ether, mp 111–112°C, Rf 0.24, $[\alpha]_D$ +9.4° (c 0.26). NMR data (CDCl$_3$): $^1$H, $\delta$ 1.86 (3H, s, CH$_3$CO); $^1$C, 97.29 (C-1β, $^{13}$J$_{CH}$ = 165.8 Hz) and 91.03 (C-1α, $^{13}$J$_{CH}$ = 165.8 Hz) in a ratio of 1:3, 20.77 (COCH$_3$).

Anal. Found: C, 70.48; H, 6.65. Calcd. for C$_{29}$H$_{26}$O$_7$: C, 70.71; H, 6.55.

4-O-Acetyl-2,3,6-tri-O-benzyl-\alpha-D-glucopyranosyl chloride (30). A solution of 29 (3.8 g) and thionyl chloride (2.0 ml) in dichloroethane (60 ml) containing dimethylformamide (0.14 ml) was stirred for 16 hr at 20°C and then filtered through silica gel (14 g). The filtrate was evaporated in vacuo to give an oil, 30 (3.8 g, quantitative yield), which was used directly for the next glycosylation, Rf 0.38 in 5:1 toluene–ethyl acetate.

3-O-(4-O-Acetyl-2,3,6-tri-O-benzyl-\alpha-D-glucopyranosyl)-1,2-O-isopropylidene-sn-glycerol (31). A mixture of 30 (3.8 g), tetrabutylammonium chloride (4.6 g), 15 (2.2 g) and N,N-diisopropylethylamine (2.2 g) in dichloroethane (50 ml) was stirred for 16 hr at 80–90°C under argon. Processing and chromatography on silica gel (300 g) in 5:1 toluene–ethyl acetate afforded 31 (3.1 g, 68.1%), Rf 0.28 in 5:1 toluene–ethyl acetate, $[\alpha]_D$ +25.3° (c 0.32). NMR data (CDCl$_3$): $^1$H, $\delta$ 1.86 (3H, s, CH$_3$CH$_2$), 1.37 and 1.43 (6H, s, 2 × CH$_3$).

Anal. Found: C, 69.10; H, 6.94. Calcd. for C$_{25}$H$_{26}$O$_6$: C, 69.29; H, 6.98.

1,2-O-Isopropylidene-3-O-(2,3,6-tri-O-benzyl-\alpha-D-glucopyranosyl)-sn-glycerol (32). A solution of 31 (1.55 g) was deacetylated as described for 10. Chromatography of the product on silica gel (100 g) in 3:1 toluene–ethyl acetate afforded 32 (1.42 g, 98.4%), Rf 0.50 in 5:1 toluene–ethyl acetate, $[\alpha]_D$ +29.7° (c 0.46). NMR data (CDCl$_3$): $^1$H, $\delta$ 1.40 (3H, s, CH$_2$CH$_3$), 1.34 (3H, s, CHCH$_3$); $^1$C, $\delta$ 109.46 (CM$_2$), 97.41 (C-1′, $^{13}$J$_{CH}$ = 165.8 Hz), 26.79 (CH$_3$), 25.51 (CH$_3$).


3-O-(4-O-Acetyl-2,3,6-tri-O-benzyl-\alpha-D-glucopyranosyl)-sn-glycerol (33). A solution of 31 (8.8 g) and camphorsulfonic acid (100 mg) in methanol (10 ml) was stirred for 1 day at 20°C and then neutralized with triethylamine (2 ml). Evaporation in vacuo and chromatography on silica gel (500 g) in 2:1 toluene–ethyl acetate afforded 33 (5.4 g, 65.5%), Rf 0.14 in 2:1 toluene–ethyl acetate, $[\alpha]_D$ +23.5° (c 0.22). NMR data (CDCl$_3$): $^1$H, $\delta$ 1.86 (3H, s, COCH$_3$).

Anal. Found: C, 67.44; H, 6.85. Calcd. for C$_{35}$H$_{38}$O$_{9}$: C,
3-O-[4-O-Acetyl-2,3,6-tri-O-benzyl-α-D-glucopyranosyl]-1,2-O-cyclohexyli dine-sn-glycerol (34). A mixture of 33 (5.3 g), 1,1-dimethoxycyclohexane (2.9 g) and p-toluenesulfonic acid (100 mg) in dimethylformamide (50 ml) was stirred for 2 hr at 40°C under reduced pressure (20 mmHg). After neutralization with triethanolamine (2 ml), the mixture was evaporated in vacuo, and then the residue was chromatographed on silica gel (500 g) in 2:1 toluene-ethyl acetate to give 34 (6.0 g, 98.3%), Rf 0.68 in 2:1 toluene-ethyl acetate, [\(\delta_{D}\) +34.8° (c 0.31). NMR data (CDCl3): \(\delta_{H}\), 5.182 (3H, s, COCH3), 1.8-1.2 (10H, m, cyclohexyl). Anal. Found: C, 71.03; H, 6.40; S, 2.73. Calcd. for C64H70O13: C, 70.98; H, 6.38; S, 2.69.

1,2-O-Cyclohexyli dene-3-O-[2,3,6-tri-O-benzyl-α-D-glucopyranosyl]-α-D-glucopyranosyl-sn-glycerol (35). Deacetylation of 34 (5.9 g) was performed as described for 31 to give 35 (5.6 g, quantitative yield), Rf 0.20 in 5:1 toluene-ethyl acetate, [\(\delta_{D}\) +34.8° (0.31). NMR data (CDCl3): \(\delta_{H}\), 4.740-7.7 (15H, m, aromatic), 1.8-1.2 (10H, m, cyclohexyl). Anal. Found: C, 71.29; H, 7.34. Calcd. for C38H46O9: C, 70.57; H, 7.17.

1,2-O-Cyclohexylidene-3-O-[2,3,6-tri-O-benzyl-α-D-glucopyranosyl]-α-D-glucopyranosyl-sn-glycerol (36). To the mixture of activated molecular sieves 4A (0.6 g) and silver trifluoromethanesulfonate (0.2 g) in dichloroethane (5 ml), a solution of 35 (0.24 g) in dichloroethane (2 ml) was added, and then the mixture was stirred for 1 hr at 20°C. After stirring for 2 hr, a solution of silver trifluoromethanesulfonate (0.1 g) in toluene (1 ml) was added, and then the mixture was stirred for 1 hr at 20°C. The reaction mixture was filtered through celite, and then the filtrate was washed with aqueous sodium bicarbonate and water, dried over anhydrous magnesium sulfate and then evaporated in vacuo. The residue was chromatographed on silica gel (60 g) in 2:1 dichloromethane-acetone to give 36 (0.34 g, 92.5%), Rf 0.40 in 2:1 toluene-ethyl acetate, [\(\delta_{D}\) +15.7° (c 0.33). NMR data (CDCl3): \(\delta_{H}\), 5.200 (3H, s, COCH3), 1.98 (3H, s, COCH3), 1.93 (6H, s, 2xCOCH3), 1.8-1.2 (10H, m, cyclohexyl); \(\delta_{C}\), 110.12 (C-1'b, \(1\delta_{C-CH}\) = 164.8 Hz), 79.43 (C-1'a, \(1\delta_{C-CH}\) = 166.0 Hz).

Anal. Found: C, 64.18; H, 6.72. Calcd. for C50H52O15: C, 64.23; H, 6.69.

1,2-O-Cyclohexyli dene-3-O-[2,3,6-tri-O-benzyl-4-O-(β-D-glucopyranosyl)-β-D-glucopyranosyl]-α-D-glucopyranosyl-sn-glycerol (37). Deacetylation of 36 (0.29 g) was performed as described for 34, and chromatography on silica gel (30 g) in 9:1 chloroform-methanol gave 37 (0.22 g, 93.5%), Rf 0.40 in 9:1 chloroform-methanol, [\(\delta_{D}\) +51.7° (c 0.29).


1,2-O-Cyclohexylidene-3-O-[2,3,6-tri-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-α-D-glucopyranosyl]-sn-glycerol (38). 37 (380 mg) was benzylated with sodium hydride (370 mg, 50% in mineral oil) and benzyl bromide (1.0 g) in dimethylformamide (20 ml) as described for 11. Chromatography on silica gel (3 g) in 9:1 toluene-ethyl acetate afforded 38 (365 mg, 64.9%), Rf 0.45 in 5:1 toluene-ethyl acetate, [\(\delta_{D}\) +37.3° (c 0.22). NMR data (CDCl3): \(\delta_{H}\), 7.4-7.0 (35H, m, aromatic), 1.85-1.20 (10H, m, cyclohexyl). Anal. Found: C, 74.59; H, 6.99. Calcd. for C70H78O13: C, 74.71; H, 6.81.

1,2-O-Cyclohexyli dene-3-O-[2,3,6-tri-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-α-D-glucopyranosyl]-sn-glycerol (39). A solution of 38 (1.14 g) in methanol (100 ml) containing p-toluenesulfonic acid (20 mg) was stirred for 1 hr at 20°C and then neutralized with triethylamine (0.5 ml). Evaporation in vacuo and chromatography on silica gel (100 g) in 2:1 toluene-ethyl acetate gave 39 (922 mg, 87.2%), mp 110-114°C (diisopropyl ether), Rf 0.24 in 2:1 toluene-ethyl acetate, [\(\delta_{D}\) +28.7° (c 0.13). NMR data (CDCl3): \(\delta_{H}\), 7.4-7.0 (35H, m, aromatic).

Anal. Found: C, 73.20; H, 6.72. Calcd. for C64H58O13: C, 73.40; H, 6.74.

1-O-p-Toluenesulfonyl-3-O-[2,3,6-tri-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-α-D-glucopyranosyl]-sn-glycerol (40). A mixture of 39 (295 mg), p-toluenesulfonyl chloride (60 mg) and 4-dimethylaminopyridine (10 mg) in pyridine (5 ml) was stirred for 1 hr at 0-5°C and then poured into ice-cold aqueous sodium bicarbonate. Processing and chromatography on silica gel (30 g) in 1:1 toluene-ethyl acetate afforded 40 (243 mg, 72.2%), Rf 0.24 in 5:1 toluene-ethyl acetate, [\(\delta_{D}\) +28.7° (c 0.23). NMR data (CDCl3): \(\delta_{H}\), 5.2.40 (3H, s, CH3).

Anal. Found: C, 71.03; H, 6.40; S, 2.73. Calcd. for C71H72O13S: C, 70.98; H, 6.38; S, 2.69.

1,2-Anhydro-3-O-[2,3,6-tri-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]-sn-glycerol (8). To a solution of 40 (204 mg) in methanol (2 ml) was added 0.5 N sodium methoxide in methanol (0.07 ml) at 0°C, and then the mixture was stirred for 4 hr at 0-5°C. After neutralization with acetic acid, processing and chromatography on silica gel (25 g) in 5:1 toluene-ethyl acetate gave 8 (150 mg, 85.0%), Rf 0.36 in 5:1 toluene-ethyl acetate, [\(\delta_{D}\) +26.4° (c 0.13). NMR data (CDCl3): \(\delta_{H}\), 5.2.9-2.5 (2H, m, H-1 and H-1b).

Anal. Found: C, 74.79; H, 6.65. Calcd. for C64H58O12: C, 74.69; H, 6.66.

1-O-[2,3,6-Tri-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-α-D-glucopyranosyl]-3-deoxy-2(R)-
glycerol (43). A solution of 8 (220 mg) in tetrahydrofuran (3 ml) was treated with lithium aluminum hydride (100 mg) in tetrahydrofuran (3 ml) as described for 4 or 5 to give 43 (215 mg, 99.9%) after chromatography on silica gel (10 g) in toluene-ethanol acetate, Rf 0.50 in 2:1 toluene-ethanol acetate, [α]D +27.7° (c 0.21). NMR data (CDCl3): 1H, δ 0.13 (3H, d, J = 6.9 Hz, CH2CH3).

Anal. Found: C, 71.49; H, 6.11. Calcd. for C78H80O16S:

C, 71.78; H, 6.09. CH2CH3).

Anal. Found: C, 75.07; H, 6.72. Calcd. for C64H68O12: C, 74.69; H, 6.66.

1-O-[2,3,6-Tri-O-benzyl-4-O-(2,3,4,6-tetra-O-benzylβ-D-glucopyranosyl)-β-D-glucopyranosyl]-3-deoxy-2(S)-glycerol (44). A solution of 9 (120 mg) in tetrahydrofuran (3 ml) was treated with lithium aluminum hydride (100 mg) as described for 18 or 21 to give 44 (86.1 mg, 70.8%) after chromatography on silica gel (10 g) in 2:1 toluene-ethanol acetate, Rf 0.50 in 2:1 toluene-ethanol acetate, [α]D +26.7° (c 0.21). NMR data (CDCl3): 1H, δ 1.14 (3H, d, J = 8.0 Hz, CH2CH3).

Anal. Found: C, 74.14; H, 6.89. Calcd. for C64H68O12: C, 74.54; H, 6.84.

Bioassay tests. Ten day old barley seedlings (second leaf stage) were cut at 1 ~2 cm above the ground, and then each cutting was placed upright in 1 ml of 10~3 ~10~4M/liter of each sample solution in a test tube. The cuttings were kept at 23°C for 2 days in a growth chamber. The tested Atlas (susceptible) leaves with 3 x 10~3 M/liter of 6 developed chlorosis and the other compounds were not toxic at 3 x 10~3 M/liter. The control and Atlas 46 (resistant) leaves remained unaffected.

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