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A REASSESSMENT OF THE PHYTOTOXIN, RHYNCHOSPOROSIDE: ATTACHMENT OF THE AGLYCON

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ABSTRACT

The aglycon of rhynchosporoside has been established as 1,2-propanediol, and its site of attachment has been reassigned from O-2 to O-1 of the aglycon. Syntheses of the corresponding cellobiosides, previously suggested to be the phytotoxic compounds, are reported.

DISCUSSION

The causal agent of scald disease in barley, *Rhynchosporium secalis*[1], produces a phytotoxin trivially named rhynchosporoside[2]. Originally proposed to be 1-hydroxy-2-propyl α-cellobioside (**1**), an enzyme-mediated synthesis was performed to prepare this phytotoxin[3]. We report here a total synthesis of this product (Scheme 1).

Hepta-*O*-acetylcellobiosyl bromide[4] (**2**) was treated with sodium benzenethioxide[5] to yield phenyl 1-thio-β-cellobiose heptaacetate[3], which was deacetylated with liquid ammonia in methanol[5] and the hydroxyl groups were reprotected by benzylation to yield **4**. Mercuric sulfate-mediated displacement of the phenylthio group by an appropriate alcohol would provide the requisite benzylated precursor.

The appropriately protected alcohol **5** was prepared from allyl alcohol by first benzylating the exposed hydroxyl group and then oxymercurating the alkene. Compounds **4** and **5** reacted to give the highly benzylated product **6**. Hydrogenolysis of the benzyl groups afforded the same product as previously obtained by the enzyme-mediated reaction, confirming the identity of this product.

Relative t.l.c. mobilities, together with biological activity of material from both sources that was considerably lower than that of the natural product, suggested that we had not, in fact, prepared the most active substance. The difference was at-

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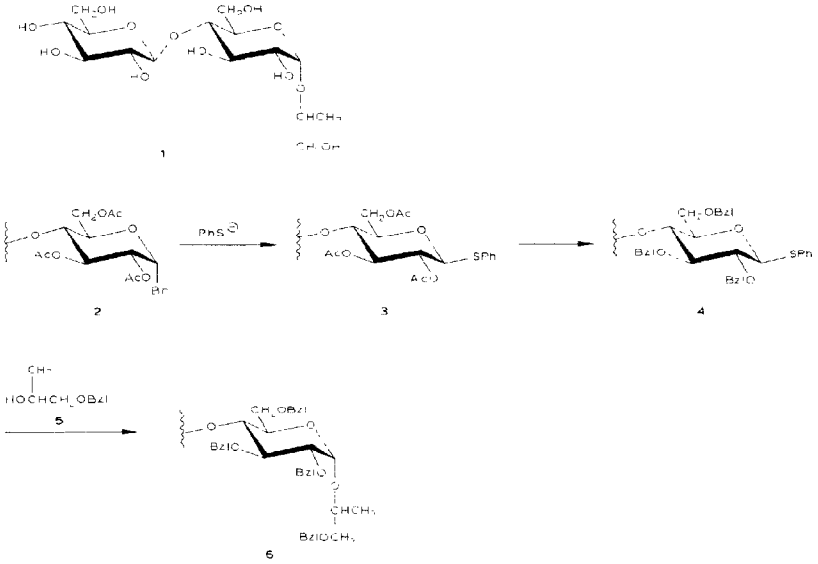


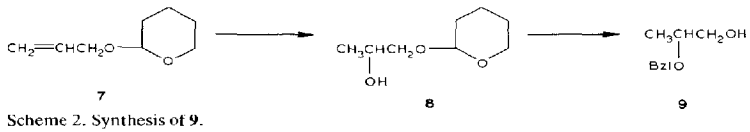
Scheme 1. Synthesis of **6**.

tributed to erroneous original assignment of the attachment of the aglycon. Hydrolysis of rhynchosporoside gave 1,2-propandiol, but it might have been attached through O-1 rather than O-2. This would not have been noted in the enzyme-mediated reaction, as we had prepared the aglycon already attached to glucose[3]. The enzyme was utilized only to attach a second D-glucosyl group to O-4 of the preformed glucosyl derivative. An unambiguous assignment of the attachment was required.

ATTACHMENT OF AGLYCON

The synthesis described in Scheme 1 gave one of the benzylated hydrolysis products (**5**). Unambiguous preparation of the other benzylated isomer (Scheme 2) and comparison of these two derivatives to the hydrolyzate of the natural product was required.

Again starting with allyl alcohol, the hydroxyl group was protected as the tetrahydropyran-2-yl derivative **7**, which was subjected in turn to oxymercuration–demercuration to yield **8**. Benzylation of the free hydroxyl group, followed by selective removal of the protecting group[6] gave the other isomer (**9**). The two, isomeric benzylated propanediol derivatives had clearly distinguishable retention times in g.l.c. (SE-30).

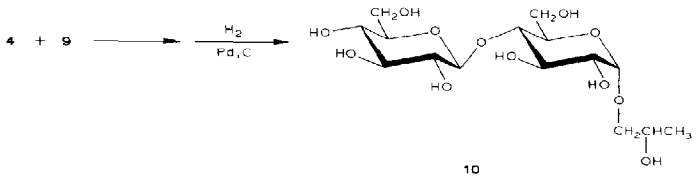


7 8 9

Scheme 2. Synthesis of **9**.

A sample of natural rhynchosporoside was exhaustively benzylated, and the aglycon released by acid hydrolysis subjected to g.l.c. analysis; only one product was observed, having the same retention-time as **9**. This result unequivocally confirmed that attachment of the aglycon had been previously misassigned, and that it was actually at O-1.

Synthesis of the actual natural product was then reexamined, by the general methodology utilized for preparation of **1**. Compound **10** thus prepared had the same R_f value as an authentic sample[7].



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BIOLOGICAL TESTING

Samples of **1** and **10** were tested on cuttings of barley (cultivar var. Hannchen C.I. 531). Each elicited symptoms characteristic of the natural rhynchosporosides; but of considerably diminished intensity as compared with the natural toxins. Part of this difference in activity certainly derives from the configurational inhomogeneity of the aglycon, as demonstrated by utilizing the *R*- and *S*-aglycon-substituted cellobiosides[7] in the same bioassay, and observing that the *R* isomer is much more active than the *S*-.

However, it is considered that the most important factor in the decreased activity resides in further differences from the natural phytotoxin. Both the D-glucosyl and cellobiosyl components possess phytotoxic activity, and there is also evidence for a cellotriosyl derivative (Fig. 1). As hydrolysis of the aglycon utilized the unpurified toxic fraction, it is most reasonable to suggest that there exists a homologous series of D-glucose oligomers, each having the 1,2-propanediol aglycon. It is possible that these act synergistically to produce the observed toxic symptoms. Alternatively, one of the higher oligomers may be a more important contributor to the biological activity; there is no evidence as yet to differentiate these hypotheses.

As the 2-hydroxypropyl α-D-glucoside, α-cellobioside, and probably the α-

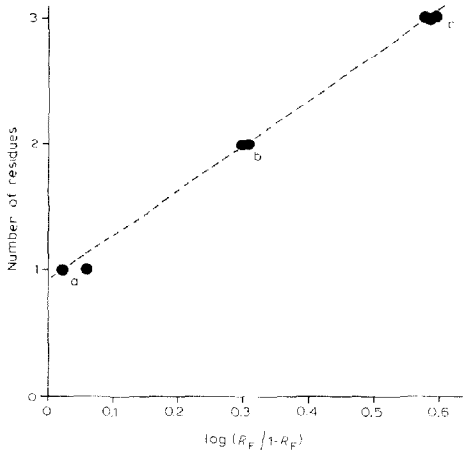

Fig. 1. Plot of number of residues *vs* R_F value a, α-D-Glucoside; b, α-cellobioside; c, the natural toxin.

cellotrioside are found in *R. secalis*, it seems appropriate to name them according to the degree of polymerization of the (1→4)-linked glycosyl residues. Accordingly, we suggest the names, 1-, 2-, and 3-rhynchosporoside.

EXPERIMENTAL

General methods. — Chromatographic solvent systems used were: *A* 84:16 carbon tetrachloride–ethyl acetate, *B* 99.3:0.7 chloroform–ethanol, *C* 4:1 carbon tetrachloride–ethyl acetate, *D* 61:13:26 ethyl acetate–water–methanol, *E* 81:13:6 butanone–ethanol–water, and *F* 89:11 carbon tetrachloride–ethyl acetate. Organic solutions were dried with anhydrous magnesium sulfate and/or potassium carbonate, and evaporated in a rotary evaporator.

Preparation of phenyl 1-thio-β-cellobioside heptaacetate (**3**). — Tetrahydrofuran (50 mL), and a solution of sodium hydride (1.92 g, 0.04 mol), and a 10% excess of thiophenol (4.41 g, 0.04 mol) were added slowly to a solution of **2** in chloroform. The mixture was warmed for 30 min at 45° and then filtered and washed three times with saturated aqueous sodium hydrogencarbonate. The chloroform layer was washed with water and dried over anhydrous calcium chloride. The chloroform was evaporated to yield the crude, crystalline product (33.5 g), which could be purified by the method of Purves[5], it had R_F 0.23 (t.l.c., *B*). This product was used directly in the next step.

Phenyl hepta-O-benzyl-1-thio-β-cellobioside (**4**). — A solution of the preceding product in 5 times its weight of ammonia-saturated methanol was stirred over-

night at room temperature and then evaporated. The residue was extracted with warm, dry ether (to remove acetamide). The product (13.9 g, 89%) had R_F 0.3 (t.l.c., *E*).

The phenyl 1-thio-β-cellobioside (1.1 g, 0.03 mol) and dry 1,4-dioxane (50 mL) were placed in a 250-mL, 3-necked flask, equipped with a reflux condenser, mechanical stirrer, and a dropping funnel. Sodium hydride (7.3 g, 0.31 mol) was added and the stirred mixture was boiled for 15 min while benzyl chloride (38.7 g, 0.306 mol) was added dropwise. Boiling was continued for 3 h, and then the cooled mixture was poured onto ice (200 mL), and ethanol (130 mL) was added. Upon stirring, a solid separated, and water (100 mL) was added. The product had R_F 0.75 (t.l.c., *C*). Recrystallization from ethanol gave pure **4** (7.5 g, 23%), m.p. 152°; ν_{max} 3440, 3050, 2880, 1463, 1362, 1220, 1072, 915, 740, and 698 cm^{-1}; n.m.r. δ 3.2–4.2 (envelope, 13 H), 4.3–5.1 (envelope, 14.8 H), 5.27 (0.33 H), and 7.3 (m, 40 H).

Anal. Calc. for $C_{67}H_{68}O_{10}S$: C, 75.54; H, 6.43; S, 3.01. Found: C, 75.49; H, 6.57; S, 3.12.

Preparation of 1-benzyloxy-2-propanol (**5**). — Allyl benzyl ether (10 g, 68 mmol) was added to a stirred mixture of mercuric acetate (21.0 g, 68 mmol) in 1:1 water–THF (80 mL), at room temperature[8]. After the yellow color had disappeared, the mixture was stirred for 15 min, and then 3M potassium hydroxide (67.5 mL) was added, followed by a solution of sodium borohydride (1.28 g) in 3M potassium hydroxide (67.5 mL). Mercury was allowed to precipitate and a solution of saturated sodium chloride (100 mL) was added. The solution was filtered through Celite, extracted with dichloromethane, and dried. The product was monitored on a column (3.05 m × 0.64 cm) of 15% Carbowax (on Chromosorb G, base-washed). At the oven temperature of 195°, and a flow-rate of 68 mL/min, the retention time was 36.7 min. The crude mixture was filtered, concentrated, and resolved on a low-pressure liquid-chromatographic system utilizing a 100 × 2.5-cm column, packed with silica gel (60–200 mesh), and solvent system *A* R_F 0.21. The void volume of the column was 325 mL. After 300 mL of effluent had passed, 19.6-mL fractions were collected. Tubes 23–68 contained the derivative (7.0 g, 62% yield); ν_{max} 3420, 2845, 1465, 1105, 745, and 705 cm^{-1}; n.m.r.: δ 1.0–1.26 (d, 3 H), 3.03–3.5 (m, 2 H), 3.63–4.2 (m, 10 H), 4.47 (s, 2 H), 7.27 (s, 5 H); *m/z* 166 (M^+).

Anal. Calc. for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49. Found: C, 72.00; H, 8.45.

Preparation of compound **1**. — Mercuric sulfate (0.6 g, 1 mmol) was added to a solution of **5** (0.54 g, 3.2 mmol), and **4** (1 g, 0.9 mmol), in dry THF (20 mL). The mixture was stirred overnight at room temperature, and the reaction was observed by t.l.c. (silica gel with solvent *A*) to be incomplete. The β anomer had R_F 0.54, and the α anomer R_F 0.47. The mixture was then boiled under reflux for 7 days (this time was arbitrary), at which time the reaction was complete. The mixture was filtered and the filtrate evaporated. The product was separated on a column (17 × 2.3 cm) of silica gel, with variations of solvent system *A*; 10:1, 200 mL; 8:1, 100 mL; and 16:3.

N.m.r. data (β anomer): δ 1.28 (d, 3 H), 3.1–5.3 (envelope, 33 H), and 7.28

(m, 40 H); (α anomer): δ 1.07–1.3 (two d, 3 H), 3.1–5.2 (envelope, 32 H), 5.28 (d, 1 H, J 4 Hz), and 7.28 (m, 40 H).

Both anomers were hydrogenolyzed overnight with equal weights of 10% palladium-on-carbon, in ethanol at a hydrogen pressure of 7.5 lb.in^{-2}. The reaction was monitored by t.l.c. on silica gel with solvent *D*; the β anomer had R_F 0.291, R_{Glc} 0.96, and the α anomer had R_F 0.317, R_{Glc} 1.05.

Preparation of 2-(allyloxy)tetrahydropyran (**7**). — Concentrated hydrochloric acid (2 drops) was added to a solution of distilled (2*H*)-dihydropyran (30 g, 0.36 mol) and distilled allyl alcohol (20.7 g, 0.357 mol), in a 100-mL flask equipped with a calcium chloride drying tube[9], and externally cooled by an ice–water bath. The mixture was stirred magnetically for 3.3 h and the reaction monitored by g.l.c. on a column (3.0 m × 0.64 cm) of 15% Carbowax (on Chromosorb G), at 120°, and a flow rate of 108 mL/min. The retention time was 12.5 min. The solution was made neutral with 10M sodium hydroxide, and dried in the presence of sodium hydrogencarbonate. The mixture was filtered and the filtrate distilled from sodium hydrogencarbonate yield 35.9 g (71%); b.p.$_{\sim 40}$ 156–159° (lit.[10] b.p.$_{760}$ 165–167°); ν_{max} 3095, 3025, 2905, 2885, 1653, 1460, 1448, 1388, 1350, 1328, 1268, 1205, 1188, 1138, 1122, 1082, 1060, 1030, 1000, 965, 921, 902, 870, 843, and 815 cm^{-1}; n.m.r.: δ 0.9–2.2 (envelope, 6 H), 3.2–4.39 (envelope, 4 H), 4.59 (s, 1 H), 4.9–5.4 (envelope, 2 H), and 5.56–6.26 (m, 1 H).

Preparation of 1-[(tetrahydropyran-2-yl)oxyl-2-propanol (**8**). — The preceding compound (26 g, 0.18 mol) was added to a stirred mixture of mercuric acetate (59.4 g, 0.183 mol) in 1:1 aqueous THF (220 mL), in a 500-mL flask at room temperature[8]. The yellow solution lost its color very quickly, and the mixture was stirred for another 0.5 h. Potassium hydroxide solution (3M, 180 mL) was added, followed by sodium borohydride (3.48 g) in 3M potassium hydroxide (180 mL). The solution was kept overnight, whereupon mercury precipitated. Saturated aqueous sodium chloride (200 mL) was added, and the mixture was extracted 4 times with dichloromethane. The reaction was monitored by g.l.c. on a column (2.74 m × 0.64 cm) of SE-30 (on Chromosorb-G) at 125° and a flow rate of 50 mL/min. The product, **4**, had a retention time of 19.6 min. The organic layer was dried in the presence of sodium hydrogencarbonate, concentrated, and distilled; yield 22.9 g (78%); b.p.$_{24}$ 121.5–122.5°; lit.[11] b.p.$_{0.3-0.4}$ 60–64°; ν_{max} 3430, 2950, 2880, 1450, 1380, 1270, 1204, 1140, 1125, 1080, 1065, 1038, 978, 904, 870, and 810 cm^{-1}; n.m.r.: δ 1.15 (d, 3 H, J 6.4 Hz), 1.3–2.3 (envelope, 6 H), 3.0–4.2 (envelope, 6 H), 4.6 (s, 1 H).

Sodium hydride (6.48 g, 0.27 mol) was added to a stirred solution of **4** (21 g, 0.131 mol) in 1,4-dioxane (40 mL). Benzyl chloride (2.9 g, 0.197 mol) was added dropwise and, after the addition was complete, the mixture was boiled for 4 h under reflux. The product was observed by g.l.c. with a column (3.05 m × 0.64 cm) of 20% SE-30 (on Anakrom) at 210° and a flow rate of 108 mL/min. The product had a retention time of 22.9 min. The resulting mixture was centrifuged, decanted, and the pellet was rinsed with 1,4-dioxane. The combined supernatant solutions

were vacuum filtered, the filtrate concentrated, and distilled from sodium hydrogencarbonate; yield 26 g (79%); b.p.$_{13}$ 186–187°; ν_{max} 3510, 3062, 2970, 2900, 1513, 1470, 1390, 1365, 1276, 1218, 1200, 1138, 1080, 986, 915, 881, 828, 747, and 708 cm^{-1}; n.m.r.: δ 1.16 (d, 3 H, J 6 Hz), 1.3–2.2 (envelope, 6 H), 3.1–4.1 (envelope, 5 H), 4.57 (s, 3 H), and 7.25 (s, 5 H).

Anal. Calc. for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 71.83; H, 8.96.

1-[(Tetrahydropyran-2-yl)oxy]-2-benzyloxypropane (1 g, 4 mmol) was added to methanol (10 mL) containing Dowex 50W-X8 resin (1.5 g). The heterogeneous mixture was shaken for ~1 h at room temperature[6], and the reaction was monitored on a column (3.05 m × 0.32 cm) of 15% SE-30 (on Chromosorb G) at 200°, and a flow rate of 11 mL/min. The mixture was filtered, and the filtrate concentrated and distilled. G.l.c. indicated 98.6% conversion; b.p.$_{11}$ 134–136°; ν_{max} 3460, 3060, 3000, 2960, 2898, 1512, 1469, 1378, 1355, 1220, 1160, 1100, 1075, 1000, 920, 868, 805, 745, and 705 cm^{-1}; n.m.r.: δ 1.09 (d, 3 H, J 6 Hz), 2.2 (m, 1 H), 3.42 (m, 3 H), 4.48 (s, 2 H), and 7.23 (s, 5 H); m/z 166 (M^+).

Anal. Calc. for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49. Found: C, 72.34; H, 8.43.

G.l.c. systems for analysis of 1-benzyloxy-2-propanol (**5**), *and 2-benzyloxy-1-propanol* (**9**). — 1-Benzyloxy-2-propanol and 2-benzyloxy-1-propanol were separated on a column (1.37 m × 0.32 cm) of 15% SE-30 (acid-washed Chromosorb W, 80–100 mesh) at 85°; after 200 s had elapsed the temperature was programmed at 1°/min to 140°. The injector temperature was 273°, and the detector temperature was 302° with a helium flow-rate of 12 mL/min. The observed retention-time for **5** was 2893 s, and that for **9** 3023 s. All results were confirmed by co-injection.

Benzylation of the carbohydrate samples from extracts of R. secalis. — Sodium hydride (84 mg, 35 mmol) was added to a solution of the carbohydrate sample (4.17 mg) and dry 1,4-dioxane (10 mL). Benzyl chloride (0.15 g, 12 mmol) was added to this vigorously stirred solution, and the mixture was boiled overnight under reflux. The cooled mixture was filtered and the filtrate evaporated.

Hydrolysis of the benzylated toxin sample. — The syrupy product from the benzylation reaction was transferred in chloroform to a flask containing 56 mL of acetic acid at 90°. Boiling M sulfuric acid (3 mL) and conc. sulfuric acid (0.5 mL) were added and, after 2 h, an additional 0.84 mL of conc. sulfuric acid was added. The mixture was maintained for 24 h at 90°, whereupon it was cooled and 50 mL of water added. The mixture was extracted with dichloromethane. The extracts were made neutral with saturated sodium hydrogencarbonate, re-extracted with dichloromethane, dried, and evaporated. The remaining sample was subjected to g.l.c. analysis.

Preparation of compound **10**. — Mercuric sulfate (0.6 g, 2 mmol) was added to a solution of 2-benzyloxy-1-propanol (0.269 g, 16.2 mmol), and **4** (1 g, 0.9 mmol) in dry THF (19 mL). The mixture was boiled overnight under reflux; t.l.c. with solvent *A* showed three products: hepta-*O*-benzylcellobiose, R_F 0.19; 2-benzyloxypropyl 2,3,6,2′,3′,4′,6′-hepta-*O*-benzyl-α-cellobioside, R_F 0.52; and the β anomer, R_F 0.62. The material having R_F 0.19, was separated from the other two

products by chromatography on a column (17 × 2.3 cm) of silica gel eluted with solvent *A*. The material from the two more-mobile spots formed a solid upon concentration*; yield 0.62 g (59%); n.m.r.: δ 1.2 (d), 3.2–4.1 (envelope), 4.2–5.1 (envelope), 5.24 (resonance typical of an α anomer), and 7.26 (m).

Anal. Calc. for $C_{71}H_{76}O_{12}$: C, 76.05; H, 6.83. Found: C, 76.37; H, 7.06.

The foregoing mixture was resolved on a column (17 × 2.3 cm) of silica gel with solvent *F*, to give the β anomer yield 0.368 g (35%); n.m.r.: δ 1.21 (m, 3 H), 2.9–5.1 (envelope, 33 H), and 7.26 (m). The α anomer, yield 0.249 g (24%) was also isolated, n.m.r.: δ 1.21 (d), 3.2–4.1 (envelope, 17 H), 4.2–5.3 (envelope, 16 H), and 7.26 (m).

Both anomers were debenzylated by dissolving the product in ethanol and adding an equal weight of 10% palladium-on-carbon. The mixture was hydrogenolyzed, the resultant **10** had the same R_f value as an authentic sample[7].

Cultures of R. secalis. — The *R. secalis* used throughout this study was isolated from naturally infected barley in Montana and was designated as the CA75 isolate. The isolate was grown and harvested according to the method of Auriol *et al.*[2].

Bioassay. — Barley cultivar var. Hannchen C.I. 531, was used for all bioassays. When the seedlings were 8–9 days old (second-leaf stage), the plants were cut with a sharp razor-blade 1–2 cm above the ground, and each cutting was placed in a miniature conical tube containing 0.2–0.6 mL of the solution to be tested. Water blanks were simultaneously conducted with all bioassays. The test results were obtained after 3 days of incubation, at room temperature. Bioassays were conducted in a plant growth-chamber, environmentally controlled at 15–24° diurnal cycle utilizing a 12-h photoperiod[3].

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*By n.m.r. spectroscopy, the free cellobiose product appeared to be totally α. As the yield of this by-product was ~41%, and that of the α anomer was 24%, the total yield of α anomer was 65%. This is significant, as the percentage of the starting material, phenyl hepta-*O*-benzyl-β-1-thiocellobioside, was 66% (n.m.r.), thus demonstrating a highly efficient and specific displacement of the sulfur-containing leaving group.

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