Wilhelm, J. A., and Haselkorn, R. (1970), *Proc. Nat. Acad. Sci. U. S. 65*, 388.

Yutani, K., Yutani, A., Imanishi, A., and Isemura, T. (1968), *J. Biochem. (Tokyo) 64*, 449.

Extracellular 2-Acetamido-2-deoxy-D-galacto-D-galactan from *Aspergillus nidulans**

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ABSTRACT: An extracellular polysaccharide from *Aspergillus nidulans* is a linear molecule containing 4-O-substituted α-D-galactopyranosyl and 4-O-substituted 2′-acetamido-2′-deoxy-α-D-galactopyranosyl units in an approximately 1.8:1 ratio. A Smith degradation yielded mainly 2-*O*-(2′-acetamido-2′-deoxy-α-D-galactopyranosyl)-D-threitol, two hydroxyethylidene acetals, D-threitol, and a trace of unidentified material. The different sugar units are distributed evenly throughout the chain, consecutive *N*-acetyl-D-galactosamino units not being detected in appreciable amounts. 3,4,6-Tri-*O*-methyl-D-galactose was prepared for use of its methyl glycoside as a gas–liquid chromatographic standard.

Initial tests on an extracellular polysaccharide produced by *Aspergillus nidulans* indicated it to be a comparatively rare fungal polymer of a galactosamine and a galactose; a sugar composition which has only once been found in a fungal polysaccharide (Trotter and Whisler, 1965). There are important relationships between cell wall (especially polysaccharide structure) and both fungal taxonomy and morphogenesis (recently reviewed by Bartnicki-Garcia (1968)) and thus a more detailed examination of the *Aspergillus* heteropolymer was carried out.

Experimental Procedure

Production of Polysaccharide. Aspergillus nidulans (Eidam) Wint. strain b 1 was supplied by M. Reever, Department of Biology, University of Saskatchewan, *via* G. Pontecorvo, Glasgow. The organism was routinely grown on Vogel's medium N (Vogel, 1964) with 1% D-glucose as a carbon source. Alternatively a medium (M. J. Johnson, personal communication) was used consisting of D-glucose, 10 g/l.; KH_2PO_4, 5.5 g/l.; $MgSO_4 \cdot 7H_2O$, 0.2 g/l.; $CaCl_2$, 0.015 g/l.; $(NH_4)_2HPO_4$, 2.0 g/l.; $(NH_4)H_2PO_4$, 1.5 g/l.; Na_2HPO_4, 10.0 g/l.; $Fe_2(SO_4)_3$, 0.6 mg/l.; $ZnSO_4 \cdot 7H_2O$, 0.2 mg/l.; $CuSO_4 \cdot 5H_2O$, 0.2 mg/l.; $MnSO_4 \cdot H_2O$, 0.2 mg/l.; biotin, 10 μg/l. Cultures were grown at 30° in erlenmeyer flasks (*e.g.*, 900 ml in each 2-l. flask) on a rotary shaker. The mycelium was filtered off using cheese cloth and the extracellular polysaccharide precipitated from the culture fluid by the addition of 1 volume of ethanol. Further soluble material could be obtained by washing the mycelium with water. A polysaccharide of a different type was precipitated on standing overnight at 5°.

General Procedures. Descending paper chromatography was carried out on Whatman No. 1 filter paper using the following solvent systems (in volume ratios): (A) butyl alcohol–ethanol–water (40:11:19), (B) 2-propanol–water (4:1), (C) ethyl acetate–acetic acid–water (9:2:2), (D) ethyl acetate–pyridine–water (10:4:3), (E) isoamyl alcohol–pyridine–water (5:5:4) (Powning and Irzykiewicz, 1965). Gas chromatography of methyl glycosides was carried out on a 12 ft × 0.25 in., outside diameter, 3% neopentylglycol succinate on Chromosorb W column maintained at 170° using helium (40 psi) as carrier gas (Lee and Ballou, 1965), while amino sugars were also characterized on a Spinco amino acid analyzer, on paper chromatograms, and by gas–liquid chromatography of their trimethylsilyl derivatives (P. A. J. Gorin, data to be published). Threitol was characterized by gas–liquid chromatography of its tetraacetate using a column containing a nitrile silicone XE-60 packing (Gorin and Spencer, 1966).

Sugars were detected on paper chromatograms with *p*-anisidine hydrochloride (Hough *et al.*, 1950), ammoniacal silver nitrate (Partridge, 1946), ninhydrin, and Elson–Morgan dimethylaminobenzaldehyde (Partridge, 1948) spray reagents.

Acid hydrolyses of the polysaccharide were carried out in 3 M HCl at 100° for 2 hr and the solution evaporated, or in 1 M H_2SO_4 at 100° for 18 hr and the solution was neutralized ($BaCO_3$), filtered, and evaporated. Partial acid hydrolysis was performed on the dispersed polysaccharide on 0.15 M HCl, at 100° for 30 min. The mobile solution was deionized with mixed Amberlite 1R120 (H^+ form) and Dowex 1-X8 (bicarbonate form) and the polysaccharide precipitated with excess ethanol.

Methylation was carried out by the successive procedures of Haworth (1915) and Kuhn *et al.* (1955). Proton magnetic resonance spectra were taken using a Varian HA-100 high-resolution nuclear magnetic resonance spectrometer. Measurements were made in D_2O at 70° with tetramethylsilane in a coaxial capillary as external standard (τ 10).

Preparation of 3,4,6-Tri-O-methyl-D-galactose. 1,2,3,4,6-Penta-*O*-acetyl-β-D-galactose (5 g) was converted into

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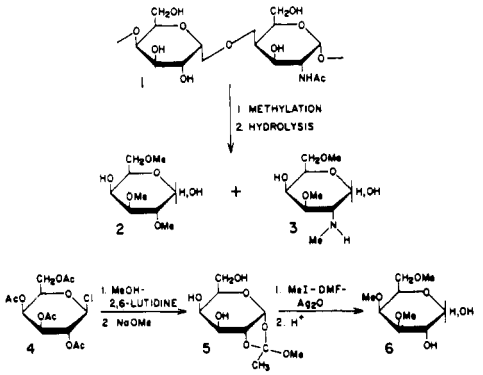

FIGURE 1: Methylation–fragmentation products from the *A. nidulans* polysaccharide and preparation of 3,4,6-tri-*O*-methyl-D-galactose.

2,3,4,6-tetra-*O*-acetyl-1-chloro-β-D-galactose (Figure 1, **4**) by the method of Korytnyk and Mills (1959). The crude syrupy product could not be induced to crystallize and was treated with 2,6-lutidine (30 ml) containing methanol (3 ml) (Mazurek and Perlin, 1965) to give the 1,2-(methyl orthoacetate) of 3,4,6-tri-*O*-acetyl-α-D-galactose. After 1 hr the solution was evaporated to a syrup. This was partitioned between chloroform and water and the chloroform layer was evaporated to a syrup. The product was deacetylated to the orthoacetate with 0.1 M sodium methoxide in methanol (60 ml), and the solution evaporated to a syrup after 1 hr. A portion of the product was deionized with Amberlite 1R120 (H^+ form) and Dowex 1-X8 (bicarbonate form) and examined on a paper chromatogram (solvent A: spray ammoniacal silver nitrate). Two spots were obtained, one corresponding to D-galactose and the other, with R_F L-rhamnose, corresponded to the 1,2-(methyl orthoacetate) (Figure 1; **5**).

One-half of the product obtained following deacetylation was methylated (Kuhn *et al.*, 1955) by shaking a mixture of methyl iodide (8 ml) and *N*,*N*-dimethylformamide (1 ml) with silver oxide (10 g). After 18 hr the solution was diluted with chloroform, filtered, and evaporated to a syrup. The product was hydrolyzed in 1 M H_2SO_4 (5 ml) at 100° for 2 hr the solution neutralized ($BaCO_3$), filtered, and evaporated to dryness. The product which consisted of tri-*O*- and tetra-*O*-methyl-D-galactose was fractionated on a cellulose column using benzene–ethanol (20:1, v/v) as eluent. The resulting sirupy 3,4,6-tri-*O*-methyl-D-galactose (Figure 1; **6**, 265 mg) had $[\alpha]_D^{25}$ +120° (*c* 0.8, H_2O) and consumed 0.81, 0.94, and 0.95 mole per mole of lead tetraacetate after 2, 5, and 15 min, respectively, in accord with its structure (Charlson and Perlin, 1956). Kuhn and Baer (1955) report $[\alpha]_D^{25}$ +110° for crystalline 3,4,6-tri-*O*-methyl-D-galactose synthesized from D-galactal. Reduction with sodium borohydride gave 1,3,4-tri-*O*-methyl-D-galactitol, mp 98–99° (from EtOAc), and $[\alpha]_D^{25}$ +5.8° (*c* 0.9, H_2O). In accord with its structure, it consumed 0.93 mole/mole of sodium periodate after 18 hr without formation of acid.

Anal. Calcd for $C_9H_{20}O_6$: C, 48.20; H, 8.99. Found: C, 48.04; 9.19%.

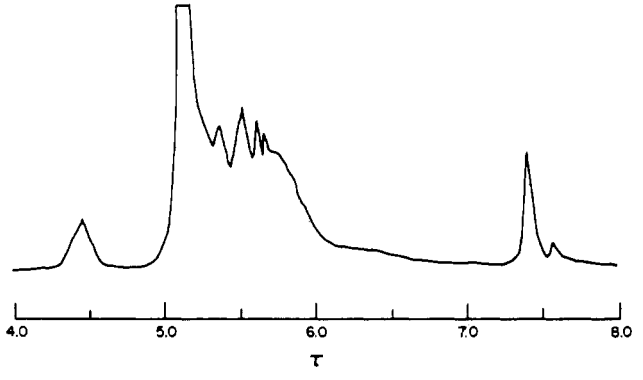

FIGURE 2: Proton magnetic resonance spectrum of partially acid-degraded *A. nidulans* polysaccharide.

Results

In an initial experiment, the viscosity of the culture medium increased and indicated the presence of a high molecular weight polymer. An extracellular polysaccharide was readily isolated from the culture medium and appeared relatively pure as only a galactose and galactosamine and negligible amounts of amino acids appeared as products of acid hydrolysis (chromatographic solvents C and D). The amount of polysaccharide in the medium increased up to 10-days culture, typical amounts being around 100 mg/l. Higher yields (*ca.* 120 mg/l.) were obtained if 4% D-glucose was used as a carbon source. The yields decreased with longer periods of incubation. An autolytic enzyme complex capable of degrading the polysaccharide and yielding a galactose and a galactosamine was recovered from the culture medium of older cultures by ammonium sulfate precipitation. Initial attempts to resolve the polysaccharide into individual galactan and galactosamine polymers by gel filtration and ion-exchange chromatography (CM-cellulose, pH 6.0) failed. Acid hydrolysis of samples taken daily showed a constant galactose to galactosamine ratio and thus it was tentatively assumed that the polysaccharide was a heteropolymer.

General Characterization. Acid hydrolysis of the polysaccharide gave the D enantiomers of galactose and galactosamine hydrochloride. The former was characterized following H_2SO_4 hydrolysis of the polysaccharide (100 mg). The hydrolysate was chromatographed on a cellulose column, solvent: butyl alcohol–water (9:2, v/v), giving D-galactose (from MeOH), mp and mmp 167° $[\alpha]_D^{25}$ +80° (*c* 0.1, H_2O; equilibrium value). D-Galactosamine was initially characterized by paper chromatography of the hydrochloride formed on hydrolysis (solvent E) using ninhydrin and Elson–Morgan spray reagents. The product was further characterized following ninhydrin degradation to lyxose (solvent B) (Stoffyn and Jeanloz, 1954). The acid hydrolysis residue was also converted into 2-deoxy-2-[(benzyloxycarbonyl)amino]-D-galactose (from H_2O), mp and mmp 175° (Heyns and Beck, 1957).

The polysaccharide had $[\alpha]_D^{25}$ +165° (*c* 0.1, aqueous 10% NaOH) suggesting that it contains predominant α-D-glycosidic linkages (Hudson, 1938). Paper chromatographic examination of the hydrolysate (0.15 M HCl at 100° for 30 min) showed that the mono- or oligosaccharides were not formed thus excluding the possibility of acid-labile furanose units in the polysaccharide (Whitehouse and Kent, 1958). A proton magnetic resonance spectrum of the polysaccharide in D_2O could not be obtained because the solution was too

TABLE I: Gas–Liquid Chromatography Characteristics of Methyl Tri-*O*-methyl-D-galactosides.

Isomer	Retention Time (min) of Peak and Percentage of Total Peak Area in Parentheses
2,3,4-	49.2 (100)
3,4,6-	30.3 (74), 45.1 (26)
2,4,6-	28.5 (40), 33.1 (60)
2,3,6-	23.8 (58), 29.4 (17), 31.9 (25)
Sample	23.6 (54), 29.2 (21), 31.8 (25)

TABLE II: Chromatographic Characteristics of 2-Deoxy-*O*-methyl-2-methylamino-D-galactoses[a] and the Methylation Fragment.

Isomer	Rate on Paper Chromatogram Compared to Rhamnose	Retention Time on Spinco Amino Acid Analyzer Compared to Phenylalanine (min)
3-*O*-	0.59	−25
4-*O*-	0.67	−18
6-*O*-	0.72	+23
3,4-Di-*O*-	0.82	−13
3,6-Di-*O*-	0.80	−26
4,6-Di-*O*-	0.89	+29
3,4,6-Tri-*O*-	1.41	0
Sample	0.80	−26

[a] P. A. J. Gorin, data to be published.

viscous. However, partial acid hydrolysis (180 mg) gave a polymer [140 mg, $[\alpha]_D^{25}$ +138° (*c* 0.3, H_2O), N, 2.8%] which dissolved in dilute NaOD in D_2O to give distinct proton magnetic resonance signals in the H-1 (τ 4.45) and *N*-acetyl (τ 7.40) regions (Figure 2). The relative sizes of the signals indicate that 39% of the sugar units contain *N*-acetyl groups.

The polysaccharide was soluble in dilute NaOH and did not reprecipitate on neutralization with acetic acid. On periodate oxidation, the sample consumed 0.66, 0.68, and 0.70 mole of periodate per mole of anhydrohexose unit after 1, 2, and 3 days, respectively. It follows that approximately 65% of the polysaccharide consisted of D-galactose units and 35% of *N*-acetyl-D-galactosamine units on a molar basis. The possibility of an appreciable proportion of unacetylated D-galactosamine units are excluded since the undegraded and degraded polysaccharides have a nitrogen content of 2.8% which corresponds to 35% of *N*-acetyl-D-galactosamine. This figure is close to 39% of N-acetylated sugar units calculated from the proton magnetic resonance spectrum of the partially degraded polysaccharide (Figure 2). The above data correspond to a molar ratio of *N*-acetyl-D-galactosamine and D-galactose units of 1:1.8.

Methylation–Fragmentation Analysis. The polysaccharide (Figure 1; **1**, 50 mg) was partially methylated with dimethyl sulfate–aqueous sodium hydroxide according to the Haworth procedure (Haworth, 1915). The reaction mixture was neutralized with acetic acid, dialyzed against water, and evaporated to a residue which was completely methylated by the Kuhn method (Kuhn *et al.*, 1955) using methyl iodide–*N*,*N*-dimethylformamide–silver oxide over a period of 5 days. Full methylation was attained since the linear methylated polymer gave 2,3,6-tri-*O*-methyl-D-galactose and 2-deoxy-3,6-di-*O*-methyl-2-methylamino-D-galactose on hydrolysis (see below).

The product was refluxed in 5% methanolic hydrochloride (5 ml) overnight. One-half of the reaction mixture was neutralized (Ag_2CO_3), filtered, and evaporated to a syrup which was hydrolyzed overnight at 100° in 10% sulfuric acid (2 ml). The solution was neutralized ($BaCO_3$), filtered, and evaporated. The product on a paper chromatogram (solvent A; spray: *p*-anisidine hydrochloride) gave a single carmine spot corresponding in R_F to a tri-*O*-methylgalactose (Figure 1; **2**). The product was converted into a methyl glycosidic mixture by boiling under reflux for 3 hr in 3% methanolic hydrogen chloride, followed by neutralization (Ag_2CO_3), filtration, and evaporation. Gas–liquid chromatography of the methyl glycosides gave 3 peaks corresponding in retention times (Table I) and relative peak areas to methyl 2,3,6-tri-*O*-methyl-$\alpha\beta$-D-galactosides. They were distinguishable from peaks arising from the methyl glycosides of 2,4,6-, 2,3,4-, and 3,4,6-tri-*O*-methyl-D-galactose (Table I). 3,4 6-Tri-*O*-methyl-D-galactose (Figure 1; **6**) was not available and was thus synthesized (see Experimental Procedures).

The methylated polysaccharide was degraded by refluxing methanolic hydrogen chloride followed by 3 M hydrochloric acid (2 ml) at 100° for 2 hr. The product gave a spot on a paper chromatogram (solvent A) using the ninhydrin spray corresponding to 2-deoxy-3,6-di-*O*-methyl-2-methylamino-D-galactose (Figure 1; **3**). The 3,6 isomer was confirmed using a Spinco amino acid analyzer (P. A. J. Gorin, data to be published) (see Table II). The methylation–fragmentation data, the resistance of the polysaccharide to hydrolysis, and its high positive specific rotation thus indicate the presence of 4-O-linked α-D-galactopyranosyl and 4-O-linked 2′-deoxy-2′-acetamido-α-D-galactopyranosyl units.

Smith Degradation of the Polysaccharide. Information on the sequence of D-galactose and *N*-acetyl-D-galactosamine units in the polysaccharide was obtained by subjecting it to a Smith degradation (Goldstein *et al.*, 1959), in which the polyalcohol was degraded at pH 2 at 100° (Gorin and Spencer, 1968a). The polysaccharide (Figure 3; **1**, 0.80 g) was dissolved in 0.05% aqueous sodium hydroxide (500 ml) with the aid of a Sorvall OmniMixer. The viscous solution was neutralized with acetic acid and sodium periodate (3.0 g) added. The viscosity was reduced after a few minutes and after 24 hr the solution was evaporated to 50 ml and acetic acid (750 ml) added. The mixture was centrifuged, the residue dispersed in acetic acid, and the suspension recentrifuged. This process was repeated and finally the residue was dispersed in acetone, filtered off, and dried (yield 0.55 g).

The polyaldehyde was dispersed in water (50 ml) using

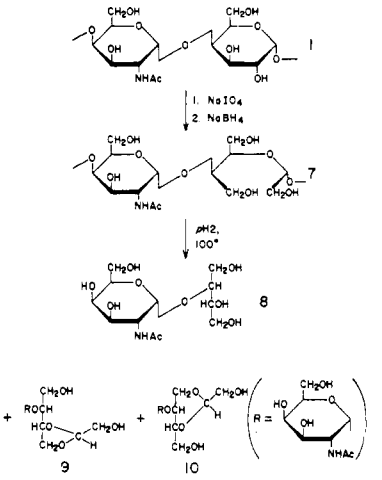

FIGURE 3: Smith degradation of *A. nidulans* polysaccharide.

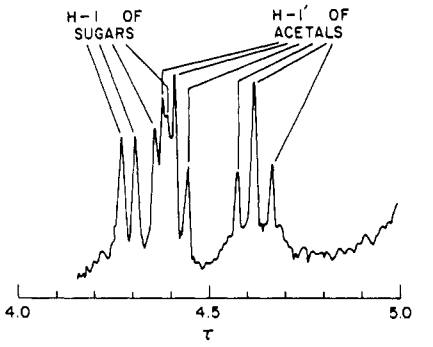

FIGURE 4: Proton magnetic resonance spectrum of mixture of two *O*-hydroxyethylidene derivatives of 2-*O*-(2′-acetamido-2′-deoxy-α-D-galactopyranosyl)-D-threitol.

the Sorvall OmniMixer and sodium borohydride (0.20 g) added. After 1 hr the solution was deionized with mixed Amberlite 1R120 (H^+ form) and Dowex 1-X8 (bicarbonate form) and filtered and the filtrate evaporated to 50 ml. The solution of polyalcohol (Figure 3; **7**) was adjusted to pH 2 with H_2SO_4, kept at 100° for 1 hr, neutralized ($BaCO_3$), filtered, and evaporated to a small volume. Addition of excess acetone to the solution showed that polysaccharide was not present since no precipitate was formed. Examination of the solution on a paper chromatogram (solvent A; spray: ammoniacal silver nitrate) showed four spots. Three of the spots have R_F values corresponding to D-threitol, D-ribose, and D-mannose, and another at 1.2 times the R_F of lactose.

Cellulose column chromatography of the mixture using butyl alcohol–water (19:1, v/v) as eluent gave D-threitol (32 mg) and a 9:1, v/v, mixture gave the material (100 mg) with the R_F of ribose. Hydrolysis of the latter material with 3 M HCl at 100° for 1 hr gave D-galactosamine and D-threitol. This fraction appeared to contain two hydroxyisopropylidene acetals of 2-*O*-(2′-acetamido-2′-deoxy-α-D-galactopyranosyl-D-threitol (Figure 3; **9** and **10**) on the basis of the proton magnetic resonance spectrum and because of the isolation of the unacetalated compound (see below). The proton magnetic resonance spectrum in the region τ 4.0–5.0 showed two triplets superimposed on two doublets (Figure 4). The doublets (τ 4.29, 4.37: both J = 4 Hz from α anomers, van der Veen, 1963) correspond to the anomeric protons of two derivatives of 2-*O*-(2′-acetamido-2′-deoxy-α-D-galactopyranosyl)-D-threitol and the triplets to H-1′ (τ 4.41, 4.62) of *O*-hydroxyethylidene groups substituting the D-threitol moiety: *O*-hydroxyethylidene derivatives are known to give proton magnetic resonance signals at τ 4.5–5.0 (Gorin and Ishikawa, 1966). The acetals should substitute the threitol moiety at the 1,3- and 3,4 positions, respectively. This follows from the above data and from the findings of Gorin *et al.* (1965) and Gorin and Spencer (1965) who isolated 2-*O*-β-D-mannopyranosyl-D-erythritol and its 1,3-*O*- and 3,4-*O*-hydroxyethylidene derivatives on Smith degradation of a mannan containing alternate 1→3- and 1→4-linked β-D-mannopyranose units.

Elution of the cellulose column with acetone–water (6:1, v/v) gave material (172 mg) with an R_F of D-mannose. Crystallization from EtOH–EtOAc gave 2-*O*-(2′-acetamido-2′-deoxy-α-D-galactopyranosyl)-D-threitol (Figure 3; **8**), mp 98°, $[\alpha]_D^{25}$ +186° (*c* 0.3, H_2O), proton magnetic resonance data: solvent D_2O τ 4.41 (J = 4 Hz; H-1); 7.35 (3 protons; NAc). Hydrolysis gave D-galactosamine and D-threitol. Periodate oxidation (1 day) resulted in uptake of 2.11 moles/mole of oxidant with formation of 0.91 mole/mole of formaldehyde; acid was not formed. An α-D linkage was assigned since it has a high positive specific rotation (Hudson, 1938) and since the proton magnetic resonance spectrum contained H-1 signals with J = 4 Hz (van der Veen, 1963). *Anal.* Calcd for $C_{12}H_{23}O_9 \cdot 0.5\ H_2O$: C, 43.20; H, 7.23; N, 4.19. Found: C, 43.45; H, 7.34; N, 4.32.

Material (29 mg) running at 1.2 times the rate of lactose was eluted using acetone–water (4:1, v/v), but was not characterized.

A Smith degradation of the polysaccharide using vigorous hydrolytic conditions gave D-galactosamine and D-threitol, the latter being identified by paper chromatography and gas–liquid chromatography of its tetraacetate (Gorin and Spencer, 1966).

The gravimetric analysis carried out on cellulose chromatography indicates that, on a molar basis, 23% of D-threitol, 71% of combined 2-*O*-(2′-acetamido-2′-deoxy-α-D-galactopyranosyl)-D-threitol and its *O*-hydroxyethylidene derivatives, and approximately 5% of unknown fragments were formed.

Discussion

The extracellular heteropolysaccharide from *A. nidulans* is linear, containing 4-O-substituted 2′-deoxy-2′-acetamido-α-D-galactopyranosyl and 4-O-substituted α-D-galactopyranosyl units in an approximately 1:1.8 ratio. This ratio is based on the nitrogen content and periodate oxidation characteristics of the polysaccharide and the relative sizes of the H-1 and *N*-acetyl proton magnetic resonance signals of partially acid-degraded polysaccharide. The Smith degradation data show that there are few, if any consecutive 4-O-linked 2′-deoxy-2′-acetamido-α-D-galactopyranose units. An apparent anomaly in the Smith degradation is the formation of 23% of D-threitol compared to 71% of combined 2-*O*-(2′-acetamido-2-deoxy-α-D-galactopyranosyl)-D-threitol and its *O*-hydroxyethylidene derivatives, figures which correspond to a ratio of 1.3:1 of D-galactose and *N*-acetyl-D-galactosamine units. However the ratio is

unreliable since it is likely that *O*-hydroxyethylidene-D-threitols were formed (Gorin and Spencer, 1965) and not accounted for in the gravimetric analysis.

Although D-galactose oxidase oxidizes several D-galactosides without their prior hydrolysis to monosaccharides (Avigad *et al.*, 1962), the *A. nidulans* polymer was oxidized to only a limited extent after overnight incubation (D. E. Eveleigh and P. A. J. Gorin, unpublished data). The chemical structure of the polysaccharide has interest since it raises the question of whether α-D-galactopyranose units are converted into 2′-deoxy-2′-acetamido-α-D-galactopyranose units at the polymeric level rather than arising from an amino sugar nucleotide.

The cell wall of *Amoebidium parasiticum* (Trotter and Whisler, 1965) has 30% of a galactosamine and 10% of a galactose forming the main polysaccharide components of the cell wall. It is not known whether these sugars form a heteropolymer, but from the general similarity to the *A. nidulans* polysaccharide, it warrants further analysis. Any comment regarding the usefulness of such polymers as a taxonomic aid must await more examples than the two instances cited, especially as *A. nidulans* (Ascomycetes) and *Amoebidium parasiticum* (a member of the ill-defined Trichomycete group in the Phycomycetes), fall into widely divergent taxonomic groups. A number of other fungal polysaccharides containing a 2-amino-2-deoxy-galactose have been observed (Bartnicki-Garcia, 1968; Gorin and Spencer, 1968b) but only the partly *N*-acetylated 2-amino-2-deoxy-D-galactan from *Apergillus parasiticus* has been examined in detail (Distler and Roseman, 1960; Roseman *et al.*, 1955) and this apparently differs widely in structure from the 2-acetamido-2-deoxy-D-galacto-D-galactan from *A. nidulans*.

The presence of a polymer giving D-galactosamine and D-galactose as a minor component of the *A. nidulans* cell wall is indicated from analysis of its hydrolysis products (D. E. Eveleigh and P. A. J. Gorin, unpublished data). A galactosamine has previously been reported in *A. nidulans* cell walls as a minor component, while temperature sensitive mutants lacking amino sugars were also reported (Cohen *et al.*, 1969). Such polymers may assume greater importance since Buck *et al.* (1969) have suggested that large amounts (18%) of a galactosamine in the cell wall may be associated with the presence of virus particles in *Penicillium stoloniferum* ATCC 14586.

Acknowledgments

The authors wish to thank Mr. A. S. Sieben and Mr. R. J. Magus for valuable technical assistance, Mr. M. Mazurek for preparation of proton magnetic resonance spectra, and Mr. W. C. Haid for elemental analysis.

References

Avigad, G., Amaral, D., Asensio, C., and Horecker, B. L. (1962), *J. Biol. Chem. 237*, 2736.
Bartnicki-Garcia, S. (1968), *Annu. Rev. Microbiol. 22*, 87.
Buck, K. W., Chain, E. B., and Darbyshire, J. E. (1969), *Nature (London) 223*, 1273.
Charlson, A. J., and Perlin, A. S. (1956), *Can. J. Chem. 34*, 1200.
Cohen, J., Katz, D., and Rosenberger, R. F. (1969), *Nature (London) 224*, 713.
Distler, J. J., and Roseman, S. (1960), *J. Biol. Chem. 235*, 2538.
Goldstein, I. J., Hay, G. W., Lewis, B. A., and Smith, F. (1959), 135th National Meeting of the American Chemical Society, Boston, Mass., April, 3D.
Gorin, P. A. J., Horitsu, K., and Spencer, J. F. T. (1965), *Can. J. Chem. 43*, 950.
Gorin, P. A. J., and Ishikawa, T. (1966), *Can. J. Chem. 44*, 1787.
Gorin, P. A. J., and Spencer, J. F. T. (1965), *Can. J. Chem. 43*, 2978.
Gorin, P. A. J., and Spencer, J. F. T. (1966), *Can. J. Chem. 44*, 993.
Gorin, P. A. J., and Spencer, J. F. T. (1968a), *Can. J. Chem. 46*, 3407.
Gorin, P. A. J., and Spencer, J. F. T. (1968b), *Advan. Carbohyd. Chem. 23*, 367.
Haworth, W. N. (1915), *J. Chem. Soc.*, 8.
Heyns, K., and Beck, M. (1957), *Chem. Ber. 90*, 2443.
Hough, L., Jones, J. K. N., and Wadman, W. H. (1950), *J. Chem. Soc.*, 1702.
Hudson, C. S. (1938), *J. Amer. Chem. Soc. 60*, 1537.
Korytnyk, W., and Mills, J. A. (1959), *J. Chem. Soc.*, 636.
Kuhn, R., and Baer, H. H. (1955), *Chem. Ber. 88*, 1537.
Kuhn, R., Trischmann, H., and Löw, I. (1955), *Angew. Chem. 67*, 32.
Lee, Y. C., and Ballou, C. E. (1965), *Biochemistry 4*, 257.
Mazurek, M., and Perlin, A. S. (1965), *Can. J. Chem. 43*, 1918.
Partridge, S. M. (1946), *Nature (London) 158*, 270.
Partridge, S. M. (1948), *Biochem. J. 42*, 238.
Powning, R. F., and Irzykiewicz, H. (1965), *J. Chromatogr. 17*, 621.
Roseman, S., Watson, D. R., Duff, I. F., and Robinson, W. D. (1955), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 14*, 312.
Stoffyn, P. J., and Jeanloz, R. W. (1954), *Arch. Biochem. Biophys. 52*, 373.
Trotter, M. J., and Whisler, H. C. (1965), *Can. J. Bot. 43*, 869.
van der Veen, J. M. (1963), *J. Org. Chem. 28*, 564.
Vogel, H. J. (1964), *Amer. Natur. 98*, 435.
Whitehouse, M. W., and Kent, P. W. (1958), *Tetrahedron 4*, 425.