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Studies on Metabolic Products of the *Penicillium luteum* Series

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Considerable attention has been devoted to the polysaccharide metabolic products of *Penicillium luteum* Zukal. Raistrick & Rintoul (1931) isolated the mucilaginous polysaccharide, luteic acid, which was shown to be a malonylpolyglucose. Alkaline or mild acidic hydrolysis of luteic acid yielded the acid-free polysaccharide, luteose, which was constituted mainly of β -glucose units linked through the 1:6-positions (Anderson, Haworth, Raistrick & Stacey, 1939). Luteic acid was elaborated by *P. luteum* when grown on a number of different hexoses, pentoses or glycerol as sole source of carbon (Birkinshaw & Raistrick, 1933). Later, Anderson & Raistrick (1936) showed that although luteic acid was the main metabolic product of *P. luteum* Zukal, other laevorotatory polysaccharides, built up of mannose, galactose and fructose units, were produced in proportions varying with the age of the culture. Anderson *et al.* (1939) isolated a laevorotatory polysaccharide which gave D-galactose and D-mannose on hydrolysis and for which the name galuteose was proposed.

The present work is concerned with the isolation of two hitherto undescribed polysaccharides, the properties and component sugar residues of which have been investigated. A study of the sedimentation of the two new polysaccharides in the ultracentrifuge is presented in the following paper (Ogston, 1949).

EXPERIMENTAL

Strains of Penicillium luteum

Six strains of *P. luteum* were used; they are distinguished by their laboratory catalogue numbers. The strains were broadly similar in their cultural characteristics and morphological appearance and clearly belong to the *P. luteum* series. Strains F 6, 39, 46, 51 and 52 agreed closely with Thom's

(1930) description of *P. luteum* Sopp and F 231 was a conidial non-ascospore form closely corresponding to *P. luteum* Zukal. Strains F 6, 39, 46, 51 and 52 give broadly spreading floccose colonies on Czapek-Dox agar which were yellow at the margin and slowly developed greyish green conidial areas in the centre. The reverse was orange to red in colour. On beer-wort agar, broadly spreading thick floccose felts developed with zonate bands of yellow, pink and green conidial areas; the reverse was orange to red. On nutrient gelatin medium, broadly spreading mainly white floccose, buckled colonies developed with some pink zones and green conidial areas. The gelatin was liquefied and became red in colour. These strains were isolated in the laboratory, F 39 from mouldy cake, F 46 from a solution of sodium carboxymethylcellulose and F 51 and 52 from an industrial acetone recovery tower. Strain F 231 was purchased from the National Collection in January 1947 as *P. luteum* Zukal N.C.T.C. 585. On Czapek-Dox agar it formed broadly spreading mainly white floccose colonies with marginal green conidial zones; the reverse was colourless. Similar colonies were formed on nutrient gelatin medium, which was liquefied and remained colourless. In all the above strains the sterigmata were acuminate, 10–11 $\times$ 2.5 μ . and conidia ovoid about 2.5 $\times$ 3.5 μ .

Cultural methods

The cultures were grown in glass culture vessels ('Glaxo' bottles) each containing 350 ml. of Czapek-Dox medium of the following composition: glucose, 50 g.; NaNO₃, 2.0 g.; K₂HPO₄, 1.0 g.; KCl, 0.5 g.; MgSO₄.7H₂O, 0.5 g.; FeSO₄.7H₂O, 0.01 g.; distilled water, 1 l. After sterilization by autoclaving, each vessel was sown with a spore suspension from 7-day-old beer-wort agar slopes. The cultures were incubated at 25° in darkness, and usually harvested when the glucose was virtually completely exhausted. Initial and final glucose concentrations were determined in samples of the culture filtrates by the Fehling-Soxhlet method in which cuprous oxide was determined iodometrically by the method of Shaffer & Hartmann (1921). Carbon was determined by the wet combustion method of Houghton (1945). Volatile neutral

compounds, volatile acids and non-volatile acids were determined by methods based on those of Birkinshaw & Raistrick (1931). Data based on single flask cultures of the six strains are summarized in Table 1, and show that from 11 to 54% of the glucose carbon fermented by the various strains was converted to soluble metabolic compounds in the filtrates. All the cultures produced acid during growth, but there was no evidence of the formation of significant quantities of volatile acids or volatile neutral compounds.

pH 4.92 and on electrometric titration (glass electrode) 25.0 ml. 0.1 N-NaOH were required to neutralize the filtrate to pH 7.0. Under corresponding conditions, luteic acid (5 g.) required 22.5 ml. N-NaOH for neutralization (Raistrick & Rintoul, 1931). The filtrate had a reducing value (R.V.) corresponding to 6.98 g./l. apparent glucose, which was equivalent to 32.8% of the crude polysaccharide (38.7% on an ash-free basis). The reducing value before hydrolysis was 3.3% as apparent glucose. The substance gave no reaction

Table 1. *Preliminary analysis of carbon compounds present in culture filtrates of strains of Penicillium luteum*

P. luteum Strain F	Time of growth (days)	pH		Carbon (g./l.)			Volatile acids (ml. N-acid/l.)	Non-volatile acids (g. Ca salt/l.)	Metabolic carbon compounds (% initial C)	Metabolic carbon compounds (% C fermented)
		Initial	Final	Initial	Final total	Final glucose				
6	12	5.8	3.5	19.12	8.83	0.60	0.11	0.0	43.0	44.4
39	18	5.5	4.7	20.20	5.02	0.90	—	—	20.4	21.4
46	25	7.0	2.9	18.51	10.62	1.24	0.46	4.2	50.7	54.3
51	24	5.5	3.3	20.20	9.78	0.80	0.69	0.0	44.4	46.3
52	18	5.5	—	20.20	2.32	0.0	—	—	11.5	11.5
231	92	5.8	3.5	18.72	11.10	8.12	—	—	15.9	28.1

Isolation of polysaccharides

Preliminary experiments showed that the culture filtrates contained polysaccharides, specimens of which were isolated, as follows. Spores of each of the six strains were seeded into groups of ten flasks, containing 350 ml. of medium (initial glucose, 49.0 g./l.). At the completion of fermentation (Table 2) the culture filtrates were separated from the mycelial felts by filtration through fluted paper. The filtrates were evaporated *in vacuo* at low temperature to 0.1 vol. and the polysaccharides precipitated by addition of 4 vol. of ethanol. The polysaccharides separated out as heavy syrupy precipitates which were desiccated with difficulty by trituration with increasing strengths of ethanol. They were finally washed with absolute ethanol and ether, and dried *in vacuo* over P_2O_5 . The yields and some of the properties of the crude products are summarized in Table 2.

with iodine. A sample of the crude product (15.0 g.) in water (30 ml.) was dialysed in a collodion sac against six changes of distilled water for 66 hr. The polysaccharide was recovered by concentration *in vacuo* to 30 ml. and addition of ethanol to 66% concentration (w/w) obtained 4.39 g. (29.3%). The low recovery on dialysis suggested that the polysaccharide may have a comparatively low molecular weight and undergo slow diffusion. The dialysed product had an ash content of 2.2%.

Tollens's phloroglucinol test was negative indicating the absence of pentose residues. The naphthoresorcinol reaction for uronic acids was negative. A negative result was also obtained with Dische's (1947) carbazole reaction for uronic acids. The crude polysaccharide gave a positive reaction with the Seliwanoff resorcinol reagent, indicating the presence of ketose sugars.

The polysaccharides from the other strains in 100 ml. aqueous solution, adjusted to pH 2 with HCl, were freed from

Table 2. *Isolation and properties of crude polysaccharides from strains of Penicillium luteum*

Strain F	Time of incubation (days)	Culture filtrate		Crude polysaccharide				Oxidation product	
		Final glucose (g./l.)	pH	Yield (g.)	Yield (as % of initial glucose)	Ash (%)	$[\alpha]_D^{18}$ (°)	Substance and m.p.	Yield (as % of polysaccharide)
6	21	1.0	3.93	18.10	10.6	15.2	+ 7.0	Mucic acid, 206°	22.8
39	21	1.4	3.11	34.43	20.1	13.8	+ 5.8	Mucic acid, 214°	17.9
46	21	1.3	2.97	30.68	17.9	11.7	+ 8.5	Mucic acid, 209–211°	12.6
51	22	0.6	3.02	41.64	24.3	10.3	+ 5.7	Mucic acid, 215°	11.0
52	22	1.6	3.10	18.54	10.8	15.0	0.0	Mucic acid absent	—
231	41	2.0	3.88	27.77	16.2	19.6	+ 12.9	Mucic acid absent	—

The properties of the crude polysaccharide from strain F 6 were examined in some detail. On ignition the substance gave 15.2% of colourless ash. Its specific rotation, $[\alpha]_D^{18}$, was +7° in water (c, 1); crude luteic acid has $[\alpha]_D - 25$ to -30° . A sample of the polysaccharide (5.32 g.) was hydrolysed with $N-H_2SO_4$ (50 ml.) at 100° for 7.5 hr. An equivalent of saturated $Ba(OH)_2$ solution was added to the cooled hydrolysate, the mixture diluted to 250 ml. and filtered. The filtrate gave

ash and low molecular impurities by dialysis in collodion sacs against repeated changes of distilled water until the dialysate was neutral (72 hr.). The dialysed polysaccharide solutions were evaporated *in vacuo* to about 10% concentration and fractionated by addition of increasing proportions of ethanol. Properties of the isolated fractions are summarized in Table 3. Acid equivalents were determined by titration of 0.02 g. of polysaccharide in 5 ml. water with 0.01 N-NaOH (CO_2 free)

Table 3. *Ethanol fractionation of crude polysaccharides from strains of Penicillium luteum*

Source of polysaccharide, etc.	Fraction no.	Conc. of ethanol (% w/w)	Weight (g.)	Acid equiv.	$[\alpha]_D^{20^\circ}$ (°)
Strain F 6 (25.02 g. dialysed; recovery, 14.50 g.; 57.9 %)	(Insol.)	0	1.28	—	—
	1	44.1	4.09	4730	—
	2	61.2	4.10	6060	+21.6
	3	70.3	2.62	530	-18.1
	4	76.0	1.06	450	-14.0
Strain F 39 (39.63 g. dialysed; recovery, 4.82 g.; 12.2 %)	(Sol.)	—	1.35	—	—
	1	50.8	2.30	1930	+ 5.0
	2	56.5	1.78	2670	+11.0
	3	63.0	0.38	1240	- 7.2
	4	70.3	0.17	890	—
Strain F 46 (38.2 g. dialysed; recovery, 11.36 g.; 29.7 %)	(Sol.)	—	0.04	—	—
	(Sol.)	—	0.15	—	—
	(Insol.)	0	1.66	—	—
	1	39.3	0.90	—	—
	2	48.5	0.12	1260	—
Strain F 51 (35.6 g. dialysed; recovery, 6.56 g.; 18.4 %)	3	53.5	0.35	1790	+ 6.9
	4	54.8	0.73	2380	+14.4
	5	56.9	1.06	2850	+16.3
	6	59.8	0.45	730	-13.8
	7	69.1	0.98	1300	+ 1.3
	8	70.0	2.85	630	-14.8
	9	70.5	1.78	620	-19.2
	10	76.0	0.05	—	—
	(Sol.)	—	0.43	—	—
	1	47.0	4.32	1440	- 2.0
Strain F 52 (41.7 g. dialysed; recovery, 15.92 g.; 38.2 %)	2	58.9	1.91	1180	-13.0
	3	63.4	0.09	—	—
	4	76.0	0.05	—	—
	(Sol.)	—	0.19	—	—
	1	54.5	8.99	2060	+17.6
Strain F 231 (19.0 g. dialysed; recovery, 1.68 g.; 8.8 %)	2	58.0	2.53	600	-12.0
	3	63.6	2.60	440	-22.5
	4	70.2	1.20	440	-22.2
	5	76.0	0.21	440	-22.3
	(Sol.)	—	0.39	—	—
	1	52.0	0.17	3320	—
	2	56.0	0.55	13000	+21.7
	3	65.7	0.64	6210	+13.9
	4	71.8	0.12	5470	—
	5	76.0	0.05	—	—
	(Sol.)	—	0.15	—	—

Table 4. *Properties of polysaccharide fractions (Strain F 6)*

Fraction	$[\alpha]_D^{20^\circ}$ (°)	Ash (%)	pH of 1 % solution	R.V. (% apparent glucose)		Mucic acid on oxidation (%)
				Before hydrolysis	After hydrolysis	
1	—	0.55	4.21	1.50	86.0	38.3 (m.p. 210°)
2	+21.6	0.20	4.91	1.49	92.8	—
3	-18.1	0.0	3.20	3.81	84.3	3.3 (m.p. 210°)
4	-14.0	0.80	3.46	8.73	79.7	—

using phenolphthalein as indicator. Specific rotation data refer to aqueous solutions, $c=1$. In the case of the fractions from strain F 6, reducing power was determined before and after hydrolysis and the products of nitric acid oxidation examined (Table 4). Reducing power before hydrolysis was determined with 0.02 g. quantities by Hanes's modification of the method of Somogyi (1937; see Hockenhull & Herbert, 1945). For determination of reducing power after hydrolysis, the polysaccharides (0.02 g.) were hydrolysed for 2 hr. at 100° with 2N-HCl in sealed tubes, neutralized, and reducing

sugars determined as above. Samples of polysaccharide (0.5 g.) were oxidized by the method of Kent & Tollens (1885), and the yield of mucic acid and its melting point recorded. (All melting points are corrected.)

Strain F 6 was shown to yield a mixture of at least two polysaccharides which were separated by ethanol fractionation into a dextrorotatory, neutral polysaccharide (F 1 and F 2) and a laevorotatory, acidic polysaccharide (F 3 and F 4). The former appeared to predominate in the crude polysaccharide. The dextrorotatory polysaccharide yielded mucic

acid (38.3%) on nitric acid oxidation, whereas the laevorotatory polysaccharide gave only an insignificant yield of the acid.

Strain F 39 gave a dextrorotatory, neutral polysaccharide, and in addition a very small amount of a laevorotatory polysaccharide.

Strain F 46 crude polysaccharide was shown to contain a dextrorotatory, neutral polysaccharide and a larger quantity of a laevorotatory, acidic polysaccharide.

Strain F 51 gave laevorotatory products only, which were weakly acidic (acid equivalent 1200).

Strain F 52 gave approximately equal quantities of a dextrorotatory, neutral polysaccharide and a laevorotatory, acidic polysaccharide ($[\alpha]_D^{20} -22^\circ$, acid equivalent 440, corresponding to approximately one equivalent of acid for every three anhydrohexose units in the polysaccharide). Fraction 1 (7.8 g.) was refractionated between ethanolic concentrations of 50 and 60% (w/w) and shown to consist mainly of a dextrorotatory, neutral polysaccharide ($[\alpha]_D^{16} +21.6$, acid equivalent 3600, 4.27 g.). Nitric acid oxidation of fraction 3 ($[\alpha]_D -22.5^\circ$) gave oxalic acid (6.3%), and no mucic acid was detected.

Strain F 231 crude polysaccharide gave a very low recovery (8.8%) on dialysis. The product consisted of dextrorotatory, neutral polysaccharides. No laevorotatory substances were detected. Nitric acid oxidation of fraction 2 gave mucic acid (m.p. 207° , yield 43.9%). This fraction had R.v. before hydrolysis of 0.5% as apparent glucose. It corresponded closely in its properties to the dextrorotatory polysaccharide isolated from strain F 6.

Dextrorotatory polysaccharide from strain F 6

Isolation and properties of the polysaccharide. Preliminary experiments showed that the most satisfactory method of isolation of the pure dextro- and laevorotatory polysaccharides was by further ethanolic fractionation of the dextro- and laevo-rotatory fractions obtained in the preliminary ethanolic fractionation of the dialysed polysaccharide. The dextrorotatory polysaccharide was normally precipitated when the ethanolic concentration reached 50–60%. The following results were obtained in a typical experiment. The crude polysaccharide (40.7 g.) was dissolved in water and the insoluble fraction centrifuged off. The latter was twice extracted with water and the extracts combined with the main extract. The dried insoluble fraction weighed 3.57 g.

The solution (150 ml.) was made acid to pH 2 with HCl and dialysed for 6 days against several changes of distilled water. The dialysed polysaccharide solution was concentrated *in vacuo* to 63 g. and ethanol added to 50% concentration (w/w). The precipitated polysaccharide was dried in the usual way; 8.18 g., acid equivalent 6000. The polysaccharide (6.93 g.) was dissolved in water (100 ml.) and divided into seven fractions by addition of increasing proportions of ethanol (Table 5). The acid equivalents and specific rotations of the fractions were determined. The main fractions (nos. 3–5, 5.25 g., 75.7% of the starting material) were practically identical, being virtually neutral and having specific rotations in the range $+23.0$ to $+29.7^\circ$. Further ethanolic fractionation showed that fraction 3 consisted essentially of polysaccharide with specific rotation of $+27.5^\circ$. Fractions with specific rotations in the range $+27.5$ to $+29.7^\circ$ were regarded as the pure dextrorotatory polysaccharide and were used in the experiments described below. The pure substance was ash-free; it contained 0.2–0.5% N, but was free from S and halogens.

Hydrolysis of polysaccharide. Preliminary experiments showed that liberation of reducing sugars from the polysaccharide on hydrolysis with $2N-H_2SO_4$ at 100° was at a maximum after 8 hr. The polysaccharide (1.93 g.) in $2N-H_2SO_4$ (100 ml.) was hydrolysed under these conditions. The ice-cold hydrolysate was carefully neutralized with hot saturated $Ba(OH)_2$, a few drops of $N-H_2SO_4$ added and finally neutralized with an excess of pure $BaCO_3$. The precipitated $BaSO_4$ was centrifuged off and washed; the combined supernatant solution and washings were evaporated *in vacuo* to 100 ml. The neutralized hydrolysate had a total reducing value corresponding to 1.50 g. apparent glucose, i.e. 77.8% of the polysaccharide (1.56 g. as apparent galactose, i.e. 80.9% of the polysaccharide); its optical rotation α_D^{16} was $+1.065^\circ$ (1 dm.) and specific rotation $[\alpha]_D^{16} +71.0^\circ$ (R.v. as glucose) and $+68.2^\circ$ (R.v. as galactose).

Identification of component sugars. Partition chromatograms on paper were made with samples of the neutralized hydrolysate described above (Partridge, 1946, 1948; Forsyth, 1948). The chromatograms were developed for 72 hr. at 20° . *n*-Butanol, ethanol, water (40, 10, 50% v/v) and *n*-butanol were used as solvents. D-Galactose, D-glucose, D-fructose and D-mannose were included as reference sugars.

Butanol-ethanol-water. The chromatograms gave only a single spot corresponding to galactose. Glucose and mannose

Table 5. *Homogeneity of dextrorotatory polysaccharide (Strain F 6)*

(Dextrorotatory fractions of dialysed polysaccharide were refractionated with ethanol and properties of the fractions determined.)

Fraction	Conc. of ethanol (%, w/w)	Weight (g.)	Acid equiv.	$[\alpha]_D^{20}$ ($^\circ$)	Nitrogen (%)
1	20.5	0.20	3100	—	—
2	49.3	0.40	6300	—	—
3	52.5	2.44	15000	$+23.0$	—
3a*	—	—	10000	$+27.5$	0.47
4	54.8	1.95	14000	$+28.9$	—
5	57.7	0.86	24000	$+29.7$	0.18
6	69.0	0.26	7000	—	—
7	Mother liquor	0.23	940	—	—
Recovery, 6.34 g., 91.5%					

* Fraction 3a was obtained by reprecipitation of fraction 3, three times between 49.5 and 53.0% alcoholic concentration.

were absent. The sensitive resorcinol reagent (Forsyth, 1948) was used to demonstrate the absence of ketose sugars.

Butanol. The chromatograms clearly showed the presence of galactose and the absence of glucose, mannose and fructose.

Quantitative determinations of galactose in the polysaccharide hydrolysate were carried out by the partition chromatographic technique described by Flood, Hirst & Jones (1947, 1948). The polysaccharide (10 mg.) and L-rhamnose (5 mg.) as reference sugar were hydrolysed with 0.4 ml. 2 N-H₂SO₄ in a sealed tube for 1 hr. at 100°. The neutralized hydrolysate was transferred to the chromatograms and developed with *n*-butanol, ethanol, water (40, 10, 50% v/v) for 72 hr. at 20°. Recoveries of galactose and rhamnose corresponding to 88% of the former in terms of the polysaccharide were obtained.

Yeast fermentation of polysaccharide hydrolysate. The chromatographic analyses described above showed that galactose was the main component sugar of the polysaccharide. The chromatograms demonstrated the absence of significant quantities of glucose, fructose and mannose. The absence of reducing sugars, other than galactose and those normally fermented by yeast, was confirmed by fermentation of the neutralized hydrolysate with a strain of *Saccharomyces carlsbergensis* Hansen (N.C.T.C. 742), which had been trained to ferment galactose. The organism was passaged three times in a medium containing 10 parts of hydrolysed lactose (5 g. lactose/l. hydrolysed with 0.5 N-HCl for 1.5 hr. at 100° and neutralized to pH 5-6) and 1 part of beer wort. The cells were washed twice with saline and suspended in the polysaccharide hydrolysate at 30° and pH 5-6 so that the cell density was about 80 × 10⁶/ml. and fermentation was allowed to proceed for 96 hr. (Wise & Appling, 1944). A parallel fermentation with a similar concentration of D-galactose was carried out as a control. Reducing value determinations were made with 0.2 ml. samples of the supernatant solutions, freed from cells by centrifugation, by Hanes's modification of Somogyi's (1937; see Hockenull & Herbert, 1945) method (Table 6). The

acetic acid (5 ml.) added to give an acetic acid concentration of about 90% and the mixture allowed to stand at 0°. After seeding with a trace of D-galactose a mass of colourless crystals was obtained which was filtered off and washed with glacial acetic acid; yield of crude D-galactose, 1.24 g. (51.0%). The crude product (m.p. 160-163°) was once recrystallized from 90% acetic acid, and almost pure D-galactose obtained, m.p. 163°, unchanged on admixture with authentic D-galactose, m.p. 166°. $[\alpha]_D^{18} + 113^\circ$ after 5 min. $\rightarrow +79^\circ$ after 19 hr.

D-Galactose methylphenylhydrazine. On treatment with 0.2 g. methylphenylhydrazine (asym. redistilled: b.p. 104° at 14 mm.) and 0.1 g. 50% acetic acid the sugar (0.25 g.) in 50% ethanol gave crystalline D-galactose methylphenylhydrazine (0.33 g.; 84% of the theoretical; Neuberg, 1907; Lüdtke, 1929). The crude product was recrystallized from hot pyridine, ethanol (1:1, v/v) mixture; after one to two crystallizations pure D-galactose methylphenylhydrazine, m.p. 189°, was obtained. The melting point was undepressed on admixture with the authentic hydrazine (m.p. 189°). (Found (Weiler and Strauss): C, 54.8; H, 7.1; N, 9.2. Calc. for C₁₅H₂₀N₂O₅: C, 54.9; H, 7.1; N, 9.9%.)

A further galactose determination on the neutralized hydrolysate was made by the methylphenylhydrazine method of Hirst, Jones & Woods (1947). A portion of the hydrolysate corresponding to 0.9637 g. of polysaccharide was treated with the methylphenylhydrazine reagent and gave 0.7767 g. galactose methylphenylhydrazine, which corresponds to 0.5358 g. galactose according to the formula of Hirst *et al.* (1947), i.e. to 55.6% of the polysaccharide.

The excess methylphenylhydrazine was removed as benzaldehyde methylphenylhydrazine from the mother liquor in the above determination as described by Morgan (1938). The volume of the residual solution was adjusted to 25 ml. The total reducing value corresponded to 0.0578 g. apparent glucose, i.e. 6.0% of the polysaccharide. The remainder (24 ml.) was evaporated to a syrup, dissolved in methanol (5 ml.) and treated with *p*-nitrophenylhydrazine (0.1 g.) as described by van Ekenstein & Blanksma (1903; Reclaire, 1908). No crystalline hydrazine was obtained under these conditions.

D-Galactose p-nitrophenylhydrazine. Neutralized hydrolysate equivalent to 2.275 g. polysaccharide was evaporated *in vacuo* to a thick syrup, which was dissolved in methanol (15 ml.) and heated with *p*-nitrophenylhydrazine (2.0 g.) for 10 min. on the water bath. A bright yellow microcrystalline solid soon separated on cooling. The product was filtered off on a sintered-glass crucible and washed with ice-cold ethanol and ether. On evaporation of the mother liquor and washings nearly to dryness *in vacuo* and addition of absolute methanol (10 ml.) further crops of the hydrazine were obtained. Yield of crude hydrazine 2.255 g., corresponding to 56.6% of the polysaccharide. The crude substance softened at 184° and melted with decomposition at 185°. It was recrystallized from boiling absolute ethanol, m.p. 194°. On admixture with authentic D-glucose *p*-nitrophenylhydrazine (m.p. 186°) the melting point was depressed to 184°. On admixture with authentic D-galactose *p*-nitrophenylhydrazine (m.p. 195°) the melting point was undepressed at 194°. (Found: C, 46.0; H, 5.3; N, 12.7. Calc. for C₁₂H₁₇N₃O₇: C, 45.7; H, 5.4; N, 13.3%.)

Acetylation of polysaccharide. Preliminary experiments were made with the crude polysaccharide to determine the optimal conditions for acetylation. The pure substance (0.545 g.) was dissolved in water (2 ml.); pyridine, redistilled

Table 6. *Fermentation of dextrorotatory polysaccharide hydrolysate by Saccharomyces carlsbergensis*

Time (hr.)	Polysaccharide hydrolysate		D-Galactose	
	Reducing value of fermentation mixture as		Reducing value of fermentation mixture as	
	apparent glucose (mg./100 ml.)	Residual R.V. (% of initial)	apparent glucose (mg./100 ml.)	Residual R.V. (% of initial)
0	383	100	243	100
72	9.5	2.5	6.5	2.7
96	8.4	2.2	2.3	0.9

galactose was rapidly fermented until less than 1% of the original reducing power remained after 96 hr. Similar results were obtained with the hydrolysate, which retained only 2.2% of the original reducing power after 96 hr. fermentation. It is concluded that less than 2.2% of the reducing substances (i.e. 1.8% of the polysaccharide) were present as sugars other than glucose, fructose, mannose and galactose.

Isolation of D-galactose and derivatives. A sample of neutralized hydrolysate corresponding to 2.435 g. polysaccharide was evaporated *in vacuo* to a thick syrup, glacial

over KOH, b.p. 115° (25 ml.), was added and followed by redistilled acetic anhydride (25 ml.); the latter was added gradually so that the temperature of the mixture did not exceed 35°. The mixture was kept at 20° for 48 hr.; at this stage a sample gave no precipitate on pouring into ice-cold water. The solution was heated under reflux at 90° for 6 hr., cooled, and poured into 500 ml. of ice-cold water giving an immediate precipitate of almost colourless acetyl polysaccharide. The latter was filtered off, washed free from acid with water and dried *in vacuo* over P₂O₅. Yield, 0.690 g., i.e. 71.2% of the theoretical yield of triacetyl compound. Fractionation of the acetyl derivative showed that it was homogeneous. The crude substance (0.531 g.) was dissolved in CHCl₃ (15 ml.) and three times reprecipitated by addition of 3 vol. ether. The third precipitate was redissolved in CHCl₃ (10 ml.) and fractionated by addition of increasing proportions of ether. The main fractions were precipitated by 59% (v/v) and 75% (v/v) of ether, respectively; their weights were 0.213 and 0.120 g., total recovery 0.405 g. (76.3%). The specific rotations of the fractions were respectively, $[\alpha]_D^{17} + 38.3^\circ$ and $+44.1^\circ$ in CHCl₃ (c, 0.4) and their acetyl contents, 46.2 and 49.2% (theory for triacetyl hexosan, 44.8%).

Benzoylation of polysaccharide. The polysaccharide (1.04 g.) was benzoylated by the Schotten-Baumann method. The crude benzoylated product (1.66 g., 54.5% of the theoretical for the tribenzoyl hexosan) separated almost immediately as an almost colourless precipitate. It was soluble in acetone and CHCl₃, but insoluble in ether and water. After fractional precipitation from CHCl₃ solution with ether the main fraction had a specific rotation of $[\alpha]_D^{18} + 18^\circ$ in CHCl₃ (c, 0.6).

Laevorotatory polysaccharide from strain F 6

Isolation and properties of the polysaccharide. After removal of the dextrorotatory polysaccharide from solutions of the crude dialysed polysaccharide by addition of ethanol to 50–60% concentration, the mother liquor consisted predominantly of laevorotatory polysaccharide. A portion of crude laevorotatory polysaccharide precipitated from these mother liquors by increasing the ethanolic concentration to 80% (w/w) (9.68 g., $[\alpha]_D^{16} - 2.1^\circ$, acid equivalent 770) was redissolved in water (200 ml.), centrifuged to remove insoluble material and the clear, pale golden-brown coloured supernatant solution divided into six fractions by addition of increasing concentrations of ethanol. (The insoluble fraction formed a thick viscous paste on addition of water, but did not dissolve. It was readily soluble in N-NaOH and slowly soluble in 2N-Na₂CO₃, but insoluble in acids. No precipitate was obtained on acidification of the alkaline solution.) After removal of the first ethanolic precipitate (51.5% ethanol), the supernatant solution was decolorized by treatment with animal charcoal (2 g.) at 20° for 18 hr. The charcoal was centrifuged off and the ethanolic fractionation continued. After precipitation and centrifugation of fraction 3, the opalescent mother liquor was evaporated *in vacuo* to 35 g. and fractionation continued by addition of sufficient ethanol (70 ml., 61.4%) to produce an appreciable further precipitate. The fractions were precipitated as clear, colourless syrups which were desiccated with difficulty by treatment with increasing concentrations of ethanol; they were finally washed with ether (dried over Na) and dried *in vacuo* over

P₂O₅. The yields and properties of the fractions are summarized in Table 7. The results showed that the main laevorotatory fractions were practically identical, having specific rotations in the range -13° to -17° and acid equivalents of 680–825 (mean 765, which corresponds to approximately one acidic group per five anhydrohexose units).

Table 7. *Homogeneity of laevorotatory polysaccharide (Strain F 6)*

(Laevorotatory fractions of dialysed polysaccharide were refractionated with ethanol and properties of the fractions determined.)

Fraction no.	Conc. of ethanol (% w/w)	Weight (g.)	Acid equiv.	$[\alpha]_D^{18}$ (°)
Insol.	0	1.294	941	-5.0 (in 0.1N-NaOH)
1	51.5	0.183	1580	—
2	59.0	2.713	1296	+ 5.8
3	68.2	2.962	793	-10.4
3a*	—	—	825	-13.3
4	61.4	0.421	682	-16.9
5	70.3	0.425	790	-14.5
6	75.9	0.088	971	—
Mother liquor	—	0.392	—	—
		Recovery, 8.478 g., 87.5%		

* Fraction 3a was obtained by fractionation of fraction 3, between 75 and 99% ethanol concentration (w/w).

The polysaccharide (10 mg.) gave no reaction for ketose sugars in the Seliwanoff test after 15 min. at 100°; under the same conditions fructose (2 mg.) gave a definite pink coloration after 2–5 min. On hydrolysis with N-H₂SO₄ (0.0577 g. polysaccharide in 25 ml.) at 100°, the initial reducing value of 5.3% as apparent glucose rapidly increased to a maximum of 67.8% as apparent glucose after 3 hr., and thereafter decreased at an approximately linear rate, reaching 60.0% after 6 hr. A sample of polysaccharide (0.4984 g.) was dissolved in 100 ml. N-H₂SO₄ and hydrolysed under these conditions. The hydrolysate (5 ml.) was treated with the equivalent of Ba(OH)₂ (12.9 ml., 0.387N) and the precipitate of BaSO₄ centrifuged off. The supernatant solution (10 ml.) had pH 8.36 and required less than 0.1 ml. 0.1N-H₂SO₄ for neutralization. It was clear that no acid was liberated on hydrolysis. The remainder of the hydrolysate ($\approx$ 0.440 g. polysaccharide) was neutralized in the usual way and the neutralized hydrolysate diluted to 50 ml. Its reducing power was 69.6% (as apparent glucose) and its specific rotation on this basis was $+47.8^\circ$. On heating at 100° for 30 min. with phenylhydrazine-HCl and sodium acetate, glucosazone was obtained. The solution of neutralized hydrolysate was used in the experiments described below.

Identification of component sugars. Chromatographic analyses of the laevorotatory polysaccharide hydrolysate were carried out as described above. The position of the isolated sugars on the chromatogram was detected by means of the ammoniacal AgNO₃ and resorcinol reagents.

Butanol-ethanol-water. The chromatograms gave only a single spot corresponding to glucose. Galactose, mannose and fructose were shown to be absent by use of the authentic sugars for reference purposes.

Quantitative determinations of glucose were made by the method of Flood *et al.* (1947, 1948). The polysaccharide (11.2 mg.) and L-rhamnose (5.0 mg.) as reference sugar were hydrolysed with 0.4 ml. 2N-H₂SO₄ in a sealed tube for 1 hr. at 100°. The chromatograms were developed with *n*-butanol, ethanol, water (40, 10, 50 v/v) for 72 hr. at 20°. The recoveries of glucose and rhamnose corresponded to 63.6% of glucose in the polysaccharide.

Acetylation of polysaccharide. The laevorotatory polysaccharide (0.529 g.) was dissolved in water (2 ml.) and pyridine (25 ml.), and acetic anhydride (25 ml.) added as described above. After 48 hr. at 20° a colourless precipitate had formed. This was filtered off, washed free from acid with water and dried *in vacuo* (0.588 g., i.e. 62.5% of the theoretical yield of triacetylpolysaccharide). The product had the unusual property of insolubility in CHCl₃, pyridine and acetone as well as in ether and water. (Found: C, 47.7; H, 5.7; acetyl, 30.7. C₁₂H₁₆O₈ (C₆H₇O₅(CH₃CO)₃) requires C, 50.0; H, 5.6; acetyl, 44.8%.)

The mother liquor was heated at 90° for 6 hr. and poured into ice-cold water. No further precipitate of acetylpolysaccharide was obtained.

Benzoylation of polysaccharide. The polysaccharide (0.55 g.) benzoylated normally by the Schotten-Baumann method giving 0.96 g. (59.6% of the theoretical yield) of crude benzoyl polysaccharide. The crude product (0.793 g.) was dissolved in CHCl₃ (5 ml.), twice reprecipitated by addition of 3 vol. ether, and redissolved in CHCl₃. The solution was fractionally precipitated with ether and shown to be homogeneous. The pure benzoyl polysaccharide had a specific rotation, $[\alpha]_D^{16} + 3.3^\circ$ in CHCl₃ (c, 0.6).

Action of enzymes on the polysaccharides. Solutions of the dextro- and laevo-rotatory polysaccharides (2%, 1 ml.) were diluted with water (18.5 ml.) and a 1% solution of a malt amylase (mixture of α - and β -amylases) preparation (0.5 ml.) added; pH 4.6 and 30°. There was no increase of reducing power after 24 hr. incubation. The polysaccharides (2%, 1 ml.) were diluted with water (18.9 ml.) and invertase (yeast autolysate preparation) (0.1 ml.) was added; pH 4.6 and 30°. There was no increase of reducing power after 19 hr. incubation. The polysaccharides (2%, 1 ml.) were diluted with water (18.5 ml.) and 1% emulsin (British Drug Houses Ltd.; 0.5 ml.) added; pH 7 and 30°. There was no change in reducing power after 42 hr. incubation. The experiments showed that the polysaccharides were not attacked by α - or β -amylases, invertase or β -glucosidase.

Growth and polysaccharide production by Penicillium luteum F 6 on various carbon sources

A series of media was prepared, based on the Czapek-Dox formula, each containing one of the following substances as carbon source: glucose, galactose, lactose, maltose, soluble starch (2%), glycerol, sodium citrate cryst. (9.7%), sodium tartrate cryst. (6.4%), sodium acetate cryst. (3.8%), sucrose, xylose (1%), dulcitol (1%), mannitol (1%), sorbitol (1%), ethanol (2.5%) (concentration 5%, unless otherwise stated.) Three 'Glaxo' bottle cultures (350 ml. each) were sown with equal volumes of a spore suspension of *P. luteum* F 6 for each carbon source. The initial pH of the media was pH 6–7. Observations on the growth rate of the fungus, the fall of reducing power in the sugar-containing media and pH changes are recorded in Table 8. The cultures were harvested after incubation at 25° for the periods stated in

Table 8, the mycelium filtered off, dried and weighed. The culture filtrates were evaporated *in vacuo* to about 0.1 vol. and the ethanol-insoluble fraction, including polysaccharides, precipitated by addition of a slight excess (2–4 vol.) of ethanol. Samples (0.5 g.) of the crude 'polysaccharides' were oxidized with nitric acid by the Kent & Tollens (1885) method; the yields and melting points of the crude oxidation products are recorded in Table 8.

The mycelial growth on the galactose medium was heavier and more vigorous than that on any other medium; it differed significantly from that of the glucose medium, which was white and slimy with a light cream coloured culture filtrate, in its production of green conidial areas. The mycelium was reddish brown on its underside and the culture filtrate reddish brown in colour. Lactose also formed early (5 days) sporulation, but mycelial growth was slow and sparse. No growth took place on citrate or tartrate as sole source of carbon. The acetate medium gave rise to a trace of colourless submerged mycelium. Similar results were obtained with dulcitol, mannitol and sorbitol. Dulcitol and mannitol were recovered unchanged in yields of 95 and 85%, respectively, after incubation for 34 days. A thick syrupy product, which did not crystallize on standing, was obtained from the sorbitol medium; it was probably crude sorbitol. Moderately heavy growth of pink-coloured mycelium was obtained on the ethanol medium, which favoured the production of isolated colonies rather than a continuous felt. The observations of mycelial growth, recorded in columns 3–5 of Table 8, were visual estimates made during growth; they were not in every case supported by the mycelial weights finally obtained.

Reducing power determinations after 19 days' incubation showed that galactose was metabolized more rapidly than glucose; at this stage the mean galactose content was 0.60 g./100 ml. and that of glucose 0.89 g./100 ml. After incubation for 19 days the pH of the glucose medium fell to 2.63 and ultimately rose again to 3.90; the corresponding values for the galactose medium were 3.96 and 5.14.

The ethanol-precipitated fractions from the glucose, galactose, maltose, starch, glycerol, xylose and ethanol media were undoubtedly constituted mainly of polysaccharides. The product from the lactose medium (10.8 g., 20.6%) was the unchanged sugar; it reduced Fehling solution and its m.p. of 200° was undepressed on admixture with authentic lactose. On heating with acetic anhydride and sodium acetate it gave 59% of the theoretical yield of lactose octa-acetate, m.p. 95–100°, undepressed on admixture with authentic lactose octa-acetate. The sparse growth with this disaccharide, as compared with that on media containing its hydrolysis products, suggests that the fungus may not produce lactase. The bulk of the substrate was, however, probably metabolized by some other route since only 21% was recovered and the rotation ($\alpha_D^{17.5} + 1.39^\circ$, 1 dm.) of the culture filtrate was not in agreement with its reducing power (4.11 g./100 ml. as apparent lactose) on the assumption that the sugar was the main component. On addition of ethanol (2 vol.) to the concentrated culture filtrate a small immediate precipitate was obtained. On standing for a few hours the supernatant solution deposited lactose as a crystalline mass.

The culture filtrate from the galactose medium was deep red in colour. The coloured component was separated by extraction with CHCl₃ and on evaporation of the solvent orange-red crystalline needles were obtained (0.02 g., 0.04% of sugar fermented, m.p. 99–104°).

Table 8. *Growth of Penicillium luteum* F 6 on various sources of carbon

Carbon source	Conc. (%)	Mycelial growth after						pH of culture filtrate	Cultures harvested after incubation (days)	Mycelium weight (g.)	Yield of polysaccharide		Oxidation product		Reducing value as glucose	
		9 days	21 days	27 days	4 days	27 days	of final conc. (%) of culture filtrate				(g.)	Percentage of substrate			Before hydrolysis	After hydrolysis with 2N-HCl in sealed tube, 2 hr.
Glucose	5.0	3	4	4	4	4	0.19*	3.90	27	6.10	3.64	6.93	Mucic acid, m.p. 202°	10.9	1.7	58.9
Galactose	5.0	4	4	4	4	4	0.15†	5.14	30	8.70	1.86	3.54	Mucic acid, m.p. 215°	23.3	1.7	28.9
Lactose	5.0	1	2	2	2	2	4.11†	7.13	30	1.04	Trace	—	—	—	55.7 (83.4§)	82.3
Maltose	5.0	3	4	4	4	4	0.34†	4.00	27	7.38	2.58	4.91	Mucic acid, m.p. 209°	20.0	2.1	50.3
Soluble starch	2.0	4	4	4	4	4	0.0	7.54	28	3.34	3.64	17.3	Mixture of mucic acid, m.p. 207° and oxalic acid, m.p. 99°	2.0	3.0	24.6
Glycerol	5.0	2	4	4	4	4	0.0	5.90	27	0.87	1.45	2.76	Mucic acid, m.p. 206°	7.6	1.1	26.2
Citrate (Na ₂ C ₆ H ₅ O ₇ · 5H ₂ O)	9.7	0	0	0	0	0	—	—	27	0.0	0.0	0.0	—	—	—	—
Tartrate (Na ₂ C ₄ H ₄ O ₆ · 2H ₂ O)	6.4	0	0	0	0	0	—	—	27	0.0	0.0	0.0	—	—	—	—
Acetate (NaC ₂ H ₃ O ₂ · 3H ₂ O)	3.8	1	1	1	1	1	—	9.13	28	Trace	(2.01)	(5.04)	No mucic acid formed	—	0.7	2.9
Sucrose	5.0†	2	2	2	3	3	2.85†	3.22	34	2.50	1.58	3.01	Oxalic acid, m.p. 102°	13.3	1.0	48.7
Xylose	1.0	2	3	4	4	4	0.04*	3.84	27	2.55	1.41	13.4	Undetermined	14.7	1.7	14.9
Dulcitol	1.0	1	1	1	1	1	—	7.40	34	0.45	0.0	0.0	—	—	—	—
Mannitol	1.0	1	3	3	3	3	0.0	6.00	34	0.50	0.0	0.0	—	—	—	—
Sorbitol	1.0	1	1	1	1	1	—	7.08	34	0.60	0.0	0.0	Undetermined	—	—	—
Ethanol	2.5	2	2	2	3	3	0.0	5.66	34	1.11	1.47	5.60	Mucic acid, m.p. 196°	5.5	0.8	15.0

Mycelial growth key: no growth, 0; trace of growth, 1; fairly good growth, 2; good growth, 3; abundant growth, 4.
* As glucose. † As sugar specified. ‡ As invert sugar. § As anhydrous lactose.

After incubation for 28 days the soluble starch medium gave no I_2 reaction, showing that *P. luteum* excretes α -amylase into the medium. After concentration to 0.05 vol. the culture filtrate was non-viscous. The yield of polysaccharide (17.3%) and presence of mucic acid in its oxidation products showed that the starch had been metabolized by the fungus.

The culture filtrate from the acetate medium (pH 9.13) required 10 ml. N-HCl for neutralization to pH 6.93. The filtrate had no detectable rotation in a 1 dm. tube. The ethanolic precipitated fraction was non-polysaccharide in character; it gave a negative Molisch reaction and did not reduce Fehling's solution.

Growth and fermentation of the sucrose medium was poor as compared with the media containing the monosaccharides and maltose. After 34 days, 2.85 g./100 ml., i.e. 57% of the initial sucrose, remained unfermented.

Isolation of mucic acid (melting point undepressed on admixture with authentic mucic acid) on oxidation of the polysaccharide fractions from the glucose, galactose, maltose, starch, glycerol, and ethanol media showed that these substrates as sole sources of carbon were metabolized by *P. luteum* with the production of galactose-containing polysaccharides.

Growth of Penicillium luteum F 6 on the dextro- and laevo-rotatory polysaccharides as sole sources of carbon

Dextrorotatory polysaccharide. A medium based on the Czapek-Dox formula, containing the dextrorotatory polysaccharide (1%) as the sole source of carbon was sterilized in two portions of 10 ml. in conical flasks (50 ml.) by intermittent steaming at 100° on 3 successive days. The medium was inoculated with a weak spore suspension of *P. luteum* F 6, precautions being taken to avoid removal of nutrients from the agar slope in transferring the spores. The cultures were incubated at 25°. (Control cultures with medium containing salts, but no added carbohydrate, gave only traces of mycelial growth after 37 days.) After incubation for 37 days the polysaccharide medium contained only a trace of imponderable, semi-submerged mycelium.

Laevorotatory polysaccharide. A medium corresponding to that described above was prepared; it contained the laevo-rotatory polysaccharide (0.5%) as sole source of carbon. Nine portions of the medium (10 ml. each) were inoculated. After incubation for 5 days there was slight superficial growth with green conidial areas, and after 37 days the medium was covered with a continuous felt of colourless slimy mycelium, which was harvested, dried and weighed. The yield of mycelium (0.0994 g., i.e. 1.105 g./l. medium) was of the same order as that obtained from media containing lactose (5%), glycerol (5%), or ethanol (2.5%), Table 8. The combined culture filtrate slightly reduced Fehling solution. It had no detectable optical rotation.

Influence of Penicillium luteum polysaccharides on aggregation of soil particles

Geoghegan & Brian (1948a, b) showed that certain bacterial and mould polysaccharides had an aggregating effect on soil particles. The crude polysaccharide from *P. luteum* F 6, described in Table 2, had a definite aggregating effect, although less than that of levans and dextrans (Geoghegan & Brian, 1948b). The crude polysaccharides from strains

nos. F 39, 46, 51, 52 and 231 and the pure dextro- and laevo-rotatory polysaccharides of strain F 6 were tested for soil aggregating properties (Table 9). The products from strains F 46 and 51 had slight aggregating effects, although small compared with the crude product from strain F 6 or levan. The pure dextro- and laevo-rotatory polysaccharides had much lower activities than the crude product from which they were obtained by alcoholic fractionation.

Table 9. *Influence of Penicillium luteum polysaccharides on aggregation of soil particles*

Polysaccharide	Percentage aggregation when 0.25% of the polysaccharide was applied to soil
Crude products:	
Strain F 6*	50.1
Strain F 39	1.8
Strain F 46	4.3
Strain F 51	14.4
Strain F 52	1.3
Strain F 231	1.6
Dextrorotatory polysaccharide, F 6	5.3
Laevorotatory polysaccharide, F 6	23.6
Levan†	90.9
Control	1.3

* Geoghegan & Brian (1948b).

† Geoghegan & Brian (1948a).

DISCUSSION

It is clear from the properties of the dextro- and laevo-rotatory polysaccharides that they are different from the *P. luteum* products hitherto described. The most important properties are summarized in Table 10 for comparison with those of luteic acid, luteose and galuteose. Anderson & Raistrick (1936) reported the formation in the metabolic products of *P. luteum* Zukal of laevo-rotatory polysaccharides, built up of mannose, galactose or fructose units in proportions varying with the age of the culture. Mannose was isolated as the phenylhydrazone (yield, 5%) from some of the crude fractions from our strain F 6 and fructose was detected by partition chromatography, but no pure polysaccharides containing these sugars were isolated. It is concluded that glucose and galactose are the predominant units for polysaccharide synthesis by *P. luteum*. The fungus synthesized a glucose-containing polysaccharide from glucose, fructose, mannose, xylose, arabinose and glycerol as sole sources of carbon (Birkinshaw & Raistrick, 1933) and a galactose-containing polysaccharide from glucose, galactose, maltose, starch, glycerol, and ethanol, but not from acetate, citrate, tartrate, sucrose, dulcitol, mannitol or sorbitol as sole sources of carbon.

The chemical evidence described above supports the conclusion that the polysaccharides are chemical entities. The ultracentrifugal data presented in the

Table 10. *Properties of Penicillium luteum polysaccharides*

Property	Luteic acid (Raistrick & Rintoul, 1931)	Luteose (Raistrick & Rintoul, 1931)	Galuteose (Anderson <i>et al.</i> 1939)	Dextrorotatory	Laevorotatory
Specific rotation, $[\alpha]_{5461}^{\circ}$	-47 (as Na salt)	-46.4	—	—	—
Specific rotation, $[\alpha]_D^{\circ}$	—	—	-25	+29	-16
Acid equivalent	434.7	Neutral	—	Neutral	800
Component units (100 g.):					
D-Glucose (g.)	83	100	—	0	64
D-Galactose (g.)	—	—	66	88	0
D-Mannose (g.)	—	—	20	0	0
Malonic acid (g.)	23.4	—	—	—	—
Viscosity of aqueous solution	Viscous	Viscous	Non-viscous	Non-viscous	Non-viscous

following paper (Ogston, 1949) shows, however, that they are polydisperse. The dextrorotatory polysaccharide consists of components of molecular weight of approximately 50,000 to 200,000 and the laevorotatory polysaccharide of components with a mean molecular weight of 15,000. The behaviour of the organism in producing a series of polysaccharides indistinguishable in their chemical properties, but of different molecular complexity, may be related to its power of producing a range of polysaccharide products according to the period of incubation as reported by Anderson & Raistrick (1936).

Stacey (1947) recorded that the biological function of the extracellular mould polysaccharide is unknown, it being generally assumed to act as a reserve carbohydrate. This may be the case with the laevorotatory polysaccharide which sustained about the same amount of mycelial growth as media containing lactose, glycerol or ethanol as sole sources of carbon. The dextrorotatory polysaccharide was not, however, attacked to any significant extent.

P. luteum is common in soils. The marked effect of the crude polysaccharide from strain F 6 on the aggregation of soil particles is of interest. The fungus may take part in producing crumb structure in soil (Geoghegan, 1947). The polysaccharides are hygroscopic and the difficulty of desiccation of their aqueous solutions has been noted; the latter may be

of some biological significance to the fungus in delaying desiccation in soil.

SUMMARY

1. Isolation of two new polysaccharides from strains of *Penicillium luteum* series, grown on Czapek-Dox glucose medium, by ethanolic fractionation of dialysed culture filtrates is described.

2. The dextrorotatory neutral polysaccharide, $[\alpha]_D^{20} + 29^{\circ}$ in water (c, 1), was non-viscous in aqueous solution. D-Galactose (88 %) was the main component sugar; glucose, mannose and fructose were absent. The acetyl and benzoyl derivatives had specific rotations of $[\alpha]_D^{17} + 41^{\circ}$ in chloroform (c, 0.4) and $[\alpha]_D^{18} + 18^{\circ}$ in chloroform (c, 0.6), respectively.

3. The laevorotatory polysaccharide, $[\alpha]_D^{18} - 16^{\circ}$ in water (c, 1), was acidic (acid equivalent, 800). D-Glucose (64 %) was the main component sugar; galactose, mannose and fructose were absent. The acetyl polysaccharide was insoluble in the common organic solvents. The benzoyl derivative had a specific rotation of $[\alpha]_D^{16} + 3.3^{\circ}$ in chloroform (c, 0.6).

4. Galactose-containing polysaccharide was synthesized by the mould from glucose, galactose, maltose, starch, glycerol, and ethanol but not from acetate, citrate, tartrate, sucrose, dulcitol, mannitol or sorbitol as sole sources of carbon.

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The Sedimentation and Diffusion of Polysaccharides from *Penicillium luteum*; Interpretation of the Results Obtained from Polydisperse Material in the Gouy Diffusiometer

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Samples of the dextro- and laevo-rotatory polysaccharides, prepared from *Penicillium luteum* by Freeman & Macpherson (1949), were submitted for the examination of their ultracentrifugal sedimentation and diffusion.

EXPERIMENTAL

The solid materials were dissolved in, and thoroughly dialysed against, buffer of composition: NaCl, 0.2M; KH_2PO_4 , 0.027M; Na_2HPO_4 , 0.027M. The final concentrations were about 1 g./100 ml. and were estimated by refractometry against the buffer.

Fig. 1. Sedimentation diagrams: (a) dextrorotatory polysaccharide 37 min. after reaching full speed; (b) laevorotatory polysaccharide 80 min. after reaching full speed (60,000 rev./min.).

Sedimentation measurements. Sedimentation was observed in a Svedberg oil-turbine ultracentrifuge by the method of Philpot (1938), using the standard conditions of running recommended by Cecil & Ogston (1948).

Both samples proved to be polydisperse. The dextro-rotatory material (Fig. 1a) was composed of two main fractions: a more slowly sedimenting fraction which appeared to be homogeneous and a more rapidly sedimenting fraction which was heterogeneous. The sedimentation constant of the faster material and the relative proportions of the two fractions could not be determined accurately because the boundaries were not clearly resolved. However, separation of the schlieren curve into two more or less symmetrical parts (Fig. 2) gave an approximate estimate of the amounts of the two components. The combined boundary represented only 0.86 of the total refracting material.

The laevorotatory material (Fig. 1b) gave a single boundary, the thickness of which showed that the polysaccharide was polydisperse; it consisted of material sedimenting over a range of rates, symmetrically distributed about a mean value. Integration of this boundary showed that it repre-

Fig. 2. Tracing from sedimentation diagram (full line) of dextrorotatory polysaccharide, 47 min. after reaching full speed (60,000 rev./min.), to show analysis into components (broken lines); gradient of concentration dc/dx against position in cell, x .

sented only 0.64 of the total refracting material; however, this value may be too low, because the lower limit of the boundary appeared to be reaching the bottom of the cell before its upper limit had fully left the meniscus.

The results are given in Table 1.

Diffusion measurements. Diffusion was measured by means of the Gouy diffusiometer (Coulson, Cox, Ogston & Philpot, 1948). Ogston (1949) has shown that this method can be used to determine the diffusion constants of a mixture of two