ALLERGENIC GLUCANS FROM DERMATOPHYTES

PART I. ISOLATION, PURIFICATION, AND BIOLOGICAL PROPERTIES

M. J. How*, M. T. Withnall**, and C. N. D. Cruickshank

Department of Chemistry and the MRC Unit on Experimental Pathology of the Skin, University of Birmingham, Birmingham 15 (Great Britain)

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ABSTRACT

Structurally similar, allergenic α-D-glucans and a mixture of glycopeptides have been isolated from the mycelia of various dermatophytes by using mild methods. The glucans, which contain (1→4)-linked residues of α-D-glucose, are structurally different from glucans previously isolated from similar sources. The ability of the glucan and glycopeptide components of the mycelia to sensitize guinea pigs for "immediate" and "delayed" skin-reactions is investigated and the significance of these results is discussed.

INTRODUCTION

Over forty years ago Bloch and his associates[1] recognised the importance of hypersensitivity reactions in dermatophyte infections and concluded that an active principle that was extracted from the mycelia of the causative fungi was a "carbohydrate–protein" complex. Previous studies in these laboratories have shown[2] that the course of dermatophyte hypersensitivity in humans can be replicated in guinea pigs and that sensitized humans and animals show both "immediate" (Type I) and "delayed" (Type IV) skin-reactions, as defined by Gell and Coombs[3], when challenged by intradermal injection of a non-irritant, ethylene glycol extract of the fungal mycelium.

Subsequent studies[4–7] showed that the ethylene glycol extracts of mycelia from a wide range of pathogenic and non-pathogenic, keratinophilic fungi contained a mixture of structurally related, allergenic glycopeptides in which the preponderant sugars were D-galactose and mannose. The glycopeptides, which were fractionated by precipitation with cetyltrimethylammonium bromide (Cetavlon) in the presence of sodium tetraborate at pH 7–11.5, elicited both "immediate" and "delayed" skin-responses in guinea pigs that were sensitized with the whole mycelium. Studies of the biological activities of chemically and enzymically modified glycopeptides indicated

*Present address: Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford, Great Britain.

**Present address: The Biological Research Laboratories, May and Baker Ltd., Dagenham, Essex, Great Britain.

that the carbohydrate portion was important in mediating the "immediate" reaction, whereas the "delayed" reaction apparently depended upon the presence of the peptide component[5].

Clear-cut separations and quantitative recoveries of the components of the ethylene glycol extracts of mycelia were not achieved by using the Cetavlon–borate method of fractionation. Furthermore, fractions which were precipitated at an alkaline pH might have contained partially degraded components of the original extract. These disadvantages of the method of fractionation, together with the fact that none of the fractions thus isolated elicited skin reactions as large as those manifested by the original mycelial extract, prompted the investigations of milder methods of fractionation which are described in this paper. These investigations led to the isolation of hitherto unreported glucans from several species of dermatophytes, and the allergenic properties of these and other components of the mycelia are reported.

MATERIALS AND METHODS

Fungal cultures and fungal products. — *Trichophyton rubrum* (N.T.C. D. 353) was grown for 3 weeks at room temperature as a surface culture[2] on a medium containing D-glucose (4%) and Panmede (Paines and Byrne Ltd., Greenford, Middlesex, 2%), and the acetone-dried mycelium was extracted with ethylene glycol (3 × 400 ml/100 g of mycelium). The extract was centrifuged and the solution was successively dialysed against water, Seitz-filtered, and freeze-dried. Mycelia from *T. mentagrophytes*, *T. schoenleinii*, *Microsporum canis*, and *Keratinomyces ajelloi*, grown in surface culture, and from *T. rubrum* that was grown for 6 days in submerged culture[8] in a medium containing D-glucose (4%), Panmede (1%), and acid-hydrolysed casein (2%), were extracted similarly.

Isolation and purification of polysaccharides from ethylene glycol extracts of fungal mycelia. — In a typical experiment, a solution of the ethylene glycol extract (1.34 g) in water (12.5 ml) was eluted with water from a column (58 × 2.8 cm) of Bio-Gel P-300, and fractions (5 ml) of the eluate were assayed for carbohydrate[9] and for reaction with ninhydrin[10]. The contents of appropriate tubes were combined and freeze-dried. A solution of the crude polysaccharide (0.59 g) thus obtained, in 5mM phosphate buffer (pH 7.0, 5 ml), was eluted from a column (39 × 2 cm) of DEAE-Sephadex A-50 (chloride form) with phosphate buffer (125 ml), followed by sodium chloride (0 to 0.1M) in phosphate buffer. Fractions (5 ml) were assayed as described, and the contents of appropriate tubes were combined, dialysed against water, and freeze-dried.

Analytical studies of polysaccharides and glycopeptides. — (*a*) *Qualitative and quantitative analyses of monosaccharides.* Samples (1–3 mg) of ethylene glycol extracts or fractions obtained from the extracts were hydrolysed (M sulphuric acid, 4 h, 100°), and the neutralised ($BaCO_3$) hydrolysates were analysed by paper chromatography (Whatman No. 1 paper), using pyridine–ethyl acetate–acetic acid–water[11] (5:5:1:3), and by paper electrophoresis[12] (Whatman 3MM paper) in acetate buffer (pH 6.0).

The amount of glucose in the neutralised ($BaCO_3$) and de-ionised (Dowex-50W x8 resin, H^+ form) hydrolysates was determined with cysteine and sulphuric acid[13]. The hydrolysates were also analysed for D-glucose by using D-glucose oxidase[14] and, in the case of the polysaccharide from *T. rubrum*, for D-galactose by using D-galactose oxidase[15]. The monosaccharide components of hydrolysates were also converted into *O*-trimethylsilyl derivatives[16] which were analysed by using a Pye 104 gas chromatograph [column packing of 10% SE-30 on siliconised Celite (100–200 mesh, Pye Unicam Ltd.) at 181°]. D-Glucose was the only monosaccharide detected in hydrolysates of the purified polysaccharides.

(*b*) *Determination of nitrogen and amino acids.* — Nitrogen was determined by using a Perkin–Elmer 240 elemental analyser. Samples of the glucan from *T. rubrum* (surface culture) were hydrolysed with 6M hydrochloric acid for 24 h at 110° in sealed glass tubes under nitrogen, and hydrochloric acid was then removed by rotary evaporation with the addition of water. Amino acids in the hydrolysate were converted into dansyl derivatives[17], and the product mixture was analysed by t.l.c. on silica gel, using benzene–pyridine–acetic acid[17] (80:20:2). The chromatograms were heated for 5 min at 110° and were examined under u.v. light. The dansyl derivative obtained from 7.5×10^{-3} μg of glycine was used as a control.

(*c*) *I.r. spectra of glucans.* I.r. spectra were determined on discs containing glucan (1.5 mg) and KBr (300 mg).

Investigation of the heterogeneity of dermatophyte glucans by elution from Porasil. — Aqueous suspensions of Porasil E and F (75–100 mesh, Waters Associates, Instruments Ltd.) were degassed under diminished pressure and packed into glass columns. In a typical experiment, a solution of the glucan (0.4 mg) in water (0.5 ml) was eluted from a column of Porasil F (95 × 1.0 cm) with degassed water at 38 ml/h/cm^2, and the column eluate was monitored continuously for glucose by using an automated modification of the cysteine–sulphuric acid reaction[13]. Similar experiments were carried out using Porasil E.

beta-Amylolysis of the glucans from T. rubrum *and* T. mentagrophytes, *and of fractions obtained from* T. rubrum *glucan by elution from Porasil E.* — Aliquots (0.5 ml) of an aqueous solution containing the glucan (2.08 mg/ml) were mixed with 0.4M acetate buffer (pH 5.8, 0.5 ml), and aliquots (1 μl, containing 20 μg of enzyme) of a suspension of sweet-potato beta-amylase (α-1,4-glucan maltohydrolase, Sigma) in ammonium sulphate were added. The solutions and control solutions of glucan without enzyme, and of buffer with enzyme alone, were heated at 37°. Aliquots (0.1 ml) of the solutions were removed during 6 h and heated for 5 min at 100°, prior to analysis for reducing sugars[18]. The reducing-sugar content of the test solutions was constant after incubation with beta-amylase for 4 h, and the increase in reducing power corresponded to 31.6% hydrolysis of the glucan to maltose. Essentially similar methods were used to compare fractions of apparently different molecular weight, which were obtained from the glucan isolated from *T. rubrum* (surface culture) by elution from Porasil E, and to measure the extent of beta-amylolysis of the glucan isolated from *T. mentagrophytes.*

Biological activity of components of the mycelial extract. — Batches of 3–6 guinea pigs were sensitized by subcutaneous injection (into the nape of the neck) of suspensions containing 5 mg of acetone-dried mycelium or of purified components of the mycelial extracts in Freund's complete adjuvant (0.2 ml). After 3 weeks, sensitized animals, as well as control animals not sensitized and animals injected with Freund's complete adjuvant only, were tested intradermally with solutions (1 mg/ml in physiological saline, 0.1 ml) of ethylene glycol extracts of the mycelia and of fractions selected at various stages of the purification of the extract. In some cases, the animals were challenged with mannans and glucans of plant and bacterial origin. The mean diameters of "delayed" reactions were recorded 24 h after injection, and the same batch of animals was tested[7] for "immediate" reactions one week later, using intracardial Evans Blue as an indicator (see below). Results of skin tests were expressed as the mean diameters of reactions ±the standard error of the mean.

Passive transfer of "immediate" hypersensitivity (passive cutaneous anaphylaxis). — Serum (0.1 ml) from a sensitized guinea pig was injected intradermally into several sites on each non-sensitized guinea pig, in batches of four to seven animals. Serum (0.1 ml) from a non-sensitized animal was injected similarly into another group of non-sensitized animals. After 24–48 h, each animal was injected intracardially with a 1% solution of Evans Blue in saline (0.9 ml). After 20 min, solutions (0.1 ml) of appropriate fractions from the dermatophyte extract and a control of saline (0.1 ml) were super-injected into the sites on each animal and, after a further 20 min, the animals were killed and the diameters of the blue stains around the injection sites were measured on the inside of the skin.

RESULTS

Fractionation of ethylene glycol extracts of dermatophytes by elution from Bio-Gel P-300. Isolation, purification, and physical properties of glucans. — The percentage (w/w) of material that was extracted from the dried mycelia with ethylene glycol ranged from 1.3% (*T. mentagrophytes*) to 3.6% (*T. rubrum*, submerged culture). A preponderantly carbohydrate component of apparently high molecular weight was separated from components ("glycopeptides") of lower molecular weight, which contained carbohydrate (glucose, galactose, mannose, and a small proportion of xylose) and peptide, by elution with water of an aqueous solution of the ethylene glycol extract of *T. rubrum* (surface culture) from Bio-Gel P-300 (Fig. 1). D-Glucose was the only monosaccharide detected in hydrolysates of the component (glucan) of high molecular weight which was further purified by elution from DEAE-Sephadex with 5mM phosphate buffer (pH 7.0) (Fig. 2). Essentially similar elution-profiles were obtained for the ethylene glycol extracts of *T. mentagrophytes* and *T. schoenleinii* (surface cultures) by using these fractionation procedures. A clear-cut separation of glucan from "glycopeptides" by elution from Bio-Gel P-300 was not obtained with the extract of *M. canis* (surface culture). A glucan was isolated, however, on elution from DEAE-Sephadex of the components of higher molecular weight thus obtained.

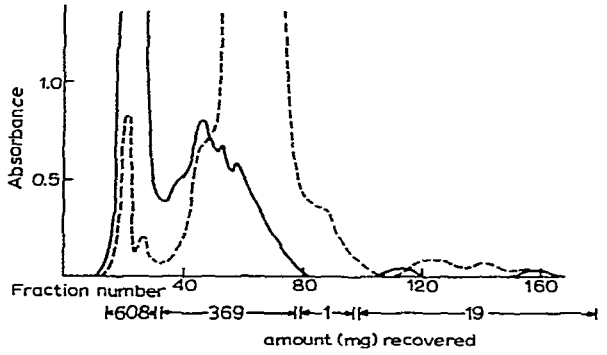

Fig. 1. Elution of ethylene glycol extract (1.34 g) of *T. rubrum* (surface culture) mycelium from a column (58 × 2.8 cm) of Bio-Gel P-300 with water. Fractions assayed with orcinol[9] (———), ninhydrin[10] (– – – –).

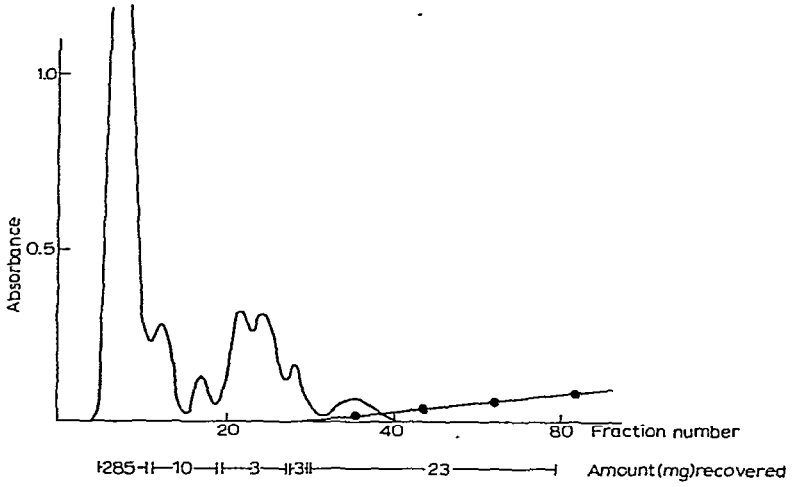

Fig. 2. Purification of crude glucan (0.586 g) from *T. rubrum* (see Fig. 1) by elution from a column (39 × 2 cm) of DEAE-Sephadex A-50 (Cl^-) with 5mM phosphate buffer (pH 7.0) and increasing concentrations of sodium chloride. Fractions assayed with orcinol[9] (———). Sodium chloride concentration (•—•).

Glycopeptides, but no glucan of high molecular weight, were detected in ethylene glycol extracts of *T. rubrum* grown in submerged culture. Glucose, galactose, mannose, and a small proportion of xylose were detected in hydrolysates of the glycopeptide components of all the dermatophytes studied. Analytical data for the glucans are summarised in Table I. D-Galactose was not detected in hydrolysates of the glucans by using D-galactose oxidase. The glucan isolated from *T. rubrum* contained less than 0.1% of nitrogen, and no amino acids were detected in hydrolysates of the glucan.

The infrared spectra of all the purified glucans showed Type 2a absorption (846 ±8 cm^{-1}), characteristic of the presence of α-D-glucosidic linkages, but no

TABLE I

ANALYTICAL DATA FOR PURIFIED GLUCANS ISOLATED FROM ETHYLENE GLYCOL EXTRACTS OF DERMATOPHYTE MYCELIA (SURFACE CULTURE)

Dermatophyte species	*Analytical data for glucan*[a]			
	$[\alpha]_D$ (*degrees*)	*Structural features from i.r. spectrum*[b]	*Maximal amount* D-*glucose liberated on hydrolysis*[c]	
			Cysteine–H_2SO_4	D-*Glucose oxidase*
T. rubrum	+186	α-(1→4) linkages	97.6 ±0.7	96.1 ±0.7
T. mentagrophytes	+204	α-(1→4) linkages	102.3 ±0.8	98.1 ±0.9
M. canis	+192	α-(1→4) linkages	Not determined	
T. schoenleinii	Not determined	α-D-glucosidic linkages	Not determined	

[a]Obtained by sequential fractionation of the appropriate ethylene glycol extract, using Bio-Gel P-300 and DEAE-Sephadex. [b] Ref. 19. [c]M H_2SO_4, 100°, 4 h. Corrected for losses on hydrolysis and neutralization. Values quoted are mean values ±standard error, for 6 analyses.

Type 2b absorption (891 ±7 cm^{-1}) which has been detected in the spectra of β-linked D-glucans[19]. Evidence for the presence, in all the glucans, of α-(1→4)-linked D-glucose residues (absorption 930 ±4, 758 ±2 cm^{-1}) was also obtained. Unequivocal assignment of other absorption peaks in the spectra was not possible.

beta-Amylolysis of the glucans from T. rubrum *and* T. mentagrophytes: *fractionation of* T. rubrum *glucan by elution from Porasil and beta-amylolysis of the fractions.* — The identification of components of the product mixture obtained by beta-amylolysis of the glucans will be described in a later paper[20]. No evidence was obtained for the presence of a disaccharide other than maltose. beta-Amylolysis of the glucans from *T. rubrum* and *T. mentagrophytes* liberated maltose equivalent to 31.6 and 18.1%, respectively, of the glucans. The elution profile of the *T. rubrum* glucan from Porasil F showed a single peak, corresponding to fractionated material, whereas elution from Porasil E showed a peak due to totally excluded material as well as a peak

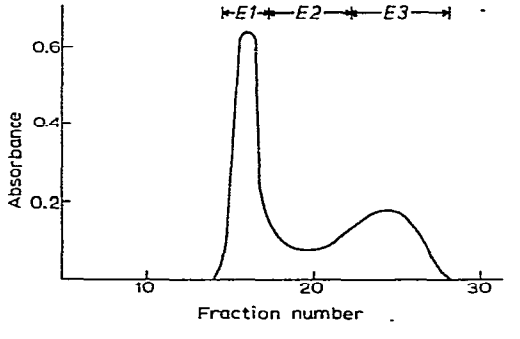

Fig. 3. Elution of purified glucan (5.8 mg) from *T. rubrum* (see Fig. 2) from a column (135 × 0.8 cm) of Porasil E with water. Fractions assayed with cysteine–sulphuric acid[11].

TABLE II

"IMMEDIATE" AND "DELAYED", DIRECT AND CROSS SKIN-REACTIONS OF THE ETHYLENE GLYCOL EXTRACT OF *T. rubrum* (SURFACE CULTURE) AND ITS COMPONENTS

Material tested	*Mean reaction-diameter (mm) ± S.E. in animals sensitized with*					
	Ethylene glycol extract		*Glycopeptides*[a]		*Glucan*[b]	
	"Immediate"	*"Delayed"*	*"Immediate"*[c]	*"Delayed"*	*"Immediate"*	*"Delayed"*
Ethelene glycol extract	13.9 ±0.5	21.2 ±1.3	15.5	20.0 ±1.0	16.3 ±0.3	18.4 ±0.5
Glycopeptide components[a]	14.5 ±0.4	18.1 ±1.7	14.5	16.8 ±1.3	15.0 ±0.3	13.7 ±0.6
Glucan components[b]	8.1 ±0.8	4.1 ±0.4	4.5	3.5 ±0.5	11.0 ±0.2	3.8 ±0.4
Saline	1.6 ±1.0	3.1 ±0.6	2.0	2.0 ±0.7	2.0 ±1.0	2.5 ±0.5

[a]Obtained by elution of ethylene glycol extract from Bio-Gel P-300. [b]From ethylene glycol extract, by sequential fractionation using Bio-Gel P-300 and DEAE-Sephadex. [c]Results from one animal only.

due to fractionated material (Fig. 3). A similar profile was obtained on elution from Porasil E of the polydisperse, homopolymer, Dextran 2000 (Pharmacia Ltd.). The amounts of maltose produced on incubation with beta-amylase of the three fractions (*E1*, *E2*, and *E3*, Fig. 3), obtained on elution of the *T. rubrum* glucan from Porasil E, corresponded to 29.4, 29.6, and 32.2%, respectively, of the glucan fractions. The similarity of these values suggests that the three fractions of the glucan, thus obtained, all contained peripheral chains of α-(1→4)-linked D-glucose residues and that the average number of D-glucose residues in these chains, relative to the total content of D-glucose in the fractions, was very similar.

Biological activity of components of dermatophyte mycelia. — The ethylene glycol extract of *T. rubrum* mycelium (surface culture), the purified glucan, and the mixture of glycopeptides, isolated from the extract, were each capable of sensitizing guinea pigs (Table II). All three groups of animals thus sensitized gave good "immediate" and "delayed" responses when challenged with the ethylene glycol extract of the mycelium, or with the glycopeptide components. The glucan, however, elicited significant "immediate" responses in animals sensitized with the glucan or the parent ethylene glycol extract, but poor or insignificant "delayed" responses in all three groups of animals. The latter results were confirmed (Table III) by using animals sensitized with *T. rubrum* glucan. Thus, these animals gave significant "immediate", but not "delayed", reactions on challenge with two batches of *T. rubrum* glucan and with the glucan from *M. canis*. No significant reaction of either type was elicited in these animals when challenged with the glucan from *T. mentagrophytes*. Animals sensitized by injection of 5, 1, or 0.2 mg of the glucan from *T. rubrum* gave essentially similar skin-reactions when tested with the ethylene glycol extract of *T. rubrum* and its glucan and glycopeptide components, whereas animals which had received Freund's adjuvant alone failed to show significant reactions when challenged with these materials.

TABLE III

"IMMEDIATE" AND "DELAYED" SKIN-REACTIONS OF GLUCANS FROM *T. rubrum*, *T. mentagrophytes*, AND *M. canis* IN ANIMALS SENSITIZED WITH *T. rubrum* GLUCAN

Material tested	*Mean reaction diameter* (mm) ±*S.E. in animals sensitized with* T. rubrum *glucan (batch* 1)	
	"Immediate"	*"Delayed"*
T. rubrum mycelium (surface culture); ethylene glycol extract	14.9 ±0.9	15.1 ±0.7
T. rubrum glucan (batch 1)	7.5 ±0.9	3.5 ±0.4
T. rubrum glucan (batch 2)	5.9 ±0.9	2.8 ±1.0
T. mentagrophytes glucan	3.8 ±0.6	3.0 ±0.4
M. canis glucan	9.8 ±1.3	4.7 ±0.8
Saline	1.6 ±1.0	2.0 ±0.7

Further information about the structure of the glucan from *T. rubrum* was obtained (Table IV) by testing glucans and mannans, whose main structural features are known, in animals sensitized with the ethylene glycol extract of *T. rubrum* and with the purified glucan component of the extract. In order to compare the results obtained on challenge with the different polysaccharides, each group of animals was

TABLE IV

"IMMEDIATE AND "DELAYED" SKIN-REACTIONS ELICITED BY PLANT AND BACTERIAL POLYSACCHARIDES AND BY COMPONENTS OF *T. rubrum* MYCELIUM IN ANIMALS SENSITIZED WITH COMPONENTS OF *T. rubrum* MYCELIUM

Material tested[a]	*Ref.*	*Mean reaction-diameter (mm) ± S.E. in animals sensitized with*			
		Ethylene glycol extract of T. rubrum		T. rubrum *glucan*	
		"*Immediate*"	"*Delayed*"	"*Immediate*"	"*Delayed*"
Nigeran	21	4.8 ±0.6[c]	7.2 ±0.8[c]	7.6 ±0.8	6.1 ±0.7
T. rubrum glycopeptides[b]		14.5 ±0.3[c]	20.5 ±1.1[c]		
T. rubrum ethylene glycol extract				15.3 ±0.8	17.4 ±1.1
Laminarin	22	2.9 ±0.7	3.8 ±0.5	5.5[d]	3.3 ±0.4
T. rubrum ethylene glycol extract		13.9 ±0.5	21.2 ±0.5	15.0[d]	14.8 ±5.4
Dextran (ex *Leuconostoc mesenteroides* NRRL B-742)	23	7.0 ±1.1	3.7 ±0.5		
T. rubrum glycopeptides[b]		15.2 ±0.5	16.2 ±1.9		
Dextran (ex *Betacoccus arabinosaceous*)	24	3.1 ±0.5	4.0 ±0.2		
T. rubrum glycopeptides[b]		15.2 ±0.5	16.2 ±1.9		
Amylose	25	3.4 ±0.7	2.8 ±0.3		
T. rubrum ethylene glycol extract		13.3 ±1.0	28.6 ±1.2		
Amylopectin	25	6.5 ±0.5	3.0 ±0.5		
T. rubrum ethylene glycol extract		13.3 ±1.0	28.5 ±1.2		
Mannan (ex *Bacillus polymyxa*)	26			6.4 ±0.3	6.2 ±0.6
T. rubrum ethylene glycol extract				16.3 ±0.3	18.4 ±0.2
T. rubrum glucan				11.0 ±0.2	
Mannan (ex *Hansenula holstii* NRRL T-2448)	27			7.3 ±0.6	6.8 ±0.5
T. rubrum ethylene glycol extract				16.3 ±0.3	18.4 ±0.2
T. rubrum glucan				11.0 ±0.2	

[a]Main structural features of non-dermatophyte materials: Nigeran, (1→3)- and (1→4)-linked α-D-glucopyranose. Laminarin, (1→3)-linked β-D-glucopyranose. Dextran ex *Leuconostoc mesenteroides*; 67% (1→6)-linked α-D-glucose, 21% "(1→4)-like", 12% "(1→3)-like". Dextran *ex Betacoccus arabinosaceous*; mainly (1→6)-linked α-D-glucose; some branching through C-3. Amylose; essentially linear polymer of (1→4)-linked α-D-glucopyranose. Amylopectin; (1→4)-linked α-D-glucose, *ca.* 1 residue in 12 branched through C-6. Mannan *ex. Bacillus polymyxa*; (1→2)-, (1→3)-, (1→2,6)-linked, and terminal non-reducing mannose residues. Mannan *ex Hansenula holstii*; sequences of at least three (1→3)-linked α-D-mannopyranose residues, some (1→2)-linked residues, and phosphate in diester linkage between C-6 of one mannose residue and C-1 of another. [b] Fraction precipitated at pH 7–9.5 by Cetavlon in the presence of sodium tetraborate (Barker *et al.*[4]). [c]Animals were sensitized with *T. rubrum* mycelium. [d]Results from one animal only.

also tested with materials from *T. rubrum*. Some of the non-dermatophyte glucans elicited significant "immediate" reactions but, with the exception of nigeran, none gave "delayed" reactions. The two mannans tested both gave significant "immediate" and "delayed" reactions in animals sensitized with the glucan from *T. rubrum*.

Passive transfer of immediate-type hypersensitivity. — Antibodies responsible for "immediate" hypersensitivity in guinea pigs are able to sensitize, passively, local areas of the skin of non-sensitized guinea pigs for "immediate" reactions. Animals that were passively sensitized by intradermal injection of serum from guinea pigs that had been actively sensitized with the glucan from *T. rubrum* produced "immediate" reactions when challenged 48 h later with that glucan and also when challenged with glycopeptide components from *T. rubrum* (surface culture). This confirmed that true, immediate-type reactions were observed in actively sensitized animals. Neither the glucan nor the glycopeptide components elicited reactions in animals that had received serum from a non-sensitized guinea pig.

DISCUSSION

The structurally similar α-D-glucans which were isolated in the present work are hitherto unreported, allergenic components of the dermatophytes. The only other allergenic components that were isolated from the mycelia investigated comprise complex mixtures of glycopeptides which are probably similar to those isolated previously from such sources. In the present work, the glucans were readily separated from the crude mycelial extract by fractionation at neutral pH. By contrast, in the "Cetavlon–borate" method previously used to fractionate the mycelial extract, such glucans would have been precipitated by alcohol at pH 11.5. Such conditions might result in partial degradation of these components. Improved methods for fractionating the mixture of glycopeptides will be described elsewhere.

The glucan was characteristic of *T. rubrum* grown in surface, rather than submerged, culture. Further work is necessary, however, to show how the types of allergenic components that are produced are related to the different physical and nutritional parameters of the two methods of culture. The infrared spectra of the glucans from *T. rubrum*, *T. mentagrophytes*, and *M. canis* are consistent with the presence of (1→4)-linked residues of α-D-glucose. Other modes of linkage cannot be excluded, but the susceptibility of the glucans from *T. rubrum* and *T. mentagrophytes* to beta-amylolysis indicates the presence, in those polysaccharides, of peripheral chains of α-(1→4)-linked D-glucose residues, although the number and/or length of such sequences in the two glucans are probably different. beta-Amylolysis also provided a means of comparing the fractions of different molecular weight that were obtained from the *T. rubrum* glucan by elution from Porasil E. Large and small molecules of this glucan were hydrolysed to approximately the same extent, thus suggesting that the glucan is probably not a mixture of discrete polysaccharides, such as amylose and amylopectin, which have different molecular sizes and differ greatly in their susceptibility to hydrolysis by beta-amylase, but rather that it is a polydisperse

homopolymer. The present results, however, do not exclude the possibility that polysaccharides with such structural differences as are present in amylose and amylopectin, but with a similar distribution of molecular weights, are present in the glucan from *T. rubrum*.

Bishop and co-workers[28–33] have isolated water-soluble polysaccharides, including galactomannans, a glucan, and, in some cases, a mannan, from surface cultures of various dermatophytes by extraction of the mycelia with hot water and hot 3% sodium hydroxide following prolonged incubation with trypsin. The glucans thus obtained, which had low specific optical rotations, contained various proportions of (1→6)- and (1→3)-linked residues and were branched through C-3 and C-6. The allergenicity of these materials was not reported. These indications that the glucans reported by Bishop and co-workers are significantly different structurally from those reported in the present work are endorsed by further structural studies which will be reported in a later paper.

One important aspect of this work has been an investigation of the role of the glucans in hypersensitivity reactions. Previous studies in these laboratories have attributed the "immediate" reactivities of glycopeptide allergens from dermatophytes to their carbohydrate moiety, and the "delayed" reactivities to the peptide component. Similar conclusions were reached by Holborow and Loewi[34] in studies of the allergenicity of the blood-group substances, and other workers[35–37] have indicated that pure polysaccharides can elicit "immediate", but not "delayed", hypersensitivity reactions. The ability of the dermatophyte glucans to elicit "immediate", but not "delayed", reactions in animals sensitized with the dermatophyte mycelium or with the glucan is consistent with such reports. Passive transfer of immediate-type hypersensitivity to the glucan from *T. rubrum* confirms that this material elicits a true "immediate" reaction in actively sensitized animals. The lack of reactivity of the glucan from *T. mentagrophytes* in animals sensitized with the glucan from *T. rubrum* indicates that these glucans do not possess the same antigenic determinants. The glucan from *M. canis* probably is antigenically similar to that from *T. rubrum*. Indirect information about the structure of the carbohydrate portion of glycopeptides from dermatophytes has been obtained[7] by testing polysaccharides of known structure for "immediate" reactions in animals sensitized with the glycopeptides. The results of similar experiments in the present work suggest that structures containing (1→3)-, (1→4)-, and (1→6)-linked residues of α-D-glucose may be involved in eliciting the allergic response to the glucan from *T. rubrum*. The ability of the two mannans to elicit skin reactions in glucan-sensitized animals is not readily explained.

Certain aspects of the allergenicity of the glucan from *T. rubrum* are not readily explained. Thus, the ethylene glycol extract of the mycelium of *T. rubrum*, and the glycopeptides obtained from it, gave "delayed" reactions, as well as larger "immediate" reactions than did the glucan from *T. rubrum* in guinea pigs that were sensitized actively or passively with the glucan. The glycopeptides did not cross-react with products of Mycobacterium which are present in Freund's adjuvant, but the possibility remained that the "delayed" reactions elicited by the glycopeptides might

be due to the presence in the glucan of undetected peptide similar to that present in the glycopeptides. The presence of 5 μg of peptide in the 5 mg of glucan used might sensitize animals to delayed reactions, since Salvin[38] has shown that 5 μg of egg albumen is sufficient to induce delayed hypersensitivity in guinea pigs. Recent work[39] has shown that a highly purified, pneumococcal polysaccharide containing 0.04% of nitrogen is an efficient sensitizer and inducer of delayed hypersensitivity reactions in the guinea pig. Experimental verification of the presence or absence of very small proportions of peptide in the glucan is very difficult by chemical methods. The reactions to the glycopeptides in animals sensitized with 5, 1, and 0.2 mg of our glucan could be due to as little as 0.2 μg or 0.1% of nitrogen. It may be that amounts of this order are sufficient to induce hypersensitivity in the presence of carbohydrate carrier but are insufficient to elicit delayed reactions on subsequent challenge with the same material, although reactions occurred to the larger amount of peptide in the glycopeptides.

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