ELSEVIER

Carbohydrate Research 272 (1995) 121–128

CARBOHYDRATE RESEARCH

Note

Chemical structure of fungal cell-wall polysaccharides isolated from *Microsporum gypseum* and related species of *Microsporum* and *Trychophyton*

Jesús Jiménez-Barbero [a], Alicia Prieto [b], Begoña Gómez-Miranda [b], Juan Antonio Leal [b], Manuel Bernabé [a,*]

[a] *Grupo de Carbohidratos, Instituto de Química Orgánica, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain*
[b] *Centro de Investigaciones Biológicas, CSIC, Velázquez 144, 28006 Madrid, Spain*

Received 23 June 1994; accepted in revised form 10 November 1994

Keywords: Fungal cell-wall polysaccharides; Microsporum; Trychophyton

Dermatophytes are a group of fungi which parasitise man and animals, and cause superficial cutaneous infections involving primarily the keratinised tissues of the epidermis, nails, and pilosebaceous follicles. The cell wall of dermatophytes is made up of chitin, a water-insoluble β-(1 → 3)-glucan, and water-soluble polysaccharides which are antigenically relevant. Bishop et al. [1–3] have isolated and partially characterised galactomannans from *Trichophyton granulosum*, *T. interdigitale*, *T. rubrum*, *T. schönleinii*, and *Microsporum quinckeanum*. Later, Takeda et al [4,5] studied the structure of a mannan isolated from *T. mentagrophytes* and *Epidermophyton floccosum*, which consists of a (1 → 6)-*O*-α-D-mannopyranosyl-(1 → 6)-*O*-[α-D-mannopyranosyl-(1 → 2)]-*O*-α-D-mannopyranosyl repeating trisaccharide. We have recently found a comb-like structure of (1 → 6)-*O*-[α-D-mannopyranosyl-(1 → 2)]-*O*-α-D-mannopyranosyl repeating units in the cell-wall polysaccharides of several *Aphanoascus* species [6] and now we wish to report on the isolation and chemical structure of polysaccharides obtained from the cell wall of several *Microsporum* and *Trichophyton* species.

* Corresponding author.

Elsevier Science Ltd.
SSDI 0008-6215(95)00023-2

1. Experimental

Growth of organisms, cell-wall preparation, isolation and chemical analyses.—Four species of *Microsporum* and two species of *Trichophyton*, from the Dr Guarro Collection (Facultat de Medicina, Universitat de Barcelona, 43201 Reus, Tarragona, Spain), were studied: *M. gypseum* (FMR 3314), *M. canis* (FMR 3373), *M. racemosum* (FMR 3315), *M. cookei* (FMR 4007), *T. rubrum* (IFO 5467), and *T. soudanense* (Pereiro 12931). Fungi culture, isolation and purification of polysaccharide fractions from hyphae, and chemical analyses have been previously reported [7]. The fractions obtained from the dry cell-wall material of the different species were as follows: the polysaccharide material extracted with 1 M NaOH at 20°C contained water-soluble polysaccharides (Fraction F1S), and water-insoluble polysaccharides (Fraction F1I). The material extracted with 1 M NaOH at 70°C (Fraction F3) was a very small fraction, and the insoluble residue (Fraction F4) was the main fraction. On refractionation of F1S, a soluble (F1S-B) and a slightly soluble fraction (F1S-A) were obtained. On re-extraction of fractions F1I with 0.5 M NaOH, a soluble (F1I-A) and a highly insoluble fraction (F1I-B) were obtained.

Methylation analysis.—The samples were methylated following a modification [8] of the Hakomori method [9]. Since complete methylation was not achieved, the samples were re-methylated according to the procedure of Purdie and Irvine [10]. The methylated polysaccharides were hydrolysed and the monosaccharides released were converted into partially methylated alditol acetates and analysed as previously described [11].

NMR analysis.—The conventional 1D-^{1}H-NMR spectra were recorded at 70°C on a Varian XL-300 spectrometer. Proton chemical shifts refer to residual HDO at δ 4.41 ppm. 2D-^{1}H-and ^{13}C-NMR experiments were carried out at 40°C on a Varian Unity 500 spectrometer (residual HDO at δ 4.61 ppm). Carbon chemical shifts refer to internal acetone at δ 31.07 ppm.

The polysaccharides (ca. 20 mg) from fractions F1S-B were dissolved in D_2O (1 mL) followed by centrifugation (10000g, 20 min) and lyophilisation. The process was repeated twice and the final sample was dissolved in D_2O (0.7 mL, 99.98% D) and degassed in the NMR tube under Ar.

The polysaccharides from fractions F1I were dissolved in 0.5 M NaOD (1 mL), followed by centrifugation, as above. The supernatant solution was submitted to NMR analysis.

The parameters used for 2D experiments were as follows. The double quantum filtered DQF-COSY experiment was performed in the phase sensitive mode using the method of States et al. [12]: 256 × 1K data matrix; zero-filled to 2K × 2K; 16 scans per increment; recycle delay, 2 s; spectral width, 1500 Hz; sine-bell filtering in both dimensions. The clean 2D-TOCSY experiment [13] was carried out in the phase sensitive mode using MLEV-17 for isotropic mixing; mixing time, 145 ms; 512 × 1K data matrix; zero-filled to 2K × 2K; squared cosine-bell functions were applied in both dimensions. Similar parameters were used for the 2D rotating frame NOE [14] (ROESY, CAMELSPIN) experiment (mixing time 300 ms) and the 2D-NOESY experiment (mixing time 300 ms). The pure absorption one-bond proton–carbon correlation experiments were collected in the ^{1}H-detection mode using the HMQC pulse sequence [15]

and a reverse probe; 256 × 1K data matrix; spectral widths of 8000 and 1500 Hz in F_1 and F_2; recycling delay, 1.5 s; delay corresponding to a J value of 145 Hz; squared cosine-bell filtering in F_2 and gaussian filtering in F_1. 16 (^{13}C-decoupled) or 32 (^{13}C-coupled) scans were accumulated per t_1 increment. The HMBC experiment [16] was performed using a delay of 80 ms and 96 scans per increment.

2. Results and discussion

The proportions of the fractions obtained from the dry cell-wall material of the different species were as follows: fraction F1S, ca. 5%; fraction F1I, around 2–9%. The fraction F3 was 1 to 3%, and the insoluble residue (fraction F4) was 58–71%. Refractionation of F1S gave F1S-B (60–70%) and the slightly soluble fraction F1S-A (10–30%). Refractionation of F1I gave F1I-A (0–20%) and F1I-B (80–95%).

Analyses of fractions F1S-B gave D-mannose as practically the only component. Fractions F1S-A and F1I-B gave mostly D-glucose.

Fractions F1I-A consisted of D-xylose, with the exception of those derived from *M. canis* and *M. cookei*, where no xylose or only a trace was detected.

Fraction F4 consisted of the glucan–chitin complex.

The conventional ^{1}H-NMR spectra for the polysaccharides F1S-B of all six species are shown in Fig 1. The general pattern demonstrated that all of them consist of almost identical skeletons, with four anomeric protons which display small differences in their relative intensities. The residues of the repeating unit have been labelled **A** to **D**, in order of increasing field of the H-1 resonances. The four anomeric signals are of the same relative intensity in *M. gypseum*, *T. rubrum*, and *T. soudanense*, indicating that the polysaccharides are composed of a tetrasaccharide repeating block. Accordingly, we carried out 2D-NMR experiments on the polysaccharide of *M. gypseum*. The resolution of the ^{1}H- and ^{13}C-NMR spectra, combined with the DQF-COSY, TOCSY, and HMQC experiments (see Fig. 2a,c), permitted the unambiguous assignment of all resonances for the mannose residues (Table 1). The relatively low-field values for C-2 and C-6 in unit **A**, and the magnitude of ^{13}C shifts in unit **B**, as compared with published values for methyl α- and β-mannopyranosides [17], indicate a 2,6-disubstituted mannose and a terminal mannopyranose for fragments **A** and **B**, respectively. Similarly, the low-field values for C-6 in units **C** and **D** indicate 6-substituted fragments for these. Methylation analysis showed the presence of terminal mannose, 6-substituted mannose, and 2,6-di-substituted mannose in the relative proportions 1:2:1, in accordance with the NMR results.

To discriminate between the two possibilities of arrangement of fragment **A**, we ran a 2D-NOESY spectrum of F1S-B (Fig. 2b). In addition to expected intraresidue signals, the spectrum showed cross-peaks for H-1**A**/H-1**B**, H-1**A**/H-6a**C**, H-1**A**/H-6b**C**, H-1**A**/H-5**C**, and H-1**B**/H-2**A**. A 2D-ROESY (CAMELSPIN) experiment led to analogous results, in support of unit **B** being (1 → 2)-linked to unit **A**, which is, in turn, (1 → 6)-linked to unit **C**. The only possibility for unit **D** is to be connected to position 6 of unit **A**. Conclusive additional evidence was provided by the long-range proton–carbon

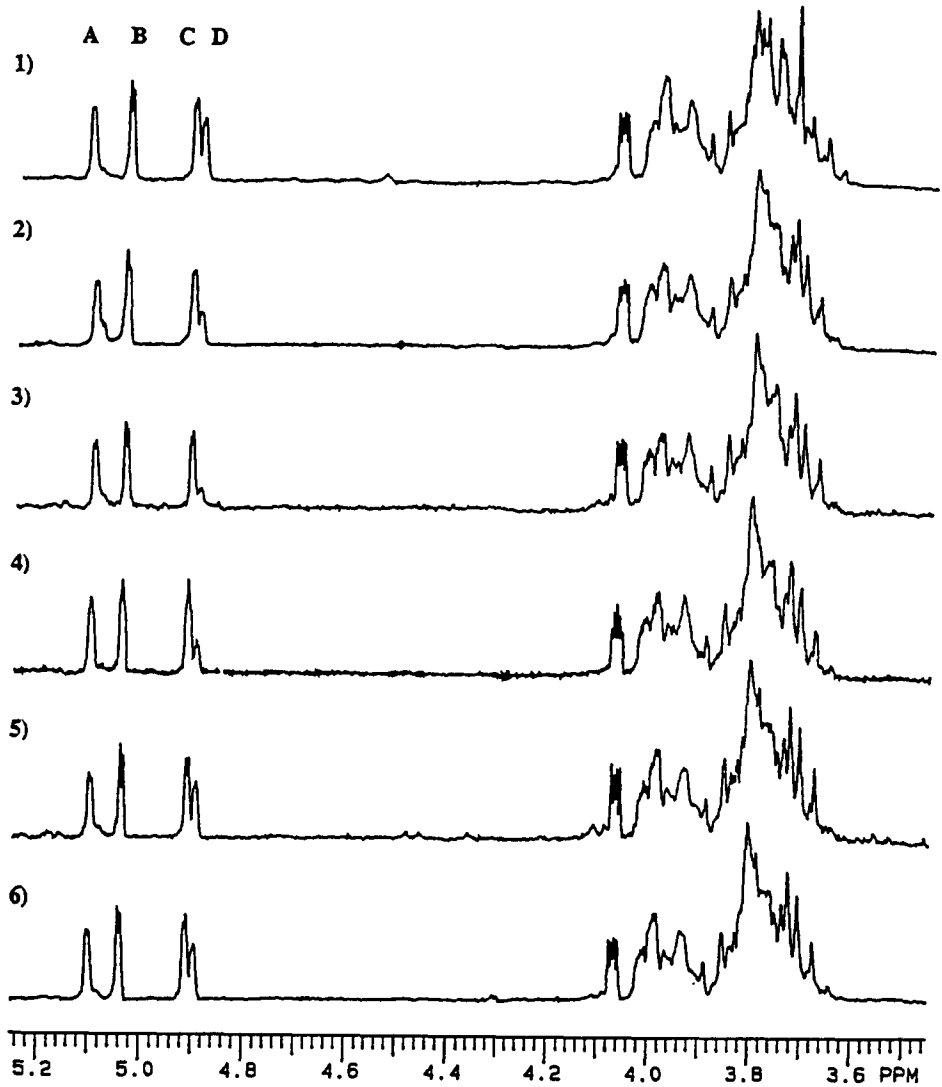


Fig. 1. ^{1}H-NMR spectra (40°C, 300 MHz) of the cell-wall F1S-B mannans isolated from: (1) *M. gypseum*; (2) *M. canis*; (3) *M. racemosum*; (4) *M. cookei*; (5) *T. rubrum*; (6) *T. soudanense*. The anomeric protons for the units of the tetrasaccharide repeating block have been labelled (**A**–**D**).

correlation HMBC experiment which shows signals for C-1**B**/H-2**A**, H-1**B**/C-2**A**, H-1**A**/C-6**C**, H-1**C**/C-6**D**, H-1**D**/C-6**A**, C-1**D**/H-6a**A**, C-1**D**/H-6b**A**, C-1**A**/H-6a**C**, and C-1**A**/H-6b**C**.

Concerning the configuration of the anomeric centres, a carbon-coupled HMQC

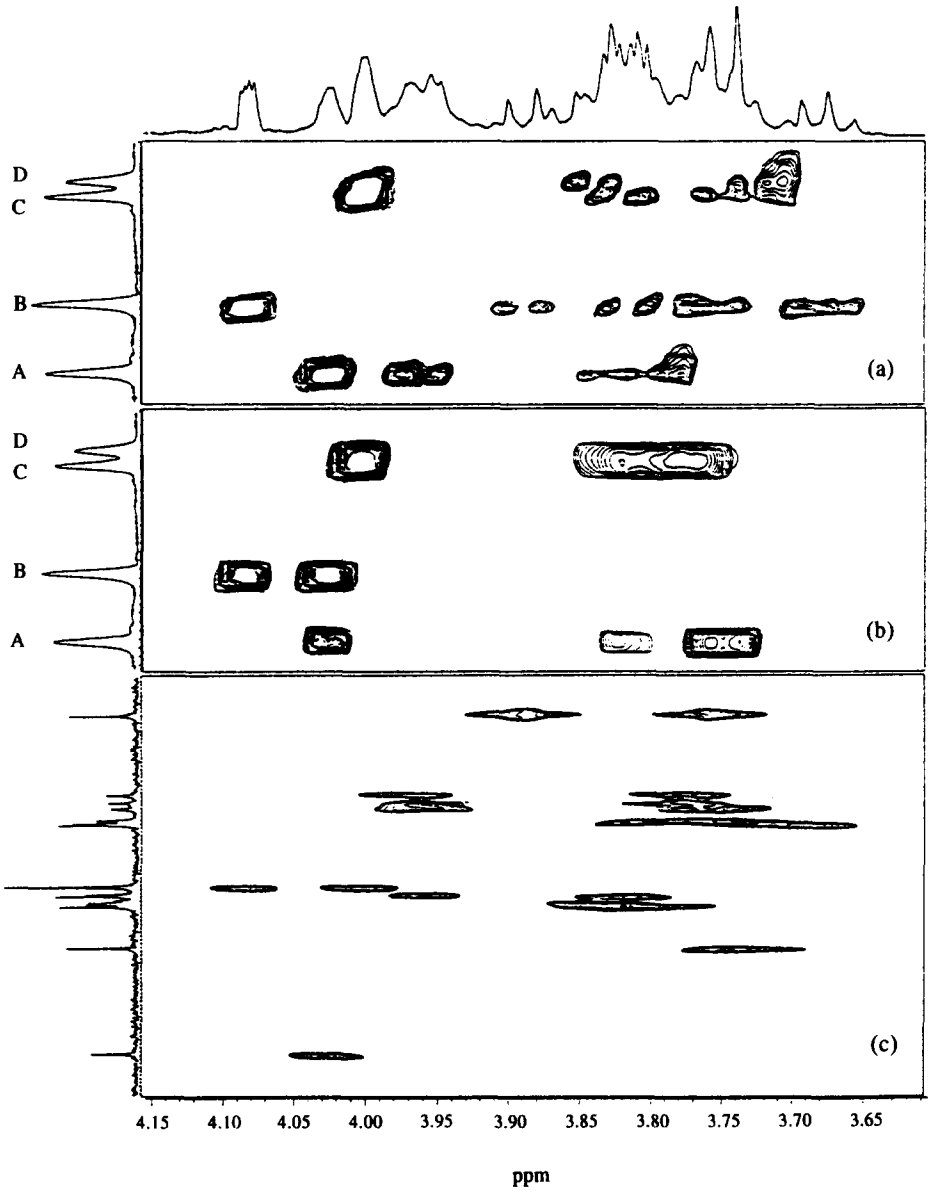


Fig. 2. 2D-NMR spectra (40°C, 500 MHz) for selected regions of the F1S-B mannan from *M. gypseum*: (a) TOCSY (HOHAHA); (b) NOESY; (c) HMQC subspectra. The anomeric protons have been labelled **A**–**D**.

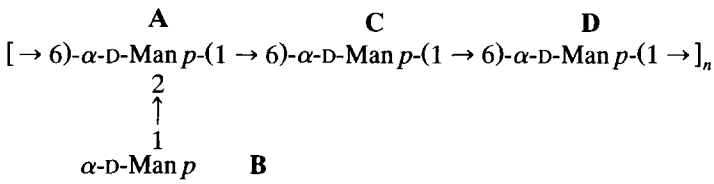
experiment allowed the measurement of the $^{1}J_{C\text{-}1,H\text{-}1}$ values, giving 176.0 Hz for units **A**, **C**, and **D** and 174.0 Hz for unit **B**, which, in accordance with the low-field values of the chemical shifts, are in favour of the anomeric configurations being α in all four

Table 1
^{1}H- and ^{13}C-NMR chemical shifts (δ, ppm) and coupling constants (Hz, in brackets) for the cell-wall polysaccharides F1S-B and F1I-A from *M. gypseum*

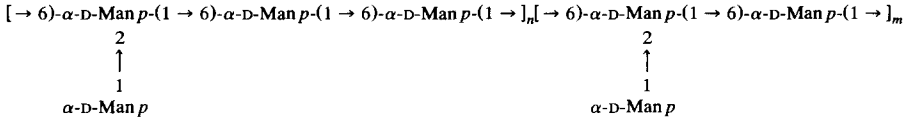
Unit	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
F1S-B							
A	5.13	4.03	3.96	3.82	3.81	3.95	3.76
B	5.05	4.08	3.80 [a]	3.68	3.73	3.89	3.73
C	4.93	4.00	3.81 [a]	3.74	3.81	3.95	3.74
D	4.91	4.00	3.81 [a]	3.74	3.81	3.97	3.76
Unit	H-1	H-2	H-3	H-4	H-5ax	H-5eq	
F1I-A							
-4-β-Xyl*p*-	4.192 [8.0]	3.059 [8.6]	3.260 [8.8]	3.529 [10.5; 4.9]	3.152	3.834 [-11.2]	
Unit	C-1	C-2	C-3	C-4	C-5	C-6	
F1S-B							
A	99.0	79.6	71.3	67.5 [b]	71.9 [c]	66.5	
B	103.1	70.9	71.4	67.6	74.1	61.9	
C	100.3	70.9	71.4	67.4 [b]	71.7 [c]	66.8	
D	100.3	70.9	71.4	67.3 [b]	71.7 [c]	66.1	
F1I-A							
-4-β-Xyl*p*-	103.5	74.5	76.1	77.2	64.6		

[a,b,c] These values may have to be interchanged.

residues. Consequently, we deduce the structure of the mannan from *M. gypseum* as being:



Comparing relative intensities for the anomeric protons of the residues **A** – **D** in the polysaccharides of the rest of the dermatophytes studied, we can see that *T. rubrum* and *T. soudanense* are practically identical to *M. gypseum*, while in *M. canis* and *M. cookei* the relative proportion of unit **D** is about one-half of that in *M. gypseum*, and the relative intensity of signal **D** in *M. racemosum* is about one-third of that in *M. gypseum*, suggesting that, in the mannans of these species, unit **D** is lower by a variable amount in comparison with that in *M. gypseum*. Hence we propose an idealised general skeleton:



where n and m differ for the different polysaccharides, being, approximately, $m = 0$ for *M. gypseum, T. rubrum*, and *T. soudanense*; $n = m$ for *M. canis* and *M cookei*; and $n = 2m$ for *M. racemosum.*

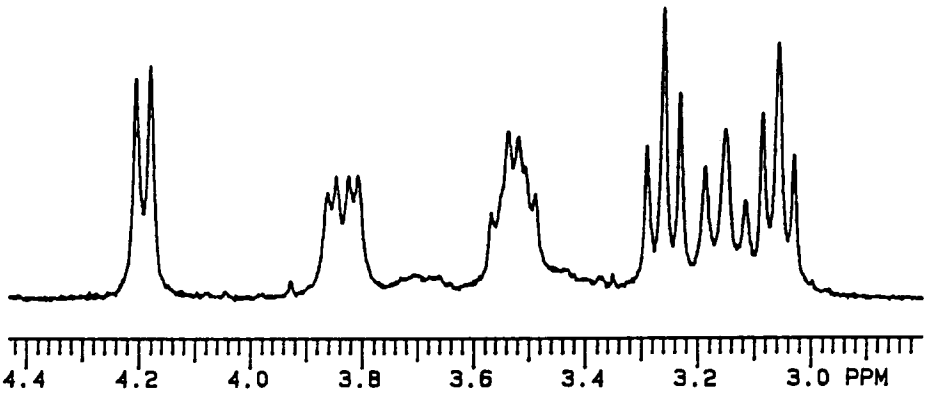


Fig. 3. ^{1}H-NMR spectrum of the xylan from fraction F1I-A of *T. soudanense*.

Concerning the alkali-soluble polysaccharide from fraction F1I-A, the analysis of the conventional simple ^{1}H-NMR spectrum (300 MHz) allowed straightforward assignment of all signals of the single unit (see Fig. 3 and Table 1). The theoretical spectrum was calculated using the PANIC program, and the values of the coupling constants are indicative of β-xylopyranose. A proton-detected ^{13}C–^{1}H correlation experiment (HMQC) allowed the assignment of the ^{13}C signals. The low value of C-4 [17] demonstrates that the structure corresponds to a β-(1 → 4)-xylan.

Acknowledgement

This work was supported by Grants PB 87/0243 and PB 91/0054 from Dirección General de Investigación Científica y Técnica.

References

[1] C.T. Bishop, F. Blank, and M. Hranisavljevic-Jakovljevic, *Can. J. Chem.*, 40 (1962) 1816–1825.
[2] C.T. Bishop, M.B. Perry, F. Blank, and F.P. Cooper, *Can. J. Chem.*, 43 (1965) 30–39.
[3] C.T. Bishop, M.B. Perry, and F. Blank, *Can. J. Chem.*, 44 (1966) 2291–2297.
[4] I. Kawarasaki, T. Takeda, Y. Ogihara, H. Shimonaka, and Y. Nozawa, *Chem. Pharm. Bull.*, 27 (1979) 2073–2075.
[5] T. Takeda, I. Kawarasaki, and Y. Ogihara, *Carbohydr. Res.*, 89 (1981) 301–308.
[6] J. Jiménez-Barbero, M. Bernabé, J.A. Leal, A. Prieto, and B. Gómez-Miranda, *Carbohydr. Res.*, 250 (1993) 289–299.
[7] J. Guarro, J. Cano, J.A. Leal, B. Gómez-Miranda, and M. Bernabé, *Mycopathologia*, 122 (1993) 69–77.
[8] P.-E. Jansson, L. Kenne, H. Liedgren, B. Lindberg, and J. Lönngren, *Chem.Commun., Univ. Stockholm*, 8 (1976) 1–75.
[9] S. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
[10] T. Purdie and J.C. Irvine, *J. Chem. Soc.*, 83 (1903) 1021.
[11] B. Gómez-Miranda, A. Prieto, and J.A. Leal, *FEMS Microbiol. Lett.*, 70 (1990) 331–336.
[12] D.J. States, R.A. Haberkorn, and D.J. Ruben, *J. Magn. Reson.*, 48 (1982) 286–292.

[13] C. Griesinger, G. Otting, K. Wüthrich, and R.R. Ernst *J. Am. Chem. Soc.*, 110 (1988) 7870–7872.
[14] A.A. Bothner-By, R.L. Stephens, J.-M. Lee, C.D. Warren, and R.W. Jeanloz, *J. Am. Chem. Soc.*, 106 (1984) 811–813; A. Bax and D.G. Davis, *J. Magn. Reson.*, 63 (1985) 207–213.
[15] A. Bax and S. Subramanian, *J. Magn. Reson.*, 67 (1986) 565–569.
[16] A. Bax and M.F. Summers, *J. Am. Chem. Soc.*, 108 (1986) 565–569.
[17] K. Bock, C. Pedersen, and H. Pedersen, *Adv. Carbohydr. Chem. Biochem.*, 42 (1984) 193–225.