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Polysaccharides from hot water extracts of roasted *Coffea arabica* beans: isolation and characterization

L. Navarini*, R. Gilli, V. Gombac, A. Abatangelo, M. Bosco, R. Toffanin

POLY-tech s.c.a.r.l., AREA Science Park, Padriciano 99, I-34012 Trieste, Italy

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Abstract

A polysaccharide fraction was obtained from the hot water extract of defatted ground dark roasted coffee (pure *Coffea arabica* blend for *espresso* brewing technique) by means of classical precipitation methods. This high molecular weight ($\langle M_w \rangle = 10\,900$ Da) product was shown to be composed of mannose, galactose, arabinose and traces of proteinaceous material. Further fractionation yielded two different carbohydrate polymers, which were structurally characterized. One polysaccharide was identified as a β-D-(1-4) mannan containing small amounts of galactose and arabinose. The second polysaccharide, obtained in low yield after removal of the mannose-containing polymeric material, was identified as an arabinogalactan. The starting fraction was found to be composed of about 80% (mol basis) of mannan and of about 20% of arabinogalactan. ^{13}C-NMR spectra revealed that the arabinogalactan has a backbone chain of β-(1-3)-D-galactopyranose units. Some of these units were substituted with either terminal β-D-galactose or terminal α-L-arabinofuranose side chains mainly in C-6 position. Both the mannan and the arabinogalactan isolated are structurally related to the polysaccharides originally present in the green coffee beans.

Keywords: Roasted *Coffea arabica*; Mannan; Arabinogalactan; NMR

1. Introduction

Coffee is one of the world's most widely consumed beverages. The soluble material that can be extracted from the roasted coffee is of particular importance to the quality of coffee brews. Under conditions normally used in the *espresso* brewing technique, relatively high quantities of soluble material can be extracted, particularly polysaccharides. These polysaccharides play an important role in the retention of volatile substances; they also contribute to the coffee brew viscosity and thus to the creamy sensation perceived in mouth, known as "body" (Illy & Viani, 1995).

Green coffee bean (*Coffea arabica*) polysaccharides have been the subject of several investigations in the 1960s (Wolfrom, Laver, & Patin, 1961; Wolfrom & Patin, 1964, 1965; Thaler & Arneth, 1967). The most recent structural study is that of Bradbury and Halliday (1990) who identified an arabinogalactan, a mannan and cellulose with only minor amounts of other polysaccharides in green coffee beans. In the same study, the major polysaccharides arabinogalactan and mannan, have been characterized in detail. The arabinogalactan on hydrolysis gave arabinose (19.8% w/w), galactose (48.2% w/w), mannose (0.8% w/w) and rhamnose (1.1% w/w). From these data it is clear that 30.1% (w/w) has not been defined. In spite of this, the polymer was described to be "principally a $\beta 1 \rightarrow 3$-linked galactan chain with frequent short side chains linked at C6 to galactose residues $1 \rightarrow 3$-linked to terminal arabinose residues. The polymer also contains some nonterminal arabinose residues (linked at C2 and C5) as well as a small proportion of $1 \rightarrow 6$-linked galactose residues". All the arabinose residues have been found to be in the furanoside form. The possibility of covalent linkages between the arabinogalactan and proteins has also been suggested. The mannan on hydrolysis gave mannose (94% w/w) galactose (3.3% w/w) and glucose (1.7% w/w) but the authors defined it essentially as a linear $\beta 1 \rightarrow 4$-linked mannan "with only about 1 one-residue galactose stub at C6 per 100 mannose residues" (Bradbury & Halliday, 1990). However, the comparison between the results of the methylation analysis of the whole-bean polysaccharides and those of the isolated mannan reveals some discrepancies. In particular in the whole-bean analysis the mannan appeared to be more branched and polymerized than the isolated one.

The value of optical rotation of the isolated mannan (in 1 N NaOH) was found to be similar to that of the ivory nut mannan (Bradbury & Halliday, 1990). In our opinion the internal inconsistencies and the discrepancies are mainly

* Corresponding author.

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due to the drastic conditions used to isolate the two polysaccharides. Therefore it is reasonable that the mannan and the arabinogalactan described by Bradbury and Halliday (1990) probably represent a simplified picture of the real situation in the green coffee beans.

Roasting strongly alters and degrades the carbohydrate polymers inducing depolymerization, structural modifications and formation of condensation complexes with proteins, protein fragments and other breakdown products. For this reason analytical study of carbohydrate polymers from processed *Coffea arabica* involves considerable difficulties in isolation and characterization. However, only a limited number of investigations have been devoted to the study of the polysaccharides or their conversion products in roasted coffee, coffee brews, extracts and instant coffee (Trugo, 1985; Viani, 1993). Wolfrom and Anderson (1967) were able to isolate from an instant coffee powder, of unknown source, arabinogalactan (only galactose and arabinose on acid hydrolysis) and mannan (94% w/w mannose and traces of galactose on acid hydrolysis); however, structural evidence for the two polymers was not produced. The value of optical rotation of the mannan was found to be similar to that of ivory nut mannan, a typical water insoluble polysaccharide, thus casting some doubts on the real water solubility of the starting coffee powder. In a more recent article, the water soluble residue obtained after a series of extractions with different solvents of instant coffee, was found to be composed of mannose, galactose and arabinose with some minerals, proteins and traces of chlorogenic acids (Pictet, 1975), but no information was provided about the possible arrangement of the sugar components.

Maier and Buttle (1973) reported the isolation of a fraction composed of polymerized material (M_W 5000–50 000). This material was shown to be composed of mannose, galactose and arabinose, and approximately from 6 to 12 aminoacid residues were found per molecule, probably arranged as peptides. In contrast to Wolfrom and Anderson (1967) and Maier and Buttle (1973) suggested that the sugar components are arranged as an arabinogalactomannan in which galactose and arabinose side chains are linked to a backbone of β-D-(1-4) mannose units. According to the authors, the arabinogalactomannan is linked to peptides or single aminoacids to form a complex. Unfortunately, in this study the term arabinogalactomannan is inferred from constituent monosaccharides only.

The isolation of a polysaccharide fraction containing mannose, galactose, arabinose and traces of proteinaceous material, from the hot (90°C) water extracts prepared from defatted dark roasted *Coffea arabica* beans, largely stimulated the present investigation. As a matter of fact, in spite of the difference of source, extraction and isolation procedures, the composition of this fraction resembles those reported in the literature. Accordingly, the study of this fraction gives the opportunity to provide further information on the fate of polysaccharides, originally present in green coffee beans, after roasting. In order to characterize the starting fraction and the derived sub-fractions, NMR spectroscopy has been chosen thanks to the several advantages offered by this technique in comparison with other classical methods. Moreover, no NMR data have been reported so far on roasted coffee polysaccharide conversion products.

2. Experimental

2.1. Materials

Extraction and isolation of fraction A. Ground dark roasted coffee (pure *Coffea arabica* blend for espresso brewing technique), ICS grade, produced by ILLYCAFFE' S.p.A., Trieste, Italy, was used. 25 g of coffee powder was heated under reflux with *n*-hexane 16 h in a Soxhlet apparatus. The defatted dried powder was extracted twice for 1 h with Milli-Q water (200 ml) at 90°C. The solid residue was removed by filtration, ammonium sulfate was added to give a saturated solution and the mixture was maintained at 4°C overnight. The resulting precipitate was collected by centrifugation, dissolved in water and extensively dialyzed against (in the order): Milli-Q water, aqueous NaCl 1 M and Milli-Q water. The non-dialyzable solution, after filtration, was freeze-dried to give a fluffy dark brown material (1.085 g). This material (1.0 g), was redissolved in Milli-Q water (50 ml), the solution was added to 2.5 volumes of isopropanol and the mixture was maintained at 4°C overnight. The resulting precipitate was filtered, dissolved in Milli-Q water and the solution was dried under reduced pressure in a Rotavapor. The solid material was redissolved in water (200 ml) and freeze-dried to give a brown fluffy material (560 mg) hereafter referred to as fraction A. The supernatant was dried under reduced pressure in a Rotavapor, dissolved in Milli-Q water and freeze-dried to give 356 mg of brown fluffy material (fraction B). This material is under investigation.

Isolation of sub-fractions Ans and As. 500 mg of fraction A were solubilized in water (100 ml) and the pH of the solution was adjusted to 12.5 by addition of 0.1 M NaOH. After 3 h under stirring at room temperature followed by neutralization, the solution was dialyzed against water. After exhaustive dialysis, the non-dialyzable solution was centrifuged (15 000 rpm. for 15 min), the precipitate and the supernatant were collected and recovered by freeze-drying, giving a light creamy-colored fraction hereafter referred to as sub-fraction Ans (62 mg) and a brown-colored fraction hereafter referred to as sub-fraction As (318 mg), respectively. The reproducibility of this behaviour has been checked on a different batch.

Isolation of sub-fraction AG. In order to further characterize the sub-fraction As, according to Wolfrom and Anderson (1967), a portion of the product (287 mg) was dissolved in 0.05 M NaOH (70 ml). To the solution was added saturated aqueous $Ba(OH)_2$ (35 ml). After standing

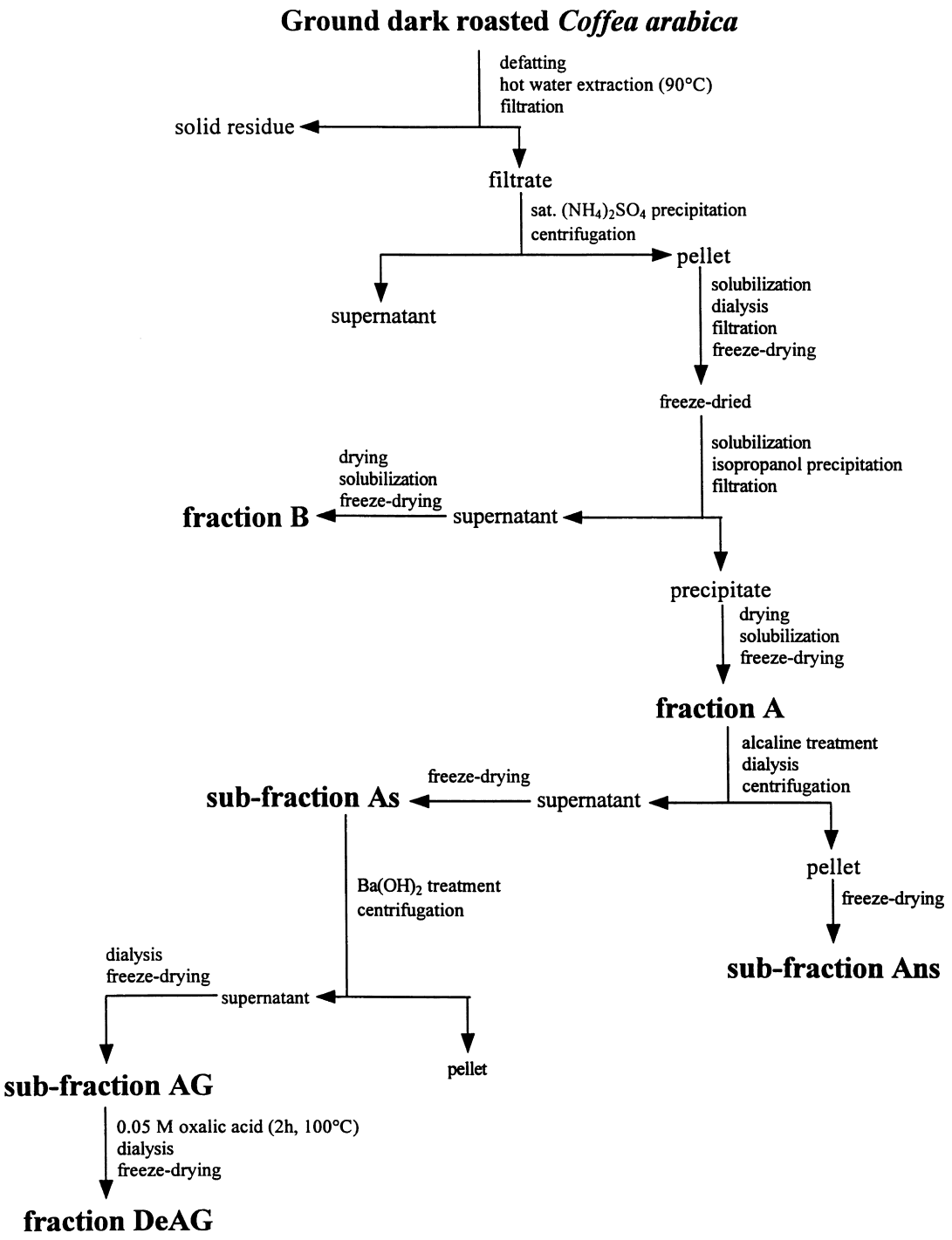

Fig. 1. Extraction and Isolation scheme.

overnight at room temperature, the brown precipitate, which had formed was removed by centrifugation and discarded. The supernatant was extensively dialyzed against water and recovered by freeze-drying to give a nearly colorless fluffy solid (80.8 mg) hereafter referred to as sub-fraction AG.

In the scheme reported in Fig. 1 the experimental procedure leading to fraction A and sub-fractions Ans, As and AG is described.

Selective hydrolysis of sub-fraction AG. Sub-fraction AG (37.8 mg) was heated in 0.05 M oxalic acid for 2 h at 100°C. The hydrolysate was then extensively dialysed against Milli-Q water and freeze-dried to give fraction DeAG (11.0 mg).

3. Methods

Total carbohydrate content in fraction A was determined

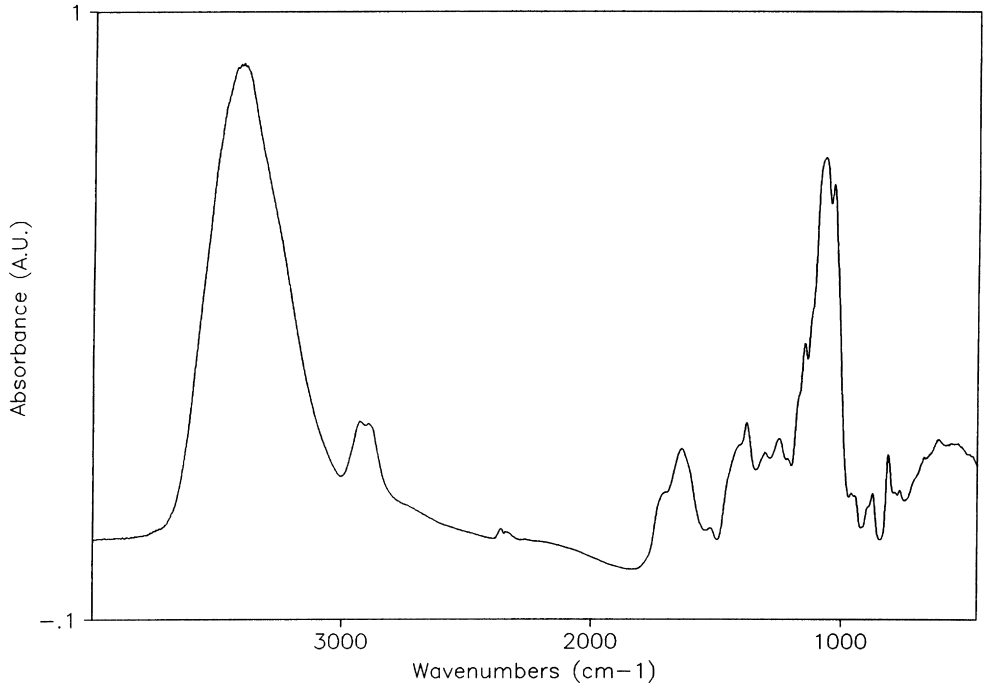

Fig. 2. FTIR spectrum of fraction A.

by phenol-sulfuric acid colorimetric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Protein content of 6.9% w/w was estimated on the basis of elemental analysis (%N × 6.25). Sugar composition was determined by gas-chromatography of the alditol acetate derivatives of the hydrolized samples (TFA 2N, 100°C, 16 h). A Hewlett–Packard model 5890A equipped with a flame ionization detector was used. The column was a 30 m × 0.25 mm SP-2330 Fused Silica (Supelco) with a temperature scan rate of 4°C/min in the range of 150–250°C. FTIR spectra were recorded using a Perkin–Elmer 1750 spectrometer interfaced with a Perkin–Elmer 7300 data processor (128 scans at 2 cm^{-1} resolution). Pellets were prepared using 200 mg of IR spectroscopic grade KBr and 2 mg of freeze dried samples previously maintained for 24 h in a desiccator under low pressure.

All NMR spectra were recorded at 4.70 T (^1H, 200.13 MHz and ^{13}C, 50.32 MHz) with a Bruker AC 200 instrument equipped with a multinuclear 5 mm probe. 10–15 mg of any sample were dissolved with 0.5 ml of D_2O directly inside the NMR tube and the analysis were performed at 60°C. The ^1H spectra were stored with a spectral width of 2 KHz and 8*K* complex data points. Broadband ^1H-decoupled ^{13}C spectra were obtained with a spectral width of 16 KHz and 16*K* complex data points; the distortionless enhancement by polarization transfer experiment (DEPT-135) was performed with the same spectral parameters using a 5.5 μs pulse to obtain a 90° flip angle for the ^{13}C spins and a 22.5 μs pulse for the ^1H spins; the delay for the evolution of the $^1J_{CH}$ coupling constant was set equal to 3.3 ms.

The (DEPT) experiments without decoupling were conducted at 50.32 MHz. The weight-average molecular weight ($\langle M_w \rangle$) was determined by high performance size exclusion chromatography (HP-SEC) using G6000, G5000 and G3000 TSK PW × 1 columns (TosoHaas) in series, with a differential refractometer (Waters 410) in line. The samples were eluted with 0.15 M NaCl (0.80 ml/min). Broad Standard Calibration procedure was performed by using a characterized sample of hyaluronic acid having the $\langle M_w \rangle$ equal to 150 000 Da and a polydispersity index ($\langle M_w \rangle / \langle M_n \rangle$) of 1.72. The choice of the calibration standard was dictated by the similarity of its backbone rigidity with that of the two component polysaccharides as it appeared from NMR linkage analysis (discussed later).

UV–vis absorption spectra and circular dichroism (CD) spectra were recorded by using a Varian Cary 3E UV–vis spectrophotometer and a Jasco J-600 spectropolarimeter, respectively. Fluorescence spectra were taken by using a Perkin–Elmer LS 30 luminescence spectrometer.

4. Results and discussion

4.1. Fraction A

The extraction and isolation procedures followed to obtain fraction A were performed under very mild conditions in order to prevent chemical changes. The yield of fraction A was about 2.2% (w/w) of the starting coffee powder.

In agreement with the chemical analysis, the FTIR spectrum of fraction A, reported in Fig. 2, clearly shows the typical signal pattern expected for a carbohydrate moiety.

Table 1
Sugar analysis of fraction A and sub-fractions Ans and As (weight basis and mol basis)

Fraction	Total weight (mg)	wt.%				mol/100 mol sugar		
		Mannose	Galactose	Arabinose	Non-carbohydrate components	Mannose	Galactose	Arabinose
A	500	74.5	19.2	3.7	2.6	75.9	19.6	4.5
Ans	62	91.8	3.5	1.0	3.7	95.1	3.6	1.2
As	318	65.7	27.7	4.2	2.4	66.7	28.1	5.1
Loss[a] (mg)	−120							
(% w/w)	−24.0	−28.6	−6.0	−24.4	−23.6			

[a] Loss (% w/w) is obtained by comparing weight of component in fraction A with weight recovered in fraction Ans plus fraction As.

In particular, the spectrum is dominated by signals in the region 3600–2800 cm^{-1} due to stretching vibrations of CH and OH and by signals in the C–O stretching region (1200–950 cm^{-1}) (Mathlouthi & Koenig, 1986). In the anomeric region (950–700 cm^{-1}) several bands are present, including absorption at 892 cm^{-1} (type 2b peak typical for β anomers) and at 875 cm^{-1} (type 2c peak) that can reveal the presence of D-mannopyranose units (Mathlouthi & Koenig, 1986). In the carbonyl stretching region, in addition to the signal due to residual water (1640 cm^{-1}), a shoulder at 1710 cm^{-1} may reveal the presence of carbonyl compounds related to Maillard reaction products (Fors, 1983), but the amide bands, which are related to proteinaceous components (6.9% (w/w) in the fraction), are overlapped by the signal from residual water.

Sugar composition of fraction A (see Table 1) confirmed the presence of mannose as the main constituent and revealed the presence of galactose, arabinose and traces of other unidentified components. The weight-average molecular weight $\langle M_W \rangle$ of the sample was 10 900 Da and the monomodal molecular weight distribution curve was characterized by a polydispersity index ($\langle M_w \rangle / \langle M_n \rangle$) of 1.56. The chromatogram, reported in Fig. 3, clearly shows a very small and broad signal in the high molecular weight region (about 270 000 Da) possibly related to the presence of aggregates. The ^{1}H-HMR spectrum, reported in Fig. 4, in full agreement with FTIR data, shows the typical pattern expected for a polysaccharide moiety. In the anomeric proton region a well-resolved signal at 4.7–4.8 ppm, typical of β anomeric forms for mannose-containing polysaccharides, can be observed. Moreover, in addition to the signal at 2.2 ppm, due to acetone used as an internal standard, minor non-carbohydrate broad signals are present in the region 3.0–0.5 ppm. On the basis of the elemental analysis and literature data (Pictet, 1975; Maier & Buttle, 1973) these signals are attributed to proteinaceous and/or nitrogen-containing (melanoidins) components. Aromatic protons were not detected.

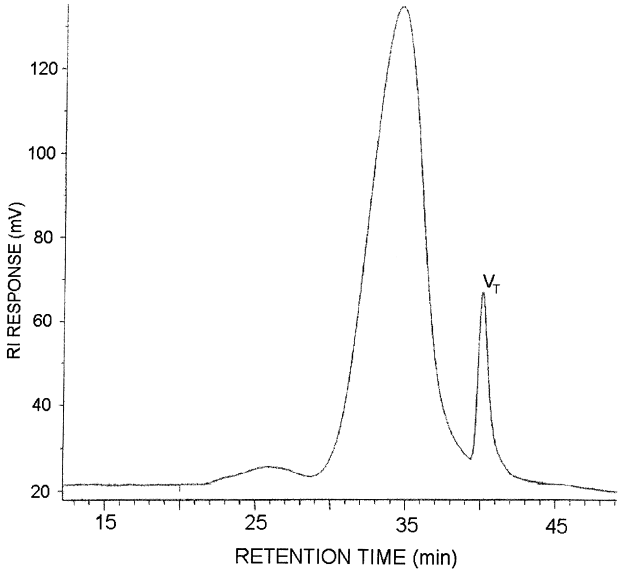

Fig. 3. HP-SEC chromatogram of fraction A.

The UV–vis absorption spectrum of fraction A in aqueous solution, reported in Fig. 5(a), shows two maximum absorptions at 285 and 329 nm with a long tail extending into the visible light region of 400–500 nm. The scattering induced by the presence of the aggregates revealed by HP-SEC analysis is likely to contribute to the strong increase in the absorption around 200 nm. The ^{13}C-NMR spectrum, reported in Fig. 6(a), is dominated by six well-resolved signals, suggesting that fraction A is mainly constituted by a relatively simple glycan. On the basis of the sugar composition and literature data, the anomeric signals at 101.0, 104.7 and 110.0 ppm (very low intensity) were assigned to D-mannopyranose (Bociek, Izzard, Morrison, & Welti, 1981; Grasdalen & Painter, 1980), β-D-galactopyranose (Bock, Pedersen, & Pedersen, 1984) and terminal α-L-arabinofuranose (Ebringerova, Hromadkova, Alfoldi, & Berth, 1992) residues, respectively. In order to identify the configuration of the principal anomeric carbon (at 101.0 ppm) assigned to D-mannopyranose, a distortionless enhancement by polarization transfer (DEPT) without the decoupling experiment was performed. The coupling constant $^1J_{CH}$ value (162.17) is indicative of β-linkages (Bock, Lundt, & Pedersen, 1973), in agreement with the ^{1}H-NMR data. Comparison with literature data (Bociek et al., 1981; Grasdalen & Painter, 1980) revealed that the ^{13}C chemical shifts of the other principal resonances in the spectrum corresponded to those observed for the linear unsubstituted β-(1-4)-D-mannopyranose units. In particular, the signals were assigned as

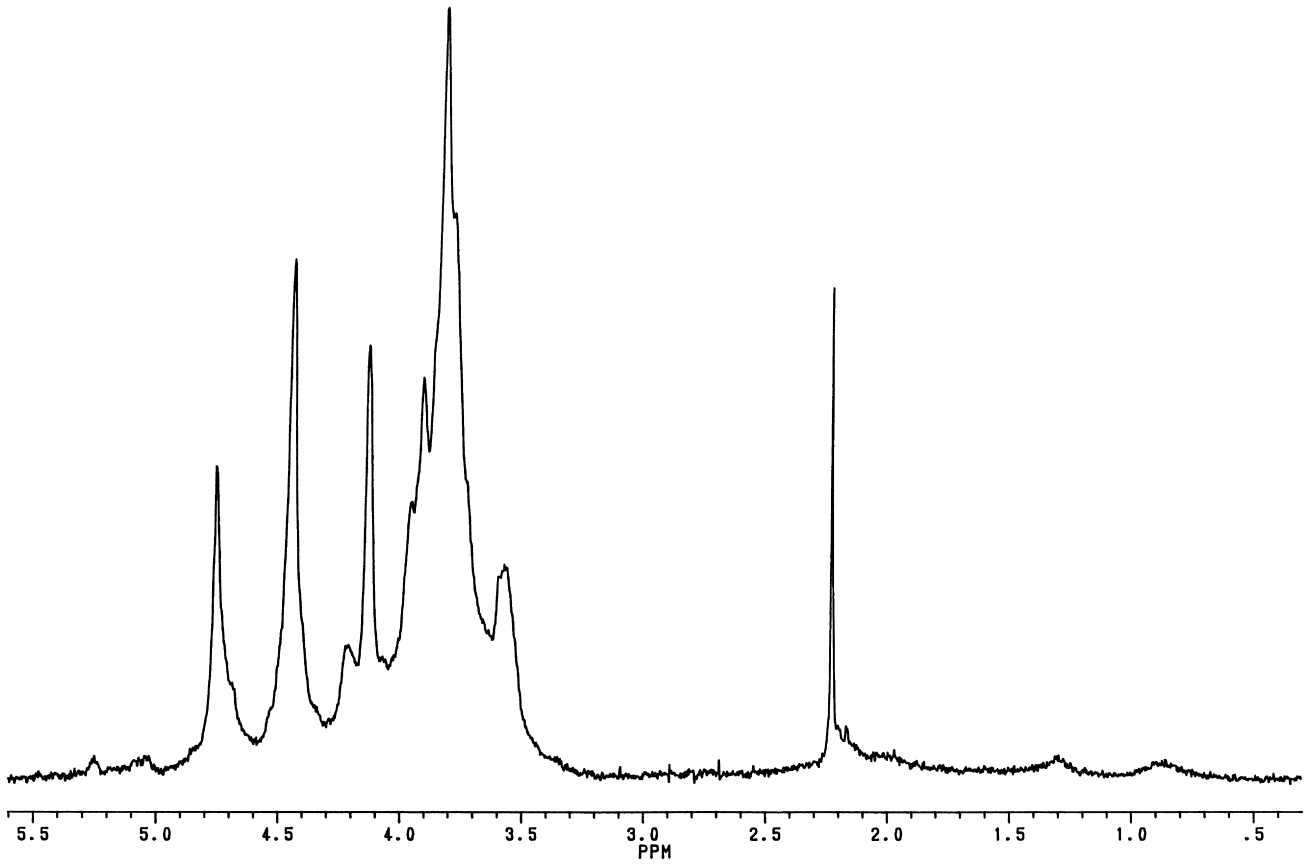

Fig. 4. ^{1}H-NMR spectrum of fraction A. The signal at 2.2 ppm is due to acetone used as an internal standard.

follows: 77.3 (C4), 75.9 (C5), 72.3(C3), 70.8 (C2) and 61.4 (C6).

The minor signals present in the spectrum are related to the other sugar components and in particular the signals in the range 80–85 ppm may be ascribed to C2 and C4 of furanosidic units and to the presence of 1-3 linkages. Moreover signals around 69 ppm reveal the presence of 1-6 linkages. These findings suggests that the arrangement of the component sugars in fraction A may resemble that found in the green coffee beans (Bradbury & Halliday, 1990): mannose as a linear β-(1-4)-D-mannan and arabinose and galactose as an arabinogalactan, however the poor resolution does not permit supporting this hypothesis or to exclude a priori the presence of the arabinogalactomannan previously reported (Maier & Buttle, 1973). Moreover, the water solubility of fraction A suggests that the linear β-(1-4)-D-mannan, if present as a separate entity, should be strongly depolimerized in comparison with that originally present in the green coffee beans (Bradbury & Halliday, 1990).

In an attempt to clarify this point, fraction A was further investigated.

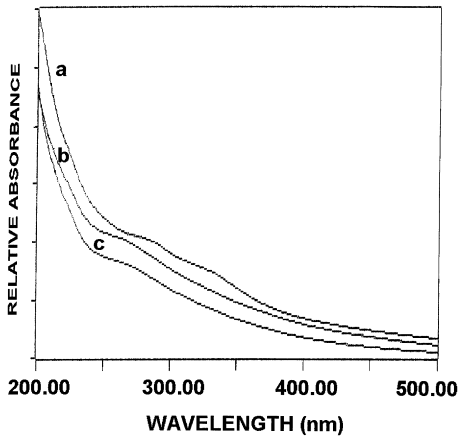

Fig. 5. UV–vis absorption spectra of fraction A (a); sub-fraction As (b); and sub-fraction AG (c).

4.2. Sub-fractions Ans and As

Fraction A was subjected to a mild alkaline treatment in order to check its chemical stability. If proteinaceous and/or nitrogen-containing components are associated to the carbohydrate moiety, as suggested in previous studies (Maier & Buttle, 1973), mild alkaline conditions should be sufficient to hydrolyze these alkali labile glycosidic linkages between carbohydrates and, for instance, peptides. After solubilization of fraction A in water and adjustment of the pH to 12.5, the resulting solution was stirred for 3 h at room

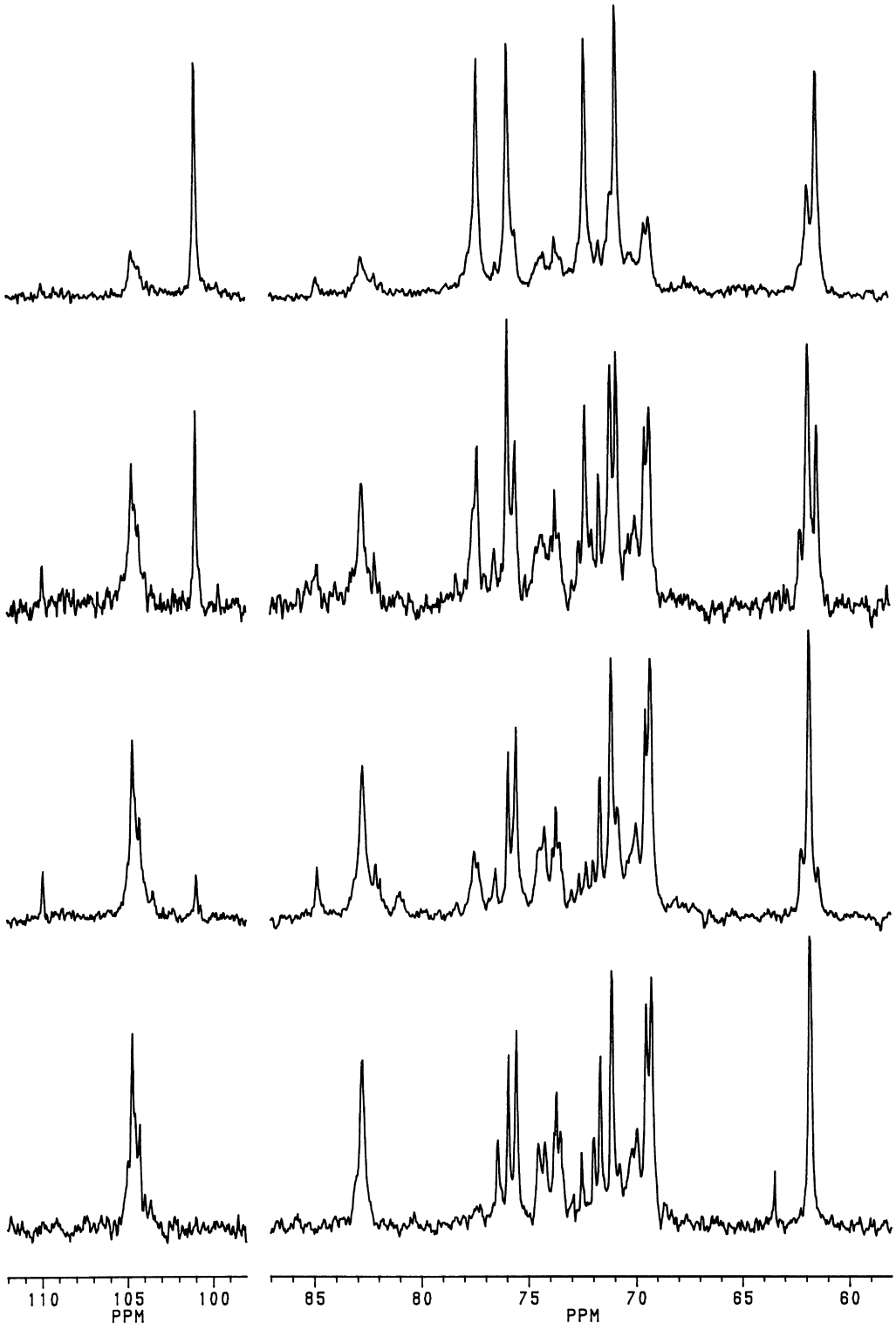

Fig. 6. ^{13}C-NMR spectra of fraction A (a); sub-fraction As (b); sub-fraction AG (c) and fraction DeAG (d).

temperature, neutralized and dialyzed against water. Unexpectedly, in the course of the dialysis, the non-dialyzable solution formed a precipitate. The precipitate and the remaining non-dialyzable solution gave sub-fractions Ans and As, respectively. The same mild alkaline treatment was repeated on sub-fraction As. In this case no precipitate was observed after extensive dialysis.

As shown in Table 1, in comparison with fraction A, the sub-fraction Ans is composed mainly of mannose and galactose plus traces of arabinose and other unidentified

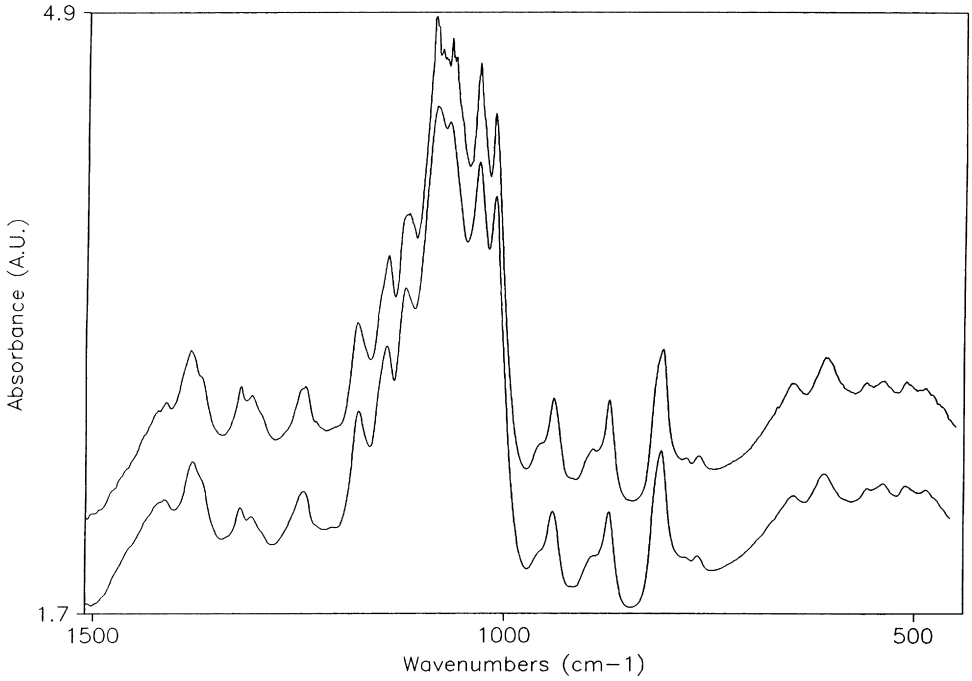

Fig. 7. Comparison between the FTIR spectra of sub-fraction Ans (below) and "ivory nut" mannan (above).

components. In contrast sub-fraction As was richer in arabinose and galactose and poorer in mannose. The $\langle M_w \rangle$ of this sub-fraction was 8250 Da with a polydispersity index of 1.40. The data reported in Table 1 indicate that the weight loss of the individual sugar components expressed as a percentage on fraction A is not the same. In particular mannose and arabinose are lost in similar percentage but galactose loss is approximately 4 times less. The weight loss percentage of non-carbohydrate components is comparable with that of the total weight loss suggesting that the non-carbohydrate components do not seem to be preferentially bound to either of the two sub-fractions. In order to further characterize sub-fraction Ans its FTIR spectrum was compared to that of ivory nut β-(1-4)-D-mannan (mannan A as described by Aspinall, Hirst, Perceival, & Williamson, 1953). The comparison, reported in Fig. 7, clearly shows agreement between the two FTIR patterns suggesting that the sub-fraction Ans is a mannan. However, the presence of small amounts of galactose and arabinose indicates a low level of substitution not detected by FTIR. This water-insoluble mannan is structurally related to that originally present in the green coffee beans. For the latter, a low level of branching and galactose in small amount have been found but arabinose may have been overlooked due to the well-known lability of this sugar residue (Bradbury & Halliday, 1990). Moreover, sub-fraction Ans seems to be structurally related to the arabinogalactomannan described by Maier and Buttle (1973) even if the term arabinogalactomannan seems to be too strict in view of the small amounts of galactose and arabinose found in sub-fraction Ans.

The comparison of the ^{13}C-NMR spectrum of fraction A and that of sub-fraction As reported in Fig. 6(b) gives additional information. In addition to the six resonances assigned to β-(1-4)-D-mannopyranose units, the spectrum of sub-fraction As shows a number of intense carbohydrate signals with the same chemical shifts as the resonances previously observed, but with a lower intensity, in the spectrum of fraction A. In the anomeric region, the intensity of signals which had been assigned to β-D-galactopyranose and terminal α-L-arabinofuranose residues in fraction A appeared to be strongly increased, in sub-fraction As, whereas the intensity of signal assigned to β-(1-4)-D-mannopyranose units is substantially decreased.

The signals in the range 80–85 ppm previously ascribed to C2 and C4 of furanosidic units and to the presence of 1-3 linkages and the signals around 69 ppm previously attributed to the presence of 1-6 linkages are still present but they are better resolved in the spectrum. These structural elements are consistent with those of the arabinogalactan originally present in the green coffee beans (Bradbury & Halliday, 1990).

Although ^{13}C-NMR spectrum is more informative, the ^{1}H-NMR spectrum of sub-fraction As (data not shown) shows, a pattern similar to that of fraction A in the region where non-carbohydrate components are expected.

The UV–vis absorption spectrum of sub-fraction As in aqueous solution, reported in Fig. 5(b), shows a maximum absorption at 263 nm with a long tail extending into the visible region. A fluorescent scan showed that when this sub-fraction was excited at 360 nm, an emission spectrum was obtained with a maximum at 440 nm (see Fig. 8(a)). Such spectroscopic properties suggest that the non-carbohydrate components can be described as melanoidins (e.g. non-enzymatically glycosylated proteins) (Lusk, Cronan,

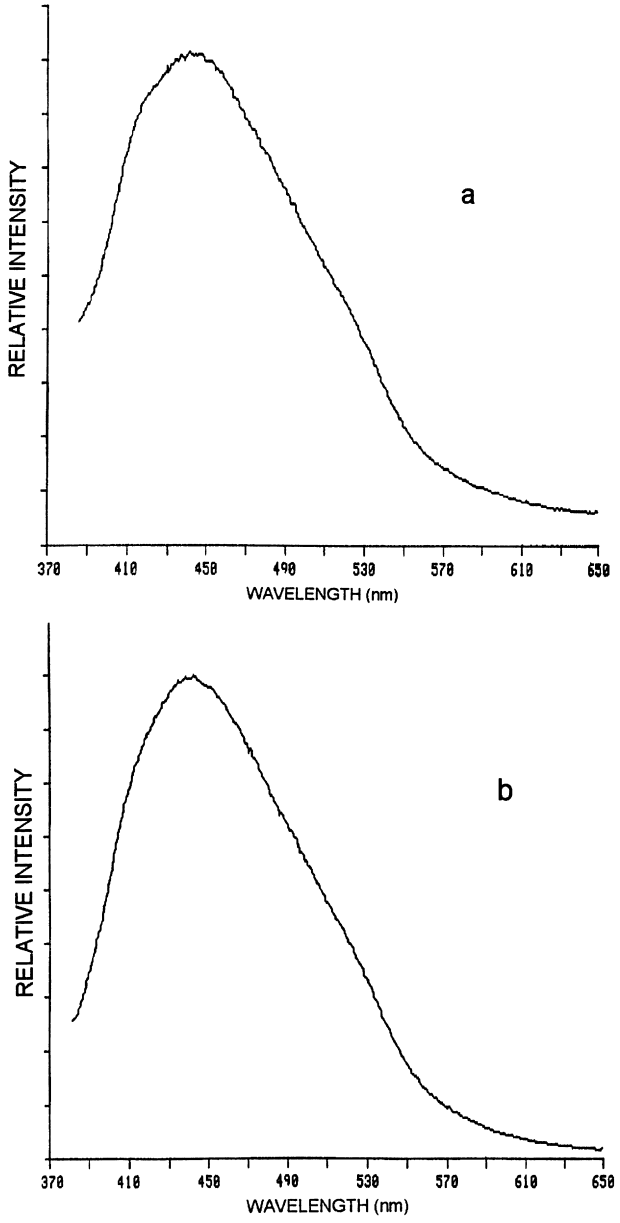

Fig. 8. Fluorescence emission spectra of sub-fraction As (a) and sub-fraction AG (b); excitation, 360 nm.

Chicoye, & Goldstein, 1987). The CD spectrum (not shown) does not show any signal in the range 200–500 nm.

The possible occurrence of depolymerized mannan in sub-fraction As, chemically identical to sub-fraction Ans is supported by the NMR data. Therefore, in the attempt to remove mannose-containing polymeric material from sub-fraction As, barium hydroxide was used as a complexing agent, as described elsewhere (Wolfrom & Anderson, 1967). The formation of an abundant precipitate after addition of barium hydroxide to a solution of sub-fraction As in 0.05 N NaOH further confirmed the presence of the mannose-containing component. From the supernatant, after exhaustive dialysis against water, sub-fraction AG was obtained.

4.3. Sub-fraction AG

This sub-fraction contained galactose as the main component, arabinose, residual mannose and traces of other not identified components (see Table 2). The $\langle M_w \rangle$ of this sub-fraction was 6800 Da with a polydispersity index of 1.35. In Table 2 it is clearly shown that more than 95% (w/w) of mannose was removed from fraction As. Comparable weight loss percentage for galactose and arabinose is observed. Also in this case the weight loss percentage of the non-carbohydrate components is comparable with that of the total weight loss. The UV–vis absorption spectrum (see Fig. 5(c)), the fluorescent scan (see Fig. 8(b)) and the CD spectrum (not shown) of sub-fraction AG in aqueous solution, do not show any difference compared to those of the sub-fraction As.

In the ^{13}C-NMR spectrum shown in Fig. 6(c), six signals assigned to the ring carbons of the β-(1-4)-D-mannopyranose units were still present but with very low intensity. Six intense resonances at 104.9, 82.9, 75.7, 71.3, 69.5 and 61.9 were also observed. By comparison with chemical shift data from the literature (Collins, Bradbury, Trifonoff, & Messer, 1981), these signals indicate the presence of β-(1-3)-D-galactopyranose units. Five minor resonances were also present at 110.1(C1), 85.03 (C4), 81.17 (C2), 77.67 (C3) and 62.40 ppm (C5) and were assigned to terminal α-L-arabinofuranose residues (Ebringerova et al., 1992). Six additional sharp resonances at 104.3, 75.9, 73.7, 71.6, 69.5 and 61.9 ppm were assigned as being due to terminal β-D-galactopyranose units in side chains by comparison with the chemical shifts of methyl-β-D-galactoside (Marohoshi, 1991). The presence of negative signals at 70.0 ppm in a DEPT-135 experiment (data not shown) demonstrated the existence of glycosylation at C6 position. The resonances at about 74 ppm were assigned to the C5 carbon atoms which experienced an upfield shift (β effect) induced by glycosylation.

Table 2
Sugar analysis of sub-fractions As and AG (weight basis and mol basis)

Fraction	Total weight (mg)	wt.%				mol/100 mol sugar		
		Mannose	Galactose	Arabinose	Non-carbohydrate components	Mannose	Galactose	Arabinose
As (part of)	287	65.7	27.7	4.2	2.4			
AG	80.8	9.7	77.1	11.2	2.0	9.7	76.9	13.4
Loss (mg)	−206.2							
(% w/w)	−71.8	−95.8	−21.6	−24.9	−76.5			

Table 3
Sugar composition of the polysaccharides present in the fraction A and in the sub-fractions Ans, As and AG

Fraction	Mol/100 mol sugar				
	Mannan			Arabinogalactan	
	Mannose	Galactose	Arabinose	Galactose	Arabinose
A	75.90	2.87	0.96	16.73	3.54
A[a]	75.90	2.17	0.91	17.43	3.59
Ans	95.10	3.60	1.20	–	–
As	66.70	2.52	0.84	25.58	4.26
AG	9.70	0.37	0.12	76.53	13.28

[a] Including weight loss.

The presence of residual mannose may be due to a small amount of mannan impurity. The experimental data strongly suggest that sub-fraction AG can be described as an arabinogalactan having a backbone chain of β-(1-3)-D-galactopyranose units to which terminal β-D-galactose and α-L-arabinofuranose side chains are linked, mainly in C6 position. These structural data reveal a very strong similarity between fraction AG and the arabinogalactan originally present in the green coffee beans (Bradbury & Halliday, 1990).

Since L-arabinofuranose residues are readily cleaved under acidic conditions, a simple partial hydrolysis of sub-fraction AG with 0.05 M oxalic acid was performed (Bradbury & Halliday, 1990). The ^{13}C-NMR spectrum of hydrolyzed sub-fraction AG (fraction DeAg) is reported in Fig. 6(d). The spectrum clearly shows the absence of the five resonances, which were assigned to the terminal α-L-arabinofuranose residues, as expected, and no signals of β-(1-4)-D-mannopyranose units were detected. The whole set of signals previously assigned to the backbone and terminal galactose units was present in the spectrum. The ratio backbone/terminal galactose units suggests a highly substituted galactan chain.

5. Conclusions

In spite of the different sources, extraction and isolation procedures, the sugar composition of the polysaccharide fraction isolated from hot water extract of roasted *Coffea arabica* beans presently investigated (fraction A), is in substantial agreement with previous reported data (Wolfrom & Anderson, 1967; Maier & Buttle, 1973). However two different interpretations were proposed as far as the arrangement of the sugar components is concerned. In one case two different polysaccharides (mannan and arabinogalactan) were found (Wolfrom & Anderson, 1967) whereas a single arabinogalactomannan was found by the latter authors (Maier & Buttle, 1973).

Comparative analysis of the previous work and data from the present investigation strongly suggests that the above interpretations may be considered as complementary rather than alternative. In fact the polysaccharides isolated from the starting polysaccharide fraction (fraction A) are indeed two: "mannan" and arabinogalactan. However the so-called mannan is not a simple linear β-(1-4)-D-mannan, as previously suggested (Wolfrom & Anderson, 1967), but a branched β-(1-4)-D-mannan substituted with small amounts of galactose and arabinose (strictly speaking: an arabinogalactomannan). This polysaccharide can be separated from the starting fraction by means of a mild alkaline treatment and it is recovered as a water insoluble material (sub-fraction Ans) whereas the arabinogalactan (sub-fraction AG) can be isolated upon the removal of the mannose-containing component from the starting fraction A. Assuming that the sugar composition of the water-insoluble material represents the sugar composition of the mannose-containing polysaccharide present in the starting fraction and in the other sub-fractions examined and taking into account the different weight loss of component sugars, it is possible to calculate the sugar composition of the two polysaccharides as shown in Table 3. By examining Table 3, it can be observed that fraction A is composed of about 80% (mol basis) of "mannan" and of about 20% of arabinogalactan and that the molar ratio galactose/arabinose in the arabinogalactan is close to 5:1. Moreover sub-fraction AG contains about 10% of mannan and about 90% of arabinogalactan. In sub-fractions As and AG, the molar ratio galactose/arabinose in the arabinogalactan is close to 6:1. The small difference in the galactose/arabinose molar ratio may reflect the lability of the arabinose residues.

Both polysaccharides are structurally related to those originally present in the green coffee beans, even if the arabinogalactan appears to be more altered by the process of roasting (Bradbury & Halliday, 1990).

Yet, it is not clear if in the starting fraction the two polysaccharides are the individual components of a physical mixture or if they are associated to form a complex assembly. Under the latter hypothesis, proteinaceous material may play an important role but this has to be supported by further studies.

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