ELSEVIER

Carbohydrate Polymers 49 (2002) 57–62

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

# Isolation of xylogalactans from the Corallinales: influence of the extraction method on yields and compositions

Diego A. Navarro, Carlos A. Stortz*

*Departamento de Química Orgánica – CIHIDECAR, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pab. 2, Ciudad Universitaria, 1428 Buenos Aires, Argentina*

Received 13 March 2001; accepted 4 July 2001

**Abstract**

An already studied seaweed, *Corallina officinalis* and two other species of the red seaweed order Corallinales, i.e. *Bossiella orbigniana* and *Jania rubens*, were extracted using three different standard procedures: the method of Usov, which destroys the calcium carbonate cover by the addition of concentrated acid, the method of Cases, which applies controlled acid degradation of this cover, and the method of Takano, which avoids an acid medium, and uses hot water. The three methods extract similar amounts of products, but those using acid give better yields of xylogalactans, without evidence for degradation. However, subsequent hot water extraction shows that the traces of acid present when applying the method of Usov yield partially degraded products at this stage. The characteristics of the xylogalactans of the newly studied seaweeds are similar to those previously studied. However, in *Jania rubens* significant amounts of 3,6-anhydrogalactose, and in *Bossiella orbigniana* small amounts of the D-enantiomer of 2-*O*-methylgalactose were found. 

*Keywords*: Rhodophyta; Corallinaceae; Extraction; Agaran; Xylogalactans; *Bossiella*; *Jania*; *Corallina*

## 1. Introduction

Sulfated galactans are distributed among most of the red seaweeds (Rhodophyceae). They consist of linear chains of alternating 3-linked β-galactose residues and 4-linked α-galactose residues. The former units always belong to the D-series, whereas the latter may occur as residues of the D- or L-series (Stortz, Cases & Cerezo, 1997). The configuration of these units has allowed classification of these polysaccharides as carrageenans, or agarans, respectively. However, the presence of hybrids carrying units with either configuration was also found in many species (Stortz & Cerezo, 2000; Stortz et al., 1997). The structures are usually masked by substitution with sulfate groups, pyruvic acid ketals, methoxyl groups, a 3,6-anhydro ring replacing the α-galactose unit, and the presence of side chains.

Red seaweeds produce large amounts of galactans. Therefore, the products from species belonging to different orders have been studied in detail (Stortz & Cerezo, 2000). However, those belonging to the Corallinales have received less attention, probably due to their calcareous cover, which diminishes the yield of polysaccharides sharply. Besides a preliminary report (Turvey & Simpson, 1965), the first detailed study was carried out with the Atlantic species *Corallina officinalis* (Cases, Stortz & Cerezo, 1992). This seaweed carries a peculiar type of agaran ('corallinan'), with methoxyl and/or sulfate groups on positions 2 and 3 of the L-galactose units, and β-D-xylosyl or sulfate groups attached at position 6 of the D-galactose units. Some fractions also carry methoxyl and 4-*O*-methyl-D-galactosyl groups at position 6 of the D-galactose units (Cases, Stortz & Cerezo, 1994; Stortz et al., 1997). Similar results have been found for three other species from the Pacific Ocean, i.e. *Corallina pilulifera* (Usov, Bilan & Klochkova, 1995; Usov, Bilan & Shashkov, 1997), *Joculator maximus* (Takano, Hayashi, Hayashi, Hara & Hirase, 1996), and *Bossiella cretacea* (Usov & Bilan, 1996, 1998), each one with minor characteristics of its own. Besides, these seaweeds were shown to produce floridean starch (Cases et al., 1992; Takano et al., 1996; Usov & Bilan, 1996; Usov et al., 1995) and, in some species the presence of alginate, uncommon in red seaweeds was also shown to occur (Usov & Bilan, 1996; Usov et al., 1995).

In their original work, Turvey and Simpson (1965) removed the calcium carbonate cover by careful addition of diluted hydrochloric acid, keeping the pH above 6.5, in order to extract the polysaccharides. Cases et al. (1992,

* Corresponding author. Tel./fax: +54-11-4576-3346.
*E-mail address:* stortz@qo.fcen.uba.ar (C.A. Stortz).

0144-8617/02/$ - see front matter 
PII: S0144-8617(01)00298-3

1994) followed a similar procedure, while Takano et al. (1996) effected the extraction with hot water, in an attempt to avoid an acidic medium, which might degrade the polysaccharides. On the other hand, Usov et al. (1995) used concentrated hydrochloric acid to destroy the calcite cover and extract the polysaccharides.

In the present work, the polysaccharides from *Corallina officinalis* were extracted using the three different methods, and the differences in yields and compositions were assessed. Besides, the same methods were used to extract the polysaccharides from the other two Atlantic corallinean seaweeds, *Bossiella orbigniana* (Decaisne) Silva, and *Jania rubens* (L.) Lamoroux. The characterization of these polysaccharides is reported for the first time.

## 2. Experimental

### 2.1. Materials

Samples of *Corallina officinalis* and *Jania rubens* were collected near Miramar (Buenos Aires Province, Argentina), while those of *Bossiella orbigniana* were collected near Comodoro Rivadavia (Chubut Province, Argentina). The seaweeds were air dried, manually cleaned and milled to a fine powder before extraction. All chemicals were of analytical grade.

### 2.2. Extraction procedures

The seaweeds were extracted using three different procedures.

#### 2.2.1. By the method of Cases et al. (1992)

15 g of milled seaweed were suspended in 90 ml of water, and 1 M HCl was added dropwise with strong mechanical stirring, until no more $CO_2$ evolution was detected (*ca* 250 ml). The pH was not allowed to drop below 6. The suspension was stirred mechanically for 24 h at room temperature. The residue was removed by centrifugation and washed ($2 \times 25$ ml). The supernatant was combined with the washings, concentrated to *ca* 50 ml and dialyzed ($M_w$ cut-off 6000–8000) against tap water and finally distilled water. After lyophilization, the products were purified by redisolution in water, centrifugation (the residue was discarded), dialysis of the supernatant, and finally isolated by lyophilization.

#### 2.2.2. By the method of Usov et al. (1995)

27 ml of concentrated HCl was added carefully, with strong stirring, to 15 g of milled seaweed suspended in 75 ml of water. The mixture was stirred mechanically for 2 h at room temperature. After centrifugation and removal of the supernatant, the residue was re-extracted for three more times with 75 ml HCl 0.1 M each and finally washed with water ($2 \times 25$ ml). The supernatants and washings were combined, and treated as described in Section 2.2.1 to yield the final products.

#### 2.2.3. By the method of Takano et al. (1996)

15 g of milled seaweed were extracted with water at 100°C, for 10 h ($2 \times 120$ ml). The final residue was washed with water ($2 \times 25$ ml), and the combined extracts were treated as stated in Section 2.2.1.

#### 2.2.4. Subsequent extractions

Each residue of the above mentioned extraction procedures was re-extracted with water (100 ml) at 100°C for 20 h. The extracts were recovered after centrifugation, to yield the final solid products obtained as described in Section 2.2.1.

### 2.3. General methods

Total carbohydrates were assayed by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers & Smith, 1956), standardized as depicted by Cases et al. (1992). Protein was quantitated by the method of Lowry, Rosebrough, Farr and Randall (1951), using bovine serum albumin as standard. Sulfate was determined by a turbidimetric method (Dodgson & Price, 1962). Molecular weights (uncorrected for the presence of non-carbohydrate components) were calculated from the reducing power, which was determined by the method of Park and Johnson (1949). Uronic acids were quantitated using the method of Filizetti-Cozzi and Carpita (1991). Optical rotations were measured using 0.4–0.6% solutions in water. Cyclizable 6-sulfate was determined by measuring the 3,6-anhydrogalactose (Yaphe & Arsenault, 1965) produced after alkaline treatment of the products (1 M NaOH, 80°C, 5 h).

The proportions of monosaccharides constituting the polysaccharides were determined by gas chromatography (GLC) of the hydrolyzates, using three different derivatives. The regular hydrolysis procedure was performed by treating the polysaccharides with 2 M trifluoroacetic acid (90 min at 120°C). The TFA was eliminated by evaporation. Aliquots of the hydrolyzates were converted to their aldononitrile acetates (Turner & Cherniak, 1981) and analyzed by GLC as described elsewhere (Cases et al., 1992). Other aliquots of the hydrolyzates were converted to the acetylated aminodeoxyalditols using (*S*)-1-amino-2-propanol and (*S*)-α-methylbenzylamine (Cases, Cerezo & Stortz, 1995) and analyzed by GLC as stated therein. In order to avoid destruction of the 3,6-anhydrogalactose, alditol acetates were obtained from the products of a reductive hydrolysis procedure (Stevenson & Furneaux, 1990). The alditol acetates were analyzed by GLC using a capillary column ($30\ m \times 0.25$ mm) coated with SP-2330 (0.20 μm), using nitrogen as the carrier with a flow rate of 1 ml/min, in the split mode (split ratio 1:100). Runs were programmed from 200°C, 2°C/min to 230°C, and then held for 20 min. Injector and detector (FID) were kept at 240°C.

Table 1
Yields and analyses of the products obtained from the three seaweeds by three different extraction procedures

| | *Corallina officinalis* | | | *Bossiella orbigniana* | | | *Jania rubens* | | |
|---|---|---|---|---|---|---|---|---|---|
| | Co-C | Co-U | Co-T | Bo-C | Bo-U | Bo-T | Jr-C | Jr-U | Jr-T |
| Yield (%) | 0.48 | 0.40 | 0.28 | 0.48 | 0.67 | 0.52 | 0.71 | 1.00 | 1.10 |
| Carbohydrates (%) | 55 | 72 | 46 | 54 | 62 | 54 | 52 | 62 | 48 |
| Protein (%) | 12 | 6 | 18 | 20 | 13 | 30 | 15 | 9 | 29 |
| Sulfate (% $SO_3Na$) | 7 | 6 | 7 | 7 | 7 | 7 | 8 | 7 | 6 |
| Uronic acids (%) | 4 | 4 | 4 | 6 | 3 | 3 | 4 | 4 | 3 |
| $[\alpha]_D$ (°) | −55 | −44 | −36 | −48 | −63 | −33 | −51 | −63 | −33 |
| Mol. Weight (kDa) | 11.7 | 13.1 | 10.7 | 5.3 | 8.6 | 3.8 | 11.3 | 10.6 | 7.9 |
| Yield of residue (%) | 40 | 11 | 92 | 52 | 4 | 92 | 45 | 18 | 86 |

## 3. Results

*Corallina officinalis*, thoroughly studied in our lab, and two other Atlantic corallinean seaweeds from the Argentine shores, i.e. *Bossiella orbigniana* and *Jania rubens* were chosen for this study. The three seaweeds were milled, and divided into three portions, which were subjected to the extraction procedures as already been reported by Cases et al. (1992), Takano et al. (1996) and Usov et al. (1995). In this way, nine extracts were obtained. The products were isolated after dialysis, freeze-drying, redissolution in water, dialysis of the supernatant and final freeze-drying. Their acronyms combine the initials of the name of the seaweed (Co, Bo and Jr, respectively) with the initial of the first author of the extraction procedure (C, U and T, respectively). The yields and analyses of the products are shown in Table 1. In order to calculate the proportions of their constituent monosaccharides, three derivatization methods have been used, as it was found that this is the only way to discriminate between all of them: aldononitrile acetates are useful to distinguish between 3- and 4-*O*-methylgalactose; reductive hydrolysis and derivatization to the alditol acetates gives information about the 3,6-anhydrogalactose present, while derivatization with chiral amines was used to determine the configuration of the sugars present. The last method cannot be used alone, as some overlapping occurs (Cases, Cerezo & Stortz, 1995). The final, averaged results are shown in Table 2.

The yields from each method are similar (Table 1). However, the analyses (Tables 1 and 2) of the hot water-extracted products (-T samples) indicate that they have larger proportions of floridean starch (higher proportion of glucose and lower optical rotation) and protein, and thus, lesser amounts of xylogalactans. On the other hand, those xylogalactans have a markedly lower proportion of xylose (Table 2). The molecular weight was not reduced by the acid treatment. Furthermore, the neutral extractions led to the products with slightly lower molecular weight, while the most severe acid extractions gave higher molecular weight products. These extractions (-U) also led to products with

Table 2
Monosaccharides constituting the products obtained from the three seaweeds by three different extraction procedures (mols/100 mols [galactose + methylated galactoses])

| | *Corallina officinalis* | | | *Bossiella orbigniana* | | | *Jania rubens* | | |
|---|---|---|---|---|---|---|---|---|---|
| | Co-C | Co-U | Co-T | Bo-C | Bo-U | Bo-T | Jr-C | Jr-U | Jr-T |
| Xylose | 34 | 29 | 23 | 35 | 30 | 23 | 38 | 35 | 29 |
| Mannose | 3 | 2 | 6 | 5 | 3 | 5 | 3 | 2 | 3 |
| Glucose | 7 | 8 | 19 | 26 | 7 | 42 | 8 | 8 | 46 |
| D-Galactose | 48 | 54 | 46 | 33 | 36 | 33 | 49 | 53 | 45 |
| 2-*O*-Me-D-Gal | – | – | – | 3 | 3 | 4 | – | – | – |
| 3-*O*-Me-D-Gal | – | – | – | – | – | – | 2 | tr. | 2 |
| 4-*O*-Me-D-Gal | 3 | 2 | 3 | – | – | – | – | – | – |
| 6-*O*-Me-D-Gal | 2 | 2 | 3 | 13 | 12 | 16 | 4 | 4 | 5 |
| L-Galactose | 22 | 22 | 25 | 30 | 28 | 30 | 26 | 23 | 23 |
| 2-*O*-Me-L-Gal | 20 | 16 | 17 | 13 | 13 | 8 | 9 | 9 | 10 |
| 3-*O*-Me-L-Gal | 5 | 4 | 4 | 7 | 7 | 8 | 5 | 6 | 5 |
| 3,6-AnGal | – | – | 2 | 1 | 1 | 1 | 5 | 6 | 11 |
| Ratios | | | | | | | | | |
| D/L[a] | 53/47 | 58/42 | 52/48 | 49/51 | 51/49 | 53/47 | 55/45 | 57/43 | 51/49 |
| Gals/Xyl | 2.9 | 3.4 | 4.4 | 2.9 | 3.3 | 4.4 | 2.6 | 2.8 | 3.5 |

[a] Assuming that the 3,6-anhydrogalactose belongs to the L-series.

Table 3
Yields, analyses and monosaccharide compositions (mols/100 mols of [galactoses + methylated galactoses]) of the products obtained by hot water extraction of the residues produced from the three seaweeds by three different extraction methods

| | *Corallina officinalis* | | | *Bossiella orbigniana* | | | *Jania rubens* | | |
|---|---|---|---|---|---|---|---|---|---|
| | Co-C2 | Co-U2 | Co-T2 | Bo-C2 | Bo-U2 | Bo-T2 | Jr-C2 | Jr-U2 | Jr-T2 |
| Yield (%) | 0.29 | 0.22 | 0.2 | 0.23 | 0.17 | 0.23 | 0.33 | 0.59 | 0.65 |
| Carbohydrates (%) | 82 | 96 | 77 | 71 | 82 | 60 | 51 | 40 | 44 |
| Protein (%) | 13 | 6 | 13 | 21 | 15 | 19 | 49 | 52 | 36 |
| Sulfate (% $SO_3Na$) | 6 | 2 | 5 | 6 | 5 | 6 | 4 | 4 | 4 |
| Uronic acids (%) | 7 | 7 | 4 | 4 | 4 | 7 | 3 | 4 | 3 |
| $[\alpha]_D$ (°) | −44 | +64 | −28 | −20 | +17 | −44 | −45 | −18 | −37 |
| Mol. Weight (kDa) | 15.2 | 2.7 | 6.7 | 5.1 | 1.6 | 7.1 | 4.5 | 3 | 7 |
| Xylose | 23 | 17 | 26 | 27 | 15 | 26 | 29 | 16 | 29 |
| Mannose | 3 | 7 | 3 | 4 | 6 | 3 | 3 | 6 | 3 |
| Glucose | 13 | 265 | 35 | 72 | 183 | 6 | 18 | 173 | 9 |
| D-Galactose | 58 | 43 | 51 | 39 | 40 | 40 | 45 | 47 | 45 |
| 2-*O*-Me-D-Gal | – | – | – | 2 | 1 | 2 | – | – | – |
| 3-*O*-Me-D-Gal | – | – | – | – | – | – | 2 | 2 | 3 |
| 4-*O*-Me-D-Gal | 2 | 2 | 3 | – | – | – | – | – | – |
| 6-*O*-Me-D-Gal | 2 | 2 | 3 | 13 | 12 | 10 | 4 | 2 | 5 |
| L-Galactose | 20 | 31 | 23 | 28 | 30 | 30 | 28 | 41 | 21 |
| 2-*O*-Me-L-Gal | 14 | 15 | 14 | 11 | 11 | 11 | 7 | 8 | 7 |
| 3-*O*-Me-L-Gal | 3 | 7 | 4 | 6 | 6 | 5 | 4 | – | 4 |
| 3,6-AnGal | – | – | 2 | 2 | – | 2 | 8 | – | 15 |
| Ratios | | | | | | | | | |
| D/L[a] | 63/37 | 47/53 | 56/44 | 54/46 | 53/47 | 53/47 | 52/48 | 51/49 | 53/47 |
| Gals/Xyl | 4.3 | 5.8 | 3.8 | 3.7 | 6.6 | 3.8 | 3.4 | 6.4 | 3.5 |

[a] Assuming that the 3,6-anhydrogalactose belongs to the L-series.

the lowest amount of protein. The extractions with careful addition of acid (-C) usually gave products with intermediate characteristics, though they had the largest xylose/galactose ratios. For *Bossiella orbigniana* this method removed an unusually high amount of glucose (Table 2). The yields of the residues are shown in Table 1. It is evident that the strong acid medium (-U) removed completely the calcium carbonate, while the hot water (-T) performed a less complete extraction, while the controlled acid extraction only removed about half of the calcite cover.

According to the analysis, the products of *Bossiella orbigniana* and *Jania rubens* are xylogalactans, as reported previously for *Corallina officinalis* and other Corallinales. They have similar amounts of D- and L-galactoses, large proportions of xylose and methoxylated galactose units. Methoxyl groups have been found to appear on every position of galactose in *Corallina officinalis*, but mainly on C-2. The products from *Bossiella orbigniana* are similar, although no methoxylation on C-4 has been found, the proportion of C-6 methoxylation is much higher, and C-2 methoxylation appears for galactoses from both enantiomeric series. On the other hand, those from *Jania rubens* are much less methoxylated (especially on C-2), and minor amounts of C-3 methoxylation of D-galactose units are found. In addition, in this seaweed moderate amounts of 3,6-anhydrogalactose were found for the first time in a corallinean polysaccharide. This sugar can be detected mainly using the hot-water extraction procedure (Table 2). No cyclizable 6-sulfate has been found in either of these polysaccharides.

The nine residues from the extractions were re-extracted with hot water. Yields, analyses and monosaccharide compositions are shown in Table 3. Their acronyms add the number 2 to the name of the parent extract. The yields of products are lower than those obtained in the first extraction, in every method. Only a slight dependence on the first extraction procedure is shown. The products carry similar amounts of uronic acids to their counterparts from the first extraction, but they have less sulfate. Only in the protein-rich *Jania rubens* was the amount of protein shown to increase markedly. However, the molecular weight of most of the products dropped considerably, especially for the products first extracted by the harsh acid procedure (-U2). The products are enriched in floridean starch, thus, their optical rotations are lower in absolute value, and can even acquire positive values (Table 3). This is true especially for the products that originated from the harsh acid treatment (-U2), and also for those originated from the other acidic procedure (-C2). As the method of Takano has removed larger amounts of floridean starch and less of xylogalactans in the first extraction, their proportions are now reversed. As for the characteristics of the xylogalactans, they are similar to those from the first extractions. However, the proportion of xylose decreases sharply in the products

extracted by the method of Usov, and also, but to a lesser extent in those extracted by the method of Cases. The degradative effect of these methods is seen in the second extraction procedure. For the xylogalactans from *Jania rubens*, the products of the second extraction arising from the harsh acid procedure (Jr-U2) did not carry any 3,6-anhydrogalactose, while those from the other procedures were slightly enriched in this sugar.

## 4. Discussion

The polysaccharides from the order Corallinales have been studied in detail for only four species. The structural resemblance between them is marked, thus, their description through a collective name ('corallinans', Cases et al., 1994) is validated. Corallinans are polysaccharides with an agaran backbone, devoid of 3,6-anhydrogalactose, in which C-6 of β-D-galactose residues is substituted almost completely, mainly by β-D-xylopyranosyl residues, and also by sulfate, while the C-2 of α-L-galactose moieties is partially substituted by methoxyl and sulfate groups, and C-3 of the same unit also carries some sulfate groups. The polysaccharides from *Joculator maximus* (Takano et al., 1996) are the simplest: they were shown to carry only these main substituents. In *Corallina pilulifera* (Usov et al., 1997), small proportions of C-3 of α-L-galactose and C-6 of β-D-galactose also appear methoxylated. In addition, in *Bossiella cretacea* (Usov & Bilan, 1998), C-4 of β-D-galactose appears partially sulfated, a feature unique for this species. On the other hand, the products from *Corallina officinalis* (Cases et al., 1994; Stortz et al., 1997) are more complex: they are similar to those of *Corallina pilulifera*, although the degree of methoxylation is higher, and small amounts of 4-*O*-methyl-D-galactose side chains appear as substituents on C-6 of D-galactoses.

The analysis of the products arising from the two seaweeds studied for the first time in the present work, i.e. *Bossiella orbigniana* and *Jania rubens* indicate that they follow similar patterns. However, the products from *Jania rubens* exhibit significant amounts of 3,6-anhydrogalactose (Tables 2 and 3), a fact uncommon within the order. This seaweed also gave rise to products with minor amounts of 3-*O*-methyl-D-galactose, indicating a possible deviation from the regular agaran structure. The polysaccharides from *Bossiella orbigniana* follow the pattern of the order. However, it is noteworthy that there is a large proportion of 6-*O*-methyl-D-galactose (Tables 2 and 3), similar to that of 2-*O*-methylgalactose, and also that part of the latter sugar belongs to the D-series. Only trace amounts of this sugar were found in some fractions from *Corallina officinalis* (Cases et al., 1995). Further work in progress will establish the fine structural details of the polysaccharides from these new seaweeds.

This work also compares the ability of the three different methods usually applied to the extraction of the corallinans. The best method will be that which avoids adsorption or clustering of the xylogalactans into the calcite cover, but produces little or no degradation, i.e. that which gives the highest yield of xylogalactans of the higher molecular weight with the lowest reduction of labile sugars. The method of Usov et al. (1995) employs concentrated hydrochloric acid in order to destroy the calcium carbonate cover. The original method, modified by Cases et al. (1992) also employs hydrochloric acid, but diluted and added with special care to avoid sudden pH drops. Alternatively, the method of Takano et al. (1997) avoids an acid medium, and uses hot water to perform the extraction. In their procedures, further purification is effected by precipitation with cationic detergents (Takano et al., 1997; Usov et al., 1995) or by anion-exchange chromatography (Cases et al., 1992; Turvey & Simpson, 1965), in order to separate the xylogalactans from the floridean starch and other contaminants. In the extraction stages, the yields of the products obtained by each method were similar (Table 1). However, the hot-water extraction method effected a better extraction of the floridean starch and protein (Tables 1 and 2). Thus, this method extracts less xylogalactans that carry lower proportions of xylose (Table 2). This fact may be attributed to a differential location of the xylogalactans within the cell wall matrices. Those carrying more xylose are less exposed to the extraction medium, unless the calcium carbonate is destroyed. A decrease in the molecular weight using this method is observed, possibly due to the fact that the starch has a lower molecular weight than the xylogalactans. The products extracted with the harsh method of Usov have molecular weights even larger than those extracted by the other methods (Table 1), indicating a more complete extraction of the larger xylogalactans, and little or no degradation. However, the proportion of xylose is lower than that using the method of Cases et al. (1992), thus suggesting partial cleavage of xylosyl side chains. The Cases procedure does not destroy completely the calcium carbonate cover as the Usov procedure does (Table 1), suggesting that the latter may have reached more 'extractable places'.

Hot-water extraction of the residues originating in the three methods of extraction gives interesting complementary data. The residues from the concentrated acid extraction have suffered a marked degradation, possibly by the effect of traces of acid remaining in the residue (even after washing), when exposed to high temperatures. This fact was not observed by Usov et al. (1995), because they effected the second extraction of the residue with sodium carbonate. In this work, the degradation in those extracts (-U2) is shown by a decrease in their molecular weights and proportions of xylose, and the disappearance of 3,6-anhydrogalactose (Table 3). However, the yield of xylogalactans in those extractions is low, when compared to that of floridean starch. The first hot-water extraction (-T products) removed nearly all the floridean starch, as shown by the low amounts of glucose found in the second-extraction products -T2. Their xylogalactans have slightly higher proportions of

xylose than those of the first extraction, as expected (see above), while those from the controlled acid extraction (-C2) carry less xylose. The extracts from *Jania rubens* appear highly enriched in protein, while their xylogalactans, are even richer in 3,6-anhydrogalactose than those obtained from the first extraction, suggesting the possible presence of products with agarose-like structures, which are less prone to be removed by water.

## 5. Conclusion

The extraction of the xylogalactans from the Corallinales can be effected by any of the three extraction procedures. The method of Takano et al. (1997) gives lower yields of xylogalactans and higher proportions of starch in the first extraction. Thus, the acid extractions may be considered preferable. However, if a second hot water extraction is carried out, the effect of acid shows detrimental effects on the xylogalactans, especially when using concentrated acid in the first extraction.

## Acknowledgements

This work was supported by grants from UBA and the Foundations Vitae-Antorchas. The authors are indebted to Drs M.R. Cases and M.L. Flores for the collection of the seaweeds, and to Dr A.S. Cerezo for helpful discussions. C.A. Stortz is a Research Member of the National Research Council of Argentina (CONICET).

## References

Cases, M. R., Stortz, C. A., & Cerezo, A. S. (1992). Methylated, sulphated xylogalactans from the red seaweed Corallina officinalis. *Phytochemistry*, *31*, 3897–3900.

Cases, M. R., Stortz, C. A., & Cerezo, A. S. (1994). Structure of the 'corallinans', sulfated xylogalactans from *Corallina officinalis*. *International Journal of Biological Macromolecules*, *16*, 93–97.

Cases, M. R., Cerezo, A. S., & Stortz, C. A. (1995). Separation and quantitation of enantiomeric galactoses as their mono-*O*-methylethers as their diastereomeric 1-deoxy-1-(2′-hydroxypropylamino)-alditols. *Carbohydrate Research*, *269*, 333–341.

Dodgson, K. S., & Price, R. G. (1962). A note on the determination of the ester sulphate content of sulphated polysaccharides. *Biochemical Journal*, *84*, 106–110.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Filisetti-Cozzi, T. M. C. C., & Carpita, N. C. (1991). Measurement of uronic acids without interference from neutral sugars. *Analytical Biochemistry*, *197*, 157–162.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, *193*, 265–275.

Park, J. T., & Johnson, M. J. (1949). A submicrodetermination of glucose. *Journal of Biological Chemistry*, *181*, 149–151.

Stevenson, T. T., & Furneaux, R. H. (1990). Chemical methods for the analysis of sulphated galactans from red algae. *Carbohydrate Research*, *210*, 277–298.

Stortz, C. A., & Cerezo, A. S. (2000). Novel findings in carrageenans, agaroids and 'hybrid' red seaweed galactans. *Current Topics in Phytochemistry*, *4*, 121–134.

Stortz, C. A., Cases, M. R., & Cerezo, A. S. (1997). Red seaweed galactans: methodology for the structural determination of corallinan, a different agaroid. In R. R. Townsend & A. T. Hotchkiss Jr., *Techniques in glycobiology* (pp. 567–593). New York: Marcel Dekker.

Takano, R., Hayashi, J., Hayashi, K., Hara, S., & Hirase, S. (1996). Structure of a water-soluble polysaccharide sulfate from the red seaweed *Joculator maximus* Manza. *Botanica Marina*, *39*, 95–102.

Turner, S. H., & Cherniak, R. (1981). Total characterization of polysaccharides by gas–liquid chromatography. *Carbohydrate Research*, *95*, 137–144.

Turvey, J. R., & Simpson, P. R. (1965). Polysaccharides from *Corallina officinalis*. *Proceedings of the International Seaweed Symposium*, *5*, 323–327.

Usov, A. I., & Bilan, M. I. (1996). Polysaccharides from algae. 49. Isolation of alginic acid, sulfated xylogalactan and floridean starch from calcareous red alga *Bossiella cretacea* (P. et R.) Johansen (Rhodophyta, Corallinaceae). *Bioorganicheskaya Khimiya*, *22*, 126–133 (in Russian).

Usov, A. I., & Bilan, M. I. (1998). Polysaccharides from algae 52. The structure of sulfated xylogalactan from the calcareous red alga *Bossiella cretacea* (P. et R.) Johansen (Rhodophyta, Corallinaceae). *Bioorganicheskaya Khimiya*, *24*, 139–146.

Usov, A. I., Bilan, M. I., & Klochkova, N. G. (1995). Polysaccharides of algae 48. Polysaccharide composition of several calcareous red algae: isolation of alginate from *Corallina pilulifera* P. et R. (Rhodophyta, Corallinaceae). *Botanica Marina*, *38*, 43–51.

Usov, A. I., Bilan, M. I., & Shashkov, A. S. (1997). Structure of a sulfated xylogalactan from the calcareous red alga *Corallina pilulifera* P. et R. (Rhodophyta, Corallinaceae). *Carbohydrate Research*, *303*, 93–102.

Yaphe, W., & Arsenault, G. P. (1965). Improved resorcinol reagent for the determination of fructose, and of 3,6-anhydrogalactose in polysaccharides. *Analytical Biochemistry*, *13*, 143–148.