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SEASONAL VARIATION IN CHEMICAL COMPOSITION OF *PADINA PAVONIA*

M. MAGDEL-DIN HUSSEIN

Laboratory of Natural Products, National Research Centre, Dokki, Cairo

and

A. ABDEL-AZIZ and H. M. SALEM

Department of Biochemistry Faculty of Agriculture, Cairo University, Giza, Egypt.

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Abstract. An investigation of the effect of seasonal variation on the chemical composition of the brown algal species, *Padina pavonia*, showed that the fluctuations of protein and amino acid content of total lipids and mannitol were noted in August and November, respectively. The presence of the free monosaccharides, glucose, xylose, glucuronic acid, and mannose was observed in November. In the other seasons, when mannose was absent, there were only traces of the first three sugars. Acid hydrolysis of the seaweed afforded mannuronic acid, guluronic acid, glucuronic acid and their respective lactones as well as galactose, glucose, mannose, xylose, and fucose. The proportions of these sugars in the algal material differed according to the collection season.

Marine brown algae have been studied intensively for the industrial importance of their constituents, like alginic acid, mannitol, and laminaran. Evironmental conditions influence, to a great extent, the types and proportions of the constituents of any algal species. The effect of seasonal changes on such algal components has been reported by many investigators.[1-4] Therefore for the better utilization of seaweeds, it is necessary to investigate their constituents in different seasons. The present work deals with the influence of seasonal variations upon the composition of the brown algal species, *Padina pavonia.*

Materials and Methods

Collection and Pretreatment of the Alga. Padina pavonia was collected periodically in 1974-1975 from the same place at Roushdy, Alexandria. The alga was thoroughly washed with running water, dried in the sun for several days and then ground. The values were calculated on a dry weight basis.

Determination of Ash and its Mineral Components. After ashing at 800°, Ca, Mg, Na, Mn, Zn, and Cu were determined using the atomic absorption spectrophotometric technique.

Determination of Total Phosphorus. The algal material was wet ashed using a mixture of sulphuric-perchloric-nitric acids (1 : 2: 3). Phosphorus content of the resultant ash was determined according to the method of Fiske and Subbarow.[5]

Total Lipids. Lipids were isolated by Soxhlet extraction with petroleum ether (b. p. 40-60°) for 12 hr.

Mannitol. This was determined titrimetrically by the direct periodate oxidation using the milled algal material and titration of the liberated iodine with standard sodium thiosulphate solution.[6]

Low Molecular Weight Carbohydrates. The algal material was extracted with boiling 85% ethanol for 24 hr in a Soxhlet apparatus. After filtration, the extract was concentrated and examined by paper chromatography using *n*-butanol-pyridine-water (6 : 4 : 3, by vol.)[7] as solvent. Spots were detected with aniline phthalate and aniline diphenylamine phosphoric acid.[8]

Determination of the Combined Sugars. Complete acid hydrolysis of the algal material, pre-extracted with 85% ethanol, was carried out adopting the method of Haug and Larsen.[9] The hydrolysis products were then chromatographed in Whatman No. 1 filter paper (50 cm length) using *n*-butanol-ethanol-water (40 : 11 : 19, by vol.)[10] as solvent. After developing the paper for 48 hr (3 times), the separated sugars were determined quantitatively.[11] Further chromatographic examination of the algal hydrolysate was done to identify the uronic acid components. The solvent mixtures used were ethylacetate-pyridine acetic acid-water (5 : 5 : 1 : 3, by vol.)[12] and pyridine-ethylacetate-water (11 : 40: 6, by vol.).[12]

Crude Protein. Organic nitrogen was determined by micro-Kjeldahl method and multiplied by 6.25.

Amino Acids. The algal material was hydrolysed in 6N HCl in a sealed tube for 10 hr at 105°. After the removal of HCl by evaporation at 100° with the occasional addition of water, paper chromatography of the hydrolysate was performed using the solvent mixture *n*-butanol-acetic acid-water (4 : 1 : 5, by vol.)[13] and phenol-water (4 : 1, w/v).[14] After developing, the papers were dipped in cadmium-

ninhydrin reagent,[15] and the amino acids and then determined, individually, according to the method of Levy and Chung.[14]

For tryptophane, the algal material was hydrolysed with 14% barium hydroxide for 24 hr at 120°. After neutralization with CO_2, the hydrolysate was analysed for its content of tryptophane adopting the method of Blauth, *et al.*[16]

Results and Discussion

The data recorded in Tables 1-2 indicated that there is a significant seasonal variation in the chemical composition of *Padina pavonia*. Nevertheless, the changes in the amounts of both of mannitol and crude protein are less extreme than those of the other algal constituents.

Seasonal fluctuations of protein content appear to correspond with those of the ash, a result which is in accordance with that obtained by Black[17] for *Laminaria saccharina*. In April the alga showed the highest amounts of Ca, P, K, Zn, and Cu in addition to the maximum content of the bound amino acids: threonine, phenylalanine, serine, glutamic acid, *iso*-leucine, arginine, tyrosine, methionine, valine, cysteinea, lysine, histidine, and proline. On the other hand, in August, *P. pavonia* was found to comprise the least amount of P and the minimum contents of the bound amino acids : glycine, serine, aspartic acid, histidine, and proline. These observations can generally be attributed to the fact that the ash constituents of the brown algae present in the living plants as salts are accumulated by the protoplasts and bases associated with intracellular sulphuric acid esters.

In accordance with the results of Abdel-Fattah and Hussein,[4] for three of the other local brown algae, the maximum contents of total lipids and mannitol were noted in August and November respectively. Detection of low-molecular weight carbohydrates, other than mannitol, was achieved by qualitative paper chromatography. In November *P. pavonia* was found to contain the free monosaccharides: glucose, xylose, glucuronic acid and mannose. In the other seasons, when mannose was absent, there were only traces of the first three sugars. As far the authors are aware, this observation is being recorded for the first time.

TABLE 1. COMPOSITION OF *PADINA PAVONIA* AS INFLUENCE BY SEASONAL VARIATION

	Date of collection			
	August 1974	Nov. 1974	Jan. 1975	April 1975
Total ash (%)	17.57	18.14	18.33	22.39
Ca (%)	2.48	1.87	1.52	3.41
Mg (%)	2.58	1.68	1.71	1.47
Na (%)	0.73	0.36	0.39	0.53
P (%)	0.38	0.38	0.63	0.77
K (ppm)	240.50	228.90	456.50	507.30
Mn (ppm)	166.00	130.00	164.00	86.00
Zn (ppm)	120.00	100.00	109.00	134.00
Cu (ppm)	50.00	50.00	32.00	81.00
Crude protein (%)	23.00	23.35	24.81	25.75
Total lipids (%)	16.94	16.35	11.38	16.74
Mannitol (%)	2.13	2.62	2.61	2.30
Combined uronic acids* (%)	16.30	13.50	11.10	11.20
Combined glucose (%)	10.40	8.20	5.90	8.00
Combined fucose (%)	3.80	3.40	3.30	4.50
Combined galactose (%)	3.30	2.50	2.80	3.20
Combined mannose (%)	2.40	2.20	4.10	4.60
Combined xyloss (%)	1.60	0.50	2.90	3.90
Total combined sugars (%)	37.80	30.30	30.10	35.40

*Determined as glucuronic acid.

TABLE 2.—THE AMINO ACID PATTERN** OF ALGAL MATERIAL IN DIFFERENT SEASONS

	Dates when collected			
Type of amino acid	August 1974	Nov. 1974	Jan. 1975	April 1975
Glycine	29	33	48	37
Threonine	16	15	14	17
Phenylalanine	16	15	16	17
Serine	15	15	16	17
Aspartic acid	15	17	18	17
Glutamic acid	12	11	11	12
Leucine	14	17	11	15
Isoleucine	11	11	11	12
Alanine	14	7	7	11
Arginine	8	8	5	12
Tyrosine	10	9	10	11
Methionine	10	9	9	10
Valine	9	9	9	10
Cysteine	3	3	2	5
Lysine	2	2	2	3
Histidine	2	2	2	3
Proline	1	2	2	2
Tryptophane	2	2	1	1
Total	189	187	194	212

**Mg. bound amino acid per g. alga.

Paper chromatography of the acid hydrolysates of the algal material showed the presence of mannuronic acid, guluronic acid, glucuronic acid, and their respective lactones as well as galactose, glucose, mannose, xylose, and fucose. These sugars are derived from alginic acid, laminaran, fucan-like polysaccharides, and cellulose. These results essentially agree much with those of Mian and Percival[18] who separated and characterized the aforementioned polymers from *P. pavonia* (collected from the south of England). The same authors found that the fucans which were present in 5 sequential extracts comprised variable proportions of fucose, xylose, glucuronic acid, galactose and sulphate half-esters.

To determine the above mentioned sugar components, as they are present in their original polymers, it was necessary to compensate for the breakdown effect of hydrolysis conditions on these substances. The correction factors[19-21], 1.304, 1.056, 1.067, 1.208, 1.147, and 1.032, were thus used for glucuronic acid, galactose, glucose, mannose, xylose, and fucose, respectively, and were obtained from identical hydolysis experiments using standard sugar samples. The data recorded in Table 1 indicate that *P. pavonia* is generally characterized by its relatively higher contents of uronic acids and glucose and lower contents of galactose, mannose, xylose, and fucose. However, it is worth noting that the amount of each sugar component varied according to the season of collection. Thus the maximum contents of uronic acids, glucose, and galactose residues were noted in August when the algal species showed its maximum contents of fucose, mannose, and xylose residues. On the other hand, the minimum contents of uronic acids, glucose, and fucose residues were noted in January when the content of total combined sugars was also minimum. Furthermore, the content of mannose, xylose, and galactose residues was minimum in November. These results, collectively, indicated that the biosynthesis of the sugar residues reached its maximum and minimum rates in the months of high and low temperatures and light intensity, respectively.

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