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COMPOSITION OF SOME BROWN ALGAE AS INFLUENCED BY SEASONAL VARIATION*

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Abstract—An investigation of the effect of seasonal variation on the composition of three brown algae showed that *Cystoseira* was richer in its mannitol content (10·7%) than *Sargassum* (6·7%). In all three species, laminarin ranged from 2·24% to 4·42% during most months but was absent in June. Alginic acid content (30–32%) was at its maximum in August for the two *Cystoseira* species but was high both in April and August in *S. linifolium*. The uronic acid composition of alginic acid as well as the fucose and galactose contents of the algal species showed definite seasonal variation.

INTRODUCTION

STUDIES on marine brown algae have been carried out for a long time because of the industrial importance of their constituents, like alginic acid, mannitol and laminarin. Ecological conditions influence, to a great extent, the types and the proportions of the constituents of any algal species. The effect of seasonal variation on such algal components have been reported by many investigators.[1–3] Recently, Motwalli[4] has made ecological studies on the local brown algae of the Alexandrian coast. These studies, however, have been confined to the alginic acid content.

The present work was undertaken to investigate the composition of three local brown algal species not previously studied, as influenced by seasonal variations. This may provide the necessary information as regards the appropriate time of harvesting the algal species for the production of one or more of their constituents.

RESULTS AND DISCUSSION

Cystoseira barbata, *C. abrotanifolia* and *Sargassum linifolium* were found in all the months investigated, except that *C. abrotanifolia* was absent in April. In June, August and November, *C. abrotanifolia* was present in especially large amounts.

The data recorded in the table indicated that the ash content reached its maximum in June and minimum in November. This result is in accordance with that obtained by Black[1] for *Laminaria cloustoni*. *C. barbata* showed, however, the highest and lowest ash content of any of the species. Also in June the amount of Mg was either equal to or higher than that of Ca. In this month, the ash was characterized by its lowest content of both metal ions. On the

* Part I in a projected series "Biochemical Studies on Marine Algal Constituents".

[1] W. A. P. BLACK, *J. Soc. Chem. Ind.* **67**, 165 (1948).
[2] N. S. VARIER and K. S. PILLAI, *Bull. Central Research Inst. Univ. Travancore*, **11**, 33 (1952); cited from *C.A.* **47**, 1871 (1953).
[3] G. K. YATSENKO, *Nauchn. Dokl. Vysshei Shkoly. Biol. Nauki* **1**, 149 (1963); cited from *C.A.* **59**, 5497 (1963).
[4] A. M. MOTWALLI, M.Sc. Thesis, Faculty of Science, Alexandria Univ. (1966).

other hand in November, at which the ash content reached its minimum, the amount of Ca was significantly higher than that of Mg, except in the case of *C. abrotanifolia* which was always characterized by a high Mg/Ca ratio.

The *Cystoseira* species proved to be richer in mannitol than *S. linifolium*. For all species, mannitol was at a minimum in spring. Both methods of mannitol estimation gave the same results, indicating that the direct periodate oxidation of the algal material did not affect constituents other than mannitol. Mannitol was readily crystallized by adding acetone to the alcoholic extracts and the algal samples were identical in m.p. and R_f with authentic material.

TABLE 1. COMPOSITION OF SOME BROWN ALGAL SPECIES AS INFLUENCED BY SEASONAL VARIATION

Algal species	Date of collection	Total ash (%)	Ca^{2+} in ash (%)	Mg^{2+} in ash (%)	Crude protein (%)	Mannitol (%)		Laminarin (%)
						Periodate	Extraction	
Cystoseira barbata	11.1967	12·17	18·91	5·76	19·44	8·19	7·99	2·69
	4.1968	15·50	5·71	9·20	14·44	2·96	2·83	2·26
	6.1968	25·57	4·32	4·38	15·88	9·03	9·32	Traces
	8.1968	17·02	9·51	5·15	17·44	8·05	8·21	2·24
C. abrotanifolia	11.1967	15·72	8·79	14·95	15·18	10·70	10·01	4·42
	6.1968	24·68	4·98	6·04	10·31	6·94	7·15	Traces
	8.1968	22·81	7·35	7·99	16·56	8·54	8·49	3·37
Sargassum tinifolium,	11.1967	17·23	20·43	5·03	15·94	6·72	6·15	2·28
	4.1968	18·46	15·53	8·25	14·56	3·12	2·91	2·26
	6.1968	24·60	7·25	9·35	13·94	3·27	3·41	Traces
	8.1968	18·49	19·48	8·37	22·44	4·74	4·82	4·29

Algal species	Date of collection	Total lipids (%)	Alginic acid (%)		M/G in alginic acid	Fucose (%)	Galactose (%)
			Gravimetrically	Colorimetrically			
Cystoseira barbata	11.1967	0·96	18·23	23·47	1·80	1·10	0·33
	4.1968	1·01	21·92	26·27	1·25	1·28	0·19
	6.1968	1·33	23·78	27·31	0·92	1·88	0·29
	8.1968	1·44	25·04	30·56	0·80	1·18	0·18
C. abrotanifolia	11.1967	0·61	17·12	23·20	1·54	0·87	0·51
	6.1968	1·03	22·55	25·68	0·93	1·52	0·93
	8.1968	1·20	23·97	30·92	0·93	1·35	0·19
Sargassum linifolium	11.1967	0·47	17·94	22·81	0·85	0·51	0·46
	4.1968	0·43	26·45	32·06	0·78	1·88	0·51
	6.1968	0·45	20·23	27·31	0·87	0·69	0·37
	8.1968	0·60	26·72	32·22	0·77	0·66	0·21

M/G: Mannuronic/Guluronic.

Contrary to the earlier findings of Cameron *et al.*,[5] *n*-butanol was found to be unsuitable for the complete extraction of mannitol from the brown algae investigated. This solvent required longer periods of successive extractions which led to the contamination of the product with much coloured substances. On the other hand, a single and continuous extraction with boiling 85 per cent ethyl alcohol for 24 hr was found sufficient to remove all the mannitol present.

[5] M. C. CAMERON, A. G. ROSS and E. G. V. PERCIVAL, *J. Soc. Chem. Ind.* **67**, 161 (1948).

Laminarin was also found in our local brown algae but in small amounts. *Cystoseira* species were similar in that their highest content of laminarin was found in November, while that of *S. linifolium* was in August. The three species were similar in lacking laminarin in June. Lipids constituted a minor component of the algal material; *Cystoseira* species were relatively richer in lipids than *S. linifolium*.

Alginic acid comprised the major component of brown algae, reaching its maximum in summer and minimum in autumn. The three species were similar in that the lowest alginic acid content was associated with the least amount of ash. The alginic acid hydrolysates revealed the presence of only mannuronic acid, guluronic acid and their respective lactones. However, the uronic acid composition of the different alginates also showed seasonal variation. Generally, the proportion between mannuronic acid and guluronic acid (M/G) in the alginates of the algal species was relatively higher in November than in August, at which months the minimal and maximal contents of alginic acid were noted. On the other hand, the alginates of *Cystoseira* species showed higher M/G values than those of *S. linifolium*.

The acid hydrolysates of the different algal species showed the presence of mannuronic, glucuronic and/or guluronic acids as well as their lactones. In addition, glucose, galactose and fucose were also detected. The results for fucose indicate that the brown algae studied contain little fucoidin. As expected, the contents of fucose and galactose in these algae varied according to the species and season.

EXPERIMENTAL

Collection and Pretreatment of Algae

The different organisms of brown algae used throughout this work were *Cystoseira barbata*, *C. abrotanifolia* and *Sargassum linifolium*. They were collected periodically in 1967–1968 from the same place at Ras-Elteen, Alexandria. The number of plants included in each sample was about 400, at the same stage of development. This number represented the sum of triplicate collections which were achieved within a week, nearly at the middle of each of the months stated. The algae were thoroughly washed with running water for about 3 min to remove foreign substances, spread and left in the sun for several days and finally milled. The values were calculated on a dry weight basis.

Determination of Ash, Calcium and Magnesium

After ashing at 800°, Ca and Mg were determined complexometrically according to the method of Flaschka.[6]

Crude Protein

Organic N was determined by Kjeldahl's method and multiplied by 6·25.

Mannitol

Mannitol was determined titrimetrically by the direct periodate oxidation using the milled algal material and titration of the liberated iodine with standard sodium thiosulphate solution[5] and gravimetrically by continuous extraction with boiling 85% ethyl alcohol for 24 hr. In the latter method, the filtered alcoholic extract was concentrated and then treated with acetone. On cooling, mannitol crystallized out. After isolation of crystalline mannitol, its m.p. and mixed m.p. were determined. It was also detected chromatographically using *n*-BuOH–HoAc–H_2O (12:3:5, v/v)[7] as solvent and the Dedonder reagent[8] as detecting agent.

Laminarin

Laminarin was determined by isolation from algae according to the method of Black *et al.*[9]

[6] H. FLASCHKA, *Mikrochim. Acta* **39**, 38 (1952).
[7] I. SMITH, *Chromatographic and Electrophoretic Techniques*, Vol. I, p. 246, William Heinemann Medical Books Ltd., London (1960).
[8] R. DEDONDER, *Bull. Soc. Chim. France* 874 (1952).
[9] W. A. P. BLACK, W. J. CORNHILL, E. T. DEWAR and F. N. WOODWARD, *J. Appl. Chem.* **1**, 505 (1951).

Acid Decomposition of Laminarin

The isolated laminarin was partially hydrolysed with 0·3 N HCl in a boiling water bath for 2–3 hr. The hydrolysate was chromatographed on Whatman No. 1 paper using the same solvent as for mannitol. The glucose produced was detected by spraying with ammoniacal $AgNO_3$, *p*-aminophenol and aniline diphenylamine phosphate.[10]

Total Lipids

Total lipids were isolated by soxhlet extraction with *n*-hexane for 12 hr. The difference in weight before and after extraction represented the total lipid content.

Alginic Acid

After extracting alginic acid with Na_2CO_3 according to the method of Cameron *et al.*[5] it was determined gravimetrically by precipitation in 5% HCl (w/v). Colorimetric determination of alginic acid was also achieved in another similar extract using the carbazole reaction according to the method of Percival and Ross.[11]

Acid Hydrolysis of Alginic Acid

The isolated alginic acid of each organism was hydrolysed in 2 N H_2SO_4[12] and the hydrolysis products compared with authentic mannuronic, guluronic, glucuronic acids and their lactones by paper chromatography using EtOAc–pyridine–HOAc–H_2O (5:5:1:3, v/v) and pyridine–EtOAc–H_2O (11:40:6, v/v)[13] and aniline phthalate and hydroxylamine hydrochloride as spray reagents.[10]

Quantitative Determination of the Uronic Acid Composition of Alginates

This was achieved according to the method of Haug and Larsen[12] in which mannuronic and guluronic acids in each hydrolysate were separated on a column of anion exchange resin, Dowex 1X8 (200–400 mesh), by linear gradient elution with 0·5 N to 2 N HOAc. Mannuronic and guluronic acids were determined in the eluates by the orcinol method.[14] The uronic acid composition of the different alginates was given as the proportion between mannuronic and guluronic acid residues (M/G).

Acid Hydrolysis of Brown Algae

The algal material of each species was hydrolysed in 2 N H_2SO_4[15] and the hydrolysate was chromatographed as for alginic acid.

Quantitative Determination of Fucose and Galactose in the Algal Hydrolysates

Quantitative paper chromatographic separation of the algal hydrolysates was achieved on Whatman No. 3MM paper using EtOAc–pyridine–HOAc–H_2O (5:5:1:3). The unstained area corresponding to the position of fucose or galactose was cut off and eluted with water. Reaction with L-cysteine and H_2SO_4 was used for the determination of fucose[16] and galactose.[17]

Preparation of Mannuronic and Guluronic Acids

Mannuronic and guluronic acids were prepared by hydrolysing alginic acid and separation of the mannurone and gulurone produced by chromatography on cellulose according to the method of Fischer and Dörfel.[15] The lactones were transformed to the uronic acids by the addition of sufficient NaOH to keep the solution at pH 8 for 1 hr. Thereafter, the solution was treated with cation exchange resin Lewatit S100.

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