Low Molecular Weight Carbohydrates in *Sargassum natans* from Puerto Rico

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***d*-Mannitol, *d*-mannitol monoacetate, and an unidentified carbohydrate were isolated from *Sargassum natans*.**

PRELIMINARY tests carried out in this laboratory indicated that an ether extract of *Sargassum natans* exhibited antibiotic properties (1). To obtain further data, aqueous and alcoholic extracts of this alga were prepared and tested for antibiosis. Negative results were obtained.

Since no work is reported on the polysaccharides found in *Sargassum natans*, we separated and identified some of the carbohydrates present in the alcoholic extract of this alga. The presence of *d*-mannitol, *d*-mannitol monoacetate, and unknown carbohydrate were evidenced.

EXPERIMENTAL

A chemical analysis of dried *Sargassum natans* from Puerto Rico gave the following results: 1.23 Gm. ether extract, 74.21 Gm. carbohydrates, 14.21 Gm. fiber, 0.77 Gm. nitrogen, 4.81 Gm. protein, 33 mg. tryptophan, 44 mg. methionine, 174 mg. lysine, 19.75 Gm. ash, 289 mg. calcium, 57.2 mg. phosphorous, 57.4 mg. iron, 0.01 mg. carotene, 0.31 mg. riboflavin, 1.46 mg. niacin, 3.70 mg. ascorbic acid, 326 calories.

An extract prepared by continuous extraction of 100 Gm. of dried alga, with 95% ethanol for 24 hours, on cooling, deposited about 3% of a white crystalline precipitate (ppt. No. 1).

The alcohol was removed from the rest of the sample under reduced pressure. The residue consisted of a crystalline, brownish mass, with an oily layer, which was removed by washing with ether. Several recrystallizations of this mass from alcohol gave ppt. No. 2. A small amount of ppt. No. 3 was obtained by the evaporation of cold alcoholic extracts.

Osazones, hydrazones, and semicarbazones were prepared of precipitate No. 1 and 2.

A second batch of 800 Gm. of dried alga was extracted for 67 hours with ether, followed by 71 hours of alcoholic extraction. A 19.8 Gm. quantity of ppt. No. 1 was obtained by decantation of the alcoholic extract. After concentration to dryness, the residue was dissolved in water and filtered. Lead acetate was added to the filtrate, the precipitate formed was removed, and the excess of lead was precipitated with hydrogen sulfide. After evaporation of the solution to dryness, the residue was dissolved in 1% ethanol and absorbed on a carbon Celite column (35 × 4.5 cm.) which was eluted with aqueous ethanol (1–15%). From 30–40 μl. of each fraction was investigated by paper chromatography prior and after hydrolysis with 0.1 *N* hydrochloric acid. Ethyl acetate:acetic acid:water (3:1:3 v/v) was used as solvent. The indicator spray used on monosaccharides was ammoniacal silver, Hough's method (2) and modified method of Trevelyn, *et al.* (3). The sugar alcohols were spotted by bromcresol purple reagent and appeared as yellow spots on a blue background owing to the pH change of the sugar-borate complex of Bradfield (4) and Hackmann (5).

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RESULTS

Sargassum natans is a brown alga, whose chemical composition revealed it to be rich in carbohydrates and low in nitrogen. It grows abundantly on the coasts of Puerto Rico.

Three pure compounds were obtained from the alcoholic extraction of this alga. Ppt. No. 1, m.p. of over 300°, contained carbon, hydrogen, and oxygen, reduced the silver nitrate sodium ethoxide reagent, gave a positive carbazol reaction for carbohydrates, did not reduce Fehling's solution and reacted with semicarbazide hydrochloride to form a compound, m.p. 119–120°. Its chromatographic strip resembles in R_f and appearance a cyclitol.

Ppt. No. 2, identified, as *d*-mannitol, by paper chromatography, and by derivatives of phenylhydrazine and semicarbazide hydrochloride, was confirmed by microanalysis.[1]

Ppt. No. 2 (pure) Found: C = 39.47; H = 7.79. Calculated as *d*-mannitol: C = 39.53; H = 7.74. Phenylhydrazine derivative of ppt. No. 2. Found: C = 64.51; H = 6.17; N = 18.80. Phenylhydrazine derivative of pure *d*-mannitol. Found: C = 64.55; H = 6.19; N = 19.00. Semicarbazide derivative of ppt. No. 2. Found: C = 20.49; H = 5.6; N = 46.55. Semicarbazide derivative of pure *d*-mannitol. Found: C = 20.46; H = 5.08; N = 46.47.

Ppt. No. 3, m.p. 124–126°, which was identified by paper chromatography as *d*-mannitol monoacetate was confirmed by microanalysis as $C_8H_{16}O_7$. Found: C = 42.8; H = 7.19. Calcd.: C = 42.9; H = 7.05. The hexacetate formed from the hydrolyzed sample of this monoacetate was also confirmed by microanalysis. Found: C = 50.84; H = 5.92. Calcd.: C = 50.21; H = 5.76.

SUMMARY

1. Chemical analysis of *Sargassum natans* indicated it to be rich in carbohydrates and low in nitrogen.

2. Three natural products were separated by solubility ratios and column chromatography from *Sargassum natans* from Puerto Rico.

3. *d*-Mannitol and *d*-mannitol monoacetate were identified among the three products obtained.

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[1] All microanalyses were performed by Dr. L. I. Duiguid at St. Louis, Mo.