Phytochemistry, Vol. 24, No. 1, pp 67–69, 1985.
Printed in Great Britain

0031–9422/85 $3.00 + 0 00

SORBITOL AND SORBITOL PHOSPHATE IN BROWN SEAWEEDS

MARCEL QUILLET, VIVIANE IMHOFF* and GENEVIEVE DE LESTANG-BRÉMOND

Laboratoire de Physiologie Végétale, Université Catholique de l'Ouest, B.P 808, 49005 Angers, France

(*Received* 8 *May* 1984)

Key Word Index—Fucales; Laminariales; Dictyotales; Sphacelariales; brown seaweeds; sorbitol; sorbitol phosphate; gulitol; alginic acid.

Abstract—Small amounts of D-sorbitol were extracted from 15 species of brown seaweeds representing four orders, independent of seasonal effects. Sorbitol phosphate was also isolated and identified.

INTRODUCTION

Following Stenhouse [1], much work has been published on mannitol, a polyalcohol found in brown seaweeds, in various amounts according to species and seasons. But a more detailed study, effected in our laboratory over several years, has enabled us to find other polyalcohols in far smaller amounts; their interest appeared first to be secondary. Yet, some, such as sorbitol, may be physiologically significant. In effect, this polyalcohol, which is indistinguishable from L-gulitol since it has the same stereochemical structure (Fig. 1), is the precursor of L-guluronic acid (one of the two components of alginic acid) as demonstrated in our accompanying paper [2].

The present publication, which contains observations recorded over numerous seasons, shows that, like alginic acid, sorbitol is always found, during the growing periods, in small amounts in all the brown seaweeds we analysed, and that its phosphorylated form exists as well.

RESULTS AND DISCUSSION

Free sorbitol

Fifteen species, belonging to four different orders, were collected along the north coast of Brittany (France) at different times of the year. The thalli were immediately fixed in boiling ethanol. After discarding resins, pigments, salts, proteins and polysaccharides, the concentrated aqueous extract afforded a crystalline mixture consisting mostly of mannitol. The small quantity of sorbitol present, about 2% of the total, was obtained by using

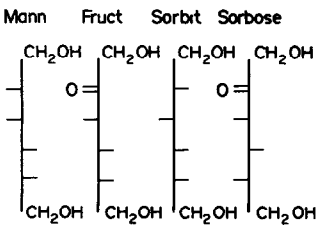

Fig. 1. Oxidation of D-mannitol and D-sorbitol to D-fructose and L-sorbose by *Acetobacter suboxydans*.

Acetobacter suboxydans, which oxidizes the mannitol to fructose and the sorbitol to sorbose [3, 4] (Fig. 1), the R_fs of which are different in aqueous phenol (fructose 0.51, sorbose 0.42). The ketohexoses are easily and specifically detected as blue spots with the phosphoric urea reagent. In addition, the fructose produced can be eliminated by fermentation using baking yeast, which leaves the sorbose unattacked. This method, simple in principle, is rather time-consuming. But it has the advantage of giving sufficient sorbose for measuring its specific optical rotation (see Table 1).

The amount of sorbose recovered (1.5%) gives an indication of the initial sorbitol concentration, but clearly material was lost during isolation.

A second method was used to isolate sorbitol by dissolving the crude crystals in solvents such as cold 95–97% ethanol (Fig. 2) or the mixture butanol–methyl ethyl ketone–water–methanol (5:2:2:1).

The soluble extract obtained was then analysed, with internal standard sorbitol: method (2a) by GLC on OV 225, after acetylation (Fig. 3): retention time of peracetylated mannitol, 34 min; of peracetylated sorbitol, 37.5 min and by (2b) by HP TLC on silica gel in the solvent *n*-butanol–methylethylketone–methanol (5:2:2:1): mannitol, R_f 0.46; sorbitol, R_f 0.43.

Using method (1), (2a) or (2b), as indicated in the following list after the month of the harvest, we found sorbitol in all the species studied, namely: Fucales: *Pelvetia canaliculata* Dcne & Thur. (February; method 1); *Fucus spiralis* L. (September; 1); *Fucus vesiculosus* L. (March; 2a); *Fucus serratus* L. (March; 2a, 2b); *Ascophyllum nodosum* Le Jol. (July; 2a); *Bifurcaria bifurcata* Huds (September; 1); *Himanthalia lorea* Lyngb. (July; 2a); *Cystoseira foeniculacea* Grev. (September; 2a); *Sargassum muticum* (Y) Fensh. (March and April; both 2a). Laminariales: *Laminaria digitata* Lamour (August; 2a); *Laminaria saccharina* Lamour (September; 1); *Saccorhiza polyschides* Ligh. (March; 2a, 2b). Dictyotales: *Dictyota dichotoma* Lamour (September; 1). Sphacelariales: *Cladostephus verticillatus* Ag. (September; 1); *Stypocaulon scoparium* Kütz (June; 1).

Sorbitol phosphate

To obtain sorbitol phosphate, it is necessary to start from the crude extract (CE) only freed of its cations. From this acidic solution the phosphated polyalcohols are

*Present address: Laboratoire de Biochimie, Université de Paris-Sud, Bat. 430, 91405 Orsay, France.

Table 1. Amount of sorbitol extracted and optical rotation of the sorbose obtained

Species and month of harvest	Crystalline extract (g)	Sorbose (mg)	$[\alpha]_D$ found*	Sorbitol A	Sorbitol B	Sorbitol C
Stypocaulon scoparium July 1966	5.2	70	−43°	71	—	1.5/100
Dictyota dichotoma September 1966	8 4	34	−42°	34	87	1.5/100
Laminaria saccharina September 1965	4.1	36	−45°	36	—	—

*Literature value: −43° [5].

(A) Amount of sorbitol (mg) calculated from the recovery of sorbose; (B) sorbitol lost in alcoholic supernatants of the crystalline extract, evaluated from the amount of HIO_4 reduced in 1 min; (C) total evaluated sorbitol (% of the dry glucidic extract).

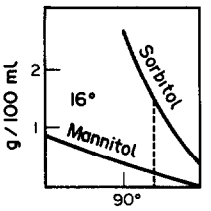

Fig. 2 Solubility of sorbitol and mannitol (g per 100 ml) in aqueous 80–99% ethanol.

obtained as their barium salts. The flocculate, centrifuged at 0° to prevent isomerization, was thoroughly washed with distilled water. Ba^{2+} was removed with sulphuric acid and a resin; the solution was neutralized with 0.1 M sodium hydroxide. The abundant brown flocculate appearing after concentration was discarded. Final purification was carried out by ascending HP TLC on silica gel in *n*-butanol–pyridine–water (2:2:1) for 2.25 hr. Most contaminants migrated rapidly. The phosphated polyols migrated more slowly with R_f 0.23 (sorbitol-P) and 0.26 (mannitol-P).

The slower band was eluted quickly in cold water to avoid dissolution of the silica. The filtrated eluate was hydrolysed by 2 M trifluoroacetic acid for 2.25 hr at 100°. Phosphorus was measured by Fiske and Subbarow's method, on a microscale, on a weighed aliquot of this hydrolysate and sorbitol by GLC (Fig. 4) with the appropriate standards run under the same conditions, for P as for polyols. A total hydrolysate of *Fucus vesiculosus* gave the following values: peracetylated sorbitol, 82.3 μg; peracetylated mannitol, 12.6 μg; phosphorus (P) found 6.1 μg (calc. 6.7 μg).

Sorbitol-P and mannitol-P have also been detected in *Sargassum muticum*. The amount of sorbitol phosphate could not be accurately determined because of the small quantities present but it seems to occur in amounts roughly equal to that of the free sorbitol.

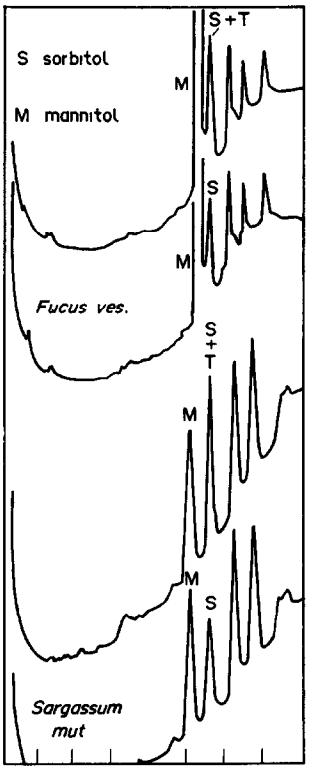

Fig. 3. GLC of acetylated extracts of *Fucus vesiculosus* and *Sargassum muticum*. (T) Authentic acetylated sorbitol, added to each acetylated extract. The peak of sorbitol was clear, even in the presence of larger amounts of mannitol.

EXPERIMENTAL

Preparation of the crude extract (CE). Extraction was effected immediately on harvest, by boiling in EtOH (1 l. per 300 g seaweed) for 15 min. Thalli were cut into a few mm^2 pieces in alcohol, using a mixer. The pulp was drained by boiling 85% EtOH (6 hr) in a BBS extractor (Bercauverre, Paris, France). This extract was added to the rest of the alcohol and evapd to dryness, then suspended in H_2O (200 ml), filtered on a polypropylene cloth and centrifuged (30 000 *g*). Major amounts of resin and lipid, as well as NaCl and proteins, were discarded.

Preparation of free sorbitol. To the crude extract (CE) was

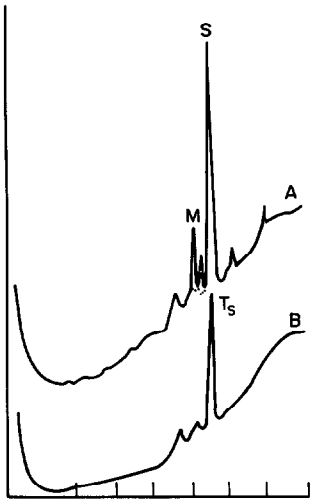

Fig. 4. (A) GLC of the sorbitol phosphate of *Fucus vesiculosus* after hydrolysis and acetylation. The peak of mannitol arose from mannitol phosphate (B) Authentic acetylated sorbitol (1.73 μg).

added 1:10 of a $ZnSO_4$ soln (*ca* 10%) and the amount of $Ba(OH)_2$ necessary to precipitate Zn and SO_4. After centrifugation at 0° and careful washing with cold H_2O, the flocculate was discarded. The supernatants were combined, freed of salts by resins (Amberlite IR 120 and IRA 410), concd to a syrup and crystallization was effected by adding 5 vols. EtOH containing some Et_2O. A second crystallization afforded a mixture of white needles in which it was difficult to recognize the short and thin crystals of sorbitol. Excess mannitol was removed as follows.

(1) *Acetobacter suboxydans* (Inst. Pasteur, No. 53162) was grown on Gasser's medium [6] containing glycerol instead of glucose. When the bacteria were in full growth, 3–4 ml of the suspension (10^7 bacteria/ml) was used to inoculate 250 ml of a new Gasser's medium is which 5 g of the mannitol + sorbitol extract took the place of glycerol or glucose. All operations were carried out under sterile conditions. Six days later, at 27–28°, the refined, de-ionized suspension was freed of fructose by carefully washed baking yeast, which consumed it in 3 days at 37°. After further flocculation and de-ionization, an aliquot was concd and spotted on Whatman No. 1 paper against authentic sorbose and fructose and developed in phenol saturated with H_2O for 12 hr by descent. Visualization: phosphoric–urea reagent. Other aliquots were used to determine the optical rotation and the reducing power [7].

(2) Using differential solubilities. A few grams of the crystalline mixture mannitol + sorbitol were stirred, for several days in 95% EtOH (*ca* 18 ml for 5 g crystals). The soluble portion, dried, was treated again with the minimum of 95% EtOH (*ca* 1 ml) and the soluble part (S) was dried under red. pres. over P_2O_5.

(2a) The soluble part (S) was acetylated with Ac_2O (0.5 ml for 5 mg) and pyridine (0.25 ml) for 24 hr at 20°. After addition of 5 ml H_2O and extraction into 2 ml $CHCl_3$, the chloroform phase was evapd to dryness to eliminate HOAc, dissolved in fresh $CHCl_3$ and injected in a FID gas chromatograph. Column. Pyrex (2 m × 2 mm), 3% OV 225 on Gas Chrom Q, 100–200 mesh. Programme. 160–277°; 1.5°/min Carrier gas N_2 (1.25 bar at 160°). Standards: sorbitol (Merck) or mannitol, fully acetylated by the above technique, was added to a sample of the extract.

(2b) HP TLC of the soluble part (S) dissolved in water (*ca* 2%, very small spots) was performed on silica gel plates (Merck). Solvent: *n*-BuOH–MeCOEt–H_2O–MeOH (5.2:2:1) by ascent for 2 25 hr. Visualization: $AgNO_3$.

Phosphated polyalcohols The crude extract was decationized by IR-120 resins then neutralized by a chilled, limpid, aqueous $Ba(OH)_2$ soln so that the medium remained alkaline for only a short time. HP TLC: Three (10 × 10) plates were run simultaneously. Bands were visualized with $AgNO_3$ and P was detected in eluates of small areas (30 mm^2) by Fiske and Subbarow's reagent, after filtration through a 0.2 μm membrane filter (Sartorius), drying and calcination, under oxidizing conditions (50 μl H_2SO_4 + 50 μl HNO_3).

GLC was performed after acetylation as above; the peracetylated products were quantitatively collected by extracting with $CHCl_3$ (× 3); evapn to dryness removed HOAc, and redissolving with minimum $CHCl_3$ (35.5 mg = 24 μl); injected 1.5 μl Authentic peracetylated sorbitol: 1.73 μg was injected just after the above sample. For maximum accuracy, areas of the peaks were measured after photographic enlargement (× 1 8).

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