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Note

The structure of agar
Part III*. Pyruvic acid, a common feature of agars from different agarophytes

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Fractionation of a commercial agar on DEAE Sephadex A-50(Cl^-) has shown the complexity of agar and indicated that the masking of the agarose repeating-unit with 4,6-*O*-(1-carboxyethylidene)-D-galactose in place of D-galactose residues occurs in regions of the molecules not heavily sulphated[1]. The result has been confirmed by hydrolysing the polysaccharide fractions with a purified, bacterial agarase[2]. How these results can be related to agars from different agarophytes is discussed in this paper.

A variety of agars were analysed for pyruvic acid, which is indicative of the extent of replacement of the D-galactose residues with 4,6-*O*-(1-carboxyethylidene)-D-galactose and sulphate. The results are shown in Table I. The criterion for calling these polysaccharides agars was that they were all degraded, to a greater or lesser extent, with the extracellular agarase from *Pseudomonas atlantica* to form neutral oligosaccharides based on the disaccharide neoagarobiose, and charged oligosaccharides which have yet to be identified.

TABLE I

SULPHATE AND PYRUVIC ACID CONTENT OF AGARS FROM DIFFERENT AGAROPHYTES

Alga	*Country of origin*	*Sulphate* (%)	*Pyruvic acid* (%)	*Polysaccharide extracted from dry alga* (%)
Gracilaria compressa	Barbados	4.1	2.92	16.4
Gelidium cartilagineum	United States	3.6	1.29	22.5
Pterocladia pinnata	Barbados	3.7	0.65	15.3
Gracilaria damaecornis	Barbados	6.5	0.17	11.6
Gracilaria foliifera	Nova Scotia, Canada	2.5	0.13	9.4
Gelidiella acerosa	Barbados	4.6	0.12	8.5
Digenia simplex	Barbados	7.0	0.11	6.0
Gelidium sesquipedale	Spain	3.2	0.04	28.5

*Part II: see Ref. 2.

Table I shows that the agars with the highest content of pyruvic acid have approximately the same amount of sulphate, and agars with lower or higher amounts of sulphate have very low, yet detectable, pyruvate levels. Until more agars are examined, it is perhaps premature to give reasons for this, but we anticipate that agars with high sulphate values would have low pyruvate levels, since our previous studies[1,2] have shown that, in a commercial agar, the pyruvic acid is always found remote from the sulphated regions of the molecule.

The three agars containing the greatest concentration of pyruvic acid were taken for further study. These were the agars from *Gracilaria compressa*, *Pterocladia pinnata*, and *Gelidium cartilagineum*. The agars were fractionated on DEAE Sephadex A-50(Cl^-) by elution, successively, with distilled water, and 0.5M, 1M, and 2.5M sodium chloride. The yield of polysaccharide in, and the analytical data for, each fraction are given in Table II.

TABLE II

FRACTIONATION OF THE AGARS ON DEAE SEPHADEX A-50 (Cl^-)

Polysaccharide fraction (%)	*Eluant*	*Pyruvic acid* (%)	*Sulphate* (%)
Gracilaria compressa			
a (12)	Distilled water	0.1	0.7
b (48)	NaCl 0.5M	3.8	3.0
c (4)	1.0M	2.9	6.7
d (2)	2.5M	2.3	9.7
Pterocladia pinnata			
a (3)	Distilled water	0.02	0.63
b (26)	NaCl 0.5M	0.88	1.60
c (17)	1.0M	0.53	9.50
d (9)	2.5M	0.42	9.62
Gelidium cartilagineum			
a (20)	Distilled water	0.01	0.10
b (20)	NaCl 0.5M	0.88	1.1
c (14)	1.0M	0.70	4.1
d (6)	2.5M	0.50	6.0

In each case, with increasing ionic strength of the eluant, the sulphate content of the eluted polysaccharide increases, and the pyruvic acid content reaches a maximum in the polysaccharide fraction eluted with 0.5M sodium chloride. This indicates that it may be a general feature of pyruvic acid-containing, agar polysaccharides that replacement of D-galactose with 4,6-*O*-(1-carboxyethylidene)-D-galactose occurs in regions of the molecule low in sulphate. When *Gracilaria compressa* agar was fractionated, the highest concentration of pyruvic acid (3.8%) was found in the polysaccharide fraction eluted with 0.5M sodium chloride. This is therefore the optimum substrate for the agarase, when preparing charged sugars containing 4,6-*O*-(1-carboxyethylidene)-D-galactose.

The elution pattern of various agar polysaccharides when fractionated on DEAE Sephadex A-50(Cl^-) indicates the suitability of the agar as a source of agarose. Agarose is that part of the whole agar complex which has the lowest charge content and therefore the highest gelling ability. A high percentage of the complete agar eluted in the water eluant will be indicative of its suitability. Values between 15–30% for the yield of polysaccharide are ideal as long as an economic amount of polysaccharide is extracted from the dry weed (Table I).

EXPERIMENTAL

Pyruvic acid was determined by the lactate dehydrogenase method[3], and sulphate (SO_4^{2-}) by the procedure of Jones and Letham[4].

Preparation of agars. — Air-dried agarophyte (40 g) was soaked in water for 3 h and then extracted with distilled water (2 litres) for 3 h at 100°. The hot extract was filtered through Celite 545 and allowed to gel. The gel was cut into strips and frozen at −10°. The frozen polysaccharide was thawed, filtered, dehydrated with ethanol, and dried *in vacuo* at 45°.

Fractionation of agars. — Agar (1 g) was soaked in distilled water for 10 h. The water was changed three times during this period. The purpose of this initial step was to ensure that the polysaccharide complex was freed of salts. The agar was then dissolved in boiling water and added at 60° to a jacketted column containing DEAE Sephadex A-50(Cl^-) which was washed at this temperature with distilled water, and 0.5M, 1M, and 2.5M sodium chloride, ensuring, by means of the phenol–sulphuric reagent[5], that the eluant had become polysaccharide-free before changing the ionic strength of the eluant. The fractions were dialysed against warm, running water overnight and concentrated, and the polysaccharide was precipitated with ethanol (4 volumes). The precipitates were removed by centrifugation, washed with ethanol, and allowed to air dry.

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