Agar Elaborated by *Gracilaria* Sp. from the Coast of British Columbia

Part I. Properties of Agars Isolated from Algae Collected at Bamfield Inlet, Wiseman's Bay and Nuttal Bay.

by
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ABSTRACT

Yields of agars in the Gracilaria sp. from three localities in British Columbia ranged from 20 to 45% of the dry weight. Apart from chemical composition and physical measurements conducted on 1% aqueous solutions, use was made of the Instron Universal Testing Machine in determining force-deformation characteristics of the gel systems to provide quality criteria for the agars isolated.

A weak-gelling polysaccharide was extracted from the alga collected in Wiseman's Bay. Agars from the Gracilaria sp. collected at the head of Bamfield inlet and Nuttal Bay generally yielded soft, elastic and non-rigid gels which fluctuated in gel strength with time of season.

RÉSUMÉ

La production d'agar-agar des Gracilaria sp. récoltées dans trois localités de la Colombie-Britannique variait de 20 à 45% de leur poids sec. En plus de mesurer la composition chimique et les paramétres physiques à l'aide de solutions aqueuses de 1%, nous avons déterminé les caractéristiques effort-déformation des géloses à l'aide d'un appareil d'essai universel Instron afin d'établir des critéres de qualité pour les types d'agar-agar isolés.

Un polysaccharide à faible pouvoir de gélification a été extrait d'une algue recueillie dans la baie de Wiseman. L'agar-agar extrait des Gracilaria sp. du fond de l'inlet Bamfield et de la baie Nuttal donnent généralement des géloses molles, élastiques et non rigides dont la résistance varie selon moment de la saison.

INTRODUCTION

Plants of the red algal genus Gracilaria are eaten as foodstuffs and as a food source in pondfish culture in Asia, Indonesia, and the South Pacific Islands (Chapman 1970; Shang 1976). However it is as a source of the commercially important polysaccharide agar that this agarophyte is becoming an increasingly valuable international commodity (Bissell 1972).

World annual exploitation of agarophytes for agar production is approximately 39,000 tonnes, composed of 59.6% Gracilaria species, 35.3% Gelidium species and 5.1% Pterocladia species; minor amounts of Ceramium, Ahnfeltia and Acanthopeltis species yield agaroid-type polysaccharides. Gracilaria currently sells for about $750/tonne and Gelidium for $1,200/tonne depending on quality of the colloid and the cleanliness of the plants. From these agarophytes some 500 tonnes of bacteriological grade agar and 6,000 tonnes of food/industrial grade agar are produced with average values of $20/Kg and $9/Kg respectively. The more neutral fraction from agar known as agarose commands the highest price at $880/Kg.

The term agar represents a complex spectrum of closely related polysaccharides possessing varying degrees of chemical substitution to a basic structure composed of alternating 1,3-linked D-galactose and 1,4-linked 3,6-anhydro-L-galactose residues. The family of galactans can range from the highly priced neutral polymer, agarose, composed essentially of the basic structure with some methoxyl substituents, to a highly acidic polymer containing a large percentage of substituent sulphate ester groups, 4,6-carboxyethylidene groups and glucuronic acid residues (Yaphe et al. 1971). Different species appear to contain mixtures of these polysaccharides which dictate the quality of the agar produced (Stoloff et al. 1946).

Apart from the examination of a Gracilaria specimen from Cherry Point (Whyte et al. 1976) little is known of the quality of agar produced by agarophytes in British Columbia. In this initial study the agar components of the Gracilaria from three completely different habitats were examined and the effect of season on the quality of agar from the Gracilaria growing vegetatively at Bamfield Inlet was established.

EXPERIMENTAL

A. COLLECTION OF GRACILARIA SP.

(1) Bamfield Inlet Alga: collections were made on June 4, July 11, August 1, September 3 and September 22, 1975, from the head of Bamfield Inlet, Fig. 1, an area consisting of fine sand and mud flats. This algal population consisted of a free-floating intertidal mass of fragments, Fig. 1, held together by byssal threads of Mytilus edulis. Specimens, 10 cm approximately in length, varied in colour from brown to yellow, depending on exposure within the mat to sunlight, and in general were vegetative in nature.

(2) Wiseman's Bay Alga: collections were made on August 7 and September 3, 1975, from the mud-gravel beach situated on the east side of Bamfield Inlet, Fig. 2. These tetrasporic plants, 32 cm approximately in length, were dark-brown in colour, irregularly and profusely branched, Fig. 2, and were unattached in the intertidal zone.

(3) Nuttal Bay Alga: collections were made on July 3 and November 7 from the rock and pebble bay on the Strait of Georgia, just south of Parksville on Vancouver Island, Fig. 3. The plants, 14 cm approximately in length, were deep red-purple in colour, moderately branched, Fig. 3, and were attached to rocks in the subtidal zone by discoid holdfasts. The texture of these plants was more rigid and prone to breakage than the "brown variety", Fig. 2, from Wiseman's Bay.

B. EXTRACTION AND PURIFICATION OF POLYSACCHARIDE

The dry alga, 10 g, was washed with cold water to remove excess salts, then extracted for 60-90 min with 400 ml water at 95-100°C. The resultant mixture was centrifuged at 3000-5000 rpm and the residue re-extracted with a further 400 ml water. Further extraction with 300 ml water yielded only minor amounts of agar. The combined centrifugates were rotary evaporated to 300 ml and 1,500 ml of ethanol added to precipitate the crude agar. Purification was performed by redissolution of the polymer in 200 ml water and evaporating off excess ethanol. If the resultant solution gelled the material was freeze-thawed, if a weak gel resulted the polysaccharide was isolated by ethanol precipitation and dried by solvent exchange with acetone and ethyl ether.

C. GELATION TEMPERATURE DETERMINATION

A hot 1% aqueous solution of the polysaccharide (10 ml) was poured into a test tube (16 x 200 mm). The tube was then clamped in a 1L beaker containing 900 ml of boiling water which was stirred constantly by a bar magnet. A precision thermometer (0.1°C divisions) was positioned off centre in the test tube and the cooling rate regulated to approximately 0.25°C per minute after the temperature had attained 50°C. Glass beads, 3 mm in diameter, were introduced periodically at the surface of the solution and the gel temperature recorded when the bead just failed to sink. The method proved to be reproducible to ±0.1°C.

D. GEL MELTING POINT DETERMINATION

A hot 1% aqueous solution of the polysaccharide (10 ml) was poured into a test tube (16 x 200 mm) and a glass rod the same diameter as the thermometer was then immersed into the solution in an off-centre location. After the gel was allowed to set overnight at room temperature the rod was carefully replaced by the precision thermometer and the test tube was clamped in a water bath heated at 1°C per minute. The melting point was recorded when a 3 mm diameter coloured glass bead, placed just below the surface of the gel, sank.

E. VISCOSITY DETERMINATION

The viscosity, in centipoise, of a 1% aqueous solution of the polysaccharide was measured at 65°C with a Brookfield Viscometer Model LVF, using the No. 1 spindle or the U.L. adapter.

F. ANHYDRO-3,6-ANHYDROGALACTOSE DETERMINATION

The content of this sugar in the agar polymers was measured by the colour reaction with resorcinol according to the method of Yaphe (Yaphe 1960), except for the modified use of synthesized methyl 3,6-anhydro-α-D-galactose (Lewis et al. 1963) as the standard sugar for calibration purposes.

G. SULPHATE DETERMINATION

The spectrophotometric procedure using 4-amino-4'-chlorodiphenyl reagent was used (Jones and Letham 1956).

H. TEXTURE PROFILE ANALYSIS

A 1% aqueous solution of agar was prepared by refluxing and stirring 100 ml distilled water with 1.0 g dry polymer until dissolved, then from the resultant solution 30 ml was poured while hot into each of three 50 ml beakers (42 x 53 mm). The samples were allowed to gel and equilibrated at room temperature overnight with the beaker covered by aluminum foil. Testing with an Instron Universal Testing Machine Model 1122, operating at a crosshead speed of 10 mm/min, a chart speed of 50 mm/min, with a 1 cm^2 stainless steel cylindrical probe, provided values for gel strength, deformation, cohesiveness and rigidity of the gels. Average reproducibility for all rheological parameters was within ± 2.0%.

The force-time plot for the texture profile analyses of these gels, Fig. 4, afforded rheological parameters which can be defined as follows:

Gel Strength (S): the maximum load required to rupture the gel matrix in the immediate vicinity of the applied load (g/cm^2).

Deformation: the total distance penetrated by the plunger into the gel matrix prior to surface rupture providing a measure of the elastic limit of the network (mm).

Cohesiveness (C): the deformation of the gel surface which corresponds to half the rupture load providing a measure of the extent to which a gel may be deformed before its elastic elements are ruptured (mm).

Rigidity (R): the load per unit distance required to deform the surface of the gel which is calculated from the slope of the first half of the load-deformation curve providing a measure of the firmness or stiffness of the gel network ($g/cm^2/mm$).

DISCUSSION OF RESULTS

Unequivocal identification and speciation of the Gracilaria genus in B.C. is impossible at present because of the confusion surrounding the taxonomy of the Gracilariaceae family. Based on the systematics of the male reproductive structures Yamamoto (Yamamoto 1978) has catagorized two distinct types of this alga and from recent biological studies of the local morphotypes tentative categorizations have been suggested (Saunders et al. 1976, Lindsay et al. 1977 and 1978).

The "chorda" type, characterized by G. sjoestedtii, occurs attached in subtidal and intertidal locations, is light red in colour and has long principal axes from which two orders of irregular branching arise. The "verrucosa" type is found attached and unattached in subtidal and intertidal locations, is deep burgandy in color and irregularly and highly branched, such as the specimen from Nuttal Bay, Fig. 3. In B.C. this latter type also occurs in another form labelled "verrucosa brown" (Lindsay et al. 1978) which is morphologically similar but tends to be yellow to brown in colour and is located in the intertidal zone. Specimens collected at Bamfield Inlet and Wiseman's Bay, Fig. 1 and 2 respectively, were characteristic of this type.

Yields of agar isolated from the alga collected from June to September at Bamfield Inlet were relatively high, ranging from 26.0% to 35.4% of the freeze dried weight. An increase from June to the highest inclusion in August was followed by a gradual decline as the season progressed, Table 1.

The 3,6-anhydrogalactose contents of the agars from the Bamfield Inlet samples ranged from 29.2% in September to the highest level 32.2% in the June specimen, Table 1. This component of agar, in general, provides a measure of the quality, with the higher inclusion levels corresponding to a stronger gelling capacity of the polymer. The strongest gels are provided by agarose molecules, which from the idealized structure in Fig. 5, contain 47% anhydrogalactose residues and are devoid of sulphate substitution. As the season progressed the sulphate content of the agar from Bamfield Inlet samples, Table 1, increased from 3.57% in June to 4.91% by the end of September. Theoretically the strongest gel based on the anhydrogalactose and sulphate contents would be that elaborated by the alga in June. Indeed, from Table 2 it can be observed that the June sample provided the strongest of all the gels tested.

Gel formation properties of agars are unique in exhibiting a hysteresis effect due to gelation occurring at temperatures significantly below the gel melting temperatures. The gelation temperatures of 1% aqueous solutions of the agar from the Bamfield Inlet alga varied little, $39.3 \pm 0.5°C$, indicating that the observed 40.7 to 47.2°C hysteresis was a function of the variable gel melting temperatures, Table 1. Gel melting temperatures which are concentration dependent are also considered to reflect the molecular weight of the polymers. Nevertheless, no correlation could be found between the temperatures and the corresponding viscosities of the agar solutions.

Measurement of the viscosities of polysaccharide solutions is the simplest method of comparing the size of polymers. Thus the values recorded, Table 2, would suggest that higher molecular weight polysaccharides were contained in the Bamfield Inlet alga towards the latter part of the season. Sulphate ester content appeared to influence only slightly the viscosities of the agar solutions.

Use was made of the Instron Universal Testing Machine to determine the gelation capacity of 1% aqueous solutions of the isolated agars. Many devices and instruments are used to determine gel parameters, consequently a lack of uniformity exists in the data contained in the literature. Standardization of a procedure using the Instron became necessary when it was noted that the gel strengths of the gels were proportional to the speed with which the plunger entered the gel matrix. Optimizing the reproducibility of results culminated in instrument operating conditions which provided accurate determinations graphically displayed in Figure 4.

This instrument also provided a measurement of applied force substantially free from friction and a force continuously exerted against the gel matrix even after rupture to provide a texture profile analysis or the force-deformation characteristics of individual agars. Objective evaluations were based on a comparison with values obtained from high quality bacteriological grade agar (BBL-Lot number 504605). Thus it is important to realize that the gel strength values quoted in this report are from 1% aqueous solutions and that under the standardized conditions of measurement a bacteriological grade commercial agar provided a value of 247 g/cm^2.

Properties of the gels provided by the agars from Bamfield Inlet alga are presented in Table 2. A strong, rigid, inelastic and firm gel was provided by the June specimen, but the following month a decrease in the rigidity and gel strength parameters indicated the formation of softer and weaker gel systems. Deformation values increased for the agar gels from August and late September specimens suggesting a more plastic matrix. In addition the increase in cohesive qualities of these agar gels also characterized them as soft, non-rigid yet strong gels. Non-rigid as applied to gels often signifies a degree of gumminess or stringiness associated with a three dimensional matrix of high molecular weight polymers. Some correlations between the viscosities of the agar solutions and the degree of cohesion of the gel state were evident in Table 2. Values for the agar isolated from the early September Gracilaria were typical of soft, weak, spongy and gummy gels. As the season progressed therefore, the gelation characteristics of the agars changed from brittle, hard and stiff systems to more soft, elastic and non-rigid systems.

Apart from the location at the head of Bamfield Inlet, a population of the "verrucosa brown" type Gracilaria existed at Wiseman's Bay, Figure 2, and for comparative purposes specimens were collected in August and September and the agar components analyzed, Tables 3 and 4.

High yields of agar of exceptionally low anhydrogalactose and high sulphate contents were obtained which on dissolution as 1% aqueous solutions provided exceptionally weak gels with about a 4 g/cm^2 strength rating. The 2°C differential in the gelation temperature and the gel

melting temperature again indicated the extremely poor gel forming capabilities of these extractives from the Wiseman's Bay population of Gracilaria. Although from the viscosity measurement a high molecular weight polysaccharide was evident in the August sample this parameter had little influence on the gelation capacity of the polymer, possibly as a result of the high sulphate content. Undoubtedly, of the two algal populations of the "verrucosa brown" type, the head of Bamfield Inlet yielded the better quality agar.

The other "verrucosa type" Gracilaria analyzed was the subtidal population at Nuttal Bay, Figure 3. Polysaccharide yields were identical from specimens collected in July and November, Table 3. The low 3,6-anhydrogalactose content combined with the high sulphate ester level of these agars from Nuttal Bay algae were factors in the polymer's structure conducive to the formation of soft gels. Indeed, the rheological properties of the agar gels, Table 4, represented soft, plastic gels which nevertheless were intrinsically strong. A higher gel strength was provided by the November sample than that from bacteriological grade commercial agar, however the harder brittle nature of the latter gel was confirmed by the high value for rigidity, Table 4.

It is important to consider that although the agars isolated to date from Gracilaria in B.C. have in many cases comparable gel strengths to that from commercial bacteriological agar, it is the classification of the cohesive qualities of the gel systems which generally dictate the ultimate uses of these polymers. Apart from the firm agar gel displayed by the June specimen from Bamfield Inlet, all the other agar gels would, without chemical modification, be considered too soft for bacteriological grades but quite suitable for food and industrial purposes.

The intertidal "verrucosa brown" type Gracilaria from Wiseman's Bay yielded an essentially non-gelling polymer of little commercial value. The vegetative mat of the intertidal "verrucosa brown" type Gracilaria collected at the head of Bamfield Inlet over the summer period consistantly provided a reasonable industrial grade agar as did the "verrucosa type" subtidal alga from Nuttal Bay. Morphologically similar Wiseman's Bay and Bamfield Inlet "verrucosa brown" type Gracilaria yielded polysaccharides with completely different qualities suggesting that environmental conditions may play a vital role in the development of these polymers *in situ* in the alga.

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The authors are deeply indebted to Mr. R.G. Saunders and Mr. J.G. Lindsay, Canadian Benthic Ltd., Bamfield, for collecting the specimens of Gracilaria studied and to the Marine Resources Branch, Government of B.C. for sanctioning this co-operation.

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Table 1. Physicochemical parameters of the agar isolated from Gracilaria sp. collected at Bamfield Inlet.

Parameter	Month Collected				
	June	July	Aug.	Sept. (early)	Sept. (late)
Polysaccharide, %†	26.0	32.2	35.4	34.8	31.0
Anhydrogalactose, %*Δ	32.2	31.0	30.1	29.2	30.5
Sulphate, %Δ	3.57	4.52	4.81	4.84	4.91
Gelation temp., °C	38.8	39.5	39.6	39.8	39.5
Gel melting temp., °C	82.8	86.7	83.7	80.5	82.4

† Percentage of dry weight.

* As anhydro-3,6-anhydrogalactose ($C_6H_8O_4$).

Δ Percentage of dry polysaccharide.

Table 2. Rheological properties of 1% solutions of the agar isolated from Gracilaria sp. collected at Bamfield Inlet.

Parameter	Month Collected				
	June	July	Aug.	Sept. (early)	Sept. (late)
Viscosity, cps	27.4	63.0	240	136	173
Gel strength, g/cm^2	190	65	126	95	160
Gel deformation, mm	3.7	3.6	6.7	5.0	6.9
Gel cohesion, mm	2.4	2.2	3.5	3.5	3.8
Gel rigidity, $g/cm^2/mm$	40.1	15.4	18.2	13.4	21.1

Table 3. Physicochemical parameters of the agar isolated from Gracilaria sp. collected at Nuttal Bay and Wiseman's Bay.

Parameter	Nuttal Alga		Wiseman's Alga		Commercial Agar
	July	Nov.	Aug.	Sept.	
Polysaccharide, %†	31.9	31.9	33.4	45.1	-
Anhydrogalactose, %*Δ	26.3	25.8	20.9	22.2	35.5
Sulphate, %Δ	7.40	12.12	12.98	9.94	2.19
Gelation temp., °C	36.9	40.0	19.6	17.4	34.9
Gel melting temp., °C	88.3	91.7	21.4	19.4	85.0

† Percentage of dry weight.

* As anhydro-3,6-anhydrogalactose ($C_6H_8O_4$).

Δ Percentage of dry polysaccharide.

Table 4. Rheological properties of the 1% solutions of the agar isolated from Gracilaria sp. collected at Nuttal Bay and Wiseman's Bay.

Parameter	Nuttal Alga		Wiseman's Alga		Commercial Agar
	July	Nov.	Aug.	Sept.	
Viscosity, cps	75.5	48.9	352	44.9	1.9
Gel strength, g/cm^2	113	259	4.2	3.9	247
Gel deformation, mm	5.9	6.5	-	-	3.0
Gel cohesion, mm	4.3	4.5	-	-	2.0
Gel rigidity, $g/cm^2/mm$	13.3	28.8	-	-	62.0

Fig.1

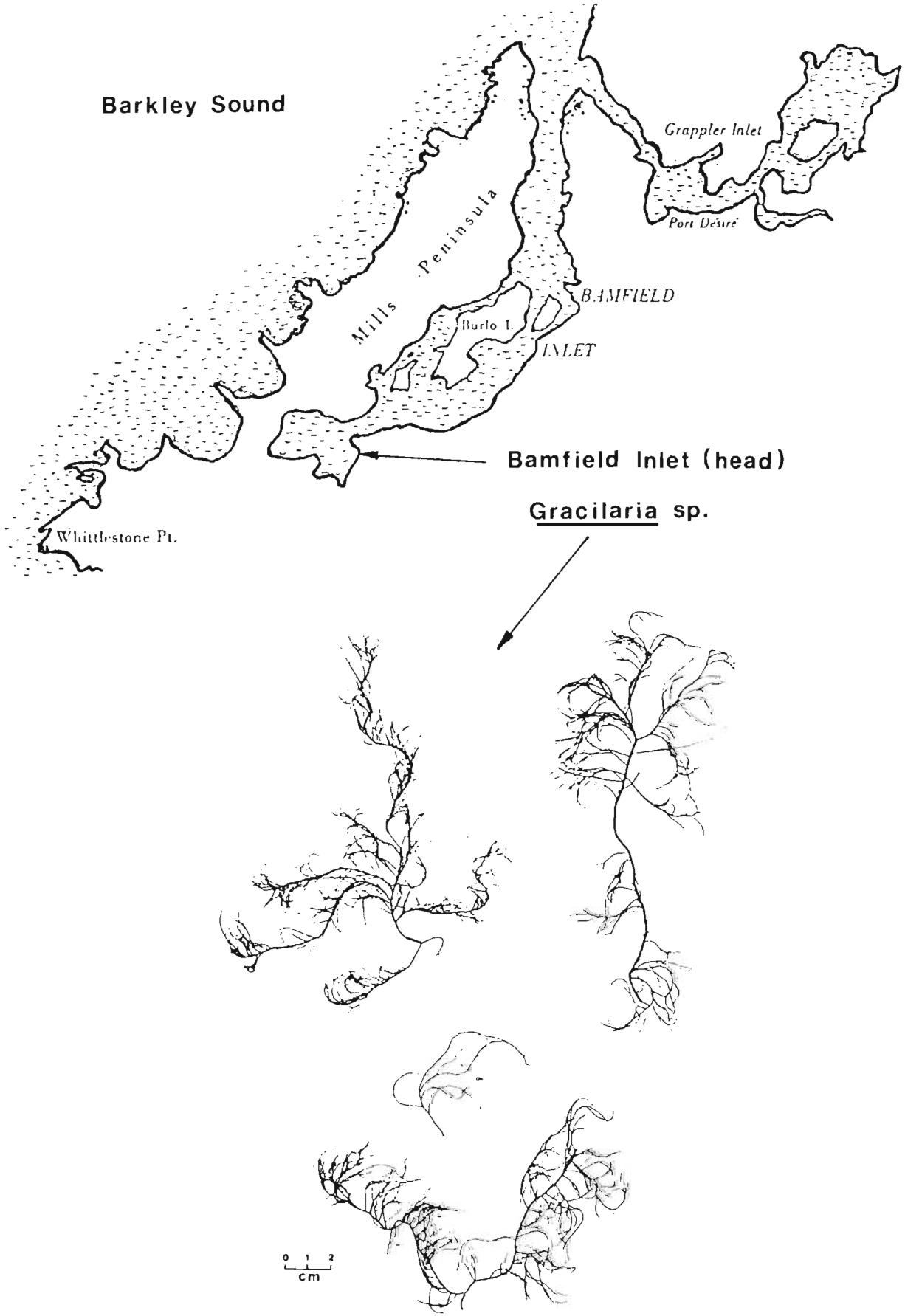
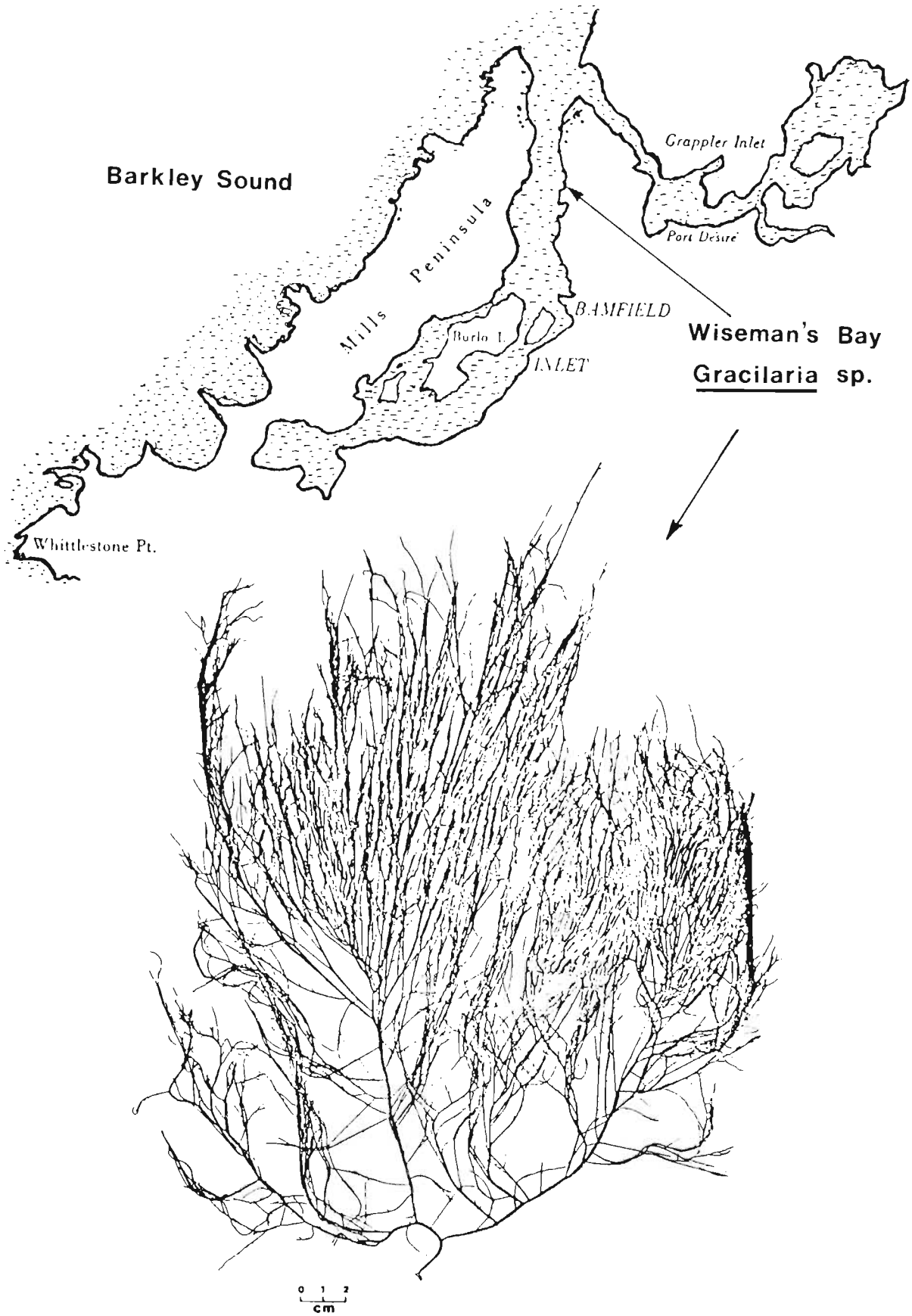

Barkley Sound
Grappler Inlet
Port Desire
Mills Peninsula
BAMFIELD
INLET
Burlo I.
Bamfield Inlet (head)
Whittlestone Pt.
Gracilaria sp.
0 1 2
cm

Fig.2


Barkley Sound
Grappler Inlet
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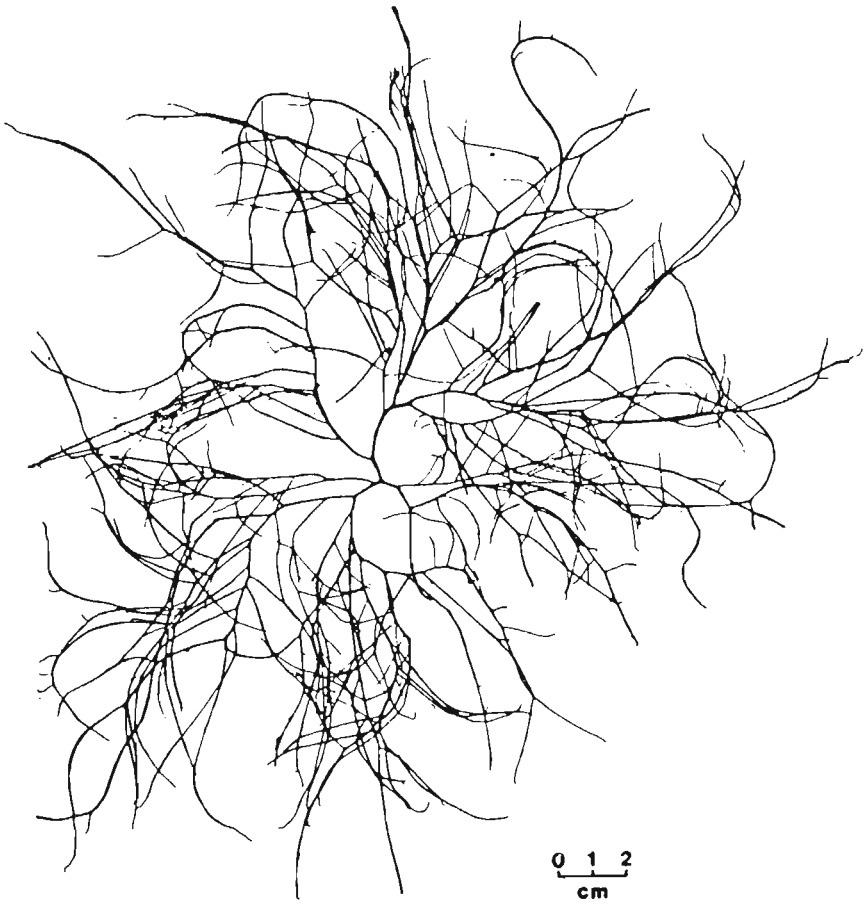
Whittlestone Pt.
0 1 2
cm

Fig.3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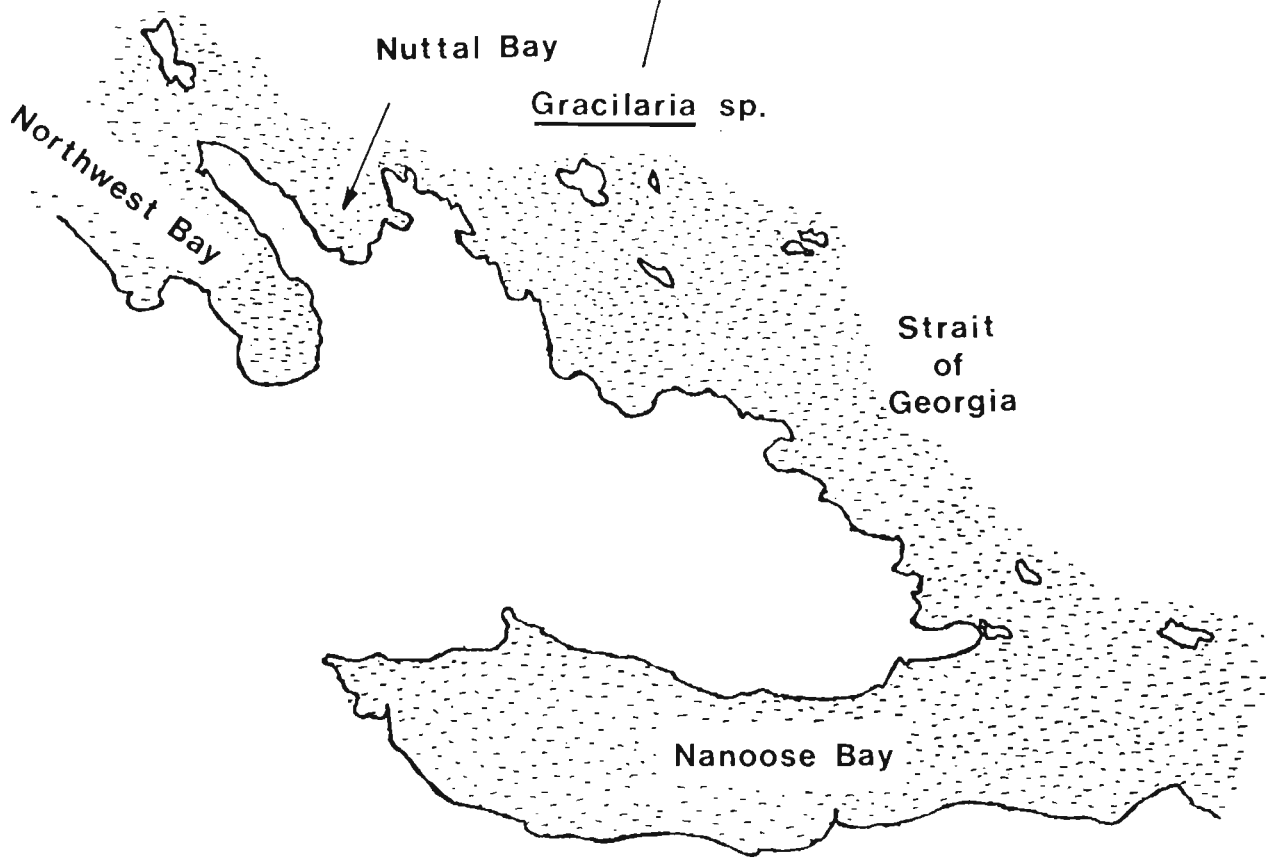
Nuttal Bay
Gracilaria sp.
Northwest Bay
Strait
of
Georgia
Nanoose Bay

Force Time Plot for Agar Gel

S = Strength
C = Cohesiveness
R = Rigidity = $\frac{S}{2C}$

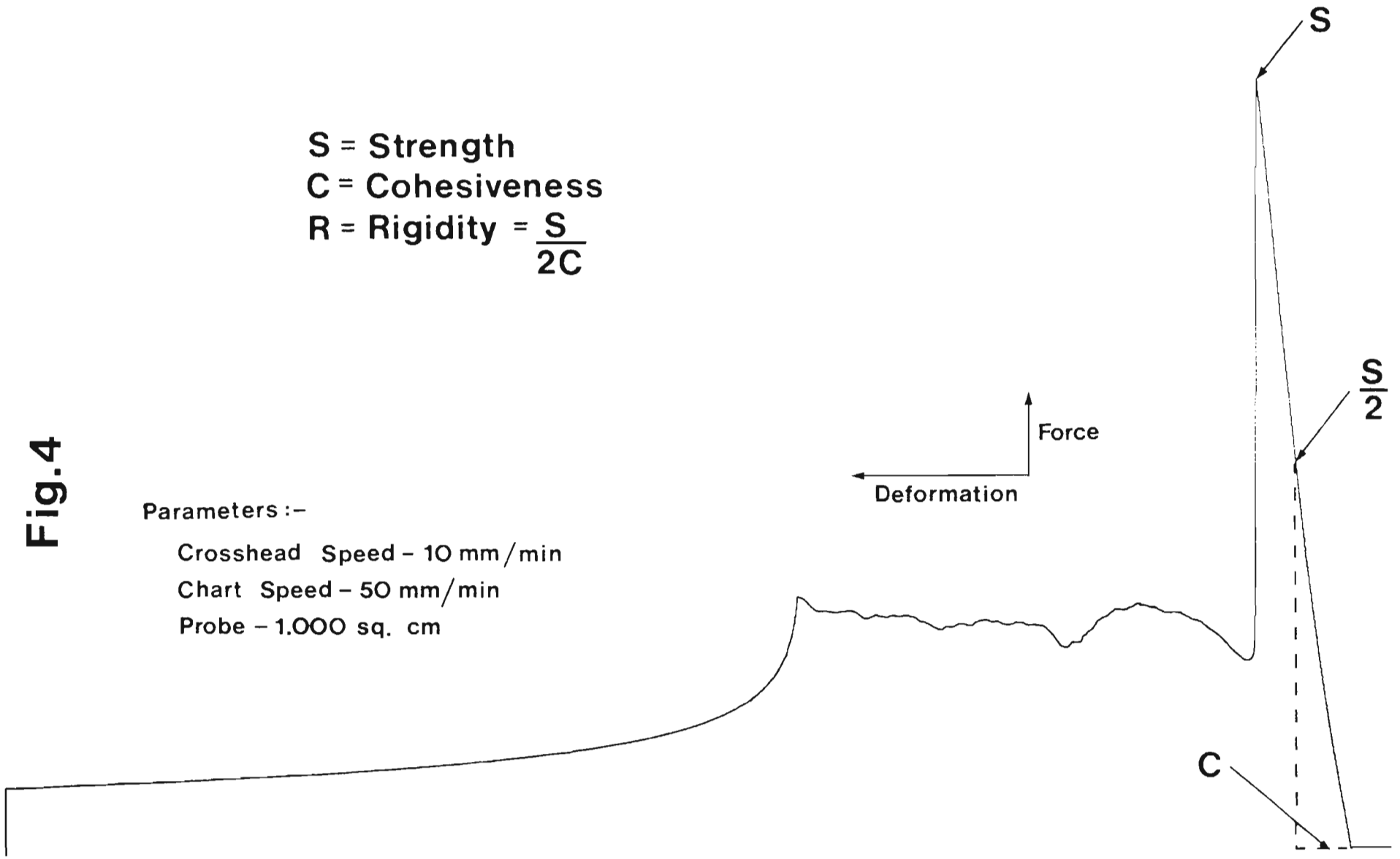

Fig.4

Parameters :-

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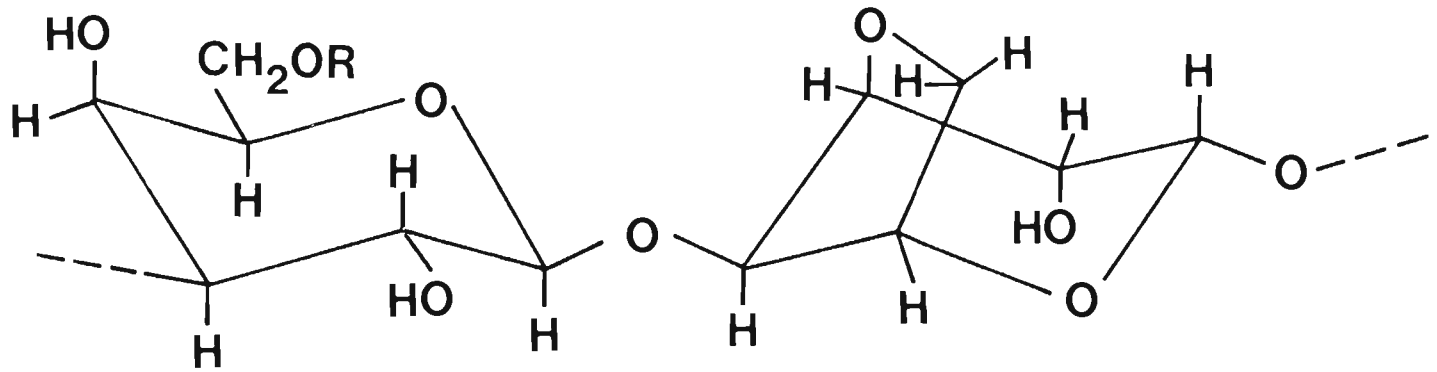
Chart Speed - 50 mm/min
Probe - 1.000 sq. cm

Fig.5



(1→3) β-D-Galactose (1→4) 3,6-anhydro-α-L-Galactose

Agarose (R = H or CH_3)