Fractionation of Alginic Acid

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Up to recently, it was generally accepted that alginic acid was a linear polymer of anhydro-β-D-mannuronic acid [1]. In 1955, however, Fischer and Dörfel [2] reported the presence of guluronic acid in hydrolysates of alginic acid. The proportion of the two uronic acids varied with the species. It has not been decided whether the alginic acid in a particular seaweed plant is a homogeneous compound, or whether it is a mixture of chain molecules of different uronic acid composition. Methods for investigation of the homogeneity of alginic acid are therefore of considerable interest.

Recently McDowell [3] reported that precipitation with manganous salts separated alginate into two fractions which not only differed markedly in molecular weight, but also seemed to differ with respect to calcium-sodium ion exchange properties. He did not investigate the uronic acid composition of the fractions.

During an investigation in this laboratory of the solubility of alginates it was observed that alginate could be separated into two fractions by salt solutions.

Materials and methods. All seaweed samples used contain at least 50 plants, collected at the same time, and from a small area. The plants are dried at room temperature and ground. Fucoidin and laminarin are removed by extraction overnight with 0.2 N sulphuric acid and the alginate is extracted by shaking the seaweed samples with 3 % sodium carbonate solution at room temperature. After filtration, the alginate is precipitated by addition of alcohol to about 30 %. The precipitated sodium alginate is washed with alcohol, and the excess alcohol pressed out. The alginate sample, which contains 60—80 % alcohol, is shaken with the salt solution for 2—3 h, left overnight, shaken again for about one hour, and then centrifuged. The volume of salt solution is chosen so that the alginate concentration is approximately 0.3 %. The gel brought down by centrifugation is washed with half the original amount of salt solution. The amounts of alginate in the gel and the solution are determined gravimetrically after precipitation with alcohol and washing with alcohol and ether.

The solubility of alginate from *Laminaria digitata* f. *stenophylla* (collected at Tarva, outside the Trondheimsfjord, 3/7 1957) in potassium chloride solutions of different strengths was investigated, and the results are given in Fig. 1. Approximately one third of the alginate is soluble and two thirds insoluble in solutions which contain between 0.6 and 1 N potassium chloride, and the shape of the curve gives evidence for the presence of two different compounds.

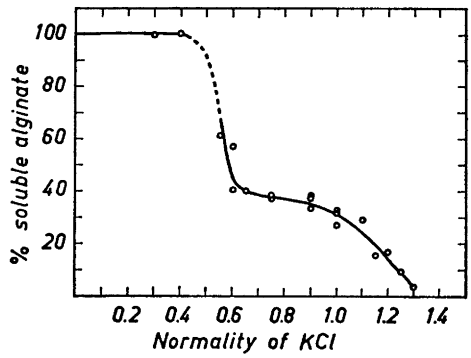

Fig. 1.

The soluble and insoluble fractions of the alginate were shaken with potassium and sodium chloride solutions of the same strength as those used in the original fractionation, and with a volume chosen to give the same alginate concentration. The results are given in Table 1 and show that there is a marked difference between the two fractions with respect to solubility in salt solutions.

Table 1.

Solvent	1st fractionation		2nd fractionation	
			Soluble	Insoluble
1 N NaCl	Soluble	29 %	84 %	16 %
	Insoluble	71 »	12 »	88 »
1 N KCl	Soluble	31 %	100 %	0 %
	Insoluble	69 »	3 »	97 »

Table 2.

	Precipitated, % 2.3 N KCl	Undissolved, % 0.75 N KCl	Undissolved, % 1.0 N KCl
L. digitata, Tarva 3/7—57	69	63	70
Ascophyllum nodosum, Lysøysund 17/9—58	58	55	60
L. hyperborea, Munkholmen 26/8—57			
Fronds	54	45	76
Stipes	—	10	27

The comparatively sharp fractionation makes it improbable that the separation is solely due to a difference in molecular weight. Determinations of viscosity gave an "apparent intrinsic viscosity" determined in a Höppler Viscometer [4] of 17.1, 18.5 and 19.3 $dl \cdot g^{-1}$ for the soluble fraction, the unfractionated alginate, and the insoluble fraction, respectively. A small part of the same *Laminaria digitata* sample as that used above was heated for 15 min to 120°C after the dry matter content had been adjusted to 60 %. The intrinsic viscosity of the alginate was thus reduced to 5.5 $dl \cdot g^{-1}$. In 1 N potassium chloride 45 % of this alginate was insoluble. These observations clearly indicate that the basis of the separation of the fractions in this way is not primarily one of molecular weight differences.

If potassium chloride was added to an alginate solution, no precipitation occurred at salt concentrations below 1.5 N. If, however, a 0.3 % solution of the *Laminaria digitata* alginate was rapidly mixed with equal volumes of saturated (4.65 N) potassium chloride, a gel-like precipitate was formed. The precipitate could be removed from the solution by centrifugation. In Table 2, the percentage of precipitated alginate is given, together with the percentage of alginate which was not dissolved in potassium chloride solution of two different strengths.

In stipes of *Laminaria hyperborea*, the gel that was formed could not be separated from the solution, even by prolonged centrifugation at 20 000 *g*. In the three other cases, the precipitated amount was approximately equal to the amount that was not dissolved in potassium chloride solution of a strength between 0.75 and 1 N. It was also shown that of the fraction that was precipitated only traces could be dissolved in 1 N potassium chloride solution, while the total amount dissolved of the fraction that was not precipitated. Fractionation by precipitation and by dissolution seems, therefore, to give the same fractions.

Alginate from *Laminaria digitata* was hydrolysed according to the method of Fischer and Dörfer [1]. Paper chromatograms of the uronic acids gave two spots with R_G values of 0.38 and 0.32, respectively, in pyridine:ethylacetate-acetic acid: water, 5:5:1:3. Chromatograms of the lactones gave two spots with R_F values 0.53 and 0.77 in pyridine:ethylacetate:water, 11:40:6. According to Fischer and Dörfel these correspond well with the movements of mannuronic and guluronic acids and their lactones. No attempts have been made to determine the uronic acids quantitatively, but on the basis of the size and intensity of the uronic acid spots, there appears to be about three times as much mannuronic as guluronic acid; this is as reported by Fischer and Dörfel.

The fractions separated by precipitation with potassium chloride solution, as described above, were hydrolysed and chromatographed. In both cases two spots with the same R_G values as those found for the unfractionated sample were observed on the uronic acid chromatograms. In the soluble fraction, however, the two spots were of approximately the same strength, while the spot corresponding to guluronic acid was weaker in the insoluble fraction than in the unfractionated sample. Even after five reprecipitations with potassium chloride solution a weak spot corresponding to guluronic acid could be observed in hydrolysates of the insoluble fraction.

The results show that alginic acid in *Laminaria digitata* is a heterogeneous compound, and can be separated into fractions which differ in solubility properties and in uronic acid composition.

The work is being continued and a detailed report will be published later.

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Note on the Crystal Structure of Trivanadium Pentoxide

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The existence of a vanadium oxide V_3O_5 has been reported in a previous communication [1]. The compound was found to be monoclinic with the symmetry $C2/c$ or Cc and with the unit cell dimensions

$$a = 9.98 \text{ Å}, \quad b = 5.03 \text{ Å}, \quad c = 9.84 \text{ Å}, \quad \beta = 138.8°.$$

Slight deviations in the parameter values have been observed indicating that the range of composition of the oxide has a finite width.

The observed density of 4.6 is in accordance with a unit cell content of 4 units of V_3O_5 (calculated density 4.75).

The crystal structure has been derived on the basis of single-crystal data registered by one of us (G.A.) using MoK radiation. The following preliminary parameter values were thus obtained:

12 V and 20 O in 8 sets of point positions 4(a) of space-group Cc (No. 9): (0,0,0; $\frac{1}{2},\frac{1}{2},0$) + x,y,z; $x,\bar{y},\frac{1}{2}+z$.

4 V_1:	$x = 0.088$,	$y = 0.250$,	$z = 0.464$
4 V_2:	$x = 0.412$,	$y = 0.250$,	$z = 0.536$
4 V_3:	$x = 0.000$,	$y = 0.750$,	$z = 0.250$
4 O_1:	$x = 0.04_4$,	$y = 0.60$,	$z = 0.10_2$
4 O_2:	$x = 0.13_9$,	$y = 0.10$,	$z = 0.31_1$
4 O_3:	$x = 0.36_1$,	$y = 0.90$,	$z = 0.18_9$
4 O_4:	$x = 0.45_6$,	$y = 0.40$,	$z = 0.39_8$
4 O_5:	$x = 0.25_0$,	$y = 0.40$,	$z = 0.00_6$

The arrangement of the metal atoms is centrosymmetrical ($C2/c$) while that of the oxygen atoms (derived by S.Å.) is not.

The structure is built up of VO_6 octahedra which are mutually joined by sharing corners, edges and faces. By means of the latter kind of connection, "double-octahedra" (containing V_1 and V_2 atoms) result which are similar to those occurring in the corundum-type structures, *e. g.* V_2O_3. The "double-octahedra" are mutually joined by edges to form parallel rows extending infinitely through the structure. These rows are coupled together by "single-octahedra" (containing V_3 atoms). The V—V distance of octahedra joined by faces is 2.74 Å, while the V—V distances of octahedra sharing edges are 2.96 and 3.10 Å. The V—O and O—O distances, which so far are less accurately known, occur within the ranges 1.9—2.2 and 2.5—3.1 Å, respectively. Refinement of the structure is in progress.

X-Ray studies at temperatures up to 1 000°C did not show any indications of phase transformations in V_3O_5.

The structure of V_3O_5 is essentially different from those of low- and high-Ti_3O_5 [2]. The V_3O_5 structural type has, however, been found to be present in a phase $TiCr_2O_5$ obtained in low yield by melting a mixture of the pure oxides in an electric arc furnace under an inert atmosphere [3]. (This phase seems to be stabilized by minor contents of aluminium.)

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