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海藻酸化學成分之研究

湯元吉

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On the Alginic Acid from Laminaria longissima Miyabe[1]

(海藻酸化學成分之研究)

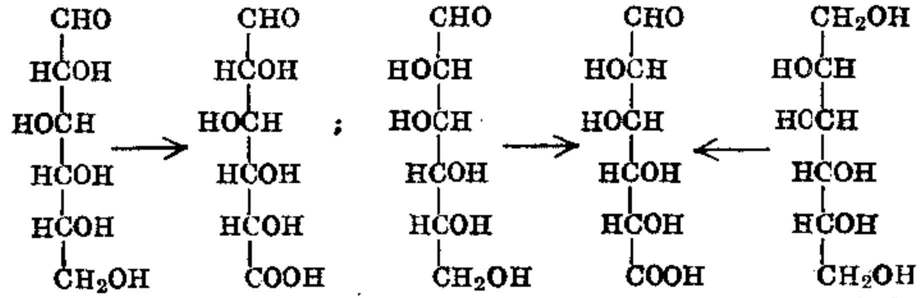
by

Yuan-chi Tang

(湯 元 吉)

The chemical nature of the cell wall constituents of the marine algae has long been a subject of investigation. Stanfort[2] extracted Laminaria with 2% sodium carbonate solution and acidified the extract with mineral acid. He called the highly polymerized acid thus obtained "alginic acid" or "algin". Later on Schmiedeberg[3] and Krafting[4] also obtained from Laminaria "laminaric acid" and "tang acid" as they are called. According to Kylin[5] all these three substances are mixture of two pectic acids, alginic and fucinic, which occur in the middle lamella of the algae as calcium salts, called algin and fucin respectively. Fucin was discovered by van Wisselingh[6] in Fucus vesiculosus. By treating with 1% sulfuric acid and iodine he found it differs from algin in giving a blue coloration. Besides this apparent distinction, they look very much alike. Owing to their gelatinous nature they serve for many industrial purposes such as adhesives, binders, mordants, sizing materials, waterproof agents, medicine, etc.

Recent investigations have brought some light on the chemical nature of alginic acid. The first attempt to investigate systematically the hydrolysis of alginic acid was undertaken by Hoagland and Lieb[7]. These authors claimed to have obtained evidence that the alginic acid prepared from Marcocystis pyrifera, after the hydrolysis with hydrochloric acid, yielded pentoses which were somewhat doubtfully identified by means of phenylhydrazine as *d*-xylose and *l*-arabinose. Several years later, Atsuki and Tomoda[8], in an examination of alginic acid from Laminaria saccharina, suggested that the alginic acid is composed of the *d*-galacturonic or *d*-glucuronic acid units. At the same time Schmidt and Vocke[9] investigated alginic acid from Fucus serratus, described the hydrolysis of this material by successive treatment with 80% sulfuric acid and finally boiling 4.5% sulfuric acid. They claimed to have established that alginic acid is a polymerized *d*-glucuronic acid by preparing a cinchonine salt having melting point 204°C, which is the melting point given by Neuberg[10] for the cinchonine salt of *d*-glucuronic acid prepared by the hydrolysis of euxanthone. Later on, Nelson and Cretcher[11] reported to have obtained from Laminaria and Macrocystis, a new aldehyde acid, which on hydrolysis yielded a hexuronic acid different from both *d*-galacturonic and *d*-glucuronic acids. Since *d*-mannose is widely distributed in nature and *d*-mannitol is found in Macrocystis about 4-5%, the authors claimed that the alginic acid is composed of *d*-mannuronic acid units, because *d*-mannuronic acid is genetically related to *d*-mannose and *d*-mannitol in the same way as *d*-glucuronic acid to *d*-glucose, as indicated by the following structural formulas.



$$\begin{array}{ccccccccc}
\mathrm{CHO} & & \mathrm{CHO} & & \mathrm{CHO} & & \mathrm{CHO} & & \mathrm{CH_2OH} \\
\mathrm{HCOH} & & \mathrm{HCOH} & & \mathrm{HOCH} & & \mathrm{HOCH} & & \mathrm{HOCH} \\
\mathrm{HOCH} & \longrightarrow & \mathrm{HOCH} & ; & \mathrm{HOCH} & \longrightarrow & \mathrm{HOCH} & \longleftarrow & \mathrm{HOCH} \\
\mathrm{HCOH} & & \mathrm{HCOH} & & \mathrm{HCOH} & & \mathrm{HCOH} & & \mathrm{HCOH} \\
\mathrm{HCOH} & & \mathrm{HCOH} & & \mathrm{HCOH} & & \mathrm{HCOH} & & \mathrm{HCOH} \\
\mathrm{CH_2OH} & & \mathrm{COOH} & & \mathrm{CH_2OH} & & \mathrm{COOH} & & \mathrm{CH_2OH}
\end{array}$$

d-Glucose *d*-Glucuronic acid *d*-Mannose *d*-Mannuronic acid *d*-Mannitol

Later they gained evidence of their suggestion from the investigation on hydrolysat of Macrocystis and Laminaria, from which they obtained a cinchonine salt of *d*-mannuronic acid

$C_{25}H_{32}O_8N_2$ having m.p. 152°C, and then the *d*-mannuronic acid lactone $C_6H_8O_6$ having m.p. 140-141°C. On oxidation, the lactone obtained yielded a dibasic acid whose diamide, $C_6H_{12}O_6N_2$, having m.p. 189°C and $[\alpha]_D^{20} = -24.46°$ and whose diphenylhydrazide having m.p. 212°C, both well agree with the corresponding derivatives of the known *d*-mannosaccharic acid dilactone.

On the other hand, Bird and Haas[13] isolated from the hydrolysat of Laminaria spp. two crystalline cinchonine salts. One of which showed a melting point 161°C and $[\alpha]_D^{20} = +154°$ figures, that are only in agreement with those reported by Nelson and Cretcher metioned above. The other salt melted at 195-197°C and showed $[\alpha]_D^{20'} = +112.8°$. But these constants are in fair agreement with those reported by Schmidt and Vocke. Corresponding to the two cinchonine salts, Bird and Haas isolated also two quinine salts of m.p. 168°C, $[\alpha]_D = -173.5°$ and of m.p. 162-163°C, $[\alpha]_D = -175.3°$ respectively. However, they showed that alginic acid on oxidation with nitric acid, yielded *d*-mannosaccharic acid and they agreed with the conclusion given by Nelson and Cretcher, that the acidic nucleus in algin is *d*-mannuronic acid.

Since it has been established that alginic acid is a uronic acid polymer exclusively[11] and again the results so far reported are not consistent, there naturally arises a question whether it is composed entirely of *d*-mannuronic acid or of a mixture of uronic acids. The author wishes to report here his result of investigation on the brown algae, Laminaria longissima Miyabe, which is also used as food in Eastern Asia.

According to his investigation so far carried out, the author obtained two forms of alginic acid from the algae after the removal of fucoidin[14], calcium, and magnesium with 0.5% hydrochloric acid. The one being water-soluble is extracted with water, and the other being not obtainable from aqueous extraction is lixiviated with 2% sodium carbonate solution and subsequently acidified with mineral acids.

Water-soluble Alginic Acid.

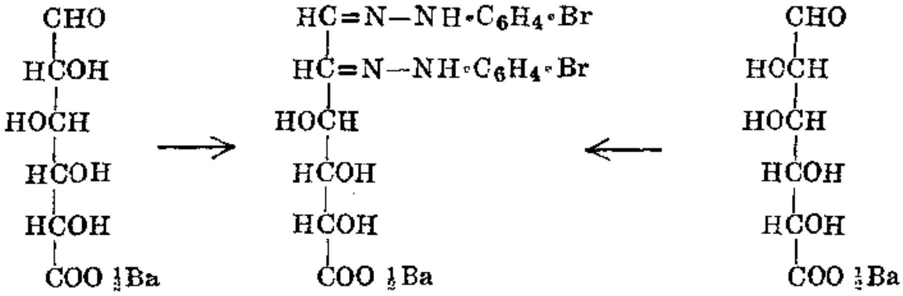
Nelson and Cretcher[15] already noted that the alginic acid is slightly soluble in water. Bird and Haas[13] also established that the cell wall of Laminaria contains alginic acid in two forms, one a water-soluble salt of calcium, magnesium and alkali metal, and the other in free state which appears to be the material extracted by cold dilute sodium carbonate. In agreement with these observations, the author isolated two forms of alginic acid from Laminaria longissima Miyabe. It is to be noted, that after the algae being treated with hydrochloric acid, the water-soluble alginic acid although in the free state is still insoluble in cold water. The extraction should therefore be made with boiling water. The acid thus isolated can then dissolve in cold water, it is now probably in hydrato form. This water-soluble alginic acid differs from the alkali-soluble one in optical rotation and neutralisation equivalent as shown:

	Spec. rotation	Neutralisation equivalent
Water-soluble alginic acid	–101.9°	260
Alkali-soluble alginic acid	–134.8°	183

The water-soluble alginic acid is nitrogen free. It may be precipitated from its aqueous solution by alcohol, glacial acetic acid, lead basic acetate, lead acetate, $FeCl_3$, $AgNO_3$ (precipitate soluble in ammonia), $CuSO_4$, $CaCl_2$ (precipitate soluble on addition of saturated NaCl solution), $BaCl_2$ and $ZnSO_4$, but not by $(NH_4)_2SO_4$, $MnSO_4$, acetic acid, $HgCl_2$, $Ni(NO_3)_2$, $CO(NO_3)_2$, and $(NH_4)_2HPO_4$. Its aqueous solution is acidic to litmus and turns congo paper to blue. It reduces hot Fehling's solution, gives strong orcinol and naphthoresorcinol reactions and dissolves in NH_3, KOH and NaOH showing immediately a densely yellow coloration which is intensified on heating. On standing the color fades gradually, probably due to the formation of the alkali salt of the acid. By hydrolysis with 2.5% sulfuric acid, we obtained, besides a small amount of sugar, always a reducing acid possessing aldehyde properties. This aldehyde acid may be isolated in form of its barium salt which shows in aqueous solution the same precipitation reaction with lead basic acetate as that produced from the barium salt of the aldehyde acid from the hydrolysat of alkali-soluble alginic acid. We have no doubt that the water-soluble alginic acid as well as the alkali-soluble one are composed of *d*-mannuronic acid. The evidence will be further illustrated in discussing alkali-soluble alginic acid. In a later publication, the author shall report that the water-soluble alginic acid here described is composed of tetra-anhydro-tetra-mannuronic acid which may exist in three forms a, b, and c. From this fact we can draw parallel between the laevo-rotatory water-soluble alginic acid from Laminaria longissima Miyabe and the dextro-rotatory pectic acid of Ehrlich[16].

Alkali-soluble alginic acid.

The alkali-soluble alginic acid may be extracted from Laminaria longissima Miyabe, after the removal of fucoidin and water-soluble alginic acid, with 2% sodium carbonate solution and subsequent acidification. It contains trace of nitrogen and gives orcinol and naphthoresorcinol reaction. It dissolves readily in sodium bicarbonate and sodium sulfite solution. Comparing with water-soluble alginic acid its ammonia and alkali solutions exhibit a pale yellow coloration, which on heating intensifies, getting an intensity as that of the water-soluble alginic acid. On standing the color also fades. By oxidation with nitric acid (sp. gr. 1.12) on water bath, it yields neither saccharic acid nor mucic acid. But on oxidation at 50°C, *d*-mannosaccharic acid is formed. It is identified as its ammonium salt having m.p. 189°C. Furthermore, by successive treatment of the alkali-soluble alginic acid with 80% sulfuric acid and finally with boiling 4-5% sulfuric acid, barium *d*-mannuronate is obtained with 26% Ba, corresponding to the formula $(C_6H_9O_7)_2Ba$. From the barium salt, the author obtained a brucine salt having m.p. 147°C, which is the melting point given by Nelson and Cretcher[17] for the brucine salt of *d*-mannuronic acid prepared by the hydrolysis of alginic acid from Macrocystis pyrifera, Laminaria saccharina and Fucus serratus. He obtained from the barium salt also p-bromophenylosazone, having m.p. 208-210°C, which is the melting point given by van der Haar[18] for the same compound of barium *d*-glucuronate. Both osazones are actually identical as indicated by the following structural formulas:



$$\begin{array}{ccccc} \mathrm{CHO} & & \mathrm{HC{=}N{-}NH{\cdot}C_6H_4{\cdot}Br} & & \mathrm{CHO} \\ | & & | & & | \\ \mathrm{HCOH} & & \mathrm{HC{=}N{-}NH{\cdot}C_6H_4{\cdot}Br} & & \mathrm{HOCH} \\ | & & | & & | \\ \mathrm{HOCH} & \longrightarrow & \mathrm{HOCH} & \longleftarrow & \mathrm{HOCH} \\ | & & | & & | \\ \mathrm{HCOH} & & \mathrm{HCOH} & & \mathrm{HCOH} \\ | & & | & & | \\ \mathrm{HCOH} & & \mathrm{HCOH} & & \mathrm{HCOH} \\ | & & | & & | \\ \mathrm{COO\ \tfrac{1}{2}Ba} & & \mathrm{COO\ \tfrac{1}{2}Ba} & & \mathrm{COO\ \tfrac{1}{2}Ba} \end{array}$$

Barium *d*-glucuronate p-bromophenylosazone of both salts Barium *d*-mannuronate

But we shall mention here, that the formation of p-bromophenylosazone of barium *d*-mannuronate often requires much longer heating than that of barium *d*-glucuronate.

The aqueous solution of the barium *d*-mannuronate, on adding a freshy filtered solution of lead basic acetate, gives a colorless floccurent precipitate, which is soluble in excess of the precipitant. The clear solution thus obtained, when heated in boiling water becomes soon turbid, and developes a orange-yellow coloration. One minute after, orange-yellow precipitate separates out and the supernatant liquid is clear. According to Ehrlich[19], aqueous solution of *d*-glucuronic acid or its salts by the same treatment, form yellow to red-brown precipitate. Owing to the lack of *d*-glucuronic acid or its salts at hand, the author did not compare the color of the precipitate he obtained with that from *d*-glucuronic acid and distinguish between them.

From the results above mentioned, it is beyond doubt that alginic acids from Laminaria longissima Miyabe consist only of *d*-mannuronic acid units.

Experimental.

Preparation of water-soluble alginic acid from Laminaria longissima Miyabe.

The brown algae brought from market, was thoroughly washed with tap water and then dried and cut into small pieces with scissors. Two hundred grams of this material were extracted by boiling in 4000 cc. of alcohol. After filtering and drying in the air, it was then immersed in 3000 cc. of 0.5% hydrochloric acid to remove the fucoidin and calcium and magnesium, the latter two are present in cell wall in combined forms with the alginic acids. After standing for twenty-two hours with frequent shaking, the material was filtered, and thoroughly washed. The washed material was extracted with 3500 cc. of distilled water by boiling two hours in a large enameled jar and the processes were repeated for five times. The residue was filtered off every time through a filtering cloth and pressed within the cloth under a handpress. The digested pulp-like residue was left for the preparation of alkali-soluble alginic acid. Afterwards, the combined filtrates were filtered repeatedly through the cloth and finally through a filter paper until the solution became clear. The solution obtained was concentrated on a water bath. The concentrated solution was vigorously stirred towards the end of the evaporation to prevent the material to form a hard cake. The residue was moistened with 70% alcohol and then ground vigorously in a mortar. It was repeatedly extracted for several times, each time for one hour, by boiling in 600 cc. of 70% alcohol under a reflux condenser. Thus we obtained from the dark brown residue a crop of crude water-soluble alginic acid. It was nearly colorless and the dried substance weighed about sixty grams. It was purified by dissolving in 1000 cc. of hot water and this brown solution was repeatedly filtered until the filtrate became clear. Then the clear solution was poured slowly into 3000 cc. of 95% alcohol, whereby the alginic acid separated out. It is necessary that 2-3 cc. of conc. hydrochloric acid are added to the solution before filtration, otherwise the colloidal alginic acid will pass through the filter. The polyuronic acid was then filtered and washed repeatedly with hot 95% alcohol until free from chloride ions. It was then transferred to ether, left over night, filtered again and ground immediately in an agate mortar in order to reduce it to fine powder. After drying at 100°C, the yield weighed 27 gms. Ash: 7.01%. One gram substance neutralizes 38.40 cc. of $\frac{N}{10}$ NaOH, with phenolphthalein as indicator corresponding to a neutralization equivalent of 260. When dissolved in the calculated amount of $\frac{N}{10}$ NaOH, it gives a specific rotation $[\alpha]_D = -101.9°$ After hydrolysis with boiling 2.5% sulfuric acid for four hours, we removed sulfuric acid from the hydrolysat with barium carbonate. The filtrate was concentrated under reduced pressure and four to five volumes of 95% alcohol were added to it. Thus we obtained a precipitate of barium salt corresponding to the formula $(C_6H_9O_7)_2Ba$. This can purified by reprecipitation from its aqueous solution with alcohol.

Preparation of alkali-soluble alginic acid from Laminara longissima Miyabe.

The algae-residue after the removal of the water-soluble alginic acid was treated with 4000 cc. of 2% sodium carbonate solution by standing for twenty-two hours with frequent stirring. The mixture was then heated to about 80°C for half an hour. On cooling it was filtered through a filtering cloth. The residue was again boiled twice with 2000 cc. water and filtered again. The substance remained weighed about 52 g. The combined filtrates were repeatedly filtered until clear and then acidified with 10% hydrochloric acid, whereby the polyuronic acid separated out in gelatinous flocculant precipitate. On standing for four hours it was filtered through a filtering cloth, washed first with dilute hydrochloric acid and then with hot 95% alcohol until free from chloride ions. It was then transferred to alcohol, stood over night and the alcohol was renewed several times until the substance shrinked. The precipitate was then soaked in ether for fourty-eight hours, and after filtration ground immediately in an agate mortar. On drying at 105°C about 26 g. of grayish-white powder were obtained. It was purified by reprecipitation from its 1% sodium carbonate solution with mineral acid. Ash: 0.78%. One gram substance neutralizes 54.73 cc. of $\frac{N}{10}$ NaOH corresponding to a neutralization equivalent of 179. When dissolved in the calculated amount of $\frac{N}{10}$ NaOH it gives a specific rotation $[\alpha]_D = -134.8°$

Oxidation of alkali-soluble alginic acid to d-mannosaccharic acid.

Three grams of dried and finely ground alkali-soluble alginic acid were covered with 4.5 g. of nitric acid (sp. gr. 1.2); this was heated under a reflux condenser for twenty-four hours at 50°C: about 50 cc. of water were now added and after thorough mixing the insoluble residue was filtered off on a Büchner funnel. The filtrate was then rapidly evaporated on a water-bath to about half its volume and then to dryness in a desiccator over sodium hydroxide, while a light lemon-yellow syrup resulted. On stirring this with concentrated ammonia it turned brown and deposited in the course of a few minutes a mass of crystals which on recrystallizing from water separated out in well formed rhombs, melting at 189°C. Owing to the limited amount of material available, no analysis was made.

Hydrolysis of the alkali-soluble alginic acid with 80% sulfuric acid.

The hydrolysis was best accomplished in the following manner. Five grams of the dry pulverized acid was thoroughly mixed with 25 g. of cold 80% sulfuric acid. The flask was stoppered and allowed to stand for three days at room temperature. The contents was then slowly poured into 500 cc. of water. After the termination of hydrolysis the solution is cooled and the water-insoluble residue was removed by filtration and washed with water and alcohol. This residue gave the naphthoresorcinol reaction and weighed 0.3 g. In order to reduce decomposition of the uronic acid, it was found advisable to remove the sulfuric acid in two steps. Approximately $^9/_{10}$ of the acid is removed by adding 58 g. of $Ba(OH)_2 \cdot 8H_2O$ dissolved in 600 cc. of water at 40°C. The solution should be added from a burette while the solution is stirred vigorously with a glass rod to prevent local overheating and alkalinity. After that 6 g. of finely powdered $BaCO_3$ (a slight excess) suspended in water are added and the solution is next heated on a water bath at 70-80°C for twenty minutes to insure the complete conversion of *d*-mannuronic acid lactone in the barium salt. Five grams of blood charcoal were now added and the heating continued for an additional five minutes. The inorganic barium salts were removed by filtration. The residue was boiled four times with 250 cc. water. The last filtrate gave only very faint naphthoresorcinol test, while this test given by the residue was very prominent. It may be inferred that a part of the *d*-mannuronic acid still exists in the residue probably as basic salt of the formula Mannur.-Ba-OH. The combined filtrates and washings were then concentrated to a volume of 150 cc. under reduced pressure. The temperature within the flask should not exceed 40°C. The solution was then filtered and treated with blood charcoal. The light yellow solution was now poured slowly into 4-5 volumes of 95% alcohol. After standing over night the barium *d*-mannuronate was filtered rapidly on a Büchner funnel, whereupon it was partially dehydrated by washing first with hot 95% alcohol and then with hot 99% alcohol and ether. The drying of the barium salt was completed by rapid grinding in an agate mortar. The yield of barium *d*-mannuronate from 5 g. of alkali-soluble alginic acid is 3.5 g. equivalent to approxim. 50% of the theoretical quantity obtainable. The freshly prepared barium *d*-mannuronate, particularly immediately filtered from the alcoholic solution is very hygroscopic, therefore it is imperative to subject the salt to the dehydration process as quickly as possible. When the salt is not handled properly in the course of the drying operation, it absorbs rapidly atmospheric moisture and forms a molasses-like substance. After it is obtained in a dry state, the tendency to be hydroscopic is much less. After it has been reprecipitated once from its aqueous solution with alcohol, it was found to be analytically pure.

Anal. Calcd. for $(C_6H_9O_7)_2Ba$: Ba,26.24. Found: Ba,25.96, 26.08.

Brucine d-Mannuronate.

Brucine *d*-mannuronate is prepared from barium *d*-mannuronate in the manner usually for the preparation of alkaloidal salts and crystallized twice from 80% alcohol. The compound crystallizes in small needles and melts at 147°C with decomposition.

Anal. Calcd. for $C_{29}H_{36}O_{11}N_2$: N,4.76. Found: N,4.45.

Barium salt of the p-bromophenylosazone of barium d-mannuronate.

Barium salt of the uronic acid 1 part, p-bromophenylhydrazine hydrochloride 3 parts, barium acetate 4 parts and water 70 parts. The mixture was heated for two minutes in a boiling water-bath, shaken well, and rapidly filtered. The turbid yellow filtrate was treated with three cc. of glacial acetic acid, and again heated in a boiling water-bath. After 15′, 45′, 75′, 120′ and 180′ heating, yellow crystals, which separated out each time, were filtered off, washed well with water, and with boiling absolute ethyl alcohol and ether. The melting point of the derivative was 208-210°C with decomposition.

Anal. Calcd. for $(C_{18}H_{17}O_5N_4Br_2)_2Ba$: N,9.37; Ba,II.49. Found: N,9.26; Ba,11.77.

Summary.

1.) Two forms of alginic acids have been for the first time isolated from Laminaria longissima Miyabe and their specific rotations and neutralization equavalents are determined.

2.) *d*-Mannosaccharic acid in form of its ammonium salt having m.p. 189°C has been obtained from the alkali-soluble alginic acid from Laminaria longissima Miyabe on oxidation with nitric acid.

3.) Pure barium *d*-mannuronate has been obtained from the alkali-soluble alginic acid by hydrolysis with 80% sulfuric acid and finally boiling 4-5% sulfuric acid. From this salt, two derivatives, brucine *d*-mannuronate having m.p. 146°C and p-bromophenylosazone of barium *d*-mannuronate having m.p. 208-210°C have been prepared.

5.) From the study of the derivatives above mentioned, the alkali-soluble alginic acid from Laminaria longissima Miyabe is for the first time confirmed to be composed of *d*-mannuronic acid.

6.) It has been shown to be possible that the nucleus of the water-soluble alginic acid from Laminaria longissima Miyabe is also *d*-mannuronic acid.

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中 文 摘 要

一八八三年 Stanfort 氏用 2% 炭酸鈉水溶液從 Laminaria (巨褐藻類 Phacosporales 植物)中提出一種多聚酸(highly polymerized acid), 而名之曰海藻酸 (alginic acid). 此酸係一種固體物質，帶膠性。可作黏料之用， 故在工業上之用途甚廣； 歐美各國用彼製成之各種專利品，不勝其數。但其化學成分， 則至近年已來， 始有若干化學家證明其爲 hexuronic acid 一類之化合物(因其呈 α-naphthoresorcinol 反應之故)；惟 hexuronic acid 種類甚多，吾人試觀下表，卽知各方面所得之實驗結果，猶未能盡趨一致也。

研究人名	供給海藻酸之海藻名稱	實驗結果(證明方法此處從略)
Atsuki and Tomoda	Laminaria Saccharina	*d*-gluronic acid or *d*-galacturonic acid
Schmidt and Vocke	Fucus serratus	*d*-glucuronic acid
Nelson and Cretcher	Macrocystis pyrifera Fucus serratus Laminaria Saccharina	*d*-mannuronic acid
Miwa	Ulopteryx pinnatifida and other algae	*d*-mannuronic acid (?)
Bird and Haas	Laminaria spp.	*d*-mannuronic acid
Y. C. Tang	Laminaria longissima Miyabe	*d*-mannuronic acid

予爲欲明瞭此問題起見，曾從市售昆布 (Laminaria longissima Miyabe) 中提出兩種海藻酸(一爲水溶解性 water-soluble, 一爲鹼溶解性 alkali-soluble.) 而研究之。所得結果，現可列舉如下：

(一) 初次由 Laminaria longissima Miyabe 中提出兩種海藻酸，並測定其比轉光率與中和等值。

(二) Laminaria longissima Miyabe 中所含鹼溶解性海藻酸用硝酸氧化後，可得到 *d*-mannuronic acid, 其鋅化合物之融點爲 189° C.

(三) 用 80% 冷硫酸及 4-5% 沸硫酸使從 Laminaria longissima Miyabe 之中提出之鹼溶解性海藻酸水分解後，可得到下列數

種誘導體： barium-*d*-mannuronate ($(C_6H_9O_7)_2$ Ba), brucine-*d*-mannuronate (融點 146° C) 及 barium-*d*-mannuronate 之 p-bromophenylosazone (融點 208-210° C).

（四） 由上述各種誘導體之研究，證明 Laminaria longissima Miyabe 中所含鹼溶解性海藻酸係一種由 *d*-mannuronic acid 所組成之多聚體。

（五） Laminaria longissima Miyabe 中所含水溶解性海藻酸之化學成分，大致亦爲 *d*-mannuronic acid, 但詳細研究，尙在繼續進行中。

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1. The author wishes to thank Prof. K. Okamura, Fishery Institute of Tokyo, and Prof. Y. Yamada, Hokkaido Imperial University, Tokyo, for the identification of the algae here examined.

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ERRATUM.

Page	Line	
3	12	for "metioned" read "mentioned".
4	16	for "neutralisation equivalent" read "neutralization equivalent".
4	18	for "Neutralisation equivalent" read "Neutralization equivalent".
4	10*	for "$CO(NO_3)_2$" read "$Co(NO_3)_2$".
6	14*	for "freshy" read "freshly".
8	15*	for "Laminara" read "Laminaria".
8	17	for "This can purified" read "This can be purified".
9	1	for "shrinked" read "shrank".
9	8	for "179" read "183".
9	2*	insert "The mixture was boiled under reflux over direct flame for four hours".
10	12	for "in the barium salt" read "into the barium salt".
13	6	"則至近年己來" 應作 "則至近年以來".
13	12	for "d-gluronic acid" read "d-glucuronic acid".
14	10*	for "(1913)" read "(1931)".

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*from bottom

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by

YUAN-CHI TANG

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