[CONTRIBUTION FROM THE LABORATORY OF PLANT NUTRITION, UNIVERSITY OF CALIFORNIA]

# The Structure of Sodium Sulfuric Acid Ester of Galactan from Irideae Laminarioides (Rhodophyceae)

BY W. Z. HASSID

In a previous paper[1] the writer reported the isolation of a new carbohydrate from a red alga, *Irideae laminarioides*, which proved to be a sodium sulfuric acid ester of galactan. A tentative formula was proposed for this compound.

In the following work the compound has been subjected to a series of chemical processes, whereby the structure and the linkages involved between its groupings were studied.

## Experimental

**Acetylation of the Sodium Sulfuric Acid Ester of Galactan.**—Haworth's method for acetylation of inulin[2] was followed with some modifications. Three grams of the finely powdered sodium sulfuric acid ester of galactan, prepared from *Irideae laminarioides* was stirred with 5 cc. of pyridine at 80° for half an hour. After cooling to room temperature, 30 cc. of acetic anhydride was slowly added with thorough mixing of the ingredients. After several hours 15 cc. of acetic acid was also added to the mixture. The substance did not, however, dissolve as in the case of acetylated inulin prepared by Haworth. This preparation was tested for its acetyl content at three or four day intervals, and it was observed that its acetyl value was gradually rising. After about two weeks, the acetyl value became constant and corresponded to two hydroxyl groups per one molecule of galactose.

For the acetyl determination the following method was used. About 20 cc. of 0.1 *N* sodium hydroxide was added to 0.1 g. of the acetylated substance in a flask and after standing for several hours was titrated with 0.1 *N* hydrochloric acid.

Duplicate determinations on 0.1 g. of material required 5.5 cc. of 0.1 *N* hydrochloric acid. The acetyl value was found to be 23.6%. Calculated acetyl content for two acetyl groups in $C_{10}H_{13}O_{10}SNa$ was 24.7%.

The hydrogen atoms of two hydroxyl groups in the compound were replaced by acetyl groups. The addition of 0.1 *N* sodium hydroxide regenerated the two hydroxyl groups using up an equivalent amount of alkali. The ethereal sulfate group was not saponified under these conditions.

**Preparation of Galactan.**—The ethereal sulfate group could be hydrolyzed off the compound either by acid or alkali.

(a) The acid hydrolysis was accomplished in the following manner. Thirty grams of the original material was hydrolyzed with 600 cc. of 0.5 *N* sulfuric acid under a reflux condenser for five hours at a temperature of 65–70°. The solution was then cooled and neutralized with barium hydroxide. The barium sulfate precipitate was filtered off and washed. The filtrate was concentrated under reduced pressure to a thick sirup and poured into an excess of 95% alcohol. The galactan precipitated as an amorphous white mass. It was dried at 50° in a vacuum oven. The yield was about 13 g. Its specific rotation (*c*, 0.4) in water, $[\alpha]_D$ was +82.2. Its reducing value, after hydrolysis of the substance with 2% sulfuric acid at 105–110° for seven hours and neutralization of the acid with barium hydroxide, was 95.8%.

(b) The alkali hydrolysis was accomplished as follows. Five grams of the original substance was placed in a flask containing 100 cc. of water and 5 g. of barium hydroxide. The mixture was heated under a reflux condenser at 70°. As the hydrolysis progressed, the sulfate ion precipitated by the barium as barium sulfate could be seen forming and settling at the bottom of the flask. The barium sulfate precipitate was then filtered off, a little hot saturated barium hydroxide added to the filtrate, and the mixture again heated under the reflux condenser at the same temperature. This operation was repeated a few times until no more barium sulfate precipitate could be seen forming. The solution was then cooled and the excess of hydroxide neutralized with dilute sulfuric acid. The precipitate was filtered off and washed, the filtrate concentrated and the galactan precipitated out from 95% alcohol. It was then dried in the vacuum oven at 50°. Its specific rotation (*c*, 0.4) in water, $[\alpha]_D$ was +78.0°.

The reducing value after hydrolysis with 2% sulfuric acid at 105–110° for seven hours and neutralization of the acid was 86.3%. This low reducing value is probably due to the incomplete removal of the sulfuric acid group or destruction of some of the carbohydrate by the alkali.

**Methylation of the Sodium Sulfuric Acid Ester of Galactan.**—Seventeen grams of the substance was methylated by the procedure described by Haworth and Learner[3] for methylation of inulin. A methylated sirup was obtained. After the addition of 30 cc. of absolute alcohol, and upon standing, a white amorphous mass was precipitated. The solid was filtered off, washed with absolute alcohol, and dried at 50°.

The methoxy content of the methylated substance was determined by Dore's method[4] with the following modification. The water in one of the two U-tubes of the apparatus was replaced by a 5% solution of cadmium sulfate. This was done in order to take care of the sulfur present in the compound which in the presence of hydrogen iodide is reduced to hydrogen sulfide and precipitated by the silver as silver sulfide. By introducing cadmium sulfate, the hydrogen sulfide was decomposed and trapped as cadmium sulfide.

A methoxy determination was made on the methylated substance. It was found to be 14.8%.

This partially methylated substance was subjected twice

---

[1] W. Z. Hassid, THIS JOURNAL, **55**, 4163 (1933).

[2] W. N. Haworth, *Helv. Chim. Acta*, **15**, 609 (1932).

[3] W. N. Haworth and A. Learner, *J. Chem. Soc.*, 619 (1928).

[4] W. H. Dore, *J. Ind. Eng. Chem.*, **12**, 472 (1920).

to further methylation with Purdie's reagents. Its methoxy content was raised to 20.0%.

A third methylation with the Purdie reagents did not increase the methoxy content. The substance was, apparently, completely methylated. The results obtained agreed well with the calculated value for two methoxy groups in the compound $C_8H_{13}O_8SNa$, 21.23%.

The specific rotation (*c*, 0.8) in chloroform, $[\alpha]_D$ of this methylated compound was +17.2.

**Hydrolysis of the Methylated Compound and Preparation of Dimethylmethylgalactoside.**—Six grams of the methylated substance was hydrolyzed with 300 cc. of 2% sulfuric acid under a reflux condenser at a temperature of 105–110° for seven hours. The solution was cooled and nearly neutralized with barium hydroxide and completely neutralized with silver carbonate. The precipitate was filtered off and extracted four times with 100-cc. portions of chloroform. The chloroform extracts were dried with anhydrous sodium sulfate. The sodium sulfate was filtered off and after evaporating the chloroform under reduced pressure, a sirup was obtained, which did not crystallize after standing for several days. This sirup reduced Fehling's solution and did not precipitate barium sulfate on the addition of barium chloride after boiling a small amount with hydrochloric acid.

This showed that the methylated polysaccharide was hydrolyzed to a methylated monosaccharide, and that the sulfuric acid group was split off.

The methylated monosaccharide was then converted into its glycoside by boiling with 75 cc. of methyl alcohol containing 2 g. of hydrogen chloride under a reflux condenser for eight hours. The acid was then neutralized with silver carbonate and the filtered solution was evaporated to a sirup which was extracted with benzene and evaporated again. The sirup distilled at 90° and 0.1 mm. pressure. This sirup was taken up in a little petroleum ether and left standing in the cold with occasional stirring. In the course of a few days the sirup gave a crystalline substance, which did not reduce Fehling's solution, and apparently was dimethylmethylgalactoside.

Its methoxy content was 39.0%; calculated $OCH_3$ content for dimethylmethylgalactoside, $C_6H_{18}O_6$ was 41.8%; $[\alpha]_D$ (*c*, 0.8) in water was +97.2.

**Preparation of Trimethylgalactan.**—Twenty-five grams of galactan prepared by the acid hydrolysis, was methylated by the method of West and Holden.[5] A methylated sirup was obtained, which, upon the addition of a little petroleum ether and stirring, set to a mass of crystals. The crystals were filtered off and dried at 40°. The specific rotation of this substance (*c*, 0.8) in water $[\alpha]_D$ was +32.4. Its methoxy content was 44.5%. The calculated value for $OCH_3$ content in trimethylgalactan $C_9H_{16}O_5$ was 45.6%.

**Preparation of Trimethylgalactose from the Methylated Galactan.**—Eighteen grams of the methylated galactan was hydrolyzed with 400 cc. of 2 *N* hydrochloric acid in a distilling flask for an hour under a reflux condenser, using a boiling water-bath. The solution was cooled, saturated with sodium sulfate and extracted four times with 150-cc. portions of chloroform. The combined chloroform extracts were dried with sodium sulfate. Five grams of carboraffin was added and filtered on a dry talc filter, prepared on a small Büchner funnel. The solution was then transferred into a distilling flask, and the chloroform distilled off under reduced pressure. The remaining sirup was then distilled under 0.1 mm. pressure. About 12 g. of the sirup distilled over at 94°. An attempt to crystallize this sirup failed.

The sirup reduced Fehling's solution, and its specific rotation (*c*, 0.8) in water, $[\alpha]_D$ was +129.0. Its methoxy content was 40.9%. The calculated value for three $OCH_3$ groups, trimethylgalactose in $C_6H_{18}O_6$ was 41.8%.

An attempt to prepare an osazone from this trimethylgalactose failed. This fact shows that the hydroxyl on the second carbon atom must be occupied by one of the methoxy groups.

**Oxidation and Esterification of the Trimethylgalactose.**—The degradative oxidation of this compound was carried out in two stages, involving first the oxidation to the lactone by bromine water, and second, its conversion into a dibasic acid. This mode of procedure in stages possesses the advantage that the final product is homogeneous, whereas this is not the case when the methylated sugar is oxidized directly with nitric acid.

Ten grams of the sirup was placed in a glass-stoppered flask to which 8 cc. of bromine and 50 cc. of water were added. The contents of the flask were placed at a temperature of 30° for seven hours with frequent shaking. At the end of that time the oxidation was not complete, as it gave a positive test with Fehling's solution. After keeping the flask at the same temperature for about seventy-two hours with occasional shaking, the solution was devoid of action toward Fehling's solution. The excess of the bromine was removed by aëration and this was followed by treatment of the solution with litharge and finally with silver oxide until neutral to litmus. The filtrate was saturated with hydrogen sulfide, again filtered, and the solution evaporated to a sirup. This sirup was extracted several times with anhydrous ether; the ether was evaporated, and a sirup was obtained.

The sirup obtained from the oxidation with bromine weighing about 7 g. was treated with 56 cc. of concentrated nitric acid and warmed for half an hour at 50–60°, whereupon oxidation commenced. The temperature was gradually raised to 90°, and so maintained for five hours. At the end of that time the reaction ceased and the solution was transferred to a distilling flask and diluted with water. The nitric acid was removed by distillation under diminished pressure with frequent additions of water. The residue was dried at 80° in the vacuum oven overnight.

This sirup was esterified by boiling with 100 cc. of methyl alcohol containing 3 g. of hydrogen chloride under a reflux condenser for eight hours. The acid was neutralized with silver carbonate and the filtered solution was evaporated to a sirup. The small quantity of mineral matter was removed by extracting the sirup in benzene and then evaporating the solution. The sirup distilled at 0.1 mm. pressure and 95°. Its specific rotation (*c*, 0.8) in water, $[\alpha]_D$ was +41.1°. Its methoxy content $OCH_3$, was 51.1%; calculated for $C_9H_{16}O_7$, dimethylarabodimethoxyglutarate was 52.5%.

**Isolation of a Dimethoxyhydroxyglutaric Acid from the Esterified Substance.**—About 4 g. of the sirup, dimethyl-

---

(5) E. S. West and R. F. Holden, THIS JOURNAL, **56**, 930 (1934).

arabodimethoxyglutarate, was hydrolyzed with 50 cc. of 2% hydrochloric acid at 90° for two hours. The acid was neutralized with silver carbonate, the solution filtered and evaporated under diminished pressure, extracted with chloroform and the chloroform again distilled off.

The sirup was then distilled at 0.1 mm. pressure. The water-bath was replaced by an oil-bath as the sirup did not distil at 100°. A small amount (not enough to analyze) distilled over at 115°. The temperature was raised but no more of the sirup would distil over. The residue was then examined for its methoxy content and this was found to be 31.1%.

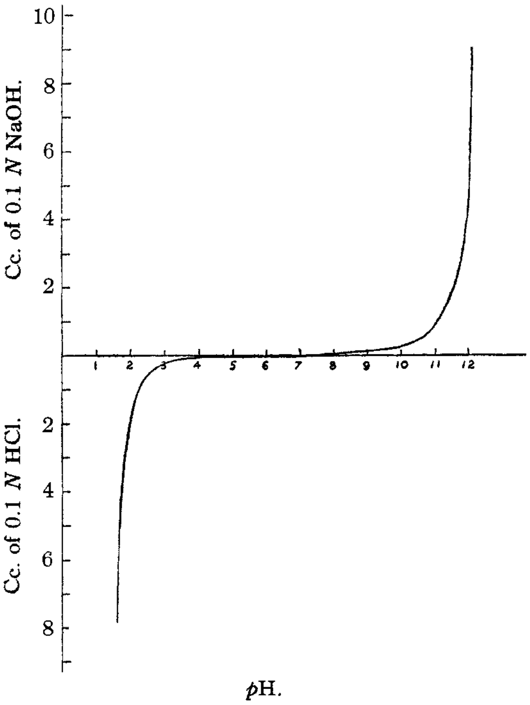


Fig. 1.—Titration curve of sodium sulfuric acid ester of galactan.

0.0921 gram of the sirup, on heating with 0.1 *N* sodium hydroxide and titrating with 0.1 *N* hydrochloric acid, required 9.3 cc. of 0.1 *N* sodium hydroxide for its neutralization. The undistilled residue was apparently dimethoxyhydroxyglutaric acid. The calculated value for the methoxy content of dimethoxyhydroxyglutaric acid, $C_7H_{12}O_7$, was 29.8% and its titration value for 0.0921 g. is 8.8 cc. of 0.1 *N* sodium hydroxide. This suggests that part of the acid is in the form of its lactone which would account for the high results obtained.

**Evidence to Show that the Sodium Sulfuric Acid Ester of Galactan is a Salt of a Strong Acid.**—The *p*H of a 1% solution of the acid previously obtained (1) was 3.6. On further continuation of the electrodialysis, the *p*H could be reduced to 2.86.

0.3 gram of this acid was titrated with 0.1 *N* sodium hydroxide. The amount required was 0.82 cc. The theoretical value for the titration of 0.3 g. was 1.24 cc. The titration, therefore, showed this acid to be of a greater equivalent weight than the theoretical one. The theoretical value of one equivalent weight of the acid $[(C_6H_9O_4)OSO_2OH]$ being 242, the calculated value from the titration was 366.

On further experimentation with this acid, it was found that part of the sulfur group was hydrolyzed off during the process of dialysis. It was noticed that by adding a solution of barium chloride to the anode compartment of the dialysis apparatus, a precipitate of barium sulfate was formed. This showed that some of the sulfate was hydrolyzed off the compound and moved through the membrane to the positive electrode. This accounts for the apparent high equivalent weight.

A 1% solution of the neutral salt itself (*p*H 6.8) was then taken and a titration curve was run by adding 0.1 *N* acid and 0.1 *N* alkali. A curve typical of that of a salt of a strong acid was obtained as shown in Fig. 1.

The specific conductance at 25° of various concentrations of the salt of the compound was then determined by using an electrical conductivity apparatus with the following results.

| Solution, % | Specific conductance at 25° |
|---|---|
| 2.0 | $2.53 \times 10^{-3}$ |
| 1.0 | $1.43 \times 10^{-3}$ |
| 0.5 | $6.88 \times 10^{-4}$ |
| .25 | $3.68 \times 10^{-4}$ |
| .125 | $1.84 \times 10^{-4}$ |

A 2% solution of sodium sulfuric acid ester of galactan, the specific conductance of which is $2.53 \times 10^{-3}$ reciprocal ohms at 25°, is equivalent to 0.076 *N*. If we compare this with the specific conductance of 0.02 *N* potassium chloride, which is $2.77 \times 10^{-3}$ reciprocal ohms at the same temperature, we find that the specific conductance of the salt is about 25% of that of the potassium chloride. The low specific conductance of the salt may be due to the fact that the solution of the compound in question is highly viscous and colloidal, and its large negative ion is probably highly hydrated and has a low mobility.

The neutrality of the compound, the shape of its titration curve and its specific conductance show that the sodium sulfuric acid ester of galactan is a salt of a strong acid.

**Determination of Molecular Weight of the Sodium Sulfuric Acid Ester of Galactan.**—The molecular weight of this compound was determined cryoscopically, the semimicrochemical method of Rieche[6] being used.

The rise in temperature when 30 mg. was dissolved in 5 cc. of water was 0.004°.

From the formula $M = 1000\ (C \times S)/S' \times \Delta$, where $M$ is molecular weight, $C$ is boiling point constant of water, $S$ is weight of substance, $S'$ is weight of solvent, $\Delta$ is rise in temperature, the value of the molecular weight of the substance was calculated as 780.

Considering that this substance is a sodium salt of a strong acid, the above value should be multiplied by two; thus, the approximate molecular weight becomes 1560. If we divide this value by the weight of one unit in the compound $[(C_6H_9O_4)OSO_2ONa]_n$ which is 264, the value $n$ becomes 5.91. The data seem to indicate that there are approximately 6 units per one molecule of the compound. We can therefore, write the empirical formula for this compound as $[(C_6H_9O_4)OSO_2ONa]_n$, where $n$ is approximately 6.

---

[6] A. Rieche, *Ber.*, **59**, 2186 (1926).

## Discussion

The empirical formula for the sodium sulfuric acid ester of galactan was established from the analytical data as $[(C_6H_9O_4)OSO_2ONa]_n$.[1] The analytical data shown in the experimental work also indicate that this non-reducing polysaccharide consists entirely of galactose units and sodium ethereal sulfate groups and that there is one unit of galactose for each ethereal sulfate group.

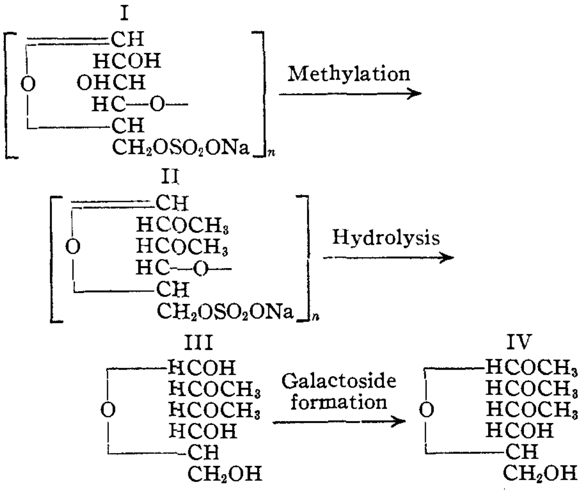
Two questions arise: (1) how are these galactose units linked together in the polysaccharide and (2) what position does the ethereal sulfate group occupy?

It is evident that the terminal carbon atom is tied up in a galactosidal linkage between the individual galactose units, from the fact that the substance is non-reducing. On complete methylation of the original compound two methoxy groups could be substituted for the same number of hydroxyls. That there are two free hydroxyl groups in one unit of galactose is confirmed by the fact that by acetylation of the compound two acetyl groups could be introduced into it.

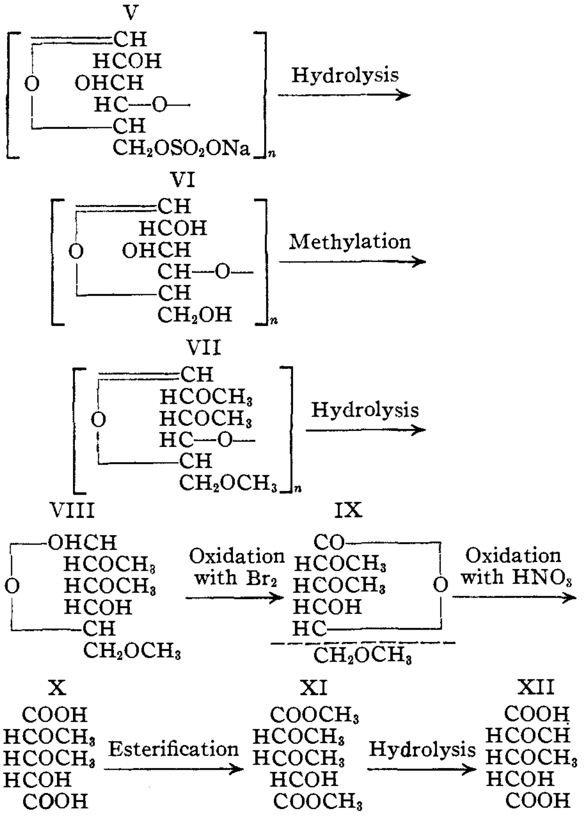
Acid hydrolysis of the methylated compound resulted in a reducing dimethylgalactose. The linkages holding together the galactose units, apparently, were broken with simultaneous splitting off of the ethereal sulfate group.

When the dimethylgalactose was boiled with methyl alcohol containing 3% hydrochloric acid, dimethylmethylgalactoside was obtained.

The above series of changes can be expressed by the equations



On hydrolyzing off the ethereal sulfate group of the original compound (V), galactan was obtained (VI). Upon methylation and subsequent hydrolysis of the trimethylgalactan (VII), trimethylgalactose was obtained (XIII). Oxidation with bromine, then with nitric acid and subsequent esterification, resulted in a dimethylarabodimethoxyglutarate. On hydrolysis of this dimethylarabodimethoxyglutarate, dimethoxyhydroxyglutaric acid was obtained.



It is evident that one of the methyl groups in the trimethylgalactose (VIII) is on the primary alcohol group, because, after splitting off the sixth carbon atom of the substance by oxidation with nitric acid, dimethoxyhydroxyglutaric acid was obtained, a substance with only two methyl groups.

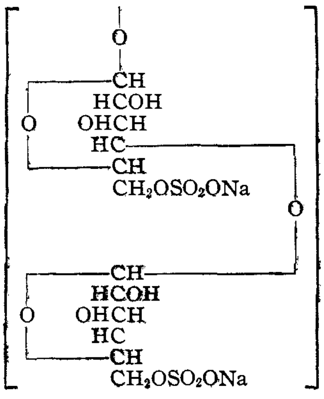
This shows that there could be no 1,6 linkage between the individual units in the original compound (V), because if we assume this to be true, the ethereal sulfate group could not have been attached to the sixth carbon atom, and should have occupied any other one of the five positions of carbon atom groups in the galactose unit. After hydrolyzing off the ethereal sulfate group and methylating the compound, a trimethylgalactan would have resulted, which had no methyl group on the sixth carbon atom. On hydrolysis of the compound and subsequent oxidation with nitric acid, a trimethoxyglutaric acid would have been obtained instead of a dimethoxyhydroxyglutaric acid (XII) as was the case. This, therefore, eliminates the possibility of 1,6 linkage.

The linkage between both of the first carbon atoms of the individual units is excluded, because a trimethylgalactan would have been obtained in (II) instead of a dimethylgalactan, and a tetramethylgalactan in (VII) instead of a trimethylgalactan.

The linkage between the first and second carbon atoms is eliminated on the basis that no osazone could be obtained from a trimethylgalactose (VIII). If there were a 1,2 linkage in the polysaccharide, a free hydroxyl should have resulted on the second carbon atom group on hydrolysis of the trimethylgalactan to the trimethylgalactose, and, consequently, the preparation of an osazone should have been possible.

The linkage of 1,5 between the units may also be eliminated, if we assume the usual amylene ring of the galactose itself. Since the four positions, 1,1, 1,2, 1,5 and 1,6 are eliminated, only the two possibilities, 1,3 and 1,4 remain. Since the 1,3 linkage has never been found, while the 1,4 is common in many disaccharides and polysaccharides, it is, therefore, reasonable to assume that the latter is more probable.

Presumably the ethereal sulfate group exists as a side chain formed by replacement of an hydroxyl group. The position of this ethereal sulfate group is uncertain. However, from consideration in a previous paper[1] this group is assigned to the sixth C atom as the most probable position. Further studies are being undertaken in attempt to establish this point.



In accordance with these considerations, the arrangement shown is tentatively proposed, indicating the linkage between two units.

The molecular weight determination indicates that the polysaccharide is made up of approximately six of these building units, perhaps similarly arranged.

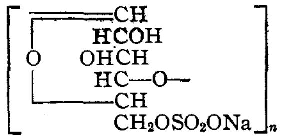
The writer wishes to express his indebtedness to Mr. W. H. Dore under whose general direction this work was carried out. The many valuable suggestions received materially aided the progress of the work.

## Summary

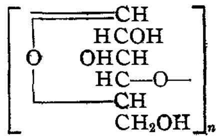
1. The sodium sulfuric acid ester of galactan isolated from *Irideae laminarioides* indicates that it is a salt of a strong acid. This is shown from the titration curve of the substance and also its conductivity data.

2. When the sodium sulfuric acid ester of galactan was subjected to electrodialysis, the sodium was removed and the substance initially neutral became acid, *p*H of about 3. This resulting substance, apparently, was sulfuric acid ester of galactan. It could not be obtained in pure form because the sodium could not be entirely dialyzed out and also because part of the sulfuric acid group was split off in the process of dialysis.

3. A tentative structural formula shown is proposed for the substance in which *n* seems to be approximately 6.



4. The sulfuric acid group in this compound was removed by hydrolysis and a polysaccharide was thus obtained which, in the chemical sense, is a pure galactan.



This is, apparently, the first time that a homogeneous galactan, consisting entirely of galactose units, was obtained from a plant.

Berkeley, California Received June 14, 1935