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

The Isolation of a Sodium Sulfuric Acid Ester of Galactan from Irideae Laminarioides (Rhodophyceae)

BY W. Z. HASSID

Several investigators,[1] working on marine algae reported ethereal sulfates in these plants. However, in no case was the complete structure of these compounds determined with any degree of certainty.

In the present work the writer has isolated from *Irideae laminarioides* a comparatively simple ethereal sulfate, the complete chemical nature of which has been determined.

Extraction.—The plants *Irideae laminarioides* were collected at Moss Beach, California, washed immediately with distilled water, dropped into boiling 95% alcohol and boiled for ten minutes. The plants were brought to the laboratory, the alcohol poured off, the contents ground and extracted in a Soxhlet extractor, first with methyl and then with ethyl alcohol, until free of chlorophyll. The extracted residue was then dried at 50° in a vacuum oven.

Thirty grams of this material was heated with four liters of water on a steam-bath for several hours, until a colloidal solution was formed. The solution was filtered on a large Buchner funnel through a silk filter. The residue on the silk filter was taken up with about three liters of water, heated for several hours on the steam-bath and filtered again. The combined filtrates were placed in a large evaporating dish and evaporated to about 800 cc. The solution was then poured into two liters of 90% alcohol. A milky colloidal solution was formed and was allowed to stand for several hours. The clear supernatant solution was decanted from the residue, which settled at the bottom, and concentrated under reduced pressure to about 500 cc. It was then slowly poured with continual stirring into 10 volumes of 95% alcohol, whereupon a precipitate of white flocculent threads formed. After an hour this was filtered and washed, using a silk filter, first with 95% and then with absolute alcohol and finally dried in a vacuum oven at 50°. About 10 g. of a snow white substance was obtained, which did not reduce Fehling's solution. It formed a colloidal solution when dissolved in water. Its specific rotation $[\alpha]_D$ was 69.2 after two reprecipitations from alcohol. When dissolved in water its P_H was 6.8.

Hydrolysis.—A solution of 10 g. of the dry material in 500 cc. of 2% sulfuric acid was heated under a reflux condenser at a temperature of 105–110° for seven hours, cooled and nearly neutralized with a hot saturated solution of barium hydroxide and completely neutralized with barium carbonate. The mixture was then heated to 80° and allowed to stand overnight, filtered, the precipitate washed with hot water and the filtrate diluted to one liter. Aliquot portions of this were concentrated under reduced pressure. The reducing value of four 10-cc. aliquots was determined in terms of glucose with Fehling's solution, using the Munsen Walker tables. The mean value, when arbitrarily calculated to galactose applying Browne's factor 0.898[2] to the value for glucose, indicated hydrolysis of 54.0%.

Identification of Galactose.—(1) An aliquot representing 2 g. of the prepared ester was evaporated under reduced pressure to a thin sirup. The concentrated solution was

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(1) P. Haas, *Biochem. J.*, **15**, 469 (1921); F. Fairbrother and H. Mastin, *J. Chem. Soc.*, **123**, 1412 (1923); W. F. Hoffman and R. A. Gortner, *J. Biol. Chem.*, **65**, 371 (1925); G. M. Bird and P. Haas, *Biochem. J.*, **25**, 403 (1931); W. L. Nelson and L. H. Cretcher, *J. Biol. Chem.*, **94**, 147 (1931).

(2) C. A. Browne, "Handbook of Sugar Analysis," John Wiley and Sons, New York, 1912, p. 421.

treated with a large volume of methyl alcohol and then 1 cc. of glacial acetic acid was added, inoculated with a minute crystal of galactose and allowed to stand for several days. The crystals which deposited in the bottom of the beaker were collected, dissolved in water, and an orange-yellow phenylosazone was prepared. Its melting point was 194–196°. This corresponds to the melting point of the phenylosazone of galactan, given by Mulliken[3] as 196°.

(2) A confirmatory test showed that the phenylosazone could be prepared from the sirupy hydrolyzate without first obtaining the actual galactose crystals.

Quantitative Determination of Galactose.—Van der Haar's modification of the Kent–Tollens–Creydt method[4] with the additional precautions suggested by Wise and Peterson[5] was used for the quantitative determination of galactose. A determination, in triplicate, on the substance under consideration showed 53.2% anhydrogalactose.

Test for Pentose Sugars.—(1) Tollens' phloroglucinol method was applied to the hydrolyzed and unhydrolyzed substance. Both samples gave a yield equivalent to 0.5% of furfural. However, this result was probably due to the hydroxymethylfurfural obtained from the galactose, as it is in fair agreement with the results of Wise and Peterson,[5] who report 0.47% hydroxymethylfurfural due to galactose, and Schorger and Smith,[6] who report 0.55%.

(2) Samples of the hydrolyzed and unhydrolyzed substance were also examined for pentose sugars by the thiobarbituric acid method of Dox and Plaisance.[7] No precipitate of furfural malonyl-thiourea was obtained by the addition of the thiobarbituric acid to the hydrochloric acid distillate.

(3) Attempts were also made to determine whether any pentose or methylpentose sugar was present in the hydrolysis products of the substance, using various phenylhydrazine derivatives. No evidence was obtained for the presence of either pentoses or methylpentoses.

Test for Uronic Acids.—Dore's[8] modification of the Le Fèvre method for determination of uronic acid was applied to this substance. The results were negative.

Determination of the Sodium Ethereal Sulfate Group.—Ignition of the substance in an electric muffle at red heat showed the ash content to be 25.4%. The ash was analyzed for sulfur from the weight of barium sulfate obtained when the ash was dissolved and the sulfate precipitated with barium chloride. The sulfur content was 5.84%.

The original substance was analyzed for its sulfur content in a Parr bomb by oxidation with sodium peroxide and potassium chlorate; 11.5% sulfur was found by this method.

The substance was also hydrolyzed for two hours with 1:4 hydrochloric acid and the products of hydrolysis were examined for sulfur: 12.4% sulfur was found.

Sulfur in the substance by the Parr bomb method	11.5%
Sulfur in the substance by the hydrolysis with hydrochloric acid	12.4%
Sulfur in the ash	5.8%

From the above results it is evident that the sulfur content in the substance is approximately double the quantity of that in the ash.

Tests for sulfate ions were also made by dissolving the substance in warm water and adding barium chloride. There were no sulfate ions present in the solution.

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(3) S. P. Mulliken, "Determination of Pure Organic Compounds," John Wiley and Sons, New York, 1911, Vol. I, p. 30.

(4) A. W. Van der Haar, "Nachweis zur Trennung und Bestimmung der Monosaccharide und Aldehydsäuren," Gebrüder Borntraeger, Berlin, 1920, pp. 124–126.

(5) L. E. Wise and F. C. Peterson, *Ind. Eng. Chem.*, **22**, 362 (1930).

(6) A. W. Schorger and D. F. Smith, *ibid.*, **8**, 494 (1916).

(7) A. W. Dox and G. P. Plaisance, This Journal, **38**, 2156 (1916).

(8) W. H. Dore, unpublished data.

Fifty-mg. portions of the ash were analyzed for sulfate, calcium, magnesium and sodium: 34.8 mg. of sulfate was found, 13.5 mg. of sodium and 1 mg. each of calcium and magnesium.

These results are in close agreement with the assumption that the ash is chiefly sodium sulfate.

Preparation of the Sulfuric Acid Ester of Galactan.—(1) Two grams of the substance was dissolved in 200 cc. of water and placed in an electrodialysis apparatus described by Greenberg and Greenberg.[9] The electrodialysis proceeded for seventy-two hours. The solution was then concentrated to a small volume and poured into 95% alcohol. The physical appearance of the precipitated substance thus obtained was quite different from that of the original. The fibrous structure had disappeared and the appearance was that of an amorphous granular powder. It gave an acid reaction; its *P*H was 3.6.

(2) Another 2-g. portion of the substance was dissolved in 200 cc. of water, placed in an 800-cc. capacity collodion bag. The bag with the solution was placed in a vessel in which water at 55° was continuously circulating. The dialysis proceeded for seventy-two hours. At the end of that time the solution in the bag was concentrated to a small volume and poured into alcohol as before. The acid ester was obtained as in (1).

Since no pentose sugar and no uronic acid could be found in the isolated substance, galactose appears to be the only sugar present. This conclusion is also supported by the fact that the reducing value corresponding to 54% of the substance, when arbitrarily calculated to galactose, was in satisfactory agreement with the value 53.2% calculated as anhydrogalactose by the modified Van der Haar method.

That the substance is a sodium ethereal sulfate was apparent from the fact that it contained approximately double the quantity of sulfur found in its ash, and from the fact that most of the ash consisted of sodium sulfate.

Comparison of actual results with calculated values, as shown in the table, indicates that there is one unit of galactose in the substance for each sodium ethereal sulfate group.

	Calculated, %	Found, %
Sulfur	12.1	12.0
Carbohydrate portion	54.8	54.0
Sodium ethereal sulfate	45.0	44.7

The table shows a close agreement between the percentages found and percentages calculated for the sulfur content, carbohydrate fraction and the sodium ethereal sulfate group.

Corresponding to the results which have been obtained for various polysaccharides, it might be expected that the structure of this substance is represented by a number of anhydrogalactose units united by glucosidal linkages or oxygen bridges to form chains or rings. The fact that this substance is fibrous strongly suggests that its structure may be a chain rather than a ring structure. Presumably the ethereal sulfate groups exist as side chains formed by replacement of hydroxyl groups. It is impossible of course to determine with certainty the position of the linking oxygen bridges, but assuming that the galactose units possess the usual

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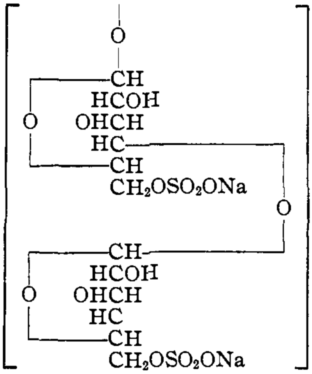
(9) D. M. Greenberg and L. D. Greenberg, *J. Biol. Chem.*, **99**, 1 (1932).

1:5 ring, it is perhaps not unreasonable to propose a chain structure similar to that put forward by Sponsler and Dore[10] for cellulose.

The position of the ethereal sulfate group is in doubt, but one may anticipate that it would be found attached to the sixth C atom. The following consideration upholds the validity of this suggestion: the galactose unit has a 1:5 ring structure, and therefore the group attached to the sixth C atom must protrude from the ring. Owing to the steric position of the groups attached to this atom, they will tend to be more reactive than other groups. Hence the ethereal sulfate group is assigned to the sixth C atom as the most probable position.

In accordance with these ideas the arrangement shown is tentatively proposed showing the linkage between two units.



The polysaccharide itself is made up of an unknown number of these building units, perhaps similarly arranged.

The sulfur in this substance is apparently united with the carbohydrate portion, since it is not precipitated as sulfate when barium chloride is added to an aqueous solution of this substance.

The sodium, however, appears to exist in ionic form, since it moves freely and passes through a collodion membrane when the substance is subjected to electrodialysis or plain dialysis.

The rest of the substance may be considered as a large negatively charged colloidal micelle and does not pass through the membrane.

The writer wishes to acknowledge his indebtedness to Mr. W. H. Dore for the valuable advice received during the course of the experimental work.

Summary

1. A sodium sulfuric acid ester of galactan has been isolated, apparently for the first time, from *Irideae laminarioides*.

2. When subjected to dialysis the sodium was practically all removed from the substance and an acid sulfuric ester of galactan resulted.

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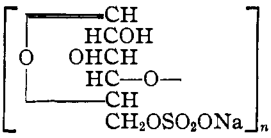
(10) O. L. Sponsler, and W. H. Dore, "Colloid Symposium Monograph," Chemical Catalog Company, Inc., New York, 1926, Vol. IV, p. 174.

3. No sulfur was removed by dialysis.
4. The physical properties of this acid were different from those of the original substance. It had the appearance of an amorphous granular powder in contrast with the fibrous structure of the original substance.
5. A 1% solution of the acid had a P_H of 3.6.
6. The tentative structural formula shown is proposed for the substance in which n is unknown.



BERKELEY, CALIFORNIA

RECEIVED MAY 29, 1933
PUBLISHED OCTOBER 6, 1933

[COMMUNICATION FROM THE LABORATORY OF ORGANIC CHEMISTRY, UNIVERSITY OF WISCONSIN]

The Synthesis and Reactions of Certain Nitrogen Ring Compounds over Nickel

BY CHARLES F. WINANS AND HOMER ADKINS

In this group of three papers we are surveying the application of nickel as a catalyst for reactions which result in the formation of amines.[1] These reactions are of four general types. In the first type are those in which an amine reacts with an alcohol or amine with the elimination of water or ammonia

$$ROH + RNH_2 \longrightarrow R_2NH + H_2O \qquad (I)$$

$$RNH_2 + RNH_2 \longrightarrow R_2NH + NH_3 \qquad (II)$$

In the second group a cyano, oximino, nitro or nitroso group is hydrogenated successively to an imine and an amine

$$RC{\equiv}N \xrightarrow{H_2} RCH{=}NH \qquad (III)$$

$$R_2C{=}NOH \xrightarrow{H_2} R_2C{=}NH + H_2O \qquad (IV)$$

$$RCH{=}NH \xrightarrow{H_2} RCH_2NH_2 \qquad (V)$$

In the third group a nitrogen to nitrogen or nitrogen to carbon bond undergoes hydrogenolysis with the formation of amines

$$RCH(N{=}CHR)_2 \xrightarrow{H_2} RCH_2NH_2 + (RCH_2)_2NH \qquad (VI)$$

$$RNHNHR \xrightarrow{H_2} 2RNH_2 \qquad (VII)$$

In the fourth group an imine, aldehyde or ketone adds an amine, followed in most cases by hydrogenolysis with the elimination of ammonia or water

$$RCH{=}NH + R'NH_2 \longrightarrow RCH(NH_2)NHR \xrightarrow{H_2} RCH(R)NH + NH_3 \qquad (VIII)$$

$$RCH{=}O + R'NH_2 \longrightarrow RCH(OH)NHR \xrightarrow{H_2} RCH(R)NH + H_2O \qquad (IX)$$

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(1) Winans and Adkins, THIS JOURNAL, (a) **54**, 306 (1932); (b) **55**, 2051 (1933).