[Contribution from the Division of Plant Nutrition, College of Agriculture, Berkeley, and the U. S. Department of Agriculture, Agricultural Research Administration, Bureau of Plant Industry, Soils and Agricultural Engineering, Guayule Research Project]

Inulin in Guayule, Parthenium argentatum Gray

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McRary and Traub[3] have shown that the rubber producing plant guayule contains a polysaccharide having properties of a fructosan. Although Guglielminetti[4] states that guayule plants contain inulin, his statement is not supported by experimental data. It was therefore of interest to study the chemical structure of the guayule fructosan and to establish whether or not it is the same as that of inulin.

In the present investigation the properties of this fructosan and its acetylated and methylated derivatives were studied. They were found to be similar to those of inulin. The polysaccharide was soluble in hot water, had a specific rotation $[\alpha]\text{D}\ -35°$, and gave the same X-ray diffraction pattern as inulin (Table II). The triacetyl derivative had a specific rotation in chloroform $[\alpha]\text{D}\ -33°$; the trimethyl derivative had a specific rotation in the same solvent $[\alpha]\text{D}\ -49.8°$. These results are in agreement with the data of Haworth, *et al.*,[5,6] for inulin. Complete methylation and subsequent hydrolysis of the fructosan produced 3,4,6-trimethylfructofuranose as the main product. The high methoxyl content of the first two fractions (Table I, fractions I and II) indicated the probable presence of tetramethylfructose. Inasmuch as the quantity of this tetramethyl derivative was too small to make its isolation practicable, the less accurate method of determining this product on the basis of the methoxyl content of the distilled fractions was employed. The tetramethylfructose thus determined amounted to 3.0%, corresponding to a chain length of approximately 38 fructose units. Considering the limitations of the method, the result is in satisfactory agreement with that of Haworth, *et al.*,[6] who found a chain length of 30 fructose units for inulin by the end group method.

The residue from the distillation, and also the last fraction of the distillate (Table I, fraction V), reduced Fehling solution. A similar observation was made by Haworth, *et al.*,[6] in the case of distillation of the hydrolysis products of methylated inulin. The residue in the still, when further distilled under diminished pressure, also reduced Fehling solution.

When the trimethylfructofuranose was treated with phenylhydrazine an osazone was obtained, which retained the three methyl groups originally present in the methylated sugar, showing that positions 1 and 2 in the latter could not have been occupied by methyl groups. It must be assumed, therefore, that the fructofuranose residues in the fructosan are linked through positions 1 and 2.

Evidence that the fructose residues of the guayule fructosan exist as fructofuranose was obtained from oxidation of the polysaccharide with sodium periodate. In a fructosan consisting of fructofuranose residues glycosidically united through positions 1 and 2, each residue in the chain possesses a pair of free hydroxyls on contiguous carbon atoms 3 and 4, which can be oxidized with periodate consuming one mole of the latter. A fructosan with a fructopyranose configuration would have three free adjacent hydroxyls on carbon atoms 3, 4 and 5, and when similarly oxidized would consume two moles of periodate and form one mole of formic acid. Since in the oxidation of the guayule fructosan only one mole of the oxidant was used for each fructose anhydride residue and since no formic acid was formed, it is concluded that the residue in the fructosan possesses the fructofuranose configuration.

In connection with the demonstration of the occurrence of inulin in guayule, it may be noted that inulin also occurs in a number of members of Compositae, such as dahlia, Jerusalem artichoke, chicory, dandelion, and the Russian dandelion *Taraxacum kok-saghyz*, which is also a rubber-producing plant.[7] Also fructose polysaccharides other than inulin are wide-spread in many grasses, barley and other cereals.[8] The chief distinction between inulin and other fructosans resides in their molecular constitutions. Haworth, *et al.*,[5,6] showed that, whereas the fructose units in inulin are glycosidically linked through the first and second carbon atoms, the linkage in the other fructosans usually occurs through the second and sixth carbon atoms of the contiguous fructose units.[9,10] Schlubach, *et al.*,[11] also report the existence of a fructosan, irisin, in the rhizomes of *Iris* species, which probably consists of a difructofuranose polymer having the junction between the second and fourth carbon atoms.

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Experimental

Preparation of Fructosan.—One hundred grams of coarsely ground guayule tissue was extracted in a Soxhlet apparatus with 80% ethanol until the percolate was colorless. The tissue was air dried and extracted with five 300-ml. portions of water at the temperature of the boiling water-bath. The combined dark-water extract was then treated with an excess of neutral lead acetate, centrifuged, and deleaded with hydrogen sulfide. The solution was adjusted to *p*H 6, decolorized with charcoal at about 80° and concentrated under reduced pressure to 100 ml. Addition of 3 volumes of acetone to the concentrate caused the formation of a white precipitate, which was allowed to flocculate in an ice-bath. After centrifuging the precipitate, it was taken up in hot water, further decolorized with charcoal, and reprecipitated from aqueous solution with acetone four successive times. The final product was dried *in vacuo* at 60°.

Properties of the Fructosan.—The white powdered fructosan was practically insoluble in water at room temperature, but dissolved readily in hot water, forming a clear solution, which did not flocculate on standing. It had an ash content of 1.2%, it did not reduce Fehling solution but gave a positive Seliwanoff reaction. The specific rotation (*c*, 2) of the fructosan in water, calculated on an ash-free basis, was $[\alpha]_D$ −35°. The fructosan hydrolyzed completely with 0.1 *N* hydrochloric acid at 100° in thirty minutes. The reducing value, determined on the neutralized hydrolyzate by oxidation with ferricyanide and titration with ceric sulfate,[12] was 93%, calculated as glucose. When the hydrolyzed solution was analyzed for fructose by Roe's method[13] a value of 95% for ketose sugar was obtained.

The intrinsic viscosity,[14] which is the ratio η_{sp}/C (η_{sp} = specific viscosity, C = concentration) as C approaches zero, was η_i 0.057; inulin under the same conditions had an intrinsic viscosity of η_i 0.066. The viscosities of the materials may be regarded as not significantly different and the two carbohydrates may therefore be considered as having approximately the same molecular shape.

Acetylation.—Eighteen grams of the fructosan was dissolved in a small amount of hot water, cooled to room temperature and reprecipitated by the addition of two volumes of ethanol. The precipitate was collected on a Büchner funnel, washed with ethanol, and the slightly moist polysaccharide transferred to a flask and stirred mechanically with 180 ml. of pyridine for twelve hours. One hundred and eighty ml. of acetic anhydride was gradually added with stirring of the solution in the course of an hour and the mixture continued to be stirred for five more hours. The solution was then slowly poured into an excess of cold water and left standing for several hours. The precipitate was filtered, washed with water until free of pyridine and acetic acid and dried *in vacuo* at 40°. Further purification of the product was effected by dissolving in ten times its weight of hot methanol; the solution was filtered hot, and the filtrate was heated to redissolve the prematurely separated product, which was again allowed to separate slowly on cooling. A yield of 25 g. was obtained. The acetylated fructosan was soluble in chloroform and acetone. Its specific rotation (*c*, 2) in chloroform was $[\alpha]_D$ −33°. The acetyl content, $COCH_3$, 44.3% (calculated $COCH_3$ content for the triacetate, $(C_6H_7O_5(CH_3CO)_3)_n$, 44.8%).

Methylation.—Twenty grams of the acetylated fructosan was simultaneously deacetylated and methylated according to the method of Haworth and Streight.[15] The methylated product was dissolved in chloroform and the solution filtered, evaporated to a small volume and reprecipitated by the addition of petroleum ether. A yield of 10.5 g. of methylated product was obtained (74% of the theoretical). The specific rotation (*c*, 2) of the methylated fructosan in chloroform was $[\alpha]_D$ −51°. Its methoxyl content, OCH_3, was 44.7% (calculated for $(C_6H_7O_2(OCH_3)_3)_n$, 45.6%).

Hydrolysis of Methylated Fructosan.—A sample of 8 g. of the trimethyl fructosan was treated with 300 ml. of methanol and 100 ml. of water containing 4 g. of oxalic acid. The mixture was kept at 80° until the rotation of the solution became constant (twenty-four hours). The solution was neutralized with calcium carbonate, filtered, evaporated to a sirup under diminished pressure, extracted with chloroform and dried with anhydrous sodium sulfate. After removal of the chloroform under diminished pressure, 100 ml. of methanol, containing 0.25% of dry hydrogen chloride, was added to the sirup and allowed to remain at room temperature for forty-eight hours. The solution was then neutralized with barium carbonate, concentrated under diminished pressure at 35° in the presence of barium carbonate and the product extracted with ether. After evaporation of the ether, the yield of methylfructosides was 7.95 g. The methylfructosides were fractionally distilled at 10^{-4} mm. pressure into fractions shown in Table I.

TABLE I

HYDROLYSIS PRODUCTS OF METHYLATED GUAYULE FRUCTOSAN

Fraction	Temp., °C.	Weight, g.	$[\alpha]_D$ in water	$n^{18}D$	OCH_3
I	100	0.877	54.3	1.4502	54.3
II	100	0.840	80.4	1.4521	53.3
III	120–130	1.290	23.7	1.4534	52.4
IV	130–140	2.370	10.5	1.4596	50.3
V	140–170	1.588	14.1	1.4611	41.2
		6.965			

The distilled fractions, except for fraction V and the residue left in the flask (0.99 g.), did not reduce Fehling solution. The five fractions were analyzed for their methoxyl contents. Fraction III, having a methoxyl content of 52.4%, is obviousy trimethylmethylfructoside (calculated OCH_3 content for $C_6H_8O_2(OCH_3)_4$, 52.6%). Fraction II also appears to be chiefly trimethylmethylfructoside. Calculated on the basis of a 62.0% theoretical methoxyl content for tetramethylmethylfructoside, fraction I with a methoxyl content of 54.3% and fraction II with that of 53.3% would contain 18% and 7% of tetramethymethylfructoside, respectively. Fractions I and II would thus contain a total of 0.217 g. of tetramethylmethylfructoside, which (on the basis of 86% recovery) is equivalent to 0.239 g. of tetramethylfructose, or to 3% of the weight of hydrolyzed methylated polysaccharide. Such proportion of tetramethylfructose corresponds to a chain length of 38 fructose units. Inasmuch as this chain length is based on analysis of the methoxyl content and not on the actual separation of the tetramethylfructose, this value can be only considered as a rough approximation.

Preparation of a Phenylosazone from Trimethylfructose.—A 0.6-g. sample of fraction III was hydrolyzed with 0.1 *N* hydrochloric acid, neutralized, and treated with phenylhydrazine in dilute acetic acid and heated for half an hour at 100°. The orange-red sirup that separated was washed with water, and, after the addition of dilute ethanol, it set to a hard semicrystalline mass. The properties of this osazone agreed with those of the hydrated osazone prepared by Haworth and Learner[8] from the hydrolysis products of methylated inulin.

Anal. Calcd. for $C_{21}H_{28}O_4N_4 \cdot H_2O$: C, 60.3; N, 13.4; OCH_3, 22.2. Found: C, 60.7; N, 12.8; OCH_3, 23.0.

Oxidation of the Fructosan with Sodium Periodate.—A dry 0.25-g. sample of the polysaccharide was dissolved in 10 ml. of hot water. After cooling to 25°, 5 ml. of 0.5 *M* sodium periodate was added and diluted to 25 ml. The initial specific rotation of the fructosan $[\alpha]_D$ −35° grad-

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[12] W. Z. Hassid, *Ind. Eng. Chem., Anal. Ed.*, **9**, 228 (1937).

[13] J. H. Roe, *J. Biol. Chem.*, **107**, 15 (1934).

[14] E. O. Kraemer, *Ind. Eng. Chem.*, **30**, 1200 (1938).

[15] W. N. Haworth and H. R. L. Streight, *Helv. Chim. Acta*, **15**, 609 (1932).

ually diminished until after three hours it came to a constant rotation of $[\alpha]_D$ −83°. A similar sample of commercial inulin oxidized with sodium periodate in the same manner reached the same constant rotation within a similar period of time. The absence of formic acid was shown by the fact that no titratable acidity was produced at the end of the reaction. The amount of periodate consumed in the reaction[16] was estimated by treating 5-ml. samples with an excess of 0.1 *N* sodium arsenite in the presence of potassium iodide and sodium bicarbonate buffer and back titrating with 0.1 *N* iodine. The results showed that 1.0 and 1.03 moles of the oxidant were used for each fructose anhydride unit of the guayule fructosan and commercial inulin, respectively.

X-Ray Diffraction Data.—The sample of guayule fructosan that was used for the chemical studies was subjected to an X-ray diffraction examination by the powder method. The material was packed into a thin-walled glass tube having an internal diameter of about 0.6 mm., and exposed to molybdenum radiation filtered through a zirconium oxide screen. A pattern was obtained which contained five distinct lines on a considerably darkened background, indicating that the material was partially crystalline, but that much of it existed in random orientation capable of producing only amorphous scattering.

Apparently there are no crystal structure data for inulin available in the literature with which the above lines can be compared. Reference patterns for comparison were obtained from three authentic samples of inulin: (1) inulin from dahlia prepared by one of us (W. L. McR.), (2) commercial inulin "c. p.," Pfanstiehl and (3) inulin "pure" Pfanstiehl. All three inulin samples gave patterns identical with that obtained from the guayule fructosan, except that one of the weaker lines was lacking in the pattern from the last inulin sample.

The interplanar spacings found in the X-ray diffraction produced by guayule fructosan and by the three inulin samples are given in Table II. The close agreement between corresponding spacings in both magnitude and intensity of reflections afford independent confirmation of the conclusion, based on chemical evidence, that the fructosan from guayule has a structure indistinguishable from that of inulin.

Table II

X-Ray Diffraction Patterns of Guayule Fructosan and Inulin

Fructosan from guayule		Inulin from dahlia		Inulin "C. P." Pfanstiehl		Inulin "pure" Pfanstiehl	
d	*I*	*d*	*I*	*d*	*I*	*d*	*I*
7.3	Strong	7.3	Strong	7.3	Strong	7.3	Strong
5.6	Weak	5.6	Weak	5.6	Weak	...	...
5.0	Weak	5.0	Weak	5.0	Weak	5.0	Weak
4.1	Strong	4.1	Strong	4.1	Strong	4.1	Strong
3.7	Weak	3.62	Weak	3.62	Weak	3.62	Weak

d is interplanar distances expressed in ångström units. *I* is intensity of reflections.

The authors are indebted to Dr. H. S. Owens for the determination of viscosities of the inulins.

Summary

1. The properties and the chemical structure of the fructosan isolated from the guayule plant have been studied.

2. Methylation and hydrolysis of the polysaccharide produced chiefly 3,4,6-trimethylfructofuranose, showing that the fructose units are combined through positions 2 and 1. Data obtained from the oxidation of the fructosan with sodium periodate, show that the fructose units possess the furanose configuration.

3. The X-ray diffraction pattern of guayule fructosan is identical with that of inulin, providing additional support to the chemical evidence.

4. It is concluded that the guayule fructosan is identical with inulin.

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[16] E. L. Jackson, "Organic Reactions," edited by R. Adams, John Wiley and Sons, Inc., New York, N. Y., 1944, Vol. II, pp. 341–375.

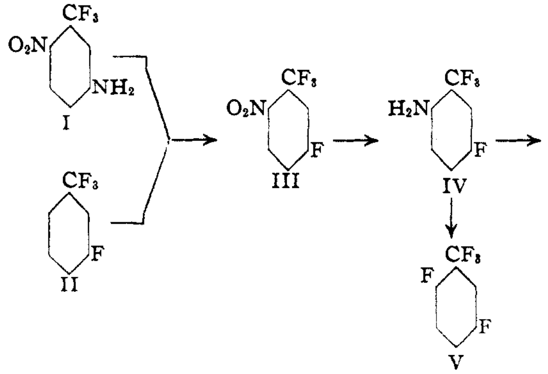
[Contribution from the Geochemical Section of the Illinois State Geological Survey]

Aromatic Fluorine Compounds. I. The Synthesis of 2,5- and 3,5-Difluorobenzotrifluorides[1]

By G. C. Finger[2] and F. H. Reed[3]

As part of a study in this Laboratory, of the synthesis and properties of aromatic fluorine compounds, 2,5- and 3,5-difluorobenzotrifluoride have been synthesized.

A Schiemann reaction[4] on 2-nitro-5-aminobenzotrifluoride (I) gave 2-nitro-5-fluorobenzotrifluoride (III), but this compound was found to be more conveniently prepared by the nitration of 3-fluorobenzotrifluoride (II); the structure of the nitro compound in this synthesis was established by the identity of the acetyl derivatives at (IV). An iron reduction[5] of III gave a quantita-



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[1] Presented before the Organic Division at the 107th Meeting of the American Chemical Society, Cleveland, Ohio, April 5, 1944. Published with the permission of the Chief of the Illinois State Geological Survey.

[2] Chemist.

[3] Chief Chemist.

[4] Schiemann and Winkelmüller, *Ber.*, **60**, 1186 (1927).

[5] Lukasevich and Vorshilova, *Compt. rend. acad. sci. U. R. S. S.*, **2**, 344 (1935).