AN ABSTRACT OF THE THESIS OF

Keane Paul Nimick for the Ph. D. in Chemistry
(Name) (Degree) (Major)

Date Thesis presented- November 27, 1942

Title-- The Constitution of A New Fructosan Isolated From Yucca Mohavensis, Sarg.

Abstract Approved:---
(Major Professor)

A new carbohydrate has been isolated from the stem of the yucca plant, and has been designated as yuccan.

Yuccan comprises about 15% of the stem and is made up entirely of fructose anhydride units. It has a molecular weight of about 1660, which corresponds to 10 fructose units. Its empirical formula is $(C_6H_{10}O_5)_{10}$.

The above conclusions were arrived at from the following information:

1. Carbon and hydrogen values on yuccan, yuccan acetate, and the methyl ether derivative.

2. The acetyl content and the methoxyl content of the corresponding derivatives.

3. The molecular weight of yuccan and of yuccan acetate.

4. The quantitative increase in weight when yuccan was acetylated.

5. Fructose determinations on the hydrolyzed yuccan:

a. By optical rotation

b. By the reduction method (Schaffer-Hartmann)

c. By direct fructose determination (Friedman and Klaas).

Yuccan is a non- reducing carbohydrate (boiling fehling's solution). It is hygroscopic, tasteless and very soluble in water. It forms addition products with both barium hydroxide and with ethyl alcohol. It is extremely labile in acid solutions.

A new fructosan was isolated from another yucca stem. This compound has a molecular weight equivalent to 20 fructose units. This new fructosan seems to decompose into two 10 unit fragments when the acetate of the compound is melted in camphor. (Rast determination of molecular weight).

THE CONSTITUTION OF A NEW FRUCTOSAN ISOLATED FROM YUCCA MOHAVENSIS, SARG.

by

KEENE PAUL DIMICK

A THESIS

submitted to the

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

DOCTOR OF PHILOSOPHY

June 1943

APPROVED:

[redacted]

Associate Professor of Chemistry

In Charge of Major

[redacted]

Head of Department of Chemistry

[redacted]

Chairman of School Graduate Committee

[redacted]

Chairman of State College Graduate Council

TABLE OF CONTENTS

I.	PURPOSE	1
II.	INTRODUCTION.	3
	Structure of Fructosans	5
	Yuccan	6
III.	EXPERIMENTAL.	9
	Isolation of Yuccan	9
	Properties of Yuccan.	11
	Molecular Weight of Yuccan.	12
	Hydrolysis of Yuccan.	13
	Preparation of the Barium Salt.	14
	Preparation of the Acetate.	15
	Methylation of the Fructosan.	17
	Fructosan From Another Yucca Stem	18
	A Comparison of the Two Yucca Stems	20
IV.	SUMMARY	21
V.	CONCLUSION.	22
VI.	BIBLIOGRAPHY.	23

ACKNOWLEDGMENT

I wish to express my appreciation to Dr. Bert E. Christensen for his help, advice and encouragement in this work.

THE CONSTITUTION OF A NEW FRUCTOSAN ISOLATED FROM YUCCA MOHAVENSIS, SARG.

I PURPOSE

This work was undertaken as an outgrowth of a Ph. D. thesis done by Dr. Benjamin Stark (24) on the plant Yucca mohavensis, Sarg. In his thesis he was mainly concerned with the saponin content of this plant. He gave in his work the approximate analysis of the plant, and the references to work already done on the yuccas.

In his analysis he observed that the reducing sugar content of an aqueous extract of the stem was about 4% (based on dry weight), and after acid hydrolysis or enzymatic hydrolysis, the reducing sugar content increased to some 45%. He ascribed this increase to be due to sucrose.

Preliminary experiments carried out by the writer proved this not to be the case, but that the increase in reducing sugars on acid hydrolysis was due to the presence of a polyfructosan which--like sucrose--showed no reducing properties. A literature survey showed that many compounds of this general type occured widely spread in nature--several having already been characterized. Inulin is the

best known example of such a compound, and corresponds to starch which is built up of glucose units.

It was thought to be worth while to extend this study, and to characterize this compound as fully as possible.

A short report of the existance of this compound, along with some of its properties, has been published in the J. Am. Chem. Soc. (5).

II INTRODUCTION

The fructosans, also known as levulosans, anhydrofructoses, poly-fructosans, fructose anhydrides, and levans, have been known since the middle of the nineteenth century. They are compounds formed from the polymerizations of fructose, and are composed of from 2 to 30 units of fructose. Their carbon and hydrogen analysis agree with the general formula $(C_6H_{10}O_5)_X$, and are therefore complete anhydrides. That is, every molecule of fructose present has lost one molecule of water in the condensation. As mentioned, inulin is the best known example of such a compound. Haworth and coworkers (6,10) have shown this compound to be a polymerized anhydro fructofuranose, the linkages being at positions 1 and 2 of the fructose chain. See Figure 1.

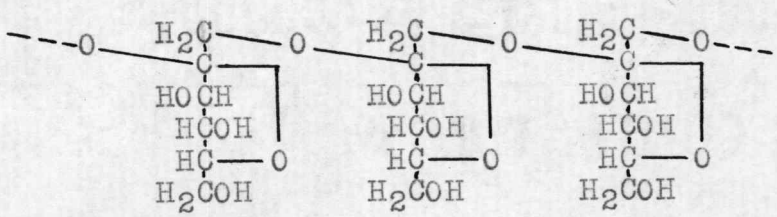


Figure 1

Pasteur (13) is possibly the first worker to isolate and analyze one of these substances. He isolated it from a carbohydrate culture of a slime producing bacteria and assigned to it the formula $C_{12}H_{20}O_{10}$.

The presence of these compounds is not confined to any one group of plants. The fructosans have been reported to occur in cereals, bulbs, grains, leaves, fruits, trees and grasses. They have also been produced by enzymatic synthesis (2,9,25).

These compounds are thought to serve as food material for the plant. Colin and Belval (3) while investigating the changes occuring in the stem of the wheat plant, observed that after the plant had formed spikes, that the stem contained an alcohol insoluble substance which on acid hydrolysis yielded fructose. Immature wheat grains contained 6% fructosan and an equal amount of starch. As the grain approaches maturity, the starch content increases to 50-60%, and the fructose content drops to about 0.4%. This is in agreement with the findings of other workers. Schlubach and Koenig (2 0) found the fructosan "Graminin" in the unripe rye grain. According to these authors the ripe grain contains only traces of this material.

The formation of these compounds in a plant seems to arrive from the glucose molecule which is converted to the fructose molecule and then condensed in the anhydro-

fructo-furanose form. This possible mechanism has not as yet been proven.

Bacteria are known to produce these compounds, and the enzyme responsible for the production of one such fructosan, "levan" has been isolated from Bacillus mesentaricus by Hibbert and coworkers (9,12). It has been repeatedly shown that the bacterial production of these compounds necessitates the presence of fructose in the furanose form, such as exists in the sucrose molecule. See Figure 2. A mixture of glucose and fructose as a medium will not give rise to the fructosans.

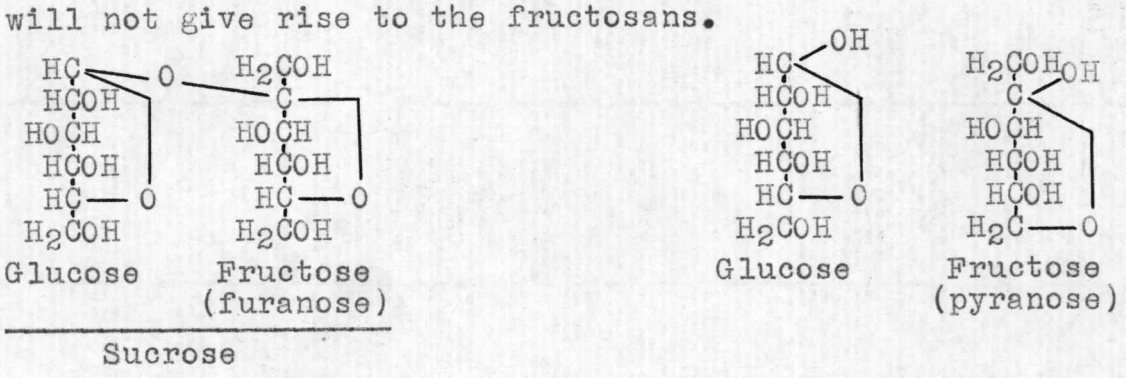


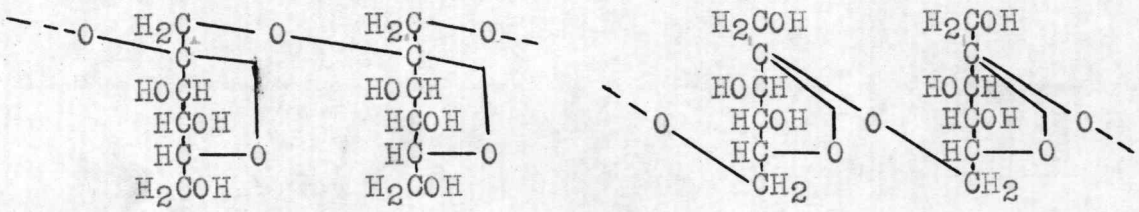
Figure 2

Structure of Fructosans

Hans Schlubach and his coworkers (14-23) have been outstanding in this particular field of carbohydrate chemistry. They have published a great number of papers on their findings, and have summarized their work in two articles (16,17).

Schlubach contends that there are two main classes of these compounds; the "inulin" group and the "Phlein" group. These groups differ in the manner in which the fructose molecules are linked together. In the "inulin" group, which consists of the fructosans, asparagosin (15), sinistrin (22), and irisin (19), the fructose molecules are connected through the 1 and 2 positions.

In the "Phlein" group (16), which consists of secalin (14) and levan (9,12), the fructose molecules are connected through the 2 and 6 positions. See Figure 3.



Inulin Type Phlein Type

Figure 3

The structures of these compounds are still not completely understood, but as the methods and tools for attacking such a problem are extended, new light will be thrown on the structure of these complex carbohydrates.

Yuccan

The compound which we have isolated from the yucca

plant appears different from any compound of this type reported in the literature. We have named this compound "yuccan" in agreement with the plan suggested by Hibbert (12), thus keeping it analogous to type compounds--araban, xylan, levan, dextran and etc.

In general, the properties of yuccan are similar to the properties of other fructosans. Thus, it is a hygroscopic, white, tasteless powder with no crystalline properties. It is soluble in water, pyridine and 70% alcohol and insoluble in all general organic solvents. It forms--as do the other fructosans--addition products with barium hydroxide and ethyl alcohol. It does not form an addition product with calcium hydroxide. It will not reduce boiling fehling's solution. It is hydrolyzed very rapidly to fructose by boiling 0.03 N acid and much more slowly by the enzyme invertase.

In comparing the physical properties of yuccan with the physical properties of other fructosans reported in the literature, it was found to compare most closely with the fructosan, "graminin" isolated by Schlubach and Koenig (20) from immature rye kernels. It has the same general properties and is composed of ten fructose units. The optical rotation of yuccan and of yuccan acetate, however, serve to differentiate it from graminin. A comparison of

the properties of graminin and yuccan is given in Table I.

TABLE I

	Yuccan	Graminin (20)
Melting point	170-185°C.	---
$[\alpha]_D^{25}$	-30.0°	-40.0°
Molecular weight*	1660	1494
Solubility in water	very soluble	very soluble
Solubility in piridine	soluble	soluble
Exposed to air	white hygroscopic powder	white hygroscopic powder
Addition product with ethanol	positive	positive
% Ba in the Barium salt	22.4-23.5	---
Max. degree of hydrolysis	96% (#)	91% (')
Reduction with Fehling's solution	negative	negative
Fructose determination	100%	---
Taste	Tasteless	Tasteless
$[\alpha]_D^{25}$ of the acetate in chloroform	-14.6°	-7.2°
Melting point acetate	70-85°C.	---
Mol. Wt. of acetate	2941 (")	2736 (-)

(*) Determined cryoscopically in water.
(#) Determined by the Schaffer-Hartmann method.
(') Determined by Bertrand's copper method.
(") Determined cryoscopically in camphor.
(-) Determined cryoscopically in benzene.

III EXPERIMENTAL

Isolation of Yuccan

Dr. Benjamin Stark (24) in 1940 had subjected a sample of yucca stem * to the following treatment:

The stem was stripped of its bark, cut into small pieces, dried at 60°C. and then ground in a small burr mill. The dry meal thus obtained was exhaustively extracted with petroleum ether, ether, absolute alcohol and 70% alcohol, in the order given. The extract from the 70% alcohol, constituting 40% of the dry stem was used in this study.

When a sample of this extract was treated with hot concentrated barium hydroxide, and a small amount of alcohol was added, a copius white precipitate immediately formed.

The following procedure was then set up in order to purify and to test the homogeneity of the material from the 70% alcoholic extract:

Twenty grams of the extract were dissolved in water and the solution made up to 100 ml. To this was added an equal volume of hot concentrated barium hydroxide suspension containing 20 g. of the hydrated base. When 200 ml.

---

* Samples furnished by Truesdail Laboratories, Los Angeles, California.

of 95% ethanol were added to this mixture a heavy precipitate settled out. After cooling, the precipitate was removed and washed with 10% ethanol.

This material was suspended in 100 ml. of water, the barium removed with carbon dioxide, and the filtrate decolorized with 1 g. of charcoal. The compound was again precipitated with barium hydroxide and the treatment repeated.

To remove the last traces of barium, the solution was treated with a small amount of dilute sulfuric acid until one drop would cause no further turbidity. By increasing the alcohol content of the aqueous solution, the product was fractionally precipitated into three gummy fractions. It is important to precipitate the compound with alcohol in order to separate it from the small excess of H_2SO_4. If the solution is left even slightly acidic, the compound will completely hydrolyze to fructose.

The precipitate of each fraction was dissolved in a minimum of water and placed in a vacuum oven at 60°C. When the solutions reached a syrupy stage, ethanol was added, causing the formation of a gummy mass which dried to white powder. These fractions all behaved similarly on the melting point block, had the same molecular weight, and the same optical rotation. Yield 42%. See Table II.

TABLE II

	Fraction I	Fraction II	Fraction III
Weight gm.	1.84	3.24	3.37
% alcohol	76%	81%	88%
M. P.	180-190	180-190	180-190
Mol. weight	1690	1660	1630
$\alpha]_D^{25}$	31.0°	30.0°	30.0°

(The molecular weights and optical rotations are corrected for alcohol and water content.)

Properties of Yuccan

Yuccan is a tasteless, white powder which contains 7-8% moisture when dried under average conditions of humidity. This was determined by heating a sample at 60-70°C. in a vacuum oven until there was no further loss in weight. This required about 48 hours.

It is very soluble in water, giving a clear solution. It is insoluble in most organic solvents, with the exception of pyridine. Yuccan gives a negative test in boiling Fehling's solution, and gives no coloration with iodine. It is readily hydrolyzed by dilute acids to give fructose, as indicated by the Seliwanoff test. No hydrolysis was observed when it was treated with a heavy suspension of

Fleischman's yeast for 20 minutes at 30°C. Sucrose under similar conditions was hydrolyzed in a matter of 10 seconds.

Molecular Weight of Yuccan

Initial molecular weight measurements gave a value of 700 (cryoscopic - H_2O) for the fructosan and 2,800 (cryoscopic - camphor) for its acetate. Since these values were not in harmony, it appeared that yuccan had formed an addition product with ethyl alcohol. This was confirmed by chemical analysis according to the method of Friedman and Klaas (7). The molecular weight when corrected for the alcohol content (4.0%), was 1660.

This method is essentially the distillation of the alcohol into standard potassium permanganate, oxidizing the alcohol by heating, adding potassium iodide to the excess permanganate, and titrating the liberated iodine with standard potassium thiosulfate. The difference between the amount of thiosulfate used in a sample with alcohol in it and a blank represents the amount of alcohol present.

The alcohol was found to be easily liberated from yuccan by evaporation of its aqueous solution. Samples of the fructosan from this treatment gave a molecular weight of 1720. Since the compound was labile, it was necessary to carry out all evaporations in a vacuum oven, at 60°C. The dry alcohol-fructosan complex was quite stable. After heating in a vacuum oven at 70°C. for 24

hours, the compound still contained 4% ethanol. Further heating at 120°C. for 24 hours in an oven reduced the alcohol content to 1.2%.

Analysis:

	Calculated $(C_6H_{10}O_5)_{10}$	Found
C	44.4	43.9
H	6.23	6.29
Mol. Wt.	1621	1660

Hydrolysis of Yuccan

In order to obtain optimum conditions for hydrolysis the following was conducted:

Twenty milligrams of yuccan were made up to 100 ml. of solution. Aliquots containing 0.4 mg. of the fructosan were placed in tubes and made 0.03 N. with H_2SO_4. These tubes were heated at 100°C. for definite intervals of time, and the amount of reduction then measured by the Schaffer-Hartman method. Maximum hydrolysis (96%) was found to occur in 20 minutes while longer boiling decreased the reduction value. In 60 minutes the reduction value was 88%.

Fructose determinations of the hydrolysate using the method of Corley (4) indicated 100% conversion at the time of maximum reduction. Measurements of the optical

rotation of the hydrolysate gave a value of 91% of the fructose calculated to be present. Comparable runs with both sucrose and fructose, undergoing the same treatment gave values of 93% and 94% of the theoretical.

Glucuronic acid was suspected as one of the hydrolysis products on the basis of carbon and hydrogen values, and other considerations (8). Determinations of the acid content, after hydrolysis with standard acid, indicated no formation of acidic compounds as a result of hydrolysis. This was determined by back titration with standard base.

A preparation of the osazone of the hydrolysate disclosed the presence of only glucosazone. M.P. 205-206°C.

Preparation of the Barium Salt

The barium salt was prepared free from barium carbonate in the following manner:

Pure fructosan was dissolved in a small amount of water and an excess of saturated barium hydroxide was added. The slightly turbid solution was filtered by suction, taking precaution against the passage of air through the filter. Alcohol was added to the now clear aqueous filtrate, and the snow-white granular precipitate was filtered immediately by suction. The precipitate was washed first with 50% ethanol, and finally with 95% ethanol.

The barium salt was then allowed to air dry and was finally dried completely in a vacuum oven at 50°C.

Two different samples prepared in like manner contained 22.4% and 23.5% barium. Assuming 3 BaO units per molecule the molecular weight of the fructosan appears to be 1500 and 1600 respectively. This is in fair agreement with the values determined cryoscopically in water. The barium salt of the fructosan is soluble in water.

Preparation of the Acetate

The acetylation was carried out by slightly modifying the method of Haworth (11). Three grams of the dried fructosan were added to 35 ml. of pyridine and shaken at 30°C. until dissolved. Thirty ml. of acetic anhydride were added slowly with stirring. The white precipitate which formed was easily redissolved. The mixture was allowed to stand at room temperature for eighteen hours. The straw colored mixture was then poured into 500 ml. of ice water. The white precipitate was filtered, washed, dried over phosphorous pentoxide, and again acetylated.

Two experiments were attempted using 1 and 3 gram samples respectively. The weight of the products obtained from the esterification indicated 30 free hydroxal groups per molecule. Yields calculated on this basis were 101.6% and 100.2%.

Acetyl determinations were performed using a modification of Armstrong and Arup's method (21). About thirty mg. of the acetate were dissolved in 5 ml. of alcohol and 7.5 ml. of 0.1 N sodium hydroxide (CO_2 free) were added. After several hours of shaking at room temperature, the excess base was titrated with 0.1 N sulfuric acid. The results obtained using this procedure are given in TableIII.

TABLE III

Compound	No. of Detns.	Time of Shaking	Theoretical % CH_3CO	Found %CH_3CO	Average Deviation
Glucose pentacetate	6	5 hrs.	55.1	57.0	0.2
Glucose	1	3 hrs.	0.0	1.2	---
Glucose	1	5 hrs.	0.0	2.2	---
Sucrose octacetate	4	4-8 hrs.	50.7	50.6	0.1
Sucrose	4	3-24 hrs.	0.0	0.0	---
Yuccan acetate	5	4-15 hrs.	45.9	45.4	0.2
Yuccan	3	3-5 hrs.	0.0	0.0	---

Analysis:

	Calculated $(C_6H_{10}O_5)_{10}(CH_2CO-)_{30}$	Found
C	50.0	49.8
H	5.59	5.61
(CH_3CO-)	44.8	45.4
Mol. Wt.	2881	2790

Methylation of the Fructosan

The methylation was carried out according to the method of Haworth and Percival (11). 4.17 g. were dissolved in 50 ml. of acetone and heated at 55°C with 26 ml. of methyl sulfate and 72 ml. of 30% sodium hydroxide for 90 min. with vigorous stirring. Both reagents were added simultaneously in 10% portions at 10 minute intervals. The acetone was removed in vacuo. The remaining solution was extracted with four 25 ml. portions of chloroform, and the extract then evaporated in vacuo. Fifty ml. of acetone were added to the partially methylated chloroform extract. The methylation was repeated five times before the methoxyl content became constant.

To obtain the pure methylated derivative, the final chloroform extract was washed repeatedly with distilled water and dried over anhydrous sodium sulfate. The salt was then filtered off and the chloroform removed by evaporation in vacuo. The product was a heavy yellow oil which was soluble in ether, chloroform, and alcohol. Yield 1.6 g.

Analysis:

	Calculated $(C_6H_{10}O_5)_{10}(CH_2)_{30}$	Found
C	52.9	52.6
H	7.88	8.01
CH_3O-	45.5	45.6

Fructosan From Another Yucca Stem

In order to compare the fructosan obtained from Stark's yucca stem another sample was obtained from the desert in Southern California. This was subjected to the following treatment:

The bark was stripped from the stem and it was sliced into pieces about 1/4 inch in thickness. The pieces were weighed wet and were then dried in a vacuum oven at 70-80°C. The moisure content was found to be 72.9%.

The dried material was then ground in a burr mill and the meal was extracted with boiling water. The slightly yellow aqueous extract was decolorized with charcoal, filtered and evaporated in vacuo to a syrup. The syrup was treated with a large amount of alcohol which precipitated a gummy brown material. This was dried in a vacuum oven. The tan powder obtained represented 58% by weight of the original dry meal. It was further purified by the barium hydroxide treatment. Molecular weight and optical rotation were determined on the now almost white fructosan as well as the molecular weight of the prepared fructosan acetate. See Table IV.

The molecular weight of the fructosan was determined by the cryoscopic method in water. The molecular weight of the acetate was determined by the Rast method in

TABLE IV

Mol. Wt. Fructosan	3150
Calculated $(C_6H_{10}O_5)_{20}$	3244
Mol. Wt. Fructosan Acetate *	4500
Calculated $(C_6H_{10}O_5)_{20}(CH_2CO-)_{60}$	5760
Fructosan $[\alpha]_D^{25}$	-36.0°

camphor. The high temperature necessary to melt the mixture probably caused the Fructosan acetate to decompose into two ten unit fragments. This was substantiated by repeated melting of the mixture. The molecular weight fell from 4500 to 2650 where it remained constant. This value is in good agreement for a 10 unit molecule. See Table V . The molecular weight determinations on Yuccan

TABLE V

No. of times melted	Mol. Wt.
1	4500
2	3600
3	3250
6	2720
9	2650
11	2650

* Value obtained from the first melt.

acetate did not show this variation in molecular weight.

A Comparison of the Two Yucca Stems

The dry meal from both yucca stems was used for the analysis. The two samples will be designated as yucca 1 and yucca 2, referring to Stark's material and to the new yucca stem.

Five gm. of the dry meal was suspended in 250 ml. of distilled water. This was warmed to 70-80°C. and the mixture was shaken to insure thorough extraction of the fructosan from the pulp. After cooling, the mixture was filtered and 10 ml. of the filtrate was made up to 100 ml. This solution was analyzed for reducing sugars before hydrolysis and after hydrolysis and for fructose after hydrolysis. See Table VI . The water content of both yucca stems was almost the same.

TABLE VI

	"Yucca 1"	"Yucca 2"
Reducing sugar Unhydrolized	3.6%	5.0%
Reducing sugar Hydrolized	59.0%	52.7%
Fructose content	55.0%	52.5%

IV SUMMARY

1. A new fructosan has been isolated from the plant, Yucca mohavensis, Sarg., and has been designated as, "yuccan."

2. The physical properties of this compound have been studied and compared with the fructosan graminin.

3. Analysis of yuccan, its acetyl derivative, and its methyl ether derivative, indicates that it is a compound composed of ten fructose units. These units are connected together in such a manner as to produce a complete anhydride which has the general formula $(C_6H_{10}O_5)_X$.

4. The water content and sugar content of the yucca plant is given, and a comparison is made between two different plants.

5. A second yucca plant yielded a new fructosan which had twice the molecular weight of yuccan. It is therefore composed of 20 fructose units.

V CONCLUSION

This study could be extended by exhaustively methylating yuccan, hydrolizing this derivative, and studying the methylated fructose units. This work, when completed, would place yuccan in one of the two groups suggested by Schlubach.

The compound isolated from the new yucca stem might be further studied in the same manner.

Commercially, the yucca plant could serve as a source of fructose. The sugar content of the wet stem amounts to about 15%, and since the stem weighs between three and four pounds, each stem would yield about 0.5 lbs. of fructose.

VI BIBLIOGRAPHY

1. Armstrong, E. F. and Arup, J. Chem. Soc. 85, 1043, (1904)

2. Challinor, S. W., Haworth, W. N. and Hirts, E. L., J. Chem. Soc., 676 and 1560, (1934)

3. Colin, H. and Belval H., Bull. soc. chim. biol., 7, 155, (1925)

4. Corley, R. C., J. Biol. Chem., 81, 81, (1929)

5. Dimick, K. P. and Christensen, B. E., J. Am. Chem. Soc., 64, 2501, (1942)

6. Drew, H. D. K. and Haworth, W. N., J. Chem. Soc., 2690-2697, (1942)

7. Friedman, T. E. and Klaas, R., J. Biol. Chem., 115, 47, (1936)

8. Gisvold, Ole and Rogers, C. H., The Chemistry of Plant Constituents, Burgess Publishing Press, Minneapolis, Minn., (1939)

9. Harrison, F. C. and Hibbert, H., Can. J. Res., 3, 449, (1930)

10. Haworth, W. N. and Learner, A., J. Chem. Soc., 619-625, (1928)

11. Haworth, W. N. and Percival, E. G. V., J. Chem. Soc., 2277, (1932)

12. Hibbert, H. and Tipson, R. S., Can. J. Res., 4, 221 (1931)

13. Pasteur, L., Bull. soc. chim. 30-31, (1861)

14. Schlubach, H. H. and Bandmann, C., Ann., 540, 285, (1939)

15. Schlubach, H. H. and Boe, H., Ann., 532, 191, (1937)

16. Schlubach, H. H. and Ketu Sinh, Ann., 544, 101, (1940)

17. Schlubach, H. H. and Ketu Sinh, Ann., 544, 111, (1940)

18. Schlubach, H. H. and Knoop, H. and Mao Yin Liu, Ann., 504, 30, (1933)

19. Schlubach H. H. and Knoop, H, Ann., 497, 208, (1932)

20. Schlubach H. H. and Koenig, K., Ann., 514, 182, (1934)

21. Schlubach H. H. and Lendzian, H., Ann., 532, 200, (1937)

22. Schlubach H. H. and Loop, W., Ann., 523, 130, (1936)

23. Schlubach H. H. Peitzner, H., Ann., 530, 120, (1937)

24. Stark, B., Ph. D. Thesis, on file at the Oregon State College Library, Corvallis, Oregon, (1941)

25. Veibel, S., Biochem. J., 32, 1949, (1938)