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Title A Study of the Chemical Constituents of Yucca mohavensis, Sarg.

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The development of methods used in plant analysis is described.

The glycosides are discussed according to their classification, isolation, structure, hydrolytic products, functions, synthesis, and physiological action.

The general properties of the saponins are given with their structure, methods of isolation, and hydrolytic products.

A review was made of the work on other species of Yucca.

The results of the quantitative extraction of Yucca mohavensis with various solvents are given in addition to ash, sugar, and protein determinations.

The presence of a saponin of unknown structure has been established and some of its properties are described. Glucose was identified as one of the hydrolytic products.

A glycoside was isolated from the aqueous alcohol extract. Its molecular weight and percentage composition were determined. From these data the formula $C_{28}H_{46}O_{10}$ was established for the glycoside. The per cent sugar formed on hydrolysis and water of hydration were also determined.

A STUDY OF THE CHEMICAL CONSTITUENTS OF *Yucca mohavensis*, Sarg.

by

JOHN BENJAMIN STARK

A THESIS

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A STUDY OF THE CHEMICAL CONSTITUENTS OF *Yucca mohavensis*, Sar.

INTRODUCTION

The problem of isolating and identifying the constituents of a plant is extremely complex. In order to appreciate the magnitude of the problem let us consider some of the primary obstacles to plant analysis, such as the great variety of compounds which may be present. A list of the more important substances found in plants is presented in Table I. This problem is further complicated by the complexity of the individual components, the changes due to enzyme action, air oxidation, interaction of the plant substances and reactions which may occur during analysis. The simultaneous occurrence of two or more compounds of similar structure is a frequent situation while many times these compounds are present in low concentrations.

The final step in the identification of a given component is dependent to a large degree on the purity of the compound. Due to the nature of the material and the difficulties of purification it is often impossible to adjudge definite criteria of purity for many of the complex substances found in plants. This is especially true of complex amorphous compounds as gums, starches and saponins.

Table I

- Carbohydrates
  - Simple sugars
  - Oligosaccharides
  - Polysaccharides
    - pentosans
    - methyl pentosans
    - hexosans
      - starches
      - cellulose
      - levulosans
      - mannosans
      - galactosans
    - mixed pentosans
      - gums
      - mucilages
      - hemicelluloses
      - pectins
    - mixed hexosans
      - lignocelluloses
      - pectocelluloses
      - adipocelluloses
  - Sugar alcohols
- Nitrogen bases
  - Alkaloids
  - Amines
- Natural glycosides of
  - Phenols
  - Alcohols
  - Aldehydes
  - Acids
  - Oxycumarin
  - Oxyanthraquinone
  - Oxyflavone
  - Sulfur derivatives
  - Anthocyans
  - Triterpenes
  - Miscellaneous
- Fats and Oils
- Alcohols
  - Simple alcohols
  - Phytosterols
- Acids
- Esters
- Terpenes
- Plant pigments
- Resins

Methods of Plant Analysis

The work on plants, previous to the beginning of the eighteenth century, was of little value, due in part to the methods employed and to the interpretation of the results.

The earliest studies of the chemical composition of plants were undertaken in the latter part of the seventeenth century (18). During this period several hundred species of plants were subjected to dry distillation. The information obtained from these experiments gave little indication of the composition of the plants, since dry distillation decomposes the original plant materials and yields nearly the same products in all cases. Material advances in plant analysis began with the introduction of solvent extraction procedures. This method has proven to be of great value in plant studies, but earlier investigators were prone to treat these extracts as chemical individuals rather than as a mixture of the plant constituents. Even today we treat many of our plant products such as fats as individuals, although it is realized that they are mixtures. This is mainly due to the difficulties encountered in separating the different components.

It is probable that Scheele (18) was one of the first investigators to adopt chemical methods in the detection of vegetable materials. He isolated several acids through the

formation of their calcium salts, and subsequent decomposition of these salts with sulfuric acid. The use of the lead ion in the form of lead, or basic lead acetate, is now preferred to the calcium ion since the lead compounds can be completely decomposed with hydrogen sulfide, yielding a precipitate of lead sulfide to be filtered from the solution. An additional reason is that some separations can be made on basis of the formation of an insoluble compound with basic lead acetate but not with lead acetate. Purification by compound formation is not limited to the use of inorganic reagents. Esters, oximes, osazones, and phenylhydrazones are a few examples of organic derivatives that may be formed and then decomposed to yield the original substances.

At present a great number of methods are open to use in plant studies but the choice of procedures depends largely on the type of compound that is desired. Generally a simple extraction is the first step in any analysis. This extraction may be made with a large variety of solvents. Ether is used for the extraction of fat materials while alcohol and water are solvents for glycosides, alkaloids, salts and many other substances. For certain substances it is necessary to use basic or acidic solutions for the extraction.

After these various extracts are obtained one is faced with the problem of their purification as only

occasionally will a relatively pure compound be obtained. In many cases purification can be accomplished by extraction with two immiscible solvents. Steam distillation and fractional distillation under vacuum are valuable in isolating essential oils. Recrystallization or the use of differential solubility is one of the most important tools we have in the purification of organic compounds. It is one of the few existing methods for the separation of many glycosides. One of the most important of the recent advances in purification methods has been the development of chromotographic adsorption (22). This tool is especially valuable in the studies of plant pigments since compounds which show only a dissimilarity in the position of the double bond can be differentially adsorbed.

There are numerous reasons for the difficulties often encountered in applying these methods to the isolation of glycosides from plant materials. The isolation of a glycoside is especially difficult when several closely allied ones are present in low concentrations. The presence of enzymes and oxidation by the air are frequently interfering factors. The amorphous precipitates formed by many of the glycosides make it extremely difficult to remove the foreign matter. We are also limited in the use of compound formation by the decomposition of the glycoside itself or by its neutral character which prohibits the formation of

salts.

The first step in the isolation of a glycoside is generally extraction from the plant with alcohol or water; then purification is attempted by precipitation with ether, recrystallization from various solvents, or the formation of an insoluble compound with lead acetate or basic lead acetate. Ordinary dialysis or electrodialysis may be used to remove the last of the mineral matter if previous methods have not accomplished this. The choice of these methods depends on the characteristics of the glycoside that is under consideration. Later in this paper the results of the application of these methods to a study of a saponin and other glycosides present in Yucca mohavensis will be shown, but first it is desirable to present a short discussion of the glycosides in order that a better idea may be had concerning their composition, structure, occurrence, and function.

DISCUSSION

Glycosides

The glycosides are a class of compounds that yield glucose or some other sugar on hydrolysis with enzymes or dilute acids. Perhaps the best division that can be made of glycosides is to divide them into the holosides or carbohydrates which yield only sugar in hydrolysis and the heterosides which yield some other portion besides the sugar on hydrolysis. The classification is not absolute in the case of the carbohydrates since many of the more complex ones yield slight amounts of other materials besides sugars on hydrolysis. Studies of potato starch have shown that phosphoric acid is an integral part of the molecule. The isolation of fatty acids from rice starch is another case in which a holoside yielded products in addition to sugars on being hydrolyzed.

Before discussing the glycosides in more detail some knowledge of their general structure is valuable. The glycosides may be considered as having the acetal type of linkage. This is shown quite well in the probable formation of sucrose from glucose and fructose (Fig. 1) for the holosides and salicin from saligenin and glucose for the heterosides (Fig. 2).

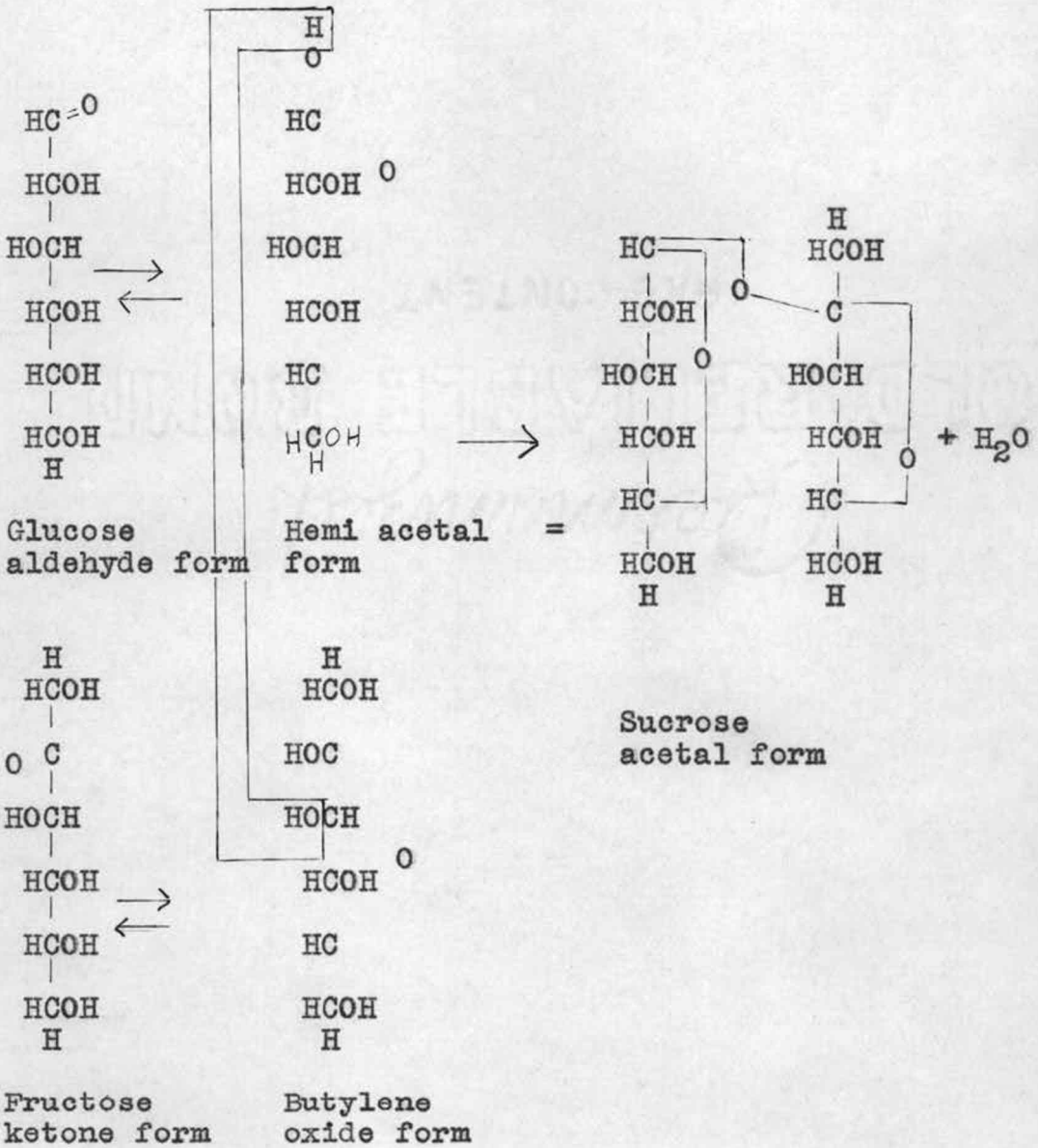
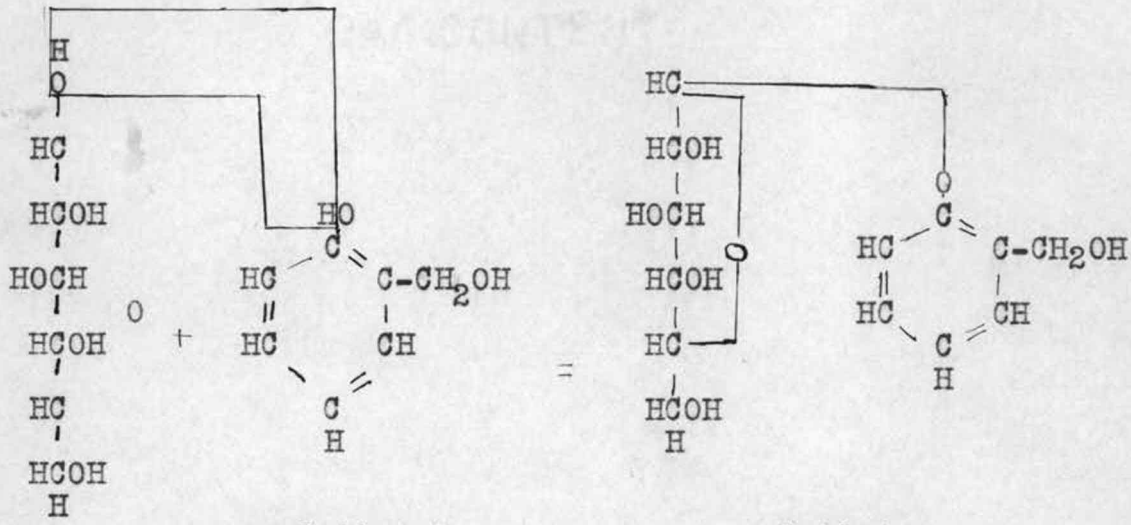

Glucose aldehyde form
Hemi acetal form
Sucrose acetal form
+ H_2O
Fructose ketone form
Butylene oxide form

Fig. 1



Saligenin

Salicin

Glucose
hemi-acetal
form

Fig. 2

Holosides

The holosides may be classified into two main groups. The first group includes the oligosaccharides while the second group consists of the polysaccharides. The oligosaccharides are relatively simple compared with the polysaccharides. They may be formed from two, three or four molecules of the same or different sugars linked together in the same manner as sucrose. Many of the oligosaccharides occur only as decomposition products of more complex glycosides.

The other main division of the holosides, the polysaccharides, are high molecular weight compounds consisting mainly of condensed simple sugars or oligosaccharides. They are very difficult to purify due to their formation of colloidal solutions. In nearly all cases there is not sufficient data to permit the placement of the individual polysaccharides in other than a broad group. These groups are the pentosans, hexosans, mannans, galactans and mixed polysaccharides. The mixed polysaccharides comprise the hemicelluloses, gums, mucilages, and pectins. The arabans and xylans are natural pentosans. The former yields arabanose and the latter xylose on hydrolysis of the respective polysaccharides. A large number of the hexosans are known. The starches are the most

important members of this class. Cellulose and inulin are two other important hexosans. The former yields glucose on hydrolysis while the latter is composed of fructose units. The mannans and galactans are apparently less important polysaccharides. The hemicelluloses are polysaccharides associated with cellulose but differ from it in their ease of hydrolysis and the mixture of sugars produced. The gums and mucilages are very similar to the hemicelluloses but yield galatose and arabinose instead of glucose, mannose, and xylose. The pectins are another division of the mixed polysaccharides yielding principally arabinose on hydrolisis.

Heterosides

The heterosides are glycosides that yield one or more molecules of sugar on hydrolysis in addition to some non-sugar portion termed the aglucene. The glycosides are so very widely distributed in nature that there is probably one or more in every plant. A particular class of glycosides may be quite widely distributed or it may occur only in some particular order of plants. The mustard oil glycosides are present chiefly in the cruciferae. The glycoside coniferin is found in various coniferous trees but is absent in nearly all other plants.

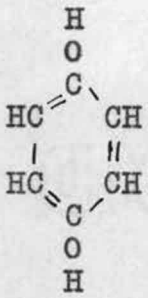
With our present knowledge it is impossible to give

a simple classification of the glycosides. They may be classed according to the sugar residue, the aglucene, the plant function, the physiological action, or some combination of these. Generally the sugar residue is the least complex, but in many cases the sugar fraction has not been identified or is poorly characterized. Division on the basis of plant function and physiological activity are at present poor methods as they are not sufficiently understood. Classification according to the aglucone present is generally preferred since it is the common determining factor in the properties of the glycoside but it is not entirely satisfactory since there are many aglucones whose composition and structure are practically unknown. In Table II a classification is made of the glycosides from their aglucones according to Gortner (7) listing in addition an example of each with the products of hydrolysis. The structural formulas of the aglucones are found in Figs. 3 and 4.

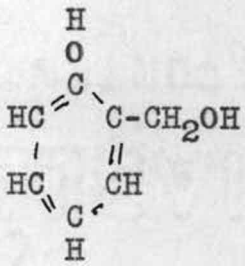
The functions performed by the different glycosides in the plant are not very well understood. Many theories have been advanced to explain the purpose of the glycosides but due to their diverse nature it does not seem likely that any one theory will fit all cases. Very likely many of the glycosides function as reserve food materials. This view is supported by the variations in the amount of

Table II

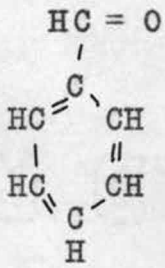
Name	Hydrolytic Products	Class
Arbutin	Glucose, Hydroquinone	Phenols
Salicin	Glucose, Saligenin	Alcohols
Amygdalin	Glucose, Benzaldehyde (HCN)	Aldehyde
Gaultherin	Glucose, Methyl salicylate	Acids
Aesculin	Glucose, Aesculetin	Oxycumarin
Ruberythric acid	Glucose, Alizarin	Oxyanthraquinone
Quercetrin	Rhamnose, Quercetin	Oxyflavone
Sinigrin	Glucose, Allyliso-thiocyanate ($KHSO_4$)	Mustard oil
Pelargonin	Glucose, Pelargonidin	Anthocyans
Digilanide A	Digitoxose, Glucose, Acetic acid, Digitoxigenin	Digitalis
Gitonin	Galactose, pentose, Gitogenin	Saponins



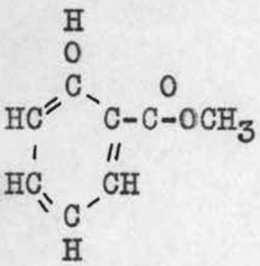
Hydroquinone



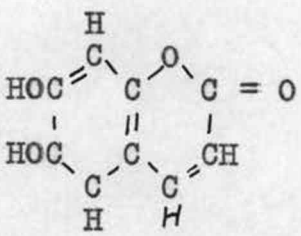
Saligenin



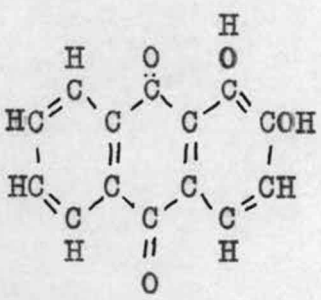
Benzaldehyde



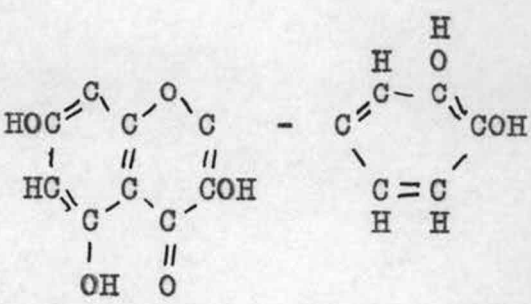
Methyl-salicylate



Aesculetin

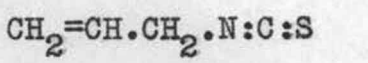


Alizarin

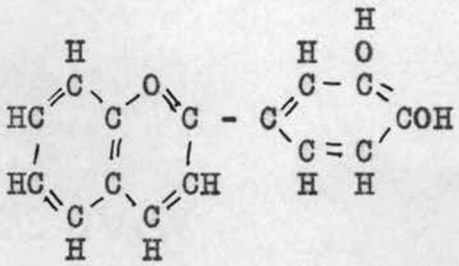


Quercetin

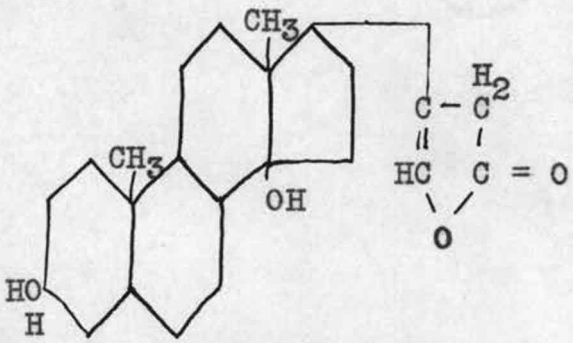
Fig. 3



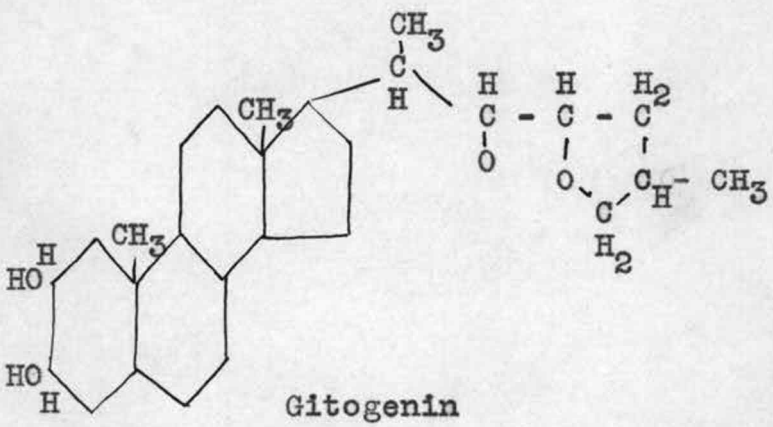
Allyl isothiocyanate



Pelargonidin



Digitoxigenin



Gitogenin

Fig. 4

salicin in the willow (24). The glycoside salicin is formed during the day but at night it is broken down by the enzyme present into sugar and saligenin. The glucose is removed and the saligenin remains to form more salicin the next day. According to some investigations (18) the cyanogenetic glycosides are not reserve food materials. They may function as a deterrent to the removal of green seeds by animals since the amount decreases in the ripening fruit. Instead of protecting the plant from animals some glycosides may function to attract animals that aid in fertilization. The aglucones of many of the glycosides have antiseptic properties. This may be of value in protecting the plant from disease in damaged parts. Glycosides may result from the union of toxic substances with sugars forming inert substances in a manner similar to the union of uronic acids with toxic substances in the animal body. The glycosides may also be useful as a storehouse for easily oxidized substances holding them in a stable form until they are needed. The chromogen producing glycosides may have the function of oxygen carriers in plant oxidative processes. Knowledge of the functions of the glycosides is far from complete but there is no doubt that they are of extreme importance in the metabolic processes of the plant.

The synthesis of glycosides may be accomplished by

various means. The glycoside may be formed from the sugar and aglucone by the action of the enzyme that hydrolyzes the glycoside. This reaction has been accomplished in vivo (11) and is probably the method employed by the plant. One laboratory method is to condense the proper aglucone with an acetobromo derivative of the sugar in the presence of silver oxide or sodium hydroxide (14). Methyl glucoside was prepared by Fisher by dissolving glucose in cold methyl alcohol saturated with hydrogen chloride. That plants may be employed to synthesize glycosides is shown by the formation of salicin in maize plants inoculated with saligenin (2).

Many of the glycosides have a definite physiological action that has proven of great value. The glycosides from Digitalis purpurea have valuable heart stimulating properties. They decrease the rate and increase the intensity of the heart beat. Many other glycosides also have cardiac action but they are not as important. Phlordzin has been of great importance in assisting the studies of sugar metabolism by the production of glucosuria.

Saponins

The saponins are an important class of glycosides which are very widely distributed in the plant kingdom. The term saponin appears to have been employed first by

Gmelin and Grothus (6). It was originally restricted to the glycosides obtained from the root of Saponaria rubra and Saponaria alba but it is now used to designate a large number of glycosides that have some few properties in common. The aglucones of many of the saponins are triterpene in nature and yield Diels hydrocarbon (4) (methyl cyclopentenophenanthrene) on dehydrogenation.

The most characteristic property of the saponins is their ability to produce a very stable foam when shaken with a water air mixture. They also produce very stable emulsions and even gels when their water solutions are shaken with fats, resins, hydrocarbons, and other water insoluble compounds. Saponins act as protective colloids preventing the precipitation of suspended solids. Most of the saponins are neutral but a few have acid properties. The acid saponins can be precipitated from solution with lead acetate, some of the neutral ones with basic lead acetate and the most of them with barium hydroxide. The saponins are generally soluble in alcohol, aqueous alcohol and water but insoluble in chloroform, ether, acetone, and hydrocarbon solvents. The toxicity of the saponins towards cold-blooded animals varies but for many of these substances a dilution of one part in 100,000 is fatal to fish. This property depends on their ability to haemolyze blood and this in turn on the marked lowering in surface tension

they produce in solution.

Saponins are generally hydrolyzed with dilute mineral acids although they may be hydrolyzed in some cases with enzymes. The aglucone produced is known as the sapogenin. Saponins will form acetyl derivatives with acetic anhydride but the product formed on decomposition of the acetyl compound by treating with alcoholic potassium hydroxide is not identical with the original.

The function of saponins in plant metabolism is unknown but it is likely that they play some important part. Their ability to hold insoluble substances in solution and the marked lowering of surface tension they show in water solution must have some influence on the plant metabolism.

The Yuccas

In the previous discussion it has been shown that there is considerable variation in the composition of different plants. This fact has made it necessary to make a study of as many different plant species as possible in order to obtain a more perfect picture of plant chemistry. In addition to increasing our knowledge of plant chemistry there is always the possibility that the investigation will find new and valuable substances. These plant investigations are usually furthered by a personal interest in particular plants or in particular types of compounds.

The present investigation was undertaken because of an increasing interest in plant chemistry. Yucca mohavensis was selected for the investigation since it was thought to contain glycosides which were substances of primary interest.

Yucca mohavensis, Sargent (9) belongs to the order of Lilliaceae. It is one of the taller Yuccas and has its habitat in the southwestern United States ranging from western Texas north to Monterey, California, south to northern Mexico. As a rule the trunk is from 7 to 15 feet tall, but sometimes it is very short or even entirely lacking. All samples of the Yucca stem used in this investigation were furnished by Truesdail Laboratories, Inc., Los Angeles, California, through the courtesy of The Desert Products Laboratories, Inc., Los Angeles, California.

Before the investigation of this Yucca is discussed in detail mention should be made of the work that has been carried out on other species of Yucca.

From a survey of the literature it was found that investigations had been made on three different species of Yucca. The most extensive research was by Helen Abbot Michael (15) on the different parts of Yucca angustifolia. Since the study on the wood of the root is more nearly parallel to this research problem it will be used for a comparison. The quantitative results are tabulated in

Table III.

Table III

Moisture	11.67%
Ash	15.75%
Petroleum ether extract	0.55%
Ether extract	1.70%
Alcohol extract	14.30%
Water extract	16.10%
Albuminoids	4.74%
Glucose	6.0 %
Saponin	9.4 %

The petroleum ether extract was identified as a fixed oil admixed with volatile fatty acids. A resin was the principal product of the ether extract. A saponin was present in the alcoholic and aqueous extracts but identification was not attempted. There were no alkaloids or tannins present.

In 1916 Arno Viehoever and others (23) published a report on the presence of a saponin in Yucca angustifolia. The method of isolation and purification is not given. The formula $C_{36}H_{56}O_{20}$ was assigned to the saponin from calculations made on the basis of the molecular weight and combustion data. Galactose was indicated as the sugar formed on hydrolysis.

In the same journal Carl O. Johns and others (10) report that a saponin was isolated from Yucca radiosa on extracting the ground stem with alcohol. The formula was given as $C_{37}H_{58}O_{20}$. The sugar formed on hydrolysis was

either glucose or mannose.

L. H. Chernoff and others (1) report the isolation of a saponin from Yucca filamentosa. The dried ground Yucca was extracted with 95 per cent alcohol which was then concentrated and dried after mixing with magnesium oxide. This material was then extracted with absolute alcohol which precipitated the saponin on standing. Further purification was made by dissolving the saponin in hot absolute alcohol, cooling and washing the precipitate with ether. This saponin did not form an insoluble compound with lead acetate, basic lead acetate or barium hydroxide. It would not form a compound with cholesterol. The formula $C_{24}H_4O^0l^4$ was assigned to the saponin. Hydrolysis of the saponin with 6 per cent sulfuric acid gave a compound soluble in dilute alcohol and acetone but insoluble in 10 per cent acid or base. Glucose was produced on hydrolysis and there were indications of the presence of glucuronic acid.

EXPERIMENTAL

Introduction

The present investigation was directed towards a study of the stem since it was most available and was thought to be of greater value than other portions of the plant.

The stem was received in pieces about a foot long. These were stripped of the bark, cut into small pieces, dried at 60° C. in an air oven, ground in a burr mill and then stored until used.

With some modifications (19) the method of extraction followed was the same as that proposed by Dragendorff (5). Since the primary purpose was a study of the glycosides present in the alcohol and aqueous alcohol extracts only a few tests were made on other extracts.

The extractor shown in Fig. 5 was designed for the continuous extraction of larger amounts of material. The flask A was a 500 cc. round-bottom flask. The tubes B and F were 8 mm. while H and E were 6 mm. in diameter. The adapter D was made from an 8 inch Pyrex test tube. The open tube E was to allow for the escape of air and to maintain an equal pressure in the system. The bottle G was an ordinary four liter chemical container.

To operate the extractor a layer of cotton was placed

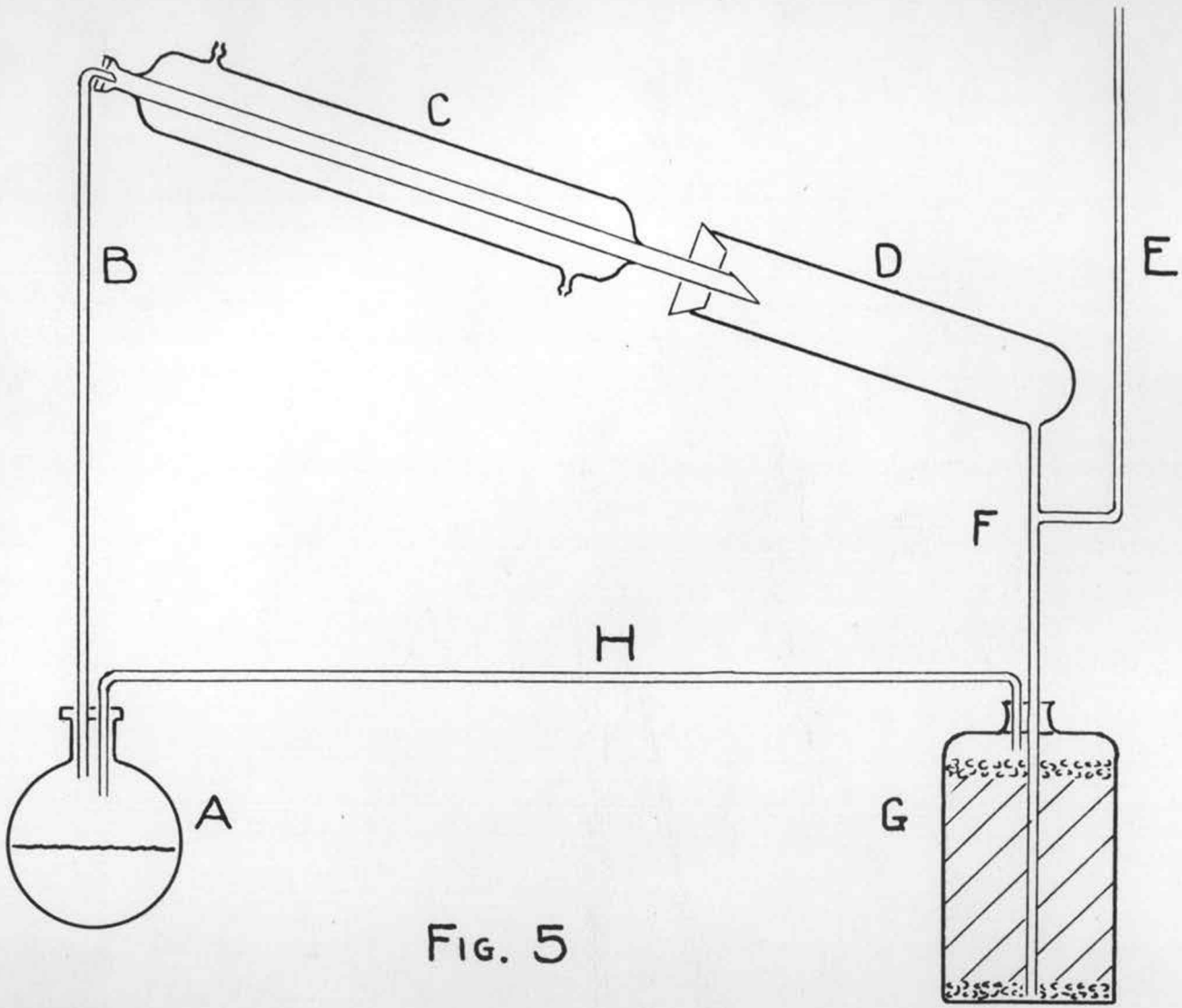


FIG. 5

in G, the tube F placed in position, the ground material to be extracted was introduced, covered with another layer of cotton and then the bottle was filled with solvent. More solvent was placed in A which was then attached to the apparatus. The cork stoppers were coated with water glass which was allowed to dry before the flask was heated with a steam bath or hot plate. The solvent would be vaporized, flow through B to the condenser. The liquid would then enter the bottle G extracting the soluble substances and return to the flask through H. For extraction with hot solvents the bottle was placed in a container of water maintained just below the boiling point of the solvent.

For the successive extractions made with petroleum ether, ether and hot absolute alcohol the material was placed in the continuous extractor and then extracted for several days. In one sample the ether extraction was followed by chloroform but this solvent was discarded as the amount extracted was negligible. Following the hot alcohol extraction the material was extracted ten times with 70 per cent alcohol. After each extraction the material was removed from the extractor and dried before another solvent was introduced.

Results

The quantitative results of the extracts are included in Table IV with ash, sugar and protein determinations. Ten gram samples of the dried ground stem were extracted with each solvent until no further loss in weight occurred. The original reducing substances were determined by the Shaffer-Hartmann (21) method and then calculated as glucose. The difference between reducing power before and after hydrolysis with invertase was calculated as sucrose. The total nitrogen was determined by the Kjeldahl method and calculated as protein.

Table IV

Determination	Per cent
Petroleum ether extract	0.1
Ether extract	0.2
Absolute alcohol extract	34.2
70% alcohol extract	40.2
Water extract	8.4
Insoluble remainder	17.9
Ash	2.12
Protein	2.36
Glucose	4.0
Sucrose	24.0

Tests were made of the various extracts to detect the presence of alkaloids. No precipitate was obtained with phosphomolybdic acid, phosphotungstic acid, Wagner's, Meyer's, and Marme's reagents, therefore it was concluded

that alkaloids were absent. Tannins and their decomposition products were tested for by the addition of ferric chloride. This test indicated not only the absence of tannins but also of other phenolic type compounds. The test for cyanogenetic glycosides was negative. Tests made on the alcohol extract indicated the presence of saponins and reducing sugars. No free acids were present but the slight amount of ash indicated the probability of salts. The 70% alcohol extract contained some of the saponin, other glycosides and sugars including sucrose. The aqueous extract contained salts and probably gums but no starch or dextrins.

A study was made of the methods used in purifying saponins (3, 12, 13, 20). Most of these methods depend on recrystallizations, formation of compounds with lead or barium ions, or adsorption of the impurities. These various methods were tried and the best results were obtained with recrystallizations from absolute alcohol. It was possible to obtain a saponin with about 3.5 per cent sugar by this method but further recrystallizations were not effective as the saponin would hydrolyze on drying, forming more sugar. As no precipitate was formed on adding lead acetate, basic lead acetate, or barium hydroxide to a saponin solution this method could not be used for further purification. An attempt was made to remove the remaining sugar through the use of bacteria. One per cent solutions

of the saponin were treated with cell suspensions of Bacterium coli, Streptococcus lactus and Lactobacillus casei. No acids were produced by these bacteria even when more sugar was added indicating that the saponin was toxic to their action.*

The following investigations were made with the crude saponin. It was soluble in water, methyl alcohol, and ethyl alcohol, slightly soluble in propyl alcohol, butyl alcohol, and amyl alcohol, insoluble in ether, acetone, chloroform, dioxane, ethyl acetate, and benzene. The saponin would not form an insoluble compound with lead acetate, basic lead acetate, barium hydroxide or cholesterol. The best material obtained by recrystallization from alcohol melted at 193-197° C. with decomposition. It contained 0.5 per cent ash. Micro-molecular weight determinations by the Rast (16) method using camphor gave an average value of 620. Hydrolysis with 5 per cent aqueous hydrogen chloride produced 20 per cent reducing sugar which was identified by the phenylosazone as glucose. No other sugars could be detected. The amount of sugar produced on hydrolysis indicates that there is one sugar molecule in every molecule of saponin. Hydrolysis could be produced by a concentration of acid as low as 0.1N but the saponin would

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* The assistance of Dr. F. J. Rudert of the Bacteriology Department was obtained in this experiment.

hydrolyze only very slowly in a neutral or basic solution. On hydrolysis 18 per cent water insoluble substances were formed. A small portion of this crude material was soluble in acetone, dioxane, chloroform, or secondary butyl alcohol but the major part was insoluble in all solvents tried. The acetone soluble fraction was acid in character as it would dissolve in basic solutions and be reprecipitated on acidification. Many attempts to obtain a crystalline sapogenin met with failure. This is an exception as the sapogenins usually crystallize very readily.

A very small amount of a glycoside was isolated from the 70 per cent alcohol extract. This material was crystalline in nature forming rosettes of fine needles. It was insoluble in water, chloroform, benzene, dioxane and ether, slightly soluble in hot ethanol or methanol and quite soluble in aqueous methanol or ethanol. It was purified by several recrystallizations from aqueous alcohol. The pure material melted at 247-250° C. with slight decomposition. It was identified as a glycoside by the Molish test using alpha naphthol and concentrated sulfuric acid. Tests for elements showed that nitrogen, sulfur, and the halogens were absent.

The glycosides formed a hydrate when exposed to air. The amount of water in the hydrate appeared to be a function of the humidity. This forced the use of a special technique

in the weighing for the molecular weight and micro combustion determinations. For the latter the glycoside was first dried until no further loss in weight occurred. It was then exposed to the air until equilibrium with the water vapor was reached. A sample was then weighed out and the amount of water present was subtracted to obtain the true weight of the material. For the ebullioscopic molecular weight determinations the glycoside was dried, pelletted and dried again. The weight of the pellet was calculated as the loss in weight of the weighing bottle when the pellet was removed.

The molecular weight was determined by the ebullioscopic and cyroscopic methods. For the ebullioscopic determination Rieche's apparatus (17) was used with absolute alcohol as the solvent. The average of four determinations was 556 with an estimated error of 10 per cent. The micro Rast method (16) was used for the cyroscopic determination with benzoic acid as a solvent. The molal freezing point depression constant for benzoic acid was determined as 7.2 using triphenylcarbinol as the known. An average value of 539 was obtained for the molecular weight by this method. The average of three micro-combustions gave values for carbon of 61.9 per cent and hydrogen 8.47 per cent. From these data the formula for the glycoside was calculated as $C_{28}H_{40}O_{10}$. The theoretical values for this compound are

carbon 62.0 per cent, hydrogen 8.49 per cent and molecular weight 542.

The water of hydration was found to be 7.18 per cent which corresponds to approximately two and one half molecules of water of hydration.

A portion of the glycoside was hydrolyzed with 0.6N sulfuric acid at 240° F. for 10 hours. The amount of sugar produced, determined by the micro Shaffer-Hartmann method (21), was 27.3 per cent. According to the formula given for the compound one molecule of sugar would be 32.2 per cent of the total. Due to the difficulties in obtaining complete hydrolysis and preventing the decomposition of the sugar the value of 27.3 is a good agreement with the theoretical.

Table V summarizes the information obtained on this glycoside.

Table V

Molecular weight (cyroscopic)	539
Molecular weight (ebullioscopic)	556
Molecular weight (theoretical)	542
Per cent carbon (experimental)	61.9
Per cent carbon (theoretical)	62.0
Per cent hydrogen (experimental)	8.49
Per cent hydrogen (theoretical)	8.47
Per cent water of hydration	7.18
Molecules of water of hydration	2.5
Per cent sugar on hydrolysis (experimental)	27.3
Per cent sugar on hydrolysis (theoretical)	32.2
Formula for glycoside	$C_{28}H_{46}O_{10}$

Since a report of a glycoside of similar composition and properties could not be found in the literature it is likely that this one has hitherto been unknown. It is purposed that this glycoside be known as mohavenin in order to designate its source and to distinguish it from other glycosides present in the Yuccas.

SUMMARY

1. The results of the quantitative extraction of Yucca mohavensis with various solvents are given in addition to ash, sugar, and protein determinations.

2. The presence of a saponin of unknown structure has been established and some of its properties described. Glucose was identified as one of the hydrolytic products.

3. A glycoside was isolated from the aqueous alcohol extract. Its molecular weight and percentage composition were determined. From these data the formula $C_{28}H_{46}O_{10}$ was established for the glycoside. The per cent sugar formed on hydrolysis and water of hydration was also determined.

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