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PART I: IDENTITIES OF THE POLYSACCHARIDES IN SAGUARO CACTUS.

PART II: IDENTIFICATION OF THE MONOTERPENOIDS IN THE ESSENTIAL OIL OF SAND SAGE BRUSH

ARTEMISIA FILIFOLIA (TORR.)

by

Michael James Onore

A Dissertation Submitted to the Faculty of the
DEPARTMENT OF CHEMISTRY
In Partial Fulfillment of the Requirements For the Degree of
DOCTOR OF PHILOSOPHY
In the Graduate College
THE UNIVERSITY OF ARIZONA

1967
I hereby recommend that this dissertation prepared under my direction by Michael James Onore entitled

**PART I: IDENTITIES OF THE POLYSACCHARIDES IN SAGUARO CACTUS. PART II: IDENTIFICATION OF THE MONOTERPENOIDS IN THE ESSENTIAL OIL OF SAND SAGE BRUSH ARTEMISIA FILIFOLIA (TORR.)*

be accepted as fulfilling the dissertation requirement of the degree of Doctor of Philosophy

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ABSTRACT

Part I

An investigation of the polysaccharides of the saguaro cactus (*Carnegiea gigantea*) was undertaken. Composition of the polysaccharides of the pulp and the polysaccharides of the callus tissue were established and compared. It was found that the major sugar galactose appearing in the pulp polysaccharides was not found to appear in the callus polysaccharides. This leads to the conclusion that galactose plays an important role in the wound response of the plant and may be a possible precursor to the formation of lignin in the saguaro cactus.

Part II

An investigation of the essential oil of *Artemisia filifolia* (sand sage brush) was undertaken. Isolation of four pure terpenoid compounds in the low-boiling fraction was accomplished by preparative VPC. These compounds were identified as 1,8-cineole, filifolone (a new monoterpene), camphor, and isophorone by spectral and derivative comparison. The stereochemistry of filifolone was established. Possible biosynthetic pathways were also postulated for the formation of the terpenoids found.
PART I

IDENTITIES OF THE POLYSACCHARIDES
IN SAGUARO CACTUS
INTRODUCTION

Historical Background

Polysaccharides are important to every living organism. In plants, polysaccharides serve as a structural support, as a food source, in wound healing, in disease response, in frost resistance, as precursors for other chemical compounds, and in many other functions. In the animal world they are found abundantly in exoskeletons of insects and crustaceans, cartilage, in animal joint fluids, in fluid cancer, and in skin and mucosa.¹

In wound healing and disease response, plants react usually by exuding gums from the injured area.² Gums are sticky fluids made up of polysaccharides which tend to cover and seal the incision. The fluid thickens on drying in air, and may finally harden to a brittle, translucent glassy mass.³ A large body of evidence indicates that the gum is a natural exudant of the tree. However, Lutz⁴ found that by treating species of Acacia with the fungus Asterula grumipara, a water soluble gum was formed. He then found by treating with other fungi that gums, which were practically insoluble in water, were produced. He concluded that the gum produced, as well as its solubility, depended entirely upon the organism which produces the wound or infection.

The monosaccharide units of gums have been investigated. Generally gums are highly branched structures and are usually composed of two to five types of monosaccharides. Mesquite gum, for example, was found
to contain units of D-galactose, L-arabinose, and 4-monomethyl-D-glucuronic acid in the ratios of 4:2:1 (Figure I-1).^5

Gum Arabic, an exudant from the shrublike trees of the genus Acacia, has a possible structure shown in Figure I-2.\textsuperscript{6}

In the animal world polysaccharides play an important part in many functions of the living organisms. A great number of these polysaccharides have been investigated in detail. Chitin, which occurs in the lower animal world, is the principal frame substance.\textsuperscript{7} Chitin is a long unbranched molecule composed entirely of N-acetyl-D-glucosamine linked by beta 1-4 bonds.\textsuperscript{8}

Chondroitin sulfuric acid, a mucopolysaccharide which is one of the major constituents of cartilage, is widespread throughout the animal world. It yields on hydrolysis approximately equimolar quantities of 2-desoxy-2-amino-D-galactose, D-glucuronic acid, sulfuric acid, and acetic acid. Heparin, a blood anticoagulant, is also a polysaccharide. Figure I-3 shows its probable repeating unit.\textsuperscript{9}

In animal tissues, the polysaccharide hyaluronic acid is commonly found. It is found in mesenchymal tissue and in the capsules of some bacteria. The monomeric building units consist of N-acetyl-D-glucosamine and D-glucuronic acid in a 1—4 linkage.\textsuperscript{10} Figure I-4 shows its probable repeating unit.

Bacteria are known to produce a number of polysaccharides which can either be helpful or harmful to humans. Dextrans are produced by a wide variety of microorganisms. These dextrans find use as blood plasma substitutes and consist of D-glucose units. Polysaccharides of the salmonella bacilli have been isolated and studied chiefly because of
Figure I-1. Possible Structure of Mesquite Gum

---6Gal---
\[3\]

\[3\]

| 1 |
Me G.A.

\[2\]

\[1\]

\[Araf_2\]

| 1 |

\[Araf\]

Figure I-2. Possible Structure of Gum Arabic

\[Araf_3-1Galp\]

\[1\]

\[6\]

\[G.A.\]

\[4\]

\[1\]

\[Araf\]

\[G.A.\]

\[4\]

\[1\]

\[Araf\]

\[Araf\]

\[G.A.\]

\[G.A.\]

\[1\]

\[G.A.\]

\[G.A.\]

\[G.A.\]

\[1\]
Figure I-3. Repeating Unit of Heparin

Figure I-4. Repeating Unit of Hyaluronic Acid
deleterious effects of these organisms on human health, e.g., typhoid fever and certain types of food poisoning. Specific polysaccharides from several salmonella organisms have been found to contain 32-44% glucose, 8-21% galactose, 19-25% mannose and hexosamine.

Many polysaccharides have shown an active and beneficial influence in man. Acetoxane is a polysaccharide isolated from Acetobacter xylinum. This polysaccharide increases unspecific resistance to staphylococci and to irradiation. Acetoxane also gives a slight increased resistance to influenza virus and depresses exuding during inflammation. Acetoxane was found to contain glucose, mannose, ribose, and rhamnose. The absence of amino acids has been verified. Antibacterial activity was found in a polysaccharide isolated from paniculate wormwood. A polysaccharide isolated from Seriatia marcesuns inhibited growth of the Sarcoma 180 or Sarcoma 37 tumor by about 50-53% or with increased doses about 70-73%. A polysaccharide isolated from bamboo grass was found to be effective against Ehrlich sarcoma. The composition of this polysaccharide was xylose, arabinose, and galactose in an equimolar ratio.

Polysaccharides are of value in plants for their own adaptive and healing mechanisms. Cristofor Simionescu used D-glucose containing $^{14}C$ to trace the pathway of the sugar in the biosynthesis of cellulose and lignin in both tumorous and normal tissue. He found that there was a greater radioactive count in the lignin than in the cellulose of both the tumorous and healthy tissue. Also, there was a greater concentration of radioactivity in the healthy tissue. This indicates that the
glucose to a great degree contributes to lignin biosynthesis in the normal plant.

Simionescu found that attack of fir trees by Melampsorella cerastii causes abnormal growth and changes in the chemical composition of the wood. The cellulose content of the attacked portion was found to decrease by an amount ranging from 3.18 to 7.11%. This decrease was accompanied by a corresponding increase in the lignin content. The concentration of pentosans and of water soluble compounds also decreased while changes in the resin and tannin content was negligible. Koblitz has shown that growth stimulating materials affect increased production of lignin at the expense of polysaccharide. The total concentration of lignin and polysaccharides remains constant.

Cold acclimation also brings about a change in the polysaccharide content. I. V. Ogalevets found that starch and hemicellulose accumulated in the bark of birch, oak, and apple trees throughout the fall. With the coming of winter, these stored carbohydrates underwent gradual hydrolysis. The more frost resistant varieties underwent the greatest changes. The dogwood underwent similar changes. It was found that the starch content of the dogwood increased rapidly, then decreased rapidly during cold acclimation while reducing sugars increased. Raffinose became the major carbohydrate following cold acclimation; the lowest limit of survival was -125°F.

**Statement of the Problem**

The saguaro, being somewhat unique in its wound responses, is a good model for studying lignin formation. Its massive dimensions and
rapid formation of large callus sections permit easy isolation of the callus, as well as permitting direct instrumental measurements of such factors as pH and temperature. Further, the cortical tissue is almost 100% polysaccharide in contrast to the callus (30% lignin); this provides an excellent plant model for studying lignification processes. Previous investigations of the phenolic compounds involved has been carried out by Steelink et al. Also, Steelink et al. have investigated many of the other chemical components present. Polysaccharides obviously play a major role in the wound response and the formation of the lignin.

Simionescu compared the sawdust obtained from a plum tree and the crown gall tumor obtained from the same tree. The crown gall tumor showed a decrease of pentosans and the sugars were harder to hydrolyze. There was also a marked decrease in the cellulose content and a corresponding increase in lignin content of 19.16%. The total amount of cellulose-lignin remains constant for both tumor and healthy woods. Therefore, in the saguaro, a search for the polysaccharide which is primarily responsible for the wound response will give an insight into the mechanism of the callus formation.

Knowledge of the wound responding polysaccharide would lead to further studies in the biosynthesis of lignin. The use of tracer studies using radioactive carbohydrates corresponding to those disappearing would be interesting. This necessitates the study of both the water soluble and the water insoluble polysaccharides in both the pulp and the callus tissue.
Previous Chemical Work

Chemical investigations of the saguaro was first done by Heyl in 1928. The alkaloid carnegine (Figure 1-5) was found to be present in a 0.7% yield. Hodgkins more recently found the arenaline analog which is similar to alkaloids generally found in the cactaece. Greene, in 1936, analyzed the pulp and seeds for their sugar, protein, ash, and acid content. The sterol beta-sitosterol has been isolated from the pulpy cortex by Kircher.

The lignin fractions have been investigated by Steelink et al.; the lignin content of the callus (30.4%) was much higher than that of the ribs (21.9%). This increase in lignin content is similar to that found by Simionescu for injured fir trees (see page 6). The pulp was found to be devoid of lignin. Products from nitrobenzene oxidation of the lignin of both callus and rib were 4-hydroxybenzaldehyde, vanillin, and syringaldehyde. Recent work by Steelink and Caldwell showed that the callus contained the following phenolic acids: 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, and 3-methoxy-4-hydroxybenzoic acid. Small amounts of 4-hydroxycinnamic acid and 3-methoxy-4-hydroxycinnamic acid were also found in the callus extracts. The flavonoid quercetin (Figure 1-5) and a second unidentified flavonoid were found to occur in the callus in 0.097% yield. Waxes and lipids in concentrations up to 1.17% were found in the callus; however, no attempt was made to identify them.

Compared to the callus, the pulp (cortex) was singularly devoid of phenolics. Only dopamine (Figure 1-5) 3,4-dihydroxy-o-phenylethylamine was detected in significant quantities. When the healthy tissue
Figure 1-5. Structures of (a) Carnegine, (b) Quercetin, and (c) Dopamine
(cortex) was mechanically wounded, dopamine accumulated at the site of the wound. This was followed by a slow formation of the ligniferous callus tissue. The dopamine appeared to be the precursor to the melanin (blackening) at the site of wounding; however, it may also be a precursor to the alkaloids in the plant, as well as acting as a hormone.29

Whether dopamine or the cortical sugars are direct precursors to all the phenolic compounds and lignin remains to be determined. Biosynthetic experience would predict that injury to the plant stimulates a rapid metabolic conversion of certain monosaccharides to lignin, cellulose, and other callus constituents. The fats of the sugars in the cortex must await (a) a quantitative determination of the various saccharides in the pulp and callus tissue and (b) a C14 tracer study of the changes occurring on wounding.

**Approach to the Problem**

The purpose of this investigation was to determine the polysaccharide constituents of healthy tissue and wound tissue (callus). From this one could deduce which sugars are utilized to form wound tissue. In addition, such a study would provide a basis for future biosynthetic experiments with radioactive compounds.

VPC was chosen as the best analytical method for a quantitative determination of the sugars. The method was perfected by Sloneker30 for analysis of monosaccharide units. It is based on the reduction of free sugars to the alditols, their conversion to acetates, and subsequent analyses by VPC. It represents a significant advance in the analysis of sugars. There are three major limiting factors to this technique:
(a) analysis of uronic acid is not possible, (b) analysis of amino sugars is not possible, and (c) some methoxy and deoxy sugars overlap the hexoses and pentoses hindering quantitative analysis.

Recently analysis by TLC and paper chromatography has been carried out by Steelink et al.\textsuperscript{31} on the various hydrolyzed polysaccharide fractions, as an additional verification of the VPC studies.
EXPERIMENTAL

Methods and Materials

Apparatus

Melting points were determined in capillary tubes with a Mel-Temp apparatus. A Perkin-Elmer Infracord Model 137B spectrophotometer was used for infrared spectra. All gas chromatography analyses were done on an F & M Model 609 flame ionization chromatograph equipped with a disc integrator. Column temperatures were 190°C. Gas chromatographic columns were 1/4 in. o.d. x 10 ft. packed with 3% ECNSS-M an organo-silicone polyester phase, consisting of ethylene glycol succinate chemically combined with a silicone of cyanoethyl type (Applied Science Laboratories State College, Pa.).

Procedures

Preparation of the Sample. The pulp and callus material were obtained from the desert regions around Tucson, Arizona. The areas and dates of collection will be noted with the sample analysis.

The pulpy cortex material was cut away from the outer skin and inner rib sections in the minimum time possible after getting the sample to the laboratory. It was ground up in a Waring blender for approximately two minutes with enough hot ethanol to give a 60-80% ethanol-water mixture to remove free sugars. The ground pulp and ethanol solution was filtered, and the alcohol portion saved for further analyses. The ground pulp was washed several times with excess 80% ethanol.
The pulp was air-dried and was ready for extraction of the water soluble polysaccharides.

The callus material was taken from the cactus and cleaned thoroughly until all pulp and black oxidized material had been removed. It was broken up into small pieces and placed in a Waring blender for two minutes with 80% hot ethanol. The ethanol solution was filtered off and saved for future analyses. The ground callus was washed thoroughly with 80% ethanol. The callus was air-dried and was ready for extraction of the water soluble polysaccharides.

**Extraction of the Sample.** The pulp and callus were extracted in the same manner for water soluble polysaccharides. The air-dried ground material was suspended in a large volume of water (50 ml. per gram). The water was brought to reflux temperature and held there for several hours with constant stirring. The water mixture was allowed to cool and then was filtered. Considerable difficulty was encountered when filtering the pulp. A gelatinous mass usually resulted with the water extraction. The insoluble material (consisting mainly of water insoluble polysaccharides and lignin in the case of the callus) was washed with water and air-dried. The water filtrate was concentrated in a flash evaporator until a thick viscous solution was obtained. The polysaccharide was precipitated by the addition of 95% ethanol, filtered off, washed with 95% ethanol, and stored in 95% ethanol until used in analysis. When the sample was needed, the ethanol was filtered off and the sample was air-dried.
Hydrolysis, Reduction, and Acetylation of the Sample. One gram of the dried polysaccharide was mixed with 10 ml. of 72% sulfuric acid. This mixture was allowed to stand until a homogeneous solution was obtained. This solution now contained oligosaccharides which were further degraded by diluting the solution to a 1% sulfuric acid solution and refluxing for 6 to 8 hours. The solution containing the free sugars was mixed with barium carbonate to get rid of the excess sulfuric acid present. The solution was filtered and washed with acid ion exchange resin \(\text{Amberlite IR-120(H}^+\text{)}\) overnight to remove excess barium ions. The solution was filtered and neutralized using sodium bicarbonate. The sugars were immediately reduced using excess sodium borohydride (see page 17). The polyhydric alcohols were washed again with acid ion exchange resin \(\text{Amberlite IR-120 (H}^+\text{)}\) to break up the excess sodium borohydride and the borate esters present. The solution was filtered and reduced to dryness in a flash evaporator. The resulting polyhydric alcohols were mixed with acetic anhydride-pyridine solution (1.0 ml. of solution per 100 mg. of alcohol) and refluxed for 4 hours. The solution was evaporated to dryness, and the resulting alditol acetates were taken up in excess tetrahydrofuran (THF). This solution was filtered and reduced to a small volume prior to analysis by VPC.

The ethanol extract of the pulp and callus was concentrated and reacted with excess sodium borohydride directly. The objective here was to analyze the free sugars present in the saguaro. The acetylation and analysis were conducted in the same manner as the other polysaccharide analysis.
Delignification and Alkaline Extraction of the Callus Tissue.

After vapor phase chromatography of the callus tissue, a further study of the insoluble polysaccharide fraction was undertaken. It was necessary to establish whether the xylan was co-polymerized with glucose or if a true xylan polymer was present.

The procedure used for separation of the cellulose from the other polysaccharides was as follows. The ground callus material, 39.2 gm., was placed in a soxlet and extracted for three days with petroleum ether (b.p. 60-100°). This removed the lipids from the callus. The material was air-dried and extracted again in a soxlet with a 2:1 benzene-ethanol mixture for three days to remove phenolics from the callus. A total of 1.3 gm. of material was extracted from the callus. A total of 1.3 gm. of material was extracted from the callus leaving 37.9 gm. of callus remaining. The air-dried callus, 37.9 gm., was suspended in 800 ml. of hot water in a 2 l. Erlenmeyer flask. To the suspension, 3 ml. of glacial acetic acid and 7.5 gm. of sodium chlorite were added. The flask was stoppered with an inverted 50 ml. Erlenmeyer flask (prevents pressure build-up) and heated on a steam bath. The flask was heated for one hour. The heating is then stopped and fresh portions of acetic acid and sodium chlorite were added and heating was resumed. This process was repeated three more times. The delignified callus was washed with 2 l. of cold water and air-dried to give 27.5 gm. of holocellulose. The callus contained, therefore, 27.5% lignin.

The holocellulose, 18 gm., was added to a 3-necked 2 l. round-bottomed flask along with 1 l. of 12% sodium hydroxide. The sodium
hydroxide solution had high purity nitrogen bubbled through it for two minutes to expell the oxygen present. The liquid with the suspended holocellulose was mechanically stirred while a stream of nitrogen was passed through the vessel. The first extraction was for a period of 48 hours. The mixture was filtered and a minimum of air was sucked through the filter. The volume of the filtrate was noted. The residue was transferred back to the flask, and 1 l. of 7.1% sodium hydroxide was added (excluding the volume retained by the swollen sample). The same procedure was repeated. A third l. of 7.1% sodium hydroxide was added, again minus the volume retained by the swollen sample, and the same procedure was repeated. The combined filtrate was neutralized using concentrated hydrochloric acid. The xylan polymer was precipitated by the addition of 95% ethanol. The recovered xylan polymer was air-dried and gave 5.3 gm. or a 34% yield.

The solid residue was washed with 2 l. of water and gave 8.8 gm. of cellulose or a 49% yield. The remaining 3.9 gm. of material was lost due to fragmentation to alcohol soluble oligosaccharides. The xylan polymer was hydrolyzed according to the procedure given on page 15 and analyzed further by paper chromatography.\textsuperscript{35}

**Preparation of the Standards for VPC Analysis.** In the preparation of the standards, the method of A. Thompson\textsuperscript{36} was used in the reduction step. To a solution of the sugar (10 gm. in 30 ml. of water), sodium borohydride was added dropwise with constant mechanical stirring. The addition took approximately 10 minutes and the temperature was regulated to stay below 50°. The mixture was allowed to stand for an additional 10 minutes. Amberlite IR-120(H\textsuperscript{+}) was added slowly until no
more hydrogen was liberated. An additional 50 ml. of Amberlite IR-120(H+) was added and the solution was stirred for 15 minutes. The mixture was filtered, and the solution was evaporated to a thin syrup with a flash evaporator. Methanol was added and flash evaporated off several times. This removed the remaining boric acid as the volatile methyl borate. The syrup was dissolved in a minimum amount of water and alcohol was added to precipitate the alditol. The alditol was recrystallized from a water-ethanol solution if it was a solid and washed with a water-ethanol solution if it was a liquid.

The purified alditol was mixed with a 1:1 acetic anhydride-pyridine solution (1.0 ml. of solution per 100 mg. of alditol). The solution was refluxed for 4 hours and cooled. The excess acetic anhydride-pyridine was stripped off using the flash evaporator. The crude product was recrystallized from a water-n-propyl alcohol solution.

Results

Preparation of Derivatives

The compounds listed in Table I-1 were all prepared by the sequence of reactions described on pages 19 and 20.

Accuracy of the Analytical Technique

A standard solution was prepared containing known amounts of the acetates of xylitol, arabinitol, mannitol, galactitol, and glucitol. This mixture was injected into a gas chromatograph. Using xylitol as one, the ratio of the weight in grams of each acetate to xylitol and the ratio of each peak area to xylitol was calculated (Table I-2).
Table I-1
Results From Preparation of Derivatives

<table>
<thead>
<tr>
<th>Product</th>
<th>Starting Compound</th>
<th>Grams Reacted</th>
<th>Actual Yield</th>
<th>Theoretical Yield</th>
<th>Percent Yield</th>
<th>Melting Point, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactitol hexaacetate</td>
<td>D-Galactose</td>
<td>10</td>
<td>12.4</td>
<td>24.1</td>
<td>51.5</td>
<td>171-172.5</td>
</tr>
<tr>
<td>Arabinitol pentaacetate</td>
<td>L-Arabinose</td>
<td>10</td>
<td>17.2</td>
<td>24.0</td>
<td>71.6</td>
<td>76-77</td>
</tr>
<tr>
<td>Glucitol hexaacetate</td>
<td>D-Glucose</td>
<td>10</td>
<td>13.2</td>
<td>24.1</td>
<td>54.8</td>
<td>98.5-99.5</td>
</tr>
<tr>
<td>Mannitol hexaacetate</td>
<td>D-Mannose</td>
<td>10</td>
<td>6.1</td>
<td>24.1</td>
<td>25.3</td>
<td>124-125.5</td>
</tr>
<tr>
<td>Xylitol pentaacetate</td>
<td>D-Xylose</td>
<td>10</td>
<td>17.4</td>
<td>24.0</td>
<td>72.3</td>
<td>61</td>
</tr>
<tr>
<td>Rhamnitol pentaacetate</td>
<td>L-Rhamnose</td>
<td>5</td>
<td>6.1</td>
<td>11.4</td>
<td>53.7</td>
<td>None*</td>
</tr>
<tr>
<td>Ribitol pentaacetate</td>
<td>D-Ribose</td>
<td>3.3</td>
<td>5.8</td>
<td>8.0</td>
<td>72.2</td>
<td>None*</td>
</tr>
<tr>
<td>L-Inositol hexaacetate</td>
<td>L-Inositol</td>
<td>2</td>
<td>3.1</td>
<td>4.8</td>
<td>64.5</td>
<td>216-218</td>
</tr>
<tr>
<td>Lyxitol pentaacetate</td>
<td>D-Lyxose</td>
<td>1</td>
<td>1.8</td>
<td>2.4</td>
<td>75.0</td>
<td>73-74</td>
</tr>
<tr>
<td>Product</td>
<td>Starting Compound</td>
<td>Grams Reacted</td>
<td>Actual Yield</td>
<td>Theoretical Yield</td>
<td>Percent Yield</td>
<td>Melting Point, °C</td>
</tr>
<tr>
<td>---------</td>
<td>-------------------</td>
<td>---------------</td>
<td>--------------</td>
<td>-------------------</td>
<td>---------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>2-Desoxy Glucitol pentaacetate</td>
<td>2-Desoxy Glucose</td>
<td>1</td>
<td>1.5</td>
<td>2.3</td>
<td>65.0</td>
<td>82-83</td>
</tr>
<tr>
<td>3-Methoxy Glucitol pentaacetate</td>
<td>3-Methoxy Glucose</td>
<td>1</td>
<td>1.7</td>
<td>2.2</td>
<td>80.5</td>
<td>None*</td>
</tr>
</tbody>
</table>

* Material was a syrup which was purified by activated charcoal.
Table I-2
Results of the Synthetic Mixture

<table>
<thead>
<tr>
<th>Run I</th>
<th>Xylose</th>
<th>Arabinose</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight in grams</td>
<td>0.092</td>
<td>0.085</td>
<td>0.100</td>
<td>0.095</td>
<td>0.094</td>
</tr>
<tr>
<td>Ratio of weight comp. to Xylitol</td>
<td>1</td>
<td>0.920</td>
<td>1.089</td>
<td>1.034</td>
<td>1.015</td>
</tr>
<tr>
<td>Ratio of peak area comp. to Xylitol</td>
<td>1</td>
<td>0.890</td>
<td>1.053</td>
<td>1.007</td>
<td>0.939</td>
</tr>
<tr>
<td>Error(^a)</td>
<td>0</td>
<td>0.036</td>
<td>0.036</td>
<td>0.024</td>
<td>0.076</td>
</tr>
<tr>
<td>Percent Error</td>
<td>0</td>
<td>3.32</td>
<td>3.30</td>
<td>2.30</td>
<td>7.48</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run II</th>
<th>Xylose</th>
<th>Arabinose</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio of peak area</td>
<td>1</td>
<td>0.083</td>
<td>1.082</td>
<td>1.043</td>
<td>1.043</td>
</tr>
<tr>
<td>Error(^a)</td>
<td>0</td>
<td>0.037</td>
<td>0.007</td>
<td>-0.009</td>
<td>-0.028</td>
</tr>
<tr>
<td>Percent Error</td>
<td>0</td>
<td>4.06</td>
<td>0.62</td>
<td>-0.87</td>
<td>-3.17</td>
</tr>
</tbody>
</table>

\(^a\) Ratio of weight - ratio of peak area.
accuracy for this experiment was relatively good and was comparable to that found by Sloneker.37

Hydrolysis and Analysis of a Known Polysaccharide

A polysaccharide of known composition was obtained: Stractan AF #2, an arabinogalactan (Stein, Hall & Co., Inc.). The polysaccharide was hydrolyzed and worked up in the same manner as the polysaccharide from the saguaro. The object of this experiment was to determine if any changes took place in the sugar ratios under the drastic conditions used in hydrolysis. The results of this experiment are shown in Table I-3.

Table I-3

Results of a Known Polysaccharide

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Peak Area</th>
<th>Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arabinose</td>
<td>Galactose</td>
</tr>
<tr>
<td>1</td>
<td>170</td>
<td>980</td>
</tr>
<tr>
<td>2</td>
<td>300</td>
<td>1605</td>
</tr>
<tr>
<td>3</td>
<td>380</td>
<td>2070</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Percent of Total Peak Area</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arabinose</td>
<td>Galactose</td>
</tr>
<tr>
<td>1</td>
<td>14.78</td>
<td>85.22</td>
</tr>
<tr>
<td>2</td>
<td>15.75</td>
<td>84.25</td>
</tr>
<tr>
<td>3</td>
<td>15.51</td>
<td>84.49</td>
</tr>
</tbody>
</table>

Average Percent of Total Peak Area

<table>
<thead>
<tr>
<th>Arabinose</th>
<th>Galactose</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.35</td>
<td>84.65</td>
</tr>
</tbody>
</table>
The known Stractan AF #2 has an approximate 1:6 arabinose to galactose ratio. This means that 12.2% of the polymer is arabinose and 87.8% of the polymer is galactose. This experiment was in close agreement with known results as an average of 15.35% arabinose and 84.65% galactose was found.

A theoretical yield of alditol acetate was calculated for the polysaccharide using a 1:6 ratio. It was found to be 2.49 grams for 1 gram of hydrolyzed polysaccharide. The actual yield found was 1.1 grams or an overall yield of 44.3% was achieved.

Preliminary Analysis of the Polysaccharides in the Pulp of the Saguaro Cactus

A medium size arm of a saguaro, approximately 15 kilograms, was taken from a mature cactus on August 15, 1965. The location of the cactus was about 4 miles east of the Desert Museum outside of Tucson, Arizona. It was processed according to the procedure given on page 15. The results of the water soluble fraction of the pulp are shown in Table I-4. The unknowns I and II were not identified; N.M.R. analysis of these were not definitive in showing them to be desoxy sugars. Use of thin layer and paper chromatography did not lead to an identification, although it is felt that the two unknowns are probably desoxy sugars or methylated sugars.

Preliminary Analysis of the Pulp

The polysaccharide (5 grams) obtained from the saguaro cactus collected on August 15, 1965, was slurried in 500 ml. of water. After the polysaccharide was dissolved, varying amounts of 95% ethanol were
Table I-4

Results of the Water Soluble Fraction in the Pulp of the Saguaro Cactus

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Peak Area</th>
<th>Percent of Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rhamnose</td>
<td>Arabinose</td>
</tr>
<tr>
<td>1</td>
<td>714</td>
<td>418</td>
</tr>
<tr>
<td>2</td>
<td>346</td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td>948</td>
<td>582</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Percent of Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rhamnose</td>
</tr>
<tr>
<td>1</td>
<td>10.98</td>
</tr>
<tr>
<td>2</td>
<td>11.25</td>
</tr>
<tr>
<td>3</td>
<td>11.19</td>
</tr>
</tbody>
</table>

Average Percent of Total Peak Area

<table>
<thead>
<tr>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Unknown I</th>
<th>Xylose</th>
<th>Unknown II</th>
<th>Galactose</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.14</td>
<td>5.13</td>
<td>11.29</td>
<td>9.48</td>
<td>0.99</td>
<td>61.80</td>
</tr>
</tbody>
</table>
added to precipitate out the different fractions of polysaccharides. As the alcohol to water ratio was increased, the higher molecular weight polysaccharides precipitated. After the addition of recorded amounts of ethanol, the solution was filtered to obtain that fraction of polysaccharide.

Seven fractions of polysaccharides were obtained from this method. The first fraction, 1.2 gm., consisted of the polysaccharides which were not soluble in the 500 ml. of water. Fractions two, three, and four resulted from consecutive additions of 25 ml. of ethanol and yielded, respectfully, 0.1 gm., 0.5 gm. and 0.3 gm. of polysaccharides. Fraction five, 0.5 gm., was taken after an additional 60 ml. of ethanol and fraction six, 1.1 gm., resulted from an additional 50 ml. of ethanol. The material remaining in solution was taken as the seventh fraction, 0.7 gm. of dried material. These samples were then hydrolyzed and worked up in the normal manner described on page 15.

Fraction two was too dilute to be analyzed. Fraction seven was worked up but did not give any calculable results. Tables 1-5 to 1-9 list the results on the various fractions. Figure I-6 shows the changes which occurred in the various sugars as the polysaccharide fractions became more alcohol soluble.

Preliminary Analysis of the Pulp Tissue of the Saguaro Cactus

Two saguaro arms were obtained from two different cacti on May 3, 1966, about 3 miles west of the Desert Museum outside of Tucson, Arizona. The first arm, sample II, was a small arm weighing approximately 7 kilograms and had a very slimy pulp. The second arm, sample
Table I-5

Results of Fraction One From the Fractionated Polysaccharide

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Peak Area</th>
<th>Xylose</th>
<th>Galactose</th>
<th>Glucose</th>
<th>Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>182</td>
<td>336</td>
<td>883</td>
<td>858</td>
<td>184</td>
<td>2443</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>155</td>
<td>371</td>
<td>970</td>
<td>836</td>
<td>120</td>
<td>2452</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>172</td>
<td>316</td>
<td>1050</td>
<td>950</td>
<td>227</td>
<td>2715</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>167</td>
<td>306</td>
<td>1057</td>
<td>950</td>
<td>202</td>
<td>2682</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Xylose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.45</td>
<td>13.75</td>
<td>36.14</td>
<td>35.12</td>
<td>7.53</td>
</tr>
<tr>
<td>2</td>
<td>6.32</td>
<td>15.13</td>
<td>39.55</td>
<td>34.09</td>
<td>4.89</td>
</tr>
<tr>
<td>3</td>
<td>6.34</td>
<td>11.64</td>
<td>38.67</td>
<td>34.99</td>
<td>8.36</td>
</tr>
<tr>
<td>4</td>
<td>6.23</td>
<td>11.41</td>
<td>39.41</td>
<td>35.42</td>
<td>7.53</td>
</tr>
</tbody>
</table>

Average Percent of Total Peak Area

<table>
<thead>
<tr>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Xylose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.58</td>
<td>12.98</td>
<td>38.44</td>
<td>34.90</td>
<td>7.08</td>
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</tbody>
</table>
Table I-6
Results of Fraction Three From the Fractionated Polysaccharide

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Peak Area Unknown I</th>
<th>Xylose</th>
<th>Galactose</th>
<th>Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1480</td>
<td>224</td>
<td>150</td>
<td>244</td>
<td>1212</td>
<td>3310</td>
</tr>
<tr>
<td>2</td>
<td>1455</td>
<td>203</td>
<td>130</td>
<td>209</td>
<td>1236</td>
<td>3233</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Percent of Total Peak Area</th>
<th>Unknown I</th>
<th>Xylose</th>
<th>Galactose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44.71</td>
<td>6.77</td>
<td>4.53</td>
<td>7.37</td>
<td>36.62</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>45.00</td>
<td>6.28</td>
<td>4.02</td>
<td>6.46</td>
<td>38.23</td>
<td></td>
</tr>
</tbody>
</table>

Average Percent of Total Peak Area

<table>
<thead>
<tr>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Unknown I</th>
<th>Xylose</th>
<th>Galactose</th>
</tr>
</thead>
<tbody>
<tr>
<td>44.86</td>
<td>6.53</td>
<td>4.28</td>
<td>6.92</td>
<td>37.43</td>
</tr>
</tbody>
</table>
### Table I-7

**Results of Fraction Four From the Fractionated Polysaccharide**

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Unknown I</th>
<th>Peak Area</th>
<th>Xylose</th>
<th>Unknown II</th>
<th>Galactose</th>
<th>Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>336</td>
<td>351</td>
<td>70</td>
<td>340</td>
<td>3238</td>
<td>4335</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>366</td>
<td>366</td>
<td>80</td>
<td>418</td>
<td>3362</td>
<td>4592</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>351</td>
<td>331</td>
<td>55</td>
<td>366</td>
<td>3357</td>
<td>4460</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Percent of Total Peak Area</th>
<th>Unknown I</th>
<th>Xylose</th>
<th>Unknown II</th>
<th>Galactose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.75</td>
<td>8.10</td>
<td>1.61</td>
<td>7.84</td>
<td>74.69</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>7.97</td>
<td>7.97</td>
<td>1.74</td>
<td>9.10</td>
<td>73.21</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7.87</td>
<td>7.42</td>
<td>1.23</td>
<td>8.21</td>
<td>75.27</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Average Percent of Total Peak Area</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhamnose</td>
<td>7.86</td>
<td>7.83</td>
</tr>
<tr>
<td>Unknown I</td>
<td>1.53</td>
<td>8.38</td>
</tr>
<tr>
<td>Xylose</td>
<td>8.38</td>
<td>8.38</td>
</tr>
<tr>
<td>Galactose</td>
<td>74.39</td>
<td>74.39</td>
</tr>
</tbody>
</table>
Table I-8

Results of Fraction Five From the Fractionated Polysaccharide

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Peak Area</th>
<th>Galactose</th>
<th>Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Unknown I</td>
<td>Unknown II</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>694</td>
<td>868</td>
<td>1972</td>
<td>162</td>
<td>5724</td>
</tr>
<tr>
<td>2</td>
<td>771</td>
<td>1022</td>
<td>2412</td>
<td>120</td>
<td>5784</td>
</tr>
<tr>
<td>3</td>
<td>699</td>
<td>928</td>
<td>2116</td>
<td>100</td>
<td>5356</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Percent of Total Peak Area</th>
<th>Galactose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Unknown I</td>
<td>Unknown II</td>
</tr>
<tr>
<td>1</td>
<td>7.37</td>
<td>9.21</td>
<td>20.93</td>
<td>1.72</td>
</tr>
<tr>
<td>2</td>
<td>7.63</td>
<td>10.11</td>
<td>23.86</td>
<td>1.19</td>
</tr>
<tr>
<td>3</td>
<td>7.60</td>
<td>10.09</td>
<td>23.00</td>
<td>1.09</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Average Percent of Total Peak Area</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Unknown I</th>
<th>Unknown II</th>
<th>Galactose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.53</td>
<td>9.80</td>
<td>22.60</td>
<td>1.33</td>
<td>58.73</td>
</tr>
<tr>
<td>Run Number</td>
<td>Ribose</td>
<td>Arabinose</td>
<td>Peak Area</td>
<td>Percent of Total Peak Area</td>
<td>Average Percent of Total Peak Area</td>
</tr>
<tr>
<td>------------</td>
<td>--------</td>
<td>-----------</td>
<td>-----------</td>
<td>---------------------------</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Unknown I</td>
<td>Unknown II</td>
<td>Galactose</td>
</tr>
<tr>
<td>1</td>
<td>162</td>
<td>110</td>
<td>1838</td>
<td>80</td>
<td>920</td>
</tr>
<tr>
<td>2</td>
<td>152</td>
<td>150</td>
<td>1890</td>
<td>142</td>
<td>883</td>
</tr>
<tr>
<td>3</td>
<td>147</td>
<td>120</td>
<td>2044</td>
<td>85</td>
<td>985</td>
</tr>
</tbody>
</table>
Figure 1-6
Graph of the Constituents of the Polysaccharides Fraction
III, had a more coarse and slightly moist pulp. Both samples were worked up in the normal manner described on page 15. The results of water soluble and water insoluble polysaccharides from the pulp are listed in Tables I-10 to I-14.

Preliminary Analysis of the Callus Tissue of the Saguaro Cactus

Callus tissue (62.5 grams) donated by R. Caldwell was worked up according to the procedure described on page 15; however, the black oxidized surface of the callus tissue was not completely removed. This would account for the presence of a large number of sugars which were not found when the black oxidized surface was removed. The results are listed in Tables I-15 to I-17.

Primary Analysis of the Pulp and Callus Tissue of a Single Saguaro Cactus

In an attempt to get a comparative analysis, a medium size saguaro cactus, approximately 15 ft. tall, containing no arms, was cut down on September 23, 1966. The saguaro cactus was located about 15 miles east of Tucson, Arizona, on the road to Reddington. The cactus contained excellent callus tissue and a healthy pulp.

Pulp Fraction. Two pulp fractions were collected and worked up according to the procedures given on page 15. The pulp tissue from the first sample (sample IV) was worked up quantitatively. (All of the polysaccharide solutions were extracted with petroleum ether (b.p. 30-60°) and then with ether. This removed most of the compounds that were soluble in organic solvents, leaving the polysaccharides in solution.) From 851 grams of fresh pulp, the following fractions were obtained:
### Table I-10
Results of the Water Soluble Polysaccharides From the Pulp of Sample II

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Peak Area</th>
<th>Xylose</th>
<th>Galactose</th>
<th>Glucose</th>
<th>Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>355</td>
<td>380</td>
<td>140</td>
<td>2970</td>
<td>490</td>
<td></td>
<td>4335</td>
</tr>
<tr>
<td>2</td>
<td>330</td>
<td>420</td>
<td>150</td>
<td>3160</td>
<td>460</td>
<td></td>
<td>4520</td>
</tr>
<tr>
<td>3</td>
<td>760</td>
<td>900</td>
<td>275</td>
<td>6590</td>
<td>1200</td>
<td></td>
<td>9725</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Xylose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.19</td>
<td>8.77</td>
<td>3.23</td>
<td>68.51</td>
<td>11.30</td>
</tr>
<tr>
<td>2</td>
<td>7.30</td>
<td>9.29</td>
<td>3.32</td>
<td>69.91</td>
<td>10.18</td>
</tr>
<tr>
<td>3</td>
<td>7.82</td>
<td>9.25</td>
<td>2.83</td>
<td>67.76</td>
<td>12.34</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Xylose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.77</td>
<td>9.10</td>
<td>3.13</td>
<td>68.73</td>
<td>11.27</td>
</tr>
<tr>
<td>Run Number</td>
<td>Rhamnose</td>
<td>Arabinose</td>
<td>Peak Area</td>
<td>Glucose</td>
</tr>
<tr>
<td>------------</td>
<td>----------</td>
<td>-----------</td>
<td>-----------</td>
<td>---------</td>
</tr>
<tr>
<td></td>
<td>Rhamnose</td>
<td>Arabinose</td>
<td>Xylose</td>
<td>Galactose</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>325</td>
<td>1240</td>
<td>2330</td>
</tr>
<tr>
<td>2</td>
<td>280</td>
<td>370</td>
<td>1280</td>
<td>2830</td>
</tr>
<tr>
<td>3</td>
<td>340</td>
<td>390</td>
<td>1340</td>
<td>2890</td>
</tr>
<tr>
<td>4</td>
<td>240</td>
<td>190</td>
<td>640</td>
<td>1450</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Percent of Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rhamnose</td>
<td>Arabinose</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>6.00</td>
</tr>
<tr>
<td>2</td>
<td>4.20</td>
<td>5.55</td>
</tr>
<tr>
<td>3</td>
<td>4.92</td>
<td>5.64</td>
</tr>
<tr>
<td>4</td>
<td>6.70</td>
<td>5.31</td>
</tr>
</tbody>
</table>

Average Percent of Total Peak Area

<table>
<thead>
<tr>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Xylose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.95</td>
<td>5.62</td>
<td>19.84</td>
<td>41.96</td>
<td>28.63</td>
</tr>
</tbody>
</table>
## Table I-12
Results of the Alcohol Soluble Polysaccharides From the Pulp of Sample II

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Unknown I</th>
<th>Xylose</th>
<th>Unknown II</th>
<th>Peak Area</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Glucose</th>
<th>Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>920</td>
<td>600</td>
<td>4920</td>
<td>290</td>
<td>12,110</td>
<td>18,920</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>150</td>
<td>660</td>
<td>340</td>
<td>3190</td>
<td>180</td>
<td>8010</td>
<td>12,530</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>520</td>
<td>230</td>
<td>2250</td>
<td>80</td>
<td>5380</td>
<td>8550</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>130</td>
<td>710</td>
<td>440</td>
<td>3275</td>
<td>180</td>
<td>8280</td>
<td>13,015</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Unknown I</th>
<th>Xylose</th>
<th>Percent of Total Peak Area</th>
<th>Unknown II</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.42</td>
<td>4.86</td>
<td>3.17</td>
<td>26.00</td>
<td>1.53</td>
<td>64.00</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.20</td>
<td>5.27</td>
<td>2.71</td>
<td>25.46</td>
<td>1.44</td>
<td>63.93</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.02</td>
<td>6.08</td>
<td>2.69</td>
<td>26.32</td>
<td>0.94</td>
<td>62.92</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.00</td>
<td>5.46</td>
<td>3.38</td>
<td>25.16</td>
<td>1.38</td>
<td>63.62</td>
<td></td>
</tr>
</tbody>
</table>

Average Percent of Total Peak Area

<table>
<thead>
<tr>
<th>Unknown I</th>
<th>Xylose</th>
<th>Unknown II</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.91</td>
<td>5.42</td>
<td>2.99</td>
<td>25.74</td>
<td>1.32</td>
<td>63.62</td>
</tr>
</tbody>
</table>
Table I-13
Results of the Water Soluble Polysaccharides
From the Pulp of Sample III

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Peak Area</th>
<th>Percent of Total</th>
<th>Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Xylose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>670</td>
<td>360</td>
<td>515</td>
<td>1430</td>
<td>3320</td>
</tr>
<tr>
<td>2</td>
<td>680</td>
<td>430</td>
<td>530</td>
<td>1630</td>
<td>3770</td>
</tr>
<tr>
<td>3</td>
<td>680</td>
<td>370</td>
<td>640</td>
<td>1760</td>
<td>3900</td>
</tr>
<tr>
<td>4</td>
<td>650</td>
<td>320</td>
<td>620</td>
<td>1510</td>
<td>3500</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Xylose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.18</td>
<td>10.84</td>
<td>15.51</td>
<td>43.07</td>
<td>10.39</td>
</tr>
<tr>
<td>2</td>
<td>18.03</td>
<td>11.41</td>
<td>14.06</td>
<td>43.24</td>
<td>13.26</td>
</tr>
<tr>
<td>3</td>
<td>17.45</td>
<td>9.48</td>
<td>16.41</td>
<td>45.12</td>
<td>11.54</td>
</tr>
<tr>
<td>4</td>
<td>18.57</td>
<td>9.14</td>
<td>17.71</td>
<td>43.14</td>
<td>11.43</td>
</tr>
</tbody>
</table>

Average Percent of Total Peak Area

<table>
<thead>
<tr>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Xylose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.56</td>
<td>10.22</td>
<td>15.92</td>
<td>43.64</td>
<td>11.66</td>
</tr>
</tbody>
</table>
Table I-14
Results of the Alcohol Soluble Polysaccharides
From the Pulp of Sample III

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Unknown I</th>
<th>Xylose</th>
<th>Unknown II</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Glucose</th>
<th>Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>365</td>
<td>210</td>
<td>1180</td>
<td>170</td>
<td>2810</td>
<td>4335</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>960</td>
<td>580</td>
<td>2630</td>
<td>205</td>
<td>6360</td>
<td>10,735</td>
</tr>
<tr>
<td>3</td>
<td>65</td>
<td>710</td>
<td>540</td>
<td>2340</td>
<td>140</td>
<td>5530</td>
<td>9325</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Unknown I</th>
<th>Xylose</th>
<th>Percent of Total Peak Area</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>7.71</td>
<td>4.44</td>
<td>24.92</td>
<td>3.59</td>
<td>59.35</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>8.94</td>
<td>5.40</td>
<td>24.50</td>
<td>1.91</td>
<td>59.25</td>
</tr>
<tr>
<td>3</td>
<td>0.69</td>
<td>7.61</td>
<td>5.79</td>
<td>25.10</td>
<td>1.50</td>
<td>59.30</td>
</tr>
</tbody>
</table>

Average Percent of Total Peak Area

<table>
<thead>
<tr>
<th>Unknown I</th>
<th>Xylose</th>
<th>Unknown II</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.69</td>
<td>8.09</td>
<td>5.21</td>
<td>24.84</td>
<td>2.33</td>
<td>59.30</td>
</tr>
</tbody>
</table>
### Table I-15

Results of the Water Soluble Polysaccharides
From the Callus Tissue Sample I

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Xylose</th>
<th>Peak Area</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Glucose</th>
<th>Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1020</td>
<td>826</td>
<td>1589</td>
<td>224</td>
<td>1380</td>
<td>562</td>
<td>5601</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1067</td>
<td>836</td>
<td>1694</td>
<td>224</td>
<td>1440</td>
<td>592</td>
<td>5873</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1005</td>
<td>848</td>
<td>1694</td>
<td>165</td>
<td>1256</td>
<td>448</td>
<td>5416</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Xylose</th>
<th>Percent of Total Peak Area</th>
<th>Galactose</th>
<th>Glucose</th>
<th>Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18.21</td>
<td>14.75</td>
<td>28.37</td>
<td>4.00</td>
<td>24.64</td>
<td>10.03</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>18.17</td>
<td>14.23</td>
<td>28.84</td>
<td>4.15</td>
<td>24.52</td>
<td>10.18</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>18.55</td>
<td>15.66</td>
<td>31.28</td>
<td>3.05</td>
<td>23.19</td>
<td>8.27</td>
<td></td>
</tr>
</tbody>
</table>

Average Percent of Total Peak Area

<table>
<thead>
<tr>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Xylose</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.31</td>
<td>14.88</td>
<td>29.50</td>
<td>3.73</td>
<td>24.12</td>
<td>9.46</td>
</tr>
</tbody>
</table>
Table I-16

Results of the Water Insoluble Polysaccharides
From the Callus Tissue Sample I

<table>
<thead>
<tr>
<th>Run No.</th>
<th>Unknown I</th>
<th>Arabinose</th>
<th>Unknown II</th>
<th>Peak Area</th>
<th>Xylose</th>
<th>Unknown III</th>
<th>Unknown IV</th>
<th>Mannose</th>
<th>Glucose</th>
<th>Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>831</td>
<td>147</td>
<td>1564</td>
<td>694</td>
<td>254</td>
<td>75</td>
<td>80</td>
<td>1594</td>
<td>5239</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>703</td>
<td>184</td>
<td>1502</td>
<td>714</td>
<td>264</td>
<td>100</td>
<td>70</td>
<td>1500</td>
<td>5037</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>679</td>
<td>167</td>
<td>1450</td>
<td>664</td>
<td>229</td>
<td>80</td>
<td>75</td>
<td>1506</td>
<td>4850</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run No.</th>
<th>Unknown I</th>
<th>Arabinose</th>
<th>Unknown II</th>
<th>Xylose</th>
<th>Unknown III</th>
<th>Unknown IV</th>
<th>Mannose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.86</td>
<td>2.80</td>
<td>29.85</td>
<td>13.25</td>
<td>4.85</td>
<td>1.43</td>
<td>1.53</td>
<td>30.43</td>
</tr>
<tr>
<td>2</td>
<td>13.96</td>
<td>3.65</td>
<td>29.82</td>
<td>14.18</td>
<td>5.24</td>
<td>1.99</td>
<td>1.39</td>
<td>29.78</td>
</tr>
<tr>
<td>3</td>
<td>14.00</td>
<td>3.44</td>
<td>29.82</td>
<td>13.69</td>
<td>4.72</td>
<td>1.65</td>
<td>1.55</td>
<td>31.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run No.</th>
<th>Unknown I</th>
<th>Arabinose</th>
<th>Unknown II</th>
<th>Xylose</th>
<th>Unknown III</th>
<th>Unknown IV</th>
<th>Mannose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.60</td>
<td>3.30</td>
<td>29.85</td>
<td>13.71</td>
<td>4.94</td>
<td>1.69</td>
<td>1.49</td>
<td>30.42</td>
</tr>
</tbody>
</table>
Table I-17

Results of the Alcohol Soluble Polysaccharides
From the Callus Tissue Sample I

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Arabinose</th>
<th>Unknown I</th>
<th>Xylose</th>
<th>Peak Area</th>
<th>Unknown II</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Glucose</th>
<th>Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>380</td>
<td>40</td>
<td>130</td>
<td>580</td>
<td>820</td>
<td>0</td>
<td></td>
<td></td>
<td>8870</td>
</tr>
<tr>
<td>2</td>
<td>530</td>
<td>50</td>
<td>220</td>
<td>840</td>
<td>1130</td>
<td>100</td>
<td></td>
<td></td>
<td>10,870</td>
</tr>
<tr>
<td>3</td>
<td>205</td>
<td>60</td>
<td>140</td>
<td>370</td>
<td>580</td>
<td>90</td>
<td></td>
<td></td>
<td>6050</td>
</tr>
<tr>
<td>4</td>
<td>230</td>
<td>30</td>
<td>100</td>
<td>375</td>
<td>680</td>
<td>90</td>
<td></td>
<td></td>
<td>6280</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Arabinose</th>
<th>Unknown I</th>
<th>Xylose</th>
<th>Percent of Total Peak Area</th>
<th>Unknown II</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.51</td>
<td>0.37</td>
<td>1.20</td>
<td>5.35</td>
<td>7.58</td>
<td>0</td>
<td></td>
<td>81.98</td>
</tr>
<tr>
<td>2</td>
<td>3.87</td>
<td>0.36</td>
<td>1.60</td>
<td>6.11</td>
<td>8.22</td>
<td>0.73</td>
<td></td>
<td>79.11</td>
</tr>
<tr>
<td>3</td>
<td>2.74</td>
<td>0.80</td>
<td>1.87</td>
<td>4.94</td>
<td>7.74</td>
<td>1.20</td>
<td></td>
<td>80.72</td>
</tr>
<tr>
<td>4</td>
<td>2.95</td>
<td>0.38</td>
<td>1.28</td>
<td>4.82</td>
<td>8.73</td>
<td>1.16</td>
<td></td>
<td>80.67</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Average Percent of Total Peak Area</th>
<th>Arabinose</th>
<th>Unknown I</th>
<th>Xylose</th>
<th>Unknown II</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.27</td>
<td>0.48</td>
<td>1.49</td>
<td>5.31</td>
<td>8.07</td>
<td>0.77</td>
<td>80.62</td>
</tr>
</tbody>
</table>
a. 17 grams of water soluble polysaccharides (2.00% yield),
b. 14 grams of water insoluble polysaccharides (1.65% yield),
c. 15.4 grams of alcohol soluble polysaccharides (1.81% yield).

(Note: Calcium oxalate can be present in the pulp of the saguaro cactus in concentrations up to 30% of the total dry weight; however, it was not isolated in this experiment.)

The second pulp sample (sample V) was obtained from the same cactus as sample IV. This sample was processed two days after sample IV due to the difficulty of working up two samples at the same time. In the alcohol extract of the pulp from samples IV and V, a quantitative analysis was not possible. The pulp collected in this sample contained a large quantity of unidentifiable sugars. These sugars interfered completely with the analysis. A qualitative analysis did give tentative identifications for rhamnose, ribose, mannose, and glucose. No further attempt was made to identify the unknown sugars. The results of samples IV and V are shown in Tables I-18 to I-21.

Callus Tissue Fraction. Two samples of callus tissue were obtained from the same saguaro cactus harvested on the 23rd of September, 1966. Both samples were worked up according to the procedures on page 15. The following fractions were obtained from 62.7 grams of callus tissue which was extracted in the same manner as the pulp fraction:

a. 0.3 grams of water soluble polysaccharides (0.48% yield),
b. 40.7 grams of water insoluble material (64.71% yield),
c. 3.5 grams of alcohol soluble polysaccharides (5.58% yield).

The 40.7 grams of water insoluble material consisted approximately of
Table I-18

Results of the Water Soluble Polysaccharides From the Pulp of Sample IV

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Peak Area</th>
<th>Galactose</th>
<th>Glucose</th>
<th>Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>398</td>
<td>821</td>
<td>324</td>
<td></td>
<td>1593</td>
</tr>
<tr>
<td>2</td>
<td>169</td>
<td>1097</td>
<td>2442</td>
<td>1104</td>
<td></td>
<td>4812</td>
</tr>
<tr>
<td>3</td>
<td>196</td>
<td>1204</td>
<td>2783</td>
<td>1132</td>
<td></td>
<td>5315</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Percent of Total Peak Area</th>
<th>Percent of Total Peak Area</th>
<th>Percent of Total Peak Area</th>
<th>Percent of Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.51</td>
<td>22.80</td>
<td>50.75</td>
<td>22.94</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3.69</td>
<td>22.65</td>
<td>52.36</td>
<td>21.30</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.21</td>
<td>24.98</td>
<td>51.54</td>
<td>20.34</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rhamnose</th>
<th>Average Percent of Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.45</td>
<td>23.48</td>
</tr>
<tr>
<td></td>
<td>51.55</td>
</tr>
<tr>
<td></td>
<td>21.53</td>
</tr>
</tbody>
</table>
Table I-19
Results of the Water Insoluble Polysaccharides From the Pulp of Sample IV

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Peak Area</th>
<th>Xylose</th>
<th>Galactose</th>
<th>Glucose</th>
<th>Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>85</td>
<td>202</td>
<td>294</td>
<td>786</td>
<td>830</td>
<td></td>
<td>2197</td>
</tr>
<tr>
<td>2</td>
<td>49</td>
<td>316</td>
<td>498</td>
<td>1137</td>
<td>1144</td>
<td></td>
<td>3144</td>
</tr>
<tr>
<td>3</td>
<td>105</td>
<td>204</td>
<td>308</td>
<td>651</td>
<td>714</td>
<td></td>
<td>1982</td>
</tr>
<tr>
<td>4</td>
<td>157</td>
<td>311</td>
<td>465</td>
<td>1004</td>
<td>1012</td>
<td></td>
<td>2949</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Percent of Total Peak Area</th>
<th>Xylose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.87</td>
<td>9.19</td>
<td>13.38</td>
<td>35.78</td>
<td>37.78</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.56</td>
<td>10.05</td>
<td>15.84</td>
<td>36.16</td>
<td>36.39</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5.30</td>
<td>10.29</td>
<td>15.54</td>
<td>32.85</td>
<td>36.02</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5.32</td>
<td>10.55</td>
<td>15.77</td>
<td>34.05</td>
<td>34.32</td>
<td></td>
</tr>
</tbody>
</table>

Average Percent of Total Peak Area

<table>
<thead>
<tr>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Xylose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.01</td>
<td>10.02</td>
<td>15.13</td>
<td>34.71</td>
<td>36.12</td>
</tr>
</tbody>
</table>
Table I-20

Results of the Water Soluble Polysaccharides
From the Pulp of Sample V

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Peak Area</th>
<th>Glucose</th>
<th>Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70</td>
<td>286</td>
<td>828</td>
<td>252</td>
<td>1436</td>
</tr>
<tr>
<td>2</td>
<td>296</td>
<td>1127</td>
<td>3193</td>
<td>1092</td>
<td>5708</td>
</tr>
<tr>
<td>3</td>
<td>346</td>
<td>1204</td>
<td>3332</td>
<td>1065</td>
<td>5947</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Percent of Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rhamnose</td>
</tr>
<tr>
<td>1</td>
<td>4.87</td>
<td>19.92</td>
<td>57.66</td>
</tr>
<tr>
<td>2</td>
<td>5.19</td>
<td>19.74</td>
<td>55.94</td>
</tr>
<tr>
<td>3</td>
<td>5.82</td>
<td>20.25</td>
<td>56.63</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rhamnose</th>
<th>Average Percent of Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rhamnose</td>
</tr>
<tr>
<td>5.29</td>
<td>19.97</td>
</tr>
</tbody>
</table>
Table I-21

Results of the Water Insoluble Polysaccharides From the Pulp of Sample V

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Peak Area</th>
<th>Xylose</th>
<th>Galactose</th>
<th>Glucose</th>
<th>Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>423</td>
<td>898</td>
<td>1067</td>
<td>3220</td>
<td>2944</td>
<td>8552</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>212</td>
<td>433</td>
<td>555</td>
<td>1656</td>
<td>1582</td>
<td>4438</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>132</td>
<td>318</td>
<td>468</td>
<td>1141</td>
<td>1104</td>
<td>3163</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>363</td>
<td>736</td>
<td>876</td>
<td>2502</td>
<td>2328</td>
<td>6805</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Percent of Total Peak Area</th>
<th>Xylose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>495</td>
<td>10.50</td>
<td>12.48</td>
<td>37.65</td>
<td>34.42</td>
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</tr>
<tr>
<td>2</td>
<td>4.78</td>
<td>9.76</td>
<td>12.51</td>
<td>37.31</td>
<td>35.65</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4.17</td>
<td>10.05</td>
<td>14.80</td>
<td>36.07</td>
<td>34.90</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5.33</td>
<td>10.82</td>
<td>12.87</td>
<td>36.77</td>
<td>34.21</td>
<td></td>
</tr>
</tbody>
</table>

Average Percent of Total Peak Area

<table>
<thead>
<tr>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Xylose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.81</td>
<td>10.28</td>
<td>13.13</td>
<td>36.95</td>
<td>34.80</td>
</tr>
</tbody>
</table>
30% lignin and 70% holocellulose. This gives a yield of 28.5 grams of holocellulose or a 45.3% yield. The results on these two samples (samples II and III) are given in Tables 1-22 to I-27.
Table I-22

Results of the Water Soluble Polysaccharides From the Callus Tissue Sample II

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Xylose</th>
<th>Peak Area</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Glucose</th>
<th>Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>147</td>
<td>545</td>
<td>1007</td>
<td>1748</td>
<td>1196</td>
<td>644</td>
<td>5282</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>130</td>
<td>134</td>
<td>751</td>
<td>1336</td>
<td>766</td>
<td>522</td>
<td>3983</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>120</td>
<td>540</td>
<td>602</td>
<td>1338</td>
<td>816</td>
<td>398</td>
<td>3709</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Xylose</th>
<th>Percent of Total Peak Area</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.78</td>
<td>10.22</td>
<td>19.06</td>
<td>33.09</td>
<td>22.64</td>
<td>12.19</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3.26</td>
<td>12.00</td>
<td>18.86</td>
<td>33.54</td>
<td>19.23</td>
<td>13.11</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.24</td>
<td>11.67</td>
<td>16.23</td>
<td>36.07</td>
<td>22.00</td>
<td>10.73</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Average Percent of Total Peak Area</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Xylose</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.09</td>
<td>11.30</td>
<td>18.05</td>
<td>34.23</td>
<td>21.30</td>
<td>12.01</td>
</tr>
</tbody>
</table>
Table I-23

Results of the Water Insoluble Polysaccharides From the Callus Tissue Sample II

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Arabinose</th>
<th>Xylose</th>
<th>Peak Area Mannose</th>
<th>Glucose</th>
<th>Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>162</td>
<td>2698</td>
<td>346</td>
<td>5980</td>
<td>9186</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>1266</td>
<td>204</td>
<td>2708</td>
<td>4258</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>1565</td>
<td>167</td>
<td>2708</td>
<td>4470</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Arabinose</th>
<th>Percent of Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arabinose</td>
<td>Xylose</td>
</tr>
<tr>
<td>1</td>
<td>1.76</td>
<td>29.37</td>
</tr>
<tr>
<td>2</td>
<td>1.88</td>
<td>29.73</td>
</tr>
<tr>
<td>3</td>
<td>2.01</td>
<td>33.67</td>
</tr>
</tbody>
</table>

Average Percent of Total Peak Area

<table>
<thead>
<tr>
<th>Arabinose</th>
<th>Xylose</th>
<th>Mannose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.88</td>
<td>30.92</td>
<td>4.10</td>
<td>63.09</td>
</tr>
</tbody>
</table>
Table I-24

Results of the Alcohol Soluble Polysaccharides
From the Callus Tissue Sample II

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Peak Area</th>
<th>Unknown I</th>
<th>Glucose</th>
<th>Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>1580</td>
<td>1925</td>
<td>3505</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>1241</td>
<td>1390</td>
<td>2631</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Percent of the Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unknown I</td>
</tr>
<tr>
<td>1</td>
<td>45.08</td>
</tr>
<tr>
<td>2</td>
<td>47.17</td>
</tr>
</tbody>
</table>

Average Percent of the Total Peak Area

<table>
<thead>
<tr>
<th>Unknown I</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>46.13</td>
<td>53.89</td>
</tr>
</tbody>
</table>
Table I-25
Results of the Water Soluble Polysaccharides
From the Callus Tissue Sample III

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Peak Area</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Glucose</th>
<th>Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>480</td>
<td>1070</td>
<td>1260</td>
<td>1910</td>
<td>3200</td>
<td>1800</td>
<td>9720</td>
</tr>
<tr>
<td>2</td>
<td>150</td>
<td>650</td>
<td>1010</td>
<td>1140</td>
<td>2130</td>
<td>960</td>
<td>6040</td>
</tr>
<tr>
<td>3</td>
<td>290</td>
<td>710</td>
<td>750</td>
<td>1100</td>
<td>1900</td>
<td>800</td>
<td>5550</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Percent of Total Peak Area</th>
<th>Xylose</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.94</td>
<td>11.01</td>
<td>12.96</td>
<td>19.65</td>
<td>32.92</td>
<td>18.52</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.48</td>
<td>10.76</td>
<td>16.72</td>
<td>18.87</td>
<td>35.26</td>
<td>15.89</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5.23</td>
<td>12.79</td>
<td>13.51</td>
<td>18.01</td>
<td>34.23</td>
<td>14.41</td>
<td></td>
</tr>
</tbody>
</table>

Average Percent of Total Peak Area

<table>
<thead>
<tr>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Xylose</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.22</td>
<td>11.52</td>
<td>14.40</td>
<td>18.84</td>
<td>34.14</td>
<td>16.27</td>
</tr>
</tbody>
</table>
Table I-26

Results of the Water Insoluble Polysaccharides
From the Callus Tissue Sample III

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Xylose Peak Area</th>
<th>Glucose Peak Area</th>
<th>Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1070</td>
<td>3200</td>
<td>4270</td>
</tr>
<tr>
<td>2</td>
<td>1870</td>
<td>4970</td>
<td>6840</td>
</tr>
<tr>
<td>3</td>
<td>1350</td>
<td>3611</td>
<td>4961</td>
</tr>
<tr>
<td>4</td>
<td>1600</td>
<td>4400</td>
<td>6000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Percent of Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Xylose</td>
</tr>
<tr>
<td>1</td>
<td>25.05</td>
</tr>
<tr>
<td>2</td>
<td>27.34</td>
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<tr>
<td>3</td>
<td>27.21</td>
</tr>
<tr>
<td>4</td>
<td>26.67</td>
</tr>
</tbody>
</table>

Average Percent of Total Peak Area

<table>
<thead>
<tr>
<th></th>
<th>Xylose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>26.57</td>
<td>73.43</td>
<td></td>
</tr>
</tbody>
</table>
Table I-27
Results of the Alcohol Soluble Polysaccharides
From the Callus Tissue Sample III

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Peak Area</th>
<th></th>
<th>Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unknown I</td>
<td>Glucose</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2740</td>
<td>5698</td>
<td>8430</td>
</tr>
<tr>
<td>2</td>
<td>2470</td>
<td>4930</td>
<td>7400</td>
</tr>
<tr>
<td>3</td>
<td>2900</td>
<td>5370</td>
<td>8270</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Run Number</th>
<th>Percent of the Total Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unknown I</td>
</tr>
<tr>
<td>1</td>
<td>32.50</td>
</tr>
<tr>
<td>2</td>
<td>33.38</td>
</tr>
<tr>
<td>3</td>
<td>35.06</td>
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</tbody>
</table>

Average Percent of the Total Peak Area

<table>
<thead>
<tr>
<th>Unknown I</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>33.65</td>
<td>66.35</td>
</tr>
</tbody>
</table>
DISCUSSION

Procedures for the isolation and identification of polysaccharides are diverse and many times are designed to suit only the polysaccharide under investigation. In characterizing polysaccharides, two problems are extremely important: (1) isolation of a pure polysaccharide, and (2) minimization of changes in the sugar during the isolation and identification.

The purity of the isolated polysaccharide is extremely difficult to establish. The purity with respect to inorganic salts and protein present can be easily determined. The purity in the case where there exist two or more sugars in the polysaccharide is much harder to determine. It must be determined, for example, whether the isolated material consists of two distinct polymers, each based on a single sugar, or if the sugars are chemically combined in a single polymer. In many cases this question can be answered by partial degradation of the polysaccharide to yield disaccharides. If each disaccharide is homogeneous with respect to the other disaccharide, then two distinct polymers, each based on a single sugar, could be assumed. For example, if a polysaccharide containing both glucose and galactose was hydrolyzed to disaccharides, the expected results would be three kinds of disaccharides. The first disaccharide would be composed of two glucose units; the second would be composed of two galactose units; and the third would be composed of one glucose unit and one galactose unit. If there were an absence of a disaccharide containing both a glucose and a galactose unit, then the
polysaccharide was in reality two distinct polymers. One polysaccharide would be a pure glucan and the other a pure galactan.

Under normal conditions it is desirable to purify polysaccharides by fractional precipitation. This can be done by using the difference in solubility of different polysaccharides in water-organic solvent mixtures or by varying the pH of the aqueous media. Schlubach and Peitzner found that they had to precipitate a polysaccharide called tricitin 300 times before being able to obtain a constant optical rotation. Other methods of precipitation involve complexing the polysaccharides out of solution. For example, starch can be isolated by being precipitated as the iodine complex. Amylose can be separated from amylpectin by forming insoluble complexes with a number of compounds. The mannan in yeast can be precipitated from solution with copper. Electrophoresis can be used to fractionate a polymer or charged polysaccharide from a neutral polysaccharide.

During hydrolyses of polysaccharides, structural changes frequently occur. Polyfructoses require extremely mild treatment since fructose is rapidly destroyed by strong acids. Barium sulfate must be used with caution. Laidlaw and Reid found that one can get epimerization of some sugars with barium sulfate. Also in the uronic acid fractions of the polymer, there is always the danger of not getting complete hydrolysis with 1% sulfuric acid. Usually a much greater concentration of acid is needed which may lead to extensive degradation of the sugars present.

The problems of analysis of the polysaccharides vary directly with the complexity of the polysaccharide present. If there exist
pentosans in combination with cellulose, there will occur some degradation of the pentoses with 72% sulfuric acid. From previous work by Simonescu, it seems apparent that hexoses play an important part in the biosynthesis of the lignin material. Therefore, in this investigation we chose to use the more strenuous conditions of 72% sulfuric acid to insure the hydrolysis of the hexoses at the expense of some of the pentoses present.

To determine the comparative degradation of pentoses and hexoses, the arabinogalactan, Stractan AF #2, was hydrolyzed with 72% sulfuric acid. Analysis showed an average percentage of 15% arabinose (see page 22). The company that produces the Stractan AF #2 reports that the polymer is in a 1:6 ratio of arabinose to galactose or 12.2% arabinose. So, in this case, the destruction of the pentose has not taken place.

The percentages of each sugar in the water soluble and water insoluble polysaccharide fraction for both the pulp and callus tissue are shown in Figure 1-7. Galactose appears to undergo the most significant change from healthy to wound tissue. The comparisons are made on the dry weights. The saguaro pulp is on the average 90-95% water so the analysis on page 32 would show that the 851 grams consists of 85.1 grams of solid material (using 90% water). Of this 85.1 grams, 46.1 grams of recovered polysaccharides were obtained, giving a 54.5% yield. The water soluble polysaccharides in the pulp consist of 17 grams, of which 51.5% (8.9 grams) is galactose. The water insoluble polysaccharide in the pulp consists of 14 grams, of which 34.7% (4.86 grams) is galactose. Since the alcohol soluble fraction didn't give a good analysis due to its complexity, it will be estimated from sample III where galactose
Figure I-7.
Graph of the Constituents of the Water Soluble and Water Insoluble Fractions for Both the Pulp and Callus Tissue
was found to be 2-3% of the total fraction (page 37). The alcohol soluble fraction was found to be 15.4 grams which would give 0.35 grams of galactose. The total yield of galactose is 14.1 grams, or 16.6% of the total weight of solids is galactose in the pulp.

In the callus, galactose does not appear in the water soluble polysaccharides, but this is only 0.48% of the callus material. The water insoluble material of the callus is composed of 27.5% lignin and 72.5% holocellulose. In this material, which accounts for 64.7% of the total weight, there has been found two uronic acids by Steelink et al. One uronic acid was not identified; the other was galacturonic acid. This accounts for some of the disappearance of the galactose as galacturonic acid. At this time, it is very hard to be sure whether all of the galactose is transformed into uronic acids or if a portion is being incorporated into lignin.

Also, during the investigative TLC work done by Steelink and Riser, sucrose was found in some of the samples, mainly in the alcohol soluble portions of the pulp. Sucrose yields on reduction mannitol and glucitol in equal amounts. Therefore, a correction factor should be made for the sucrose present to obtain an accurate analysis. In the saguaro, however, the alcohol extract is the least significant in terms of meaningful data, and the sucrose concentration was not determined.

Further experiments to resolve whether galactose is involved in lignin formation could be done by radioactive tracer studies. A solution of radioactive galactose can either be injected into or painted on the wounded area of the cactus. After callus tissue formation, both the lignin and holocellulose would be investigated to see where the
radioactivity is incorporated. If galactose is being transformed into lignin, then a method for studying the biosynthetic steps in the conversion is found.

Also, further investigations of the pentose to hexose ratio would be interesting. Simonescu reported a decrease in pentose in the crown gall tumor of plum trees. A slight decrease in the pentose to hexose ratio was noted in the saguaro callus compared to the pulp. However, the strenuous hydrolysis conditions may account for this difference. The pentosans should be hydrolyzed under much milder conditions and then an accurate check could be made on this ratio.

In conclusion, the sugars found in the saguaro cactus pulp are listed as follows: The alcohol soluble polysaccharides contained xylose (5.42%), unknown II (2.99%), mannose (25.74%), galactose (1.32%), and glucose (63.62%). The water soluble polysaccharides contained rhamnose (5.38%), arabinose (20.30%), galactose (55.05%), and glucose (19.26%). The water insoluble polysaccharides contained rhamnose (4.81%), arabinose (10.28%), xylose (13.13%), galactose (36.95%), and glucose (34.80%).

The sugars found in the saguaro cactus callus are listed as follows: The alcohol soluble polysaccharides contained unknown I (33.65%) and glucose (66.35%). The water soluble polysaccharides contained rhamnose (4.22%), arabinose (11.52%), xylose (14.40%), mannose (18.84%), galactose (34.14%), and glucose (16.27%). The water insoluble polysaccharides contained xylose (26.57%) and glucose (74.43%).
PART II

IDENTIFICATION OF THE MONOTERPENOIDs IN THE ESSENTIAL OIL
OF SAND SAGE BRUSH ARTEMISIA FILIFOLIA (TORR.)
INTRODUCTION

Historical Background

Essential oils, the volatile odoriferous substances found in many plants, have been used for thousands of years and were known to the ancient Greeks. With the advent of modern chemistry, the essential oils were found to be complex mixtures of compounds. In general, the essential oils contain acyclic, alicyclic, aromatic, and heterocyclic compounds. The compounds may be classified generally as: (a) nitrogen and sulfur containing compounds, (b) aromatic compounds, (c) terpenes, and (d) miscellaneous compounds, including unbranched, long-chain compounds. Terpenes usually consist of two or more isoprene units combined together, which contain carbon and hydrogen or carbon, hydrogen, and oxygen.

All terpenes are derived from a common biosynthetic pathway in the beginning stages as shown in Figure II-1. From geranyl pyrophosphate, the biosynthesis of the rest of the terpenes can proceed as shown in Figure II-2.

Statement of the Problem

Artemisia species occur in great number in the Southwestern region. Artemisia tridentata or common sagebrush, the state flower of Nevada, is perhaps the best known. Other Artemisia species, Artemisia frigida, Artemisia filifolia, Artemisia bigelovii, and Artemisia spinos-cens are valuable as browse plants, although some can be toxic if eaten
Figure II-1. Biosynthetic Pathways for the Synthesis of Terpenes
Figure II-2. Biosynthetic Pathways for Higher Terpenes
in excess. Early white settlers and Indians used *Artemisia filifolia*, *Artemisia frigida*, and *Artemisia tridentata* for medicinal purposes. The leaves of *Artemisia frigida* were used by Hopi Indians to flavor roasted sweet corn.\(^5\)

In the course of an investigation of terpene-bearing plants of the Southwest,\(^6,7\) it was found that *A. filifolia* had a large, steam distillable terpene content. *Artemisia filifolia* had a distinctive odor, and its use by Indians and early white settlers for medicinal purposes was of great interest. Preliminary spectrophotometric investigations indicated that some unusual structures were represented in the oil.

**Approach to the Problem**

The purpose of this study was to identify the constituents of the oil and to deduce biosynthetic and taxonomic relations therefrom. The investigation of the plant was limited to just the essential oils from the leaves and stems. The heavier branches and the roots were not investigated for essential oils. Vapor phase chromatography, N.M.R., and I.R. were used for identification. Derivatives were then prepared for a complete confirmation of each compound.
EXPERIMENTAL

Methods and Materials

Apparatus

Melting points were determined in capillary tubes with a Mel-Temp apparatus. A Perkin Elmer Infracord Model 137B spectrophotometer was used for infrared spectra. An A-60 Varian N.M.R. was used for all N.M.R. spectra. Gas chromatography was carried out on an Aerograph instrument and also on an F & M Model 609 flame ionization gas chromatograph equipped with a disc integrator. All VPC work was carried out at column temperatures between 140° and 190°. The main gas chromatographic column used was a 3/8 in. o.d. x 12 ft. copper tube packed with 20% Carbowax 20-M on a 30/60 mesh gas Chrom R support in series with a 1/4 in. o.d. x 12 ft. copper tube packed with 20% Carbowax 20-M on a 30/60 mesh gas Chrom R support. All optical rotations were taken on a Bendix Automatic polarimeter Type 143A equipped with a 500 watt light source and a filter to isolate the sodium D line.

Procedures

Extraction of Sample. Artemisia filifolia (Torr.) was collected about 2 miles east of Wilcox, Arizona, several times during June and July, 1966. The feathery leaves and stems were collected, leaving the main trunk and roots. The whole wet plant was placed in a ten-gallon can fitted with an outlet tube. Water was added to cover the plants. The can was heated with a bunsen burner. The outlet was connected to a
series of two condensers and the distillate collected in an ice-cooled beaker. Salt was added to the distillate and several extractions were made with petroleum ether (b.p. 30-60°). A 0.52% yield of yellow oil was obtained from the leaves and stems.

**Analysis of Sample.** Inadequate separation was obtained with thin layer chromatography, although a variety of different solvents were investigated. Vapor phase chromatography of the oil at 190° on a Carbowax column gave a reasonable separation of the injected oil into four major compounds. Collection of the samples was carried out on an Aerograph using the above mentioned column. Reinjections of the fractions collected gave quite pure compounds for spectral analysis. A typical VPC chromatogram is shown in Figure II-3.

**Results**

**Compound I**

The infrared spectrum of the purified material called compound I is shown in Figure II-4. The most significant bands in the I.R. spectrum of this compound were a doublet at 1385 cm.⁻¹ signifying a gem dimethyl grouping and the ether bands at 1200 cm.⁻¹. The N.M.R. of this compound showed three protons at 9.05 T, 6 protons at 8.9 T and 9 protons at 8.3 T. These spectral results are identical to those of 1,8-cineole whose I.R. spectra appears in Figure II-5. Figure II-6 shows the structure of 1,8-cineole.

Preparation of an oxonium salt was attempted on both the compound I and 1,8-cineole. Several types of derivatives were tried and found unsatisfactory. The o-cresol derivative of 1,8-cineole was then
Figure II-3. VPC Chromatogram of Essential Oils
Figure II-4. Infrared Spectrum of Compound I

Figure II-5. Infrared Spectrum of 1,8-Cineole
Figure II-4.

Figure II-5.
made by shaking an alcohol solution of o-cresol with 1,8-cineole. A melting point of 54-55° was obtained. The o-cresol derivative for the unknown was made and a melting point of 54-55° was obtained. The mixed melting point of the two derivatives was not depressed and the unknown was identified as 1,8-cineole.

**Compound II**

The infrared spectrum of the purified material called compound II is shown in Figure II-8 (see page 70). Several significant bands were found in the I.R. spectrum. The bands at 1650 cm.⁻¹ and at 810 cm.⁻¹ indicate that a tri-substituted ethylene group is present. The doublet band at 1385 cm.⁻¹ indicates the presence of a gem dimethyl grouping. The N.M.R. spectrum, using TMS as an internal standard, gave a doublet at 9°C containing 6 protons. There were 5 protons at 8.3°C to 8.5°C, 2 protons at 7.5°C, and 1 proton at 4.7°C. Two possibilities for this spectral data are shown in Figure 7.
The author is deeply indebted to Dr. S. Pakniker* for his interpretation of the spectrum as compound IIb and also for the pure sample of compound IIb that he furnished.** The infrared spectrum of compound IIb (Figure II-9) and its N.M.R. spectrum were identical to the spectra of the compound II. The retention times on a 24 ft. 20% Carbowax column and also on a 10 ft. 20% Carbowax column of both compounds were identical.

The semicarbazone of both the compound II and compound IIb were made. The melting points of each were 193°. The mixed melting point produced no depression. Compound II was assigned the structure IIb.

The optical rotation of compound IIb was taken. Compound IIb, 0.056 grams, was taken up in 5 ml. of absolute ethanol. The observed rotation was = - .210°. From the formula, \[ \alpha^o = 100/\text{L.C} \], where \( \alpha \) is the observed rotation, - .210°, L is the length of the cell, 0.1 decimeter, and C is concentration, 1.116 grams/100 ml.; an optical rotation

*Postdoctoral fellow under Dr. R. Bates at University of Arizona, 1965-66.

**Compound IIb was prepared according to the procedure given by J. J. Beereboom, J. Org. Chem., 30, 4230 (1965).
Figure II-8. Infrared Spectrum of Compound II

Figure II-9. Infrared Spectrum of Compound IIb
Figure II-8.

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.

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Figure II-9.

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.
of -188.2° was calculated. The correction factor for the instrument was 
\[ \alpha = \alpha^\circ (C)(L)(1.035) \]. This gives the corrected optical rotation as 
-181.8°. The rotation \(^8\) for the pure isomer would be -307° so the com-
pound is partially racemic. For the sake of convenience this compound 
IIb will be called filifolone through the rest of the thesis.

The absolute configuration of filifolone was determined by re-
acting it with 20% alcoholic potassium hydroxide to form \(\alpha\)-fencholenic 
acid. \(^9\) An optical rotation, \(^{10,11}\) \(\alpha = -9.1^\circ\), was found which denotes 
the structure shown in Figure II-10. Therefore, filifolone has the 
structure shown in Figure II-10.

![Figure II-10. Stereochemistry of \(\alpha\)-Fencholenic Acid and Filifolone](image)

**Compound III**

The infrared spectrum of the compound III is shown in Figure 
II-11. Several significant bands were found in the infrared spectrum. 
The band at 1740 cm.\(^{-1}\) signifies a strained ring system, probably a 
cyclopentanone. The doublet at 1385 cm.\(^{-1}\) signifies that a gem dimethyl 
grouping is present. The N.M.R. spectrum using TMS as an internal 
standard showed 2 peaks at 9\(\tau\) containing 9 protons. At 8.4\(\tau\), 5
Figure II-11. Infrared Spectrum of Compound III

Figure II-12. Infrared Spectrum of Camphor
Figure II-11.

COMPOUND III

PHASE — NUJOL

Figure II-12.

CAMP'HOR

PHASE — NUJOL
protons were located and at 7.6T, 2 protons were found. Compound III was also a solid and was melted in a sealed capillary tube at 176-177°. This spectral data is identical to that of camphor whose I.R. is given in Figure II-12, page 72.

Preparation of the oxime of camphor and compound III was undertaken. The melting points of both were identical at 116°. The mixed melting point of the two compounds was 115.5-117°. The semicarbazone of both compounds was prepared and both gave identical melting points of 237-241°. The mixed melting point of the two compounds was 237-241°. Compound III was identified as camphor (Figure II-13).

![Figure II-13. Structure of Camphor](image)

The optical rotation of compound III was taken and found to be -42.2°. The corrected optical rotation is then -40.8°. The rotation for pure l-camphor is -44.26° at 20°; therefore, compound III is l-camphor.

**Compound IV**

The infrared spectrum of compound IV is shown in Figure II-14. Several significant bands were found in the infrared spectrum. The
Figure II-14. Infrared Spectrum of Compound IV

Figure II-15. Infrared Spectrum of Isophorone
Figure II-14

Figure II-15
bands at 1670 cm.\(^{-1}\) and 1635 cm.\(^{-1}\) signified a conjugated ketone. The doublet band at 1385 cm.\(^{-1}\) signified a gem dimethyl grouping and the band at 820 cm.\(^{-1}\) signified a trisubstituted ethylene grouping. The N.M.R. spectrum, using TMS as an internal standard, showed a singlet at 9\(\tau\) containing 6 protons. At 8.3\(\tau\), there occurred 3 protons and at approximately 8\(\tau\), 4 more protons were located. One proton was found at 4.3\(\tau\). This spectral data was found to be identical to that of isophorone, whose infrared spectrum is given in Figure II-15 (see page 74).

The semicarbazones of isophorone and compound IV were prepared and both were found to melt at 184-185°. The mixed melting point was not depressed. The VPC retention times on a 24 ft. 20% Carbowax column for both compounds were identical. The unknown IV was identified as isophorone. The structure is shown in Figure II-16.

![Figure II-16. Structure of Compound IV](image)

A quantitative determination was carried out on the essential oil which boiled below 120° at 30 mm. All work was done on an F & M gas chromatograph equipped with a disc integrator. Cineole, filifolone,
Camphor and isophorone were found to be present in the respective percentages: 34.25%, 11.10%, 13.52%, and 40.88%.
DISCUSSION

The finding of camphor and 1,8-cineole in *Artemisia filifolia* is not unexpected. A large number of Artemisia species have been found which contain these two compounds and may account for the widespread use in folk medicine. The other two compounds, isophorone and filifolone, are both unusual and rare terpenoid type compounds.

The compound filifolone is an extremely rare terpene. It has been found only once before in the species *Zieria smithii* in Australia.\textsuperscript{12,13,14} The compound in *Zieria smithii* differs only in the optical rotation, $\alpha = +307^\circ$ from that found in *Artemisia filifolia*, $\alpha = -182^\circ$.

Isophorone, a common synthetic product derived from acetone, has not been found in nature before. The fact that it is a C\textsubscript{9} compound instead of a C\textsubscript{10} compound means that a decarboxylation step, possibly from $\alpha$-cyclocitral, is involved. The term terpene is used to describe a compound which is a constituent of an essential oil and which contains carbon and hydrogen or carbon, hydrogen, and oxygen and is not aromatic in character.\textsuperscript{15} In this respect, isophorone can be classified as a terpene.

A possible biosynthetic pathway to account for the occurrence of these compounds in nature is shown in Figure II-17. There is evidence for at least one more cyclobutanone compound and possibly more in the essential oil that are yet to be identified. In view of the rare compounds found, a further investigation of the extractives should prove interesting. In particular, the biosynthesis of the unusual isophorone
Figure II-17. Possible Biosynthetic Pathway for the Four Terpenes Found in the Essential Oil of *Artemisia filifolia*
fraction, by $^{14}C$ labelling experiments, should be illuminating. Also, since citral may be a common intermediate for isophorone and filifolone, its role in the biosynthetic sequence should be evaluated.
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