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(ENGELMAN) FAMILY LILIACEAE.

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A PHYTOCHEMICAL INVESTIGATION OF YUCCA SCHOTTII
(ENGEIMAN) FAMILY LILIACEAE

by

Ronald Charles Backer

A Dissertation Submitted to the Faculty of the
COLLEGE OF PHARMACY
In Partial Fulfillment of the Requirements
For the Degree of
DOCTOR OF PHILOSOPHY
WITH A MAJOR IN PHARMACEUTICAL CHEMISTRY
In the Graduate College
THE UNIVERSITY OF ARIZONA

1970
I hereby recommend that this dissertation prepared under my direction by RONALD CHARLES BACKER entitled A PHYTOCHEMICAL INVESTIGATION OF YUCCA SCHOTTII (ENGELMAN) FAMILY LILIACEAE be accepted as fulfilling the dissertation requirement of the degree of Doctor of Philosophy.

Dissertation Director

Date

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SIGNED: Ronald Becker
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In the course of a continuing search for more active anti-inflammatory substances, the saponin-containing fraction of the leaves of *Yucca schottii* was found to possess significant activity against the Carrageenin-induced Edema Test. The saponin fraction has been investigated and the isolation and characterization of the constituents have been accomplished.

The active anti-inflammatory butanol fraction, obtained by partitioning a water extract with butanol, formed a persistent foam on shaking and showed appreciable hemolytic activity. These properties indicated that the fraction was probably saponin as supported by the infrared spectrum which showed typical saponin absorptions.

Preliminary investigations of the sapogenins were carried out utilizing thin-layer chromatography. Hecogenin, kammogenin, tigogenin, and yuccagenin were isolated from an acid hydrolyzed ethanolic extract of the leaves. They were identified by their melting points and their infrared and mass spectra.

The active fraction contained seven saponins which were separated by thin-layer and column chromatography of their acetates. The parent saponins were regenerated by alkaline hydrolysis of their acetates.
Each of the seven saponins was separated into its aglycone and sugar moiety by acid hydrolysis. The aglycone of saponins 1-5 and 7 was yuccagenin. The sugar moiety associated with each of these saponins was identified by thin-layer and gas-liquid chromatography of its trimethylsilyl ether to be galactose. Saponin 6 possesses a ketone group as indicated by its infrared spectrum. Its aglycone was identified as kammogenin. The sugar moiety associated with it was identified by thin-layer and gas-liquid chromatography to be 2-deoxyribose.

A preparative isolation procedure, utilizing the water soluble Girard derivative formed by reacting the saponin acetate mixture with Girard Reagent "T", was developed for the ketone-containing saponin 6. The Girard derivative was decomposed by refluxing with hydrochloric acid to regenerate saponin acetate 6. The acetate, when subjected to alkaline hydrolysis, yielded saponin 6.

A carbon-hydrogen determination indicated that saponin 6 contains 5 moles of 2-deoxyribose and its molecular weight is 1025. Its molecular formula is C52H80O20. Methylation of saponin 6 and subsequent acid hydrolysis yielded 2-methoxykammogenin. The glycosidic linkage occurs at the 3 position of kammogenin as further indicated by a series of oxidation reactions, Oppenauer and Jones, and isomerizations to yield $\Delta^4$-3-keto-2-methoxykammogenin.
The active fraction has been shown to contain seven saponins. Six of these have yuccagenin as their aglycone and galactose as their sugar moiety. The other has kammo- genin as its aglycone and 5 moles of 2-deoxyribose attached at the 3 position as its sugar moiety. The saponins of the active fraction are to be subjected to further pharmacological studies to evaluate their potential use as anti-inflammatory agents.
CHAPTER 1

INTRODUCTION

Yucca schottii, family Liliaceae, also known by the common name Hoary yucca, is a plant native to the Southwestern United States. Historically, yucca plants have served as an important basic material for the Indians of the Southwest. Fiber from the leaves was used to produce rope, mats, baskets, cloth, and sandals. The roots, referred to as amole, have a saponifying action and were used by the Indians as a type of soap and as a laxative. The buds, flowers, and stalks were boiled, roasted, or eaten raw. A fermented beverage was made from the fruit.

In tests performed on various extracts of the ground leaves of Yucca schottii at The University of Arizona, College of Pharmacy, Tucson, Arizona, it was discovered that the saponin-containing fraction of the plant showed anti-inflammatory properties, specifically against carrageenin-induced edema. An investigation of this fraction was begun in order to attempt to identify the material responsible for the anti-inflammatory activity.
History of Anti-inflammatory Testing

Preliminary extracts of Yucca schottii were prepared and tested by Dr. Mary Caldwell, et al., of The University of Arizona, College of Pharmacy, Tucson, Arizona. Botanical identification was confirmed by Robert Barr, Research Associate at The University of Arizona. This experiment was done as part of a project to screen plants indigenous to Arizona and the Southwest for their possible anti-inflammatory properties.

Carrageenin-induced Edema Testing Method

To test the anti-inflammatory properties of the plant extracts, young adult male Holtzman rats of 125 to 165 g body weight were used. Extracts were administered in aqueous suspension by gastric gavage. Controls received only tap water. This treatment was given 1 hour before injection of the phlogistic agent, carrageenin, into the foot. Carrageenin, an extract of Chondrus obtained from Algin Corporation of America, was prepared as a 1% suspension in a 0.9% sodium chloride solution. A volume of 0.05 ml was injected into the plantar tissue of the right hind paw. Immediately thereafter, the volume of the injected foot was measured. The method of measuring the volume of the injected foot was by immersing it in mercury exactly to
an ink mark on the skin over the lateral malleolus. The mercury column was connected to a galvanometer. The galvanometer was calibrated in terms of milliliter displacement of mercury. The method is reproducible and very rapid.

Swelling of the paw reaches a peak in 3 to 5 hours, then it retains about the same degree of edema for several hours. For extract testing, increase in foot volume 3 hours after the injection of the phlogistic agent was adopted as a measure of effect. The preliminary water extracts were lyophilized and tested for anti-inflammatory properties. They were tested in doses of 3.5, 4.5, and 5.5 g/kg. The test results are summarized in Table I. At all three dose levels there was a significant level of activity.

Due to the positive test results obtained on Yucca schottii, further fractionation studies were pursued, both from a purely phytochemical viewpoint, and also if possible, to isolate the anti-inflammatory constituent present in the plant.

A literature search of Yucca schottii revealed the fact that this plant contains steroidal saponins. Prior to this investigation the only saponin isolated from the leaves of Yucca schottii was kammonin (Fig. 1a). When subjected to acid hydrolysis, kammonin yielded the aglycone, kammogenin (Fig. 1b) and an unidentified sugar moiety.
Table I. Preliminary Anti-inflammatory Testing

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<tr>
<th>Plant Extract</th>
<th>No. Controls</th>
<th>No. Test Animals</th>
<th>Dose (g/kg)</th>
<th>Average % Inhibition</th>
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</thead>
<tbody>
<tr>
<td>H₂O Soluble</td>
<td>3</td>
<td>3</td>
<td>3.5</td>
<td>44</td>
</tr>
<tr>
<td>H₂O Soluble</td>
<td>3</td>
<td>3</td>
<td>4.5</td>
<td>77</td>
</tr>
<tr>
<td>H₂O Soluble</td>
<td>3</td>
<td>3</td>
<td>5.5</td>
<td>90</td>
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</table>
Fig. 1a. Proposed Structure of Kammonin
R = Sugar Units

Fig. 1b. Kammogenin
It has been established previously that triterpenoid saponins possess anti-inflammatory properties. Therefore, an investigation of the saponin fraction was pursued as the possible source of the anti-inflammatory activity.

**Saponin Extraction**

A 10 lb. sample of the dried leaves of the plant was ground into a meal-like material in a Wiley mill equipped with a 4-mm size screen. The dry ground material was macerated with hot water for 3 hours. A dark green extract was obtained which displayed foaming properties, indicating the probable presence of saponins. The water extract was concentrated by air evaporation from a volume of 4 l. to 1 l.

The concentrated water extract was partitioned in a separatory funnel with butanol. The butanol extract was washed with water until the final washing gave a negative Benedict's test, indicating that all reducing sugars had been removed from the butanol extract. The butanol extract was dried by air evaporation. The butanol insoluble fraction was lyophilized (Fig. 2).

The dried butanol extract and the lyophilized butanol insoluble fraction were tested at The University of Arizona, College of Pharmacy, Tucson, Arizona for anti-inflammatory properties by means of the Carrageenin-induced
Dried Leaves

Water

Marc (Discard)

BuOH Insoluble

Lyophilized

BuOH Insoluble Extract

BuOH Soluble

1. Wash with water until negative to Benedict's solution

2. Evaporate to dryness

BuOH Soluble Extract

Fig. 2. Saponin Extraction
Edema Test. The butanol insoluble fraction was tested at a dose of 500 mg/kg. The butanol soluble extract was tested at doses of 500 mg/kg and 5 g/kg. The results are summarized in Table II. All the anti-inflammatory activity was present in the butanol soluble extract.

**Preliminary Chemical Analysis**

The butanol extract was subjected to a series of qualitative tests in order to obtain some information about its chemical nature. The following tests were employed:

1. **Ferric Chloride Test.** One drop of a 10% ferric chloride solution was added to 0.5 g of the butanol extract in 5 ml of ethanol. The lack of formation of a blue-black color indicated that phenols were probably not present.

2. **Liebermann-Burchard Test.** A 0.5 g sample of the butanol extract dissolved in ethanol was treated with 5 drops of acetic anhydride and 2 drops of sulfuric acid. Color changes occurred indicating the probable presence of steroids.

3. **Mayer's Test.** Reagents:
   a. Mercuric Chloride (1.35 g in 60 ml of water)
   b. Potassium Iodide (3.0 g in 10 ml of water)
Table II. Anti-inflammatory Testing of Butanol Soluble and Insoluble Fractions

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<th>No. Test Animals</th>
<th>Dose g/kg</th>
<th>Average % Inhibition</th>
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<td>3</td>
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<td>-47</td>
</tr>
<tr>
<td>BuOH Soluble</td>
<td>9</td>
<td>9</td>
<td>0.5</td>
<td>32</td>
</tr>
<tr>
<td>BuOH Soluble</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>99</td>
</tr>
</tbody>
</table>
The two solutions were mixed and water was added to make 100 ml of solution. Five milliliters of the test solution was added to 0.5 g of the butanol extract in 5 ml of water to which one drop of 6 N hydrochloric acid had been added. No precipitate formed, indicating the probable absence of alkaloids.

4. **Solubility Test.** The extract was found to be soluble in water, ethanol, butanol, and pyridine. It was insoluble in all other common non-polar solvents.

5. **Saponin Hemolysis.** The butanol extract was chromatographed on two thin-layer plates (prepared with Silica Gel G obtained from Brinkman Instruments, Inc.) using the chromatographic system chloroform: methanol:water (65:25:10 lower phase only) (System 1). A piece of #1 Whatman paper was attached to the top of each thin-layer plate and folded over the back in order to extend the developing time of the chromatogram. The chromatograms were removed after 24 hours and allowed to air dry. They were visualized in the following manner:

   Chromatogram A was visualized by means of charring with ceric sulfate. The developed
chromatogram revealed that there were seven major components in the butanol extract (Fig. 3).

Chromatogram B was developed with a blood-gelatin solution. A 0.9% sodium chloride solution (100 ml) was added to 4.5 g of gelatin powder, and allowed to stand at room temperature for 30 minutes. The mixture was heated to 80°C, in a water bath. The solution was cooled to 45°C and 6 ml of cow's blood was added. The blood-gelatin suspension was poured onto chromatogram B. It was left in a horizontal position until the film had set. The film became transparent in the area corresponding to the seven components visualized in chromatogram A. Since all seven major substances caused the hemolysis of red blood cells, it was assumed that they were probably saponins.

Infrared Analysis of the Saponin Mixture

A Perkin-Elmer spectrophotometer was used to obtain an infrared spectrum of the saponin fraction (Fig. 4). The spectrum was obtained utilizing a potassium bromide pellet. The spectrum showed a broad absorption at 2.9 microns, a
Fig. 3. Chromatogram A - Saponin Mixture
Fig. 4 Infrared Spectrum of Saponin Fraction
long band at 3.4 microns, a weak band at 5.89 microns, a small peak at 10.2 and 10.9 microns, a large peak at 11.15 microns and another small peak at 11.6 microns.

According to detailed studies on infrared spectra of steroidal saponins, the spiroketal structure of the side chain shows a characteristic spectrum whose major absorption peaks correlate exactly with those peaks at 10.2, 10.9, 11.15, and 11.6 microns. In addition to the saponin nature of the spectrum, the peak at 5.89 microns indicated the possible presence of a ketone.
CHAPTER 2

SAPOGENINS FROM THE LEAVES OF YUCCA SCHOTTI

In reviewing the literature, numerous references are found describing the methods of isolating sapogenins. The most frequently used method is to make an ethanolic extract and hydrolyze it under acidic conditions. The sapogenins are then extracted from the reaction mixture with ether.

Seasonal variations cause changes in the sapogenins found in the plant. In order to study these variations in *Yucca schottii*, leaves collected from the plant before flowering and at the time of fruiting were investigated.

**Ethanolic Extract of the Leaves Collected before Flowering**

One pound of the dried ground leaves of *Yucca schottii* collected before flowering was macerated with 4 l. of 95% ethanol for 24 hours. The extract was filtered and concentrated to 2 l. by air evaporation. Five hundred milliliters of concentrated hydrochloric acid was added to the ethanolic extract. The solution was refluxed on a steam bath for 3 hours. After cooling, 2 l. of water was added
and the excess ethanol removed by air evaporation. The remaining solution was extracted three times with a liter of ether each time. The ether layers were combined and the aqueous layer was discarded. The ether extract was washed with 1 l. of water and 1 l. of a 5% solution of sodium bicarbonate. The ether extract was then dried with anhydrous magnesium sulfate, filtered, and evaporated to dryness under reduced pressure.

Thin-layer Chromatography of the Sapogenins from the Leaves Collected from the Plant before Flowering

The residue from the evaporated ether extract was chromatographed on a thin-layer plate using the chromatographic system chloroform:methanol:water (188:12:1) (System 2). Visualization was accomplished by means of charring with ceric sulfate. Three major substances were present (Fig. 5).

Infrared Analysis of the Sapogenins

The spectrum was obtained utilizing a potassium bromide pellet. The major absorptions again demonstrated the presence of sapogenins by the correlations of the well-defined spiroketal peaks at 10.2, 10.9, 11.15, and 11.6 microns. Also still prominent was the ketone peak at 5.89 microns (Fig. 6).
Fig. 5. Thin-layer Chromatogram of the Sapogenins from the Leaves Collected from the Plant before Flowering
Fig. 6. Infrared Spectrum of Sapogenin Mixture
**Isolation of Ketogenins from the Leaves Collected before Flowering**

The infrared spectrum of the sapogenins isolated from the hydrolyzed ethanol extract of the leaves of *Yucca schottii* collected before flowering showed a prominent ketone absorption. An experiment was carried out in an attempt to isolate the ketogenins using Girard Reagent "T".

Girard Reagent "T", trimethylaminoacetohydrazide chloride, whose structure is similar to that of a semicarbazide, condenses with carbonyl compounds in absolute ethanol containing a small amount of glacial acetic acid as a catalyst to give a derivative of the type

\[(\text{CH}_3)_3\text{N-CH}_2-\text{C-N}=\text{CR}_2 (\text{Cl}^-)\].

These are soluble in water because of the presence of the dipolar ionic grouping. Therefore, they can be extracted from an ether solution with water. The derivative is easily hydrolyzed with excess water under catalysis by mineral acid to regenerate the water-insoluble carbonyl compound.

The sapogenin mixture (500 mg), 20 g of Girard Reagent "T", and 20 ml of absolute ethanol were refluxed in a round bottom flask for 1 hour on a steam bath. The solution was cooled and added to a separatory funnel containing 100 ml of water, 50 g of crushed ice, 15.9 g of sodium carbonate, and 50 ml of ether. The mixture was shaken
vigorously for 5 minutes. The ether layer was removed and 50 ml of fresh ether was added and the procedure was repeated.

The ether layers were combined, washed with 100 ml of water and with 100 ml of a 5% sodium bicarbonate solution, dried with anhydrous magnesium sulfate, and evaporated to dryness under reduced pressure. The residue contained the ketone-free genins that were present. The residue was labeled Girard ether 1 (GE₁) and set aside for thin-layer chromatographic investigation.

The aqueous layer contained the Girard derivatives of the ketone-containing sapogenins. To catalyze the hydrolysis of the derivatives, 11.4 ml of concentrated hydrochloric acid was added to the aqueous phase. Then 50 ml of ether was added and the two phases were left undisturbed for 1 hour. The ether layer was removed and the aqueous layer was washed with an additional 50 ml of ether. The ether layers were combined, washed with 100 ml of water and with 100 ml of a 5% sodium bicarbonate solution, dried with anhydrous magnesium sulfate, and evaporated to dryness in vacuo. The residue should contain the sapogenins possessing a ketone group. It was labeled Girard ether 2 (GE₂) and set aside for thin-layer chromatographic investigation.
Thin-layer Chromatography of Girard Ether 2

GE₂ was chromatographed on a thin-layer plate using System 2. Reference samples of chlorogenin, hecogenin, tigogenin, and yuccagenin (obtained from Syntex, Palo Alto, California) were also applied to the thin-layer plate. Visualization of the chromatogram was accomplished by means of charring with ceric sulfate (Fig. 7). GE₂ contained one major substance which corresponded to substance 1 of the original genin mixture from the leaves of Yucca schottii collected from the plant before flowering. Its Rf value was not identical with any of the authentic reference samples.

Identification of Substance 1

Substance 1 was recrystallized from methanol to yield a white crystalline material which melted at 242-244°C. An infrared spectrum showed absorptions in all areas of the spiroketal side chain and a large absorption at 5.86 microns (Fig. 8). In addition to the distinctive spiroketal side chain peaks in the infrared spectrum of saponins and sapogenins, information is also revealed concerning the stereochemical form of the saponin and sapogenin side chain. The spiroketal side chain can exist in two different stereochemical forms, neo and iso (Fig. 9). The relative intensities of the peaks at 11.1 and 10.9 microns distinguish between the two stereochemical forms. If the peak at 11.1 microns is of greater intensity than the peak
**Fig. 7.** Thin-layer Chromatogram of Girard Ether 2
Fig. 8. Infrared Spectrum of Substance 1
Fig. 9. Neo and Iso Forms of Saponin Side Chain
at 10.9 microns, the spiroketal side chain is in the iso form. Examination of the infrared spectrum of substance 1 revealed that it exists in the iso form.

Mass Spectrum of Substance 1

The mass spectrum was obtained on a Hitachi Perkin-Elmer, RMU-6e Spectrometer. The mass spectrum of substance 1 had a parent peak at 444 m/e. The base peak was at 139 m/e (Fig. 10).

Formation of the Base Peak

The base peak of 139 m/e is a very common occurrence for steroidal sapogenins. The presence of adjacent oxygen atoms in these molecules confines most ionization to the spiro system. If the molecular ion is represented by a, the following scheme shows how the base peak may form, a→b→c→d→e (Fig. 11).

Reduction of Substance 1

Substance 1 appeared to be kammogenin from its melting point, infrared and mass spectrum. However, no authentic sample of kammogenin was available for comparison with substance 1. Since kammogenin is 12-keto-yuccagenin, reduction of the ketone group of substance 1 should convert it to yuccagenin. Therefore, substance 1 was subjected to the Huang-Minlon modification of the Wolff-Kishner reaction (Fig. 12).
Fig. 10. Mass Spectrum of Substance 1
Fig. 11. Base Peak Formation
Fig. 12. Reduction of Substance 1 by Huang-Minion Reaction
Ten milligrams of substance \( \text{I} \) was refluxed in an atmosphere of nitrogen with 2 ml of ethanol, 2 ml of diethylene glycol, and 0.36 ml of 85% hydrazine hydrate for 25 minutes. Then 200 mg of potassium hydroxide was added to the reaction. It was refluxed for an additional 35 minutes and then the condenser was removed. The temperature of the mixture was allowed to rise to 190°C and the reaction was refluxed for another 2.5 hours. After cooling, the reaction mixture was poured into water saturated with sodium chloride and left to stand overnight. After 12 hours, the salt solution was extracted with ether. The ether layer was washed with water, dried with anhydrous magnesium sulfate, and evaporated to dryness in vacuo. The ether residue was recrystallized from acetone. A white crystalline substance was obtained which melted at 248-250°C.

Thin-layer Chromatography of Huang-Minlon Reduction Product

The Huang-Minlon reduction product was chromatographed on a thin-layer plate using System 2. A reference sample of yuccagenin was also applied to the thin-layer plate. Visualization was accomplished by means of charring with ceric sulfate. The \( R_f \) value of the Huang-Minlon reduction product was identical with that of yuccagenin.
Infrared Analysis of Huang-Minlon Reduction Product

The infrared spectrum of the Huang-Minlon product was obtained utilizing a potassium bromide pellet. It showed major absorptions at 10.2, 10.9, 11.15, and 11.6 microns. No absorption was detected in the ketone region of the spectrum (Fig. 13). Comparison of the infrared spectrum of the Huang-Minlon product and that of yuccagenin showed them to be identical. It was therefore concluded that substance 1 is kammogenin.

Thin-layer Chromatography of Girard Ether 1

GE1 was chromatographed on a thin-layer plate using System 2. Authentic samples of chlorogenin, hecogenin, tigogenin, and yuccagenin were also applied to the thin-layer plate. Visualization was accomplished by means of charring with ceric sulfate. The extract contained two major substances (Fig. 14). They corresponded to substances 2 and 3 in the original genin mixture from the leaves of Yucca schottii collected before flowering. Their Rf values were identical with those of yuccagenin and tigogenin, respectively.

Separation and Identification of Substances 2 and 3

Substances 2 and 3 were separated by preparative thin-layer chromatography using System 2. The edges of the
Fig. 13. Infrared Spectrum of Huang-Minlon Product
Fig. 14. Thin-layer Chromatogram of Girard Ether 1
chromatograms were visualized by means of charring with ceric sulfate. The two substances were removed from the plates and each one was eluted from the silica gel with acetone. In each case the acetone solution was filtered and evaporated to dryness in vacuo.

Substance 2 was recrystallized from acetone to yield a white crystalline substance which melted at 247-250°C. The infrared spectrum showed major absorption peaks at 10.2, 10.9, 11.15, and 11.6 microns (identical to Fig. 13). The mass spectrum had a parent peak at 430 m/e and a base peak at 139 m/e. Comparison of the infrared and mass spectra of substance 2 showed them to be identical to those of yuccagenin. A mixed melting point determination of substance 2 and yuccagenin showed no depression.

Substance 3 was recrystallized from methanol to yield a white crystalline material which melted at 206-208°C. The infrared spectrum was obtained utilizing a potassium bromide pellet. It possessed the well-defined spiroketal peaks and lacked any absorption in the ketone absorption region (Fig. 15). Comparison of the infrared spectrum of substance 3 with that of tigogenin showed them to be identical. A mixed melting point determination of substance 3 and tigogenin showed no depression.
Fig. 15. Infrared Spectrum of Substance 3
Ethanolic Extract of the Leaves Collected at the Time of Fruiting

One pound of the dried ground leaves of *Yucca schottii*, collected at the time of fruiting was extracted and hydrolyzed as previously described on pages 15 and 16 for the leaves collected before flowering.

Thin-layer Chromatography of the Sapogenins of the Leaves Collected at the Time of Fruiting

The ether residue from the above described hydrolysis was chromatographed on a thin-layer plate using System 2. Visualization was accomplished by means of charring with ceric sulfate. Four major substances were present. They were labeled a, b, c, and d (Fig. 16).

Infrared Analysis of the Sapogenins of the Leaves Collected at the Time of Fruiting

The infrared spectrum was obtained utilizing a potassium bromide pellet. The infrared spectrum revealed a ketone absorption at 5.89 microns as well as the spiroketal absorptions at 10.2, 10.9, 11.15, and 11.6 microns (Fig. 17).

Isolation of the Ketogenins from the Leaves Collected at the Time of Fruiting

Isolation of the ketogenins of the leaves collected at the time of fruiting was accomplished with Girard Reagent "T" in the same manner as the ketogenin of the leaves.
Fig. 16. Thin-layer Chromatogram of the Sapogenins from the Leaves Collected at the Time of Fruiting
Fig. 17. Infrared Spectrum of the Sapogenins of the Leaves Collected at the Time of Fruiting
collected before flowering was isolated. Two ether fractions were obtained: Girard ether 1a (GE1a) which contained the sapogenins without a ketone and Girard ether 2a (GE2a) which contained the sapogenins with a ketone.

Thin-layer Chromatography of Girard Ether 1a

GE1a was chromatographed on a thin-layer plate using System 2. A reference sample of GE1 was applied to the thin-layer plate. Visualization was accomplished by means of charring with ceric sulfate. Two substances were present which corresponded to substances b and d of the original genin extract of the leaves collected at the time of fruiting. Substances b and d had identical Rf values with the two substances present in GE1, i.e., yuccagenin and tigogenin, respectively.

Separation and Identification of Substances b and d

Substances b and d were separated by preparative thin-layer chromatography using System 2. The edges of the thin-layer plates were visualized by means of charring with ceric sulfate. Each substance was removed from the silica gel with acetone. In each case the acetone solution was filtered and evaporated to dryness in vacuo.
Substance b was recrystallized from acetone to yield a white crystalline substance which melted at 247-249°C. The infrared spectrum possesses no ketone absorption (identical to Fig. 13). Comparison of the infrared spectra of substance b and substance 2, yuccagenin, from GE1 showed them to be identical. A mixed melting point showed no depression.

Substance d was recrystallized from methanol to yield a white crystalline material which melted at 206-208°C. The infrared spectrum had no ketone peak (identical to Fig. 15). Comparison of the infrared spectrum of substance d with that of substance 3, tigogenin, from GE1 showed them to be identical. A mixed melting point showed no depression.

Thin-layer Chromatography of Girard Ether 2a

GE2a was chromatographed on a thin-layer plate using System 2. A reference sample of GE2 was applied to the thin-layer plate. Visualization was accomplished by means of charring with ceric sulfate. Two substances were present which corresponded to substances a and c of the original genin extract of the leaves collected at the time of fruiting (Fig. 18). Substance a had an identical Rf value with substance 1, kammogenin, from GE2.
Fig. 18. Thin-layer Chromatogram of Girard Ether 2a
Separation and Identification of Substances a and c

Substances a and c were separated by preparative thin-layer chromatography using System 2. The edges of the thin-layer plates were visualized by means of charring with ceric sulfate. Each substance was removed from the silica gel with acetone. In each case the acetone solution was filtered and evaporated to dryness in vacuo.

Substance a was recrystallized from methanol to yield a white crystalline material which melted at 242-244°C. Its infrared spectrum showed absorption in all areas of the spiroketal side chain and a large absorption at 5.86 microns. Comparison of the infrared spectrum of substance a with the spectrum of substance 1, kammogenin, showed them to be identical. A mixed melting point showed no depression.

Substance c was recrystallized from methanol to yield a white crystalline material which melted at 262-265°C. The mass spectrum had a parent peak at 430 m/e and a base peak at 139 m/e. The infrared spectrum showed the spiroketal side chain absorptions and a significant ketone peak. Comparison of the infrared and mass spectra of substance c with those of hecogenin showed them to be identical. A mixed melting point showed no depression.
Summary of Sapogenin Investigation

Prior to this investigation, the only sapogenin isolated from the leaves of *Yucca schottii*, collected at the time of fruiting or from before flowering, was kammogenin. This investigation found hecogenin, kammogenin, tigogenin, and yuccagenin at the time of fruiting and kammogenin, tigogenin, and yuccagenin in the leaves collected from before flowering (Fig. 19).
Fig. 19. Sapogenins Isolated from the Leaves of *Yucca Schottii*
CHAPTER 3

SEPARATION OF THE SAPONIN MIXTURE OF THE LEAVES COLLECTED BEFORE FLOWERING

Attempts were made to separate the saponin mixture utilizing preparative thin-layer chromatography using the chromatographic system chloroform:methanol:water (65:35:10 lower phase only) (System 3). System 3 was useful for small quantities but did not give satisfactory resolution with large quantities.

In an attempt to isolate the saponins, their acetates were prepared. The acetates have a much lower polarity and this technique has been successful in other investigations.\textsuperscript{15}

**Preparation of the Saponin Acetates**

One hundred grams of the saponin mixture from the leaves collected before flowering was acetylated by refluxing for 2 hours with 100 ml of pyridine and 100 ml of acetic anhydride. The reaction mixture was cooled and diluted with 200 ml of water and extracted with three 100 ml portions of ether. The combined ether extract was washed with three 100 ml portions of 2% hydrochloric acid solution. The ether fraction was then washed with two 100
ml portions of 5% sodium bicarbonate solution and with two 100 ml portions of water. It was dried with anhydrous magnesium sulfate and evaporated to dryness under reduced pressure.

Thin-layer Chromatography of the Saponin Acetate Mixture

The saponin acetates were chromatographed on a thin-layer plate using ether as the solvent system (System 4). Visualization was accomplished by means of charring with ceric sulfate. There were seven major substances in the saponin acetate mixture (Fig. 20).

Resolution of the Saponin Acetate Mixture by Silica Gel G Dry Column

A column was prepared in the following manner: one end of cellophane dialysis tubing No. 4465-A2 (Arthur H. Thomas Co., Philadelphia, Pennsylvania), 1 m long and 9 cm in diameter, was attached to a constricted glass joint and tied with a string held by a clamp. The other end was moistened around the lip to a depth of about 2.5 cm. A Buchner funnel fitted with filter paper of about the same diameter as the tubing was introduced into the lower part of the tubing and was tied with a string. In order to prevent the column from collapsing when packed, the funnel was supported by a ring. Compressed air was applied gently on the upper part of the constricted glass joint in order to
Fig. 20. Thin-layer Chromatogram of the Saponin Acetate Mixture
open the flat tubing into a cylindrical shape. Approximately 1.5 kg of Silica Gel G, activated overnight in an oven at 120°C, was poured into the column. Compressed air was applied occasionally to aid in packing. The column was patted and vibrated manually in order to obtain a homogeneous packing.

After packing the column, 15 g of the saponin acetate mixture was dissolved in 100 ml of chloroform and absorbed on 50 g of silica gel. The mixture was allowed to dry in the air. It was poured slowly and carefully into the column so as not to upset the upper surface of the Silica Gel G. A large piece of cotton was then placed on top of the silica gel-acetate mixture so that the solvent dripping into the column would not disturb the silica gel-acetate mixture (Fig. 21) (a graphic representation of the dry column by Allen Davidson, University of Arizona, College of Pharmacy, Tucson, Arizona).

The chromatographic solvent system was ether: petroleum ether:acetone (9:2:1) (System 5). A 2-l. funnel was placed over the column, filled with the chromatographic liquid, and allowed to drip at a rate preventing overflow on the upper part of the column. The funnel was refilled and allowed to continue dripping overnight. When approximately
Fig. 21. Graphic Representation of the Dry Chromatographic Column
3 l. of the liquid had entered the column, the liquid began to drip from the funnel at the bottom end of the column and was collected.

Since the distribution of the mixture in the column should be the same as the distribution of the mixture on thin-layer plates, the collected liquid was periodically chromatographed using the original saponin acetate mixture as a reference. The liquid was allowed to drip until the eluate from the column when chromatographed on a thin-layer plate showed all of the less polar material which precedes saponin acetate 7. At this point about 6 l. of the chromatographic liquid had been added to the column.

The column was allowed to stand for 48 hours. Eighteen exploratory samples starting at the top at a distance of approximately 2.5 cm from each other were taken in the following manner: a small window was cut in the column with the top and the sides opened and the bottom uncut. The flap was pulled down and a small amount of the wet silica gel was taken from the opening with a spatula and placed in a 50-ml Erlenmeyer flask. Ten milliliters of acetone was added to the flask. The flap was then pulled up and covered with cellophane tape in order to prevent further evaporation of the chromatographic liquid. After 2 hours, the contents of the flasks were filtered and the acetone evaporated to dryness.
Thin-layer Chromatography of Exploratory Samples

The residue of each exploratory sample was dissolved in a few drops of chloroform and chromatographed on a thin-layer plate using System 4. Visualization was accomplished by means of charring with ceric sulfate. Samples 1-4 remained at the origin, samples 5 and 6 were void of any material, samples 7-10 contained saponin acetates 1-4, samples 11-15 contained saponin acetates 4-7, sample 16 contained saponin acetates 5-7, and samples 17 and 18 contained saponin acetates 6 and 7 (Fig. 22).

Cutting of the Dry Column

On the basis of the exploratory samples, the dry column was cut in such a manner as to combine sections 1-6, 7-10, 11-15, and 17 and 18. The combined sections and section 16 were each placed in a 1-1 Erlenmeyer flask containing 500 ml of acetone. The acetone-silica gel mixture was stirred for 24 hours, filtered, and air evaporated.

Preparative Thin-layer Chromatography of the Combined Dry Column Sections

Each combined section was chromatographed on preparative thin-layer plates using System 4. The edge of each plate was visualized by means of charring with ceric sulfate. Each individual saponin acetate was scraped off and combined with the same acetate from another section.
Fig. 22. Thin-layer Chromatogram of Exploratory Samples
The seven saponin acetates were eluted from the silica gel with acetone. Each acetone solution was filtered and evaporated to dryness under reduced pressure.

**Thin-layer Chromatography of Saponin Acetates 1-7**

Each saponin acetate was chromatographed on a thin-layer plate using System 4. The original saponin acetate mixture was spotted on the thin-layer plate as a reference. Visualization was accomplished by means of charring with ceric sulfate (Fig. 23).

**Alkaline Hydrolysis of Saponin Acetates 1-7**

Saponin acetates 1-7 were each treated in the following manner: To 100 mg of the saponin acetate was added 2 ml of saturated methanolic potassium hydroxide solution and 2 ml of 75% methanol. The mixture was refluxed for 2 hours, 10 ml of water was added to the reaction mixture, and the methanol was evaporated by passing a stream of air over the reaction mixture. The liquid was neutralized with concentrated hydrochloric acid and diluted with water to twice its volume. It was extracted with three 10 ml portions of n-butanol saturated with water. The combined n-butanol extracts were washed with two 5 ml portions of water saturated with n-butanol. The n-butanol layer was evaporated to dryness.
Fig. 23. Thin-layer Chromatogram of Saponin Acetates 1-7
Thin-layer Chromatography of Saponins 1-7

The alkaline hydrolysis product of each acetate was chromatographed on a thin-layer plate using System 1. To increase the resolution the thin-layer plate was developed five times before visualization. The plate was visualized by means of charring with ceric sulfate (Fig. 24). Comparison of the saponin of each acetate was achieved by spotting the original saponin extract on the thin-layer plate. The following results were observed:

- Saponin acetate 1 yielded saponin 1
- Saponin acetate 2 yielded saponin 2
- Saponin acetate 3 yielded saponin 3
- Saponin acetate 4 yielded saponin 5
- Saponin acetate 5 yielded saponin 4
- Saponin acetate 6 yielded saponin 6
- Saponin acetate 7 yielded saponin 7

Mild Acid Hydrolysis of the Saponins

The hydrolysis of the saponins under strongly acidic conditions could cause the decomposition of their sugars. Each saponin was therefore hydrolyzed in the following manner: Each saponin was dissolved in 10 ml of a 1:1 mixture of dioxane and 4 N sulfuric acid. The mixture was refluxed on a steam bath for 3 hours and then 25 ml of cold water was added. The mixture was allowed to cool to room
Fig. 24. Thin-layer Chromatogram of Saponins 1-7
temperature and was then extracted with three 10 ml portions of ether. The aqueous layer was neutralized with barium carbonate. The insoluble barium sulfate formed from the addition of the barium carbonate was filtered off and the aqueous filtrate was concentrated to a syrup. The syrup was dissolved in ethanol, filtered, and evaporated to dryness. The dried sugar residue was labeled and set aside for investigation. The ether solution was dried with anhydrous magnesium sulfate and evaporated to dryness under reduced pressure. The residue from each saponin was dissolved in chloroform and passed through an aluminum oxide column (Grade IV). The colorless eluates from each saponin ether residue yielded a white crystalline material upon evaporation.

Thin-layer Chromatography of Saponin Aglycones

The seven saponin aglycones were chromatographed on a thin-layer plate using System 2. The genins hecogenin, kammogenin, tigogenin, and yuccagenin were also applied to the thin-layer plate as references. The chromatogram was visualized by means of charring with ceric sulfate. The saponin aglycones from saponins 1-5 and 7 had the same $R_f$ value as yuccagenin. The saponin aglycone from saponin 6 had the same $R_f$ value as kammogenin (Fig. 25).
Fig. 25. Thin-layer Chromatogram of Saponin Aglycones
Identification of Saponin Aglycones 1-5 and 7

Each aglycone was recrystallized from acetone to yield a white crystalline substance which melted at 247-249°C. The infrared spectra of aglycones 1-5 and 7 were identical. A comparison of them with the infrared spectrum of yuccagenin showed them to be identical. A mixed melting point determination was made for each aglycone with yuccagenin and none showed a depression.

Identification of Saponin Aglycone 6

The aglycone was recrystallized from methanol to yield a white crystalline material which melted at 242-244°C. The infrared spectrum showed absorption in all areas of the spiroketal side chain and a ketone absorption at 5.86 microns. A comparison of the infrared spectrum with that of kammogenin showed them to be identical. A mixed melting point determination with kammogenin showed no depression.

Thin-layer Chromatography of the Seven Saponin Sugar Moieties

The seven saponin sugar moieties were chromatographed on a thin-layer plate using the chromatographic system chloroform:glacial acetic acid:water (3:3.5:0.5) (System 6). Reference samples of arabinose, 2-deoxyribose, fucose, galactose, and glucose (obtained from Aldrich Chemical Company, Milwaukee, Wisconsin) were applied to the
thin-layer plate. Visualization was accomplished by means of charring with ceric sulfate. The sugar moieties of saponins 1-5 and 7 had identical Rf values, which were identical to that of the galactose reference. The sugar moiety from saponin 6 had the same Rf value as the authentic sample of 2-deoxyribose (Fig. 26).

Gas Chromatographic Analysis of Sugars

The analysis of sugar components in steroidal glycosides and polysaccharides is often carried out by paper chromatography separation followed by colorimetric methods. A more rapid as well as a more accurate method has been developed for such studies in these fields. This method developed by Sweeley utilizes the trimethylsilyl ethers (TMS€) of the sugars for analysis by gas-liquid chromatography (glc).

Preparation of Trimethylsilyl Ethers

Ten milligrams of each saponin sugar moiety was treated at room temperature for 5 minutes with 1 ml of TMS reagent (obtained from Perco Supplies, San Gabriel, California). After 5 minutes the reaction mixture was evaporated to dryness under reduced pressure. The residue was extracted with 1 ml of n-hexane. The hexane extract was
Fig. 26. Thin-layer Chromatogram of the Seven Saponin Sugar Moieties
submitted to glc. The TMSE derivatives of 2-deoxyribose and galactose were prepared by treating them in the same manner as described for the saponin sugar moieties.

Gas Chromatographic Analysis

The gas chromatographic analysis was carried out using a Perkin-Elmer Model 880, with flame ionization detector, employing a coiled Pyrex column packed with 2.5% SE-30 on Chromosorb W (80-100 mesh). The column temperature was 140°C. The injector and detector temperature was 200°C. Nitrogen was used as the carrier gas. The nitrogen rate was held constant throughout all determinations.

A complex chromatogram was obtained from each monosaccharide, due to the formation of equilibrium mixtures of monosaccharides during hydrolysis. Identification of the saponin sugar moieties were made by comparing their chromatograms with those of the reference samples. The chromatograms of the TMSE derivatives of the saponin sugar moieties 1-5 and 7 were identical with that of galactose. The chromatogram of saponin sugar moiety 6 was identical with that of 2-deoxyribose.

Summary of Saponin Investigation

Seven saponins were isolated from the butanol fraction. Six of these had yuccagenin as their aglycone. The
sugar moiety associated with each of them was galactose. Since the saponins of yuccagenin display different physical properties, the number of galactose units or the glycosidic linkages are probably different. Either of these two factors could cause sufficient differences in the physical properties of the saponins to allow for their separation by chromatographic techniques. Saponin 6 had kammogenin as its aglycone. The sugar moiety associated with it was 2-deoxyribose.

The sapogenin investigation of an ethanolic extract of the leaves of \textit{Yucca schottii} collected before flowering revealed the presence of kammogenin, yuccagenin, and tigogenin. This is in contrast to the saponin investigation of the butanol extract of the leaves which contained only the saponins of yuccagenin and kammogenin. Since the extracts were prepared by different extraction procedures, it is possible that this is the primary cause of the difference. Another possibility is that the saponins of tigogenin remained in the water solution upon partitioning with butanol. It is also possible that tigogenin exists in the leaves as the genin and therefore would be soluble in ethanol but not in water.
CHAPTER 4

STRUCTURE ELUCIDATION STUDIES OF SAPONIN 6

In 1952 Wall and Eddy, using crude saponin preparations, proved in contradiction to the proposed structure of Marker and Lopez (Fig. 1a) that saponin side chains exist in spiroketals. They used the infrared spectra of the saponins as the basis of their proof. The well-defined spiroketal peaks present in the spectra of the saponin aglycones were also present in the spectra of the parent saponins. They also showed that the infrared spectra of saponins whose aglycones possess a ketone group have an absorption at 5.89 microns. Lithium aluminum hydride reduction caused a decrease in the intensity of the peak at 5.89 microns. It was therefore concluded that these saponins possess a ketone group.

It has been shown that the aglycone of saponin 6 is kammogenin. Kammogenin has a ketone at the 12 position. A Girard derivative was used to isolate it, since water insoluble ketones react with Girard Reagent "T" to form water soluble derivatives. Since saponin acetate 6 is water insoluble it should be possible to isolate it utilizing Girard Reagent "T".
Isolation of Saponin Acetate 6 with Girard Reagent "T"

Eighty grams of the saponin acetate mixture, 320 g of glacial acetic acid, 160 g of Girard Reagent "T", and 3.2 l. of absolute ethanol were refluxed for 1 hour. The reaction mixture was cooled and poured into a separatory funnel containing 5 l. of water, 2.5 kg of ice, 2.5 l of ether, and 265 g of sodium carbonate. The separatory funnel was shaken for 5 minutes. The ether layer was removed and 1 l. of additional ether was added. The separatory funnel was shaken again and the ether layer was again removed. The two ether layers were combined, washed with water, washed with a 5% solution of sodium bicarbonate, dried with anhydrous magnesium sulfate, and evaporated to dryness in vacuo. The residue was labeled Girard insoluble acetates and set aside.

Two liters of concentrated hydrochloric acid was added to the aqueous phase and 2.5 l. of ether was added to the acid solution. The mixture was allowed to stand undisturbed for 1 hour. It was shaken for 5 minutes and then left undisturbed for 15 minutes. This procedure was repeated four times. The ether layer was washed with water and with a 5% solution of sodium bicarbonate, dried with anhydrous magnesium sulfate, and evaporated to dryness under reduced pressure. The residue was labeled Girard soluble acetate.
Thin-layer Chromatography of Girard Soluble and Insoluble Acetates

The Girard soluble and insoluble acetate residues were chromatographed on a thin-layer plate using System 4. The original acetate mixture was spotted on the thin-layer plate as a reference. The chromatogram was visualized by means of charring with ceric sulfate. The Girard insoluble fraction contained acetates 1-5 and 7. The Girard soluble fraction contained only acetate 6 (Fig. 27).

Alkaline Hydrolysis of Acetate 6

Two grams of the Girard soluble fraction was hydrolyzed by the procedure described on page 52. The alkaline hydrolysis product was chromatographed on a thin-layer plate using System 1. The original saponin mixture was used as a reference. The chromatogram was visualized by means of ceric sulfate charring. The Rf value of the alkaline hydrolysis product was identical with that of saponin 6 (Fig. 28).

Infrared Analysis of Saponin 6

An infrared spectrum of the alkaline hydrolysis product, saponin 6, was obtained utilizing a potassium bromide pellet. The spectrum indicated that the product was a saponin of the iso series, possessing a ketone absorption peak at 5.89 microns (Fig. 29).
Fig. 27. Thin-layer Chromatogram of Girard Soluble and Insoluble Acetates
Fig. 28. Thin-layer Chromatogram of Alkaline Hydrolysis Product of Acetate 6
Fig. 29. Infrared Spectrum of Saponin 6
Acid Hydrolysis of the Ketone Containing Saponin

The ketone containing saponin (365 mg) was refluxed in 10 ml of 4 N sulfuric acid:dioxane (1:1) for 3 hours. The reaction mixture was diluted with water and extracted with three 25-ml portions of ether. The combined ether extract was washed with water and with a 5% solution of sodium bicarbonate, dried with anhydrous magnesium sulfate, and evaporated to dryness in vacuo. The ether residue which contained the aglycone of saponin 6 weighed 155 mg. It was recrystallized from methanol to yield a white crystalline substance which melted at 244-245°C.

Thin-layer Chromatography of the Aglycone of Saponin 6

The aglycone of saponin 6 was chromatographed on a thin-layer plate using System 2. A sample of kammogenin was spotted on the thin-layer plate as a reference. The chromatogram was visualized by means of charring with ceric sulfate. The aglycone had an Rf value identical with that of the reference sample, kammogenin (Fig. 30).

Infrared Analysis of the Aglycone of Saponin 6

The infrared spectrum of the aglycone of saponin 6 was obtained utilizing a potassium bromide pellet. The infrared spectrum was identical with that of kammogenin (identical to Fig. 8).
Fig. 30. Thin-layer Chromatogram of the Aglycone of Saponin 6
Thin-layer Chromatography and Gas-liquid Chromatography of the Sugar Moiety of Saponin 6

Examination of the sugar moiety of saponin 6 on thin-layer and gas-liquid chromatography gave identical results with those previously found for saponin 6 on pages 59 and 61. The sugar moiety was 2-deoxyribose.

Molecular Weight Estimation

An attempt was made to calculate the molecular weight of saponin 6 by the amount of kammogenin obtained from the hydrolysis of 365 mg of saponin 6. The hydrolysis is described on page 69. The yield of kammogenin was 155 mg. The molecular weight was estimated to be 1043. If the molecular weight of saponin 6 were 1043, the number of moles of 2-deoxyribose associated with saponin 6 would be 5.17.

The molecular formula of saponin 6, assuming it contains 5 moles of 2-deoxyribose would be $C_{52}H_{80}O_{20}$. This formula corresponds to a molecular weight of 1025. Analytical: Calculated for $C_{52}H_{80}O_{20}$: C, 60.90; H, 7.86; Found: C, 60.90; H, 8.50; (The carbon-hydrogen determination was carried out by Huffman Laboratories, Inc., Wheatridge, Colorado.)
Determination of the Location of the Glycosidic Linkage

Kammogenin, the aglycone of saponin 6, has hydroxyl groups at the 2 and 3 position. The 5 moles of 2-deoxyribose could be located at either the 2 or 3 position or divided between the two positions. A group of experiments was performed to determine the location of the sugar moieties.

Methylation of Saponin 6

A mixture of 3.14 g of saponin 6 and 150 ml of dimethyl sulfoxide was stirred for 30 minutes at room temperature under a nitrogen atmosphere. Sodium hydride (1.5 g) was added to the solution and stirring was continued under a nitrogen atmosphere for another 30 minutes, and then 45 ml of methyl iodide was added with further stirring for 30 minutes. After the reaction mixture was diluted with 900 ml of water, the methylated product was extracted with ether, washed with water, dried with anhydrous magnesium sulfate, and evaporated to dryness in vacuo to yield 3.27 g of methylated saponin.

Acid Hydrolysis of Methylated Saponin 6

Thirty milliliters of a mixture of hydrochloric acid and ethanol (3:97) was added to a 50-ml round-bottom flask containing 3.27 g of methylated saponin 6. The mixture was refluxed for 3 hours. It was then diluted with
75 ml of water. The excess ethanol was removed by evaporation induced by a jet of air passing over the top of the evaporating dish. The aqueous mixture was extracted with three 50-ml portions of ether. The combined ether extract was washed with water and with a 5% solution of sodium bicarbonate, dried with anhydrous magnesium sulfate, and taken to dryness in vacuo. The residue was dissolved in benzene and adsorbed on a short alumina column (10-15 g). The first eluent, benzene, removed the low polar impurities. The second eluent was chloroform. The chloroform upon evaporation yielded 435 mg of residue which was recrystallized from acetone to yield 208 mg of product melting at 228-229.5°C. The mass spectrum had a parent peak at 458 m/e and a base peak at 139 m/e. This molecular weight corresponds to a monomethoxykammogenin. It can therefore be concluded that the sugar moieties of saponin 6 are located at only one position on the aglycone, either at the 2 or 3 position.

Oppenauer Oxidation of Methoxykammogenin

Kammogenin possesses a double bond at the 5 position and a hydroxyl group at both the 2 and 3 positions (Fig. 1b). Therefore, methoxykammogenin must also have a double bond at the 5 position. The oxidation of methoxykammogenin would produce either 2- or 3-keto-methoxykammogenin.
Under the Oppenauer conditions the C-5 double bond would be isomerized to the 4 position if the keto group was at the 3 position. The resulting $\Delta^4$-3-keto-2-methoxy-kammogenin would possess an ultraviolet absorption. If, however, the oxidation product was 2-keto-3-methoxykammogenin, it would not have an ultraviolet absorption.

Methoxykammogenin (100 mg) was dissolved in a mixture of 9 ml of toluene and 1.8 ml of cyclohexanone. The solution was heated to boiling and 100 mg of aluminum isopropoxide in 0.9 ml of toluene was added. The mixture was refluxed for 60 minutes then poured into water and made basic with 1 N sodium hydroxide solution. The organic layer was separated and the aqueous layer was extracted with ether. The organic layer and the ether layer were combined and evaporated. The residue was purified by dissolving it in benzene and passing it through a small alumina column (Grade II). The benzene eluate was evaporated to dryness to yield a white crystalline material.

The infrared spectrum of the benzene residue was obtained utilizing a potassium bromide pellet. The spectrum possesses two absorptions in the carbonyl region, 5.89 and 5.95 microns. The carbonyl absorption at 5.89 microns was the normal C-12 carbonyl of kammogenin. The carbonyl absorption at 5.95 microns corresponds to that of an $\alpha, \beta$-unsaturated carbonyl group. Therefore, the methoxy
group of methoxykammogenin is probably located at the 2 position. This conclusion is further supported by the strong double bond absorption observed at 6.21 microns.\textsuperscript{17} In addition to the two carbonyl peaks were the well-defined absorption peaks of the spiroketal side chain (Fig. 3). An ultraviolet spectrum of the benzene residue possessed a maximum at 240 nm ($\log \varepsilon \approx 4.25$).

On the basis of the spectral data of the Oppenauer oxidation product, the possibility of the sugar units being located at the 3 position was highly supported.

**Jones Oxidation of Methoxykammogenin**

The Jones oxidation reaction is carried out under mild conditions allowing oxidation to occur without causing isomerization of double bonds.\textsuperscript{14} The product of this reaction will not possess an ultraviolet absorption. However, if the oxidation takes place at the 3 position as suspected, subsequent treatment with base will cause the isomerization of the C-5 double bond to the conjugated 4 position, therefore confirming that the sugar moieties of saponin 6 are located at the 3 position.

To a stirred cold solution (8-9°C) of 100 mg of methoxykammogenin in 40 ml of acetone (distilled over potassium permanganate) was added dropwise a solution of Jones' reagent (26.72 g of chromium trioxide in 23 ml of concentrated sulfuric acid diluted with water to a volume of
Fig. 31. Infrared Spectrum of Oppenauer Oxidation Product
100 ml) until the mixture turned slightly orange. After 10 minutes, 0.2 ml of isopropyl alcohol, a small amount of sodium bicarbonate, and anhydrous magnesium sulfate were added. The reaction mixture was filtered through a sintered-glass filter and the filtrate was evaporated to dryness in vacuo. The residue was recrystallized from acetone to yield 44 mg of ketomethoxykammogenin melting at 179-181°C.

An infrared spectrum was obtained of the Jones oxidation product utilizing a potassium bromide pellet. The infrared spectrum possessed two carbonyl absorptions, at 5.81 and 5.90 microns (Fig. 32). The product did not possess an ultraviolet absorption. The mass spectrum had a parent peak at 456 m/e and a base peak at 139 m/e.

Isomerization of the Double Bond of Ketomethoxykammogenin

Ketomethoxykammogenin (33 mg) was dissolved in 1.9 ml of hot methanol, and 1 drop of 10% methanolic potassium hydroxide solution was added. The solution was heated on a steam bath for 5 minutes, and a few drops of methanol were added to replace any solvent lost by evaporation. The reaction mixture was then poured into water, it was neutralized with 6 N acetic acid, and then extracted with ether. The ether layer was dried with anhydrous magnesium sulfate and finally evaporated to dryness under reduced pressure. The residue was recrystallized from acetone to yield 24 mg of
Fig. 32. Infrared Spectrum of Jones Oxidation Product
product melting at 225-227°C. The infrared spectrum had two carbonyl absorptions, at 5.89 microns (carbonyl at C-12) and at 5.95 microns (conjugated carbonyl at C-3) (identical to Fig. 31). The product possessed an ultraviolet absorption maximum at 240 nm (log ε 4.25). The mass spectrum possessed a parent peak at 456 m/e and a base peak at 139 m/e. The isomerized product was concluded to be Δ⁴-3-keto-2-methoxy-kammogenin. Therefore, saponin 6 must have all 5 units of 2-deoxyribose linked to the 3 position of its aglycone, kammogenin (Fig. 33).

Summary of Structure Elucidation of Saponin 6

It has been established that saponin 6, C₅₂H₈₀O₂₀, contains 5 units of 2-deoxyribose and has a molecular weight of 1025. Its aglycone is kammogenin and all 5 units of 2-deoxyribose are linked to the 3 position.
Fig. 33. Saponin 6
R = 5 units of 2-deoxyribose
SUMMARY OF THE PHYTOCHEMICAL INVESTIGATION OF YUCCA SCHOTTII

The saponin-containing fraction of the leaves of Yucca schottii has been shown to possess anti-inflammatory properties against the Carrageenan-induced Edema Test.

A preliminary investigation of the sapogenins present in the leaves of Yucca schottii was undertaken. Ethanolic extracts of the leaves collected before flowering and at the time of fruiting were subjected to acid hydrolysis. The sapogenins were isolated by thin-layer chromatography and identified by comparison of their infrared and mass spectra with those of authentic samples. Kaempogenin, tigogenin, and yuccagenin were isolated from the acid-hydrolyzed ethanolic extract of the leaves collected from before flowering and hecogenin, kaempogenin, tigogenin, and yuccagenin from the ethanolic extract of the leaves collected at the time of fruiting.

The active saponin fraction was shown to contain seven saponins by thin-layer chromatography. Separation of the seven saponins was accomplished by a modified dry silica
gel column technique and preparative thin-layer chromatography. Saponins 1-5 and 7 were shown to possess yuccagenin as their aglycone and galactose as their sugar moiety. Saponin 6, whose infrared spectrum revealed the presence of a ketone peak, was shown to possess kammogenin as its aglycone and 2-deoxyribose as its sugar moiety.

A preparative isolation method was developed for saponin 6 utilizing Girard Reagent "T". Saponin 6 was shown to possess 5 units of 2-deoxyribose attached to the 3 position of its aglycone, kammogenin. The molecular formula of saponin 6 has been determined to be C_{52}H_{80}O_{20}.


