



A study of the chemical constituents and of the physiological properties of *Rivea corymbosa* (L.)
Hallier filius
by W Eugene Keeland

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree
of Doctor of Philosophy in Chemistry at Montana State College
Montana State University
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Abstract:

The seed of *Rivea Corymbosa* (L.) Hallier filius have been examined chemically and physiologically. The seed were extracted with solvents to remove lipid materials. The extracted lipids were fractionated and identified insofar as possible, Two. unknown fatty" acids were in the lipid fraction, The defatted seed,"were exhaustively extracted with absolute ethanol, .From the ethanolic extract there have been isolated two crystalline products, one has- been characterized as hydroquinone, and the other is a glucoside to which the molecular formula of (formula not captured in OCR) has been"assigned, The sugar, moiety has been shown to be glucose? The aglucone has been assigned a molecular formula of (formula not captured in OCR) Based upon experimental data a structural formula has been postulated for the aglucone. and the glucoside. The glucoside has been found to possess central nervous system activity. Efforts to isolate a crystalline alkaloid have met with failure. However, positive alkaloidal test have been obtained under conditions which ,should have precluded interfering substances

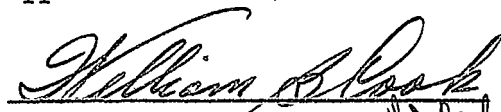
A STUDY OF THE CHEMICAL CONSTITUENTS AND OF THE PHYSIOLOGICAL
PROPERTIES OF RIVEA CORYMBOSA (L.) HALLIER FILIUS.

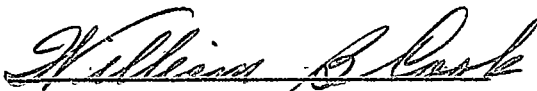
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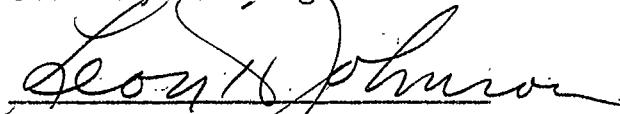
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Montana State College

Approved:


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August, 1960

ACKNOWLEDGEMENTS

The author would like to acknowledge the professional and personal aid so sincerely offered by the instructional staffs and graduate students of Baylor University and Montana State College. I would like to especially thank the members of my graduate committee, Drs. Anacker, Binder, Cook, Froeschner, Reed, and Roehm for their interest as shown by their direction of my graduate training.

I would like to thank the Geschickter Fund for Medical Research and the Medical Science Foundation for their financial support of this project and for obtaining the starting materials that were used.

I would like to especially thank Dr. William B. Cook for his personal and professional aid and guidance which has so contributed to the successful completion of this thesis. In appreciation for this aid and guidance, I would like to dedicate this thesis to him.

TABLE OF CONTENTS

	Page
LIST OF TABLES	6
LIST OF FIGURES	7
ABSTRACT	8
I. INTRODUCTION	9
A. Historical	
B. Identification and Taxonomy of <u>Rivea corymbosa</u>	
C. Chemical Constituents of <u>Rivea corymbosa</u>	
II. SOURCE AND DESCRIPTION OF THE SEED OF <u>RIVEA CORYMBOSA</u>	20
III. EXTRACTION OF THE SEED OF <u>RIVEA CORYMBOSA</u>	21
A. Preparation of the seed and the methods used in the extraction.	
B. Qualitative Relationships	
C. Quantitative Relationships	
IV. COMPOSITION OF THE OILS OF <u>RIVEA CORYMBOSA</u>	24
V. FRACTIONATION OF THE ETHANOLIC EXTRACT OF THE SEED OF <u>RIVEA CORYMBOSA</u>	26
A. General Considerations	
B. Tetrahydrofuran Fractionation	
C. Water Fractionation	
D. Figures	
VI. DETERMINATION OF THE ALKALOID CONSTITUENTS IN THE SEED OF <u>RIVEA CORYMBOSA</u>	45
A. Introduction	
B. Method I	
C. Method II	
D. Stass-Otto Method	
E. Discussion	
VII. PHYSIOLOGICAL EFFECTS OF THE SEED OF <u>RIVEA CORYMBOSA</u>	50
A. Introduction	
B. Experimental	

VIII.	SUGGESTIONS FOR FURTHER RESEARCH	52
IX.	SUMMARY	53
X.	APPENDIX	54
XI.	LIST OF REFERENCES	67

LIST OF TABLES

Table	Page
I. Fatty Acid Composition of <u>Rivea corymbosa</u>	25
II. Differentiation of the genus <u>Rivea</u> from the genus <u>Ipomoea</u>	59

LIST OF FIGURES

Figure	Page
1. Infrared Spectrum of Hydroquinone	38
2. Infrared Spectrum of Unknown	38
3. Infrared Spectrum of Glucoside recrystallized from Absolute Methanol	39
4. Infrared Spectrum of the Glucoside recrystallized from Water-Methanol	39
5. Infrared Spectrum of the Glucoside Hepta-acetate	40
6. Infrared Spectrum of the Aglucone Isolated from the Acid Hydrolysis of the Glucoside	40
7. Reproduction of the Sugar Chromatogram using the Solvent System n-Butanol:Ethanol:Water::10:1:2	41
8. Reproduction of the Sugar Chromatogram using the Solvent System n-Butanol:Pyridine:Water::6:4:3	42
9. Infrared Spectrum of the Aglucone	43
10. Infrared Spectrum of the Aglucone Tetra-acetate	43
11. Infrared of Phenanthrene	44
12. Infrared Spectrum of Unknown	44
13. Illustration of <u>ololiuqui</u> by Hernandez	55
14. Illustration of the seed <u>Rivea corymbosa</u>	57
15. Illustration of the plant of <u>Rivea corymbosa</u>	58
16. Distribution of <u>Rivea corymbosa</u>	60
17. Scheme of the Extraction Process	61

ABSTRACT

The seed of Rivea Corymbosa (L.) Hallier filius have been examined chemically and physiologically. The seed were extracted with solvents to remove lipid materials. The extracted lipids were fractionated and identified insofar as possible. Two unknown fatty acids were in the lipid fraction. The defatted seed were exhaustively extracted with absolute ethanol. From the ethanolic extract there have been isolated two crystalline products, one has been characterized as hydroquinone, and the other is a glucoside to which the molecular formula of $C_{28}H_{46}O_{12}$ has been assigned. The sugar moiety has been shown to be glucose. The aglucone has been assigned a molecular formula of $C_{21}H_{36}O_7$. Based upon experimental data a structural formula has been postulated for the aglucone and the glucoside. The glucoside has been found to possess central nervous system activity. Efforts to isolate a crystalline alkaloid have met with failure. However, positive alkaloidal test have been obtained under conditions which should have precluded interfering substances.

I. INTRODUCTION

A. Historical Introduction

The original impetus for the study of the chemical constituents of the Central American narcotic, ololiuqui, was the unexplained basis for the miraculous effects reported from the use of the drug by the pre-Columbian natives of certain central Mexican areas. The term ololiuqui was originally used only to designate the seed of a trailing vine, which has since been classified as Rivea corymbosa (L.) Hallier filius.⁷ In time the term was applied to the trailing vine from which the seed was obtained. The term ololiuqui, which means roundish and hemp-like in shape, was given to the seed because of its appearance.⁹ Actually, the term ololiuqui is the Aztec equivalent of our word roundish. The Aztecs gave the name caotl-xoxouqui to the plant Rivea corymbosa; this term may be roughly translated as "greensnake".⁹ This designation was probably derived from the fact that the vine twines along the ground or up trees or shrubs. After the arrival of the Spanish clerics to Mexico, the term piule was used to designate the seed of Rivea corymbosa. Piule appears to be a distortion of the word peyote, which is a term used by the natives of northern Mexico to designate a locally used narcotic. According to Dr. B. P. Reko, an archaeologist and an etymologist, the term peyote is derived from the Aztec work piyautli; the root pi means small or little, and yautli is a general term used for the plants having a stunning odor or a narcotic action.⁹ Since the Aztec narcotic ololiuqui possessed similar physiological action to peyote, such a distortion occurred.

The drug from Rivea Corymbosa was only one of many used by the pre-Columbia natives as a narcotic. Some of the other herbs used by these natives, because of their narcotic properties, were: (1) the cactus button, peyote (Lophophora Williamii (Lem.) Cauter), (2) the mushroom, teonanactl (Panaeolus campanulatus L. var. sphinctrinus (Fr.) Bresadda).

These drugs were used by the natives as general medicinals, narcotics, and in connection with their religious ceremonies. The use of these drugs as general medicinals has been reported by numerous authors.^{3,4,7,8,9} In a letter to Dr. C. G. Santesson, a chemist and a pharmacologist of the Carolinian Medico-Surgical Institute in Stockholm, Sweden, Dr. B. P. Reko reported an example of the use of Rivea corymbosa seed as a medicinal to mitigate a feverous condition.* The natives also used the drug for the treatment of gout, syphilis and putrid abscesses.

It has been reported that the ingestion of these drugs results in an intoxicating and deranging effect. Because of the deranging effect, the seed of Rivea corymbosa was used as a divining potion to predict future events, and to ascertain things that had happened in the past. Reko has reported that because of its intoxicating effect, ololiuqui was used by the Indian priests to communicate with the spirits.⁹ The clairvoyant effects were used by diviners to determine guilt and innocence when a crime was committed.

*See Appendix I for the complete text.

For centuries before the conquest of the Aztecs by Spaniards, the Aztec priests used a brew of the seed of Rivea corymbosa in their religious activities. It is believed that they would administer the brew to humans who were to be sacrificed to their gods in order to stun them into submitting to the ordeal.

Most of the literature which is available and which concerns the use of the drug from Rivea corymbosa indicates that the greatest use of the drug was in central Mexico. By the time of the Spanish conquest it appears to have been localized mainly in the area of the Aztec Empire. This is true despite the fact that the plant grows in other areas surrounding the Caribbean Sea and the Gulf of Mexico.* This restriction of area is probably the result of the jealous manner with which the priests of one area guarded their means of creating and maintaining their superstitious hold upon local charges. Although the drug Rivea corymbosa achieved its greatest use in the central Mexican area of the Aztec Empire, it is believed that the use of this drug actually originated with tribes in southern Mexico. The Mextecos and the Zapotecos are the tribes which are believed to have originated the use of Rivea corymbosa in their daily lives.⁷ Berendt¹ and Standley² have recorded in their works that the plant was known by the Mayas, who designated it xtabentum, however, its properties were not known by the Mayas.

* A map of the distribution of Rivea corymbosa in central and southern Mexico will be found in Appendix VI.

B. History of the identification of ololiuqui

The Spanish physician Francisco Hernández, was the first European to make a study of the native drugs of the Central American area.^{3,9} Hernández was sent to Mexico by Phillip II of Spain between 1570 and 1575 to study the medicines used by natives of the Aztec Empire. It was Hernández who first described the seed of Rivea corymbosa and illustrated the plant. He described its use by the local natives rather completely. However, it was not until 1649 that his complete ethnobiological notes were published. His notes, which had been partially translated into Spanish by Francisco Ximénez in 1615, were not published until 1649.⁴

At about the same time that the work of Hernández was published, Bernadino de Sahagún studied and reported on the botanical materials which the natives of Mexico used in their daily lives. It was Sahagún who first pointed out that the natives of this area possessed three plants which they called ololiuqui. Only one of these plants was used by them as a narcotic.⁵ Sahagún also pointed out that the plant which possessed the narcotic principle produced only a single seed. In 1629, Hernado Ruiz de Alarcón described in further detail the Aztec method of using the drug ololiuqui.⁶ He called attention to the fact that, when made into a brew and ingested, it deprives one of his senses.

Although all of these early publications described the effect upon and the use of the drug ololiuqui by the natives of central Mexico, they did not describe the plant or illustrate it so that others would be able to examine it. With Hernández's publication in 1649, a complete

description with illustrations of ololiuqui was provided.*

Ololiuqui was one of the chief narcotics and general medicinals of the Aztecs and of the other Central American natives, yet a definite botanical classification required approximately 90 years. The plant was not classified until the latter half of the nineteenth century and a classification was not fully accepted until the middle of the twentieth century.

In 1854 Oliva classified the plant from which ololiuqui is obtained as Convolvulus microcalyx.¹⁰ The classification of Oliva's was accepted by many of the botanists in the latter half of the nineteenth century,¹¹ while others considered his classification open to doubt.¹²

It has since been shown that this designation is correct only with respect to the family, Convolvulaceae.

In an article published in 1903, Dr. Manuel Urbina identified ololiuqui as Rivea corymbosa (Ipomoea sidaefolia (HBK) Choisy).¹³ However, many eminent botanists did not accept the classification of Urbina. One of those who did not accept the designation of Urbina was W. E. Safford, a botanist with the United States Department of Agriculture.¹⁴ Safford concluded in 1915 that the correct designation for the plant from which ololiuqui is obtained was Datura meteloides Dunal ex. D. C., and that it is of the family Solanaceae.¹⁵ Because of his eminent position as a botanist, this designation was accepted by many anthropologists and botanists. Two of the reasons why Safford identified the plant as a species of Datura were: (1) Datura was used by the natives of northern

*His illustration and description will be found in Appendix II.

Mexico and of North America for the purposes of divination,¹⁶ (2) Safford suspected that Hernández erroneously gave a figure of a Convolvulaceous plant rather than a Datura as he should have. Safford argued as follows:

" . . . it is not surprising that it should have been so confused (i.e. Datura meteloides with Rivea corymbosa), for its trumpet-shaped flowers, like that of the closely allied Datura discolor, strongly suggests a morning-glory."¹⁵

It should be noted that the plant from which ololiuqui is obtained was indirectly linked with the Solanaceae by Hernández.³ Hernández compared the physiological action of ololiuqui to that of Solanum maniacum of Dioscorides* but he did not classify the plant as such. Reko has reported evidence which tends to refute the conclusions of Safford and to support the designation of Urbina.⁹

The following taken from the monograph of Schultes, sums up the present position as to the identity of the plant from which ololiuqui is obtained.

"In summarizing the problem of the identification of ololiuqui (that is the plant), therefore, we may state that all of the available early reports, the field observations of Reko, and my own ethnobotanical studies indicates that Urbina was correct in referring to ololiuqui as Rivea corymbosa and that Safford was wrong in suggesting that it was derived from a species of Datura. Furthermore, recent pharmacological work, in demonstrating the presence of an intoxicating principle in the seed of Rivea corymbosa, removes the most important argument which Safford advanced in favor of identification."⁷

*This is the designation used by Hernández and does not represent a modern classification based upon the principles of Linnaeus.

C. Nomenclature and Taxonomy of Rivea corymbosa.*

Schultes gives the following list of designations for the plant from which ololiuqui is obtained:⁷

1. Rivea corymbosa (L) Hallier filius in Engler Bot. ahrb. 8 (1893) 158.
2. Convolvulus corymbosus Lineæus syst. Nat. ed. 10, 2 (1759) 923.
3. Convolvulus domingensis Desrousseau in Lamarck Encyl. 3 (1791) 554.
4. Convolvulus sidaefolius Humboldt. Bonplaud and Kunth Nov. Gen. and Sp. 3 (1818) 99.
5. Ipomoea** corymbosa (L.) Roth Nov. Pl. Sp. Ind. Orient. (1821) 109.
6. Ipomoea sidaefolia (HBK) Choisy in Mem. Soc. Phys. Hist. Nat. Geneve 6 (1833) 459.
7. Turbina corymbosa*** (L) Rafinesque Fl. Teller 4 (1838) 81.
8. Ipomoea burmanni Choisy in De Candolle Prodr. 9 (1845) 350.
9. Ipomoea antillana Millspough in Field Mus. Nat. His. But. Ser. 2, pt. 1 (Plantas Utowanae), Publ. No. 43 (1900) 84.
10. Ipomoea domingensis (Desr.) House in Mullenbergia 3 (1907) 38.

Schultes has given the following description of the plant:⁷

"The plant is a large, scandent, twining woody vine which has leaves 5-9 cms. long and 2.5-4 cms. wide. The leaves are broadly cordate or ovate-cordate, entire, glabrous or very sparingly pubescent, and long-petiolate. Auxillary peduncles and usually many-flowered. The flowers are borne in congested cymes. The corolla are gamopetalous and the infundibuliform as hypocrateri morphous, 2-4 cms. long and white or whitish. The lobes are entirely globose. It has two stigmas and the stamen are included. The ovary are glabrous and 2-celled. The sepals are ovate to ovate-lanceolate and the enlarged in fruit, scarious, somewhat ligneous, about 1 cm. long. The fruit is ellipsoidal, buc-

* An illustration of the plant of Rivea corymbosa will be found in Appendix III.

**In Appendix V will be found a differentiation between Ipomoea and Rivea.

***The Western Regional Research Laboratory used this classification until a sample was sent to them. Now they use the classification Rivea corymbosa.

cate, indehiscent, 1-celled and 1-seeded.* The seeds are roundish, minutely puberulent, and rather woody."

D. The Chemical Composition of Rivea corymbosa.

Prior to this work there was no definitive chemical investigation of the seed of Rivea corymbosa reported in the literature.** The literature revealed two reports by C. G. Santesson.^{8,17} Working with a sample of seed supplied by B. P. Reko, Santesson reported that he discovered a narcotic principal in the seed of Rivea corymbosa.

Santesson's first experiment (he carried out several which includes both chemical and physiological studies) is briefly outlined. He extracted a portion of the crushed seed of Rivea corymbosa with alcohol. The extract was a clear, yellow solution. Santesson observed that the addition of water to this clear yellow solution resulted in the solution becoming turbid. After some time a heavy, fine-grained, white precipitate resulted. The alcoholic solution when reduced to dryness upon a steam bath, left a residue which was yellow and tasted extremely bitter. The yellow extract was combined with some distilled water and heated on a water bath. Upon cooling, a mucilaginous mass was obtained. This mucilaginous condition probably resulted from the protein and mucus which are present in the drug. Consequently, the filtration process was extremely difficult. The filtrate was brown, turbid, bitter, slightly acidic, and exhibited a negative Fehling's sugar reaction. Upon refluxing the fil-

*The Appendix IV gives an illustration of the seed of Rivea corymbosa.

**A recent article by Perez-Amador and Herran has been published in Tetrahedron Letters, No. 7, pp.30-32, 1960.

trate with hydrochloric acid there resulted a solution which gave a positive Fehling's sugar reaction. This demonstrated that the drug contains a glycoside which is readily cleaved with hydrochloric acid giving an aglycone and a reducing sugar. This result is not unexpected since it is known that the Convolvulaceae contains many species which produce glycosides.

Santesson carried out some physiological experiments using an aqueous extract of the seed of Rivea corymbosa. As test animals he used frogs (Rana temporaria). To the frogs he gave a subcutaneous injection of the drug. According to Santesson:⁸

". . . They (the frogs) soon lay on their backs without turning round by themselves; movement, even on excitation, did not succeed at all or only with difficulty. If the frogs were turned on their bellies, they could jump initially fairly well, but later made only shorter and less skillful jumps. In the dorsal position the spontaneous breathing was interrupted for a long time. Occasionally movements to croak were observed, but without sound. The animals had in some sense lost all initiative. A partial paralysis of the brain, as a form of narcosis seemed to exist."

Santesson also reported the use of white mice and a rabbit in his physiological experiments. As a result of subcutaneous injection, the animals exhibited dullness and sleepiness. The mice moved with difficulty and breathed extremely heavily. The rabbits showed signs of increased salivation.

Reko, in his publication, suggested that ololiuqui actually contained an inactive glycoside and that it could be rendered effective only after splitting.⁹ In order to test this hypothesis, Santesson hydrolyzed

an aqueous extract with hydrochloric acid. The hydrolysate was filtered to remove a gray-brown flocculent precipitate which formed during the hydrolysis. This filtrate was neutralized with sodium carbonate and the neutralized solution was used in additional animal physiological tests. Since this solution contained an excess of sodium chloride, it was necessary to run some controlled experiments using a solution of sodium chloride. The effects upon the test animals of the hydrolyzed material were approximately the same as the unhydrolyzed material, except more intense. In fact, some of the animals died as a result of the injected material which had been hydrolyzed.

Santesson attempted to ascertain if the physiologically active principle was an alkaloid. He treated an aqueous solution of the seed with acetic acid, boiled the resulting solution to remove most of the protein and mucilage materials from the solution as a red-brown mass and then filtered the solution. The filtrate gave a negative Fehling's sugar reaction. This indicated that the glucoside was unhydrolyzed. The solution was also tested with several alkaloidal detection reagents. In each case the results were negative.

The above solution was then refluxed with hydrochloric acid. After this treatment the solution was filtered to remove additional protein and mucilage which had precipitated during the refluxing with the hydrochloric acid. The resulting filtrate was tested with a sugar detecting reagent (Fehling's solution) and was found to give a positive reaction with the test solution. The solution was found to give a positive

test reaction for the presence of an alkaloid, also. These reactions indicate that the seed of Rivea corymbosa contains a gluco-alkaloid and that the alkaloid is released upon hydrolysis with hydrochloric acid.

The following summary of the chemical constituents of Rivea corymbosa is taken from the paper by Santesson:

1. "The drug probably contains a glycoside, a glycoalkaloid, with an alkaloid linked to a sugar, 2. Animal experiments with frogs, white mice, and rabbits show both before and after the hydrolysis of the glycoside with boiling hydrochloric acid solution, that the drug has a depressing principle which acts upon the central nervous system."

II: SOURCE AND DESCRIPTION OF THE SEED OF RIVEA CORYMBOSA

Two shipments of seed have been received. The first shipment was obtained indirectly from the Botanical Gardens of Harvard University. The seed were grown in Cuba.

The second shipment of seed was collected in Mexico for this research project, and obtained through the agency of Dean Willis R. Brewer of the College of Pharmacy, University of Arizona. The following description of the seed was included:

"Rivea corymbosa is a low running vine with cordate, alternate, leaves with abundant white tubulous campanulate flowers; with a small, light brown fruit, each one with one seed; seeds brown or orange gray, hairy pubescent, round. The seeds were mature when collected at 40 km. from Tuxtla Gutierrez to a town named San Fernando (Chiapas) and was collected growing in limey, rocky soils at 500 meters over sea level."

The research workers are deeply indebted to Dr. Richard E. Schultes of the Botanical Museum of Harvard University for a final verification of the authenticity of the second shipment of seed.

III. EXTRACTION OF THE SEED OF RIVEA CORYMBOSA.*

A. Preparation of the seed and methods used.

Many of the seed were obtained with the husks still on them. These husks were removed by rubbing the seed on a rough surface, such as a washboard. The husks were then blown from the seed using compressed air. Many of the seed were worm eaten. These were very carefully removed. After sorting, the seeds were washed with distilled water and then allowed to dry. The seeds were then pulverized using a burr mill.** The pulverized seeds were then extracted with organic solvent. Three different methods for the extraction of the seed of Rivea corymbosa have been employed. These methods are: soxhlets, toogood, and a large Waring blender.***

The most generally acceptable method was the use of the large Waring blender. This method combined the speed of the soxhlets with the quantity factor of the toogood method.

B. Qualitative Relationship.

The pulverized seed of Rivea corymbosa was extracted with a non-polar solvent. This initial extraction was to remove fats and waxes which are present in the original seed. Lipid materials, if not removed, would result in increased difficulties at later stages of the extraction process. A variety of solvents have been used. These include diethyl-

* A scheme for the extraction will be found in Appendix VII.

** The burr mill used here was a used coffee grinder.

***The Waring blender was one-gallon capacity and constructed of stainless steel.

ether, petroleum ether (boiling point range 60-110°), Skelly F (this is a petroleum fraction which has a boiling point range of 30-60°), 1,2-dichloroethane, and carbon tetrachloride. The most generally satisfactory solvent, on the basis of solvent power, expense and the nonexplosive nature of the chemical, was 1,2-dichloroethane.

Following extraction with the nonpolar solvent, the marc was air dried to remove residual solvent.

The dried marc was then extracted with a polar solvent. Absolute methanol and ethanol were used. It was believed that the absence of water would prevent the extraction of much of the protein and mucilaginous material which are present in the seed. Although methanol and ethanol are approximately equal as solvents, ethanol was used for three reasons: 1) it is less toxic than methanol; 2) it has a higher vapor pressure;* 3) the explosive hazard is reduced.

C. Quantitative Relationships.

The quantitative relationships were determined using a soxhlets extractor. The percentage of lipid material in the seed of Rivea corymbosa was determined as follows (1,2-dichloroethane was used as the extracting solvent):

Weight of the seed extracted	276.1 g
Weight of lipid materials removed	26.3 g
Percent lipid material in the seed	9.5%

*This was especially true when the large Waring blender was used, as the friction of the blades rotating at high speed produced large quantities of heat.

The percentage of the alcohol soluble material was determined as follows (absolute ethanol was used as the extracting solvent):

Weight of the seed extracted	276.1 g
Weight of the alcoholic soluble materials ..	20.6 g
Per cent alcohol soluble material	7.6 %

IV. THE COMPOSITION OF THE OILS OF RIVEA CORYMBOSA*

A sample of the oil of Rivea corymbosa was sent to Dr. I. A. Wolff, Chief, Industrial Crops Laboratory, Peoria, Illinois. The work done at the Regional Laboratory consisted in determining the percent of the fatty acids in the oil. The seed contains 9.45% oil.

Table I shows percentages based on the total fatty acid present, with the exception of the percentages of linoleic and lenolenic acids after alkaline isomerization. These percentages are based on the total oil examined. These values would be larger if the percentages were based on the fatty acids present.

TABLE I

This distribution does not represent an unusual oil according to those who did the analysis. It is noted that the seed of Rivea corymbosa does contain two unidentified fatty acids.

*The fatty acid determination was done at the Industrial Crops Laboratory, Northern Utilization Research and Development Division, Peoria, Illinois.

TABLE I
Fatty Acid Composition of Rivea corymbosa

Fatty Acid	Percentage	Percentage after alkaline isomerization
Myristic	0.3	----
Palmitic	22.0	----
Palmitoleic	0.3	----
Stearic	9.1	----
Oleic	12.4	----
Linoleic	45.7	37.5
Linolenic	2.2	2.4
C ₂₀ (saturated)	2.0	----
C ₂₀ (1 double bond)	0.0	----
C ₂₀ (2 double bonds)	0.0	----
C ₂₀ (3 double bonds)	0.0	----
C ₂₂ (saturated)	1.0	----
C ₂₂ (1 double bond)	0.0	----
C ₂₂ (2 double bonds)	0.0	----
C ₂₂ (3 double bonds)	0.0	----
C ₂₄ (saturated)	Trace	----
C ₂₄ (1 double bond)	0.0	----
Unknown	0.8	----
Unknown	0.4	----

V. FRACTIONATION OF THE ETHANOLIC SOLUBLE CONSTITUENTS
OF RIVEA CORYMBOSA.

A. General Considerations.

The material which was obtained from the ethanolic extraction of the seed of Rivea corymbosa consists of a multiplicity of chemical compounds. Several attempts have been made to effect some separation of this mixture. Some of these attempts have met with success, while others have not. Two methods have been used to fractionate the extract: 1) chromatography,* 2) solvent fractionation.

In the chromatographic fractionation several solvents, solvent systems, and adsorbents were employed; however, little success was achieved using this process.

In the solvent fractionation process two devices have been used, 1) a magnetic mixer, and 2) a small Waring blender. As most of the solvents were inflammable, the magnetic mixer was the final choice of the two methods.

A great variety of organic solvents, varying in their polarity, have been used in the solvent fractionation. The solvents used, were: 1) tetrahydrofuran, 2) diethylether, 3) n-butyl ether, 4) acetone, 5) cellosolve, 6) 1,2-dimethoxyethane, 7) ethyl acetate, 8) bis-(2-ethoxy)-ethyl ether, 9) isopropyl ether, 10) methyl-ethyl ketone, 11) chloroform, 12) carbon tetrachloride, 13) n-butyl alcohol, and 14) water. Most of these solvents removed only a small amount of resinous material, which

*Much of the chromatography was done by Mr. Ben R. Condray, while at Baylor University, Waco, Texas.

could not be crystallized or sublimed to give a crystalline produce. However, as a result of the use of two solvents, 1) tetrahydrofuran and 2) water, it was possible to isolate two crystalline products.

B. Tetrahydrofuran Fractionation.

The ethanolic extract of the seed of Rivea corymbosa was stirred at room temperature with tetrahydrofuran for a period of twelve hours. The suspension was filtered to remove insoluble material. The filtrate was reduced to dryness using a Rinco evaporator. A small amount of yellow amorphous material was extracted by the tetrahydrofuran. The amount of material removed by the extraction was dependent upon the quantity of tetrahydrofuran used in the extraction. The yellow material yielded a white, crystalline product, when it was sublimed at a temperature between 125 and 135°. At this temperature the yellow material decomposed, and the crystalline sublimate was contaminated with some of the decomposition products. It was necessary, therefore, to resublime the product which was obtained from the first sublimation several times in order to obtain a pure product. The white, crystalline product was characterized as follows. 1) Since the compound sublimed at 125-135°, it was not possible to obtain a good melting point using an open capillary. In a sealed capillary, the compound melted at 169-170°.* 2) The acetate of the compound was prepared.** The acetate was purified by re-

* All melting points are corrected.

**The method used in the preparation of the acetate is given in Appendix VIII-A.

crystallization from absolute methanol, followed by sublimation. The melting point of the purified acetate was 120-121°. 3) The crystalline compound was found to give a positive test for a phenol, in a solution of 5 per cent sodium carbonate, with Folin-Ciocalteu phenol reagent.* 4) An aqueous solution of the crystalline compound slowly turned a rose color when exposed to the air. 5) From the carbon-hydrogen analysis and the molecular weight, the molecular formulas of the original compound and of the acetate of the compound were determined to be: (a) the original compound $C_6H_6O_2$, (b) the acetate of the original compound $C_{10}H_{10}O_4$. 6) The infrared and the ultraviolet spectra of the compound were determined.** These spectra were found to be identical with the spectra of an authentic sample of hydroquinone. See Figures 1 and 2 for the comparison of the infrared spectrum of the known and the unknown samples.

Elemental Analysis:*** Calculated for hydroquinone:

C = 65.44%, H = 5.49%, Molecular weight 110.11;

Found, C = 65.83%, H = 4.93%, Molecular weight 507,
(high, due to association).

Calculated for hydroquinone diacetate:*** C = 61.84%,

H = 5.19%, Molecular weight 194.18, Found: C = 61.95%,

H = 5.09%, Molecular weight 182.

* The preparation of this reagent and the method of its use is given in Appendix VIII-B.

** The infrared spectra was run by Samuel P. Sadtler Research Laboratories, Philadelphia, Pa.

***The carbon-hydrogen analysis were run by Clark Microanalytical Laboratory, Urbana, Illinois.

C. Water Fractionation.

It was found that water solubility furnished a means of fractionating the ethanolic extract of Rivea corymbosa. The longer the solution was allowed to set, the larger the quantity of material which precipitated. The precipitate was washed with large quantities of distilled water to remove soluble material from the original precipitation. The insoluble material was then recrystallized. After trying several solvent systems, it was found that the best solvent for recrystallization was hot, absolute methanol. After several recrystallizations, the crystalline product had a melting point of 241-241.6°. The material was found to recrystallize from water and methanol to give large, well-defined crystals. These crystals melted at 230-232°. Because of the difference in the melting points of these two compounds, the infrared spectra of the two were compared (see Figures 3 and 4).^{*} The only difference in the spectra of the two materials was the presence of two bands in the spectra of the material obtained from water and methanol at 1620 Cm^{-1} and 1660 Cm^{-1} . These bands are characteristic for compounds which have well-defined water of crystallization. The material obtained by recrystallizing from water and methanol contained 4.54 per cent water of crystallization. This was determined by the weight-loss method.^{**}

Functional group analysis indicates the following:¹⁹ 1) The presence of hydroxyl groups was indicated by the following positive tests,

* The infrared spectra were run on a Beckman IR-4

** Determined by Geller Laboratory, Bardonia, N. Y.

(a) periodate; (b) Lucas' reagent; and (c) acetyl chloride. 2) The absence of any carbonyl functional group. 3) The absence of any alkene functional group by the following negative tests: (a) bromine addition; (b) tetranitromethane; and (c) ozonolysis.*

The infrared spectrum of the compound (see Figure 5) exhibits several bands, one at 3450 cm^{-1} and the other between 1000 and 1100 cm^{-1} . The first, at 3450 cm^{-1} is characteristic for compounds which have hydroxyl functional groups. The magnitude of this band is determined by the number of hydroxyls which are present in the compound. The bands which appear between 1000 and 1100 cm^{-1} are characteristic of glycosides. The band at 1120 cm^{-1} suggests the possibility of ether functional groups. The infrared spectrum indicates that the compound is not aromatic. The absence of any band in the 1800 - 1700 cm^{-1} region indicates the absence of carbonyl functional groups in the molecule. Functional group analysis and infrared spectrum analysis, therefore, indicates that the compound is a glycoside with only hydroxyl and possibly ether functional groups.

Because of the difficulty in obtaining an accurate molecular weight of the glycoside, as a result of the insolubility of the compound in suitable solvents for the Rast or ebullioscopic molecular weight determinations, it was decided to determine the molecular formula of the glycoside acetate. From this information, by back calculation, it is possible to assign a molecular formula to the glucoside. The acetate

* We are indebted to Dr. John S. Belew of Baylor University, Waco, Texas for the ozonolysis.

was prepared by the method given in Appendix VIII-B. The acetate was recrystallized from hot, absolute methanol to a constant melting point of 247-248°. The infrared spectrum of the glycoside acetate is shown in Figure 5. From the carbon-hydrogen analysis and the molecular weight, the glycoside acetate was assigned a molecular formula of $C_{42}H_{60}O_{19}$.

Elemental analysis: Found, C = 58.21%, H = 7.16%;

Molecular weight 840; Calculated for $C_{42}H_{60}O_{19}$,

C = 58.05%, H = 6.96%, molecular weight 868.90.*

The number of acetyl groups in the glycoside acetate was determined and found to be seven.

Analysis: Found 36.28%; calculated for seven acetyl groups 35.82%.

Using the molecular formula of the glycoside acetate and the fact that the acetate contains seven acetyl groups, it is reasonable to assign a molecular formula of $C_{28}H_{46}O_{12}$ to the glycoside.

Elemental analysis:* Found C = 59.08%, H = 8.36%;

$[\alpha]_D^{23} = 49.8 \pm 0.3^\circ$ (C = 1.079mg/ml in pyridine),**C-methyl = 0.96%***; H = 8.07%, 4 C-methyls = 0.95%.

The next problem was the hydrolysis of the glycoside and the identification of the sugar moiety and of the aglycone. Because the glycoside is only slightly soluble in water and dilute acid, hydrolysis of the acetal sugar linkage was quite difficult. A large number of hydro-

* Analysis run by Geller Laboratories, Bardonia, New York

** All optical rotations were run by Schwarzkopf Micro-Analytical Laboratory, Woodside, New York

*** See Appendix IX for a discussion of C-methyl group analysis.

lytic processes were attempted. Those hydrolytic methods which split the glycoside also substantially degraded the aglycone. The degradation resulted in the formation of a carbonyl functional group. Figure 6 shows the infrared spectrum of the product isolated from acid hydrolysis of the glycoside. It reveals a band at 1715 cm^{-1} which is characteristic for a carbonyl functional group. The 2,4 dinitrophenylhydrazone of the carbonyl compound was prepared according to the method given in Appendix V VIII-C. After recrystallization the melting point of the phenylhydrazone was $123-125^\circ$. The molecular formula of the 2,4 dinitrophenylhydrazone is $\text{C}_{24}\text{H}_{34}\text{N}_4\text{O}_6$.

Elemental analysis: Found, C = 60.72%, H = 7.23%,

N = 11.81%, molecular weight 446; calculated for

$\text{C}_{24}\text{H}_{34}\text{N}_4\text{O}_6$, C = 60.74%, H = 7.22%, N = 11.81%,

molecular weight 474.54.*

It is apparent that the glycoside suffered the loss of 10 carbons, 16 hydrogens, and 10 oxygens during the acid hydrolysis. Since the net loss due to the glucose moiety is $\text{C}_6\text{H}_{11}\text{O}_5$, the degradation of the aglycone resulted in the additional loss of 4 carbons, 5 hydrogens, and 5 oxygens. The exact nature of these losses are unknown.

The sugar moiety was identified by paper chromatography. The unknown sugar solution was chromatographed with glucose, and found to have the same Rf value as glucose. Two different solvent systems were

* Analyses run by Geller Laboratories, Bardonia, New York

** Analyses run by Berkeley Analytical Laboratory, University of California, Berkeley, California

employed in the chromatography. See Figures 7 and 8 for a reproduction of these chromatograms. The chromatogram shown in Figure 7 was run using the solvent system n-butanol-Ethanol-water:: 10-1-2, and the chromatogram shown in Figure 8 was run using the solvent system n-butanol-pyridine water:: 6-4-3. Further confirmation of the sugar as glucose was supplied by the preparation of the phenylosazone and the penta-acetate.

It was determined that the glucoside could be hydrolyzed by suspending it in a solution of water and methanol (75% water and 25% methanol) with emulsin or β -glucosidase. The aglucone was isolated and recrystallized from water and methanol to a constant melting point of 166-168°. Figure 9 shows the infrared spectrum of the aglucone. The acetate was prepared by the method given in Appendix VIII-A. The aglucone acetate was recrystallized from hot, absolute methanol to a constant melting point of 208-210°. The infrared spectrum of the aglucone acetate is shown in Figure 10.

The molecular formula assigned to the aglucone acetate is

$C_{30}H_{44}O_{11}$. Elemental analysis: Calculated for $C_{30}H_{44}O_{11}$,

C = 62.05%, H = 7.64%, molecular weight 580.65; Found:

C = 62.02%, H = 7.79%, molecular weight 580*, $[\alpha]_D^{23.5} =$

$-52.4 \pm 0.5^\circ$ (C = 1.132 mg/ml in pyridine).

The number of acetyl groups in the aglucone acetate was determined

* Analyses run by Berkeley Analytical Laboratory, University of California Berkeley, California.

and found to be four.

Analysis: Found 28.90%; Calculated for four, 29.67%.

Using the molecular formula of the aglucone acetate and the number of acetyl groups, it is possible to assign the molecular formula of $C_{22}H_{36}O_7$ for the aglucone.

Elemental analysis: * Found, C = 64.12%, H = 9.07%;

$[\alpha]_D^{23} = -40.8 \pm 0.6$ (C = 1.006 mg/ml in methanol),

C-methyl 4.3%; Calculated for $C_{22}H_{36}O_7$, C = 64.05%,

H = 8.79%, 1 C-methyl 3.63.

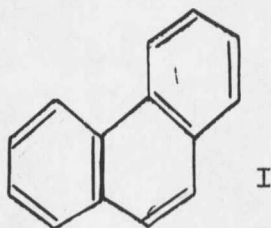
If one adds to the molecular formula of the aglucone $C_6H_{10}O_5$ (the empirical formula of glucose in a glucoside), the molecular formula of the glucoside is obtained, $C_{28}H_{46}O_{12}$. As shown earlier, this formula agrees with the analytical data and with the formula which was postulated on similar evidence obtained from the glucoside hepta-acetate.

The determination of the carbon skeleton of the aglucone was accomplished through dehydrogenation studies.^{32,33,34,35} The aglucone was heated for 6 hours, as an intimate mixture, with an excess of palladium on charcoal (5 percent) at 350°. During the heating a stream of dry nitrogen was used to sweep out the hydrogen which was formed. After heating for six hours, the reaction mixture was cooled and extracted

* Analyses run by Berkeley Analytical Laboratory, University of California Berkeley, California.

with cyclohexane. The cyclohexane was removed, at reduced pressure, with the Rinco evaporator.

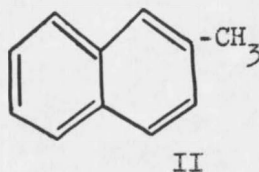
The residual oil was further purified by chromatographing it on a column of alumina*³³ using n-hexane as the eluting solvent. Three cuts were taken. From the first cut, a white, crystalline product was obtained. This product was characterized as phenanthrene (I) by comparing the melting point, ultraviolet and the infrared spectra of the unknown with that of an authentic sample of phenanthrene (see Figures 11 and 12 of infrared spectra). The ϵ values of phenanthrene are:^{22,27} λ_{\max} 251 mu, $\epsilon = 63,000$; λ_{\max} 330 mu, $\epsilon = 252$. The values for the unknown are: λ_{\max} 251 mu, $\epsilon = 65,200$; λ_{\max} 330 mu, $\epsilon = 247$.



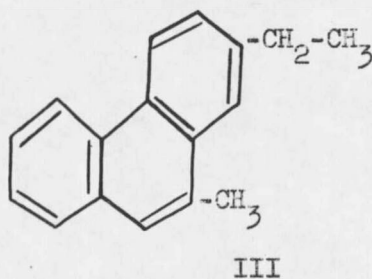
After extraction with cyclohexane, the reaction mixture was extracted with benzene. Upon removal of the benzene using the Rinco evaporator, a yellow, oily material was obtained. This oil was chromatographed on silica gel,³⁵ using n-hexane as the eluting solvent. Two fractions were obtained. The first fraction did not fluoresce in ultraviolet light. However, the ultraviolet spectrum of the fraction was similar to those reported for naphthalene derivatives.²² A picrate was prepared which after several recrystallizations from methanol, exhibited

* The method of preparation of the alumina is described in Appendix VIII-D

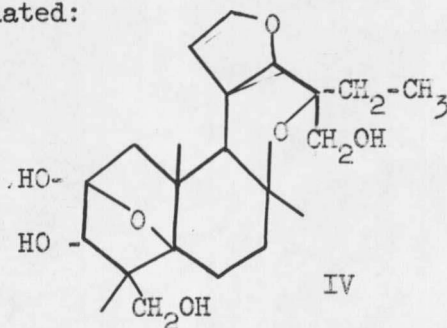
a constant melting point of 115-116°. This melting point corresponds to the picrate of 2-methyl naphthalene (II).



The second fraction, which fluoresced under ultraviolet irradiation, was found to have an ultraviolet spectrum similar to those reported for other phenanthrene derivatives.^{20,22} It had the following maxima: 299 mu, 288 mu, and 277 mu. The picrate of the aromatic material was prepared. After several recrystallizations, a constant melting product was obtained. The picrate was orange and had a melting point of 107-108°. This is the melting point which has been recorded for 10-methyl-2-ethyl phenanthrene (III).²¹

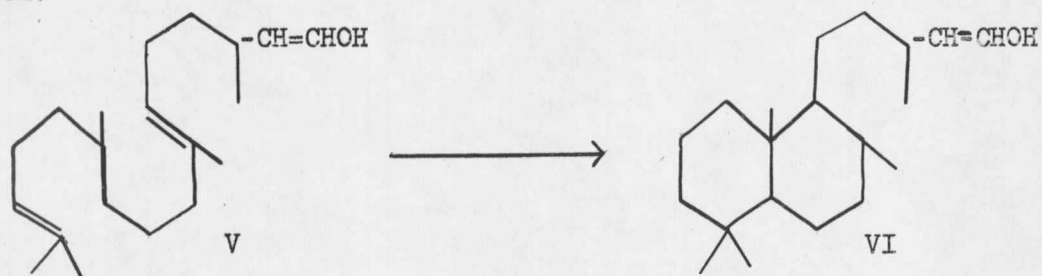


On the basis of the evidence, the following structure for the aglucone is postulated:



The exact positions of the oxygens cannot, on the basis of the evidence thus far available, be assigned. However, in the above proposed structure, the oxygens are placed at those positions most generally found to be occupied in similar or related compounds. The carbon skeleton and the dehydrogenation products obtained from it are amply justified by the available literature.^{23,24,25,26}

The biogenetic relationship of geranyl geraniol (V), with the carbon skeleton of structure (VI) affords a ready synthesis of the carbon skeleton.



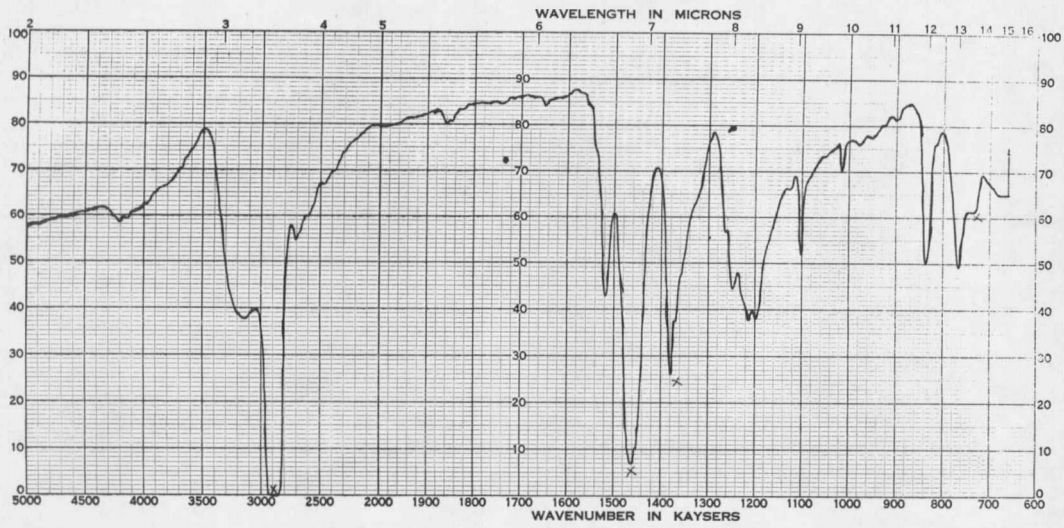


Figure 1

Infrared Spectrum of Hydroquinone

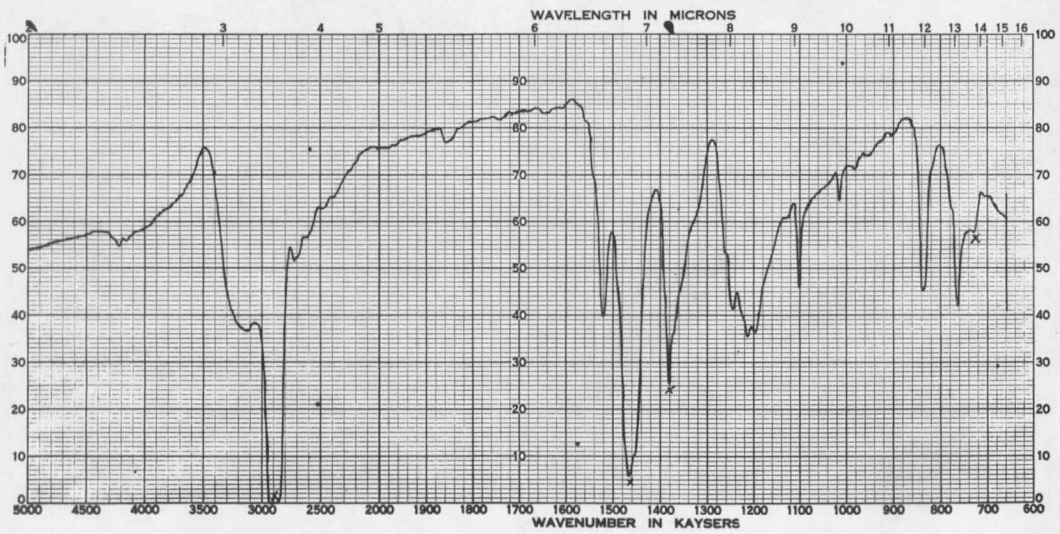


Figure 2

Infrared Spectrum of Unknown

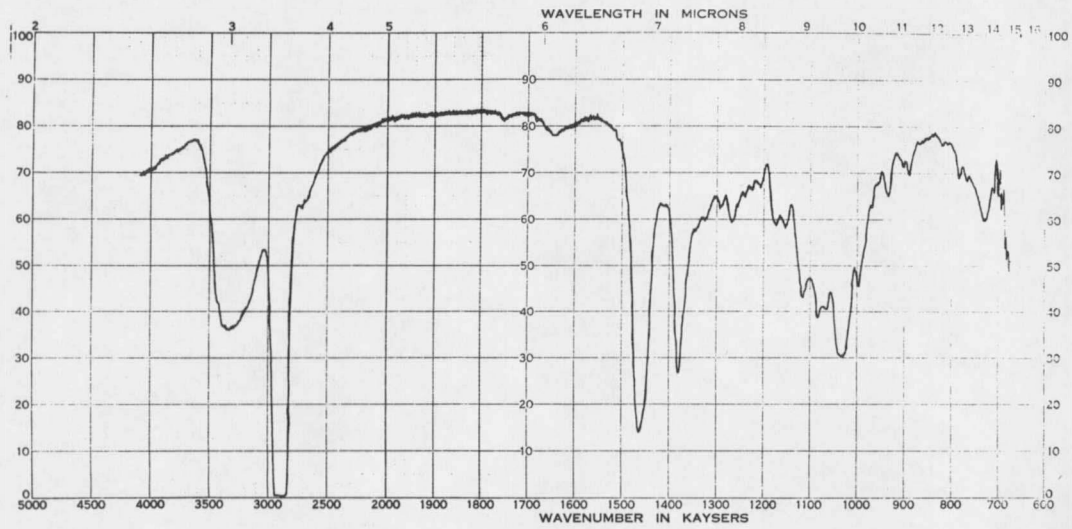


Figure 3

Infrared Spectrum of Glucoside recrystallized
from absolute alcohol

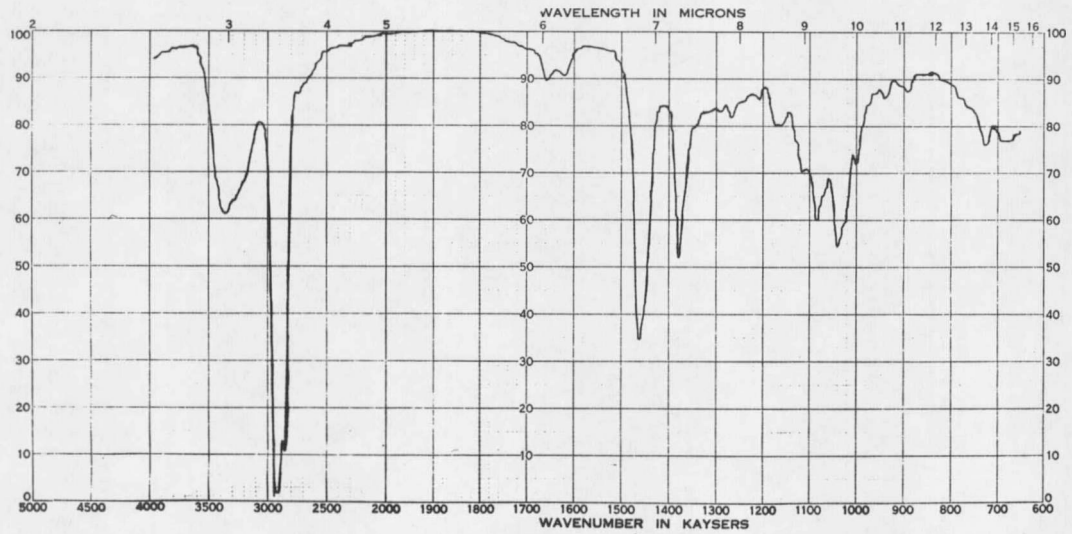


Figure 4

Infrared Spectrum of Glucoside recrystallized
from water-methanol

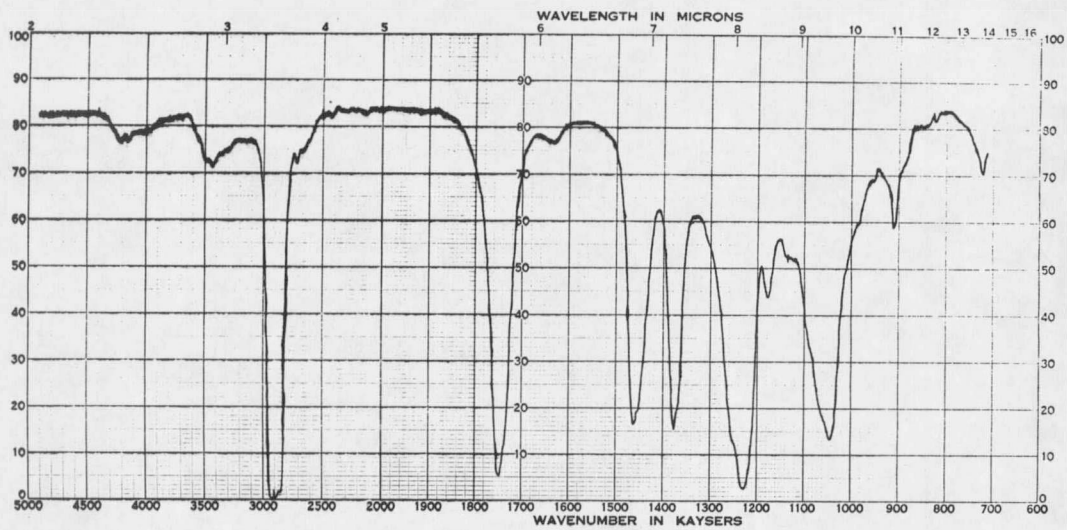


Figure 5

Infrared Spectrum of Glucoside Hepta Acetate

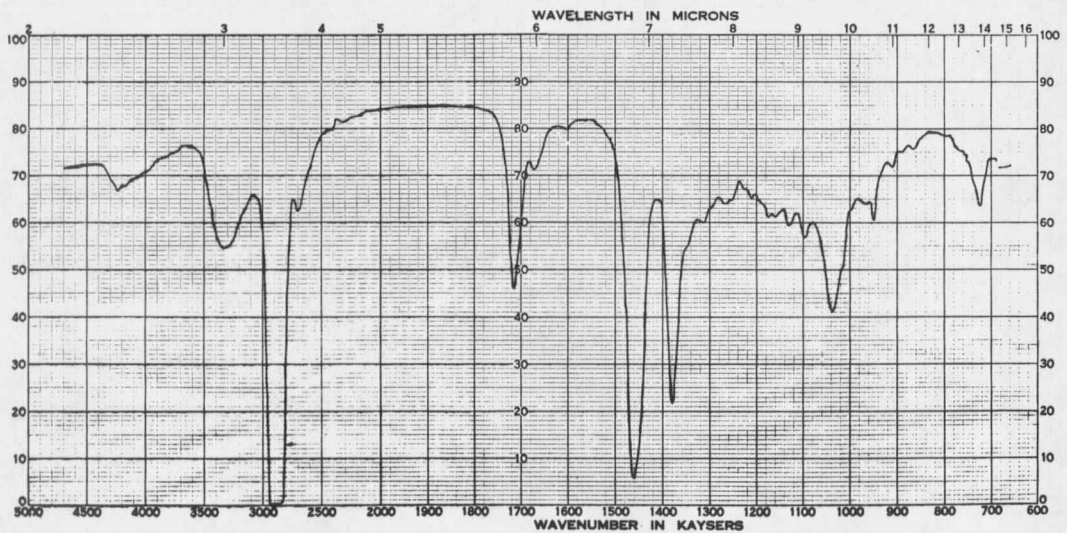


Figure 6

Infrared Spectrum of Aglucone isolated from the acid hydrolysis of the Glucoside

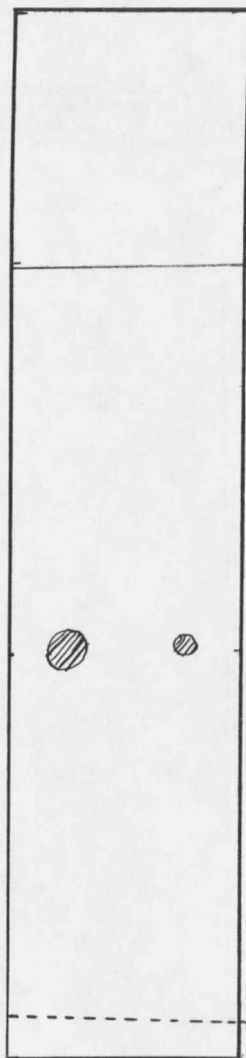


Figure 8

Reproduction of the sugar chromatogram
using the Solvent System n-butanol:
pyridine: water = 6:4:3.

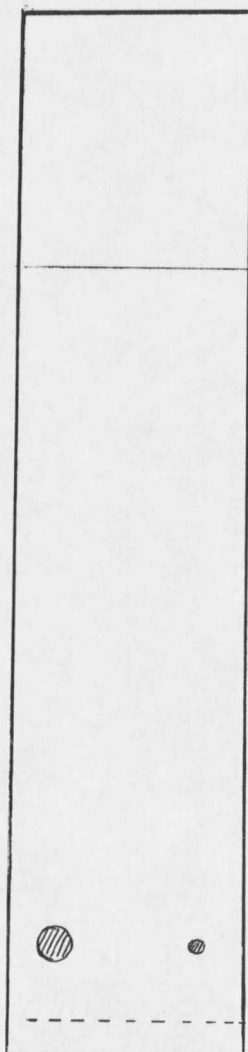


Figure 7

Reproduction of the Sugar Chromatogram
using the solvent system n-butanol:
Ethanol: Water = 10:1:2.

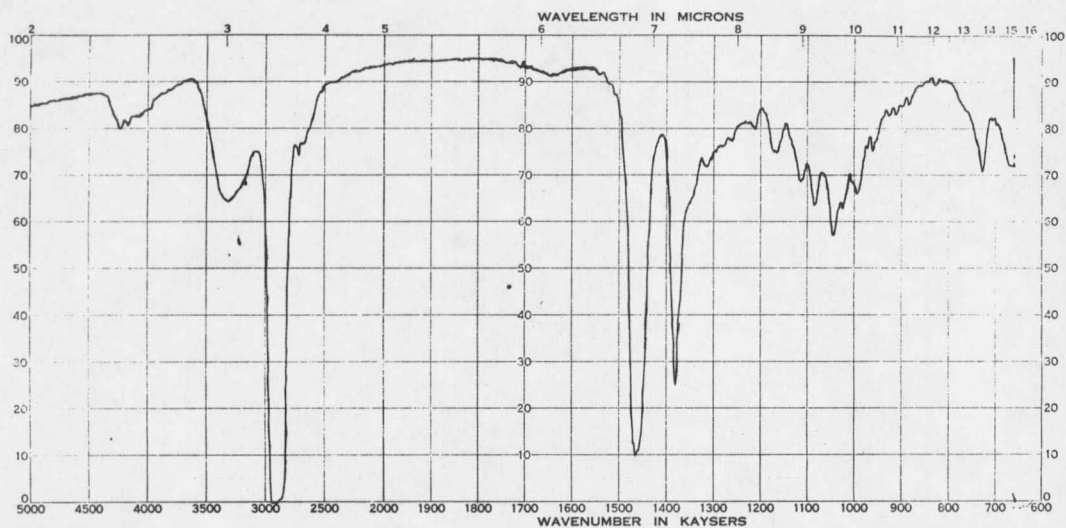


Figure 9
Infrared Spectrum of the Aglucone

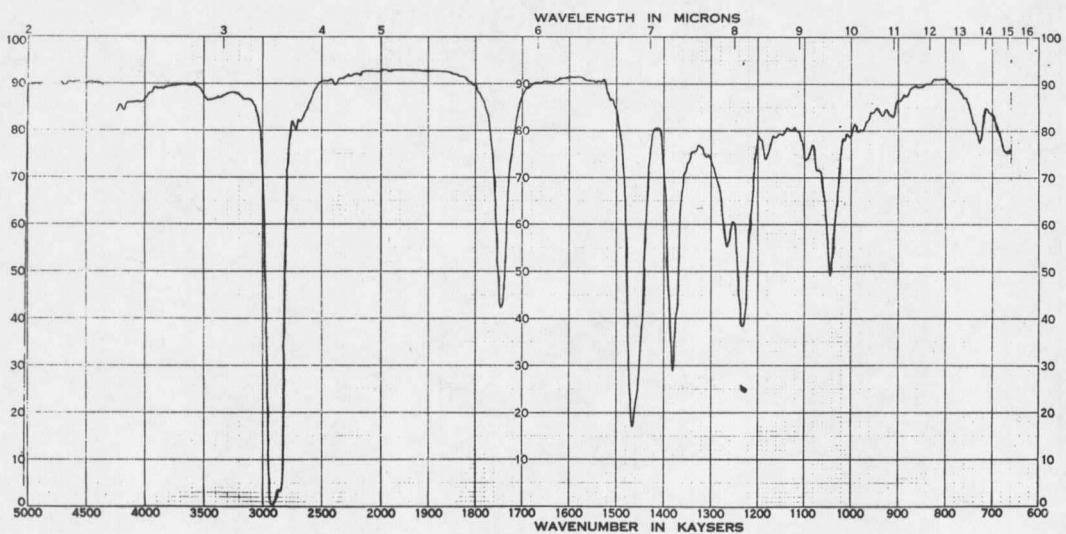


Figure 10
Infrared Spectrum of the Aglucone tetra-acetate

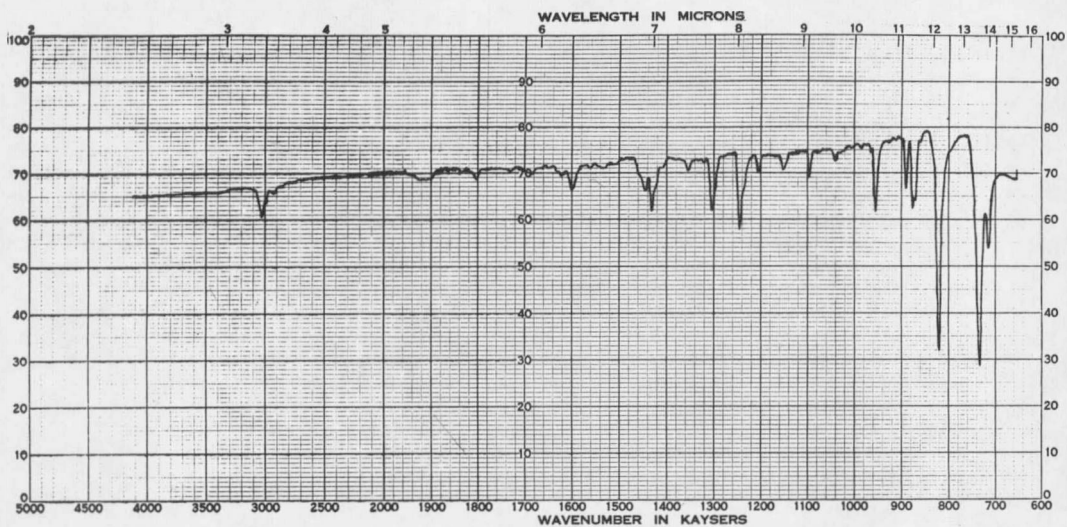


Figure 11

Infrared of Phenanthrene

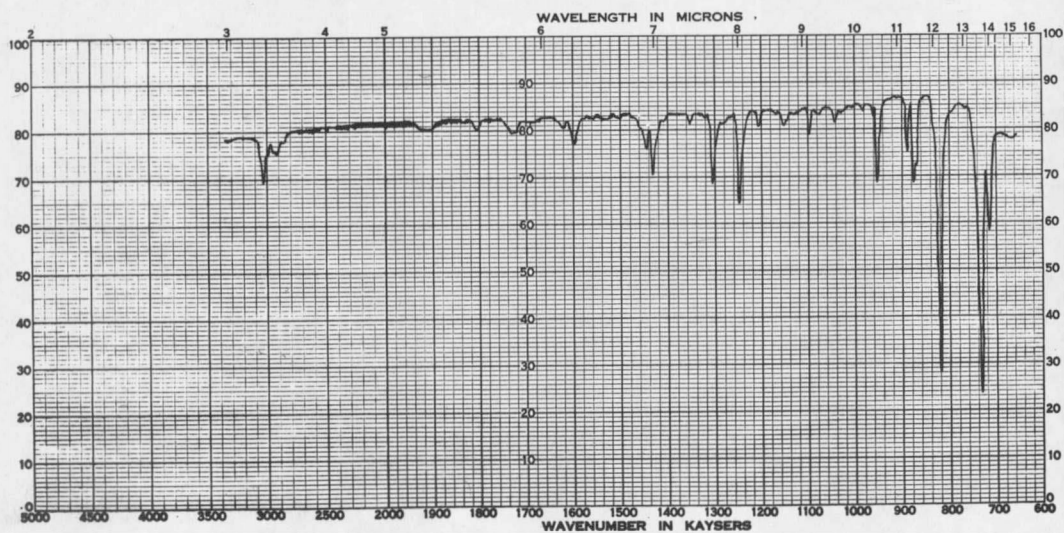


Figure 12

Infrared of Unknown

VI. DETERMINATION OF THE ALKALOIDAL CONSTITUENTS
IN THE SEED OF RIVEA CORYMBOSA.

A. Introduction.

The original interest in the study of the drug ololiuqui was its reported use by the Central American natives as a narcotic. Since the drug possessed physiological action characteristic of that produced by many alkaloids, repeated attempts have been made to isolate the alkaloidal materials. Of the methods attempted in this investigation, three will be described.

B. Method I

15.9 g of pulverized ololiuqui was extracted with 250 ml. of methanol and hydrochloric acid (225 ml. of absolute methanol and 25 ml. of concentrated hydrochloric acid) for 25.5 hours in a soxhlet extractor. After this time the hydrochloric acid was neutralized with an equivalent amount of KOH. The neutral solution was filtered to remove the KCl which formed and precipitated. The light brown filtrate was reduced in volume using the Rinco evaporator. As the solution was concentrated a resinous material separated, together with additional KCl. These were removed by filtration. After removal of inorganic salts the solution was reduced to dryness. The residue was a resinous, brown material. This material was triturated for eight hours with CHCl_3 using a magnetic mixer. The CHCl_3 extract was evaporated to dryness. The residue was then taken up in H_2O , treated with Darco to remove residual resinous materials, and the following tests were made on the resulting solution.

1. Scheibler's alkaloid test ³⁰..... Positive
2. Mayer's alkaloid test ³⁰ Positive
3. Folins-Ciocoltec Phenol Test* Positive
4. Molisch sugar test Positive
5. Protein and Amono acids Negative

C. Method II.

110.6 grams of pulverized ololiuqui, which had been previously defatted with 1,2-dichloroethane, was extracted for 28 hrs. in a soxhlets extractor with 900 ml. of a 1 N methanol-sulfuric acid solution. Spot checks indicated the reflux temperature to be $64^{\circ} + 3^{\circ}$. The sulfate ions were precipitated with $Ba(OH)_2$. The $BaSO_4$ was removed by filtration. The solution was then made slightly acid with sulfuric acid and water added. The methanol which remained was removed with the Rinco evaporator. As methanol was removed a resinous material deposited on the sides of the flask, the flask was changed periodically to remove the resins. The residue of the methanol-sulfuric acid extract was dissolved in distilled water and adjusted to a pH of 11 with NaOH. The basic solution was steam distilled. The steam distillate was evaporated to dryness with the Rinco evaporator. The residue gave the following reagent tests:³⁰

*For details of the reaction test see Appendix VIII-B.

1. Folins-Ciocoltec phenol reagent Positive
2. Schribler's Alkaloid reagent Positive
3. Mayer's Alkaloid reagent Negative

The solution was then adjusted to a pH of 1 with H_2SO_4 and steam distilled. The steam distillate gave negative alkaloid reagent tests. The acid solution was extracted with $CHCl_3$. The material extracted gave the following tests:

1. Scheibler's Alkaloid Reagent Positive
2. Mayer's Alkaloid Reagent Negative

The solution was then made basic with NH_4OH and extracted with $CHCl_3$. The material extracted gave the following tests:

1. Scheibler's Alkaloid Reagent Positive
2. Mayer's Alkaloid Reagent Positive
3. Folins-Ciocoltic Phenol Reagent Positive

D. Stass-Otto Method³⁰

30 grams of the crude ethanolic extract was dissolved in 800 ml. of absolute ethanol. This solution was made acidic with tartaric acid and refluxed for 22 hours. The ethanol was then removed with the Rinco evaporator. The residue was dissolved in water and filtered. The filtrate was reduced to dryness, and the residue was dissolved in 450 ml. of absolute ethanol and kept cold overnight. The cold solution was filtered and the filtrate reduced to dryness. The residue was dissolved in water and filtered. This process was repeated three times. The resulting solution was made acidic (pH 3) with HCl and extracted with ether. The ether solution was

over anhydrous Na_2SO_4 and reduced to dryness. The residue gave the following tests.³⁰

1. Scheibler's Alkaloid Reagent Positive
2. Mayer's Alkaloid Reagent Positive

The solution was then made basic with NaOH and again extracted with ether. The ether solution was dried over anhydrous Na_2SO_4 and reduced to dryness on a Rinco evaporator. The residue gave the following reagent tests:³⁰

1. Scheibler's Alkaloid Reagent Positive
2. Mayer's Alkaloid Reagent Negative

E. Discussion

The original purpose of this investigation was to isolate an alkaloidal moiety. The methods employed failed to result in the isolation of a crystalline alkaloid. The reagent tests were conducted on samples obtained under conditions which should have precluded interfering substances. Since the tests were positive, it is assumed that the seed of Rivea corymbosa does contain alkaloidal materials. The difficulty encountered in attempting to isolate an alkaloid or alkaloids indicates that they are present in very small quantity. It is believed, on the basis of experimental evidence, that the alkaloids may contain a phenolic hydroxyl group. Such a group, under the basic conditions which were used, would not allow the alkaloid to be extracted with organic solvents. Also, because of the basic nature of the alkaloids it would not be extracted from an acid solution.

In a recent private communication with Dr. Hofmann of Sandoz, Basel, Switzerland, it was learned that the Sandoz group has recently isolated two indole alkaloids from the drug ololiuqui in milligram quantities.

VII. PHYSIOLOGICAL EFFECTS OF THE SEED OF RIVEA CORYMBOSA

A. Introduction.

The presence of a physiological principle in the drug ololiuqui has been reported in several articles.^{3,4,5,7,17,37} The use of the drug by the natives of central Mexico has been well established.³⁶ The natives are reported to have used the drug as a general medicinal and as a narcotic. As a general medicinal the drug appears to have been a "cure-all". As a narcotic the drug was used in their religious activities and as a means to divine past and future occurrences. More recently, Osmond has reported on the physiological effects of the drug ololiuqui.³⁸ His report tends to substantiate the earlier reports.

B. Experimental.

Two research laboratories have investigated the physiological properties of the drug ololiuqui during the preparation of this thesis.

1) Under the direction of Dr. Kenross-Wright, a physiologist at Baylor University School of Medicine, an investigation of the physiological action of the drug ololiuqui was carried out using human and animal subjects. With the animals the drug was found to possess physiological activity. However, when the drug was administered to the human subjects, they became nauseated and no definite results were obtained.

2) More extensive investigations have been run at Granger Research Laboratories, Washington, D. C. It has been found there that the drug acts as a central nervous system stimulant. It was further found that the crystalline glucoside is approximately five times as effective as a central nervous system stimulant

as is the initial ethanolic extract of the drug.

VIII. SUGGESTIONS FOR FURTHER RESEARCH

- A. Research should be undertaken to identify the two unknown fatty acids.
- B. The relationship of the hydroquinone to other constituents should be determined.
- C. Selective degradation of the aglucone should be undertaken. This could be done by chemical and catalytic oxidation. Knowledge as to the exact position of the oxygens could thus be obtained.
- D. Degradation products of the aglucone obtained upon acid hydrolysis should be characterized.
- E. The alkaloidal material(s) should be isolated. It is believed this could be done by hydrolyzing the ethanol soluble material with dilute acid, followed by extraction with organic solvents, using conditions of basicity which would allow the extraction of a possible alkaloid which contains a phenolic hydroxyl group. A larger quantity of seed than has been available in this investigation should be used.

IX. SUMMARY

- A. The drug ololiuqui has been examined chemically and physiologically.
- B. The oil of Rivea corymbosa has been examined. The oil possesses two unknown fatty acids; otherwise, the fatty acid distribution is not unusual.
- C. From the ethanolic extract of the defatted drug, material has been isolated which upon sublimation will give a white, crystalline compound which has been characterized as hydroquinone.
- D. A white, crystalline glucoside has been isolated from the ethanolic extract of the defatted drug; the glucoside has been assigned the molecular formula of $C_{28}H_{46}O_{12}$. The glucoside was shown to contain no unsaturation or carbonyl functional groups. It was shown to contain seven hydroxyls and three ether functional groups. All ethers are cyclic or bridged ethers.
- E. The glucoside was hydrolyzed with emulsin.
- F. The sugar moiety was shown to be glucose.
- G. The aglucone was assigned a molecular formula of $C_{22}H_{36}O_7$. The aglucone contains four hydroxyls and three cyclic or bridged ethers. The aglucone was found to react with periodate, thus indicating at least two vicinal hydroxyl groups. On the bases of dehydrogenation studies, a hydrogenated pyranonaphthalene nucleus has been postulated for the carbon skeleton of the aglucone.
- H. Infrared spectra of the glucoside and aglucone are presented.
- I. Physiological studies of the drug are presented.
- J. Suggestions for further research are presented.

