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 filus 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 interferring substances
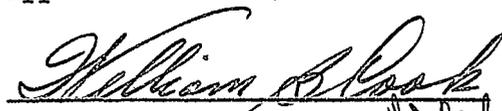
A STUDY OF THE CHEMICAL CONSTITUENTS AND OF THE PHYSIOLOGICAL
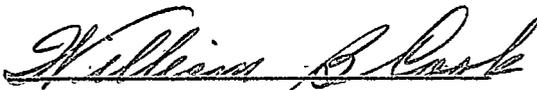PROPERTIES OF <u>RIVEA</u> <u>CORYMBOSA</u> (L.) HALLIER FILIUS.

by

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ABSTRACT

The seed of Rivea Corymbosa (L.) Hallier filus 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 interferring 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 <u>Rivea Corymbosa</u> 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, <u>peyote</u> (<u>Lophophora Williamii</u> (Lem.) Caulter), (2) the mushroom, <u>teonanactl</u> (<u>Paneolus campanulatus</u> L. var. <u>sphinctrinus</u> (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 pharmocologist of the Carolinian Medico-Surgical Institute in Stockholm, Sweden, Dr. B. P. Reko reported an example of the use of <u>Rivea corymbosa</u> 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 <u>Rivea corymbosa</u> 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, <u>ololiuqui</u> 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.

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*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] Berendt[1] and Standley[2] 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.

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*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 Euro-
pean 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 illus-
trated the plant. He described its use by the local natives rather
completely. However, it was not until 1649 that his complete ethnobio-
logical notes were published. His notes, which had been partially
translated into Spanish by Francisco Ximénez in 1615, were not pub-
lished 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

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[*]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 Diosorides* 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]

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*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 Lineaeus 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. Impomoea** corymbosa (L.) Roth Nov. Pl. Sp. Ind. Orient.
   (1821) 109.
6. Impomoea 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 infundilbuliform as hypocrateri morphous, 2-4 cms.
long and white or whitish. The lobes are entirely globrous.
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-

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* An illustration of the plant of Rivea corymbosa will be found in
Appendix III.
**In Appendix V will be found a differentiation between Impomoea 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 liter-
ature 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 extract-
ed a portion of the crushed seed of Rivea corymbosa with alcohol. The
extract was a clear, yellow solution. Santesson observed that the addi-
tion 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 mucila-
ginous 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-

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*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 con-

tains 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 gly-

cosides.

Santesson carried out some physiological experiments using an

aqueous extract of the seed of Rivea corymbosa. As test animals he used

frogs (Rana tempararia). 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 in-
> itially 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 diffi-

culty and breathed extremely heavily. The rabbits showed signs of increas-

ed salivation.

Reko, in his publication, suggested that ololiuqui actually con-

tained 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 hy-
drolysis. 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 neces-
sary to run some controlled experiments using a solution of sodium chloride.
The effects upon the test animals of the hydrolyzed material were approx-
imately 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 solu-
tion 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 pro-
tein and mucilage which had precipitated during the refluxing with the
hydrochloric acid. The resulting filtrate was tested with a sugar de-
tecting 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 corym-

bosa 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 hydrochlor-
> ic 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 Gutierres to a town named San Ferancdo (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-

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* 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 satis-

factory solvent, on the basis of solvent power, expense and the nonexplo-

sive 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 corym-

bosa_ was determined as follows (1,2-dichloroethane was used as the ex-

tracting 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%

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*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 %
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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.

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 inflamable, 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

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*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) tetrahydroforan 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 tempera-

ture between 125 and 135°. At this temperature the yellow material de-

composed, 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 capil-

lary. In a sealed capillary, the compound melted at 169-170°.*   2) The

acetate of the compound was prepared.** The acetate was purified by re-

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* 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 Folins-Ciocaltec 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 authenic 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.

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* 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,

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* 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) ozonalysis.*

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 embullioscopic 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

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

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± 0.3° (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.